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Synthesis, characterization and biological evaluation of novel series of 2-(benzylamino)-2-oxoethyl]-2-oxo-2*H*-1-benzopyran-3-carboxamide derivatives

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A novel series of benzopyran-3-carboxamide derivatives have been designed and synthesized using a smooth and linear multistep synthesis. Amidation of coumarin-3-carboxylic acid with glycine ethyl ester in the presence of EDC.HCl and HOBt as coupling agent followed by the hydrolysis results in the formation of key synthon, [(2-oxo-2*H*-1-benzopyran-3-carbonyl) amino] acetic acid **7** which is further coupled with substituted aryl amines using HATU in combination with Hünig's base to get the target compounds **8(a-h)**. The synthesized compounds have been screened for their *in vitro* antibacterial and antioxidant activity and the results are expressed as MIC and IC_{50} values respectively. Further, the binding ability of synthesized compounds with different proteins have been examined by molecular docking studies.

Keywords: EDC.HCl, HOBt, carboxamide, HATU, diisopropylamine, molecular docking

Coumarin and their derivatives were considered to be an elite class of compounds because of their potential beneficial effects on human health[1]. Among numerous classes of biologically potent chemical moieties, coumarin derivatives are recognized as key building blocks in drug research. The synthesis of heterocyclic compounds containing coumarin moiety has attracted the attention of organic and medicinal chemist as members of this family exhibits wide spectrum of biological activities such as anticancer[2], anti-oxidant[3], analgesic[4], antihistamine[5], anti-HIV[6], anti-coagulant[7], antibacterial[8], anti-inflammatory[9], and antituberculosis[10] and enzyme inhibitors[11]. Apart from its medicinal applications, coumarins have widely used in food and cosmetics industries[12], fluorescent transthyretin folding sensors[13] and photosensitizers[14].

Coumarins, a class of benzopyrone family[15] constitutes one of the largest and most widespread classes in the realm of the plant as secondary metabolites and have important effects in plant biochemistry like control of respiration, photosynthesis, involved in the actions of plant growth hormones[16]. A large number of natural products containing coumarin nucleus as a structural core is extensively used important scaffolds in medicinal and agricultural chemistry. For examples, Warfarin and Acenocoumarol are anticoagulant agents, 7-hydroxycoumarin also known as umbelliferone used as a sunscreen, a fluorescence indicator, and a dye indicator[17], Osthole, a natural *o*-methylated coumarin shows antifungal activity[18].

A variety of methods have been documented for the synthesis of coumarins which can be further exploited as the molecular template for the synthesis of pharmocogicaly active heterocyclic compounds. The substitution at C-3 and C-4 position of the coumarin nucleus offers a high degree of diversity and useful for the development of new therapeutic agents. Several compounds with high insecticidal activity have been obtained by the modification of the amide bridge part as linker of two aryl rings. Sub structures of the acetamido group found in many insecticides have received considerable attention in pesticide and medicinal chemistry[19]. Ghaneiand co-workers reported the potency of coumarin-

carboxamide derivatives bearing indole amine scaffold as new cholinesterase (AChE) inhibitors[20]. Coumarin and 3-carboxamide coumarin derivatives were described as FXIIa inhibitors[21]. Hao Liu and co-workers well documented the antimicrobial activity and Inhibitory effects against Topo II and Topo IV by introducing the pyrazole ring and binding it to coumarin with an amide bond to get novel set of carboxamide derivatives[22]. On the basis of SAR studies, Franco Chimenti and his co-workers pointed out that carboxamide functionality on the coumarin nucleus displayed a good anti-inflammatory effect in H. pylori-induced gastric inflammation[23].

Enlightened by the aforementioned remarkable and synthetic applications, we presented herein our research findings on development of efficient methodology to facilitate the synthesis of novel (benzylamino)-2-oxoethyl]-2-oxo-2*H*-1-benzopyran-3-carboxamide derivatives by the reaction of [(2-oxo-2*H*-1-benzopyran-3-carbonyl) amino] acetic acid with substituted benzyl amines and the synthesized compounds have been evaluated for their different pharmacological activities.

Result and Discussion

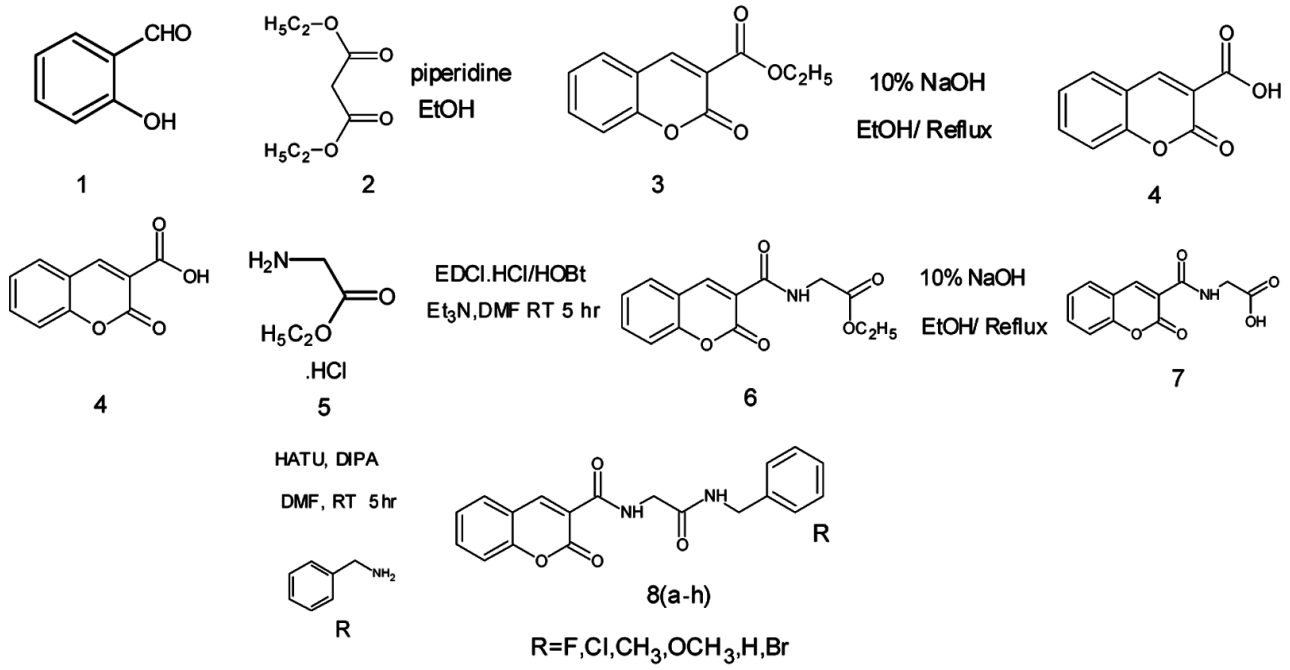
Chemistry

The synthetic pathway leading to the new carboxamide derivatives accessed in the present study have been described in Scheme I. The key intermediate, 2-oxo-2*H*-1-benzopyran-3-carboxylic acid (**4)** required for the synthesis of target compounds have been synthesized by the saponification of ethyl 2-oxo-2*H*-1-benzopyran-3-carboxylate which was synthesized by the reported procedure[24].

The coupling of the carboxylic acid with amines for the formation of amide linkage is performed using different several efficient and selective catalysts such as DCC, HBTU, TPTU and PS-TBTU which satisfies the chemistry protocol. In order to attain optimum reaction conditions such as yield of product and reaction time, we have screened with several coupling reagents. To best of our knowledge, we found that amides can be obtained in good yield with a span of reaction time using EDC.HCl and HOBt.

In the first step, ethyl [(2-oxo-2*H*-1-benzopyran-3-carbonyl) amino] acetate (**6)** was obtained by the treatment of coumarin carboxylic acid (**4**) with glycine ethyl ester hydrochloride (**5**) in presence of EDC.HCl and HOBt at RT which was subsequently hydrolysed in presence of 10% NaOH to give the corresponding benzopyran-3-carbonyl) amino] acetic acid (**7**). The final step involves the reaction of compound **7** with substituted aromatic amines in the presence of N, N-diisopropylethylamine and HATU to furnish *N*-[2-(benzylamino)-2-oxoethyl]-2-oxo-2*H*-1-benzopyran-3-carboxamide derivatives (**8a-h**).

The structures of all newly synthesized compounds have been characterised by IR, ^{1}H NMR, ^{13}C NMR



Scheme I — Synthesis of benzopyran-3-carboxamide derivatives **8a-h**

and mass analysis. The spectral data have been included in experimental part.

Postulated structures of synthesized ethyl [(2-oxo-2*H*-1-benzopyran-3-carbonyl) amino] acetate **6** was confirmed by IR, ^{1}H and ^{13}C NMR, and mass analysis. The FTIR spectrum of compound **6** showed a broadband at 3314.37 cm^{-1} which confirms the –NH stretching vibration of the amide. Three other peaks were observed at 1757.43 $cm^{-1,}$ 1713.32 cm^{-1} and 1637.17 cm^{-1} for carbonyl group of amide carbonyl, carbonyl lactone of coumarin and ester carbonyl stretching respectively. The ^{1}H NMR spectrum of compound **6** contained a triplet with a chemical shift of 1.16-1.19 ppm range, which consistent with the ethyl ester, as well as a singlet with a chemical shift of 9.03 ppm which consistent with the protons of the amide group. The ^{13}C NMR spectrum of the compound **6** contained a signal at 169.86 ppm which was consistent with the carbonyl carbon of the amide group. The mass analysis of compound **6** gave a molecular ion peak with an *m/z* value of 276.03 for (M+H) which was consistent with the molecular formula $C_{14}H_{13}NO_5$.

The structure of compound **7** was confirmed by IR, ^{1}H NMR, ^{13}C NMR, and mass analysis. The FTIR spectrum of compound **7** showed characteristic broadband at 3313.20 cm^{-1} which attributed to –NH stretching vibration of the amide. Three other peaks were observed at 1735.75 cm^{-1}, 1711.80 cm^{-1} and 1656.70 cm^{-1} for carbonyl group of amide carbonyl, coumarin carbonyl, and ester carbonyl respectively. The broad absorption band at 3642.91 cm^{-1} attributed to –OH stretching vibration of carboxylic group. The ^{1}H NMR spectrum of **7** contained a singlet with chemical shift of 9.03 ppm, which consistent with the protons of the amide group, and a singlet at δ 12.83 which consistent with the protons of the carboxylic group. The ^{13}C NMR spectrum of the compound **7** contained a signal at 171.24 ppm which was consistent with the carbonyl carbon of the carboxylic functional group. The mass analysis of compound **7** gave a molecular ion peak with an *m/z* value of 246.0018 (M-H) which was consistent with the molecular formula $C_{12}H_9NO_5$.

The structure of carboxamide derivatives (**8a-h)** was confirmed by IR, ^{1}H NMR, ^{13}C NMR, and mass analysis. The FTIR spectrum of compound **8b** displayed two peaks at 3271.64 cm-^{1} and 3329.31 cm^{-1} which were attributed to –NH stretch of amide group of both benzyl amide and glycine ester amide respectively. The ^{1}H NMR spectrum of **8b** showed a doublet with the chemical shift of 4.05-4.66 ppm range corresponds to protons of benzyl $–CH_{2,}$ another doublet at δ 4.31-4.32 range, which consistent with the protons of glycine- $CH_{2.}$ The absence of carboxylic -OH proton of compound **7** in ^{1}H NMR at 12.83 ppm can be observed in the series **8 (a-h)** and a singlet with a chemical shift of 8.54 ppm, which consistent with the protons of the benzyl amide -NH group indicates the formation of new amide bond. Another singlet with a chemical shift of 9.12 ppm which consistent with the protons of glycine amide -NH group. The ^{13}C NMR spectrum of the compound **8b** contained a signal at δ 168.65 which consistent with the carbonyl carbon of coumarin. The mass spectrum of compound **8b** gave a molecular ion peak with an *m/z* value of 355.02 (M+H) which consistent with the molecular formula $C_{19}H_{15}FNO_4$.

Biological activity

Antibacterial activity

All the newly synthesized compounds were evaluated for *in-vitro* antibacterial activity against a gram-negative bacterium *Klebsiella pneumonia* and gram-positive bacterium *Bacillus subtilis*taking Tetracycline as a standard drug. The results have been summarized in Table I. From the results, it can be noted that all the synthesized compounds exhibited significant antibacterial activity against tested strains.

To further quantify the antibacterial potency of the synthesized compounds **8(a-h)**, the MIC was determined using Microtitre plate assay and the results are as shown in Table II. The results of MIC of tested compounds reveals that compound **8h** with –Br substitution on *p*-position is potent against *Bacillus subtilis*and *Klebsiella pneumonia* with MIC value of 0.078 mg/mL, followed by **8e** with –Cl substitution on 4-position of aryl group which exhibited activity with MIC value of 0.078 mg/mL and 0.156 mg/mL against *Bacillus subtilis* and *Klebsiella pneumonia* respectively. Further, the compounds **8c** and **8g** showed considerable potency towards the tested strains whereas **8a** and **8d** showed a low response. From this data, it is found that electron-withdrawing groups such as Br, F, Cl plays a key role in enhancing the potency of synthesized compounds against the tested pathogens. From the close observation of primary antibacterial screening, it is revealed that the structural and electronic parameters may have a greater impact on varying the efficacy of antimicrobial activity[25]. Further, the difference in MIC noted in **8b** and **8f** (Table II) clearly indicated

Table I — Antibacterial activity data of synthesized compounds **8a-h**

Compounds	Zone of Inhibition in (mm)			
	Gram positive		Gram Negative	
	Bacillus subtilis		*Klebsiellapneumoniae*	
8a	2.0±0.707	2.4±0.707	0.8±0.141	1.4±0.353
8b	4.4±0.565	5±0.622	6.5±0.141	8±0.848
8c	2.0±0.282	2.5±0.424	1.0±0.212	1.5±0.424
8d	1.9±0.565	2.4±0.282	0.6±0.212	1.25±0.141
8e	6.8±1.41	8±1.241	7.5±0.424	9±1.55
8f	4.5±0.565	5.5±0.848	4±0.282	12.5±0.707
8g	2.3±0.282	2.7±0.424	6±0.565	2.0±0.424
8h	7.2±0.424	8.5±0.707	8.0±1.55	9.5±0.424
+ control- Tetracycline (std)	22.5±0.35	-	24±0.70	-

Zone of inhibition is expressed in millimetres Values are averages ± standard deviations calculated from the data of two independent measurements at two different concentration (10 and 20 mg/mL).

Table II — MICs of synthesized compounds **8a-h**

	MICs of synthesized compounds (mg/mL)								
Organisms	**8a**	**8b**	**8c**	**8d**	**8e**	**8f**	**8g**	**8h**	std
K. pneumonia	1.25	0.312	0.625	1.25	0.156	0.312	0.625	0.078	0.0097
Bacillus subtilis	1.25	0.156	0.625	1.25	0.078	0.312	0.625	0.078	0.0097

Std: Tetracycline

Table III — DPPH radical activity results of the compounds **8a-h**

Concentration (mg/mL)	Percentage inhibition (%)			IC_{50}(mg/mL)
	10	20	30	
Std/Galic acid	60.32±	86.14±	93.71±	18.144
8a	36.71±1.852	42.51±4.44	49.60±2.420	20.333
8b	15.23±0.31	029.41±1.00	40.05±3.21	019.564
8c	15.86±2.680	33.14±1.923	45.20±1.555	19.413
8d	9.32±1.930	20.59±2.715	42.15±1.555	21.718
8e	14.76±3.097	34.05±1.371	44.13±4.454	18.976
8f	32.19±3.691	35.61±2.192	47.40±2.262	21.914
8g	8.20±1.994	16.55±2.064	34.62±3.620	21.273
8h	23.11±1.060	30.71±3.973	42.15±2.220	20.665

that the *Bacillus subtilis* strain is quite susceptible compared to *Klebsiella pneumonia* strain. These can be associated with the presence of the peptidoglycan cell wall which makes it exhibit a varied mode of susceptibility[26].

Antioxidant activity

In the present study, the newly synthesized compounds were screened for their antioxidant properties using metal chelating assay as well as DPPH assay at different concentrations and results were expressed as a percentage and also in terms of IC_{50} (mg/mL).

The synthesized compounds were screened for 1,1diphenyl-picrylhydrazyl (DPPH) radical scavenging activity having Butylatedhydroxytoluene (BHT) as standard and the results have been shown in Table III. According to the tabulated data, among the synthesized compounds, **8e** with chloro substitution at *para* position displayed high degree of radical scavenging activity with a lowest IC_{50} value of 18.976 mg/mL, followed by the compounds **8b**, **8h** with halogen-substituted (fluoro, bromo) and compound **8a** with no substitution showed moderate antioxidant activity. Furthermore, compound **8c** with electron-donating methyl group on phenyl ring coupled with amide nucleus showed significant oxidative stress-reducing activity with IC_{50} value of 19.564 mg/mL. We also observed that compounds **8f**, **8g** with two electron withdrawing groups on phenyl ring drastically

reduced the activity with a higher concentration of 21.91 and 21.27 mg/mL respectively.

The chelating capacity of test compounds with Fe^{+2} ion varies significantly on the basis of substitution on the aromatic ring. The chelating ability of the synthesized compounds with ferrous ion is represented in Table IV.

The results obtained from this method revealed that the compound **8a** without any substitution as well as **8h** with bromo substitution on aryl group showed the highest activity with IC_{50} values 18.521, 18.740 mg/mL respectivelywhereas moderate scavenging capacity was exhibited by the same compounds in DPPH method. The compounds **8d**, **8c**, **8e**, **8f,** and **8g** showed significant chelating capacity with ferrous ion compared to EDTA with lower concentration. Though the compound **8b**, which exhibited moderate free radical scavenging activity showed lower chelating capability with ferrous ion compared to standard (EDTA).

From both the assays results, it is revealed that the compounds showed a varying degree of oxidative stress reducing capacity. The compounds which showed moderate radical scavenging activity have shown least metal chelation capacity and compounds with moderate radical scavenging capacity presented good ferrous ion chelation property.

Molecular Docking studies

The enzyme GlcN-6-P synthase leads to the formation of UDP-N-acetyl-glucosamine which is the major intermediate in the biosynthesis of all amino sugars containing macromolecules both in prokaryotic and eukaryotic cells. This concerns also the cell walls macromolecules. The Peroxisome proliferator-activated receptor α (PPARα), is a ligand-activated transcription factor and member of the superfamily of nuclear hormone receptor. This receptor plays key roles in maintaining glucose and lipid homeostasis by modulating gene expression of hepatocytes. Transforming growth factor beta (TGF-β) is the most potent pro-fibrogenic cytokine and its expression is increased in almost all of fibrotic diseases. TGF-β1 increases ROS production and suppresses antioxidant enzymes, leading to a redox imbalance. ROS, in turn, induce/activate TGF-β1 and mediate many of TGF-β's fibrogenic effects, forming a vicious cycle. Hence inhibition of these proteins has important implications for chemotherapy[27-29].

The results of docking assess the quality and energy of binding of the structures with the molecules of a bio target. The consensus function allows us to create a rating of compounds and analyze the data regarding the choice of potential agonists/antagonists of the biological target selected. The results obtained were compared with the known antibiotic, Streptomycin and presented in Table V and Figure 1.

Based on the results of docking studies, it was observed that all the synthesized ligand molecules showed higher binding energy and the least number of hydrogen bonding with protein molecule than compared to standard. Among the tested ligands, the molecules **8c**, **8d**, and **8e** showed good binding interaction (−8.0, −7.8 and −7.8 kcal/mol) and establishes three hydrogen bonding with the amino acid molecules in relation to the GlcN-6-P synthase protein. Compound **8g** was found to have a least binding interaction (−4.2 kcal/mol) and establishes one hydrogen bonding with the binding receptor.

The results obtained were compared with the known antioxidant, Silymarine and presented in Table VI, Figure 2 and Figure 3.

The docking of synthesized ligand molecules with protein PPARα and TGF-β1 showed good binding interaction and hydrogen bonding formed between ligand and protein molecules were comparable with the standard. Compound **8f** showed the highest binding interaction (-10.8 kcal/mol) and five hydrogen bonding with different amino acids of

Table IV — Metal chelating results of the compounds **8a-h**

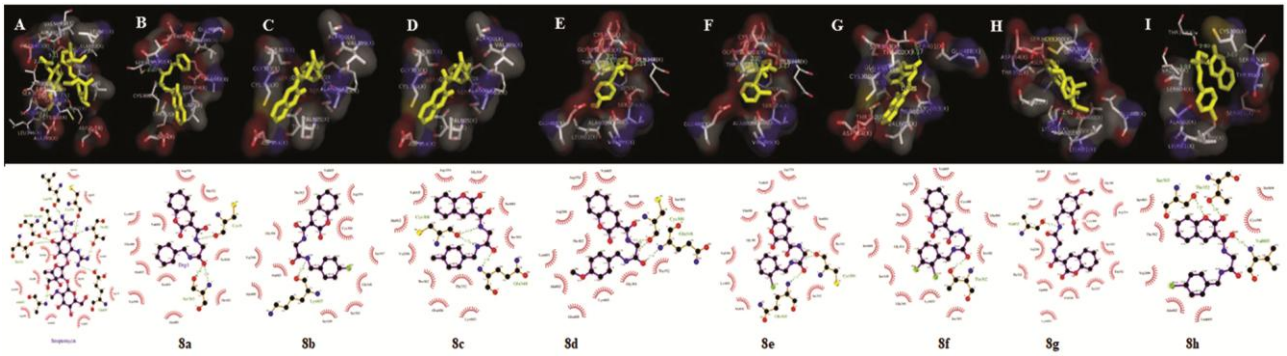
Concentration (mg/mL)	Percentage inhibition (%)			IC_{50}(mg/mL)
	10	20	30	
Std/EDTA	66.26±4.553	76.17±2.121	96.40±3.606	20.995
8a	38.19±2.80	59.39±3.408	67.73±2.347	18.521
8b	19.87±3.63	23.15±4.087	32.16±1.569	22.778
8c	11.55±3.224	15.88±1.866	20.90±3.153	20.271
8d	23.12±1.527	27.83±2.729	32.06±2.941	19.844
8e	7.14±1.654	15.98±2.729	28.04±0.848	20.460
8f	0.60±0.367	10.75±2.559	25.32±0.947	20.542
8g	14.02±0.735	18.78±2.390	25.48±2.094	20.657
8h	22.11±2.022	48.64±3.733	61.40±1.640	18.740

Table V — Binding energy, H- Bonds, H- Bond length, H-Bond with and hydrophobic interactions of ligands with receptors

Ligands	Target Protein	Affinity (kcal/mol)	H-bonds	H-bond length (Å)	H-bond with	Hydrophobic interactions
Streptomycin	GlcN-6-P synthase	- 3.7	10	2.80 2.52 2.99 2.80, 2.83 2.65 2.79 2.79 3.11	Ala 602 Thr352 Ser349 Ser303 Leu346 Cys300 Thr302 Glu488	Leu601,Val399, Lys603, Ala400, Ser604, Ser347, Ala299, Ser401, Gly301, Asp354, Val605
8a		- 7.6	3	2.80, 3.25 3.07	Ser303 Cys300	Ala400, Val399, Ser405, Ala602, Glu488, Lys603, Val605, Asp354, Thr352, Ser604, Thr302
8b		- 7.2	1	3.28	Lys603	Ala400, Ala602, val399, Gly301, Thr352, Val605, Asp354, Cys300, Ser347, Glu348, Ser303, Ser349
8c		- 8.0	3	3.18 3.23, 3.13	Gln348 Cys300	Lys603, Thr352, Glu488, Thr302, Val399, Ala602, Val605, Asp354, Gly301, Ser604, Ser303
8d		- 7.8	3	3.14, 3.19 3.11	Gln348, Cys300	Thr352, Gly301, Lys603, Glu488, Ala602, Leu601, Thr302, Val399, Asp354, Val605, Ser604, Ser303
8e		- 7.8	3	3.17, 3.5 3.09	Gln348 Cys300	Ala602, Lys603, Val399, Gly301, Glu488, Val605, Asp354, Thr302, Ser604, Thr352, Ser303
8f		- 7.6	1	3.7	Thr302	Lys603, Ser303, Gln348, Ser349, Ser604, Gly301, Thr352, Asp354, Val605, Cys300, Glu488, Ser401
8g		- 4.2	1	2.92	Ala602	Leu601, Glu488, Val605, Gly301, Asp354, Cys300, Ser303, Thr352, Ser347, Glu348, Lys603, Ala400, Thr302, Val399
8h		- 5.4	3	3.29 2.80 3.03	Ser303 Thr352 Val605	Leu601, Ala602, Val399, Thr302, Ser401, Ser604, Cys300

PPARα protein molecule and compound **8c** establishes three hydrogen binding with Ser241 and Agr240 amino acid in the active site of target protein TGF-β1 with highest binding affinity -7.3 kcal/mol. Other ligand molecules showed good interaction and hydrogen bonding with the target receptor.

Overall it is predicted that, since the test synthesized compounds have a significant binding affinity, hydrogen boding and hydrophobic interactions with target proteins, the inhibition efficiency of the compounds **8(a-h)** (Table VII) is comparatively higher.



2D Ligplot analysis and docking results showing crystal structure of GlcN-6-P synthase, ligand Streptomycin (Standard drug), **B, C, D, E, F, G, H, I**.: 2D Ligplot analysis and docking results showing crystal structure of GlcN-6-P synthase with the ligand **8a, 8b, 8c, 8d, 8e, 8f, 8g and 8h** respectively.

Figure 1 — Molecular docking studies with respect to receptor GlcN-6-P synthase

Table VI — Binding energy, H- Bonds, H- Bond length, H-Bond with and hydrophobic interactions of ligands with receptors

Ligands	Target Protein	Affinity (kcal/mol)	H-bonds	H-bond length (Å)	H-bond with	Hydrophobic interactions
Silymarin	PPARα	-	6.5	3 3.29 2.94 3.13	Cys275 Ser280 Thr283	Cys276, Met355, Phe318, Leu321, Met330, Ile317, Met220, Phe218, Glu286, Val324, Met320, Ala333, Val332, Thr279, Leu331
8a		-	10.3	3 2.80, 2.82 3.08	Ser280 His440	Val324, Met220, Leu331, Thr279, Leu321, Cys276, Met355, Gln277, Phe273, Leu456, Val444, Leu460, Tyr314, Phe318, Ile317, Thr283
8b		-	10.6	3 2.80, 2.96 3.11	Ser280 His440	Met220, Val324, Ile317, Thr279, Met355, Cys276, Leu456, Gln277, Leu460, Phe273, Val444, Phe318, Tyr314, Leu331, Thr283
8c		-	10.7	3 2.80, 2.84 3.11	Ser280 His440	Leu331, Val324, Lea321, Cys276, Thr279, Met355, Gln277, Phe273, Val444, Leu456, Tyr465, Leu460, Phe318, Ile317, Thr283, Tyr314, Met220
8d		-	10.4	4 2.97 2.81, 2.98 3.09	Thr279 Ser280 His440	Gly335, Val324, Leu331, Met355, Phe273, Cys276, Leu456, Gln277, Val444, Leu460, Tyr314, Phe318, Ile317, Thr283, Leu321, Met220
8e		-	10.6	4 2.94 3.02, 2.83 3.10	Thr279 Ser280 His440	Phe318, Tyr314, Leu460, Leu456, Phe273, Val444, Gln277, Cys276, Met355, Ile317, Val324, Leu331, Leu321, Thr283
8f		-	10.8	5 2.91, 2.80 3.12, 2.97 3.07	Thr279 Ser280 His440	Val324, Leu321, Met355, Cys276, Leu456, Phe273, Glu277, Val444, Tyr314, Phe318, Leu460, Ile317, Thr283, Met220

(*Contd*.)

Table VI — Binding energy, H- Bonds, H- Bond length, H-Bond with and hydrophobic interactions of ligands with receptors

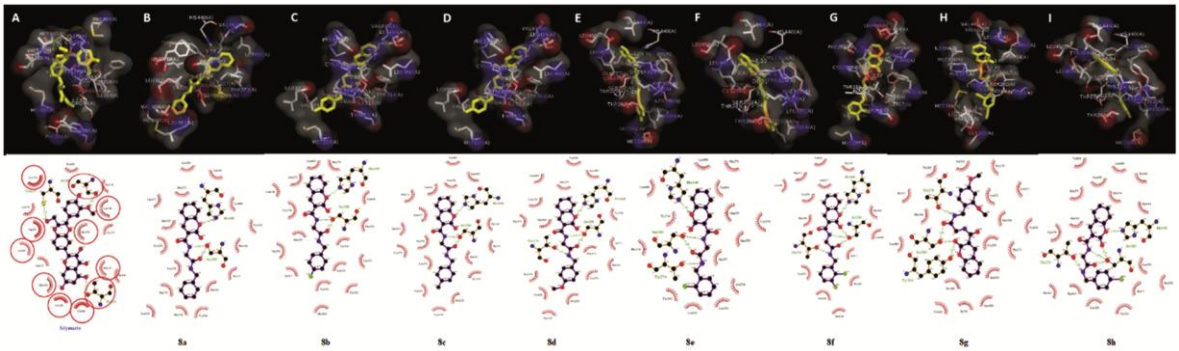
Ligands	Target Protein	Affinity (kcal/mol)		H-bonds	H-bond length (Å)	H-bond with	Hydrophobic interactions
8g		-	10.7	4	2.85 2.70, 2.80 3.04	Tyr314 Ser280 Thr279	Leu460,Phe318, Ile317, Thr283, Val324, Gly335, Leu321, Leu331, Met330, Val332, Cys276, Phe273, Met355, Leu456, Ile354, Val444, His440
8h		-	10.6	5	2.89 2.97, 2.86, 3.10 3.04	Thr279 Ser280 His440	Cys276, Met355, Gln277, Val444, Leu456, Phe273, Leu460, Tyr464, Phe318, Leu331, Met220, Ile317, Val324, Leu321, Tyr314, Thr283
Silymarin	TGF-β1	-	6.8	2	3.17 3.18	Arg240 Ser241	Glu247, Phe243, Ile367, Arg244, Gly353, Leu354, Val373, Phe216, Glu238, Arg237
8a		-	7.0	--	--	--	Arg332, Leu354, Val373, Arg244, Phe243, Arg240, Ser241, ile367, Gly353
8b		-	7.0	--	--	--	Arg244, Ile367, Arg332, Val373, Gly353, Leu354, Ser241, Arg240, Phe243
8c		-	7.3	3	3.14 3.18, 3.17	Ser241 Arg240	Arg244, Glu247, Phe216, Gly353, Val373, Arg215, Gly374, Leu354
8d		-	7.1	1	3.13	Gly374	Phe216, Val373, Arg240, Arg244, Phe243, Ile367, Glu247, Leu354, Arg215
8e		-	7.1	2	3.24, 2.92	Arg240	Glu247, Ile367, Arg332, Val373, Leu354, Ser241, Arg244
8f		-	7.2	2	3.13, 3.15	Arg240	Glu247, Arg244, Ile367, Arg332, Val373, Leu354, Ser241, Phe243
8g		-	7.1	1	2.96	Arg240	Val373, Arg332, Ile367, Arg244, Phe243, leu354, Arg215, Phe216
8h		-	7.2	2	3.7, 3.18	Arg240	Ser241, Gly353, Val373, Leu354, Arg332, Phe243, Ile367, Arg244, Ile367, Glu247

Experimental Section

All chemicals and solvents were analytical grade and purchased from commercial suppliers Sigma-Aldrich, Spectrochem, India and used without purification. Thin-layer chromatography (TLC) experiment was performed on Aluminium backed silica gel 40 F254 plates to monitor the progress of the reaction. Electro thermal apparatus was used to record the melting point of synthesized compounds and are uncorrected. The FT-IR spectra were recorded in KBr pellets using a Shimadzu FT-IR spectrophotometer. ^{1}H and ^{13}C NMR spectra were recorded on the Bruker 400 MHz spectrometer. The chemical shifts with reference to TMS as an internal standard are reported in δ (ppm) and the coupling constants (*J*) values are expressed in Hertz (Hz). The mass spectra of synthesized compounds were recorded using Agilent LC-MS instrument.

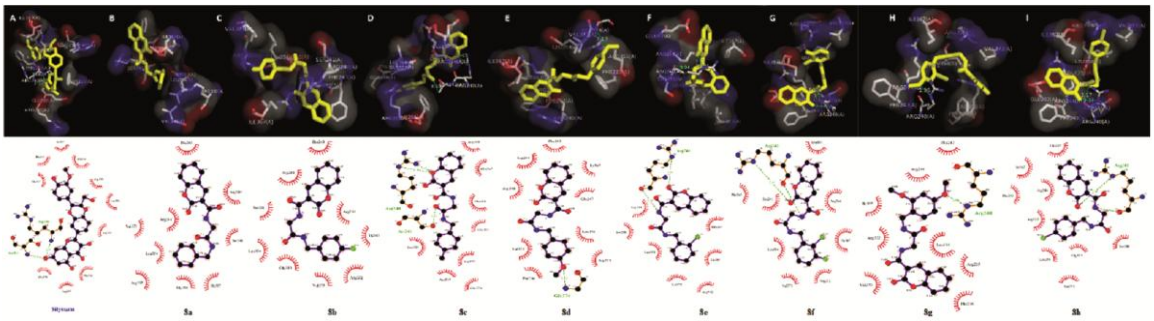
Synthesis of ethyl [(2-oxo-2*H*-1-benzopyran-3-carbonyl) amino] acetate, 6

To the stirred solution of 2-oxo-2*H*-1-benzopyran-3-carboxylic acid (10g, 52.59 mmol), glycine ethyl ester hydrochloride (10.94g, 78.43mmol) and HOBt (12g, 78.43mmol) in 140 mL of DMF, N-methyl morpholine (13.23g, 130.74mmol) was added drop wise and the stirring was continued for 10 min. at the



2D Ligplot analysis and docking results showing crystal structure of PPARα, ligand Silymarin (Standard drug), **B, C, D, E, F, G, H, I**.: 2D Ligplot analysis and docking results showing crystal structure of PPARα with the ligands **8a, 8b, 8c, 8d, 8e, 8f, 8g and 8h** respectively

Figure 2 — Molecular docking studies with respect to receptor PPARα.



2D Ligplot analysis and docking results showing crystal structure of TGF-β1, ligand Silymarin (Standard drug), **B, C, D, E, F, G, H, I**.: 2D Ligplot analysis and docking results showing crystal structure of TGF-β1with the ligands **8a, 8b, 8c, 8d, 8e, 8f, 8g and 8h** respectively.

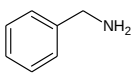
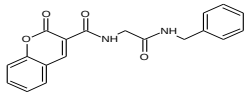
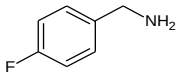
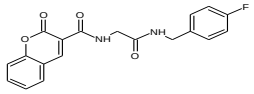
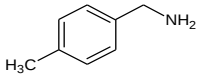
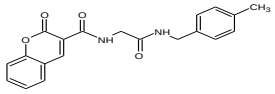
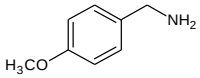
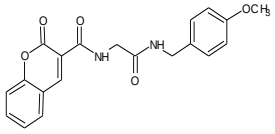
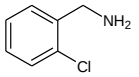
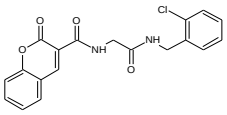
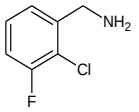
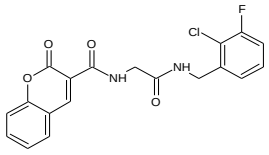
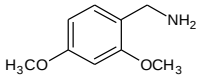
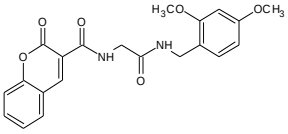
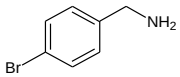
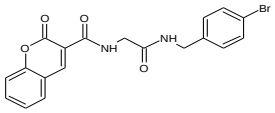
Figure 3 — Molecular docking studies with respect to receptor TGF-β1.

same temperature. To this, 15.03g of EDC.HCl (78.43mmol) was added and the resulted reaction mixture was stirred for 5-6 hr at RT. Progress of the reaction was monitored by TLC and after completion of the reaction, whole reaction mixture was quenched with 500 mL of ice cold water and stirred for 30 min. The precipitate formed was filtered, washed with water and dried under vacuum to afford ethyl [(2-oxo-2*H*-1-benzopyran-3-carbonyl) amino] acetate (**6**) as off white solid. (88 %). m.p. 171-173°C. R_f =0.39 ($CHCl_3$-MeOH 9:1); IR (KBr): 1656.70 (C=O), 1711.80 (C=O), 1735.75 (C=O), 3314.37 cm^{-1} (N-H); ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.181 (3H, t, J = 6Hz, ester-CH_3), 4.09 (2H, d, J = 5.5 Hz, glycine-CH_2), 4.27 (2H, q, J_1 = 5.4 Hz, J_2 = 7.4 Hz, ester-CH_2) 7.48-8.87 (5H, Ar-H) 9.03 (1H, s, amide NH); ^{13}C NMR (100 MHz, DMSO-d_6): δ 14.51 (ester-CH_3), 41.92 (amide-CH_2), 61.01 (ester-CH_2), 116.598, 118.533, 118.826, 125.597, 130.860, 134.772, 148.63, 154.43 (coumarin C-Ar), 160.73, 161.82 (coumarin –C=O) and 169.86 (glycine ester-C=O). MS: *m/z* 276.03 (M+H). Anal. Calcd for $C_{14}H_{13}NO_5$ (275.25).

Synthesis of [(2-oxo-2*H*-1-benzopyran-3-carbonyl) amino] acetic acid, 7

35 mL Sodium hydroxide solution (10%) was added to 10 g of ethyl [(2-oxo-2*H*-1benzopyran-3-carbonyl) amino] acetate **6** (36.32 mmol), and the resulted solution was refluxed at 100°C for about 2hr. The reaction mixture turns to a yellowish solution during this period. After completion of the reaction (checked by TLC), the reaction mass was cooled to RT, diluted with 50 mL of ice and acidified with hydrochloric acid (6 N) to p*H* 3 under constant stirring for about 30 min. The precipitate obtained was collected by filtration, washed with water and finally dried under vacuum to afford [(2-oxo-2*H*-1benzopyran-3-carbonyl) amino] acetic acid **7** as off pale yellow solid (84%). m.p. 247-250°C. R_f =0.28($CHCl_3$-MeOH 9:1); IR (KBr): 1637.17 (C=O), 1713.32 (C=O), 1757.43 (C=O), 3313.20 (N-H), 3462.91 cm^{-1} (O-H); ^{1}H NMR (400 MHz, DMSO-d_6): δ 4.08 (2H, d, J = 5.6 Hz, glycine-CH_2), 7.54-8.93 (5H, Ar-H), 9.06 (1H, t, J = 5.2 Hz, -NH), 12.80 (1H, s, -COOH); ^{13}C NMR (100 MHz, DMSO-d_6): δ

Table VII — Physicochemical data of substituted derivatives **8a-h**

Compd	R1	Product	MF/M.Wt	Yeld(%)	m.p. (°C)
8a			$C_{19}H_{16}N_2O_4$ /336.34	85%	200-202
8b			$C_{19}H_{15}FNO_4$ /354.33	87%	208-210
8c			$C_{20}H_{18}N_2O_4$ /350.36	88%	228-230
8d			$C_{20}H_{18}N_2O_5$ /366.36	85%	198-202
8e			$C_{19}H_{15}FClN_2O_4$ /370.78	78%	215-216
8f			$C_{19}H_{14}ClFN_2O_4$ /388.77	88%	220-222
8g			$C_{21}H_{20}N_2O_6$/396.39	74%	194-196
8h			$C_{19}H_{15}BrNO_4$ /415.23	83%	225-227

41.937 (amide-CH_2), 116.590, 118.602, 118.602, 118.845, 125.597, 130.851, 134.733, 148.538, 154.424 (coumarin C-Ar), 160.806, 161.595 (coumarin –C=O), 171.246 (acid –C=O); MS: *m/z* 246.0018 (M-H). Anal. Calcd for $C_{12}H_9NO_5$ (247.20).

General procedure for the synthesis of substituted N-[2-(benzylamino)-2-oxoethyl]-2-oxo2*H*-1- benzopyran-3-carboxamide derivatives, 8a-h

To the suspension of hypuric acid **7** (1 mmol) and substituted aryl amine (1 mmol) in DMF (5 mL), N-ethyl diisopropylamine (3 mmol) was added drop wise at 0°C and followed by the addition of HATU (1.3 mmol) during a period of 15 min. The reaction medium was allowed to reach RT and stirred for 4 hrs at the same temperature. After completion of the reaction, the reaction mixture was poured into ice cold water and stirred at RT for 30 min. The precipitated solid was filtered, washed with water and kept for drying under vacuum.

N-[2-(Benzylamino)-2-oxoethyl]-2-oxo-2*H*-1-benzopyran-3-carboxamide, 8a: White solid. m.p. 200-202°C. IR (KBr): 1654.76 (C=O), 1622.34 (C=O), 3276.41(N-H), 3327.31 cm^{-1} (N-H); ^{1}H NMR (400 MHz, DMSO-d_6): δ 4.23 (2H, d, *J* = 3.6 Hz, glycine-CH_2), 4.46 (2H, d, *J* = 4 Hz, benzyl-CH_2), 7.48-8.87 (5H, Ar-H), 8.7 (1H, s, amide-NH-benzyl), 8.97 (1H, d, *J* = 6.8Hz, Ar-H), 9.2 (1H, s, amide-NH-glycine); ^{13}C NMR (100MHz, DMSO-d_6): δ 43.8 (amide-CH_2), 43.5(benzyl–CH_2), 126.8, 127.3, 128.6, 128.6, 127.1, 141.7 (Ar-C), 114.1, 121.5, 122.3, 125.5, 126.8, 128.4, 138.6, 150.3, 159.5, 159.8 (coumarin –C=O), 171.1 (benzyl amide –C=O); MS: *m/z* 337.23 (M-H). Anal. Calcd for $C_{19}H_{16}N_2O_4$ (336.34).

N-(2-{[(4-Fluorophenyl)methyl]amino}-2-oxoethyl)-2-oxo-2*H*-1-benzopyran-3 carboxamide, 8b: White solid, m.p. 208-210°C. IR (KBr): 1100 (C-F), 1658.05 (C=O), 1716.59 (C=O), 3271.64 (N-H), 3329.31 cm^{-1} (N-H); ^{1}H NMR (100 MHz, DMSO-d_6): δ 4.06 (2H, d, *J* = 3.6 Hz, glycine-CH_2), 4.32 (2H, d, *J* = 4

Hz benzyl-CH_2), 7.18-8.03 (8H, Ar-H), 8.54 (1H, s, amide-NH-benzyl), 8.93 (1H, s, ArH), 9.12 (1H, s, amide-NH-glycine); ^{13}C NMR (100 MHz, DMSO-d_6): δ 41.84 (amide-CH_2), 43.24 (benzyl –CH_2), 115.28, 115.49, 116.57, 118.86, 118.98, 125.59, 129.56, 129.65, 130.79, 134.63, 135.89, 148.14, 154.37, 160.40, 160.77, 161.57 (coumarin –C=O), 162.82 (Ar–C-F) 168.65 (benzyl amide –C=O); MS: *m/z* 355.02 (M+H). Anal. Calcd for $C_{19}H_{15}FNO_4$ (354.33).

N-(2-{[(4-Methylphenyl)methyl]amino}-2-oxoethyl)- 2-oxo-2*H*-1-benzopyran-3-carboxamide, 8c: White solid, m.p. 228-230°C. IR (KBr): 1658.60 (C=O), 1718.99 (C=O), 3332.91 cm^{-1} (NH); ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.24 (3H, s, Ar-CH3), 4.01 (2H, d, *J* = 4.4 Hz glycine-CH2), 4.23 (2H, d, *J* = 5.2 Hz, benzyl-CH2), 7.13-7.98 (8H, Ar-H), 8.44 (1H, s, amideNH-benzyl), 8.88 (1H, s, Ar-H), 9.07 (1H, s, amide-NH-glycine); ^{13}C NMR (100 MHz, DMSO-d_6): δ 25.63 (Ar-CH3), 41.84 (amide-CH2), 43.24 (benzyl –CH2), 115.28, 115.49, 116.57, 118.86, 118.98, 125.59, 129.566, 129.65, 130.79, 134.63, 135.89, 148.14, 154.37 (coumarin C-Ar), 160.77, 161.57 51 (coumarin –C=O), 168.82 (benzyl amide –C=O); LCMS: *m/z* 335.04 (M-H). Anal. Calcd for $C_{20}H_{18}N_2O_4$ (350.36).

N-(2-{[(4-Methoxyphenyl)methyl]amino}-2-oxoethyl)-2-oxo-2*H*-1-benzopyran-3carboxamide, 8d: White solid, m.p. 198-202°C. IR (KBr): 1611.20 (C=O), 1657 (C=O), 1720.74 (C=O), 3279.33 (N-H), 3340.74 cm^{-1} (N-H); ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.73 (3H, s, Ar-OCH_3), 4.24 (2H, d, *J* = 4.8Hz, glycine-CH_2), 4.26 (2H, d, *J* = 4.6 Hz, benzyl-CH_2), 6.94-8.03 (8H, Ar-H), 8.49 (1H, t, *J* = 12Hz, amide-NH-benzyl), 8.93 (1H, s, Ar-H), 9.13 (1H, t, *J* = 5.2Hz, amide-NH-glycine); ^{13}C NMR (100 MHz, DMSO-d_6): δ 42.02 (amide-CH_2), 43.22 (benzyl –CH_2), 55.50 (Ar-OCH_3), 114.12, 116.57, 118.87, 118.98, 125.59, 129.03, 130.78, 131.57, 134.63, 148.12, 154.37, 158.68, 160.77, 161.51 (coumarin –C=O), 168.41 (benzyl amide –C=O), MS: *m/z* 367.04 (M+H). Anal. Calcd for $C_{20}H_{18}N_2O_5$ (366.36).

N-(2-{[(2-Chlorophenyl)methyl]amino}-2-oxoethyl)-2-oxo-2*H*-1-benzopyran-3-carboxamide, 8e: White solid. m.p. 215-216°C. IR (KBr): 781.23 (C-Cl), 1608.62 (C=O), 1658.05 (C=O), 1716.59 (C=O), 3271.64 (N-H), 3329.31 cm^{-1} (N-H); ^{1}H NMR (400 MHz, DMSO-d_6): δ 4.06 (2H, d, *J* = 3.6 Hz, glycine-CH_2), 4.32 (2H, d, *J* = 4 Hz, benzyl-CH_2), 7.18-8.03 (8H, Ar-H), 8.54 (1H, s, amide-NH-benzyl), 8.93 (1H, s, Ar-H), 9.12 (1H, s, amide-NH-glycine); ^{13}C NMR (100 MHz, DMSO-d_6): δ 41.84 (amide-CH_2), 43.24 (benzyl –CH_2), 115.284, 115.498, 116.578, 118.865, 118.985, 125.597, 128.82 (Ar–C-Cl), 129.566, 129.654, 130.792, 134.635, 135.8900, 148.149, 154.376, 160.40, 160.77, 161.57 (coumarin –C=O), 168.658 (benzyl amide –C=O); HRMS: *m/z* 371.28 (M+H). Anal. Calcd for $C_{20}H_{18}N_2O_5$ (370.78).

N-(2-{[(2-Chloro-3-fluorophenyl) methyl] amino}-2-oxoethyl)-2-oxo-2*H*-1-benzopyran-3-carboxamide, 8f: White solid. m.p. 220-222°C. IR (KBr): 783.21 (C-Cl), 1021.34 (C-F), 1655.76 (C=O), 1619.34 (C=O), 3345.61 cm^{-1} (N-H); ^{1}H NMR (400 MHz, DMSO-d_6): δ 4.05 (2H, d, *J* = 5.2 Hz, glycine-CH_2), 4.38 (2H, d, *J* = 5.6 Hz, benzyl-CH_2), 7.22-8.02 (7H, Ar-H), 8.61 (1H, t, *J* = 6.8Hz, amide-NH-benzyl), 8.92 (1H, s, Ar-H), 9.11 (1H, t, *J* = 5.2Hz, amide-NHglycine); ^{13}C NMR (400MHz, DMSO-d_6): δ 35.7 (amide-CH_2), 42.8 (benzyl –CH_2), 119.4, 119.4, 124.0, 128.3, 138.6, 144.1 (Ar-C), 114.2, 120.5, 122.3, 125.4, 126.8, 128.2, 138.5, 150.4, 159.5, 159.5 (coumarin –C=O), 171.1(benzyl amide –C=O). LCMS: *m/z* 389.34 (M+H). Anal. Calcd for$C_{19}H_{14}ClFN_2O_4$ (388.77).

N-(2-{[(2,4-Dimethoxyphenyl)methyl]amino}-2-oxoethyl)-2-oxo-2*H*-1-benzopyran-3carboxamide, 8g: White solid, m.p. 194-196°C. IR (KBr): 1611.20 (C-O), 1657 (C=O), 1720.74 (C=O), 3279.33 (N-H), 3340.74 cm^{-1} (N-H); ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.73 (3H, s, Ar-OCH_3), 3.73 (3H, s, Ar- OCH_3), 4.2(2H, d, *J* = 4.8 Hz, glycine-CH_2), 4.26 (2H, d, *J* = 4.6 Hz, benzyl-CH_2), 7.18-8.03 (8H, Ar-H), 8.49 (1H, t, *J* = 12Hz, amide-NH-benzyl), 8.93 (1H, s, Ar-H), 9.13 (1H, t, *J* = 5.2Hz, amide-NH-glycine); ^{13}C NMR (100 MHz, DMSO-d_6): δ 42.02 (amide-CH_2), 43.22 (benzyl –CH_2), 55.50 (Ar-OCH_3), 55.51 (Ar-OCH_3),114.12, 116.57, 118.87, 118.98, 125.59, 129.03, 130.78, 131.57, 134.63, 148.12, 154.37, 158.68, 160.77, 161.51 (coumarin – C=O), 168.41 (benzyl amide –C=O); HRMS: *m/z* 397.24 (M+H). Anal. Calcd for $C_{20}H_{18}N_2O_5$ (396.39).

N-(2-{[(4-Bromophenyl)methyl]amino}-2-oxoethyl)-2-oxo-2*H*-1-benzopyran-3-carboxamide, 8h: White solid. m.p. 194-196°C. IR (KBr): 1611.20 (C-O), 1657 (C=O), 1720.74 (C=O), 3279.33 (N-H), 3340.74 cm^{-1} (N-H); ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.73 (3H, s, Ar-OCH_3), 3.73 (3H, s, Ar- OCH_3), 4.2 (2H, d, *J* = 4.8 Hz, glycine-CH_2), 4.26 (2H, d, *J* = 4.6 Hz, benzyl-CH_2), 6 7.32-8.03 (8H, Ar-H), 8.49 (1H, t, *J* = 12Hz, amide-NH-benzyl), 8.93 (1H, s, Ar-H), 9.13(1H, t, *J* = 5.2Hz, amide-NH-glycine); ^{13}C NMR (100 MHz, DMSO-d_6): δ 42.02 (amide-CH_2), 43.22 (benzyl –CH_2), 55.50 (Ar-OCH_3), 55.51 (Ar-OCH_3), 114.12, 116.57, 118.87, 118.98, 125.59,

129.03, 130.78, 131.57, 134.63, 148.12, 154.37, 158.68, 160.77, 161.51 (coumarin – C=O), 168.41 (benzyl amide –C=O), HRMS: *m/z* 397.24 (M+H). Anal. Calcd for $C_{20}H_{18}N_2O_5$ (396.39).

Antibacterial activity

The antibacterial activities of the synthesized compounds were performed by the agar well diffusion method as previously described[30]. Nutrient agar was poured on the Petri dish and allowed to solidify. Overnight grown bacterial strains (*Klebsiella pneumonia*, and *Bacillus subtilis*) were seeded on to Petri dish plates and wells of 6.0-mm diameter were aseptically bored and 50 µL of the synthesized compounds 8(a-h) were added to the wells. Petri dish plates were kept for incubation for 24 hr at 37°C. The resulting zone of inhibition flanging the wells was measured in millimetre (mm) and used as criterion for the antibacterial activity. DMSO was used as a negative control while tetracycline as standard.

Absorbance based Microtitre Plate Assay (MPA)

Microtitre plate assay was performed as previously described[31-33] with slight modification. 100 µL of sterile nutrient broth media was pipette into wells of the 96-microtitre plate. Subsequently, 100 µL aliquot of synthesized compounds of stock concentration 10mg/mL were added and then serially diluted in each well. After serial dilution, 100 µL of grown bacterial culture of *Klebsiella pneumonia* and *Bacillus subtilis* media of 0.4 OD at 600 nm was added into all wells. Positive control used contain sterile growth media and grown bacterial strains whereas the negative control contains synthesized compounds and sterile growth media only. The absorbance was recorded by using EnSpire Multimode PlateReader using 96-well plate at 600 nm before incubation and after incubation at 37°C for 24 hr. The MIC value for the synthesized compounds was expressed as the lowest concentration that inhibits bacterial growth. All tests were performed in duplicate.

Antioxidants activity

DPPH scavenging activity

Radical scavenging activity was determined according to Blois[34] with some modification. An aliquot of 25 µL with different concentrations of 10, 20, 30 mg/mL of synthesized compounds dissolved in DMSO was put in a microplate and mixed with 175 µL (0.5 mM) of ethanolic solution of DPPH. The mixture was incubated at room for 30 minutes. The gallic acid was used as the standard. The decolourization of DPPH solution was determined by measuring the absorbance at 517 nm using EnSpire Multimode Plate Reader against blank containing only DPPH. The percentage inhibition activity was calculated using the formula given below and IC50 (Inhibitory concentration) was displayed in Table IV. % inhibition of DPPH radical= [(Abr-Aar)/ Abr× 100]. Where Abr is the absorbance before reaction and Aar is the absorbance after the reaction has taken place. All the experiments were performed in duplicate and average were calculated.

Metal Chelating assay The Fe (II) binding ability of the chelators was determined according to the method of Dinis[35] with some modification according to Adjimani[36] by substituting ferrous chloride by ferrous sulphate. Ferrozine can quantitatively form complexes with ferrous iron yielding a red colour. However, in the presence of chelating agents, there is a disruption of the formation of the complex which leads to a decrease in the red color. Measurement of colour reduction gives an estimation of the binding ability of the coexisting chelator. An aliquot of 25 µL with different concentrations of 10, 20, 30 mg/mL of synthesized compounds dissolved in DMSO was mixed with 0.125 µL of 0.2 mM ferrous sulphate in a microplate. The reaction is started by the addition of 50 µL of 5 mMferrozine, mixed properly and incubated at RT for 10 min. The ferrous ion was monitored by measuring the formation of a red ferrous ion-ferrozine complex at 562 nm. The percentage inhibition activity was calculated by the formula: % Inhibition of metal chelating activity= [(Ac-As)/ Ac× 100].

Where Ac is the absorbance of control before reaction and As is the absorbance of iron chelator. IC_{50} (Inhibitory concentration) was also determined and represented in Table V. All the experiments were performed in replicate and average were calculated.

Molecular Docking studies

The structure of the target glucosamine-6-phosphate synthase (GlcN-6-p synthase), Peroxisome Proliferator-Activated Receptor Alpha (PPARα) and Transforming growth factor β1 (TGF-β1), was obtained from Protein Data Bank (PDB ID; 2VF5, 5HYK, and 1VJY). Structures of synthesized compounds **8(a-h)** were drawn and analyzed using ChemDraw Ultra 12.0. 3D coordinates were obtained using PRODRG online server[37]. Active pockets for proteins were obtained from CASTpserver[38].

Intermediary steps, such as energy minimization, protein and ligands preparation and grid box creation were completed using Graphical User Interface program AutoDock Tools (ADT). AutoDock/Vina employs iterated local search global optimizer[39,40]. During the docking procedure, both the protein and ligands are considered as rigid. The results less than 1.0 Ao in positional root-mean-square deviation (RMSD) was clustered together and represented by the result with the most favourable free energy of binding. The pose with lowest energy of binding or binding affinity was extracted and aligned with receptor structure for further analysis[41,42].

Conclusion

We have designed and synthesized a novel series of 2-(benzylamino)-2-oxoethyl]-2-oxo-2*H*-1-benzopyran-3-carboxamide derivatives using acid amine coupling reaction with suitable coupling reagents. All the synthesized compounds were characterized by IR, ^{1}H NMR, ^{13}C NMR, and mass spectrometry analysis. Further, synthesized compounds were screened for their anti-bacterial and anti-oxidant activity. Out of eight derivatives, compounds **8c**, **8e,** and **8a**, **8h** exhibited efficient anti-oxidant activity in DPPH and metal ion chelating assay. On the other hand inhibition efficacy of synthesized compounds against the test pathogens showed moderate activity.

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Conflict of Interest

There are no conflict of interest.

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