A 3D-Quantitative Structure–Activity Relationship Study of Benzamide Type Serotonin 5-HT$_4$ Receptor Agonists Based on a Comparative Molecular Field Analysis Model, and the Design and Synthesis of Potent Agonists

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A 3D-quantitative structure–activity relationship (3D-QSAR) study was carried out using comparative molecular field analysis (CoMFA) of the 5-HT$_4$ agonistic activity of benzamide type compounds, which had been already synthesized and reported to show 5-HT$_4$ agonistic activity. The chosen alignment yielded a good cross-validated result (r^2_{cv}=0.628). This CoMFA model was able to predict the 5-HT$_4$ agonistic activity of three structurally different compounds. Consequently, 5-amino-*N*-[2-(1-azabicyclo[3.3.0]octan-5-yl)ethyl]-6-chloro-3,4-dihydro-2*H*-1-benzopyran-8-carboxamide (22) was obtained as the most potent 5-HT$_4$ agonist.

Key words comparative molecular field analysis; 5-HT$_4$ agonistic activity; 2*H*-1-benzopyran-8-carboxamide

The 5-HT$_4$ receptor, which is a serotonin receptor subtype, has been identified in the central nervous system (CNS),[2)] the heart,[3)] and the gastrointestinal tract[4)] which could indicate a wide therapeutic use for a modulator of this receptor. Metoclopramide[5)] (**1**) and cisapride[6)] (**2**) are clinically used as gastroprokinetic agents. Their gastroprokinetic action is postulated to be due to agonistic activity at a serotonin receptor subtype (5-HT$_4$).[7)] These agents, however, have dopamine D$_2$ receptor antagonistic activity, which is responsible for unfavorable side effects such as extrapyramidal disorder and cryptorrhea (lactation and prolactinemia). To moderate these side effects and to obtain more potent compounds, the modification of the *tert*-amine side chain of metoclopramide (**1**) was studied and various benzamide derivatives, for instance, mosapride[8)] (**3**), zacopride[9)] (**4**), and so on, have been synthesized (Chart 1). From the study of the *tert*-amine side chains of the benzamide series, it was found that the azabicyclo moiety was effective to reveal potent and selective serotonin 5-HT$_4$ agonism.[10)] In contrast, the study of the substituent effects at the 3-position of the benzamide derivatives on 5-HT$_4$ agonistic activity has hardly been reported except for SB 204070[11)] (**5**). Furthermore, no pharmacophore models for the 5-HT$_4$ receptor agonists have been reported in the literature.

Recently, we reported the synthesis and the 5-HT$_4$ agonistic activity of some 3-substituted benzamide, benzo[*b*]furan-7-carboxamide, 2,3-dihydrobenzo[*b*]furan-7-carboxamide, and indole-5-carboxamide derivatives having the 2-(1-azabicyclo[3.3.0]octan-5-yl)ethyl group as the azabicyclo moiety.[12)]

This paper describes a 3D-quantitative structure–activity relationship (3D-QSAR) study on the 5-HT$_4$ agonistic activity of these derivatives and shows that the comparative molecular field analysis (CoMFA) technique is useful to explain the 5-HT$_4$ agonistic activity of these compounds and to design new potent 5-HT$_4$ agonists.

Methods

3D-QSAR Methodology Table 1 lists the structure and the observed and calculated biological activity values of compounds **6**—**9** used to derive the CoMFA model and to test the predictivity of the model itself. The 5-HT$_4$ receptor agonistic activity of these compounds was checked in accordance with the method described by Baxter *et al.*[4)] using cisapride (**2**) as a control compound, as reported in our previous papers.[12)] All the activity values are in the range of 5.34 (the least active compound) to 7.89 (the most active compound).

The conformational analysis of each compound were conducted through the Random Search module in SYBYL with Tripos force field and partial atomic charges were calculated by Gesteiger–Huckel method. Some local minimum conformers with an energy up to 10 kcal/mol above the global minimum were collected to make the conformer set. The conformer sets of chiral compound **8b** and **8c** contain one stereoisomer in order to be able to

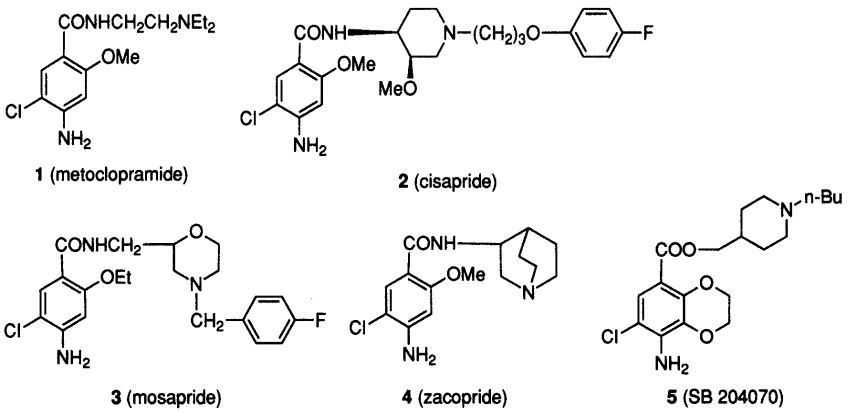

Chart 1

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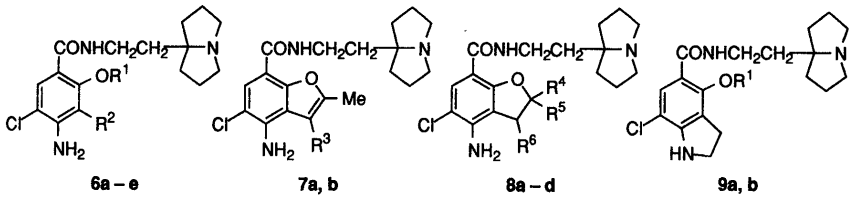
Table 1. The Physicochemical and Pharmacological Data for Synthesized Compounds (**6**—**9**)



No.	Substrate	pEC_{50}[a]		
		Obsd[b]	Calcd[c]	Diff[d]
6a	R^1=CH_3 R^2=H	5.72	5.77	−0.05
6b	R^1=CH_3 R^2=CH_2CHCH_2	6.32	6.34	−0.02
6c	R^1=CH_2CH_3 R^2=CH_2CHCH_2	6.35	6.31	0.04
6d	R^1=CH_3 R^2=$CH_2CH_2CH_3$	6.26	6.30	−0.04
6e	R^1=CH_2CH_3 R^2=$CH_2CH_2CH_3$	6.44	6.42	0.02
7a	R^3=H	7.43	7.38	0.05
7b	R^3=Cl	6.10	6.10	0.00
(*R*)-**8a**	R^4=CH_3 R^5=R^6=H	7.49	7.49	0.00
(*S*)-**8a**	R^4=CH_3 R^5=R^6=H	7.85	7.82	0.03
8b	R^4=CH_2CH_3 R^5=R^6=H	7.74	7.77[e]	−0.03
8c	R^4=R^6=CH_3 R^5=H	7.89	7.94[f]	−0.05
8d	R^4=R^5=R^6=H	6.52	6.48	0.04
9a	R^1=CH_3	5.34	5.34	0.00
9b	R^1=CH_2CH_3	5.35	5.34	0.01

a) pEC_{50} is defined as the concentration (M) of 50% relaxation of the tested compounds taken from ref 4. *b*) Experimental data taken from ref. 12. *c*) Values calculated according to the calibration model. *d*) Difference between observed and calculated values. *e*) *R* isomer. *f*) 2*S*,3*S* isomer.

select one conformer from both isomers.

As the active conformation of the 2-(1-azabicyclo[3.3.0]-octan-5-yl)ethyl group is unknown and we have synthesized no compound having other azabicyclo moieties, it is difficult to optimize the active conformation of the azabicyclo moiety. However, the X-ray crystallographic study[13] of (*S*)-**8a** hemifumarate showed that the 2-(1-azabicyclo[3.3.0]octan-5-yl)-ethyl group was extended to the opposite side of the benzamide moiety. On the other hand, the 2-(1-azabicyclo[3.3.0]-octan-5-yl)ethyl group of (*S*)-**8a**, which is a free base, is shrunk by the result of hydrogen bonding with *tert*-amine, amide proton, and the oxygen atom in the 2,3-dihydro-2-methylbenzo[*b*]furan ring.[12b] Generally, it is thought that the conformation of the 2-(1-azabicyclo[3.3.0]octan-5-yl)ethyl group is similar to (*S*)-**8a** hemifumarate in the active site, because its *tert*-amine is a strong base and forms a salt with an acid. We temporarily referred to this extended form of 2-(1-azabicyclo[3.3.0]octan-5-yl)ethyl group as the active conformation.

In this CoMFA analysis, we decided to define the alignment criterion based the pharmacophoric group of the studied compounds. The key substructures in benzamides, which are thought to play an important role in the interaction with binding site, are (i) the centroid of the condensed benzene ring, (ii) the nitrogen atom on the benzene ring, and (iii) the chlorine atom at the 5-position. In order to discover the best CoMFA model, we tried many alignments over 1500 patterns. An alignment was made by overlapping each conformer, which was randomly selected from its own conformer set using our alignment criterion. The CoMFA calculations were performed using the QSAR module of SYBYL with default setting. Steric and electrostatic interaction energy were calculated using a carbon sp^3 probe atom with a +1 charge, a distance-dependent dielectric constant ($1/r$), and an energetic cut off of 30 kcal/mol with no electrostatic interactions at steric contacts.

Regression analyses were performed using SYBYL implementation of PLS algorithm. Initially, PLS with cross-validation (leave-one-out technique) was performed to obtain cross-validated r^2 (r^2_{cv}) and optimal number of components. Next, PLS (non-cross-calidated model) calibration equations were then derived by using the optimal number of components. We used CoMFA standard default scaling option to weigh the steric and electrostatic field columns. As a result of this method, we chose some alignments with $r^2_{cv}>0.6$ as a good model. And, to find the best model from these alignments, we performed non-cross-validated PLS analysis for each chosen alignment with 2 of the number of components (data not shown). Then, we selected an alignment by comparing the best r^2 value from the previous chosen alignments as the best correlation. The statistics of this model with optimal number of components are given in Table 2. This model, having 6 optimal components, kept the highest correlation (r^2=0.909) with PLS regression using components=2. We consider that our CoMFA model is suitable because the alignment keeps high correlation regardless of reducing the number of components.

Table 2. The Statistics of the Cross-Validated CoMFA Analysis and the Calibration CoMFA Model

Principal components	6
r^2_{cv}	0.628
r^2	0.999
S_{PRESS}	0.609
SEE	0.047
Steric and electrostatic contribution (%)	51.1, 48.9
F value	796.680
Number of compounds	14

CoMFA coefficient contour maps of the coefficients of each grid point were also genetated by following the standard procedure in SYBYL. These maps show lattice points where the QSAR strongly associates changes in the steric and electrostatic field values with changes in biological activity in order to obtain chemical informations.

Chemistry

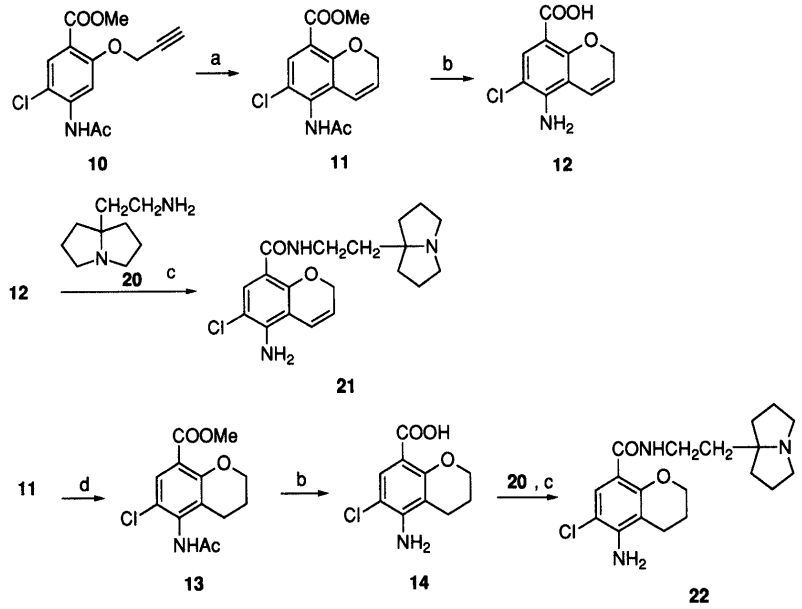
The calculated methyl 5-acetylamino-6-chloro-2*H*-1-benzopyran-8-carboxylate derivatives (**21**—**23**) were prepared as follows (Charts 2 and 3). The Claisen type rearrangement of methyl 4-acetylamino-5-chloro-2-propargyloxybenzoate (**10**)[12] in diphenyl ether gave methyl 5-acetylamino-6-chloro-2*H*-1-benzopyran-8-carboxylate (**11**) accompanied with methyl 4-acetylamino-5-chloro-2-methylbenzo[*b*]furan-7-carboxylate.[12] The alkali hydrolysis of **11** gave 5-amino-6-chloro-2*H*-1-benzopyran-8-carboxylic acid (**12**). The hydrogenation of **11** gave methyl 5-acetylamino-6-chloro-3,4-dihydro-2*H*-1-benzopyran-8-carboxylate (**13**) and then the alkali hydrolysis of **13** gave 5-amino-6-chloro-3,4-dihydro-2*H*-1-benzopyran-8-carboxylic acid (**14**). Next, methyl 4-acetylamino-2-hydroxy-3-(2-butenyl)benzoate (**15**)[12] was converted to the methyl 5-acetylamino-3,4-dihydro-2-methyl-2*H*-1-benzopyran-8-carboxylate (**16**) by cyclization under acidic conditions. Chlorination of **16**, followed by alkali hydrolysis, gave the 5-amino-6-chloro-3,4-dihydro-2-methyl-2*H*-1-benzopyran-8-carboxylic acid (**19**).

Finally, the reactions of **12**, **14**, and **19** with 5-(2-aminoethyl)-1-azabicyclo[3.3.0]octane[14] (**20**) in the presence of 1,1′-carbonyldiimidazole (CDI) afforded the corresponding amides **21**, **22**, and **23** which were then converted to the hemifumarate for the biological tests.

Results and Discussion

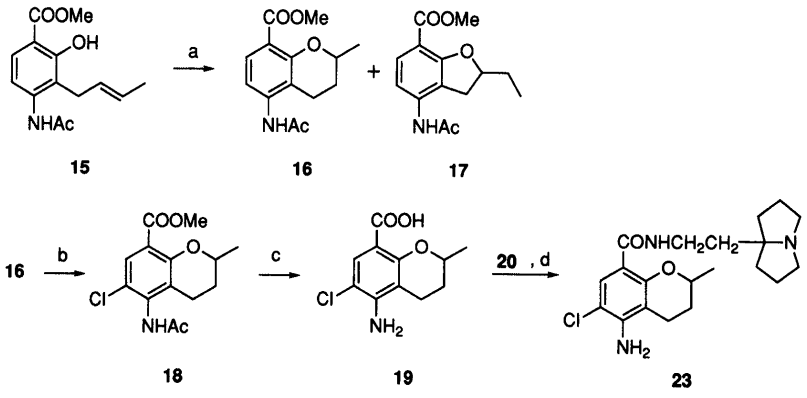
The chosen alignment yielded a good cross-validated result (r^2_{cv}=0.628) with the optimal number of components found equal to be 6 (Table 2). In this model, both the steric and electrostatic fields contribute to the QSAR equation by 51.1 and 48.9%, respectively. Figure 1 depicts a plot of the calculted *vs.* the observed 5-HT_4 agonistic activity values of compounds using the optimal non-cross-validated model.

The CoMFA steric and electrostatic contour maps plotted using compounds **6c**, **7b**, (*S*)-**8a**, and **9a** as reference structures are shown in Fig. 2. The green and yellow polyhedra represent regions of space whose occupancy by the ligands increases or decreases the 5-HT_4 agonistic activity, respectively. The yellow contours basically surround a portion of



Reagents: a diphenyl ether, Δ; b 4N NaOH; c CDI; d H_2, 5% Pt/C

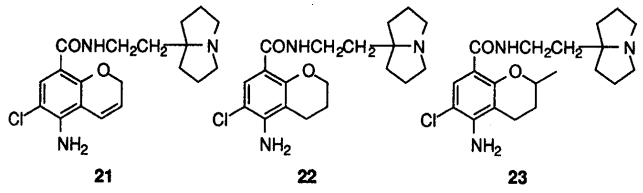
Chart 2



Reagents: a 97% H_2SO_4; b NCS; c 4N NaOH; d CDI

Chart 3

Table 3. The Observed and Predicted 5-HT_4 Receptor Agonistic Activity Values of Compounds (**21**—**23**)



21 **22** **23**

No.	pEC_{50}[a]		
	Obsd[b]	Pred[c]	Diff[d]
21	7.62	7.65	−0.03
22	8.31	7.64	0.67
23	7.74	7.71[e]	0.03

a, *b*) See the corresponding footnotes of Table 1. *c*) Values predicted by the CoMFA model. *d*) Difference between observed and predicted values. *e*) *S* isomer.

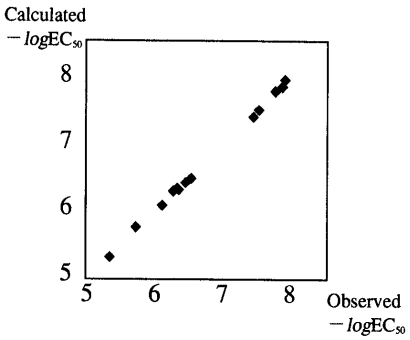


Fig. 1. The Calculated *vs.* the Observed pEC_{50} Value for the CoMFA Analysis of the 14 Compounds of the Training Set

The Model was derived using six principal components yielding a cross-validated $r^2_{cv}=0.628$.

the superimposed molecules where there was a field a little apart from a space between the nitrogen atom on the benzen ring and the 3-position of the benzamide moiety. Substantially, the model suggested the presence of no more than one substituent on the basic nitrogen. In fact, both the indole derivatives (**9a**, **b**) are less active compounds.

The red and blue polyhedra describe regions where a high electron density within the ligand structure enhances or diminishes activity, respectively.

A test of the robustness of our CoMFA model dealt with the assessment of its general applicability in predicting the activity of compounds belonging to different classes, such as a related new family of compounds (**21**—**23**). These new compounds were designed to fit to green region of CoMFA model for the purpose of increasing activity by altering benzo[*b*]furan to a benzopyran ring. The corresponding local minimum energy conformers of compound **21**, **22**, and **23** were aligned as described above and then predicted the activity. Each conformer which had the lowest energy conformation the highest activity predicted. Each dihydropyran ring which has the lowest energy comformer shows a half-chair comformation. In the case of compound **23**, the lowest energy conformer of the *S* and *R* isomer predicted the high activity (7.71, 7.54), respectively. This slight difference of both predicted activity suggests that both isomers have similar activity. The observed and predicted pEC_{50} values for the 2*H*-1-benzopyran-8-carboxamide derivatives are listed in Table 3 and plotted in Fig. 3. The CoMFA steric and electrostatic contour maps plotted using compounds **21**, **22**, and **23** as predicted structures are shown in Fig. 4. All of compounds fit in the CoMFA model. And the C2 methylene and the C3 methyne protons in compound **21**, the C2 and the C3 methylene protons in compound **22**, and the methyl group at the C2 position and the C3 methylene protons in compound **23** occupied the green polyhedra which represent regions of space whose occupancy by the ligands increases the 5-HT_4 agonistic activity. It was predicted that these compounds would show potent 5-HT_4 agonistic activity. It can be seen that the 5-HT_4 agonistic activity of all the examined compounds are predicted within 0.670 log units of their experimentally observed activity with an average absolute error of 0.24 log units. There was a slight difference compared with the training set in these parameters, but a good predictive ability was obtained. This CoMFA model can be applied to structurally different compounds. It is particularly interesting to observe that even the 5-HT_4 agonistic activity of the 2*H*-1-benzopyran-8-carboxamide type derivatives has been correctly predicted.

In conclusion, a 3D-QSAR model has been developed using the CoMFA methodology for a set of 14 compounds showing different 5-HT_4 agonistic activity values. The model is able to predict the 5-HT_4 agonists of three structurally similar compounds not used in the construction of the cross-validated model. The most active compound is **22** of which pEC_{50} value is 8.31 (EC_{50} is 4.9 nM). It was impossible to extend our study to the active conformation of other types of azabicyclo moieties except for the 2-(1-azabicyclo[3.3.0]-octan-5-yl)ethyl group, because no variation in the azabicyclo moiety was used in our model. It is thought that not only the benzamide moiety but also the azabicyclo moiety is important for 5-HT_4 agonistic activity. In fact, many derivatives, which were modified at the azabicyclo moiety, for example, cisapride (**2**), mosapride (**3**), and zacopride (**4**) *etc.*, appeared to have high 5-HT_4 agonistic activity. It is expected that a 3D-QSAR model including both the benzamide and azabicyclo moieties will be constructed to expand our CoMFA model.

Experimental

Chemistry All melting points were determined using a Yanagimoto micromelting point apparatus and are uncorrected. IR spectra were recorded using a Perkin Elmer 1600 spectrometer. Mass spectra were obtained using a JEOL JMS-SX 120A spectrometer. ^{1}H-(270 MHz) NMR spectra were recorded using a JEOL JNM-GSX 270 in $CDCl_3$ or DMSO-d_6. Chemical shifts are expressed as δ values (ppm) with tetramethylsilane as an internal standard, and coupling constants (*J* values) are given in hertz (Hz).

Methyl 5-Acetylamino-6-chloro-2*H*-1-benzopyran-8-carboxylate (11) A mixture of methyl 4-acetylamino-5-chloro-2-propargyloxybenzoate (**10**)[12] (10.0 g, 35.5 mmol) in diphenyl ether (40 ml) was stirred for 1 h at 240—250 °C. The cooled reaction mixture was poured into hexane (200 ml). After the resultant top liquid was removed, the residual substance was purified by silica gel column chromatography (AcOEt : hexane = 1 : 1) and recrystallized from EtOH–CH_2Cl_2 to give 2.67 g (26.7%) of **11** as prisms accompanied with methyl 4-acetylamino-5-chloro-2-methylbenzo[*b*]furan-7-carboxylate (**24**, 5.0%). **11**: mp 196—197 °C. ^{1}H-NMR ($CDCl_3$) δ: 2.25 (3H, s, CH_3CO), 3.88 (3H, s, CH_3), 4.90 (2H, dd, *J*=3.4, 2.0 Hz, CH_2), 5.90 (1H, br s, C3-H), 6.33 (1H, br, C4-H), 7.13 (1H, br s, NH), 7.72 (1H, s, C7-H). IR (KBr) cm^{-1}: 1708, 1654 (CO). HRMS *m/z*: Calcd for $C_{13}H_{12}ClNO_4$: 281.0455. Found: 281.0423. The spectral data of **24** coincided with the compound in our recent report.[12]

Methyl 5-Acetylamino-6-chloro-3,4-dihydro-2*H*-1-benzopyran-8-carboxylate (13) A mixture of **11** (2.31 g, 8.20 mmol), and 5% Pt–C, in a

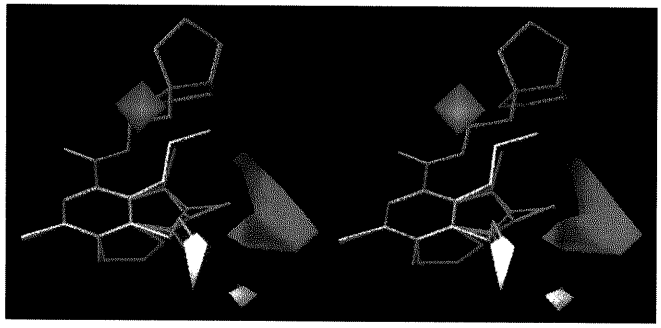


Fig. 2. The CoMFA Steric and Electrostatic Contour Plot from the Analysis Based on the 3D-QSAR Model with No Cross-Validation

The sterically favored area is represented by green polyhedra. The sterically unfavored area is represented by yellow polyhedra. The negative charge favored area is represented by red polyhedra. The negative unfavored area is represented by dark blue polyhedra. Compounds **6c**, **7b**, (*S*)-**8a**, and **9a** are also represented as white, blue, yellow, and cyan, respectively.

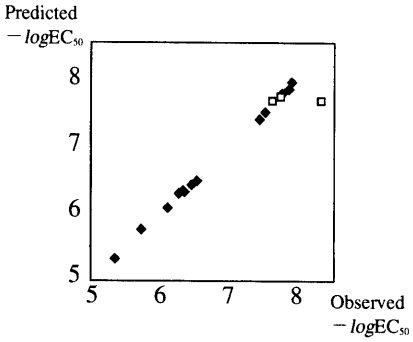


Fig. 3. The Predicted *vs.* the Obserbed pEC_{50} Value for the CoMFA Analysis of Compounds **21**, **22**, and **23**

The open square means predicted compounds and closed square means training set compounds.

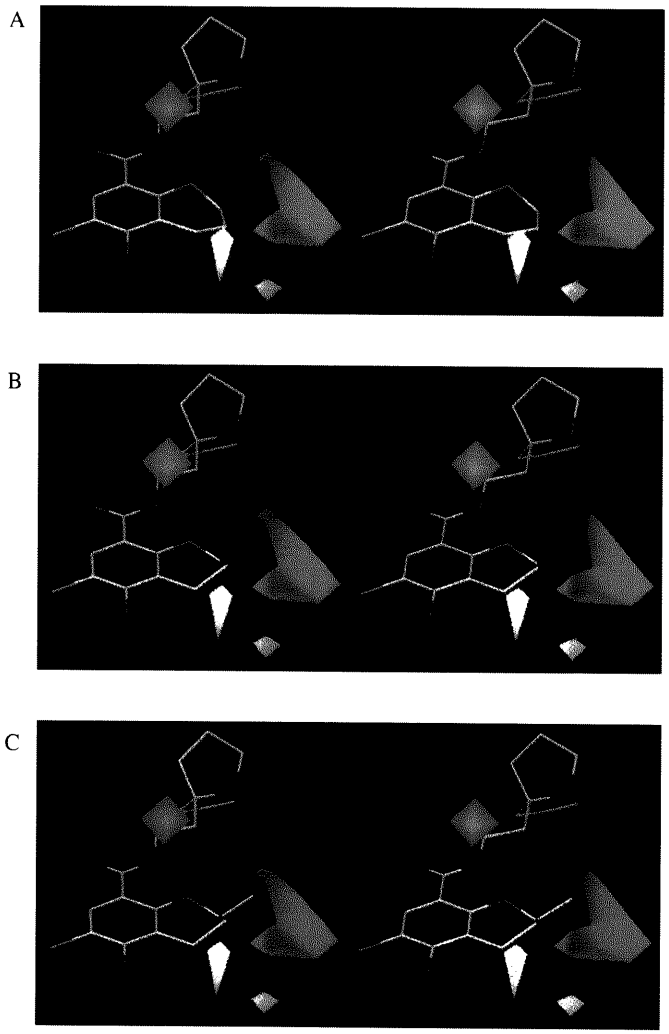


Fig. 4. The CoMFA Steric and Electrostatic Contour Plot with Compounds **21** (A), **22** (B), and **23** (C) as the Predicted Compounds

The meaning of green, yellow, red, and dark blue polyhedra are described in Fig. 2.

mixture of $CHCl_3$ (21 ml) and EtOH (7.0 ml) was stirred under H_2 gas for 3 h at room temperature and then fiiltered. The fiiltrate was concentrate*in vacuo*, and EtOH was added to the residue. The resultant precipitate was collected by fiiltration to give 1.9 g (85.1%) of **13** as a powder. mp 184—186 °C. ^{1}H-NMR ($CDCl_3$) δ: 1.97 (2H, m, C3-H), 2.24 (3H, s, CH_3CO), 2.71 (2H, t, J=6.6 Hz, C4-H), 3.87 (3H, s, CH_3), 4.28 (2H, t, J=5.4 Hz, C2-H), 7.03 (1H, br s, NH), 7.71 (1H, s, C7-H). IR (KBr) cm^{-1}: 1728, 1659 (CO). HRMS *m/z*: Calcd for $C_{13}H_{14}ClNO_4$: 283.0611. Found: 283.0620.

Methyl 5-Acetylamino-3,4-dihydro-2-methyl-2*H*-1-benzopyran-8-carboxylate (16) A mixture of methyl 4-acetylamino-2-hydroxy-3-(2-buten-1-yl)benzoate (**15**)[12] (3.10 g, 11.8 mmol) in 97% H_2SO_4 (30 ml) was stirred for 30 min at 20—25 °C, poured onto ice (500 g), and extracted with $CHCl_3$. The extract was washed with water, dried over Na_2SO_4, and concentrated *in vacuo*. The residue was purifiied by silica gelcolumn chromatography (AcOEt : hexane=2 : 1) to give 1.25 g (40.3%) of **16** as a powder accompanied with methyl 4-acetylamino-2,3-dihydro-2-ethylbenzo[*b*]furan-7-carboxylate (**17**) (11.3%). **16**: mp 163—164 °C. ^{1}H-NMR ($CDCl_3$) δ: 1.45 (3H, d, J=6.4 Hz, 2-CH_3), 1.7—1.8 (1H, m, CH of C2), 2.1—2.2 (1H, m, CH of C2), 2.20 (3H, s, $COCH_3$), 2.6—2.7 (2H, m, CH_2), 3.86 (3H, s, OCH_3), 4.1—4.2 (1H, m, CH), 7.00 (1H, br s, NH), 7.53 (1H, d, J=6.3 Hz, C6-H), 7.70 (1H, d, J=6.3 Hz, C7-H). IR (KBr) cm^{-1}: 1731, 1656 (CO). HRMS *m/z*: Calcd for $C_{14}H_{17}NO_4$: 263.1157. Found: 263.1151. **17**: mp 85—87 °C. ^{1}H-NMR ($CDCl_3$) δ: 1.04 (3H, t, J=7.3 Hz, CH_3), 1.7—1.8, 1.9—2.0 (each 1H, m, 2-CH_2), 2.21 (3H, s, $COCH_3$), 2.76 (1H, dd, J=7.3, 15.6 Hz, CH_2), 3.19 (1H, dd, J=9.3, 15.6 Hz, CH_2), 4.9—5.0 (1H, m, CH), 3.93 (3H, s, OCH_3), 6.99 (1H, br s, NH), 7.33 (1H, d, J=6.3 Hz, C5-H), 7.74 (1H, d, J=6.3 Hz, C6-H).

Methyl 5-Acetylamino-6-chloro-3,4-dihydro-2-methyl-2*H*-1-benzopyran-8-carboxylate (18) Compound **16** (1.00 g, 3.80 mmol) was chlorinated with *N*-chlorosuccinimide in a similar procedure as employed in the synthesis of **10**[12] to give 1.07 g (94.7%) of **18** as a powder. mp 220—223 °C. ^{1}H-NMR ($CDCl_3$) δ: 1.44 (3H, d, J=6.4 Hz, 2-CH_3), 1.66, 1.98 (each 1H, m, C3-H), 2.24 (3H, s, CH_3CO), 2.6—3.0 (2H, m, C4-H), 3.87 (3H, s, CH_3), 4.26 (1H, m, C2-H), 6.98 (1H, br s, NH), 7.71 (1H, s, C7-H). HRMS *m/z*: Calcd for $C_{14}H_{16}ClNO_4$: 297.0768. Found: 297.0753.

5-Amino-6-chloro-2*H*-1-benzopyran-8-carboxylic Acid (12) A mixture of compound **11** (413 mg, 1.47 mmol) in 4 N NaOH (16 ml) was refluxe for 3 h. The cooled reaction mixture was neutralized with 4 N HCl, and the resultant precipitate was collected by fiiltration to give 13 mg (54.0%) of **12** as a powder. mp 163—165 °C. ^{1}H-NMR ($CDCl_3$) δ: 4.65 (2H, br s, NH_2), 4.90 (2H, dd, J=3.9, 2.0 Hz, CH_2), 5.95 (1H, dt, J=10.3, 3.9 Hz, C3-H), 6.48 (1H, dt, J=10.3, 1.9 Hz, C4-H), 7.95 (1H, s, C7-H), 10.3 (1H, br, OH). IR (KBr) cm^{-1}: 1714, 1645 (CO). HRMS *m/z*: Calcd for $C_{10}H_8ClNO_3$: 225.6311. Found: 225.6308.

5-Amino-6-chloro-3,4-dihydro-2*H*-1-benzopyran-8-carboxylic Acid (14) Compound **13** (1.98 g, 6.98 mmol) was similarly hydrolyzed with alkali to give 1.48 g (93.2%) of **14** as a powder. mp 231 °C (dec.). ^{1}H-NMR ($CDCl_3$) δ: 2.18 (2H, m, C3-H), 2.54 (2H, t, J=6.8 Hz, C4-H), 4.37 (2H, t, J=5.1 Hz, C2-H), 4.52 (2H, br s, NH_2), 7.98 (1H, s, C7-H), 10.5 (1H, s, COOH). IR (KBr) cm^{-1}: 1708, 1630 (CO). HRMS *m/z*: Calcd for $C_{10}H_{10}ClNO_3$: 227.0349. Found: 227.0352.

5-Amino-6-chloro-3,4-dihydro-2-methyl-2*H*-1-benzopyran-8-carboxylic Acid (19) Compound **18** (1.00 g, 3.34 mmol) was similarly hydrolyzed with alkali to give 686 mg (85.1%) of **19** as a powder. mp 263 °C (dec.). ^{1}H-NMR ($CDCl_3$) δ: 1.53 (3H, d, J=6.4 Hz, 2-CH_3), 1.87, 2.19 (each 1H, m, C3-H), 2.56 (2H, m, C4-H), 4.33 (1H, m, C2-H), 4.51 (2H, br s, NH_2), 7.99 (1H, s, C7-H), 10.7 (1H, s, COOH). IR (KBr) cm^{-1}: 1708, 1628 (CO). HRMS *m/z*: Calcd for $C_{11}H_{12}ClNO_3$: 241.0506. Found: 241.0509.

5-Amino-*N*-[2-(1-azabicyclo[3.3.0]octan-5-yl)ethyl]-6-chloro-2*H*-1-ben-

zopyran-8-carboxamide (21) 1,1-Carbonyldiimidazole (71.8 mg, 0.443 mmol) was added to a solution of **12** (100 mg, 0.443 mmol) in dry THF (1.0 ml) portionwise, and the mixture was stirred for 1 h. Then, a solution of 5-(2-aminoethyl)-1-azabicyclo[3.3.0]octane (**20**)[14] (62.8 mg, 0.443 mmol) in dry THF (0.2 ml) was added to it. The whole was refluxed for 1 h, cooled, and concentrated *in vacuo*, The residue was dissolved in $CHCl_3$. The solution was washed with saturated $NaHCO_3$ and then water, and concentrated *in vacuo*. The residue was purified by alumina column chromatography ($CHCl_3$) to give 135 mg (84.4%) of **21** as a powder, which was converted to the hemifumarate in the usual manner.[12] **21** hemifumarate: mp 216 °C (dec.). ^{1}H-NMR (DMSO-d_6) δ: 1.6—1.9 (10H, m, CH_2), 2.5—2.6, 2.9—3.1, 3.4—3.5 (each 2H, m, CH_2), 4.84 (2H, dd, J=3.9, 2.0 Hz, C2-H), 5.50 (2H, br s, NH_2), 5.6—5.9 (1H, m, C3-H), 6.2—6.4 (1H, m, C4-H), 6.45 (1H, s, CH, fumaric acid), 7.60 (1H, s, C7-H), 8.43 (1H, s, CONH). *Anal.* Calcd for $C_{19}H_{24}ClN_3O_2 \cdot 1/2C_4H_4O_4$: C, 60.07; H, 6.24; N, 10.01. Found: C, 59.89; H, 6.12; N, 9.87. IR (KBr) cm^{-1}: 3387(NH), 1621(CO). EIMS *m/z*: 361 (M^+), 110 (base peak).

5-Amino-*N*-[2-(1-azabicyclo[3.3.0]octan-5-yl)ethyl]-6-chloro-3,4-dihydro-2*H*-1-benzopyran-8-carboxamide (22) Compound **14** (1.00 g, 4.39 mmol) was similarly converted to 1.60 g (100%) of **22** as a powder, which was converted to the hemifumarate in the usual manner. **22** hemifumarate: mp 221 °C (dec.). ^{1}H-NMR (DMSO-d_6) δ: 1.6—2.0 (12H, m, CH_2), 2.4—2.5, 2.7—2.8, 3.2—3.3, 3.3—3.4 (each 2H, m, CH_2), 4,19 (2H, t, J=5.1 Hz, C2-H), 5.50 (2H, br s, NH_2), 6.46 (1H, s, CH, fumaric acid), 7.62 (1H, s, C7-H), 8.40 (1H, s, CONH). *Anal.* Calcd for $C_{19}H_{26}ClN_3O_2 \cdot 1/2C_4H_4O_4$: C, 59.78; H, 6.69; N, 9.96. Found: C, 59.66; H, 6.68; N, 9.71. IR (KBr) cm^{-1}: 3395 (NH), 1635, 1621 (CO). EIMS *m/z*: 363 (M^+), 110 (base peak).

5-Amino-*N*-[2-(1-azabicyclo[3.3.0]octan-5-yl)ethyl]-6-chloro-3,4-dihydro-2-methyl-2*H*-1-benzopyran-8-carboxamide (23) Compound **19** (100 mg, 0.413 mmol) was similarly converted to 135 mg (90.1%) of **23** as a powder, which was converted to the hemifumarate in the usual manner. **23** hemifumarate: mp 220 °C (dec.). ^{1}H-NMR (DMSO-d_6) δ: 1.39 (3H, d, J=6.4 Hz, 2-CH_3), 1.5—2.1 (12H, m, CH_2), 2.4—3.5 (8H, m, CH_2), 4.1—4.2 (1H, m, C2-H), 5.51 (2H, br s, NH_2), 6.45 (1H, s, CH, fumaric acid), 7.60 (1H, s, C7-H), 8.02 (1H, s, CONH). *Anal.* Calcd for $C_{20}H_{28}ClN_3O_2 \cdot 1/2C_4H_4O_4$: C, 60.61; H, 6.64; N, 9.94. Found: C, 60.56; H, 6.68; N, 9.71. IR (KBr) cm^{-1}: 3384 (NH), 1635, 1621 (CO). EIMS *m/z*: 377 (M^+), 110 (base peak).

Serotonin 5-HT$_4$ Receptor Agonistic Activity The 5-HT$_4$ agonistic activity was tested by using the methodology of Baxter *et al.*[4] Briefly, the tunica muscularis mucosae (TMM) preparation was obtained from rat esophagus, and the responses to the cumulative addition of the compounds were expressed as percent relaxation of the carbachol-induced tone. The potency of agonistic activity was estimated in terms of the concentration giving 50% relaxation (EC_{50}).

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