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Synthesis, Antioxidant, Antibacterial and Cytotoxic Activity of Novel Chromone Derivatives

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ABSTRACT

The present work was aimed to synthesize novel chromone derivatives to target estrogen receptor positive breast cancer. About 80% of all breast cancers are "ER-positive. The chromone scaffold is a privileged scaffold for exploration of anticancer agents. 3(4-oxo-4H-chromen-3-yl)acrylic acid amides derivatives designed, synthesized by employing the molecular hybridization approach between different aromatic, aliphatic amines and 3(4-oxo-4H-chromen-3-yl)acrylic acid. The docking study of 3(4-oxo-4H-chromen-3-yl)acrylic acid amides were performed using Schrodinger 2015 (maestro 10.1) on human estrogen receptor α-Ligand-Binding domain (1XP6), Tyrosyl-t-RNA synthetase protein (1JIK), DNA gyrase protein (4DUH), nitric oxide synthase (3NLE) and evaluated in vitro antioxidant activity, antibacterial activity, cytotoxicity against human Breast Cancer Cell Line (MCF-7). The in silico studies indicated that 3(4-oxo-4H-chromen-3-yl)acrylic acid amides derivatives exhibited comparable docking score and good hydrogen bond interactions with the amino acids present in the active site of 3NLE and 1XP6. Many of the synthesized compounds exhibited potent antioxidant and cytotoxic activity. The most potent antioxidant activity was observed for compound A_5 with IC_{50} value of 0.5 µg/ml, most potent anticancer activity was observed for compound A_1 with IC_{50} value of 37.13 µg/ml and potent antibacterial activity was observed for compound A_1 with Minimum Inhibitory Concentration (MIC) value of 100 µg/ml against Escheriea coli and Proteus vulgaris.

Keywords: 3(4-oxo-4H-chromen-3-yl)acrylic acid amides, Estrogen receptor, Breast cancer, Antioxidant activity, Antibacterial activity

INTRODUCTION

Estrogen receptor-positive (ER+) breast cancer is the most common type of breast cancer diagnosed today. According to the American Cancer Society, about two out of every three cases of breast cancer are hormone receptor positive. Most of these cases are ER+ or receptive to both estrogen and progesterone [1]. In Estrogen receptor positive breast cancer the level of Estrogen is a key factor for the initiation and progression of breast cancer [2-5]. In the mammary epithelial, estrogen controls many cellular activities such as proliferation, differentiation and migration [6,7]. There are two genetically distinct and functional Estrogen Receptors (ERs), ERα and ERβ, belonging to the superfamily of nuclear receptors for steroid/thyroid hormones. The structural differences between the two ERs indicate that they serve distinct actions [8]. Estrogen exert its functions in different tissues by binding with its receptors, including alpha and beta (ERα and ERβ), the former is the major one involved in breast cancer and chosen as an important target for endocrine therapy in clinic [2,9].

Heterocycles play an important role in the design and discovery of new physiological/pharmacologically active compounds [10]. Chromone (1) (4H-chromen-4-one, 4H-1-benzopyran-4-one) is an important class of oxygen-containing heterocyclic compounds with a benzoannelated γ-pyrone ring and they are part of the flavonoid family. The chromone and related compounds are widespread in the plant kingdom from algae to conifers. Chromones have found to be active in a number of plant cycles, including growth regulation, indole acetic acid oxidation and dormancy inhibition as well as exhibiting cytokinin-type behavior and stimulating oxygen uptake in plant tissue [11]. Chromone derivatives are abundant in nature and exhibit a wide range of pharmacological activity like antibacterial, antifungal [12,13], anticancer [14], antioxidant [15], anti-HIV [16], antiulcer [17], immunostimulators [18], biocidal [19], wound healing [20], antiinflammatory [21], and immune stimulatory [22]. Many chromone derivatives are also photoactive and can be used easily in various photo induced reactions affording diverse heterocyclic compounds [23]. Chromone derivatives are also active at benzodiazepine receptors [24] and on lipoxygenase and cyclooxygenase [25]. In addition to this, they have been shown to be possessing antimutagenic properties [26] as well as the ability to inhibit electron transport through inhibition at Nicotinamide Adenine Dinucleotide Hydrogen (NADH): Ubiquinone oxidoreducatase and phorbol ester-induced ornithine decarboxylase [27,28]. Chromones may also have application in cystic fibrosis treatment, as they activate the cystic fibrosis transmembrane conductance regulator. These compounds also possess Low mammalian toxicity and are present in large amounts in the diet of humans due to their origin in plants [25]. Acrylic acid derivatives have wide range of therapeutically importance such as, Anti-tumor activity [29], antioxidant activity [30] and antibacterial [31] activity.

In recent years styryl chromones have been reported for breast cancer activity by Bhatnagar et al. An emerging strategy within medicinal chemistry and drug discovery is the combination of two distinct pharmacophores into a single molecule, well documented as Molecular Hybridization (MH). Based on the more diverse biological activities of chromone and chromone derivatives, in the present study, we made an attempt to synthesize some novel 3(4-oxo-4H-chromen-3-yl)acrylic acid Amides by employing hybridization approach with an aim to obtain possible novel breast cancer agents and potent antioxidants.

MATERIALS AND METHODS

Chemistry

All chemicals and dry solvents were purchased from the local manufacturers and S.D Fine Chem. Ltd, Mumbai, India. All the chemicals used in the synthesis were obtained from standard commercial sources. All Reactions were monitored by Thin Layer Chromatography (TLC) carried out on EMerck silica gel plates ($60F_{254}$) with UV light, iodine as probing agents. Column chromatography separation was performed using Avra Synthesis Pvt. Ltd. Silica gel 60, 0.140-0.25 mm (60-120 mesh) using combination of Ethyl acetate and Hexane. Melting points were determined on an digital melting point apparatus (Jain Scientific glass works) by open capillary method and are uncorrected. Proton Nuclear Magnetic Resonance (^{1}H-NMR) and Carbon-13 Nuclear Magnetic Resonance (^{13}C-NMR) spectra were recorded on Varian Unity 400 or Varian Inova 500 or Bruker Avance 300 MHz. Chemical shifts are relative to Tetramethylsilane (TMS) as an internal standard. Mass spectra recorded on Agilent LC/MSD trap SL 1100 series spectrometer with a 70 eV (ESI probe). Infrared (IR) spectra were recorded a Thermo nicolet Nexus 670 FTIR spectrometer, Perkin-Elmer Infrared-683 or 1310 with NaCl optics. The names of all compounds given in the experimental section were taken from Chemdraw Ultra, Version 8.0. All the reactions were carried out in dried glassware under an atmosphere of nitrogen.

General procedure for the synthesis of 4-oxo-4H-chromene-3-carbaldehyde (1)

To the dry dimethylformamide (121 ml) in a three necked flask, phosphorus oxychloride (0.49 mol) was added slowly with intensive stirring at 50°C. Heating and stirring was continued for 2 h at 45-55°C. The solution of 2-hydroxyacetophenone (0.12 mol) in Dimethyl Formamide (DMF) (25 ml) was then slowly added under stirring at 50°C. The stirring was continued for 2 h at 55-60°C. After cooling the mixture was kept overnight at room temperature and diluted slowly by adding crushed ice (500 g) and stirred again for 6 h. The crystals were filtered off and recrystallized from alcohol [32].

General procedure for the synthesis of (2E)-3-(4-oxo-4H-chromen-3-yl)acrylic acid (2)

A mixture of 3-formyl chromone (10 mmol) and malonic acid (20 mmol), in the presence of pyridine (5 ml) was refluxed in 50 ml round bottom flask for 30-45 min with vigorous stirring. After completion of reaction (monitored by TLC), the reaction mixture was cooled to room temperature, the pH was adjusted to 1.0 with conc. HCl and the reaction mass was again stirred for 30 min. The yellow coloured solid thus obtained was filtered and washed with 15 ml 2 1 N HCl and dried [33].

General procedure for the synthesis of different (2E)-3-(4-oxo-4H-chromen-3-yl)acrylamides (3a-e)

Acid (1 eqv.) and Amine (1 eqv.) were dissolved in a dimethylformamide (10 ml). The solution was cooled to 0°C under an atmosphere of nitrogen, then Hydroxybenzotriazole (HOBt, 1.2 eqv.) and 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC·HCl, 1.2 eqv.) were added, followed by N-methyl morpholine (3 eqv). The mixture was warmed to room temperature and stirred for 24 h, then partitioned between saturated aqueous sodium bicarbonate (100 ml) and ethyl acetate (100 ml). The aqueous phase was extracted with ethyl acetate (3 × 100 ml) and the combined organic phases were washed with water (100 ml) and brine (2 × 100 ml) then dried ($MgSO_4$). The solvent was removed under reduced pressure. The residue was purified by column chromatography on silica gel (Table 1) [34].

Synthesis of (2E)-N-(4-hydroxyphenyl)-3-(4-oxo-4H-chromen-3-yl)acrylamide (A_1)

Yield: 70%; mp: 178-180°C; ^{1}H-NMR ($CDCl_3$), δ=5.17 (m, 1H), 6.84 (d, J=8.6 Hz, 3H), 7.05-6.90 (m, 5H), 7.28 (d, J=12.2 Hz, 1H), 7.39 (t, J=8.4 Hz, 1H), 7.50 (s, 1H), 7.88 (d, J=7.7 Hz, 1H); ^{13}C-NMR ($CDCl_3$) δ=181.6, 161.6, 158.1, 153.7, 140.9, 133.4, 131.816, 125.6, 123.7, 122.7, 120.6, 117.7, 117.4, 116.9, 115.9, 104.5; IR (KBr) cm^{-1} 3289.36, 3254.03, 3137.45, 3091.66, 1662.81, 1616.54, 1560.22, 1412.67, 1246.19, 826.30.

Synthesis of (2E)-N-(3-hydroxyphenyl)-3-(4-oxo-4H-chromen-3-yl)acrylamide (A_2)

Yield: 72%; mp: 184-186°C; ^{1}H-NMR($CDCl_3$), δ=4.27 (s, 1H), 5.75 (s, 3H), 6.55-6.03 (m, 7H), 7.04 (s, 1H), 10.84 (s, 1H); ^{13}C-NMR ($CDCl_3$), δ=183.9, 171.0, 159.5, 157.8, 141.7, 140.7, 138.0, 135.0, 131.2, 127.0, 125.2, 123.4, 121.9, 118.1, 111.6, 108.7, 106.9, 104.1; IR (KBr) cm^{-1} 3274.06, 2923.10, 1783.14, 1651.32, 1463.84, 1222.69, 768.67.

Synthesis of (2E)-N-methyl-3-(4-oxo-4H-chromen-3-yl)acrylamide (A_3)

Yield: 80%; mp: 170-172°C; ^{1}H-NMR ($CDCl_3$), δ=3.64 (s, 3H), 6.63 (d, 1H, J=9.460 HZ), 6.94 (t, 1H, J=7.477HZ), 7.08 (d, 1H, J=8.240 HZ), 7.51-7.58 (m, 2H), 7.78 (d, 1H, J=9.308 HZ), 7.98 (s, 1H), 11.42 (s, 1H); ^{13}C-NMR ($CDCl_3$), δ=32.7, 117.2, 117.6, 123.5, 125.8, 126.1, 133.5, 133.6, 146.3, 151.1, 157.9, 176.9, 179.8; IR (KBr) cm^{-1} 3065.33, 2923.44, 1670.48, 1624.89, 1583.49, 1453.83, 1222.90, 1150.77.

Synthesis of (2E)-N-butyl-3-(4-oxo-4H-chromen-3-yl)acrylamide (A_4)

Yield: 75%; mp: 181-183°C; ^{1}H-NMR($CDCl_3$), δ=0.88 (t, 3H, J=7.324HZ), 1.21-1.33 (m, 2H), 1.41 (d, 2H, J=6.409HZ), 1.46-1.52 (m, 2H), 5.03-5.07 (m, 1H), 6.87 (t, 1H, J=8.087 HZ), 6.94 -7.00 (m, 1H), 7.13 (d,1H, J=12.817 HZ), 7.36-7.48 (m, 2H), 7.70 (dd, 1H, J=1.831 HZ), 9.99 (t, 1H, J=6.256 HZ); ^{13}C-NMR ($CDCl_3$), δ=13.6, 19.2, 32.7, 47.9, 117.2, 117.6, 120.9, 123.5, 125.8, 126.1, 133.5, 146.3, 151.1, 157.9, 176.9, 179.8; IR (KBr) cm^{-1} 3289.17, 3202.80, 3050.59, 2981.99, 1683.45, 1602.33, 1534.76, 1410.04, 1233.68, 1178.32;

Synthesis of (2E)-3-(4-oxo-4H-chromen-3-yl)-N-phenylacrylamide (A_5)

Yield: 75%; mp: 182-184°C; ^{1}H-NMR ($CDCl_3$) δ=7.27-7.48 (m, 9H), 7.57 (s, 1H), 7.68-7.71 (m, 1H), 8.10 (s, 1H), 8.26 (d, J=8.087 Hz, 1H); ^{13}C-NMR ($CDCl_3$), δ=118.0, 119.0, 121.3, 123.5, 124.1, 125.8, 126.2, 128.1, 128.7, 129.1, 134.0, 134.3, 136.3, 143.4, 155.4, 157.6, 166.9, 175.7; IR (KBr) cm^{-1} 3297.30, 3202.17, 3044.53, 2872.42, 1678.65, 1598.33, 1457.21, 1232.83.

Docking studies

Docking was performed using Schrodinger 2015 (maestro 10.1) version software on HP Compaq 6200 Pro MT PC workstation (Intel(R) Core(TM) i7 CPU 2600 @3.40 GHz; 8 GB Ram, 500 GB Hard disk). The typical structure file from the Protein Data Bank (PDB) was not suitable for immediate use in molecular modelling calculations. A typical PDB structure file consists only of heavy atoms and may include a co-crystallized ligand, water molecules, metal ions, and cofactors. In a few PDB files, the interatomic distances in the backbone differ substantially from standard values, causing PDB reading functionality to miss some connectivity's and break the molecules in multiple chains. Some PDB structures were multimeric, and may need to be reduced to a single unit. Schrodinger had therefore assembled a set of tools to prepare proteins in a form that was suitable for modelling calculations. The protein preparation allows to download a protein from its raw state, (Which may be having missing hydrogen atoms and incorrect bond order assignments, charge states, or orientations of various groups) and to convert to a state in which it was properly prepared for calculations. Tyrosyl-t-RNA synthetase protein (1JIK), DNA gyrase protein (4DUH), nitric oxide synthase (3NLE), Human Estrogen Receptor Alpha Ligand-Binding Domain (1XP6) were selected as the Targets.

BIOLOGICAL ACTIVITY

Antioxidant activity

The free radical scavenging activity of all the samples was evaluated by 1,1-diphenyl-2-picryl-hydrazyl (DPPH) according to the previously reported method by Shen et al. Briefly an 0.1 mM solution of DPPH in ethanol was prepared and 1 ml of this solution was added to 3 ml of the solution of all samples in ethanol at different concentration (1, 2, 3, 4, 5 and 10 µg/ml). The mixtures were shaken vigorously and allowed to stand at room temperature for 30 min. Then the absorbance was measured at 517 nm using a UV-Visible spectrophotometer. Ascorbic acid was used as the reference. Lower absorbance values of reaction mixture indicate higher free radical scavenging activity [35].

Breast cancer activity

The monolayer cell culture was trypsinized and the cell count was adjusted to 1.0×10^5 cells/ml using Eagle's Minimal Essential Medium (EMEM) and Dulbecco's Modified Eagle's Medium (DMEM) containing 10% FBS. To each well of the 96-well microtitre plate, 0.1 ml of the diluted cell suspension (Approximately 10,000 cells) was added. After 24 h, when a partial monolayer was formed, the supernatant was flicked off, washed the monolayer once with medium and 100 µl of different concentrations of test drug were added on to the partial monolayer in microtitre plates. The plates were then incubated at 37°C for 3 days in 5% CO_2 atmosphere, and microscopic examination was carried out and observations were noted every 24 h interval. After 72 h, the drug solutions in the wells were discarded and 50 µl of 3-(4,5-dimethythiazol-. 2-yl)-2,5-diphenyl Tetrazolium Bromide (MTT) in Phosphate Buffer Solution (PBS) was added to each well. The plates were gently shaken and incubated for 3 h at 37°C in 5% CO_2 atmosphere. The supernatant was removed and 100 µl of propanol was added and the plates were gently shaken to solubilize the formed formazan. The absorbance was measured using a microplate reader at a wavelength of 540 nm. The percentage growth inhibition was calculated using the following formula and concentration of test drug needed to inhibit cell growth by 50% (CTC_{50}) values is generated from the dose-response curves for each cell line [36].

Antibacterial activity

The ability of the compounds to inhibit growth of clinical bacteria was determined using the agar disc diffusion method. Sterile filter paper discs, 11 mm in diameter were impregnated with each compound concentration and dried at 30°C in the static incubator. They were then carefully placed aseptically with a forceps on the surface of the Nutrient Agar (NA) plates that were preinoculated with the 24 h culture of bacteria and 0.1 ml spore suspension (1 × 105 spores/ml). The control antibiotics disc containing gentamicin (40 µg/ml) was placed on each of the inoculated plates of nutrient agar. The plates were left on the bench undisturbed for few minutes, after which the bacterial culture plates were incubated at 37°C for 24 h. The external diameters of visible zones of growth inhibition were measured after incubation [37].

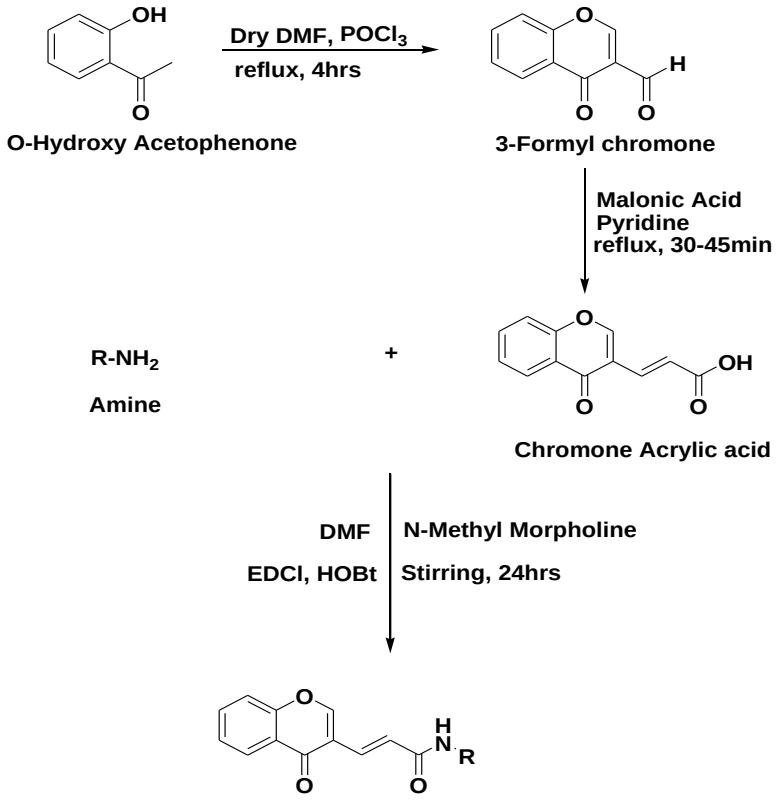
RESULTS AND DISCUSSION

Chemistry

The synthesis of 3(4-oxo-4H-chromen-3-yl)acrylic acid amides (3a-e) is shown in Scheme 1. In the first step 4-oxo-4H-chromene-3-carbaldehyde (1) was synthesized by refluxing dry dimethylformamide, Phosphorus oxychloride, 2-hydroxyacetophenone for 4 h. Compound 1 was refluxed with malonic acid in the presence of pyridine for 30-45 min to give (2E)-3-(4-oxo-4H-chromen-3-yl)acrylic acid (2). Compound 2 was then coupled with various aromatic and aliphatic amines by using EDC·HCl and HOBt to give (2E)-3-(4-oxo-4H-chromen-3-yl)acrylamides (3a-e). The Structures of all the synthesized compounds were confirmed on the basis of ^{1}H-NMR, ^{13}C-NMR, and IR spectral data.

All the compounds (3a-e) exhibited characteristic absorption bands in the IR spectra (cm^{-1}) i.e., 3254.03 (N-H amide), 3137.45 (C-H alkene), 3091.66 (C-H aromatic), 1662.81 (C=O keto), 1616.54 (C=O amide), 1560.22 (C=C alkene), 1412.67 (C=C aromatic) and at other regions of the spectrum depending upon the specific substituents present in each compound. The ^{1}H-NMR spectra of the compounds (3a-e) revealed the characteristic protons in between δ=0.88 and 8.26. The spectra also showed the peaks accounting for the aromatic protons and for the different substituent protons in between the corresponding regions of the spectrum. The ^{13}C-NMR spectra of the compounds (3a-e) exhibited the characteristic peaks of the carbonyl carbon in between δ=165-170, apart from the peaks corresponding to the other carbons.

The ^{1}H-NMR spectrum (400 MHz, $CDCl_3$) of A_3 showed the characteristic signals in between δ=3.64 and 11.42. The singlets at δ=3.64, 6.63, 7.78, 7.98, 11.42 indicates the presence of methyl, ethylenic, dihydro pyran, NH protons respectively. Signals at δ=6.94, 7.08, 7.51-7.58 indicates the presence of aromatic protons. ^{13}C-NMR spectrum showed methyl, ethylenic, aromatic, carbonyl carbons signals at δ=32.748, 146.381, 123.513, 157.949, 151.156, 133.660, 133.529, 126.105, 125.867, 117.655, 117.239, 176.916, 179.853.



Scheme 1: Synthesis of 3(4-oxo-4H-chromen-3-yl)acrylic acid amides (3a-e)

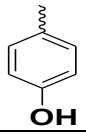
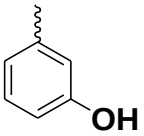
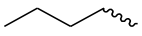
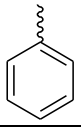
Compound	R
3a	
3b	
3c	
3d	
3e	

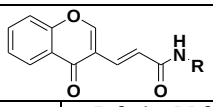
Table 1: Physical characterization data of compounds (A_1-A_5)

Docking and biological activity

***In vitro* cytotoxic activity**

Results indicate that all the chromone linked amides synthesized, showed good cytotoxic activity (Table 2). Among the compounds tested, A_1 with 4"-phenol substitution was found to be the most potent compound and having a IC_{50} value of 37.13 µg/ml. The chromone linked amides A_2 having a 3"-phenol substitution (Figure 1), A_4 having butyl substitution, A_5 having phenyl substitution were also found to be potent with a IC_{50} value of below 50 µg/ml. Cytotoxicity was reduced in A_3 with an IC_{50} value >100 µg/ml when the butyl side chain replaced with methyl.

XP docking studies indicates that chromone linked amides have good binding ability with Human Estrogen Receptor Alpha Ligand-Binding Domain in complex with compound 16 (PDB ID: 1XP6) (Tables 3 and 4). They shows good docking score and also showing good hydrogen bonding interaction with amino acids present in the active site of 1XP6. Among the compounds A_5 and A_1 was found to have more docking score i.e., -8.787, -7.99 respectively. Remaining compounds in this series was found to have good docking score and showing hydrogen bond interactions with LYS 531, ASP 351 (Figure 2).



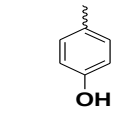
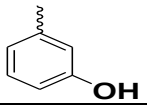
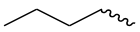
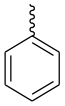
Compounds	R	Molecular formula	Relative Molecular Mass (RMM)	Melting point (°C)	Yield %
A_1		$C_{18}H_{13}NO_4$	307.3	178-180	70
A_2		$C_{18}H_{13}NO_4$	307.3	184-186	72
A_3		$C_{13}H_{11}NO_3$	229.23	170-172	80
A_4		$C_{16}H_{17}NO_3$	271.31	181-183	75
A_5		$C_{18}H_{13}NO_3$	291.3	182-184	75

Table 2: Cytotoxic activity of chromone linked amides (A_1 to A_5). Expressed as CTC_{50} in µg/ml

Compound	CTC_{50}
A_1	37.13
A_2	40.83
A_3	> 100
A_4	42.10
A_5	41.24
Tamoxifen	10.15

Table 3: Extra precision (XP) docking results of Schrodinger: 1XP6 (Human Estrogen Receptor Alpha Ligand-Binding Domain in complex with compound 16). G Score, H-Bond, Glide energy values of docked ligands

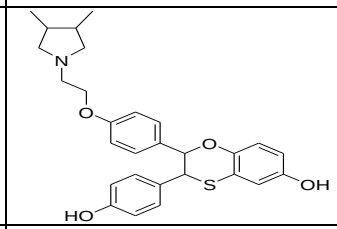
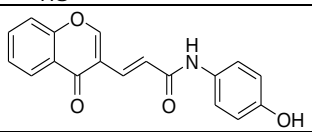
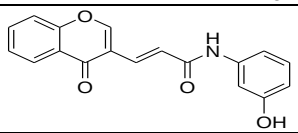
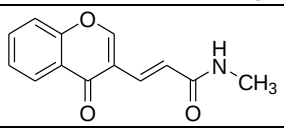
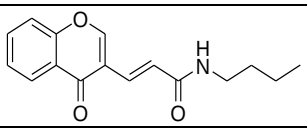
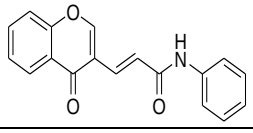
Compound	Structure	G Score	H-Bond	Glide energy (Kcal/mol)
Existing ligand		-14.246	-2.155	-75.647
A_1		-7.99	0	-40.391
A_2		-7.644	-0.7	-39.432
A_3		-6.721	-0.359	-31.203
A_4		-7.455	0	-36.284
A_5		-8.787	0	-40.18

Table 4: Extra precision (XP) docking results of Schrodinger: Hydrophilic and hydrophobic interactions of compounds with amino acids of 1XP6

S. No.	Compound	Interacting residues
1.	A_1	LYS 531, TRP 383
2.	A_2	LYS 531, TRP 383, PHE 404
3.	A_3	PHE 404
4.	A_4	ASP 351
5.	A_5	TRP 383, LYS 531

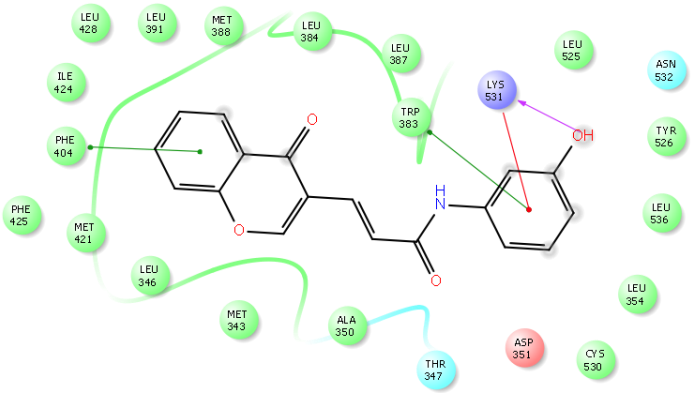


Figure 1: Interactions (Hydrophobic, hydrophilic) of A_2 with amino acids present in the active site of 1XP6

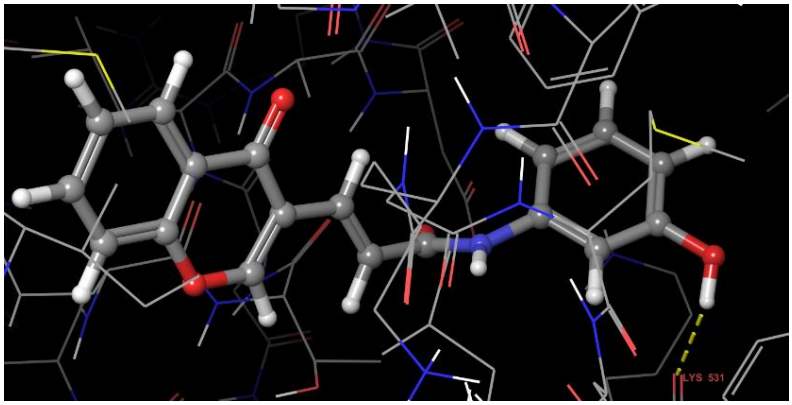


Figure 2: H-Bonding interactions of A_2 with amino acids present in the active site of 1XP6

Antioxidant activity

Results showed that all the chromone linked amides synthesized, showed most potent antioxidant activity, comparable with that of the standard (Table 5). Among the compounds tested, A_5 with phenyl moiety and A_1 with 4-phenol moiety were found to be the most potent antioxidants and having an IC_{50} value of 0.5 µg/ml and 0.6 µg/ml respectively. A_2 having a 3-phenol, A_3 having methyl substitution (Figure 3) and A_4 having butyl substitution were also found to be equipotent with a IC_{50} value of 1 µg/ml. All compounds were found to be more potent than well-known antioxidant ascorbic acid in the DPPH-assay. Individually chromone and amino phenols were showed antioxidant activity. In present work we are linking the chromone with amines by using a linker so the resulting compounds are showing more potent antioxidant activity.

Docking results also indicates that compounds have good binding ability with endothelial nitric oxide synthase (Tables 6 and 7). All compounds was found to have more docking score than existing ligand and also showing good hydrogen bonding interaction with Amino acids present in the active site of 3NLE. Among the compounds A_1 and A_5 was found to have more docking score i.e., -8.644, -9.003 respectively and showing strong hydrogen bond interactions with SER 428, TRP 358 and GLY 188. Remaining compounds in this series was found to have good docking score and showing hydrogen bond interactions with PHE 475, GLY 188 and TRP 358 (Figure 4).

Table 5: Anti-oxidant activity of chromone linked amides (A_1 to A_5). (Expressed as IC_{50} in µg/ml)

Compound	IC_{50}
A_1	0.6
A_2	1
A_3	1
A_4	1
A_5	0.5
Standard (Ascorbic acid)	3

Table 6: Extra precision (XP) docking results of Schrodinger: 3NLE (Endothelial nitric oxide synthase). G Score, H-Bond, Glide energy values of docked ligands

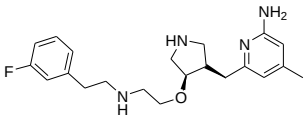
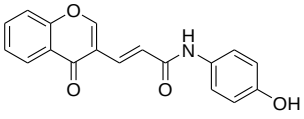
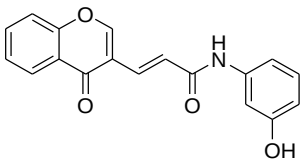
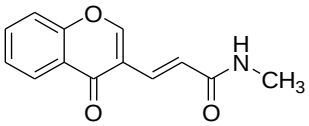
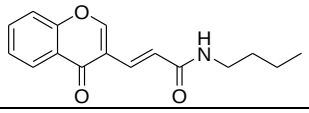
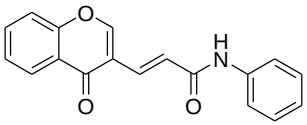
Compound	Structure	G Score	H-Bond	Glide energy (Kcal/mol)
Existing ligand		-6.537	0	-29.457
A_1		-8.644	-0.299	-40.325
A_2		-8.517	-0.7	-39.453
A_3		-7.026	-0.379	-27.349
A_4		-7.743	1.045	-31.527
A_5		-9.003	-0.7	-36.025

Table 7: Extra precision (XP) docking results of Schrodinger: Hydrophilic and hydrophobic interactions of compounds with amino acids of 3NLE

S. No.	Compound	Interacting residues
1	A_1	SER 428. TRP 180. PHE 355
2	A_2	PHE 475, TRP 180
3	A_3	GLY 188, TRP 358, TRP 180, PHE 475
4	A_4	TRP 358, GLY 188, PHE 355, PHE 475
5	A_5	TRP 358, GLY 188, TRP 180, PHE 355

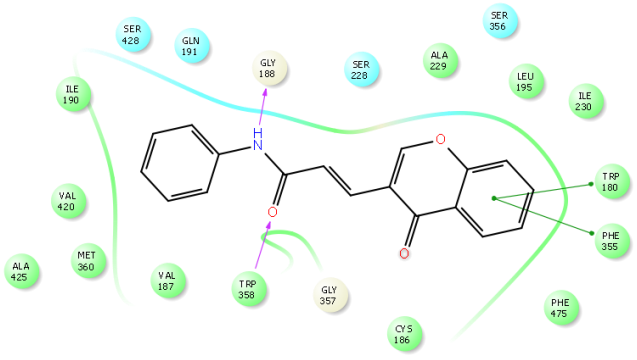


Figure 3: Interactions (Hydrophobic, hydrophilic) of A_5 with amino acids present in the active site of 3NLE

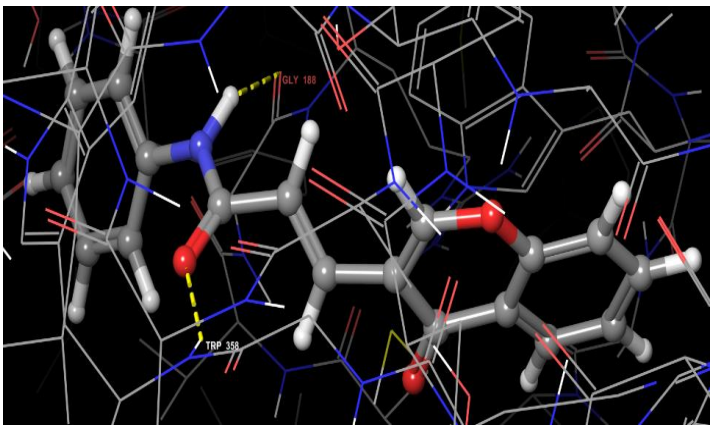


Figure 4: H-Bonding interactions of A_5 with amino acids present in the active site of 3NLE

Antibacterial activity

Gram-positive organism (*Staphylococcus aureus*): From the results it is evident that the chromone linked amides synthesized, showed antibacterial activity against the tested organism, but not comparable with that of the standard. Among the compounds tested, A_1 having a 4"-phenol substitution, A_2 having 3"-phenol substitution and A_4 having 1"-butyl substitution were found to be potent against *S. aureus* with a MIC value of 500 µg/ml. All the other chromone linked amides i.e., A_3 having 1"-methyl substitution, A_5 having 1"-phenyl substitution, were found to inactive against *S. aureus* (Tables 8-12).

XP docking indicates that compounds have good binding ability with *S. aureus* tyrosyl-tRNA synthetase (PDB ID: 1JIK). Most potent compounds in this series i.e., A_1, A_2 and A_4 was found to have good docking score and showing hydrogen bond interactions with ASP 177, TYR 36, GLY 49, GLY 193 and GLY 38 amino acids present in the active site of *S. aureus* tyrosyl-tRNA synthetase (Figures 5 and 6).

Gram-negative organisms (*E. coli*): Among the compounds tested, A_1 having a 4"-phenol substitution was found to be most potent against *E. coli* with a MIC value of 100 µg/ml. Some of the chromone linked amides i.e., A_2 having 3"-phenol substitution and A_5 having 1"-phenyl substitution, were found to potent against *E. coli* with a MIC of 500 µg/ml. Most potent compounds in this series i.e., A_1, A_2, and A_5 were found to have good docking score and showing hydrogen bond interactions with GLY 77, ASP 73, GLY 101, ARG 136 amino acids present in the active site of *E. coli* DNA gyrase B (PDB ID: 4DUH) (Figures 7 and 8).

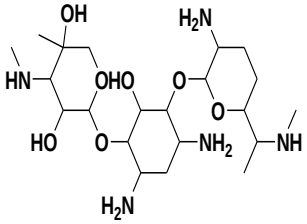
***Pseudomonas aeruginosa*:** Among the compounds tested, A_5 having a 1"-phenyl substitution, A_4 having a 1"-butyl substitution, were found to be potent against *P. aeruginosa* with a MIC value of 500 µg/ml, 1000 µg/ml respectively.

***P. vulgaris*:** Among the compounds tested, A_1 having a 4"-phenol substitution was found to be most potent against *P. vulgaris* with a MIC value of 100 µg/ml. Some of the chromone linked amides i.e., A_2 having 3"-phenyl substitution, A_4 having 1"-butyl substitution, A_5 having 1"-phenyl substitution were found to potent against *P. vulgaris* with a MIC of 500 µg/ml.

Table 8: Antibacterial activity of chromone linked amides (A_1 to A_5). (Expressed as MIC in µg/ml)

Compounds	*Staphylococcus aureus*	*Escheriea coli*	*Pseudomonas aeruginosa*	*Proteus vulgaris*
A_1	500	100	-	100
A_2	500	500	-	500
A_3	-	-	-	-
A_4	500	1000	1000	500
A_5	-	500	500	500
(Gentamycin)	< 1	< 2	< 2	< 2

Table 9: Extra Precision (XP) docking results of Schrodinger: 1JIK (*Staphylococcus aureus* tyrosyl-tRNA synthetase. G Score, H-Bond, Glide energy values of docked ligands

		XP descriptor terms		
Entry name	**Structure**	**G Score**	**H-Bond**	**Glide energy (Kcal/mol)**
Gentamycin		-6.058	-1.758	-37.529

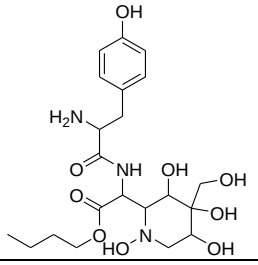
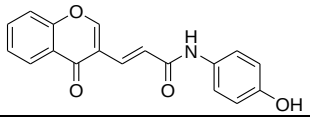
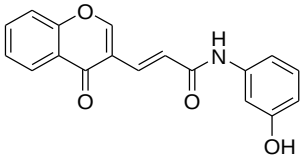
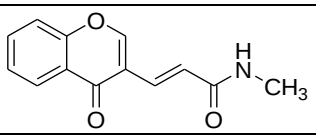
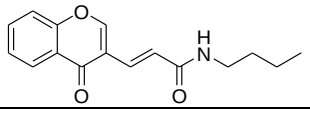
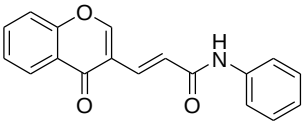
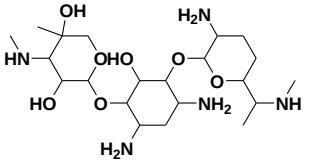
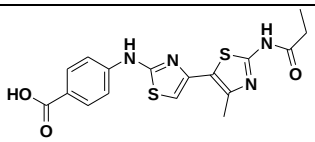
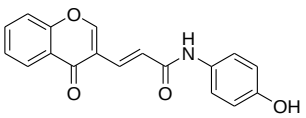
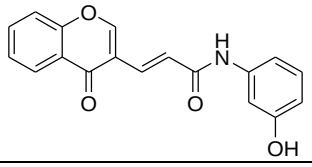
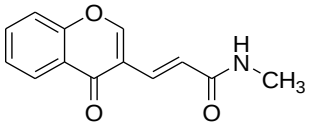
Existing ligand		-12.309	-3.907	-58.71
A_1		-6.654	-0.987	-50.134
A_2		-5.323	-0.7	-45.584
A_3		-4.269	-0.301	-36.971
A_4		-5.2	-0.611	-40.833
A_5		-5.18	-0.86	-41.011

Table 10: Extra Precision (XP) docking results of Schrodinger: 4DUH (*Escheriea coli* DNA gyrase B) G Score, H-Bond, Glide energy values of docked ligands

Entry name	Structure	G Score	H-Bond	Glide energy (Kcal/mol)
Gentamycin		-6.058	-1.758	-37.529
Existing ligand		-7.473	-2.34	-43.222
A_1		-5.35	-0.156	-35.98
A_2		-5.036	-0.519	-35.886
A_3		-5.867	-0.35	-35.008
A_4		-5.87	-0.992	-39.445

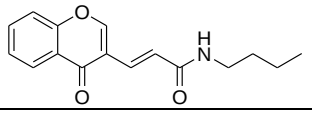
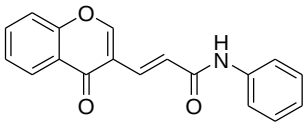
				
A_5		-3.765	0	-34.691

Table 11: Extra Precision (XP) docking results of Schrodinger: Hydrophilic and hydrophobic interactions of compounds with amino acids of 1JIK

S. No.	Compound	Interacting residues
1	A_1	ASP 177, TYR 36
2	A_2	GLY 49
3	A_3	GLY 193
4	A_4	GLY 193, GLY 38
5	A_5	GLY 193, GLY 38

Table 12: Extra Precision (XP) docking results of Schrodinger: Hydrophilic and hydrophobic interactions of compounds with amino acids of 4DUH

S. No.	Compound	Interacting residues
1	A_1	GLY 77, ASP 73
2	A_2	GLY 101, ARG 136
3	A_3	ALA 100, ASN 46, LYS 103
4	A_4	LYS 103, ASP 73, ARG 76
5	A_5	ARG 76, ARG 136

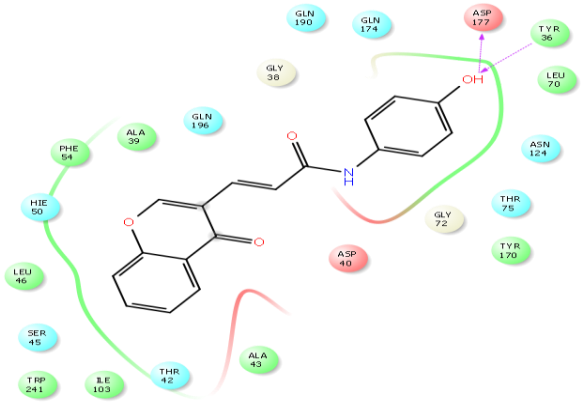


Figure 5: Interactions (Hydrophobic, hydrophilic) of A_1 with amino acids present in the active site of 1JIK

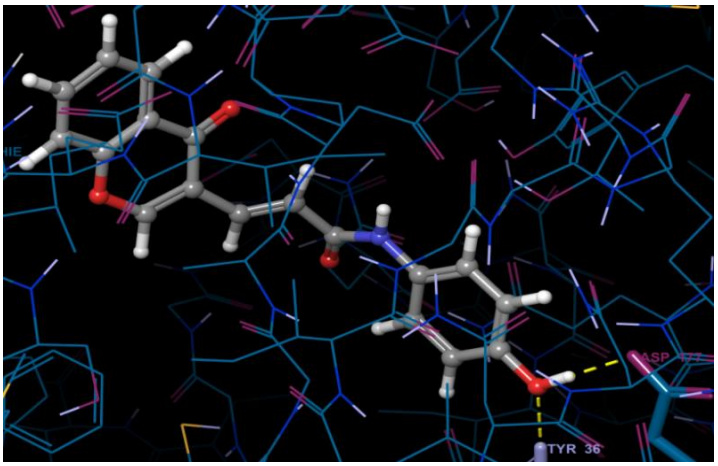


Figure 6: H-Bonding interactions of A_1 with amino acids present in the active site of 1JIK

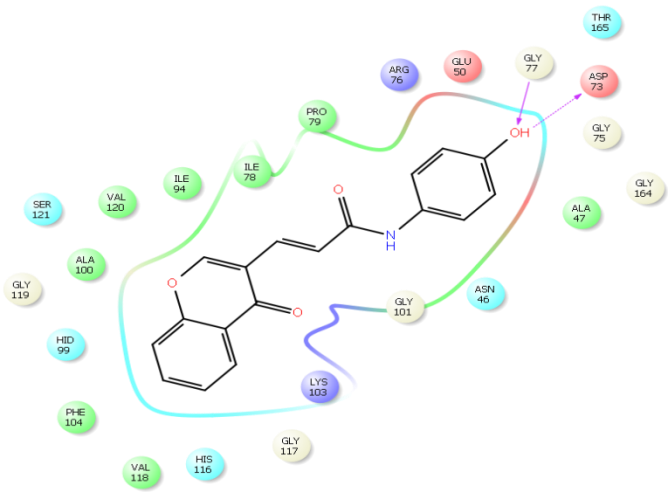


Figure 7: Interactions (Hydrophobic, hydrophilic) of A_1 with amino acids present in the active site of 4DUH

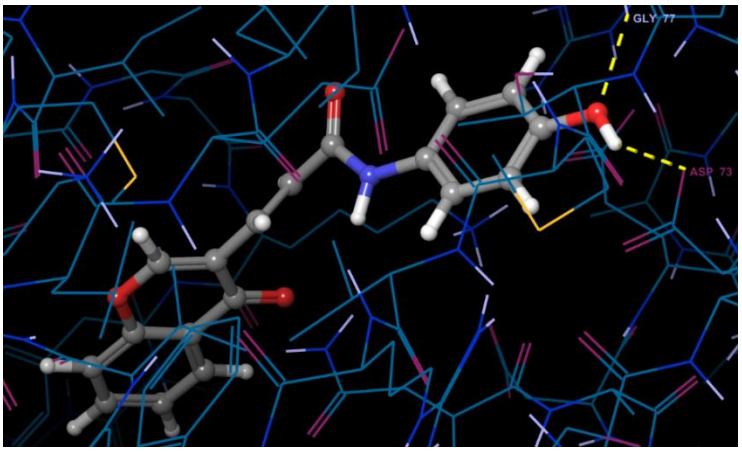


Figure 8: H-Bonding interactions of A_1 with amino acids present in the active site of 4DUH

CONCLUSION

In summary a new series of 3(4-oxo-4H-chromen-3-yl)acrylic acid amides were synthesized by employing molecular hybridization approach to target estrogen receptor alpha ligand binding domain. The *in silico* studies showed that 3(4-oxo-4H-chromen-3-yl)acrylic acid amides was found to have good docking score and also showing good hydrogen bonding interactions with amino acids present in the active site of estrogen receptor alpha ligand binding domain (1XP6), nitric oxide synthase (3NLE). *In vitro* cytotoxicity of these novel compounds are performed on MCF-7 breast cancer cell line using MTT Assay. Many of the synthesized compounds exhibited potent cytotoxic activity and A_1 showed most potent cytotoxicity with IC_{50} value of 37.13 µg/ml respectively. A_2, A_4 and A_5 compounds showed potent cytotoxicity with IC_{50} value < 43 µg/ml. All compounds showed most potent antioxidant activity with IC_{50} value ≤1 µg/ml and they were found to be more potent than well-known antioxidant Ascorbic acid in the DPPH-assay. Individually chromone and amino phenols were showed antioxidant activity. In present work we are linking the chromone with amines by using a linker so the resulting compounds are showing more potent antioxidant activity. Antibacterial activity results showed that compounds are less potent towards both the gram positive and gram negative organisms.

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