RECEPTOR-BASED 3D QSAR ANALYSIS OF SEROTONIN 5-HT$_{1D}$ RECEPTOR AGONISTS

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A three-dimensional quantitative structure–activity relationship study (3D QSAR) has been successfully applied to explain the binding affinities for the serotonin 5-HT$_{1D}$ receptor of a triptan series. The paper describes the development of a receptor-based 3D QSAR model of some known agonists and recently developed triptans on the 5-HT$_{1D}$ serotonergic receptor, showing a significant correlation between predicted and experimentally measured binding affinity (pIC$_{50}$). The pIC$_{50}$ values of these agonists are in the range from 5.40 to 9.50. The ligand alignment obtained from dynamic simulations was taken as basis for a 3D QSAR analysis applying the GRID/GOLPE program. 3D QSAR analysis of the ligands resulted in a model of high quality (r^2 = 0.9895, q^2_{LOO} = 0.7854). This is an excellent result and proves both the validity of the proposed pharmacophore and the predictive quality of the 3D QSAR model for the triptan series of serotonin 5-HT$_{1D}$ receptor agonists.

Keywords: Serotonin 5-HT$_{1D}$ receptor agonist; 3D QSAR; Molecular modeling; GRID/GOLPE; Indoles; Azoles; 5-Hydroxytryptamine; Triptans.

Our knowledge and understanding of the serotonin (5-hydroxytryptamine, 5-HT) receptor system has been revolutionized in recent years through the extensive use of modern molecular biology and traditional biochemical and pharmacological techniques. In addition, the heterogeneity of this receptor (super)family[1] offers the possibility of discovering selective ligands for each of these receptor subtypes to further delineate their role in several clinical disorders[2,3]. 5-HT receptors have been classified into seven main families, 5-HT$_{1–7}$, including 15 different subtypes[4]. With the exception of the 5-HT$_3$ receptor, the other members have been shown, or are considered, to be part of the G-protein coupled receptor (GPCR) superfamily. Of the 5-HT receptor families, the 5-HT$_1$ group appears to be the most complex with the existence of at least four subtypes: 5-HT$_{1A}$, 5-HT$_{1B}$, 5-HT$_{1D}$, and 5-HT$_{1E}$ [5]. 5-HT$_{1D}$ receptors are located primarily in the CNS, but are also found in vascular

smooth muscle, mediating contraction. Despite a decade of research the precise involvement of the various receptors in the mechanism of action of the 5-$HT_{1B/1D}$ agonists is still unclear[6], though more information is expected from the clinical testing of selective 5-HT_{1D} receptor agonists[7]. Serotonin 5-HT_{1D} receptor agonists are generally called triptans (Fig. 1). In recent years several 5-$HT_{1B/1D}$ receptor agonists have been developed by pharmaceutical companies as antimigraine agents[3,8,9]. The first of these was sumatriptan[10], which has been followed by a series of other triptans[11]. These molecules such as naratriptan (log *P*: 1.40 ± 0.63) and zolmitriptan (log *P*: 1.76 ± 0.37) are in general more lipophilic than sumatriptan (log *P*: 0.79 ± 0.62) and may have improved clinical effectiveness as a result of increased blood-brain barrier penetration as well as activation of $HT_{1B/1D}$ receptor or other receptors within the CNS[12]. Although there have been many modeling studies of GPCRs, very few of them examine the agonist binding site and SAR on 5-HT_{1D} receptor ligands[13–16]. The first step in a 3D QSAR study is the generation of a reliable pharmacophore model. Many strategies have been reported for this purpose in the literature[17]. This study attempts to produce a molecular model for the $5HT_{1D}$ receptor agonist binding site and to investigate the correlation between ligand-receptor interaction and biological activity. The 5-HT_{1D} receptor affinities of the investigated compounds were taken from recent publications[8,18,19].

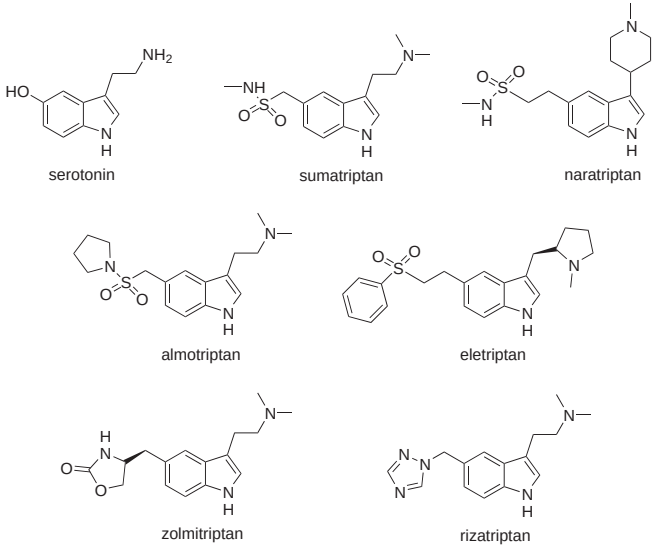
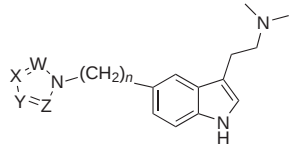


FIG. 1
Structures of some agonists for 5-HT_{1D} receptor

METHODS

Data Set

For this investigation, structure–activity data for a series of 13 serotonin 5-HT_{1D} receptor agonists, originally reported by Street et al.[8,18] were selected. In addition, the following well-known serotonin 5-HT_{1D} receptor agonists were taken into consideration: almotriptan, eletriptan, naratriptan, rizatriptan, sumatriptan and zolmitriptan[8,19]. The molecular structures of all agonists are shown in Fig. 1 and Table I. The binding affinities (pIC_{50}) values of these compounds are spread in a range from 5.40 to 9.50 and therefore allow to perform a sound 3D QSAR analysis (Table II).



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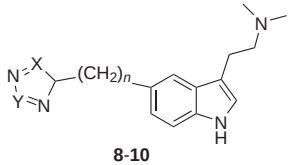

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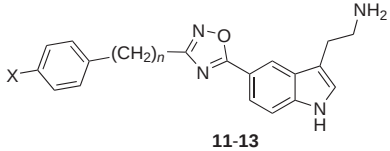

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TABLE I
Chemical structure of studied compounds **1**–**13**

Compound	*n*	W	X	Y	Z
1	1	C	N	C	C
2	1	CH_3C	N	C	C
3	1	N	C	C	N
4	1	N	N	N	C
5	1	N	N	C	N
6	0	N	C	N	C
7	0	CH_3C	N	C	C
8	0	–	O	H_2N–C	–
9	1	–	S	H_2N–C	–
10	1	–	NH	N	–
11	1	–	CH_3O	–	–
12	1	–	CH_3SO_2NH	–	–
13	0	–	CH_3NHCO	–	–

Molecular Modeling

The molecular modeling studies were performed on a Silicon Graphics Indigo2 R10000 using the SYBYL 6.5 software package[20]. The nitrogen of the side chain was always taken protonated to imitate physiological conditions. Partial atomic charges were calculated with the MOPAC/AM1 protocol. For energy minimizations and conformational analyses, the SYBYL force field was employed using the steepest descent method (500 steps) and followed by conjugate gradient to a gradient of 0.05 kcal/mol Å. Since no rigid template was available for the conformational analysis; the conformationally restricted (*R*)-eletriptan and (*S*)-zolmitriptan were chosen as

TABLE II
Experimentally determined activities and predicted activities of GOLPE

Compound	Experimental pIC_{50} value[a]	Predictive pIC_{50} value
Almotriptan	7.39	7.54
Eletriptan	8.44	7.87
Naratriptan	7.87	7.94
Rizatriptan	7.30	7.69
Sumatriptan	7.70	7.47
Zolmitriptan	8.72	8.12
1	7.50	7.56
2	7.20	7.42
3	6.60	6.96
4	7.40	7.40
5	7.40	7.30
6	7.70	7.67
7	8.10	7.63
8	7.10	6.97
9	8.70	8.23
10	5.40	6.71
11	9.10	8.66
12	9.50	8.99
13	9.10	8.98

[a] Data from refs[8,18,19].

templates for identifying the 5-HT_{1D} receptor binding conformations of the other agonists.

Extensive conformational analyses were performed to determine the putative binding conformations of the ligands. For each compound, rotatable bonds were assigned and a systematic conformational search was made, allowing the bonds to rotate stepwise with a 30° increment of dihedral angles. The low-energy conformers with extended geometry of the basic side chain were chosen.

After full minimization by the SYBYL force field (steepest descent), optimized geometries of these compounds were obtained and then aligned using the fit atom protocol in SYBYL. As fit points used for the ligand superposition, the side chain nitrogen, the indole nitrogen and the 5 position of the indole ring were chosen. These energy-minimized and superimposed structures were used for the docking study.

For the docking we have used a 3D model of the transmembrane domain of the human 5-HT_{1D} receptor which is based on the crystal structure of bovine rhodopsin. The amino acid sequence of the human 5-HT_{1D} receptor was extracted from Swissprot database[21]. The side chains were initially created using the homology utility of the InsightII molecular modeling package. All ligands were docked into the receptor manually. For this purpose Asp118 was taken as main anchor point. Additional contacts of the ligands with Thr202 as well as some aromatic amino acids from helices 3 and 7 were tried. The initial complexes then have been energy-minimized using steepest descent. Subsequently, molecular dynamics simulations (MDS), performed in vacuum by using the GROMACS 1.6 software, were utilized to find energetically favourable bioactive conformations of the ligands inside the putative binding pocket. The system was treated with backbone position restraints on the peptide for 100 ps. During MDS, the temperature was kept at T_0 = 310 K. The structures were saved for every ps. The ligand–receptor complexes obtained after MDS were optimized again by using the steepest descent. To carry out QSAR analysis, all compounds obtained after MDS were extracted from the receptor.

3D QSAR Analysis

We have used the GRID/GOLPE method to perform a 3D QSAR analysis[22]. The interaction field between the ligands and an alkyl OH probe was calculated using the GRID program[23] employing a grid spacing of 1 Å. The size of grid box used for calculation was defined in such a way that it extended approximately 4 Å beyond each of the molecules in each dimension. The in-

teraction energies obtained between each compound and the probe as well as the experimentally determined binding affinities at the serotonin 5-HT_{1D} receptor served as input for the GOLPE program[24]. The preliminary model contained 12 101 *x* variables for each compound (interaction values, *x* variables; affinities, *y* variable). Most of these variables are not meaningful for the explanation of the differences in affinity and introduced noise into the statistical PLS (partial least square) analysis. This noise was eliminated during the data pretreatment procedure by variable selection and the number of *x* variables was reduced form 12 101 per ligand to 6050. Since many grid points still do not contain information with relevance to biological data, the D-optimal preselection approach was applied which selects the most informative variables only. This method enabled a further reduction of *x* variables to 1512. In the last step, a fractional factorial design (FFD) procedure was employed to optimize the predictability of the model. The final model contains 1079 *x* variables and three principal components.

Cross validation of the model was made using the leave-one-out (LOO) method. In this procedure, one compound at a time is excluded from the data set for the generation of a new model and its affinity is predicted based on this new model. The model building and prediction cycle is repeated until each compound was left out once. A predictive correlation coefficient q^2 is calculated from the correlation between experimental and predicted pIC_{50} values.

RESULT AND DISCUSSION

The present work presents the first attempt to include some triptan molecules in a 3D QSAR model for serotonin 5-HT_{1D} receptor. The aim of this study was to obtain more detailed information on the interaction of ligands with the receptor and on their selectivity.

For this investigation, a series of 19 serotonin 5-HT_{1D} agonists were considered. Six of the compounds studied are in therapeutic use against migraine. Thirteen of them, **1**–**13**, are taken from recent publications. Examination of the structure of the agonists led us to hypothesize that the structural features necessary for binding to the $5HT_{1D}$ receptor site comprise one aromatic ring system, a basic nitrogen atom under protonated physiological conditions and a hydrogen bond acceptor function which all the ligands have in common. The three mentioned pharmacophoric elements correspond with the main characteristic features of the physiological substrate serotonin and very probably are involved in binding as well as activation of the 5-HT_{1D} receptor. As all G-protein- coupled receptors, also the 5-HT_{1D} re-

ceptor contains a conservative aspartic acid (Asp118) in helix 3 which may be engaged in a hydrogen-bond-enforced electrostatic interaction with the basis nitrogen of the serotoninergic agonists[14,21].

Similar to serotonin, all serotonin receptor agonists contain an aromatic ring at a certain distance from the basic nitrogen atom, capable of forming hydrophobic interactions[15]. The docking of the agonists in the model of the human 5-HT_{1D} receptor was carried out manually. The ligands were initially positioned in such a way that the interaction between their functional groups and the appropriate amino acid side chains, presumably primarily involved in the binding process, could be formed.

Initial molecular dynamics simulations of the complexes between the triptans and the model of 5-HT_{1D} receptor have allowed us to define those amino acid side chains of the receptor that interact with proposed pharmacophore elements of the molecules. Figure 2 shows the superimposed lowest energy complexes of the six studied triptans found as result of the dynamics simulations. As can be seen in Fig. 2, all studied triptans were always located between helices H3, H5, H6, and H7 roughly occupying the same section of space. Figure 3 illustrates in more detail the binding positions and interactions with particular amino side chains for representative agonists (sumatriptan, eletriptan and rizatriptan).

All ligands have the following binding contacts in common: (i) the ionic interaction between the protonated nitrogen and the carboxylate group of Asp118 (H3), (ii) a hydrogen bond between an electronegative element of the ligand and the hydroxy group of Thr202 (H5), (iii) the interaction of the electron-rich π systems of Trp114 (H3), Trp343 (H7), Tyr346 (H7) which

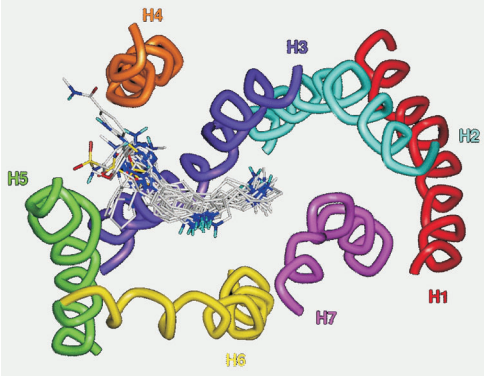

FIG. 2
The superposition of the investigated agonists in the 5-HT_{1D} receptor

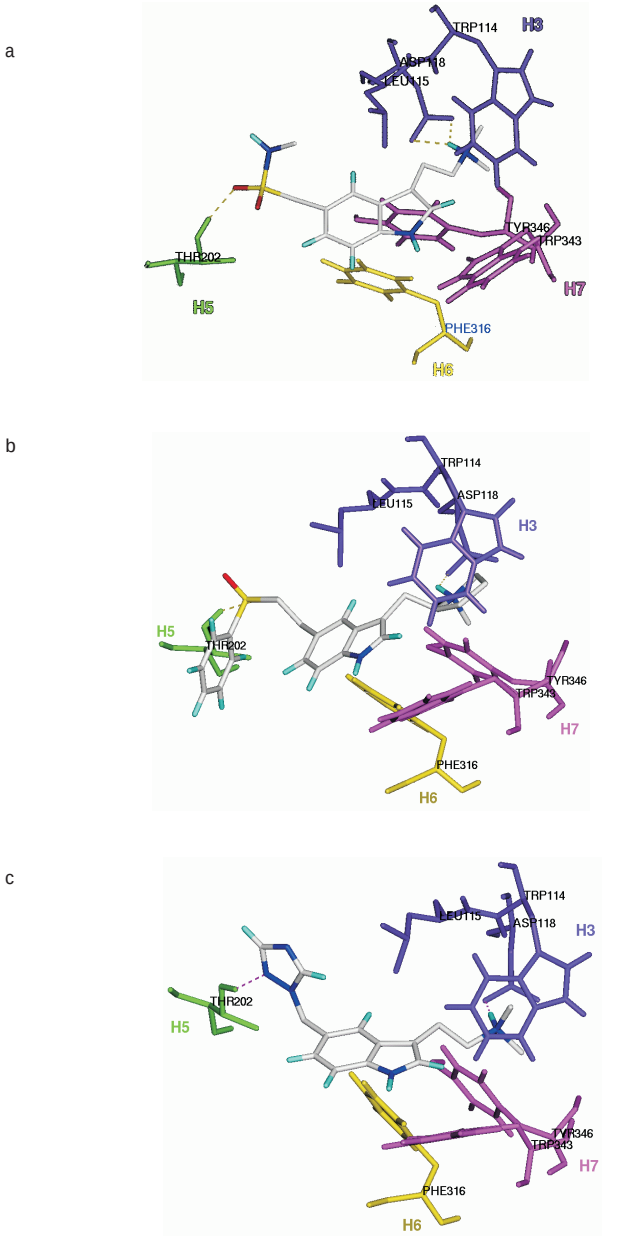

FIG. 3

The active side of the 5-HT_{1D} receptor showing the amino acid residues which interact with: a sumatriptan, b eletriptan, c rizatriptan as representative molecules

form a sort of aromatic cage and the positively charged aliphatic carbon atoms adjacent to the protonated nitrogen atom of the ligand (ion stabilization) (iv) the π–π stacking interaction between the indole ring system of the ligand and the benzene ring of Phe316 (H6) and the dispersive interaction with Leu115 (H3). The presented binding site model is almost identical with that of Hibert et al.[25] which also incorporates the aspartate on helix 3 and threonine of helix 5 as binding partners of the HT_{1D} agonists. However, in the model of Hibert the aromatic portion of the agonists is interacting with phenylalanine on helix 5 whereas the model presented here suggests that this residue is directed away from the central binding cleft towards helix 6, where it stabilizes the position of phenylalanine on helix 6, which does interact directly with the indole moiety of the agonists.

For the subsequently performed 3D QSAR analysis, the docked and superimposed agonists were extracted from the receptor binding pocket. The result of the statistical analysis yielded a correlation between physicochemical properties of the molecules and their binding affinities at the 5-HT_{1D} receptor. The GRID/GOLPE method was applied using the hydroxy probe. The 3D QSAR model is based on a set of 19 compounds. The resulting model shows a high LOO cross-validated correlation (q^2 = 0.7854) and therefore is a useful tool for predicting the 5-HT_{1D} affinities of new agonists at this receptor. In addition, the model yielded a conventional r^2 of 0.9895 (three principal components) and a low SDEP value of 0.44 (Fig. 4). It ex-

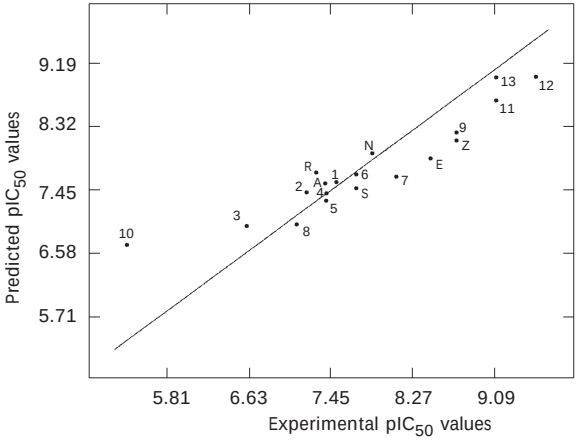

FIG. 4

Plot of the predicted pIC_{50} values versus the experimental pIC_{50} values for the 5-HT_{1D} GRID/GOLPE model (A, almotriptan; E, eletriptan; N, naratriptan; R, rizatriptan; S, sumatriptan; Z, zolmitriptan)

plains approximately 98% of the variance in ligand binding of the investigated compounds. Clearly, the theoretically predicted pIC_{50} values are in very good agreement with the experimentally determined values (Table II). The quality of the model is also illustrated in Fig. 4, which displays a plot between predicted and experimental pIC_{50} values for the whole set of compounds. Only compound **10** shows an inacceptable large difference between the predicted and experimentally determined affinity. This behavior might be explained by the acid character of the tetrazole structure which is deprotonated under physiological conditions. It can be imagined that this negatively charged fragment is not tolerated by the receptor in the same binding geometry as the other members of the series but causes a different binding mode of compound **10** which cannot be described by the QSAR model.

The study was initially undertaken to explore the binding geometries of known and new triptans at the $5HT_{1D}$ serotonergic receptor. It could be shown though that the combination of receptor modeling with 3D QSAR methods yields a much better explanation of qualitative as well as quantitative aspects of binding for the set of compounds studied. The receptor-based QSAR model is able to give detailed information about the character of the interaction sites in the binding pocket which determine the variance in biological activity. This information can be utilized for the design of structurally new 5 HT_{ID} agonists.

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REFERENCES

1. a) Zifa E., Fillion G.: *Pharmacol. Rev.* **1992**, *44*, 401; b) Hartig P. R., Adham N., Zgombick J., Weinshank R., Branchek T.: *Drug Dev. Res.* **1992**, *26*, 215; c) Beer M. S., Middlemiss D. N., McAllister G.: *Trends Pharmacol. Sci.* **1993**, *14*, 228; d) Teitler M.: *Med. Chem. Res.* **1993**, *3*, 273; e) Bruinvels A. T., Landwehrmeyer B., Probst A., Palacios J. M., Hoyer D.: *Mol. Brain Res.* **1994**, *21*, 19.
2. a) Langer S. Z., Brunello N., Racagni G. Mendlewicz J.: *Serotonin Receptor Subtypes: Pharmacological Significance and Clinical Implications*. Karger, Basel 1992; b) Olivier B., Mos J., van der Heyden J. A. M., Molewijk H. E., van Dijken H. H., Zethof T., Hest A. v., Tulp M. Th. M., Slangen J. L. in: *Trends in Receptor Research* (V. Claassen, Ed.), p. 97. Elsevier, Amsterdam 1993.
3. Napier C., Stewart M., Melrose H., Hopkins B., McHarg A., Wallis R.: *Eur. J. Pharmacol.* **1999**, *368*, 259.

4. a) Hoyer D., Martin G.: *Neuropharmacology* **1997**, *36*, 419; b) Uphouse L.: *Neurosci. Biobehav. Rev.* **1997**, *21*, 679; c) Saxena P. R., De Vries P., Villalon C. M.: *Trends Pharmacol. Sci.* **1998**, *19*, 311.
5. a) Leonhardt S., Herrick-Davis K., Titeler M.: *J. Neurochem.* **1989**, *53*, 465; b) Parsons A. A.: *Trends Pharmacol. Sci.* **1991**, *12*, 310.
6. Goadsby P. S.: *Drugs* **1999**, 361.
7. Jandu K. S., Barret V., Bruckwell M., Cambridge D., Farrant D. R., Foster C., Giles H., Glen R. C., Hill A. P., Hobbs H., Honey A., Martin G. R., Salmon J., Smith D., Wollard P., Selwood D. L.: *J. Med. Chem.* **2001**, *44*, 681.
8. Street L. J., Baker R., Davey W. B., Guiblin A. R., Jelley R. A., Reeve A. J., Routledge H., Sternfeld F., Watt A. P., Beer M. S., Middlemiss D. N., Noble A. J., Stanton J. A., Scholey K., Hargreaves R. J., Sohal B., Graham M. I., Matassa V. G.: *J. Med. Chem.* **1995**, *38*, 1799.
9. a) Martin G. R.: *Cephalalgia* **1997**, *17*, 4; b) Connor H. E., Feniuk W., North P. C., Oxford A. W., Humphrey P. P. A.: *Cephalalgia* **1997**, *17*, 145; c) Rabasseda X.: *Drugs Today* **2001**, *37*, 5.
10. Stewart W. F., Lipton R. B., Celentano D. D., Reed M. L.: *J. Am. Med. Assoc.* **1992**, *267*, 64.
11. a) Yevich J. P., Yocca F. D.: *Curr. Med. Chem.* **1997**, *4*, 295; b) Bou J., Domenech T., Puig J., Heredia A., Gras J., Fernandez-Forner D., Beleta J., Palacios J. M.: *Eur. J. Pharmacol.* **2000**, *410*, 33; c) Johnson D. E., Rollema H., Schmidt A. W., McHarg A. D.: *Eur. J. Pharmacol.* **2001**, *425*, 203.
12. Hoskin K. L., Goadsby P. J.: *Exp. Neurol.* **1998**, *150*, 45.
13. a) Trumpp-Kallmeyer S., Bruinvels A., Hoflack J., Hibert M.: *Neurochem. Int.* **1991**, *19*, 397; b) Castro J. L., Baker R., Guiblin A. R., Hobbs S. C., Jenkins M. R., Russel M. G. N., Beer M. S., Stanton J. A., Scholey K., Hargreaves R. J., Graham M. I., Matassa V. G.: *J. Med. Chem.* **1994**, *37*, 3023.
14. Glen R. C., Martin G. R., Hill A. P., Hyde R. M., Woollard P. M., Salmon J. A., Buckingham J., Robertson A. D.: *J. Med. Chem.* **1995**, *38*, 3566.
15. Bremner D. H., Ringan N. S., Wishart G.: *Eur. J. Med. Chem.* **1997**, *32*, 59.
16. Chambers M. S., Street L. J., Godacre S. W., Hobbs S. C., Hunt P., Jelley R. A., Matassa V. G., Reeve A. J., Sternfeld F., Beer M. S., Stanton J. A., Rathbone D., Watt A. P., MacLeod A. M.: *J. Med. Chem.* **1999**, *42*, 691.
17. a) Kubinyi H.: *3D QSAR in Drug Design: Theory, Methods and Applications*. ESCOM, Leiden 1993; b) Höltje H.-D., Folkers G.: *Molecular Modeling: Basic Principles and Aplications*. VCH, Weinheim 1997.
18. Street L. J., Baker R., Castro J. L., Chambers M. S., Guiblin A. R., Hobbs S. C., Matassa V. G., Reeve A. J., Beer M. S., Middlemiss D. N., Noble A. J., Stanton J. A., Scholey K., Hargreaves R. J.: *J. Med. Chem.* **1993**, *36*, 1529.
19. Deleu D., Hanssens Y.: *J. Clin. Pharmacol.* **2000**, *40*, 687.
20. SYBYL, version 6.5. TRIPOS Assoc., St. Louis (MO), USA 2002.
21. Hamblin M. W., Metcalf M. A.: *Mol. Pharmacol.* **1991**, *40*, 143.
22. a) Cruciani G., Watson K. A.: *J. Med. Chem.* **1994**, *37*, 2589; b) Pastor M., Cruciani G., Clementi S.: *J. Med. Chem.* **1997**, *40*, 1455; c) Goodford P. J.: *J. Med. Chem.* **1985**, *28*, 849.
23. *GRID*, version 16. Molecular Discovery Ltd., Oxford 1985.
24. *GOLPE*, version 4.0. Multivariate Infometric Analysis, Perugia 1999.
25. Hibert M. F., Trumpp-Kallmeyer S., Bruinvels A., Hoflack J.: *Mol. Pharmacol.* **1991**, *40*, 8.