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Design, synthesis and biological evaluation of novel 6*H*-benzo[*c*]chromen-6-one, and 7,8,9,10-tetrahydro-benzo[*c*]chromen-6-one derivatives as potential cholinesterase inhibitors

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ABSTRACT

Hydroxylated 6*H*-benzo[*c*]chromen-6-one derivatives (i.e., urolithins) are the main bioavailable metabolites, and biomarkers of ellagitannins present in various nutrition. Although these dietaries, the sources of urolithins, are employed in folk medicine as cognitive enhancer in the treatment of Alzheimer's Disease, urolithins have negligible potential to inhibit acetylcholinesterase and butyrylcholinesterase enzymes, the validated targets of Alzheimer's Disease. Therefore, within this research, a series of 6*H*-benzo[*c*]chromen-6-one, and 7,8,9,10-tetrahydro-benzo[*c*]chromen-6-one derivatives has been designed, synthesized, and their biological activities were evaluated as potential acetylcholinesterase and butyrylcholinesterase inhibitors. The compounds synthesized exerted comparable activity in comparison to rivastigmine, galantamine, and donepezil both in in vitro and in vivo studies.

1. Introduction

Alzheimer's Disease, characterized by progressive deterioration of memory and higher cortical functions that ultimately result in total degradation of intellectual and mental activities, is a complex neurodegenerative disease.[1–3] The symptomatic development of the disease starts with mild cognitive disorders, and they are followed by difficulties in the interaction with the environment, personal disorders, and finally end-stage character loss.[4] Therefore, it also has devastating effects socioeconomically to patient care-givers, and totally national health economics regarding that the incidence of the disease reaches up to 50% at ages above 85.[5]

Unfortunately, the complete etiology of the disease still remains unknown. Various physiological targets, and pathways, including but not limited to, beta-amyloid biosynthesis and degradation pathways (i.e., beta-, and gamma-secretase inhibitors, antibeta-amyloid immunization),[6–8] tau-protein hyperphosphorylating kinases (i.e., kinase inhibitors),[9] oxidative stress (i.e., antioxidants),[10] brain iron homeostasis (e.g., chelating agents)[11] have all been tried, however, none of them so far found validated targets in clinical trials. The agents targeting the cholinergic neurotransmission (i.e., namely, acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE) inhibitors) are the only clinically approved drugs, besides the NMDA receptor antagonist memantine, since acetylcholine deficiency is characteristics in the development of dementia and cognitive disorders in Alzheimer's Disease.[12] However, there are only several clinically approved drugs currently on the market as cholinesterase inhibitors (i.e., rivastigmine, galantamine, donepezil, and tacrine) and their clinical effectiveness is only available for mild to moderate level cognitive disorders, generated in the first couple years of the progressive development of Alzheimer's Disease.[12,13] Furthermore, tacrine is currently not used due to its serious hepatotoxic side effects.[14] Although the employment of cholinergic hypothesis in Alzheimer's Disease has been known for more than three decades, the number of AChE and BuChE inhibitor drugs on the market is questionable regarding the number of drugs in other pharmacological groups. Therefore, more drug candidates are expected not only to reach to market to be used, at least, in the symptomatic treatment of the disease but also to provide alternative treatment, in particular, expected to act in a longer period, and high levels of cognitive disability symptoms. That is the basic reason of observing most developed drug companies still designing AChE and BuChE inhibitor drugs and trying their effect in clinical trials.[15–17]

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Based on these, our interest on this research was to design novel AChE and BuChE inhibitor molecules. Our starting point was on the common compounds present in some dietaries (i.e., pomegranate, nuts, and some berries) since there is scientific data available on the positive effects of these nutrients in the treatment of cognitive disorders in Alzheimer's Disease.[18–20] Our findings have directed us that ellagitannins are the common, and major compounds that human are exposed to following the digestion of these dietaries.[18–20] However, ellagitannins are macromolecules and they have no bioavailability.[21] In fact, they are almost fully converted to urolithins (i.e., hydroxylated 6*H*-benzo[*c*]chromen-6-one derivatives) in the human gastrointestinal flora and these compounds are even accepted as the biomarkers of ellagitannin metabolism.[21,22] Our initial trials with one of these metabolites of ellagitannin metabolism (i.e., 3-hydroxy-6*H*-benzo[*c*]chromen-6-one, also referred to as Urolithin B) have not provided potential AChE and BuChE inhibitor molecule (i.e., IC_{50}s were found higher than 50 μM for both cholinesterase enzymes). Another disadvantage that literature suggests on urolithins is that these compounds do not have the potential to penetrate to the central nervous system.[23] Therefore, we have designated mainly two group of compounds (i.e., rivastigmine-like and donepezil-like analogues) employing 6*H*-benzo[*c*]chromen-6-one moiety, present in urolithins. Furthermore, since the central effect is required in the treatment of the disease, the 7,8,9,10-tetrahydro-benzo[*c*]chromen-6-one derivative compounds (i.e., relatively more saturated urolithin derivatives) were also designated regarding the potential of tetrahydrocannabinol (i.e., a well-known cannabinoid receptor partial agonist that also possesses tetrahydro-6*H*-benzo[*c*]chromen moiety) to penetrate the central nervous system.[24] Therefore, this study, for the first time, shows the generations of original 6*H*-benzo[*c*]chromen-6-one, and 7,8,9,10-tetrahydro-benzo[*c*]chromen6-one moiety bearing compounds that possess promising AChE and BuChE inhibitory potential. The title compounds and their generation are illustrated in Scheme 1. As seen, the rivastigmine-like title compounds possess a meta-disubstitution (i.e., a 1-(dimethylamino)ethyl moiety and a –OR substituent) on a rigid planar system as it appears on rivastigmine. On the other hand, the structural organization in donepezil (i.e., a rigid planar system connected to a tertiary amine with an alkylene spacer) is utilized in donepezil like title compounds employing 6*H*-benzo[*c*]chromen-6-one, 7,8,9,10-tetrahydro-benzo[*c*]chromen6-one moieties.

2. Results and discussion

2.1. Synthetic pathways

The synthetic strategy for the synthesis of the title compounds within the first group (i.e., **4a**, and **9**–**14**) was designated to obtain their racemic mixture except the compound **4b** (i.e., the S enantiomer of the racemate **4a**), which was obtained through directly employing the commercially available **2b** as the starting material (i.e., Scheme 2 for the title compounds **4a**–**b** and Scheme 3 for the title compounds from **9** to **14**). The synthetic strategy follows the reductive amination of acetophenone derivatives, the esterification, and finally the aryl-coupling reaction steps. Following the reactions all the title compounds were converted to their corresponding HCl salts.

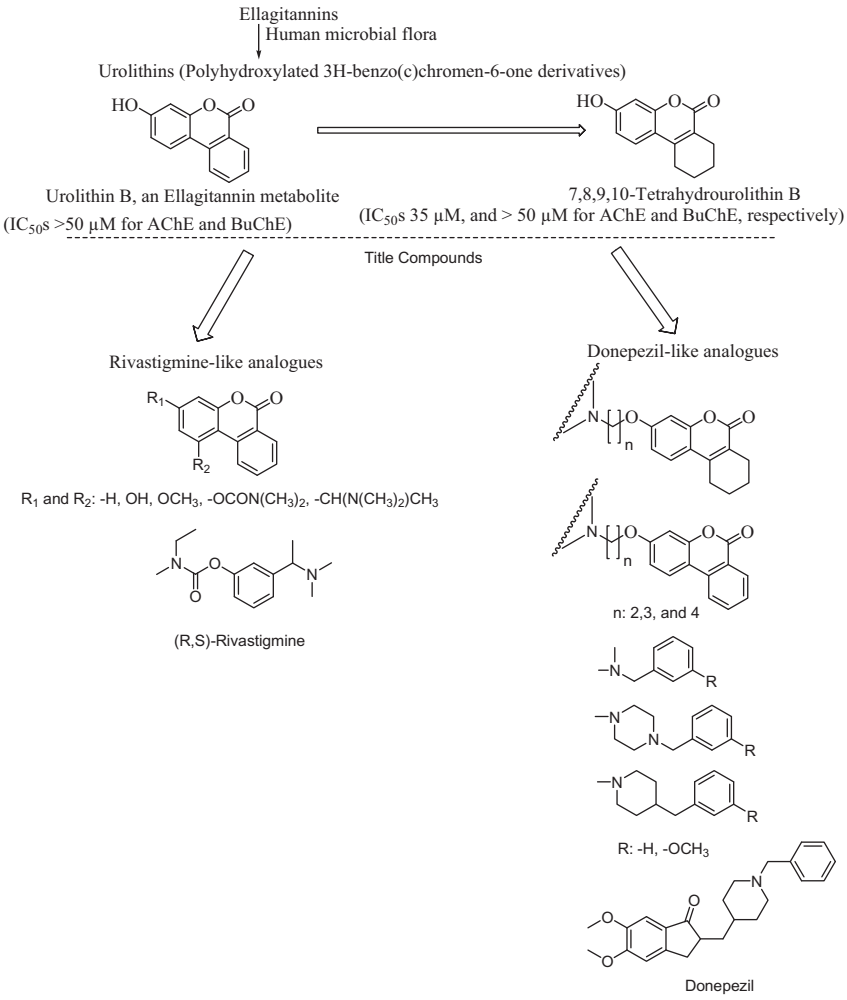
The mono-O-methylation of **5** was quite critical not only to make another substitution on the 1-(dimethylamino)ethyl substituted ring but also to protect one of the phenolic hydroxyls for the esterification reaction in the total synthesis of the compounds **9**–**14**. The reductive amination reactions (i.e., also a step pursued in the commercial synthesis of rivastigmine) employing $NaCNBH_3$ yielded the intermediates **2** and **7** in moderate yields. The esterification of **2**, and **7** employing 2-bromobenzoic acid was only successively performed in the presence of polyphosphoric acid, since other traditional methods yielded too low yields for the synthesis of the intermediates **3a**–**b**, and **8**. The intramolecular aryl-coupling was optimized employing $PdCl_2(PPh_3)_2$, NaOAc, and DMA. Other palladium sources, bases, and organic solvents utilized conditions, yielded no product formation or too poor yields. The synthesis of the title compounds **9** and **10** were achieved in the same reaction, although the reaction dominantly yielded the compound **9** as the major product. The reasonable R_f difference in the TLC monitoring of the reaction wherein acetone was used as the mobile phase led us to quantitatively separate these compounds (i.e., **9** and **10**) employing column chromatography. HBr catalyzed O-demethylation of **9**, and **10** yielded the title compounds **11**, and **12**, respectively. Finally, O-carbamylations of **11** and **12** were performed to obtain the title compounds **13**, and **14**.

The second group compounds, 3-aminoalkyloxy-substituted-6*H*-benzo[*c*]chromen-6-one (i.e., **19** series) and 3-aminoalkyloxy-substituted-7,8,9,10-tetrahydro-benzo[*c*]chromen-6-one (i.e., **20** series) derivatives, were synthesized following the strategy wherein, first, the 3-hydroxy-7,8,9,10-tetrahydro-benzo[*c*]chromen-6-one (i.e., **15**) and 3-hydroxy-6*H*-benzo[*c*]chromen-6-one (i.e., **16**) were synthesized and the consequent alkylation and amination reactions were conducted (Scheme 4). Accordingly, the procedures described in the literature for the synthesis of **15** and **16** were modified to increase the yields. Literature suggests the reaction of resorcinol and 2-oxo-cyclohexancarboxylate ethyl ester to obtain **15** in the presence of a Lewis acid.[25] Our trails gave the best results in the presence of $ZrCl_4$ without an organic solvent and heating up the reaction to 80 °C. The synthesis of **16** from resorcinol and 2-bromobenzoic acid under $CuSO_4$ catalyzed aqueous base conditions is also described well in the literature.[26] However, employing 2-iodobenzoic acid in our studies resulted in very good yields to synthesize **16**. The following reactions on the compounds **15** and **16** were all in parallel. First, the corresponding alkyl halides were prepared (i.e., **17a**–**c**, and **18a**–**c**). The synthesis of **17a** and **18a** was absolutely achieved with concomitant elimination reactions, therefore, the procedure utilizing 1,2-dichloroethane was improved to obtain around 50% yield, ideal for these synthesis intermediates. The conventional methods for the amination of **17a**–**c**, and **18a**–**c** derivatives (e.g., the refluxing of corresponding amine with an alkyl halide in the presence of base and NaI in an appropriate organic solvent) under various temperatures yielded out too low yields (e.g., mostly less than 50% conversion after 1 day reflux).[27] This was overcome employing microwave assisted amination reactions simply using the same reaction conditions of conventional methods described above. Therefore, synthesis of the title compounds **19a1**–**19c6** and **20a1**–**20c6** (i.e., totally 36 compounds) were efficiently conducted employing a single mode microwave instrument and the average yields were around 70–80% in a 32 min run time. All the title compounds were then converted to their corresponding HCl salts.

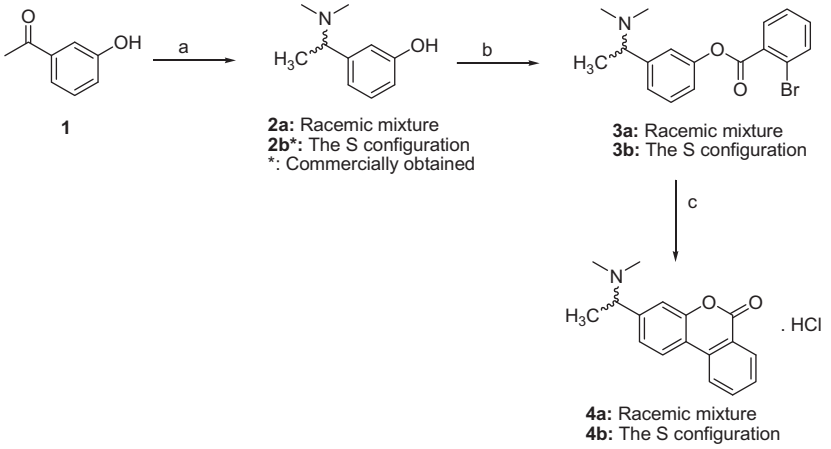
2.2. In vitro analysis of compounds: Cholinesterase assays

All the original compounds synthesized were tested for their potential to inhibit AChE and BuChE enzymes. The IC_{50}s obtained for each test compound including the reference compounds rivastigmine, donepezil, and galantamine as well as their selectivity are displayed in Table 1.

The first critical observation within the first group of compounds (i.e., **4a**–**b**, and **9**–**14**) was on the effect of stereochemistry regarding the comparison of the activities of **4a** and **4b**. The employment of the S-enantiomer (**4b**) did not result in significant difference for the inhibition of AChE and BuChE, since both the racemate (**4a**) and the S enantiomer (**4b**) appeared to possess similar activity. The compounds **9**, **11**, and **13** are *meta*-substituted

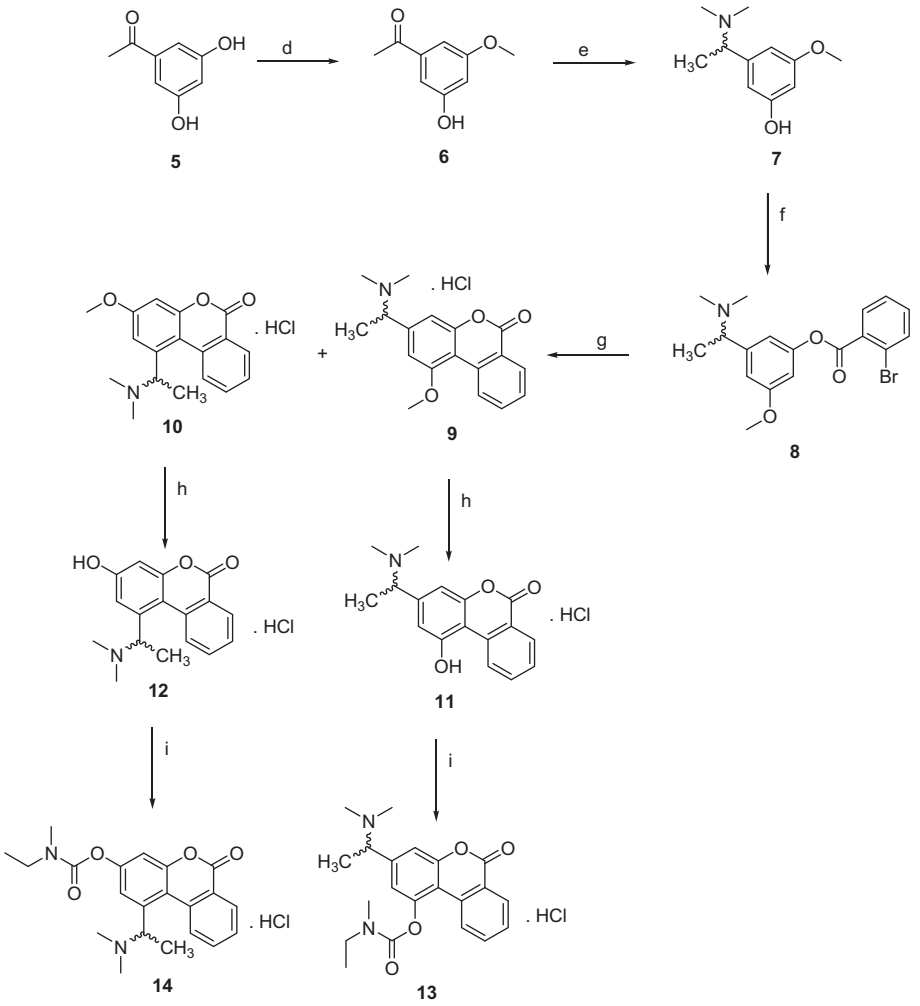


Scheme 1. The origin of development of title compounds.



Scheme 2. Synthesis of the title compounds **4a**–**b**. Reagents and conditions: (a) $NaCNBH_3$, dimethylamine HCl, methanol, reflux, 17 h; (b) 2-bromobenzoic acid, PPA, 80 °C, 15 min; (c) $PdCl_2(PPh_3)_2$, NaOAc, DMA, 130 °C, 16 h, HCl, acetone.

analogues of compound **4a**. Very interestingly, among those *meta*-substitutions, only the methoxy substituent present in **9** appeared to increase the potential to inhibit AChE, since hydroxyl (i.e., in **11**), and *O*-carbamyl (i.e., in **13**) substitutions resulted in lower potential to inhibit AChE. However, the compounds **11** and **13** displayed lower IC_{50} values for the inhibition of BuChE. These results suggest that particularly the compounds **11** and **13** display similarity in activity in comparison to rivastigmine. Therefore, *meta*-substitution was found the key position to obtain either AChE selective or BuChE selective analogues regarding the AChE selectivity of compounds **4a** and **9** and BuChE selectivity of the compounds **11** and **13**. The comparison of the activities of the compounds **10**,



Scheme 3. Synthesis of the title compounds **9**–**14**. Reagents and conditions: (d) NaOH, Dimethylsulfate, 50 °C, 2 h; (e) $NaCNBH_3$, dimethylamine HCl, methanol, reflux, 17 h; (f) 2-bromobenzoic acid, PPA, 80 °C, 15 min; (g) $PdCl_2(PPh_3)_2$, NaOAc, DMA, 130 °C, 16 h; (h) 48% aqueous HBr, 130 °C, 24 h; (i) *N*-Ethyl-*N*-methylcarbamyl chloride, pyridine, rt, 20 h.

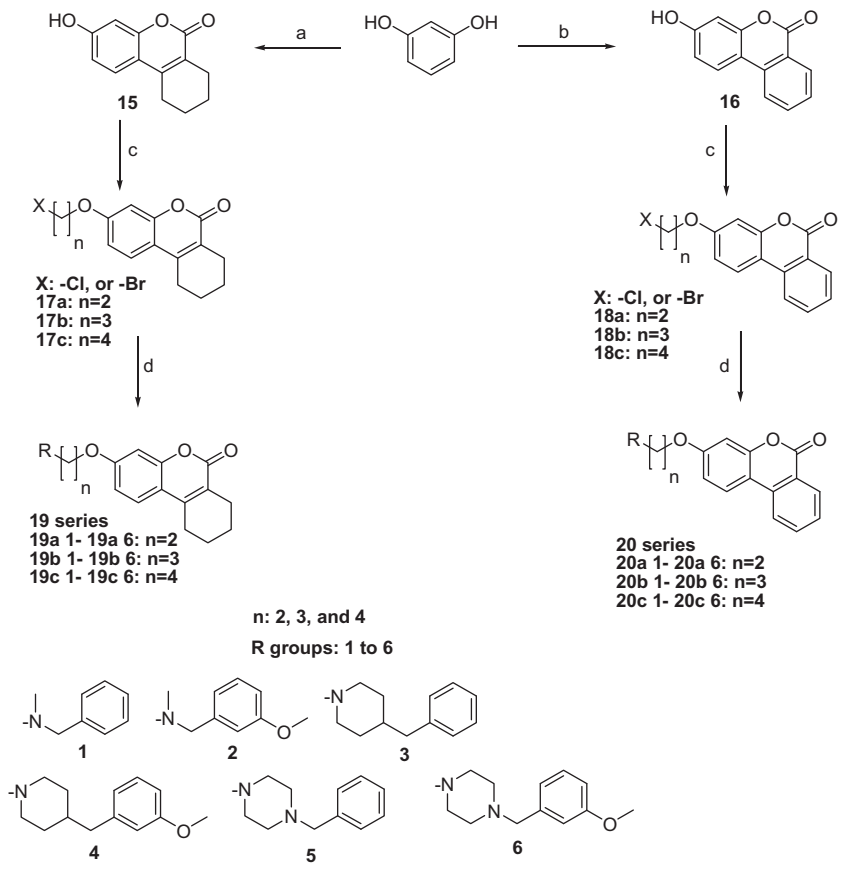
12, and **14** with their corresponding regioisomers (i.e., **9**, **11**, and **13**, respectively) has revealed out that the position of aminoalkyl moiety in the compounds **9**, **11** and **13** is better to obtain lower IC_{50}s for the inhibition of AChE. This observation could not be extrapolated to BuChE inhibition, since compound **14** exhibited a stronger potential to inhibit BuChE. Overall, the activities observed for the first group of compounds were found in the at least comparable to better range according to the activities obtained for rivastigmine. In addition, our research on this first group compounds also pointed out significant SAR to convert selectivity towards either of the two cholinesterase enzymes.

The second group of compounds (i.e., the **19** and **20** series) have 3 basic differences; the saturation level of the benzo[*c*]chromen-6-one moiety, the carbon chain bridging the benzo[*c*]chromen-6-one moiety to amine group, and the various amine substitutions. The results obtained for this group absolutely have indicated that this type of structural organization reveals out AChE-selective molecules. Indeed, all members of this category displayed selectivity for AChE. Therefore, the activity potential of these compounds might be resembled to the activities of donepezil and galantamine, since both of them are significant AChE-selective agents. However, the average selectivity ratios of the test compounds were found far behind the ones for those two drugs. Another important SAR for the second group compounds revealed out that the **19** series compounds, in general, have better potential and selectivity to inhibit AChE. This is, in fact, in parallel with our initial observation that compound **15** is more potent inhibitor of AChE in comparison to the potential of compound **16** to inhibit AChE. Therefore, the **19** series developed from **15** kept their property in terms of more potent inhibition of AChE.

It is critical to state that the compounds **19b5** and **20b5** were found the most potent AChE and BuChE inhibitors within this group. Although they appear to be more active than rivastigmine and have comparable activity in comparison to galantamine in terms of AChE inhibition, they definitely exert a better potential to inhibit both cholinesterase enzymes that is absent in the current 3 reference drugs (i.e., rivastigmine, galantamine, donepezil), used in the treatment of Alzheimer's Disease, since these drugs have higher IC_{50} values for BuChE.

An important observation was on the *meta*-methoxy substitution of the title compounds. All unsubstituted derivatives (i.e., no *meta*-methoxy substitution as seen in **a1**–**3**–**5**, **b1**–**3**–**5**, and **c1**–**3**–**5** series) were found to have better potential to inhibit AChE in comparison to the potential of their corresponding *meta*-methoxy substituted analogues (i.e., **a2**–**4**–**6**, **b2**–**4**–**6**, and **c2**–**4**–**6** series).

Among the amine derivatives utilized *N*-methylbenzylamine, and, in particular, *N*-benzylpiperazine substituted derivatives appeared to be the more active compounds in comparison to 4-benzylpiperidine containing derivatives. The *N*-benzylalkyl



Scheme 4. Synthesis of the title compounds 1**9** and **20** series. Reagents and conditions: (a) ethyl 2-oxocyclohexanecarboxylate, $ZrCl_4$, 70 °C, 1 h; (b) 2-iodobenzoic acid, NaOH, $CuSO_4$, H_2O, reflux, 40 min; (c) alkyl halide (i.e., 1,2-dichloroethane, 1-bromo-3-chloropropane, or 1-bromo-4-chlorobutane), base (i.e., NaOEt for $n = 2$, NaH, for $n = 3$, or 4); (d) K_2CO_3, NaI, Acetonitrile, amine derivative (i.e., **1**–**6**), 32 min microwave assisted reaction, 105 °C.

Table 1
Cholinesterase activities and selectivities of compounds (i.e., test compounds **4a**–**b**, **9**–**14**, **19a1**–**19c6**, and **20a1**–**20c6**)

Compounds	IC_{50} (μM)		Selectivity*	Compounds	IC_{50} (μM)		Selectivity
	AChE	BuChE			AChE	BuChE	
4a	10.9 ± 0.5	17.9 ± 1.2	~1.6	**19c4**	13.7 ± 1.0	27.8 ± 0.4	~2.0
4b	7.9 ± 0.9	16.2 ± 2.5	~2.1	**19c5**	6.4 ± 0.2	14.6 ± 0.6	~2.3
9	2.8 ± 0.2	21.1 ± 1.3	~7.5	**19c6**	8.7 ± 0.3	23.4 ± 1.5	~2.7
10	15.1 ± 0.5	24.7 ± 0.4	~1.6	**20a1**	6.7 ± 0.5	14.1 ± 0.1	~2.1
11	18.9 ± 1.0	4.2 ± 0.4	~4.5	**20a2**	18.4 ± 1.0	33.2 ± 0.3	~1.8
12	33.1 ± 0.6	>40	>1	**20a3**	20.0 ± 0.2	>40	>2
13	39.7 ± 0.4	12.7 ± 1.2	~3.5	**20a4**	29.3 ± 0.2	>40	>1
14	>40	8.5 ± 0.2	>4	**20a5**	0.9 ± 0.1	12.1 ± 0.4	~13.4
19a1	2.8 ± 0.2	>40	>14	**20a6**	11.4 ± 0.2	>40	>3
19a2	6.0 ± 0.7	>40	>6	**20b1**	8.5 ± 0.5	14.9 ± 0.5	~1.7
19a3	8.1 ± 0.1	>40	>5	**20b2**	20.9 ± 0.8	29.2 ± 0.2	~1.4
19a4	37.2 ± 0.8	>40	>1	**20b3**	4.1 ± 0.2	21.3 ± 0.2	~5.2
19a5	1.3 ± 0.1	32.0 ± 0.5	~24.6	**20b4**	18.5 ± 0.2	>40	>2
19a6	7.3 ± 0.1	>40	>5	**20b5**	0.8 ± 0.5	3.7 ± 0.4	~4.6
19b1	2.1 ± 0.3	15.9 ± 0.6	~7.6	**20b6**	18.0 ± 0.5	>40	>2
19b2	2.7 ± 0.2	>40	>14	**20c1**	1.1 ± 0.1	6.5 ± 0.1	~5.9
19b3	4.3 ± 0.3	>40	>9	**20c2**	8.3 ± 0.3	17.6 ± 1.1	~2.1
19b4	4.9 ± 0.5	>40	>8	**20c3**	14.2 ± 1.0	>40	>2
19b5	0.9 ± 0.1	3.1 ± 0.1	~3.4	**20c4**	27.4 ± 0.5	>40	>1
19b6	6.2 ± 0.2	18.4 ± 0.6	~2.9	**20c5**	8.2 ± 0.8	>40	>4
19c1	1.0 ± 0.2	18.9 ± 0.5	~18.9	**20c6**	21.3 ± 0.2	>40	>1
19c2	1.7 ± 0.1	>40	>23	Rivastigmine	35.2 ± 0.1	11.0 ± 1.0	~0.3
19c3	9.2 ± 0.1	20.1 ± 0.3	~2.2	Donepezil	0.008 ± 0	7.1 ± 0.1	~888
				Galantamine	0.7 ± 0.1	21.9 ± 0.2	~31

* Selectivity either for AchE or BuChE.

structure is, in fact, also present in donepezil. Therefore, the activity loss found in derivatives formed through the employment of 4-benzylpiperidine moiety (i.e., **a3–4**, **b3–4**, and **c3–4** series) was attributed to the absence of the *N*-benzylalkyl moiety in the structure.

In the evaluation of the number of carbon atoms that acts as the spacer between the benzo[*c*]chromen-6-one moiety and amine group within the 2nd group of compounds, the 2 or 3 carbon atom distance were found ideal since the **19c** and **20c** series were found, in general, to possess less potential to inhibit the cholinesterase enzymes. However, this does not totally reflect the required distance from the Oxygen atom bound to 3rd position of the benzo[*c*]chromen-6-one moiety and the Nitrogen atom, since *N*-methylbenzylamine moiety containing structures have a shorter distance (i.e., in **a1**, **b1**, and **c1** series) in comparison to other amine substitutions. Therefore, it is reasonable to observe that almost all **a1**, **b1**, and **c1** series compounds displayed significant potential to inhibit AChE. This also means that, considering the **19c5** and **20c5** as the least active *N*-benzylpiperazine containing structures, the 4 carbon atom space is at the board to obtain AChE inhibitory potential. In fact, this evaluation is also completely straightforward for BuChE inhibition, since the arrangement of inhibitory potential was found as **b5** > **a5** > **c5** in **19** and **20** series compounds. This is also a reflection of significance of the 3 carbon chain spacer, ideal for cholinesterase inhibition within this group of compounds.

Based on these results, 8 compounds (i.e., **4a**, **4b**, **9**, **11**, **19c1**, **19b5**, **20b5**, and **20c1**) of totally 44 new original molecules were selected to test them in in vivo experiments.

2.3. In vivo testing of compounds: scopolamine induced passive avoidance test

Although there is no ideal test in animal models for measuring the cognition, the scopolamine induce passive avoidance test is one of the most abundantly employed methods to measure the effects of drug candidates on cognition in animal models. As expressed previously, 8 of the 44 original compounds were selected and tested their activity in the scopolamine induce passive avoidance test. Following the administration of scopolamine and the test substances the time for the rats visiting the dark box (latency) was measured for 5 min. The time difference between the no scopolamine administered group and the only scopolamine administered group was taken as 100% and the effect of each compound was calculated accordingly in terms of the percentage antagonism. Each test compound was assessed employing ten animals per dose. The results are described in Table 2. Accordingly, all test compounds exhibited at least comparable activity with donepezil, and rivastigmine, since the percent antagonism was found in 50–85% range for all of them. Very interestingly, among the first series of the title compounds, the most active one in in vivo experiments appeared to be the compound **11**, a BuChE selective inhibitor. However, the activities of the other first group series compounds (i.e., **4a**, **4b**, **9**, and **13**) were all found reasonable. The difference regarding stereochemistry seen for the compounds **4a** and **4b** was found, again, negligible in in vivo studies. **19b5**, the most potent inhibitor of the second group series, also exerted a good activity in in vivo experiments. **19c1**, and **20c1**, relatively weaker cholinesterase inhibitors in comparison to **19b5** also exerted promising results in in vivo studies. The most unexpected result in in vivo studies was obtained with the compound **20b5**, since it was found as one of the most potent inhibitors of cholinesterase enzymes. However, the effect obtained with **20b5** is still comparable with rivastigmine.

Table 2
In vivo activity results of selected compounds

Compound	Antagonism (%)
4a	70
4b	71
9	71
11	49
19c1	65
19b5	58
20c1	62
20b5	85
Donepezil	58
Rivastigmine	85

3. Conclusion

Benzo[*c*]chromen-6-one moiety is present in various plant-derived nutrition, however, it has negligible inhibitory potential to inhibit cholinesterase enzymes. Therefore, this research, for the first time, displayed the generation of benzo[*c*]chromen-6-one derived compounds for the inhibition of cholinesterase enzymes. The molecules designed were subcategorized to rivastigmine-like and donepezil-like analogues. Indeed, the synthesized molecules tested within the two groups exerted similarity in terms of potential to inhibit AChE and BuChE, regarding the activity potentials of donepezil and rivastigmine. Moreover, the activities of the compounds were further shown in the in vivo experiments. The results definitely have shown that most of the original compounds designed and synthesized are promising molecules to be tested for further drug development stages. The at least comparable activity of test compounds in comparison to donepezil and rivastigmine within the in vivo experiments is another parameter to consider the oral bioavailability and activity of the molecules. However, more information (e.g., the correct salt selection, basic ADME properties, and the screening of activity in other in vivo models) is required to reach to the hit molecule for the continuation of drug-development stages. The compounds particularly **4a** and **11** from the first group, and the compounds **19b5**, **19c1**, and **20c1** appears to be the ideal molecules that might be chosen as lead compounds for further generation of benzo[*c*]chromen-6-one derivative hit molecules, since these compounds have reflected well the activity expected according to the SARs driven throughout the research.

4. Experimental

4.1. Synthesis

All chemicals, reagents, and solvents were analytical grade, purchased from commercial suppliers (i.e., Sigma–Aldrich, Acros, and Rare Chemicals), and used without purification unless otherwise specified. The reactions were monitored employing thin-layer chromatography (TLC), performed on Merck 60 F_{254} plates and visualized under UV light. Melting points were determined on a Q100 Model TA Instruments® apparatus and they are uncorrected. The purity of the test substances were analyzed through UPLC studies using UV detection both at 220 nm and 254 nm and the purity of all the test compounds were greater than 99%. In UPLC studies, a Waters Acquity Ultra Performance LC System with a PDA detector was utilized and the analytical separations were achieved using an Acquity UPLC BEH C18 column (1.7 μm 2.1 cm × 50 mm) and a mobile phase with a linear gradient formed between 25% of acetonitrile to 55% Acetonitrile both in pH 7 phosphate buffer. The column temperature was set to 25 °C and the flow rate was 0.3 mL/min. The exact mass analyses were conducted employing a Waters LCT Premier XE KE309 (TOF-MS) instrument. The analyses were performed in positive mode and the capillary,

and the sample cone voltages were set to 2500 V, and 100 V, respectively. The desolvation temperature was at 150 °C and the source temperature was set to 100 °C. ^{1}H NMR spectra were recorded on a Varian-300 NMR spectrometer using TMS as an internal standard in either DMSO-d_6 or $CDCl_3$ and the chemical shifts are reported in δ (ppm).

4.1.1. (±)-3-(1-(Dimethylamino)ethyl)phenol (2)

A mixture of 27.23 g 3-hydroxyacetophenone (0.2 mol) (**1**), 3.60 g dimethylamine hydrochloride (0.4 mol), 10 g activated molecular sieve (3 Å) and 10 g dry $MgSO_4$ was refluxed in dry methanol for 1–2 h. Then, 18.85 g $NaCNBH_3$ was added to reaction mixture and the mixture was refluxed for additional 18–20 h. After hot filtration of reaction mixture, methanol was removed under reduced pressure and the residue thus obtained was dissolved in water (250 mL). The content was acidified with concd HCl to pH 1 and extracted with ethyl acetate (3 × 100 mL). The aqueous solution was basified to pH 8–9 using $NaHCO_{3(s)}$ (i.e., solid $NaHCO_3$) and extracted with ethyl acetate (5 × 100 mL). Combined ethyl acetate extracts were washed with water dried over $MgSO_4$ and concentrated under reduced pressure to give thick oil. This oil was dissolved in isopropyl ether (120 mL) under heating and allowed to cool to 0 °C. The crystalline solid obtained was filtered and dried to obtain a light yellow crystalline solid. Yield obtained: 56.0%. Purity by HPLC: 99%. Mass spectrometry: peak at *m*/*z* 166 $(M+1)^+$. ^{1}H NMR data in CD_3OD: δ 1.34 (d, 3H, —CH—*CH*$_3$), 2.17 (s, 6H, *CH*$_3$—N—*CH*$_3$), 3.22 (q, 1H, —N—*CH*—CH_3), 6.63–6.68 (m, 1H, Ar-H), 6.69–6.79 (m, 2H, Ar-H), 7.10 (t, 1H, Ar-H).

4.1.2. (±)-3-(1-(Dimethylamino)ethyl)phenyl 2-bromobenzoate (3a)

A mixture of 1.65 g (±)-3-(1-(dimethylamino)ethyl)phenol (10.0 mmol) (**2**) and 2.2 g 2-bromobenzoic acid (11.0 mmol) was heated in polyphosphoric acid at 80 °C for 10–15 min. After complete disappearance of aminophenol derivative, checked by TLC, the reaction vessel was cooled. Then, 100 mL ethylacetate and 100 mL water were added to reaction mixture, respectively, and pH of the content was adjusted to 8–9 using $NaHCO_{3(s)}$. Organic phase was separated and concentrated under reduced pressure to give oily ester product. Yield obtained: 78.0%. Mass spectrometry: peak at *m*/*z* 348 $(M+1)^+$. ^{1}H NMR (300 MHz, $CDCl_3$, ppm): δ 1.36 (d, 3H, —CH—*CH*$_3$), 2.17 (s, 6H, *CH*$_3$—N—*CH*$_3$), 3.26 (q, 1H, —N—*CH*—CH_3), 7.04–7.21 (m, 3H, Ar-H), 7.26–7.41 (m, 3H, Ar-H), 7.63–7.70 (m, 1H, Ar-H), 7.91–7.99 (m, 1H, Ar-H).

4.1.3. (±)-3-(1-(Dimethylamino)ethyl)-6*H*-benzo[*c*]chromen-6-one hydrochloride (4a)

To a solution of (±)-3-(1-(dimethylamino)ethyl)phenyl 2-bromobenzoate (1.006 g, 4.05 mmol) (**3a**) in 35 mL of dry DMA, NaOAc (664 mg, 8.1 mmol) and $PdCl_2(PPh_3)_2$ (284 mg, 0.405 mmol) were added at 80 °C. After stirring the reaction mixture at 130 °C for 15–16 h, the solvent was evaporated under reduced pressure. The content was acidified with 1 N HCl (200 mL) and extracted with ethyl acetate (3 × 75 mL). The aqueous solution was basified to pH 8–9 using $NaHCO_{3(s)}$ and extracted with ethyl acetate (5 × 100 mL). Combined ethyl acetate extracts were dried over $MgSO_4$ and concentrated under reduced pressure to give yellow thick oil. Column chromatography on silica gel employing acetone gives desired benzo[*c*]chromen-6-one derivative (526 mg, 68.0%). (±)-3-(1-(Dimethylamino)ethyl)-6*H*-benzo[*c*]chromen-6-one (free base) dissolved in dry acetone (10 mL) was filtrated from 0.45 μm filter. HCl gas was passed through the obtained acetone solution until salt precipitation took place. The content was mixed for additional 1 h and then filtrated. Yield obtained: 81.0%, HPLC purity: 99.90%, HRMS (ESI) $C_{17}H_{18}NO_2$ calcd 268.1338 $(M+1)^+$, found 268.1330, mp: 235.4 °C, ^{1}H NMR (300 MHz, DMSO-d_6, ppm): δ 1.67 (d, 3H, —CH—*CH*$_3$), 2.52 (s, 3H, *CH*$_3$—N—CH$_3$), 2.72 (s, 3H, CH$_3$—N—*CH*$_3$), 4.56 (q, 1H, —N—*CH*—CH$_3$), 7.60–7.73 (m, 3H, Ar-H), 7.94 (dt, 1H, Ar-H), 8.22 (dd, 1H, Ar-H), 8.39–8.49 (m, 2H, Ar-H), 11.40 (br s, 1H, N-*H*).

4.1.4. (*S*)-3-(1-(Dimethylamino)ethyl)phenyl 2-bromobenzoate (3b)

The desired ester derivative was synthesized employing (*S*)-3-(1-(dimethylamino)ethyl)phenol (i.e., obtained through hydrolysis of the commercially available rivastigmine) as the starting compound according to procedure given for the compound **3a**. Yield obtained: 78.0%. Mass spectrometry: peak at *m*/*z* 348 $(M+1)^+$. ^{1}H NMR (300 MHz, $CDCl_3$, ppm): δ 1.36 (d, 3H, —CH—*CH*$_3$), 2.17 (s, 6H, *CH*$_3$—N—*CH*$_3$), 3.26 (q, 1H, —N—*CH*—CH$_3$), 7.04–7.21 (m, 3H, Ar-H), 7.26–7.41 (m, 3H, Ar-H), 7.63–7.70 (m, 1H, Ar-H), 7.91–7.99 (m, 1H, Ar-H).

4.1.5. (*S*)-3-(1-(Dimethylamino)ethyl)-6*H*-benzo[*c*]chromen-6-one hydrochloride (4b)

The procedure for synthesis and HCl salt preparation was the same as the procedure given for the compound **4a**. (i.e., yield obtained 66.0%, 83.0%, respectively, for synthesis and salt preparation). HPLC purity: 99.95%, HRMS (ESI) $C_{17}H_{18}NO_2$ calcd 268.1338 $(M+1)^+$, found 268.1341, mp 254.1 °C, ^{1}H NMR (300 MHz, DMSO-d_6, ppm): δ 1.66 (d, 3H, —CH—*CH*$_3$), 2.51 (d, 3H, *CH*$_3$—N—CH$_3$), 2.72 (d, 3H, CH$_3$—N—*CH*$_3$), 4.50–4.62 (m, 1H, —N—*CH*—CH$_3$), 7.59–7.74 (m, 3H, Ar-H), 7.92 (dt, 1H, Ar-H), 8.22 (dd, 1H, Ar-H), 8.37–8.49 (m, 2H, Ar-H), 11.36 (br s, 1H, N-*H*).

4.1.6. 1-(3-Hydroxy-5-methoxyphenyl)ethanone (6)

Procedure A: 6.2 g NaOH (0.150 mol) was dissolved in 125 mL water. 25 g 3,5-dihydroxyacetophenone (0.160 mol) (**5**) was weighted in 250 mL round bottom flask and previously prepared NaOH solution was added to the flask. After complete dissolution of acetophenone derivative in basic aqueous media, the reaction mixture was cooled to 0–10 °C. Then, 19.5 g DMS (0.156 mol) was added to reaction flask dropwise. The content was mixed at ambient temperature for one hour, and then it was heated at 50 °C for two hours. To separate 3,5-dimethoxyacetophenone impurity, pH of medium was adjusted to 14 with NaOH pellet and this solution was extracted with EtOAc (3 × 50 mL). The alkali solution was acidified with conc HCl to pH 1 and extracted with ethyl acetate (3 × 75 mL). Combined ethyl acetate extracts were dried over $MgSO_4$ and concentrated under reduced pressure to give a mixture of 3,5-dihydroxyacetophenone and 1-(3-hydroxy-5-methoxyphenyl)ethanone. To the mixture, 50 mL acetone and 25 g $NaOH_{(s)}$ was added. When, 8–10 mL cold water was put into the reaction flask slowly, sodium salt of 3,5-dihydroxyacetophenone is precipitated. The liquid part of the content was decanted to another round bottom flask. The solid part was washed with acetone (3 × 15 mL). Combined acetone parts were concentrated under reduced pressure to give 1-(3-hydroxy-5-methoxyphenyl)ethanone as off white solid. Yield obtained: 56.2%. Mass spectrometry: peak at *m*/*z* 166 (M+1). ^{1}H NMR (300 MHz, $CDCl_3$, ppm): δ 2.56 (s, 3H, *CH*$_3$—C═O), 3.79 (s, 3H, —O—*CH*$_3$), 6.62–6.67 (m, 2H, Ar-H), 7.01–7.05 (m, 1H, Ar-H), 7.07–7.11 (m, 1H, Ar-H).
Procedure B: 11.36 mL NEt_3 (82.14 mmol) was added to 137 mL water. 25 g 3,5-dihydroxyacetophenone (0.160 mol) was weighted in 250 mL round bottom flask and previously prepared NEt_3 solution was added to the flask. The reaction mixture was cooled to 0–10 °C. Then, 7.78 mL DMS (82.14 mol) was added to reaction flask dropwise. The content was mixed at ambient temperature for 4 h. After the reaction mixture was cooled 0–5 °C, the content was filtrated to remove undissolved 3,5-dihydroxyacetophenone. pH of filtrate was adjusted to 1 with concd HCl and extracted with EtOAc (3 × 50 mL). Combined ethyl acetate extracts were dried

over $MgSO_4$ and concentrated under reduced pressure to give mixture of 3,5-dihydroxyacetophenone and 1-(3-hydroxy-5-methoxyphenyl)ethanone. To the mixture, 50 mL acetone and 25 g $NaOH_{(s)}$ was added. When, 8–10 mL cold water is put into the reaction flask slowly, sodium salt of 3,5-dihydroxyacetophenone was precipitated. The liquid part of the content was decanted to another round bottom flask. The solid part was washed with acetone (3 × 15 mL). Combined acetone parts were concentrated under reduced pressure to give 1-(3-hydroxy-5-methoxyphenyl)ethanone as off white solid. Yield obtained: 44.7%.

4.1.7. (±)-3-(1-(Dimethylamino)ethyl)-5-methoxyphenol (7)

The desired aminophenol derivative was synthesized according to procedure given for the compound **2**. Yield obtained 60.6%. Mass spectrometry, peak at *m*/*z* 196 $(M+1)^+$. ^1H NMR (300 MHz, $CDCl_3$, ppm): δ 1.29 (d, 3H, —CH—*CH*$_3$), 2.13 (s, 6H, *CH*$_3$—N—*CH*$_3$), 3.15 (q, 1H, —N—*CH*—CH$_3$), 3.61 (s, 3H, O—*CH*$_3$), 6.21 (t, 1H, Ar-H), 6.24–6.27 (m, 1H, Ar-H), 6.29–6.31 (m, 1H, Ar-H), 8.32 (br s, 1H, —O—*H*).

4.1.8. (±)-3-(1-(Dimethylamino)ethyl)-5-methoxyphenyl 2-bromobenzoate (8)

The compound was synthesized according to procedure given for the compound **3a**. Yield obtained: 70.2%. Mass spectrometry: peak at *m*/*z* 378 $(M+1)^+$. ^1H NMR (300 MHz, $CDCl_3$, ppm): δ 1.33 (d, 3H, —CH—*CH*$_3$), 2.19 (s, 6H, *CH*$_3$—N—*CH*$_3$), 3.23 (q, 1H, —N—*CH*—CH$_3$), 3.75 (s, 3H, O—*CH*$_3$), 6.64 (t, 1H, Ar-H), 6.75 (d, 2H, Ar-H), 7.32–7.39 (m, 2H, Ar-H), 7.64–7.69 (m, 1H, Ar-H), 7.92–7.97 (m, 1H, Ar-H).

4.1.9. (±)-3-(1-(Dimethylamino)ethyl)-1-methoxy-6*H*-benzo[*c*]chromen-6-one hydrochloride (9)

The compound was synthesized according to the procedure given for the compound **4a**. (i.e., yield obtained 46.2%, 81.3%, respectively, for synthesis and salt preparation). HPLC purity: 99.40%, HRMS (ESI) $C_{18}H_{20}NO_3$ calcd 298.1443 $(M+1)^+$, found 298.1433, mp: 247.81 °C, ^1H NMR (300 MHz, DMSO-d_6, ppm): δ 1.68 (d, 3H, —CH—*CH*$_3$), 2.56 (d, 3H, *CH*$_3$—N—CH$_3$), 2.76 (d, 3H, CH$_3$—N—*CH*$_3$), 4.07 (s, 3H, O—*CH*$_3$), 4.51 (q, 1H, —N—*CH*—CH$_3$), 7.27 (d, 1H, Ar-H), 7.47 (s, 1H, Ar-H), 7.65 (dt, 1H, Ar-H), 7.90 (dt, 1H, Ar-H), 8.25 (dd, 1H, Ar-H), 8.93 (d, 1H, Ar-H), 11.41 (br s, 1H, —N—*H*). It is important to note that the ring formation to obtain the title compound from (±)-3-(1-(dimethylamino)ethyl)-5-methoxyphenyl2-bromobenzoate (**8**) also yields (±)-1-(1-(dimethylamino)ethyl)-3-methoxy-6*H*-benzo[*c*]chromen-6-one (**10**). Their separation was achieved through silica-gel flash chromatography employing acetone as the mobile phase (i.e., the R_f values were 0.6 and 0.2 on TLC, respectively, for the compounds **9**, and **10**).

4.1.10. (±)-1-(1-(Dimethylamino)ethyl)-3-methoxy-6*H*-benzo[*c*]chromen-6-one hydrochloride (10)

The compound was synthesized according to the procedure described for the title compound **9** (i.e., yield obtained 14.2%, 83.7%, respectively, for synthesis and salt preparation) HPLC purity: 99.90%, HRMS (ESI) $C_{18}H_{20}NO_3$ calcd 298.1443 $(M+1)^+$, found 298.1431, mp: 110.4 °C,^1H NMR (300 MHz, CD_3OD, ppm): δ 1.54 (d, 3H, —CH—*CH*$_3$), 2.18 (s, 6H, *CH*$_3$—N—*CH*$_3$), 3.86 (s, 3H, O—*CH*$_3$), 4.15 (q, 1H, —N—*CH*—CH$_3$), 6.81 (d, 1H, Ar-H), 7.24 (d, 1H, Ar-H), 7.53 (dt, 1H, Ar-H), 7.82 (dt, 1H, Ar-H), 8.20 (br s, 1H, —N—*H*), 8.28 (dd, 1H, Ar-H).

4.1.11. (±)-3-(1-(Dimethylamino)ethyl)-1-hydroxy-6*H*-benzo[*c*]chromen-6-one hydrochloride (11)

To (±)-3-(1-(dimethylamino)ethyl)-1-methoxy-6*H*-benzo[*c*]chromen-6-one (**9**) (4.60 g, 15.47 mmol) was added 48% aqueous HBr (190 mL) at room temperature. The mixture was heated at 130 °C for 20–24 h. After cooling, the aqueous solution was basified to pH 8–9 using $NaHCO_{3(s)}$ and extracted with CH_2Cl_2 (4 × 50 mL). Combined CH_2Cl_2 extracts were dried over $MgSO_4$ and concentrated under reduced pressure to give yellow-brown oil. Column chromatography on silica gel employing acetone gave desired benzo[*c*]chromen-6-one derivative (1.37 g, 31.0%). In order to prepare its HCl salt, the free base was dissolved in 30 mL dry methanol/acetone (1:3) and filtrated from 0.45 μm filter. HCl gas was passed through the obtained methanol–acetone solution. Liquid part was concentrated under reduced pressure to give yellow oil. When 10 mL dry acetone was added to resulted oil, HCl salt form of 3-(1-(dimethylamino)ethyl)-1-hydroxy-6*H*-benzo[*c*]chromen-6-one was precipitated. The content was mixed for 1 h and then filtrated. The residue was dried at 40 °C under vacuum. Yield obtained: 79.3%. HPLC purity: 99.82%, HRMS (ESI) $C_{17}H_{18}NO_3$ calcd 284.1287 $(M+1)^+$, found 284.1293, mp: 253.5 °C ^1H NMR (300 MHz, DMSO-d_6, ppm): δ 1.62 (d, 3H, —CH—*CH*$_3$), 2.54 (s, 3H, CH$_3$—N—*CH*$_3$), 2.75 (s, 3H, *CH*$_3$—N—CH$_3$), 4.43 (q, 1H, —N—*CH*—CH$_3$), 7.06 (s, 1H, Ar-H), 7.23 (s, 1H, Ar-H), 7.62 (t, 1H, Ar-H), 7.90 (dt, 1H, Ar-H), 8.24 (d, 1H, Ar-H), 9.09 (d, 1H, Ar-H), 11.07 (br s, 1H, —N—*H*), 11.55 (s, 1H, Ar-H).

4.1.12. (±)-1-(1-(Dimethylamino)ethyl)-3-hydroxy-6*H*-benzo[*c*]chromen-6-one hydrochloride (12)

To (±)-1-(1-(dimethylamino)ethyl)-3-methoxy-6*H*-benzo[*c*]chromen-6-one (1.49 g, 5.0 mmol) was added 48% aqueous HBr (62 mL) at room temperature. The mixture was heated at 120 °C for 4 h. After cooling, the aqueous solution was basified to pH 8–9 using $NaHCO_{3(s)}$ and extracted with CH_2Cl_2 (4 × 40 mL). Combined CH_2Cl_2 extracts were dried over $MgSO_4$ and concentrated under reduced pressure to give yellow solid (1.20 g, 84.5%). Its HCl salt was prepared according to procedure given for the compound **4a**. Yield obtained, 86.0%. HPLC purity: 99.07%, HRMS (ESI) $C_{17}H_{18}NO_3$ calcd 284.1287 $(M+1)^+$, found 284.1289, mp: 217.0 °C, ^1H NMR (300 MHz, CD_3OD, ppm): δ 1.50 (d, 3H, —CH—*CH*$_3$), 2.17 (s, 6H, *CH*$_3$—N—*CH*$_3$), 4.13 (q, 1H, —N—*CH*—CH$_3$), 6.62 (d, 1H, Ar-H), 7.09 (s, 1H, Ar-H), 7.48 (t, 1H, Ar-H), 7.78 (dt, 1H, Ar-H), 8.15 (br s, 1H, —N—*H*), 8.25 (dd, 1H, Ar-H).

4.1.13. (±)-3-(1-(Dimethylamino)ethyl)-6-oxo-6*H*-benzo[*c*]chromen-1-yl ethyl(methyl)carbamate (13)

960 mg (±)-3-(1-(dimethylamino)ethyl)-1-hydroxy-6*H*-benzo[*c*]chromen-6-one (**11**) (3.00 mmol) and 547.40 mg carbamoyl chloride (4.30 mmol) was mixed in pyridine (18 mL) at ambient temperature for 18–20 h. Pyridine was concentrated under reduced pressure to give yellow-brown oil. Then pH was adjusted to 8–9 using saturated $NaHCO_3$ and extracted with CH_2Cl_2 (4 × 50 mL). Combined CH_2Cl_2 extracts were dried over $MgSO_4$ and concentrated under reduced pressure to give yellow thick oil. Column chromatography on silica gel employing acetone gave desired **13** (1.04 g, 95.0%). In order to prepare its HCl salt the free base was dissolved in 30 mL dry acetone/heptane (1:3) and it was filtrated from 0.45 μm filter. HCl gas was passed through the obtained acetone-heptane solution, until salt precipitation took place. The content was mixed for 2 h and then filtrated. Yield obtained: 96.3%. HPLC purity: 99.16%, HRMS (ESI) $C_{21}H_{25}N_2O_4$ calcd 369.1814 $(M+1)^+$, found 369.1825, mp: 256.3 °C, ^1H NMR (300 MHz, DMSO-d_6, ppm): δ 1.07–1.35 (m, 3H, *CH*$_3$—CH$_2$—N—), 1.67 (d, 3H, —CH—*CH*$_3$), 2.54 (d, 3H, *CH*$_3$—N—CH$_3$), 2.71 (d, 3H, CH$_3$—N—*CH*$_3$), 3.08 (d, 3H, *CH*$_3$—N—CH$_2$—CH$_3$), 3.28–3.68 (m, 2H, CH$_3$—N—*CH*$_2$—CH$_3$), 4.57 (q, 1H, —N—*CH*-CH$_3$), 7.42 (s, 1H, Ar-H), 7.65–7.77 (m, 2H, Ar-H), 7.91–8.02 (m, 1H, Ar-H), 8.27–8.42 (m, 2H, Ar-H), 11.37 (br s, 1H, —N—*H*).

4.1.14. (±)-1-(1-(Dimethylamino)ethyl)-6-oxo-6*H*-benzo[*c*]chromen-3-yl ethyl(methyl)carbamate hydrochloride (14)

660 mg (±)-1-(1-(dimethylamino)ethyl)-3-hydroxy-6*H*-benzo[*c*]chromen-6-one (**12**) (2.33 mmol) and 424.76 mg carbamoyl chloride (3.49 mmol) was mixed in pyridine (16 mL) at ambient temperature for 17–19 h. Pyridine was concentrated under reduced pressure to give yellow-brown oil. Then pH was adjusted to 8–9 using saturated $NaHCO_3$ and extracted with CH_2Cl_2 (4×50 mL). Combined CH_2Cl_2 extracts were dried over $MgSO_4$ and concentrated under reduced pressure to give yellow thick oil. Column chromatography on silica gel (acetone/dichloromethane; 2:1) gave desired benzo[*c*]chromen-6-one derivative (511 mg, 60.0%). Then, the free base dissolved in 30 mL dry ether/acetone (1:3) was filtrated from 0.45 μm filter. HCl gas was passed through the obtained ether–acetone solution, until salt precipitation took place. The content was mixed for 2 h and then filtrated. Yield obtained: 71.7%. HPLC purity: 99.62%, HRMS (ESI) $C_{21}H_{25}N_2O_4$ calcd 369.1814 $(M+1)^+$, found 369.1808, mp: 127.9 °C, ^1H NMR (300 MHz, CD_3OD, ppm): δ 1.13–1.31 (m, 3H, *CH*$_3$—CH$_2$—N—), 1.67 (d, 3H, —CH—*CH*$_3$), 2.33 (s, 6H, *CH*$_3$—N—*CH*$_3$), 3.04 (d, 3H, *CH*$_3$—N—CH$_2$—CH$_3$), 3.34–3.57 (m, 2H, CH$_3$—N—*CH*$_2$—CH$_3$), 4.48 (br s, 1H, —N—*CH*—CH$_3$), 7.13 (s, 1H, Ar-H), 7.43 (d, 1H, Ar-H), 7.63 (dt, 1H, Ar-H), 7.89 (dt, 1H, Ar-H), 8.16 (br s, 1H, —N—*H*), 8.33 (dd, 1H, Ar-H).

4.1.15. 3-Hydroxy-7,8,9,10-tetrahydro-benzo[*c*]chromen-6-one (15)

A mixture of resorcinol (5.0 g, 45.45 mmol) and ethyl 2-oxocyclohexanecarboxylate (7.73 g, 45.45 mmol) was heated at 70 °C in the presence of zirconium(IV) chloride (1.06 g, 10 mol %) for 1 h. After completion of the reaction, the reaction mixture was cooled to room temperature and poured onto crushed ice (200 g). The solid product obtained was filtered off, and washed with ice-cold water. Yield obtained: 91%, ^1H NMR (DMSO-d_6) δ 1.68–1.73 (m, 4H, —CH$_2$—*CH*$_2$—*CH*$_2$—CH$_2$—), 2.27–2.40 (m, 2H, —CH$_2$—*CH*$_2$—C=C—), 2.62–2.76 (m, 2H, —CH$_2$—*CH*$_2$—C—COO—), 6.65 (d, 1H, Ar-H), 6.74 (dd, 1H, Ar-H), 7.47 (d, 1H, Ar-H), 10.35 (s, 1H, —O—*H*).

4.1.16. 3-Hydroxy-6*H*-benzo[*c*]chromen-6-one (16)

A mixture of 2-iodobenzoic acid (30 g, 0.12 mol), resorcinol (40 g, 0.36 mol), and NaOH (17.4 g, 0.44 mol) in water (150 mL) was heated under reflux for 30 min. After the addition of aqueous $CuSO_4$ (28%, 25 mL) the mixture was refluxed for additional 10 min, during which time the product (0.62 g) precipitated as a white powder. The precipitate was filtered and washed with cold water. Yield obtained: 80.1%, ^1H NMR (DMSO-d_6) δ 6.71 (d, 1H), 6.80 (dd, 1H, Ar-H), 7.51 (t, 1H, Ar-H), 7.83 (t, 1H, Ar-H), 8.08–8.22 (m, 3H, Ar-H), 10.32 (s, 1H, —O—*H*).

4.1.17. 3-(2-Chloroethoxy)-7,8,9,10-tetrahydro-6*H*-benzo[*c*]chromen-6-one (17a)

$Na_{(s)}$ (1.9 g, 82.6 mmol) was dissolved in 50 mL dry ethanol to give NaOEt solution. To 3-hydroxy-7,8,9,10-tetrahydro-6*H*-benzo[*c*]chromen-6-one (15 g, 69.4 mmol) solution in 150 mL Ethanol, previously prepared NaOEt solution was added at ambient temperature. Ethanol was distilled out under reduced vacuum and then 100 mL dry DMF was added onto the residue. 100 mL 1,2-dichloroethane was put into the reaction mixture and the content was refluxed for 7 h. The reaction mixture was cooled to room temperature and it was poured onto 250 mL cold 0.8 N NaOH solution. 100 mL 1,2-dichloroethane was added to the medium and organic phase was separated. Then, the aqueous phase was extracted with additional 1,2-dichloroethane (2×50 mL). Combined organic extracts were washed with water, dried over $MgSO_4$ and concentrated under reduced pressure to give the white solid product. Yield obtained: 49.6%. (300 MHz, $CDCl_3$, ppm): δ 1.69–1.82 (m, 4H, —CH$_2$—*CH*$_2$—*CH*$_2$—CH$_2$—), 2.41–2.54 (m, 2H, —CH$_2$—*CH*$_2$—C=C—), 2.62–2.73 (m, 2H, —CH$_2$—*CH*$_2$—C—COO—), 3.79 (t, 2H, Cl—*CH*$_2$—), 4.21 (t, 2H, —O—*CH*$_2$—CH$_2$—), 6.72 (d, 1H, Ar-H), 6.80 (dd, 1H, Ar-H), 7.40 (d, 1H, Ar-H).

4.1.18. 3-(3-Chloropropoxy)-7,8,9,10-tetrahydro-6*H*-benzo[*c*]chromen-6-one (17b)

3-Hydroxy-7,8,9,10-tetrahydro-6*H*-benzo[*c*]chromen-6-one (10 g, 46.2 mmol) and NaH (2.76 g, 69 mmol) were mixed in 40 mL DMF. 1-Bromo-3-chloropropane (18.17 g, 115.5 mmol) was added to this mixture and the reaction was mixed at ambient temperature for 6 h. After completion of the reaction, the reaction mixture was poured onto 200 mL cold 15% NaOH solution. The mixture was mixed for 5 min and then 50 mL of hexane was added. The solid product precipitated was filtered and washed with cold water. Yield obtained: 81.2% (300 MHz, $CDCl_3$, ppm): δ 1.68–1.82 (m, 4H, —CH$_2$—*CH*$_2$—*CH*$_2$—CH$_2$—), 2.20 (p, 2H, Cl—CH$_2$—*CH*$_2$—CH$_2$—O—), 2.47–2.51 (m, 2H, —CH$_2$—*CH*$_2$—C=C—), 2.65–2.70 (m, 2H, —CH$_2$—*CH*$_2$—C—COO—), 3.70 (t, 2H, Cl—*CH*$_2$—), 4.09 (t, 2H, —O—*CH*$_2$—CH$_2$—), 6.73–6.79 (m, 2H, Ar-H), 7.39 (d, 1H, Ar-H).

4.1.19. 3-(4-Chlorobutoxy)-7,8,9,10-tetrahydro-6*H*-benzo[*c*]chromen-6-one (17c)

3-(4-Chlorobutoxy)-7,8,9,10-tetrahydro-6*H*-benzo[*c*]chromen-6-one was synthesized according to procedure given for **17b**, except the employment of 1-bromo-4-chlorobutane (19.81 g, 115.5 mmol) instead of 1-bromo-3-chloropropane. Yield obtained: 82.5%. ^1H NMR (300 MHz, $CDCl_3$, ppm): δ 1.58–1.72 (m, 4H, —CH$_2$—*CH*$_2$—*CH*$_2$—CH$_2$—), 1.73–2.06 (m, 4H, Cl—CH$_2$—*CH*$_2$—*CH*$_2$—CH$_2$—O—), 2.37–2.56 (m, 2H, —CH$_2$—*CH*$_2$—C=C—), 2.57–2.76 (m, 2H, —CH$_2$—*CH*$_2$—C—COO—), 3.56 (t, 2H, Cl—*CH*$_2$—), 3.96 (t, 2H, —O—*CH*$_2$—CH$_2$—), 6.61–6.79 (m, 2H, Ar-H), 7.37 (d, 1H, Ar-H).

4.1.20. 3-(2-Chloroethoxy)-6*H*-benzo[*c*]chromen-6-one (18a)

3-(2-Chloroethoxy)-6*H*-benzo[*c*]chromen-6-one was synthesized according to procedure given for the compound **17a** (14.7 g, 69.4 mmol), except the employment of 3-hydroxy-6*H*-benzo[*c*]chromen-6-one instead of 3-hydroxy-7,8,9,10-tetrahydro-6*H*-benzo[*c*]chromen-6-one. Yield obtained: 46.8%. ^1H NMR (300 MHz, $CDCl_3$, ppm): δ 3.80 (t, 2H, Cl—*CH*$_2$—), 4.23 (t, 2H, —O—*CH*$_2$—CH$_2$—Cl), 6.79 (d, 1H, Ar-H), 6.87 (dd, 1H, Ar-H), 7.45 (dt, 1H, Ar-H), 7.72 (dt, 1H, Ar-H), 7.87–7.95 (m, 2H, Ar-H), 8.28 (dd, 1H, Ar-H).

4.1.21. 3-(3-Chloropropoxy)-6*H*-benzo[*c*]chromen-6-one (18b)

3-(3-Chloropropoxy)-6*H*-benzo[*c*]chromen-6-one was synthesized according to procedure given for the compound **17b**, except the employment of 3-hydroxy-6*H*-benzo[*c*]chromen-6-one (9.8 g, 46.2 mmol) instead of 3-hydroxy-7,8,9,10-tetrahydro-6*H*-benzo[*c*]chromen-6-one. The reaction was completed in 40 min. Yield obtained: 79.3%. ^1H NMR (300 MHz, $CDCl_3$, ppm): δ 2.22 (p, 2H, Cl—CH$_2$—*CH*$_2$—CH$_2$—O—), 3.71 (t, 2H, Cl—*CH*$_2$—), 4.12 (t, 2H, —O—*CH*$_2$—CH$_2$—), 6.79 (d, 1H, Ar-H), 6.84 (dd, 1H, Ar-H), 7.44 (t, 1H, Ar-H), 7.72 (tt, 1H, Ar-H), 7.86–7.94 (m, 2H, Ar-H) 8.28 (dd, 1H, Ar-H).

4.1.22. 3-(4-Chlorobutoxy)-6*H*-benzo[*c*]chromen-6-one (18c)

3-(4-Chlorobutoxy)-6*H*-benzo[*c*]chromen-6-one was synthesized according to procedure given for the compound **17b**, except the employment of 3-hydroxy-6*H*-benzo[*c*]chromen-6-one (9.8 g, 46.2 mmol) instead of 3-hydroxy-7,8,9,10-tetrahydro-6*H*-benzo[*c*]chromen-6-one. The reaction was completed in 40 min. Yield obtained: 83.6%. ^1H NMR (300 MHz, $CDCl_3$, ppm): δ 1.89–1.99

(m, 4H, Cl—CH_2—CH_2—CH_2—CH_2—O—), 3.56–3.60 (m, 2H, Cl—CH_2—), 3.98–4.01 (m, 2H, —O—CH_2—CH_2—), 6.77 (d, 1H, Ar-H), 6.83 (dd, 1H, Ar-H), 7.43 (dt, 1H, Ar-H), 7.71 (dt, 1H, Ar-H), 7.86 (d, 1H, Ar-H), 7.93 (d, 1H, Ar-H), 8.28 (dd, 1H, Ar-H).

4.1.23. General procedure for the synthesis of 19, and 20-series title compounds

A mixture of 2.73 mmol appropriate alkyl halide (i.e., **17a**–**c**, or **18a**–**c**), 0.6 g K_2CO_3 (4.34 mmol), and 0.6 g NaI (4.00 mmol) in ACN (12 mL) was mixed for 5 min in a 30 mL microwave reaction vessel. Then, 8.2 mmol appropriate amine derivative (i.e., **1**–**6**, as shown in Scheme 3) was added and the resulting content was mixed for additional 2 min. The reaction vessel was capped, and inserted inside the microwave instrument (i.e., A CEM Model Single Mode Microwave Instrument) and heated for 32 min at 105 °C at the dynamic mode of the instrument automatically calibrating the radiation and temperature balance with respect to the change in pressure. Then, acetonitrile was distilled out under vacuum and the residue thus obtained was mixed with 20 mL of K_2CO_3 solution (5.0%) and this mixture was heated at 60 °C for 1 h. Then the mixture was cooled down to room temperature and the aqueous phase was extracted 3 times with 20 mL of ethylacetate. Combined organic extracts were dried over $MgSO_4$ and concentrated under reduced pressure to give the product (i.e., free base). The free base was dissolved in 10 mL acetone and HCl gas was continuously passed until HCl salt precipitation took place.

4.1.24. 3-(2-(Benzyl(methyl)amino)ethoxy)-7,8,9,10-tetrahydro-6*H*-benzo[*c*]chromen-6-one hydrochloride (19a1)

Yield obtained 75.1%, 83.3%, respectively, for the reaction and HCl salt preparation. ^{1}H NMR (300 MHz, $CDCl_3$, ppm): δ 1.71–1.80 (m, 4H, —CH_2—CH_2—CH_2—CH_2—), 2.31 (s, 3H, —N—CH_3), 2.43–2.54 (m, 2H, —CH_2—CH_2—C≡C—), 2.62–2.73 (m, 2H, —CH_2—CH_2—C—COO—), 2.80 (t, 2H, —N—CH_2—CH_2—O—), 3.58 (s, 2H, —N—CH_2—Ph), 4.06 (t, 2H, —O—CH_2—CH_2—), 6.71 (d, 1H, Ar-H), 6.76 (dd, 1H, Ar-H), 7.15–7.31 (m, 5H, Ar-H), 7.37 (d, 1H, Ar-H). HPLC purity: 99.67%, HRMS (EI) $C_{23}H_{26}NO_3$ calcd 364.1913 (M+), found 364.1909, mp: 196.0 °C.

4.1.25. 3-(2-((3-Methoxybenzyl)(methyl)amino)ethoxy)-7,8,9,10-tetrahydro-6*H*-benzo[*c*]chromen-6-one hydrochloride (19a2)

Yield obtained 68.1%, 90.1%, respectively, for the reaction and HCl salt preparation. ^{1}H NMR (300 MHz, $CDCl_3$, ppm): δ 1.71–1.80 (m, 4H, —CH_2—CH_2—CH_2—CH_2—), 2.35 (s, 3H, —N—CH_3), 2.42–2.55 (m, 2H, —CH_2—CH_2—C≡C—), 2.63–2.72 (m, 2H, —CH_2—CH_2—C—COO—), 2.84 (br s, 2H, —N—CH_2—CH_2—O—), 3.60 (s, 2H, —N—CH_2—Ph), 3.75 (s, 3H, —O—CH_3), 4.10 (t, 2H, —O—CH_2—CH_2—), 6.71–6.79 (m, 3H, Ar-H), 6.83–6.91 (m, 2H, Ar-H), 7.18 (t, 1H, Ar-H), 7.38 (d, 1H, Ar-H). 90.1%, HPLC purity: 99.98%, HRMS (ESI) $C_{24}H_{28}NO_4$ calcd 394.2018 $[M+H]^+$, found 394.2000, mp: 176.5 °C.

4.1.26. 3-(2-(4-Benzylpiperidin-1-yl)ethoxy)-7,8,9,10-tetrahydro-6*H*-benzo[*c*]chromen-6-one hydrochloride (19a3)

Yield obtained 66.3%, 87.4%, respectively, for the reaction and HCl salt preparation. ^{1}H NMR (300 MHz, $CDCl_3$, ppm): δ 1.32–1.45 (m, 2H, —O—CH_2—CH_2—N—CH_2—$CH_{2(\mathbf{ax})}$—CH—), 1.46–1.53 (m, 1H, —O—CH_2—CH_2—N—CH_2—CH_2—*CH*—CH_2—CH_2—), 1.57 (d, 2H, —O—CH_2—CH_2—N—CH_2—$CH_{2(\mathbf{eq})}$—CH—), 1.63–1.78 (m, 4H, —CH_2—CH_2—CH_2—CH_2—), 2.05 (t, 2H, —O—CH_2—CH_2—N—$CH_{2(\mathbf{ax})}$—), 2.39–2.54 (m, 4H, —CH_2—CH_2—C≡C— and —O—CH_2—CH_2—N—$CH_{2(\mathbf{eq})}$—), 2.62–2.73 (m, 2H, —CH_2—CH_2—C—COO—), 2.81 (t, 2H, —N—CH_2—CH_2—O—), 2.99 (d, 2H, —CH—CH_2—Ph), 4.13 (t, 2H, —O—CH_2—CH_2—), 6.72 (d, 1H, Ar-H), 6.77 (dd, 1H, Ar-H), 7.06–7.15 (m, 3H, Ar-H), 7.18–7.24 (m, 2H, Ar-H), 7.37 (d, 1H, Ar-H). HPLC purity: 99.85%, HRMS (ESI) $C_{27}H_{32}NO_3$ calcd 418.2382 $[M+H]^+$, found 418.2392, mp: 153.1 °C.

4.1.27. 3-(2-(4-(3-Methoxybenzyl)piperidin-1-yl)ethoxy)-7,8,9,10-tetrahydro-6*H*-benzo[*c*]chromen-6-one hydrochloride (19a4)

Yield obtained 76.1%, 88.3%, respectively, for the reaction and HCl salt preparation.^{1}H NMR (300 MHz, $CDCl_3$, ppm): δ1.28–1.36 (m, 2H, —O—CH_2—CH_2—N—CH_2—$CH_{2(\mathbf{ax})}$—CH—), 1.44–1.52 (m, 1H, —O—CH_2—CH_2—N—CH_2—CH_2—*CH*—CH_2—CH_2—), 1.60 (d, 2H, —O—CH_2—CH_2—N—CH_2—$CH_{2(\mathbf{eq})}$—CH—), 1.71–1.79 (m, 4H, —CH_2—CH_2—CH_2—CH_2—), 2.02 (t, 2H, —O—CH_2—CH_2—N—$CH_{2(\mathbf{ax})}$—), 2.44–2.50 (m, 4H, —CH_2—CH_2—C≡C—and —O—CH_2—CH_2—N—$CH_{2(\mathbf{eq})}$—), 2.62–2.71 (m, 2H, —CH_2—CH_2—C—COO—), 2.76 (t, 2H, —N—CH_2—CH_2—O—), 2.94 (d, 2H, —CH—CH_2—Ph), 3.73 (s, 3H, —O—CH_3), 4.08 (t, 2H, —O—CH_2—CH_2—), 6.62–6.78 (m, 5H, Ar-H), 7.12 (t, 1H, Ar-H), 7.37 (d, 1H, Ar-H). HPLC purity: 99.66%, HRMS (ESI) $C_{28}H_{34}NO_4$ calcd 448.2488 $[M+H]^+$, found 448.2496, mp: 101.9 °C.

4.1.28. 3-(2-(4-Benzylpiperazin-1-yl)ethoxy)-7,8,9,10-tetrahydro-6*H*-benzo[*c*]chromen-6-one dihydrochloride (19a5)

Yield obtained 70.3%, 88.8%, respectively, for the reaction and HCl salt preparation.^{1}H NMR (300 MHz, $CDCl_3$, ppm): δ 1.69–1.81 (m, 4H, —CH_2—CH_2—CH_2—CH_2—), 2.35–2.62 (m, 10H, —CH_2—CH_2—C≡C— and piperazine protons), 2.63–2.70 (m, 2H, —CH_2—CH_2—C—COO—), 2.78 (t, 2H, —N—CH_2—CH_2—O—), 3.46 (s, 2H, N—CH_2—Ph), 4.07 (t, 2H, —O—CH_2—CH_2—), 6.71 (d, 1H, Ar-H), 6.76 (dd, 1H, Ar-H), 7.15–7.27 (m, 5H, Ar-H), 7.36 (d, 1H, Ar-H). HPLC purity: 99.70%, HRMS (ESI) $C_{26}H_{31}N_2O_3$ calcd 419.2335 $[M+H]^+$, found 419.2333, mp: 129.6 °C.

4.1.29. 3-(2-(4-(3-Methoxybenzyl)piperazin-1-yl)ethoxy)-7,8,9,10-tetrahydro-6*H*-benzo[*c*]chromen-6-one dihydrochloride (19a6)

Yield obtained 66.0%, 80.6%, respectively, for the reaction and HCl salt preparation. ^{1}H NMR (300 MHz, $CDCl_3$, ppm): δ 1.71–1.79 (m, 4H, —CH_2—CH_2—CH_2—CH_2—), 2.38–2.63 (m, 10H, m, 10H, —CH_2—CH_2—C≡C— and piperazine protons), 2.64–2.73 (m, 2H, —CH_2—CH_2—C—COO—), 2.78 (t, 2H, —N—CH_2—CH_2—O—), 3.44 (s, 2H, N—CH_2—Ph), 3.74 (s, 3H, —O—CH_3), 4.07 (t, 2H, —O—CH_2—CH_2—), 6.69–6.88 (m, 5H, Ar-H), 7.16 (t, 1H, Ar-H), 7.37 (d, 1H, Ar-H). HPLC purity: 100.0%, HRMS (ESI) $C_{27}H_{33}N_2O_4$ calcd 449.2440 $[M+H]^+$, found 449.2453, mp: 125.6 °C.

4.1.30. 3-(3-(Benzyl(methyl)amino)propoxy)-7,8,9,10-tetrahydro-6*H*-benzo[*c*]chromen-6-one hydrochloride (19b1)

Yield obtained 69.8%, 88.2%, respectively, for the reaction and HCl salt preparation.^{1}H NMR (300 MHz, $CDCl_3$, ppm): δ 1.66–1.89 (m, 4H, —CH_2—CH_2—CH_2—CH_2—), 1.90–2.08 (m, 2H, —O—CH_2—CH_2—CH_2—N—), 2.22 (s, 3H, —N—CH_3), 2.40–2.62 (m, 4H, —CH_2—CH_2—C≡C— and —N—CH_2—CH_2—), 2.63–2.79 (m, 2H, —CH_2—CH_2—C—COO—), 3.50 (s, 2H, —N—CH_2—Ph), 4.02 (t, 2H, —O—CH_2—CH_2—), 6.67–6.82 (m, 2H, Ar-H), 7.12–7.33 (m, 5H, Ar-H), 7.34–7.46 (m, 1H, Ar-H). HPLC purity: 99.00%, HRMS (ESI) $C_{24}H_{28}NO_3$ calcd 378.2069 $[M+H]^+$, found 378.2070, mp: 84.8 °C.

4.1.31. 3-(3-((3-Methoxybenzyl)(methyl)amino)propoxy)-7,8,9,10-tetrahydro-6*H*-benzo[*c*]chromen-6-one hydrochloride (19b2)

Yield obtained 60.2%, 86.4%, respectively, for the reaction and HCl salt preparation. ^{1}H NMR (300 MHz, $CDCl_3$, ppm): δ1.68–1.84 (m, 4H, —CH_2—CH_2—CH_2—CH_2—), 1.89–2.01 (m, 2H, —O—CH_2—CH_2—CH_2—N—), 2.19 (s, 3H, —N—CH_3), 2.45–2.54 (m, 4H, —CH_2—CH_2—C≡C— and —N—CH_2—CH_2—), 2.64–2.73 (m, 2H, —CH_2—CH_2

—C—COO—), 3.45 (s, 2H, —N—CH_2—Ph), 3.71 (s, 3H, —O—CH_3), 3.99 (t, 2H, —O—CH_2—CH_2—), 6.67–6.76 (m, 3H, Ar-H), 6.82 (dd, 2H, Ar-H), 7.14 (t, 1H, Ar-H), 7.37 (dd, 1H, Ar-H). HPLC purity: 99.45%, HRMS (ESI) $C_{25}H_{30}NO_4$ calcd 408.2175 $[M+H]^+$, found 408.2166, mp: 215.6 °C.

4.1.32. 3-(3-(4-Benzylpiperidin-1-yl)propoxy)-7,8,9,10-tetrahydro-6*H*-benzo[*c*]chromen-6-one hydrochloride (19b3)

Yield obtained 66.7%, 82.5%, respectively, for the reaction and HCl salt preparation. ^{1}H NMR (300 MHz, $CDCl_3$, ppm): δ 1.22–1.42 (m, 2H, —N—CH_2—$CH_{2(ax)}$—CH—CH_2—CH_2—), 1.43–1.56 (m, 1H, —N—CH_2—CH_2—*CH*—CH_2—CH_2—), 1.57–1.69 (m, 2H, —N—CH_2—$CH_{2(eq)}$—CH—CH_2—), 1.70–1.86 (m, 4H, —CH_2—CH_2—CH_2—CH_2—), 1.87–2.09 (m, 4H, —N—$CH_{2(ax)}$—CH_2—CH—CH_2— and —O—CH_2—CH_2—CH_2—N—), 2.39–2.61 (m, 6H, —CH_2—CH_2—C=C—, —N—$CH_{2(eq)}$—CH_2—CH—CH_2—, and O—CH_2—CH_2—CH_2—N—), 2.63–2.76 (m, 2H, —CH_2—CH_2—C—COO—), 2.95 (d, 2H, —CH—CH_2—Ph), 4.01 (t, 2H, —O—CH_2—CH_2—), 6.70–6.83 (m, 2H, Ar-H), 7.03–7.28 (m, 5H, Ar-H), 7.39 (d, 1H, Ar-H). HPLC purity: 99.59%, HRMS (ESI) $C_{28}H_{34}NO_3$ calcd 432.2539 $[M+H]^+$, found 432.2559, mp: 121.7 °C.

4.1.33. 3-(3-(4-(3-Methoxybenzyl)piperidin-1-yl)propoxy)-7,8,9,10-tetrahydro-6*H*-benzo[*c*]chromen-6-one hydrochloride (19b4)

Yield obtained 65.6%, 81.1%, respectively, for the reaction and HCl salt preparation.^{1}H NMR (300 MHz, $CDCl_3$, ppm): δ 1.30–1.42 (m, 2H, —N—CH_2—$CH_{2(ax)}$—CH—CH_2—CH_2—), 1.46–1.57 (m, 1H, —N—CH_2—CH_2—*CH*—CH_2—CH_2—), 1.58–1.71 (m, 2H, —N—CH_2—$CH_{2(eq)}$—CH—CH_2—), 1.72–1.88 (m, 4H, —CH_2—CH_2—CH_2—CH_2—), 1.92–2.02 (m, 4H, —N—$CH_{2(ax)}$—CH_2—CH—CH_2— and –O—CH_2—CH_2—CH_2—N—), 2.42–2.61 (m, 6H, —CH_2—CH_2—C=C—, —N—$CH_{2(eq)}$—CH_2—CH—CH_2—, and O—CH_2—CH_2—CH_2—N—), 2.66–2.78 (m, 2H, —CH_2—CH_2—C—COO—), 2.97 (d, 2H, —CH—CH_2—Ph), 3.77 (s, 3H, —O—CH_3), 4.03 (t, 2H, —O—CH_2—CH_2—), 6.63–6.83 (m, 5H, Ar-H), 7.17 (t, 1H, Ar-H), 7.42 (d, 1H, Ar-H). HPLC purity: 99.00%, HRMS (ESI) $C_{29}H_{36}NO_4$ calcd 462.2644 $[M+H]^+$, found 462.2644, mp: 119.1 °C.

4.1.34. 3-(3-(4-Benzylpiperazin-1-yl)propoxy)-7,8,9,10-tetrahydro-6*H*-benzo[*c*]chromen-6-one dihydrochloride (19b5)

Yield obtained 68.0%, 90.2%, respectively, for the reaction and HCl salt preparation.^{1}H NMR (300 MHz, DMSO-d_6, ppm): δ1.57–1.78 (m, 4H, —CH_2—CH_2—CH_2—CH_2—), 1.83 (p, 2H, —O—CH_2—CH_2—CH_2—N—), 2.14–2.52 (m, 12H, —CH_2—CH_2—C=C—, —O—CH_2—CH_2—CH_2—N— and piperazine protons), 2.64–2.78 (m, 2H, —CH_2—CH_2—C—COO—), 3.40 (s, 2H, —N—CH_2—Ph), 4.04 (t, 2H, —O—CH_2—CH_2), 6.83–6.93 (m, 2H, Ar-H), 7.15–7.34 (m, 5H, Ar-H), 7.55 (d, 1H, Ar-H). HPLC purity: 100.0%, HRMS (ESI) $C_{27}H_{33}N_2O_3$ calcd 433.2491 $[M+H]^+$, found 433.2488, mp: 96.3 °C.

4.1.35. 3-(3-(4-(3-Methoxybenzyl)piperazin-1-yl)propoxy)-7,8,9,10-tetrahydro-6*H*-benzo[*c*]chromen-6-one dihydrochloride (19b6)

Yield obtained 64.6%, 80.3%, respectively, for the reaction and HCl salt preparation.^{1}H NMR (300 MHz, $CDCl_3$, ppm): δ 1.72–1.79 (m, 4H, —CH_2—CH_2—CH_2—CH_2—), 1.93–2.10 (m, 2H, —O—CH_2—CH_2—CH_2—N—), 2.39–2.73 (m, 14H, —CH_2—CH_2—C=C—, —O—CH_2—CH_2—CH_2—N—, —CH_2—CH_2—C—COO— and piperazine protons), 3.50 (s, 2H, N—CH_2—Ph), 3.75 (s, 3H—O—CH_3), 4.00 (t, 2H, O-CH_2—CH_2—), 6.71–6.91 (m, 5H, Ar-H), 7.17 (t, 1H, Ar-H), 7.38 (d, 1H, Ar-H). HPLC purity: 98.72%, HRMS (ESI) $C_{28}H_{35}N_2O_4$ calcd 463.2597 $[M+H]^+$, found 463.2597, mp: 274.2 °C.

4.1.36. 3-(4-(Benzyl(methyl)amino)butoxy)-7,8,9,10-tetrahydro-6*H*-benzo[*c*]chromen-6-one hydrochloride (19c1)

Yield obtained 61.6%, 86.2%, respectively, for the reaction and HCl salt preparation. ^{1}H NMR (300 MHz, $CDCl_3$, ppm): δ 1.62–1.88 (m, 8H, —O—CH_2—CH_2—CH_2—CH_2—N— and C—CH_2—CH_2—CH_2—CH_2—C), 2.20 (s, 3H, —N—CH_3), 2.43 (t, 2H, —CH_2—CH_2—CH_2—N—), 2.49–2.58 (m, 2H, —CH_2—CH_2—C=C—), 2.68–2.76 (m, 2H, —CH_2—CH_2—C—COO—), 3.49 (s, 2H, —N—CH_2—Ph), 3.96 (t, 2H, —O—CH_2—CH_2—CH_2—), 6.74 (d, 1H, Ar-H), 6.79 (dd, 1H, Ar-H), 7.18–7.32 (m, 5H, Ar-H), 7.42 (d, 1H, Ar-H). HPLC purity: 99.98%, HRMS (ESI) $C_{25}H_{30}NO_3$ calcd 392.2226 $[M+H]^+$, found 392.2227, mp: 150.7 °C.

4.1.37. 3-(4-((3-Methoxybenzyl)(methyl)amino)butoxy)-7,8,9,10-tetrahydro-6*H*-benzo[*c*]chromen-6-one hydrochloride (19c2)

Yield obtained 59.4%, 89.2%, respectively, for the reaction and HCl salt preparation. ^{1}H NMR (300 MHz, $CDCl_3$, ppm): δ 1.59–1.84 (m, 8H, —O—CH_2—CH_2—CH_2—CH_2—N— and C—CH_2—CH_2—CH_2—CH_2—C), 2.19 (s, 3H, —N—CH_3), 2.41 (t, 2H, —CH_2—CH_2—CH_2—N—), 2.46–2.53 (m, 2H, —CH_2—CH_2—C=C—), 2.63–2.72 (m, 2H, —CH_2—CH_2—C—COO—), 3.46 (s, 2H, —N—CH_2—Ph), 3.73 (s, 3H, —O—CH_3), 3.92 (t, 2H, —O—CH_2—CH_2—CH_2—), 6.67–6.77 (m, 3H, Ar-H), 6.81–6.88 (m, 2H, Ar-H), 7.16 (t, 1H, Ar-H), 7.37 (d, 1H, Ar-H). HPLC purity: 99.67%, HRMS (ESI) $C_{26}H_{32}NO_4$ calcd 422.2331 $[M+H]^+$, found 422.2345, mp: 184.8 °C.

4.1.38. 3-(4-(4-Benzylpiperidin-1-yl)butoxy)-7,8,9,10-tetrahydro-6*H*-benzo[*c*]chromen-6-one hydrochloride (19c3)

Yield obtained 70.4%, 80.3%, respectively, for the reaction and HCl salt preparation. ^{1}H NMR (300 MHz, $CDCl_3$, ppm): δ 1.19–1.38 (m, 2H, —N—CH_2—$CH_{2(ax)}$—CH—CH_2—CH_2—), 1.39–1.53 (m, 1H, —N—CH_2—CH_2—*CH*—CH_2—CH_2—), 1.53–1.94 (m, 12H, —N—CH_2—$CH_{2(eq)}$—CH—CH_2—CH_2—, —C—CH_2—CH_2—CH_2—CH_2—C—, —O—CH_2—CH_2—CH_2—CH_2—N— and —N—$CH_{2(ax)}$—CH_2—CH—CH_2—), 2.34 (t, 2H, —O—CH_2—CH_2—CH_2—CH_2—N—), 2.42–2.56 (m, 4H, —N—$CH_{2(eq)}$—CH_2—CH—CH_2— and —CH_2—CH_2—C=C—), 2.62–2.73 (m, 2H, —CH_2—CH_2—C—COO—), 2.89 (d, 2H, —CH—CH_2—Ph), 3.94 (t, 2H, —O—CH_2—CH_2—), 6.70 (d, 1H, Ar-H), 6.74 (dd, 1H, Ar-H), 7.03–7.25 (m, 5H, Ar-H), 7.36 (d, 1H, Ar-H). HPLC purity: 99.71%, HRMS (ESI) $C_{29}H_{36}NO_3$ calcd 446.2695 $[M+H]^+$, found 446.2695, mp: 94.6 °C.

4.1.39. 3-(4-(4-(3-Methoxybenzyl)piperidin-1-yl)butoxy)-7,8,9,10-tetrahydro-6*H*-benzo[*c*]chromen-6-one hydrochloride (19c4)

Yield obtained 59.5%, 81.9%, respectively, for the reaction and HCl salt preparation. ^{1}H NMR (300 MHz, $CDCl_3$, ppm): δ 1.20–1.41 (m, 2H, —N—CH_2—$CH_{2(ax)}$—CH—CH_2—CH_2—), 1.41–1.55 (m, 1H, —N—CH_2—CH_2—*CH*—CH_2—CH_2—), 1.55–1.95 (m, 12H, —N—CH_2—$CH_{2(eq)}$—CH—CH_2—CH_2—, —C—CH_2—CH_2—CH_2—CH_2—C—, —O—CH_2—CH_2—CH_2—CH_2—N— and —N—$CH_{2(ax)}$—CH_2—CH—CH_2—), 2.36 (t, 2H, —O—CH_2—CH_2—CH_2—CH_2—N—), 2.45–2.57 (m, 4H, —N—$CH_{2(eq)}$—CH_2—CH—CH_2— and —CH_2—CH_2—C=C—), 2.62–2.76 (m, 2H, —CH_2—CH_2—C—COO—), 2.91 (d, 2H, —CH—CH_2—Ph), 3.75 (s, 3H, —O—CH_3), 3.96 (t, 2H, —O—CH_2—CH_2—), 6.62–6.80 (m, 5H, Ar-H), 7.14 (t, 1H, Ar-H), 7.39 (d, 1H, Ar-H). HPLC purity: 96.54%, HRMS (ESI) $C_{30}H_{38}NO_4$ calcd 476.2801 $[M+H]^+$, found 476.2816, mp: 151.9 °C.

4.1.40. 3-(4-(4-Benzylpiperazin-1-yl)butoxy)-7,8,9,10-tetrahydro-6*H*-benzo[*c*]chromen-6-one dihydrochloride (19c5)

Yield obtained 64.3%, 84.7%, respectively, for the reaction and HCl salt preparation. ^{1}H NMR (300 MHz, $CDCl_3$, ppm): δ 1.58–1.85 (m, 8H, —C—CH_2—CH_2—CH_2—CH_2—C— and —O—CH_2—CH_2-

—CH_2—CH_2—N—), 2.30–2.62 (m, 12H, —O—CH_2—CH_2—CH_2—CH_2—N—, piperazine protons and —CH_2—CH_2—C=C—), 2.63–2.74 (m, 2H, —CH_2—CH_2—C—COO—), 3.46 (s, 2H, N—CH_2—Ph), 3.95 (t, 2H, —O—CH_2—CH_2—), 6.68–6.78 (m, 2H, Ar-H), 7.14–7.29 (m, 5H, Ar-H), 7.37 (d, 1H, Ar-H). HPLC purity: 99.36%, HRMS (ESI) $C_{28}H_{35}N_2O_3$ calcd 447.2648 $[M+H]^+$, found 447.2637, mp: 270.7 °C.

4.1.41. 3-(4-(4-(3-Methoxybenzyl)piperazin-1-yl)butoxy)-7,8,9,10-tetrahydro-6*H*-benzo[*c*]chromen-6-one dihydrochloride (19c6)

Yield obtained 72.6%, 82.2%, respectively, for the reaction and HCl salt preparation. ^{1}H NMR (300 MHz, $CDCl_3$, ppm): δ 1.62–1.88 (m, 8H, —C—CH_2—CH_2—CH_2—CH_2—C— and —O—CH_2—CH_2—CH_2—CH_2—N—), 2.35–2.66 (m, 12H, —O—CH_2—CH_2—CH_2—CH_2—N—, piperazine protons and —CH_2—CH_2—C=C—), 2.67–2.78 (m, 2H, —CH_2—CH_2—C—COO—), 3.47 (s, 2H, N-CH_2—Ph), 3.76 (s, 3H, —O—CH_3), 3.97 (t, 2H, —O—CH_2—CH_2—), 6.71–6.79 (m, 3H, Ar-H), 6.82–6.88 (m, 2H, Ar-H), 7.18 (t, 1H, Ar-H), 7.39 (d, 1H, Ar-H). HPLC purity: 99.54%, HRMS (ESI) $C_{29}H_{37}N_2O_4$ calcd 477.2753 $[M+H]^+$, found 477.2762, mp: 243.4 °C.

4.1.42. 3-(2-(Benzyl(methyl)amino)ethoxy)-6*H*-benzo[*c*]chromen-6-one hydrochloride (20a1)

Yield obtained 79.2%, 83.2%, respectively, for the reaction and HCl salt preparation. ^{1}H NMR (300 MHz, $CDCl_3$, ppm): δ 2.35 (s, 3H, —N—CH_3), 2.84 (t, 2H, —N—CH_2—CH_2—O—), 3.62 (s, 2H, —N—CH_2—Ph), 4.12 (t, 2H, —N—CH_2—CH_2—O—), 6.81 (d, 1H, Ar-H), 6.85 (dd, 1H, Ar-H), 7.19–7.32 (m, 5H, Ar-H), 7.47 (t, 1H, Ar-H), 7.75 (dt, 1H, Ar-H), 7.84–7.98 (m, 2H, Ar-H), 8.32 (d, 1H, Ar-H). HPLC purity: 99.54%, HRMS (ESI) $C_{23}H_{22}NO_3$ calcd 360.1600 $[M+H]^+$, found 360.1613, mp: 221.7 °C.

4.1.43. 3-(2-((3-Methoxybenzyl)(methyl)amino)ethoxy)-6*H*-benzo[*c*]chromen-6-one hydrochloride (20a2)

Yield obtained 74.4%, 85.1%, respectively, for the reaction and HCl salt preparation. ^{1}H NMR (300 MHz, CD_3OD, ppm): δ 2.96 (s, 3H, —N—CH_3), 3.63 (br s, 2H, —N—CH_2—CH_2—O—), 3.78 (s, 3H, —O—CH_3), 4.34–4.54 (m, 4H, —N—CH_2—Ph and —N—CH_2—CH_2—O—), 6.88–7.17 (m, 5H, Ar-H), 7.37 (t, 1H, Ar-H), 7.53 (t, 1H, Ar-H) 7.81 (t, 1H, Ar-H), 7.99–8.14 (m, 2H, Ar-H), 8.21 (d, 1H, Ar-H). HPLC purity: 99.40%, HRMS (ESI) $C_{24}H_{24}NO_4$ calcd 390.1705 $[M+H]^+$, found 390.1709, mp: 205.9 °C.

4.1.44. 3-(2-(4-Benzylpiperidin-1-yl)ethoxy)-6*H*-benzo[*c*]chromen-6-one hydrochloride (20a3)

Yield obtained 70.1%, 88.6%, respectively, for the reaction and HCl salt preparation. ^{1}H NMR (300 MHz, $CDCl_3$, ppm): δ 1.16–1.40 (m, 2H, —O—CH_2—CH_2—N—CH_2—$CH_{2(\mathbf{ax})}$—CH—), 1.42–1.56 (m, 1H, —O—CH_2—CH_2—N—CH_2—CH_2—CH—CH_2—CH_2—), 1.57–1.70 (m, 2H, —O—CH_2—CH_2—N—CH_2—$CH_{2(\mathbf{eq})}$—CH—), 2.03 (t, 2H, —O—CH_2—CH_2—N—$CH_{2(\mathbf{ax})}$—), 2.50 (d, 2H, —O—CH_2—CH_2—N—$CH_{2(\mathbf{eq})}$—), 2.78 (t, 2H, —N—CH_2—CH_2—O—), 2.96 (d, 2H, —CH—CH_2—Ph), 4.12 (t, 2H, —N—CH_2—CH_2—O—), 6.74–6.91 (m, 2H, Ar-H), 7.03–7.28 (m, 5H, Ar-H), 7.46 (t, 1H, Ar-H), 7.73 (dt, 1H, Ar-H), 7.82–7.99 (m, 2H, Ar-H), 8.31 (d, 1H, Ar-H). HPLC purity: 99.37%, HRMS (ESI) $C_{27}H_{28}NO_3$ calcd 414.2069 $[M+H]^+$, found 414.2075, mp: 199.6 °C.

4.1.45. 3-(2-(4-(3-Methoxybenzyl)piperidin-1-yl)ethoxy)-6*H*-benzo[*c*]chromen-6-one hydrochloride (20a4)

Yield obtained 70.1%, 88.6%, respectively, for the reaction and HCl salt preparation. ^{1}H NMR (300 MHz, $CDCl_3$, ppm): δ 1.29–1.95 (m, 7H, —O—CH_2—CH_2—N—CH_2—CH_2—CH—, —N—CH_2—CH_2—CH—CH_2—CH_2— and —O—CH_2—CH_2—N—$CH_{2(\mathbf{ax})}$—), 2.53 (d, 2H, —O—CH_2—CH_2—N—$CH_{2(\mathbf{eq})}$—), 3.13–3.33 (m, 2H, - N-CH_2—CH_2—O—), 3.38–3.56 (m, 2H, —CH—CH_2—Ph), 3.73 (s, 3H, —O—CH_3), 4.15 (t, 2H, —N—CH_2—CH_2—O—), 6.58–6.72 (m, 3H, Ar-H), 6.78 (d, 1H, Ar-H), 6.86 (dd, 1H, Ar-H), 7.14 (t, 1H, Ar-H), 7.47 (d, 1H, Ar-H), 7.74 (dt, 1H, Ar-H), 7.86–7.98 (m, 2H, Ar-H), 8.30 (dd, 1H, Ar-H). HPLC purity: 99.49%, HRMS (ESI) $C_{28}H_{30}NO_4$ calcd 444.2175 $[M+H]^+$, found 444.2171, mp: 174.3 °C.

4.1.46. 3-(2-(4-Benzylpiperazin-1-yl)ethoxy)-6*H*-benzo[*c*]chromen-6-one dihydrochloride (20a5)

Yield obtained 71.8%, 90.8%, respectively, for the reaction and HCl salt preparation. ^{1}H NMR (300 MHz, $CDCl_3$, ppm): δ 2.35–2.71 (m, 8H, piperazine protons), 2.80 (t, 2H, —N—CH_2—CH_2—O—), 3.47 (s, 2H, —N—CH_2—Ph), 4.10 (t, 2H, —N—CH_2—CH_2—O—), 6.79 (d, 1H, Ar-H), 6.85 (dd, 1H, Ar-H), 7.16–7.29 (m, 5H, Ar-H), 7.44 (dt, 1H, Ar-H), 7.72 (dt, 1H, Ar-H), 7.87 (d, 1H, Ar-H), 7.93 (d, 1H, Ar-H), 8.29 (dd, 1H, Ar-H). HPLC purity: 99.97%, HRMS (ESI) $C_{26}H_{27}N_2O_3$ calcd 415.2022 $[M+H]^+$, found 415.2039, mp: 176.6 °C.

4.1.47. 3-(2-(4-(3-Methoxybenzyl)piperazin-1-yl)ethoxy)-6*H*-benzo[*c*]chromen-6-one dihydrochloride (20a6)

Yield obtained 75.6%, 88.2%, respectively, for the reaction and HCl salt preparation. ^{1}H NMR (300 MHz, $CDCl_3$, ppm): δ 2.36–2.73 (m, 8H, piperazine protons), 2.80 (t, 2H, —N—CH_2—CH_2—O—), 3.44 (s, 2H, —N—CH_2—Ph), 3.74 (s, 3H, —O—CH_3), 4.10 (t, 2H, —N—CH_2—CH_2—O—), 6.69–6.89 (m, 5H, Ar-H), 7.16 (t, 1H, Ar-H), 7.44 (dt, 1H, Ar-H), 7.72 (dt, 1H, Ar-H), 7.87 (d, 1H, Ar-H), 7.93 (d, 1H, Ar-H), 8.29 (dd, 1H, Ar-H). HPLC purity: 99.62%, HRMS (ESI) $C_{27}H_{29}N_2O_4$ calcd 445.2127 $[M+H]^+$, found 445.2143, mp: 282.3 °C.

4.1.48. 3-(3-(Benzyl(methyl)amino)propoxy)-6*H*-benzo[*c*]chromen-6-one hydrochloride (20b1)

Yield obtained 75.2%, 80.8%, respectively, for the reaction and HCl salt preparation. ^{1}H NMR (300 MHz, $CDCl_3$, ppm): δ 1.97 (p, 2H, —O—CH_2—CH_2—CH_2—N—), 2.19 (s, 3H, —N—CH_3), 2.52 (t, 2H, —O—CH_2—CH_2—CH_2—N—), 3.47 (s, 2H, —N—CH_2—Ph), 4.03 (t, 2H, —O—CH_2—CH_2—CH_2—N—), 6.75–6.85 (m, 2H, Ar-H), 7.17–7.25 (m, 5H, Ar-H), 7.44 (dt, 1H, Ar-H), 7.73 (dt, 1H, Ar-H), 7.87 (d, 1H, Ar-H), 7.95 (d, 1H, Ar-H), 8.30 (dd, 1H, Ar-H). HPLC purity: 99.92%, HRMS (ESI) $C_{24}H_{24}NO_3$ calcd 374.1756 $[M+H]^+$, found 374.1750, mp: 186.9 °C.

4.1.49. 3-(3-((3-Methoxybenzyl)(methyl)amino)propoxy)-6*H*-benzo[*c*]chromen-6-one hydrochloride (20b2)

Yield obtained 75.8%, 80.3%, respectively, for the reaction and HCl salt preparation. ^{1}H NMR (300 MHz, $CDCl_3$, ppm): δ 1.96 (p, 2H, —O—CH_2—CH_2—CH_2—N—), 2.20 (s, 3H, —N—CH_3), 2.51 (t, 2H, —O—CH_2—CH_2—CH_2—N—), 3.44 (s, 2H, —N—CH_2—Ph), 3.71 (s, 3H, —O—CH_3), 4.02 (t, 2H, —O—CH_2—CH_2—CH_2—N—), 6.67–6.87 (m, 5H, Ar-H), 7.14 (t, 1H, Ar-H), 7.43 (dt, 1H, Ar-H), 7.72 (dt, 1H, Ar-H), 7.85 (d, 1H, Ar-H), 7.94 (d, 1H, Ar-H), 8.29 (dd, 1H, Ar-H). HPLC purity: 99.95%, HRMS (ESI) $C_{25}H_{26}NO_4$ calcd 404.1862 $[M+H]^+$, found 404.1856, mp: 215.1 °C.

4.1.50. 3-(3-(4-Benzylpiperidin-1-yl)propoxy)-6*H*-benzo[*c*]chromen-6-one hydrochloride (20b3)

Yield obtained 70.0%, 88.8%, respectively, for the reaction and HCl salt preparation. ^{1}H NMR (300 MHz, $CDCl_3$, ppm): δ 1.30–1.75 (m, 7H, —O—CH_2—CH_2—CH_2—N—CH_2—CH_2—CH—,—N—CH_2—CH_2—CH—CH_2—CH_2— and —O—CH_2—CH_2—CH_2—N—$CH_{2(\mathbf{ax})}$—), 2.10–2.23 (m, 2H, —O—CH_2—CH_2—CH_2—N—), 2.55 (d, 2H, —O—CH_2—CH_2—CH_2—N—$CH_{2(\mathbf{eq})}$—), 2.75–3.06 (m, 2H, —O—CH_2—CH_2—CH_2—N—), 3.19–3.44 (m, 2H, —N—CH_2—Ph), 4.06 (t, 2H, —O—CH_2—CH_2—CH_2—N—), 6.77 (d, 1H, Ar-H), 6.82 (dd, 1H, Ar-H), 7.12–7.26 (m, 5H, Ar-H), 7.46 (m, 1H, Ar-H), 7.73 (dt, 1H, Ar-H), 7.89 (d, 1H, Ar-H), 7.94 (d, 1H, Ar-H), 8.3 (dd, 1H, Ar-H). HPLC purity: 99.09%, HRMS (ESI) $C_{28}H_{30}NO_3$ calcd 428.2226 $[M+H]^+$, found 428.2212, mp: 232.4 °C.

4.1.51. 3-(3-(4-(3-Methoxybenzyl)piperidin-1-yl)propoxy)-6*H*-benzo[*c*]chromen-6-one hydrochloride (20b4)

Yield obtained 76.5%, 80.4%, respectively, for the reaction and HCl salt preparation. ^{1}H NMR (300 MHz, $CDCl_3$, ppm): δ 1.20–1.35 (m, 2H, —O—CH_2—CH_2—CH_2—N—CH_2—$CH_{2(ax)}$—CH—), 1.41–1.53 (m, 1H, —N—CH_2—CH_2—*CH*—CH_2—CH_2—), 1.54–1.66 (m, 2H, —O—CH_2—CH_2—CH_2—N—CH_2—$CH_{2(eq)}$—CH—), 1.85 (t, 2H, —O—CH_2—CH_2—CH_2—N—$CH_{2(ax)}$—), 1.95 (p, 2H, —O—CH_2—CH_2—CH_2—N—), 2.37–2.53 (m, 4H, —O—CH_2—CH_2—CH_2—N—$CH_{2(eq)}$— and —O—CH_2—CH_2—CH_2—N—), 2.88 (d, 2H, —N—CH_2—Ph), 3.74 (s, 3H, —O—CH_3), 4.01 (t, 2H, —O—CH_2—CH_2—CH_2—N—), 6.59–6.73 (m, 3H, Ar-H), 6.77–6.88 (m, 2H, Ar-H), 7.14 (t, 1H, Ar-H), 7.44 (dt, 1H, Ar-H), 7.72 (dt, 1H, Ar-H), 7.87 (d, 1H, Ar-H), 7.94 (d, 1H, Ar-H), 8.29 (dd, 1H, Ar-H). HPLC purity: 99.19%, HRMS (ESI) $C_{29}H_{32}NO_4$ calcd 458.2331 $[M+H]^+$, found 458.2317, mp: 225.8 °C.

4.1.52. 3-(3-(4-Benzylpiperazin-1-yl)propoxy)-6*H*-benzo[*c*]chromen-6-one dihydrochloride (20b5)

Yield obtained 59.8%, 80.0%, respectively, for the reaction and HCl salt preparation. ^{1}H NMR (300 MHz, $CDCl_3$, ppm): δ 1.95 (p, 2H, —O—CH_2—CH_2—CH_2—N—), 2.43–2.68 (m, 10H, piperazine protons and —O—CH_2—CH_2—CH_2—N—), 3.46 (s, 2H, —N—CH_2—Ph), 4.01 (t, 2H, —O—CH_2—CH_2—CH_2—N—), 6.78 (d, 1H, Ar-H), 6.83 (dd, 1H, Ar-H), 7.14–7.29 (m, 5H, Ar-H), 7.43 (t, 1H, Ar-H), 7.71 (dt, 1H, Ar-H), 7.89 (d, 1H, Ar-H), 7.93 (d, 1H, Ar-H), 8.28 (dd, 1H, Ar-H). HPLC purity: 99.74%, HRMS (ESI) $C_{27}H_{29}N_2O_3$ calcd 429.2178 $[M+H]^+$, found 429.2171, mp: 283.8 °C.

4.1.53. 3-(3-(4-(3-Methoxybenzyl)piperazin-1-yl)propoxy)-6*H*-benzo[*c*]chromen-6-one dihydrochloride (20b6)

Yield obtained 76.8%, 81.6%, respectively, for the reaction and HCl salt preparation. ^{1}H NMR (300 MHz, $CDCl_3$, ppm): δ 1.88–2.03 (m, 2H, —O—CH_2—CH_2—CH_2—N—), 2.22–2.71 (m, 10H, piperazine protons and —O—CH_2—CH_2—CH_2—N—), 3.45 (s, 2H, —N—CH_2—Ph), 3.73 (s, 3H, —O—CH_3), 4.02 (t, 2H, —O—CH_2—CH_2—CH_2—N—), 6.69–6.89 (m, 5H, Ar-H), 7.16 (d, 1H, Ar-H), 7.44 (dt, 1H, Ar-H), 7.72 (dt, 1H, Ar-H), 7.87 (d, 1H, Ar-H), 7.94 (d, 1H, Ar-H), 8.29 (dd, 1H, Ar-H). HPLC purity: 99.52%, HRMS (ESI) $C_{28}H_{31}N_2O_4$ calcd 459.2284 $[M+H]^+$, found 459.2277, mp: 265.0 °C.

4.1.54. 3-(4-(Benzyl(methyl)amino)butoxy)-6*H*-benzo[*c*]chromen-6-one hydrochloride (20c1)

Yield obtained 78.3%, 85.6%, respectively, for the reaction and HCl salt preparation. ^{1}H NMR (300 MHz, $CDCl_3$, ppm): δ 1.66 (p, 2H, —O—CH_2—CH_2—CH_2—CH_2—N—), 1.80 (p, 2H, —O—CH_2—CH_2—CH_2—CH_2—N—), 2.16 (s, 3H, —N—CH_3), 2.39 (t, 2H, —O—CH_2—CH_2—CH_2—CH_2—N—), 3.45 (s, 2H, —N—CH_2—Ph), 3.94 (t, 2H, —O—CH_2—CH_2—CH_2—CH_2—N—), 6.76 (d, 1H, Ar-H), 6.82 (dd, 1H, Ar-H), 7.13–7.29 (m, 5H, Ar-H), 7.43 (dt, 1H, Ar-H), 7.71 (dt, 1H, Ar-H), 7.86 (d, 1H, Ar-H), 7.93 (d, 1H, Ar-H), 8.29 (dd, 1H, Ar-H). HPLC purity: 99.92%, HRMS (ESI) $C_{25}H_{26}NO_3$ calcd 388.1913 $[M+H]^+$, found 388.1911, mp: 169.2 °C.

4.1.55. 3-(4-((3-Methoxybenzyl)(methyl)amino)butoxy)-6*H*-benzo[*c*]chromen-6-one hydrochloride (20c2)

Yield obtained 80.3%, 86.1%, respectively, for the reaction and HCl salt preparation. ^{1}H NMR (300 MHz, $CDCl_3$, ppm): δ 1.64 (p, 2H, —O—CH_2—CH_2—CH_2—CH_2—N—), 1.80 (p, 2H, —O—CH_2—CH_2—CH_2—CH_2—N—), 2.16 (s, 3H, —N—CH_3), 2.38 (t, 2H, —O—CH_2—CH_2—CH_2—CH_2—N——N—), 3.42 (s, 2H, —N—CH_2—Ph), 3.73 (s, 3H, —O—CH_3), 3.94 (t, 2H, —O—CH_2—CH_2—CH_2—CH_2—N—), 6.67–6.87 (m, 5H, Ar-H), 7.15 (t, 1H, Ar-H), 7.42 (dt, 1H, Ar-H), 7.71 (dt, 1H, Ar-H), 7.85 (d, 1H, Ar-H), 7.92 (d, 1H, Ar-H), 8.28 (dd, 1H, Ar-H). HPLC purity: 99.89%, HRMS (ESI) $C_{26}H_{28}NO_4$ calcd 418.2018 $[M+H]^+$, found 418.2024, mp: 209.5 °C.

4.1.56. 3-(4-(4-Benzylpiperidin-1-yl)butoxy)-6*H*-benzo[*c*]chromen-6-one hydrochloride (20c3)

Yield obtained 68.9%, 90.8%, respectively, for the reaction and HCl salt preparation. ^{1}H NMR (300 MHz, $CDCl_3$, ppm): δ 1.21–1.39 (m, 2H, —N—CH_2—$CH_{2(ax)}$—CH—$CH_{2(ax)}$—CH_2—), 1.40–1.54 (m, 1H, —N—CH_2—CH_2—*CH*—CH_2—CH_2—), 1.55–1.86 (m, 8H, —O—CH_2—CH_2—CH_2—CH_2—N—, —N—CH_2—$CH_{2(eq)}$—CH—$CH_{2(eq)}$—CH_2— and —N—$CH_{2(ax)}$—CH_2—CH—CH_2), 2.35 (t, 2H, —O—CH_2—CH_2—CH_2—CH_2—N—), 2.48 (d, 2H, —N—$CH_{2(eq)}$—CH_2—CH—CH_2), 2.90 (d, 2H, —N—CH_2—Ph), 3.98 (t, 2H, —O—CH_2—CH_2—CH_2—CH_2—N—), 6.79 (d, 1H, Ar-H), 6.84 (dd, 1H, Ar-H), 7.03–7.26 (m, 5H, Ar-H), 7.44 (dt, 1H, Ar-H), 7.73 (dt, 1H, Ar-H), 7.88 (d, 1H, Ar-H), 7.94 (d, 1H, Ar-H), 8.30 (dd, 1H, Ar-H). HPLC purity: 99.26%, HRMS (ESI) $C_{29}H_{32}NO_3$ calcd 442.2382 $[M+H]^+$, found 442.2403, mp: 159.2 °C.

4.1.57. 3-(4-(4-(3-Methoxybenzyl)piperidin-1-yl)butoxy)-6*H*-benzo[*c*]chromen-6-one hydrochloride (20c4)

Yield obtained 75.4%, 82.2%, respectively, for the reaction and HCl salt preparation. ^{1}H NMR (300 MHz, $CDCl_3$, ppm): δ 1.20–1.38 (m, 2H, (m, 2H, —N—CH_2—$CH_{2(ax)}$—CH—$CH_{2(ax)}$—CH_2—), 1.39–1.54 (m, 1H, —N—CH_2—CH_2—*CH*—CH_2—CH_2—), 1.55–1.82 (m, 8H, —O—CH_2—CH_2—CH_2—CH_2—N—, —N—CH_2—$CH_{2(eq)}$—CH—$CH_{2(eq)}$—CH_2— and —N—$CH_{2(ax)}$—CH_2—CH—CH_2), 2.33 (t, 2H, —O—CH_2—CH_2—CH_2—CH_2—N—), 2.45 (d, 2H, —N—$CH_{2(eq)}$—CH_2—CH—CH_2), 2.88 (d, 2H, —N—CH_2—Ph), 3.73 (s, 3H, —O—CH_3), 3.98 (t, 2H, —O—CH_2—CH_2—CH_2—CH_2—N—), 6.57–6.73 (m, 3H, Ar-H), 6.75–6.88 (m, 2H, Ar-H), 7.07–7.17 (m, 1H, Ar-H), 7.45 (t, 1H, Ar-H), 7.72 (t, 1H, Ar-H), 7.82–7.99 (m, 2H, Ar-H), 8.29 (d, 1H, Ar-H). HPLC purity: 99.01%, HRMS (ESI) $C_{30}H_{34}NO_4$ calcd 472.2488 $[M+H]^+$, found 472.2490, mp: 177.8 °C.

4.1.58. 3-(4-(4-Benzylpiperazin-1-yl)butoxy)-6*H*-benzo[*c*]chromen-6-one dihydrochloride (20c5)

Yield obtained 68.3%, 84.3%, respectively, for the reaction and HCl salt preparation. ^{1}H NMR (300 MHz, $CDCl_3$, ppm): δ1.56–1.84 (m, 4H, —O—CH_2—CH_2—CH_2—CH_2—N—), 2.24–2.67 (m, 10H, piperazine protons and —O—CH_2—CH_2—CH_2—CH_2—N—), 3.46 (s, 2H, —N—CH_2—Ph), 3.97 (t, 2H, —O—CH_2—CH_2—CH_2—CH_2—N—), 6.78 (d, 1H, Ar-H), 6.83 (dd, 1H, Ar-H), 7.14–7.28 (m, 5H, Ar-H), 7.44 (dt, 1H, Ar-H), 7.72 (dt, 1H, Ar-H), 7.87 (d, 1H, Ar-H), 7.94 (d, 1H, Ar-H), 8.29 (dd, 1H, Ar-H). HPLC purity: 99.77%, HRMS (ESI) $C_{28}H_{31}N_2O_3$ calcd 443.2335 $[M+H]^+$, found 443.2350, mp: 163.6 °C.

4.1.59. 3-(4-(4-(3-Methoxybenzyl)piperazin-1-yl)butoxy)-6*H*-benzo[*c*]chromen-6-one dihydrochloride (20c6)

Yield obtained 75.2%, 78.4%, respectively, for the reaction and HCl salt preparation. ^{1}H NMR (300 MHz, $CDCl_3$, ppm): δ 1.63 (p, 2H, —O—CH_2—CH_2—CH_2—CH_2—N—), 1.77 (p, 2H, —O—CH_2—CH_2—CH_2—CH_2—N—), 2.28–2.65 (m, 10H, piperazine protons and —O—CH_2—CH_2—CH_2—CH_2—N—), 3.43 (s, 2H, —N—CH_2—Ph), 3.74 (s, 3H, —O—CH_3), 3.97 (t, 2H, —O—CH_2—CH_2—CH_2—CH_2—N—), 6.69–6.87 (m, 5H, Ar-H), 7.15 (t, 1H, Ar-H), 7.43 (dt, 1H, Ar-H), 7.72 (dt, 1H, Ar-H), 7.86 (d, 1H, Ar-H), 7.93 (d, 1H, Ar-H), 8.29 (dd, 1H, Ar-H). HPLC purity: 98.48%, HRMS (ESI) $C_{29}H_{33}N_2O_4$ calcd 473.2440 $[M+H]^+$, found 473.2448, mp: 251.2 °C.

4.2. Enzyme assays: inhibition of AChE and BuChE

AChE inhibitory activities of the test compounds were determined by the modified Ellmann's method.[28,29] Briefly, each reaction mixture (final volume of 200 μL) contained 168 μL of 50 mM of Tris HCl buffer (pH 8.0), 10 μL of 6.8 mM DTNB solution (i.e., 0.34 mM final) containing 20 mM $MgCl_2$ and 100 mM NaCl, 10 μL of ACHE solution (0.4 U/mL from Human recombinant AChE), and 2 μL of each sample solution. The reactions were initiated by the

addition of 10 μL of 10 mM acetylthiocholine iodide (i.e., 0.5 mM final). After incubation for 15 min at 27 °C, the enzyme catalyzed formation of the yellow color was measured at 412 nm using Biotech Synergy 2 Spectrophotometer. The non-enzymatic hydrolysis of acetylthiocholine iodide was also measured in enzyme-free assay systems, and the results were employed as blank (i.e., also referred to as blank in the calculation below). In control experiments, inhibitor-free assay systems were utilized to measure the full activity (i.e., also referred to as the control in the calculation below). In positive control experiments, rivastigmine, galanthamine, and donepezil were employed as the reference inhibitors. Each experiment was performed in triplicate, and the mean ± standard deviation was calculated. Absorbance values for each measurement were utilized in the percentage inhibition calculated through $[(\text{Acontrol} - \text{Ablank}) - (\text{Asample} - \text{Ablank})/(\text{Acontrol} - \text{Ablank})] \times 100$. The concentration of test compounds and reference materials which determined 50% inhibition of the AChE or BuChE activity (IC_{50}) was calculated using a sigmoidal hill slope model. BuChE inhibitory activities of the test compounds and the reference drugs were measured following the same methodology described for the AChE assays except employing 1.6 U/mL BuChE from human recombinant BuChE solution as the enzyme source and the butyrylthiocholine iodide (i.e., 1.5 mM final) as the substrate of the reaction. Each enzyme utilized hydrolyzed 1.0 μmol of the corresponding substrate under the experimental conditions.

4.3. Scopolamine induced passive avoidance test

Scopolamine induced passive avoidance test is one of the mostly applied methods to measure cognitive abilities in in vivo studies.[30] The experiments were conducted in a laboratory working according to the GLP guideliness. A two-compartment (i.e., the illuminated and dark compartments) model instrument was utilized. Male Wistar rats, at an age of approximately 2 months, and around 250 g body weight were employed. For learning, the rats were placed into the illuminated compartment while the door closed and allowed a habituation phase (30 s). Then the door automatically opened and stayed open for 5 min experimental time. Two seconds after the rat entering the dark compartment of the apparatus the door closed and after 2 s latency an electric stimulus took place lasting for 3 or 6 s. The rat stayed in the dark compartment for another 30 s delay before it was placed into the home cage.

10 rats for each test compound were employed. For testing, the rats were treated with 1 mg/kg (ip) of scopolamine, half an hour before the administration of the each test compound. One hour after the oral administration of the each compound (i.e., 1 mg/kg), the rats were placed into the illuminated compartment with the door closed again for a habituation time of 30 s. Following the opening of the door automatically, the time for the rats visiting the dark box (latency) was measured for 5 min. The time difference between the no scopolamine administered group and the only scopolamine administered group was taken as 100% and the effect of each compound of the present invention was calculated accordingly in terms of the percentage antagonism.

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