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Study of the Interaction Between Aryloxypropanolamines and Asn386 in Helix VII of the Human 5-Hydroxytryptamine$_{1A}$ Receptor

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SUMMARY

We studied the stereoselective interaction between aryloxypropanolamines and the human 5-hydroxytryptamine$_{1A}$ (5-HT$_{1A}$) receptor. *R*- and *S*-enantiomers of propranolol, penbutolol, and alprenolol were investigated for their ability to bind to human 5-HT$_{1A}$ wild-type and Asn386Val mutant receptors. Asn386 seemed to act as a chiral discriminator. Although both aryloxypropanol enantiomers displayed lower affinity for the mutant receptors, the affinities for the *S*-enantiomers were more affected. Receptor affinities of other structurally unrelated 5-HT$_{1A}$ ligands were not decreased by the mutation of Asn386 to valine. In addition, a series of analogues of propranolol with structural variation in the oxypropanolamine moiety was synthesized, and affinities for wild-type and Asn386Val mutant 5-HT$_{1A}$ receptors were determined. Both the hydroxyl and the ether oxygen atoms of the oxypropanol moiety seem to be required for binding at wild-type 5-HT$_{1A}$ receptors. The hydroxyl group of propranolol probably directly interacts with Asn386. The ether oxygen atom may be important for steric reasons but can also be involved in a direct interaction with Asn386. These findings are in agreement with the interactions of aryloxypropanolamines with Asn386 in rat 5-HT$_{1A}$ receptors that we previously proposed. The loss of affinity for propranolol by the Asn386Val mutation could be regained by replacement of the hydroxyl group of the ligand by a methoxy group. This modification of the propranolol structure has no effect on the affinity of both enantiomers for the wild-type 5-HT$_{1A}$ receptor, which provides an alternative hypothesis for the interaction of Asn386 with the oxypropanol oxygen atoms. According to this novel hypothesis, the oxypropanol oxygen atoms may both act as hydrogen bond acceptors from the NH$_2$ group of Asn386.

Aryloxypropanolamines, such as pindolol **1** and propranolol **2** (Fig. 1), are well known for their ability to antagonize β-adrenoceptor activity (1). Many members of this class also display considerable affinity for the 5-HT$_{1A}$ and the rodent 5-HT$_{1B}$ receptor subtypes (2–4). An asparagine residue in the putative helix VII of these receptors was shown to play a pivotal role in aryloxypropanolamine binding in a number of studies (5–9). Replacement of this essential asparagine abolishes the interaction with aryloxypropanolamines, which results in a dramatic loss of affinity. For example, replacement of the Asn386 by valine in the human 5-HT$_{1A}$ receptor severely reduced its affinity for pindolol **1** but hardly affected the affinity for the neurotransmitter serotonin (5-HT, **3**) (5). Moreover, introduction of this asparagine in receptors with low affinity may dramatically increase aryloxypropanolamine binding. This is illustrated by the respective mutations of phenylalanine and threonine in asparagine in the corresponding positions in α_2-adrenoceptors (4) and 5-HT$_{1D\beta}$ receptors (7, 8). In these studies, the asparagine in helix VII was identified as a very important difference between α_2-adrenoceptors and 5-HT$_{1D\beta}$ receptors, on one hand, and between β_2-adrenoceptors and rodent 5-HT$_{1B}$ receptors, on the other hand. Also, in 5-HT$_{1D\alpha}$, 5-HT$_{1E}$, and 5-HT$_{1F}$ receptors, the introduction of an asparagine at the position corresponding with that of asparagine 386 in the human 5-HT$_{1A}$ receptor increases affinity for pindolol **1** and propranolol **2** dramatically (9). Sequence analyses show that the asparagine in helix VII is the only residue that is present in all receptors with high affinity for pindolol **1** and propranolol **2** and is absent in all receptors with low affinity (10).

We previously reported a model of pindolol **1** docked into the 5-HT$_{1A}$ receptor (11), based on the high-resolution structure of bacteriorhodopsin (12). Despite the apparent flaws of bacteriorhodopsin-based G protein-coupled receptor models, our 5-HT$_{1A}$-receptor model was shown to agree very well

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ABBREVIATIONS: 5-HT, 5-hydroxytryptamine; 8-OH-DPAT, 8-hydroxy-2-dipropylaminotetralin; MeOH, methanol; EtOAc, ethyl acetate; DMSO, dimethylsulfoxide.

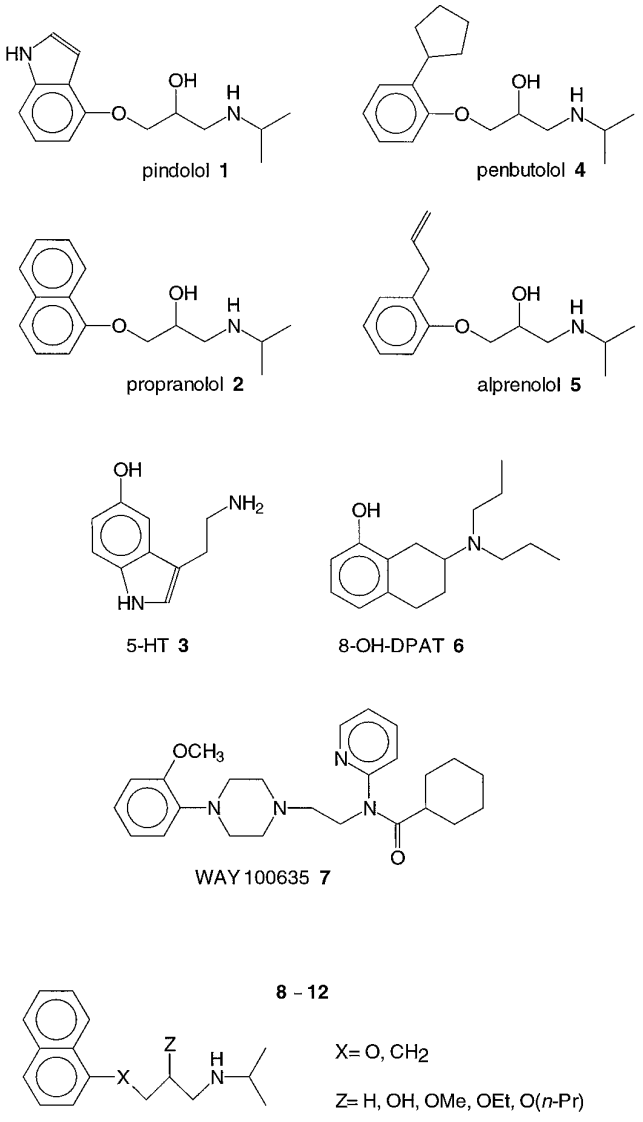


Fig. 1. Structures of 5-HT_{1A} receptor ligands and variations in the propranolol structure that were investigated in this study.

with the corresponding structure-affinity relationships (11). Therefore, it was decided to investigate this model further, keeping in mind its possible limitations, and to test its predictive value by evaluation with experimental data. In the initial model, we were the first to hypothesize a double hydrogen bond interaction between the oxypropanol moiety and the amide group of Asn386. This interaction is schematically depicted for the potent compound (*S*)-penbutolol **4** in Fig. 2. In the present study, we examined the validity of this hypothetical interaction. For this purpose, the effect of modification of the putative interacting groups in both the ligands and the receptor was investigated. We synthesized a series of congeners of propranolol with structural variations in the oxypropanol moiety (Fig. 1). These propranolol derivatives, as well as both enantiomers of unmodified propranolol **2**, penbutolol **4**, and alprenolol **5**, were investigated for their ability to bind to wild-type human 5-HT_{1A} receptors and to corresponding Asn386Val mutant receptors (5). In addition, a three-dimensional model of the atomic coordinates of the Asn386Val mutant receptor was created from the previously

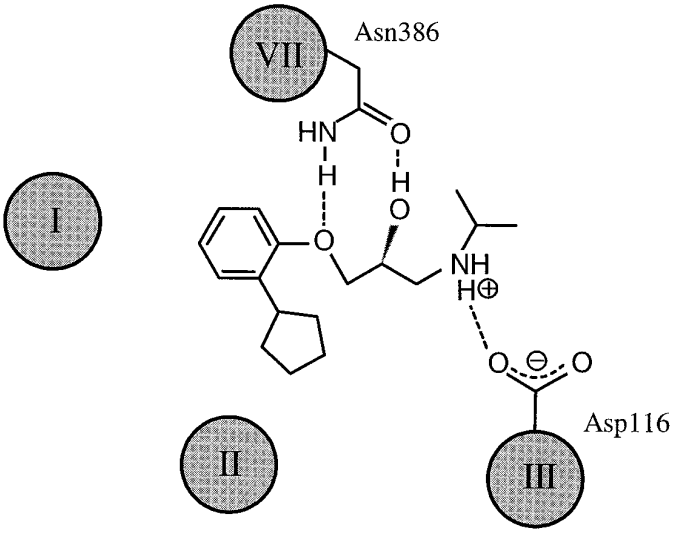


Fig. 2. Schematic representation of a hypothetical double hydrogen bonding interaction between Asn386 in the human 5-HT_{1A} receptor and the oxypropanol moiety of (*S*)-penbutolol **4**, as suggested by Kuipers *et al.* (11).

reported wild-type 5-HT_{1A} receptor model (11). An attempt was made to design a propranolol analogue with restored affinity for the mutant receptor using this model. 5-HT_{1A} ligands from classes other than aryloxypropanolamines were incorporated in the study for comparison. These ligands were the neurotransmitter serotonin (5-HT, **3**) and the 5-HT_{1A}-selective compounds 8-OH-DPAT **6** (the *R*-enantiomer being a full agonist) and WAY 100635 **7** (antagonist). For residue numbering, the slightly modified sequence reported by Chanda *et al.* (13) was used. As a consequence, the Asn385 referred to by Guan *et al.* (5) corresponds with Asn386 in this paper.

Materials and Methods

Chemistry

Melting points are uncorrected. ^{1}H-NMR spectra were recorded on a Bruker WP-200 or AM400 instrument. Chemical shifts (δ) are expressed in parts per million relative to internal tetramethyl silane; coupling constants (J) are expressed in Hertz. Elemental analyses were performed at the Mikroanalytisches Labor Pascher, Remagen-Bandorf, Germany. For flash chromatography, Merck silica gel type 60 (size 230–400 mesh) was used. Unless stated otherwise, the starting materials used were high-grade commercial products. All reactions were performed under a nitrogen atmosphere.

Compounds **2**, **3**, and **6** are commercially available. Compounds **4** and **5** were gifts from Hoechst (Amsterdam, The Netherlands) and Astra (Hässle, Mölndal, Sweden), respectively. Compound **7** (14) was synthesized and kindly provided by Dr. M. Mensonides (School of Pharmacy, Groningen, The Netherlands). The synthesis of compound **8** is described in Ref. 15, and that of compound **9** is described in Refs. 16 and 17. The physical data of previously reported compounds matched those given in the corresponding references.

The new compounds **10**, **11**, and **12** were obtained by a nucleophilic substitution reaction of propranolol **2** and the corresponding alkylbromides under basic conditions. The separate enantiomers (*R*)- and (*S*)-**10** were synthesized accordingly from the corresponding enantiomers of propranolol **2**.

1-(Isopropylamino)-3-(1-naphthyloxy)-2-methoxy-propane, (*E*)-2-butenedioate (compound 10). To a solution of 5.3 g (20.4 mmol) of racemic propranolol **2** in 85 ml of dimethylformamide,

0.82 g NaH (60% disp., 20.4 mmol) was added. The reaction mixture was stirred at 35–40° for 30 min. Then, drop-wise, 1.3 ml of MeI (20.4 mmol) was added, and the mixture was allowed to react at room temperature for 1 hr. Subsequently, the reaction mixture was poured out in 500 ml water and extracted with three 150-ml portions of EtOAc. The combined organic fractions were washed with 2 × 300 ml of water and dried on Na_2SO_4. Removal of the solvent *in vacuo* yielded 6.1 g of a light brown oil. Purification by flash chromatography (EtOAc/MeOH/triethylamine, 95:5:1) yielded 4.8 g (86%) of light brown product as a free base. The latter was converted in its fumarate salt by the addition of 2.1 g (18.0 mmol) of fumaric acid in 20 ml of absolute ethanol. The white crystals obtained were collected by filtration, washed with EtOAc and petroleum ether (40–60°), and dried over potassium hydroxide pellets. The yield was 5.33 g (78%) of racemic **10**; mp 140.5–142.5° [^{1}H-NMR (DMSO/$CDCl_3$ 4:1): δ 1.22 and δ 1.23 (2 × d, 6 **H**, NCH(CH_3)$_2$, J = 6); δ 3.08 (dd, 1 **H**, C(methoxy)C*H*N, J = 8 and 13); δ 3.18 (dd, 1 **H**, C(methoxy)C*H*N, J = 4 and 13); δ 3.2 (m, 1 **H**, NC*H*(CH_3)$_2$); δ 3.52 (s, 3 **H**, OCH_3); δ 4.04 (m, 1 **H**, C*H*OCH_3); δ 4.24 (dd, 1 **H**, napht-O-CH_2, J = 5 and 10); δ 4.33 (dd, 1 **H**, napht-O-CH_2, J = 4 and 10); δ 5.5–6.5 (broad band, interchangeable H); δ 6.57 (s, 2 **H**, fumarate HC═CH); δ 6.96 (d, 1 **H**, napht H-2, J = 7); δ 7.4 (t, 1 **H**, napht H-3, J = 7); δ 7.43–7.54 (cluster, 3 **H**, napht H-4, 6, 7); δ 7.84 (m, 1 **H**, napht H-5); δ 8.2 (m, 1 **H**, napht H-8). Anal. ($C_{17}H_{23}NO_2.C_4H_4O_4$) C, H, N].

For the syntheses of the separate *R*- and *S*-enantiomers of **10**, the same procedure was followed, taking the corresponding enantiomers of propranolol **2** as starting compounds. (*S*)-**10**: m.p. 175–176°; $[\alpha]^{25}_D$ −16° (c = 1.5 g/ml, MeOH). (*R*)-**10**: m.p. 175–176°; $[\alpha]^{25}_D$ +16° (c = 1.5 g/ml, MeOH). In contrast to the racemate, (*R*)- and (*S*)-**10** crystallized as the half-fumarate salts. ^{1}H-NMR spectra obtained for the racemate and both enantiomers were identical under the conditions used.

1-(Isopropylamino)-3-(1-naphthyloxy)-2-ethoxy-propane, (*E*)-2-butenedioate (compound 11). Compound **11** was obtained from the reaction of 5.2 g of racemic propranolol **2** (20 mmol) and 1.6 ml (21 mmol) of ethyl bromide, following the same procedure as for compound **10**. The yield was 2.92 g (36%) of **11** (white solid); mp 142.5–144.5°; [^{1}H-NMR (DMSO/$CDCl_3$ 4:1) δ 1.2 (cluster, 9H, CH_3); δ 3.02 (dd, 1 **H**, C(ethoxy)C*H*N, J = 8 and 13); δ 3.24 (cluster, 2 **H**, C*H*NC*H*(CH_3)$_2$); δ 4.09 (m, 1H, C*H*-ethoxy); δ 4.22 (dd, 1H, napht-O-CH_2, J = 5 and 10); δ 4.3 (dd, 1 *H*, napht-O-CH_2, J = 4 and 10); δ 4.6–5.8 (broad band, interchangeable H); δ 6.56 (s, 2 **H**, fumarate HC═CH); δ 6.95 (d, 1 **H**, napht H-2, J = 7); δ 7.39 (t, 1H, napht H-3, J = 7); δ 7.43–7.53 (cluster, 3 **H**, napht H-4, 6, 7); δ 7.83 (m, 1 **H**, napht H-5); δ 8.18 (m, 1 **H**, napht H-8). Anal. ($C_{18}H_{25}NO_2.C_4H_4O_4$0.0.10 H_2O) C, H, N].

1-(Isopropylamino)-3-(1-naphthyloxy)-2-n-propoxy-propane, (*E*)-2-butenedioate (compound 12). Compound **12** was obtained from the reaction of 5.2 g racemic propranolol **2** (20 mmol) with 1.9 ml (21 mmol) of *n*-propylbromide, following the same procedure as for compound **10**. The yield was 2.92 g (35%) of **12** (white solid); mp 125–127°; [^{1}H-NMR (DMSO/$CDCl_3$ 4:1) δ 0.9 (t, 3 **H**], $OC_2H_4CH_3$, J = 7); δ 1.2 (d, 6 **H**, NCH(CH_3)$_2$, J = 6); δ 1.59 (m, 2 **H**, $OCH_2CH_2CH_3$); δ 3.04 (dd, 1 **H**, C(OC_3H_7)C*H*N, J = 8 and 13); δ 3.1–3.24 (cluster, 2 **H**, C*H*NC*H*(CH_3)$_2$); δ 3.56–3.72 (cluster, 2 **H**, $OCH_2C_2H_5$); δ 4.08 (m, 1 **H**, C*H*OC_3H_7); δ 4.22 (dd, 1H, napht-O-CH_2, J = 5 and 10); δ 4.31 (dd, 1H, napht-O-CH_2, J = 4 and 10); δ 4.6–6.0 (broad band, interchangeable H); δ 6.57 (s, 2 **H**, fumarate HC═CH); δ 6.95 (d, 1 **H**, napht H-2, J = 7); δ 7.39 (t, 1 **H**, napht H-3, J = 7); δ 7.43–7.54 (cluster, 3 **H**, napht H-4, 6, 7); δ 7.83 (m, 1 **H**, napht H-5); δ 8.18 (m, 1 **H**, napht H-8). Anal. ($C_{19}H_{27}NO_2.C_4H_4O_4$0.0.10 H_2O) C, H, N].

Biochemistry

Mutagenesis and expression. The cloning and mutation of the human 5-HT_{1A} receptor was described before by Guan *et al.* (5) Both wild-type and mutant receptors were expressed in COS-7 cells by using the DEAE-dextran method. For the transfection, a kit (ProFection; Promega, Madison, WI) was used.

Ligand binding. After transfection (72 hr), the cells were scraped off the plates in Dulbecco's modified Eagle's medium (containing 5% fetal calf serum, 1% *l*-glutamine, and 1% penicillin/streptomycin) and washed two times with 50 mM Tris, pH 7.4, at 4°. The pellet was stored at +20°. Before use, the pellet was thawed quickly, diluted in 50 mM Tris, pH 7.4, containing 4 mM $CaCl_2$, and then homogenized 10–20 sec with an Ystral homogenizer. Ligand binding assays were conducted as previously described by Schlegel *et al.* (18). The competition binding studies to determine the K_i values were performed using 1 nM [^{3}H]-8-OH-DPAT. Nonspecific binding was determined with 10 μM 5-HT **3**. IC_{50} values were calculated using the Prism program (GraphPad Software, San Diego, CA) and converted to K_i values using a K_d value of 1.5 nM for [^{3}H]-8-OH-DPAT.

Modeling software and hardware. Small-ligand building and docking procedures and computations (MAXIMIN, MOPAC) were performed with the SYBYL package, version 6.1a (Tripos Associates, St. Louis, MO), running on a Silicon Graphics Iris Indigo Elan 4000 (Mountain View, CA). For MAXIMIN calculations (Tripos force field), the Powell method was chosen (default values).

Model building and docking. We used our model for the rat 5-HT_{1A} receptor, which we previously reported for docking studies of (*S*)-propranolol **2** and (*S*)-**10** (11). An Asn386Val mutant model was created from this wild-type receptor model by the straightforward replacement of Asn386 by a valine residue with the modify monomer command in the SYBYL BIOPOLYMER module. (*S*)-propranolol **2** in the wild-type receptor model, which was described in reference 11, was taken as a starting structure. A similar interaction of (*S*)-penbutolol **4** is schematically depicted in Fig. 2. Modification of the hydroxyl group was investigated at both the wild-type and the mutant receptors. The structure of (*S*)-propranolol **2** was converted in compound (*S*)-**10** by changing the hydroxyl group to methoxy using the sketch option in SYBYL. The complexes of the compounds (*S*)-**2** and (*S*)-**10** with the wild-type, and Asn386Val mutant receptor models were energy-minimized using molecular mechanics calculations (Tripos Force Field). For each docked compound, an active site was created that contained all side chains of residues within a distance of 4 Å from the ligand (e.g., Fig. 3). These side chains and the ligand itself were allowed to optimize their position and conformation; the backbone atoms were kept fixed. Initially, the hydrogen bond O—H distances between Asn386 and the ligand were constrained at 2.0 Å [force constant, 200 kcal/(mol·Å)^2]. The complexes were further minimized without these distance constraints.

Starting structures for the MOPAC AM1 calculations (19) of the complexes were obtained by extracting the Asn386 residue and the interacting ligands from the receptor models and through subsequent modification of Asn386 to an acetamide molecule using the sketch option in SYBYL. MOPAC AM1 calculations were performed on uncharged complexes of an acetamide molecule interacting with (*S*)-propranolol **2** and (*S*)-**10**, using default values, and of the separate molecules in these complexes (19). The additional keyword MMOK was used for calculations of molecules containing an amide moiety. The energy gain by complex formation was calculated as the heat of formation of the complex minus that of the separate components.

Results and Discussion

Structure Affinity Relationships

Effect of asparagine-to-valine mutation on affinity of 5-HT_{1A} reference compounds. From the results in Table 1, it seems that 5-HT **3**, the enantiomers of the 5-HT_{1A}-selective compound 8-OH-DPAT **6**, and the selective antagonist WAY 100635 **7** all have high affinity for the wild-type human 5-HT_{1A} receptor. The mutation of Asn386 to valine only slightly decreases affinity for these compounds, with the

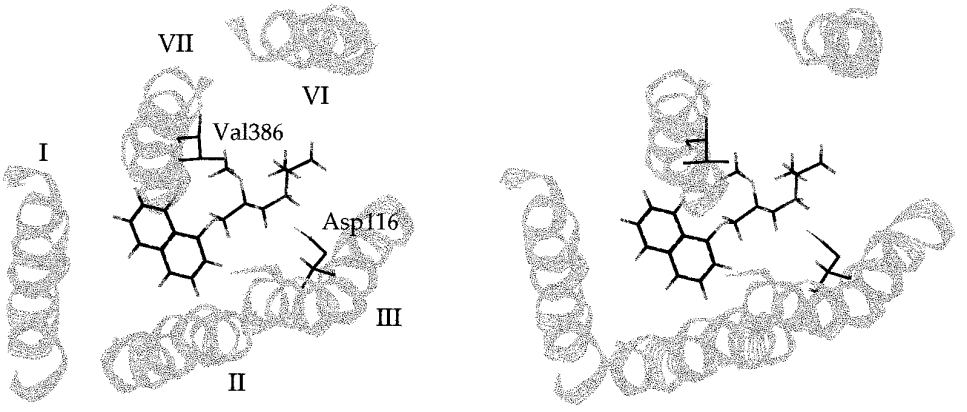


Fig. 3. Resulting position of (*S*)-**10** docked into the Asn386Val mutant receptor model, viewed from the extracellular side. The putative α-helical transmembrane domains I, II, III, VI, and VII are schematically represented in gray. The methoxy group of (*S*)-**10** has a favorable contact with Val386. For clarity, other residues have been omitted.

TABLE 1

K_i values for the displacement of [^{3}H]-8-OH-DPAT from human 5-HT$_{1A}$ wild-type receptors and corresponding Asn386Val mutant receptors by a series of reference compounds and enantiomers of aryloxypropanolamines

Compound	Number	K_i[a]		Mutant/wild-type ratio
		Wild-type	Asn386Val mutant	
		nM		
Reference compounds				
5-HT	**3**	3.3 ± 1.7	14 ± 6	4.2
8-OH-DPAT	(R)-**6**	3.8 ± 1.4	0.5 ± 0.2	0.13
	(S)-**6**	4.6 ± 2.4	9 ± 3	2.0
WAY-100635	**7**	2.8 ± 0.7	6.5 ± 1.1	2.3
Aryloxypropanolamines				
Penbutolol	(S)-**4**	3.3 ± 0.7	500 ± 100	151
	(R)-**4**	80 ± 20	3400 ± 1700	43
Alprenolol	(S)-**5**	60 ± 20	7200 ± 700	120
	(R)-**5**	5400 ± 1800	56000 ± 9000	10
Propranolol	(S)-**2**	160 ± 20	3600 ± 1000	23
	(R)-**2**	2100 ± 500	9800 ± 1600	4.7

[a] K_i values are based on 3 determinations, each using four to six concentrations in duplicate and are presented as mean ± standard error.

exception of the agonist (*R*)-8-OH-DPAT **6**. This enantiomer displays 8-fold higher affinity for the mutant than for the wild-type receptor. The valine at position 386 may provide an extra interacting group for (*R*)-**6**. In our 5-HT$_{1A}$ receptor model (11), one of the propyl chains of (*R*)-8-OH-DPAT **6** is directed toward helices VI and VII, although helix VII is somewhat distant for a direct contact between the propyl chain and the valine residue at position 386. A favorable contact of the latter with the propyl chain of the ligand may account for the higher affinity of (*R*)-**6** for the mutant receptor. The data from Table 1 show that the Asn386Val mutant is still capable of binding 5-HT$_{1A}$ compounds that are structurally unrelated to aryloxypropanolamines. Apparently, Asn386 is not essential for recognition of these compounds by 5-HT$_{1A}$ receptors.

Effect of asparagine-to-valine mutation on stereoselectivity of aryloxypropanolamines. In contrast to the low sensitivity of other structural classes, the affinity of aryloxypropanolamines is strongly affected by the Asn386Val mutation, especially that of the *S*-enantiomers (Table 1). For penbutolol **4**, the most potent of the compounds investigated, the affinity of the *S*-enantiomer is decreased 150-fold by this replacement. The affinity of the *R*-enantiomer is also decreased but less dramatically (42-fold). As a result, the stereoselectivity of the *S*- compared with the *R*-enantiomer of penbutolol **4** decreased from 24-fold at the wild-type to 6.8-fold at the mutant receptor. Similar effects are observed for the less potent compounds alprenolol **5** and propranolol **2**. The affinities for the *S*-enantiomers of these compounds decreased 120-fold and 22.5-fold, respectively, by the mutation of Asn386 to valine. The affinities of the corresponding *R*-enantiomers are less affected: 10-fold and 4.7-fold decreases for alprenolol **5** and propranolol **2**, respectively. Thus, the stereoselectivity of *S*- compared with *R*-enantiomers of alprenolol **5** is reduced from 90-fold at wild-type receptors to 7.8-fold at Asn386Val mutant receptors. A similar but less dramatic decrease in stereoselectivity, from 13-fold to 2.7-fold, was observed for propranolol **2**. Receptor stereoselectivity for *S*- compared with *R*-enantiomers decreased by the Asn386Val mutation because it affected the binding of (*S*)-aryloxypropanolamines more severely than that of the corresponding *R*-enantiomers. Apparently, Asn386 acts as a chiral discriminator for these compounds. The hydroxyl group of the *S*-enantiomers may be directly involved in the interaction with Asn386 in helix VII. The *R*-enantiomers probably bind in a similar region, because their affinities are also decreased by the Asn386Val mutation.

The results from Table 1 are in agreement with those obtained by Guan *et al.* (5) and confirm their conclusion that Asn386 is responsible for high 5-HT$_{1A}$ receptor affinity of aryloxypropanolamine racemates but is not required for binding other structural classes of compounds. In addition, we have shown that their observation for aryloxypropanolamine racemates is dominated by effects on the *S*-enantiomers.

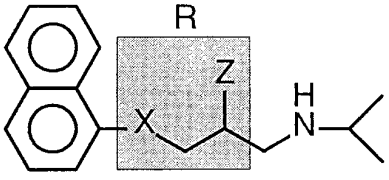
Effect of modification of the propranolol oxypropanol moiety. In compound **8**, the hydroxyl group of propranolol **2** has been removed (Table 2). This compound is a factor 9.4 times less potent than (*S*)-propranolol **2** at the wild-type human 5-HT_{1A} receptor, although its affinity for the mutant receptor is 2.8-fold higher than that of (*S*)-propranolol **2**. These results may be explained by the lack of a favorable interaction of the hydroxyl group with the asparagine group in the wild-type receptor and the absence of a unfavorable contact with the valine group at the corresponding position in the mutant receptor. These findings confirm that the hydroxyl group in (*S*)-propranolol **2** interacts with Asn386, probably via a hydrogen bond. In contrast, the hydroxyl group of (*R*)-propranolol **2** seems unfavorable for interaction with both the wild-type and the mutant receptors. Its removal, as in compound **8**, increases affinity by a factor of 1.4 and 7.5 for wild-type and mutant receptors, respectively.

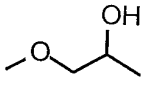
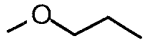
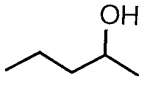
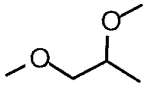
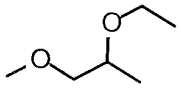
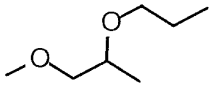
Replacement of the ether oxygen atom by a methylene group, as in compound **9**, is highly unfavorable for binding at the wild-type 5-HT_{1A} receptors. This racemate has 25-fold lower affinity for the wild-type receptor than (*S*)-propranolol **2**. These findings are in agreement with the involvement of the ether oxygen in a hydrogen bond interaction, as previously hypothesized in our 5-HT_{1A} receptor model (11). However, steric factors may also contribute to the dramatic loss of affinity by replacement of the oxygen atom in propranolol **2** by a methylene group in **9**. The methylene bridge may prevent **9** from adopting the bioactive conformation or, as observed in our 5-HT_{1A} receptor model, cause steric hindrance with Asn386.

Replacement of the hydroxyl group of propranolol **2** with a methoxy group, as in compound **10**, has no effect on affinity for the wild-type receptor for either of the two enantiomers. This observation provides evidence for an alternative hydrogen binding hypothesis, which is schematically depicted in Fig. 4a. The methoxy group of the *S*-enantiomer is important for binding because its removal, as in compound **8**, reduces affinity for the wild-type and mutant receptors 10-fold and

TABLE 2

K_i values for the displacement of [^{3}H]-8-OH-DPAT from human wild-type 5-HT_{1A} receptors and corresponding Asn386Val mutant receptors by propranolol 2 and a series of analogues



R	Number	K_i[a] nM	
		Wild-type	Asn386Val mutant
	(*S*)-**2**	160 ± 20	3600 ± 1000
	(*R*)-**2**	2100 ± 500	9800 ± 1600
	**8**	1500 ± 600	1300 ± 500
	**9**	4000 ± 900	5187, 6634[b]
	**10**	170 ± 30	140 ± 60
	(*S*)-**10**	150 ± 40	160 ± 60
	(*R*)-**10**	1700 ± 300	1300 ± 500
	**11**	1400 ± 200	2000 ± 500
	**12**	3800 ± 1600	7200 ± 1900

[a] K_i values are based on 3 determinations, each using four to six concentrations in duplicate and are presented as mean ± standard error.
[b] Based on 2 determinations.

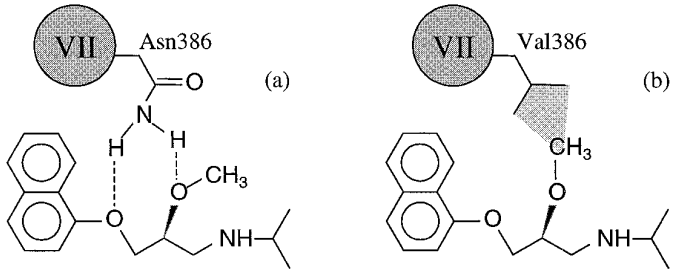


Fig. 4. Schematic representation of putative interactions of compound (*S*)-**10** with the residue in position 386 of 5-HT_{1A} wild-type receptors and the corresponding Asn386Val mutant receptors. a, *Dashed lines,* hydrogen bonding interactions in the wild-type receptor, with the oxygen atoms of (*S*)-**10** acting as proton acceptors. b, *Shaded area,* a favorable lipophilic contact of Val386 with the methoxy group of (*S*)-**10** in the mutant receptor.

8.1-fold, respectively. In the wild-type receptor, one or both oxygen atoms of (*S*)-**10** may act as hydrogen bond acceptors from the amide NH_2 group. In principle, this hypothesis is also valid for the binding of (*S*)-propranolol **2**. The loss of this interaction in the Asn386Val mutant may be compensated by a favorable lipophilic contact of Val386 with the methoxy group of **10** (Fig. 4b). In contrast, the contact of the hydroxyl group in propranolol **2** with the lipophilic mutant Val386 is unfavorable. This hypothesis accounts for the 22.5-fold preference of the mutant receptor for (*S*)-**10** compared with (*S*)-propranolol **2**. Although in the hypothesis of Fig. 4b, the phenoxy oxygen of (*S*)-**10** is not involved in a direct interaction with the mutant receptor, it may still be important for steric reasons. The methoxy group of (*R*)-**10** is not important for affinity for the 5-HT_{1A} wild-type or Asn386Val mutant receptors because its removal in compound **8** does not affect the corresponding affinities.

Elongation of the methoxy substitution of **10** to ethoxy or *n*-propoxy in **11** and **12**, respectively, decreases affinity for both the wild-type and the mutant receptor. Apparently, such large substitutions cannot be accommodated by either of the two receptors. These results are in agreement with observations in the mutant receptor model.

Modeling

The methoxy group of (*S*)-**10** docked into the Asn386Val mutant receptor model showed a favorable contact with Val386 (Fig. 3). Observed C—C distances between the methoxy carbon atom of the ligand and the three carbon atoms of the valine side chain were 3.7, 4.2, and 5.1 Å, respectively (torsion angles Val386 $C'C_\alpha C_\beta C$ −63° and 174°, respectively). Thus, according to the model, compound **10** might have higher affinity for the mutant receptor than propranolol 2. Therefore, compound 10 was synthesized and pharmacologically evaluated as was described in the previous section. The uncertainty with respect to the exact atomic coordinates is a well-known deficit of G protein-coupled receptor models. Therefore, two additional analogues of compound **10** with longer alkoxy side-chains were also incorporated in the study. The position of compound **10** in the receptor model does not allow for elongation of the methoxy side chain, as in compounds **11** and **12**. Chain elongation would cause either steric hindrance with the backbone of helix VII or internal strain in the molecule structure. Indeed, compounds **11** and **12** display a low affinity for Asn386Val mutant receptors. Thus, the results of binding experiments at Asn386Val mu-

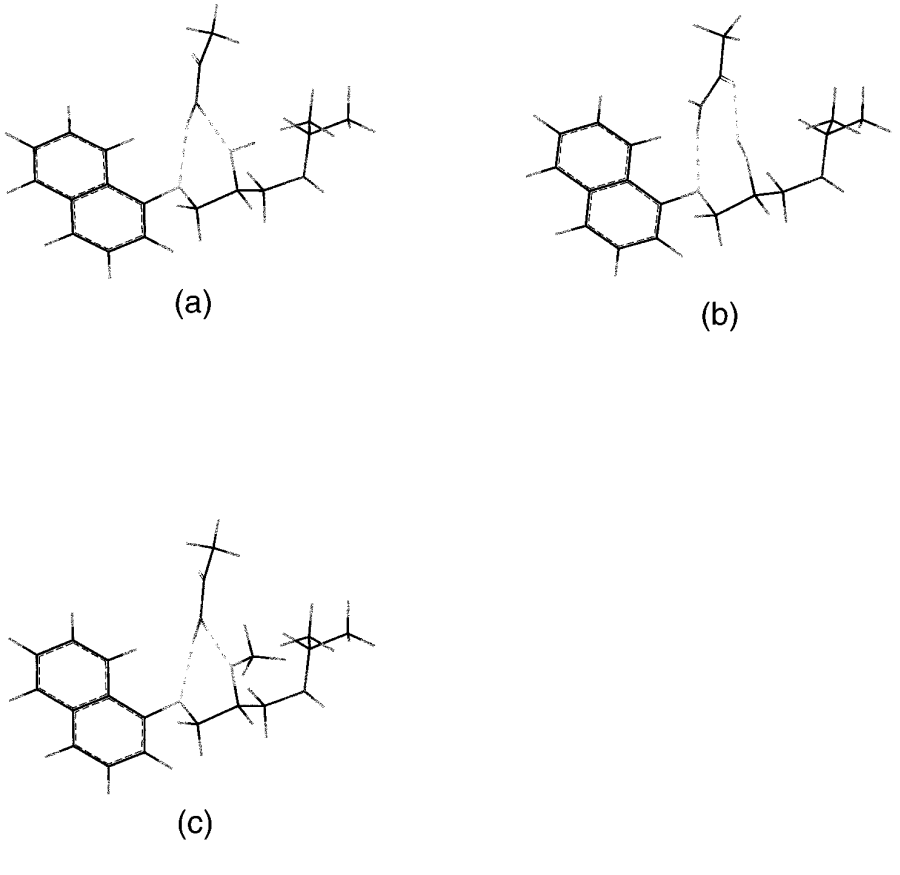


Fig. 5. Structures of complexes of acetamide, mimicking an asparagine side chain, with (*S*)-propranolol **2** and (*S*)-**10** as calculated using MOPAC AM1. For (*S*)-propranolol **2**, two putative binding modes with double hydrogen bonding interactions (A and B) are found, whereas the oxygen atoms of (*S*)-**10** can only act as hydrogen bond acceptors (C).

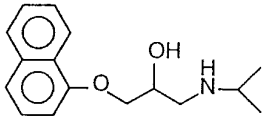
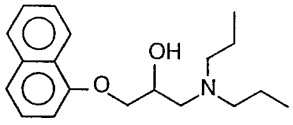
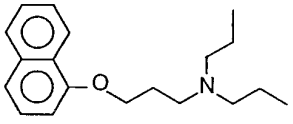
tant receptors are in good agreement with the observations from the model. The equally high affinity of compound **10** for wild-type 5-HT_{1A} receptors was, however, not predicted from the double hydrogen bond interaction depicted in Fig. 2 (11). From the indifference of compound **10** to the Asn386Val mutation, it might be argued that it binds in an unexpected manner. However, the affinity ratio of a factor 8 for the *R*- and *S*-enantiomers of compound **10** at the mutant receptor is similar to that of propranolol **2** at the wild-type receptor (factor 13), which indicates that both compounds address similar binding sites at mutant and wild type receptors. The alternative interaction hypothesis for compounds (*S*)-**10** and (*S*)-propranolol **2** with Asn386 in Fig. 4a may account for the observed structure-affinity relationships. Both compounds could be fitted into the receptor model having such double-hydrogen-bond interactions with the NH_2 group of Asn386.

The result of this docking experiment was supported by MOPAC AM1 calculations of complexes of acetamide, mimicking the Asn386 residue, with (*S*)-propranolol **2** and (*S*)-**10**. The structures of the stable complexes that resulted from these calculations are shown in Fig. 5. Thus, AM1 calculations indicate that (*S*)-**10** can interact with the amide side chain of Asn386, as depicted in Fig. 3a. For (*S*)-propranolol **2**, both binding modes seem to be possible (Figs. 2 and 3a).

Glennon *et al.* (14) recently published a study with a hypothesis for aryloxypropanol binding that is similar to our previously published interaction model of these compounds at 5-HT_{1A} receptors (11). They studied the interaction of propranolol **2** with 5-$HT_{1D\beta}$ Thr355Asn mutant receptors. Asn355 in the latter receptor is located at the same position as Asn386 in rat and human 5-HT_{1A} receptors. This residue seems to play a pivotal role in receptor recognition of propranolol (7, 8). Effects on receptor affinity found by the removal of the hydroxyl group of propranolol in **8** and the introduction of the methylene group in compound **9** were similar to the observations from this study.

The present findings concerning the importance of the aryloxypropanolamine hydroxyl group for 5-HT_{1A} receptor affinity are different from previous results of Pierson *et al.* (20). In their study, removal of the hydroxyl group of the propranolol analogue **13**, yielding **14**, slightly (3-fold) increased affinity for the rat 5-HT_{1A} receptor (Table 3). We found that removal of the hydroxyl group of (*S*)-propranolol **2** itself, as in compound **8**, decreases 5-HT_{1A} receptor affinity approximately 10-fold. The *N*-di-*n*-propyl substitutions in **13** and **14** probably force these compounds to bind to 5-HT_{1A} receptors in an orientation different from that of propranolol **2**. Replacement of the *N*-isopropyl substitution in propranolol **2** by *N*-di-*n*-propyl in **13** decreases 5-HT_{1A} receptor affinity approximately 15-fold. Apparently, *N*-di-*n*-propyl substitution is favorable for affinity of the agonist (*R*)-8-OH-DPAT **6** but not for the antagonist propranolol **2**. A different binding mode of compound **13** at the 5-HT_{1A} receptor implies that the surrounding of its hydroxyl group differs from that of propranolol **2**. Such a different binding mode may also account for the observed selectivity of **13** and **14** for 5-HT_{1A} versus rat 5-HT_{1B} receptors, which is lacking in propranolol **2** (20). In our model for the 5-HT_{1A} receptor (11), the two hydrogen atoms at the protonated basic nitrogen atom of pindolol **1** and propranolol **2** are directed toward the backbone of helix III (compounds docked according to Fig. 2). One interacts with Asp116 (Fig. 2). Conversion of the secondary amine to a tertiary amine would introduce steric hindrance with the backbone of helix III. This observation in the model is in agreement with the loss of 5-HT_{1A} receptor affinity of compound **13** with respect to propranolol **2** (Table 3).

TABLE 3
5-HT_{1A} (rat) receptor affinities of propranolol and two analogues

Compound	Number	K_i[a]
		nM
	**2**	90 ± 15
	**13**	1325 ± 200
	**14**	450 ± 20

[a] Data taken from Pierson et al. (20), presented as mean ± standard error.

Conclusions. The interaction between an asparagine residue in putative helix VII of the human 5-HT_{1A} receptor and aryloxypropanolamines was studied. For this purpose, the mutual effects of replacement of Asn386 with valine and modification of the oxypropanol moiety were investigated.

The *S*-enantiomers of propranolol **2**, penbutolol **4**, and alprenolol **5** are more potent at wild-type and Asn386Val mutant human 5-HT_{1A} receptors than the corresponding *R*-enantiomers. Mutation of Asn386 to valine lowered affinity for both enantiomers, although affinities for the *S*-enantiomers were most affected. Receptor affinities for other structural classes were not lowered by this receptor mutation.

Modifications of the oxypropanol moiety of propranolol **2** were studied at the wild-type and Asn386Val mutant 5-HT_{1A} receptors. Both oxygen atoms of the oxypropanol moiety were shown to be essential for the interaction of propranolol **2** with the wild-type 5-HT_{1A} receptors. The hydroxyl group probably has a hydrogen bond interaction with Asn386. The ether oxygen may be important for steric reasons but can also be involved in a direct interaction with Asn386. These findings are in agreement with interactions of arylpropanolamines with the essential asparagine in helix VII as previously hypothesized by Kuipers *et al.* (11) and Glennon *et al.* (14). However, the high affinity for the wild-type receptor was not influenced by replacement of the hydroxyl group of (*S*)-propranolol **2** by methoxy in the *S*-enantiomer of compound **10**. This indicates that one or both ether oxygen atoms of (*S*)-**10** may act as hydrogen bond acceptors from the amide NH_2 group of Asn386. (*S*)-**10** also displays high affinity for the Asn386Val mutant receptors as a result of a favorable lipophilic contact of Val386 with its methoxy group.

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