

Original Research Article

Design and Development of Halogenated Chalcone Derivatives as Potential Anticancer Agents

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Abstract

Purpose: To design and develop halogenated chalcone derivatives and evaluate them as anticancer agents using different cancer cell lines.

Methods: Based on *in silico* design and docking on known target, crystal structure of the complex of interleukin-1beta converting enzyme (ICE) with a peptide based inhibitor, (3S)-N-Methanesulfonyl-3-({1-[N-(2-naphthyl)-l-valyl]-l-prolyl}amino)-4-oxobutanamide (1BMQ), novel halogenated chalcone derivatives were designed (7a-h) employing LigandFit module of Accelrys (Discovery Studio, 2.1 version). Standard protocols for ligand and protein preparation were employed and their binding orientation validated using (3S)-N-Methanesulfonyl-3-({1-[N-(2-naphthyl)-l-valyl]-l-prolyl}amino)-4-oxobutanamide (MNO 601), a caspase inhibitor as reference standard. Energy minimized conformers with best dock scores were considered for the identification of interacting amino acid residues with ligands. Selected derivatives were synthesized and analyzed by melting point, ¹H NMR, IR and mass spectroscopy. Their evaluation for anticancer activity was carried out using adriamycin, paclitaxel and 5-fluorouracil as reference standards on prostate (PC-3), colon (COLO-205), ovary (OVCAR-5), liver (HEP-2) and neuroblastoma (IMR-32) cancer cell lines, and % growth inhibition and half maximal inhibitory concentration (IC₅₀) values were calculated.

Results: Among synthesized compounds, 7b showed the most promising cytotoxic activity with an IC₅₀ of 49.9 μM on colon cancer cell lines (Colo-205), followed by 7d with an IC₅₀ of 66.6 μM against ovarian cancer cell lines (OVCAR-5).

Conclusion: We report the successful synthesis, spectral characterization and *in vitro* anticancer evaluation of a series of novel halogenated chalcone derivatives against a number of human cancer cell lines. The findings indicate the emergence of new anticancer compounds.

Keywords: Halogenated chalcones, Dock scores, Anticancer activity, Interleukin-1beta converting enzyme.

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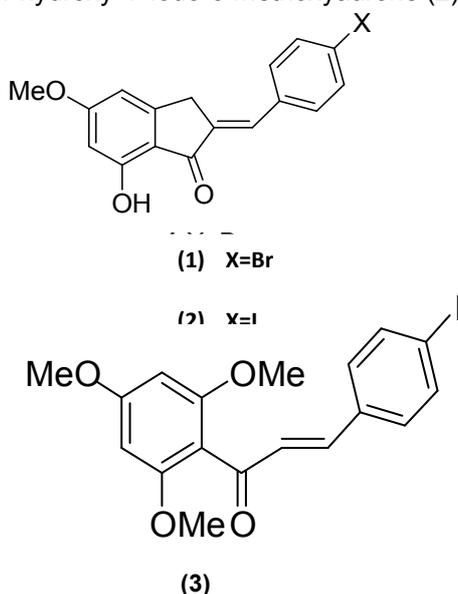
INTRODUCTION

Chalcones constitute one of the important classes of anticancer agents that have shown promising therapeutic efficacy in the

management of human cancers. They are considered as the precursors of flavonoids and isoflavonoids and are abundantly available in edible plants. They show a wide variety of activities, including anticancer [1], anti-

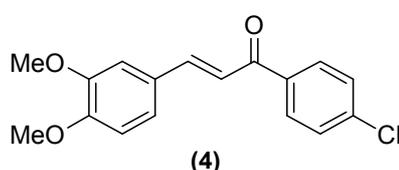
inflammatory [2] and antiparasitic [3] activities. A number of modifications on chalcone chromophore have been reported including hydroxyl [4], methoxy [5], and amino [6] groups as substituents with promising anticancer activity.

A series of 4-hydroxy-6-methoxyaurones and 4,6-dimethoxyaurones, which have binding affinity toward the nucleotide-binding domain of P-glycoprotein, an ABC (ATP-binding cassette) transporter that mediates the resistance of cancer cells to chemotherapy, have been synthesized [7]. These compounds differ by the nature of the substituent on the aurone B-ring. The most active compounds were halogenated aurones: 4-bromo-4-hydroxy-6-methoxyaurone (1) and 4-hydroxy-4-iodo-6-methoxyaurone (2).



Further, they reported a strategy of opening ring B to generate diaryl chalcones and succeeded in getting 2,4,6-trihydroxy-4-iodochalcone (3) with higher affinity with a K_D value in nanomolar range [8].

A number of chalcone derivatives have been evaluated for multidrug resistance (MDR) reversal activity on mouse lymphoma cells and it was found that their activity decreased when the hydrophobicity of the substituent on ring B was lowered, it is usually at 4-position (para to the carbonyl group) and the rank order of activity was found to be: chloro > dimethylamino > methoxyl group. One of them (4) exhibited activity at nanomolar concentrations *in vitro* [9].



These observations prompted us to design and synthesize halogenated chalcone derivatives based on *in silico* modeling and docking on known target 1BMQ and evaluated for their anticancer activity on different cell lines.

EXPERIMENTAL

Software tools

Molecular modeling was performed using Dell Precision work station T3400 running Intel Core2 Duo Processor, 4GB RAM, 250 GB hard disk, and NVidia Quadro FX 4500 graphics card. The software tool employed for the study was LigandFit module of Accelrys (Discovery Studio 2.1 version) [10].

Materials and equipment

Starting materials, reagents and solvents were purchased from commercial suppliers and purified/distilled/crystallized before use. All the melting points were recorded in open glass-capillaries on a Veego MP-D digital melting point apparatus and are uncorrected. Bruker Avance II (400 MHz) Nuclear Magnetic Resonance (NMR) spectrometer was used to record ^1H spectra. Chemical shifts (δ) are reported as downfield displacements from Tetramethylsilane (TMS) used as internal standard. Infrared (IR) spectra were recorded with Shimadzu FT-IR-8400S spectrophotometer on KBr pellets. Mass spectra (ESI-method), were recorded on Bruker Daltonics Esquire 300 mass spectrometer.

Molecular modeling studies

Design of ligands

The design of the target molecules was based on docking studies with the intention to gain an insight into the binding mode of the most active compounds of the series.

Docking protocols

It can be described as two components: a search strategy and an evaluation of docking results (scoring function). The search algorithm generates optimum number of poses including experimentally determined binding mode. The docked poses were scored using Dock score (Accelrys) to find the better docking pose.

Preparation of proteins

Docking was carried out against caspase-1 protein (1BMQ) which is the possible anticancer target of chalcone derivatives. The selected

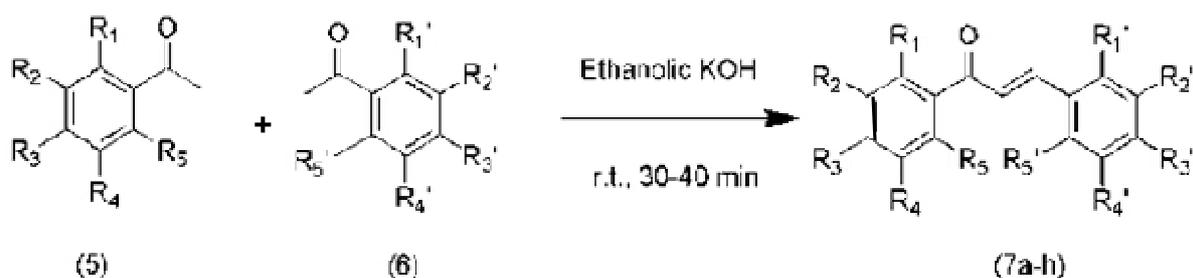


Figure 1: Scheme of synthesis

target protein was retrieved from Protein Data Bank (PDB) [13].

All ligands, bound water molecules and co-factors were removed from the proteins which were taken in .pdb format. CHARMM force field [14] for minimization of the protein {(steepest descent (gradient < 0.1) and conjugate gradient algorithms (gradient < 0.01)}. An appropriate active-site was defined to constitute amino acid residues (PDB: 1BMQ) of 10 Å from the centroid of the bound ligand (if required, partitioned up to 5 levels). Stochastic conformational searching was applied to the ligands with a number higher than the default number of Monte Carlo search steps, to ensure extensive conformational sampling.

Preparation of ligands

2-D Structures of the ligands were imported by sketching the structures from Chem Draw Ultra - 5.0 version [15]. The ligands were minimized by CHARMM Force Field with default setting. 'Prepare Ligands' module was used for the final preparation of ligands for docking run.

Ligand Fit docking

Ligand Fit (a shape-based method) employs a cavity detection algorithm. A shape comparison filter was combined with Monte Carlo technique to generate ligand conformations which were then docked into the active-site of a protein. Caspase-1 protein was chosen as a target for docking because the sensitivity of U937 monocytic cells to apoptosis induced by etoposide and that the apoptotic process involves the activity of members of the caspase-1 subfamily [16-19].

A grid resolution was set at 0.5 Å (default). The ligand-accessible grid was defined such that the minimum distance between a grid point and the protein is 2.0 Å for hydrogen and 2.5 Å for heavy atoms. The grid extended from the defined active site to a distance of 5 Å in all directions. This grid

was used to calculate the non-bonded interactions between the ligands and protein residues. Non-bonded cutoffs were set at 10 Å while using a distance-dependent dielectric constant. Ten iterations of minimization were performed before considering the next iterations and the selected conformers were minimized further to 100 iterations. The ligand conformational space was explored with different initial poses. To avoid identical conformations, RMSD cutoff of 1.5 and a score cutoff of 20 Kcal/mol were maintained while recording the final conformations. Docking run generated 10 poses for each ligand and assigned scores.

Synthesis

General procedure

Acetophenone (5) 1.1 mol was dissolved in ethanol with stirring and ethanolic KOH (6 mol) was added to this solution at 0 °C. The mixture was stirred for 10-30 min followed by the addition of 1 mol of the appropriate aldehyde (6). The completion of the reaction was monitored by TLC (CHCl₃:CH₃OH, 95:5) and the disappearance of aldehyde spot was considered as completion of the reaction. The solid was separated from the reaction mixture as a precipitate, filtered and re-crystallized using rectified spirit to obtain pure product (7).

Pharmacology

In vitro cytotoxicity against human cancer cell lines

The human cancer cell lines were procured from National Cancer Institute, Frederick, U.S.A. The cells were grown in tissue culture flasks in complete growth medium (RPMI-1640 medium with 2mM glutamine, pH 7.4, supplemented with 10 % fetal calf serum, 100 µg/ml streptomycin and 100 units/ml penicillin) in a carbon dioxide incubator (37 °C, 5 % CO₂, 90 %RH). The cells at subconfluent stage were harvested from the flask by treatment with trypsin {0.05 % in PBS

(pH 7.4) containing 0.02% EDTA}. Cells with viability of more than 98 %, as determined by trypan blue exclusion, were used for the determination of cytotoxicity. The cell suspension of 1×10^5 cells/ml was prepared in complete growth medium.

Stock solutions (2×10^{-2} M) of compounds 7a-h were prepared in Dimethyl sulfoxide (DMSO). The stock solutions were serially diluted with complete growth medium containing 50 µg/ml of gentamycin to obtain working test solutions of the required concentrations.

In vitro cytotoxicity against five human cancer cell lines was determined using 96-well tissue culture plates. Cell suspension (100 µL) was added to each well of the 96-well tissue culture plate. The cells were allowed to grow in carbon dioxide incubator (37 °C, 5 % CO₂, 90 %RH) for 24 h. Test materials in complete growth medium (100 µL) were added after 24 h of incubation to the wells containing cell suspension. The plates were further incubated for 48 h in a carbon dioxide incubator. The cell growth was stopped by gently layering trichloroacetic acid (50 %, 50 µL) on top of the medium in all the wells. The plates were incubated at 4 °C for one hour to fix the cells attached to the bottom of the wells. The liquid of all the wells was gently pipetted out and discarded. The plates were washed five times with distilled water to remove trichloroacetic acid, growth medium, low molecular weight metabolites, serum proteins, etc, and air-dried. The plates were stained with sulforhodamine B dye (0.4 % in 1 % acetic acid, 100 µL) for 30 min. The plates were washed five times with 1 % acetic acid and then air-dried. The adsorbed dye was dissolved in Tris-HCl buffer (100 µL, 0.01M, pH 10.4) and the plates were gently stirred for 10

min on a mechanical stirrer. The optical density (OD) was recorded on an ELISA reader at 540 nm.

The cell growth was determined by subtracting the mean OD value of the respective blank from the mean OD value of the experimental set. Growth (%) in the presence of the test material was calculated, considering the growth in the absence of any test material as 100 %; in turn, growth inhibition (%) in the presence of the test material was calculated [11,12].

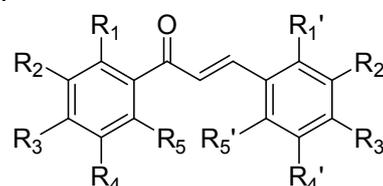
Statistical analysis

Values reported are the mean values of three experiments each carried in triplicate. IC₅₀ values were calculated using non-linear regression analysis with the aid of Prism (GraphPad Inc, USA). $P < 0.05$ was considered statistically significant in all cases.

RESULTS

Dock scores

Energy minimized conformer with best Dock scores (Table 1) was considered for the identification of interacting amino acid residues with ligands.



(7a-h)

Table 1: Docking studies of halogenated chalcones

Compound	R1	R2	R3	R4	R5	R1'	R2'	R3'	R4'	R5'	1BMQ Dock score
Standard (MNO601)											57.5
7a	H	H	Br	H	H	OCH ₃	H	OCH ₃	H	H	48.5
7b	H	H	OC ₆ H ₅	H	H	F	H	H	H	OCH ₃	48.6
7c	H	H	Br	H	H	Cl	OCH ₃	OCH ₃	H	Cl	51.87
7d	H	H	OCH ₃	H	H	Br	H	H	OCH ₃	H	49.62
7e	H	H	OC ₆ H ₅	H	H	H	H	OCH ₃	H	F	48.71
7f	H	H	Br	H	H	OCF ₃	H	H	H	H	47.3
7g	H	H	Br	H	H	Br	H	H	OCH ₃	H	46.05
7h	H	H	OCH ₃	H	H	Cl	OCH ₃	OCH ₃	H	Cl	49.39
7i	H	H	H	H	OCH ₃	H	OC ₂ H ₅	H	H	Cl	47.3
7j	H	H	H	H	H	H	H	H	H	Br	38.32
7k	H	H	H	H	H	H	H	H	H	H	35.89
7l	H	H	Br	H	H	H	H	H	H	H	37.36
7m	H	H	Cl	H	H	H	H	H	H	H	36.62
7n	H	H	H	H	H	H	Cl	H	H	H	37.73

Chemistry

In the present investigation eight new chalcone derivatives (**7a-h**) were prepared by the Claisen–Schmidt condensation of different ketones (**5**) and appropriately substituted aldehydes (**6**) using reported method (Figure 1, Table 2) [20].

The compounds were re-crystallized using rectified spirit. All the compounds were characterized by detailed spectroscopic (IR, ¹H NMR, Mass) analyses.

Table 2: Reaction time and yield of products 7a-h

Compound	Reaction time	
	(min)	Yield (%)
7a	16	94
7b	24	93
7c	12	90
7d	17	90
7e	30	89
7f	25	92
7g	13	82
7h	28	87

1-(4-Bromo-phenyl)-3-(2,4-dimethoxy-phenyl)-propenone (7a)

Yellow solid, m.p.: 89-91 °C, IR (KBr) 2852, 1652, 1463, 1453, 967 cm⁻¹; ¹H NMR (CDCl₃, 400 MHz, δ, ppm) 3.90 (3H, s, OCH₃), 3.86 (3H, s, OCH₃), 6.4 (1H, d, Ar), 6.5 (1H, dd, Ar), 7.4 (1H, d, Ar), 7.5 (1H, d, =CH-CO-), 7.6 (2H, d, Ar), 7.8 (2H, d, Ar), 8.0 (1H, d, =CH-Ar); MS (ESI, *m/z*) = 347 (M⁺+1, 81%), 349 (M⁺+2, 72%).

3-(2-Fluoro-6-methoxy-phenyl)-1-(4-phenoxy-phenyl)-propenone (7b)

Light yellow solid, m.p.: 92-95 °C, IR (KBr) 2951, 2851, 1659, 1462, 1454, 972 cm⁻¹; ¹H NMR (CDCl₃, 400 MHz, δ, ppm) 3.8 (3H, s, OMe), 6.65 (2H, m, Ar), 7.01 (4H, m, Ar), 7.1 (1H, d, =CH-CO-), 7.2 (4H, m, Ar), 7.8 (1H, d, =CH-Ar), 7.97 (2H, m, Ar); MS (ESI, *m/z*) = 350 (M⁺+1) (73%).

1-(4-Bromo-phenyl)-3-(2,6-dichloro-3,4-dimethoxy-phenyl)-propenone (7c)

Yellow solid, m. p.: 155-157 °C, IR (KBr) 2923, 2853, 1664, 1490, 1458, 974, 960 cm⁻¹; ¹H NMR (CDCl₃, 400 MHz, δ, ppm) 3.7 (3H, s, OMe), 3.85 (3H, s, OMe), 6.9 (1H, s, Ar), 7.56 (1H, d, =CH-CO-), 7.64 (2H, d, Ar), 7.78 (2H, d, Ar), 7.81 (1H, d, =CH-Ar); MS (ESI, *m/z*) = 416 (M⁺+1) (63%), 417 (M⁺+2) (65%).

3-(2-Bromo-5-methoxy-phenyl)-1-(4-methoxy-phenyl)-propenone (7d)

Yellow solid, m.p.: 121-123 °C, IR (KBr) 2922, 2852, 1658, 1462, 1455, 968 cm⁻¹; ¹H NMR (CDCl₃, 400 MHz, δ, ppm), 3.85 (3H, s, OMe), 3.89 (3H, s, OMe), 6.81 (1H, dd, Ar), 6.97 (2H, d, Ar), 7.23 (1H, d, =CH-CO-), 7.38 (1H, d, Ar), 7.51 (1H, d, Ar), 8.01 (1H, d, =CH-Ar), 8.06 (2H, d, Ar); MS (ESI, *m/z*) = 347 (M⁺+1) (75%), 349 (M⁺+2) (77%).

3-(2-Fluoro-4-methoxy-phenyl)-1-(4-phenoxy-phenyl)-propenone (7e)

Yellow solid, m.p.: 125-127 °C, IR (KBr) 2972, 2942, 2881, 2837, 1680, 1657, 1585, 1488, 1469, 988 cm⁻¹; ¹H NMR (CDCl₃, 400 MHz, δ, ppm), 3.85 (3H, s, OMe), 6.65 (2H, m, Ar), 7.01 (4H, m, Ar), 7.1 (1H, d, =CH-CO-), 7.2 (4H, m, Ar), 7.8 (1H, d, =CH-Ar), 7.94 (2H, m, Ar); MS (ESI, *m/z*) = 349 (M⁺+1) (62%).

1-(4-Bromo-phenyl)-3-(2-trifluoromethoxy-phenyl)-propenone (7f)

Yellow solid, m.p.: 180-183 °C, IR (KBr) 2852, 1654, 1596, 1552, 1462, 1455, 1376, 1306, 1154, 1078, 966 cm⁻¹; ¹H NMR (CDCl₃, 400 MHz, δ, ppm), 7.13 (1H, d, Ar), 7.17 (1H, d, =CH-CO), 7.38 (1H, m, Ar), 7.47 (2H, m, Ar), 7.57 (1H, d, =CH-Ar), 7.8 (2H, m, Ar), 7.95 (2H, m, Ar); MS (ESI, *m/z*) = 371 (M⁺+1) (62%), 373 (M⁺+2) (60%).

3-(2-Bromo-5-methoxy-phenyl)-1-(4-bromo-phenyl)-propenone (7g)

Yellow solid, m.p.: 192-194 °C, IR (KBr) 2842, 1678, 1582, 1481, 1394, 1245, 1072, 1014, 817 cm⁻¹; ¹H NMR (CDCl₃, 400 MHz δ, ppm), 3.85 (3H, s, OMe), 6.81 (1H, dd, Ar), 6.97 (2H, d, Ar), 7.23 (1H, d, =CH-CO-), 7.38 (1H, d, Ar), 7.51 (1H, d, Ar), 8.01 (1H, d, =CH-Ar), 8.06 (2H, d, Ar); MS (ESI, *m/z*) = 394 (M⁺+1) (66%), 396 (M⁺+2) (64%).

3-(2,6-Dichloro-3,4-dimethoxy-phenyl)-1-(4-methoxy-phenyl)-propenone (7h)

Yellow solid, m.p.: 131-133 °C, IR (KBr) 2945, 2877, 2837, 1669, 1606, 1490, 1434, 961 cm⁻¹; ¹H NMR (CDCl₃, 400 MHz, δ, ppm), 3.57 (3H, s, OMe), 3.7 (3H, s, OMe), 3.85 (3H, s, OMe), 6.9 (1H, s, Ar), 7.56 (1H, d, =CH-CO-), 7.59 (2H, d, Ar), 7.78 (2H, d, Ar), 7.81 (1H, d, =CH-Ar); MS (ESI, *m/z*) = 368 (M⁺+1) (82%).

Pharmacology

The *in vitro* cytotoxic activity of compounds 7a-h are reported in terms of % inhibitory concentration (IC₅₀) and the data are shown in Table 3.

The synthesized compounds showed good anticancer activity against colon and ovarian cancer cell lines. The results of anticancer activity revealed that in the case of colon cell line (COLO-205), the maximum inhibition 71% (50 µM) was observed for 7b with IC₅₀ = 49.9 followed by 37 % and 36 % for 7d and 7a at the same concentration. For ovarian cancer cell line (OVCAR-5) also the maximum inhibition 42 % (50 µM) and 39 % (10 µM) were observed for 7d (IC₅₀ = 66.6), followed by 38 % at the same concentration for 7a.

Against liver cell line (HEP-2) the maximum inhibition of 32% (50 µM) and 31% (50 µM) for

7b and 7d were observed. The inhibitory effect on CNS was also evaluated using (IMR-32) cell lines. In this case maximum inhibition of 35 % for 7d and 30 % for 7c (50 µM) were observed. In the case of prostate cancer cell line (PC-3) none of the compounds showed significant activity. Compounds 7b and 7d, however, exhibited 11 % inhibition at 50 µM.

DISCUSSION

In the present investigation, *in silico* docking studies were performed using the crystal structure of interleukin-1beta convertase (caspase-1) (PDB ID: 1BMQ) [13, 21] to recognize the hypothetical binding mode of the ligands with the receptor in order to design a series of novel halogenated chalcone derivatives (7a-h) as possible anticancer agents. To investigate the ability of molecular docking to

Table 3: *In vitro* cytotoxicity including IC₅₀ values of compounds 7(a-h) against different human cancer cell lines

Compound	Conc. / IC 50 (µM)	% Growth inhibition ^a / IC50 ^b				
		PC-3	Colo-205	OVCAR-5	Hep-2	IMR-32
7a	10	0	20	22	9	0
	50	8	36	38	19	0
	IC50	>100	>100	>100	>100	>100
7b	10	2	20	33	22	9
	50	11	71	34	32	25
	IC50	>100	49.9	>100	>100	>100
7c	10	1	32	11	16	8
	50	3	33	21	19	30
	IC50	>100	>100	>100	>100	>100
7d	10	6	18	39	7	9
	50	11	37	42	31	35
	IC50	>100	>100	66.56	>100	>100
7e	10	2	13	36	13	9
	50	2	18	37	15	13
	IC50	>100	>100	>100	>100	>100
7f	10	2	19	16	5	11
	50	5	22	24	7	20
	IC50	>100	>100	>100	>100	>100
7g	10	0	6	24	7	0
	50	6	10	34	11	20
	IC50	>100	>100	>100	>100	>100
7h	10	1	7	20	15	0
	50	1	9	22	23	9
	IC50	>100	>100	>100	>100	>100
Adriamycin	10	73	81	79	86	47
Paclitaxel	10	10	78	53	54	21
5 FU	2	9	39	32	10	11

^a % inhibition caused by the compounds and standard drugs at various concentrations.

^b 50 % inhibitory concentration represents the mean from dose response curves of number of experiments

reproduce an experimentally observed ligand binding mode, the co-crystallized ligand MNO 601 (3s)-n-methanesulfonyl-3-({1-[n-(2-naphthyl)-l-valyl]-l-prolyl}amino)-4-oxobutanamide has been used as reference ligand ($IC_{50} = 38$ nM). Subsequently, MNO 601 was docked back into its binding site of the crystal structure of the caspase-1 using Discovery Studio, Version 21 (2007), Accelrys Inc. [10].

The docking pose closely resembled the co-crystallized conformation. The naphthyl group showed significant π -stacking interaction with His342 and Arg383 that was proposed to be crucial for having the enhanced binding affinity of the co-crystal ligand [21]. The carbonyl oxygen atom formed a strong hydrogen bond with the side chains of Arg341 at a distance 2.70 Å. Moreover, the nitrogen atom of the amide group seems to be deprotonated and have a salt bridge with Arg341. The oxygen atom of methanesulfonylaminocarbonyl group was found to form a hydrogen bond with the nitrogen atom of guanidine moiety of Arg341 and pyrazole ring of His237 at a distance of 2.47 and 3.10 Å respectively. The aldehyde group of the ligand formed strong covalent bond with the sulphur atom of Cys285. All the designed compounds were docked following the same docking protocol and analyzed for their interactions with the binding site (active site) amino acids of caspase-1. Binding orientation of the most active compound, **7b**, is also presented (Figure 2).

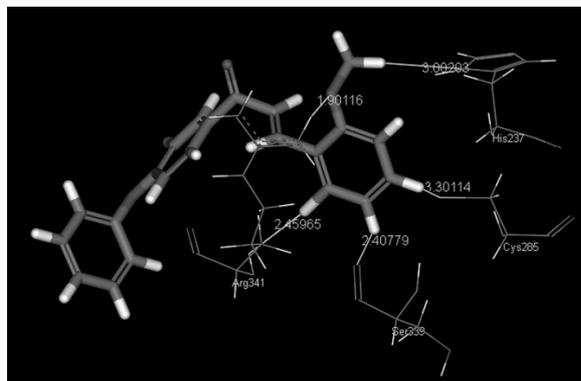


Figure 2: Binding orientation of the most active compound (**7b**)

The designed compounds were found to accommodate the binding pocket of the receptor showing the important interactions with the crucial amino acid residues. In particular, the O-methoxy moiety was found to form a significant hydrogen bond interaction with His237 and Arg341 at a distance of 3.0 Å and 1.9 Å respectively. Interestingly, no salt bridge formation was observed with the designed compounds. The fluoro group of the phenyl ring

formed crucial hydrogen bond with Arg341. Weak hydrogen bond is also observed between phenyl ring and Cys 285. Additional interaction with Ser339 was also seen.

All the compounds with predicted good binding affinities (docking score) as compared to the reference standard (MNO 601) (Table-1) were selected for synthesis (Figure 1, Table 2) and later on evaluated for anticancer activity. The synthesized compounds showed good anticancer activity against colon and ovarian cancer cell lines. The *in vitro* results (Table 3) showed that these molecules may potentially be used for targeting caspase-1 for the management of cancer expressing caspase dependent apoptosis pathway. Therefore, the synthesized compound, 3-(2-Fluoro-6-methoxy-phenyl)-1-(4-phenoxy-phenyl)-propenone (**7b**) may possess essential candidature to be studied *in vivo* for proving anticancer efficacy.

CONCLUSION

The new halogenated chalcones designed and synthesized, based on the bioisosteric replacement of a known ligand, were well docked onto the binding pocket of caspase enzyme and interacted with crucial amino acid residues. Compounds **7a**, **7b**, **7d** are active on colon cancer line COLO-205, and **7b** showed IC_{50} value of 49.9. These molecules may serve as useful 'lead' compounds for further development.

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