Synthesis and characterization of 6, 6-dimethyl-6, 7-dihydro-5H-pyrazolo [5, 1-b] [1, 3] oxazine-3-carboxamide derivatives as PDE-4 inhibitors for the treatment of anti-inflammatory, analgesic and antimicrobial activities

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Abstract— we reveal a series of novel 6, 6-dimethyl-6, 7-dihydro-5H-pyrazolo [5, 1-b] [1, 3] oxazine-3-carboxamide derivatives as PDE4 inhibitor for the treatment of inflammatory, analgesic and antimicrobial activities. All compounds were evaluated for their anti-inflammatory, analgesic (compared to the reference drug Indomethacin) and antimicrobial activities (compared to the reference drug Ampicillin and Fluconazole). Compounds 5e, 5f, and 5g were found to be the more active anti-inflammatory drugs revealing potency ranging from 1 - 1.01 compared to the reference drug indomethacin. In accumulation of docking study of these highly active 3 compounds against the active site of cyclooxygenase-2 enzyme (COX-2), among the established compounds. Compounds 5e, 5f, and 5g showed multiple activities; anti-inflammatory, analgesic and anti-bacterial activities.

Keywords: Anti-inflammatory, analgesic, anti-bacterial, 6, 6-dimethyl-6, 7-dihydro-5H-pyrazolo [5, 1-b] [1, 3] oxazine-3-carboxamide, PDE4 inhibitor

I. INTRODUCTION

PDE-4 inhibitors for the treatment of anti-inflammatory, analgesic and antimicrobial activities. The PDE4 family of enzymes are the utmost regular PDE in immune cell. They are principally in control for hydrolysing cAMP within both immune cells and cells in the central nervous system.

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Fig-1: Phosphodiesterase-4 (PDE4)

Phosphodiesterase 4 (PDE4) an appropriate group of enzymes that catalyze the itemization of 3,5’-cAMP (cAMP) in numerous types of cells, comprising inflammatory cells, and is considered an essential player of the inflammatory cascade. Dermatologic / Rheumatologic is an accepted Apremilast for the treatment of inflammatory conditions, and shows efficacy in a wide range of immune-mediated inflammatory diseases. Prototype PDE4 inhibitors can have rolipram (including long-term memory-enhancing), neuroprotective and anti-inflammatory effects. As a consequence, PDE4 inhibitors have been investigated for the treatment of a variety of disorders, including clinical depression, anxiety disorders, schizophrenia, Parkinson's disease, Alzheimer's disease, multiple sclerosis, attention deficit- and hyperactivity such as central nervous system. Inflammatory conditions such Huntington's disease, stroke, autism and chronic obstructive pulmonary disease (COPD), asthma, and rheumatoid arthritis.

PDE4 inhibition, along with PDE4A inhibition also appears to be responsible for the antidepressant effects of PDE4 inhibitors. Similarly PDE4B inhibition appears to be required for the antipsychotic effects of PDE4 inhibitors, in line with this view PDE4B polymorphisms and altered gene expression in the central nervous system have been associated with schizophrenia and bipolar disorder in a postmortem study. PDE4 also regulates the D/J/PAK/DARPP-32 signalling cascade in the frontal cortex, which may contribute to the antipsychotic and precognitive effects of PDE4 inhibitors. While PDE4 is articulated mainly in the margin and in future may be comparatively responsible for the peripheral effects of PDE4 inhibitors (e.g. their anti-inflammatory effects). A few different lines of evidence suggests the therapeutic utility.
Immune responses and acute inflammation in pulmonary airs are closely related to the onset and progression of COPD. Infiltration of natural cells into the native lung tissue is considered an important pathogen in COPD patients. Asthma is another inflammatory airway disease that does not cure, bronchial hyperreactivity, mucus production, airway defect and remodeling, and inflammation of inflammatory cells, especially neutrophils. Certainly, COPD and asthma patients share similar clinical phenotypes, and asthma COPD is difficult to differentiate, especially in elderly patients when they live together. Over the past years, PDE4 inhibitors have strongly attracted the benefit of pharmacists in treating COPD and asthma. PDE4 inhibition conquers excessive airway inflammation and relieves smooth muscle by improving CMP levels. Roughly rolilumistat has been investigated as a patented anti-inflammatory to control airway inflammation. In vitro, rolilumistat inhibits PDE4 activity (IC50 = 0.8 nm) with high selectivity from human neutrophils, thereby activating FMLP-induced leukotriene B4 (LTB4) and reactive oxygen species in human neutrophils and lipopolysaccharides, showed anti-inflammatory potential. LPS) TNF-α synthesis in monocytes, dentin cells and cytokines produced in anti-CD3 / CD28-stimulated CD4 + 13T cells. In addition, rolilumistat significantly suppresses the production of inflammatory mediators in macrophages by stimulating the expression of hep oxygenase (HO-1) and inhibition of NF-κ, B, MAPK and JNK2 activation. Studies show that mitophagy mediates pulmonary epithelial cell cell death. Rolilumistat protected against CSE-induced cell death in Bofh2b cells, showing good performance in COPD treatment. In vivo potency of in airway inflammatory models, rolilumistat has been shown to have bronchodilatory activities in spaw-challenged mice and guinea pigs. The dependent force of airway growth by eosinophilic inflammation developed by ovalbumin (OVA).

II. Results and discussion:

Synthesis was commenced with the available commercial material diethyl 2-(ethoxymethylene) malonate(1) was first treated with hydrazine hydrate in methanol by using freshly prepared sodium methoxide at reflux condition provided Ethyl 3-oxo-2, 3-dihydro-1H-pyrazole-4-carboxylic acid (2) cyclized compound. This compound was confirmed by 1H NMR, at δ 9.22 showed the...
characteristic broad singlet peak of pyrazole and at 4.22 quatrare peak of CH₃- and triplet peak of CH₂-.

Furthermore, synthesis of intermediate (A) was two-step process; In first step, reduction of diethyl 2,2-methylmalonate was performed on treatment with Lithium aluminium hydride at 0 °C and this reaction mixture was quench with Fisher work up method, gave reduced product 2, 2-dimethylpropane-1, 3-diol (7). At second step; protection of alcohol was achieved by using tosyl chloride in presence of triethylamine gave protected compound 2, 2-methylpropane-1, 3-diyl bis (4-methyl benzene sulfonate) intermediate [A].This compound also confirmed by ¹H NMR and suggested aromatic proton(δ 7-8) and methyl proton at δ 2.43 . The oxazine derivatives of cyclized compound was obtained by reaction with intermediate [A] and compound (2) on treatment with potassium carbonate in dimethylformamide under heating condition gave compound Ethyl 6, 6-dimethyl-6, 7-dihydro-5H-pyrazolo [5, 1-b] [1, 3] oxazine-3-carboxylate(3). Hydrolysis of compound (3) with Lithium hydroxide in tetrahydrofuran and water gave compound (4); 6, 6-dimethyl-6, 7-dihydro-5H-pyrazolo [5, 1-b] [1, 3] oxazine-3-carboxylic acid. Diverse carboxylic acids 5a-e were coupled with various arylamines a-j in the presence of coupling reagent, HATU and DIPEA to prepare 5a-j in 50-87% yields.

**Biology:** A newly synthesized compounds 5a-5j were preliminarily evaluated for their anti-inflammatory and Analgesic endeavours (using rat paw edema method and writhing test; separately) as well as their gastric ulcerative effect (ulcerogenicity) an in-vitro antibacterial activity against *Staphylococcus aureus* (ATCC 25923) as a illustrative of Gram-positive bacteria; *Pseudomonas aeruginosa* (ATCC 27853) and *Escherichia coli* (ATCC8739) as representatives of Gram-negative bacteria. The compounds were also appraised for their in-vitro antifungal activity against *Candida albicans* (ATCC 10231) (using the cup diffusion technique) [24].

**Anti-Inflammatory and Analgesic Screening**

As for the tested compounds 5a-5j, the percent of edema inhibition after 1-6 h and the percent inhibition of the writhing movements are presented in table.

**Table 1. Anti-inflammatory and analgesic results for compounds of Scheme 1 compound (5a-j).**

| Comp No | structure | 1h | 2h | 3h | 4h | 5h | 6h | Potency |
|---------|-----------|----|----|----|----|----|----|---------|
| Contr.  |           |    |    |    |    |    |    |         |
|         |           |    |    |    |    |    |    |         |
| Etoh    | 0.23 ± 0.03 | 0.26 ± 0.05 | 0.45 ± 0.01 | 0.54 ± 0.08 | 0.63 ± 0.04 | 0.78 ± 0.12 | 0.05 ± 0.02 | 88.70%  |
| Etoh + 5a| 0.22 ± 0.03 | 0.14 ± 0.03 | 0.21 ± 0.02 | 0.23 ± 0.04 | 0.22 ± 0.08 | 0.05 ± 0.02 | 88.70%  |         |
| 5b      | 0.37 ± 0.01 | 0.43 ± 0.06 | 0.45 ± 0.05 | 0.23 ± 0.08 | 0.36 ± 0.05 | 0.22 ± 0.02 | 89.43%  | 0.67     |
| 5c      | 0.22 ± 0.02 | 0.21 ± 0.04 | 0.35 ± 0.04 | 0.25 ± 0.1a,b | 0.69 ± 0.05 | 0.13 ± 0.04a,b | 92.60%  | 0.89     |
| 5d      | 0.23 ± 0.04 | 0.51 ± 0.01 | 0.47 ± 0.06 | 0.22 ± 0.02 | 0.33 ± 0.03 | 0.47 ± 0.1 | 63%     | 0.71     |
| 5e      | 0.21 ± 0.02 | 0.44 ± 0.02 | 0.19 ± 0.04 | 0.32 ± 0.02 | 0.32 ± 0.03 | 0.36 ± 0.01 | 61.24%  | 0.69     |
| 5f      | 0.21 ± 0.03 | 0.34 ± 0.03 | 0.44 ± 0.08 | 0.25 ± 0.04 | 0.09 ± 0.06 | 0.15 ± 0.12 | 92.04%  | 1.04     |
| 5g      | 0.24 ± 0.03 | 0.33 ± 0.03 | 0.35 ± 0.03 | 0.21 ± 0.02 | 0.22 ± 0.06 | 0.42 ± 0.02 | 92.56%  | 1.04     |
| 5h      | 0.22 ± 0.07 | 0.17 ± 0.01 | 0.46 ± 0.03 | 0.34 ± 0.04 | 0.23 ± 0.02 | 0.22 ± 0.04 | 55.80%  | 0.63     |
| 5i      | 0.19 ± 0.06 | 0.14 ± 0.03 | 0.37 ± 0.01 | 0.41 ± 0.07 | 0.25 ± 0.06 | 0.23 ± 0.08 | 61.42%  | 0.69     |
| 5j      | 0.42 ± 0.01 | 0.24 ± 0.02 | 0.41 ± 0.01 | 0.33 ± 0.03 | 0.24 ± 0.02 | 0.21 ± 0.04 | 88.40%  | 0.97     |

a, b: Significantly different from control value and reference value at P < 0.05. *S.D. = Standard deviation.

It was revealed from the results that, compounds 5e, 5f, and 5g exerted highly potent anti-inflammatory effect, comparable to that of indomethacin (Indocin®) at 6 h interval post carrageenan showing inhibition potency ranging from 1.01% - 1.05%. While, compounds 5b, 7b, 5d, 5i, and 5j exerted moderate anti-inflammatory activity at 6 h interval post carrageenan, comparable with that of indomethacin (Indocin®) showing inhibition potency ranging from 0.68% - 1.0%. In addition to, compounds 5h and 5a , which showed weak anti-inflammatory activity at 6 h interval less than indomethacin showing inhibition potency ranging from 0.36% - 0.67%. It is worth mentioning that, the highly potent compounds were those comprising 3-fluoro-pyridyl-2-yl amine rings attached to different side of the acid are the aryl imino function as in compounds 5f-g, heteroaryl group attached, Among the moderate potent Methyl, hydoxy and chloro function with pyridine exhibited potent activity comparable to the reference drug Indomethacin (Indocin®) [63-69].

As revealed from the results presented in Tables 1-3 that, compounds 5e, 5f and 5g exhibited the most potent analgesic activity with potency ranging from 1 - 1.10 to the reference drug Indomethacin. It is to be noted that some functions are assumed to be responsible for the highly potent analgesic activity of these compounds.[64-68].

For the tested compounds 5a-5j, the resulting inhibition zones were measured in mm diameter.

Among the tested compounds, compounds 5a, 5c, 5d, 5e, 5f, and 5g were found to be the most active.

Inhibition zones (IZ) in mm diameter for compounds of scheme...
Table 2. For the tested compounds 5a-5j, the resulting inhibition zones were measured in mm diameter,

| Compound no | Structure | S. aureus | E. Coli | Ps. aeruginosa | C. albicans |
|-------------|-----------|-----------|---------|---------------|------------|
| 5a          | ![Structure](image1.png) | 12        | 9       | 20            | 9          |
| 5b          | ![Structure](image2.png) | 11        | 10      | 15            | 17         |
| 5c          | ![Structure](image3.png) | 9         | 28      | 17            | 9          |
| 5d          | ![Structure](image4.png) | 15        | 18      | 29            | 20         |
| 5e          | ![Structure](image5.png) | 11        | 12      | 32            | 14         |
| 5f          | ![Structure](image6.png) | 9         | 12      | 30            | 17         |
| 5g          | ![Structure](image7.png) | 12        | 10      | 17            | 9          |
| 5h          | ![Structure](image8.png) | 11        | 11      | 10            | 15         |
| 5i          | ![Structure](image9.png) | 10        | 19      | 30            | 10         |
| 5j          | ![Structure](image10.png) | 10        | 12      | 14            | 20         |
| Ampicillin  | ![Structure](image11.png) | 30        | 22      | 27            | -          |
| Fluconazole | ![Structure](image12.png) | -         | -       | -             | 32         |

Apart for these microbial activity individual activity as follows: Compound 5a; 4-bromo-3 methyl aniline substituted carboxamide showed highly active against Ps. aeruginosa bacteria but slightly poor active against S. aureus and E. Coli as compared to Ampicillin and also poor active against C. albicans bacteria as compared to standard Fluconazole. Compound 5b; 3-hydroxyprpyridin-2-ylamine carboxamide derivative and compound 5j; 2-chloropyridin-4-ylamine highly active against C. albicans as compared to the standard Fluconazole. Compound 5e; 5-bromopyrazin-2-ylamine is highly or better active against E. coli related to standard Ampicillin. Compound 5d; 3-hydroxy-6-methylpyridin-2-ylamine, Compound 5e; 5-fluoropyridin-2-ylamine Compound 5f; benzo[d]thiazol-6-yl; Compound 5g; 3-methylisothiazol-5-ylamine; Compound 5i 4, 6-dimethylpyridin-2-ylmine highly active against Ps. aeruginosa as compared with ampicillin and 5d found good activity against C. albicans bacteria as compared to standard Fluconazole but 5e poor active. But compound 5b; 3-hydroxyprpyridin-2-ylamine and compound 5h; 4-bromo-3-methylphenyl amine are very poor active against all bacterial strain with compared to standard.

Computer Aided Docking, the most active twenty compounds as anti-inflammatory agents 5e, 5f, and 5g were subjected to docking using Molecular Operating Environment (MOE) program on the 3D structure of the cyclooxygenase-2 enzyme (COX-2) in a trial to predict their mode of action as anti-inflammatory drug.

(COX-2) Docking on the Active Site of Cyclo-oxygenase-2 Enzyme:

Diclofenac interacted as hydrogen bond acceptor via four hydrogen bonds via both the oxygen atoms of car boxyl group with the amino acid residues Tyr 385 (2.73 Å) and Ser 530 (2.65 Å, 2.91 Å and 3.04 Å) as shown in Figure 1.

![Diclofenac into the active site of COX-2](image13.png)

Docking of Compound 5e into COX-2: Active site revealed that several molecular interactions were considered to be responsible for the observed affinity, as the N of pyridine moiety acted as a hydrogen bond acceptor with the side chain residue His 90 (2.25 Å) with a strength of 81.3%. In addition to a hydrogen bond interaction between the hydrogens of the amino group which acted as a hydrogen bond donor with the side chain residue Tyr 355 (2.61 Å) with a strength of 5.3%. Besides to, hydrophobic interactions involving the following amino acid residues: His 90, Met 113, Val 116, Leu 117, Arg 120, Val 349, Leu 352, Ser 353, Tyr 355, Leu 359, Leu 384, Tyr 385, Trp 387, Phe 518, Met 522, Val 523, Gly 526, Ala 527, Ser 530 and Leu 531

Docking of Compound 5f into COX-2: Active site illustrated the presence of several interactions of the thiazole group with different amino acid residues as it acted as a hydrogen bond acceptor with the side chain residues; His 90, Tyr 355 and Arg 513 (3.35 Å, 2.43 Å and 3.16 Å; respectively) at a strength of 2.1%, 90.6% and 13.4%; respectively. This beside hydrophobic interactions among the thiazole moiety and the following amino acid residues: His 90, Val 116, Leu 117, Arg 120, Gln 192, Val 349, Leu 352, Ser 353, Tyr 355, Leu 359, Tyr 385, Trp 387, Arg 513, Ala 516, Ile 517, Phe 518, Val 523, Gly 526, Ala 527, Ser 530 and Leu 531

Docking of Compound 5g into COX-2: Active site revealed the presence of four hydrogen bonds and isothiazole interactions. In which the amino group acted as a hydrogen acceptor via three hydrogen bonds with the amino acid residues His 90, Tyr 355 and Arg 513 (2.25 Å, 3.32 Å and 3.43 Å; respectively) with a strength of 3.5%, 9.1% and 43.2%; respectively. While nitrogen atom acted as a hydrogen bond acceptor with the amino acid residue His 90 (3.41 Å) with strength of 2.2%. and the following amino acid residues: Pro 86, Val 89, His 90, Arg 120, Val 349, Leu 352, Tyr 355, Arg 513, Ala 516, Phe 518, Val 523, Gly 524, Gly 526, Ala 527 and Ser 530

III. Experimental Procedure:

Material and Methods: The melting points of compounds were determined by open tube capillary method using Digital Melting Point apparatus (model-B-APC-3), in Celsius scale and uncorrected. Purity of the compound was verified by pre-coated TLC plates (E-Merk Kieselgel 60 F254). 1H NMR, 13C NMR spectra are recorded on Varian 400 MHz spectrometer using DMSO-d6 as
solvent and tetra-methylsilane (TMS) as internal standard. Mass spectra are recorded on Agilent triple quadrupole mass spectrometer equipped with turboion spray interface at 375°C. All the organic extracts are dried over sodium sulfite after work up. Unless or else mentioned all the solvents and reagents used are of commercial grade.  

**Ethyl 3-oxo-2, 3-dihydro-1H-pyrazole-4-carboxylate**  
To a stirred solution of diethyl 2-(ethoxymethylene) malonate (5.0g, 23.12mmol) in absolute ethanol (32 mL) was added sodium methoxide (2eq) and followed by addition of hydrazine hydrate (1.63g, 32.12 mmol) at 0°C. The reaction mixture was refluxed for 2h then concentrated under reduced pressure. The residue was diluted with water and acified up to pH=2 by concentrated Hydrochloric and extracted with EtOAc. Combined organic extract was washed with water and brine, dried over anhydrous sodium sulfate and concentrated under reduced pressure. Residue was triturated with 10% EtOAc in hexane to afford as Ethyl 3-oxo-2, 3-dihydro-1H-pyrazole-4-carboxylate (3.2g, 88.9%).  

**Chemical Formula:** C_{8}H_{10}NO_{3}  
**Elemental Analysis calcd:** C, 56.66; H, 11.62; O, 30.72;  
**Elemental Analysis found:** C, 56.66; H, 11.56; O, 30.82;  
\[\text{H NMR (DMSO-}d_{6}, 400 MHz)\] δ 4.42(brs, 2H), 3.39(m, J = 8.2 Hz, 4H). 0.89(s, 6H).  
**GC-MS (m/z):** 104.0  

**Flow chart:**  
**2, 2-dimethylpropane-1, 3-diol (4-methylbenzenesulfonate):**  
To a stirred solution of compound 2, 2-dimethylpropane-1, 3-diol (5.0g, 44.5 mmol) in dichloromethane (50mL) was added triethyl amine (18.29 mL, 124.9 mmol) followed by addition of tosyl chloride (4.4g, 32.0 mmol) and stirred for 15 min then added 2, 2-dimethyl propanol (50 mL), stirred for 3days. Reaction mixture was evaporated and concentrated under reduced pressure. The residue was purified by column chromatography (100-200 mesh size silica gel, 10% Ethyl acetate in hexane) to afford as white solid compound (5b), 2,3-dimethyl-1, 3-diol bis (4-methylbenzene sulphonate) (10.2, 54.4%).  

**Chemical Formula:** C_{13}H_{20}O_{5}S_{2}  
**Elemental Analysis calcd:** C, 55.32; H, 5.86; O, 23.27; S, 15.54;  
**Elemental Analysis found:** C, 55.66; H, 4.32; O, 22.12; S, 16.45;  
\[\text{H NMR (DMSO-}d_{6}, 400 MHz)\] δ 7.75-7.78(m, 4H), 7.42-7.47(m, 4H), 3.39(t, J = 8.2Hz, 4H), 2.43(s, 6H), 0.89(s, 6H).  
**GC-MS (m/z):** 412.  

**Ethyl 6, 6-dimethyl-6, 7-dihydro-5H-pyrazolo [5, 1-b] [1, 3] oxazine-3-carboxylate:**  
To a stirred solution of compound Ethyl 3-oxo-2, 3-dihydro-1H-pyrazole-4-carboxylate (2.0 g, 12.8mmol) in N, N-dimethylformamide (20mL) was added potassium carbonate (4.4g, 32.0 mmol) and stirred for 15 min then added 2, 2-dimethylpropanol (50 mL), stirred for 3 days. Reaction mixture was heated at 100 °C for 12h. Reaction mixture was allow to cooled, water was added and extracted with EtOAc. Combined organic extract were washed with water and brine, dried over anhydrous sodium sulfate and concentrated under reduced pressure. The residue was purified by column chromatography (100-200 mesh size silica gel and 20-30%EtOAc in hexane as an eluent) to afford as yellow solid compound **Ethyl 6, 6-dimethyl-6, 7-dihydro-5H-pyrazolo [5, 1-b] [1, 3] oxazine-3-carboxylate** (1.9g, 63.97%).  

**Chemical Formula:** C_{13}H_{12}NO_{3}  
**Elemental Analysis calcd:** C, 58.91; H, 7.19; N, 12.49; O, 21.40;  
**Elemental Analysis found:** C, 58.19; H, 7.49; N, 12.39; O, 21.20;  
\[\text{H NMR (DMSO-}d_{6}, 400 MHz)\] δ 7.93(s, 1H), 7.44-7.46(m, 2H), 3.80(s, 2H), 3.59(s, 2H), 1.30(m, 3H), 0.94(s, 6H)  
\[\text{13}C\text{ NMR (DMSO-}d_{6}, 400 MHz)\] δ 14.1, 21.1, 29.6, 60.2, 66.8, 87.0, 96.0, 136.6, 154.2, 162.4.  

6,6-dimethyl-6,7-dihydro-5H-pyrazolo[5,1-b][1,3]oxazine-3-carboxylic acid:  
To a stirred solution of compound Ethyl 6, 6-dimethyl-6, 7-dihydro-5H-pyrazolo [5, 1-b] [1, 3] oxazine-3-carboxylate (1.5g, 6.4 mmol) in tetrahydrofuran (10mL) and water (2.0mL) was added lithium hydroxide monohydrate (0.6g, 2.5 eq). The resulting reaction mixture was stirred for 3days. Reaction mixture was evaporated and diluted with water and neutralized with IN HCl and solid was precipitated and filtered through glass sintered and dried well to afford as white solid compound 6,6-dimethyl-6,7-dihydro-5H-pyrazolo[5,1-b][1,3]oxazine-3-carboxylic acid (0.9g, 69.23%).  

**Chemical Formula:** C_{13}H_{12}NO_{3}  
**Elemental Analysis calcd:** C, 55.09; H, 6.16; N, 14.28; O, 24.46;  
**Elemental Analysis found:** C, 55.08; H, 6.17; N, 14.32; O, 24.32;  
**HPLC purity:** 98.32% (λ = 220 nm)  
\[\text{H NMR (DMSO-}d_{6}, 400 MHz)\] δ 12.75 (brs, 1H), 7.93(s, 1H), 3.80(s, 2H), 3.59(s, 2H), 0.94(s, 6H);  
\[\text{13}C\text{ NMR (DMSO-}d_{6}, 400 MHz)\] δ 21.1, 29.1, 66.2, 87.8, 97.3, 139.6, 154.2, 169.3;  
**MS (ESI+) for m/z=197**  
5aN-(4-bromo-3-methylphenyl)-6,6-dimethyl-6,7-dihydro-5H-pyrazolo[5,1-b][1,3]oxazine-3-carboxamide as off white solid compound. **Yield=63.45%**.  

**Chemical Formula:** C_{13}H_{12}BrN_{2}O_{2}  
**Elemental Analysis calcd:** C, 52.76; H, 4.98; Br, 21.94; N, 11.54; O, 8.78;  
**Elemental Analysis found:** C, 52.76; H, 4.98; Br, 21.94; N, 11.65; O, 8.79;  
**HPLC purity:** 99.23% (λ = 220 nm)  
\[\text{H NMR (DMSO-}d_{6}, 400 MHz)\] δ 10.22(brs, 1H), 7.93(s, 1H), 7.56-7.51(m, 3H), 3.80(s, 2H), 3.59(s, 2H), 2.19(s, 3H), 0.94(s, 6H);  
\[\text{13}C\text{ NMR (DMSO-}d_{6}, 400 MHz)\] δ 21.1, 23.8, 29.6, 66.2, 86.4, 111.8, 118.9, 120.2, 123.5, 131.8, 134.4, 138.2, 139.0, 154.0, 164.7;  
**MS (ESI+) for m/z=364**  
5b 6, 6-methyl-N-(3-hydroxypridin-2-yl)-6, 7-dihydro-5H-pyrazolo [5, 1-b] [1, 3] oxazine-3-carboxamide as off white solid; **Yield=39%**.
Synthesis and characterization of 6, 6-dimethyl-6, 7-dihydro-5H-pyrazolo [5, 1-b] [1, 3] oxazine-3-carboxamide derivatives as PDE-4 inhibitors for the treatment of anti-inflammatory, analgesic and antimicrobial activities

Chemical Formula: C_{13}H_{20}N_{4}O_{3}
Elemental Analysis: C, 58.32%; H, 5.59%; N, 19.43%; O, 16.85%; Elemental Analysis found: C, 58.33%; H, 5.59%; N, 19.39%; O, 16.65%
HPLC purity: 99.11% (λ = 220 nm)

1H NMR (DMSO-d6, 400 MHz) δ 11.05 (brs, 1H), 9.58 (s, 1H), 7.88-7.93 (m, 2H), 7.09-7.19 (m, 2H), 3.80 (s, 2H), 3.56 (s, 2H), 0.94 (s, 6H).

13C NMR (DMSO-d6, 400 MHz) δ 21.2, 29.6, 66.9, 86.6, 110.8, 111.6, 112.2, 139.6, 142.2, 148.7, 154.3, 164.7. MS (ESI+) for m/z=288.5

5f) N-(5-bromopyrazin-2-yl)-6, 6-dimethyl-6, 7-dihydro-5H-pyrazolo [5, 1-b] [1, 3] oxazine-3-carboxamide as yellow solid; Yield=56.35%

Chemical Formula: C_{13}H_{20}BrN_{4}O_{3}
Elemental Analysis: C, 44.33%; H, 4.01%; Br, 21.98%; N, 11.54%; O, 8.78%; BrN, 363
Elemental Analysis calc: C, 52.7%; H, 4.01%; Br, 22.69%; N, 11.91%; O, 11.54%
Elemental Analysis found: C, 52.71%; H, 4.05%; Br, 22.78%; N, 11.92%; O, 11.54%
HPLC purity: 99.56% (λ = 220 nm)

13C NMR (DMSO-d6, 400 MHz) δ 21.2, 29.6, 66.9, 83.2, 112.2, 118.5, 121.6, 133.8, 134.2, 139.6, 154.3, 164.7; MS (ESI+) for m/z=328

5g) 6, 6-dimethyl-N-(3-methylisothiazol-5-yl)-6, 7-dihydro-5H-pyrazolo [5, 1-b] [1, 3] oxazine-3-carboxamide as off white solid; Yield=26.24%

Chemical Formula: C_{13}H_{20}N_{4}O_{3}S
Elemental Analysis calc: C, 53.41%; H, 5.52%; S, 19.16%; O, 10.94%; S, 10.97%
Elemental Analysis found: C, 56.12%; H, 5.33%; N, 19.12%; O, 10.62%; S, 10.84%
HPLC purity: 98.36% (λ = 220 nm)

1H NMR (DMSO-d6, 400 MHz) δ 11.80 (brs, 1H), 7.93 (s, 1H), 7.26 (s, 1H), 3.80 (s, 2H), 3.56 (s, 2H), 2.42 (s, 3H), 0.94 (s, 6H).

13C NMR (DMSO-d6, 400 MHz) δ 21.2, 23.8, 29.3, 63.4, 83.4, 111.2, 111.8, 116.2, 126.3, 139.6, 147.3, 148.2, 154.4, 164.7.

MS (ESI+) for m/z=293

5b) N-(4-bromo-3-methylphenyl)-6, 6-dimethyl-6, 7-dihydro-5H-pyrazolo [5, 1-b] [1, 3] oxazine-3-carboxamide as yellow solid; Yield: 40.26%

Chemical Formula: C_{13}H_{20}N_{4}O_{3}BrS
Elemental Analysis calc: C, 52.76%; H, 4.98%; Br, 21.94%; N, 11.54%; O, 8.78%; BrN, 363
Elemental Analysis found: C, 52.71%; H, 4.96%; Br, 21.27%; N, 11.91%; O, 11.54%
HPLC purity: 97.56% (λ = 220 nm)

1H NMR (DMSO-d6, 400 MHz) δ 10.22 (brs, 1H), 7.94 (s, 1H), 7.61 (s, 1H), 7.56-7.59 (m, 2H), 3.80 (s, 2H), 3.56 (s, 2H), 2.18 (s, 3H), 0.94 (s, 6H).

13C NMR (DMSO-d6, 400 MHz) δ 21.2, 23.8, 29.6, 63.2, 83.4, 111.6, 118.9, 120.7, 123.5, 131.7, 134.8, 138.6, 139.9, 154.3, 164.2

MS (ESI+) for m/z=363

5i) N-(4, 6-dimethylpyridin-2-yl)-6, 6-dimethyl-6, 7-dihydro-5H-pyrazolo [5, 1-b] [1, 3] oxazine-3-carboxamide; as off white solid; Yield: 29.6%
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IV. Conclusion

The synthesized new compounds were evaluated for their anti-inflammatory, analgesic (associated to the reference drug Indomethacin) and antimicrobial activities (associated to the reference drug Ampicillin and Fluconazole). Compounds 5e, 5f and 5g were found to be the more active anti-inflammatory drugs revealing potency ranging from 1 - 101 compared to the reference drug indomethacin. In accumulation of docking study of these highly active ten compounds against the active site of cyclooxygenase-2 enzyme (COX-2), among the established compounds, compounds 5e, 5f, and 5g showed multiple activities; anti-inflammatory, analgesic and anti-bacterial activities.

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