Synthesis, pharmacological evaluation and structure-activity relationship of recently discovered enzyme antagonist azoles

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**ABSTRACT**

Global people are suffering from the legion of diseases. Cytotoxic property of the chemical compound would not solely influence effective drug properties and reduce unnecessary side effects. Proteins/enzymes responsible for microbe proliferation or survival are specifically targeted and inhibited successfully making the cells to undergo apoptosis. Furthermore, isoforms of essential enzymes have distinct physiological functions; thereby inhibition of essential enzyme isoforms is an apt way to the clinical approach of disease neutralization. Drugs are designed so as to play significant roles such as signaling pathways in the oncogenic process including cell proliferation, invasion, and angiogenesis. The present review comprises collective information of the recent synthesis of various organic drug compounds in brief, which could inhibit particular enzyme. The review also covers the correlation of the structure of a drug molecule designed and its inhibitory activity. Also, the most significant enzyme inhibitors are highlighted and structural moieties/core units responsible for remarkable inhibitory values are emphasized.

1. Introduction

Enzyme inhibitors are drug molecules capable of reducing the enzymatic activity by binding to the enzyme. Inhibition of an enzyme is crucial for pathogen's survival that could make a pathogen weak and destroy it subsequently. In addition to this, the enzyme inhibitors are also used to maintain metabolic balance in case of inhibition of metabolic enzymes when they are overexpressed. Besides this, some drug molecules bind to enzymes and enhance enzymatic activity. The mechanism of enzyme inhibition involves the reversible or irreversible binding of the drug molecule to an enzyme thereby ceasing substrate to enter enzyme's active site. The non-covalent type of interactions prevail in between reversible inhibitors and enzyme while covalent bonding is observed for irreversible inhibitor-enzyme complex; wherein modification of key amino acid residues required for enzymatic activity is usually noticed. In spite of the cruciality of enzyme inhibitors, these have been designed and synthesized continuously and it has been a potential area of research in biochemistry and pharmacology. The apt enzyme inhibitors should possess characteristics such as high specificity and potency. In the case of reversible inhibitors, some of the inhibitors have a remarkably similar structure with that of substrates. For instance, inhibitors of DHFR and protease inhibitors mimic the structures of the substrate. One more strategic design involves the design of enzyme inhibitors as transition state mimics possessing higher binding interaction than substrate-based designs.

The irreversible enzyme inhibitors most often possess reactive functional groups such as nitrogen mustards, aldehydes, haloalkanes, alkenes, Michael acceptors, phenyl sulfonates and fluorophosphonates. Hence the researchers have utilized the above mentioned information in the design and development of drug compounds as enzyme inhibitors. This derivative work includes synthesis of many series of compounds possessing enzyme inhibitory properties [5,6]. Since diverse chemical pharmacores prevail that could inhibit various enzymes, the enzyme inhibitors are described in different classes depending upon the designed structural entity [7,8]. The type of enzyme being inhibited is also accounted for in the classification. In each class of enzyme inhibitors, pharmacology, in particular, enzyme inhibition properties are described. Additionally, the structure and activity relationship (SAR) of potent enzyme inhibitors is discussed. The structural motifs attributed to noteworthy inhibitory results have been identified and highlighted in order to encourage further research and develop more efficient enzyme inhibitors.

1.1. [1,4] Dioxino [2,3-f] quinazoline derivatives

**c-Met and VEGFR-2** are the tyrosine kinases that are responsible for the signaling pathways in the oncogenic process including regulation of...
cell proliferation, invasion, and angiogenesis. Hence, the design strategy involves the preparation of the enzyme inhibitors that could inhibit c-Met and VEGFR-2 effectively. Dioxino [2,3-f] quinazoline derivatives have been designed as dual c-Met and VEGFR-2 inhibitors taking the consideration of pharmacological importance of cyclopropane-1,1-dicarboxamide moiety of cabozantinib or foretinib and the dihydro [1,4] dioxino [2,3-f] quinazoline core structure towards enzyme inhibition [1].

Synthetic route involves hydrolysis of one of the ester group of compound 1 by LiOH to get carboxylic acid derivative 2, followed by amidation at the carboxylic acid end with aniline/substituted anilines leading to β-amido ester 3a-j and the sequence of reactions was repeated wherein second ester functionality was cleaved resulting into β-amino ester 4a-j followed by amidation with various 3-substituted-4-hydroxyanilines rendering diamide derivative 5a-k to yield [1,4] dioxino [2,3-f] quinazoline derivatives 7a-m (Scheme 1).

The lead compounds 7a-7m were tested for enzyme inhibitory activity against VEGFR-2 kinase wherein good inhibitory potencies were exhibited by 7a (IC\textsubscript{50} = 18.9 nM), 7k (IC\textsubscript{50} = 3.5 nM), 7l (IC\textsubscript{50} = 8.8 nM) and 7m (IC\textsubscript{50} = 4.8 nM). The most potent inhibitors 7k and 7m (Figure 1) possessed comparable activities with that of reference compound cabo- zantinib (IC\textsubscript{50} = 3.6 nM) in which derivative 7k has almost identical inhibitory potential with that of the reference compound. The other derivatives have shown the IC\textsubscript{50} values in the range of 139–834 nM. Compounds 7e and 7f were totally inactive at the evaluated drug concentration. Based on the remarkable VEGFR-2 kinase inhibitory activity; compounds 7a, 7k, 7l, and 7m were chosen to investigate c-Met enzyme inhibitory activity. Surprisingly the selected derivatives are also active towards c-Met and IC\textsubscript{50} values were found to be 18.5 nM, 7.3 nM, 9.9 nM and 5.8 nM for compounds 7a, 7k, 7l, and 7m respectively. The derivative 7m stood atop amongst c-Met inhibitors and has higher potency compared to cabozaNTinib (IC\textsubscript{50} = 6.8 nM). The structure-activity relationship inferred that the substituent attached to the phenyl ring of cyclopropane-1,1-dicarboxamide moiety has a great impact on inhibitory activity wherein the derivatives with electron-withdrawing atom fluoro at p-position were notable inhibitors. While the presence of electron-withdrawing moieties at p-position has paved to reduced activities. All the four potent compounds have exhibited dual inhibitory activity. However when it comes to selectivity, compound 7k having methyl motif.

![Scheme 1. Synthesis of dual c-Met and VEGFR-2 inhibitors [1]; Reagents and conditions: (a) LiOH, MeOH/H\textsubscript{2}O, rt. 1h; (b) EDC, HOBt, DCM, aniline or substituted aniline; (c) LiOH, MeOH/H\textsubscript{2}O, rt.; (d) EDC, DMA, substituted or unsubstituted 4-aminophenol; (e) K\textsubscript{2}CO\textsubscript{3}, isopropanol.]

![Figure 1. Structures of most potent dual c-Met and VEGFR-2 inhibitors.](image-url)
at quinazoline 7-oxygen atom has selectivity towards VEGFR-2 while compound 7m possessing methoxethyl moiety at quinazoline 7-oxygen atom inclines towards c-Met.

1.2. 1,2,3-1H-Triazoles-linked 4H-pyrano[2,3-d]pyrimidine

The important classes of enzymes, namely, protein tyrosine phosphatases and protein tyrosine kinases are crucial for signal transduction of cellular processes such as immune response, metabolism, growth and gene transcription. Likewise, the other two protein kinase phosphatases (PtpA and PtpB) are reported to be secreted by MTB in infected human macrophages which take prime responsibility for the cause of mycobacterium tuberculosis. Further, these enzymes can be utilized as clinical targets for the design and development of potential drug molecules. Taken together with the medicinal properties of pyrano[2,3-d]pyrimidine moiety towards anti-tubercular activity and decent MTB PtpB inhibitory activity of 1,2,3-1H-triazole, a structural framework is designed anticipating better MTB PtpB inhibitory activity [2].

The synthetic route goes in this way; firstly three-component cyclization reaction of ester 8, malononitrile 9 and various aromatic aldehydes 10a-y has produced cyclic amino nitrile 11a-y wherein the adjacent amino and nitrile groups are utilized for intramolecular cyclization with acetic anhydride to form 4[H]-pyranopyrimidines 12a-y. Propargyl motif of propargyl bromide is appended to NH group of intermediate 12a-y by nucleophilic substitution yielding 13a-y which is subsequently tethered with D-glucose via triazole formation paving to lead compounds 15a-y (Scheme 2).

The synthesized derivatives are allowed to inhibit the MTB PtpB enzyme. Except for inactive compounds 15i-k, other derivatives have exhibited weak to remarkable inhibitory activity. The compounds 15g, 15t, and 15u yielded moderate activity with IC50 values of 9.52 ± 1.13 μM, 7.94 ± 0.23 μM and 7.51 ± 0.33 μM respectively. Further, remarkable inhibitory activity is observed for the compounds 15v (2.22 ± 0.23 μM), 15x (3.53 ± 0.19 μM) and 15y (1.56 ± 0.21 μM) (Figure 2) wherein most significant inhibitory activity is bestowed for compound 15y possessing –OMe, –OH, and –NO2 at 3-, 4- and 5-positions of phenyl ring respectively. SAR studies revealed that electron-donating –OH group at p-position of phenyl ring has the strongest impact on inhibitory activity excluding compound 15l which exhibited weak activity. A combination of the –OH and alkoxy moieties has resulted in noteworthy potencies (15v and 15x). Besides, the presence of the 5-nitro group along with –OMe and –OH groups at 3- and 4-positions elicited the highest activity.

Figure 2. Illustration of structures of remarkable MTB PtpB inhibitors.
1.3. 1,2,4-Triazole-5-one derivatives

Inhibition of tumoral carbonic anhydrases (CA) could lead to slowdown of metastasis and reduced cancer symptoms. However, most of the discovered CA inhibitors have also inhibited isoforms of CA and thereby reduced efficiencies are observed. In order to enhance the efficiency of the CA inhibitors, the pharmacologically significant phenolic motif and 1,2,4-triazole ring are brought together in a framework of 1,2,4-triazole-5-one derivatives [3].

Two series of 1,2,4-triazole derivatives have been designed wherein synthesis of the first series of compounds 18a-e commences from 1,2,4-triazole-N-amine 16. In the first step, triazole 16 is alkylated at N4 to give 4-heptyl-1,2,4-triazole 17 which on condensation with various 4-halosalicylaldehyde yielded hydrazone analogs 18a-e. In the second series, 4-heptyl-1,2,4-triazole 17 is deaminated to corresponding 1,2,4-triazole 19 (Scheme 3).

The deaminated derivative 19 is treated with ethyl bromoacetate to afford N-substituted-1,2,4-triazole derivative 20 followed by cleavage at ester linkage with hydrazine yielded semicarbazide analog 21. Ultimately the semicarbazide 21 is condensed with various 4-substituted salicylaldehyde to form compounds 22a-e.

The designed derivatives are tested for bovine carbonic anhydrase II potentials using reference compound sulfanilamide. Among the two series of inhibitors, hydrazine analogs 18a-e have shown significant inhibitory activity. Amongst them, 4-bromophenol analog 18c (Figure 3) has exhibited the most potent activity (67.07 \( \pm \) 0.01%) with IC\(_{50}\) value of 60.80 \( \mu \)M. However, its activity is moderate compared to sulfanilamide (93.00 \( \pm \) 0.04%) with IC\(_{50}\) value of 3 \( \mu \)M. The other derivatives have percentage inhibitions in the range of 18.41 \( \pm \) 0.03% to 64.97 \( \pm \) 0.05%. Moderate electron-withdrawing -Br atom at the p-position of the phenolic ring in addition to semicarbazide linker flanked by 4-alkylated 1,2,4-triazole and 4-bromophenol have enhanced its inhibitory property to a greater extent.

1.4. 1,2,4-Triazol-based benzothiazole/benzoxazole derivatives

The activated macrophages produce pro-inflammatory cytokines such as interleukins and TNF-\( \alpha \) which mediate the inflammation. p38 MAP, one of the isoforms of pro-inflammatory kinases is also included under the class of kinases, inhibition of which would be an efficient approach for reducing inflammation and chronic inflammatory diseases. In this conjunction anti-inflammatory and p38 MAP kinase inhibitory activities of heteroaryl scaffolds, benzothiazole and benzoxazole are utilized for the synthesis of 1,2,4-triazole-based benzothiazole/benzoxazole derivatives as p38 MAP kinase inhibitors [4].

Preparation of the final compounds 27a-n entails synthesis of benzothiazole/benzoxazole derivatives 23a-b and reserved for further use in Scheme 4. The scheme starts from esterification of phenoxy acetic acid to get ethyl 2-phenoxy acetate which is then treated with hydrazine affording semicarbazide 24. The reaction of compound 24 with various aryl isothiocyanates gave thiourea derivatives 25a-g and cyclization of these thiourea derivatives yielded triazole 2-thiols 26a-g. Initially, prepared compound 23a-b is appended to SH of triazole 2-thiol leading to 1,2,4-triazole-based benzothiazole/benzoxazole derivatives (Scheme 4).

The title compounds have been screened for p38 MAP kinase inhibitory activity using reference compound SB203580. Alongside percent inhibition of the protein is also carried out. The tested derivatives have found to inhibit in the range of 47.97–85.36% and the corresponding derivatives have p38 MAP kinase inhibitory activity in the range of 0.031–1.273 \% . Out of the evaluated compounds, noteworthy results were shown by the compounds 27b, 27d, 27i, and 27k (Table 1). These derivatives have potentials comparable to the SB203580; particularly compounds 27b and 27i (Figure 4) have greater inhibitory values than that of the reference compound.

SAR studies infer that in general electron-withdrawing groups have a higher impact on inhibitory activity. Again position of the electron-withdrawing group and its electronegativity are responsible for the

![Figure 3. Structure of 1,2,4-triazole-5-one derivative with significant CA II inhibitory activity.](image-url)
The strongest inhibition. The compounds 27b and 27i possessed fluoro group at p-position of the benzene ring directly attached to triazole have inhibited to a larger extent. In particular, combination of 4-fluoro group and benzothiazole (27b) has the perfect molecular structure for greatest inhibition. Decreased electronegativity of the group attached at benzene 4-position led to abated inhibitory values wherein 27d and 27k yielded decreased inhibitions. The derivatives with electron-donating groups have exhibited the least inhibitory activities.

1.5. 1,2,4-Trisubstituted imidazolinones

Hypoxia induces human carbonic anhydrase hCA IX expression in solid tumors viz., glioma, breast cancer, and colon cancer. Inhibition of hCA IX could be an attractive drug target approach; as such an act strongly suppresses the growth of both primary tumor phases as well as metastasis. Combining the CA inhibitory activity of benzenesulfonamide derivatives with the p38α MAPK inhibitory activity possessed by the imidazole resulted in 1,2,4-trisubstituted imidazolinones [5].

Initially amido acid functionality of benzamide acid derivatives 28a, b is cyclized in presence of p-substituted benzaldehydes to oxazolone ring derivatives 29a-d (Scheme 5); further, these derivatives are utilized as intermediates for the design of the target molecules. Among the four intermediates, first two intermediate compounds 29a and 29b are ring inserted with p-amino benzene sulfonamide producing compounds 31a and 31b. Target compounds 33a-f are obtained on the treatment of derivatives 31a and 31b with compounds 32a-c (Scheme 7).

All the synthesized molecules are evaluated for their p38α MAPK inhibitory activity using sorafenib. Most of the derivatives have exhibited
decent inhibition in comparison to reference. Out of them, the most potent inhibitory activity (IC$_{50} = 0.056 \mu M$) is bestowed by the compound 30h; about 28 fold highly potent compared to sorafenib (IC$_{50} = 1.58 \mu M$). Slightly lower inhibitory activity yet stronger activity amongst the evaluated molecules is shown by the derivatives 30c, 30g 31a, and 33e with identical activity (IC$_{50} = 0.14 \mu M$). These derivatives have 11-fold higher inhibitory potency than that of sorafenib. Compound 33c (IC$_{50} = 0.134 \mu M$) could also be included in the significant p38MAPK inhibitors list. Correlation of structure and activity revealed that most significant p38MAPK inhibitor 30h possesses 3,4-dimethoxybenzene and 4-(N-acetyl benzenesulfonamide motifs on its imidazolinone ring.

The designed derivatives are tested for CA inhibitory activity towards hCA isoforms, I, II, IV, and IX using acetazolamide (AAZ) and inhibitory values are represented as K$_i$ values. Surprising inhibitory activity is observed towards the four isoforms of CA wherein compound 31a (Figure 5) rendered most potent inhibitory activity towards all four CA isoenzymes CA I (K$_i$ = 95.0 nM), II (K$_i$ = 0.83 nM), IV (K$_i$ = 6.9 nM) and IX (K$_i$ = 12.4 nM). Dual inhibition is also observed in the case of compound 31a. Removal of the 3-methoxy moiety, removal of methyl 4-methoxybenzylidene group and deacetylation of sulfonamide of p38αMAPK inhibitor have converted into the dual p38αMAPK/CA inhibitory. This result indicated that primary benzenesulfonamide, 4-hydroxybenzylidene, and 4-methoxyphenyl moieties are most crucial for its dual inhibitory properties.

1.6. 1H-Pyrazolo [3,4-b] pyridine derivatives

Acetylcholinesterase (AChE) inhibition is the most attractive approach for the design of anti-AD drugs. 1H-Pyrazolo [3,4-b] pyridine derivatives have been reported to exhibit cyclin-dependent kinases, GSK 3 inhibition properties, and other prominent pharmacological activities. Considering this, novel 1H-pyrazolo [3,4-b] pyridine derivatives are designed as AChE inhibitors [6].

2-Chloro-3-cyano-pyridine 34 is cyclized to pyrazolopyridine-3-amine 35 using hydrazine which is subsequently treated with chloroacetyl chloride gave useful scaffold 36 for target compound design. p-Substituted aniline is treated with diester 37 to form a substituted product 38a, b and on cyclization yielded 4-chloroquinoline 39a, b which is allowed to undergo nucleophilic substitution reaction with piperazine affording 4-piperazinoquinolines 40a, b. The intermediate compounds 40a, b are converted into target compounds 41a, b using the initially synthesized compound 36 (Scheme 8).

Hydroxyl-triazolopyrimidine derivative 43 is chlorinated to get chloro analog 44 which on substitution reaction with piperazine has produced piperazinized triazolopyrimidine 45. The final compound 46 is synthesized from compound 45 using compound 36 (Scheme 9). 4,7-Chloroquinoline 47 on nucleophilic substitution with piperazine formed piperazine analog 48 and followed by second nucleophilic substitution with compound 36 has resulted in final compound 49 (Scheme 9). Similarly, the target compound 51 is synthesized from compound 34 with two successive nucleophilic substitutions using piperazine and compound 36 and first and second nucleophiles (Scheme 9). Finally, treatment of compound 36 with morpholine/N-methyl piperazine/piperidine resulted in target compounds 52–54 (Scheme 10).

The designed molecules are screened for AChE inhibitory activity using donepezil as a reference compound. Most of the derivatives have decent inhibitory activity, out of which compound 42 (IC$_{50} = 0.045 \pm 0.010 \mu M$), 46 (IC$_{50} = 0.022 \pm 0.010 \mu M$) have inhibitory activity as

![Scheme 5. Synthesis of intermediate compounds 29a-d; Reagents and conditions: 1) acetic anhydride, fused sodium acetate (100 °C).](image)

![Scheme 6. Synthetic route for the preparation of final compounds 30a-l [5]; Reagents and conditions: 1) glacial acetic acid, fused sodium acetate, water bath (100 °C).](image)
good as the donepezil (IC50 = 0.049 ± 0.05 μM). Significant inhibitory
tivity is shown by 7-chloro-quinoline analog 49 (IC50 = 0.012 ± 0.006
μM). To the surprise, simple molecules possessing pyrazolo-pyridine
moiety tethered to 6-membered alicyclic heterocycles via amide link-
age bestowed the most potent AChE inhibitory values. Compound
52 (IC50 = 0.0048 ± 0.001 μM) with pyrazolo-pyridine moiety linked to
piperidine is found to have 10-fold higher inhibitory potency compared
to donepezil. Alongside, the morpholine analog 54 (IC50 = 0.0049 ±
0.001 μM) has almost similar IC50 value with that of 52. Structure and
activity correlation inferred that inactiveness of compounds 41a
and 41b might be because of the presence of the ester functionality at
quinolone 3-position. However, modification of 41a, b involving removal of ester
group and simultaneous introduction of -Cl at quinolone 7-position paved
to the best inhibition properties of 49. A high negative inductive effect of
2-cyanopyridine could be the reason for the least inhibition property of
compound 51.

1.7. 2,4,5-Trisubstituted-1,2,4-triazole-3-one

Tyrosinase, a copper-containing metalloenzyme plays a prominent
role in the biosynthetic pathway of melanin pigment. Melanin is an
important pigment reported to be found in various animal parts such as
eyes, hair, and skin; it has been reported to protect human skin against
UV radiation. However, a higher amount of melanin production and
hyperpigmentation are responsible for dermatological disorders like
melasma, chloasma, freckles, etc. Thereby the drugs that inhibit tyrosi-
nase could reduce the problems associated with its hyperactivity. 1,2,4-

Triazole compounds have been reported as excellent tyrosinase in-
hibitors and are being used in cosmetics and pharmaceutical industry.
This tyrosinase inhibitory history of 1,2,4-triazoles has inspired to en-
gineer novel 2,4,5-trisubstituted-1,2,4-triazole-3-one scaffolds [7].

The design of the target compounds comprises, heptylation of com-
pound 16 to form compound 17 which is deaminated to afford 1,2,4-tri-
zazole derivative 19. Nucleophilic substitution of compound 19
with various substituted benzyl bromides resulted in N-benzyl triazolone
scaffolds 55a–d (Scheme 11).

In the tyrosinase inhibitory activity performed on target compounds
with kojic acid as a reference compound, compared to kojic acid (97.2 ±
0.2%) at 1M concentration, compounds 55a and 55b have exhibited
maximum tyrosinase inhibition percent of 52.9 ± 0.1% at 30 mM and
56.5 ± 0.9% at 6 mM concentration respectively. The corresponding IC50
values are 25 mM and 5 mM respectively for compounds 55a and 55b
respectively wherein compound 55a is 3.6 fold higher potent compared
to kojic acid (18 mM). As the maximum solubility of compound 55a is
6 mM, it cannot inhibit tyrosinase beyond 56.5%. The structure-activity
relationship shows that the 4-bromobenzyl motif has rendered
maximum tyrosinase inhibitory potency. While the exchange of the
substituent with higher electronegative groups at benzyl 4-position of
55a has decreased the inhibitory properties to a larger extent.

1.8. Aryl carboximidamides and 3-aryl-1,2,4-oxadiazoles

COX-1 and COX-2 are the cyclooxygenase isoenzymes that could
catalyze the synthesis of prostaglandins, thromboxane, and

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\text{Figure 5. Structure of significant p38αMAPK inhibitor 30h and p38αMAPK inhibitor/CA dual inhibitor 31a.}
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Scheme 8. Synthesis of target compounds 41a, b and 42 [6]; Reagents and conditions: (i) Hydrazine hydrate, EtOH, 60 °C; (ii) glacial AcOH, NaOAc, chloroacetyl chloride, RT; (iii) Benzene, 83 °C; (iv) POCl₃, 110 °C; (v) piperazine, MeOH, reflux; (vi) TEA, THF, reflux; (vii) piperazine, TEA, THF, 60 °C.

Scheme 9. Synthetic route for preparation of final compounds 49 and 51 [6]; Reagents and conditions: i) POCl₃, 110 °C; ii) piperazine, MeOH, reflux; iii) TEA, THF, reflux.
levuloglandins. Inhibition of COX enzymes would provide relief from inflammatory, pyretic, thrombotic, neurodegenerative disorders. In the literature, amidoxime and 1,2,4-oxadiazole possessing scaffolds have antihyperglycemic activity, anti-inflammatory properties, and other pharmacological effects. Thus, such pharmacologically significant moiety is made use in the design of aryl carboximidamides and 3-aryl-1,2,4-oxadiazoles as COX inhibitors [8].

Naproxen is appended to N'-hydroxy benzamidine affording aryl carboximidamides as one synthetic route. While the carboxylic acid and N'-hydroxyamidine functionalities of reactants respectively are used for cyclization to render oxadiazole derivatives (Scheme 12).

The COX-2 enzyme inhibitory activity is determined using celecoxib as a reference compound. All the aryl carboximidamides exhibited higher inhibitory potency compared to a reference compound (IC\textsubscript{50} = 42.60 nM). Among these evaluated derivatives, 58a, 58b, 58c, and 58f are the derivatives having noteworthy inhibitory potencies (Table 2); wherein compound 58c is approximately 6.6-fold higher potent than reference compound. Accordingly, the same derivatives have also rendered good 15-LOX inhibitory properties and the compound 58a (Figure 6) has bestowed with the strongest inhibitory activity. The structural significance of the potent inhibitor 58c includes the presence of the electron-donating -OCH\textsubscript{3} groups at 3,4-positions of benzamidine motif which bestowed that particular compound with the greatest inhibitory value. The replacement of the electron-donating groups with electron-withdrawing moieties has reduced the inhibitory activity gradually. In the case of LOX inhibitory activity, compound 58a is the most potent molecule possessing chloro group at 4-position of benzamidine moiety. However, the most prominent LOX inhibitor has a negative inductive group –Cl at benzamidine p-position. While the compound 58c stands next to the compound 58a probably due to the presence of two methoxy moieties (possessing negative inductive effect) at 3- and 4-positions of benzamidine motif. But an increase in electronegativity to a

### Table 2. COX-2 and 15-LOX inhibitory values of potent final compounds.

| Compound | COX-2 IC\textsubscript{50} (nM) | 15-LOX IC\textsubscript{50} (nM) |
|----------|-----------------|-----------------|
| 58a      | 7.15            | 1.77            |
| 58b      | 7.48            | 4.91            |
| 58c      | 6.40            | 2.07            |
| 58f      | 8.13            | 3.34            |
| celecoxib| 42.60           | 8.05            |

The COX-2 enzyme inhibitory activity is determined using celecoxib as a reference compound. All the aryl carboximidamides exhibited higher inhibitory potency compared to a reference compound (IC\textsubscript{50} = 42.60 nM). Among these evaluated derivatives, 58a, 58b, 58c, and 58f are the derivatives having noteworthy inhibitory potencies (Table 2); wherein compound 58c is approximately 6.6-fold higher potent than reference compound.

### Scheme 10. Synthesis of pyrazolo-pyridine derivatives 52–54 [6]; Reagents and conditions: Piperazine analogs, THF, 65 °C.

### Scheme 11. Synthetic route for design of 2,4,5-trisubstituted-1,2,4-triazole-3-one scaffolds [7]; Reagents and conditions: i) absolute ethanol, NaOEt/1-bromoheptane, reflux; ii) H\textsubscript{3}PO\textsubscript{2} solution, NaNO\textsubscript{2}, room temperature; iii) NaOEt/substituted benzyl bromides.

### Scheme 12. Schematic representation of the design of the target compounds 58a-f and 59a-f [8]; Reagent and reaction conditions: i) CDI, Acetonitrile, r.t. 3 h; ii) CDI, Acetonitrile, r.t. 3 h, then reflux 24 h.
larger extent in the case of compound 58e has resulted in weak inhibitory activity. When compared the aryl carboximidamides derivatives have displayed stronger inhibitory activity than that of oxadiazoles scaffolds towards both COX-2 and 15-LOX enzymes.

1.9. N1-(4-((7-(3-(4-Ethyl piperazine-1-yl)propoxy)-6-methoxyquinolin-4-yl)oxy)-3,5-difluorophenyl)-N3-(2-(2,6-difluorophenyl)-4-oxothiazolidin-3-yl)urea

Tyrosine kinases are expressed in both normal and malignant cells; wherein c-Met, Ron, c-Kit, AXL, and IGF-1R could be quoted as few tyrosine kinases whose inhibition properties have been determined. Therefore, the drugs with tyrosine kinase inhibition properties would be attractive chemotherapeutic agents. To develop newer and efficient chemotherapeutics, urea derivatives are designed as multi-tyrosine kinase inhibitors [9].

4-Chlorquinoline derivatives 59a-d are allowed to undergo nucleophilic substitution with substituted 4-nitrophenol or 4-nitro-1-naphthol to give ethereal derivatives of quinoline 60a-d in which nitro group is reduced to form corresponding amine scaffolds 61a-d. The carbamate derivatives 62a-d have resulted upon the treatment of compounds 61a-d with phenyl carbonchloridate. The carbamates 62a-d are hydrolyzed with hydrazine yielding semicarbazide analogs 63a-d which are on condensation with 2,6-difluoro benzaldehyde afforded benzylidine-semicarbazide hybrids 64a-d followed by cyclization with mercaptopropanic acid gave title compounds 65a-d (Scheme 13). In continuation, the intermediate compound 64d is utilized for the design of cyclized products. The use of 2-methyl mercaptopropanic acid, 3-mercaptopropionic acid

![Figure 6. Illustration of the structure of most potent LOX inhibitor (58a) and COX-2 inhibitor (58c).](image-url)

**Scheme 13.** Design and synthesis of multi-tyrosine kinase inhibitors [9]; i) substituted 4-nitrophenol or 4-nitro-1-naphthol, PhCl, reflux; ii) Fe, Conc. HCl, 95% EtOH–H2O, reflux; iii) phenyl carbonchloridate, CH2Cl2, pyridine, rt; iv) 80% NH2NH2⋅H2O, xylene, 70 °C; v) 2,6-difluorobenzaldehyde, i-PrOH, HOAc, reflux; vi) SiCl4, mercaptopropanic acid, CH2Cl2, reflux.
and 2, 2-dimethyl mercaptoacetic acid for cyclization of 64d produced 66a, 66b, and 66c respectively (Scheme 14).

Compound 61d is reused as intermediate wherein the amine functionality is converted to isothiocyanate yielding compound 67 which is reacted with hydrazine to produce corresponding thiourea derivative 68. The compound 68 is allowed to undergo a condensation reaction with 2,6-difluorobenzaldehyde, followed by cyclization with mercaptoacetic acid to yield compound 66d (Scheme 15).

4-Hydroxyquinoline derivatives 70a-c are allowed to undergo nucleophilic substitution with various secondary amines produced 71a–j which upon chlorination gave 4-chloroquinoline analogs 72a–j. Second nucleophilic substitution of compounds 72a–j with 2,6-difluoro-4-nitrophenol formed 4-aryloxy quinoline derivatives 73a–j which yielded corresponding amines 74a–j on reduction. Carbamates 75a–j have been resulted by the treatment of 74a–j with phenyl carbonchloridate and subsequent hydrolysis with hydrazine hydrate yielded 76a–j. Condensation products 77a–j are resulted by the reaction of compounds 76a–j with 2,6-difluorobenzaldehyde. The intermediate compounds 77a–j are cyclized with mercaptoacetic acid and 3-mercaptocrotonic acid to form final compounds 78a–j and 79a–c respectively (Scheme 16).

Considering cabozaunit as a reference compound, the designed derivatives are screened for c-Met kinase inhibitory activity against the cancer cell lines A549, HT-29, and MDA-MB-231. In the case of compounds 65a–d, identical percent inhibition towards c-Met is exhibited by compound 65d (Table 3) having 3,5-difluorobenzene moiety to that of reference. The compound 66c with thiazinone ring has shown better

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**Scheme 14.** Synthetic route for the preparation of the compounds 66a–c [9]; **Reagents and conditions:** i) SiCl₄, 2-methylmercaptoacetic acid, 3-mercaptoacetic acid or 2, 2-dimethyl-mercapto acetic acid, CH₂Cl₂, reflux.

**Scheme 15.** Synthesis of the thiourea derivative 66d [9]; **Reagents and conditions:** i) CSCl₂, NaHCO₃, H₂O, rt; ii) 80% NH₂NH₂⋅H₂O, CH₂Cl₂, rt; iii) 2,6-difluorobenzaldehyde, i-PrOH, HOAc, reflux; iv) SiCl₄, mercaptoacetic acid, CH₂Cl₂, reflux.
inhibition properties. Meanwhile, compound 78a possessing N-ethyl piperazine motif & thiazolone ring and compound 78f with diethylamine & thiazolone motifs have elicited excellent inhibitory properties towards all cancer cell lines.

Structure-activity relationship reveals that the significant c-Met inhibitory activity of compound 65d is attributed to the 3,5-difluoro-4-oxyaniline moiety. The particular compound has exhibited activity higher than previously reported 3-F analog which infers that second -F atom has boosted the inhibitory activity. Change in the halogen atom and its position resulted in diminished activity than that of compound 65d. In the 66a-d series of compounds, thiazinone ringed molecules displayed the most prominent activity. Other molecules of this series with methyl

Scheme 16. Synthesis of compounds 78a-j and 79a-c; Reagents and conditions [9]: i) amine, CH₃CN, reflux; ii) POCl₃, reflux; iii) 2, 6-difluoro-4-nitrophenol, PhCl, reflux; iv) Fe, Conc. HCl, 95% EtOH–H₂O, reflux; v) phenyl carbonochloridate, CH₂Cl₂, pyridine, rt; vi) 80% NH₂NH₂⋅H₂O, xylene, 70 °C; vii) 2,6-difluorobenzaldehyde, i-ProH, HOAc, reflux; viii) SiCl₄, mercaptoacetic acid or 3-mercaptopropanolic acid, CH₂Cl₂, reflux.
thiazole (66a) and dimethylthiazole (66b) ring shown reduced inhibitory activity. In other series of derivatives 78a-j, N-propyl pyrroline ring at 7-position of quinoline moiety is substituted by various cyclic secondary amines in a combination of different alkyl chains. The maximum inhibitory properties have been elicited by the compound 78a (Figure 7) with N-ethyl-N-propyl piperazine motif. Likewise, compound 78f possessing N, N-diethylyamine exhibited decent inhibitory effects whose potential is next to the potency of 78a. When it comes to structural modification, varying of secondary amines and alkyl chains for remaining derivatives shown weak activity. Even with identical moiety N-ethyl-N-propyl piperazine in the compounds 78i and 78j, they failed to exhibit good inhibitory activity as they have ethyl and butyl chains respectively. Also abated inhibitory effects are observed with N-methyl piperazine motif in case of compound 78d with propyl chain. In the series 78a-j, semicarbazide functionality is transformed to thiosemicarbazide to check the c-Met inhibitory properties. Unfortunately, no single compound has enough inhibitory effect which could be comparable to compound 78a. Out of all the final compounds, designed compound 78a is found to be more apt as a c-Met inhibitor wherein it is 27-fold and 157-fold higher potent towards A549 and HT-29 respectively compared to the reference compound. While approximately 4.3-fold stronger inhibitory activity is observed for the MDA-MB-231 cell line. In the same way compound 78f has 21-fold, 126-fold and 3.5-fold greater potencies towards A549, HT-29, and MDA-MB-231 cell lines respectively compared to cabozantinib.

Considering the most potent activity of compound 78a, it is chosen for inhibitory screening towards other tyrosine kinases such as c-Met, Ron, c-Kit, KDR, HER-2, ALK, c-Src, IGF-1R, EGFR, and AXL. Except for a few kinases such as HER-2, ALK, and IGF-IR, the compound 78a met with good expectations (Table 4). Amongst them, the compound 78a has elicited the most potent activity towards the kinase Ron. Thereby it can be a perfect multi-tyrosine kinase inhibitor.

### 2. 5,6-Dichloro-2-methyl-1H-benzimidazole derivatives

Urease is a redox metalloenzyme present in most of the plants and animals; wherein it causes diseases such as pylonephritis, urolithiasis, duodenal ulcer, chronic gastritis, etc. Hence to counteract these negative effects of urease, pharmaceutical drugs with urease inhibitory effects are most essential and thereby such inhibitors are designed and synthesized. Compounds such as imidazoles, hydroxamic acid, phosphoramidites, humic acid and 1,4-benzoquinone can be included as some of the important urease inhibitors. Further, urease inhibitory potency of 5,6-dichlorobenzimidazole scaffolds, benzyl derivatized benzimidazoles and 4,5-dichlorobenzimidazole analogs possessing cyclopropyl ring at C2 position has triggered to design and investigate novel 2-methyl benzimidazole derivatives [10].

5-Chloro-2-methyl benzimidazole 80 is nucleophilically treated with bromoethyllacetate forming N-substituted benzimidazole 81 in which ester group is transformed to semicarbazide 82 and on subsequent reaction with substituted thiocyanates afforded thiosemicarbazide derivatives 83a-g. Finally, the cyclization reaction of the derivatives 83a-g with NaOH/NaHCO3 yielded N1-methylene triazole benzimidazoles 84a-g (Scheme 17).

All the synthesized benzimidazole derivatives are tested for their anti-urease activity in the presence of the standard urease inhibitor thiourea and found to be good urease inhibitors (IC50 = 0.0294 ± 0.0015 to 0.1494 ± 0.0041 μM). Unsubstituted benzimidazole 80, N-ester substituted compound 81, its semicarbazide derivative 82 and compounds 84a, 84b have weak inhibitory activity compared to subsequent analogs. Other derivatives have shown significant effects. The most potent urease inhibitory property is displayed by the compounds 83e (IC50 = 0.0354 ± 0.0017 μM) and 84e (IC50 = 0.0294 ± 0.0015 μM). Compound 84g can also be included in potential urease inhibitors list with IC50 value of 0.0357 ± 0.0015 μM.

Correlation between evaluated structures and their inhibitory values show that compounds 80, 81, and 82 do not yield any fruitful results as they lack the pharmacological significant functionality. Attachment of substituted thiosemicarbazide functionality has resulted in enhanced inhibitory activity in the case of compounds 83a-j. Particularly compound 83e (Figure 8) possessing 4-nitrobenzene connected to thiosemicarbazide moiety has bestowed with the greatest activity. Alkyl and simple phenyl as well 4-OCH3, 4-F and 3-I phenyl analogs exhibited little reduced activity. The corresponding cyclized molecules triazole-thiol derivatized benzimidazoles 84a-j could reach up to the mark except for N-alkyl triazolethiol-benzimidazoles 84a and 84b. Out of the potent molecules of this series, compound 84e has turned out as a noteworthy molecule. Again electron-withdrawing 4-nitrobenzene motif has rendered significant urease inhibitory activity.

#### 2.1. 5-Arylisothiazol-3(2H)-one-1,(1)-(di)oxides

Overexpression of carbonic anhydrases such as hCA IX and XII have shown in certain cancer types. A cyclic secondary sulphonamide, 1,2-benzoisothiazol-3(2H)-one-1,1-dioxide has been described as a selective hCA IX inhibitor. Inspired by the CA inhibitory activity of N-substituted

### Table 3. c-Met inhibitory properties of most potent compounds.

| Compd. | c-Met Inhibition (%) | IC50 (μM) |
|--------|----------------------|-----------|
|        |                      | A549      | HT-29 | MDA-MB-231 |
| 65d    | 79.06                | 0.56 ± 0.048 | 0.23 ± 0.022 | 8.35 ± 0.86 |
| 66c    | 68.95                | 0.40 ± 0.058 | 0.27 ± 0.019 | 9.65 ± 0.78 |
| 78a    | 83.82                | 0.35 ± 0.029 | 0.073 ± 0.0086 | 3.10 ± 0.47 |
| 78f    | 78.57                | 0.45 ± 0.051 | 0.091 ± 0.0096 | 3.79 ± 0.51 |
| Cabozantinib | 80.76 | 9.52 ± 0.91 | 11.5 ± 1.1 | 13.4 ± 1.2 |

### Table 4. Multi-tyrosine kinase profile of compound 78a.

| Kinase   | IC50 (μM) | Kinase   | IC50 (μM) |
|----------|-----------|----------|-----------|
| e-Met    | 0.015     | ALK      | >10       |
| Ron      | 0.0029    | c-Src    | 0.24      |
| c-Kit    | 0.064     | IGF-1R   | 2.1       |
| KDR      | 0.85      | EGFR     | 0.52      |
| HER-2    | >10       | AXL      | 0.053     |

![Figure 7. Structure of potent multi-tyrosine kinase inhibitor.](image-url)
saccharin derivatives, a series of N-tert-butylisothiazolones are reported to have CA inhibitory activities [11].

**Scheme 17.** Synthetic route for the preparation of the final compounds 84a-g [10]; Reagents and conditions: i) bromoethyl acetate, K₂CO₃, acetone; ii) NH₂NH₂, EtOH, reflux; iii) R-CN, EtOH, reflux; iv) 2N NaOH/1M NaHCO₃.

![Synthetic pathway](image)

Figure 8. Structures of the remarkable urease inhibitors.

**Scheme 18.** Synthetic pathway for the preparation of the compounds 90 and 91 [11]; Reagents and conditions: i) SOCl₂, dry CH₂Cl₂, rt (10 min), 80 °C (2.5–3h); ii) tBu-NH₂, dry CH₂Cl₂, 80 °C (12h); iii) SO₂Cl₂, CH₂Cl₂CH₂Cl, rt (24h); iv) m-CPBA (1.4 eq), CH₂Cl₂; v) m-CPBA (3.0 eq).

The newly designed derivatives are allowed to inhibit isoenzymes hCA I, hCA II, hCA IX and hCA XII of CA using reference compound AAZ. No single compound has possessed significant inhibitory activity towards hCA I, hCA II isoenzymes. A poor activity is shown by the compound 90 and 95i towards hCA I and hCA II enzymes respectively. However, few derivatives are successful in exhibiting remarkable inhibitory activity towards hCA IX and hCA XII. Compared to reference (Kᵢ = 12 nM) compound 93i (Kᵢ = 8.8 nM) is significant hCA IX inhibitor. Besides these, derivatives 93a and 95i have been reported to possess excellent hCA IX inhibitory properties with Kᵢ values of 4.5 nM and 4.3 nM respectively. The inhibitory properties of compounds 93a and 95i are approximately threefold higher potent than the reference compound. While the CA inhibition towards XII, some derivatives have displayed decent activity. In that, compound 93a (Kᵢ = 4.3 nM) has exhibited slightly higher activity than the reference compound. The excellent inhibitory activity is elicited by the compound 93i (Figure 9) with Kᵢ value of 0.76 nM whose inhibitory activity is 7.6 times stronger than that of AAZ. Other significant inhibitors include compounds 95a and 95i (Figure 9) having Kᵢ values of 0.97 nM and 0.94 nM respectively; corresponding Kᵢ values are 5.8 fold stronger compared to AAZ.
Whatever the structure and its modification of the inhibitor, no significant inhibition is observed toward both hCA I and hCA II enzymes. Among the isothiazole monoxides, the derivative 93i possessing (N-sulfonylphenyl)-benzimidazole connected via 3-position shown stronger inhibitory effects than the standard compound. The identical motif attachment to the isothiazole ring via 2-position (compound 93h) has shown fivefold diminished activity. Other heterocycles containing isothiazoles with heteroatoms such as S (93e) or O (93f) displayed abated inhibitory activity. Also, the substitution of the phenylsulfonyl moiety with –Boc (93g) has not resulted in a good inhibitory property. Surprisingly, simple phenyl substituted isothiazole scaffold 93a has turned to be the most potent IX inhibitor. Further oxidation of compound at sulfur led to the formation of derivative 95i in which the inhibitory activity ($K_i = 4.3 \text{ nM}$) is doubled compared to that of compound 93i. When it comes to structure-activity relationship towards hCA XII, simple phenyl substituted analog 93a ($K_i = 4.3 \text{ nM}$) is successful in inhibition of hCA XII and its activity is comparable to reference compound ($K_i = 5.7 \text{ nM}$). Compound 93i with decent hCA IX inhibitory activity has got its inhibitory activity extended to the hCA XII enzyme also. A little reduced activity is rendered by the dioxide analog 95i of compound 93i which reveals that little significance has prevailed with monoxide and dioxide isothiazole derivatives. However, a difference in inhibitory activity is seen in between simple phenyl isothiazole monoxide 93a and its dioxide analog 95a. It indicates that the dioxide fragment is crucial for potential hCA XII inhibitory activity. Finally, N-debutylated isothiazole scaffolds are not found to be good hCA inhibitors.

### 2.2. Anti-fungal azoles

Fungal infections have become most common in animals and plants in which *Candida albicans* is a most general fungal pathogen. To address these serious issues, azoles are the chemical agents that have been engineered. Lanosterol-14α-demethylase, a prominent enzyme of fungus (a major fungal plasma membrane component) is essential for the synthesis of ergosterol. Lanosterol-14α-demethylase has been targeted for the design of the potent anti-fungal agents. Considering this, reported antifungal agents are assessed for their lanosterol-14α-demethylase inhibitory activity [12]. Here the enzyme inhibitory activity is correlated with the molecular descriptors; wherein the principal component analysis method is employed. Using this method, lanosterol-14α-demethylase inhibitory activity (IC$_{50}$ values) of the various anti-fungal agents is judged with proper justification.

In this method, the anti-fungal activity of eachazole anti-fungal agent is analyzed by three major factors/components; wherein the first principal component demonstrates the molecular complexity of the anti-fungal agents. The second principal component indicates hydrogen bond interactions, topological polar surface area, and lipophilicity. Finally, the third principal component explains the undefined atom stereocenter in addition to lipophilicity. The first, second and third components are accounted for in the proportion of 51.79:25.50:11.38 respectively. The assessment of the various anti-fungal agents indicated that Itraconazole (Figure 10) is found to have better comprehensive value and lowest IC$_{50}$ value among the other anti-fungal agents (Table 5). The

![Scheme 19. Synthetic route for preparation of target 5-arylisothiazole oxide scaffolds; Reagents and conditions: i) Ar-B(OH)$_2$ (2.0 eq), K$_2$CO$_3$ solid, 80°C/GPA: Pd(PPh$_3$)$_4$, THF or GPA: PdCl$_2$(dpdf), CH$_2$Cl$_2$, DME, sealed tube; ii) TFA, MW, sealed tube.](image_url)

![Figure 9. Structures of 5-arylisothiazoles as most significant CA IX and XII inhibitors.](image_url)
itraconazole is followed by the flucloxazole and ketocoxazole in the potential comprehension values list. The results inferred that IC_{50} is not only the factor to look for the design of a potent drug molecule but it is the comprehensive factor, the balance of the three principal components. Although the Flusilazole, Imazalil, Miconazole, and Penconazole have decent IC_{50} values but ranked 14, 15, 16 and 17 respectively that reveals that a great significance lies with the first principal component.

2.3. 1,2-Benzisothiazol-3(2H)-one-1,1-dioxide derivatives

The COX inhibition properties of saccharin and pyrazole derivative celecoxib have been demonstrated. Similarly, pyrazolyl benzene sulfonamides connected to thiazolidinones and pyrazoles exhibited higher COX-2 selectivity. Here in 1,2-benzoisothiazol-3(2H)-one-1,1-dioxide derivatives have been designed and synthesized as COX-1/COX-2 inhibitors [13].

The sodium salt of saccharin, benzylated at N-position formed N-benzylated saccharin 98, which on hydrolysis using hydrazine given hydrazide analog 99. Subsequently, compound 99 is utilized as intermediate for the design of target compounds. In one way, compound 99 is allowed to undergo intramolecular cyclization to produce oxadiazole thiol 100, followed by ethyl/benzyl substitution on thiol functionality gave oxadiazole thiol derivatives 101a-b. Again, intermediate 99 is utilized in one more way, wherein thiosemicarbazide derivative 102 is synthesized from compound 99 using CS_{2} intra-molecular cyclization of which resulted in triazole-thiol 103. The condensation of compound 103 with various 4-substituted benzaldehydes paved to triazole imines 104a-d (Scheme 20).

N-Isopropylation of saccharin produced compound 105 followed by hydrolysis with hydrazine has given hydrazide derivative 106. Hydrazide functionality is utilized for intramolecular cyclization with benzoylacetoacetate and benzoyl acetone to render the final compounds 107 and 108 respectively (Scheme 21). Diethyl malonate is attached to saccharin forming N-substituted saccharin 109. Diaster functionality is transformed into dihydrate analog 110, in which the hydrazide fragments are cyclized to pyrazole modified saccharin 111 as a first part. The second part involves condensation of the dihydrate analog with 4-substituted benzaldehydes to form diimine derivatives of saccharin 112a-c (Scheme 22).

The designed compounds have been allowed to inhibit COX-1/COX-2 enzymes in the presence of the standard COX inhibitor celecoxib. Almost every single compound synthesized has exhibited higher potential (IC_{50} = 1.98–12.23 μM) in comparison with celecoxib (IC_{50} = 14.8 μM). However, amongst the competing inhibitors, the isothiazole imines 112a (Figure 11) and 112b exhibited the most potent COX-1 inhibitory activity with IC_{50} values 1.98 μM and 2.78 μM respectively. However, not a quite good inhibitory activity is displayed by the evaluated molecules towards the enzyme COX-2 wherein the inhibitory activity is in the range of IC_{50} = 0.05–0.71 μM. Compound 107 has shown some significance (IC_{50} = 0.09 μM) yet half the activity exhibited by reference. The derivatives 108 (IC_{50} = 0.06 μM) and 111 (IC_{50} = 0.05 μM) are successful in rendering strongest inhibitory activity which are almost similar to that of standard compound (IC_{50} = 0.05 μM).

The compound 103 possessing benzylated sulfonamide and triazole-thiol moiety exhibited decent activity (IC_{50} = 2.98 μM). Further structural modification comprising condensation products 104a-d displayed diminished inhibitory effects. However, unexpectedly gradual increased inhibitory activity is observed for the compounds 104a-d with an increase in bulkiness with respect to benzene 4-position. The activity is desperately reduced in the case of pyrazole derivatives 107, 108 and 111. It indicates that pyrazole derivatives are not apt candidates for COX-1 inhibitory activity. Benzyliyde-hydrazide derivative of saccharin 112a is found to be the most potent COX-1 inhibitor. But the inhibitory properties are reported to be reduced a little for compound 112b with –F

Table 5. Principal component analysis of azoles as anti-fungal inhibitors.

| Anti-fungal drug | Principal Component Score | Component 1 | Component 2 | Component 3 | Comprehensive | Rank | IC_{50} (μM) |
|------------------|---------------------------|-------------|-------------|-------------|---------------|------|-------------|
| Itraconazole     | 11.0063                   | 1.4266      | 3.1907      | 6.4271      | 1             | 0.039 |
| Fluconazole      | 7.3476                    | 3.8262      | 0.1646      | 4.7998      | 2             | 0.051 |
| Ketoconazole     | 8.1369                    | 0.9626      | 1.7690      | 4.6609      | 3             | 0.064 |
| Bitertanol       | 6.7464                    | 1.3138      | 2.9667      | 3.8957      | 4             | 0.330 |
| Triadimethol     | 6.2233                    | 1.3138      | 2.9667      | 3.8957      | 5             | 0.330 |
| Propiconazole    | 6.3124                    | 0.5664      | 3.3113      | 3.7700      | 6             | 0.150 |
| Triadimethol     | 6.2432                    | 0.9526      | 1.9976      | 3.7036      | 7             | 0.130 |
| Thibucanazole    | 6.3006                    | 0.6124      | 2.3679      | 3.6887      | 8             | 0.350 |
| Hexaconazole     | 6.2842                    | 0.6124      | 2.3679      | 2.3679      | 9             | 0.066 |
| Cyproconazole    | 5.8822                    | 1.0154      | 2.8885      | 3.6336      | 10            | 0.100 |
| Mycolbutanil     | 6.2899                    | 0.5310      | 2.0388      | 3.6249      | 11            | 0.140 |
| Epoxiconazole    | 5.9337                    | 0.6880      | 3.0079      | 3.5908      | 12            | 0.220 |
| Prochloraz       | 6.3856                    | 0.3572      | 1.8924      | 3.4314      | 13            | 0.098 |
| Flusilazole      | 5.8708                    | 0.3585      | 1.5222      | 3.3051      | 14            | 0.085 |
| Imazalil         | 5.8969                    | -0.4431     | 2.4090      | 3.2151      | 15            | 0.082 |
| Miconazole       | 6.0850                    | -1.1988     | 3.0261      | 3.1901      | 16            | 0.072 |
| Penconazole      | 5.4482                    | -0.7162     | 2.6559      | 2.9412      | 17            | 0.076 |
| Bifonazole       | 4.8339                    | -1.4026     | 2.8204      | 2.4668      | 18            | 0.300 |
| Clotrimazole     | 4.8796                    | -1.5034     | 2.0570      | 2.3779      | 19            | 0.091 |
substitution at benzylidine 4-position. Again the activity lowered greatly for \textit{112c} with –Cl substitution inferring that inhibitory activity reduced with elevated bulkiness at benzylidine 4-position. In the case of COX-2 inhibition properties, only the pyrazole analogs \textit{107}, \textit{108}, and \textit{111} have shown good COX-2 inhibitory effects. Comparatively compound \textit{107} possessing pyrazolone ring in combination with \textit{N}-isopropyl sulfonylamine fragment rendered half the inhibitory activity to that of pyrazole analog \textit{108}. But the compound \textit{111} having two pyrazole rings and an intact saccharin ring has bestowed strongest COX-2 inhibitory activity. These facts indicate that COX-2 selectivity is observed for the pyrazolone ring compounds and benzylidine-hydrazide analogs are selective COX-1 inhibitors.

2.4. Benzene sulfonamides linked quinazoline scaffolds

Carbonic anhydrases \textit{hCA} IV, IX and XII have been reported for their involvement in rheumatoid arthritis. The transmembrane isoforms \textit{hCA} IX/XII are demonstrated to have overexpressed in many hypoxic tumors. The quinazolinone pharmacore has been utilized for the design of the CA inhibitors. 4-(Quinazolin-4-ylamino) benzene sulfonamides and 4-(quinazolin-4-yl oxy) benzenesulfonamide exerted decent cytosolic \textit{hCA} I & II and the transmembrane \textit{hCA} IX & XII inhibitory properties. Based on the above rationale, a new series of 2-substituted-mercapto-3-substituted-4(3H)-quinazolinones appending benzenesulfonamide moiety to it \textit{[14]} is designed.
The synthesis of compounds 114a-j and 115a-j comprises treatment of N3-benzyl-quinazolinone thiol scaffolds 113a-e with sulfonamide derivatives possessing chloroanilide functionality at 4-position to get corresponding derivatives 114a-i. In the second part, compounds 113a-e have been allowed to undergo nucleophilic substitution with sulfonamide derivatives tethered to chloroanilide functionality via ethylene chain producing compounds 115a-j (Scheme 23). Secondly, quinazolinone-2-thiol connected with 3,4,5-trimethoxybenzyl moiety at N3-position 116 is reacted with 4-chloroanilide substituted benzene-sulfonamides to produce compounds 117a-b; while its nucleophilic substitution with sulfonamide derivatives linked to chloroanilide functionality via methylene chain resulted in compounds 118a-b (Scheme 24). C6, N3-Substituted quinazolinone-2-thiol derivatives 119a-g

Scheme 22. Synthesis of the final compounds 111 and 112a-c [13]; Reagents and conditions: i) diethyl 2-bromomalonate, DMF, reflux (2h); ii) NH2NH2, rt (20min); iii) benzoyl acetone, EtOH, AcOH, reflux (12h); iv) Ar-CHO, EtOH, AcOH, reflux (2h).

Figure 11. Structure of compound 112a possessing COX-1 inhibitory activity.

Scheme 23. Preparation of target compounds 114a-j and 115a-j [14].
In the pharmacological activity towards CA, the compound 114f has elicited the most potent hCA I inhibitory activity (Ki = 39.4 nM) sixfold higher potency than the reference compound AAZ (Ki = 250.0 nM). While the compounds 115a (Ki = 78.2 nM), 120a (Ki = 86.6 nM), and 120c (Ki = 83.9 nM) have almost similar inhibitory activities which are significantly greater than AAZ. The remarkable hCA II inhibitory effect (Ki = 3.30 nM) is displayed by the compound 114f. Only a few of the evaluated compounds could inhibit hCA II effectively. To mention, compounds 115i (Ki = 6.90 nM) and 115j (Ki = 5.20 nM) exhibited noteworthy inhibitory properties. Similar activity is shown by the derivative 120c (Ki = 6.20 nM). The excellent inhibitory activity (Ki = 0.73 nM) is bestowed by the compound 120a which possessed 16.5-fold stronger inhibitory effect in comparison with AAZ (Ki = 12.0 nM). The most of screened molecules have exhibited almost similar hCA IX inhibitory values compared to AAZ (Ki = 25.0 nM). Out of those compounds, 120a (Ki = 1.80 nM) and 120c (Ki = 1.60 nM) (Figure 12) are reported to possess the strongest inhibitory potentials with 14- and 15-fold better inhibitory potentials respectively than that of reference. Not so good hCA XII inhibitory effects are exerted by the synthesized derivatives. However, a few compounds could reach the inhibitory activity of the standard compound AAZ (Ki = 5.70 nM). Amongst them, compounds 114e (Ki = 7.60 nM) and 115g (Ki = 5.20 nM) stood top of the hCA XII inhibitors.

The compound 114f possessing 6,7-dimethoxy quinazolinone-2-thiol and propanilide benzenesulfonamide moiety has the greatest hCA I inhibitory activity. However, removal or substitution of –OCH3 groups by other groups has significantly reduced the activity. Amongst the few potent hCA II inhibitors, 120a has turned out to be strongest inhibitory molecule. Structurally it possesses benzene sulfonamide hydrazone of ketone thiol at 2-position and benzene at N3-position of simple quinazolinone motif. In this series, substitution at 6-position of quinazolinone ring shown abated activity except for 6-methoxy substituted compound 120c which exhibited satisfactory inhibitory value. Except for compounds 114a & 114b, all the molecules of 114a-j and 115a-j have displayed satisfactory inhibitory properties (Ki = 5.0–65.2 nM). While deteriorated activities are observed for compounds 117a-b and 118a-b.

[Scheme 24. Synthetic route for the preparation of the compounds 117a-b and 118a-b [14].]

[Scheme 25. Schematic representation of compounds 119a-g and 120a-g [14].]
which might be attributed to sterically hindered 3,4,5-trimethoxybenzyl moiety connected at quinazolinone N3-position. Excluding for a few compounds, all the tested derivatives possessed decent hCA IX inhibitory properties irrespective of their structural variation. However, compound 120a and 120c appended with phenyl ring at N3-position and benzenesulfonamide hydrazone of ketone thiol rendered finest inhibitory activity. In case of inhibitory activity towards hCA XII, no more than a few derivatives displayed good inhibitory potencies; particularly 115g has shown strong impact which is attributed to the 6-iodoquinazolinone tethered to benzenesulfonamide via N-ethylthioacetamide fragment. Decreased/increased fragment chain length or change in the substitution at 6-/or 7-positions of quinazolinone resulted in lowered activities. An overall observation of the structure and its activity reveals that 114a-b, 117a-b, and 118a-b have shown inactivity towards all isoforms of CA. While the compounds 120a and 120c have good inhibitory potential towards all isoforms of the enzyme which infer that hydrazone of keto-thiol is crucial for CA inhibitory activity.

2.5. Carbazole and hydrazone derivatives

Tyrosinase enzyme is involved in the hyperpigmentation of the skin; melasma, flecks, lentigo, nevus, ephealis, and melanoma of pregnancy are few skin disorders to be mentioned. To address these issues, tyrosinase inhibitors are being designed and synthesized. Carbazole-substituted chalcone urea derivatives are demonstrated as decent tyrosinase inhibitors. Also, hydrazone-bridged thiazole-pyrazole scaffolds have shown to possess inhibition properties towards tyrosinase. In this context, tyrosinase inhibition properties of the previously synthesized [15, 16, 17] carbazole and hydrazone-bridged thiazole-pyrazole derivatives are described [18].

The synthesized derivatives are screened for their mushroom tyrosinase inhibitory activity using kojic acid as standard inhibitor (Scheme 26). Out of the twelve derivatives prepared, compound 121a (Ki = 1.64 ± 0.03 μM) has exhibited most potent tyrosinase inhibitory activity with 2.8-fold higher potency compared to kojic acid (Ki = 4.43 ± 0.20 μM). Almost similar inhibitory effect (Ki = 4.34 ± 0.04 μM) is exerted by the derivative 121c compared to that of the reference compound. Compound 121d (Ki = 3.46 ± 0.07 μM) could also exhibit significant inhibitory activity. The potential activity is observed in the case of carbazole analogs; particularly compound 121a possessing N-ethyl carbazole appended to benzimidazole via thiopropanamide linker exhibited most potent inhibitory activity. The substitution of benzimidazole with benzothiazole resulted in compound 121b with reduced inhibitory activity. While benzoxazole derivative 121c has some improvement but yet diminished activity compared to compound 121a. Further improved activity is exhibited by thiazole derivatized carbazole 121d. Another series of

![Diagram of potent CA inhibitors](image-url)

**Figure 12.** Structures of potent CA inhibitors.

![Scheme 26. Illustration of the structures of carbazole and hydrazone-bridged thiazole-pyrazole derivatives](image-url)
carbazoles 122a-c synthesized wherein the 4-substituted aromatic rings are connected through propanamide oxide have shown moderate inhibitory activity. It indicates that thiopropanamide fragment bestowed the strongest inhibitory effects. The series of molecules entailing pyrrole ring connected to thiazole by hydrazone-bridge 123a-d have also failed to satisfactory results. In these derivatives, compound 123d possessing 4-chlorophenyl ring attached to thiazole at 3-position shown inhibitory activity almost similar to that of the reference compound.

2.6. Carbazole-imidazole derivatives

α-Glucosidase, a digestive enzyme functions in the cleavage of the α-1,4-glycosidic bonds of polysaccharides with subsequent conversion into glucose. Control of blood glucose levels in type 2 diabetes patients is very necessary; in this regard monitor of α-glucosidase function is the foremost objective. Hence, α-glucosidase is an apt target for the design and discovery of therapeutic drugs. Based on the α-glucosidase inhibitory activity of carbazole analogs and pharmacological importance of imidazole, novel fused carbazole-imidazole derivatives are designed as potent anti-α-glucosidase agents [19].

Synthesis of the final compounds has commenced from indole derivatives. 3-Ethylamino-5-substituted indole derivatives 124a-b are cyclized with aryl ketodiol 125a-g to afford carbazole derivatives 126a-k which upon further cyclization at 2,3-positions of carbazole using substituted aromatic aldehydes and ammonium acetate gave fused imidazole-carbazole scaffolds 127a-k (Scheme 27).

All the designed molecules are evaluated for their α-glucosidase inhibitory activity using acarbose as a standard inhibitor. All the tested derivatives have exhibited better inhibitory activity (IC₅₀ = 74 ± 0.7–298.3 ± 0.9 μM) compared to acarbose (IC₅₀ = 750 ± 1.5μM). Amongst the tested compounds, few derivatives namely compounds, 127c, 127f, 127k, 127n, and 127r have displayed significant inhibitory activity (Table 6). Furthermore, the carbazole-imidazole scaffolds such as 127o (Figure 13), 127t, 127v, and 127w rendered promising inhibitory effects. Particularly compound 127w resulted in a striking activity. In the structure-activity correlation studies, the potent inhibitory activity of all the four compounds 127o, 127t, 127v, and 127w is attributed to the 4-Cl/4-Br-phenyl ring appended to 2-position of carbazole-imidazole derivatives. In these compounds, the derivative 127v with simple phenyl ring at the 4-position of carbazole-imidazole moiety has shown remarkable inhibitory activity. However, replacement of phenyl ring with 4-electron donating/electron-withdrawing substituted phenyl rings led to diminished activity and the greater extent of reduction in the inhibitory activity is observed for 4-F substituted phenyl analog 127w.

2.7. Coumarin-1,3,4-oxadiazole hybrids

Overexpression of carbonic anhydrases is associated with the proliferation, angiogenesis, and metastasis in a variety of cancers. CA inhibition would be an efficient approach for the treatment of cancer. Coumarin can form favorable interactions with active sites of the proteins and enzymes; thereby possessing a wide variety of pharmacological activities including cancer. CA IX and XII inhibitory activities of the 7-hydroxycoumarin and N-acyl benzene sulfonamide dihydro-1,3,4-oxadiazole hybrids has prompted to design a novel series of CA inhibitors wherein coumarin motif is connected to 1,3,4-oxadiazole ring [20].

7-Hydroxy-4-methyl coumarin 128 is prepared from resorcinol and ethyl acetoacetate which is transformed into corresponding coumarin ester 129 upon treatment with ethyl bromoacetate. The ester 129 is hydrolyzed with hydrazine to form semihydrazide 130 which is subsequently cyclized with CS₂ yielding oxadiazole-2-thiol coumarin scaffold.
131. Various arylalkyl halides/alkyl halides substitution of compound 131 has resulted in final compounds 132a–t (Scheme 28).

Using AAZ as a reference compound, hCA inhibitory activity of the synthesized compounds is evaluated. In the inhibitory activity towards hCA I & II, no single derivative exhibited decent activity. All the evaluated derivatives have possessed moderate inhibitory activity (IC₅₀ > 100 μM). Also, the designed derivatives failed to reach the inhibitory boundary of AAZ (IC₅₀ = 0.025 μM) in the case of hCA IX. However, compound 132n (IC₅₀ = 2.34 μM) managed to exhibit some significance yet 100-fold lower activity compared to AAZ. Surprisingly, the derivatives 132a–h displayed prominent inhibitory potentials towards hCA XII (Table 7). Amongst these, compound 132b elicited excellent activity with 25-times lower activity compared to AAZ.

As per the observation of the drug molecule aimed to inhibit the hCA enzymes, most of the compounds were inactive towards hCA I & II; similar effects are observed here in case of 1,3,4-oxadiazole coumarin analogs 132a–t. The compound 132b (Figure 14) possessing coumarin appended with methylene oxadiazole thiol and in turn benzoyl moiety attached to thiol functionality of oxadiazole has bestowed with remarkable inhibitory activity. Corresponding benzyl analog 132a has shown little diminished activity. Also, insertion of methylene fragment in case of compound 132c lowered inhibitory activity is observed. Only two 4-substituted benzyl derivatives namely compound 4-ethoxycarbonyl benzyl derivative 132f and 132h possessing 4-nitrobenzyl motif have displayed significant activity; another 4-substituted benzyl has not met the requirements. These facts reveal that substitution at 4-position of benzyl ring could not be considered as a useful structural modification. Alongside, alkyl chain modified derivatives namely; isobutyl analog 132d, chlorobutylene derivative 132e, and ethylacetate modified compound 132g resulted in good inhibitory activity. When it comes to modification of phenacil analog 132c at 4-position; no single compound has turned out to be a potent CA inhibitor. It indicated that irrespective of the nature of the group, substitution at 4-position of phenacil moiety would end up with negative results.

### 2.8. C-β-D-Glucopyranosyl azole derivatives

Type-2 diabetes caused as a result of abnormal insulin secretion or insulin resistance is the most common clinical issue to be addressed. Elevated glucose production is responsible for high glucose levels in the body. Conversion of liver glycogen to glucose through liver glycogenolysis is catalyzed by glycogen phosphorylase enzyme (GPase). Thereby inhibition of the GPase might be a validated approach to treat type-2 diabetes. Based on previously designed GPase inhibitors, novel C-β-D-glucopyranosyl azole derivatives are prepared in order to enhance the effectiveness of GPase inhibitors [21].

Glucosyl bromomethyl ketone 133 is cyclized with arylthioamides to give corresponding thiazone derivatives 134a–c which upon debenzylation resulted in final compounds 135a–c. The compound 133 is again utilized for cyclization with various carboxamides to yield imidazole analogs 136a–c and subsequent removal of benzoyl groups has produced 137a–c (Scheme 29). In another series of derivatives, formamide salt 138 is treated with α-bromoacetyl naphthalene to undergo cyclization process affording imidazole derivative 139 and finally yielded compound 140 on debenzylation (Scheme 30).

The glucosyl derivatives prepared are evaluated for their GPase inhibitory activity using 1,4-dideoxy-1,4-imino-D-arabinitol (DAB). Although the inhibitory activity is not up to the mark compared to previously designed derivatives, two compounds have succeeded to display significant activity. In that compound 137b (IC₅₀ = 4.58 μM) (Figure 15) has exhibited moderate GPase inhibitory activity. The most potent inhibitory activity is elicited by the compound 140 (IC₅₀ = 1.97 μM) amongst the other derivatives. Inhibitory potentials displayed by both the potent inhibitors 137b and 140 are comparatively lower than that of DAB (IC₅₀ ≤ 1.0 μM). Except for 2-(2-naphthyl) thiazole derivative 135b (IC₅₀ = 26.2 μM); no thiazole glycosyl derivative has shown expected inhibitory activity. Similarly, imidazole analogs have shown weak inhibitory activity excluding compound 137b which entails 1-naphthyl moiety and glycosyl motif at 2- and 4-positions of imidazole. Appendage of naphthyl moiety at 1-position of imidazole (137c) diminished up to 15-fold compared to 2-naphthyl derivative 137b.

### 2.9. Diaryl-1,5-diazoles

Overexpression of COX-2 and 5-LOX enzymes responsible for inflammation leads to elevation of downstream prostaglandin PGΕ₂ and LTΒ₄ levels respectively. PGE₂ could increase the metastasis of tumor cells; while the COX-2 promotes tumor cell survival. COX-2 and 5-LOX pathways are two principal pathways of metabolism of arachidonic acid. Inhibition of COX-2/5-LOX would become the best anti-tumor agents. Diarylpyrazole scaffolds have been reported to possess dual COX-2/5-LOX inhibitory properties. This prompted to design molecules by incorporating diaryl-1,5-diazoles and morphonine motifs [22].

Here 3,4-disubstituted acetophenones 141a–k are transformed to diketoesters 142a–k upon reaction with dimethyl oxalate, 1,3-
Diketoester functionality of compounds 142a-k is allowed to undergo intermolecular cyclization with 4-hydrazonyl benzenesulfonamide to obtain pyrazole sulfonamide derivatives 143a-k; corresponding pyrazole carboxylic analogs 144a-k are yielded by ester hydrolysis of 143a-k.

Nucleophilic substitution reaction of compounds 144a-k with morpholine/thiazine produced final compounds 145a-v (Scheme 31). Alongside the predesigned pyrazole carboxylic acids 146a-k are reacted with morpholine-N-alkylamines/alkyhydroxides affording compounds 147a-s (Scheme 32).

The designed derivatives are tested for COX-2 and 5-LOX inhibitory activities using celecoxib and zileuton as reference compounds respectively. Compared to celecoxib (IC₅₀ = 0.25 ± 0.03 μM), most of the evaluated compounds exhibited good COX-2 inhibitory activity (IC₅₀ = 0.17–7.64 μM). Amongst these, few derivatives have possessed the best activity (Table 8); particularly compound 147h (Figure 15) has bestowed with most potent COX-2 activity. While the decent IC₅₀ values (IC₅₀ = 0.68–3.41 μM) are displayed for the tested compounds towards 5-LOX. The derivatives that exhibited remarkable IC₅₀ values better than zileuton are depicted in Table 8. The compound 147k (Figure 15) has resulted in most potent 5-LOX inhibitory effect.

When the structure of the derivatives and their inhibitory values are correlated, the following conclusions can be drawn. The remarkable COX-2 inhibitor 147h (Figure 16) possesses benzenesulfonamide and 4-fluorobenzene at 1- and 5-positions of pyrazole respectively; morpholine

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Table 7. Potent hCA XII inhibitory values of the coumarin derivatives.

| Compd | hCA XII activity |
|-------|------------------|
|       | IC₅₀ (μM)        |
| 132a  | 0.28             |
| 132b  | 0.16             |
| 132c  | 0.41             |
| 132d  | 0.52             |
| 132e  | 0.74             |
| 132f  | 0.60             |
| 132g  | 0.60             |
| 132h  | 0.82             |
| AAZ   | 0.0057           |

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Scheme 28. Synthesis of the target molecules 132a-t [20]; Reagents and conditions: i) Conc. H₂SO₄, 0–10 °C (2–3h); ii) ethyl bromoacetate, K₂CO₃, acetone, reflux (6h); iii) NH₂NH₂•H₂O, THF, reflux (4h); iv) CS₂, NaOH, EtOH, reflux (12h); v) R-X, K₂CO₃, acetone, reflux (3–5h).
is tethered to 3-position of pyrazole ethyl carboxamide fragment. Exchange of 4-fluorobenzene with 4-trifluoromethyl benzene (147k) and increased alkyl chain of carboxamide fragment by one unit has diminished the activity a little. There is no precise correlation between structure and activity that could be established as wide structural modification has resulted in almost similar activity. Compound 147k having benzene sulfonamide and 4-trifluoromethylbenzene at 1- and 5-positions of pyrazole in addition to propylcarboxamide flanked by pyrazole and morpholine moiety is reported to render powerful 5-LOX inhibitory activity among the other derivatives. However, varying the substituents on pyrazole could not improve the activity.

3. Dihydroquinazoline-2-amines

Reverse transcriptase (RTase) has been reported to have its involvement in life cycle of human immunodeficiency virus type 1 (HIV-1) whereby the RTase functions reverse transcription of its RNA genome to cDNA which is followed by integration of DNA into the host cell genome. Hence the RTase has been a major therapeutic target for HIV-1. Diarylpyrimidine scaffolds are demonstrated as second generation NNRTIs; based on structural features of these compounds, dihydroquinazoline-2-amine derivatives are designed and synthesized [23].

Partial protection of 2-amino-fluorinated acetophenone 148 with 4-methoxy-benzene methanol gave compound 149 which upon intramolecular cyclization with potassium cyanate afforded intermediate 150 and then on reflux conditions resulted in compound 151. Cyclopropylacetylene is connected at 4-position of intermediate 151 leading to compound 152 in which protecting group is removed to obtain derivative 153. Chlorination at 2-position of compound 153 is achieved using POCl₃ to yield 2-chloroquinazoline analog 154; finally, various aromatic amines are utilized for nucleophilic substitution at 2-position of compound 154 to afford 2-arylaminoquinazoline derivatives 155a-x (Scheme 33).

All the synthesized molecules are tested for various strains of HIV-1 RTase using nevirapine (NVP), delavirdine (DLV), EFV and ETV as reference compounds.

In case of the RTase of IIIB strain, most of the evaluated derivatives have exhibited inhibitory activity only up to micromolar level; however a few performed up to nanomolar level, which is an encouraging result and indicates the potential of these molecules as anti-HIV agents (Scheme 34).

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\text{Scheme 29. Synthetic route for the synthesis of the derivatives 135a-c and 137a-c [21]; Reagents and conditions: i) Arylthioamides, dry DMF, 140 °C; ii) 1M NaOMe, MeOH, rt; iii) Carboxamidines, 4 Eq. K₂CO₃, THF-H₂O (4:1), rt.}
\]

\[
\text{Scheme 30. Synthesis of the glucopyranosyl azole derivative 140 [21]; Reagents and conditions: i) 4 Eq. K₂CO₃, THF-H₂O (4:1), rt; ii) 40 Eq. EtSH, 20 Eq. BF₃·Et₂O, dry CH₂Cl₂, rt.}
\]

\[
\text{Figure 14. Structures of remarkable CA XII inhibitors.}
\]

\[
\text{Figure 15. Structures of remarkable GPase inhibitors.}
\]
few compounds are successful in exhibiting inhibitory values at as low as nanomolar drug concentration (Table 9). Among those, compound 155b has displayed most significant inhibitory property towards IIIB strain RTase. Its activity is twice the activity of the reference compound EFV (EC50 = 0.0016 \mu M) and ETV (EC50 = 0.0022 \mu M). Meanwhile, compound 155b (EC50 = 0.0035 \mu M) could show nanomolar inhibitory activity towards E138K RTase strain compared to EFV (EC50 = 0.0020 \mu M) and ETV (EC50 = 0.0063 \mu M). The other derivatives have shown moderate activity. Almost similar RES056 strain RTase inhibitory activity is observed for the compound 155b (EC50 = 0.066 \mu M) compared to EFV (EC50 = 0.055 \mu M).

The most potential RTase inhibitory activity of compound 155b (Figure 17) towards all the HIV-1 strains is attributed to its remarkable structure. In this case, the pharmacore responsible for the strongest inhibitory properties is 4-cyanophenylamine appended to 2-position of quinazoline moiety. Electronegativity is not the sole factor to be accounted for potent activity; the position of the substituent on the phenyl ring also plays a prominent role. For instance, 3-CN-phenyl

Scheme 31. Synthesis of the diarylpyrazoles 155a-v [22]; Reagents and conditions: i) dimethyl oxalate, MeOH, reflux (6h); ii) 4-hydrazonyl benzenesulfonamide, MeOH, reflux (6H); iii) KOH, MeOH, reflux (2h); iv) EDCiHCl, HOBT, DMAP, CH2Cl2, 0 °C, Revitalite, 0.5h.

Scheme 32. Synthetic route for preparation of final compounds 147a-s [22]; Reagents and conditions: i) EDCiHCl, HOBT, DMAP, CH2Cl2, 0 °C, Revitalite, 0.5h, RT, overnight.
analogue 155d (EC\textsubscript{50} = 0.44 μM) has shown very weak activity compared to reference; wherein 523-fold diminished activity is observed. Besides this, 3-NO\textsubscript{2}-phenyl derivative 155e has not reached up to the mark although a strong electron-withdrawing group is present. Notwithstanding the above statement, the compound 155f possessing 4-CF\textsubscript{3} has displayed a decent inhibitory effect. In general consensus, the activity of the molecule and its structure may not be correlated efficiently. The linearity of the substituent CN and its para-position at phenyl ring would be beneficial for noteworthy inhibitory activity of the compound 155b.

Table 8. Potent COX-2 and 5-LOX inhibitory values of diarylpyrazole derivatives.

| Compd  | COX-2 activity (μM) | 5-LOX activity (μM) |
|--------|---------------------|---------------------|
| 145a   | 0.19 ± 0.09         | 145s                |
| 145s   | 0.19 ± 0.02         | 147i                |
| 147b   | 0.16 ± 0.02         | 147k                |
| 147f   | 0.19 ± 0.04         | 147l                |
| 147k   | 0.17 ± 0.07         | 147q                |

Figure 16. Structures of pyrazole benzenesulfonamides as COX-2 and 5-LOX inhibitors.

Scheme 33. Synthetic route for preparation of final compounds 155a-x [23]; Reagents and conditions: i) 4-methoxy-benzenemethanol, p-Toluenesulfonic acid, acetonitrile, 60 °C (8h); ii) Potassium cyanate, AcOH, H\textsubscript{2}O, 60 °C (5h); iii) Xylene, reflux (8h); iv) Cyclopropylacetylene, n-BuLi, tetrahydrofuran, -50 °C (1h); v) ceric ammonium nitrate, acetonitrile, H\textsubscript{2}O, rt (4h); vi) POCl\textsubscript{3}, reflux (9h); vii) ArNH\textsubscript{2}, n-BuOH, reflux (5–8h).
3.1. Dioxino [2,3-f]quinazoline derivatives

VEGF family ligands bind to cell receptor tyrosine kinases VEGFR-1, VEGFR-2, and VEGFR-3 which lead to activation of downstream signaling pathways to perform biological functions such as vascular development during angiogenesis. VEGFR-2 inhibitors have been emerging as potent antiangiogenic agents for the treatment of a large number of cancer types. Since previous studies have described that dioxine quinazoline scaffolds as EGFR inhibitors; phenylurea structural unit in lenvatinib is appended to dioxane quinazoline core by O-Bridge [24].

The synthesis of final compounds commences from 2,3,4-trihydroxy benzoic acid 156 which is esterified to corresponding ester 157. The hydroxy groups of the ester 157 are protected by benzylation followed by debenzylation of OH groups at 2- and 3-positions retaining benzyl protection at 4-position to yield partially protected compound 159. Dioxane derivative 161 is prepared by intermolecular cyclization using dibromomethane with subsequent debenzylation at 4-position. Compound 161 is alkylated at oxygen atom at 4-position giving derivatives 162a-l which are nitrated at 6-position with subsequent –NO₂ group reduction resulted in amino dioxane derivatives 164a-l. Intermolecular cyclization of compounds 164a-l with formamidine acetate produced compounds 165a-l which are chlorinated upon treatment with POCl₃ rendered 4-chloroquinazoline scaffolds 166a-l. Nucleophilic substitution of 4-chloro group of compounds 166a-l with aromatic ring substituted urea analogs 167a-l produced final derivatives 168a-l (Scheme 34).

All the synthesized derivatives have been analyzed for VEGFR-2 kinase inhibitory activity using lenvatinib as a reference compound. In this activity compound, 168h is found to be totally inactive. All the other derivatives have displayed excellent inhibitory activity except for a few derivatives compared to the reference compound (IC₅₀ = 0.0007 μM).

Most of the compounds have exhibited significant inhibitory activity; among those, few derivatives (Table 10) are enough potent to reach the inhibitory effects of lenvatinib. Compound 168j (Figure 18) has exerted the best VEGFR-2 inhibitory activity which has shown slightly diminished activity compared to the reference compound. The most remarkable VEGFR-2 kinase inhibitor 168j possesses N-propylmorpholine moiety connected to oxygen at 7-position of quinazoline and 2-fluoro-4-trifluoromethyl benzene appended to the NH end of urea fragment. In addition, benzene urea is substituted by –F at its 3-position. This combination of pharmacocores has bestowed it with the most potent activity.

Replacement of –F group with hydrogen, retaining other structural features same, has resulted in compound 168i with reduced activity. Chloro analog 168k has displayed decreased inhibitory properties compared to compound 168i. Reduction in the alkyl chain length from propyl to ethyl of morpholine paved to weakest inhibitory activity in case of compound 168h; wherein the inhibitory activity is lowered to 1000-fold. Overall observation revealed that 2-fluoro-5-trifluoromethyl benzene moiety is crucial for potent inhibitory activity. Alongside, the compound 168c and 168f with methyl and methoxymethylene fragments respectively in addition to 2-fluoro-5-trifluoromethyl benzene moiety are prominent inhibitors yet lower activity compared to compound 168j. Hence no significant inhibitor is devoid of the 2-fluoro-5-trifluoromethyl benzene moiety.

3.2. 1,2,3-Triazole analogs

Receptor tyrosine kinases such as VEGFR-2, Tie, and EphB4 are demonstrated to be overexpressed in endothelial cells which play a significant role in both vasculogenesis and angiogenesis. These also function in the process of tumor development. In order to treat metastasis and reduce angiogenesis, multi-tyrosine kinase inhibitors are designed and synthesized by incorporating 1,2,3-triazole moiety in between 3-phenyl pyridine and cyclopropylamine motifs [25] (Scheme 35).

The designed compounds are allowed to inhibit the VEGFR-2, Tie, and EphB4 kinases in presence of the standard tyrosine kinase inhibitor sorafenib. In the inhibitory activity towards VEGFR-2 kinase, few derivatives such as compound 174a (IC₅₀ = 0.00163 μM), 174g (IC₅₀ = 0.00185 μM), and 174m (IC₅₀ = 0.00163 μM) have elicited decent inhibitory activity. Particularly compound 175 (IC₅₀ = 0.00052 μM) (Figure 19) is successful in the inhibition of VEGFR-2 with most efficiency compared to reference compound (IC₅₀ = 0.00017 μM). While a good number of designed derivatives have exerted inhibitory properties stronger than sorafenib (IC₅₀ = 0.00039 μM) towards Tie-2 kinase. To mention, compound 174a (IC₅₀ = 0.00036 μM), 174d (IC₅₀ = 0.00026 μM) (Figure 19), 174f (IC₅₀ = 0.00030 μM), and 174l (IC₅₀ = 0.00028 μM) are 1,2,3-triazole derivatives with potent inhibitory effects. However, only single compound 174l (IC₅₀ = 0.00044 μM) could reach the boundary of inhibitory effect shown by sorafenib (IC₅₀ = 0.00022 μM) towards EphB4 kinase. Most of the other derivatives are totally inactive at this drug concentration. The most significant VEGFR-2 inhibitor 175 is appended with a 3-cyclopropylcarboxamidophenyl ring at 4-position of 1,2,3-triazole ring. Also, the decent inhibitory activity of 3-chlorophenyl analog with 6-methoxy pyridine 174a, a 3-aminophenyl derivative with 6-methoxy pyridine moiety 174g, and 3-methylphenyl analog with pyridine ring 174m reveals that 3-substituted phenyl rings play an essential role in the VEGFR-2 inhibitory activity. 6-Fluorophenyl in combination with 5-methoxy pyridine and pyridine moieties diminished inhibitory activity a lot. While the compound 174d possessing strongest Tie-2 activity has 4-methylphenyl ring and 6-methoxy pyridine motif. Slight change of position of methyl position from 4- to 3-position of benzene ring resulted in extreme low activity (compound 174e). Further, removal of methoxy group from pyridine 6-position in case of compound 174l led to a greatly reduced activity. 3-Chlorophenyl moiety and pyridine combination (compound 174i) exhibited one of the best potent activities; while its 6-methoxy pyridine analog (174a) showed little diminished inhibitory effects. Additionally, a 4-trifluoromethylphenyl derivative with 6-methoxy pyridine moiety (174f) can also be accounted for in most significant inhibitors. In contrast to these, only single compound 174i bearing 4-methylphenyl and pyridine moieties could reach up to the mark with two times lower activity than that of reference compound; it reveals that it is a selective EphB4 kinase inhibitor.

3.3. Aromatic ring linked-hydroxyazole scaffolds

The disease malaria, one of the biggest infectious diseases is killing most of the people every year; wherein parasitic Plasmodium species
remain the main culprit. The best approach to cure malaria is to inhibit *Plasmodium falciparum* dihydroorotate dehydrogenase (pfDHODH) enzyme which is essential for the biosynthesis of pyrimidine and in turn survival of the parasite. The recent discovery of acidic hydroxazoles with potent pfDHODH inhibitory activity has inspired to design novel derivatives of aromatic ring linked hydroxyazole scaffolds [26].

In the synthesis of compound 1,2,5-oxadiazole derivative 177, the benzoxyl group at 4-position of 1,2,5-oxadiazole 176 is hydrolyzed with subsequent nucleophilic substitution reaction using 2,2-

![Scheme 34. Synthesis of quinazoline derivatives 168a-l [24]; Reagents and conditions: i) CH3I, KHCO3, DMF, overnight, rt; ii) BnCl, K2CO3, KI, 60 °C; iii) AcOH/HCl, 45 °C (8h); iv) CH2Br2, K2CO3, DMF, 70 °C; v) Pd/C, H2, EtOH, rt; vi) R1X, K2CO3, DMF, 70 °C; vii) HNO3/AcOH, 0 °C; viii) Pd/C, H2, EtOH; ix) formamidine acetate, EtOH, reflux; x) POCl3, reflux; xi) triphosgene, THF, triethylamine, 0 °C; xii) K2CO3, isopropanol, reflux.](image-url)
diphenylethanolamine at 4-position of oxadiazole. The cyano group at 4-position of 1,2,5-thiazole is esterified to obtain corresponding ester 179 which on nucleophilic substitution with 2,2-diphenylethanamine and benzylamine yielded final compounds 180 and 181 respectively. The 3-benzylxoy-4-pyrazole carboxylic acid derivatives 182a-b transformed to diphenylethylamide derivatives of pyrazole 183a-b and then benzylxoy group is hydrolyzed to corresponding 4-hydroxy pyrazole analogs 184a-b (Scheme 36).

3-Hydroxy group of pyrazole 185 is protected using the butyloxycarbonyl group to form compound 186 in which methyl moiety at 5-position is brominated to produce compound 187. Nucleophilic substitution of compound 187 with aromatic amines/substituted phenols produced compounds 188a-f. Removal of -Boc group from compound 188a-f followed by ester hydrolysis resulted in 3-hydroxy-4-pyrazole carboxylic acid scaffolds 190a-f (Scheme 37).

The intermediate compound 189a (one of its tautomeric forms) is made use in the design of the compounds 192a-c (Scheme 37). The compound 189a is alkylated at its 3-OH moiety to produce O-alkylated pyrazoles 191a-c in which ester group at 4-position is hydrolyzed yielding corresponding 3-hydroxy-4-carboxylic acid derivatives 192a-c. One of the compounds in this series, 192c is transformed into corresponding amide 193, followed by benzylxoy moiety hydrolysis rendered the compound 194 (Scheme 38).

In the pDHODH and aDHODH inhibitory activity investigation using standard inhibitor DSM1, no single evaluated compound has shown the capability to inhibit the aDHODH enzymes. However, the designed derivatives exhibited good aDHODH inhibitory activity with the IC50 values in the range of 2.8–75 μM. Compared to DSM1 (IC50 = 0.065 μM), compound 190e (IC50 = 2.8 ± 0.3 μM) (Figure 20) is most significant pDHODH inhibitor amongst the derivatives. While compound 190f possessed remarkable activity (IC50 = 5.3 ± 1.2 μM). The derivatives such as 181 (IC50 = 19 ± 1 μM), 190a (IC50 = 19 ± 1 μM), and 190b (IC50 = 16 ± 1 μM) have displayed moderate inhibitory activity.

In the case of structure and its activity correlation studies, the series 192a-c is found to be totally inactive and accordingly the compound 194 finds a place in this list. The most significant pDHODH inhibitor 190e bears 3-trifluoromethylbenzophenone linked compound at 5-position of the pyrazole in addition to hydroxyl and carboxylic acid moieties at 3- and 4-positions of pyrazole ring. The presence of 3-trifluoromethyl group rendered its most potent activity; shift of 3-trifluoromethyl from 3- to 4-position (compound 190f) has reduced activity to half the activity of compound 190e. Besides this, replacement of the phenol moiety with aromatic amines resulted in largely diminished inhibitory activity. In common, the 3-trifluoromethyl group makes a compound more efficient in the pDHODH inhibitory properties. Although most of the pyrazole derivatives have exhibited moderate to good inhibitory activity, pyrazole could not be regarded as sole pharmacore for inhibitory properties; since, the pyrazole amide 194 has failed to show good activity. Probably the particular research team would have tried to design 3-trifluoromethylthiophenol and 3-trifluorobenzeneamine analogs to check the further improvement of the inhibitory activity.

### 3.4. Phenythiazoles

Undecaprenyl Pyrophosphatase (UppFase), one of the enzymes present in the bacterial cell wall functions as a membrane protein in biosynthesis of peptidoglycan and offers resistance to chemical entities. Bacterial diseases have become most common and problematic as they have developed antibiotic resistance with most of the existed antibiotics. In this regard, a large pool of chemical entities has been engineered to encounter antibiotic resistance. Many phenylthiazole scaffolds are designed against multidrug-resistant bacteria in addition to their potential anti-MRSA properties. Targeting UppFase, novel phenylthiazoles are prepared alongside accounting for aqueous solubility property [27].

The synthetic route involves the appending of ethynylsilyl moiety to the benzene 4-position which connected at 2-position of thiazole ring (compound 195) by sonogashira coupling to form ethynylsilyl derivative of thiazole 196. The silyl group is cleaved to produce corresponding molecule 197 and then the free ethynyl end carbon is coupled with various aromatic/aliphatic moieties yielding compound 198. Conden- sation of the intermediate 198 with aminoguanidine resulted in the final compounds 199a-z and 200a-d (Scheme 39).

The title compounds are tested for their anti-UppFase activity using linezolid and vancomycin as reference compounds. The most potent inhibitory activity is elicited by the compounds 199f, 199o, 199q and 199x (Figure 21) with an identical MIC value of 2 μM. The activity of most significant inhibitors is almost comparable with linezolid/vanco- mycin (MIC = 1 μM). The derivatives such as 199a, 199c, 199d, 199s, and 199z have possessed remarkable inhibitory potentials with identical MIC value of 4 μM.

The noteworthy MIC values of the most significant inhibitors are attributed to a distinct functional group –OH present preferably at meta-position (compound 199f). It indicates that the hydroxy group at meta-position offers the least steric hindrance and perfect fit into the enzyme active site. While the meta- and para-amin substituted derivatives have exhibited less striking activity (199a and 199c). Also, the meta-methoxy substituent (199d) exhibited diminished activity compared to compound 199f. If p-OH-phenylthiazole derivative was prepared, it would have been a furthermore potent molecule. Thiophen-2y and 5-methyl thiophene-2yl substitutions in the case of 199o and 199q analogs bestowed remarkable inhibitory activity. However, activity is reduced to a greater extent by appending thioephene with its 3-position (199p). Chirality might have played a significant role in the case of noteworthy inhibitory potency of 2-hydroxypropyl substituted analog 199x.

Almost half of the evaluated derivatives are weak UppFase inhibitors and exhibited inhibitory activity at drug concentration higher than 64 μM. Some of these derivatives with high MIC values possess bulkier ortho substituents that can be observed in the derivatives such as 199b, 199g, 199i, and 200b-c. Also, the carbonyl substituents such as amide and acetyl in compounds 199l and 199m respectively rendered weak activity. Compounds derivatized with pyridine and pyrimidine substituents also failed to show good inhibitory activity.
3.5. Pyrazole derivatives

COX-2 is an isoenzyme of the cyclooxygenases responsible for the formation of inflammatory prostaglandins such as PGE2. Since PGE2 functions as an efficient inflammatory mediator, inhibition of the PGE2 would be an effective clinical approach for inflammation treatment. Taken together the inhibitory properties of pyrazole scaffolds as COX inhibitors, novel pyrazole derivatives bearing benzenesulfonamide moiety are designed and synthesized [28].

The synthetic route commences with 4-cyano-5-amine-N1-benzene sulfonamidepyrazole 171. The –NH2 group of the compound 171 is treated with chloroalkane carbonyl chloride to obtain amide analogs 172a-c which on nucleophilic substitution with morpholine afforded final compounds 173a-b. The amine moiety of compound 171 is acylated/benzoylated in addition to acylation of the sulfonamide –NH2 in one of the compounds yielded compounds 174a-b. Condensation of compound 171 with various substituted aromatic amines produced compounds 175 (Scheme 40).

All the prepared derivatives have been evaluated for COX-1 and COX-2 inhibitory activities using celecoxib. Except for a few compounds, all the evaluated derivatives exhibited decent COX-1 inhibitory effects. Compared to celecoxib, compounds 203a (IC50 = 0.064 μM) (Figure 22) can be mentioned as strongest COX-1 inhibitor. Alongside, compound 204b (IC50 = 0.073 μM) finds a place in significant inhibitors list. Other derivatives have exhibited inhibitory activity (IC50 = 0.104–0.169 μM) as potent as celecoxib (IC50 = 0.131 μM). Regarding inhibitory activity towards COX-2 enzyme, except for compounds 202c, 203c, and 205e, all the evaluated compounds have elicited activity almost similar to the reference compound (IC50 = 0.035 μM). However, most potent inhibitory potency is bestowed by compound 202a (Figure 22).

Compound 203a stood atop in significant inhibitors list; wherein its structural features include benzene sulfonamide connected to pyrazole N1-position and morpholine appended to pyrazole 5-position via methylene amide fragment. Extension of methylene amide to ethylene amide (compound 203b) diminished COX-1 inhibitory activity 13-times compared to compound 203a. The second most significant inhibitor 204b possessed benzamide at 5-position of pyrazole ring in addition to benzenesulfonamide at N1-position. Compound 204a obtained by connecting acetamide at pyrazole ring 5-position and N-methylation of sulfonamide functionality. Since benzenesulfonamide moiety is a prominent pharmacore for the exhibition of pharmacological activity, probably acetylation of pyrazole 5-amine would not have affected the inhibitory activity much. Hence, the design of 5-acetamide analog would be a potent try to check improvement in the activity. In the case of inhibitory activity of 202a-c series compounds, compound 202a (IC50 = 0.104 μM) has shown promising results and activity (IC50 = 0.123 μM) reduced a little with an extension of alkyl chain length (202b). Reduction of the

Scheme 35. Synthetic route for the preparation of compounds 174a-p and 175 [25]; i) NaN3, DMF, rt (12h); ii) L-sodium ascorbate, copper sulfate pentahydrate, EtOH, H2O, rt; iii) dioxane, H2O, pd(pddf)Cl2, K2CO3, reflux; iv) 0 °C, CH2Cl2, triethylamine, 30min, cyclopropyl carbonyl chloride, rt.

Figure 19. Structures of potent tyrosine kinase inhibitors.
activity to a large extent is observed for isopropyl amide analog 202c. A similar effect has been observed in the case of compounds 203a-c. These facts infer that longer alkyl chain lengths deteriorated the COX-1 inhibitory activity. While compounds of 205a-f series comprising aromatic imines of pyrazole 5-amine displayed moderate to potent inhibitory activities; amongst them, 4-bromobenzylidine derivative 205d shown maximum inhibitory activity (IC$_{50}$ = 0.115 μM).

Meanwhile, in the assessment of the COX-2 inhibitory activity, the most striking inhibitory activity is observed for the compound 202a which entails chloroacetamide fragment at 5-position of pyrazole along with benzene sulfonamide at pyrazole N$_1$-position. However, the inhibitory activity abated gradually for compounds 202b and 202c with the increased bulkiness of chloroalkylamide groups. Moderate COX-2 inhibitor 204b has displayed diminished activity when sulfonamide amine
is methylated and pyrazole 5-amine is acetylated (204a). Pyrazole derivatives in which benzylidine substituted by both electron-withdrawing –F (205b) and electron-donating –OCH3 (205e) are successful in exhibiting remarkable COX-2 inhibitory activity which is almost similar to that of the reference compound. Hence correlation of structure and activity, in this case, could not be established.

3.6. Phthalimide-1,2,3-triazole hybrid compounds

The hydroxylation of L-tyrosine to L-DOPA and oxidation of L-DOPA to dopaquinone are carried out in melanin biosynthesis by multifunctional metalloenzyme tyrosinase. However, abnormal secretion of the melanin enzyme leads to skin disorders and esthetic problems in human beings. Thereby inhibition of the tyrosinase in such conditions is crucial to address these problems. Considering the prominent pharmacological properties of phthalimide and antityrosinase effects of triazole scaffolds, the design of phthalimide-1,2,3-triazole hybrid compounds is accomplished [29].

In the synthesis of target molecules, phthalimide 206 connected to propargyl fragment to form N-propargyl phthalimide 207. Substituted benzyl chlorides 208a–m are transformed into substituted benzyl azides 209a–m in which azide functionality is utilized to create a triazole with alkyn moiety of compound 207 to render final compounds, triazole linked phthalimide scaffolds 210a–m (Scheme 41).

The designed derivatives are allowed to inhibit tyrosinase using kojic acid as a standard inhibitor. More than half of the evaluated molecules have not shown good tyrosinase inhibitory activity compared to kojic acid. Methyl, methoxy and fluoro benzyl substituted triazole phthalimides (210a–f) and dichlorobenzyl scaffolds 210i and 210j would be included in the inactive molecules list with the inhibitory activity >50 μM. While –Br, –Cl and –NO2 benzyl substituted triazole phthalimides displayed decent inhibitory activities. Amongst them, compound 210k (IC50 = 26.55 ± 2.31 μM) bearing 2-bromobenzyl moiety and its 2-NO2 analog 210m (IC50 = 26.20 ± 1.55 μM) (Figure 23) are having almost similar inhibitory potencies but comparatively threefold lower activity than that of kojic acid (IC50 = 9.28 ± 1.15 μM). In both the potent inhibitors bulky electron-withdrawing substituents such as –Br, –NO2 groups have exhibited favorable effects. Less bulky groups 2-Cl and 3-Cl benzyl substituted derivatives 210g and 210h rendered moderate activity.

3.7. Purine-Pyrazole hybrids

15-LOX, one of the isoenzymes of lipoxigenases promotes invasion of tumor cells into lymphatic vessels and induction of lymph node metastasis. Through the LOX pathway hydroxyeicosatetraenoic acids (HETEs) or leukotrienes (LTs) are produced from Arachidonic acid (AA). Hence best chemotherapeutic agents include 15-LOX inhibitors. Purines have been reported as first-class 15-LOX inhibitors. Previously demonstrated purine derivatized pyrazoles possessing anticancer properties and antioxidiant effects triggered the design of a new series of purine-pyrazole hybrid derivatives [30].

In the first series, purine derivative 211 is treated with various substituted acetophenones to yield benzylidine derivatives of pyrazole 212a–d which upon cyclization using POCl3/DMF resulted in pyrazole carbaldehyde analogs 213a–d (Scheme 42).

The pyrazole carbaldehydes 213a–d are reacted with thiazolone-benzensulfonamide and thiazolone acetic acid to obtain the corresponding thiazoline incorporated pyrazole-purine derivatives 214a–d and 215a–d respectively (Scheme 43).

Likewise, the compounds 213a–d on condensation with substituted thiosemicarbazide resulted in substituted thiosemicarbazone analogs 216a–h. The thiosemicarbazone fragment is utilized in the formation of thiazole ring using p-bromo phenacyl bromide and ethyl bromoacetate to produce finally purine-pyrazole scaffolds (217a–h and 218a–h) linked to thiazole moiety through hydrazone spacer (Scheme 44).

Some selected compounds were investigated for their 15-LOX inhibitory activity using zileuton, quercetin, and meclofenamate sodium. All the evaluated derivatives have exhibited higher potential values compared to reference compounds. However, with respect to the standard inhibitor zileuton (IC50 = 3.98 μM), except for a few derivatives, all...
other screened molecules have shown potent inhibitory properties (IC\textsubscript{50} = 1.76–3.42 μM). The first series of compounds 213a-d have failed to reach a decent inhibitory level mark. It indicates that pyrazole carbalddehydes would not fit exactly into the enzyme active site. Whereas, compounds of purine-pyrazole linked to benzenesulfonamide 214a-d have displayed the striking inhibitory activities. In those, compound 214a (IC\textsubscript{50} = 1.96 μM) bearing phenyl ring at pyrazole 3-position elicited excellent activity. But the inhibitory activity reduced gradually with an increase in bulkiness at phenyl 4-position. Similar effects are observed in the case of the compounds in the 215a-d series. In these derivatives, purine-pyrazole scaffolds are appended to thiazole acetic acid moiety, wherein simple phenyl analog 215a (IC\textsubscript{50} = 2.62 μM) is the most potent inhibitor in the series. The 4-substituted phenyl analogs have exhibited diminished activity. Out of the thiosemicarbazones derivatized purine-pyrazoles, compound 216a (IC\textsubscript{50} = 2.81 μM) possessing simple phenyl ring connected to pyrazole 3-position exhibited promising inhibitory effects. Again inhibitory effects of 4-substituted phenyl analogs could be repeatedly observed to be similar to previous series compounds. A good account of inhibitory properties is displayed by 217a-h series derivatives. Amongst these derivatives, compound 217d (IC\textsubscript{50} = 1.76 μM) bearing 4-methoxyphenyl ring at pyrazole 4-position has bestowed with the most remarkable activity out of all evaluated molecules. Contrary to previous series compounds, the bulkiness at benzene 4-position appended to pyrazole 3-position has led to moderate activity.

Final series of compounds 218a-h wherein purine-pyrazole scaffolds are linked to thiazolone via diazo spacer has exerted decent inhibitory activity.

Scheme 39. Synthetic route for the preparation of thiazole derivatives 199a-z and 200a-d [27]; Reagents and conditions: i) PdCl\(_2\)(PPh\(_3\))\(_2\), ethylnyltrimethylsilane (2 Equiv), 50 °C (24h); ii) PdCl\(_2\)(PPh\(_3\))\(_2\) (5% mol), CuI (7.5% mol), Et\(_3\)N for 6–24h; iv) aminoguanidine HCl, EtOH.
activity. Even in this series of compounds, 4-substituted phenyl analog 218d (IC\textsubscript{50} = 1.98 μm) exhibited better activity.

3.8. Pyrazole and pyrazolo [1,5-\(\alpha\)] pyrimidine scaffolds

Protein kinases function transfer of \(\gamma\)-phosphate group wherein transfer of \(\gamma\)-phosphate from a nucleoside triphosphate (ATP) to the side chain of an amino acid residue in the substrate proteins such as serine, threonine, histidine and tyrosine residues. The cyclin-dependent kinases (CDK) related to serine/threonine kinase family are responsible for the initiation and succession of each cell cycle phase; amongst these, CDK2 is essential for progress through G1 to S phase. However, hyperactivation of CDK2 in a large number of human cancer types is attributed to the
overexpression of the particular enzyme. Pyrazolo [1,5-α] pyrimidines have been reported as pharmacologically important scaffolds especially in case of inhibition of CDK2/Cyclin A. Based on these facts novel pyrazolo-pyrimidine derivatives are designed and synthesized [31].

Synthesis of the first series of compounds involves condensation of ethyl acetoacetate with phenylisothiocyanate to give compound 219 which on cyclization using hydrazine hydrate produced pyrazole derivative 220. The compound 220 is utilized in the synthesis of the derivatives 221a-g and 222a-c by reacting with triethyl orthoformate and aromatic amines and aromatic aldehydes respectively (Scheme 45).

Further, cyanoacetamide 223 is transformed into compound 224 using phenylisothiocyanate followed by treatment with dimethyl sulfate. The compound 224 is allowed to undergo intermolecular cyclization with hydrazine pyrazole amide analog 225 and subsequent fusion of pyrimidine ring pyrazole 1,5-position produced pyrazol-pyrimidine derivatives 226a-d (Scheme 46). Finally, in the synthesis of compounds 229a-e, similar reaction sequences with similar reaction conditions are observed; wherein malononitrile is used as a starting material (Scheme 47).

The final derivatives designed are investigated for their anti-CDK2 inhibitory activity using dinaciclib. The inhibition percentage of the reference compound is considered as 100%. Most of the evaluated derivatives have exhibited poor inhibition percentages. In the list of potent inhibitors list, compounds 221d (Inhibition = 60%) (Figure 25) and 221g (Inhibition = 44%) could be mentioned. While compound 226c (Inhibition = 28%) has shown some significance. The compound 221d bearing phenylamine moiety at pyrazolone 3-position and 4-hydroxyphenyl ring through methylene amine fragment at 4-position bestowed the significant activity. Most of the poor CDK2 inhibitors possessed bulky groups at benzene amino methylene moiety, while the –OH functionality is small and distinct group. However, the compound 221g with bulky and hindered pyrimidine sulfonamide moiety has elicited the most promising activity; it might be attributed to the prominent pharmacological activity of pyrimidine sulfonamide. No single pyrazolopyrimidine derivative has displayed good inhibitory activity except for compound 226c. The two significant inhibitors 221d and 221g pertain to the series wherein pyrazolone is derivatized with a benzene amine group and phenylamino-methylene moiety at 3- and 4-positions respectively.

3.9. Dihydropyran fused pyrazole derivatives

Non-steroidal anti-inflammatory drugs (NSAIDs) are being used for inhibition of inflammation wherein cyclooxygenases play a significant role in the mediation of inflammation through the release of prostaglandins. Since such drugs exhibit gastrointestinal and renal toxicity, design of new NSAIDs is crucial for inhibition of cyclooxygenases. In view of promising pharmacological activity of pyrazole scaffolds such as celecoxib, deracoxib towards COX-2 enzyme, dihydropyran derivatized pyrazole derivatives are synthesized [32].

The synthetic route commences from ethyl acetoacetate wherein it is cyclized with substituted hydrazines 230a-b to produce N-substituted pyrazolone 231a-b and fusion of dihydropyran to the pyrazolone at its 4- and 5-positions via multicomponent reaction to render fused derivatives 232a-o. The amine group present at fused derivatives 232a-o is condensed with various aromatic aldehydes to obtain the final compounds 233a-o (Scheme 48).

The target molecules have been tested for COX-1 and COX-2 inhibitory properties using celecoxib. Compared to the reference compound (IC50 = 0.34 μM), no single investigated derivative has exhibited potent

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Scheme 41. Synthesis of phthalimide-triazoles as COX inhibitors [29]; Reagents and conditions: i) K2CO3, DMF, 80 °C (2h); ii) NaN3, H2O/t-BuOH, NEt3; ii) intermediates 209a-m, Cul.

Scheme 42. Design and synthesis of the compounds 212a-d [30]; Reagents and conditions: i) 4-substituted C6H5COCH3; ii) POCl3/DMF.

Figure 23. Structures of remarkable tyrosinase inhibitors.
Scheme 43. Synthetic route for the preparation of the compounds 214a-d and 215a-d [30]; Reagents and conditions: i) Dry dioxane, piperidine (catalyst) ii) Dry dioxane, CH₃COONH₄ (catalyst).

Scheme 44. Synthesis of the purine-pyrazolothiazoles 216a-h and 217a-h [30]; Reagents and conditions: i) N-substituted thiosemicarbazide, dry dioxane, glacial acetic acid (catalyst), reflux; ii) Dry dioxane, anhyd. CH₃COONa, reflux; iii) Dry dioxane, anhyd. CH₃COONa, reflux.
Scheme 45. Strategic design and synthesis of final compounds 221a-g and 222a-c [31]; Reagents and conditions: i) Na, CH₃OH, phenyl isothiocyanate, reflux 1/2 h, cool; ii) Hydrazine hydrate, reflux 1 h; iii) Aryl amine, triethyl orthoformate, DMSO, Oil Bath, 2.5 – 4.5 h, iv) Aromatic aldehyde, sodium acetate, AcOH, reflux 6 h.

Scheme 46. Synthesis of the pyrazolopyrimidines 226a-d [31]; Reagents and conditions: i) KOH, DMF, phenyl isothiocyanate, stirring, RT, 24 h; ii) Dimethyl sulfate, stirring, RT, 8 h, iii) Hydrazine hydrate, 3–4 h, iv) Hydrochloric acid, aromatic aldehyde, ethyl acetoacetate, ethanol, reflux, 4–8 h.

Figure 24. Demonstration of the structure of significant 15-LOX inhibitor.
inhibitory activity. However, compound 233j (IC50 = 2.56 μM) stood top in the inhibitors list with IC50 value sevenfold weaker compared to the standard inhibitor. Although, the activity of derivative 233h (IC50 = 4.32 μM) is poor; however, its activity is second to the strongest COX-2 inhibitor 233j. When structural modification of the derivatives is correlated with their pharmacological activity the following conclusions are made; the most significant inhibitor 233j bears phenyl ring at fused scaffold N1-position, 3,4,5-triOCH3-phenyl moiety and 4-hydroxyphenylmethanimine entity at 4- and 6-positions of dihydropyran-fused pyrazolone. Meanwhile, compound 233h is appended with phenyl rings and phenylmethanimine moiety at N1, 4 and 6-positions of the fused pyrazolone-dihydropyran moiety. Within the inhibitors list, the derivatives with 4-hydroxyphenyl rings at 4- and 6-positions possessed somewhat good inhibitory properties. In the case of COX-1 inhibitory properties, few compounds exhibited inhibitory activity stronger than celecoxib (IC50 > 50.0 μM). The strongest inhibitory activity (IC50 = 33.58 μM) is elicited by the compound 233n (Figure 26). Likewise the compound 233c (IC50 = 37.72 μM) stood next to potent COX-1 inhibitors list. While the compound 233e (IC50 = 51.32 μM) shown activity as potent as the standard inhibitor. The inhibitory values of these compounds infer that these derivatives are selective COX-1 inhibitors. The remarkable COX-1 inhibitor 233n possessed with phenyl ring at N1-position in addition to a 3-chlorophenyl moiety and 4-hydroxyphenylethanmine at 4- and 6-positions respectively. However, the derivative 233c comprising unsubstituted phenyl and phenylmethanimine moieties but 2-NO2-phenyl rings at N1, 4- and 6-positions respectively bestowed the best result. Similar to COX-2 inhibitors, compounds having 4-H-phenyl rings either at N1 or 4- or 6-positions of fused pyrazolone scaffold have displayed moderate inhibitory activity.

4. Heterocycle-fused pyrazole analogs

Acetylcholinesterase enzyme’s involvement in the dementia disease is confirmed by the cholinergic hypothesis; in this regard, cholinesterase inhibitors have been synthesized to treat Alzheimer’s and its sister diseases. Human carbonyl anhydrases catalyze the reversible conversion of carbon dioxide and bicarbonate. Isoenzyme of hCA functions in the tumorigenesis in addition to pH regulation, bone resorption, and some biosynthetic reactions. Thereby, based on the pharmacological history of pyrazoles towards cholinesterases and carbonyl anhydrases, fused pyrazole derivatives are engineered and tested for corresponding biological activity [33].

Pyrazole-3,4-dicarboxylic acid 234 is transformed to acyl chloride 235 with SOCl2 and then treatment with ammonia lead to pyrazole-diamide 236. Dehydration of dihydride 236 produced dinitrile 237 and subsequent cyclization rendered the final compound 238. One of the intermediate 235 is converted to corresponding diester 239 which upon cyclization with hydrazine yielded the fused pyrazolopyridazinone 240.

The starting material pyrazole dicarboxylic acid 234 is utilized in the design of another series of molecules. Here fused cyclic anhydride 241 is synthesized from dicarboxylic acid using a mixture of SOCl2 and DMF followed by primary amine insertion into cyclic anhydride to give fused-pyrazolo cyclic imide 242a-b (Scheme 49).

The engineered molecules are evaluated for hCA and AChE inhibitory activity using acetazolamide and tacrine respectively. A good account of carbonyl anhydrase-I & II inhibitory activity has been reported. All the synthesized derivatives have shown hCA inhibitory activity higher than reference compounds. In the case of hCA I inhibitory activity, compound 235 (IC50 = 0.83 μM) (Figure 27) is found to be the most significant hCA I inhibitor exhibiting 25-fold greater potency compared to reference compound (IC50 = 21.13 μM). The remarkable hCA I inhibitors include compound 234 (IC50 = 3.54 μM) (Figure 27), 238 (IC50 = 6.66 μM), 239 (IC50 = 5.04 μM), and 241 (IC50 = 6.01 μM). Surprisingly, the most significant hCA I inhibitor 235 (IC50 = 1.36 μM) has succeeded in eliciting the most potent hCA II activity; wherein its inhibitory activity is 21-fold stronger than the acetazolamide (IC50 = 28.55 μM). Meanwhile the derivatives 234 (IC50 = 5.52 μM), 238 (IC50 = 9.02 μM), and 239 (IC50 = 7.88 μM) managed to exhibit noteworthy hCA II potency. When the inhibitory activities towards both hCA I and hCA II are observed, it is evident that dual inhibitory effect has prevailed in the molecules 234, 235, 238, and 239. The most significant hCA I & hCA II dual inhibitor 235 comprises 3,4-dimethyl benzene and phenyl ring at pyrazole N1 and C5 positions of pyrazole. Alongside, 3- and 4-positions of pyrazole are occupied by acyl chloride moieties. The structural modifications infer that acid chlorides are more potent than corresponding carboxylic acids and again inhibitory activity reduced to a large extent for diamide analog 236. Gradual increment in CA inhibitory activity is observed for dinitrile 237, followed by pyrazolo-
pyridazine diamine 238. The huge diminished activity has been observed for the diester derivative 239 and the effects are continued to the pyrazole pyridazinone derivative 240.

In one more series of derivatives, cyclic anhydride formation (241) possessed decent activity although its activity is less than that of dicarboxylic acid 234; further, decrement in the activity is observed for derivatives (242a and 242b) wherein primary amine is inserted into cyclic anhydride.

In spite of the large IC50 values of the evaluated derivatives in the AChE inhibitory investigation, comparatively the compounds are reported to be better inhibitors than tacrine. Amongst these compounds, derivative 239 (IC50 = 60.26 μM) stood atop in the inhibition activity with 2.76-fold higher inhibitory effect than tacrine. The diester derivative 237 (IC50 = 64.04 μM) has exhibited almost similar inhibitory potential compared to compound 239. Further, dicarboxylic acid 234, pyridazineone analog 240, pyrazole derivitized with fused cyclic imide 242b have displayed slightly reduced inhibitory activity.

4.1. Pyrazole linked-benzothiazole-β-naphthol derivatives

Topoisomerases are enzymes responsible for the process of DNA replication. As the DNA is most essential for highly proliferative cells, inhibition of topoisomerase leads to cell apoptosis. Hence topoisomerases are effective chemotherapeutic candidates for corresponding drug discovery. In addition, topoisomerase I functions the process of cleavage and stitching back the single strands of double-stranded DNA. Pyrazole derivatives are demonstrated to possess DNA binding ability and anticancer properties. In addition to this, remarkable anticancer activity and DNA binding ability of benzothiazoles and potent cytotoxicity of β-naphthol scaffolds towards breast cancer have prompted to design benzothiazole-β-naphthol derivatives linked to pyrazoles [34].

In a multi-component reaction, pyrazole-3-aldehydes 243, substituted benzothiazole-2-amines 244 and β-naphthol are reacted to bestow final compounds 245a-ad (Scheme 50).

Employing circular dichroism (CD) the conformational changes in DNA with derivatives is studied; wherein the CD spectrum of the calf

Scheme 48. Design and synthesis of the target compounds 233a-o [32]; 
Reagents and conditions: i) stirring at room temperature ii) substituted aromatic aldehydes, malononitrile, piperidine, 2 h iii) substituted aromatic aldehydes, EtOH, AcOH, 3 h, Reflux.

Figure 26. Illustration of structures of molecules with remarkable COX-1 inhibitory activity.
Thymus DNA (CT-DNA) gives a positive band at 175 nm and a negative band at 245 nm due to \( \pi \)-base stacking and right-hand helicity. In this study, the addition of compounds 248d and 250a-f, the pyrazole NH is free that might be essential for apt delivery of the DNA-derivative complex. Further, the positive band at 275 nm got reduced in its intensity on the increase of concentration of the derivatives, revealing that further unwinding of DNA on derivative interaction. In addition to this, negative band intensity is altered at 245 nm; it indicates the ability of the designed derivatives to entail melting of the DNA-derivative complex. Further, the positive band at 245 nm which indicates slight hypochromicity; this, in turn, is a positive indication of melting of the DNA-derivative complex.

All the designed final derivatives have been evaluated for COX-I and COX-2 inhibitory properties in the presence of standard COX inhibitors celecoxib, diclofenac sodium, and indomethacin. No single evaluated derivative has enough COX-I potency \( (IC_{50} = 4.9–13.2 \mu M) \) to reach an inhibitory value of indomethacin \( (IC_{50} = 0.041 \mu M) \). Compounds such as 248d \( (IC_{50} = 4.9 \mu M) \), 250a \( (IC_{50} = 5.1 \mu M) \), and 250f \( (IC_{50} = 4.9 \mu M) \) have displayed COX-I activity almost similar to that of diclofenac sodium \( (IC_{50} = 3.8 \mu M) \). However, all the tested compounds could exert stronger inhibitory potentials compared to that of celecoxib \( (IC_{50} = 15.1 \mu M) \). On keen observation of inhibitory values of the compounds of three series, the derivatives of 249a-l \( (IC_{50} = 3.8 \mu M) \) to reach an inhibitory value of indomethacin \( (IC_{50} = 0.041 \mu M) \). Compounds such as 248d \( (IC_{50} = 4.9 \mu M) \), 250a \( (IC_{50} = 5.1 \mu M) \), and 250f \( (IC_{50} = 4.9 \mu M) \) have displayed COX-I activity almost similar to that of diclofenac sodium \( (IC_{50} = 3.8 \mu M) \). However, all the tested compounds could exert stronger inhibitory potentials compared to that of celecoxib \( (IC_{50} = 15.1 \mu M) \). On keen observation of inhibitory values of the compounds of three series, the derivatives of 249a-l \( (IC_{50} = 3.8 \mu M) \) to reach an inhibitory value of indomethacin \( (IC_{50} = 0.041 \mu M) \). Compounds such as 248d \( (IC_{50} = 4.9 \mu M) \), 250a \( (IC_{50} = 5.1 \mu M) \), and 250f \( (IC_{50} = 4.9 \mu M) \) have displayed COX-I activity almost similar to that of diclofenac sodium \( (IC_{50} = 3.8 \mu M) \). However, all the tested compounds could exert stronger inhibitory potentials compared to that of celecoxib \( (IC_{50} = 15.1 \mu M) \). On keen observation of inhibitory values of the compounds of three series, the compounds of 248d-f and 250a-f have exhibited stronger potentials compared to derivatives of 249a-l series. In the derivatives of 249a-l, the pyrazole NH is utilized for the design of mannich products with subsequent reduction of pyrazole basicity. Whereas in other series, 248a-f and 250a-f, the pyrazole NH is free that might be essential for apt fit into the active site of COX-I enzyme and hence these derivatives are the selective COX-I inhibitors.

In the case of COX-2 inhibitory effects, a collection of potent inhibitors are noticed \( (IC_{50} = 0.046–0.055 \mu M) \) (Table 11); wherein the \( IC_{50} \) values are almost identical with that of standard COX-2 inhibitor \( (IC_{50} = 0.049 \mu M) \). All tested derivatives have exhibited remarkable inhibitory values \( IC_{50} = 0.046–0.34 \mu M \). Contrary effects are noticed in the case of COX-2 inhibitory properties.

The strongest inhibitory properties are exerted by mannich derivatives 249a-l. Although a good account of COX-2 inhibitory properties has been observed for all the synthesized derivatives, comparatively the derivatives of the series 249a-l have elicited excellent inhibitory properties. The results infer that the mannich products of pyrazolones have COX-2 selectivity. It also indicates that \( N_1 \)-substituted pyrazoles and particular \( N \)-mannich bases of pyrazoles have essential pharmacores. Amongst the potent inhibitors, compound 249j (Figure 29) possessing 3-
fluoroaniline, 4-bromophenylhydrazone moiety at 3, 4-positions respectively in addition to N-methylene 2,6-dimethylaniline at N1-position has excelled in exhibiting most significant activity which is higher activity compared to celecoxib. Similarly, almost identical inhibitory effects are noticed for the compound 248b (Figure 29) wherein 4-bromo-phenylhyrdazone motif and N-methylene aniline are appended to pyrazole at 4- and N1-positions respectively. Only slight increment/decrement is observed on the variation of substituents on phenyl of phenylhydrazone and N-methylene aniline. Irrespective of the substituents on these phenyl rings, all mannich derivatives possessed remarkable COX-2 inhibitory properties.

4.3. Pyrazoles-containing thiophene, thienopyrimidine, and thienotriazolopyrimidine

Inflammation is a common symptom in most of the pathological diseases. COX-2 enzyme plays a prominent role in the mediation of inflammation through prostaglandins, thromboxanes, and leukotrienes. It is more fruitful to inhibit the COX-2 enzyme without interfering with COX-1 activity. Pyrazoles and pyrazolines are the most efficient choice of starting material to design potent anti-inflammatory agents. Inspired by the significant COX inhibitory of active thiophene-pyrazoles, design of pyrazoles incorporating thiophene, thienopyrimidine, and thienotriazolopyrimidine is accomplished [36].

The synthetic route involves the conversion of thiophene ester 251 to corresponding thiophene hydrazide 252 which is used as intermediate for the design of its derivatives. The –NH–NH2 fragment of compound 258 is utilized in the intermolecular cyclization with ethoxymethylene.
malononitrile and ethyl ethoxymethylene cyanoacetate producing fused thiophene-pyrimidine pyrazoles 259 and 260 respectively. Likewise, treatment of the intermediate 258 with diethyl ethoxymethylene malonate and acetylacetone has resulted in the formation of compounds 261 and 262 respectively. Condensation of compound 258 with pyrazole aldehyde 263 has produced corresponding hydrazone derivative 264 which on intramolecular cyclization rendered compound 265 (Scheme 55).

In the COX inhibitory activity investigation of the designed derivatives, good inhibitory activity is obtained towards the COX-1 enzyme; and all the evaluated molecules have exhibited stronger inhibitory potentials (IC$_{50}$ = 7.52–11.91 μM) compared to celecoxib (IC$_{50}$ = 14.70 μM). Comparatively, compound 253 (IC$_{50}$ = 7.52 μM) and 262 (IC$_{50}$ = 7.53 μM) are noteworthy derivatives to be mentioned. However, no single derivative evaluated has enough inhibitory potency that could be compared with standard COX inhibitor indomethacin (IC$_{50}$ = 0.10 μM). Regarding COX-2 inhibitory effects, a set of molecules (Table 12) has produced IC$_{50}$ values of high impact. Amongst them, compound 264 (Figure 30) has bestowed with the strongest inhibitory value which is almost comparable to that of celecoxib (IC$_{50}$ = 0.048 μM).

Compound 253 has exhibited the most significant COX-1 inhibitory activity wherein its structural features comprise 3-amino-4-phenylthiophene and 4-carbethoxy-5-hydroxypyrazole connected via the carbonyl group. Besides this, most of the derivatives (except for a few) have displayed almost similar COX-1 inhibitory properties irrespective of their structural differences. Regarding COX-2 activity, compound 264 possessing 7-phenylthienopyrimidine moiety appended to N$_1$-phenyl-3-(4-chlorophenyl) pyrazole at 4-position through methylene-hydrazine fragment has elicited promising activity. Unfortunately, cyclization (compound 265) carried out in order to check the further improvement of the activity is in vain. This fact shows that methylene-hydrazine fragment is essential for potent activity. It is also evident from the striking COX-2 inhibitory activity of 256c (Table 12) bearing methylene-hydrazine fragment. However similar analogy could not hold good for the compound 254 probably attributed to the presence of electron-withdrawing –CN and –COOEt groups at the methylene-hydrazine fragment end. Triplet of derivatives 259–261 has exhibited decent activity wherein these derivatives possessed 3,4-substituted pyrazole at thienopyrimidine 4-position. Particularly compound 260 (Figure 30) having carboxethoxy moiety and –NH$_2$ at 3- and 4-positions of pyrazole has shown nice activity. While the compounds 259 and 261 in which carboxethoxy and –NH$_2$ groups are substituted by –CN and –OH moieties respectively resulted in lowered inhibitory activity compared to compound 260.

Scheme 51. Synthesis of final compounds 248a-f [35]; Conditions and reagents: i) HCl/H$_2$O, reflux (3h); ii) substituted benzene diazonium chlorides, EtOH, CH$_3$COONa.

Scheme 52. Synthetic route for the preparation of the molecules 249a-l [35]; Reagents and conditions: i) HCHO/EtOH, stirring, 60 °C (6h).

Scheme 53. Design and synthesis of the pyrazole derivatives 250a-f [35]; Reagents and conditions: i) CH$_3$COONH$_4$, EtOH, reflux (15h).
observation reveals the essence of the carboxethoxy and –NH₂ groups at pyrazole 3- and 4-positions.

4.4. Pyrazoline benzene sulfonamides

The membrane-bound enzyme, acetylcholinesterase found in many types of tissues is responsible for the termination of cholinergic signaling wherein the enzyme acetylcholinesterase hydrolyzes acetylcholine. This subsequently leads to AD and inhibition of a particular enzyme would be an effective approach for the cure of AD. Celecoxib possessing pyrazoline and sulfonamide has been demonstrated to have stimulatory effects on brain AChE levels and β-amyloid peptide in addition to COX-2 inhibitory properties. Alongside, pyrazole, pyrazoline, and sulfonamide derivatives have exhibited carbonic anhydrase and AChE inhibitory properties. In light of these observations, a research team has aimed at a new series of pyrazolines derivatized with benzene sulfonamides [37].

The synthetic pathway involves condensation of 4-substituted acetophenones 266 with trisubstituted benzaldehydes 267 to obtain corresponding chalcones 268a-h. An α,β-unsaturated fragment in chalcones 268a-h is utilized in the intermolecular cyclization producing dihydropyrazole-sulfonamide derivatives 269a-h (Scheme 56).

All the final compounds are tested for hCA I, hCA II and AChE inhibitory properties using acetazolamide and tacrine as the reference compounds for CA and AChE enzymes respectively. In case of hCA I and hCA II, all the evaluated molecules displayed decent inhibitory activity (hCA I: IC₅₀ = 0.0347–0.0769 μM, hCA II: IC₅₀ = 0.0301–0.491 μM) which are better than that of AAZ (hCA I: IC₅₀ = 0.169 μM, hCA II: IC₅₀ = 0.149 μM). In particular compound 269d (IC₅₀ = 0.0347 μM) has exhibited the most significant hCA I inhibitory activity. Except for compound 269e (IC₅₀ = 0.0769 μM) (Figure 31), other derivatives have almost similar hCA I inhibitory properties. While compound 269a (Figure 31) stood atop of the hCA II inhibitors with IC₅₀ value 0.0301 μM. Analogous to hCA I inhibitors, all the evaluated molecules have shown almost similar IC₅₀ values towards hCA II. Observation of the hCA I and hCA II inhibitory properties infer selective inhibition. Except for compound 269e, all other derivatives have selectivity towards only one CA type. Alongside, in general, with some exceptions, the compounds with the 2,4-dimethoxy phenyl ring attached to dihydropyrazole 5-position have excelled in hCA II inhibitory potencies. In that, compound 269a possessing phenyl ring connected to dihydropyrazole 3-position bestowed the strongest activity. However, the substitution of phenyl ring at dihydropyrazole 3-position with 4-fluorobenzene/4-chlorobenzene rings has diminished the activity gradually.

| Compd | IC₅₀ (μM) |
|-------|----------|
| 249b  | 0.048    |
| 249c  | 0.055    |
| 249d  | 0.051    |
| 249j  | 0.046    |
| 249l  | 0.054    |
| Celecoxib | 0.046 |

The synthetic pathway involves condensation of 4-substituted acetophenones 266 with trisubstituted benzaldehydes 267 to obtain corresponding chalcones 268a-h. An α,β-unsaturated fragment in chalcones 268a-h is utilized in the intermolecular cyclization producing dihydropyrazole-sulfonamide derivatives 269a-h (Scheme 56).

All the final compounds are tested for hCA I, hCA II and AChE inhibitory properties using acetazolamide and tacrine as the reference compounds for CA and AChE enzymes respectively. In case of hCA I and hCA II, all the evaluated molecules displayed decent inhibitory activity (hCA I: IC₅₀ = 0.0347–0.0769 μM, hCA II: IC₅₀ = 0.0301–0.491 μM) which are better than that of AAZ (hCA I: IC₅₀ = 0.169 μM, hCA II: IC₅₀ = 0.149 μM). In particular compound 269d (IC₅₀ = 0.0347 μM) has exhibited the most significant hCA I inhibitory activity. Except for compound 269e (IC₅₀ = 0.0769 μM) (Figure 31), other derivatives have almost similar hCA I inhibitory properties. While compound 269a (Figure 31) stood atop of the hCA II inhibitors with IC₅₀ value 0.0301 μM. Analogous to hCA I inhibitors, all the evaluated molecules have shown almost similar IC₅₀ values towards hCA II. Observation of the hCA I and hCA II inhibitory properties infer selective inhibition. Except for compound 269e, all other derivatives have selectivity towards only one CA type. Alongside, in general, with some exceptions, the compounds with the 2,4-dimethoxy phenyl ring attached to dihydropyrazole 5-position have excelled in hCA II inhibitory potencies. In that, compound 269a possessing phenyl ring connected to dihydropyrazole 3-position bestowed the strongest activity. However, the substitution of phenyl ring at dihydropyrazole 3-position with 4-fluorobenzene/4-chlorobenzene rings has diminished the activity gradually.
dihydropyrazole analogs with 3,4-dimethoxy phenyl moiety at 5-position have led to potential hCA I inhibitors. Further, the AChE inhibitory activity (IC\textsubscript{50} = 0.082 – 0.233 μM) of the final compounds indicates that every tested molecule is an efficient inhibitor compared to tacrine (IC\textsubscript{50} = 0.698 μM). However, the compounds 269b (IC\textsubscript{50} = 0.0822 μM) and 269d (IC\textsubscript{50} = 0.086 μM) possessed most remarkable AChE inhibitory activities. When the inhibitory values of hCA I, hCA II and AChE are checked and correlated, an interesting observation is made wherein there is direct synchronization of hCA I, and AChE inhibitory values for compounds 269a-e. These may suggest that a potent hCA I inhibitor may be also a potent AChE inhibitor.

4.5. Pyrazolopyrimidine scaffolds

The cyclooxygenases are mediators of inflammation many times. The drugs for reduction of the risks that arise with the usage of NSAIDs to cure inflammatory-related diseases are being designed and discovered. Besides this, selective COX-2 inhibitors have been developed to reduce adverse effects. The pyrazole scaffolds possessing fused pyrazolopyrimidine core are described as potent anti-inflammatory agents alongside possessing other prominent pharmacological properties. In this regard, pyrazolopyrimidine scaffolds are synthesized to check the improvement in COX inhibitory properties [38]. Synthetic pathway commences from compound 270 wherein dinitrile ethylene 270 is allowed to undergo intermolecular cyclization with phenylhydrazine or its 4-methylsulfonyl derivative to form pyrazole derivatives 271a-b. The intermediates 275a-b are cyclized with CS\textsubscript{2} to obtain oxadiazoles linked to fused pyrazole-pyrimidine derivatives 279a-b. While cyclization of intermediates 275a-b with ethyl acetoacetate resulted in fused pyrazolo-chloropyrimidines 273a-b followed by nucleophilic substitution of –Cl with α-aminoester yielding compounds 274a-b. Hydrolysis of the ester functionality with hydrazine produced corresponding hydrazides 275a-b (Scheme 57). Hydrazides of fused pyrazole-pyrimidines 275a-b are used intermediates to design condensed products 276a-f with various aldehydes. The intermolecular cyclization of compounds 275a-f with ethyl isothiocyanate produced pyrazole-pyrimidines-linked triazole thiol 277a-b. α-Amino hydrazide fragment is utilized in which it is cyclized with appropriate phenyl or 4-substituted phenyl isothiocyanate to obtain corresponding triazole analogs 278a-f (Scheme 58).

The intermediates 275a-b are synthesized from compound 270 wherein dinitrile ethylene 270 is allowed to undergo intermolecular cyclization with phenylhydrazine or its 4-methylsulfonyl derivative to form pyrazole derivatives 271a-b which are transformed into fused pyrazolopyrimidine scaffolds using formic acid to yield 272a-b. Fused pyrimidinones 272a-b are transformed into fused pyrazolopyrimidines 273a-b followed by nucleophilic substitution of –Cl with α-aminoester yielding compounds 274a-b (Scheme 57). Hydrazides of fused pyrazole-pyrimidines 275a-b are used intermediates to design condensed products 276a-f with various aldehydes. The intermolecular cyclization of compounds 275a-f with ethyl isothiocyanate produced pyrazole-pyrimidines-linked triazole thiol 277a-b. α-Amino hydrazide fragment is utilized in which it is cyclized with appropriate phenyl or 4-substituted phenyl isothiocyanate to obtain corresponding triazole analogs 278a-f (Scheme 58).
pyrazole-pyrimidines linked to pyrazole 280a-b (Scheme 59). The expected regioisomers 281 and 282 are not formed. Except for a few derivatives, all the compounds have inhibitory activities with small differences. In structure-activity studies, it is evident that the hydrazide analogs 276a-f (except for compound 276a) are reported to possess comparatively weak COX-1 activity indicating that the hydrazide fragment is not favorable for potent activity.

Using standard COX inhibitors celecoxib, diclofenac disodium and indomethacin, COX-1 and COX-2 inhibitory properties are evaluated. Although COX-1 inhibitory properties of final compounds (IC\textsubscript{50} = 5.28–13.11 μM) are poor compared to indomethacin (IC\textsubscript{50} = 0.041 μM); compared to celecoxib (IC\textsubscript{50} = 8.1 μM) and diclofenac sodium (IC\textsubscript{50} = 3.8 μM) potent activities have been exhibited by the target molecules. The most significant COX-I inhibitory activity (IC\textsubscript{50} = 5.28 μM) is displayed by the compound 278e. Meanwhile, in the case of triazole thiol analogs, compound 277a (IC\textsubscript{50} = 7.35 μM) rendered significant activity; its methylsulfonyl analog exhibited diminished inhibitory property. When it comes to 278a-f series of compounds, all the derivatives (except for compound 278a) have shown decent COX-1 inhibitory properties. In these compounds, the presence of triazole thiol N\textsubscript{1}-(4-aryl) substituted moiety or methylsulfonyl motif must be crucial. Particularly, compound 278e possessing 4-fluorophenyl ring at triazole thiol N\textsubscript{1}-position and 4-methylsulfonylphenyl ring on fused pyrazole-pyrimidine moiety is most remarkable among evaluated molecules. Fortunately, potential IC\textsubscript{50} values are observed towards COX-2 activity. All the tested compounds have exerted noteworthy inhibitory properties which are higher than that of diclofenac sodium (IC\textsubscript{50} = 0.84 μM) and indomethacin (IC\textsubscript{50} = 0.51 μM). However, the inhibitory values are weak compared to standard inhibitor celecoxib (IC\textsubscript{50} = 0.049 μM). In these, 276a-f series compounds (except for compound 276a) have excelled in the COX-2 inhibitory activity (Table 13). Compounds of other series possessed more or less similar potencies. The aromatic hydrazide analogs of the series 276a-f have exhibited excellent COX-2 inhibitory activity which might be attributed to hydrazide fragment as well as the methylsulfonyl moiety. Particularly these compounds with methylsulfonyl moiety have resulted in a well-established activity. Besides this series, the triazole thiol analog 278a bearing phenyl ring at triazole N\textsubscript{1}-position possessed identical inhibitory activity compared to the 276a-f series. However, the substitution of 4-fluorophenyl/4-chlorophenyl or presence of methylsulfonyl

| Compd | COX-2 inhibitory activity IC\textsubscript{50} (μM) |
|-------|-----------------------------------------------|
| 256c  | 0.071                                         |
| 259   | 0.063                                         |
| 260   | 0.059                                         |
| 261   | 0.098                                         |
| Celecoxib | 0.045                        |

**Table 12. Inhibitory values of potent COX-2 inhibitory values.**

![Figure 30. Structures of remarkable COX-2 inhibitors.](image)

**Scheme 56. Synthesis of dihydropyrazole-benzensulfonamide derivatives [37]; Reagents and conditions: i) EtOH, aq. NaOH (20%), rt. ii) p-hydrazinobenzene sulfonamide hydrochloride, EtOH, glacial acetic acid, reflux (4–19h).**
moiety at \(N_1\)-position of the fused pyrazole-pyrimidine scaffold has diminished the activity to a greater extent. Along with these, compound 280a has managed to exhibit the strongest inhibitory activity which might be attributed to the pyrazole ring linked to pyrimidine moiety in addition to 4-sulfonyl-methyl phenyl ring at fused pyrazole-pyrimidine \(N_1\)-position. In general consensus, the evaluated derivatives are selective inhibitors.

4.6. Pyrazolyl pyrimidinetriones and thioxopyrimidinediones

Ecto-nucleotidases perform P2 purinergic signaling in addition to maintaining cell function. Cell activation, apoptosis, proliferation, and degenerative neurological and immunological responses are some of the disorders attributed to the overexpression of the ectonucleotidases. Hence to address such problems, the design of ectonucleotidase inhibitors is achieved. Ecto-nucleotide pyrophosphatases/phosphodiesterases (NPPs) entail seven various subtypes (NPP1-7). Among these, NPP-1 and NPP-3 perform the hydrolysis of pyrophosphatases and phosphodiester in various nucleotides. NPP1-3 are associated with a number of physiological functions such as nucleotide recycling, stimulating cell motility and modulation of purinergic receptor signaling. Particularly NPP1 performs the biological processes including insulin receptor signaling, bone mineralization, and immune modulation; however overexpression of the enzyme NPP1 results in the ectopic calcification, calcium pyrophosphate dehydrate crystal deposition and cancer cell proliferation. While the NPP3 is reported as tumor marker since its overexpression led to carcinogenesis and cancer cell metastasis. Alkaline phosphatases catalyze the dephosphorylation of nucleotide phosphates and phosphomonoesters. In the alkaline phosphatase family of enzymes, tissue-nonspecific alkaline phosphatases (TNAP) are an important type of enzymes. Overexpression of human alkaline phosphatases in cancer cells, Paget’s disease, and osteoblastic bone metastasis. Pyrazole pharmacore has been in limelight because of its versatile pharmacology alongside ectonucleotidase inhibitory properties. To address all these problems of alkaline phosphatases and nucleotide pyrophosphatases, novel pyrazolyl pyrimidinetriones and thioxopyrimidinediones are designed and synthesized [39].

The synthetic route involves condensation of phenylhydrazine with 4-substituted acetophenones 281a-g giving corresponding hydrazones 282a-g. Cyclization of the hydrazine fragment using formylating mixture yielded pyrazole derivatives 283a-g followed by condensation with pyrimidine triones/thiopyrimidine dioxanes resulted in final compounds 284a-n (Scheme 60).

**Scheme 57.** Synthetic pathway for preparation of compounds 275a-b [38]; **Reagents and reaction conditions:** i) phenylhydrazine hydrochloric (R = H) and p-methane sulfonyl hydrochloride (R = SO₂CH₃), sodium acetate, 95% ethanol, reflux (5h); ii) formic acid (85%), reflux (10h); iii) POCl₃, DMF, reflux (4h); iv) glycine ethyl ester hydrochloride, TEA, absolute ethanol, reflux (5–6h); v) hydrazine hydrate, EtOH, reflux (10h).
Scheme 58. Synthesis of the pyrazolo-pyrimidine derivatives [38]; Reagents and conditions: i) appropriate aldehyde, absolute EtOH, gl. Acetic acid, reflux (4h); ii) ethyl isothiocyanate, absolute ethanol, TEA, reflux (3h); iii) appropriate phenyl or 4-substituted phenyl isothiocyanate, absolute ethanol, TEA, reflux (3h).

Scheme 59. Preparation of target molecules 279a, b and 280a, b [38]; Reagents and conditions: i) CS₂, KOH, absolute ethanol, reflux (3h); ii) ethyl acetoacetate, absolute ethanol, reflux (10h).
The designed derivatives are investigated for their alkaline phosphatases and nucleotide phosphatase inhibitory properties using sumarin, levamisole and L-phenylalanine as reference compounds (Table 14). Almost half of the synthesized molecules could exhibit strong inhibitory values compared to corresponding standard inhibitors. Among the potent h-TNAP inhibitors, compound 284a in which N1, N3-dimethyl-2,4,6-pyrimidinetrione is tethered to pyrazole 4-position via methylene group alongside phenyl ring at pyrazole 3-position is found to possess most significant IC50 value; while the compound 284n possessing 4-nitrophenyl ring and N1, N3-diethyl-2-thioxo-4,6-pyrimidinetrione at 3- and 4-positions of pyrazole stands next to it in exhibition of h-TNAP inhibitory activity. Compound 284a has shown 61 times greater inhibitory potential than that of levamisole. In the case of N1, N3-dimethyl-2,4,6-pyrimidinetrione analogs, compounds with a 4-bulky group (irrespective of electron environment) substituted phenyl ring at pyrazole 3-position have failed to exhibit good inhibitory values. Meanwhile, the activity could not be correlated with the N1, N3-diethyl-2-thioxo-4,6-pyrimidinetrione derivatives. Surprising facts are observed in the case of h-IAP inhibitory activities. All the potent h-IAP inhibitors are h-TNAP inactive revealing that the synthesized molecules are selective inhibitors. The most potent inhibitor 284d comprises N1, N3-dimethyl-2,4,6-pyrimidinetrione connected to pyrazole 4-position and 4-bromophenyl ring at 3-position of pyrazole; it is ~ 175 fold higher activity compared to the standard inhibitor. The results described in this way; the presence of pyrimidinetrione and 4-bulky electron-withdrawing group substituted phenyl ring is a must for remarkable h-IAP inhibitory activity.

Regarding h-NPP1 activity, some compounds have rendered promising activity; amongst them, compound 284b (Figure 32) has bestowed with most striking inhibitory activity. Its significant activity is attributed to the N1, N3-dimethyl-2,4,6-pyrimidinetrione analogs of pyrazole have excelled in eliciting higher alkaline phosphatase/nucleotide pyrophosphatase inhibitory potentials compared to N1, N3-diethyl-2-thioxo-4,6-pyrimidinetrione derivatives.

### Table 13. COX-2 inhibitory values of most potent inhibitors.

| Compd | COX-2 inhibitory activity IC50 (μM) |
|-------|-----------------------------------|
| 276b  | 0.19                              |
| 276c  | 0.11                              |
| 276d  | 0.10                              |
| 276e  | 0.12                              |
| 276f  | 0.10                              |
| 278a  | 0.10                              |
| 280a  | 0.10                              |

Scheme 60. Synthesis of the target molecules 284a-n [39]: Reagents and conditions: i) AcOH, EtOH, 70 °C; ii) a. DMF, POCl3, 0→70 °C (3h); b. aq NaOH/aq NaHCO3; III) DCM/MeOH (8:1), EDA (0.2 mmol), AcOH (2 mM).
4.7. Pyridinylimidazoles

Glycogen synthase kinase $\beta$ (GSK3$\beta$) is an enzyme of synthase kinase enzyme family, associated with hyperphosphorylation tau protein and its overexpression has been led to increased production of $\beta$-amyloids. The $p38$ $\alpha$ mitogen-activated protein (MAP) kinase plays a significant role in the biosynthesis of proinflammatory cytokines at translational and transcriptional levels. Overactivity of $p38$ $\alpha$ MAP kinase is responsible for the tau protein hyperphosphorylation in addition to neuroinflammation. The research revealed that selective $p38$ $\alpha$ MAP kinase inhibitors have diminished tau phosphorylation. In this regard, pyridinyl imidazoles have been demonstrated as versatile pharmacores that target several kinases including $p38$ $\alpha$ MAP kinases. Hence, in order to reduce the problems associated with the enzymes GSK3$\beta$ and $p38$ $\alpha$ MAP kinase, a pool of pyridinyl imidazoles are designed and synthesized [40]. In the synthetic pathway, Boc-protected 4-methyl-2-aminopyridine is again protected by 4-methoxybenzyl moiety to form compound which is treated with ethyl-4-fluorobenzoate to yield a carbonyl compound. Hydroxylamine moiety is introduced adjacent to the carbonyl group in compound using sodium nitrite to render

| Comp | h-TNAP $IC_{50}$ (μM) | h-IAP | h-NPP1 | h-NPP3 |
|------|------------------|-------|-------|-------|
| 284a | 0.33             |       |       |       |
| 284b | 2.21             |       | 0.61  | 0.66  |
| 284c |               | 0.86  |       |       |
| 284d |               | 0.57  | 1.21  | 2.27  |
| 284e | 5.83             |       | 1.86  |       |
| 284f |               |       |       |       |
| 284g |               | 12.7  |       | 2.49  |
| 284h | 2.99             |       | 4.61  | 0.57  |
| 284i |               |       |       | 2.03  |
| 284j |               | 33.6  | 1.01  |       |
| 284k | 3.07             |       |       | 6.36  |
| 284l | 5.26             | 6.33  |       | 4.55  |
| 284m |               | 1.31  |       | 2.86  |
| 284n | 1.78             | 21.2  |       | 2.11  |
| Sumarin |             |       | 8.67  | 1.27  |
| Levamisole |     | 20.2  |       |       |
| L-Phenylalanine | | 100   |       |       |

Table 14. Depiction of alkaline phosphatase and nucleotide phosphatase inhibitory values of final compounds.

Figure 32. Representation of structures of potent alkaline phosphatase/nucleotide pyrophosphatase inhibitors.
compound 288 followed by reduction of hydroxylamine functionality into corresponding ammonium salt 289. α-Aminocarbonyl fragment of compound 289 is utilized for cyclization using potassium thiocyanate to produce imidazole-2-thione 290 with subsequent nucleophilic substitution with methyl chloride/benzyl chloride to obtain compounds 291-a-b. Finally, the removal of NH-Boc gave the molecules 292a-b (Scheme 61).

Pyridine-carboxamide derivatives 294a-u and 295a-e are prepared from commercially available compounds 292a-b/293a-b using the reagents mentioned in the Scheme 60. The alkyl thiol moiety is oxidized to thioxane analogs 296a-b (Scheme 62).

The commercially available compounds 297 is reacted with cyclopropanecarbonyl chloride to design pyridine-2-cyclopropylcarboxamide of imidazole thiol derivative 299; likewise, compound 298 is coupled with cyclopropanecarbonyl producing carboxamide derivative 300 (Scheme 63).

The pyridine carbonyl intermediate 287 is oxidized at the adjacent position of the carbonyl group to produce diketo analog 301 from which removal of NH-Boc led to the formation of diketo derivative of pyridine-2-cyclopropylcarboxamide derivative 303 and N. N'-dicyclopentylcarboxamide derivative 304 are obtained from diketone derivative 301 using cyclopropanecarbonyl chloride in which diketone pharmacore of compound 304 is utilized in the design of imidazole ring subsequently yielding final compounds 305a-g (Scheme 64).

NH group protection of 2-methylimidazole 306 gave compound 307; wherein tributyltin moiety is appended at imidazole 5-position to obtain compound 308. N-(4-Bromopyridin-2-yl)cyclopropane carboxamide is coupled with 2-methylimidazole at 5-position followed by hydrolysis of 2-(trimethylsilyl) ethoxy methyl protection group bestowed target molecule 310 (Scheme 65).

All the synthesized molecules are evaluated for their GSK3β and p38α MAP kinase inhibitory activities using SB203580 (IC50 = 0.041 μM) and SB217673 (IC50 = 0.089 μM) as reference compounds for p38α MAP kinase and GSK3β respectively. In the first series of pyrimidineimidazole derivatives 294a-u, an excellent account of p38α MAP kinase inhibitors molecules is found to exhibit potent p38α MAP kinase inhibitory activity. Amongst them 294e (IC50 = 0.013 μM), 294k (IC50 = 0.015 μM) and 294l (IC50 = 0.015 μM) shown most remarkable inhibitory activity. Except for compound 294a, all other derivatives displayed almost similar inhibitory potencies. This series of molecules comprise imidazole-2-thiomethane in addition to 4-fluorobenzene at imidazole 4-position and pyridine carbazole at imidazole 5-position to which various moieties are attached. Compound 294e (Figure 33) finds its place in this series as the most significant inhibitor wherein the structural significance includes 3,4,5-trimethoxy-methylene benzencarboxamide. An increase in the alkyl chain length from methylene to ethylene (compound 294a) resulted in a large extent of reduced activity (IC50 = 0.088 μM). Alongside, the removal of methoxy groups (compound 294f) has led to diminished activity (IC50 = 0.055 μM). The carboxamides with cyclopropyl ring and cyclobutyl rings have possessed decent inhibitory activity. The derivatives of the sub-series 295a-e which contain 2-benzylimidazole ring are only moderate inhibitors. However, compound 295e possessing 4-fluoro-methylene benzene motif appended to carbazole moiety is strongest p38α MAP kinase inhibitory value (IC50 = 0.003 μM) among all the pyrimidineimidazole scaffolds which has 30-fold higher potency compared to reference compound and compound 295e is found to be selective p38α MAP kinase inhibitor. The series of compounds 305a-g, pyridine-cyclopropyl carboxamides bearing 2-substituted imidazole rendered finest inhibitory activities which are almost similar with respect to IC50 values (IC50 = 0.016–0.027 μM). N-Methylimidazolopyridine-cyclopropylcarboxamide derivatives 299 and 300 are out of the boundary of the good inhibitory potencies.

In the case of GSK3β inhibitory properties, most of the derivatives are poor inhibitors compared to the standard inhibitor. In the 294a-u series, all the molecules have displayed a very weak inhibitory property except for compound 294c (IC50 = 0.040 μM) entailing cyclopropylcarboxamide attached to pyridine possessed noteworthy inhibitory effects. Along with it, compound 294q (IC50 = 0.073 μM) could also exhibit the finest inhibitory property wherein cyclopentanone ring is appended to carboxamide fragment. 2-Thiobenzylimidazole derivatives 295a-e have failed to exhibit good inhibitory effects. Half of the compounds of 305a-g series exerted remarkable GSK3β inhibitory properties; particularly compound 305c (IC50 = 0.035 μM) (Figure 33) is reported to have the strongest inhibitory potency which is better than that of standard inhibitor. Structurally compound 305c comprises pyridine-cyclopropyl amide connected to 2-ethylimidazole at 5-position. Hence pyridine-cyclopropyl amide and 2-alkyl substituted imidazole motifs favor for GSK3β/p38α MAP kinase dual inhibitory potentials. The compound devoid of 4-fluorobenzene at imidazole 4-position (compound 310) has shown complete wash out with respect to both inhibitory properties which confirms mandate of 4-fluorobenzene at imidazole 4-position for good inhibitory activity.

4.8. Quinazolin-4(3H)-one derivatives

Urease, a metalloenzyme associated with nickel performs hydrolysis of urea to carbon and ammonia; increased pH attributed to liberated ammonia paves for the survival of Helicobacter pylori. It has been reported that Helicobacter pylori is responsible for many gastroduodenal disorders such as peptic ulcer, gastric cancer, and duodenal ulcers and so forth. Hence, the need of the hour is to design the urease inhibitors. In order to counter such issues, the pleura of heterocyclic molecules are being synthesized including quinazolinones that have possessed anti-cancer properties along with other prominent pharmacological properties. In
this regard, urease inhibitory investigation is continued with the design and preparation of quinazolinone derivatives linked to thiadiazole/triazole moiety [41].

Pyrimidinone appended with 4-substituted benzyl at pyrimidinone 2-position 311a-e are treated with bromoethylacetate to get corresponding ester 312a-e. The ester functionality of compounds 312a-e is transformed to hydrazide 313a-e with subsequent treatment using ethyl isothiocyanate rendered thiosemicarbazide analogs 314a-e. The thiosemicarbazide fragment of compounds 314a-e is utilized for cyclization to produce final compounds 315a-e and 316a-e in presence of NaHCO$_3$ and H$_2$SO$_4$ respectively (Scheme 66).

The prepared molecules are evaluated for urease inhibitory effects in the presence of urea and acetohydroxamic acid. Compared to reference compounds, all the tested compounds have exhibited strong urease inhibitory properties. In the three series of compounds, the thiosemicarbazide analogs 314a-e displayed comparatively weak activity (IC$_{50}$ = 6.00–6.42 μM); indicating that thiosemicarbazide fragment is not favorable for anti-urease activity. Again pyrimidinone-thiadiazole 316a-e derivatives could show higher inhibitory activity (IC$_{50}$ = 2.24–2.98 μM) compared to thiosemicarbazide analogs but inferior inhibitory effects with respect to triazole thione derivatives 315a-e. Fortunately, pyrimidinone-linked thiadiazoles 315a-e exerted most significant

Scheme 62. Synthetic route for preparation of compounds 295a-u [40]; Reagents and conditions: i) carboxylic acid, PyBOP, DIPEA, DCM, rt; ii) carboxylic acid, HATU, DIPEA, DCM, rt; iii) acyl chloride, pyridine, 0 °C, rt; iv) amide, Pd$_2$(dba)$_3$, XantPhos, Cs$_2$CO$_3$, DMF, 100 °C (16h); v) H$_2$O$_2$, MeCN, rt.
inhibitory potencies (IC\textsubscript{50} = 1.90–2.00 \textgreek{M}). These observations show that pyrimidine linked to triazole-thiones have a beneficiary influence on urease inhibitory properties. An interesting thing observed in these pyrimidinone derivatives is a similarity in the inhibitory effects in a particular series. This similarity within a series of compounds infers that pyrimidine linked to triazole-thiones have a beneficial second-generation EGFR inhibitors. Taking consideration of anticancer properties of quinoline along with other pharmacological and properties and pyrazolylthiazole derivatized naphthalene, quinoline based 4,5-dihydropyrazoles are synthesized [42].

Synthesis of target molecules commenced with 2-Chloro-3-formyl-6-methoxyquinoline 317 wherein compound 317 is condensed with 2-hydroxyacetophenone to yield analogous chalcone 318 followed by hydrolysis with glacial acetic acid to produce chalcone of 2-hydroxyquinoline 319 (Scheme 67). An \(\alpha,\beta\)-unsaturated moiety of intermediate chalcone 318 is made to undergo intermolecular cyclization with semicarbazide/thiosemicarbazide yielding dihydropyrazole-quinoline derivatives 320a-b. In continuation, cyclization of the chalcone 318 with hydrazine and acetic acid rendered compound 321 and anilide moieties in the case of compounds 322a-d (Scheme 69). Again reaction of compound 320b with appropriate 2-oxo-N-arylpropanehydrazonyl chloride resulted in compounds 324a-d (Scheme 69).

All the synthesized molecules are first tested for their cytotoxicity on cancer cell lines such as MCFC7, HeLa, DLD1, and WT-38. Based on cytotoxicity of derivatives, some compounds which exhibited excellent activity have been selected for inhibition of EGFR. Among the chosen compounds, derivative 322b (Figure 34) has exhibited the most significant EGFR potency (IC\textsubscript{50} = 0.0318 \textgreek{M}). The molecule entails 4-tolyl and 2-chloro-6-methoxyquinoline dihydropyrazole 3- and 5-positions in addition to substituted thiazole-2yl motif at N\textsubscript{1}-position as a basic structure; besides, compound 322b is connected to 4-fluorophenyl ring at thiazole 5-position. Its activity is almost identical with that of the reference compound gefitinib (IC\textsubscript{50} = 0.0291 \textgreek{M}). Substitution of the 4-fluorophenyl ring at dihydropyrazole 5-position with phenyl ring led to the total washout of the EGFR inhibitory activity. Slightly diminished activity is observed with substitution of the 4-fluorophenyl ring with ester and anilide moieties in the case of compounds 323b and 323c. The

4.9. Quinoline based 4,5-dihydropyrazoles

Out of a large number of tyrosine kinases, epidermal growth factor receptor (EGFR) is one of the enzymes involved in the regulation of several cellular functions like cell growth, survival, proliferation, and apoptosis. Downstream activation of EGFR would occur with the binding of ligands as EGF, which leads to MAP kinase activation and in turn phosphorylation of protein will result. Many of cancer types have been demonstrated due to significant mutations through MPAPK or EGFR pathways. Drugs like afatinib and dacomitinib are designed as irreversible second-generation EGFR inhibitors. Considering the anti-cancer properties of quinoline along with other pharmacological properties and pyrazolylthiazole derivatized naphthalene, quinoline based 4,5-dihydropyrazoles are synthesized [42].

Synthesis of target molecules commenced with 2-Chloro-3-formyl-6-methoxyquinoline 317 wherein compound 317 is condensed with 2-hydroxyacetophenone to yield analogous chalcone 318 followed by hydrolysis with glacial acetic acid to produce chalcone of 2-hydroxyquinoline 319 (Scheme 67). An \(\alpha,\beta\)-unsaturated moiety of intermediate chalcone 318 is made to undergo intermolecular cyclization with semicarbazide/thiosemicarbazide yielding dihydropyrazole-quinoline derivatives 320a-b. In continuation, cyclization of the chalcone 318 with hydrazine and acetic acid rendered compound 321 (Scheme 68). Further, the thioamide functionality on dihydropyrazole 320b on cyclization with appropriate phenacyl bromides obtained corresponding thiazole derivatives 322a-d. Treatment of compound 320b with appropriate 3-chloropentane-2,4-diones yielded compounds 323a-c. Again reaction of compound 320b with appropriate 2-oxo-N-arylpropanehydrazonyl chloride resulted in compounds 324a-d (Scheme 69).

All the synthesized molecules are first tested for their cytotoxicity on cancer cell lines such as MCFC7, HeLa, DLD1, and WT-38. Based on cytotoxicity of derivatives, some compounds which exhibited excellent activity have been selected for inhibition of EGFR. Among the chosen compounds, derivative 322b (Figure 34) has exhibited the most significant EGFR potency (IC\textsubscript{50} = 0.0318 \textgreek{M}). The molecule entails 4-tolyl and 2-chloro-6-methoxyquinoline dihydropyrazole 3- and 5-positions in addition to substituted thiazole-2yl motif at N\textsubscript{1}-position as a basic structure; besides, compound 322b is connected to 4-fluorophenyl ring at thiazole 5-position. Its activity is almost identical with that of the reference compound gefitinib (IC\textsubscript{50} = 0.0291 \textgreek{M}). Substitution of the 4-fluorophenyl ring at dihydropyrazole 5-position with phenyl ring led to the total washout of the EGFR inhibitory activity. Slightly diminished activity is observed with substitution of the 4-fluorophenyl ring with ester and anilide moieties in the case of compounds 323b and 323c. The
molecule 323c possessing anilide at thiazole 4-position has shown remarkable inhibitory activity (IC₅₀ = 0.0425 μM). However, the exchange of aniline with ethyl carboxylate at thiazole 4-position (323b) has led to further reduced activity (IC₅₀ = 0.063 μM). Chalcone 318 has stood second in the potent inhibitors list with IC₅₀ value of 0.037 μM. Replacement of 2-chloro group of compound 318 with –OH resulted in a diminished activity. Diazophenyl thiazole scaffold 324a could only render a weak EGFR inhibitory activity.

5. Radiiodinated benzo[d] imidazole-quinoline derivatives

Among the transmembrane receptor tyrosine kinases, platelet-derived growth factor receptor β (PDGFRβ) is one that assumes responsibility with highly regulated cell expression. The enzyme is associated with angiogenesis and embryonic growth in addition to other functions such as the formation of blood vessels, kidneys, adipocytes. A large number of cancer types are associated with overactivity of PDGFRβ functions such as the formation of blood vessels, kidneys, adipocytes. Aβ-glycosidases are also involved in angiogenesis and embryonic growth in addition to other functions. 8-Hydroxyquinoline linked to benzimidazole derivative 325 is tri-flated using N-phenyl-bis(trifluoromethanesulfonylimide) yielding compound 326. Substitution of triflate of compound 326 followed by coupling of secondary amines at quinoline 8-position has produced compounds 327a-g (Scheme 70). 8-Hydroxy group of compound 325 is coupled with various mesylates to render compounds 328a-b (Scheme 71).

In another series, the precursor 327a is iodinated at quinoline 5-position to give compound 329 followed by NH protection by –Boc group (329). Iodine at quinoline 5-position is replaced by butyltin moiety to obtain compound 331. Final compound 333 is synthesized by radiiodinating the compound 331 to get compound 332 with subsequent NH –Boc protection removal (Scheme 70). In a similar fashion, 327d is radiiodinated at quinoline 5-position to form compound 333 (Scheme 72) (see Scheme 73).

The non-iodinated benzimidazole-quinoline derivatives 327a-g and 328a-b have been tested for the ability to reduce PDGFRβ-overexpressed cell line viability. Out of the derivatives evaluated, compounds 327a and 327d (Figure 35) exhibited enhanced inhibitory potencies on PDGFRβ positive cells. Hence, these compounds are selectively radiiodinated at quinoline 5-position to check the further improvement of the inhibitory activity; unfortunately, the radiiodinated derivatives could hardly express good binding to PDGFRβ. The results indicate that the introduction of iodine diminished the affinity towards PDGFRβ. The correlation between PDGFRβ affinity and structural versatility of the designed molecules goes in this way: the presence of piperazone and morpholine at quinoline 8-position in compounds 327a and 327d respectively bestowed them the most remarkable inhibitory potentials. Replacement of piperazone with N-methyl piperazone in case of compound 327e abated affinity is observed. Alongside, diamino-piperazone analogs 327b and 327c have lost activity. Further, diaminoethylene derivatized benzimidazole-quinolines 327f and 327g have failed to elicit potent affinity properties. All these results infer that the increased size of the secondary amine at quinoline 8-position has led to the conformation that lowered activity.

5.1. SLC-0111 thiazole and thiadiazole analogs

Carbonic anhydrases play significant roles such as lipogenesis, gluconeogenesis, ureagenesis, and tumorigenicity. Carbonic anhydrases have become versatile drug targets for AD treatment. SLC-0111 is a selective inhibitor of CA isoforms IX and XII; it possesses ureido substituted benzene sulfonamide motif. Considering the potentiality of SLC-0111 as CA inhibitors, the design and synthesis of novel SLC-0111 thiazole and thiadiazole derivatives are accomplished [44].

The synthesis of the thiazole-benzensulfonamide derivatives commences from compound 337. Carboxylic acid group of compound 337 is chlorinated to corresponding benzoyl chloride analog 338 which is converted to benzoyl azide derivative 339. The Curtius rearrangement of the compound 339 led to the formation of isocyanate derivative 340 which is used as intermediate for the preparation of urea derivative of thiazole 341a-d and thiadiazole derivatives 342a-d using thiolas and thiadiazoles respectively (Scheme 74).

The synthesis of the thiazole-benzensulfonamide derivatives commences from compound 337. Chlorination of carboxylic acid group of compound 337 and attachment of N, N-dimethyl methylene group to the NH of sulfonamide moiety resulted in corresponding benzoyl chloride analog 343 which is converted to benzoyl azide derivative 344. The Curtius rearrangement of the compound 344 led to the formation of isocyanate derivative 345 which is used as intermediate for the preparation of urea derivative of thiazole 346a-d and thiadiazole derivatives 347a-d using thiolas and thiadiazoles respectively (Scheme 75).

The synthesized derivatives are subjected to inhibition of CA isoforms using SLC-0111 and AAZ as reference compounds. Compared to SLC-0111 (hCA I: Kᵢ = 5.08 μM, hCA II: Kᵢ = 0.250 μM) thiazole derivatives of benzensulfonamide have shown potent hCA I and hCA II inhibitory activity (hCA I: Kᵢ = 0.162–0.713 μM; hCA II: Kᵢ = 0.009–0.833 μM). However, in comparison with AAZ (Kᵢ = 0.250 μM) towards hCA I, compounds 341c (Kᵢ = 0.191 μM) and 342b (Kᵢ = 0.162 μM) (Figure 36) are reported to have remarkable hCA I inhibitory agents. The significant hCA I inhibitor 342b comprises benzensulfonamide on one end of the urea and 3-phenylthiazole-5-yl on the other end. Amongst the thiazole analogs, compound 341b possessing 4-fluorophenyl moiety at thiazole 2-position has exhibited notable activity. Removal of –F or substitution with –Cl on thiazole and substitution of phenyl with 4-F/4-Cl phenyl ring have led to slightly diminished inhibitory activity. However, plane thiazole/thiadiazole derivatives of urea have shown very poor hCA I activity. 4-Fluorophenyl derivatization at 5- and 3-positions of thiazole 341c (Kᵢ = 0.0092 μM) and thiadiazole 342c (Kᵢ = 0.009 μM) respectively resulted most potent hCA II inhibitory potentials compared to AAZ (Kᵢ = 0.0125 μM). Remaining derivatives have not shown good activity. In the case of hCA IX and hCA XII isozymes, thiadiazole analogs have...
shown excellent inhibitory activity; whereas, thiazole derivatives exhibited comparatively less potent activity. In the hCA IX activity, phenyl (compound 342b) and 4-chlorophenyl (compound 342d) ring connected at thiadiazole 3-position has elicited decent inhibitory activities with $K_I$ values of 0.0083 μM and 0.0079 μM respectively. The same molecules 342b and 342d could also exhibit the strongest hCA XII inhibitory properties with $K_I$ values of 0.0094 μM and 0.0099 μM respectively. Keen observation of the structure and activity inferred that 4-fluoro/4-chlorophenyl appendants on thiazole and thiadiazole ring bestowed with decent CA inhibitory activity and the further improvement may be checked with the design of -fluoro and -chloro disubstituted phenyl thiazole/thiadiazole derivatives. Unfortunately, the thiazole and thiadiazole derivatives in which NH of benzenesulfonamide substituted with $N$, $N$-methyl methylene have totally failed to show CA inhibitory activity. It indicates that free sulfonyl amide pharmacore is crucial for CA inhibitory activity.

5.2. Tetrazole-peptidomimetics

Neutral M1-alanyl aminopeptidase (ePepN) is a bacterial protease from a gram-negative bacterium (E. Coli) studied mostly. It catalyzes the removal of polypeptide N-terminal amino acid throughout the nucleophilic attack of a water molecule activated by a Zn$^{2+}$ cation. ePepNs are not only crucial for bacterial survival, but they are also essential for E. Coli and Plasmodium falciparum. Hence, ePepN could be an attractive therapeutic target for the efficient control of bacterial diseases. Recently, tetrazole scaffolds have been highlighted to possess inhibitory properties against pathogenic bacteria and protozoa. Having considered these facts, tetrazole-peptidomimetics are designed and synthesized [45].

Amino acid derivatives protected with Fmoc is deprotected to yield resin-bound amino acid derivatives [45]. Ugi reaction of compound 349 with substituted aldehydes, isocyanides, and TMSN$_3$ resulted in tetrazole derivatives 350 followed by treatment with TFA to produce amino acid derivatives of tetrazole 351a-u (Scheme 76) (see Figure 36).

All the prepared tetrazole derivatives are investigated for ePepN inhibitory properties using bestatin. Here, compound 351b (Figure 37) and 351d are reported as remarkable ePepN inhibitors with $IC_{50}$ values of 1.4 ± 0.2 μM and 2.2 ± 0.3 μM respectively possessing threefold and fivefold activities respectively compared to bestatin (IC$_{50}$ = 7 ± 4 μM). Further compound 3511 has possessed good inhibitory activity (IC$_{50}$ = 7.2 ± 0.9 μM) as potent as a reference compound. Surprisingly, derivative 351k (Figure 37) has elicited strongest ePepN inhibitory activity (IC$_{50}$ = 0.00026 μM) possessing a 27-fold higher inhibitory activity compared to bestatin. Strongest ePepN inhibitor 351k entails benzyl moiety at the

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**Scheme 66.** Design and synthesis of quinazolinone moiety linked to thiadiazole derivatives [41]; Reagents and conditions: i) BrCH$_2$COOC$_2$H$_5$, K$_2$CO$_3$, acetone; ii) NH$_2$NH$_2$/C$_{15}$H$_2$O, EtOH; iii) C$_2$H$_5$NCS, EtOH, reflux; iv) 1M NaHCO$_3$, reflux; v) dil. H$_2$SO$_4$.

**Scheme 67.** Synthesis of compound quinoline derivative 319 [42]; Reagents and conditions: i) NaOH, CH$_3$COC$_6$H$_4$CH$_3$, RT (4h); ii) gl. AcOH, reflux (10h).
tetrazole \( \mathbf{N} \), furan-2-yl appended to methine & amino acid groups and indole-3-yl pharmacore attached to propionic acid 3-position. Similarly, another significant inhibitor \( \mathbf{351d} \) possesses almost similar structural components except for cyclohexyl ring in place of benzyl moiety on tetrazole \( \mathbf{N} \)-position. Hundredfold diminished activity (IC\(_{50} = 25 \pm 1 \) \( \mu \)M) is observed with substitution of the furan-2-yl ring of compound \( \mathbf{351l} \) with imidazole-2-yl moiety (compound \( \mathbf{351m} \)). Besides, the substitution of indole moiety in compound \( \mathbf{351l} \) with phenyl ring led to decreased inhibitory activity (IC\(_{50} = 17 \pm 1.9 \) \( \mu \)M). Further, the compound \( \mathbf{351b} \) bearing cyclohexyl ring on tetrazole \( \mathbf{N} \)-position, 4-methoxyphenyl methine moiety flanked by tetrazole and aminoacid groups in addition to 4-(benzyloxy) phenyl motif connected to propionic acid 3-position has turned out to be the best ePepN inhibitor. The research team might have prepared \( \mathbf{N} \)-benzyltetrazol analog of compound \( \mathbf{351b} \) to check the improvement in the ePepN inhibitory activity.

5.3. Thiazoles linked to diaryl ethers

Cancer has become a serious threat for the public worldwide in which large number of signaling pathways are reported to prevail in human
The newly prepared compounds 354a-j along with compound intermediate 353 are screened for Akt inhibitory effects using Cisplatin as a standard compound. Firstly, the derivatives are checked for their cytotoxicity on cancer cell lines A549, C6, and NIH/3T3 comparing with standard anticancer agent cisplatin (IC50 = 17.33 ± 2.08 μM). In this activity, compound 354f (Figure 38) has exhibited the most remarkable inhibition (IC50 = 12 ± 1.73 μM) towards A549 cell line and other derivatives have totally failed to show decent cytotoxicity. Whereas, the same compound 354f has produced enough activity (IC50 = 3.83 ± 0.76 μM) to become the most toxic derivative towards the C6 cell line. Along with this, the compound 354h (Figure 38) has found its place in the notable cytotoxic compounds list with IC50 value of 5.83 ± 0.76 μM. While compounds 353 (IC50 = 26.33 ± 1.53 μM) and 354g (IC50 = 16 ± 5.66 μM) have shown moderate cytotoxicity. Unfortunately, no single designed derivative has rendered good cytotoxicity towards the cell line NIH/3T3. The compound which exhibited notable cytotoxicity towards both A549 and C6 cell lines possesses 4-benzonitrile at thiazole 4-position; while the derivative having the strongest cytotoxicity towards C6 cell line yet comparatively lower than compound 354f bearing 4-hydroxypyrenyl moiety at thiazole 4-position. The precise correlation between the structure of the compound and its cytotoxicity cannot be established as both electron-withdrawing groups and electron-donating groups have produced good cytotoxic effects. The compounds which have exhibited decent cytotoxicity towards the cancer cell lines have been chosen for inhibition of Akt on the cell lines A549 and C6 cell lines. The intermediate compound 353 has elicited excellent percent inhibition (68.08 ± 2.48%) which is twofold stronger compared to cisplatin (31.01 ± 3.18%). Annoyingly, most A549 cell line cytotoxic molecule 354f has displayed good activity but lower activity (45.77 ± 10.58%) compared to compound 353. Besides, 4-hydroxypyrenyl analog has exhibited decent inhibition percentage (57.37 ± 17.30%). Regarding inhibition of the C6 cell line, most cytotoxic compound 354f bestowed strongest inhibition percentage (71.66 ± 4.09%) which is a slightly diminished activity compared to cisplatin (77.25 ± 5.75%). Compound 354g is also one of the finest C6 inhibitors with percentage inhibition of 70.42 ± 10.37%.

5.4. Thiazol-hydrazono-coumarin

Out of a large number of signaling pathways that are essential for the proliferation of cancer cell lines, cyclin-dependent kinases (CDKs) play a significant role. Hence CDKs are attractive molecular targets for efficient cancer therapy; thereby extensive study of these is being taken up. Many research outputs have proved the overexpression of CDKs in malignancies such as lung, ovarian, and pancreatic carcinoma and so forth. Considering the CDKs inhibitory properties of thiazole scaffolds and coumarin hydradiza hydrzone derivatives, the design and synthesis of thiazol-hydrazono-coumarin analogs are achieved [47].

In the synthesis of title compounds, 3-acetyl-6-halocoumarin 355 is condensed with thioureas to yield intermediate thiosemicarbzone of coumarin 356. Further, the intermediate 356 on intramolecular cyclization with the reagents CH3COCH2Cl, phenacyl bromide, 4-bromo phenacyl bromide, 2-bromoacetyltetralin, 3-chloroacetyltetralin, and ethyl-2-chloroacetoacetate formed the thiazol-hydrazone-coumarins 357a-c, 358a-c, 359a-c, 360a-c, 361a-c, and 362a-c respectively. The intramolecular cyclization of intermediate 356 with ethyl bromoaacetate has resulted in thiazolidinone derivatives 363a-c (Scheme 78).

The title molecules are subjected to anticancer activity followed by evaluation of CDK2 inhibitory properties of some selected compounds. In the anti-proliferative activity of designed derivatives on Hela cell line considering doxorubicin as a standard compound, all the tested compounds have shown decent anti-proliferative activity. Compared to

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Scheme 70. Preparation of benzo[d]imidazole-quinoline derivatives [43]; Reagents and conditions: i) N-phenyl-bis(trifluoromethanesulfonimide), rt (2d); ii) tris(dibenzylideneacetone) dipalladium(0), cesium carbonate, rac-BINAP, reflux (3d); iii) TFA.

Scheme 71. Synthetic route for the preparation of compounds 327a-b [43]; Reagents and conditions: i) R-OMs, Cesium carbonate, reflux, overnight; ii) TFA.
doxorubicin compound 362c (Figure 39) can be mentioned as a compound with the highest activity (Table 15). Little diminished activity is observed for the compound 362b and derivatives 361c and 362a have exhibited almost similar anti-proliferative activity. Among the various thiazole derivatives designed, thiazole-4-methyl-5-ethyl carboxylate 362a-c have been found to be significant anti-proliferative compounds; particularly compound 362c with –Br atom at coumarin 6-position stood atop of the potent anti-proliferative molecules possessing approximately 115-fold greater activity compared to doxorubicin. Replacement of –Br in compound 362c with –Cl (362b) led to twofold reduced activity and substitution of halogen with –H (363a) has resulted in a slightly diminished anti-proliferative property.

The molecules with the dominant anti-proliferative property are chosen for inhibition of the enzyme CDK2 using staurosporine as standard compound CDK2 inhibitor (Table 15). Compound 361c and 362a have exhibited weak inhibitory potentials compared to staurosporine. However, synchronization of anti-proliferative property and inhibitory activity is observed in the case of compounds 362b and 362c wherein compound 362c is bestowed with excellent inhibitory activity. Its activity is twofold stronger in comparison with standard inhibitor. Moderate inhibitory CDK2 activity is shown by compound 362b. These observations reveal the importance of size and electronegativity of –Br. Decreased halogen size and increased electronegativity diminished the CDK2 inhibitory activity.

Scheme 72. Synthesis of radioiodinated compound $^{[125]}$333 $^{[43]}$; Reagents and conditions: i) NCS, NaI, 50 °C, overnight ii) Boc$_2$O, TEA, rt (3d) iii) hexabutyldistannane, Pd[P(C$_6$H$_5$)$_3$]$_4$, reflux (24h) iv) $^{[125]}$[NaI, NCS, acetic acid, rt (15min) v) TFA, rt (30min).

Scheme 73. Synthetic pathway for preparation of radioiodinated compound 336 $^{[43]}$; Reagents and conditions: i) i) NCS, NaI, 50 °C, overnight ii) hexabutyldistannane, Pd[P(C$_6$H$_5$)$_3$]$_4$, reflux (48h) iii) $^{[125]}$[NaI, NCS, acetic acid, rt (15min).

doxorubicin compound 362c (Figure 39) can be mentioned as a compound with the highest activity (Table 15). Little diminished activity is observed for the compound 362b and derivatives 361c and 362a have exhibited almost similar anti-proliferative activity. Among the various thiazole derivatives designed, thiazole-4-methyl-5-ethyl carboxylate 362a-c have been found to be significant anti-proliferative compounds; particularly compound 362c with –Br atom at coumarin 6-position stood atop of the potent anti-proliferative molecules possessing approximately 115-fold greater activity compared to doxorubicin. Replacement of –Br in compound 362c with –Cl (362b) led to twofold reduced activity and substitution of halogen with –H (363a) has resulted in a slightly diminished anti-proliferative property.

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Figure 35. Representation of structures of molecules with strong PDGFRβ affinity.
5.5. Thiazolidine-2,4-dione-azole derivatives

Impairment of pancreas β-cells would lead to abnormal metabolism in carbohydrate and involved in diabetes development. In this situation, impairment of insulin secretion is also observed and clinically it results in diabetes. The enzymes α-glucosidase and α-amylase function in the digestion of starch, absorption of glucose. Alongside α-amylase is involved in the breakdown and absorption of insoluble starch molecules. Hence inhibition of these enzymes might be an affordable and appreciable approach to control diabetes. In this regard, the thiazolidine-2,4-dione class of molecules has been in the limelight as they have blood glucose level normalizing effect. Derivatives of thiazolidine-2,4-dione and azole have not been subjected to α-amylase and α-glucosidase inhibitory properties; hence a series of thiazolidine-2,4-dione-azole derivatives are synthesized [48].

Scheme 74. Synthetic way for the preparation of the thiazolo-benzene sulfonamides [44]; Reagents and conditions: i) SOCl₂, reflux (12hr); ii) NaN₃/Ice bath/-stirring (2hr); iii) Dry toluene/reflux (1hr); iv) Dry toluene/reflux (4hr).

Scheme 75. Synthesis of the final compounds 346a-d and 347a-d [44]; Reagents and conditions: i) SOCl₂/DMF, reflux (5hr); ii) NaN₃/Ice bath/stirring (2hr); iii) Dry toluene/reflux (1hr); iv) Dry toluene/reflux (4hr).
The synthetic route for the design of thiazolidine-2,4-dione derivatives involves the preparation of thiazolidine-2,4-dione from chloroacetic acid and thiourea. Substitution reaction of 4-fluorobenzaldehyde with compounds 366a-e gave 4-substituted benzaldehyde derivatives 367a-e which subsequently condensed with thiazolidine-2,4-dione to produce 5-benzylidene-thiazolidine-2,4-dione derivatives 368a-e (Scheme 79).

Treatment of compound 368a with bromoethyl acetoacetate yielded N-ethylacetate substituted thiazolidine-2,4-dione analog 369 followed by hydrolysis rendered N-ethanoic acid derivative 370. Again compound 368a is utilized as intermediate wherein pyrrolopyridine moiety of compound 368a coupled with aromatic amines 371a-c at 4-position to form 4-arylamino substituted pyrrolopyridine derivatives 372a-c (Scheme 80).

The α-amylase and α-glucosidase inhibitory activities are conducted on all the synthesized molecules using Acarbose as a standard inhibitor. The inhibitory properties are depicted in terms of percentages. Compared to Acarbose (α-amylase: 43.0%, α-glucosidase: 40.91%), more than half of tested compounds have possessed potent inhibitory percentages. The compounds of the series 372a-c (Figure 40) exhibited closest inhibitory percentages compared to Acarbose in the case of α-amylase activity (Table 16). In those, compound 372c having the strongest inhibitory percentage is only a little less potential compared to Acarbose. An almost similar inhibitory percentage is observed for compound 372b; however, diminished activity is shown by compound 372a probably due to large OCF3 moiety. Besides, compounds 368b-c and compound 370 rendered moderate α-glucosidase inhibitory percentages.

The same course of inhibitory percentages is observed for designed compounds towards the α-glucosidase enzyme (Table 16). Inhibitory results reveal the importance of substitution of fluorinated aromatic amines at pyrrolopyridine 8-position of derivatives 372a-c. In combination with a fluorinated aromatic amine at pyrrolopyridine 8-position, the presence of free thiazolidine-2,4-dione NH group allows the compounds of 372a-c series to exhibit inhibitory properties at maximum level.

5.6. Thiazolylpyrazolyl coumarin derivatives

Angiogenesis is the physiological process through which essential nutrients and oxygen are supplied subsequently enhancing tumor progression and metastasis. VEGFR-2 kinase is one of the enzymes/factors responsible for angiogenesis. Overexpression of the VEGFR-2 kinase is noticed in most cancer types. Therefore, inhibition of VEGFR-2 kinase signaling pathway suppresses the tumor growth. The literature revealed coumarin derivatives have attracted great attention owing to their inhibition of VEGFR-2 kinase signaling pathway. Considering VEGFR-2 kinase inhibitory activity of coumarin scaffolds along with thiazolyl-pyrazoline motif, hybrid compounds of coumarin and thiazolyl-pyrazoline have been engineered [49].

In the synthesis of title compounds, coumarin-linked amines via chalcone fragment 373a, b are cyclized with thiosemicarbazide in the intermolecular fashion to form intermediate compounds pyrazole-coumarin derivatives 374a, b. The thioamide fragment of pyrrole is utilized in the design of thiazole-pyrrole derivatives 375a, b, 376a, b and 377a, b with chloroacetone, phenacyl bromide and chloroethylacetoacetate respectively (Scheme 81). Intermolecular cyclization of pyrazole-thioamide derivatives 374a, b with bromoethylacetate and bromoethylpropionate to yield coumarin tethered thiazolones through pyrazole ring 378a, b, and 379a, b respectively (Scheme 82). The intermediate compounds 374a, b could also be transformed into...
corresponding azo-thiazole-pyrazole derivative by reacting with \( \text{CH}_3\text{CO(Cl)C-} \text{NNHAr} \) (Scheme 83). The title compounds 376a, b are further derivatized with aryldiazo group to render compounds 381a, b (Scheme 84).

The synthesized molecules are subjected to anticancer activity using doxorubicin as a standard compound. Most of the title compounds have exhibited moderate anti-proliferative activity towards MCF-7 cell lines except few derivatives which shown poor inhibitory activity.

The pyrazolo-thioamide derivatives 374a, b have failed to render decent anti-proliferative activity revealing that simple pyrazoline ring with thioamide functionality is not enough for good MCF-7 anti-proliferation. A slight increment in the activity is observed for the 4-phenyl-thiazolo-pyrazole derivatives 376a, b; and 4-hydroxycoumarin analog 376b has comparatively higher activity than 376a. Further enhanced activity is reported for the compounds thiazole 5-ethyl carboxylate scaffolds 377a, b. Again the hydroxycoumarin analog 377b has exhibited threefold stronger activity compared to 377a. A diversified pyrazolo-thiazolones 378a, b have not qualified as good candidates for MCF-7 anti-proliferative activity indicating thiazolone motif is not apt pharmacore for the activity. However, the 5-methylthiazolone derivatized pyrazoles 379a, b resulted in the finest inhibitory values. In that, compound 379a (Table 17) is the best between the two. Aryldiazo modified thiazolones 380a-d have shown improved MCF-7 anti-proliferation activity. In this series, compound 380c (Table 17 & Figure 41) elicited the closest inhibitory activity compared to the standard compound. It entails methyl and 4-chlorophenyldiazo structural units at thiazole 4- and 5-positions respectively and plane coumarin 3-yl moiety at pyrazole 3-position. The p-tolylidiaz analogs 380a and 380b have resulted in diminished activity which infers that the 4-chloro group has much impact on anti-proliferative activity. Twofold reduced activity is noticed for the compound 380d which is 4-hydroxycoumarin analog.

The most significant anti-proliferative activity has been exhibited by compounds of 381a-d series. Except for compound 381a (moderate inhibitor), the other three derivatives bestowed the most significant activity and comparatively greater activity than doxorubicin. In particular, 381d (Figure 41) has elicited excellent activity; structurally the compound possesses phenyl and 4-chlorophenyldiazo motif at 4- and 5-positions of thiazole ring respectively. An almost similar activity is rendered by compounds 381b and 381c. All the potent anti-proliferative molecules have been allowed to inhibit VEGFR-2 kinase using sorafenib as a standard VEGF-R-2 kinase inhibitor. Out of these compounds, derivatives 379a, 380b and 380c could only show moderate inhibitory activity. The significant anti-proliferative molecule 381d has also shown the strongest activity. Alongside the significant activity is exhibited by the compound 380c.

5.7. Tropinone-thiazole derivatives

Melanoma is most common in skin malignancies. Statistics revealed that approximately 30000 new cases are reported worldwide. The dysregulation of melanin melanocytes is the main cause of melanoma; subsequently, the dysregulation leads to uncontrolled melanocyte proliferation followed by observation of high melanin content.
Tropinone derivatives are explored in the medicinal field and have been found to be pharmaceutically significant. Tropinone scaffolds 381 (Figure 42) and 382 are reported to possess strong activity towards HL-60 cell lines and HCT116 cell lines respectively. Recently designed hydrazinyl triazoles have exhibited decent activity towards MV4-11 cells. All these observations have led to the design of tropinone-thiazole derivatives [50].

Tropinone 384 on condensation with thiosemicarbazide gave thiosemicarbazone of tropinone 385. The thioamide fragment of the derivative 385 is made use in intermolecular cyclization with p-substituted phenacyl bromides yielded tropinone compounds of thiazole linked via hydrazone spacer 386a-h (Scheme 85).

The tropinone derivatives are tested for their anti-proliferative properties on cancer cell lines such as MV4-1, A549, MCF-7, B16–F10, and BALB/3T3 using chlorambucil as a positive control. In this activity, almost all the tested molecules have shown better anti-proliferative properties.

**Scheme 78.** Synthetic route for the preparation of thiazol-hydrazolo-coumarin derivatives [47]; Reagents and conditions: i) EtOH, Anhyd. CH₃COONa, CH₃COCH₂Cl, reflux; ii) EtOH, Anhyd. CH₃COONa, Phenacyl bromide, reflux; iii) EtOH, Anhyd. CH₃COONa, 4-Bromo phenacyl bromide, reflux; iv) EtOH, Anhyd. CH₃COONa, 2-bromoacetyl tetralin, reflux; v) EtOH, Anhyd. CH₃COONa, 3-bromoacetylacetone, reflux; vi) EtOH, Anhyd. CH₃COONa, ethyl-2-chloroaactoacetate, reflux; vii) EtOH, Anhyd. CH₃COONa, ethyl bromoacetate, reflux.

**Table 15.** Anti-proliferative (Hela) activity and CDK2 inhibitory activity of designed molecules.

| Compd | Anti-proliferative activity IC₅₀ (μM) | CDK2 inhibitory activity IC₅₀ (nM) |
|-------|-------------------------------------|----------------------------------|
| 361c  | 0.0654 ± 0.0038                     | 1.546 ± 0.021                    |
| 362a  | 0.0596 ± 0.0026                     | 1.629 ± 0.012                    |
| 362b  | 0.0236 ± 0.0011                     | 0.216 ± 0.014                    |
| 362c  | 0.0091 ± 0.0007                     | 0.022 ± 0.002                    |
| Doxorubicin | 1.1073 ± 0.0062                  | -                                |
| Staurosporine | -                                    | 0.044 ± 0.002                  |

Figure 39. Demonstration of potent anti-proliferative (Hela) and anti-CDK2 inhibitory compound.
activity. However, compounds 386c, 386g, and 386h are reported to possess stronger inhibitory activity compared to chlorambucil and among other derivatives of the series.

The designed molecules have also been subjected to tyrosinase inhibitory activity using ascorbic acid and kojic acid as reference compounds. Compared to ascorbic acid all evaluated derivatives have possessed higher activity. However, when inhibitory values are compared with kojic acid, half of the synthesized molecules have decent tyrosinase inhibitory activity (Table 18). The compound 386f has shown activity as potent as kojic acid and twofold higher inhibitory activity is shown by the compound 386e. Whereas compounds 386g and 386h (Figure 43) with similar activities are approximately 21-fold greater potencies compared to kojic acid.

Among the various substituted phenylthiazole derivatives, compound 386g possessing 3,4-dichlorophenyl ring at thiazole 4-position has bestowed the most significant activity. The 2,4-dichlorophenyl analog bearing has exhibited slightly reduced activity. A very poor inhibitory activity is rendered by the compounds 386a and 386b possessing p-F/p-Cl phenyl moieties. These facts reveal two chloro atoms on the phenyl ring either at 2,4- or 3,4-positions have been attributed to remarkable inhibitory activity. However, the monosubstituted phenyl rings connected at thiazole 4-positions would not give expected inhibitory properties.

The research team would have tried the dibromo and difluoro disubstituted phenyl analogs for further improvement in the tyrosinase inhibitory activity.

6. Discussion and conclusion

Whole classified content entails the schematic representation of the synthesis of enzyme inhibitors, followed by pharmacological evaluation of synthesized molecules. The relation between the structure of a remarkable enzyme inhibitor and the corresponding inhibitory property is discussed qualitatively highlighting responsible structural pharmacores. (1,4] Dioxino [2,3-\(f\)] quinazoline derivatives have been synthesized and the compounds have shown c-Met and VEGFR-2 inhibitory activity even at a nanomolar concentration wherein compound 7k is found to be as potent as cabozantinib. SAR revealed the presence of p-F-phenyl ring of cyclopropane-1,1-dicarboxamide moiety has a great impact on inhibitory activity. The compound 15y has exhibited the most significant MTB PtpB inhibitory activity; which is one of the compounds of 1,2,3-1\(H\)-triazoles linked to 4\(H\)-pyrano [2,3-\(d\)] pyrimidine. The significant activity is attributed to –OMe, –OH, and –NO2 at 3, 4 and 5-positions of phenyl ring respectively. Moderate carbonic anhydrase inhibitory activity is observed for the compound 18c amongst the evaluated 1,2,4-triazole-5-one derivatives. Out of the 1,2,4-triazole-based benzothiazole/benzoxazole derivatives prepared, compound 27b has elicited stronger p38α MAB kinase inhibitory activity than that of

Scheme 79. Synthesis of a series of thiazolidine-2,4-dione-azole derivatives [48]: Reagents and conditions: i) \(\text{H}_2\text{O}, \text{Conc. HCl, reflux (10h)}\); ii) \(\text{K}_2\text{CO}_3, \text{DMF, 100 °C (5h)}\); iii) piperidine, PhCOOH, 100 °C (1h).

Scheme 80. Synthetic route for the design of pyrrolo-pyridine derivatives [48]: Reagents and conditions: i) Bromoethylacetoacetate, \(\text{K}_2\text{CO}_3, \text{DMF, 100 °C (30 min)}\); ii) HCl/\(\text{CH}_3\text{COOH}; \text{iii) Pd(dba)}_3 \text{Xanthophos, CsCO}_3, \text{dioxane, 100 °C (1h)}\).
SB203580 which might be attributed to the combined effect of 4-fluoro group and benzothiazole moieties. 1,2,4-Trisubstituted imidazolinones have also been designed in order to possess p38α MAB kinase inhibitory activity wherein compound 31a rendered the most potent inhibitory activity towards all four CA isoenzymes. The 3,4-dimethoxy entity is thought to be responsible pharmacore for such an activity. Compound 52 exhibited the most potent AChE inhibitory activity among the 1H-pyrazolo [3,4-b] pyridine derivatives synthesized. The tenfold higher activity might be due to piperidine-linked pyrazolo-pyridine moiety. Decent tyrosinase inhibitory activity is exhibited by compound 55b possessing 4-bromobenzyl motif among the 2,4,5-trisubstituted-1,2,4-triazole-3-one derivatives. In the pharmacological evaluation of aryl carbboximidamides and 3-aryl-1,2,4-oxadiazoles, compound 58c is found to be a potent inhibitor. COX-2/15-LOX dual inhibitor entails electron-donating OCH3 groups at 3,4-positions of benzamidine motif. Compound 78a is reported as a potent multi-tyrosine kinase inhibitory compound. 5,6-Dichloro-2-methyl-1H-benzimidazole derivatives have proven to be good anti-urease agents wherein compound 84e bearing 4-nitrobenzene motif stood top in the inhibitory properties. The designed 5-arylisothiazol-3(2H)-one-1,1-(di)oxides have been shown to possess potent hCA effects; particularly derivatives 95a and 95i exhibited CA IX and XII inhibitory properties at nanomolar concentration. The reported anti-fungal agents are subjected to lanosterol-14α-demethylase inhibitory activity in which itraconazole has shown potent activity. Compound 111 and 112a among 1,2-benzisothiazol-3(2H)-one-1,1-dioxide derivatives turned out to be strong COX-1 and COX-2 inhibitors. The two

| Compd  | Percentage inhibition (250 μg/ml) | α-amylase | α-glucosidase |
|--------|----------------------------------|-----------|--------------|
| 368b   | 22.51                            | 20        |
| 368c   | 21.65                            | 20        |
| 370    | 20.23                            | 19.9      |
| 372a   | 33.05                            | 32.2      |
| 372b   | 36.18                            | 35.2      |
| 372c   | 37.04                            | 36        |
| Acarbose| 43.05                            | 40.91     |

Table 16. Inhibitory percentages of potent α-amylase & α-glucosidase inhibitory compounds.

Scheme 81. Preparation of final compounds 375a & b, 376a & b and 377a & b [49]; Reagents and conditions: i) NH2CSNH2, EtOH, HCl, reflux; ii) CH3COCH2Cl, CH3COONa, EtOH, reflux; iii) PhCOCH2Br, CH3COONa, EtOH, reflux; iv) CH3COCH(Cl)COOC2H5, CH3COONa, EtOH, reflux.
pyrazole rings and an intact saccharin ring in compound 111 have bestowed the strongest activity. In the synthesized benzenesulfonamides-linked quinazoline scaffolds, compound 120c has elicited 15-fold higher activity compared to AAZ. The tyrosinase inhibitory activity of carbazole and hydrazone derivatives revealed compound 121a as the most significant inhibitory compound; the potent activity is thought to be due N-ethyl carbazole appended to benzimidazole via thiopropanamide linker. Carbazole-imidazole derivatives are synthesized to render α-glucosidase inhibitory effects and pharmacological evaluation infers simple phenyl ring at the 4-position of carbazole-imidazole moiety has attributed remarkable inhibitory activity for compound 127v. Synthesis and biological evaluation of coumarin-1,3,4-oxadiazole hybrids revealed compound 132b, a remarkable CA XII inhibitor. Coumarin appended with methylene oxadiazole thiol and in turn, benzoyl moiety attached to thiol functionality of oxadiazole has bestowed compound 132b with remarkable inhibitory activity. C-β-D-Glucopyranosyl azole derivatives designed to check glycoprotein phosphorylase inhibitory potential exhibited moderate activity; particularly compound 140 reported as a most potent inhibitor. While diaryl-1,5-diazoles have been synthesized and their COX-2 and 5-LOX inhibitory activities are performed; wherein compound 147h having benzenesulfonamide and 4-trifluoromethyl benzene at 1- and 5-positions of pyrazole in addition to propylcarboxamide flanked by pyrazole and morpholine moiety rendered decent activity. Dihydroquinazoline-2-amines revealed promising reverse transcriptase inhibitory activity in which compound 155b with p-cyanobenzeneamine ring shown to possess noteworthy activity. Compound 168j of dioxino [2, 3-f] quinazoline derivatives has resulted in striking VEGFR-2 inhibitory activity.

Scheme 82. Synthetic route for the design of the compounds 378a & b and 379a & b [49]; Reagents and conditions: i) BrCH2COOC2H5, CH3COONa, EtOH, reflux; ii) CH2CH(Br)COOC2H5, CH3COONa, EtOH, reflux.

Scheme 83. Synthesis of the compounds 380a,b [49]; Reagents and conditions: i) CH3CO(Cl)C=NNHAr, Dioxane, Et3N, reflux.

Scheme 84. Design and preparation of compounds 381a, b [49]; Reagents and conditions: i) ArN2Cl, EtOH, CH3COONa•3H2O.
to moderate UppPase inhibitory activity. Pyrazole derivatives witnessed significant COX inhibitory activity; wherein compound 203a stood atop in the list comprising benzene sulfonamide connected to pyrazole

Table 17. Anticancer properties (MCF-2) and VEGFR-2 inhibitory properties of potent molecules.

| Compd | Anticancer activity (MCF) | VEGFR-2 activity |
|-------|---------------------------|------------------|
|       | IC₅₀ μM (μg/ml)            |                  |
| 379a  | 10.75 (4.80 ± 0.47)        | 0.169 ± 0.010    |
| 380c  | 8.61 (4.90 ± 0.50)         | 0.081 ± 0.003    |
| 381b  | 6.56 (4.11 ± 0.60)         | 0.212 ± 0.014    |
| 381c  | 6.51 (4.11 ± 0.60)         | 0.582 ± 0.021    |
| 381d  | 5.41 (3.50 ± 0.50)         | 0.034 ± 0.002    |
| Doxorubicin | 6.73 (3.66 ± 0.42) |            |
| Sorafenib | -                        | 0.019 ± 0.002    |

Figure 41. Demonstration of structures of potent molecules with anti-proliferation/VEGFR-2 activity.

Figure 42. Structures of tropinone derivatives having anticancer properties.

Table 18. Tyrosinase inhibitory values of tropinone derivatives.

| Compd | Tyrosinase inhibitory activity |
|-------|-------------------------------|
|       | IC₅₀ ± SD (μM)                |
| 386e  | 33.74 ± 1.42                 |
| 386f  | 72.30 ± 7.55                 |
| 386g  | 3.22 ± 0.24                  |
| 386h  | 3.51 ± 0.15                  |
| Ascorbic acid | 386.5 ± 11.95             |
| Kojic acid | 72.27 ± 3.14                 |

$\text{N}_1$-position and morpholine appended to pyrazole 5-position via methylene amide fragment. Further moderate tyrosinase inhibitory activity is exhibited among phthalimide-1,2,3-triazole hybrid compounds. Synthesis and evaluation of 15-LOX inhibitory properties of purine-pyrazole hybrids revealed the noteworthy inhibitory potential of the compound 217d possessing 4-methoxyphenyl ring at pyrazole 4-position. Promising anti-CDK2 effects are exhibited by the compound 221d of pyrazole and pyrazole [1,5-a] pyrimidine scaffolds. COX-1 Inhibitory property investigation of dihydropryan fused pyrazole derivatives inferred moderate effects on the enzyme. Whereas, the strongest hCA I inhibitory activity is bestowed by the compound 235 of heterocycle fused pyrazole analogs; it is 25-fold stronger potent compared to the reference and possesses 3, 4-dimethyl benzene and phenyl ring at pyrazole N₁ and C₅ positions of pyrazole. Compounds 254k and 254l have been found to be decent topoisomerase I inhibitors; these are the part of pyrazole-linked benzothiazoïle-β-naphthol derivatives. The remarkable COX-2 inhibitory values are observed for compounds of pyrazoles and pyrazolo[3,4-b] pyridines in which compound 249j possessing 3-fluorooanilene, 4-bromophenylhydrazone moiety at 3, 4-positions respectively in addition to N-methylene 2,6-dimethylanilene at N₁-position has excelled in exhibiting most significant activity. In continuation, excellent COX inhibitory properties are shown by the pyrazoles containing thiophene, thienopyrimidine, and thienotriazolopyrimidine derivatives. hCA and AChE dual inhibitory properties have been elicited by derivatives of pyrazoline benzensulfonamides. Compound 269d is reported to be the most significant hCA I inhibitor. Pyrazolopyrimidine scaffolds are synthesized and evaluated for COX-2 inhibitory properties and found to be only moderate inhibitors. The compound 284d has exhibited NPP1/NPP3 dual inhibitory activity and is part of the series of the pyrazolyl pyrimidinetrienes and thioxopyrimidinediones. The GSK3β/p38α dual inhibitory potential of compound 305c among pyridinyl imidazoles might be due to pyridine-cyclopropyl amide and 2-alkyl substituted imidazole motifs. Quinazolin-4(3H)-one derivatives are reported to exhibit moderate urease inhibitory properties. In the pharmacological evaluation of quinoline based 4,5-dihydropyrrozoles, compound 322b possessing 4-tolyl and 2-chloro-6-methoxyquinoline at dihydropyrazole 3- and 5-positions respectively in addition to substituted thiazoïle-2yl motif at N₁-position as a basic structure witnessed strongest EGFR inhibitory activity. Radiodinated benzol [d] imidazoquinoline derivatives have failed to exhibit improved PDGFRβ inhibitory potentials compared to their

Scheme 85. Design and synthesis of tropinone derivatives 386a-h [50]; Reagents and conditions: i) H₂NNHCSNH₂, AcOH, EtOH, reflux (20h); ii) p-substituted phenacyl bromide EtOH, reflux (20h); iii) H₂NNHCSNH₂, p-substituted phenacyl bromide EtOH, reflux.
Thiazolidine-2,4-dione-azole derivatives are reported to possess approximately 115-fold greater activity compared to doxorubicin. The compound 351k entailing benzyl moeity at the tetrazole N1 position, furan-2-yl appended to methine flanked by tetrazole & amino acid groups and indole-3yl pharmacore attached to propionic acid 3-position. Thiazoles linked to diaryl ethers are designed to exhibit good Atk inhibitory properties wherein compound 354f bearing 4-hydroxyphenyl moiety at thiazole 4-position bestowed with most significant Atk inhibitory potential. Furthermore, thiazoi-hydrazone-coumarin derivatives have been synthesized in order to exhibit cyclin-dependent kinases inhibitory effects. The compound 362 with –Br atom at coumarin 6-position stood top of the potent anti-proliferative molecules possessing approximately 115-fold greater activity compared to doxorubicin. Thiazoline-2,4-dione-azole derivatives are reported to possess promising α-glucosidase and α-amylase inhibitory properties. Compound 381d structurally comprising phenyl and 4-chlorophenylidazole motifs at 4- and 5-positions of thiazole ring respectively resulted in most VEGF-2 kinase inhibitory property among thiazolopyrazolyl coumarin derivatives. Finally, tropinone-thiazole derivatives have been synthesized and found to be potent towards tyrosinase inhibitory activity wherein the derivative 386g possessed the strongest inhibitory activity with 21-fold higher potential compared to kojic acid.

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References

[1] D. Wei, H. Fan, K. n Zheng, X. Qin, L. Yang, Y. Yang, Y. e Duan, Q. Zhang, C. Zeng, L. Hu, Synthesis and anti-tumor activity of [1,4] dioxino [2,3,4-fl quinazoline derivatives as dual inhibitors of c-met and VEGFR-2, Bioorg. Inside Chem. 88 (2019) 10291.
[2] N.D. Thanh, D.S. Hai, N.T.T. Ha, D.T. Tung, C.T. Le, H.T.K. van, V.N. Toan, D.N. Toan, L.H. Dang, Synthesis, biological evaluation and molecular docking study of 1,2,3-1H-triazoles having 4H-pyran[2,3-d]pyrimidine as potential Mycobacterium tuberculosis protein tyrosine phosphatase B inhibitors, Bioorg. Med. Chem. Lett. 29 (2019) 164–171.
[3] Safak Akin, Hasan Ayaloglu, Ergun Gultekin, Ahmet Colak, Olcay Bekircan, Melike Yildirim Akatin, Synthesis of 1,2,4-triazole-5-on derivatives and determination of carbonic anhydrase II isoenzyme inhibition effects, Bioorg. Chem. 81 (2019) 170–175.
[4] S. Tarig, P. Kamboji, O. Alam, M. Amir, 1,2,4-Triazole-Based benzothiazole/ benzoxazole derivatives: design, synthesis, p38 MAP kinase inhibition, anti-inflammatory activity and molecular docking studies, Bioorg. Chem. 81 (2018) 630–641.
[5] H.H. Georgely, F.M. Manhi, W.R. Mahmood, N.A. Mohamed, E. Berrino, C.T. Supuran, 1,2,4-Trisubstituted imidazolinones with dual carbonic anhydrase and p38 mitogen-activated protein kinase inhibitory activity, Bioorg. Chem. 82 (2019) 109–116.
[6] T. Umar, S. Shalini, M.K. Raza, S. Gusain, J. Kumar, P. Seth, M. Tiwari, N. Hoda, A multifunctional therapeutic approach: synthesis, biological evaluation, crystal structure and molecular docking of diversified 1H-pyrazol[3,4-b]pyridine derivatives against Alzheimer's disease, Eur. J. Med. Chem. 175 (2019) 2–19.
[7] S. Akin, E.A. Demir, A. Colak, Y. Kocoglu, N. Yildirim, O. Bekircan, Synthesis, biological and molecular docking studies of some novel 2,4-trisubstituted-1,2,4-triazole-3-one derivatives as potent tyrosinase inhibitors, J. Mol. Struct. 1175 (2019) 280–286.
[8] B.G.M. Youssif, M.F.A. Mohamed, M.M. Al-Sanea, A.H. Moustafa, A.A. Abdelhamid, H.A.M. Gomaa, Novel aryl carboximidamides and 3-aryl-1,2,4-oxadiazoles analogs of naproxen as dual selective COX-2/15-LOX inhibitors: design, synthesis, and Docking studies, Bioorg. Chem. 85 (2019) 577–584.
[9] B. Qi, Y. Yang, G. Gong, H. He, X. Yue, X. Yu, H. Ji, L. Chen, X. Han, A. Zhang, G. Zhou, Discovery of Ni-(4-(7-(3-(4-ethylpiperazin-1-yl)propoxy)-6-methoxyquinolin-4-yl)oxy)-3,5-di methoxycarbonyl-2-(2,5-difluorophenyl)-4-oxothiazolidin-4-yl)urea as a multi-tyrosinase kinase inhibitor for drug-sensitive and drug-resistant cancers treatment, Eur. J. Med. Chem. 163 (2019) 10–27.
[10] E. Mentese, E. Emrik, B.B. Sokmen, Design, molecular docking and synthesis of novel 5,6-dichloro-2-methyl-1Hbenzimidazole derivatives as potential uropeptidase enzyme inhibitors, Bioorg. Chem. 86 (2019) 151–158.
[11] B. Cornelio, M. Laronze-Cochard, R. Miambos, M. De Grandis, R. Riccioni, B. Borisova, D. Dontchev, C. Machado, M. Ceruso, A. Fontana, C.T. Supuran, Synthesis of benzensulfonamides linked to quinazoline scaffolds as novel carboline carboximides as potential selective enzyme inhibitors, Bioorg. Chem. 87 (2019) 40–48.
[12] D.E.P. Sumalapao, N.G. Gloriano, Hierarchically weighted principal component analysis evaluation of antifungal azoles inhibitory potency on lanosterol-14α-demethylase in Candida albicans, Curr. Res.Environ. Appl. Mycol. 9 (2019) 44–52.
[13] E.S. Taher, T.S. Ibrahim, M. Fares, A.M.M. AL-Mahmoudy, A.F. Radwan, K.Y. Orabi, O.I. El-Salibagh, Novel benzenesulfonamide and 1,2-benzothiazol-3(2H)-one-1,1-dioxide derivatives as potential selective CA II inhibitors, Eur. J. Med. Chem. 171 (2019) 382–392.
[14] A.S. El-Azab, A.A. Abdel-Aziz, S. Bua, A. Nocentini, M.A. El-Gendy, M.A. Mohamed, T.Z. Shawer, N.A. AlSaif, C.T. Supuran, Synthesis of benzensulfonamides linked to quinazoline scaffolds as novel carboline carboximides inhibitors, Bioorg. Chem. 87 (2019) 78–90.
[15] Z.A. Kaplanikili, Synthesis of some novel Carbazole derivatives and evaluation of their antimicrobial activity, Marmara Pharm. J. 15 (2011) 105–109.
[16] Z.A. Kaplanikili, et al., Synthesis, antimicrobial activity and cytotoxicity of some new carbazole derivatives, J. Enzym. Inhib. Med. Chem. 27 (2012) 868–874.
[17] L. Yurttas, et al., Synthesis and antimicrobial activity of some new hydrazine bridged thiazole-pyrole derivatives, J. Enzym. Inhib. Med. Chem. 28 (2013) 830–835.
[18] U. Ghani, Carbazole and hydrazine derivatives as new competitive inhibitors of tyrosinase: experimental clues to binuclear copper active site binding, Bioorg. Chem. 63 (2015) 220–226.
[19] M. Adib, P. Peytam, R. Shoureshy, M.M. Khansapohtzani, M. Jahani, S. Imamzad, M.A. Faramarzi, B. Larijani, A.A. Moghadamnia, E.N. Esfahani, Synthesis and anti-tumor activity of [1,4] dioxino [2,3,4-fl quinazoline derivatives as dual inhibitors of c-met and VEGFR-2, Bioorg. Insid Chem. 88 (2019) 10291.
F. Bandarian, M. Mahdavi, Design and synthesis of new fused carbazole-imidazole derivatives as antidiabetic agents: in vitro a-glucosidase inhibition, kinetic, and in silico studies, Bioorg. Med. Chem. Lett. (2019).

[20] N.S. Goud, S.M. Ghouse, M. Alavva, A. Angeli, C.T. Supuran, Synthesis and biological evaluation of coumarin-1,3,4-oxadiazole hybrids as selective carbonic anhydrase IX and XII inhibitors, Bioorg. Chem. 87 (2019) 765–772.

[21] B. Barr, E. Serymny, E. Bokor, Z.H. Al-Oanzi, C. Moffatt, S. Ranjbar, A. Foroumadi, M. Khoshneviszadeh, Phthalimide-functionalized phenylthiazoles with potent undecaprenyl pyrophosphatase inhibitory activity, Bioorg. Med. Chem. 87 (2019).

[22] Z. Li, Z.-C. Wang, X. Li, M. Abbas, S.-Y. Wu, S.-Z. Ren, Q.-X. Liu, Y. Liu, P.-W. Chen, Discovery of Dixin (2,3,5)-quinoline derivative VEGFR-2 inhibitors exerting significant antiproliferative activity in HUVEds and mice, Eur. J. Med. Chem. 175 (2019) 11–20.

[23] H. Fan, D. Wei, K. Zheng, X. Qin, L. Yang, Y. Yang, Y. Duan, Y. Xu, L. Hu, Discovery and biological evaluation of dihydroquinazoline-2-aminos as potent non-nucleoside reverse transcriptase inhibitors of wild-type and mutant HIV-1 strains, Eur. J. Med. Chem. 176 (2019) 1–9.

[24] X. Pan, L. Liang, R. Si, J. Wang, Q. Zhang, H. Zhou, L. Zhang, Discovery of novel anti-angiogenesis agents. Part 10: multi-target inhibitors of VEGFR-2, Tie-2, and EphB4 incorporated with 1,2,3-triazole, Eur. J. Med. Chem. 163 (2019) 1–9.

[25] M.E. Elbarbary, H. Mohammad, A. Samir, N.S. Abtaleh, A.B. Norvil, A.R. Michie, M.M. Moustafa, H. Samy, H. Gohwer, M.N. Suleeman, A.S. Mayhoub, Lipophilic efficient phenylthiazoles with potent undecaprenyl pyrophosphatase inhibitory activity, Eur. J. Med. Chem. 175 (2019) 49–62.

[26] G.S. Hasan, D.E. Abdel Rahman, E.A. Abdelmalek, R.H. Refayy, M. Alaraby Salem, Y.M. Niasan, New pyrazole derivatives: synthesis, anti-inflammatory activity, cyclooxygenase inhibition assay and evaluation of mpGes, Eur. J. Med. Chem. 171 (2019) 332–342.

[27] M.B. Tewfik, P. Emani, Z. Rezei, M. Khoshnevizadeh, M. Ebrahim, M. Ebrahimi, N. Emdahi, M. Mahdavi, B. Larijani, S. Ranjbar, A. Foroumadi, M. Khoshneviszadeh, Pthihalimide-1,2,3-triazole hybrid compounds as tyrosinase inhibitors: synthesis, biological evaluation, and X-ray structural studies, Eur. J. Med. Chem. 163 (2019) 266–280.

[28] M.M. Elbarbary, H. Mohammad, A. Samir, N.S. Abtaleh, A.B. Norvil, A.R. Michie, M.M. Moustafa, H. Samy, H. Gohwer, M.N. Suleeman, A.S. Mayhoub, Lipophilic efficient phenylthiazoles with potent undecaprenyl pyrophosphatase inhibitory activity, Eur. J. Med. Chem. 175 (2019) 49–62.