Synthesis and evaluation of coumate analogues as estrogen receptor inhibitors for breast cancer therapy

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ABSTRACT
A sulphatase inhibitor 667 COUMATE is in clinical trials for estrogen-positive breast cancer therapy for postmenopausal women, while there are a number of similar sulphatase inhibitors under development. Schiff's bases are versatile pharmacophores in which the N atom involves in hydrogen bonding with active cell centers interfering in normal cell biology. A library of novel coumate analogues with Schiff bases were designed, and structural based drug design was performed with human estrogen receptor (PDB ID: 2IOG) (Already reported). Based on the in-silico outcomes, seven coumate-schiff bases were synthesized. The compounds were obtained in good yield. The novelty had been ascertained by sciFinder software. The synthesized molecules were consistent with their assigned spectra such as IR, Mass and NMR spectral data which confirmed their formation. The cytotoxicity study was performed by MTT assay for all the synthesized compounds. Most of the compounds have good IC50 values (below 100 μg/ml). Two of the synthesized compounds COU-2 and COU-5 have shown good IC50 values such as 19 μg/ml and 39 μg/ml, respectively. They suppressed the proliferation of estrogen receptor overexpressed MCF-7 cells.

INTRODUCTION
Breast cancer is the superlative ordinary identified malignant neoplasm in women worldwide (22%) and in India (18.5%) (cancer registries report and Oct 31, 2010). The estrogen receptor (ER) which is a cellular receptor that produces ligand-inducible intracellular transcription, which in turn regulates the biological consequence of estrogens at the caliber of gene regulation (Siddiqi et al., 2001). In the cell organ, the ER upstream or downstream modulates the aspect of objective genes by relating over its site-specific DNA and through additional co-regulatory proteins that contain co-activators as well as co-repressors (Nandakumar et al., 2005; Gronemeyer, 1991; Kumar and Thompson, 1999). The ER which is in ligand-bound ties as a homo dimer to explicit DNA orders named Estrogen Reaction components (EREs) and manages translation over correspondence with interpretation modulators and selection of the general translation machinery (Parker, 1998). The structures of ER, intra/intermolecular communications, post-translational alterations, and various different viewpoints influencing to the ER activities have been emerged (Muramatsu and Inoue, 2000; Lumachi et al., 2011). Comparing benefi-
The sulfated steroid hormone cleavage antecedents, for example, estrone sulfate, to the dynamic hormones by steroidal sulphatase enzyme (STS) speaks to the initial phase in the neighborhood generation of sexual hormones primarily estrogen. Along these lines, the restraint of this compound (STS), which should diminish the biosynthesis of dynamic hormones (Reed et al., 2005). Since the hydrolysis of sulfate monoester securities catalysed by sulphatase in an extent of physiological substrates, the combination of a sulfamate ester accumulate associated with a phenyl ring was seen as a key method in the enhancement of solid sulphatase inhibitors (Purohit et al., 2003). Numerous STS inhibitors persist only in preclinical times of headway, with only 667 COMUATE (Figure 2). In addition, try to recognize non-steroidal STS inhibitors provoked the enhancement of a number of bicyclic/tricyclic coumarin sulphamates, which are dynamic both in-vitro and in-vivo (Figure 3).

A progression of sulphamate derivatives of various diverse ring frameworks, one of which was phenolic, was along these lines combined. From this arrangement, 4-methylcoumarin-7-O-sulphamate was recognized as an intense non-steroidal inhibitor with helpful potential. Coumate had no proliferative impact on MCF-7 cell development, showing that it was not estrogenic. In the ovariectomised rodent, Coumate did not invigorate uterine development, affirming that it is without estrogenic action.

The Schiff bases are an important class of compounds due to their flexibility, structural similarities with natural biological substances and also due to their presence of imine (-N=CH-) which imparts in elucidating the mechanism of transformation in a biological system (Jubie et al., 2011b). Keeping in mind the estrogenic receptor inhibitory properties of coumarin nucleus, and continuation of our previous work (Begam et al., 2017), it is proposed to design and develop novel pharmacophores containing coumates and Schiff bases as estrogen receptor inhibitors. Earlier we reported the in-silico docking work (Begam et al., 2018) and base on the results it has been decided to synthesize seven novel ana-
Figure 1: Drugs used for breast cancer

Tamoxifene (1)  
Fulvestrant (2)

Anastrazole (3)  
Letrozole (4)  
Exemestane (5)

Figure 2: Structure of COUMATE

Figure 3: Coumarin based STS inhibitors
logues of COUMATES, i.e. COU-02, COU-05, COU-31, COU-36, COU-08, COU-14 & COU-39.

**MATERIALS AND METHODS**

The reagents were of business-grade and utilized as provided. liquefying focuses were resolved in open glass vessels which were uncorrected. The response advancement and virtue of the mixes were analyzed by thin-layer chromatography (TLC). The last blend was performed in IKA Fundamental Metler Tolloed Parallel synthesizer. IR spectra of mixes were recorded on BRUKER (400MHz FT-NMR) utilizing CDCl$_3$ as dissolvable and TMS as inner standard. Mass spectra were recorded by Shimadzu LC-MS. Table 1 shows.

**Table 1: Substitution pattern of the synthesized compounds**

| S.NO | Compound | -R |
|------|----------|----|
| 1.   | COU-02   | -2Cl |
| 2.   | COU-05   | -4NO$_2$ |
| 3.   | COU-31   | -4Cl |
| 4.   | COU-36   | -4OH |
| 5.   | COU-08   | -3Br |
| 6.   | COU-14   | -2NO$_2$ |
| 7.   | COU-39   | -4N, N (CH$_3$)$_2$ |

**Synthesis of 7-hydroxy-4-methyl coumarin**

A blend of resorcinol (11.14g) and ethyl acetoacetate (13.27ml) was kept in a round base flagon and kept up at 0°C. To this blend, conc. H$_2$SO$_4$ (100 ml) is included and kept at 10°C for 2h. At that point, the blend was kept at RT for 18h. The response advance was observed by TLC. On completion of response, powdered ice was included and blended. The encourage was gathered and blended with 5% sodium hydroxide (150ml), separated and 2M H$_2$SO$_4$ was included with mixing until the arrangement was acidic. The encourage was washed with water, dried and made for the following stride. The strong along these lines got and recrystallized from ethanol. Yield 85 %; m.p 130-132°C. TLC dissolvable framework, chloroform &ethyl acetic acid derivation (4:1); Rf esteem (0.54).

**Synthesis of coumate**

To an answer of 7-hydroxy-4-methyl coumarin (3g) in 30 ml of dry DMF, 672 mg of sodium hydride was included and kept in ice shower for 0°C. After this, 2eq.of sulfamoyl chloride (3.5698g) was included. The blend was kept at room temperature overnight with mixing under nitrogen air. The response advance was observed by TLC. On completion of response, the accelerate coumate was isolated by filtration and utilized for the following stage. The strong accordingly got and recrystallized from ethanol. Yield 85%; m.p 180-182°C. TLC dissolvable framework, chloroform &ethyl acetic acid derivation (4:1); Rf esteem (0.71).

**Synthesis of COUMATE -Schiff bases.**

To a well ground blend of coumate (1mmol, 02538mg) and P$_2$O$_5$ (0.536g) in a round base carafe associated with a reflux condenser was included aldehyde (0.14ml). The blend was mixed in an oil-shower (110°C) for 2h. After 2h, the response blend was cooled to RT and the strong blend was poured on ice and washed with (CH$_3$)$_2$CO (2ml). The dissolvable was vanished and the rough item was disintegrated in warm ethyl acetic acid derivation (2ml), treated with n-hexane (6ml) and was permitted to remain at RT for 5-6h. Amid this time, the Schiff bases were created and after that gathered by filtration, washed with n-hexane and dried. The stepwise reactions are depicted in the scheme 1. The response was observed by TLC (Chloroform: ethyl acetic acid derivation 4:1).

**Table 2: IC$_{50}$ values of synthesized compounds**

| S.NO | Sample Code | IC$_{50}$(μg/ml) |
|------|-------------|-----------------|
| 1.   | COU-02      | 19.17041        |
| 2.   | COU-05      | 39.8525         |
| 3.   | COU-08      | 46.46148        |
| 4.   | COU-14      | 67.14783        |
| 5.   | COU-31      | 91.8234         |
| 6.   | COU-36      | 73.39237        |

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Scheme 1: Synthetic route for the synthesis of Coumate - Schiff bases (Reagents and conditions:

- **a.** Conc. H$_2$SO$_4$;
- **b.** 18 h/RT;
- **c.** HCOOH/ACN/NH(CH$_3$)$_2$;
- **d.** 4 h/RT;
- **e.** DMF/NaH;
- **f.** RT/overnight/N$_2$ atm;
- **g.** P$_2$O$_5$;
- **h.** heat at 110$^\circ$ C)

IR (cm$^{-1}$): 1532.65 (-NO$_2$), 1007.00 (-C-O-C), 849.23 (p-substituted benzene), 1343.12 (O=S=O), 1605.71 (N=C), 1704.19 (C=O), 677.68 (Ar-CH).

$^1$H NMR (CDCl$_3$): $\delta$ 10.173 (d, 1H, N=CH), 8.417-7.274 (m, 8H, Ar-H).

$^{13}$C NMR (CDCl$_3$): ppm 190.34 (CH), 151.13-124.34 (Ar-C), 77.30-76.80 (CH$_3$). MS: m/z 393 (M-3).

**Coumarin sulfamate / Coumate**

(4aR,8aR)-4-methyl-2-oxo-4a,8a-dihydro-2H-chromen-7-yl[(E)-(4-chlorophenyl) methylidene] sulfamate (COU-31)

Yield 85%; m.p 100-102$^\circ$ C. TLC solvent system: chloroform:ethyl acetate (4:1); Rf value (0.54). IR (cm$^{-1}$): 3162.60 (O-H), 1314.90 (O=S=O), 1665.98 (C=O), 1591.19 (C=N), 1110.36 (C=O-C). $^1$H NMR (CDCl$_3$): $\delta$ 9.040 (d, 1H, N=CH), 7.786-6.962 (m, 8H, Ar-H), 2.394-1.976 (m, 3H, CH$_3$).

**Biological Assay**

The cytotoxicity screening was performed according to the previous reported method (Jubie et al., 2011a).

**RESULTS AND DISCUSSION**

**Synthesis**

7-Hydroxy-4-methyl coumarin were set up by Pechmann response among resorcinol and ethyl acetoacetate within the sight of conc. Sulphuric corrosive at room temperature. The sulfamoyl chloride was readied utilizing chlorosulfonylisocynate within the sight of formic corrosive and acetonitrile and N, N-dimethyl acetamide. The incorpor-
rated 7-Hydroxy-4-methyl coumarin was responded with sulfamoyl chloride within sight of DMF and sodium hydride at room temperature with nitrogen environment and kept medium-term to shape coumarinsulfamate/coumate. The last mixes which are Schiff bases of coumate were combined by responding coumarinsulfamate/coumate with various sweet-smelling aldehydes within sight of Phosphorus pentoxide at 110°C. All the integrated mixes were first refined by progressive recrystallization utilizing suitable solvents. The virtue of the combined mixes was checked by performing slim layer chromatography and deciding softening focuses. At that point, the combined mixes were exposed to the otherworldly examination, for example, IR, $^1$H NMR, and $^{13}$C NMR to affirm the structures.

**In-Vitro Cytotoxicity Screening**

All the six mixes were evaluated by MTT measure technique on human bosom malignant growth cell lines (MCF-7), and the outcomes were delineated in the (Table 2). All orchestrated mixes indicated huge cytotoxicity impact of underneath 100μg/ml anyway the mixes COU-02 and COU-05 displayed critical consequences for stifling MCF-7 cells in the centralization of 19 μg/ml and 39 μg/ml individually. The coumate Schiff bases COU-02 and COU-05 essentially down-managed MCF-7 cells practicality in a portion subordinate way. The information here proposed that Coumate Schiff bases had an enemy of tumor job in bosom malignancy cells multiplication without causing cytotoxicity in ordinary cells.

**CONCLUSIONS**

The lack of selective binding is the major drawback of existing drugs used for estrogen-positive breast cancer therapy. Coumate and its analogues have an inhibitory effect against the estrogen receptor. To increase the selective binding of estrogen receptor alpha, it was proposed to synthesize and screen for in-vitro cytotoxicity of Schiff bases of coumate. Two of the synthesized compounds COU-2 and COU-5 have shown good $IC_{50}$ values such as 19μg/ml and 39μg/ml, respectively. They suppressed the proliferation of estrogen receptor overexpressed MCF-7 cells. However, further studies may be explored for the anticancer potentials of these two compounds.

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