



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 sci finder 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.

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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 in-

tracellular 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-

cial partners of the nucleo receptor (NHR) group, the ER is gathered of different useful spaces that help distinct roles (Beato, 1989). Starter from NH₂- to COO-end, the significant spaces are N-terminal area (NTD); DNA-restricting space (DBD); ligand-restricting space (LBD).

Paired actuation work (AF) spaces, AF-1 and AF-2, situated inside the LBD and NTD, correspondingly, are at risk for ER transcription. AF-1 purposes as hormone-free, while AF-2 reason requires the nearness of hormone/steroid. The physiological impacts of estrogen are set up over ER-alpha and ER-beta. Both receptors have too been presented in hetero dimers framing on EREs. In view of homology between two receptors, the ER-beta has shown a high similarity to ER alpha in the DNA Binding domain (> 95% amino corrosive singularity) and in the Ligand Binding Domain (~55% amino corrosive independence). Among the two ER receptors have been involved, the character of ER- β in bosom malignancy remainders immaterial. ER- α is enunciated in a subgroup of customary bosom epithelial cells, and ER- α holding epithelial cells don't for the most part, increase in response to estrogen (Buzdar, 2000). In this connection, estrogen energizes the ordinary mammary epithelium to stash developing components that energize nearby ER-negative epithelial cells to multiply in a porcine way. In difference to the conventional bosom, most extreme pre-threatening bosom scratches brief high phases of ER- α , and ER- α articulating bosom malignant growth cells are hormone-subordinate and embrace crumbling when estrogen is unconcerned. So, ER- α is an entrenched anticipating sign of hormone empathy in bosom malignancy just as a positive prognostic sign. In the dynamic ailment circumstance, a few patients with ER- α -positive bosom tumors will answer promisingly, in any event fundamentally, to endocrine treatments. Since ER-positive bosom malignant growth is estrogen-needy, dropping estrogen levels or moving the activity of the receptor can urge these diseases to decline (Buzdar, 2002; Buzdar et al., 1998).

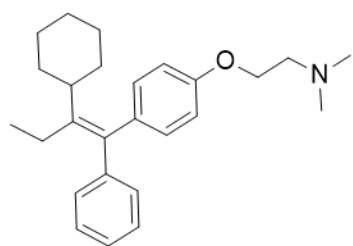
Current procedures expected to diminish measurements of coursing estrogen fuse explicit sulphatase and aromatase inhibitors. The estrogen receptor antagonists fall into two critical classes. These fuse Selective Estrogen Receptor Modulators (SERMs), for instance, tamoxifen (1) and the unadulterated adversaries of estrogens, i.e. Selective Estrogen Receptor Degradars (SERDs), for instance, fulvestrant (2), which decline ER centers. Tamoxifen passes on treatment benefits like those with oophorectomy. Anastrozole (3) was the essential aromatase substance endorsed for second-line therapy of cutting

edge carcinoma in natural time ladies; Later the drugs letrozole (4) and exemestane (5) were found to claim comparable efficacy (Baum et al., 2002; Pasqualini et al., 1989). As of late, each anastrozole and letrozole were appeared to claim superior viability than estrogen enemy as a first-line therapeutic guide for patients with cutting edge ER-positive tumors (Poirier et al., 1999). The concoction structures of medications utilized in bosom malignant growth treatment are delineated in Figure 1.

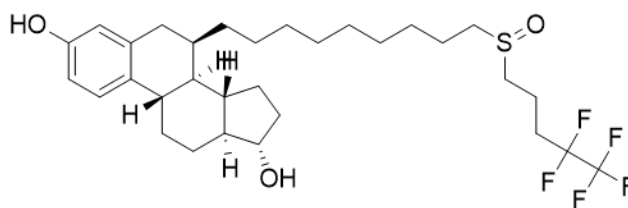
The sulfated steroid hormone cleavage antecedents, for example, estrone sulfate, to the dynamic hormones by steroidal sulphatase enzyme (STS) speaks to the initial phase in the neighborhood generation of sexual hormones primarily estrogen. Along these lines, the restraint of this compound (STS), which 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 COUMATE (Figure 2). In addition, try to recognize non-steroidal STS inhibitors provoked the enhancement of a number of bicyclic/tricyclic coumarin sulfamates, 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.

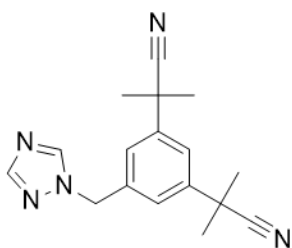
The Schiff bases are an important class of compounds due to their flexibility, structural similarities with natural biological substances and also due to their presence of imine (-N=CH-) which imparts in elucidating the mechanism of transformation in a biological system (Jubie et al., 2011b). Keeping 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-



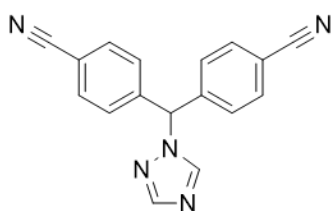
Tamoxifene (1)



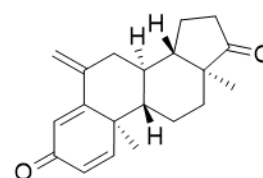
Fulvestrant (2)



Anastrozole (3)



Letrozole (4)



Exemestane (5)

Figure 1: Drugs used for breast cancer

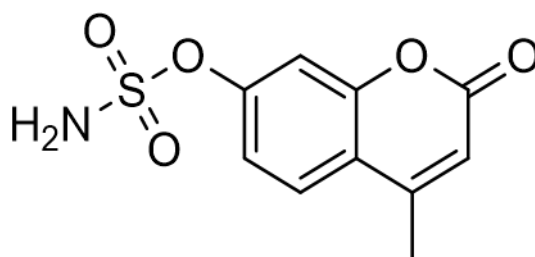


Figure 2: Structure of COUMATE

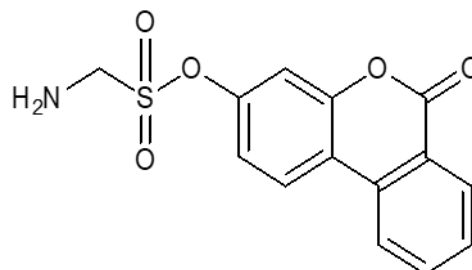
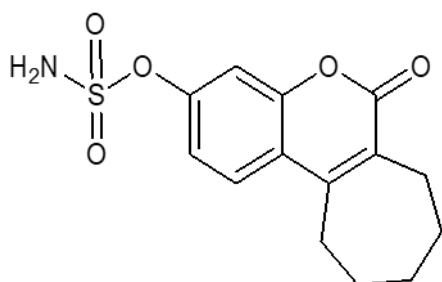


Figure 3: Coumarin based STS inhibitors

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 Toledo Parallel synthesizer. IR spectra of mixes were recorded on Perkin-Elmer FT-IR spectrometer and are communicated in cm^{-1} . $^1\text{H-NMR}$ and $^{13}\text{C-NMR}$ spectra were recorded on BRUKER (400MHz FT-NMR) utilizing CDCl_3 as dissolvable and TMS as inner standard. Mass spectra were recorded by Schimadzu 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_2SO_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, pounded ice was included and blended. The encourage was gathered and blended with 5% sodium hydroxide (150ml), separated and 2M H_2SO_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 190-192°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 tempera-

ture 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, 0.2538mg) and P_2O_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 $(\text{CH}_3)_2\text{CO}$ (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).

(4aR,8aR)-4-methyl-2-oxo-4a,8a-dihydro-2H-chromen-7-yl[(E)-(2-chlorophenyl) methylidene] sulfamate (COU-02)

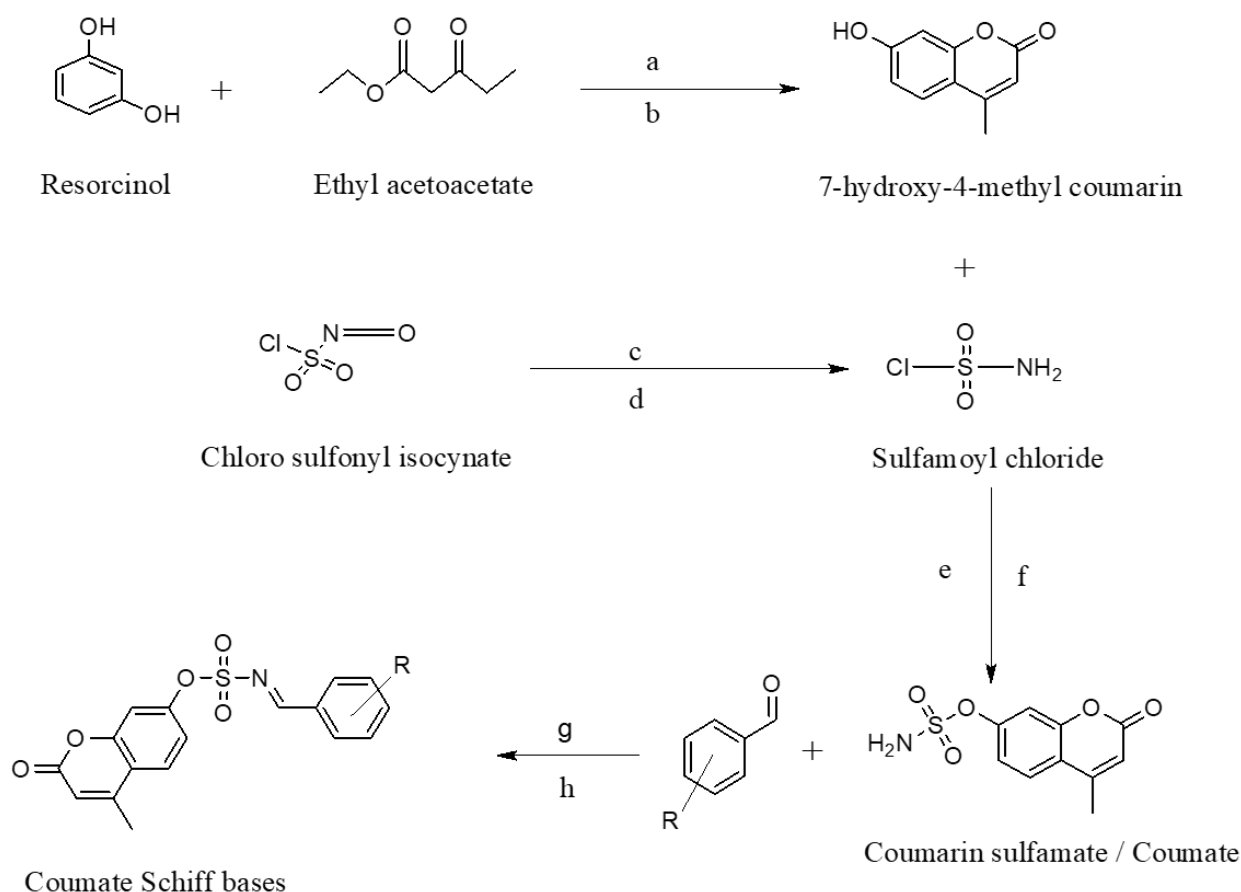
Yield 85 %; m.p 130-132°C. TLC solvent system, chloroform & ethyl acetate (4:1); Rf value (0.63). IR (cm^{-1}): 1310.76 (O=S=O), 742.32 (C-Cl), 1678.82 (C=O), 2820.22 (Ar-CH), 1042 (-C-O-C-), 814.74 (p-substituted benzene), 1678.82 (C=N). $^1\text{H NMR}$ (CDCl_3): δ 10.496 (d, 1H, N=CH), 8.038-7.261 (m, 8H, Ar-H), 3.521 (m, 3H, CH₃). $^{13}\text{CNMR}$ (CDCl_3): ppm 170.65 (CH), 134.74-126.62 (Ar-C), 77.27-76.76 (CH₃). MS: m/z 381 (M-2).

Table 2: IC₅₀ values of synthesized compounds

S.NO:	Sample Code	MCF-7 (IC ₅₀ $\mu\text{g/ml}$)
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

(4aR,8aR)-4-methyl-2-oxo-4a,8a-dihydro-2H-chromen 7yl[(E)(4nitrophenyl)methylidene] sulfamate (COU-05)

Yield 85 %; m.p 90-92°C. TLC solvent system, chloroform:ethyl acetate (4:1); Rf value (0.68).



Scheme 1: Synthetic route for the synthesis of Coumate - Schiff bases (Reagents and conditions a. Conc. H_2SO_4 ; b. 18 h/RT; c. $HCOOH/ACN/NH(CH_3)_2$; d. 4h/RT; e. DMF/NaH ; f. RT/over night/ N_2 atm; g. P_2O_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). 1H NMR ($CDCl_3$): δ 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).

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

Yield 85 %; m.p $130-132^\circ C$. TLC solvent system, chloroform: ethyl acetate (4:1); Rf value (0.69). IR (cm^{-1}): 760.84 (C-Cl), 1369.28 ($O=S=O$), 1606.10 ($N=C$), 1091.17 ($-C-O-C-$), 1676.83 ($C=O$), 843.22 (p-substituted benzene), 682.22 (Ar-CH). 1H NMR ($CDCl_3$): δ 2.381 (d, 1H, $N=CH$), 8.000-6.075 (m, 8H, Ar-H), 1.285-0.899 (m, 3H, CH_3). ^{13}C NMR ($CDCl_3$): ppm 77.22-76.72 (CH=N), 103.01 (Ar-C), 67.96 (p-substituted benzene). MS: m/z 368 (M-3).

3-(4-hydroxyphenyl)-4-methyl-2-oxo-2H-chromen-7-yl sulfamate (COU-36)

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

($-C-O-C-$), 832 (p-substituted benzene), 788.72 (Ar-CH). 1H NMR ($CDCl_3$): δ 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 chlorosulfonylisocyanate within the sight of formic corrosive and acetonitrile and N, N-dimethyl acetamide. The incorpo-

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 pentaoxide 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, ¹H NMR, and ¹³C 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 IC₅₀ 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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