Journal of Medicinal Chemistry

Volume 30, Number 9 September 1987

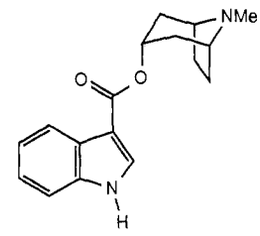
Communications to the Editor

Indazoles as Indole Bioisosteres: Synthesis and Evaluation of the Tropanyl Ester and Amide of Indazole-3-carboxylate as Antagonists at the Serotonin $5HT_3$ Receptor

Sir:

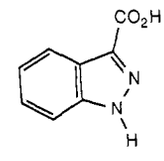
Bioisosterism has always played an important role in medicinal chemistry.[1] The replacement of one moiety with a bioequivalent is an important strategy to circumvent problems of metabolism, toxicology, absorption, or other obstacles to in vivo drug activity. Thus, bioisosterism can play an important role in the development of new and novel pharmacological agents from existing ones. Since 1979, tremendous progress has been made in the classification of receptor subtypes for the neurotransmitter serotonin, prompting the development of new potent and specific pharmacological agents at these receptor subtypes.[2] The identification of a bioisostere to the indole moiety of serotonin could play an important role in the development of such agents.

Attention has been focused recently on serotonin $5HT_3$ receptors, in part due to the report by Richardson et al. on a series of compounds that are potent and selective antagonists at this receptor.[3] These compounds might play a therapeutic role in mediating the painful effects of migraine,[4] as well as provide the necessary pharmacological tools to determine the exact role of $5HT_3$ receptors in cardiovascular and gastrointestinal systems.[5] One member of this series, **1** (ICS 205-930, indole-3-carboxylic acid, tropanyl ester), conceptually joins the indole portion of serotonin to the tropanyl moiety of cocaine, a weak antagonist at the $5HT_3$ receptor.[6] The result is a tremendous increase in $5HT_3$ receptor antagonist activity for **1** over that of cocaine.[3] Since the indole moiety conferred such a marked increase in the activity of **1**, this molecule provides an excellent framework for testing potential candidates as indole bioisosteres.

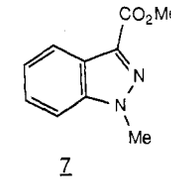


1

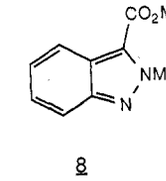
Several indazole analogues of **1** were prepared to examine the role of indazole as an indole bioisostere (Table I).[7] Indazole-3-carboxylic acid (**6**) was a pivotal intermediate for the synthesis of these indazole analogues, and was readily available in a one-pot synthesis from isatin in 50–60% yield.[8] Compound **6** was directly coupled to tropine or 3α-tropanylamine with carbonyldiimidazole in DMF (under N_2, room temperature) to give **2** or **3**, respectively (30–40% after recrystallization). Alternatively, **6** was alkylated with MeI/K_2CO_3 in DMF (50 °C for 4 h) to give a 4/1 mixture of N-1/N-2 alkylated products as the corresponding methyl esters **7** and **8**, which were readily separable by flash chromatography (40% of **7**, 10% of **8**). Hydrolysis of the esters (1 N NaOH in THF, room temperature, overnight, quantitative), preparation of the acid chlorides ($SOCl_2$ in refluxing $CHCl_3$), followed by rotary evaporation of the $CHCl_3$, dissolution in dioxane, and treatment with 3α-tropanylamine, followed by Et_3N (1 equiv) gave **4** and **5** (40–50% after recrystallization).[9]



6



7



8

The regiochemistry of alkylation in esters **7** and **8** was not readily apparent on the basis of ^1H NMR or IR. However, comparison of the ^{13}C NMR spectra of **7** and **8** with literature values for the parent 1-methyl- or 2-methylindazole was helpful.[10] As expected, in **7**, C-3 was

(1) Thornber, C. W. *Chem. Soc. Rev.* **1979**, 8, 563.

(2) Bradley, P. B.; Engel, G.; Feniuk, W.; Fozard, J. R.; Humphrey, P. P. A.; Middlemiss, D. N.; Mylecharane, E. J.; Richardson, B. P.; Saxena, P. R. *Neuropharmacology* **1986**, *25*, 563.

(3) Richardson, B. P.; Engel, G.; Donatsch, P.; Stadler, P. A. *Nature (London)* **1985**, *316*, 126. See also: Iversen, L. L. *Nature (London)* **1985**, *316*, 107.

(4) (a) Fozard, J. R.; Loisy, C.; Tell, G. *Migraine. Proc. 5th Int. Migraine Symp.* **1984**, 264. (b) Loisy, C.; Beorchia, S.; Centonze, V.; Fozard, J. R.; Schechter, P. J.; Tell, G. P. *Cephalalgia* **1985**, *5*, 79.

(5) Richardson, B. P.; Engel, G. *Trends Neurosci.* **1986**, 424.

(6) Fozard, J. R.; Mobarok Ali, A. T. M.; Newgrosh, G. *Br. J. Pharmacol.* **1977**, *61*, 130P.

(7) Ainsworth originally explored the concept of indazole as an indole bioisotere by preparing the indazole analogue of serotonin. See: (a) Ainsworth, C. *J. Am. Chem. Soc.* **1957**, *79*, 5242. (b) Ainsworth, C. *J. Am. Chem. Soc.* **1957**, *79*, 5245.

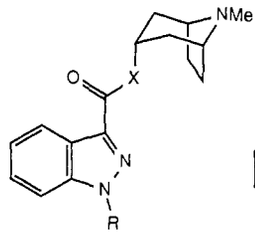
(8) Snyder, H. R.; Thompson, C. B.; Hinman, R. L. *J. Am. Chem. Soc.* **1952**, *74*, 2009.

(9) ^1H NMR data were consistent with the assigned structures.

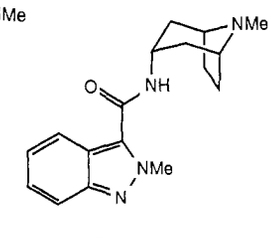
(10) Elguero, J. In *Comprehensive Heterocyclic Chemistry*; Katritsky, A. R., Rees, C. W., Eds.; Pergamon: Elmsford, NY, 1984; Vol. 5, 194.

0022-2623/87/1830-1535$01.50/0

Table I. Indazoles



2, 3, 4

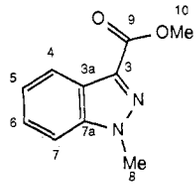


5

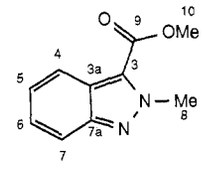
no.	R	X	formula[a]	mp, °C	recrystn solvent
2	H	O	$C_{16}H_{19}N_3O_2$	231–233	$CHCl_3/Et_2O$
3	H	NH	$C_{16}H_{20}N_4O$	223–225	*b*
4	Me	NH	$C_{17}H_{22}N_4O$	123–124	Et_2O
5			$C_{17}H_{22}N_4O$	250–251	$CHCl_3/Et_2O$

[a] All compounds gave satisfactory (±0.4%) C, H, N analysis. [b] Triturated with CH_2Cl_2.

Table II. ^{13}C NMR Data of Compounds 7 and 8



7



8

carbon	chemical shift (ppm from Me_4Si, in Me_2SO) 7	8
3	134.7	125.0
3a	124.2	124.2
4	124.5[a]	122.3[b]
5	122.4[a]	126.2[b]
6	128.0	127.6[b]
7	112.1	119.2
7a	142.1	147.9
8	37.7	42.6
9	163.7	161.4
10	53.0	53.4

[a,b] These assignments may be reversed.

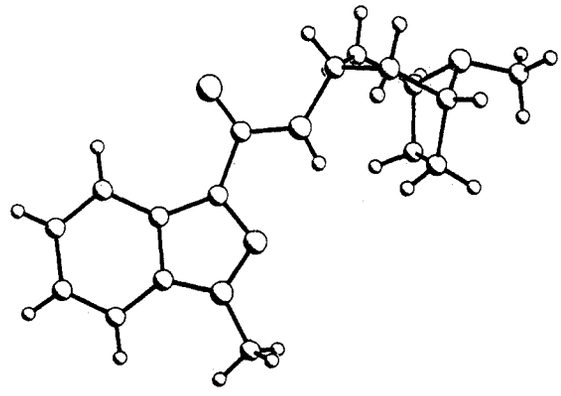


Figure 1. ORTEP plot of compound 4.

shifted downfield, and C-7 and C-7a were shifted upfield, relative to the corresponding carbons in 8. Furthermore, the *N*-methyl in 8 is shifted downfield relative to the *N*-methyl in 7 because of its proximity to the deshielding region of the ester carbonyl (Table II). The structure assignments were ultimately confirmed by X-ray crystallographic analysis of the final products 4 (Figure 1) and 5 (Figure 2).[11]

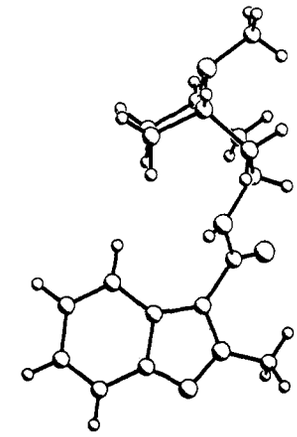


Figure 2. ORTEP plot of compound 5.

Table III. Inhibition of the von Bezold-Jarisch Reflex in Rats

no.[a]	ED_{50},[b] mg/kg iv	po	po/iv ratio
1	0.004	0.2	50
2	0.003	0.2	66
3	0.002	0.02	10
4	0.002	0.03	15
5	>0.01	ND[c]	

[a] Compounds were tested as the free bases and were dissolved in dilute acid (≤1% HCl). [b] Dose needed for 50% inhibition of the von Bezold-Jarisch reflex. The ED_{50} was measured from mean values determined at three doses. Standard error of the mean ranged from approximately 5% to 20% with $n \geq 3$ at each dose. [c] Not determined.

Serotonin, injected as a bolus into the femoral vein of anesthetized rats, induces a vagally mediated reflex bradycardia known as the von Bezold–Jarisch reflex. This effect is mediated by activation of serotonin $5HT_3$ receptors located in the wall of the right ventricle.[12] Blockade of serotonin-induced reflex bradycardia is an excellent measure of antagonist activity at $5HT_3$ receptors.[3,4] Intravenous (iv) experiments were performed as follows: urethane-anesthetized rats were given a bolus injection of serotonin (0.03 mg/kg iv) to establish the control bradycardiac response. Once the heart rate returned to base line (within 5 min), the rats were given the test compound (iv), followed by a 15-min interval and then another bolus injection of serotonin (0.03 mg/kg iv). Oral (po) experiments were performed as follows: overnight fasted rats were dosed by gavage (test compound diluted to 5 mL/kg) and then, after a 45-min interval, anesthetized with urethane.

(11) Compound 4 crystallized from ethyl ether in the space group I 2/a, $Z = 8$, with unit cell dimensions of $a = 14.078$ (3) Å, $b = 11.514$ (2) Å, $c = 19.422$ (3) Å, $\alpha = 90.00°$, $\beta = 93.189$ (1)°, $\gamma = 90.00°$. The calculated density was 1.26 g/cm^3. A total of 2124 reflections with 2θ less than 116.0° were measured on an automated four-circle diffractometer using monochromatic copper radiation. Compound 5 crystallized from chloroform-/ethyl ether in the space group P 1 Bar, $Z = 2$, with unit cell dimensions of $a = 8.560$ (2) Å, $b = 8.890$ (2) Å, $c = 11.118$ (3) Å, $\alpha = 112.706$ (2), $\beta = 91.048$ (2), $\gamma = 98.367$ (2). The calculated density was 1.29 g/cm^3. A total of 2099 reflections with 2θ less than 116.0° were measured as before. The structures were solved by using the Random Tangent routine RANT of the SHELXTL program library (G. M. Sheldrick, 1981) and were refined by the least-squares method with anisotropic temperature factors for all atoms except hydrogen. Hydrogen atoms were included with isotropic temperature factors at calculated positions. The final *R* factors were 0.0473 for 1935 unique observed reflections (compound 4) and 0.0613 for 1885 unique observed reflections (compound 5).

(12) Paintal, A. S. *Physiol. Rev.* **1973**, *53*, 159.

Fifteen minutes after anesthesia, rats were given a bolus injection of serotonin (0.03 mg/kg iv). For the po experiments, animals dosed with saline served as control. For both the iv and po experiments, percent inhibition of serotonin-induced reflex bradycardia was measured at three doses for each compound with $n \geq 3$ at each dose (Table III).

Compound **2**, the direct indazole analogue of **1**, showed comparable activity to that of **1** after both iv and po administration. In addition, 0.1 mg/kg iv of **2** did not inhibit equivalent bradycardia induced by carbamylcholine (0.01 mg/kg iv) indicating that inhibition of serotonin-induced bradycardia is due to $5HT_3$ receptor blockade and not to anticholinergic activity. Of greater interest, however, is compound **3**, which showed a modest twofold increase in activity iv, but a highly significant 10-fold increase in activity po relative to both **1** and **2**. The more stable amide linkage in **3** apparently increased oral bioavailability of the compound. Methylation of **3** at N-1 to give **4** did not change activity via iv administration, but led to a minor diminution of activity via po administration. However, methylation of **3** at N-2 to give **5** led to a substantial loss of activity.

Examination of the X-ray structures of **4** and **5** (Figures 1 and 2) indicates a significant conformational difference between the two molecules in the solid state. Compound **4** adopts an extended conformation, whereas compound **5** adopts a folded conformation. Examination of molecular models reveals that when **5** is placed in an extended conformation, there exists a prohibitive steric interaction between the N-2 methyl group on the indazole and the amide NH as well as the ethylene bridge of the tropane moiety. The folded conformation of **5** confers two major changes: it removes the tropanyl nitrogen from a presumed distal site of interaction with the receptor, and it reorients the direction of the amide carbonyl by approximately 120°. Presumably, one or both of these effects account for the diminution of activity in **5**.

Nevertheless, the potent activity of **2**–**4** as $5HT_3$ receptor antagonists indicates that indazole is a viable bioisostere for indole.

Acknowledgment. We thank Karla McKenney for her expert assistance in the preparation of this manuscript.

Registry No. **2**, 107007-94-3; **3**, 109216-57-1; **4**, 109216-58-2; **5**, 109216-59-3; **6**, 4498-67-3; **7**, 109216-60-6; **8**, 109216-61-7; tropine, 120-29-6; 3α-Tropanylamine, 87571-88-8.

Supplementary Material Available: Tables of atomic coordinates, bond lengths, bond angles, and anisotropic temperature factors for the X-ray analyses of compounds **4** and **5** (10 pages). Ordering information is given on any current masthead page.

Pawel Fludzinski,* Deborah A. Evrard
William E. Bloomquist, William B. Lacefield
William Pfeifer, Noel D. Jones
Jack B. Deeter, Marlene L. Cohen
Lilly Research Laboratories
Eli Lilly and Company
Lilly Corporate Center
Indianapolis, Indiana 46285

Received April 17, 1987