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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Synthesis, evaluation of 6,8-dibromo-2-aryl-2,3-dihydroquinolin-4(1*H*)-ones in MCF-7 (breast cancer) cell lines and their docking studies

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Abstract A series of novel 6,8-dibromo-2-aryl-2,3-dihydroquinolin-4(1*H*)-ones have been synthesized and evaluated in vitro (in MCF-7 breast cancer cell lines). Compounds **5a**, **5d**, **5e**, and **5g** exhibited potent GI_{50} and TGI values compared with reference standard and compounds **5b** and **5c** showed moderate activity. The docking studies (in silico) were conducted to recognize the hypothetical binding motif of the title compounds within the active site of aromatase enzyme employing GOLD docking software. The binding mode and SAR of the title compounds has been proposed based on the docking studies.

Keywords Dihydroquinolin-4(1*H*)-ones · MCF-7 cell line · Nonsteroidal aromatase inhibitors · Docking studies

Introduction

Breast cancer is one of the most common forms of female cancers and currently considered as the leading cause of death among women (accounting for 35% of all cancers and 20% of all cancer deaths) worldwide (Bandi *et al*., 2010). Excluding cancers of skin, breast cancer is the most frequently diagnosed cancer in women. The Indian Council of Medical Research (ICMR) registry records an whooping figure of 1,00,000 every year as new cases in Indian women and 40% of them die either of late detection or shyness of subjects to get examined (ICMR, 2008). In a high percentage of cases, it proves to be hormone-dependent because tumor progression is dependent on high levels of circulating estrogens, which play a critical role in cancer cell proliferation. Two pharmacological strategies have been employed essentially to control or alleviate the distress due to the pathological events of estrogen (Murthy *et al*., 2004). These include drugs which either act through estrogen receptor (ER) antagonism (antiestrogens) or interfere with the synthesis of steroidal hormones by inhibiting the enzymes controlling the interconversion from androgenic precursors, i.e., aromatase inhibitors (AIs). The third generation AIs, such as letrozole, anastrozole, and exemestane are now considered a valid alternative to tamoxifen as first line treatment option for advanced breast cancer (Needleman and Tobias, 2008).

Besides the development of synthetic compounds, the potential of various classes of natural products to inhibit aromatase was evaluated in order to discover novel therapeutic agents for breast cancer. As a result, several naturally occurring and synthetic flavonoids that mediate a host of biological activities were found to demonstrate inhibitory effects on aromatase (Carlo *et al*., 1999; Jeong *et al*., 1999; Kao *et al*., 1998). It has been demonstrated that several flavonoid derivatives are potent AIs and effective antiproliferative agents against MCF-7 breast cancer cell lines (Le-Bail *et al*., 1998; Pouget *et al*., 2001). This substantiates our hypothesis that the flavonoidal scaffold could churn out to be a milestone in developing the next generation of safer AIs (Rao and Murthy, 2009).

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Flavonoids, which are structurally similar to estrogens, are able to bind to the ER and possess antiestrogenic activities. Based on the data obtained from site-directed mutagenesis and ligand-docking studies into the aromatase enzyme, a binding orientation was predicted in which the A and C rings of the flavonoid mimic the D and C rings of the androstendione, respectively, while the B ring is oriented in a similar position to that of the androstendione ring A and points toward the extrahydrophobic pocket within the active site (Kao *et al*., 1998; Pelissero *et al*., 1996). This analysis places the flavonoid 4-keto functionality in the same position as the androstendione C-19 angular methyl group which interacts with the heme iron of the aromatase enzyme (Fig. 1).

With an objective of designing new and potent AIs (Murthy *et al*., 2006; Rao and Murthy, 2009), we relied on flavonoid nucleus as a structural scaffold and by bioisosteric modification on ring oxygen with nitrogen led to a series of 6,8-dibromo-2-aryl-2,3-dihydroquinolin-4(1*H*)-one derivatives. We took clue from the most active bromo derivative YM511 (Okada *et al*., 1996) and introduced bromo group in our compounds. In the patent (Rao and Murthy, 2009), which was filed from our laboratories, the human placental microsomal aromatase inhibition potencies of the final compounds, i.e., imidazole and triazole derivatives were reported. In continuation of our ongoing research, we investigated the antiproliferative activity against MCF-7 breast cancer cell lines and the aromatase inhibitory activity of 2-aryl-2,3-dihydroquinolin-4(1*H*)-ones to get the lead molecules (unpublished data from these laboratories). A series of such analogs have been designed and synthesized with a view to develop as new AIs. In this communication, we report the synthesis, in vitro biological evaluation of a series of new 6,8-dibromo-2-aryl-2,3-dihydroquinolin-4(1*H*)-ones in MCF-7 breast cancer cell lines. Further, we propose the molecular interactions and the binding mode of the synthesized compounds using the X-ray crystal structure of human placental microsomal aromatase (PDB ID: 3EQM) (Ghosh *et al*., 2009) through in silico docking studies.

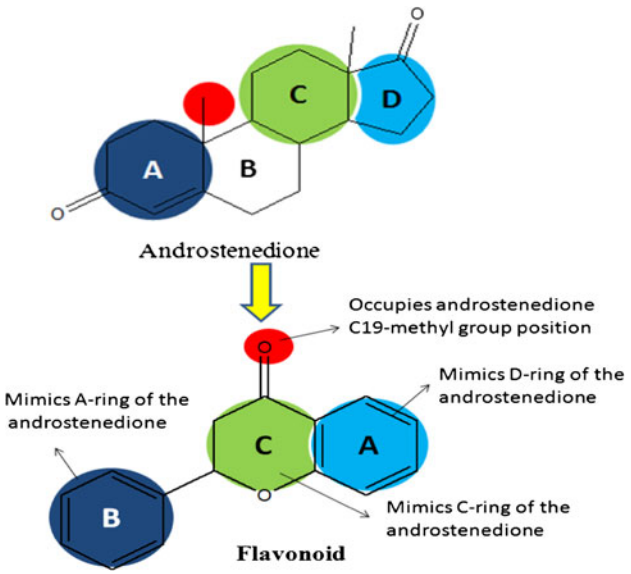


Fig. 1 Comparison of the chemical features between the model framework of androstenedione and flavonoids

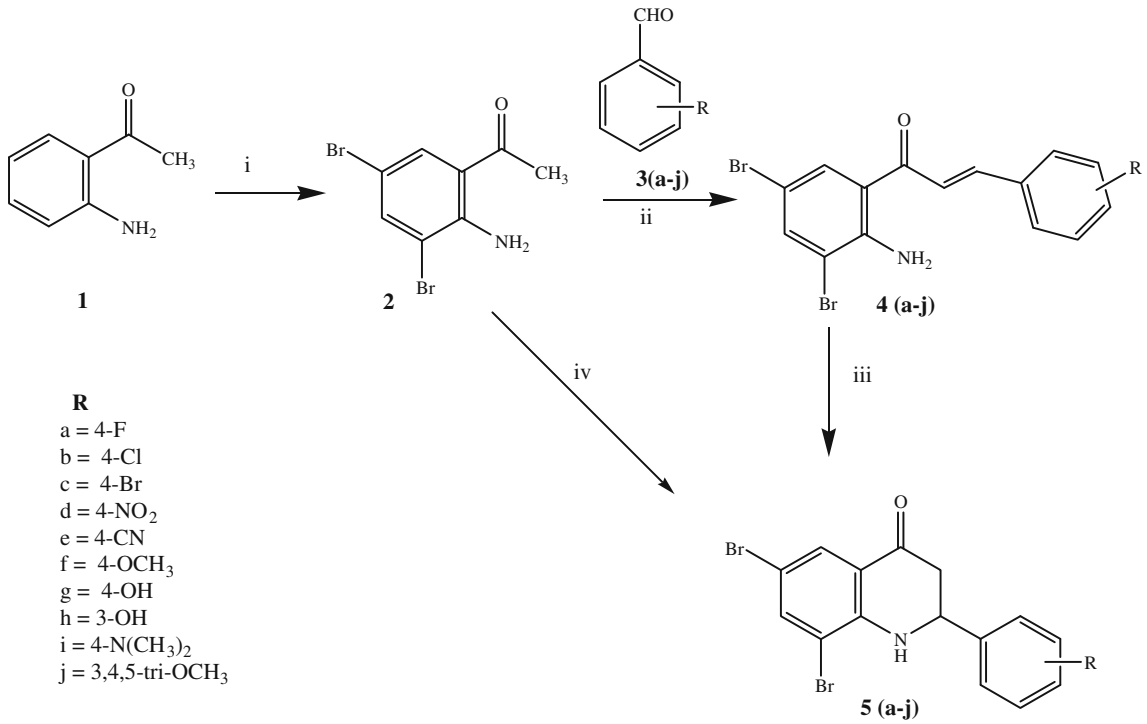
Results and discussion

Chemistry

As illustrated in Scheme 1, the title compounds were synthesized from 2-aminoacetophenone (**1**). On bromination using bromine in dichloromethane at 0–5°C, a mixture of 1-(2-amino-3,5-dibromophenyl)ethanone (**2**) and 1-(2-amino-5-dibromophenyl)ethanone was obtained. It was interesting to observe that maintaining the reaction temperature at 0–5°C and increasing the time period of stirring to 7 h were found to be crucial in the formation of compound **2** as major product. In ^{1}H NMR spectrum, the presence of two doublet peaks at δ 6.67 (J = 2.2 Hz) and δ 7.79 (J = 2.2 Hz) confirmed the formation of compound **2**. It was characterized by comparing with the literature data (Baker *et al*., 2001).

Compound **2** was subjected to Claisen–Schmidt condensation using different substituted aromatic aldehydes (**3a–j**) in the presence of sodium hydroxide and absolute ethanol keeping the temperature 0–5°C for 35–40 h results in the formation of different chalcones (**4a–j**). The chemical structures were confirmed through physical and spectral data. The ^{1}H NMR spectrum showed the presence of a broad singlet at δ 6.9 (–NH_2), a signal at δ 7.4 (J = 15.5 Hz) and δ 7.6 (J = 15.5 Hz). The higher magnitude of coupling constants (J value) for both protons indicate *trans* configuration.

Chalcones (**4a–j**) were cyclized by using a mixture of orthophosphoric acid and glacial acetic acid, where the addition of glacial acetic acid was considered to increase the acidity of the medium, thereby it readily donates the proton to chalcone to undergo intramolecular cyclization, resulting in the facile formation of title compounds (**5a–j**). The compounds were purified by column (hexane, ethyl acetate; 99:1). Formation of the title compounds was confirmed by physical and spectral data. IR spectra of **5** exhibited sharp characteristic secondary amine peak at 3390.4 cm^{-1} (absence of primary amine double band peak), while in the ^{1}H NMR spectrum showed the disappearance of vinyl and primary amine protons and the presence of additional signals at δ 2.8, at δ 4.5 (–NH,



Scheme 1 Reaction conditions (i) Br_2, DCM, 0–5°C, 7 h, (ii) absolute ethanol, NaOH, 0–5°C, 24 h, (iii) glacial acetic acid, orthophosphoric acid, 100°C, 2–3 h, and (iv) **3a–j**, L-proline, methanol, 55–60°C, 48 h

secondary amine) and at δ 4.8, providing evidence for the formation of the title compounds.

Alternatively, a one pot synthesis of title compounds was also attempted using 1-(2-amino-3,5-dibromophenyl)ethanone (**2**) and different aromatic aldehydes **(3a–j)** in equimolar quantities in the presence of L-proline (30 mol%). The reaction took place at 55–60°C, while stirring the reaction mixture in methanol for 48 h. The workup of reaction was conducted by treating the reaction mixture with saturated ammonium chloride solution followed by repeated extraction of the product with dichloromethane. The combined organic layers were dried over anhydrous sodium sulfate, and purified by column chromatography (hexane, ethyl acetate; 99:1) gave the pure products (**5a–j**). The percentage yields were found to be comparable to that of synthesis via chalcones.

In vitro screening using MCF-7 breast cancer cell lines

The synthesized compounds were evaluated for their inhibition potential on MCF-7 breast cancer cell lines (ACTREC, Mumbai, India). The current research program is the design and development of AIs and the objective is to identify some lead molecules with potent MCF-7 inhibitory activity. As a preliminary study only antiprolifirative activity in MCF-7 cell lines is reported. The percentage growth inhibitions of the compounds were compared with adriamycin (ADR) as standard at different (molar) concentrations. The results obtained from in vitro screening expressed as molar concentration at three assay end points: the 50% growth inhibitory power (GI_{50}), the cytostatic effect (TGI = total growth inhibition), and the cytotoxic effect (LC_{50}) Table 1.

All the compounds **5a–j** showed comparable growth inhibitory potencies to that of the standard drug ADR at a

Table 1 In vitro screening in MCF-7 breast cancer cell lines, percentage growth as compared to control

S. no.	Percentage growth as compared to control at μM concentrations						
	10^{-7} M	10^{-6} M	10^{-5} M	10^{-4} M	LC_{50}	TGI	GI_{50}
5a	30.1	9.2	−3.6	−48.1	>100	0.145	<0.1
5b	60.2	61.7	34.5	−40.0	>100	2.87	0.146
5c	69.7	69.7	12.5	−43.0	>100	2.6	0.151
5d	50.5	8.6	−4.4	−52.3	>100	0.132	<0.1
5e	43.1	8.2	−5.1	−58.5	>100	0.126	<0.1
5f	168.1	140.5	87.8	7.1	>100	>100	>100
5g	3.7	9.6	−2.0	−45.3	>100	0.151	<0.1
5h	117.7	107.8	25.6	14.7	>100	>100	>100
5i	164	143.8	130.5	43.0	>100	>100	>100
5j	129.5	110.5	99.6	34.3	>100	>100	>100
ADR	53.5	−13.9	−23.7	−33.5	35.5	0.183	<0.1

All the values derived from average of three (3) experiments

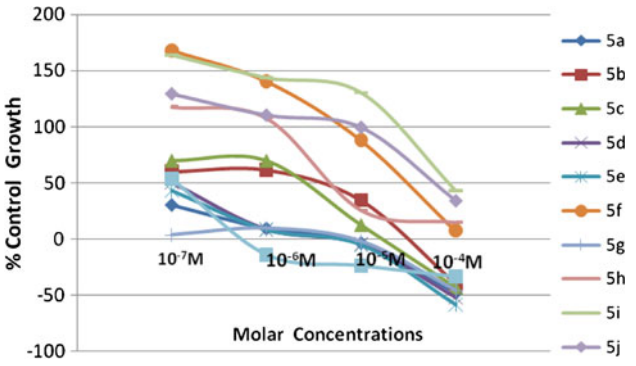


Fig. 2 Percentage growth control versus molar concentrations of compounds

concentration of 10^{-4} M. Compounds **5a**, **5d**, **5e**, and **5g** exhibited potent GI_{50} and TGI values compared with reference standard and compounds **5b** and **5c** showed moderate GI_{50} and TGI values, whereas compounds **5f**, **5h**, **5i**, and **5j** did not show significant GI_{50} and TGI values at molar concentrations tested (Fig. 2).

The final compounds (**5a**–**j**) were evaluated in silico (molecular docking) to recognize their hypothetical binding mode using the X-ray crystal structure of human placental microsomal aromatase and also to rationalize their structure–activity relationships. To investigate the ability of molecular docking to reproduce an experimentally observed ligand-binding mode, the co-crystallized ligand androstenedione has been used as reference ligand. It was docked back into its binding site (Fig. 3a) of the crystal structure of human placental microsomal aromatase using GOLD molecular docking program. The top docked conformations (poses) closely resembled the co-crystallized conformation with a root-mean-square deviation (RMSD) between 0.48 and 0.50 Å of non-hydrogen atomic positions of the ligand (androstenedione).

All the compounds were docked well into the active site of aromatase enzyme. The cyclic ketone group interacts with the heme (Fe^{3+}) of the aromatase with a distance of 2.0–2.7 Å. The potent compounds **5a**, **5d**, **5e**, and **5g** are forming H-bonding with Ser 478 or Asp 309 amino acids of the active site, which probably account for their better activity, whereas this interaction is not possible with other compounds. Fluoro derivative (**5a**) may form a H-bonding with Ser 478 (3.09 Å, Fig. 3b) and nitro derivative (**5d**) may form a H-bonding with Ser 478 (2.65 Å, Fig. 3c). Compound (**5e**) may form a H-bonding with Ser 478 (3.68 Å, Fig. 3d), unlike other derivatives H-bond forming nitrogen atom is not directly attached to aromatic ring which might here resulted in the docking orientation with a distance of 3.68 Å between the nitrogen and HO-Ser 478 amino acid of the active site. Hydroxy derivative (**5g**) may form a H-bonding with Asp 309 amino acid of the active site of aromatase (2.62 Å, Fig. 3e). In case of compound **5b** (4.213 Å, Fig. 3f) and compound **5c** (4.241 Å, Fig. 3g) are showing the same binding mode (docking orientation), these derivatives are forming week H-bonding with Ser 478 amino acid of the active site.

Conclusion

A series of novel 6,8-dibromo-2-aryl-2,3-dihydroquinolin-4(1*H*)-ones (**5a**–**j**) was synthesized, characterized, and evaluated for in vitro screening in MCF-7 breast cancer cell lines. Compounds **5a**, **5d**, **5e**, and **5g** exhibited potent GI_{50} and TGI values compared with reference standard and compounds **5b** and **5c** showed moderate activity. The binding mode of the title compounds has been proposed based on the docking studies. Further in silico docking studies were undertaken to gain an insight into the molecular interactions and binding mode of the target compounds into aromatase enzyme. All the final compounds were docked well into the active site and interacted with the cyclic ketone group with the heme (Fe^{3+}) of the aromatase with a distance of 2.0–2.7 Å. The most active compounds **5a**, **5d**, **5e**, and **5g** are forming a H-bonding with Ser 478 or Asp 309 amino acids of the active site, which probably account for their better activity compared to other analogs of the series. The docking results revealed useful information to understand the interaction mode between dihydroquinolin-4(1*H*)-ones and aromatase and will facilitate the next cycle of drug design to explore the newer lead molecules. Efforts are currently being taken up to optimize the lead structure and the results of which will be the basis of our future research endeavor.

Experimental

Chemistry

Melting points were recorded in open capillaries on LABINDIA melting point apparatus (MEPA MP08050204) and were uncorrected. IR spectra were recorded on Perkin Elmer FT-IR Spectrometer (Spectrum RX I) using KBr pellet technique. ^{1}H and ^{13}C NMR spectra were recorded on Bruker Avance II 400 MHz spectrometer in $CDCl_3$ using TMS as internal standard. Mass spectra (ESI) were recorded on Waters Micromass Q-TOF Micro and elemental analyses were performed using Thermo EA 2110 series elemental analyser. All chemicals used were of analytical grade and commercially available from E. Merck, Mumbai. Solvents were used without further purification. Silica gel (100–200 mesh; E. Merck, Mumbai)

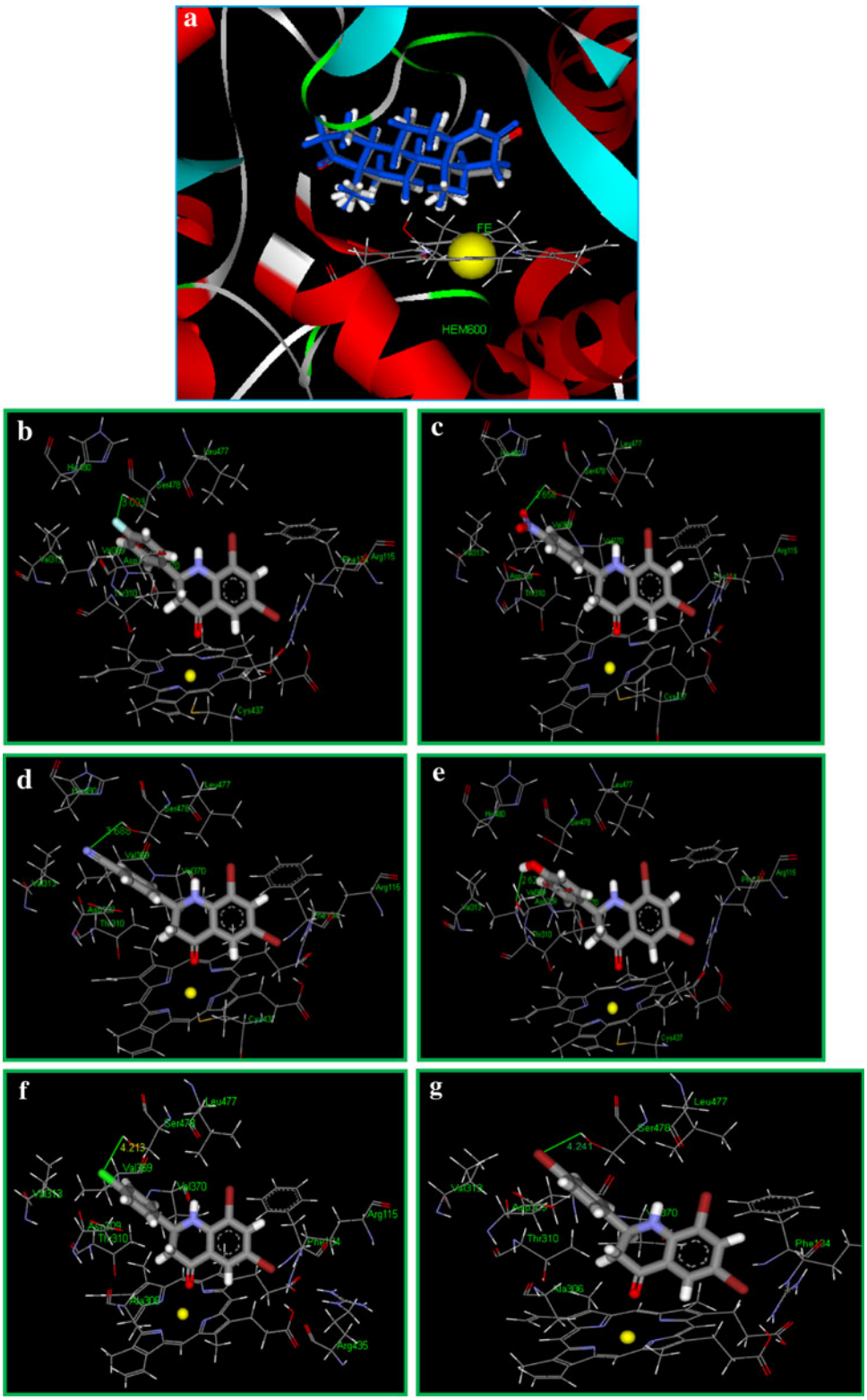


Fig. 3 **a** Superposition of top docked configurations of androstenedione on crystal structure within the active site of aromatase, **b** Docking orientation of **5a**, **c** Docking orientation of **5d**, **d** Docking orientation of **5e**, **e** Docking orientation of **5g**, **f** Docking orientation of **5b**, and **g** Docking orientation of **5c**

was used for column chromatography. All the reactions were monitored by thin layer chromatography (TLC) on precoated silica gel 60 F_{254} (mesh) (E. Merck, Mumbai) and spots were visualized under UV light (254 nm).

*Synthesis of 1-(2-amino-3,5-dibromophenyl)ethanone (**2**)*

Bromine (0.16 g, 1 mmol) dissolved in dichloromethane (10 ml) was added dropwise to a solution containing

2-aminoacetophenone (**1**) (0.135 g, 1 mmol) in dichloromethane (10 ml). The resulting mixture stirred for 7 h at 0–5°C. The solid mass obtained was filtered and the product was purified using column chromatography (hexane, ethyl acetate; 95:5). The purified compound was recrystallized from methanol (Baker *et al*., 2001; Leonard and Boyd, 1946).

Yield, 80%; mp. 120–122°C [lit. 123–124°C (Leonard and Boyd, 1946)]; IR (KBr) cm^{-1}: 3449.4, 3315.1 (–NH_2), 1674.5 (C=O). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 2.58 (s, 3H), 6.90 (br s, 2H), 6.67 (d, $J = 2.2$ Hz, 1H), 7.79 (d, $J = 2.2$ Hz, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 199.1, 147.2, 140.1, 134.1, 119.6, 112.1, 106.0, 31.5. ES^+ [*m/z*, (% relative intensity)] 295.9 (M^+, +4, 14), 293.9 (M^+, +2, 27), 291.9 (M^+, 15), 277.9 (50), 275.9 (100), 273.9 (51), 253.9 (7), 251.9 (14), 249.9 (8), 215.0(49), 213.0 (52), 197.0 (55), 195.0 (53).

*Synthesis of 1-(2-amino-3,5-dibromophenyl)-3-(4-aryl)prop-2-en-1-ones (**4a–j**)*

General procedure 1-(2-Amino-3,5-dibromophenyl)ethanone (**2**) (0.294 g, 1 mmol) was stirred with absolute ethanol (8 ml) and sodium hydroxide (750 mg) mixture at room temperature. To the mixture different aromatic aldehydes (**3a–j**) (1 mmol) were added and further stirred for 35–40 h, the temperature being maintained at 0–5°C. The solid products were separated by filtration and recrystallised from aqueous ethanol (Donnelly and Farrell, 1990b; Wattanasin and Murphy, 1980).

*1-(2-Amino-3,5-dibromophenyl)-3-(4-fluorophenyl)prop-2-en-1-one (**4a**)*

Yield, 62%; mp. 118–120°C; IR (KBr) cm^{-1}: 3457.3, 3314.2 (–NH_2), 1647.4 (C=O). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 6.89 (br s, 2H), 7.34 (d, $J = 2.4$ Hz, 1H), 7.39 (d, $J = 6.4$ Hz, 2H), 7.48 (d, $J = 15.8$ Hz, 1H), 7.56 (d, $J = 6.4$ Hz, 2H), 7.65 (d, $J = 15.6$ Hz, 1H), 7.91 (d, $J = 2.4$ Hz, 1H). Anal. Calcd for $C_{15}H_{10}Br_2FNO$: C, 45.15; H, 2.53; N, 3.51. Found: C, 44.92; H, 2.56; N, 3.44.

*1-(2-Amino-3,5-dibromophenyl)-3-(4-chlorophenyl)prop-2-en-1-one (**4b**)*

Yield, 65%; mp. 124–128°C; IR (KBr) cm^{-1}: 3458.3, 3313.2 (–NH_2), 1645.4 (C=O). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 6.92 (br s, 2H), 7.35 (d, $J = 2.4$ Hz, 1H), 7.40 (d, $J = 8.2$ Hz, 2H), 7.49 (d, $J = 15.6$ Hz, 1H), 7.58 (d, $J = 8.2$ Hz, 2H) 7.69 (d, $J = 15.6$ Hz, 1H), 7.92 (d, $J = 2.4$ Hz, 1H). Anal. Calcd for $C_{15}H_{10}Br_2ClNO$: C, 43.36; H, 2.43; N, 3.37. Found: C, 43.18; H, 2.61; N, 3.18.

*1-(2-Amino-3,5-dibromophenyl)-3-(4-bromophenyl)prop-2-en-1-one (**4c**)*

Yield, 68%; mp. 130–135°C; IR (KBr) cm^{-1}: 3459.0, 3336.2 (–NH_2), 1643.0 (C=O). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 6.90 (br s, 2H), 7.35 (d, $J = 2.4$ Hz, 1H), 7.51 (m, 5H), 7.68 (d, $J = 15.6$ Hz, 1H), 7.91 (d, $J = 2.4$ Hz, 1H). Anal. Calcd for $C_{15}H_{10}Br_3NO$: C, 39.17; H, 2.19; N, 3.05. Found: C, 39.34; H, 2.10; N, 3.16.

*1-(2-Amino-3,5-dibromophenyl)-3-(4-nitrophenyl)prop-2-en-1-one (**4d**)*

Yield, 62%; mp. 142–146°C; IR (KBr) cm^{-1}: 3459.4, 3346.6 (–NH_2), 1603.4 (C=O). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 6.99 (br s, 2H), 7.39 (d, $J = 2.4$ Hz, H), 7.49 (d, $J = 15.8$ Hz, 1H), 7.53 (d, $J = 8.8$ Hz, 2H), 7.79 (d, $J = 15.8$ Hz, 1H), 7.84 (d, $J = 8.8$ Hz, 2H), 7.91 (d, $J = 2.4$ Hz, 1H). Anal. Calcd for $C_{15}H_{10}Br_2N_2O_3$: C, 42.29; H, 2.37; N, 6.57. Found: C, 41.98; H, 2.41; N, 6.77.

*4-(3-(2-Amino-3,5-dibromophenyl)-3-oxoprop-1-enyl)benzonitrile (**4e**)*

Yield, 72%; mp. 190–192°C; IR (KBr) cm^{-1}: 3451.4, 3328.9 (–NH_2), 2222.1(C≡N), 1647.0 (C=O). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 6.42 (br s, 2H), 7.36 (d, $J = 2.2$ Hz, 1H), 7.49 (d, $J = 15.6$ Hz, 1H), 7.58 (d, $J = 15.6$ Hz, 1H), 7.73 (m, 4H), 7.89 (d, $J = 2.2$ Hz, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 189.7, 150.0, 141.2, 139.3, 137.4, 132.9, 132.7, 128.7, 125.5, 119.7, 119.2, 118.5, 113.3, 106.9. Anal. Calcd for $C_{16}H_{10}Br_2N_2O$: C, 47.32; H, 2.48; N, 6.90. Found: C, 47.56; H, 2.24; N, 6.98.

*1-(2-Amino-3,5-dibromophenyl)-3-(4-methoxyphenyl)prop-2-en-1-one (**4f**)*

Yield, 82%; mp. 130–135°C; IR (KBr) cm^{-1}: 3460.2, 3331.2 (–NH_2), 1638.6 (C=O), 1259 (C–O–C assym str), 1024.7 (C–O–C symm str). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 3.82 (s, 3H), 6.89 (br s, 2H), 6.96 (d, $J = 8.76$ Hz, 2H), 7.31 (d, $J = 15.4$ Hz, 1H), 7.60 (d, $J = 8.8$ Hz, 2H), 7.72 (d, $J = 2.12$ Hz, 1H), 7.75 (d, $J = 15.4$ Hz, 1H), 7.91 (d, $J = 2.12$ Hz, 1H). Anal. Calcd for $C_{16}H_{13}Br_2NO_2$: C, 46.75; H, 3.19; N, 3.41. Found: C, 46.71; H, 3.42; N, 3.49.

*1-(2-Amino-3,5-dibromophenyl)-3-(4-hydroxyphenyl)prop-2-en-1-one (**4g**)*

Yield, 60%; mp. 120–124°C; IR (KBr) cm^{-1}: 3520 (–OH), 3458.6, 3334.5 (–NH_2), 1637.2 (C=O). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 6.86 (br s, 2H), 6.95 (d, $J = 8.72$ Hz, 2H), 7.35 (d, $J = 15.6$ Hz, 1H), 7.62 (d, $J = 8.8$ Hz, 2H), 7.71

(d, $J = 2.16$ Hz, 1H), 7.76 (d, $J = 15.4$ Hz, 1H), 7.90 (d, $J = 2.12$ Hz, 1H), 7.97 (s, 1H). Anal. Calcd for $C_{15}H_{11}Br_2NO_2$: C, 45.37; H, 2.79; N, 3.53. Found: C, 45.22; H, 2.53; N, 3.41.

*1-(2-Amino-3,5-dibromophenyl)-3-(3-hydroxyphenyl)prop-2-en-1-one (**4h**)*

Yield, 58%; mp. 112–114°C; IR (KBr) cm^{-1}: 3518 (–OH), 3459.6, 3335.5 (–NH_2), 1637.2 (C=O). ^1H NMR ($CDCl_3$, 400 MHz): δ 6.82 (br s, 2H), 7.12 (m, 4H), 7.34 (d, $J = 15.6$ Hz, 1H), 7.72 (d, $J = 2.16$ Hz, 1H), 7.77 (d, $J = 15.4$ Hz, 1H), 7.92 (d, $J = 2.12$ Hz, 1H), 7.95 (s, 1H). Anal. Calcd for $C_{15}H_{11}Br_2NO_2$: C, 45.37; H, 2.79; N, 3.53. Found: C, 45.28; H, 2.59; N, 3.44.

*1-(2-Amino-3,5-dibromophenyl)-3-(4-(dimethylamino)phenyl)prop-2-en-1-one (**4i**)*

Yield, 45%; mp. 146–148°C; IR (KBr) cm^{-1}: 3462.1 (–NH_2), 1606.6 (C=O). ^1H NMR ($CDCl_3$, 400 MHz): δ 3.05 (s, 6H), 6.83 (br s, 2H), 6.71 (d, $J = 8.88$ Hz, 2H), 7.20 (d, $J = 15.68$ Hz, 1H), 7.6 (d, $J = 2.16$ Hz, 1H), 7.50 (d, $J = 8.88$ Hz, 2H), 7.77 (d, $J = 15.24$ Hz, 1H), 7.91(d, $J = 2.16$ Hz, 1H). Anal. Calcd for $C_{17}H_{16}Br_2N_2O$: C, 48.14; H, 3.80; N, 6.60. Found: C, 47.96; H, 4.10; N, 6.85.

*1-(2-amino-3,5-dibromophenyl)-3-(3,4,5-trimethoxyphenyl)prop-2-en-1-one (**4j**)*

Yield, 55%; mp. 128–130°C; IR (KBr) cm^{-1}: 3458.2 (–NH_2), 1645 (–C=O), 1248 (C–O–C asym str), 1113 (C–O–C symm str). ^1H NMR ($CDCl_3$, 400 MHz): δ 3.90 (s, 9H), 6.91 (br s, 2H), 6.86 (s, 2H) 7.3 (d, $J = 15.4$ Hz, 1H), 7.6 (d, $J = 15.4$ Hz, 1H), 7.71 (d, $J = 2.16$ Hz, 1H), 7.91 (d, $J = 2.2$ Hz, 1H). Anal. Calcd for $C_{18}H_{17}Br_2NO_4$: C, 45.89; H, 3.64; N, 2.97. Found: C, 45.93; H, 3.42; N, 3.18.

*Synthesis of 6,8-dibromo-2-aryl-2,3-dihydroquinolin-4(1H)-one (**5a–j**)*

General procedure 1-(2-Amino-3,5-dibromophenyl)-3-(4-aryl)prop-2-en-1-ones **(4a–j)** (3 mmol) were heated with orthophosphoric acid (12 ml) and acetic acid (12 ml) at 100°C for 2–3 h. The reaction was monitored by TLC. After cooling, the reaction mixture was added to 100 ml of ice cold water and the resultant precipitate was filtered. Crude product was purified by column chromatography (hexane, ethyl acetate; 99:1) (Donnelly and Farrell, 1990a; Tokes *et al*., 1992).

*6,8-Dibromo-2-(4-fluorophenyl)-2,3-dihydroquinolin-4(1H)-one (**5a**)*

Yield, 62%; mp. 135°C; IR (KBr) cm^{-1}: 3353.2 (–NH), 1654.4 (C=O). ^1H NMR ($CDCl_3$, 400 MHz): δ 2.81 (m, 2H), 4.55 (br s, 1H), 4.79 (m, 1H), 7.29 (m, 4H), 7.44 (d, $J = 2.6$ Hz, 1H), 7.95 (d, $J = 2.6$ Hz, 1H). Anal. Calcd for $C_{15}H_{10}Br_2FNO$: C, 45.15; H, 2.53; N, 3.51. Found: C, 45.19; H, 2.46; N, 3.37.

*6,8-Dibromo-2-(4-chlorophenyl)-2,3-dihydroquinolin-4(1H)-one (**5b**)*

Yield, 58%; mp. 138–140°C; IR (KBr) cm^{-1}: 3384.5 (–NH), 1656.7 (C=O). ^1H NMR ($CDCl_3$, 400 MHz): δ 2.75 (m, 2H), 4.50 (br s, 1H), 4.72 (dd, $J = 4.8$, 12.8 Hz, 1H), 7.38 (m, 4H), 7.41 (d, $J = 2.4$ Hz, 1H), 7.97 (d, $J = 2.4$ Hz, 1H). Anal. Calcd for $C_{15}H_{10}Br_2ClNO$: C, 43.36; H, 2.43; N, 3.37. Found: C, 42.98; H, 2.66; N, 3.52.

*6,8-Dibromo-2-(4-bromophenyl)-2,3-dihydroquinolin-4(1H)-one (**5c**)*

Yield, 67%; mp. 146–148°C; IR (KBr) cm^{-1}: 3391.5 (–NH), 1674.3 (C=O). ^1H NMR ($CDCl_3$, 400 MHz): δ 2.79 (m, 2H), 4.52 (br s, 1H), 4.70 (dd, $J = 4.8$, 12.4 Hz, 1H), 7.32 (d, $J = 8.8$ Hz, 2H), 7.40 (d, $J = 2.4$ Hz, 1H), 7.52 (d, $J = 8.8$ Hz, 2H), 7.97 (d, $J = 2.4$ Hz, 1H). Anal. Calcd for $C_{15}H_{10}Br_3NO$: C, 39.17; H, 2.19; N, 3.05. Found: C, 40.06; H, 2.47; N, 3.18.

*6,8-Dibromo-2-(4-nitrophenyl)-2,3-dihydroquinolin-4(1H)-one (**5d**)*

Yield, 58%; mp. 144–148°C; IR (KBr) cm^{-1}: 3384.5 (–NH), 1656.7 (C=O). ^1H NMR ($CDCl_3$, 400 MHz): δ 2.81 (m, 2H), 4.56 (br s, 1H), 4.81 (m, 1H), 7.44 (d, $J = 2.2$ Hz, 1H), 7.72 (d, $J = 8.24$ Hz, 2H), 7.58 (d, $J = 8.24$ Hz, 2H), 7.96 (d, $J = 2.2$ Hz, 1H). Anal. Calcd for $C_{15}H_{10}Br_2N_2O_3$: C, 42.29; H, 2.37; N, 6.57. Found: C, 42.11; H, 2.39; N, 6.42.

*4-(6,8-Dibromo-4-oxo-1,2,3,4-tetrahydroquinolin-2-yl)benzonitrile (**5e**)*

Yield, 72%; mp. 242–246°C; IR (KBr) cm^{-1}: 3352.7 (–NH), 2225.2 (C≡N), 1671.7 (C=O). ^1H NMR ($CDCl_3$, 400 MHz): δ 2.82 (m, 2H), 4.58 (br s, 1H), 4.82 (m, 1H), 7.43 (d, $J = 2.2$ Hz, 1H), 7.59 (d, $J = 8.24$ Hz, 2H), 7.71 (d, $J = 8.24$ Hz, 2H), 7.97 (d, $J = 2.2$ Hz, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 190.8, 149.7, 145.8, 138.2, 132.9, 130.0, 127.4, 120.3, 118.3, 117.9, 112.6, 111.5, 57.9, 45.6.

Anal. Calcd for $C_{16}H_{10}Br_2N_2O$: C, 47.32; H, 2.48; N, 6.90. Found: C, 47.12; H, 2.44; N, 6.56.

*6,8-Dibromo-2-(4-methoxyphenyl)-2,3-dihydroquinolin-4(1H)-one (**5f**)*

Yield, 68%; mp. 162–166°C; IR (KBr) cm^{-1}: 3376.4 (–NH), 1665.8 (C=O), 1247.9 (C–O–C assym str), 1029.2 (C–O–C symm str). ^1H NMR ($CDCl_3$, 400 MHz): δ 2.81 (m, 2H), 3.82 (s, 3H), 4.57 (br s, 1H), 4.72 (dd, $J = 3.8$, 13.36 Hz, 1H), 6.96 (d, $J = 6.72$ Hz, 2H), 7.39 (d, $J = 6.72$ Hz, 2H), 7.71 (d, $J = 2.8$ Hz, 1H), 7.92 (d, $J = 2.28$ Hz, 1H). Anal. Calcd for $C_{16}H_{13}Br_2NO_2$: C, 46.75; H, 3.19; N, 3.41. Found: C, 46.52; H, 3.47; N, 3.21.

*6,8-Dibromo-2-(4-hydroxyphenyl)-2,3-dihydroquinolin-4(1H)-one (**5g**)*

Yield, 58%; mp. 122–125°C; IR (KBr) cm^{-1}: 3375.2 (–NH), 3596 (–OH), 1661.8 (C=O). ^1H NMR ($CDCl_3$, 400 MHz): δ 2.75 (m, 2H), 4.68 (br s, 1H), 4.81 (m, 1H), 7.19 (m, 4H), 7.69 (d, $J = 2.6$ Hz, 1H), 7.89 (d, $J = 2.6$ Hz, 1H), 7.97 (s, 1H). Anal. Calcd for $C_{15}H_{11}Br_2NO_2$: C, 45.37; H, 2.79; N, 3.53. Found: C, 45.21; H, 2.86; N, 3.33.

*6,8-Dibromo-2-(3-hydroxyphenyl)-2,3-dihydroquinolin-4(1H)-one (**5h**)*

Yield, 55%; mp. 125–128°C; IR (KBr) cm^{-1}: 3350 (–NH), 3245 (–OH), 1661 (C=O). ^1H NMR ($CDCl_3$, 400 MHz): δ 2.81 (m, 2H), 4.53 (br s, 1H), 5.01 (m, 1H), 7.42 (m, 4H), 7.73 (d, $J = 2.24$ Hz, 1H), 7.91 (d, $J = 2.36$ Hz, 1H), 8.01 (s, 1H). Anal. Calcd for $C_{15}H_{11}Br_2NO_2$: C, 45.37; H, 2.79; N, 3.53. Found: C, 45.52; H, 2.54; N, 3.50.

*6,8-Dibromo-2-(4-(dimethylamino)phenyl)-2,3-dihydroquinolin-4(1H)-one (**5i**)*

Yield, 66%; mp. 138–140°C; IR (KBr) cm^{-1}: 3363.0 (–NH), 1681.8 (C=O). ^1H NMR ($CDCl_3$, 400 MHz): δ 2.80 (m, 2H), 4.52 (br s, 1H), 4.74 (dd, $J = 3.68$, 13.76 Hz, 1H), 6.73(d, $J = 8.6$ Hz, 2H), 7.31 (d, $J = 8.76$ Hz, 2H), 7.69 (d, $J = 2.28$ Hz, 1H), 7.89 (d, $J = 2.22$ Hz, 1H). Anal. Calcd for $C_{17}H_{16}Br_2N_2O$: C, 48.14; H, 3.80; N, 6.60. Found: C, 48.42; H, 3.65; N, 6.79.

*6,8-Dibromo-2-(3,4,5-trimethoxyphenyl)-2,3-dihydroquinolin-4(1H)-one (**5j**)*

Yield, 60%; mp. 152–154°C; IR (KBr) cm^{-1}: 3390.4 (–NH), 1676.3 (C=O). ^1H NMR ($CDCl_3$, 400 MHz): δ 2.89 (m, 2H), 3.91(s, 9H), 4.55 (br s, 1H), 4.69 (dd, $J = 4.08$, 13.16 Hz, 1H), 6.67 (s, 2H), 7.71 (d, $J = 2.24$ Hz, 1H), 7.90 (d, $J = 2.08$ Hz, 1H). Anal. Calcd for $C_{18}H_{17}Br_2NO_4$: C, 45.89; H, 3.64; N, 2.97. Found: C, 46.12; H, 3.84; N, 2.91.

*L-Proline catalyzed cyclization of 1-(2-amino-3,5-dibromophenyl)ethanone (**2**)*

1-(2-Amino-3,5-dibromophenyl)ethanone (**2**) and aromatic aldehydes (**3a**–**j**) in equimolar quantities were stirred in the presence of L-proline (30 mol%) in methanol for 48 h, the temperature being maintained at 55–60°C (Chandrasekhar *et al.*, 2007). After completion of reaction (monitored by TLC), reaction mixture was treated with saturated ammonium chloride solution and extracted with dichloromethane. The combined organic layers were dried over anhydrous sodium sulfate, concentrated under reduced pressure and purified by column chromatography (hexane, ethyl acetate; 95:5). The percentage yields were found to be comparable to that of synthesis via chalcones.

In vitro screening using MCF-7 breast cancer cell lines

The cell lines were grown in RPMI 1640 medium containing 10% fetal bovine serum and 2 mM L-glutamine. For present screening experiment, cells were inoculated into 96-well microtiter plates in 100 µl at plating densities as shown in the study details above, depending on the doubling time of individual cell lines. After cell inoculation, the microtiter plates were inoculated at 37°C, 5% CO_2, 95% air and 100% relative humidity for 24 h prior to addition of test compounds (Vanicha and Kanyawim, 2006).

After 24 h, one 96-well plate containing 5×10^3 cells/well was fixed in situ with TCA, to represent a measurement of the cell population at the time of drug addition (T_z). Test compounds were initially solubilized in dimethyl sulfoxide at 1 mg/ml and stored frozen prior to use. At the time of drug addition, an aliquot of frozen concentrate (1 mg/ml) was thawed and diluted to 100, 200, 400, and 800 µg/ml with complete medium containing test article. Aliquots of 10 µl of these different drug dilutions were added to the appropriate microtiter wells already containing 90 µl of medium, resulting in the required final drug concentrations (10, 20, 40, and 80 µg/ml) (Skehn *et al.*, 1990).

End point measurement

After the sample addition, plates were incubated at standard condition for 48 h and assay was terminated by the addition of cold TCA. Cells were fixed in situ by the gentle addition of 50 µl of cold 30% (w/v) TCA (final concentration, 10% TCA) and incubated for 60 min at 40°C. The

supernatant was discarded; the plates were washed five times with tap water and air dried. Sulforhodamine B (SRB) solution (50 μl) at 0.4% (w/v) in 1% acetic acid was added to each of the wells, and plates were incubated for 20 min at room temperature. After staining, unbound dye was recovered and the residual dye was removed by washing five times with 1% acetic acid. The plates were air dried. Bound stain was subsequently eluted with 10 mM trizma base, and the absorbance was read on a plate reader at a wavelength of 540 nm with 690 nm reference wavelength. Percent growth was calculated on a plate-by-plate basis for test wells relative to control wells. Percent growth was expressed as the ratio of average absorbance of the test well to the average absorbance of the control wells × 100.

Using the six absorbance measurements [time zero (T_z), control growth (C), and test growth in the presence of drug at the four concentration levels (T_i)], the percentage growth was calculated at each of the drug concentration levels. Percent growth inhibition was calculated as:

$[(T_i - T_z)/(C - T_z)] \times 100$

for concentrations for which $T_i \geq T_z$ $(T_i - T_z)$ positive or zero

$[(T_i - T_z)/T_z] \times 100$

for concentrations for which $T_i < T_z (T_i - T_z)$ negative

The dose–response parameters were calculated for each test article. Growth inhibition of 50% (GI_{50}) was calculated from $[(T_i - T_z)/(C - T_z)] \times 100 = 50$, which is the test compound concentration resulting in a 50% reduction in the net protein increase (as measured by SRB staining) in control cells during the test compound incubation. The drug concentration resulting in TGI was calculated from $T_i = T_z$. The LC_{50} (concentration of test compound resulting in a 50% reduction in the measured protein at the end of the test compound treatment as compared to that at the beginning) indicating a net loss of cells following treatment is calculated from

$[(T_i - T_z)/T_z] \times 100 = -50.$

Values were calculated for each of these three parameters if the level of activity was reached; however, if the effect was not reached or was exceeded, the values for that parameter were expressed as greater or less than the maximum or minimum concentration tested.

Docking

Computational details

Molecular modeling investigations were carried out using Dell Precision work station T3400 running Intel Core2 Duo Processor, 4 GB RAM, 250 GB hard disk, and NVidia Quodro FX 4500 graphics card. Genetic optimization for ligand docking (GOLD v4.0) molecular docking program used in this study, which was obtained from Cambridge Crystallographic Data Centre (CCDC), Cambridge (GOLD, 2008).

Protein preparation

The crystal structure of human placental microsomal aromatase with its bound natural substrate androstenedione was taken from the Protein Data Bank (PDB ID: 3EQM) (Ghosh *et al*., 2009) for protein preparation using Schrodinger protein preparation wizard tool (PPrep) (Protein preparation (PPrep) Wizard, 2009). This performs the following steps: assigning of bond orders, addition of hydrogens, and optimization of hydrogen bonds by flipping amino side chains, correction of charges, and minimization of the protein complex. All the bound water molecules, ligands, and cofactors were removed (preprocess) from the proteins which were taken in '.mae' format. The tool neutralized the side chains that are not close to the binding cavity and do not participate in salt bridges. This step is then followed by restrained minimization of co-crystallized complex, which reorients side chain hydroxyl groups and alleviates potential steric clashes. The complex obtained was minimized using OPLS_2005 force field with Polack-Ribiere Conjugate Gradient (PRCG) algorithm. The minimization was terminated either by completion of 5,000 steps or till the energy gradient converged below 0.05 kcal/mol.

Ligand preparation

Structures of the ligands were sketched using built panel of Maestro and taken in '.mae' format. LigPrep was used for final preparation of ligands, LigPrep is a utility of Schrodinger software suit that combines tools for generating 3D structures from 1D (Smiles) and 2D (SDF) representation, searching for tautomers, steric isomers and perform a geometry minimization of the ligands. The ligands were minimized by means of molecular mechanics force fields (OPLS_2005) with default setting.

GOLD docking

GOLD is a ligand-docking application that utilizes a genetic algorithm (GA) to explore ligand conformation flexibility and orientation with partial flexibility of the protein, and satisfy ligand-binding requirements. One advantage of GOLD over many other docking algorithms is that it allows for both unconstrained ligand flexibility and partial flexibility of the binding pocket thus affording a more realistic environment for ligand–receptor associations. For each of the 10 independent GA runs, a maximum number of 100

GA operations were performed. The standard set parameters were used in all the calculations. User defined default operator weights were used for crossover, mutation, and migration of 95, 95, and 10, respectively. Default cutoff values of 2.5 Å (for hydrogen bonds) and 4.0 Å (for vdW) were employed. Pop. Size = 100; max ops = 100,000; niche size = 2 were also employed. To further speed up the calculation, the GA docking was terminated when the top three solutions were within 1.5 Å RMSD of each other. Astex statistical potential (ASP) score recorded on each binding mode using a fitness function that accounts for the frequency of interactions between ligand and receptor atoms (heme) (Mooij and Verdonk, 2005). ASP score is the atom–atom potential derived from a database of protein–ligand complexes. Traditional scoring functions are based on force field or on regression, where parameters are derived from a set of experimental binding affinities and structures. ASP score uses a different approach; information about the frequency of interaction between ligand and receptor atoms (heme) is gathered by analyzing existing ligand–protein structures in the PDB and this information is used to generate statistical potentials. The empirical parameters used in the scoring function are hydrogen bond energies, atom radii, polarisabilities, torsion potentials, and hydrogen bond directionalities. The top 10 ranked solutions of the ligands were taken for further observation of binding orientation and H-bond interactions.

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