Pergamon

Bioorganic & Medicinal Chemistry Letters, Vol. 5, No. 20, pp. 2391-2396, 1995

0960-894X/95 $9.50+0.00

0960-894X(95)00417-3

5-CYANO-1-[3-(N-METHYLPYRROLIDIN-2R-YLMETHYL)INDOL-5-YL] BENZIMIDAZOLE (CP-161,242): A POTENT, CENTRALLY ACTIVE 5-HT$_{1D}$ RECEPTOR AGONIST AND BENZODIAZEPINE PARTIAL AGONIST

John E. Macor,*[1] David H. Blank, Kishor Desai, Carol B. Fox, B. Kenneth Koe, Lorraine A. Lebel, Ronald J. Post, Anne W. Schmidt, David W. Schulz, and Patricia A. Seymour

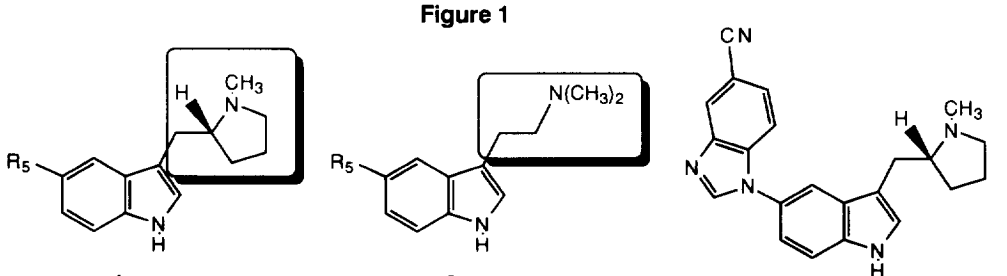
Departments of Medicinal Chemistry and Neurosciences
Central Research Division, Pfizer Inc, Groton, Connecticut 06340

[1]*PRESENT ADDRESS*: Astra Arcus USA, P.O. Box 20890, Rochester, NY 14602

Abstract: The discovery of CP-161,242 (5-cyano-1-[3-(N-methylpyrrolidin-2R-ylmethyl)indol-5-yl]benzimidazole), a potent, selective, orally active central serotonin (5-HT$_{1D}$) agonist [IC$_{50}$ (binding) = 1.3 nM, EC$_{50}$ (inhibition of adenylate cyclase) = 42 pM] and benzodiazepine ligand [IC$_{50}$ (binding) = 3.3 nM], is presented.

The large family of serotonin [5-hydroxytryptamine, 5-HT] receptors provides a plethora of medicinally important targets, and the neurotransmitter serotonin has been implicated in a wide variety of disease states ranging from depression and anxiety to migraine and sexual dysfunction.[2] The search for agents which are effective at only a single subtype within the large 5-HT receptor family should lead to new tools for understanding the function of the individual receptors and, possibly, new medicinal agents. From the clinical utility of Buspar®, a selective agonist at somatodendritic and postsynaptic 5-HT$_{1A}$ receptors, the anxiolytic activity associated with this receptor subtype has been demonstrated.[3] Even more recently, sumatriptan [Imigran®, **2** in Figure 1, where R_5= -$CH_2SO_2NHCH_3$], a 5-HT$_{\text{"1-like"}}$ agonist has been approved as a novel treatment for migraine headaches, and its selectivity for the 5-HT$_{1D}$ receptor has been proposed as the source of its anti-migraine activity.[4] The success of sumatriptan has initiated the search for other 5-HT$_{1D}$ receptor selec-

Figure 1

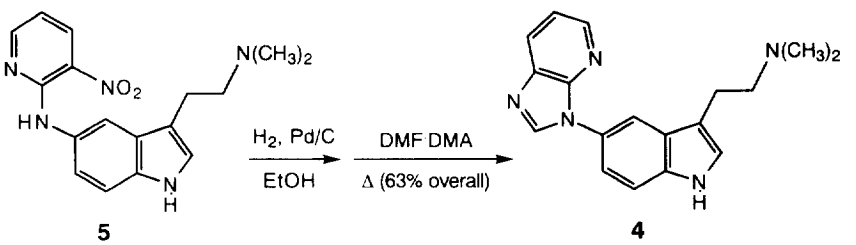


tive agonists with the purpose of further clarifying the mechanism of action of these compounds in relation to their anti-migraine activity.[5] Furthermore, since sumatriptan does not readily cross the blood/brain barrier, the role and function of 5-HT_{1D} receptors within the mammalian central nervous system has not yet been elucidated.

During the course of our studies in the area of conformationally restricted agonists of serotonin,[6] we discovered that the 3-(pyrrolidin-2R-ylmethyl) group in **1** was a novel, potent, and stereogenic replacement for the 3-(2-aminoethyl) group of serotonin and other tryptamines (**2**, Figure 1).[6g, 6h] At the same time, we also found a novel C5 substituent [Figure 1, **1** and **2**, R_5 = 5-(3-nitropyrid-2-ylamino)] on the indole nucleus which imparted reasonable selectivity for the 5-HT_{1D} receptor.[6k] Further SAR studies exploring this functionality led to 1-[3-(2-dimethylaminoethyl)indol-5-yl]pyrido[2,3-b]imidazole (**4**, Scheme 1). Compound **4** possessed improved 5-HT_{1D} receptor selectivity when compared to the 5-(3-nitropyrid-2ylamino)tryptamine[6k] (**5**). Therefore, the combination of our potent C3 substituent with a modified C5-pyrido[2,3-b]imidazole led to the discovery of 5-cyano-1-[3-(N-methylpyrrolidin-2R-ylmethyl)indol-5-yl]benzimidazole (**3**, CP-161,242, Figure 1). CP-161,242 was found to be a potent, orally active, 5-HT_{1D} receptor selective agonist. During the course of general receptor binding studies with CP-161,242, it was discovered that the compound also possessed remarkable affinity for the benzodiazepine receptor as measured by displacement of [^{3}H]flunitrazepam binding. This combination of potent and selective serotonergic agonism for the 5-HT_{1D} receptor, coupled with its high affinity for the benzodiazepine receptor made CP-161,242 a unique pharmacological agent. In this report we detail the discovery and pharmacology of 5-cyano-1-[3-(N-methylpyrrolidin-2R-ylmethyl)indol-5-yl]benzimidazole (**3**, CP-161,242).

Chemistry. Reaction of 5-aminoindole derivatives with electron poor aryl halides in the presence of a base at elevated temperature affords a smooth nucleophilic addition/halide elimination reaction leading to 5-(arylamino)indoles (Schemes 1 and 2). Accordingly, reaction of 5-amino-N,N-dimethyltryptamine[7] with 2-chloro-3-nitropyridine in the presence of triethylamine in refluxing ethanol afforded **5** as has been previously reported.[6k] Reduction of the nitro group followed by cyclization of the resulting arenediamine moiety using a formic acid synthon (dimethyl formamide dimethyl acetal) afforded the pyridoimidazole **4**.[8]

Scheme 1

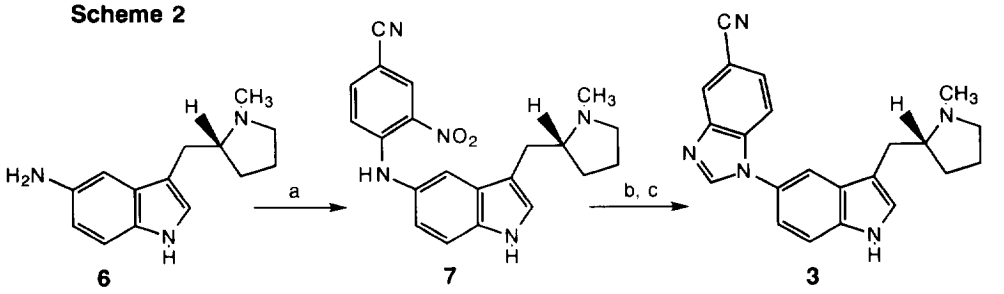


5 **4**

Analogously, condensation of the 5-amino-3-(N-methylpyrrolidin-2R-ylmethyl)indole (**6**)[6l] with 4-chloro-3-nitrobenzonitrile in refluxing ethanol in the presence of triethylamine yielded the 5-(4-cyano-2-nitrophenylamino)indole (**7**, Scheme 2). Catalytic hydrogenation of the nitro group in **7** afforded the

arenediamine which was cyclized using ethoxymethylenemalononitrile[9] (an alternate, more effective formic acid synthon) leading to 5-cyano-1-[3-(N-methylpyrrolidin-2R-ylmethyl)indol-5-yl]benzimidazole (**3**, CP-161,242).[10]

Scheme 2



a = 4-chloro-3-nitrobenzonitrile, triethylamine, EtOH, Δ (76%)

b = H_2, Pd on C, EtOH (97%)

c = ethoxymethylenemalononitrile, *i*-PrOH, Δ (85%)

Biology. Table 1 summarizes the *in vitro* pharmacology of compounds **4** and **3** (CP-161,242) along with relevant data for 5-HT and diazepam (Valium®). Binding measurements were performed using previously published protocols.[11] Specifically, 5-HT_{1A} receptor binding was performed using rat cortex and [^{3}H]OH-DPAT; 5-HT_{1D} receptor binding was performed using bovine caudate and [^{3}H]5-HT; benzodiazepine receptor binding was performed using guinea pig cerebellum and [^{3}H]flunitrazepam.[12]

The ability of compounds **3** and **4** to mimic 5-HT agonist activity at 5-HT_{1A} and 5-HT_{1D} receptors (i.e., inhibition of forskolin-stimulated adenylate cyclase) was measured using methods described previously.[11] In short, inhibition of forskolin-stimulated adenylate cyclase was measured at 5-HT_{1A} receptors in guinea pig hippocampus and at 5-HT_{1D} receptors in guinea pig substantia nigra. Both **3** (CP-161,242) and **4** were equally efficacious as 5-HT at maximal doses, demonstrating full agonism in the tissues tested.

While tryptamine (**4**) did not bind as potently as 5-HT at either 5-HT_{1A} or 5-HT_{1D} receptors, it bound preferentially to the 5-HT_{1D} receptor. This selectivity for the 5-HT_{1D} receptor also translated to a similar functional selectivity for that receptor while acting as a full agonist. CP-161,242 had equivalent binding affinity for the 5-HT_{1D} receptor as 5-HT, but CP-161,242 was significantly more selective for the 5-HT_{1D} receptor (versus the 5-HT_{1A} receptor) than 5-HT. While CP-161,242 was more potent than **4** in binding to the 5-HT_{1D} receptor, CP-161,242 appeared to be slightly less selective for the 5-HT_{1D} receptor than **4**. When the functional response of CP-161,242 was measured, it was found to be dramatically more potent than 5-HT at the 5-HT_{1D} receptor, and its functional selectivity (620-fold versus the 5-HT_{1A} receptor) is unparalleled. CP-161,242 did not bind with any appreciable affinity to 5-HT_{2A} and 5-HT_{2C} receptors (data not shown, IC_{50}'s > 10,000 nM).

General screening of CP-161,242 revealed potent affinity for the benzodiazepine (BZD) receptor. CP-161,242 was approximately equivalent to diazepam in its binding at the BZD receptor. Initial studies using GABA shift[13] of binding to BZD receptors suggest that CP-161,242 is a poorly efficacious partial agonist with a GABA shift ratio of 1.3 - 1.4 compared with 2.6 - 2.9 for diazepam in our hands (data not shown).

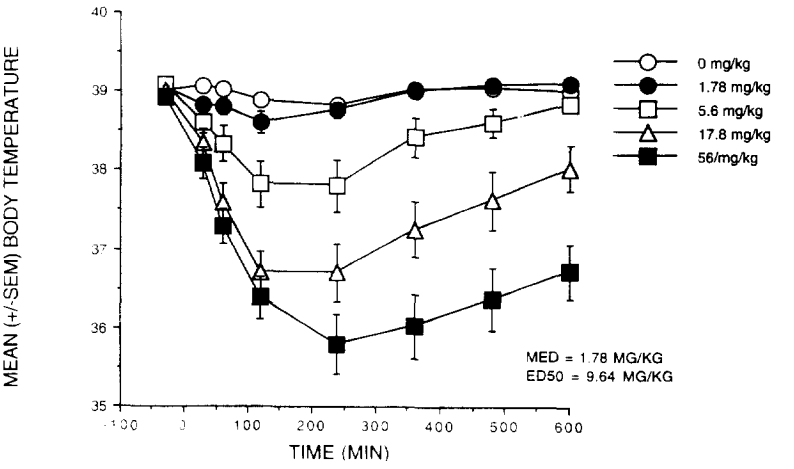
Table 1 - *In Vitro* Pharmacology[a]

	BINDING, IC_{50} (nM) ± SEM				**Inhibition of Adenylate Cyclase**, EC_{50} (nM) ± SEM		
Compound	5-HT_{1A}	5-HT_{1D}	1A/1D	BZD	5-HT_{1A}	5-HT_{1D}	1A/1D
5-HT	5.2±1.5	3.0±0.3	2	NT	43±29	8.5±6.2	5
4	210±60	11±4	19	89±9	460 [2]	14±15	32
CP-161,242 (**3**)	20±4	1.3±0.2	15	3.3±0.42	26 ±21	0.042±0.035	620
diazepam	NT	NT	NT	14±1	NT	NT	NT

[a] Values presented represent a minimum of three independent determinations and are arithmetic means. Brackets following mean indicate number of independent determinations *if less than three*. NT = not tested.

Figure 2 presents the effect of orally administered CP-161,242 on guinea pig body temperature. Previously, it has been shown that 5-HT_{1D} agonists produce hypothermia in guinea pigs,[14] and this effect has been blocked by GR127935, a selective antagonist at the 5-HT_{1D} receptor. CP-161,242 when given via oral or intraperitoneal (data not shown) administration produces a dose-dependent hypothermia in guinea pigs.[15] The minimally effective oral dose for this response is 1.78 mg/kg and the EC_{50} for this response is 9.6 mg/kg. The maximum depression in guinea pig body temperature was approximately 3 °C (at 56 mg/kg and 4 h post administration). The hypothermic effect of orally administered CP-161,242 appears to be relatively long lasting as demonstrated by significant body temperature depression at the two highest doses 10 h post administration.

Figure 2 - Dose Dependent Decrease in Body Temperature after P.O. Administration of CP-161,242 in Guinea Pigs



Discussion Skingle[14a] and Seymour[14b] have shown that 5-HT_{1D} receptor agonists lower guinea pig body temperature upon i.p. and oral administration via a centrally mediated mechanism. This effect has been shown to be blocked by 5-HT_{1D} receptor antagonists.[14] Figure 2[15] shows the effects of *orally administered* CP-161,242 on guinea pig body temperature. These results demonstrate that CP-161,242 is an orally active 5-HT_{1D} receptor agonist.

The *in vitro* data presented for CP-161,242 confirms that the compound is a potent and selective agonist for the 5-HT_{1D} receptor versus other 5-HT receptors tested (binding studies for the 5-HT_{1A} receptor utilized rat membranes, while the 5-HT_{1D} receptor binding assay used bovine caudate). Compound **4**, and CP-161,242 (**3**) showed moderate selectivity in binding experiments for the 5-HT_{1D} receptor versus the 5-HT_{1A} receptor. Of the three, only CP-161,242 shared a similar (if not better) potency to serotonin itself in the binding experiments. Examination of the compounds' ability to function as agonists produced an unusual result. While the binding selectivity and agonist selectivity for 5-HT and **4** paralleled reasonably well, the functional potency and selectivity of CP-161,242 were stunning. Acting as an agonist, CP-161,242 displayed significantly more potency and selectivity for the 5-HT_{1D} receptor than it had demonstrated in the binding experiments. There is no simple explanation for this divergence. However, research involving other G-protein coupled receptors systems might shed light on this phenomena. Specifically, within the family of muscarinic receptors (another member of the G-protein coupled superfamily of receptors), the presence of two separate recognition sites within the receptor has been proposed:[16] one region representing the binding (or capture) site and the other region responsible for receptor activation (agonist site). If two such separate sites could exist within muscarinic receptors, it might also seem possible that serotonin receptors (as well as dopamine and adrenergic systems) might also share this characteristic. One might expect that these two recognition sites would have slightly different ligand recognition requirements, thus predicting that some compounds could possess different potencies between the two sites. This would lead to the possibility of compounds with a higher degree of receptor recognition in the "efficacy site" versus the binding or "capture site," leading to compounds with extraordinary agonist potency (i.e., CP-161,242). Conversely, this would also allow for compounds with better recognition at the "binding (capture) site" versus the "efficacy site" leading to reduced efficacy compounds (i.e., partial agonists and antagonists). We are presently exploring various means to test this hypothesis for 5-HT receptors.

Finally, both CP-161,242 and **4** possessed unexpectedly potent affinity for the benzodiazepine receptor. It is not clear from a cursory examination of these two compounds what molecular features are the source of this potent affinity. However, the indol-5-ylbenzimidazole and indol-5-ylpyridoimidazole portions of these molecules clearly are the novel, common component in these serotonergics, and we have done studies (the following paper in this journal) which demonstrate that the indol-5-ylbenzimidazole moiety of CP-161,242 and the indol-5-ylpyridoimidazole of **4** are responsible for their potent BZD receptor affinities. The combined, potent activity of CP-161,242 at both 5-HT_{1D} and BZD receptors makes CP-161,242 a unique pharmacological tool. We are presently exploring potential therapeutic areas where a combined 5-HT_{1D} receptor agonist and benzodiazepine receptor partial agonist might demonstrate synergistic benefits for disease treatment.

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8. The physical and spectral data for **4** is as follows: white foam; IR ($CHCl_3$) 1600, 1494, 1460, 1406 cm^{-1}; ^1H NMR ($CDCl_3$) δ 9.97 (br s, NH), 8.44 (dd, J=1.4 and 4.7 Hz, 1H), 8.35 (s, 1H), 8.14 (dd, J=1.4 and 8.1 Hz, 1H), 7.76 (br s, 1H), 7.35-7.25 (m, 3H), 6.98 (d, J=2.0 Hz, 1H), 2.95-2.88 (m, 2H), 2.66-2.60 (m, 2H), 2.31 (s, 6H); ^{13}C NMR ($CDCl_3$) δ 147.6, 144.8, 144.4, 135.9, 135.7, 128.2, 127.9, 126.8, 123.8, 118.7, 118.6, 115.0, 114.4, 112.2, 60.0, 45.3, 23.5; FAB LRMS (m/z, relative intensity) 306 ($[MH]^+$, 19), 155 (67), 135 (32), 119 (100), 103 (44). Anal. calcd for $C_{18}H_{19}N \cdot 1/8\ CHCl_3$: C, 67.98; H, 6.02; N, 21.86. Found: C, 68.33; H, 5.65; N, 21.84.
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10. The spectral and physical properties of **3** are as follows: off-white solid; mp, 204-205 °C; IR (KBr) 2226, 1614, 1495, 1473, 1460, 1455, 1440; ^1H NMR (DMSO-d_6) d 11.2 (br s, NH), 8.76 (s, 1H), 8.35 (s, 1H), 7.78 (d, J=1.9 Hz, 1H), 7.72-7.65 (m, 2H), 7.57 (d, J=8.5 Hz, 1H), 7.35 (s, 1H), 7.30 (dd, J=8.5 and 2.0 HZ, 1H), 3.07 (dd, J=14.1 and 3.4 Hz, 1H), 2.98-2.93 (m 1H), 2.56 (dd, J=14.1 and 9.0 Hz, 1H), 2.44-2.32 (m, 1H), 2.32 (s, 3H), 2.10 (dd, J=17.0 and 8.4 Hz, 1H), 1.77-1.63 (m, 1H), 1.63-1.41 (m, 3H); ^{13}C NMR (DMSO-d_6) d 147.2, 143.6, 137.5, 136.0, 128.5, 126.9, 126.8, 125.8, 125.3, 120.2, 118.0, 115.2, 113.5, 113.0, 112.5, 104.8, 66.5, 57.4, 40.9, 31.2, 29.5, 22.1; FAB LRMS (m/z, relative intensity) 357 (29), 356 ($[MH]^+$, 100), 309 (16), 275 (5), 233 (9), 195 (14), 177 (40), 155 (69); HRMS calculated for $C_{22}H_{21}N_5$ 355.1799, found 355.1889; $[a]^{25}_{Na}$ = +111° (methylene chloride, c=1). Anal. calcd for $C_{22}H_{21}N_5$: C, 74.34; H, 5.96; N, 19.70. Found: C, 74.15; H, 6.07; N, 19.76.
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15. For an experimental procedure see reference 14. In short, male Hartley guinea pigs (300-600 g) are used. Drugs are administered as a solution (1 mL/kg). All temperatures are taken rectally. Thirty minutes prior to drug administration, a baseline temperature reading is taken. Upon drug administration, rectal temperature is assessed at 30, 60, 120, 240, 360, 480, and 600 minutes post administration. Data are analyzed with analysis of variance with repeated measures and Newman-Keuls post hoc analysis. EC_{50} was calculated using 56 mg/kg p.o. as the maximally efficacious dose and linear regression to predict the dose that would yield 50% of the maximal effect.
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(*Received in USA* 28 *July* 1995; *accepted* 13 *September* 1995)