Bioorganic & Medicinal Chemistry Letters 20 (2010) 4945–4950

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Synthesis and docking studies on styryl chromones exhibiting cytotoxicity in human breast cancer cell line

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ARTICLE INFO

Article history:
Received 9 April 2010
Revised 27 May 2010
Accepted 31 May 2010
Available online 10 June 2010

Keywords:
Estrogen receptor
Styryl chromones

ABSTRACT

The search for small molecules that preferentially target the functionally important surfaces of estrogen receptor and disrupt the transcriptional activity in the cell has emerged as a promising area towards rationale based drug design. Herein, we report substituted styryl chromones as a new class of compounds that exhibit selectivity for ERβ binding at the second binding site of HT and antiproliferative activity in human breast cancer cell line.

Estrogen receptor (ER) is a member of the nuclear hormone receptor superfamily and functions as ligand-activated transcription factor to regulate genetic networks controlling cell growth and differentiation, inflammatory responses, and metabolism. The ligand binding domain (LBD) of ER is the primary target for nuclear receptor (NR) drug discovery. Traditional approaches comprise of type I and type II antiestrogens which were designed to modulate NR activity based on competitive binding to the cognate ligand-binding domain. The recent discovery of the second binding site for tamoxifen in the ER[1] has provided additional avenues worth exploring towards better understanding of mode of action of type I antiestrogens and targeted drug discovery. The ability of small molecules to modulate nuclear receptor-dependent gene expression has made NR super family a favoured target for drug discovery. Targeting the functionally important surfaces of the receptor which disrupt transcriptional activity in the cell is emerging as a promising area towards rationale based drug design. In this context several heterocyclic ring systems such as triazenes, pyrimidines, trithianes, and cyclohexanes have recently been explored which have been reported to act as coactivator binding inhibitors (CBI) in ERα.[2] Among the heterocyclic ring systems studied; the pyrimidine-based CBIs appear to be the first small molecule inhibitors of NR coactivator binding. Among oxygen heterocycles there are several reports implicating the benzopyran scaffold as selective estrogen receptor (ER) β agonists.[3] Flavonoids such as genestien and quercetin which also share the benzopyran scaffold have been reported to possess anti cancer properties and behave in a biphasic manner at different concentrations.[4]

The above facts prompted us to evaluate the estrogenicity of styryl chromones that are a rare class of oxygen heterocycles that have been reported to possess a wide spectrum of biological activity. The natural and synthetic analogs of styryl chromones have been reported to exhibit antineoplastic, antiallergic, antihepatoprotective, and estrogenic activity.[4] Herein, we report substituted styryl chromones as a new class of compounds that exhibit selectivity for ERβ binding along with antiproliferative and cytotoxic activity on human breast cancer cell line.

Chromones can be considered as substituted flavones that behave as masked 1,3-dicarbonyl systems.[5] Although chromones are known to be Michael acceptors towards nucleophiles; the nature of the nucleophile attacking the pyrone ring determines whether attack will be at the carbonyl carbon or at the β carbon of the enone system. Also, the susceptibility of Cα=Cβ bond of styryl chromones to the attack by electron donor[6] and acceptor systems[6] posed a formidable challenge in our goal towards regioselective functionalization of C-3 and C-4 of the chromone ring. The synthesis of selected derivatives of styryl chromones was done according to Scheme 1.

E-3-Hydroxy-2-styryl chromone **1**(**a**–**c**) on reaction with malononitrile led to a mixture of products (Scheme 1).

The possible explanation could be the susceptibility Cα=Cβ bond of styryl chromones to the attack of electron donor systems; as well as the electrophilicity of the carbonyl carbon due to alpha

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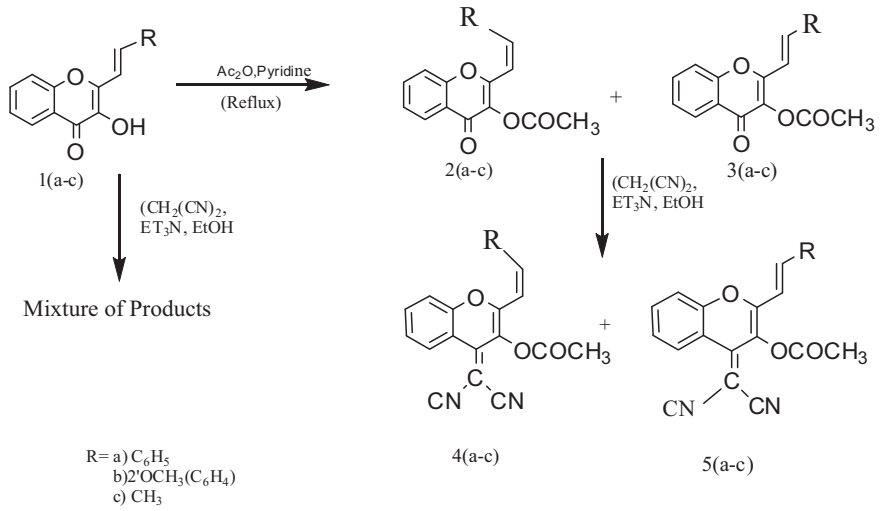
doi:10.1016/j.bmcl.2010.05.108



Scheme 1.

Table 1
Conformational energy of *E,Z* isomers of compounds

Compound	Conformation	Conformational energy of the (Kcal/mol)
Ia	*E*	20.161
1b	*E*	22.931
Ic	*E*	12.439
2a	*Z*	22.4210
2b	*Z*	23.688
2c	*Z*	17.90
3a	*E*	22.959
3b	*E*	25.820
3c	*E*	15.302
4a	*Z*	31.3308
4b	*Z*	34.322
4c	*Z*	29.741
5a	*E*	32.357
5b	*E*	36.078
5c	*E*	25.859
6a	*Z*	17.205
7a	*E*	19.850

hydroxyl group which might be leading to multiple products. Therefore, as an alternative strategy compounds **1**(**a**–**c**) were first reacted with acetic anhydride and pyridine. A racemic mixture of *E*, *Z* 3-acetoxy-2-styryl chromones was obtained in which the *Z* isomers **2**(**a**–**c**) were formed in minor amounts while *E* isomers **3**(**a**–**c**) predominated. Detailed spectroscopic and conformational energy studies (Table 1) were undertaken to assign the stereochemistry

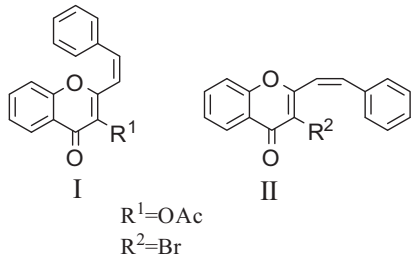
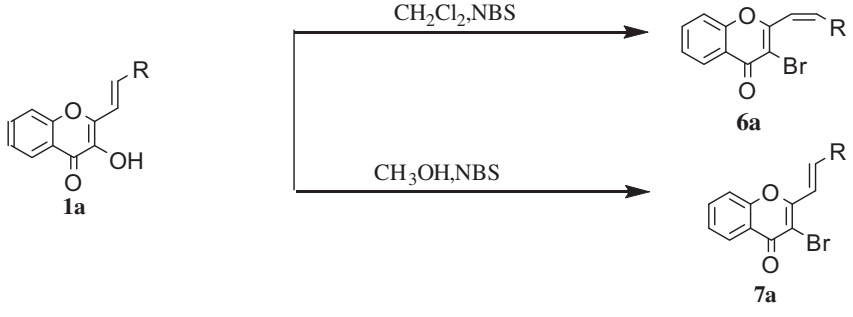


Figure 1.

of compounds **2**(**a**–**c**). Compound **2b** was chosen as the representative compound for this purpose. Compound **2b** can exist in two possible conformations **I** and **II** depicted in Figure 1.

It was observed that H-8 in compound **2b** appeared up field at δ 6.60. Previous reports where compounds of similar stereochemistry were studied suggested the presence of shielding environment in the vicinity of H-8 proton by the phenyl of styryl group that led to the up field shifting of H-8 proton.[7] A comparison of the chemical shifts obtained for H-8 in case of compound **6a** where *Z* isomer existed in conformation **II** (Fig. 1) further validated our results as in this case the H-8 proton appeared downfield at δ 7.85 (in press).

Therefore, the proposed stereochemistry of the compounds **2**(**a**–**c**) was proposed as **I**. The coupling constant value obtained for Hα and Hβ protons in compounds **2**(**a**–**c**) was 12 Hz which indicated *Z* vinylic protons. COSY experiments depicted cross peaks



Scheme 2.

Table 2
Binding energy of the complexes

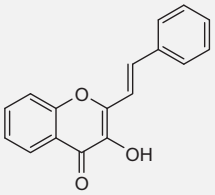
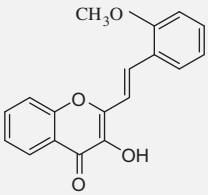
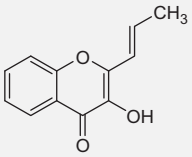
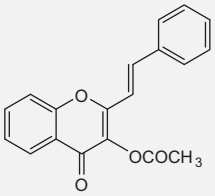
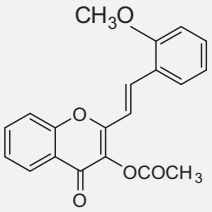
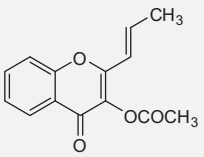
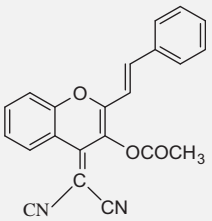
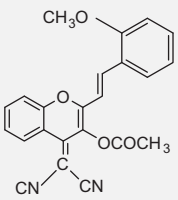
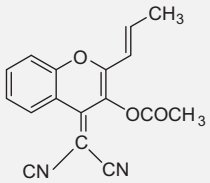
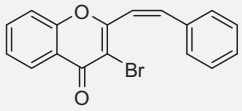
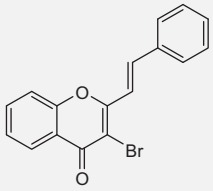
Compound	Structure	Docking score	Glide energy of the model (Kcal/mol)
Ia		−4.434	−28.239
1b		−4.431	−31.924
Ic		−4.264	−24.449
3a		−3.881	−30.842
3b		−4.124	−36.469
3c		−4.801	−30.663
5a		−3.686	−33.427
5b		−4.172	−33.956

Table 2 (*continued*)

Compound	Structure	Docking score	Glide energy of the model (Kcal/mol)
5c		−3.307	−18.610
6a		−3.768	−26.325
7a		−4.523	−39.391

between Hα and Hβ protons at δ 7.43 and 7.45 ppm; 7.26 (H-7) and 6.60 (H-8); 7.40 (Hβ) and 7.20 (H-6′); 6.89 (H-6) and 7.26 (H-7). Another interesting observation made was that in case of compounds **3a** and **3b** the Hβ proton was remarkably shielded and appeared upfield at δ 5.83 ppm probably due to the shielding effect of the phenyl ring of the styryl group. Such an effect was absent in case of compound **3c** and the Hβ proton appeared downfield at 6.93 ppm. Compounds **2** and **3**(**a**–**c**) on reaction with malononitrile stereoselectivly furnished **4** and **5**(**a**–**c**) which were again a mixture of *E*, *Z* isomers and the *E* isomer predominated as the product. The *Z* isomer was identified on the basis of the upfield shift in the position of the H-8 proton as well as COSY experiments. The significant correlations obtained in case of compound **4b** were between

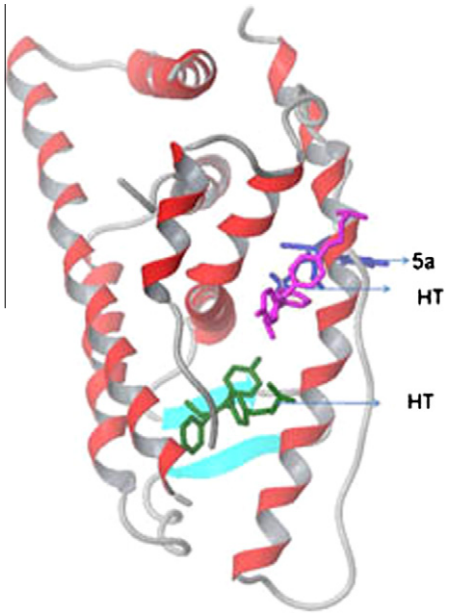


Figure 2. Three dimensional structure of ERβ docked with compound **5a**. Also shown here is HT complexed with the receptor. The compound **5a** does not bind to the cognate binding site but preferentially binds in the coactivator binding groove.

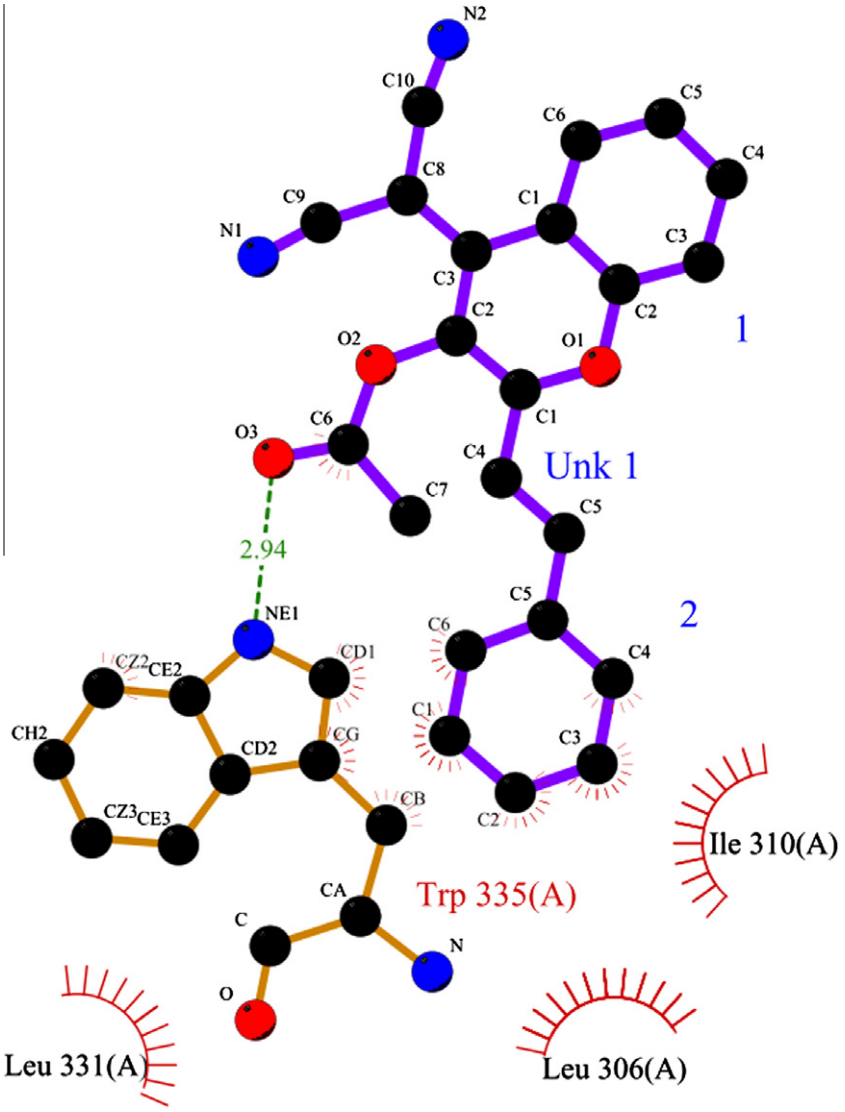


Figure 3. Interaction of compound **5a** with receptor using Ligplot.

δ 7.70 (H α) and 7.15 (Hβ); 7.15 (Hβ) and 7.38 (H-6′); 7.00 (H-7) and 6.50 (H-8′). In case of the *E* isomer **5b** a significant downfield shift in the position of H-8, H-β were observed. H-8 appeared downfield at δ 7.90. COSY experiments on compound **5a** reveal correlation peaks between 7.72 (Hα) and 7.63(Hβ); 7.90 (H-8) and 7.58 (H-7); 7.90(H-8) and 6.98 (H-6). Compounds **6a** and **7a** were prepared by reaction of **1a** with NBS (Scheme 2). The stereochemistry of compounds **6a** and **7a** were assigned on the basis spectroscopic techniques and energy calculations (in press).

Preliminary evaluation of the estrogenicity of the synthesized compounds was carried out by docking studies. The compounds were modeled using BUILD application of Maestro 8.0. The molecular modeling studies were performed on Schrodinger Maestro[8] and the binding affinities of the synthesized compounds with the receptor were compared using ligplot. The pdb entries chosen for the above studies were 2QTU[3] and 2FSZ.[1] The selection of 2QTU entry was based on structural similarity of the synthesized ligands with benzopyranones. Due to the absence of native ERβ pdb entry: 2FSZ was selected which is a complex of hydroxytamoxifen with ERβ. This pdb entry was selected as a dataset in order to compare the binding of synthesized molecules with that of hydroxytamoxifen (HT) which is a well established ER agonist /antagonist. Further, to validate our docking protocol the pdb entry 3ERT[9] which is a complex of ERα with HT, was also taken. Docking studies by defining the grid as well as blind docking of HT with ERα gave the same results as reported earlier. Docking of compounds was also done with ERα and the results not only validated our docking protocol but also established the following findings.

The docking studies showed that the compounds **1**–**5**(**a**–**c**) were binding to the second binding site of HT in the co activator groove. The docking scores and energies of the models have been shown in (Table 2). Amongst the analogs synthesized significant interactions were seen in few cases. Foremost amongst them was compound **5a**. The comparison of compound **5a** docked with ERβ shows that it has a binding site similar to HT in the coactivator groove (Fig. 2). Majority of the poses share the following common residues: Trp335, Leu331, Glu332, and Leu306 as compared with HT. Whereas Trp335 of ERβ was involved in the interaction with the phenyl ring and the ethyl group in case of HT; in our case we see interactions with the carbonyl oxygen of the acetate side chain in compound **5a**.

Also in case of compound **5a** the Van der Waals interactions were seen with Leu331, Leu306, and Ile 310 were with the phenyl ring of the styryl group as compared to Van der Waals interaction of Glu332 with phenolic ring of HT. Few other poses were also obtained but they did not have any significant interactions.

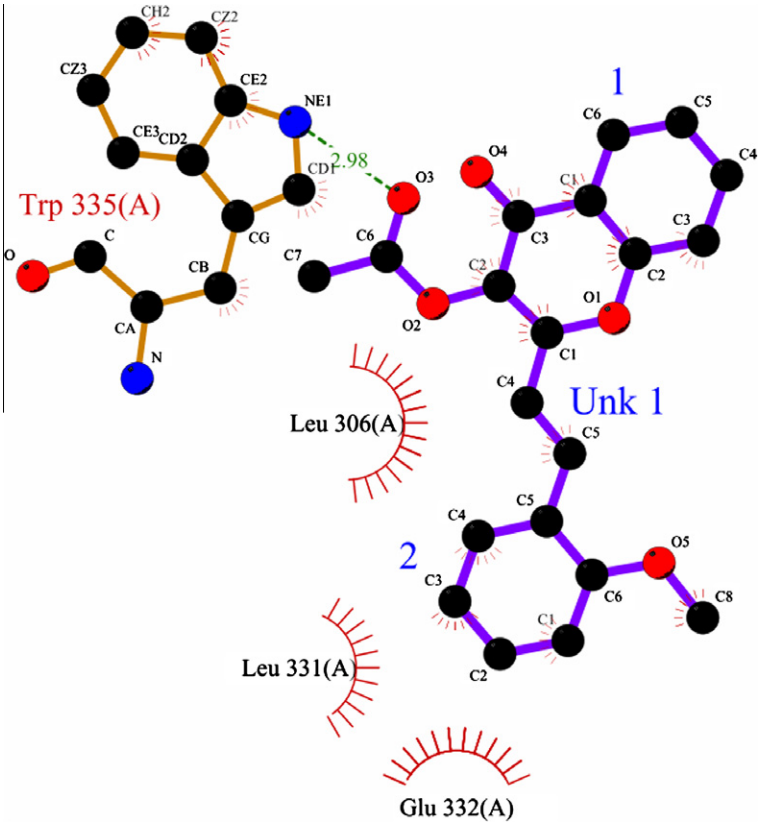


Figure 4. Interaction of compound **3b** with receptor using Ligplot.

Compound **5a** also depicted specific non-bonded interactions with the following residues; Val307, Leu306, Ile310, Leu331, Gln327, and Val328 (Fig. 3). The glide energy for the docked complex was −33.427 K cal/mol. In case of compound **5b** specific interactions were seen with the following common residues as compared to HT Val328, Ile310, and Leu331. On the other hand compound **5c** did not show any specific interaction either with the LBD or the coactivator groove. There were few conformations which showed interactions with Lys401, Tyr397, and His279. Previous studies based on structural prerequisites required for the binding in ERβ indicate that presence of phenyl group facilitated binding in the receptor which may probably be the reason why compounds **5c** and **1c** did not exhibit any significant interactions. The other analog exhibiting specific interaction in the receptor was **3a** which had specific interactions with Trp335, Leu331, Glu332, and Leu306. Few conformations were also seen to have interactions with His 279. Similarly **3b** had specific interactions with Trp335, Leu331, and Glu332 (Fig. 4). Compound **3c** had specific interaction with Trp335.

Compound **1a** had specific interactions with Trp335 and compound **1b** showed specific interaction with Trp335, Ile310, and Leu306. However, Compound **1c** did not show any specific interaction with the residues although it occupies the second coactivator groove. In case of compound **6a** the only interactions seen were with Tyr397. Compound **7a** showed interactions with Trp345. This

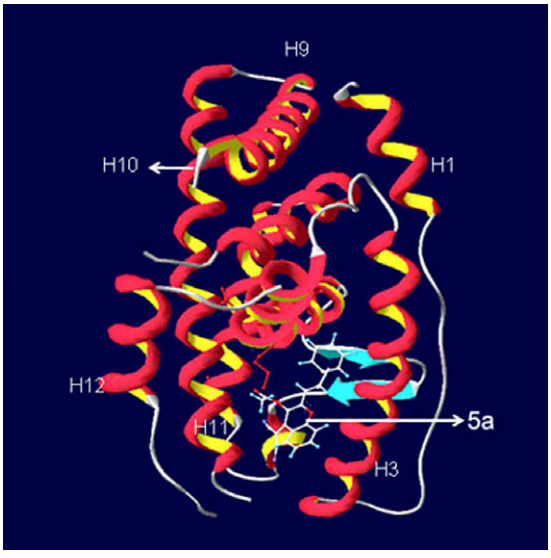


Figure 5. Three dimensional structure of ERβ docked with compound **5a** using Maestro. Beta sheet is shown in blue color and the compound is shown as ball and stick model.

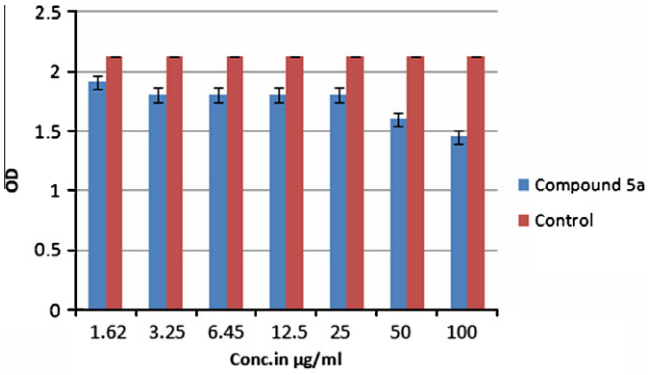


Figure 6. Comparative study between control and compound **5a** at different concentrations using MTT assay.

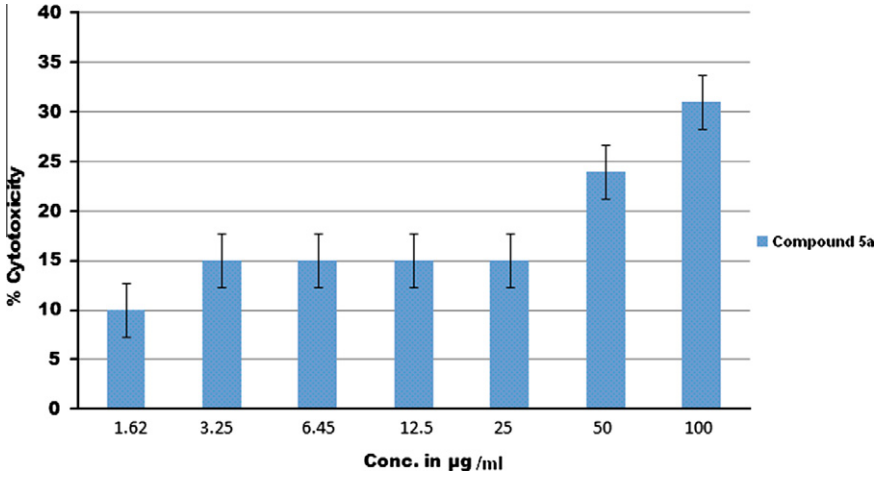


Figure 7. %Cytotoxity of compound **5a** at different concentrations.

could be probably because of a bulky substituent at C-3 due to which compounds **6a** and **7a** did not exhibit binding in the coactivator groove. Based on the above results it was hypothesized that phenyl ring at position C-2 was essential for binding to the active site. Substitution/removal of the phenyl ring by alkyl group in the side chain resulted in weaker binding affinity and no significant interactions with the receptor were observed. Although the interaction energy of compound **7a** was lowest (−39.39 Kcal/mol) but it neither exhibited binding to the second binding site of HT nor the ligand binding site. Substitution of hydroxy group at C-3 in compounds **1**(**a**–**c**) by a bulky substituent like a bromo group instead of an acetate group hindered the ligand from binding to the second binding site. Interaction energies and interactions between the ligand and the receptor residues clearly indicated that none of the compounds studied had affinity for the ligand binding domain of ERβ (Fig. 5), however, the above data suggests binding affinity to the second binding site of HT.

Recent literature reports indicate that the interaction of HT at second binding site is of considerably low affinity and ligand binding at this site is responsible for antagonist activity.[10] Accordingly, if styryl chromones exhibited binding at this site they may be expected to exhibit antagonistic activity. Cytotoxicity studies were therefore carried out using MTT assay protocol on candidate compound **5a**. The MTT assay revealed that compound **5a** was cytotoxic and antiproliferative on MCF-7 cell line (Fig. 6) in a dose dependent manner (Fig. 7), which was in accordance with antagonistic role of second binding site of HT.

Further studies will however be required to evaluate the relationship between structure, docking score and biological potency of molecules.

Acknowledgements

The author S.B. gratefully acknowledges the financial assistance provided by the Department of Science and Technology for the study. The authors thank Research Associate S.K., and students M.K.D., A.S., and A.G. who have equally contributed in the synthesis and purification of compounds. The invaluable contribution of Dr. V. Pooja and Ms. Sonali Kumari for the cytotoxicity studies is gratefully acknowledged. The assistance provided by Indian Institute of Technology, New Delhi; Central Drug Research Institute, Luck now for analytical analysis of the samples are gratefully acknowledged. All authors gratefully acknowledge infrastructure facilities provided by Amity Institute of Biotechnology, Amity University, Noida.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.05.108.

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