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Design, synthesis, and docking studies of novel benzopyrone derivatives as H_1-antihistaminic agents

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ABSTRACT

Two new series of 2*H*-1-benzopyran-2-one derivatives substituted at C-6 and/or C-7 with propanolamines, and/or piperazine propanol derivatives have been synthesized and assayed for the H_1-histamine antagonist. Twelve of the 20 newly synthesized 4- substituted benzopyrones have shown potent antihistaminic H_1 activity. In addition, molecular modeling and docking of the tested compounds into high affinity histamine binding protein (HBP) and histamine *N*-methyltranseferase (HNMT) active site in complex with its bound inhibitor (diphenhydramine) was performed in order to predict the affinity and orientation of these compounds at the active sites. The ICM score values show good agreement with predicted binding affinities obtained by molecular docking studies as verified by pharmacological screening. The results showed similar orientation of the target compounds at HBP, and HNMT active sites compared with reported histamine H_1 antagonist. Also, it was concluded that in order for the compounds to be active, they must bind with both active sites of HNMT enzyme (two pockets) to inhibit it. Compounds **8c**, **8i**, **11g**, **11i**, and **11k**; observe the maximum activities.

1. Introduction

Histamine is an intercellular chemical messenger and plays a critical role in several diverse physiological processes. Four human G-protein coupled histamine receptor subtypes (H_{1-4}) are currently recognized to mediate various actions of monoamine histamine. Among the four subtypes, the histamine H_1 receptor has been an attractive target for drug discovery for several years and H_1 receptor antagonist have proved to be effective therapeutic agents for respiratory distress, thus contributing to an important class of drugs today.[1]

In mammals, histamine action is terminated mainly through metabolic inactivation by histamine *N*-methyltransferase (HNMT) by transferring a methyl group from *S*-adenosyl-L-methionine (AdoMet) to the N-2 atom of the imidazole ring, yielding methyl histamine and *S*-adenosyl-L-homocysteine (AdoHcy),[2] HNMT has two-domain structure including a histamine-binding domain and the methyl donor (AdoMet) binding domain.[3] Histamine receptors and histamine binding sites in HNMT may be similar as both are inhibited by many H_1-antagonists.[4]

It was reported that histamine receptor H_1 antagonist diphenhydramine, the antimalarial drug amodiaquine, the antifolate drug metoprine, and the anticholinesterase drug tacrine are surprisingly all potent HNMT inhibitors, having inhibition constants in the range of 10–100 nM. Determining the structural mode of interaction of the inhibitor with HNMT, they all occupy the histamine-binding site, thus blocking access to the enzyme's active site.[5] Near the N terminus of HNMT; several aromatic residues (Phe9, Tyr15, and Phe19) adopt different rotamer conformations or become disordered in the enzyme–inhibitor complexes, accommodating the diverse, rigid hydrophobic groups of the inhibitors. The maximized shape complementarily between the protein aromatic side chains and aromatic rings of the inhibitors are responsible for the tight binding of the inhibitor.[6] Also, visualization of diphenhydramine bound to HNMT will assist in the development of new generation H_1-receptor antagonists with 'dual' actions.[6]

Quinacrine antimalarial drug is a potent inhibitor of HNMT, and a competitive inhibitor with respect to histamine.[3]

A common feature of first generation H_1 antihistaminic compounds includes two aryl or two heteroaryl rings linked to an aliphatic tertiary amine via a side chain (diphenhydramine and pheniramine). Also the second generation compounds (terfenadine and cetirizine) also contain many of the structural features of first generation compounds.[7]

The key pharmacophoric moieties consist of the same common feature functions of two ring aromatic features which are essential for significant H_1-receptor affinity which must be capable of adopting a noncoplanar conformation relative to each other for optimal interaction and a basic amine function which represent the positive ionizable feature.[1]

The piperazines or cyclizines can also be considered to be cyclic ethylenediamines (cyclizines), where terminal amine functionality

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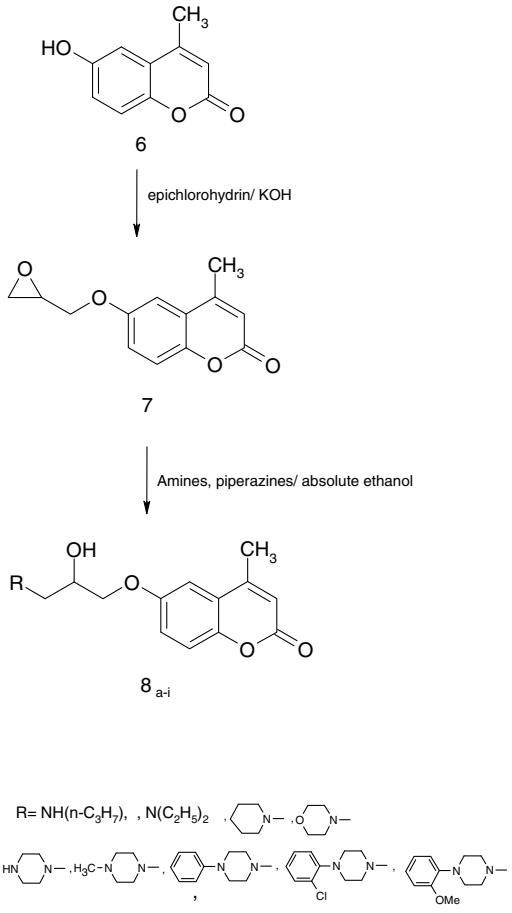
as well as the nitrogen atom of the connecting group are all part of a piperazine moiety. Both nitrogen atoms in these compounds are aliphatic and thus display comparable basicities. The piperazines are moderately potent antihistaminics with a lower incidence of drowsiness and exhibit antimuscarinic activity and may be responsible for antiemetic and antivertigo effects. The activity of the piperazine-type antihistaminics is characterized by a slow onset and long duration.[7]

Furthermore, the works of Wieland et al.[8] on the active antagonistic site region of histamine H_1 receptor prove that, one of the aromatic rings of the antagonists forms favorable aromatic π–π staking interactions with Phe 433 and Phe 436, the other ring establishes aromatic π–π staking with trp 167, additionally, the nitrogen establishes a salt bridge interaction with ASP 116.

A literature survey reveals excellent antihistaminic activity in quinazolines and condensed quinazolines.[9,10] Cromolyn sodium and nedocromil sodium which are chromone derivatives have been shown to inhibit the release of the mediators of allergic reaction.[11–13] Furthermore, it has been observed that certain benzopyranone derivatives possess significant antihistaminic activity.[11–20]

Encouraged by these findings, we thought of preparing new derivatives **8a–i**, **11a–k** of benzopyran-2-ones substituted at C-6 and/or C-7 with different propanolamines and/or piperazine propanol derivatives in order to screen them for H_1 antihistaminic activity and to study the systematic evaluation of the positional isomers in the benzopyranone nucleus, also docking the prepared and tested compounds into histamine binding protein (HBP) and histamine *N*-methyltransferase (HNMT) active site in order to predict the affinity and orientation of these compounds at the active sites in both enzymes.



Scheme 1.

2. Results and discussion

2.1. Chemistry

In this study we synthesized two new series of 4-methyl-6-substituted-2*H*-1-benzopyran-2-one **8**, and 4-phenyl-7-substituted-2*H*-1-benzopyran-2-one 11 substituted with 2-hydroxy propanolamines, 2-hydroxy propanol piperazines to be tested against H_1-antihistaminic activity.

The synthesis of our target compounds is outlined in Schemes 1 and 2. The starting compounds, 6-hydroxy-4-methyl-2*H*-1-benzopyran-2-one **6**, and 7-hydroxy-4-phenyl-2*H*-1-benzopyran-2-one 9 were first condensed with epichlorohydrin in alcoholic potassium hydroxide to give the corresponding epoxides 6-(2,3-epoxypropoxy)-4-methyl-2*H*-1-benzopyran-2-one **7**, 7-(2,3-epoxypropoxy)-4-phenyl-2*H*-1-benzopyran-2-one **10**, then **7**, 10 were reacted smoothly with appropriate amine and/or piperazine derivatives to give 1-(4-methyl-2*H*-1-benzopyran-2-one-6-yloxy)-3-(substituted amino) propan-2-ol **8a–d**, 1-(4-methyl-2*H*-1-benzopyran-2-one-6-yloxy)-3-(substituted piperazino) propan-2-ol **8e–i**. Scheme 1, and 1-(4-phenyl-2H-1-benzopyran-2-one-7-yloxy)-3-(substituted amino) propan-2-ol **11a–f**, 1-(4-phenyl-2*H*-1-benzopyran-2-one-7-yloxy)-3-(substituted piperazino) propan-2-ol **11g–k** Scheme 2.

2.2. Pharmacology

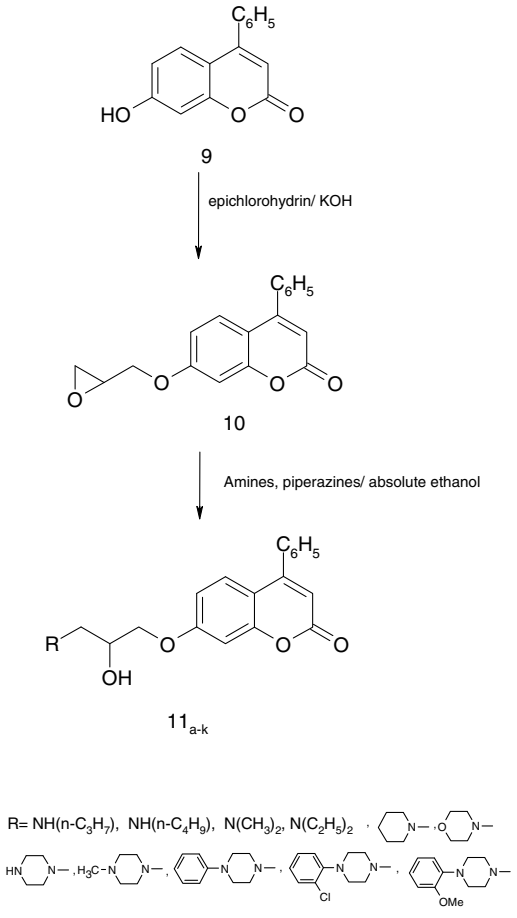
The compounds **8b**, **8c**, **8f**, **8g**, **8i**, **11a**, **11d**, **11e**, **11f**, **11g**, **11i**, and **11k** showed promising activity among the newly synthesized compounds.

Histamine diphosphate elicited contractile responses from the ileum. The threshold value for the contractile effect of histamine on the ileum was obtained at a concentration of 2×10^{-3} μg mL^{-1}, while the maximal response was attained at 9×10^{-3} μg mL^{-1} of histamine (Table 1). The potency of histamine-induced contraction of isolated guinea pig ileum decreased when pretreated with pheniramine maleate and the test compounds.

Cumulative concentration–response curves of histamine diphosphate showed that the test compounds **8b**, **8c**, **8f, 8g, 8i, 11a, 11d, 11e, 11f, 11g**, **11i**, and **11k** inhibited the histamine-induced contraction of isolated guinea pig ileum in a concentration-dependent fashion. The standard antihistaminic pheniramine maleate and three different concentrations of compounds **8b**, **8c**, **8f, 8g, 8i, 11a, 11d, 11e, 11f, 11g**, **11i**, and **11k** showed clear rightward shifts compared to that produced with saline. As shown in Figures. 1 and 2, the test compounds as **11g** and **11k** produced competitive inhibition at low concentrations and non-competitive inhibition at high concentrations of histamine-induced contractions of isolated guinea pig ileum.

The EC_{50} of histamine obtained in the presence of pheniramine maleate (5 μg mL^{-1}) and two concentrations (0.5 and 1.0 mg mL^{-1}) of compounds **8b**, **8c**, **8f, 8g, 8i, 11a, 11d, 11e, 11f, 11g**, **11i**, and **11k** were significantly higher than those for saline (Table 2). However, there were no significant differences among the EC_{50}s obtained for 0.1 mg mL^{-1} of the same compounds.

The maximum response to histamine diphosphate obtained in the presence of 1.0 mg mL^{-1} of compounds **8b**, **8c**, **8f, 8g, 8i, 11a, 11d, 11e, 11f, 11g**, **11i**, and **11k** were significantly lower than those of saline. The maximum responses to histamine obtained in the presence of pheniramine maleate (5 μg mL^{-1}) and 1.0 mg mL^{-1} of compounds: **8b**, **8c**, **8f, 8g, 8i, 11a**, **11d**, **11e**, **11f**, **11g**, **11i**, and **11k** were significantly lower than that of saline (Table 2). However, the maximum response to histamine obtained in the presence of 0.1 and 0.5 mg mL^{-1} of the test compounds were not significantly different from that of saline.



Scheme 2.

Table 1
A measure of sensitivity of isolated guinea pig ileum to histamine diphosphate

Concentration ($\times 10^{-3}$ μg mL^{-1})	Response of isolated ileum	Average height of contraction (cm)	
<1	No response	0.00	(5)
2*	Contraction	0.28	(5)
3	Contraction	0.65	(5)
4	Contraction	0.96	(5)
5	Contraction	1.25	(5)
6	Contraction	1.42	(5)
7	Contraction	1.48	(5)
8	Contraction	1.54	(5)
9**	Contraction	1.62	(5)
10	Contraction	1.62	(5)

() Number of observations.
* Threshold value (T.R.).
** Maximal response (M.R.).

2.3. Docking studies

To understand the pharmacological data on structural basis, we evaluate the titled compounds (two different classes of benzopyrones) through docking techniques using Molsoft ICM 3.4-8C program. We docked our titled compounds on one of the crystal structures of histamine-binding protein (HBPs) available through the RCSB Protein Data Bank (PDB entry 1QFT),[22] which reveals a lipocalin fold novel in containing two binding sites for the same ligand (histamine), and on another crystal structures of histamine *N*-methyltransferase (HNMT) in complex with its bound inhibitor diphenhydramine (1H *n*-[(2-bezhydroxy) ethyl] *n*,*n*-dimethylamine) available through the RCSB Protein Data Bank (PDB entry 2AOT).[6]

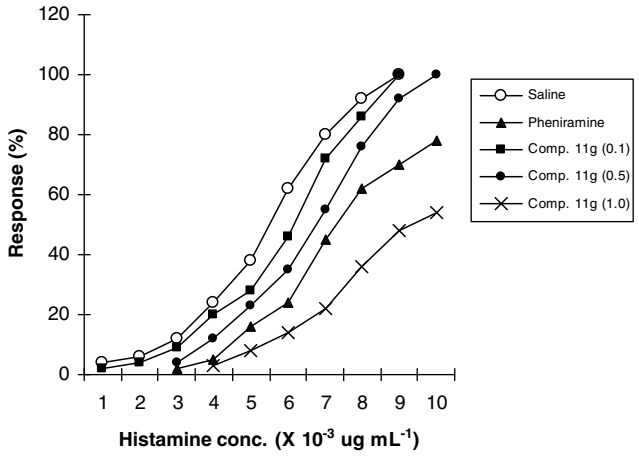


Figure 1. Cumulative concentration–response curves of histamine-induced contraction of guinea pig ileum in the presence of saline, pheniramine maleate (5 μg mL^{-1}) and three increment concentrations (0.1, 0.5, and 1.0 mg mL^{-1}) of compound **11g**. Number of observation = 5.

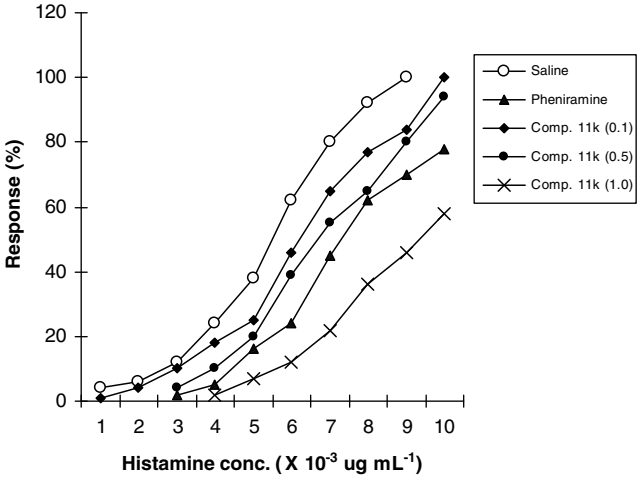


Figure 2. Cumulative concentration–response curves of histamine-induced contraction of guinea pig ileum in the presence of saline, pheniramine maleate (5 μg mL^{-1}) and three increment concentrations (0.1, 0.5, and 1.0 mg mL^{-1}) of compound **11k**. Number of observation = 5.

The ICM score values obtained by docking our titled compounds on histamine *N*-methyltransferase (HNMT) shows more accurate result than that on histamine binding protein. It also shows good agreement with the predicted binding affinities obtained by molecular docking studies on (2AOT), and as verified by pharmacological screening (Table 3 and Fig. 10).

Figure 3 shows the docking solutions of **8b, 8c, 8f, 8g, and 8i** with histamine binding protein (1QFT), Figure 4 shows the docking solutions of **11a, 11d, 11e, 11f, 11g, 11h, 11i, and 11k** with histamine binding protein (1QFT), Figures 5–8 shows the docking solutions with the highest predicted binding affinity for histamine *N*-methyltransferase (2AOT) and different benzopyrone derivatives 8a, **8i**, **11c**, and 11k, respectively, while Figure 9 shows orientations of diphenhydramine, amodiaquine, tacrine and metoprine at HNMT active site at (2AOT).

With respect to ICM scores on histamine *N*-methyltransferase (2AOT), it reveals that compound **8i** is pharmacologically active and has the least score in this series −95.75, while **8a** is inactive pharmacologically and has the highest score in this series −79.96, additionally, it shows that compound **11k** is the most ac-

Table 2
EC_{50} and E_{max} of histamine diphosphate in the presence of saline, pheniramine maleate and the test compounds ($n = 5$)

Solutions	Concentration	EC_{50} ($\times 10^{-3}$ μg mL^{-1})	E_{max} (%)
Saline	—	5.1 ± 0.28	100 ± 0.00
Pheniramine maleate	5 μg mL^{-1}	6.8 ± 0.30***	78 ± 3.82***
8b	0.1 mg mL^{-1}	5.2 ± 0.34	100 ± 0.00
	0.5 mg mL^{-1}	6.0 ± 0.31*	96 ± 4.46
	1.0 mg mL^{-1}	8.5 ± 0.32***	58 ± 3.28***
8c	0.1 mg mL^{-1}	5.5±0.14	100 ± 0.00
	0.5 mg mL^{-1}	6.5 ± 0.31*	98 ± 4.46
	1.0 mg mL^{-1}	8.3 ± 0.28***	55 ± 3.85***
8f	0.1 mg mL^{-1}	5.8 ± 0.30	100 ± 0.00
	0.5 mg mL^{-1}	6.0 ± 0.31*	98 ± 5.06
	1.0 mg mL^{-1}	8.1 ± 0.31***	54 ± 3.11***
8g	0.1 mg mL^{-1}	5.2 ± 0.34	100 ± 0.00
	0.5 mg mL^{-1}	6.3 ± 0.31*	95 ± 5.72
	1.0 mg mL^{-1}	8.5 ± 0.28***	60 ± 3.82***
8i	0.1 mg mL^{-1}	5.8 ± 0.29	100 ± 0.00
	0.5 mg mL^{-1}	6.2 ± 0.30*	96 ± 5.40
	1.0 mg mL^{-1}	8.5 ± 0.28***	58 ± 3.82***
11a	0.1 mg mL^{-1}	5.6 ± 0.27	100 ± 0.00
	0.5 mg mL^{-1}	6.3 ± 0.30*	100 ± 0.00
	1.0 mg mL^{-1}	8.5 ± 0.32***	53 ± 2.86***
11d	0.1 mg mL^{-1}	5.9 ± 0.29	100 ± 0.00
	0.5 mg mL^{-1}	6.4 ± 0.33**	95 ± 5.72
	1.0 mg mL^{-1}	8.9 ± 0.32***	60 ± 4.18***
11e	0.1 mg mL^{-1}	5.5 ± 0.29	100 ± 0.00
	0.5 mg mL^{-1}	6.2 ± 0.30*	95 ± 5.00
	1.0 mg mL^{-1}	8.3 ± 0.32***	58 ± 3.85***
11f	0.1 mg mL^{-1}	5.6 ± 0.32	100 ± 0.00
	0.5 mg mL^{-1}	6.0 ± 0.31*	94 ± 4.96
	1.0 mg mL^{-1}	8.9 ± 0.30***	60 ± 3.74***
11g	0.1 mg mL^{-1}	5.8 ± 0.29	100 ± 0.00
	0.5 mg mL^{-1}	6.3 ± 0.29**	100 ± 0.00
	1.0 mg mL^{-1}	8.6 ± 0.27***	53 ± 3.22***
11i	0.1 mg mL^{-1}	5.4 ± 0.13	100 ± 0.00
	0.5 mg mL^{-1}	6.3 ± 0.29**	94 ± 4.95
	1.0 mg mL^{-1}	8.3 ± 0.4.3***	54 ± 2.18***
11k	0.1 mg mL^{-1}	5.7 ± 0.29	100 ± 0.00
	0.5 mg mL^{-1}	6.2 ± 0.30*	96 ± 5.27
	1.0 mg mL^{-1}	8.9 ± 0.31***	52 ± 3.18***

Significant at: $^{*}P \leqslant 0.05$; $^{**}P \leqslant 0.01$; $^{***}P \leqslant 0.001$.

tive compound and has nearly the least score in this series −99.68, while **11c** is inactive pharmacologically and has the highest score in this series −81.46. Also orientation of compounds **8a**, **8i**, **11c**, 11k, and diphenhydramine at the active site reveals that the molecule of the active compounds must bind with both active sites diphenhydramine (pocket 2, red), and *S*-adenosyl-L-homocysteine (AdoHcy) (pocket 1, blue) in the enzyme to inhibit it (Figs. 5–8).

It was mentioned earlier that histamine action is terminated through metabolic inactivation by histamine *N*-methyltransferase (HNMT), and diphenhydramine, amodiaquine, metoprine, and tacrine and they are surprisingly all potent HNMT inhibitors.[6] From our Docking studies, it was revealed that they all occupy the histamine-binding site and give moderate ICM scores (Table 3), and all of them occupy only the histamine binding site pocket (red pocket) not *S*-adenosyl-L-homocystein pocket (blue one) Figure 9.

Also, structural comparisons reveal that benzopyrane-2-one moiety may share common interaction pattern with the protein binding pockets, despite their different substituents.

3. Conclusion

From the literature, we knew about the importance of the two aromatic rings feature and the basic amine for the H_1-histamine antagonist. In our studies, we focused on substitution of benzopyran-2-ones at C-6 and/or C-7 with different propanol amines and/or propanol piperazines derivatives **8a–i**, **11a–k** in order to screen them for H_1 antihistaminic activity.

Table 3
ICM Scores of the tested compounds

Compound	ICM docking scores of 2AOT	No. of hydrogen bonds	ICM docking scores of 1QFT	No. of hydrogen bonds
Diphenhydramine	−79.47	0	−61.98	1
Pheniramine	−79.48	0	−60.49	1
Cyclizine	−78.04	0	−60.08	0
Amodiaquine	**−91.00**	**0**	—	—
Metoprine	**−76.94**	**5**	—	—
Tacrine	**−6.76**	**0**	—	—
8a	**−79.96**	**5**	**−75.42**	**6**
8b	−84.67	7	−66.78	6
8c	−87.26	1	−71.42	6
8d	**−80.98**	**6**	**−64.29**	**4**
8e	**−79.55**	**3**	**−67.39**	**5**
8f	−86.24	1	−70.23	6
8g	−94.16	2	−70.15	4
8h	**−91.22**	**2**	**−70.83**	**4**
8i	−95.75	1	−74.51	7
11a	−85.09	3	−74.76	4
11b	**−93.24**	**1**	**−78.38**	**4**
11c	**−81.46**	**5**	**−64.83**	**5**
11d	−83.99	4	−73.25	4
11e	**−91.09**	**2**	**−75.94**	**5**
11f	−86.00	5	−69.20	5
11g	−84.72	6	−69.30	5
11h	**−87.22**	**3**	**−68.52**	**3**
11i	−100.03	4	−72.41	6
11j	**−96.48**	**2**	**−65.88**	**7**
11k	−99.68	2	−71.37	6

Molecular docking studies on the two crystal structures of histamine binding protein (1QFT), and histamine *N*-methyltransferase (2AOT) reveals that the results of docking is more accurate with histamine *N*-methyl transferase enzyme, and we conclude that:

(1) The only active amine derivatives in which its molecule is bound with both pockets in the enzyme are **8b, 8c, 11a, 11d, and 11f**, while the inactive amine derivatives occupy the histamine binding site only not *S*-adenosine-L-homocysteine binding site.
(2) Molecular docking and test results showed that, all the piperazine derivatives bind with both pockets of the enzyme, *N*-phenyl substitution of piperazine moiety retain activity, replacement of ortho proton of phenyl piperazine by chloro group abolish the activity, while its substitution with methoxy group significantly increase the activity in both series.

The experimental findings are in good agreement with predicted binding affinities obtained by molecular docking studies on histamine *N*-methyltransferase. The present work may be allowed an at least ranking of the new synthesized and tested H_1-antihistaminic agents.

4. Experimental

4.1. General

Remarks: All melting points are uncorrected and determined by the open capillary method using Gallen Kamp melting point apparatus (MFB-595-010M). Microanalysis was carried out at the microanalytical unit, Faculty of Science, Cairo University. Infrared spectra were determined (KBr) using Schimadzu Infrared Spec-

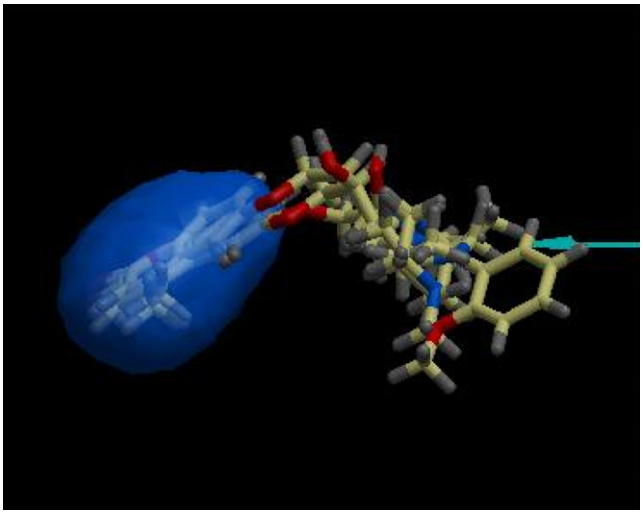


Figure 3. Orientation of active compounds of 8 at HBP active site.

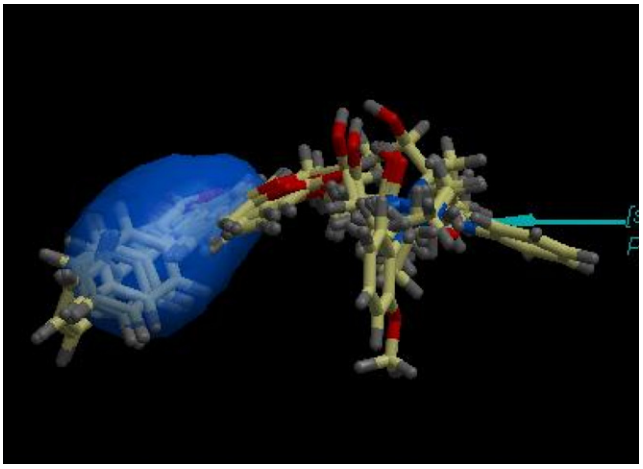


Figure 4. Orientation of active compounds of 11 at HBP active site.

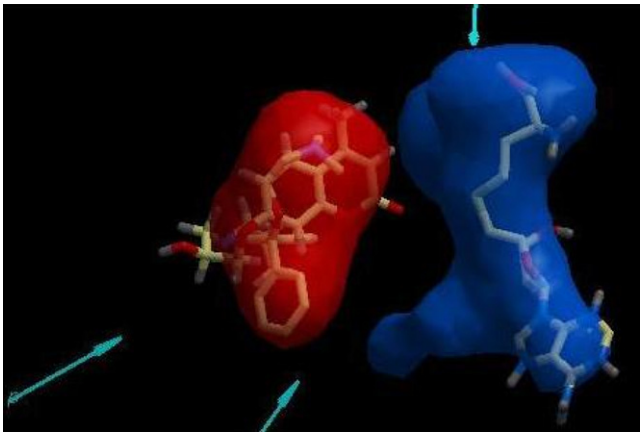


Figure 5. Orientation of **8a**, diphenhydramine (red pocket), and *S*-adenosyl-L-homocysteine (blue pocket) at HNMT active site.

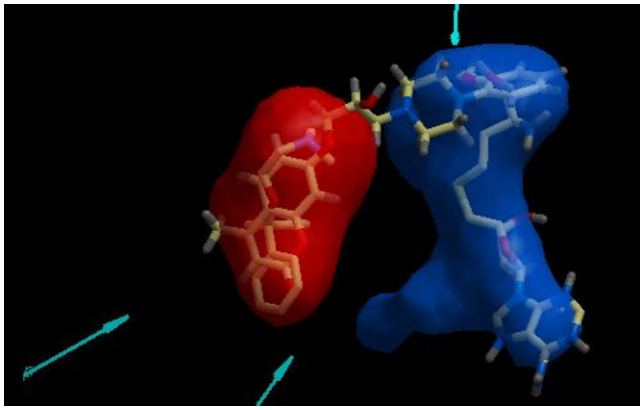


Figure 6. Orientation of **8i**, diphenhydramine (red pocket), and *S*-adenosyl-L-homocysteine (blue pocket) at HNMT active site.

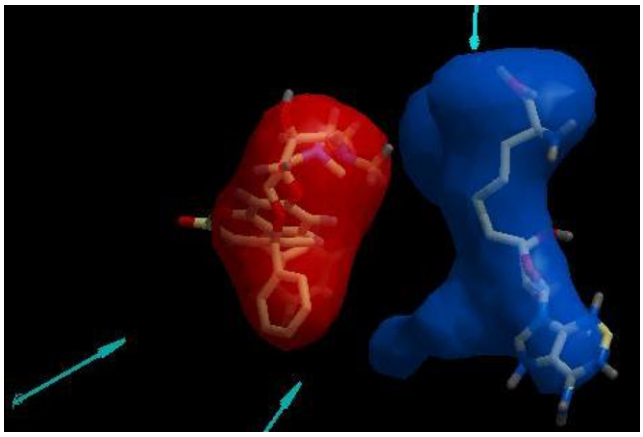


Figure 7. Orientation **11c**, diphenhydramine (red pocket), and *S*-adenosyl-L-homocysteine (blue pocket) at HNMT active site.

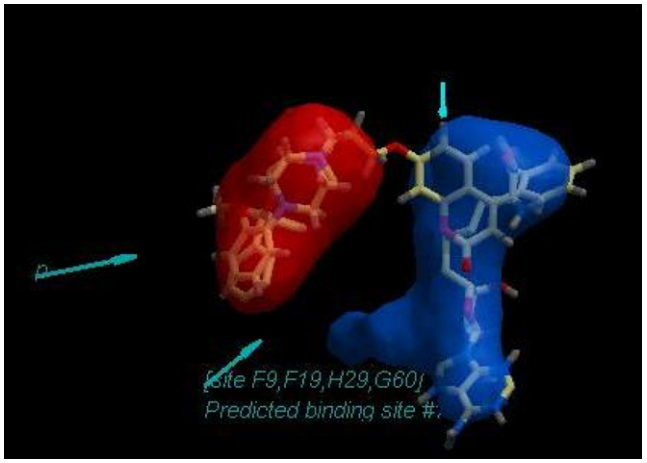


Figure 8. Orientation of **11k**, diphenhydramine (red pocket), and *S*-adenosyl-L-homocysteine (blue pocket) at HNMT active site.

trometer (IR-435) and FT-IR 1650 (Perkin Elmer). ^{1}H NMR Spectra were carried out using Joel, FX 90Q, NMR Spectrometer at 200 MHz and Fourier transform EM-390, 300 MHz NMR Spectrometer. Mass spectra were carried out using Finnigan SSQ 7000 Gas Chromatograph–Mass spectrometer. TLC was carried out using Art. 5735, DC-Plastikfolien, Kieselgel 60 F254 sheets (Merck), the developing solvents were CCl_4/CH_3OH (9:1) and the spots were visualized by UV 366 and 254 nm.

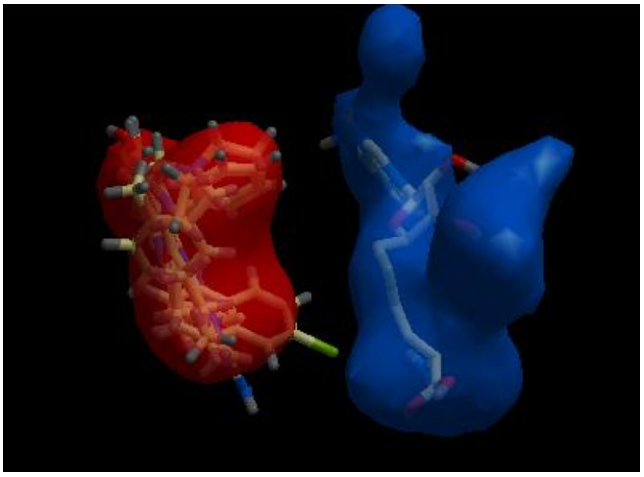


Figure 9. Orientation of diphenhydramine, amodiaquine, tacrine, and metoprine (red pocket), and *S*-adenosyl-L-homocysteine (blue pocket) at HNMT active site.

4.2. Chemistry

4.2.1. Synthesis of 6-hydroxy-4-methyl-2*H*-1-benzopyran-2-one (6).

Was previously reported.[23-25]

4.2.2. Synthesis of 7-hydroxy-4-phenyl-2H-1-benzopyran-2-one (9).

Was previously reported.[23-25]

4.2.3. Synthesis of 6-(2,3-epoxypropoxy)-4-methyl-2*H*-1-benzopyran-2-one (7).

Was previously reported.[23-25]

4.2.4. 7-(2,3-epoxypropoxy)-4-phenyl-2*H*-1-benzopyran-2-one (10)

Epichlorohydrin (2.8 mL, 0.03 mol) was added carefully while stirring to a solution of 7-hydroxy-4-phenyl-2*H*-1-benzopyran-2-one (**9**, 2.38 g, 0.01 mol) in ethanol 50 mL and potassium hydroxide (0.56 g, 0.01 mol). The mixture was refluxed for 2 h, filtered while hot, the solvent was removed under reduced pressure and the residue partitioned between water and ethyl acetate. Evaporation of the dried magnesium sulfate organic phase gave the product. Recrystallization from ethanol afforded 2.4 g, yield 81%, mp: 126 °C.

^{1}H NMR ($CDCl_3$-d_6) δ ppm: 2.76–2.73 (d, 2H, CH_2, J = 2.25 Hz), 4.05–2.79 (m, 1H, CH), 4.30–4.27 (d, 2H, OCH_2, J = 2.52 Hz), 6.208 (s, 1H, C_3-H), 7.70–6.75 (m, 8H, ArH). Anal. Calcd for $C_{18}H_{14}O_4$ (294): C, 73.47; H, 4.76. Found: C, 73.38; H, 4.59.

4.2.5. General method for preparation of 6-[2-hydroxy-3-(substituted) propoxy]-4-methyl-2*H*-1-benzopyran-2-one (8a–i)

A solution of 6-(2,3-epoxypropoxy)-4-methyl-2*H*-1-benzopyran-2-one (**7**, 2.32 g, 0.01 mol) and the appropriate amine or piperazine (0.01 mol) in ethanol 50 mL was stirred at reflux for 90 min and allowed to cool, The precipitate obtained was collected, dried and crystallized from ethanol.

4.2.5.1. 6-[2-hydroxy-3-(propylamino)propoxy]-4-methyl-2*H*-1-benzopyran-2-one (8a). Yield 63%; mp: 130 °C. ^{1}H NMR (DMSO-d_6, D_2O) δ ppm: 1.00 (t, 3H, –N–CH_2– CH_2–CH_3), 2.20 (m, 2H, –N–CH_2– CH_2–CH_3), 2.43 (s, 3H, C_4–CH_3), 2.64–3.04 (m, 4H, –CH_2–N–CH_2– CH_2–CH_3), 3.89 (m, 1H, –CH–CH_2–N–pr), 4.19 (m, 2H, OCH_2), 6.38 (s, 1H, C_3–H), 7.22–7.31 (m, 3H, ar-H), 9.57 (s, 1H, OH, or NH, exch.).

Anal. Calcd for $C_{16}H_{21}NO_4$: C, 65.98; H, 7.22; N, 4.81. Found: C, 66.47; H, 7.35; N, 4.94.

4.2.5.2. 6-[2-Hydroxy-3-(diethylamino)propoxy]-4-methyl-2H-1-benzopyran-2-one (8b). Yield 74%; mp: 125 °C. ^{1}H NMR (DMSO-d_6, D_2O) δ ppm: 1.00 (t, 6H, –N–(CH_2–CH_3)$_2$), 2.43 (s, 3H, C_4–CH_3), 2.48 (q, 4H, –N–(CH_2–CH_3)$_2$), 2.64–3.03 (m, 2H, –CH_2–N–(CH_2–CH_3)$_2$), 3.98 (m, 1H, –CH–CH_2–N–(CH_2–CH_3)$_2$), 4.19 (m, 2H, OCH_2), 6.38 (s, 1H, C_3–H), 7.21–7.50 (m, 3H, ar-H), 9.60 (s, 1H, OH, or NH, exch.). Anal. Calcd for $C_{17}H_{23}NO_4$: C, 67.11; H, 7.54; N, 4.59. Found: C, 67.33; H, 7.57; N, 4.79.

4.2.5.3. 6-[2-Hydroxy-3-(piperidino-1-yl)propoxy)]-4-methyl-2*H*-1-benzopyran-2-one (8c). Yield 67%; mp: 128 °C. ^{1}H NMR (DMSO-d_6, D_2O) δ ppm: 1.93–2.23 (m, 6H, 3*CH_2), 2.43 (s, 3H, C_4–CH_3), 2.54–2.61 (m, 6H, CH_2–N–(CH_2)$_2$-), 3.77 (m, 1H, –CH–CH_2–N–), 4.21 (m, 2H, OCH_2), 6.38 (s, 1H, C_3–H), 7.10–7.48 (m, 3H, ar-H), 9.60 (s, 1H, OH, or NH, exch.). Anal. Calcd for $C_{18}H_{23}NO_4$: C, 68.14; H, 7.56; N, 4.42. Found: C, 67.83; H, 7.65; N, 4.78.

4.2.5.4. 6-[2-Hydroxy-3-(morpholin-1-yl)propoxy)]-4-methyl-2*H*-1-benzopyran-2-one (8d). Yield 65%; mp: 144 °C. ^{1}H NMR (DMSO-d_6, D_2O) δ ppm: 2.43 (s, 3H, C_4–CH_3), 2.76–2.79 (m, 6H, CH_2-N-(CH_2)$_2$), 3.42–3.66 (m, 4H, O–(CH_2)$_2$), 3.81–3.94 (m, 1H, –CH–CH_2–N–), 4.39 (m, 2H, OCH_2), 6.38 (s, 1H, C_3–H), 7.10–7.48 (m, 3H, ar-H), 9.70 (s, 1H, OH, or NH, exch.). Anal. Calcd for $C_{17}H_{21}NO_5$: C, 63.95; H, 6.58; N, 4.39. Found: C, 63.68; H, 6.26; N 4.60.

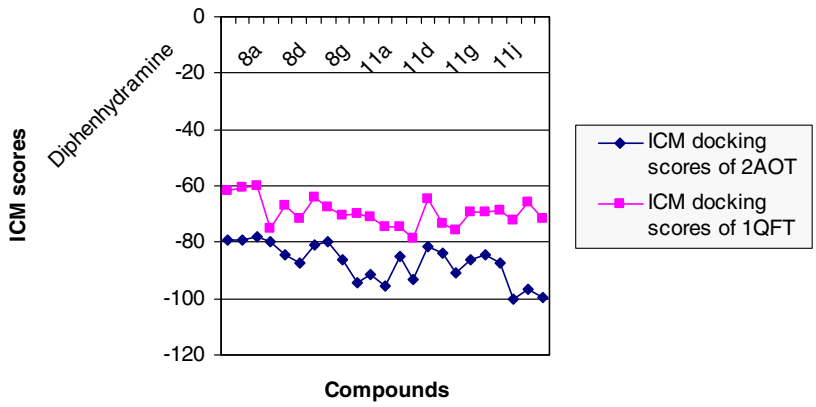


Figure 10. Chart representation of Table 3.

4.2.5.5. 6-(2-Hydroxy-3-(piperazin-1-yl)propoxy)-4-methyl-2*H*-1-benzopyran-2-one (8e). Yield 95%; mp: 159 °C. ^{1}H NMR ($CDCl_3$-d_6, D_2O) δ ppm: 2.44 (s, 3H, C_4–CH_3), 2.73–3.07 (m, 10H, N–$(CH_2)_5$), 3.45, 3.99 (m, 1H, –*CH*–CH_2–N–), 4.20 (s, 2H, OCH_2), 6.37 (s, 1H, C_3–H), 7.25–7.28 (m, 3H, ar-H), 9.58 (s, 1H, OH and/or NH). Anal. Calcd for $C_{17}H_{22}N_2O_4$: C, 64.15; H, 6.92; N, 8.81. Found: C, 64.22; H, 6.71; N, 8.85.

4.2.5.6. 6-(2-Hydroxy-3-(methylpiperazin-1-yl)propoxy)-4-methyl-2*H*-1-benzopyran-2-one (8f). Yield 80%; mp: 207 °C. ^{1}H NMR ($CDCl_3$-d_6, D_2O) δ ppm: 2.41 (s, 3H, C_4–CH_3), 2.49 (s, 3H, –N–CH_3), 2.51–3.11 (m, 10H, N–$(CH_2)_5$), 3.39, 3.67 (m, 1H, –*CH*–CH_2–N–), 4.20 (s, 2H, OCH_2), 6.37 (s, 1H, C_3–H), 7.25–7.28 (s, 3H, ar-H), 9.55 (s, 1H, OH and/or NH). Anal. Calcd for $C_{18}H_{24}N_2O_4$: C, 65.06; H, 7.23; N, 8.43. Found: C, 65.35; H, 7.62; N 8.80.

4.2.5.7. 6-(2-Hydroxy-3-(phenylpiperazin-1-yl)propoxy)-4-methyl-2*H*-1-benzopyran-2-one (8g). Yield 93%; mp: 137 °C. ^{1}H NMR (DMSO-d_6, D_2O) δ ppm: 2.43 (s, 3H, C_4–CH_3), 2.62–3.65 (m, 10H, N–$(CH_2)_5$), 3.73, 3.93 (m, 1H, –*CH*–CH_2–N–), 4.05 (s, 2H, OCH_2), 6.38 (s, 1H, C_3–H), 6.56–7.29 (m, 8H, ar-H), 15.68 (s, 1H, OH and/or NH). Anal. Calcd for $C_{23}H_{26}N_2O_4$: C, 70.05; H, 6.60; N, 7.11. Found: C, 70.11; H, 6.62; N, 7.15.

4.2.5.8. 6-(2-Hydroxy-3-(2′-chloropiperazin-1-yl)propoxy)-4-methyl-2*H*-1-benzopyran-2-one (8h). Yield 75%; mp: 100 °C. MS: *m*/*z* (%) = 428.15 (M^+, 1.59), 429.65 (0.78), 430 (0.78), 395.4 (0.63), 209 (100), 176 (50.16).[1] H NMR (DMSO-d_6, D_2O) δ ppm: 2.44 (s, 3H, C_4–CH_3), 3.33 (m, 10H, N–$(CH_2)_5$), 3.56–3.78 (m, 1H, –*CH*–CH_2–N–), 4.06 (s, 2H, OCH_2), 6.38 (s, 1H, C_3–H), 7.24–7.30 (m, 7H, ar-H), 9.59 (s, 1H, OH and/or NH). Anal. Calcd for $C_{23}H_{25}N_2O_4Cl$: C, 64.41; H, 5.83; N, 6.53. Found: C, 64.58, H 5.36, N 6.37.

4.2.5.9. 6-(2-hydroxy-3-(2′-methoxypiperazin-1-yl)propoxy)-4-methyl-2*H*-1-benzopyran-2-one (8i). Yield 95%; mp: 125 °C. ^{1}H NMR (DMSO-d_6, D_2O) δ ppm: 2.26 (s, 3H, C_4–CH_3), 2.45–3.29 (m, 10H, N–$(CH_2)_5$), 3.44 (m, 1H, –*CH*–CH_2–N–), 3.80 (s, 3H, O CH_3), 4.08 (s, 2H, OCH_2), 6.41 (s, 1H, C_3–H), 6.77–7.34 (m, 7H, ar-H), 9.57 (s, 1H, OH and/or NH). Anal. Calcd for $C_{24}H_{28}N_2O_5$: C, 67.92; H, 6.60; N, 6.60. Found: C, 68.14; H, 6.46; N, 6.63.

4.2.6. General method for preparation of 7-2-hydroxy-(3-substituted) propoxy)-4-phenyl-2*H*-1-benzopyran-2-one (11a–k)

A solution of 7-(2,3-epoxypropoxy)-4-phenyl-2*H*-1-benzopyran-2-one (**10**, 2.94 g, 0.01 mol) and the appropriate amine or piperazine (0.01 mol) in ethanol 50 mL was stirred at reflux for 90 min and allowed to cool, The residue obtained was collected, dried and crystallized from ethanol.

4.2.6.1. 7-(2-Hydroxy-(3-propylamino)propoxy)-4-phenyl-2*H*-1-benzopyran-2-one (11a). Yield 65%; mp: 230 °C. ^{1}H NMR (DMSO-d_6, D_2O) δ ppm: 1.00 (t, 3H, –N–CH_2–CH_2–*CH_3*), 2.64 (m, 2H, –N–CH_2–CH_2–CH_3), 2.85–3.12 (m, 4H, –*CH_2*–N–*CH_2*–CH_2–CH_3), 3.64 (m, 1H, –*CH*–CH_2–N–pr), 4.21 (m, 2H, O*CH_2*), 6.22 (s, 1H, C_3–H), 7.09–7.54 (m, 8H, ar-H), 9.57 (s, 1H, OH, or NH, exch.). Anal. Calcd for $C_{21}H_{23}NO_4$: C, 71.39; H, 6.52; N, 3.97. Found: C, 71.66; H, 6.31; N, 3.98.

4.2.6.2. 7-(3-Butylamino)-2-hydroxypropoxy)-4-phenyl-2*H*-1-benzopyran-2-one (11b). Yield 55%; mp: 228 °C. ^{1}H NMR (DMSO-d_6, D_2O) δ ppm: 1.00 (t, 3H, –N–CH_2–CH_2–CH_2–*CH_3*), 1.70–1.92 (m, 4H, –N–CH_2–*CH_2*–*CH_2*–CH_3), 2.43 (s, 3H, C_4–CH_3), 2.85–3.12 (m, 4H, –*CH_2*–N–*CH_2*–CH_2– CH_2–CH_3), 3.74 (m, 1H, –*CH*–CH_2–N–bu), 4.23 (m, 2H, O*CH_2*), 6.22 (s, 1H, C_3–H), 7.012–7.56 (m, 8H, ar-H), 9.55 (s, 1H, OH, or NH, exch.). Anal. Calcd for $C_{22}H_{25}NO_4$: C, 71.93; H, 6.81; N 3.81. Found: C, 71.72; H, 6.47; N, 3.83.

4.2.6.3. 7-(3-(Dimethylamino)-2-hydroxypropoxy)-4-phenyl-2*H*-1-benzopyran-2-one (11c). Yield 65%; mp: 150 °C. ^{1}H NMR ($CDCl_3$, D_2O) δ ppm: 2.33 (s, H, CH_2–$N(CH_3)_2$), 3.44–3.52 (m, 1H, –*CH*–CH_2–N–), 4.03 (m, 2H, OCH_2), 6.21 (s, 1H, C_3–H), 6.90–7.29 (m, 8H, ar-H), 9.29 (s, 1H, OH, or NH, exch.). Anal. Calcd for $C_{20}H_{21}NO_4$: C, 70.79; H, 6.19; N, 4.13. Found: C, 70.57; H, 6.24; N, 4.23.

4.2.6.4. 7-(3-(Diethylamino)-2-hydroxypropoxy)-4-phenyl-2*H*-1-benzopyran-2-one (11d). Yield 80%; mp: 229 °C. ^{1}H NMR (DMSO-d_6, D_2O) δ ppm: 1.00 (t, 6H, –N–(CH_2–*CH_3*)$_2$), 2.33 (q, 4H, –N–(*CH_2*–CH_3)$_2$), 2.64–3.30 (m, 2H, –*CH_2*–N–(CH_2–CH_3)$_2$), 4.03 (m, 1H, –*CH*–CH_2–N–(CH_2–CH_3)$_2$), 4.19 (m, 2H, O*CH_2*), 6.21 (s, 1H, C_3–H), 7.52–7.85 (m, 8H, ar-H), 9.29 (s, 1H, OH, or NH, exch.). Anal. Calcd for $C_{22}H_{25}NO_4$: C, 71.93; H, 6.81; N, 3.81. Found: C, 71.66; H; 6.31; N, 3.85.

4.2.6.5. 7-(2-Hydroxy-3-(morpholin-1-yl)propoxy)-4-phenyl-2*H*-1-benzopyran-2-one (11e). Yield 85%; mp: 226 °C. ^{1}H NMR (DMSO-d_6, D_2O) δ ppm: 1.93–2.23 (m, 6H, 3 $^*CH_2$), 3.31–3.10 (m, 6H, –CH_2–N–(CH_2)$_2$–), 3.73 (m, 1H, –*CH*–CH_2–N-), 4.23 (m, 2H, OCH_2), 6.21 (s, 1H, C_3–H), 6.89–7.74 (m, 8H, ar-H), 9.60 (s, 1H, OH, or NH, exch.). Anal. Calcd for $C_{23}H_{25}NO_4$: C, 72.81; H, 6.59; N, 3.69. Found: C, 73.11; H, 5.92; N, 3.66.

4.2.6.6. 7-(2-Hydroxy-3-(piperidin-1-yl)propoxy)-4-phenyl-2*H*-1-benzopyran-2-one (11f). Yield 75%; mp: 218 °C. ^{1}H NMR (DMSO-d_6, D_2O) δ ppm: 2.71–2.77 (m, 6H, *CH_2*–*N*–*(CH_2)$_2$*), 2.85–3.45 (m, 4H, O–(CH_2)$_2$), 3.85–3.98 (m, 1H, –*CH*–CH_2–N–), 4.43 (m, 2H, OCH_2), 6.24 (s, 1H, C_3–H), 6.88–7.56 (m, 8H, ar-H), 9.70 (s, 1H, OH, or NH, exch.). Anal. Calcd for $C_{22}H_{23}NO_5$: C, 69.29; H, 6.04; N, 3.67. Found: C, 69.30; H, 6.17; N, 3.69.

4.2.6.7. 7-(2-Hydroxy-3-(piperazin-1-yl)propoxy)-4-phenyl-2*H*-1-benzopyran-2-one (11g). Yield 75%; mp: 210 °C. ^{1}H NMR ($CDCl_3$-d_6, D_2O) δ ppm: 2.46–2.84 (m, 10H, N–$(CH_2)_5$), 3.37, 3.63 (m, 1H, –*CH*–CH_2–N–), 4.10 (s, 2H, OCH_2), 6.21 (s, 1H, C_3–H), 7.29–7.55 (m, 8H, ar-H), 9.55 (s, 1H, NH, exch.), 9.57 (s, 1H, OH, exch.). Anal. Calcd for $C_{22}H_{24}N_2O_4$: C, 69.47; H, 6.32; N, 7.37. Found: C, 69.70; H, 6.33; N, 7.32.

4.2.6.8. 7-(2-Hydroxy-3-(4-methylpiperazin-1-yl)propoxy)-4-phenyl-2*H*-1-benzopyran-2-one (11h). Yield 85%; mp: 233 °C. ^{1}H NMR (DMSO-d_6, D_2O) δ ppm: 2.49 (s, 3H, –N–CH_3), 2.51–3.11 (m, 10H, N–$(CH_2)_5$), 3.39, 3.67 (m, 1H, –*CH*–CH_2–N–), 4.20 (s, 2H, OCH_2), 6.37 (s, 1H, C_3–H), 7.25–7.28 (s, 3H, aromatic protons), 9.55 (s, 1H, OH and/or NH, exch.). Anal. Calcd for $C_{23}H_{26}N_2O_4$: C, 70.05; H, 6.59; N, 7.11. Found: C, 69.79; H, 6.27; N, 7.35.

4.2.6.9. 7-(2-Hydroxy-3-(4-phenylpiperazin-1-yl)propoxy)-4-phenyl-2*H*-1-benzopyran-2-one (11i). Yield 90%; mp: 91 °C. ^{1}H NMR (DMSO-d_6, D_2O) δ ppm: 2.50–3.28 (m, 10H, N–$(CH_2)_5$), 3.69–3.89 (m, 1H, –*CH*–CH_2–N–), 4.22 (s, 2H, OCH_2), 6.22 (s, 1H, C_3–H), 6.89–7.75 (m, 13H, ar-H), 15.69 (s, 1H, OH and/or NH, exch.). Anal. Calcd for $C_{28}H_{28}N_2O_4$: C, 73.68; H, 6.14; N, 6.14. Found: C, 73.59; H, 5.91; N 6.11.

4.2.6.10. 7-(2-Hydroxy-3-(2′-chlorophenylpiperazin-1-yl)propoxy)-4-phenyl-2*H*-1-benzopyran-2-one (11j). Yield 95%; mp: 139 °C. MS: *m*/*z* (%) = 490.25 (M^+, 1.05), 294.15 (2.44), 238 (6.64), 209 (100). ^{1}H NMR ($CDCl_3$-d_6, D_2O) δ ppm: 2.62–2.90 (m, 10H, N–$(CH_2)_5$), 3.07–3.12 (m, 1H, –*CH*–CH_2–N–), 4.10 (s, 2H, OCH_2), 6.23 (s, 1H, C_3–H), 6.77–7.49 (m, 12H, ar-H), 9.21 (s, 1H, OH and/or NH, exch.). Anal. Calcd for $C_{28}H_{27}N_2O_4Cl$: C, 68.50; H, 5.50; N, 5.71. Found: C, 68.36; H, 5.74; N 5.84.

4.2.6.11. 7-(2-Hydroxy-3-(2′-methoxyphenylpiperazin-1-yl)propoxy)-4-phenyl-2*H*-1-benzopyran-2-one (11k). Yield 85%; mp: 129 °C. ^{1}H NMR ($CDCl_3$-d_6, D_2O) δ ppm: 2.62–2.87 (m, 10H, N–$(CH_2)_5$), 3.31–3.60 (m, 1H, –*CH*–CH_2–N–), 3.87 (s, 3H, O CH_3), 4.11 (s, 2H, OCH_2), 6.22 (s, 1H, C_3–H), 6.951 (s, 4H, 2-methoxyphenyl-H), 6.77–7.34 (m, 8H, ar-H), 9.24 (s, 1H, OH and/or NH, exch.). Anal. Calcd for $C_{29}H_{30}N_2O_5$: C, 71.60; H, 6.17; N, 5.76. Found: C, 71.46; H, 5.86; N, 5.66.

4.3. Pharmacology

4.3.1. Materials and methods

Twenty representative new compounds, **8a**, **8b**, **8c**, **8d**, **8e**, **8f**, **8g**, **8h**, **8i**, **11a**, **11b**, **11c**, **11d**, **11e**, **11f**, **11g**, **11h**, **11i**, **11j** and **11k** were tested for their antihistaminic activity.

4.3.1.1. Animals. Male guinea pigs (250–400 g) were used for all experiments.

4.3.1.2. Tissue preparation. Male guinea pigs were sacrificed by a blow to the base of the skull and cervical dislocation. Clean ileal segments of 2–3 cm long were prepared from the guinea pigs. [25] One end was fixed to a glass aerating tube and the other to isotonic transducer (Bioscience, England). The whole preparation was set up in 10-mL organ bath containing aerated Tyrode solution (NaCl: 1.0; $CaCl_2$: 0.2; KCl: 0.2; $MgCl_2{\cdot}6H_2O$: 0.2; NaH_2PO_4: 0.05; $NaHCO_3$: 1, and glucose: 2.0 g/dL). The Tyrode solution was maintained at 37 °C.

4.3.1.3. Protocols.

(1) The possible in vitro antihistaminic activity of the 20 test compounds was examined by producing a cumulative concentration–response curve of histamine-induced contraction of guinea pig ileum after exposing tissue to each experimental solution.[26] In addition to histamine diphosphate solution, sixty two experimental solutions were used:
   (a) Three increment concentrations of each of the 20 test compounds (0.1, 0.5, and 1.0 mg mL^{-1}).
   (b) Pheniramine maleate (5 μg mL^{-1}).
   (c) 0.01 mL normal saline. The threshold value (T.R) is the concentration of histamine ($\times 10^{-3}$ μg mL^{-1}) at which the strip of ileum recorded the first contraction, whereas the maximal response (M.R) is the concentration at which further increases in concentration does not produce higher height of contractions. Consecutive concentrations of histamine were added every 2 min (between the threshold value and the maximal response). The percentage of maximum contraction obtained with each concentration in proportion to the maximum contraction obtained with saline was plotted against the concentration of histamine.[27] The effective concentration of histamine causing 50% of maximum response (EC_{50}) in each experiment was extrapolated from the concentration–response curve of the corresponding experiment.[28] The shift of the cumulative concentration–response curves obtained in the presence of different concentrations of the test compounds and pheniramine maleate was examined by comparing the EC_{50} of each solution with that of saline.

(2) In addition, the maximum response (E_{max}) to histamine obtained in the presence of all test compound concentrations and pheniramine maleate was compared with that of saline. The effect of different concentrations of the test compounds and pheniramine maleate was tested on different ileum strips ($n = 5$).

4.3.2. Statistical analysis

The heights of the contractions were determined and the cumulative concentration–response curves were plotted for all the recordings. EC_{50} and maximum response to histamine data were expressed as means ± SEM. The EC_{50} and maximum response to histamine in the presence of pheniramine maleate and different concentrations of test compounds were compared with those obtained in the presence of saline using Student's *t*-test.[29]

4.4. Docking studies

All docking studies were performed using "Internal Coordinate Mechanics (Molsoft ICM 3.4-8C)".

To understand the obtained pharmacological data on a structural basis, we evaluate compounds (two different classes of benzopyrone derivatives) through molecular modeling and docking techniques using program.

ICM docking is probably the most accurate predictive tool of binding geometry today.[30–33]

The aim of the flexible docking calculations is prediction of correct binding geometry for each binder. The goal is to have an adequate 3D-model of the receptor pocket we are planning to dock compounds to. Usually dock the tested compounds to determine ICM scores.

In order to compare the binding affinity of the newly synthesized benzopyrone analogs, we docked the compounds **8a–i**, **11a–k** into the empty binding site of the experimentally known Histamine-binding protein (HBPs) which reveals two binding sites for the same ligand (histamine) (1QFT) and histamine *N*-methyltransferase (HNMT) complexes with Diphenhydramine (2AOT),[6] (Table 3) which offer a new strategy for controlling histamine-based diseases.[21]

4.4.1. Generation of ligand and enzyme structures

The crystal structure of histamine-binding protein (HBPs) (1QFT),[21] and the crystal structure of histamine *N*-methyltransferase (HNMT) complexed with diphenhydramine (2AOT)[6] were downloaded through the Protein Data Bank PDB/RCSB site and saved as *.pdb file.

A set of benzopyrone analogs synthesized to inhibit (HBP) and (HNMT) was compiled by us earlier.

Molecular modeling of the target compounds were built using ChemDraw Ultra version 8.0.3 and minimized their energy through Chem3D Ultra version 8.0.3/MOPAC, Jop Type: Minimum RMS Gradient of 0.010 kcal/mol and RMS distance of 0.1 Å , and saved as MDL MolFile (*.mol).

4.4.2. Docking using Molsoft ICM 3.4-8C program

(1) Convert our PDB file (1QFT) and (2AOT) into an ICM object: This conversion involves addition of hydrogen bonds, assignment of atoms types, and charges from the residue templates.

Click on MolMechanics/Convert/Protein, and then delete water molecules.

(2) To perform ICM small molecule docking:
   (a) *Setup docking project:*
      (1) *Set project name:* Click on docking/set project name, press OK.
      (2) *Setup the receptor:* Click on docking/receptor setup, enter the receptor molecule in the receptor molecule data entry box (a_*) will do, then click on identify the binding sites button to identify the potential ligand binding pockets, press OK. After the receptor

setup is complete, the program normally displays the receptor with selected binding site residues highlighted in yellow xstick presentation.

(3) *Review and adjust binding site:* ICM makes a box around the ligand binding site based on the information entered in the receptor setup section. The position of the box encompasses the residues expected to be involved in ligand binding. Click on the menu docking/review/adjust ligand/box.

(4) *Make receptor maps: the* step now is to construct energy maps of the environment within the docking box. Click on menu docking/make receptor maps, select the resolution of the map by entering a value into the grid cell size data entry box which is 0.5, this step takes few minutes.

(b) *Start docking simulation:* Use interactive docking to dock one ligand at a time. Click on menu docking/interactive docking/mol table ligand, use the drop down arrow to find the table of ligand and/or Compounds we wish to dock, and then enter the thoroughness which represent the length of simulation. Generally 1 is reasonable value, select Calc ICM Score, then select Display run which display the ligand sampling the energy in the ligand binding project.

(3) *Display the result:* Click docking/browse/stack conformations. ICM stochastic global optimization algorithm attempts to find the global minimum of the energy function that include five grid potentials describing interaction of the flexible ligand with the receptor and internal conformational energy of the ligand, during this process a stack of alternative low energy conformations is saved (Table 3 and Fig. 10).

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