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THE DISCOVERY OF A NOVEL AND POTENT BENZODIAZEPINE RECEPTOR PHARMACOPHORE

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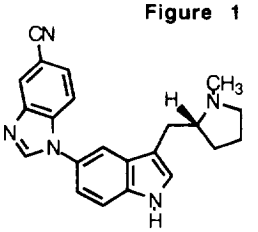
Abstract: 1-(Indol-5-yl)pyrido[2,3-b]imidazoles and 1-(indol-5-yl)benzimidazoles (**1**) have been found to be unique, novel templates for potent benzodiazepine receptor affinity. The "molecular switch" for this activity lies in the imidazole N3 atom: replacement of this nitrogen for carbon [i.e., as in 5-(indol-1-yl)indoles (**2**)] affords compounds devoid of affinity for the benzodiazepine receptor (Figure 1).

Introduction. In the preceding communication[2] in this journal, we presented our discovery of CP-161,242 (**1f**), an 1-(indol-5-yl)benzimidazole (Figure 1) which was a potent, orally active 5-HT_{1D} receptor agonist also possessing remarkable affinity for the benzodiazepine (BZD) receptor. While it has yet to be determined whether such a combination of pharmacological actions are desirable in a potential drug, we embarked on a study of CP-161,242 and its analogs aimed at understanding the source of their affinity for the BZD receptor. In this communication, we present the results of an initial study of the SAR surrounding this series.

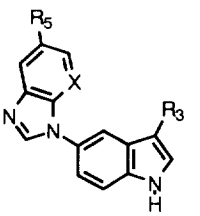
Ligands for the benzodiazepine receptor have been under intense scrutiny in recent years because of the potential array of therapeutic uses purported for this class of compounds. BZD receptor agonists have been successfully used in the treatment of anxiety (i.e., Valium®), while inverse agonists have been examined as potential drugs for the treatment of cognitive deficiencies (i.e., Alzheimer's Disease).[3] While there are a number of different structural types which possess BZD affinity, the major structural classes which possess potent BZD receptor activity are benzodiazepines (i.e., Valium®), β-carbolines, pyridodiindoles, and pyrazoloquinolines.[4]

In an effort to understand the dramatic benzodiazepine activity of CP-161,242 and its analogs, we also embarked on a modeling study of CP-161,242. Comparison of CP-161,242 and its analogs with other benzodia-

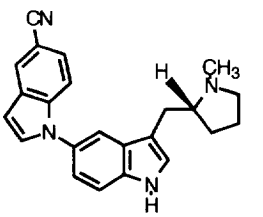
Figure 1



CP-161,242 (**1f**)
BDZ IC_{50} = 2.8 nM



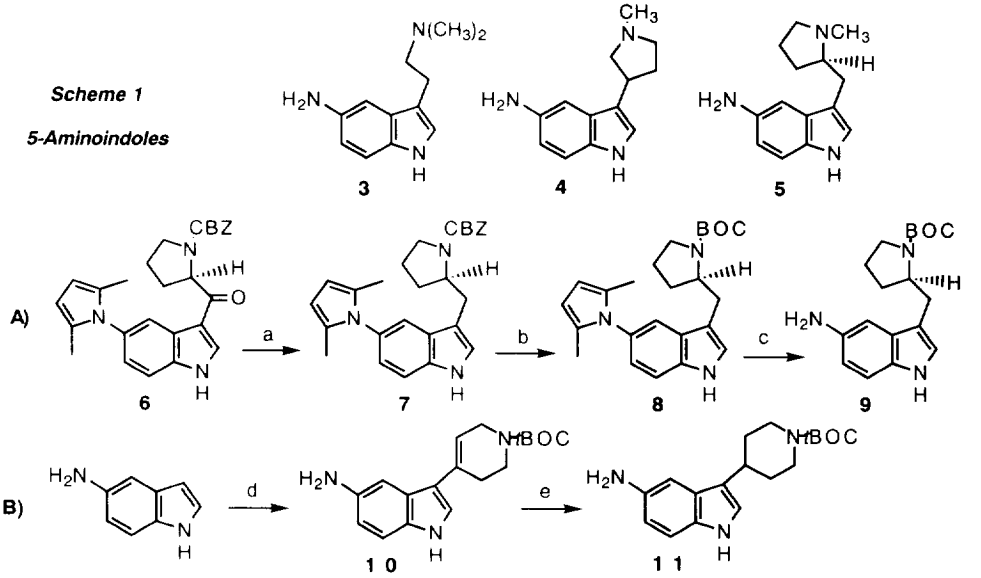
1 (X = N or C)



CP-294,879 (**2f**)
BDZ IC_{50} > 10,000 nM

zepine receptor ligands, specifically examining the areas of conserved hydrogen bonding regions within the series of ligands, strongly suggested that the imidazole nitrogen (N3) was crucial to the BZD activity of CP-161,242 and its analogs. Accordingly, the results of this modeling study directed us to examine compounds in which the imidazole (N3) nitrogen was replaced by carbon [i.e., 5-(indol-1-yl)indoles (**2**)]. The resulting analogs were, in fact, found to be devoid of BZD receptor affinity, identifying N3 of the fused imidazoles (**1**) as an extremely crucial element for BZD receptor affinity.

Chemistry.[5] Schemes 1 and 2 summarize the methods used to obtain the desired compounds for this study. Central to all the syntheses was the formation of the benzimidazole ring in **1a** to **1z** (Scheme 2A, Table 1), which resulted directly from a series of 5-aminoindole derivatives whose origins are depicted in Scheme 1. 5-Aminotryptamine (**3**),[6] 5-amino-3-(pyrrolidin-3-yl)indole (**4**),[7] and 5-amino-3-(pyrrolidin-2-ylmethyl)indole (**5**),[8] were prepared using published procedures (Scheme 1). 5-Aminoindole (**9**) was obtained through a straightforward series of protection/deprotection operations commenced with the reduction of the keto carbonyl in **6** (Scheme 1A).[8] The synthesis of **9** from **8** represents one of the few examples of the use of a 2,5-dimethylpyrrole as a $-NH_2$ protecting group.[8] The 5-amino-3-(tetrahydropyrid-4-yl)indole (**10**) was obtained using methods common in the synthesis of other 3-(tetrahydropyrid-4-yl)indoles (Scheme 1B).[9]

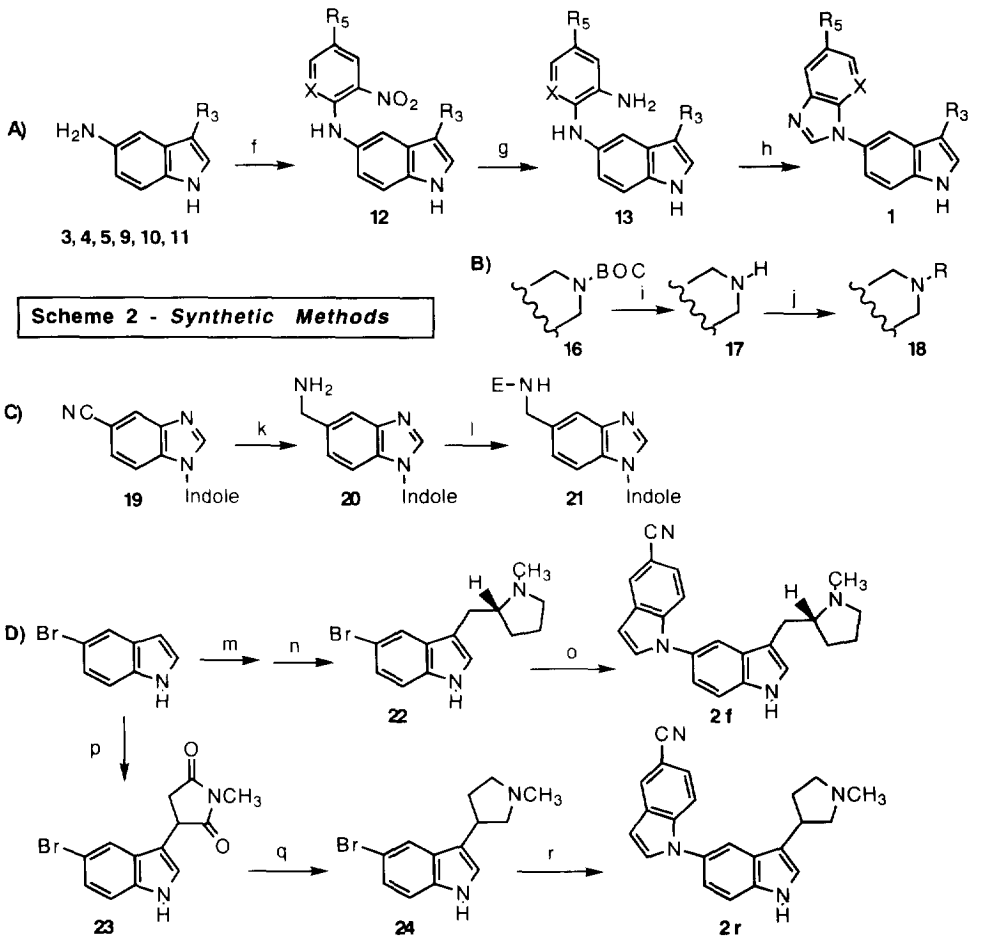


Conditions: (a) $LiBH_4$, THF, Δ (59%). (b) 1. Pd/C, H_2, EtOH; 2. $(BOC)_2O$, THF (91%). (c) $NH_2OH{\cdot}HCl$, Et_3N, *i*PrOH, H_2O, Δ (78%). (d) 4-*t*-BOC-piperidone, NaOMe, MeOH, Δ (70%). (e) Pd/C, H_2, EtOH (78%).

Displacement of halogen from the appropriate 1-halo-2-nitrobenzene (X=carbon) or 2-halo-3-nitropyridine (X=nitrogen) by the amine of the various 5-aminoindoles afforded **12** (Scheme 2A). Reduction of the nitro group in **12** afforded the diamines (**13**), which were cyclized using an appropriate formic acid synthon. It should be noted that the use of ethoxymethylenemalononitrile as the formic acid synthon[10] for the construction of the imidazole portion of benzimidazoles and pyridoimidazoles became the method of choice for

the cyclization of the diamines (**13**, Scheme 2A). Schemes 2B and 2C summarize other minor modifications (i.e., alkylations and acylations of amines) used to obtain some of the compounds of this study.

Crucial to this study (as discussed previously) was the synthesis of the 5-(indol-1-yl)indoles (**2f** and **2r**), which arose from an unprecedented (but low yielding) copper(I) promoted displacement of bromide from **22** and **24** with the sodium anion of 5-cyanoindole (Scheme 2D). The requisite 5-bromoindoles (**22**) and (**24**) were synthesized using similar methodology outlined previously.[11, 12]



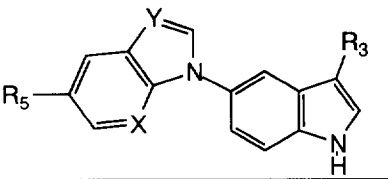
Scheme 2 - *Synthetic Methods*

Conditions: (f) 1-halo-2-nitroarene, EtOH, Et_3N, Δ (16-88%). (g) H_2, Pd/C, EtOH (used directly) or $FeSO_4$, NH_4OH, H_2O, EtOH (used directly). (h) ethoxymethylenemalononitrile, *i*PrOH, Δ (10-85%) or $(EtO)_3CH$, HCO_2H, Δ (22-75%) or DMF·DMA, TsOH (24-71%). (i) HCl(g), CH_2Cl_2, rt (64-100%). (j) alkyl halide, Et_3N, CH_2Cl_2 (34-60%). (k) $LiAlH_4$, THF, Δ (51%, R_3=D) or Raney nickel, H_2, NH_3, EtOH (89%, R_3=B). (l) Ph=N=C=O, EtOH, rt (57%) or CBZ-TRY-OH, carbonyldiimidazole, CH_2Cl_2 (54%). (m) 1. EtMgBr, benzene; 2. (R)-CBZ-proline acid chloride, benzene (89%). (n) $LiAlH_4$, THF, Δ (66%). (o) CuBr, 5-cyanoindole-sodium salt, pyridine, Δ (12%). (p) N-methylmaleimide, acetic acid, Δ (62%). (q) $LiAlH_4$, THF, Δ (48%). (r) CuBr, 5-cyanoindole-sodium salt, pyridine, Δ (13%).

Biology. Binding experiments were performed using methods outlined previously.[13] In short, guinea pig cerebellum was the tissue source and [^{3}H]flunitrazapam (0.5 nM) was used as the radioligand and chlordiazepoxide (10 μM) was used to determine non-specific binding. The pIC_{50} for diazepam (Valium®) was -7.85±0.03 (14.1 nM, n=4). The binding affinities of the compounds under study are summarized in Table 1.

TABLE 1 [a]

Benzodiazepine Binding versus [^{3}H]Flunitrazepam



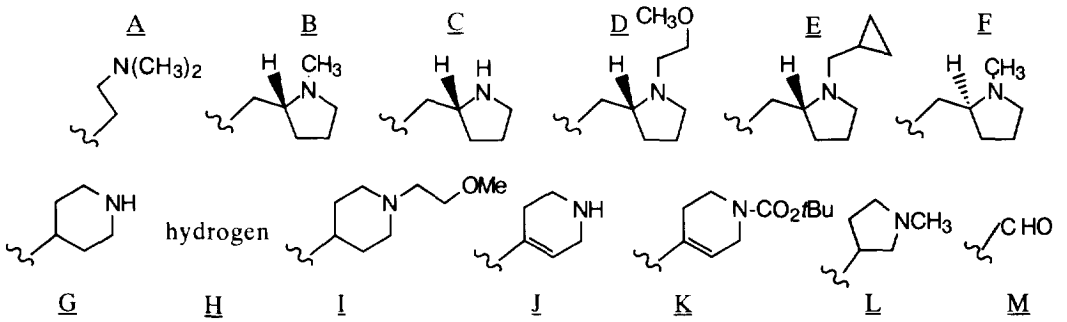
Compound	X	Y	R3	R5	IC_{50} (nM)	pIC_{50} (nM±SEM)
1a	N	N	A	-H	89	-7.05±0.04
1b	N	N	M	-H	209	-6.68±0.11
1c	N	N	B	-H	27	-7.57±0.06
1d	N	N	C	-H	110	-6.95±0.05
1e	C	N	B	-H	16	-7.81±0.13
1f	C	N	B	-CN	2.8	-8.55±0.05
2f	C	C	B	-CN	37% @ 10 uM	---
1g	C	N	B	-Me	0.16	-9.79±0.37
1h	C	N	B	-Cl	1.1	-8.97±0.28
1i	C	N	B	$-CH_2NH_2$	13	-7.89±0.08
1j	C	N	D	$-CH_2NH_2$	55	-7.26±0.09
1k	C	N	D	UREA [b]	17	-7.77±0.13
1l	C	N	D	-CN	24	-7.62±0.18
1m	C	N	E	-CN	27	-7.57±0.09
1n	C	N	B	TRYPT [c]	33	-7.48±0.18
1o	C	N	G	-CN	0.24	-9.62±0.09
1p	C	N	J	-CN	0.46	-9.34±0.17
1q	C	N	I	-CN	0.60	-9.22±0.18
1r	C	N	L	-CN	2.2	-8.66±0.11
2r	C	C	L	-CN	34% @ 10 uM	---
1s	C	N	H	-CN	18	-7.75±0.06
1t	C	N	M	-CN	19	-7.72±0.04
1u	C	N	F	-CN	1.8	-8.75±0.14
1v	C	N	H	-Me	0.79	-9.10±0.24
1w	C	N	G	-Me	0.49	-9.31±0.12
1x	C	N	K	-Me	31	-7.51±0.11
1y	N	N	B	-CN	9.8	-8.01±0.01
1z	N	N	B	4-Me	12	-7.91±0.09
CP-135,807	-	-	-	-	9600	-5.02±0.05

[a] Values presented are a result of at least three independent determinations, and are geometric means ± SEM. Diazepam was found to have an IC_{50} = 14 ± 1 nM under the identical binding conditions. Me = $-CH_3$ (i.e., methyl). ***R_3 substituents shown in Figure 2.***

[b] "UREA" is -NH(C=O)NHPh

[c] "TRYPT" is -NH-tryptophan-NH-CBZ

Figure 2 - *R3 Substituents*



Discussion. Figure 3 graphically summarizes the results of our SAR study. The 1-(indol-5-yl)benzimidazole (**1**, X=carbon) and 1-(indol-5-yl)pyrido[2,3-b]imidazole (**1**, X=nitrogen) templates possess high inherent affinity for the benzodiazepine receptor. The most potent compound within our series (**1g**, IC_{50}=0.16 nM) was approximately 100-fold more potent than diazepam (IC_{50}=14 nM) in its binding to benzodiazepine receptors. It should be noted that the immediate isolated chemical precursor to **1c** was CP-135,807 [5-(3-nitropyrid-2-ylamino)-3-(N-methylpyrrolidin-2R-ylmethyl)indole][14] which was approximately 350x less potent than **1c** (Table 1), thus indicating that the BZD receptor affinity was unique to **1**.

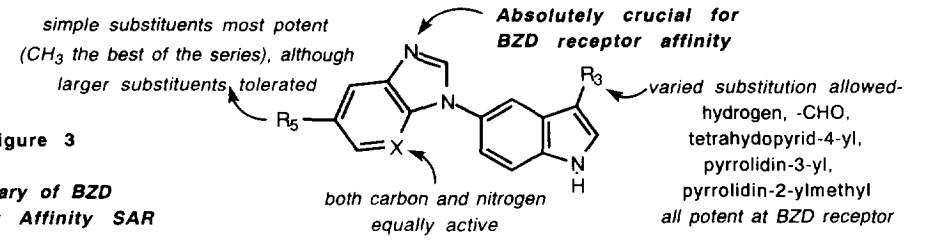


Figure 3

Summary of BZD Receptor Affinity SAR

A variety of substituents and substitution patterns are tolerated within these novel BZD receptor ligand templates (**1**). Notably, substitution at C3 of the indolic component can range from simple hydrogen (**1s** and **1v**) to much larger and basic groups,[15] with nanomolar affinity maintained throughout the substitutions. In fact, two of the most potent compounds (IC_{50} < 1 nM) in this study (i.e., **1v** and **1q**) define opposite ends of this substitution spectrum. However, affinity does diminish when the size of the indole-C3 substituent becomes too great (i.e., **1w** and **1q** versus **1x**). It should be noted that there was no receptor stereodifferentiation between the enantiomers **1f** and **1u**.

Substitution at the C5 position of the benzimidazole (and pyridoimidazole) component of the template appears to increase BZD receptor affinity (i.e., **1e** versus **1f** versus **1g**). While the most potent compounds studies possessed simple C5 substitution (i.e., -CN, $-CH_3$, and -Cl), even large C5 substituents afforded compounds (i.e., **1k**, **1i**, and **1n**) with BZD receptor affinities similar to that of diazepam (14±1 nM), despite the fact that these compounds (**1k** and **1n**) are approximately 100-fold less potent than their smaller C5 substituted analogs (i.e., **1g**).

Most striking in this study is the *absolute necessity* of the imidazole (N3) nitrogen for benzodiazepine receptor affinity. Comparison of pairs of compounds [(**1f** and **2f**) and (**1r** and **2r**)] which are identical with the exception of the crucial nitrogen, unequivocally demonstrates the unique receptor recognition role played by the N3 benzimidazole nitrogen. The potency difference within both pairs of compounds (**1f** versus **2f** and **1r** versus **2r**) is greater than 3500-fold (Table 1). The 5-(indol-1-yl)indoles (**2f** and **2r**) are effectively devoid of affinity for the benzodiazepine receptor. *The presence or absence of the N3 nitrogen in this series of compounds is the "molecular switch" which turns BZD receptor affinity "on" in the benzimidazoles and pyridoimidazoles (1) and which turns BZD receptor affinity "off" in the 5-(indol-1-yl)indoles (2).*

In conclusion, the 1-(indol-5-yl)benzimidazole and 1-(indol-5-yl)pyrido[2,3-b]imidazole are novel templates for extremely potent benzodiazepine receptor affinity. A wide degree of substitution is accepted for continued potent activity, with the single exception of replacement of the imidazole (N3) nitrogen for carbon, which leads to compounds lacking any significant affinity for the BZD receptor. We are continuing to define the SAR of these novel BZD receptor ligand templates. Furthermore, studies are presently in progress examining the level of intrinsic efficacy across our active series of compounds, their subtype specificity, and potential pharmacological uses for such a series of BZD ligands. The results of these studies will be presented in due course in their appropriate forums.

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15. The serotonergic (5-HT_{1D} receptor) activity of the C3 basic nitrogen containing groups (i.e., A, B, C, D, E, F, G, I, J, and L in Figure 2) was maintained throughout these series (i.e., **1** and **2**), with IC_{50}'s for the 5-HT_{1D} receptor generally less than 10 nM. However, 5-HT_{1D} versus 5-HT_{1A} receptor selectivity was compromised in **2** versus **1**.

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