Pergamon

Bioorganic & Medicinal Chemistry Letters, Vol. 5, No. 24, pp. 2963-2968, 1995

0960-894X/95 $9.50+0.00

0960-894X(95)00534-X

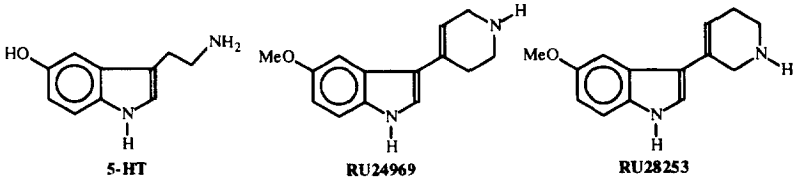
SYNTHESIS AND SEROTONIN RECEPTOR BINDING PROPERTIES OF 5-SUBSTITUTED 3-(1′,2′,5′,6′-TETRAHYDROPYRIDIN-3′-YL) INDOLES.

T. Dahlgren, R. L. Dean, L. A. Gharat, A. M. Johansson, G. Lambert,
H. B. Li, D. L. Nelson, Y. Yang, and A. R. Martin*

Department of Pharmacology and Toxicology, College of Pharmacy, University of Arizona, Tucson, AZ 85721, U. S. A.

Abstract: A semi-rigid 5-hydroxytryptamine (5-HT) analogue, RU28253 [5-methoxy-3-(1′,2′,5′,6′-tetrahydropyridin-3′-yl) indole], is a potent 5-HT_1 and 5-HT_2 agonist. It is isomeric to RU24969 [5-methoxy-3-(1′,2′,5′,6′-tetrahydropyridin-4′-yl) indole], a conformationally restricted 5-HT homologue, which has been extensively used in the study and classification of 5-HT receptors. A series of RU28253 derivatives with diverse substituents on indole 5-position were synthesized and their dissociation constants determined at the 5-HT_1 and 5-HT_2 receptors.

RU24969 and its isomer, RU28253, represent conformationally restricted analogues of 5-hydroxytryptamine or serotonin (5-HT) with high potency at 5-HT_{1A} receptors. Being partially constrained, they also provide fewer possible conformations for recognition by the receptor than 5-HT itself with its relatively flexible amino-ethyl side chain. This property is potentially useful in studies to determine the structural features essential for the high potency and selectivity between various types and subtypes of 5-HT receptors. A considerable amount of research has been published on RU24969 and its derivatives by us[1,2] and others,[3-6] but very little has been reported on RU28253 with a few exceptions.[7,8] RU28253 was also found to be more potent than RU24969 at the 5-HT_2 receptors,[7] in addition to having potency at 5-HT_{1A} receptor. In this paper we report the synthesis and the results on binding studies at the central 5-HT_{1A} and 5-HT_2 receptors of a series of RU28253 derivatives (Table 1).



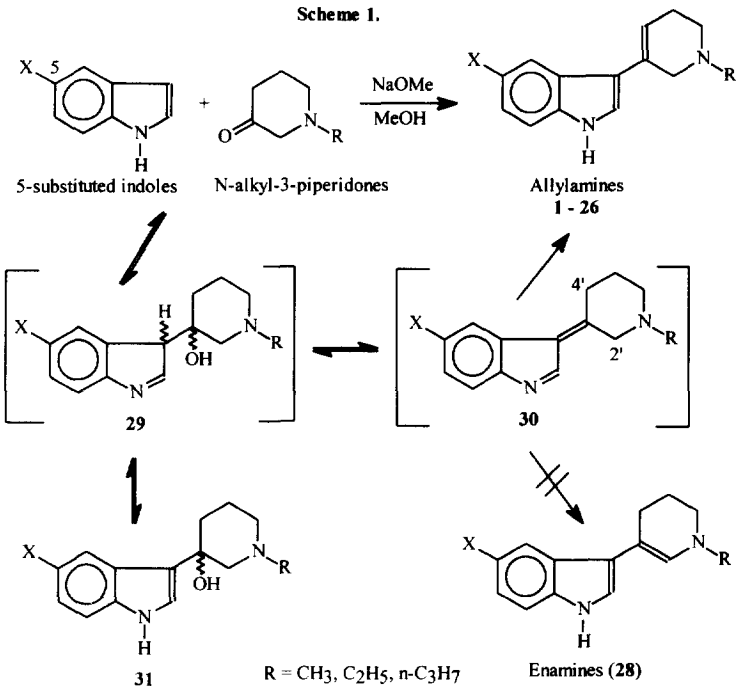
5-HT **RU24969** **RU28253**

Chemistry

Analogues **1- 26** were synthesized by direct base catalyzed condensation of the appropriate 5-substituted indole with N-methyl-, N-ethyl- or N-propyl-3-piperidone (Scheme 1).[2] All the final products isolated from these reactions were the desired allylamines [3-(1′,2′,5′,6′-tetrahydropyridin-3′-yl)indoles; **1-26**] identified by the characteristic vinyl proton chemical shift of 6.1- 6.2 ppm in the proton NMR.[9] No enamine products [3-(1′,4′,5′,6′-tetrahydropyridin-2′-yl)indoles; **28**] were observed suggesting that the base-catalyzed dehydration of the hypothetical intermediate **29** is regioselective (Scheme 1). While the tertiary alcohol **29** is assumed to be formed as an intermediate,[3] neither it nor its more stable tautomer **31** could not be isolated as a product. A minimum of 24 h for complete dehydration of **31a** (X = H, R = CH_3), synthesized separately,[10] to the corresponding allylamine (**1**) on exposure to sodium methoxide in

refluxing methanol was required. Thus, regioselective dehydration of **29** involving the more acidic indolyl-3-proton, would be expected, to form the exocyclic olefin **30**. Regioselective removal of the 4′-proton from **30**, followed by reprotonation of the indolic nitrogen would give the observed allylamine products **1-26**. Apparently, deprotonation of the 2′-proton of **30** is disfavoured due to the unfavourable interaction between the generated anion and the nitrogen lone pair. Molecular mechanics calculations indicate that the enamine is around 2.5 Kcal more stable than the allylamine, thus ruling out A 1,2 strain as an important factor in product determination.

Scheme 1.



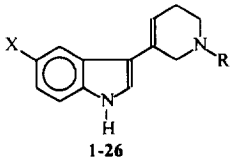
Pharmacology *(In vitro binding)*

The ability of the RU28253 derivatives (**1-26**) and a conformationally defined rigid analogue (**27**) to displace the radio-labeled ligands, [^{3}H]-8-OH-DPAT for 5-HT$_{1A}$ and [^{3}H]-Ketanserin for 5-HT$_2$, from receptor sites in homogenized rat brain tissue was assessed in vitro (Table 1).

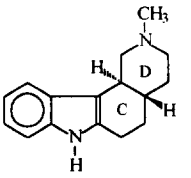
Results and Discussion

As can be seen from Table 1, compounds **9-12** and **24-26** have higher affinities for 5-HT$_{1A}$ receptor compared to the 5-HT$_2$ receptor. A 5-carboxyamido or 5-acetamido group seems to be particularly favourable for binding selectively to the 5-HT$_{1A}$ receptor sites. The overall trend shows a higher affinity for 5-HT$_{1A}$ compared to 5-HT$_2$ receptor with a few exceptions like **1**, **3**, **15**, and **20**, suggesting that the size of the 5-substituent may be a factor in determining their ability to bind well to 5-HT$_2$ receptor.

Table 1. Physical and 5-HT Receptor Binding Data for 3-(1′,2′,5′,6′-Tetrahydropyridin-3′-yl)indoles



1-26



27

Compd No.	X	R	% yield	Formula	M.P. °C	5-HT_{1A} Ki (nM)[a]	5-HT_2 Ki(nM)[b]
RU 24969	CH_3O	H	___	$C_{14}H_{16}N_2O$	___	10.9	912
RU 28253	CH_3O	H	___	$C_{14}H_{16}N_2O$	___	5.7	230
1	H	CH_3	28	$C_{14}H_{16}N_2$	189-91	261	139
2	CH_3	CH_3	29	$C_{15}H_{18}N_2$	165-7	72	194
3	F	CH_3	30	$C_{14}H_{15}FN_2$	203-5	124	43
4	Cl	CH_3	24	$C_{14}H_{15}ClN_2$	182-4	113	107
5	Br	CH_3	15-41	$C_{14}H_{15}BrN_2$	201-3	50	82
6	OCH_3	CH_3	22-32	$C_{15}H_{18}N_2O$	167-9	79	716
7	OH	CH_3	26	$C_{14}H_{16}N_{20}$	235-7	145	237
8	CN	CH_3	20	$C_{15}H_{15}N_3$	197-9	129	1035
9	$COCH_3$	CH_3	12	$C_{16}H_{18}N_2O$	216-8	91	3383
10	CO_2CH_3	CH_3	5	$C_{16}H_{18}N_2O_2$	203-5	102	5360
11	$CONH_2$	CH_3	47	$C_{15}H_{17}N_3O$	204-6	3.8	>10,000
12	$NHCOCH_3$	CH_3	24	$C_{15}H_{17}N_3O$	204-6	16	14,536
13	H	C_2H_5	22	$C_{16}H_{19}N_3O$	148-9	213	165
14	CH_3	C_2H_5	20	$C_{16}H_{20}N_2$	155-7	42	246
15	F	C_2H_5	24	$C_{15}H_{17}FN_2$	189-91	142	67
16	Cl	C_2H_5	16	$C_{15}H_{17}ClN_2$	181-3	47	150
17	Br	C_2H_5	16	$C_{15}H_{17}BrN_2$	178-80	24	125
18	OCH_3	C_2H_5	16	$C_{16}H_{20}N_2O$	153-4	71	1130
19	CN	C_2H_5	24	$C_{16}H_{17}N_3$	177-9	186	1360
20	H	nC_3H_7	26	$C_{16}H_{20}N_2$	136-7	556	146
21	Br	nC_3H_7	31	$C_{16}H_{19}BrN_2$	157-9	192	139
22	OCH_3	nC_3H_7	33	$C_{17}H_{22}N_2O$	140-2	226	1070
23	CN	nC_3H_7	47	$C_{17}H_{19}N_3$	185-7	303	958
24	CO_2CH_3	nC_3H_7	19	$C_{18}H_{22}N_2O_2$	159-61	214	>50,000
25	$CONH_2$	nC_3H_7	48	$C_{17}H_{21}N_3O$[c]	132-5	4.7	19,152
26	$NHCOCH_3$	nC_3H_7	29	$C_{18}H_{23}N_3O$[d]	89-91	205	>50,000
27[e]			5.5	$C_{16}H_{20}N_2$•HCl[f]	295 (dec)	2360	325

a. Displacement of ^{3}H-8-Hydroxy-DPAT. Each value represents at least three separate determinations with standard errors within ± 35 %.

b. Displacement of ^{3}H-Ketanserin. Each value represents at least 3 separate experiments with standard errors within ± 35 %.

c. Analysis calculated on the monohydrate.

d. Analysis calculated on the hemi-methanolate (½ CH_3OH).

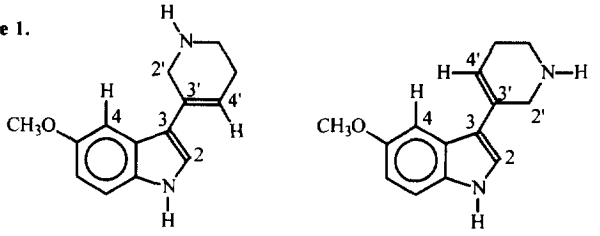
e. Compound **27** was synthesized and tested as a racemate.

f. Analysis calculated on the quartiahydrate (¼ H_2O).

There is not much difference in the binding affinity for 5-HT_{1A} receptor when the N-alkyl substituent in the piperidine ring is methyl or ethyl but an n-propyl substituent seems to decrease it. These results are parallel to those of the homologous RU24969 analogues[2] except for the general higher binding affinity of RU24969 analogs to both 5-HT_{1A} and 5-HT_2 receptor sites.

The tetracyclic analogue **27** shows a very low affinity for both 5-HT_{1A} and 5-HT_2 receptors. This result may indicate that 5-HT receptors prefer the **B** (ring coplanar) conformation of RU28253 or a non-coplanar conformation (Figure 1). Alternatively, the ethano linkage of the C-ring of **27** may sterically interfere with the receptor site. In the homologous 4-THPI series, a methyl group at the indole 2-position decreased activity at both 5-HT_{1A} and 5-HT_2 sites.[1,2]

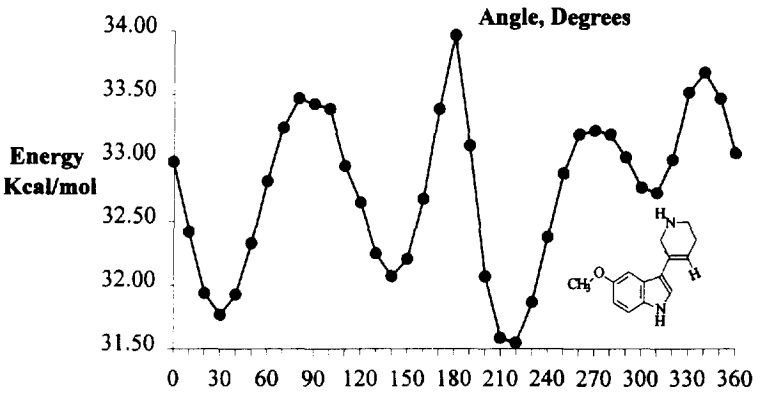
Figure 1.



We performed conformational analysis on RU28253 using molecular mechanics (PC-Model3) to determine the possible minimum energy conformations (Figure 2). The most stable conformation for the tetrahydropyridine (THP) ring is a twist chair form. The THP ring was rotated clockwise about the C3-C3′ bond through 10° increments and the corresponding total energy was calculated in kcal/mol. We start at 0° when the bonds C2-C3 and C3′-C4′ are *cis* (conformer **A**). The first energy minimum is at about 40° where the bonds C2-C3 and C3′-C4′ are intermediate between *cis* and *orthogonal*. The first energy maximum is at 90° where the bonds C2-C3 and C3′-C4′ are *orthogonal* to each other. The relatively high energy at this maximum is probably due to minimal π overlap between the C4′-C3′ π bond and the C3-C2 π bond. The next minimum at 140° is intermediate between *orthogonal* and *trans* similar to the first minimum in energy. The next maximum at 180° (conformer **B**) is the global maximum and the high energy is attributed to the steric interaction between the vinylic 4′ H and the aromatic proton on C4. At this point the C2-C3 and C3′-C4′ bonds are *trans* to each other. The global minimum at 220° is again where the bonds C2-C3 and C3′-C4′ are nearly halfway between the maxima at 180° (eclipsed) and at 270° (orthogonal). At the global minimum some π overlap of C4′-C3′ and C3-C2 π bonds is possible and steric interactions between the 4′-proton with 4-proton and 2′-proton with 2-proton are reduced. The next maximum at 270° is again due to the orthogonality of the C2-C3 and C3′-C4′ bonds where π overlap is minimized. The last energy minimum at 310° is again attributed to the bonds C2-C3 and C3′-C4′ being intermediate between planar and orthogonal conformations. The energy rises up to a maximum at 340° where the 2′-proton is nearer to the 4-proton and then drops down as it moves away at 360°. The barrier to rotation between the highest and the lowest energy conformations is about 2.5 kcal/mol. In the lowest energy conformation

(the one at 220°), the nitrogen atom of the THP ring is away from the benzene ring of indole which apparently explains the preference for the **B** conformation of RU28253 as discussed above. Because the barriers to rotation in RU28253 are so small, the combined forces of interaction between it and the receptor could allow any possible conformation of RU28253 to be the active one at a given 5-HT receptor.

Figure 2. Conformational Analysis of RU28253



Experimental

Chemicals and Method

Melting points were determined with an Electrothermal Capillary melting point apparatus and are uncorrected. ^{1}H-NMR were obtained for all the compounds on JEOL FX-90Q (90 MHz) instrument. Elemental analysis were performed by Desert Analytics, Tucson, AZ. N-methyl and N-propyl-3-piperidone were synthesized in our laboratory.[11] N-ethyl-3-piperidone hydrochloride was obtained from Aldrich Chemical Co. Those 5-substituted indoles that were not commercially available were prepared and characterized by us previously.[12,13] The tetracyclic analog **27** was synthesized in a seven step synthesis beginning with 3-acetyl-2-methylindole featuring an intramolecular Diels-Alder reaction similar to the one reported earlier for the synthesis of a homologue,[14] in our laboratory.[15] All the condensation reactions were carried out in AR-grade methanol purchased from the Aldrich Chemical Co. The following procedure for the preparation of **6** is typical of the reaction conditions employed.

5-Methoxy-(1′,2′,5′,6′-tetrahydropyridin-3′-yl) indole (**6**)

To a solution of NaOMe in AR-grade methanol, prepared by reacting 46 mg (2 mmol) of sodium in 1 ml AR-grade methanol, was added 147 mg (1 mmol) of 5-methoxyindole. The solution was refluxed for about 30 min and then a solution of N-methyl-3-piperidone (226 mg, 2 mmol) in 5 ml of AR-grade methanol was added dropwise to the above refluxing solution over a period of 15 min. The reaction mixture was refluxed for 72 h and then cooled and concentrated *in vacuo* (aspirator). Cold water was added to the reddish brown residue which was extracted with ether

concentrated *in vacuo* (aspirator). Cold water was added to the reddish brown residue which was extracted with ether (10 mL x 3). The ether extracts were combined, washed with water and brine, dried over $MgSO_4$, concentrated to a very small volume and cooled in icebath to obtain 61 mg (25%) of pale yellow crystalline solid, mp 167-169 °C.

Pharmacology*(5-HT Receptor Binding Assays)*

These were performed as described previously.[16] In brief, tissue was obtained from male Sprague-Dawley rats, which were killed by decapitation; the brains were then rapidly removed and dissected over ice. For the 5-HT_1 and the 5-HT_{1A} assays, the cortex dorsal to the rhinal sulcus was used; the 5-HT_2 assay was done using frontal cortex alone. Final tissue suspensions were in buffer of 50 mM Tris at pH 7.6. For 5-HT_1 assay, [^{3}H]-5-HT to a final concentration of about 2 nM was used as ligand, and unlabeled 5-HT at 10 μM was used to define nonspecific binding. [3H]-Ketanserin to the final concentration of about 0.4 nM was used as the 5-HT_2 ligand, and nonspecific binding was defined using 1 μM methysergide. The assay tubes were incubated at 37 °C for 10 min (15 min for the 5-HT_2 assay) and filtered through Whatman GF/B filters using a Brandel cell harvester. For the 5-HT_{1A} and 5-HT_2 assays, the GF/B filters were pretreated with a 0.1 %, v/v, solution of poly(ethyleneimine) for 2 h and allowed to dry (this was found to reduce nonspecific binding to the filters). For a similar reason, the 5-HT_2 assays were performed in disposable polypropylene rather than glass tubes. For all three binding assays, potencies of inhibiting drugs are reported as the apparent K_i values, calculated from inhibitor IC_{50} values using the Cheng-Prusoff equation.[7]

Acknowledgement

Research support from grant #NS16605 from the National Institutes of Health is gratefully acknowledged.

References

1. Taylor, E. W.; Nikam, S. S.; Lambert, G.; Martin, A. R; Nelson, D. L. *Mol. Pharmacol.* **1988**, 34, 42.
2. Agarwal, A.; Pearson, P. P.; Taylor, E. W.; Li, H. B.; Dahlgren, T.; Herslof, M.; Yang, Y.; Lambert, G.; Nelson, D. L.; Regan, J. W.; Martin, A. R. *J. Med. Chem.* **1993**, 36, 4006.
3. Guillaume, J.; Dumont, C.; Laurent, J.; Nedelac, L. *Eur. J. Med. Chem.* **1987**, 22, 33.
4. Macor, J. E.; Burkhart, C. A.; Heym, J. H.; Ives, J. L.; Lebel, L. A.; Newman, M. E.; Nielsen, J. A.; Ryan, K.; Schulz, D. W.; Togersen, L. K.; Koe, K. B. *J. Med. Chem.* **1990**, 33, 2087.
5. Hunt, P.; Nedelac, L.; Euvrard, C.; Boissier, J. R. *Abstracts of the 8th International Congress Of Pharmacology Abs. No.* **1981**, 1434, 659, abstract.
6. Euvrard, C.; Boissier, J. R. *Eur. J. of Pharmacol.* **1980**, 63, 65.
7. Taylor, E. W.; Nikam, S. S.; Weck, B.; Martin, A. R; Nelson, D. L. *Life Sciences* **1987**, 41, 1961.
8. Dumuis, A.; Bouhelal, R.; Sebben, M.; Cory, R.; Bockaert, J. *Mol. Pharmacol.* **1988**, 34, 880.
9. Zheng, Q.; Yang, Y.; Martin, A. R. *Heterocycles* **1994**, 37, 1761-1772.
10. Dean, R. L.; Gharat, L. A.; Yang, Y.; Martin, A. R. (*submitted*)
11. Lyle, R. E.; Adel, R. E.; Lyle, G. G. *J. Org. Chem.* **1959**, 24, 342.
12. Yang, Y.; Martin, A. R., Nelson, D. L; Regan, J. W. *Heterocycles* **1992**, 34, 1189.
13. Yang, Y.; Martin, A. R. *Heterocycles* **1992**, 34, 1395.
14. Herslof, M.; Martin, A. R. *Tetrahedron Lett.* **1987**, 28, 3423.
15. Details regarding the synthesis and spectroscopic properties of **27** will be published elsewhere.
16. Taylor, E. W.; Nikam, S. S.; Lambert, G.; Martin, A. R; Nelson, D. L. *Mol. Pharmacol.* **1988**, 34, 42.
17. Cheng, Y.; Prusoff, W. H. *Biochem. Pharmacol.* **1973**, 22, 3099.

(*Received in USA* 29 *September* 1995; *accepted* 7 *November* 1995)