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Synthetic Communications

An International Journal for Rapid Communication of Synthetic Organic Chemistry

ISSN: 0039-7911 (Print) 1532-2432 (Online) Journal homepage: http://www.tandfonline.com/loi/lsyc20

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To cite this article: Sumit Kumar, Nishant Verma, Nikhil Kumar, Alok Patel, Partha Roy, Vikas Pruthi & Naseem Ahmed (2016): Design, Synthesis, Molecular Docking and Biological Studies of Novel Phytoestrogen-Tanaproget Hybrids, Synthetic Communications, DOI: 10.1080/00397911.2016.1144768

To link to this article: http://dx.doi.org/10.1080/00397911.2016.1144768

Design, synthesis, molecular docking and biological studies of novel phytoestrogen-tanaproget hybrids

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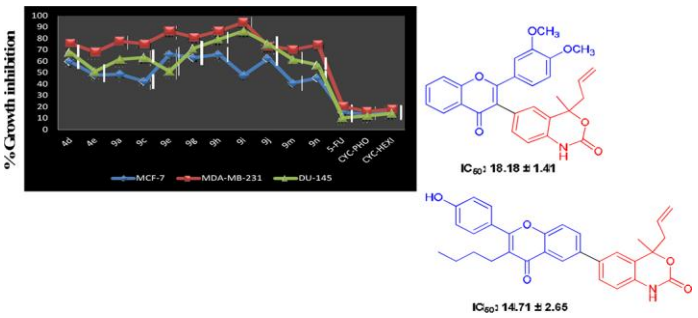
Supplemental data for this article can be accessed on the publisher's website.

Abstract

A diverse range of novel and highly functionalized flavonoids based tanaproget hybrids were synthesized and evaluated *in vitro* for their antimicrobial and anti-proliferative activities. Novel products were synthesized in good yields (81-95%) under Pd-catalysed reaction from bromo flavones and tanaproget boronic acids within 18-20 min at 60 ^{0}C. Bioassay results exhibited excellent activities against both hormone-dependent and hormone-independent human breast cancer cells (MCF-7, MDA-MB-231, DU-145, PC-3 and HeLa). Among them, compounds **4e**, **9a**, **9c**, **9e**, **9g**, **9h**, **9m** and **9n** displayed excellent activity. Compounds **4d**, **4o** and **9o** were found equally potent against *C. albicans* compared to fluconazole. Compound **5c** showed better antibacterial activity against *S. aureus*. Compounds **5a**, **9i**, **9o** and **10c** have shown admirable antibacterial activity against *E.coli*.



KEYWORDS: Phytoestrogen-Tanaproget hybrids, Estrogen and progesterone receptors, Anti-cancer activity, antimicrobial activity, Docking study

INTRODUCTION

Cancer is a generic term for a large group of diseases that can affect all parts of the body. There are 32.6 million people living with cancers in 2012 worldwide and 14.1 million new cases were reported every year in which 8.2 million led to cancer death. Therefore, the search for anticancer agents that display broader spectrum of cytotoxicity to the tumor cells is of great interest [1, 2]. Natural products obtained from plants, marines and microbes are the major resources for the bioactive agents and play a vital role in the discovery of lead molecules for new drugs. Flavonoids are the polyphenolic phytochemicals that exist ubiquitously in the plant kingdom, which can be found in several food sources such as fruits, vegetables, whole grains, and legumes [3, 4]. Earlier studies have explored flavone and flavanone moeities as an extensive variety of biological activities such as antioxidant [5], anti-inflammatory [6], antibacterial [7], antifungal [8], anticancer activities [9], antiestrogenic [10], interaction with estrogen receptors [11] and inhibitory activities against aromatase enzyme [12]. Due to their broad spectrum of pharmacological activities, the flavonoids have been extensively studied as

therapeutic agents, mainly of relevance to our study as anticancer and antimicrobial agents. In particular, due to their structural and functional resemblances to endogenous estrogens, flavonoids have attracted noteworthy interest as alternative estrogens, termed phytoestrogens and broadly studied for their potential role in many estrogen-dependent diseases including breast cancer [13].

Tanaproget drug is used as non-steroidal progesterone receptor agonist with high affinity and selectivity for the progesterone receptor [14] (Fig. 1). Generally due to the four-ring structural motif found in natural steroid receptor ligands, steroidal progestins have higher cross-reactivity with other steroid hormone receptors. Currently, available progestins have side effects due to their lack of selectivity with other steroid hormone receptors. Recently, the novel classes of non-steroidal progesterone receptor (PR) modulators with improved receptor selectivity have been reported [15-18]. These new compounds exhibit the potential for safer and wider pharmacological use as progestins.

Molecular hybridization is an effective tool to design and synthesize highly active novel molecules by covalently bonding two or more pharmacophores into a single entity [19-20] (Fig. 2). Moreover, the hybrid molecules may also minimize the undesirable side effects and express synergic action [21]. These findings had encouraged us to investigate the potential synergistic effect of phytoestrogen and tanaproget analogue scaffolds on esterogen and progesterone receptors with respect to their parent core structures.

Herein, we report a series of hybrid compounds of these two pharmacophores with an intention to synergize the anti-cancer activity against human cancer cell lines (MCF-7, MDA-MB-231, DU-145, PC-3 and Hela). Our designed compounds have three parts, namely flavonoid structure as a chief backbone, tanaproget-like moiety for enhancing desired pharmacophore behaviour with drug like properties and non-polar butyl or pentyl chain for lipophilicity control. The excellent cytotoxicity of various compounds for harmone- dependent and harmone-independent breast cells pointed them as promising lead molecules for anticancer drug design. Furthermore, molecular modelling of these novel molecules was done to provide a comprehensive guide for further understanding of their synergistic effects on estrogen and progesterone receptors.

2. RESULTS AND DISCUSSION

2.1. Chemistry

The general route for the synthesis of the target phytoestrogen-tanaproget hybrids (**4a-r**, **5a-c**, **6a-c**, **9a-o**, **10a-c**) is depicted in Scheme 1 and 2. Briefly, the key starting bromo flavonoids (**1a-c, 2a-c, 5, 6, 7a, 7b, 8a-c, 10**) and boronic acids (**3a-c**) were prepared by the literature procedures [22, 23]. The Scheme 1 and 2 illustrates the routes for the synthesis of the target hybrid compounds using bromo flavonoids, boronic acids of tanaproget analogue, Pd-complex and K_2CO_3 in ethanol at 60 ^{0}C under microwave irradiation. Generally, the products were obtained in high yields (81-95%) in 18-20 min. Compounds **4a-r**, **5a-c** and **6a-c** were prepared from 3-bromo flavone derivatives (**1a-c, 2a-c, 5** and **6)** and the appropriate boronic acids of tanaproget analogues (**3a-3c**) using

Suzuki cross-coupling reaction which gave the products **4a-r**, **5a-c** and **6a-c** in good to excellent yields (Scheme 1).

Compounds **9a-o** and **10a-c** were synthesized from 3-butyl, 6-bromo flavone or flavanone derivatives (**7a, 7b**, **8a-c** and **10**) and the appropriate boronic acids of tanaproget analogs (**3a-c**) using Suzuki reaction which furnished the products **9a-o** and **10a-c** in 74-87% yields (Scheme 2).

The assigned structure of new products (**4a-r**, **5a-c**, **6a-6c**, **9a-o**, **10a-c)** were established from their spectroscopic data (IR, ^{1}H, ^{13}C NMR, HRMS). For example, compound **4e** was obtained as a brown solid. IR spectrum of **4e** showed absorptions at 3265, 1713, 1652, 1621 and 1555 cm^{-1} for –NH, >C=O, >C=C< (tanaproget analogue moeity), >C=O and >C=C< (flavone moeity). HR-MS of **4e** supported a molecular composition of $C_{29}H_{25}NNaO_6$ $[M+Na]^+$, representing 18 degrees of unsaturation. In the ^{1}H-NMR spectra of **4e**, peak at δ_H 9.40 (s, D_2O exchangeable, 1H) confirms the presence of -NH group, methyl group occurred at δ_H 1.68 (s, 3H), two methylene protons ($-CH_2$) resonating at δ_H 2.67-2.63 (m, 2H), two $-OCH_3$ groups resonates at 3.93, three olefinic protons appears at δ_H (m, 1H) and 5.14-5.09 (m, 2H). In ^{13}C-NMR spectrum, δc 173.5 and 162.0 confirms the presence of carbonyl group of flavone and tanaproget moiety respectively. Peaks at δc 84.6, 56.3, 56.2, 45.5 and 26.3 also confirm the presence of quaternary carbon, two $-OCH_3$, methylene (CH_2) and $-CH_3$ groups.

2.2. Biological Evaluations

2.2.1. *In Vitro* Anticancer Activity

All compounds were screened for *in vitro* antiproliferative activities against the estrogen-responsive breast cancer cell line (MCF-7), estrogen-independent breast cancer cell line (MDA-MB-231), prostate cancer cell line (DU-145, PC-3) and cervix (HeLa) where 5-FU, cyclophosphamide and cycloheximide were taken as positive control using MTT assay. The results are reported in terms of % cell growth inhibition and IC_{50} values (Table 1 and 2). For a preliminary SAR evaluation, the initial series of compounds (**4a-r**, **5a-c**, **6a-c**) was first evaluated against the above mentioned cell lines to investigate the effects of different number of methoxy groups at different positions on the B-ring of oxa- and aza-flavones core and vinyl, allyl or pentyl substitution on tanaproget scaffold. Among this series, compound **4d**, **4e** and **6b** with the methoxy groups at the C-3 and 4 positions on ring-B and furan and allyl substitution showed the highest antiproliferative potency against MCF-7, MDA-MB-231 and DU-145 cell lines. Among the tested cell lines, a differential behaviour was detected when the cytotoxic effect of the active drugs was comparatively studied in all cell lines; a higher cell growth inhibition was found in breast cells (Table 1). Thus, whereas MCF-7, MDA-MB-231 and DU-145 cell proliferation was inhibited $\geq 50\%$ by compounds **4d**, **4e**, **9a**, **9c**, **9e**, **9g**, **9h**, **9i**, **9j**, **9m** and **9n**, only compounds **5b, 6a** and **6c** resulted to be cytotoxic in PC-3 cells. The synthesized compounds were found to be weakly antiproliferative (inhibition $\leq 50\%$) for HeLa cells except **6c** and **9k**. Compounds **4h, 4q**, **4r** against DU-145 cells and **4f**, **4j**, **4l**, **4m**, **5c**, **6a**, **9a**, **9n**, **10c** against HeLa cells failed to exhibit significant cytotoxicity.

In case of PC-3 cells, most of the compounds were not showing any considerable cytotoxicity except compounds **5b**, **6a**, and **6c**. Compound **9i** exhibited excellent cytotoxicity upto 94.29% with IC_{50} 19.51 ± 1.49 µM against MDA-MB-231 cells as compared to the reference drugs. Compound **9h** showed maximum cytotoxicity against MDA-MB-231 and DU-145 cells (IC_{50} 13.49 ± 2.72 and 11.09 ± 1.32 µM) respectively. In the structure –activity relationship (SAR), it was found that the introduction of hydroxyl, methoxy or amino group in the aza-flavone core with the vinyl or allyl substituent in tanaproget motif greatly enhanced their anticancer activities might be due to some carbon type and π-alkyl type interaction of electron donating groups with the amino acids which is supported by docking study also. Compounds (**4d, 4e, 9a, 9c, 9e, 9g, 9h, 9i, 9j, 9m** and **9n**) that inhibited ≥ 50% cell proliferation in at least two different cell lines are shown in Fig. 3.

The anti-proliferative activities expressed as IC_{50} values are summarized in Table 2. This table includes IC_{50} values of those compounds that at a 10 µM concentration caused a reduction ≥ 50% in the growth of breast tumor cell lines. However, IC_{50} values corresponding to compounds that inhibited tumour cell proliferation less than 50% at 10 µM were not evaluated (Fig. 4).

We also compared the antitumor activity of two related prostate cell lines, DU-145 and PC-3 cells. A comparative analysis between these two cells showed that both cell lines behaved differently. On the other hand, HeLa cells were less responsive to the antiproliferative action of almost all the synthesized compounds except **4k**, **4q**, **4r**, **5a**, **6c**,

9c, **9f**, **9k**, **9l**, **9o** and **10a**. Hence, the inspection of these results indicates the idea that cancerous tumours generally contain cell sub-populations with different biological properties.

2.2.2. Antimicrobial Activity

Synthesized compounds (**4a-r**, **5a-c**, **6a-c**, **9a-o**, **10a-c**) were screened for their antimicrobial activity. Yeast (*Candida albicans*), Gram +ve bacteria (*S. aureus*) and Gram -ve bacteria (*E. coli*) were selected to evaluate the effectiveness of the test compounds (details in Experimental section).

2.2.2.1. Antibacterial Activity

All the hybrid compounds were preliminary screened for the antibacterial assay and some of them were subjected to MIC (minimum inhibitory concentration) determination by microdilution method[24] and the results are given in Table 3. Reference drugs Cefadroxil and Ampicillin were chosen as positive controls in antibacterial activity assay.

As seen in the Table 3, most of the compounds have shown lower activity than positive control (Cefadroxil) while compounds **5c** (MIC: 31.25 µg/ml) have shown excellent antibacterial activity against *S. aureus*. Moreover, compounds **4r, 9e**, **9o** (MIC: 62.5 µg/ml) demonstrated equal potency against *S. aureus* than Cefadroxil. Compounds **4i, 4p, 5a, 6c, 9h, 9i** and **9n** were found to be the least active (MIC: 250µg/ml) against *S. aureus* compared to the reference drug. Compounds **5a**, **9i, 9o** and **10c** exhibited comparable antibacterial activity (MIC ≤ 62.5 µg/ml) than positive control against *E. coli*.

Compounds **4p, 4r, 5c, 6c, 9n** were found to be the least potent (MIC: 250 µg/ml) against *E. coli*. as compared to positive control Ampicillin (MIC: 62.5 µg/ml).

2.2.2.2. Antifungal Activity.

Some previously screened compounds for the antifungal activity were subjected to MIC determination by microdilution method [24] and the results are given in Table 3. Reference drug Fluconazole was selected as positive control in antifungal activity assay.

Compounds **4d**, **4o** and **9o** have shown equal potency (MIC: 31.25 µg/ml) as Fluconazole against *C. albicans.* Compounds **4c**, **4p**, **9b** and **9h** exhibited moderate antifungal activity (MIC: 62.5 µg/ml) while others displayed lower activity (MIC: 125-625 µg/ml).

2.3. Molecular Docking Study

In order to examine the synergistic effect of the hybrid molecules on binding with estrogen and progesterone receptors, an extensive docking study was done. Also, the majority of the flavonoid-tanaproget hybrid compounds with significant antiproliferative activities had shown distinct selectivity for the MCF-7 ER+ve breast cancer cell line that expresses high levels of ER receptor. Therefore, to investigate whether the antiproliferative activity of these synthesized derivatives (against the MCF-7 cell line) is estrogen receptor-mediated, the binding interactions of the products with the ER receptor were studied using molecular modelling (see supporting information). Genetic algorithm was implemented using AutoDock4.2 and all the final results were visually inspected using Discovery studio visualizer.

After the validation of the docking procedure (Fig. 5), the docking simulation suggested that the binding affinity of **9a-i** and **10a-c** were higher than that of **4a-r**, **5a-c** and **6a-c**.

Out of all the synthesized compounds **5b** (PDB ID: 1ZUC) and **9h** (PDB ID: 3EQM) were found to be the most potent hit with AutoDock binding energy of -10.77 and -12.09 kcal/mol respectively which was significantly greater than the references used in the present study (Table 4).

Visual analysis of docking pose indicated that compound **5b** was fully buried inside the active site of progesterone receptor (Fig. 6). The aromatic ring of tanaproget, flavone and furan ring seemed to interact completely with hydrophobic residues likes Met759, Leu763, Gly722, Leu718, Leu797 and Cys891 by π -alkyl interaction. The carbonyl and *sec*-amine group in tanaproget moiety of compound **9h** was found to be hydrogen bonded with residue Arg145 and Arg435 of 3EQM. π -alkyl interaction between aromatic ring of tanaproget and flavone moieties and residues likes Cys437, Phe430, Val369 and Met364 were also inspected and studied. π-sulphur interaction between the aromatic ring of flavone and Met364 was also apparent by visualizers.

The present *in-silico* work clearly indicated that all the tested molecules displayed higher affinity towards estrogen receptor (3EQM) than progesterone receptor (1ZUC). These docking results are also sync with the outcome of *in vitro* antiproliferative activity against a panel of five human cancer cell lines.

3. CONCLUSION

We have reported a novel series of phytoestrogen-tanaproget hybrids which exhibited excellent *in vitro* cytotoxicity against human cancer cell lines (MCF-7, MDA-MB-231, DU-145, PC-3 and HeLa). Among them, compounds **4d, 4e, 9a, 9c, 9e, 9g, 9h, 9i, 9j, 9m** and **9n** were found to exhibit excellent antiproliferative activities against the MCF-7, MDA-MB-231 and DU-145 cells. Compounds **4d**, **4o** and **9o** with MIC: 31.25µg/ml were found equally potent against *C. albicans* compared to fluconazole. Compound **5c** with MIC 31.25 µg/ml showed better antibacterial activity compared to reference drug against *S. aureus* (MIC: 62.5 µg/ml). Compounds **5a**, **9i, 9o** and **10c** with MIC: 62.5µg/ml were equally effective as standard drug (ampicillin) against *E.coli*. The molecular modelling clearly indicates that all the hybrid compounds have higher affinity towards ER (3EQM) than PR receptors (1ZUC). Compound **5b** had improved binding affinity (Ki) at PR of 12.86 nM with docking score -10.77 kcal/mol compared to tanaproget. As a non-steroidal PR antagonists, phytoestrogen-tanaproget hybrids (**5a**, **5b**, **6a**, **6b**) were found more potent as compared to tanaproget.

4. EXPERIMENTAL PROTOCOLS

4.1. Chemistry

All the required chemicals were purchased from Merck and Aldrich Chemical Company. Pre-coated aluminium sheets (silica gel 60 F254, Merck) were used for thin-layer chromatography (TLC) and spots were visualized under UV light. IR spectra were recorded with KBr on Thermo Nicolet FT-IR spectrophotometer. ^{1}H NMR and ^{13}C NMR

spectra were recorded respectively on Bruker Spectrospin DPX 500 and Jeol Resonance ECX 400II spectrometer using $CDCl_3$ or DMSO as solvent and trimethylsilane (TMS) as an internal standard. Spectra were processed using Bruker Topspin® 3.0.b.8. Melting points were performed with Ambassador® and Digital Melting point apparatus (Nutronics), Popular India. Splitting patterns are designated as follows; s, singlet; d, doublet; m, multiplet. Chemical shift (δ) values are given in ppm.

4.2. Microwave Irradiation Experiment

All microwave experiments were carried out in a dedicated Anton Paar Monowave-300 reactor, operating at a frequency of 2.455 GHz with continuous irradiation power of 0 to 850 W. The reactions were performed in a G-10 Borosilicate glass vial sealed with Teflon septum and placed in a microwave cavity. Before microwave heating, oxygen was purged into the reaction vial. Initially, microwave of required power was used and temperature was being ramped from room temperature to a desired temperature. Once this temperature was attained, the process vial was held at this temperature for required time. The reactions were continuously stirred. Temperature was measured by an IR sensor. After the experiments a cooling jet cooled the reaction vessel to ambient temperature.

4.3. General Procedure For Synthesis Of Compounds (4a-R, 5a-C, 6a-C, 9a-O, 10a-C)

To a G-4 process vial capped with Teflon septum was added 3-bromo flavone (0.5 mmol), boronic acid (0.6 mmol), Pd-complex C (0.2 mol%, 1.0 mg), TBAB (0.12 mmol),

K_2CO_3 (1.2 mmol) and 6 ml of ethanol. After a pre-stirring for one minute, the vial was subjected to microwave irradiation time of 18-20 minute at 60 °C. It was then cooled to room temperature, diluted with water, and extracted with EtOAc for three times. The organic phase was dried with Na_2SO_4 and concentrated to yield the product. The crude material was chromatographed on a silica gel column eluting with a mixture of hexane and ethyl acetate (8:2) to afford the product in (81-95%) yield. The purified products were identified by FTIR, NMR and HRMS spectra.

6-(2-(4-Methoxyphenyl)-4-Oxo-4H-Chromen-3-Yl)-4-Methyl-4-Vinyl-1H-Benzo[D][1,3] Oxazin-2(4H)-One (4a)

Brown solid, mp 160-168 ^oC, Yield 95%.^1H NMR (500 MHz, $CDCl_3$, ppm): δ 9.08 (s, 1H, D_2O exchangeable), 8.27 (d, *J* = 8Hz, 1H), 7.80 (d, *J* = 9Hz, 1H), 7.70 (t, *J* = 8.5 Hz, 1H), 7.48 (d, *J* = 8 Hz, 1H), 7.44 (t, *J* = 7.5 Hz, 1H), 7.37 (dd, *J* = 1.5, 8.5 Hz, 1H), 7.13 (d, *J* = 8Hz, 1H), 7.08-7.02 (m, 3H), 6.87 (d, *J* = 8Hz, 1H), 6.06-5.97 (m, 1H), 5.26-5.06 (m, 2H), 3.90 (s, 3H), 1.82 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$, ppm): δ174.8, 164.5, 161.8, 155.9, 153.0, 139.4, 134.2, 132.3, 131.5, 129.4, 127.5, 127.3, 126.8, 125.9, 124.5, 123.6, 120.0, 117.7, 115.7, 114.8, 113.8, 113.7, 87.7, 55.6, 25.7.FTIR (KBr, ν = cm^{-1}): 3256, 2955, 1709, 1655, 1615, 1587, 1505, 1463, 1345, 1333, 1306. HRMS (ESI+): m/z calcd. for $C_{27}H_{22}NO_5$ $[M+H]^+$: 440.1492 found : 440.1495.

4.4. Biology

4.4.1. *In Vitro* Antitumor Activity Assays

4.4.1.1. Cell Culture

Human breast (MCF-7, MDA-MB-231), prostate (PC-3, DU-145) and cervical (HeLa) cancer cell lines were all obtained from National Center for Cell Science (NCCS), Pune, India. All cell culture reagents were from GIBCO (Invitrogen, USA). Penicillin, streptomycin, MTT (3-(4,5-dimethyl-2-thiazolyl)2,5diphenyl-2H-tetrazoliumbromide), cell culture grade dimethyl sulphoxide (DMSO), 5-fluorouracil (5-FU), cyclophosphamide and actidione (cycloheximide) were from HiMedia (Mumbai, India). All the cells were maintained in Dulbecco's modified Eagle's media (DMEM) supplemented with 10% fetal bovine serum (heat inactivated) and 1% antibiotic (100 U/ml of penicillin and 100 µg/ml streptomycin) (Himedia, Mumbai, India) mix at 37 ºC in humidified atmosphere in a CO_2 incubator.

4.4.1.2. MTT Assay

MTT assay was carried out as described previously [25]. In brief, 5×10^3 cells in 200 µl of medium were seeded in 96-well plates (Griener, Germany). After 24 h, the cells were treated with various concentrations (0.1, 1, 10, 100 and 1000 µM) of test compound dissolved in DMSO. The final DMSO concentration for all dilutions was 0.1% which was used as vehicle control. The cultures were assayed after 24 h by the addition of 20 µl of 5 mg/ml MTT and incubating for 4 h at 37 ^{o}C. The MTT-containing medium was then aspirated and 200 µl of DMSO (Himedia, Mumbai, India) was added to lyse the cells and solubilize the water insoluble formazone. The absorbances of the lysates were determined on a Fluostar optima (BMG Labtech, Germany) microplate reader at 570 nm.

The percentage inhibition was calculated as

= 100 - [(Mean OD of treated cell X 100)/mean OD of vehicle treated cells (negative control)]

The dose response curve and IC_{50} values were obtained by nonlinear regression analysis [non-linear regression (sigmoidal dose response with variable slope)] using Graph Pad Prism, version 5.02 software (Graph Pad Software Inc., CA, USA).

4.4.2. *In Vitro* Antimicrobial Assay/Studies

4.4.2.1. Test Strain

The clinical isolates of C. *albicans* were obtained from the Department of Microbiology, IITR, India. The isolate was sub-cultured on Sabouraud Dextrose Agar at 37 ^{o}C for 48-72h. Both Gram (+) bacteria (*S. aureus*) and Gram (-)bacteria (*E. coli*) were also obtained from the Department of Microbiology, IITR, India, and used to evaluate the antibacterial effectiveness of the test compounds.

4.4.2.2. Microdilution Assay

Minimum inhibitory concentration (MIC) is the lowest concentration of an antimicrobial compound that inhibits the visible growth of a microorganism. MIC values of the compounds against bacterial and fungal isolates were determined on the basis of micro-well dilution method following NCCLS recommendations [26]. In this method we made stock of chemically synthesized compounds at a concentration of 10 mg/ml in DMSO, which was converted to working solution of concentration 1 mg/ml solution in methanol. Using a multichannel micropipette, 100 µl of media into all wells of pre-sterilized

microtitre plate was dispensed (experiment was done in triplicate). Two fold serial dilutions were carried out from the well 1 to the well 10 and excess media (100 µl) was discarded from the last well (No. 10). Liquid culture of test organisms were grown to a suitable phase in corresponding medium (YPD; yeast extract peptone dextrose, for fungal growth and LB; Luria Bertani Broth, for bacterial growth) for 12-18 h at 37 ^{o}C. Then optical density (OD) of liquid culture was determined at 600 nm and diluted in such a way that each well received 10^4 cfu/100 µl of fungal suspension and 10^7 cfu/100 µl of bacterial culture. Appropriate positive and negative control was also included in the study. Positive control contained only microbial cells whereas negative control contained only standard drug solution (Fluconazole for fungus and Ampicillin, Cifadroxil for bacteria). All experimental procedures were performed under sterile condition using bio-safety hood (ESCO, USA). The plates were incubated at 30 ^{o}C (fungal culture) and 37 ^{o}C (bacterial culture) for 12-16 h of growth phase.

4.5. Docking Studies

AutoDock4.2 was used for docking simulation [27]. In AutoDock, we employed Lamarckian genetic algorithm (LGA), which is the hybrid of genetic algorithm (GA) and local search algorithm (LSA), for conformation searching. The RMSD between the docked conformation, as generated by the docking algorithm and the native co-crystallised ligand conformation which is well within the grid spacing used in the docking procedure, showing that the docking method to be used was trustworthy and valid. For protein preparation, the crystal structure of molecular and pharmacological properties of a potent and selective novel non-steroidal Progesterone receptor agonist

tanaproget, at resolution 1.80 was retrieved from protein data bank (PDB code: 1ZUC) and Structural basis for androgen specificity and oestrogen synthesis in human aromatase (PDB code: 3EQM). The inhibitor, progesterone ligand and all water molecules were removed, leaving only the residues of the receptor. Preparation of the target protein with ADT involved the addition of polar hydrogens to the macromolecule, an essential step to correct the calculation of partial charge. Finally, Gasteiger charges were calculated for each atom of the macromolecule. The docking area was assigned visually around the apparent active site. A grid of 80 Å × 80 Å × 80 Å with grid spacing of 0.375 Å was positioned around the active site with all the ligand atom types using AutoGrid. All parameters were set to defaults except ga_run (100). The docking results were clustered on the basis of root mean square deviation (rmsd) and were ranked on the basis of free energy of binding. All compounds were drawn and then converted to 3D by ChemDraw(Cam-bridgesoft Inc.). Each structure was then energy minimized by AM1 force field. Discovery Studio Visualizer (DSV) was used for 3D visualization [28].

SUPPLEMENTAL MATERIAL

Characterization data ^{1}H, and ^{13}C NMR spectra associated with this article are available on the publisher's website.

ACKNOWLEDGEMENTS

The authors wish to express their gratitude to the BRNS, India for financial support, DST, New Delhi for providing HRMS facility and CSIR, New Delhi for awarding SRF to S.K.

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Table 1. *In vitro* anti-proliferative activity (% growth inhibition) at a concentration of 10 μM[a]

S. No.	Compounds	MCF-7	MDA-MB-231	DU-145	PC-3	HeLa
1	**4a**	-	19.66	15.62	6.32	21.45
2	**4b**	17.28	28.48	31.23	5.21	9
3	**4c**	20.22	22.55	25.41	14.53	22.36
4	**4d**	**59.66**	**75.95**	**67.86**	35.66	27.64
5	**4e**	**47.89**	**68.05**	**51.12**	20.01	13.25
6	**4f**	28.16	6.91	25.48	30.02	4.82
7	**4g**	12.72	21.41	32.52	14.57	15.27
8	**4h**	-	-	9.63	18.34	26.49
9	**4i**	15.66	-	16.33	36.25	19.84
10	**4j**	16.83	37.79	32.10	41	0.82
11	**4k**	18.11	34.41	42.35	29.65	39.45
12	**4l**	-	28.59	25.34	18.09	7
13	**4m**	14	31.45	24	24.05	0.67
14	**4n**	18.5	24.88	30.21	15	33.57
15	**4o**	14.39	41.16	19.25	10.20	30.64
16	**4p**	-	16.47	23.94	8	25.73
17	**4q**	-	38.86	7.62	3.53	41.01
18	**4r**	-	-	4.28	5.34	36.26
19	**5a**	25.44	35.97	45.28	19	37.27
20	**5b**	9.94	48	35.37	**56.08**	11.24

21	**5c**	19.22	16.68	22.57	26.54	5.69
22	**6a**	27.27	42.36	44.38	**51.23**	4.61
23	**6b**	37.11	**70.13**	**55.32**	32.59	14.60
24	**6c**	22.55	31.95	42.78	**44.64**	55.04
25	**9a**	**48.72**	**77.73**	**61.62**	34.58	7.09
26	**9b**	24.22	40.5	45.32	15	34.49
27	**9c**	**42.22**	**75.22**	**63.35**	11.54	36.26
28	**9d**	19.11	20.54	28.16	33.5	29.52
29	**9e**	**66.16**	**86.43**	**51.24**	23.47	18.5
30	**9f**	-	19.61	21.54	41	35.76
31	**9g**	**63.05**	**81.06**	**71.52**	39.06	16
32	**9h**	**66.16**	**86.43**	**79.29**	33.85	12.38
33	**9i**	**47.77**	**94.29**	**86.75**	31.28	26.58
34	**9j**	**62.77**	**73.06**	**75.19**	36.87	23.47
35	**9k**	14.55	35.06	29.38	0.95	**54.72**
36	**9l**	19.05	43.88	39.65	20.28	42.85
37	**9m**	**40.94**	**70.31**	**61.45**	24.67	15.27
38	**9n**	**45.5**	**74.43**	**56.87**	17.61	2.54
39	**9o**	17.38	27.86	19.64	9.27	47
40	**10a**	16.33	33.59	26.34	13.27	38.28
41	**10b**	19.05	36.52	38.40	0.89	23
42	**10c**	25.5	15.95	37.51	38.41	5.68
43	5-FU	15.54	20.22	10.32	30.43	13.00

44	CYC-PHO	13.17	15.84	12.10	25.02	9.19
45	CYC-HEXI	15.22	17.96	14.30	33.25	11.11

Bold values represent compounds showing good antiproliferative activity *5-FU* 5-fluorouracil, *CYC-PHO* cyclophosphamide, *CYC-HEXI* cycloheximide, [a]Compounds tested in triplicate, data expressed as mean value of three independent experiments.

Table 2. IC_{50} values[a,b] of *in vitro* antiproliferative activity of active compounds.

S. No.	Compound	MCF-7	MDA-MB-231	DU-145	PC-3	HeLa
				IC_{50} in µM		
1	**4d**	27.35 ± 1.91	23.30 ± 2.83	45.00 ± 1.82	59.51 ± 1.11	75.09 ± 1.25
2	**4e**	**18.18 ± 1.41**	**20.96 ± 1.14**	39.03 ±1.94	46.17 ± 1.09	57.51 ± 2.83
3	**9a**	**17.42 ±1.85**	28.06 ± 3.49	31.92 ±2.32	46.36 ± 3.02	57.51 ± 2.04
4	**9c**	**14.71 ± 2.65**	23.01 ± 2.19	43.59 ±3.23	78.70 ± 2.11	66.03 ± 2.56
5	**9e**	26.38 ± 1.74	**18.12 ± 3.16**	33.64 ± 2.42	55.87 ± 1.92	**38.62 ± 3.10**
6	**9g**	**19.24 ± 3.24**	**14.31 ± 2.67**	30.25 ± 3.38	40.19 ± 3.11	42.34 ± 2.59
7	**9h**	31.69 ± 2.13	**13.49 ± 2.72**	**11.09 ± 1.32**	41.32 ± 2.69	43.68 ± 2.45
8	**9i**	23.03 ± 1.76	**19.51 ± 1.49**	40.44 ±1.09	82.50 ± 1.89	43.23 ± 1.69
9	**9j**	29.59 ± 1.48	33.47 ± 2.46	47.97 ± 2.97	69.38 ± 1.56	44.16 ± 2.93

10	**9m**	**18.42 ± 2.53**	21.43 ± 2.49	30.32 ± 2.11	65.01 ± 1.12	68.81 ± 1.38
11	**9n**	21.29 ± 3.25	**18.06 ± 0.61**	**24.45 ± 1.77**	65.30 ± 2.40	55.87 ± 2.17
12	5-FU	30.80 ± 1.25	25.80 ± 1.48	40.01 ± 1.75	16.65 ± 2.09	42.87 ± 2.13
13	CYC-PHO	29.12 ± 2.14	29.00 ± 2.27	36.32 ± 1.12	22.77 ± 1.27	52.30 ± 1.95
14	CYC-HEXI	35.29 ± 2.07	25.13 ± 2.31	43.30 ± 2.02	18.46 ± 1.45	40.12 ± 1.78

5-FU 5-fluorouracil, *CYC-PHO* cyclophosphamide, *CYC-HEXI* cycloheximide.

[a]50% growth inhibition as determined by MTT assay (24h drug exposure). [b]Compounds tested in triplicate, data expressed as mean value ± SD of three independent experiments.

Table 3. Minimum inhibitory concentration (MIC) in µg/ml of synthesized compounds against bacterial strains evaluated by microdilution method.

Sr. No.	Compounds	Gram positive	Gram negative	
		S. aureus (µg/ml)	*E. Coli* (µg/ml)	*C. albicans* (µg/ml)
1	**4a**	-	-	125
2	**4b**	-	-	250
3	**4c**	-	-	**62.5**
4	**4d**	-	-	**31.25**
5	**4f**	-	-	125
6	**4g**	-	-	250
7	**4i**	250	125	-
8	**4j**	-	-	250
9	**4l**	-	-	125
10	**4o**	125	125	**31.25**
11	**4p**	250	250	**62.5**
12	**4r**	**62.5**	250	-
13	**5a**	250	**62.5**	-
14	**5c**	**31.25**	250	125
15	**6a**	125	125	625
16	**6c**	250	250	-
17	**9a**	-	-	500
18	**9b**	125	-	**62.5**
19	**9d**	-	-	125

20	**9e**	**62.5**	-	-
21	**9g**	125	-	-
22	**9h**	250	-	**62.5**
23	**9i**	250	**62.5**	-
24	**9l**	-	-	125
25	**9m**	-	-	125
26	**9n**	250	250	-
27	**9o**	**62.5**	**62.5**	**31.25**
28	**10b**	-	-	500
29	**10c**	125	**62.5**	-
30	Cefadroxil	62.5	-	-
31	Ampicillin	-	62.5	-
32	Fluconazole	-	-	31.25

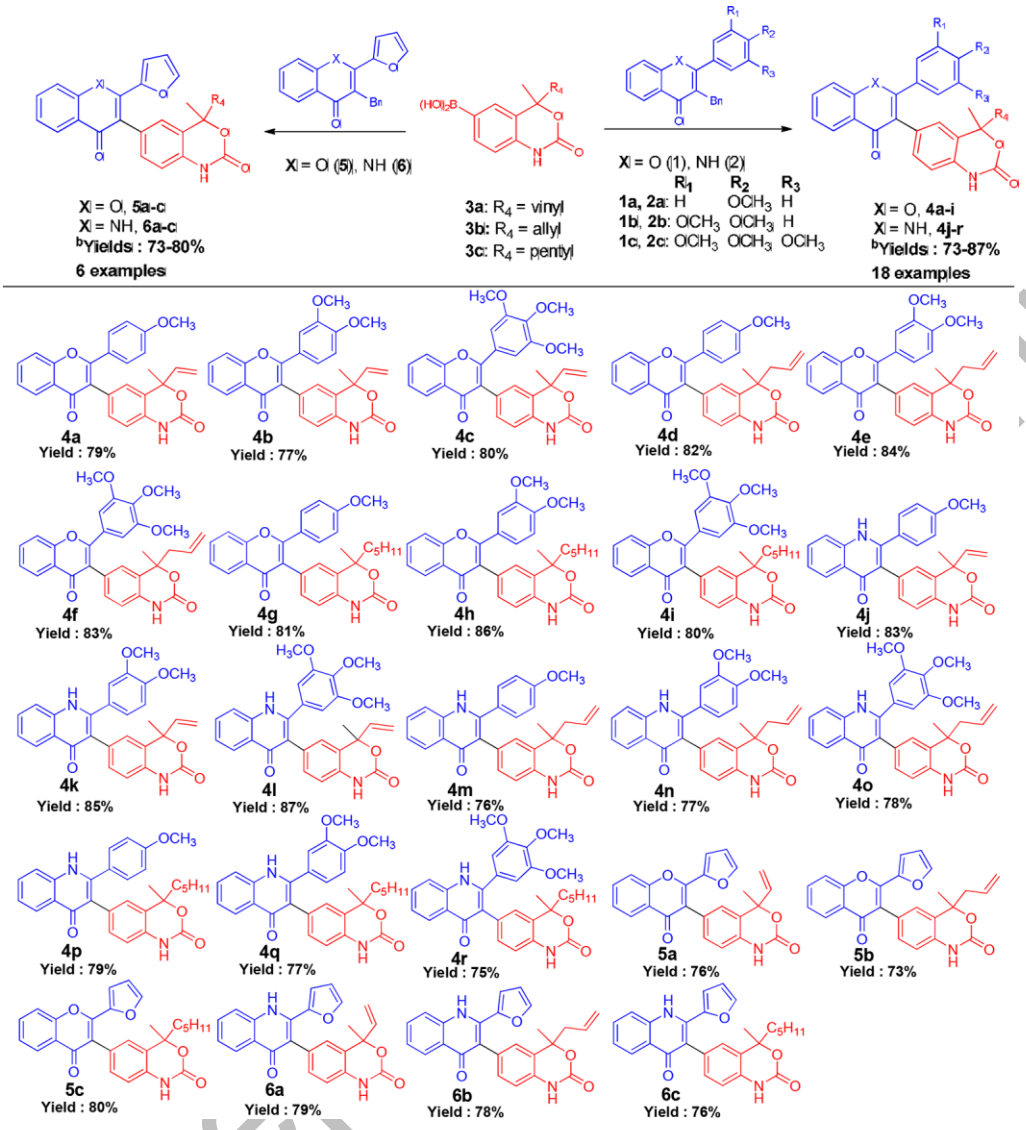
Table 4. Comparative data of Binding energy and Ki for Progesterone (PDB Code: 1ZUC) and Estrogen receptor (PDB Code: 3EQM) of most potent compounds.

Sr. no.	Compound	Progesterone receptor (PDB Code: 1ZUC)		Estrogen receptor (PDB Code: 3EQM)	
		Binding energy (kcal/mol)	*Ki* (nM)	Binding energy (kcal/mol)	*Ki* (nM)
1	4d	**-10.18**	**34.52**	-11.07	7.70
2	4e	**-10.21**	**32.66**	-11.47	3.89
3	5a	-10.44	22.10	-10.07	41.31
4	5b	**-10.77**	**12.86**	-10.25	30.48
5	9a	-9.32	146.53	-11.75	2.43
6	9c	-9.63	87.99	-11.79	2.29
7	9e	-9.96	49.85	**-11.97**	**1.67**
8	9g	-9.34	143.20	**-12.01**	**1.58**
9	9h	-9.21	176.78	**-12.09**	**1.37**
10	9i	-9.05	232.18	**12.02**	**1.55**
11	9j	-9.40	128.42	**-11.88**	**1.96**
12	9m	**-10.01**	**45.80**	-11.53	3.54
13	9n	**-10.35**	**25.88**	**-11.90**	**1.88**
14	Tanaproget	-10.39	24.20	-	-
15	Flavone	-	-	-9.31	150.34
16	5-FU	-4.67	378.20	-5.37	115.16
17	CYC-PHO	-5.75	61.31	-6.19	29.26

18	CYC-HEXI	-9.04	236.31	-9.0	254.88

Flavone = 6-bromo-3-butyl-2-(4-hydroxyphenyl)-4H-chromen-4-one, *5-FU* = 5-fluorouracil, *CYC-PHO* = cyclophosphamide, *CYC-HEXI* = cycloheximide.

Scheme 1. Synthetic routes for the compounds (**4a-r**, **5a-c**, **6a-c**).[a]



Scheme 2. Synthetic routes for the compounds (**9a-o**, **10a-c**).[a]

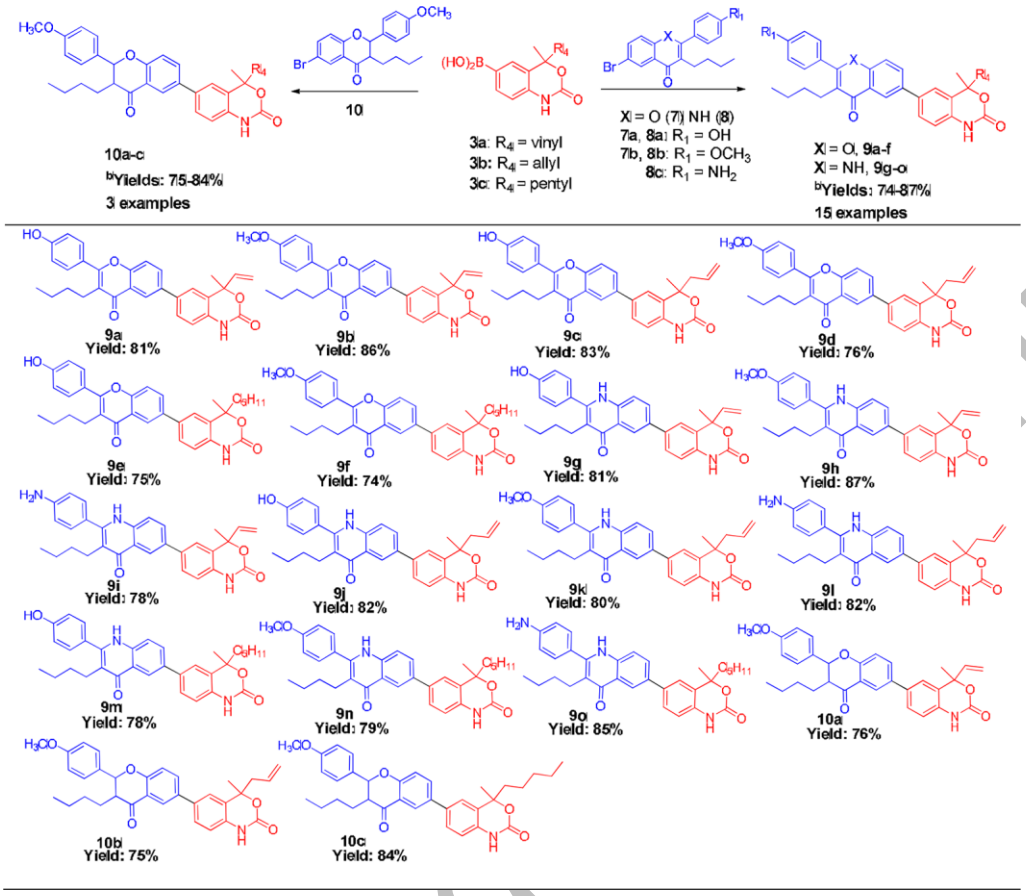


Fig. 1 Modification in Tanaproget.

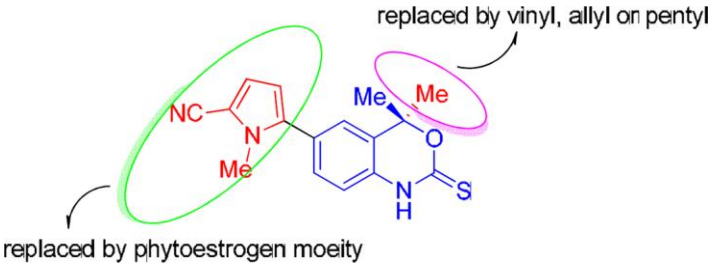


Fig. 2 Design of target compound.

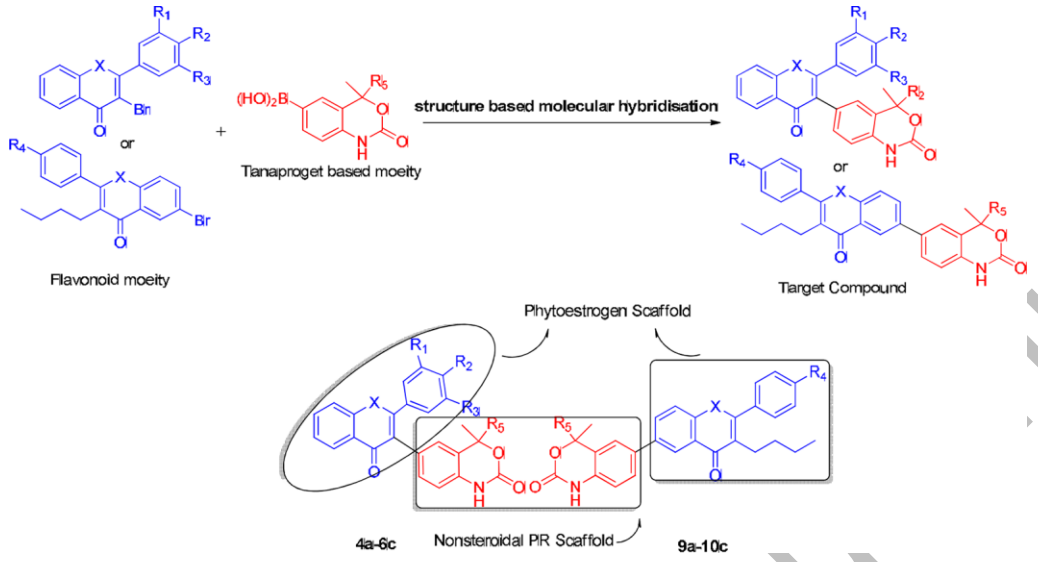


Fig. 3 Effect of most potent compounds on the proliferation of different human cell lines (MCF-7, MDA-MB-231, DU-145, PC-3 and Hela) were incubated in the presence or absence of 10 µM of different compounds for 72h at 37 ^{0}C.

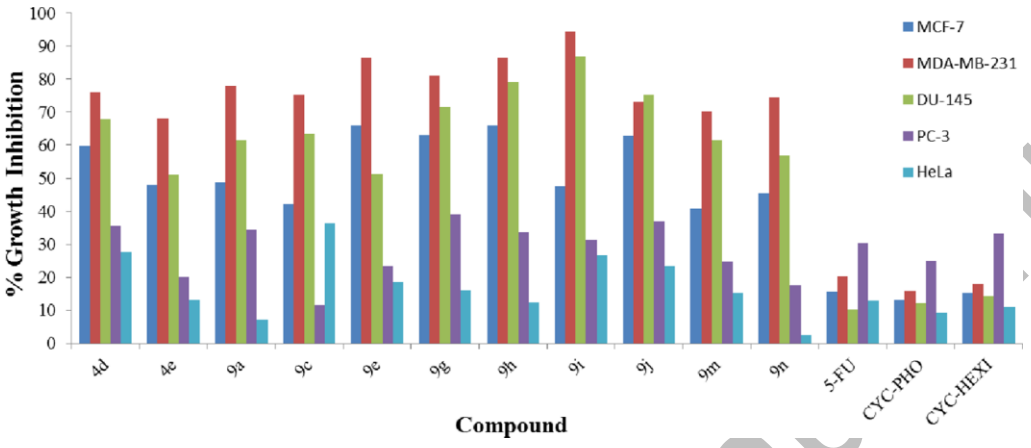


Fig. 4 Antiproliferative activities of most potent compounds against MCF-7, MDA-MB-231, DU-145, PC-3, and HeLa cell line.

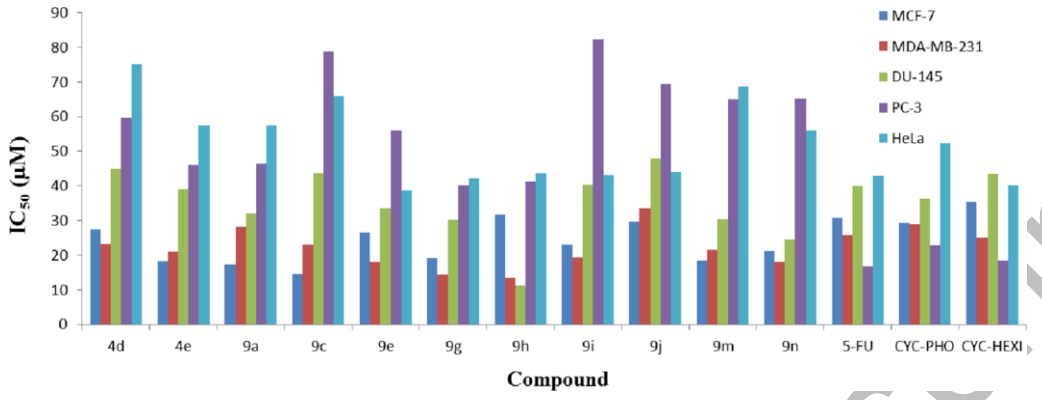
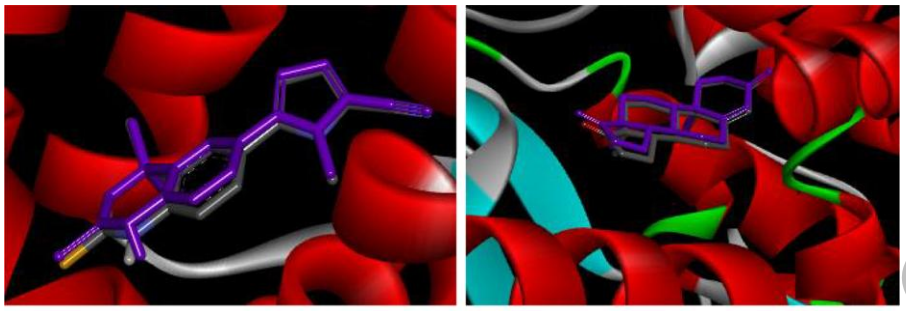


Fig. 5 Superimposition of crystal (X-ray) (a) progesterone (1ZUC) and (b) estrogen (3EQM) versus redocked conformation (*in purple*) with rmsd = 1.09 Å and 1.43 Å respectively.



(a) (b)

Fig. 6 Docked poses of most potent compounds complex with Progesterone (PDB Code: 1ZUC) and Estrogen receptor (PDB Code: 3EQM).