Rational Design, Synthesis, Biological Evaluation, Homology and Docking Studies of Coumarin Derivatives as α_1-Adrenoceptor Antagonists

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According to a three-point pharmacophore for some uro-selective α_1-adrenoceptor (AR) antagonists, a novel class of coumarin (=2*H*-1-benzopyran-2-one) derivatives have been successfully designed and synthesized with high efficacies for α_1-AR. These synthesized coumarin derivatives exhibited high efficacies towards α_1-AR in *in vitro* pharmacological assays. Compared with prazosin (pK_i value of 8.77), among those coumarins, tolylpiperazine-substituted derivatives, **7** and **8**, have comparable pK_i values of 8.81 and 8.77, respectively. The trend in efficacies of these coumarin derivatives towards α_{1A}-adrenoceptor was further rationalized by intensive molecular docking. Our work demonstrated that the designed coumarin derivatives can inhibit α_1-AR *in vitro*. These findings will provide a guide for further studies of the medical therapy of benign prostatic hyperplasia (BPH).

Introduction. – The α_1-adrenoceptors (α_1-ARs) regulate several functions of the sympathetic nervous system, whose divergent antagonists have primarily been developed for the treatment of cardiovascular diseases and benign prostatic hyperplasia (BPH) [1–5]. So far, drug therapy for BPH has been classified into two categories: 5α-reductase inhibitors acting by reducing the size of the prostate; α_1-AR antagonists acting by relaxing prostate muscle. The α_1-AR antagonists have an advantage over 5α-reductase inhibitors, since they can provide effective relief of symptoms in a short time. Analysis of a number of chemical structures of various newly synthesized α_1-AR antagonists [6–13] indicates that a large group of active compounds contain arylpiperazine moieties, such as prazosin [14][15], 5-methylurapidil, BMY-7378, REC-1512739, and RA36 (*Fig. 1*) [6]. So, to further develop α_1-AR antagonists for treating BPH, a rational strategy on the basis of prazosin was applied as depicted in *Scheme 1* with double objectives: *a*) to further clarify the structure–activity relationship with respect to the heterocyclic moiety presented in the upper part of the molecule and to explore the nature of the α_1-AR binding pocket, and *b*) to introduce a naturally occurring heterocycle, the coumarin backbone, which was perceived as a critical physicochemical characteristic to enhance the efficacy towards α_1-AR.

At the same time, the construction of a three-dimensional pharmacophore model of α_1-AR antagonists have been studied [11][12], which shared some characteristics: an aromatic ring (A); a positive ionizable center (P); and a H-bond donor (HBD); and the distances of A–P, A–HBD, and P–HBD are 5.296–5.477, 5.429–6.823, and 3.000 Å,

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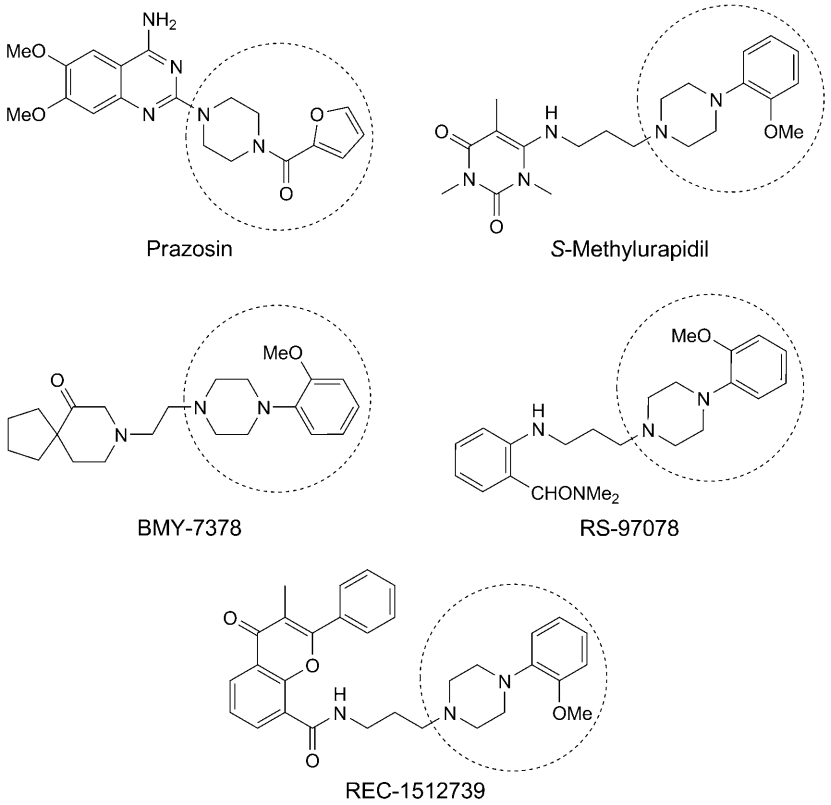
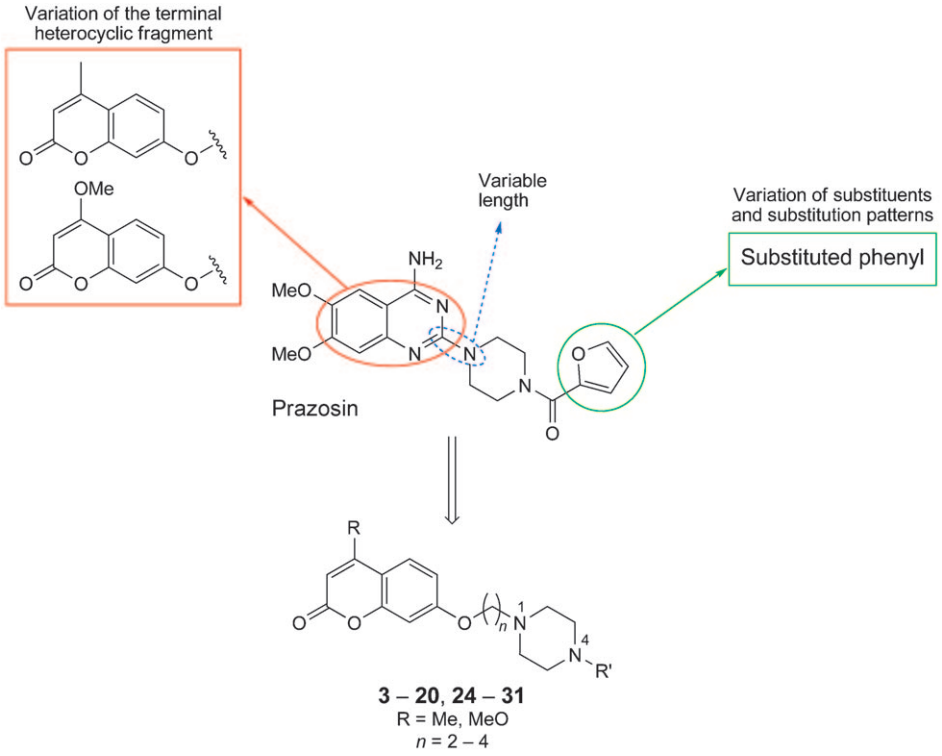


Fig. 1. *Structures of potent α_1-adrenoceptor antagonists*

respectively. Considering those factors, a series of new compounds on the basis of the piperazinyl-quinazoline molecular scaffold were designed and synthesized. The piperazinyl-quinazoline moiety in prazosin was replaced by a coumarin ring, a naturally occurring phytochemical with a wide range of biological activities, such as anti-inflammatory, antitumor [16–18], anti-allergic, and anti-HIV-1 properties [19][20]. Furthermore, in the designed coumarin derivatives, the following chemical modifications were also considered: *i*) some groups such as Cl, MeO, or Me were chosen as the substituents on the Ph ring linked to the piperazine nucleus; *ii*) the alkanediyl chain acting as a spacer contained 2–4 C-atoms. The effect of substitutions in the *o*-, *m*-, and *p*-positions of the Ph group bound to the piperazine ring was also considered. The target coumarin derivatives **3**–**20** and **24**–**31** were synthesized as depicted in *Scheme 1*.

Here, the design, preparation, and *in vitro* pharmacological characterization of a series of coumarin derivatives are reported. Moreover, recent studies had demonstrated that the α_{1A}-subtype was the predominantly expressed α_1-AR in human prostate and in the lower urinary tract (LUT). So, to further study the α_1-ARs antagonists to treat BPH, the docking behavior of the synthesized compounds has been studied based on α_{1A}-AR. Owing to the unavailable structure of the human α_{1A}-AR, the homology

Scheme 1. *Design Strategy for the Target Compounds*

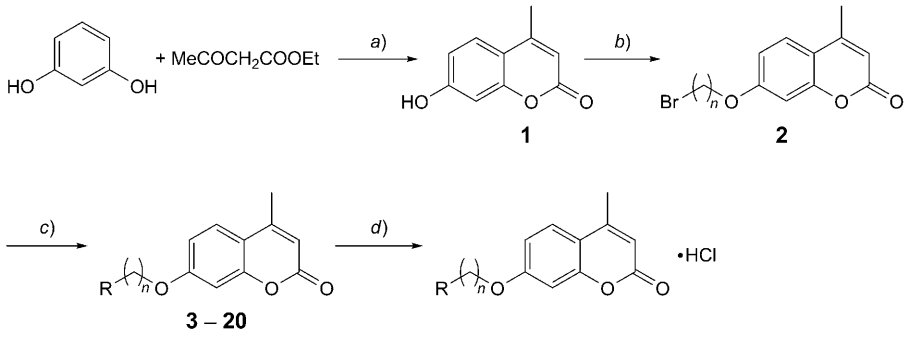


models of α_{1A}-AR was constructed. Based on these models, the intensive molecular docking was performed. The interaction mode of the designed coumarin derivatives with α_{1A}-AR was also investigated.

Results and Discussion. – 1. *Chemistry.* Several possible chemical variations were considered on the basis of piperazine derivatives. The synthesis of the target compounds is shown in *Schemes 2* and *3* (and *Tables 1* and *2*). The key intermediate, compound **1**, was prepared starting from resorcinol and ethyl acetoacetate with concentrated H_2SO_4 as the catalyst and 1,4-dioxane as solvent. Then, **1** was reacted with $Br(CH_2)_nBr$ to give the key intermediates **2**. Compounds **2** were reacted with correspondingly substituted phenylpiperazines to give the target compounds **3**–**20**. The target compounds could be transformed with HCl in MeOH into the hydrochlorides (*Scheme 2*).

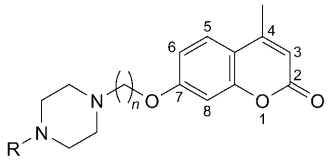
The second series of compounds were prepared by the reaction of resorcinol with malonic acid to give the intermediate compound **21**, which was methylated with MeOH and concentrated H_2SO_4 to afford **22**. Compound **22** was reacted with $Br(CH_2)_nBr$ and K_2CO_3 to give the key intermediates **23**. Then, compounds **23** were reacted with correspondingly substituted phenylpiperazines to give the target compounds **24**–**31**.

Scheme 2. *Synthesis of Compounds* **3**–**20**[1])



a) H_2SO_4, 60°. *b*) $Br(CH_2)_nBr$, K_2CO_3, reflux. *c*) RH, K_2CO_3, reflux. *d*) MeOH, HCl, reflux.

Table 1. *Structures of Compounds* **3**–**20**



Compound	*n*	R	Compound	*n*	R
3	2	Ph	**12**	2	4-MeO–C_6H_4–CO
4	2	2-MeO–C_6H_4	**13**	2	2,5-Cl_2–C_6H_3
5	2	4-MeO–C_6H_4	**14**	4	4-Me–C_6H_4
6	2	4-Cl–C_6H_4	**15**	4	2-Me–C_6H_4
7	2	4-Me–C_6H_4	**16**	4	4-MeO–C_6H_4
8	2	2-Me–C_6H_4	**17**	4	2-MeO–C_6H_4
9	2	3-MeO–C_6H_4	**18**	3	4-Me–C_6H_4
10	2	4,6-Dimethoxypyrimidin-2-yl	**19**	3	2-Me–C_6H_4
11	2	4-Me–C_6H_4–CO	**20**	3	4-MeO–C_6H_4

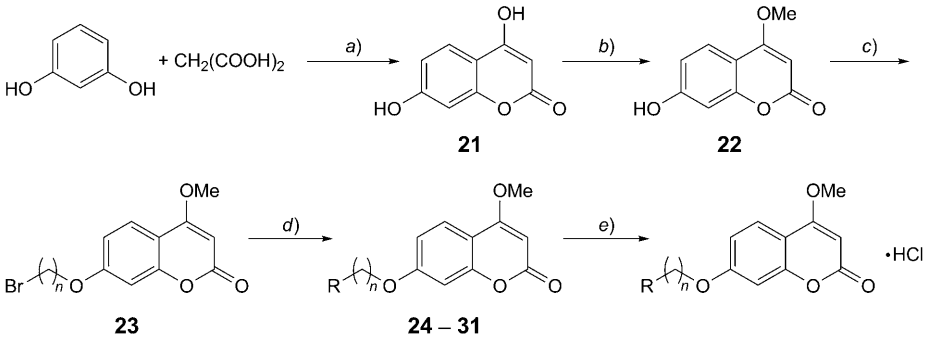
The target compounds could be transformed with HCl in MeOH into the hydrochlorides (*Scheme 3*).

The structures of all the new compounds were characterized by analytical and spectroscopic methods (^{1}H- and ^{13}C-NMR; *cf.* the *Exper. Part*). The molecular weights of all compounds synthesized were confirmed by ESI-MS, and their purity was also determined by HPLC analysis.

2. In vitro *Activities.* Antagonist efficacies were expressed as apparent pK_i values calculated from the following equation: $pK_i = -\log[B] + \log(r-1)$, where [B] is the molar concentration of antagonists, and *r* is the ratio of agonist EC_{50} determined in the presence and absence of antagonist [21]. The α_1-AR antagonist efficacies of (arylpiperazinyl)alkyl-coumarin derivatives **3**–**20** and **24**–**31**, expressed as pK_i values,

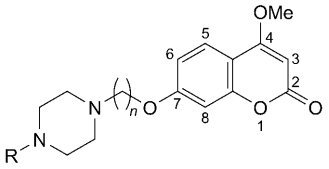
[1]) For the structure of compounds **3**–**20**, see *Table 1*.

Scheme 3. *Synthesis of Compounds* **24**–**31**[2])



a) $ZnCl_2$, $POCl_3$. *b*) MeOH, H_2SO_4. *c*) $Br(CH_2)_nBr$, K_2CO_3, reflux. *d*) RH, K_2CO_3, reflux. *e*) MeOH, HCl, reflux.

Table 2. *Structures of Compounds* **24**–**31**



Compound	*n*	R	Compound	*n*	R
24	2	Ph	**28**	2	2-MeO–C_6H_4
25	2	4-Me–C_6H_4	**29**	2	4-Cl–C_6H_4
26	2	2-Me–C_6H_4	**30**	3	4-Cl–C_6H_4
27	2	4-MeO–C_6H_4	**31**	4	4-Cl–C_6H_4

have been determined *in vitro* and compiled in *Table 3*. All these newly designed coumarin derivatives exhibit high efficacies (*Table 3*) towards α_1-AR compared with prazosin. Different substituents on the phenyl group linked to the piperazine nucleus led to diverse binding potencies. For example, 2,5-dichlorophenyl-substituted derivative **13**, and monochlorophenyl-substituted analogs **6**, **29**, **30**, and **31** exhibited moderate efficacies compared to the Me- and MeO-substituted ones. The efficacies of the compounds that bear propane-1,3-diyl or butane-1,4-diyl spacers were weaker than those with a ethane-1,2-diyl spacer (compare **14** and **18** to **7**, **15** and **19** to **8**, and **20** and **16** to **5**), indicating that the increase in the length of the alkanediyl chain lowered the activity. Moreover, the *p*-substituents on the phenylpiperazine moiety were found to be more relevant for activity. Particularly, in our study, compounds **7** and **8** with Me groups on the benzene ring are the most potent examples of this series with pK_i values of 8.81 and 8.77, respectively.

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[2]) For the structure of compounds **24**–**31**, see *Table 2*.

Table 3. *Apparent* pK_i *Values of the Synthesized Compounds*[a])

Compounds	*N*	pK_i	Compounds	*N*	pK_i
3	3	7.88 ± 0.12	**16**	3	6.61 ± 0.11
4	3	6.86 ± 0.21	**17**	3	8.14 ± 0.15
5	3	8.34 ± 0.11	**18**	3	6.76 ± 0.19
6	3	7.14 ± 0.17	**19**	3	7.31 ± 0.11
7	3	8.81 ± 0.13	**20**	3	6.65 ± 0.11
8	3	8.77 ± 0.09	**24**	3	6.91 ± 0.14
9	3	7.39 ± 0.15	**25**	3	6.78 ± 0.15
10	3	7.47 ± 0.18	**26**	3	7.88 ± 0.13
11	3	7.47 ± 0.20	**27**	3	7.12 ± 0.17
12	3	7.06 ± 0.14	**28**	3	7.78 ± 0.09
13	3	6.34 ± 0.13	**29**	3	7.33 ± 0.15
14	3	7.78 ± 0.18	**30**	3	6.88 ± 0.17
15	3	7.35 ± 0.16	**31**	3	7.30 ± 0.18
Prazosin[b])	4	8.79 ± 0.07			

[a]) Data are means ± standard deviation of *N* independent experiments. [b]) Prazosin was used as positive control.

3. *The Homology and Docking Studies.* By using the homology models of α_{1A}-AR built, a pharmacophore (*Fig. 2*, *a*) was generated with LIGANDSCOUT 2.0 (*Inte:ligand*, Vienna), based on the hypothetic binding mode between the receptor and compound **7**. This pharmacophore displayed that compound **7** mainly had five features including four hydrophobic regions and one H-bond interaction. The C=O group on the coumarin ring acted as an H-bond acceptor, which occurred also in other potent compounds (such as **5**, **8**, and **17**) and interacted with N–H group on the backbone of Phe289. The residues Phe308, Leu290, Val291, Ile178, and Val185 shaped a hydrophobic pocket which can accommodate the 4-Me group and the benzo moiety of the coumarin moiety. The 4-MeO-coumarin derivatives exhibited limited reduction in receptor-binding potency compared with the 4-Me-coumarin derivatives, revealing that small substituents at C(4) of coumarin ring might favor more potent binding capacity. This is in agreement with the result that the 4-Me group fits the hydrophobic feature better than the 4-MeO group (compare **26** to **8**, **25** to **7**, and **28** to **4**). *Fig. 2*, *b*, revealed that the spatial resistance between the MeO group and residues Val291 and Leu290 could be the reason for the lower efficacy of the 4-MeO-substituted derivatives (*i.e.*, **24**–**31**). For the phenylpiperazine moiety, the phenyl group and the 4-Me group occupied the narrow hydrophobic groove formed by the residues Val282, Leu197, Phe193, and Phe281 (*Fig. 2*, *b*). Decreases in efficacies (compare **5** and **6** to **7**, **16** to **15**, and **20** to **18**) were attributed to the Cl and MeO substituents. Whereas the spatial resistance resulted in low efficacies for MeO substituents, the presence of the Cl substituents weakened the efficacies due to its source of binding energy: *a*) lipophilic interactions; *b*) a positive electrostatic potential at the tip of the Cl-atom, which was not suitable here, although it had fairly similar size and hydrophobicity as the Me group. The *p*-substituents were sensitive to the length of the alkanediyl chain (compare **7** to **14**, **8** to **15**), and *p*-substitution turned out to be crucial for the efficacy. The piperazine N-

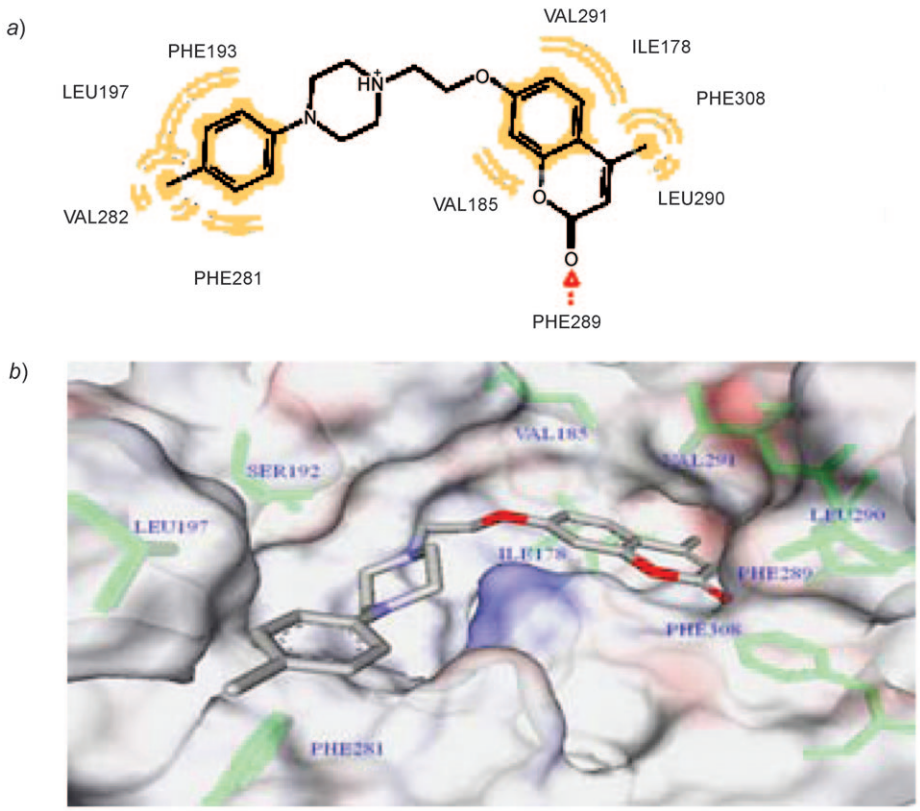

Fig. 2. *a*) *The pharmacophore based on the hypothetic binding mode of compound* **7** *with five features: one H-bond acceptor* (deep red arrow) *and four hydrophobic group*s (yellow). *b*) *The surface model for* **7** *bound to the active site of* α_{1A}*-AR, of which the active site fragment are colored according to the electrostatic potentials, and the active-site amino acid residues* (green) *of compound* **7** (gray) *shown as stick models.*

atom, which was considered as a positive ion center, displayed a weak H-bond interaction with Ser192.

Conclusions. – A novel class of coumarin derivatives with high efficacies for α_1-AR have been successfully designed and synthesized. These coumarin derivatives inhibited α_1-AR *in vitro*. All of these compounds shifted the concentration–response curves for phenylephrine in parallel without diminishing the maximum contraction, which suggested reversible antagonistic effects on α_1-AR, particularly when the substituent of phenyl ring was in *o*-position. Therefore, it can be concluded that coumarin with a phenylpiparazinyl substitution may show favorable anti-α_1-receptor activities. At the same time, the interaction mode of the designed coumarin derivatives with the α_{1A}-AR was investigated.

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Experimental Part

General. Chemicals: *Shanghai Chemical Reagent Company.* Column chromatography (CC): silica gel *60* (SiO_2, 200–300 mesh; *Qingdao Ocean Chemical Company*, P. R. China). TLC: *60 F254* silica-gel plates (250 μm, *Qingdao Ocean Chemical Company*, P. R. China). HPLC: *Agilent 1100 LC*. M.p.: *RY-1* melting-point tester; in cap. tube; uncorrected. IR Spectra: *Shimadzu FTIR-8400S* spectrometer; $\tilde{\nu}$ in cm^{-1}. ^{1}H- and ^{13}C-NMR spectra: *Bruker ACF-300* and *−500* NMR instruments; δ in ppm, J in Hz. LR-MS: *Hewlett-Packard 1100 LC/MSD* spectrometer.

*7-Hydroxy-4-methyl-2*H-*chromen-2-one* (**1**). Conc. H_2SO_4 (2 ml) was dropped slowly into 0.05 mol of resorcinol dissolved in dioxane (10 ml). The mixture was kept under 25°. After the addition of H_2SO_4, ethyl acetoacetate (=ethyl 3-oxobutanoate; 7 ml) was added dropwise within 20 min. Then, the mixture was heated to 60° and refluxed for 4 h. The mixture was then poured into H_2O (300 ml), and the precipitate was filtered off. The residue was purified by CC (cyclohexane/AcOEt 7:3) to give **1** (2.2 g, 70%).

*7-(2-Bromoethoxy)-4-methyl-2*H-*chromen-2-one* (**2a**). A mixture of **1** (0.005 mol), $BrCH_2CH_2Br$ (0.04 mol) and K_2CO_3 (1.0 g, 0.01 mol) in dry EtOH was refluxed for 24 h. The mixture was filtered, and the org. phase was evaporated under reduced pressure. The residue was purified by CC (cyclohexane/AcOEt 8:2) to give **2a** (55%). White crystals.

*7-(3-Bromopropoxy)-4-methyl-2*H-*chromen-2-one* (**2b**) and *7-(4-bromobutoxy)-4-methyl-2*H-*chromen-2-one* (**2c**) were prepared according to the same method using **1** and 1,3-dibromopropane or 1,4-dibromobutane, resp.

General Procedure for the Preparation of Compounds **3**–**20**. To a mixture of acetone and EtOH (15 ml each) were added 0.01 mol of **2**, 0.01 mol of substituted piperazine derivative, and 0.02 mol K_2CO_3. The mixture was refluxed for 48 h and then evaporated. The flash chromatography (FC) was performed to afford the target compound.

*4-Methyl-7-[2-(4-phenylpiperazin-1-yl)ethoxy]-2*H-*chromen-2-one* (**3**). Yield 41%. Colorless powder. M.p. 154–156°. HPLC 96.7% (MeOH/H_2O 75:25 (*v/v*); t_R 13.78 min). IR (KBr): 3546, 1699, 1618, 1386, 1299, 1147, 1070, 692. ^{1}H-NMR ($CDCl_3$, 300 MHz): 7.51 (*d*, J=8.8, H–C(5)); 6.90–6.98 (*m*, 5 arom. H); 7.27–7.30 (*m*, H–C(6), H–C(8)); 6.15 (*s*, H–C(3)); 4.27–4.29 (*m*, CH_2O); 3.26–3.29 (*m*, 2 CH_2N(4)); 2.78–2.80 (*m*, 2 CH_2N(1)); 2.94–2.97 (*m*, N(1)–CH_2CH_2O); 2.38 (*s*, Me–C(4)). ESI-MS: 365.0 ($[M+H]^+$).

*7-{2-[4-(2-Methoxyphenyl)piperazin-1-yl]ethoxy}-4-methyl-2*H-*chromen-2-one* (**4**). Yield 45%. Colorless powder. M.p. 156–157°. HPLC 96.3% (MeOH/H_2O 75:25 (*v/v*); t_R 14.58 min). IR (KBr): 3529, 1697, 1618, 1510, 1386, 1070, 823. ^{1}H-NMR ($CDCl_3$, 300 MHz): 7.51 (*d*, J=8.8, H–C(5)); 6.84–7.26 (*m*, 4 arom. H, H–C(6), H–C(8)); 6.14 (*s*, H–C(3)); 4.24–4.28 (*m*, CH_2O); 3.16–3.19 (*m*, 2 CH_2N(4)); 2.85–2.88 (*m*, 2 CH_2N(1)); 2.96–2.99 (*m*, N(1)–CH_2CH_2O); 2.38 (*s*, Me); 3.87 (*s*, MeO). ^{13}C-NMR ($CDCl_3$, 125 MHz): 161.8; 161.3; 155.3; 152.5; 152.3; 141.2; 125.5; 123.0; 121.0; 118.2; 113.7; 112.7; 112.0; 111.3; 101.6; 66.5; 56.9; 55.3; 53.9; 50.5; 18.6. ESI-MS: 395.3 ($[M+H]^+$).

*7-{2-[4-(4-Methoxyphenyl)piperazin-1-yl]ethoxy}-4-methyl-2*H-*chromen-2-one* (**5**). Yield 45%. Colorless powder. M.p. 156–157°. HPLC 98.7% (MeOH/H_2O 80:20 (*v/v*); t_R 6.35 min). IR (KBr): 2943, 1719, 1608, 1511, 1450, 1389, 1269, 1030, 710. ^{1}H-NMR ($CDCl_3$, 500 MHz): 7.51 (*d*, J=8.8, H–C(5)); 6.84–6.92 (*m*, 4 arom. H, H–C(6), H–C(8)); 6.14 (*s*, H–C(3)); 4.19–4.22 (*m*, CH_2O); 3.11–3.15 (*m*, 2 CH_2N(4)); 2.75–2.78 (*m*, 2 CH_2N(1)); 2.90–2.93 (*m*, N(1)–CH_2CH_2O); 2.39 (*s*, Me); 3.76 (*s*, MeO). ^{13}C-NMR ($CDCl_3$, 125 MHz): 161.8; 161.2; 155.3; 153.9; 152.5; 125.5; 118.3; 145.6; 114.5; 113.7; 112.7; 112.1; 101.6; 66.5; 56.9; 55.5; 53.8; 50.5; 18.6. ESI-MS: 395.2 ($[M+H]^+$).

*7-{2-[4-(4-Chlorophenyl)piperazin-1-yl]ethoxy}-4-methyl-2*H-*chromen-2-one* (**6**). Yield 35%. Colorless powder. M.p. 125–127°. HPLC 98.3% (MeOH/H_2O 80:20 (*v/v*); t_R 5.65 min). IR (KBr): 3452, 1726, 1498, 1392, 1284, 1234, 1141, 673. ^{1}H-NMR ($CDCl_3$, 500 MHz): 7.50 (*d*, J=8.8, H–C(5)); 7.20–7.23 (*m*, H–C(6), H–C(8)); 6.82–6.90 (*m*, 4 arom. H); 6.13 (*s*, H–C(3)); 4.21 (*t*, J=5.7, CH_2O); 3.17–3.20 (*m*,

2 $CH_2N(4)$); 2.74–2.77 (*m*, 2 $CH_2N(1)$); 2.91 (*t*, *J* = 5.7, N(1)–CH_2CH_2O); 2.39 (*s*, Me). ^{13}C-NMR ($CDCl_3$, 125 MHz): 161.3; 155.4; 152.5; 141.2; 125.7; 125.5; 18.7; 117.5; 114.7; 112.6; 112.3; 101.8; 66.5; 56.9; 53.5; 48.9. ESI-MS: 399.2 ($[M+H]^+$).

*4-Methyl-7-{2-[4-(4-methylphenyl)piperazin-1-yl]ethoxy}-2*H*-chromen-2-one* (**7**). Yield 42%. Colorless powder. M.p. 141–143°. HPLC 97.6% (MeOH/H_2O 80:20 (*v*/*v*); t_R 4.75 min). IR (KBr): 3530, 1708, 1620, 1514, 1451, 1387, 1304, 1008, 529. ^{1}H-NMR ($CDCl_3$, 500 MHz): 7.49 (*d*, *J* = 8.8, H–C(5)); 7.06–7.08 (*m*, H–C(6), H–C(8)); 6.83–6.89 (*m*, 4 arom. H); 6.13 (*s*, H–C(3)); 4.20 (*t*, CH_2O, *J* = 5.7); 3.16–3.19 (*m*, 2 $CH_2N(4)$); 2.74–2.77 (*m*, 2 $CH_2N(1)$); 2.91 (*t*, *J* = 5.7, N(1)–CH_2CH_2O); 2.39 (*s*, Me); 2.67 (*s*, Me). ^{13}C-NMR ($CDCl_3$, 125 MHz): 161.8; 161.3; 155.3; 152.5; 149.2; 129.7; 125.5; 116.5; 113.7; 112.7; 112.1; 101.6; 66.6; 56.9; 53.7; 49.6; 20.4; 18.6. ESI-MS: 379.2 ($[M+H]^+$).

*4-Methyl-7-{2-[4-(2-methylphenyl)piperazin-1-yl]ethoxy}-2*H*-chromen-2-one* (**8**). Yield 45%. Colorless powder. M.p. 112–113°. HPLC 97.5% (MeOH/H_2O 80:20 (*v*/*v*); t_R 5.36 min). IR (KBr): 2927, 1710, 1617, 1492, 1393, 1289, 1223, 835. ^{1}H-NMR ($CDCl_3$, 300 MHz): 7.49 (*d*, *J* = 8.8, H–C(5)); 7.13–7.16 (*m*, H–C(6), H–C(8)); 6.84–7.04 (*m*, 4 arom. H); 6.13 (*s*, H–C(3)); 4.19–4.21 (*m*, CH_2O); 2.97–3.00 (*m*, 3 $CH_2N(1)$); 2.77–2.81 (*m*, 2 $CH_2N(4)$); 2.39 (*s*, Me); 2.31 (*s*, Me). ^{13}C-NMR ($CDCl_3$, 125 MHz): 161.3; 155.3; 152.5; 147.3; 132.7; 131.1; 126.6; 125.6; 123.3; 112.7; 112.1; 101.7; 66.6; 56.9; 54.2; 51.5; 18.6; 17.8. ESI-MS: 379.2 ($[M+H]^+$).

*7-{2-[4-(3-Methoxyphenyl)piperazin-1-yl]ethoxy}-4-methyl-2*H*-chromen-2-one* (**9**). Yield 37%. Colorless powder. M.p. 134–136°. HPLC 96.9% (MeOH/H_2O 80:20 (*v*/*v*); t_R 4.56 min). IR (KBr): 2928, 1715, 1612, 1501, 1389, 1362, 1258, 1065, 1020, 749. ^{1}H-NMR ($CDCl_3$, 300 MHz): 7.51 (*d*, *J* = 8.8, H–C(5)); 6.84–7.26 (*m*, 4 arom. H, H–C(6), H–C(8)); 6.14 (*s*, H–C(3)); 4.24–4.27 (*m*, CH_2O); 3.14–3.18 (*m*, 2 $CH_2N(4)$); 2.85–2.89 (*m*, 2 $CH_2N(1)$); 2.97–2.99 (*m*, N(1)–CH_2CH_2O); 2.38 (*s*, Me); 3.87 (*s*, MeO). ^{13}C-NMR ($CDCl_3$, 125 MHz): 161.8; 161.2; 160.6; 155.3; 152.6; 152.4; 129.8; 125.5; 113.7; 112.6; 112.1; 108.9; 104.5; 101.6; 66.5; 56.9; 55.2; 53.6; 48.9; 18.6. ESI-MS: 395.3 ($[M+H]^+$).

*7-{2-[4-(4,6-Dimethoxypyrimidin-2-yl)piperazin-1-yl]ethoxy}-4-methyl-2*H*-chromen-2-one* (**10**). Yield 34%. Colorless powder. M.p. 112–114°. HPLC 96.8% (MeOH/H_2O 80:20 (*v*/*v*); t_R 5.77 min). IR (KBr): 2943, 1718, 1603, 1579, 1529, 1361, 1282, 1194, 1157, 1008. ^{1}H-NMR ($CDCl_3$, 500 MHz): 7.51 (*d*, *J* = 8.8, H–C(5)); 6.83–6.89 (*m*, H–C(6), H–C(8)); 6.14 (br. *s*, 1 H, pyrimidine); 5.37 (*s*, H–C(5)); 6.14 (*s*, H–C(3)); 4.20–4.23 (*m*, CH_2O); 3.83–3.89 (*m*, 2 MeO, 2 $CH_2N(4)$); 2.85–2.88 (*m*, $CH_2N(1)$); 2.63–2.66 (*m*, N(1)–CH_2CH_2O)); 2.39 (*s*, Me); 2.88–2.91 (*m*, $CH_2N(1)$). ESI-MS: 427.1 ($[M+H]^+$).

*4-Methyl-7-{2-[4-(4-methylbenzoyl)piperazin-1-yl]ethoxy}-2*H*-chromen-2-one* (**11**). Yield 36%. Colorless powder. M.p. 135–137°. HPLC 98.8% (MeOH/H_2O 80:20 (*v*/*v*); t_R 5.37 min). IR (KBr): 1720, 1616, 1429, 1391, 1156, 1070, 750. ^{1}H-NMR ($CDCl_3$, 500 MHz): 7.49 (*d*, *J* = 8.8, H–C(5)); 6.81 (*d*, *J* = 2.5, H–C(8)); 6.81 (*dd*, *J* = 2.5, 8.8, H–C(6)); 7.19–7.31 (*m*, 4 arom. H); 6.13 (*s*, H–C(3)); 4.16 (*t*, *J* = 5.6, CH_2O); 3.76–3.81 (*m*, $CH_2N(4)$); 3.48–3.51 (*m*, $CH_2N(4)$); 2.98 (*t*, *J* = 5.6, N(1)–CH_2CH_2O); 2.48–2.52 (*m*, 2 $CH_2N(1)$); 2.39 (*s*, Me); 2.37 (*s*, Me). ESI-MS: 407.2 ($[M+H]^+$).

*7-{2-[4-(4-Methoxybenzoyl)piperazin-1-yl]ethoxy}-4-methyl-2*H*-chromen-2-one* (**12**). Yield 35%. Colorless powder. M.p. 113–115°. HPLC 99.1% (MeOH/H_2O 80:20 (*v*/*v*); t_R 4.87 min). IR (KBr): 3436, 1702, 1618, 1511, 1453, 1423, 1393, 1249, 1004, 841. ^{1}H-NMR ($CDCl_3$, 500 MHz): 7.49 (*d*, *J* = 8.8, H–C(5)); 6.81 (*d*, *J* = 2.4, H–C(8)); 6.86 (*dd*, *J* = 2.4, 8.8, H–C(6)); 7.37–7.40 (*m*, 2 arom. H); 6.90–6.93 (*m*, 2 arom. H); 6.13 (*s*, H–C(3)); 4.16 (*t*, *J* = 5.5, CH_2O); 2.85–2.89 (*m*, N(1)–CH_2CH_2O); 3.64–3.68 (*m*, 2 $CH_2N(4)$); 2.48–2.53 (*m*, 2 $CH_2N(1)$); 2.39 (*s*, Me); 3.87 (*s*, MeO). ESI-MS: 423.2 ($[M+H]^+$).

*7-{2-[4-(2,5-Dichlorophenyl)piperazin-1-yl]ethoxy}-4-methyl-2*H*-chromen-2-one* (**13**). Yield 36%. Colorless powder. M.p. 134–135°. HPLC 98.1% (MeOH/H_2O 80:20 (*v*/*v*); t_R 4.07 min). IR (KBr): 2946, 1713, 1615, 1475, 1389, 1158, 1071, 808. ^{1}H-NMR ($CDCl_3$, 500 MHz): 7.50 (*d*, *J* = 8.8, H–C(5)); 6.84 (*d*, *J* = 2.5, H–C(8)); 6.89 (*dd*, H–C(6), *J* = 2.5, 8.8); 7.28 (*s*, 1 arom. H); 6.95–6.97 (*m*, 2 arom. H); 6.14 (*s*, H–C(3)); 4.21–4.23 (*m*, CH_2O); 3.10–3.13 (*m*, 2 $CH_2N(4)$); 2.92–2.96 (*m*, N(1)–CH_2CH_2O); 2.77–2.81 (*m*, 2 $CH_2N(1)$); 2.39 (*s*, Me). ^{13}C-NMR ($CDCl_3$, 125 MHz): 161.3; 155.4; 152.5; 133.2; 131.5; 127.0; 125.7; 120.9; 112.7; 112.2; 101.7; 66.5; 56.9; 53.7; 50.9; 18.7. ESI-MS: 433.1 ($[M+H]^+$).

*4-Methyl-7-{4-[4-(4-methylphenyl)piperazin-1-yl]butoxy}-2*H*-chromen-2-one* (**14**). Yield 37%. Colorless powder. M.p. 141–142°. HPLC 98.4% (MeOH/H_2O 80:20 (*v*/*v*); t_R 5.13 min). IR (KBr): 3412, 1715, 1614, 1491, 1445, 1386, 1146, 1124, 996, 557. ^{1}H-NMR ($CDCl_3$, 500 MHz): 7.49 (*d*, *J* = 8.8, H–C(5)); 6.82 (*d*, *J* = 2.5, H–C(8)); 6.86 (*d*, H–C(6), *J* = 2.5, 8.8); 7.15–7.18 (*m*, 2 arom. H); 6.98–7.01 (*m*, 2 arom.

H); 6.13 (*s*, H–C(3)); 4.14–4.17 (*m*, CH_2O); 2.94–2.98 (*m*, 2 $CH_2N(4)$); 2.63–2.66 (*m*, 2 $CH_2N(1)$); 2.49–2.52 (*m*, N(1)–CH_2(CH_2)$_2$$CH_2O$)); 2.39 (*s*, Me); 2.31 (*s*, Me); 1.87–1.90 (*m*, CH_2); 1.73–1.75 (*m*, CH_2). ^{13}C-NMR ($CDCl_3$, 125 MHz): 162.2; 161.3; 152.5; 146.6; 132.6; 131.1; 126.6; 125.5; 123.1; 119.1; 113.5; 112.7; 111.9; 101.4; 68.3; 58.2; 53.8; 51.7; 27.0; 23.3; 18.6; 17.8. ESI-MS: 407.3 ([*M*+H]$^+$).

*4-Methyl-7-{4-[4-(2-methylphenyl)piperazin-1-yl]butoxy}-2*H-*chromen-2-one* (**15**). Yield 39%. Colorless powder. M.p. 101–103°. HPLC 96.8% (MeOH/H_2O 80:20 (*v*/*v*); t_R 6.03 min). IR (KBr): 3425, 1716, 1614, 1552, 1366, 1294, 1146, 749. ^{1}H-NMR ($CDCl_3$, 500 MHz): 7.48 (*d*, *J* = 8.8, H–C(5)); 7.05–7.07 (*m*, 2 arom. H); 6.81–6.86 (*m*, 4 arom. H, H–C(6), H–C(8)); 6.13 (*s*, H–C(3)); 4.05–4.07 (*m*, CH_2O); 3.15–3.19 (*m*, 2 $CH_2N(4)$); 2.62–2.66 (*m*, 2 $CH_2N(1)$); 2.47–2.50 (*m*, N(1)–CH_2(CH_2)$_2$$CH_2O$); 2.39 (*s*, Me); 2.68 (*s*, Me); 1.88–1.91 (*m*, CH_2); 1.74–1.76 (*m*, CH_2). ^{13}C-NMR ($CDCl_3$, 125 MHz): 162.2; 161.3; 152.5; 149.3; 129.6; 129.3; 125.5; 116.5; 113.5; 112.7; 111.9; 101.4; 68.3; 58.1; 53.3; 49.7; 27.0; 23.3; 20.3; 18.6. ESI-MS: 405.3 ([*M*+H]$^+$).

*7-{4-[4-(4-Methoxyphenyl)piperazin-1-yl]butoxy}-4-methyl-2*H-*chromen-2-one* (**16**). Yield 40%. Colorless powder. M.p. 108–109°. HPLC 96.5% (MeOH/H_2O 80:20 (*v*/*v*); t_R 7.11 min). IR (KBr): 3420, 1729, 1608, 1511, 1451, 1386, 1155, 1135, 874, 819. ^{1}H-NMR ($CDCl_3$, 500 MHz): 7.48 (*d*, *J* = 8.8, H–C(5)); 6.81–6.91 (*m*, 4 arom. H, H–C(6), H–C(8)); 6.12 (*s*, H–C(3)); 4.05–4.07 (*m*, CH_2O); 3.76 (*s*, MeO); 3.09–3.13 (*m*, 2 $CH_2N(4)$); 2.62–2.65 (*m*, 2 $CH_2N(1)$); 2.46–2.49 (*m*, N(1)–CH_2(CH_2)$_2$$CH_2O$); 2.39 (*s*, Me); 2.68 (*s*, Me); 1.87–1.90 (*m*, CH_2); 1.72–1.75 (*m*, CH_2). ^{13}C-NMR ($CDCl_3$, 125 MHz): 162.2; 161.3; 155.4; 153.9; 152.5; 145.7; 125.5; 118.2; 114.5; 113.5; 112.6; 111.9; 101.4; 68.3; 58.1; 55.6; 53.4; 50.6; 27.0; 23.3; 18.6. ESI-MS: 423.3 ([*M*+H]$^+$).

*7-{4-[4-(2-Methoxyphenyl)piperazin-1-yl]butoxy}-4-methyl-2*H-*chromen-2-one* (**17**). Yield 48%. Colorless powder. M.p. 98–100°. HPLC 98.2% (MeOH/H_2O 80:20 (*v*/*v*); t_R 6.71 min). IR (KBr): 3426, 1716, 1612, 1389, 1145, 1070, 754. ^{1}H-NMR ($CDCl_3$, 500 MHz): 7.48 (*d*, *J* = 8.8, H–C(5)); 6.81–6.94 (*m*, 4 arom. H, H–C(6), H–C(8)); 6.12 (*s*, H–C(3)); 4.05–4.08 (*m*, CH_2O); 3.86 (*s*, MeO); 3.09–3.13 (*m*, 2 $CH_2N(4)$); 2.66–2.70 (*m*, 2 $CH_2N(1)$); 2.50–2.53 (*m*, N(1)–CH_2(CH_2)$_2$$CH_2O$); 2.39 (*s*, Me); 1.88–1.91 (*m*, CH_2); 1.72–1.75 (*m*, CH_2). ^{13}C-NMR ($CDCl_3$, 125 MHz): 162.2; 161.3; 155.4; 152.4; 141.4; 125.5; 122.9; 121.0; 118.3; 113.5; 112.7; 111.9; 111.3; 101.4; 68.3; 58.2; 55.3; 53.5; 50.6; 27.0; 23.3; 18.6. ESI-MS: 423.3 ([*M*+H]$^+$).

*4-Methyl-7-{3-[4-(4-methylphenyl)piperazin-1-yl]propoxy}-2*H-*chromen-2-one* (**18**). Yield 38%. Colorless powder. M.p. 146–147°. HPLC 97.6% (MeOH/H_2O 80:20 (*v*/*v*); t_R 5.67 min). IR (KBr): 3409, 1711, 1611, 1513, 1388, 1201, 1139, 927, 813. ^{1}H-NMR ($CDCl_3$, 500 MHz): 7.48 (*d*, *J* = 8.8, H–C(5)); 6.83–7.08 (*m*, 4 arom. H, H–C(6), H–C(8)); 6.12 (*s*, H–C(3)); 4.10–4.12 (*m*, CH_2O); 3.14–3.17 (*m*, 2 $CH_2N(4)$); 2.64–2.66 (*m*, 2 $CH_2N(1)$); 2.57–2.61 (*m*, N(1)–C$H_2$$CH_2CH_2O$); 2.39 (*s*, Me); 2.27 (*s*, Me); 2.02–2.05 (*m*, CH_2). ^{13}C-NMR ($CDCl_3$, 125 MHz): 162.1; 161.3; 155.4; 152.5; 149.2; 129.7; 129.6; 129.3; 125.5; 116.4; 113.6; 112.6; 111.9; 101.5; 66.8; 54.9; 53.3; 49.7; 26.5; 20.3; 18.6. ESI-MS: 393.3 ([*M*+H]$^+$).

*4-Methyl-7-{3-[4-(2-methylphenyl)piperazin-1-yl]propoxy}-2*H-*chromen-2-one* (**19**). Yield 40%. Colorless powder. M.p. 108–110°. HPLC 97.8% (MeOH/H_2O 80:20 (*v*/*v*); t_R 5.65 min). IR (KBr): 3428, 1713, 1618, 1555, 1293, 1013, 770. ^{1}H-NMR ($CDCl_3$, 500 MHz): 7.49 (*d*, *J* = 8.8, H–C(5)); 6.84 (*d*, *J* = 2.5, H–C(8)); 6.87 (*d*, *J* = 2.5, 8.8, H–C(6)); 7.16–7.18 (*m*, 2 arom. H); 6.96–6.99 (*m*, 2 arom. H); 6.12 (*s*, H–C(3)); 4.11–4.13 (*m*, CH_2O); 2.95–2.98 (*m*, 2 $CH_2N(4)$); 2.63–2.66 (*m*, 3 $CH_2N(1)$); 2.39 (*s*, Me); 2.31 (*s*, Me); 2.04–2.07 (*m*, CH_2). ^{13}C-NMR ($CDCl_3$, 125 MHz): 162.2; 161.3; 155.4; 152.5; 151.5; 132.6; 131.1; 126.6; 125.5; 123.2; 119.1; 113.6; 112.6; 111.9; 101.5; 66.8; 55.0; 53.8; 51.6; 26.5; 18.6. ESI-MS: 393.3 ([*M*+H]$^+$).

*7-{3-[4-(4-Methoxyphenyl)piperazin-1-yl]propoxy}-4-methyl-2*H-*chromen-2-one* (**20**). Yield 37%. Colorless powder. M.p. 144–145°. HPLC 96.8% (MeOH/H_2O 80:20 (*v*/*v*); t_R 5.34 min). IR (KBr): 3439, 3079, 1709, 1610, 1510, 1461, 1202, 1140, 840. ^{1}H-NMR ($CDCl_3$, 500 MHz): 7.48 (*d*, *J* = 8.8, H–C(5)); 6.83–6.92 (*m*, 4 arom. H, H–C(6), H–C(8)); 6.12 (*s*, H–C(3)); 4.10–4.13 (*m*, CH_2O); 3.11–3.14 (*m*, 2 $CH_2N(4)$); 2.64–2.67 (*m*, 2 $CH_2N(1)$); 2.57–2.61 (*m*, N(1)–C$H_2$$CH_2CH_2O$); 2.39 (*s*, Me); 3.87 (*s*, MeO); 2.04–2.06 (*m*, CH_2). ^{13}C-NMR ($CDCl_3$, 125 MHz); 162.2; 161.3; 155.4; 152.6; 152.5; 145.8; 125.6; 118.3; 114.6; 113.6; 112.7; 112.6; 112.1; 112.0; 101.6; 66.8; 55.6; 54.7; 53.5; 50.7; 26.6; 18.6. ESI-MS: 409.2 ([*M*+H]$^+$).

*4,7-Dihydroxy-2*H-*1-benzopyran-2-one* (**21**). Resorcin (0.07 mol), malonic acid (0.07 mol), and $ZnCl_2$ (30 g) were dissolved in 20 ml of $POCl_3$. The mixture was kept for 24 h at 60°. Then, the mixture

was poured into 250 ml of ice-H_2O and deposited overnight. The precipitate was filtered and then recrystallized with 5% EtOH to give **21** 5.91 g (48%).

*7-Hydroxy-4-methoxy-2*H-*chromen-2-one* (**22**). Compound **21** (1.25 g) and 7 ml of oil of vitriol was dissolved in 70 ml of MeOH. The mixture was refluxed for 1.5 h and then cooled to r.t. The crystals formed were filtered to give **22** (1.19 g, 88%).

*7-(2-Bromoethoxy)-4-methoxy-2*H-*chromen-2-one* (**23**). A mixture of **22** (0.005 mol), $BrCH_2CH_2Br$ (0.04 mol), and K_2CO_3 (1.0 g, 0.01 mol) in dry EtOH was refluxed for 24 h. The mixture was filtered, and the org. phase was evaporated. The residue was purified by CC (SiO_2; cyclohexane/AcOEt 8:2) to give **23** (55%) as white crystals. The *7-(3-bromopropoxy)-4-methoxy-2*H-*chromen-2-one* and *7-(4-bromobutoxy)-4-methoxy-2*H-*chromen-2-one* were prepared by the same method using **1** and corresponding 1,3-dibromopropane or 1,4-dibromobutane.

General Procedure for the Preparation of Compounds **24**–**31**. To 15 ml of acetone and 15 ml of alcohol were added 0.01 mol **23**, 0.01 mol of substituted piperazine derivative, and 0.02 mol of K_2CO_3. The mixture was refluxed for 48 h and then evaporated. The FC provided the target compounds.

*4-Methoxy-7-[2-(4-phenylpiperazin-1-yl)ethoxy]-2*H-*chromen-2-one* (**24**). Yield 42%. Colorless powder. M.p. 115–117°. HPLC 96.5% (MeOH/H_2O 80:20 (*v*/*v*); t_R 7.35 min). IR (KBr): 2948, 1746, 1716, 1618, 1492, 1147, 1001. ^{1}H-NMR ($CDCl_3$, 500 MHz): 7.70 (*d*, *J* = 8.8, H–C(5)); 6.81–7.25 (*m*, 5 arom. H, H–C(6), H–C(8)); 5.57 (*s*, H–C(3)); 4.20–4.23 (*m*, CH_2O); 3.22–3.25 (*m*, 2 $CH_2N(4)$); 2.74–2.77 (*m*, 2 $CH_2N(1)$); 2.91–2.93 (*m*, N(1)–CH_2CH_2O); 3.97 (*s*, MeO). ^{13}C-NMR ($CDCl_3$, 125 MHz): 166.8; 163.4; 162.4; 155.1; 151.3; 129.1; 119.8; 116.2; 112.6; 109.1; 101.2; 87.7; 66.6; 56.9; 56.2; 53.7; 49.1. ESI-MS: 381.3 ($[M+H]^+$).

*4-Methoxy-7-{2-[4-(4-methylphenyl)piperazin-1-yl]ethoxy}-2*H-*chromen-2-one* (**25**). Yield 36%. Colorless powder. M.p. 162–164°. HPLC 97.8% (MeOH/H_2O 80:20 (*v*/*v*); t_R 8.15 min). IR (KBr): 2948, 1746, 1716, 1618, 1492, 1241, 1147, 766. ^{1}H-NMR ($CDCl_3$, 500 MHz): 7.70 (*d*, *J* = 8.8, H–C(5)); 7.06–7.08 (*m*, H–C(6), H–C(8)); 6.81–6.87 (*m*, 4 arom. H); 5.57 (*s*, H–C(3)); 4.20–4.22 (*m*, CH_2O); 3.17–3.21 (*m*, 2 $CH_2N(4)$); 2.89–2.92 (*m*, N(1)–CH_2CH_2O); 2.75–2.78 (*m*, 2 $CH_2N(1)$); 3.97 (*s*, MeO); 2.67 (*s*, Me). ^{13}C-NMR ($CDCl_3$, 125 MHz): 166.8; 163.4; 162.4; 155.1; 149.2; 129.6; 129.4; 124.1; 116.5; 112.6; 109.1; 101.2; 87.7; 66.5; 56.9; 56.2; 53.7; 49.6; 20.3. ESI-MS: 395.2 ($[M+H]^+$).

*4-Methoxy-7-{2-[4-(2-methylphenyl)piperazin-1-yl]ethoxy}-2*H-*chromen-2-one* (**26**). Yield 36%. Colorless powder. M.p. 122–124°. HPLC 98.1% (MeOH/H_2O 80:20 (*v*/*v*); t_R 8.64 min). IR (KBr): 2931, 1718, 1633, 1392, 1295, 917. ^{1}H-NMR ($CDCl_3$, 500 MHz): 7.70 (*d*, *J* = 8.8, H–C(5)); 6.81 (*d*, *J* = 2.4, H–C(8)); 6.87 (*dd*, *J* = 8.8, 2.4, H–C(6)); 6.98–7.18 (*m*, 4 arom. H); 5.57 (*s*, H–C(3)); 4.21–4.24 (*m*, CH_2O); 2.95–2.98 (*m*, 2 $CH_2N(4)$, N(1)–CH_2CH_2O); 2.76–2.79 (*m*, 2 $CH_2N(1)$); 3.97 (*s*, MeO); 2.67 (*s*, Me). ^{13}C-NMR ($CDCl_3$, 125 MHz): 166.8; 163.4; 162.4; 155.1; 151.4; 132.6; 131.1; 124.1; 119.1; 112.6; 109.0; 101.2; 87.7; 66.6; 56.9; 56.2; 54.2; 51.6; 17.8. ESI-MS: 395.2 ($[M+H]^+$).

*4-Methoxy-7-{2-[4-(4-methoxyphenyl)piperazin-1-yl]ethoxy}-2*H-*chromen-2-one* (**27**). Yield 37%. Colorless powder. M.p. 141–143°. HPLC 98.4% (MeOH/H_2O 80:20 (*v*/*v*); t_R 8.19 min). IR (KBr): 2951, 1754, 1716, 1617, 1392, 1254, 831. ^{1}H-NMR ($CDCl_3$, 500 MHz): 7.70 (*d*, *J* = 8.8, H–C(5)); 6.83–6.94 (*m*, 4 arom. H, H–C(6), H–C(8)); 5.59 (*s*, H–C(3)); 4.23–4.25 (*m*, CH_2O); 3.14–3.17 (*m*, 2 $CH_2N(4)$); 2.79–2.83 (*m*, 2 $CH_2N(1)$); 2.92–2.95 (*m*, N(1)–CH_2CH_2O); 3.97 (*s*, MeO); 3.99 (*s*, MeO). ^{13}C-NMR ($CDCl_3$, 125 MHz): 166.8; 163.4; 162.4; 155.1; 153.9; 145.6; 124.1; 118.3; 114.6; 114.5; 112.6; 109.1; 101.2; 87.7; 66.6; 56.9; 56.2; 53.8; 50.5. ESI-MS: 411.2 ($[M+H]^+$).

*4-Methoxy-7-{2-[4-(2-methoxyphenyl)piperazin-1-yl]ethoxy}-2*H-*chromen-2-one* (**28**). Yield 36%. Colorless powder. M.p. 228–229°. HPLC 97.3% (MeOH/H_2O 80:20 (*v*/*v*); t_R 7.35 min). IR (KBr): 2929, 1720, 1636, 1613, 1394, 1296, 810. ^{1}H-NMR ($CDCl_3$, 500 MHz): 7.70 (*d*, *J* = 8.8, H–C(5)); 6.81 (*d*, *J* = 2.4, H–C(8)); 6.85 (br. *s*, H–C(6)); 6.87–7.02 (*m*, 4 arom. H); 5.57 (*s*, H–C(3)); 4.19–4.21 (*m*, CH_2O); 3.12–3.15 (*m*, 2 $CH_2N(4)$); 2.78–2.82 (*m*, 2 $CH_2N(1)$); 2.90–2.93 (*m*, N(1)–CH_2CH_2O); 3.97 (*s*, MeO); 3.87 (*s*, MeO). ^{13}C-NMR ($CDCl_3$, 125 MHz): 166.8; 163.4; 162.4; 155.1; 152.3; 141.3; 124.0; 122.9; 121.0; 118.2; 112.6; 111.3; 109.0; 101.2; 87.7; 66.6; 56.9; 56.2; 53.9; 50.6. ESI-MS: 411.3 ($[M+H]^+$).

*7-[2-[4-(4-Chlorophenyl)piperazin-1-yl]ethoxy]-4-methoxy-2*H-*chromen-2-one* (**29**). Yield 39%. Colorless powder. M.p. 168–170°. HPLC 96.7% (MeOH/H_2O 80:20 (*v*/*v*); t_R 7.33 min). IR (KBr): 2950, 1718, 1624, 1497, 1295, 1244, 1027, 811. ^{1}H-NMR ($CDCl_3$, 500 MHz): 7.73 (*d*, *J* = 8.8, H–C(5)); 6.83–7.24 (*m*, 4 arom. H, H–C(6), H–C(8)); 5.59 (*s*, H–C(3)); 4.23–4.25 (*m*, CH_2O); 3.22–3.25 (*m*,

2 $CH_2N(4)$); 2.88–2.93 (*m*, 3 $CH_2N(1)$); 3.99 (*s*, MeO). ^{13}C-NMR ($CDCl_3$, 125 MHz): 166.8; 163.4; 162.3; 155.1; 149.8; 141.9; 129.0; 128.9; 124.7; 124.1; 117.3; 112.6; 109.1; 101.2; 87.7; 66.6; 56.8; 56.2; 53.5; 49.1. ESI-MS: 415.2 ($[M+H]^+$).

*7-{3-[4-(4-Chlorophenyl)piperazin-1-yl]propoxy}-4-methoxy-2*H*-chromen-2-one* (**30**). Yield 33%. Colorless powder. M.p. 164–166°. HPLC 97.3% (MeOH/H_2O 80:20 (*v*/*v*); t_R 6.75 min). IR (KBr): 2944, 1712, 1611, 1497, 1139, 1070. ^{1}H-NMR ($CDCl_3$, 500 MHz): 7.48 (*d*, $J=8.8$, H–C(5)); 6.83–7.21 (*m*, 4 arom. H, H–C(6), H–C(8)); 6.13 (*s*, H–C(3)); 4.10–4.13 (*m*, CH_2O); 3.18–3.22 (*m*, 2 $CH_2N(4)$); 2.61–2.64 (*m*, 3 $CH_2N(1)$); 3.97 (*s*, MeO); 2.04–2.06 (*m*, CH_2). ESI-MS: 430.2 ($[M+H]^+$).

*7-{4-[4-(4-Chlorophenyl)piperazin-1-yl]butoxy}-4-methoxy-2*H*-chromen-2-one* (**31**). Yield 41%. Colorless powder. M.p. 114–116°. HPLC 97.7% (MeOH/H_2O 80:20 (*v*/*v*); t_R 7.32 min). IR (KBr): 2935, 1738, 1610, 1497, 1241, 1199, 1153, 1070. ^{1}H-NMR ($CDCl_3$, 500 MHz): 7.50 (*d*, $J=8.8$, H–C(5)); 7.17–7.27 (*m*, H–C(6), H–C(8)); 6.83–6.88 (*m*, 4 arom. H); 6.14 (*s*, H–C(3)); 4.07–4.09 (*m*, CH_2O); 3.16–3.19 (*m*, 2 $CH_2N(4)$); 2.62–2.65 (*m*, 2 $CH_2N(1)$), 2.46–2.49 (*m*, N(1)–$CH_2(CH_2)_2CH_2O$); 3.97 (*s*, MeO); 1.90–1.92 (*m*, CH_2); 1.72–1.75 (*m*, CH_2). ^{13}C-NMR ($CDCl_3$, 125 MHz): 162.1; 161.3; 155.3; 152.5; 149.9; 126.9; 125.5; 124.5; 117.2; 113.5; 112.6; 111.9; 101.4; 58.0; 53.1; 48.1; 26.9; 23.3; 18.6. ESI-MS: 427.2 ($[M+H]^+$).

Biological Studies [22]. The *Ethical Committee for Conduct* of *Animal Studies* at China Pharmaceutical University (CPU) approved the exper. protocol, and all animals were taken care of in accordance with the Act to Administer the Care and Use of Experimental Animals of the Jiangsu Province of China. Male *Sprague–Dawley* rats (*Shanghai Sipper-BK Lab Animal Co., LTD.*, Shanghai, P. R. China), eight weeks of age and weighing 180 ± 20 g each, were used. The anococcygeus muscles were isolated after cervical dislocation under anesthesia with Et_2O. Preparations were suspended in an organ bath (*Model 832*, *HSE Co., Ltd.*, Germany) filled with *Krebs–Henseleit* soln. (20 ml) of the following composition [mmol/l]: NaCl, 118.4; KCl, 4.7; $CaCl_2$, 2.52; $MgSO_4$, 1.2; KH_2PO_4, 1.2; $NaHCO_3$, 25; and glucose, 11.1. Each bath was maintained at 37° and continuously bubbled with a gas mixture consisting of 95% O_2 and 5% CO_2. The responses of the preparations were isometrically recorded with an automatic magnus system (*IM-400C*, *Japan Tobacco*, Tokyo, Japan) under a resting tension of 1.0 g. The preparations were equilibrated for *ca.* 1 h and contracted twice by 3×10^{-5} M of phenylephrine, and the maximum contraction of the second time was taken as 100%. Cumulative concentrations of phenylephrine (3×10^{-9}–10^{-4} M) were then added to the organ bath as a α_1-AR agonist, and concentration–response curves obtained to determine the relationship between agonist concentrations and contractile responses. After a successive concentration–response curve for the agonist had been obtained, the antagonist prazosin (10^{-8}–10^{-7} M) or the compound to be tested (10^{-6}–10^{-7} M) was added to the bath. Responses for the agonist in the presence of the antagonists were calculated as the percentage of the maximal response. *Schild* plots were constructed, and pK_i values were determined from the intercept on the abscissa scale [23].

α_{1A}-ARs Homology Model and Molecular Docking. As known, G-protein-coupled receptors (GPCRs) share similar transmembrane (TM) boundaries and overall topology. Moreover, the homology models have been used in the studies of adrenergic receptors in many cases, which have proved to be considerably effective. Here, we chose the human β_2-adrenergic receptor's crystal structure (PDB ID: 2RH1, $R=2.4$ Å) as a template to construct α_{1A}-AR homology model by Discovery Studio 2.0 (*Accelrys software Inc*, San Diego) on the basis of the assumption that α_{1A}-AR may be more homologous with β_2-AR also (identity 25%) from human sources than bovine rhodopsin (identity 21%), which was used for the modeling of GPCRs as the unique template of this sort of receptors, before the crystal structure of β_2-AR was reported. The modeling process was divided into three steps: first, the C(α) backbones of the conserved 7TM domains were built with loop modeling using the DOPE energy function which represents an improved energy function from potentials extracted from a library of non-redundant high-resolution crystal structures, and has been shown to provide higher quality models. Second, the model was verified using the Profile-3D method, evaluating the likelihood that an amino acid should be present within its current environment, and the verify scores used for further refinement were obtained for all amino acid residues. Third, we performed the CHARMm-based structural refinement of loops and side chains with LOOPER and ChiRotor algorithm, resp. In particular, the side chains of all residues were refined first, followed by the residues around the active site of the receptor identified, using the binding-

site searching tool according to the viewpoint that the active site could be influenced by the rest residues. Finally, the minimization was carried out by 1000 steps of steepest descent and then conjugate gradient minimization until, the rms gradient of potential energy was less than 0.1 kcal mol^{-1} $Å^{-1}$ using minimization protocol.

With the homology model of α_{1A}-AR, molecular docking was carried out by GOLD 3.1.1 (*CCDC*, Cambridge). We constructed the ligands using the SYBYL 7.1 molecular-modeling package (*Tripos*, Missouri), followed by minimization with MMFF94s force field applied. The ligands including prazosin and the newly designed coumarin derivatives **3**–**31** were docked into the active sites of α_{1A}-AR, and then we obtained the putative binding modes of α_{1A}-AR and the ligands. The GOLDScore fitness was used to rank top ten conformations for each compound, taking into account H-bond and hydrophobic interactions. The docking process was terminated, while the three top-ranked answers obtained were within 1.5 Å rms deviation from another, according to the suggestion that these three poses represented the most probable docking conformations. We analyzed docking results based on the scoring functions of the software or a visual inspection of the docked poses of each compound.

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