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POTENTIAL ANXIOLYTIC AGENTS. 2. IMPROVEMENT OF ORAL EFFICACY FOR THE PYRIDO[1,2-*a*]BENZIMIDAZOLE (PBI) CLASS OF GABA-A RECEPTOR MODULATORS

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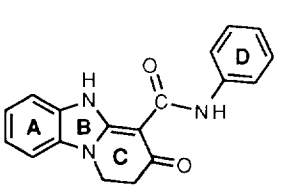
Summary: We have further explored the structure-activity relationships of pyrido[1,2-*a*]benzimidazole (PBI) derivatives (viz. prototype **1**), a novel series of central GABA-A receptor modulators, with the intent of enhancing oral efficacy. A study involving the introduction of acidic or basic groups led to the identification of RWJ-38293 (**3a**) as a potential anxiolytic agent.

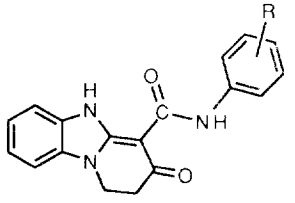
Effective therapeutic agents for the treatment of anxiety have been vigorously sought since the exciting advent in the 1960's of chlordiazepoxide, an archetype of the benzodiazepine (BZD) structural class.[1] During the intervening years, it has been established that such drugs exert their anxiolytic effects via high affinity binding to a specific "BZD" site on the GABA-A (γ-aminobutyric acid-A) receptor complex.[2] Thus, the anxiolytic drugs modulate the effects on certain brain neurons of GABA binding, including GABA-induced chloride ion influx, membrane hyperpolarization, and desensitization to excitatory stimuli.

The benzodiazepine class embraces many anxiolytic agents of varied structures. Furthermore, there are other important and useful classes such as the β-carbolines, imidazopyridines, pyrazoloquinolines, and imidazoquinoxalines. Generally speaking, the drugs in clinical use, such as diazepam, possess high affinity for the BZD binding site and exhibit agonist-type anxiolytic pharmacology.[1b,2c,2e,2h] In addition to their anxiolytic properties, most of these drugs tend to possess anticonvulsant, sedative, and muscle relaxant effects, while they are usually plagued by an adverse interaction with ethanol and a liability for physical dependence.

In our search for novel anxiolytic agents devoid of undesirable side effects, we have been intensively exploring the unique pyrido[1,2-*a*]benzimidazole (PBI) structural series, for which **1** is a prototype.[3] Because compounds in this class have biological properties ranging from pure agonists to antagonists, diverse structural



1 (RWJ-16979)



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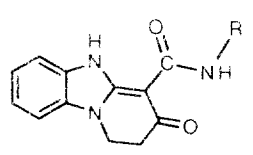

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modifications have been assessed in order to identify a suitably balanced agent for clinical development in the treatment of anxiety disorders. A key problem that surfaced in the early stage of this project was a lack of oral bioavailability: although many of the more potent compounds in vitro were also potent in vivo by intraperitoneal administration, they were relatively weak by oral administration. Thus, we sought to address this problem by the design, synthesis, and testing of structural analogues with specifically altered physicochemical properties. Our initial approach entertained the use of acidic or basic groups, either attached to or inserted in the parent ring system, for enhancing water solubility. This study culminated in the discovery of RWJ-38293 (**3a**), an orally effective agent in animal models of anxiety with minimal sedative or muscle relaxant side effects. Herein, we report highlights of this investigation.

Biology. Our early structure-activity studies[3,4] indicated that potency is highly dependent on the nature of substituents on the anilide phenyl ring ("D-ring") of **1**, particularly on stereoelectronic factors. For example, the receptor binding affinity of D-ring analogues for the BZD site on GABA-A receptors[5] is strongly reduced with a variety of simple, conventional groups, such as 4-fluoro, 4-chloro, 4-butyl, 4-nitro, 4-cyano, 4-methylthio, 2-methoxy, 2-methylthio, and 2-methyl, but largely retained with other simple groups, such as 2-fluoro, 2-chloro, 4-methoxy, and 3-methoxy. For readily introduced acidic functionalities, we considered carboxylic acid and hydroxy groups on the D-ring. As expected from the above substitution trends, the 4-carboxy derivative, **2a**, shows strongly diminished receptor affinity, whereas the 4-hydroxy compound, **2b**, shows reasonably high affinity (see Table 1); however, the in vivo activity[6] for **2b** is rather modest. By contrast, our results with basic substitution proved to be much more interesting.

Although the 4-amino compound, **2c**, has poor receptor affinity, the 2-amino isomer, **2d**, is much better. However, the N,N-dimethyl derivatives show a different binding profile with the 3-position isomer (**2f**) better than 4-position isomer (**2e**), which is much better than the 2-position isomer (**2g**). In vivo activity for these amino compounds in the metrazol test is in the same dose range except for **2g**, which appears to be less potent. A modest increase in receptor binding and metrazol potency accompanied additional 2-fluoro substitution (cf. **2h** and **2e**), whereas 2-methyl substitution decreased potency (cf. **2i** and **2e**). Although **2e** (RWJ-38137) has reduced receptor affinity, it was selected for further evaluation based on in vivo data (vide infra; see Table 2). The analogue with an N-5 methyl group, **5**, shows a nearly 10-fold enhancement of receptor binding (cf. **2e**), greater than expected,[3] but this is not translated into improved in vivo potency.

Pyridyl analogues **3a-3c** were found to have good receptor binding and in vivo potency (Table 1). Since the 4-pyridyl analogue, **3a** (RWJ-38293), exhibited the best in vivo potency in both assays, it was selected for further evaluation (vide infra; Table 2).[7] The methyl quaternary salt of **3a** (i.e., **3d**) is not very interesting. For substitution of the pyridyl 3-position, fluoro (**3g**) was better than chloro (**3e**), which was better than methyl (**3f**). As seen with **2e** (Table 1) and **1**,[3] such fluoro substitution increases receptor affinity by 2-4 times relative to the parent compound (cf. **3g** and **3a**); whereas, the in vivo results may not improve. Spacing of the pyridyl ring by one methylene (**3i** and **3j**) afforded receptor binding of the same magnitude, but in vivo activity was absent. This suggests that **3i** and **3j** may have significant antagonist properties, which is consistent with their GABA shift values of 0.9 and 1.1, respectively.[3] Of three pyrazinyl and pyrimidinyl compounds studied, **3k-3m**, 2-pyrimidinyl derivative **3l** has reasonable potency in vitro and in vivo. C-Ring unsaturation (**4**) is accompanied by less attractive activity, whereas N-5 ethyl substitution (**6**) imparts a 2-fold enhancement of receptor binding (cf. **3a**), as might be expected, but with in vivo potency unchanged.[3]

Table 1. Chemical Properties and Biological Data for PBI Derivatives[a]

Cpd No.	R	mp, °C (solv)[b]	IC_{50} (nM) no GABA	IC_{50} (nM) GABA	G.S.[c]	Metrazol, i.p. ED_{50} (mg/kg)[d]	Conflict, i.p. MED (mg/kg)[e]
1		224-225 (C/E)	9.1 (7.8-10.6)	5.9 (5.0-7.0)	1.5	5.2 (2.2-13.3)	10
2a	4-(HO_2C)	305-306 (F/D)	>10,000	>10,000	--	>30	10
2b	4-HO	298-301 (M/C)	46 (36-59)	31 (27-37)	1.5	>30	10
2c	4-H_2N	280-281 (M)	13,000	3,400	3.8	2.6 (0.59-12.0)	>10
2d	2-H_2N	212-213 (D/M/E)	50 (40-62)	33 (25-44)	1.5	2.1 (0.024-7.9)	10
2e	4-Me_2N	213-214 (D/E)	270 (160-440)	210 (120-380)	1.2	3.1 (1.8-5.2)	3
2f	3-Me_2N	239-241 (D/E)	37 (22-61)	25 (14-43)	1.5	2.8 (0.59-6.8)	>10
2g	2-Me_2N	215-217 (D/E)	10,000	--	--	4.9[f]	10
2h	2-F-4-Me_2N	232-234 (D/E)	120 (72-190)	87 (50-150)	1.4	1.0[f]	30[g]
2i	2-Me-4-Me_2N	210-211 (D/E)	4,000	1,500	2.7	3.2[f]	>10
3a	4-Py	274-276 (D/E)	160 (100-260)	78 (48-130)	2.1	3.1 (0.42-6.7)	3
3b	3-Py	258-263 (F/EE)	51 (44-59)	48 (41-56)	1.1	2.6 (0.59-12.0)	10
3c	2-Py	272-274 (A)	59 (46-88)	36 (31-41)	1.7	11.2[f]	10
3d	4-(1-MePy)$^+$	280-282 (D/M)	6,300	3,100	2.0	>10	>10
3e	4-(3-ClPy)	257-259 (D/E)	220 (130-320)	100 (65-140)	2.2	2.6 (0.76-7.2)	>10
3f	4-(3-MePy)	269-272 (D/M/E)	7,100	12,000	0.6	>10	>10
3g	4-(3-FPy)	283-285 (D/M/E)	53 (38-70)	20 (13-29)	2.6	1.3[f]	10
3h	3-(2-ClPy)	262-265 (C/M)	68 (33-140)	65 (33-130)	1.1	3.7 (1.4-11.0)	10
3i	CH_2(4-Py)	260-262 (D/M/E)	65 (46-86)	69 (42-95)	0.9	>10	>10
3j	CH_2(3-Py)	227-229 (AC)	150 (130-190)	140 (120-170)	1.1	>10	>10
3k	2-pyrazinyl	297-300 (F/C)	360 (210-620)	400 (240-670)	0.9	>10	>10
3l	2-pyrim.	236-238 (D/M/E)	54 (36-80)	50 (34-73)	1.1	2.7 (0.021.59-8.6)	10
3m	4-pyrim.	290-291 (D/E)	480 (260-910)	370 (210-660)	1.3	>10	10
4		313-315 (D/M/E)	260 (150-380)	150 (90-220)	1.7	>10	>10
5		177-179 (D/EA)	29 (11-76)	16 (6-48)	1.8	1.3 (0.50-8.1)	10
6		192-194 (D/EA)	80 (53-109)	27 (17-39)	3.0	1.2 (0.94-1.8)	3
diazepam			28 (18-39)	13 (8-21)	2.2	0.11 (0.058-0.15)	5

a. Under the heading "R", Py = pyridyl and pyrim. = pyrimidinyl. Compounds were isolated and purified in unadducted form (i.e., no acid or base addition salts) except for **2c**, which is a monohydrochloride salt. Pyridinium salt **3d** is an iodide salt solvated with 0.1 mol of CH_2Cl_2. Microanalytical data (C, H, N) were within the accepted range (±0.4%). ^1H NMR spectral data were consistent with the assigned structures. Information on biological testing is provided in the text and/or in ref 4.

Table 1. Continued

b. Mp values are corrected to a set of standards. The recrystallization solvent is given in parentheses: A = acetone, AC = acetonitrile, C = $CHCl_3$, D = CH_2Cl_2, E = EtOH, EA = ethyl acetate, EE = ethyl ether, F = dimethylformamide, M = MeOH.

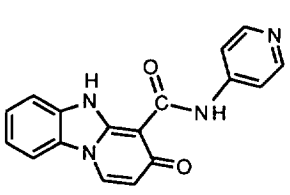
c. G.S. = GABA shift = IC_{50}(no GABA)/IC_{50}(GABA).

d. Antagonism of seizures in mice induced by metrazol (pentylenetetrazole); 95% confidence interval is given in parentheses.

e. Inhibition of conflict behavior in rats in the Vogel conflict paradigm; MED = minimum effective dose.

f. The confidence limits could not be calculated because the slope of the dose-response curve was not linear.

g. This result is for oral administration.



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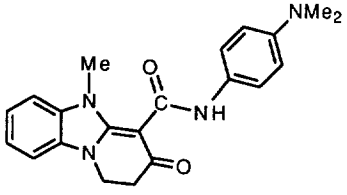

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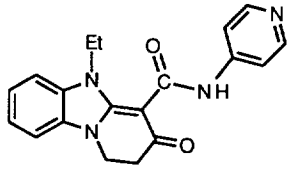

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Table 2. In Vivo Biological Data for Selected Compounds[a]

Cmpd	Metrazol ED_{50}[b]		Conflict MED[b]		EtOH sleep MED[c]		Horiz screen ED_{50}[d]	
	p.o.	i.p.	p.o.	i.p.	p.o.	i.p.	p.o.	i.p.
1	10-30	5.2	>10	10	30	3	>30	~10
2e	14	3	10	3	>60	10	>30	~10
3a	7.8	2.2	10	3	>60	>60	>30	>30
6	10	3	3	3	10[e]	--	30	3
diazepam	0.3	0.11	5	5	1	0.1	1	0.1

a. Data are given in mg/kg and are derived from studies in mice, except for the conflict test in rats.

b. See relevant footnotes in Table 1.

c. Potentiation of ethanol-induced sleep in mice; MED = minimum effective dose.

d. Activity in the horizontal screen test in mice.

e. This result is for p.o. dosing in rats.

As mentioned above, **2e** and **3a** have pharmacological attributes that warranted further investigation. In fact, these analogues, especially **3a**, display good oral efficacy in both the metrazol and conflict assays, while having a useful separation from ethanol interaction and muscle relaxant and side effects, according to two assays: potentiation of ethanol sleeping time and the horizontal screen (see Table 2). Although analogue **6** also displays good oral efficacy, it possesses a less desirable separation from side effects (Table 2). Given that the results for **3a** compare quite favorably relative to those for prototype **1** and diazepam, this compound was identified as a potential anxiolytic agent for evaluation in more advanced pharmacological models.

Synthetic Chemistry. Most of the compounds in Table 1 were synthesized by the methods previously described by us.[3] Acid **2a** was prepared from the corresponding ethyl ester, synthesized in the standard manner, by alkaline hydrolysis (NaOH) in refluxing 95% ethanol. The aniline required for **2h** was prepared from dimethylamine and 2,4-difluoronitrobenzene[8] followed by catalytic (Pd/C) hydrogenation of the resultant nitro aniline. The aniline for **2i**, as well as the aminopyridines for **3e** and **3f**, were prepared in good yield from the appropriate pivaloylamido derivatives according to the method of Turner.[9] Treatment of **3a** with iodomethane in methylene chloride afforded **3d** as a white solid (90%). Nitration of 3-fluoropyridine N-oxide[10] followed by catalytic hydrogenation (Pd/C) gave the aminopyridine (21%) needed for **3g**.

Oxidation of **3a** with MnO_2 in refluxing xylenes[11] gave **4** (65%). Reaction of the previously prepared enones[3] with the appropriate isocyanates[12] gave N-alkylated targets **5** (85%) and **6** (53%).

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4. Unpublished results from our laboratories. See: Maryanoff, B. E.; McComsey, D. F.; Ho, W. World Patent application WO9404532-A1, 3 Mar 94, 74 pp.
5. Affinity of test compounds (IC_{50} value) for the BZD site of the GABA-A receptor was determined by

competition with the radioligand [^{3}H]-flunitrazepam;[13] compounds were tested at five concentrations in a tissue preparation from rat cerebral cortex. Binding was measured in the absence and presence of GABA (1 mM) to obtain the GABA shift (G.S.) value, from which one can estimate agonist, antagonist, and inverse agonist activity for a ligand.[3] A "full agonist" would have a G.S. greater than or equal to 2.0, an antagonist would have a G.S. in the vicinity of 1.0, an inverse agonist would have a G.S. of less than or equal to 0.7, and a partial agonist would have a G.S. in the range of 1.0 to 1.5.[3]

6. For the in vivo data in Tables 1 and 2, we measured the ability of test compounds to block the tonic-clonic component of seizures induced by metrazol (pentylenetetrazole, PTZ) in mice,[14] and to release (i.e., disinhibit) behavior that had been suppressed by punishment in rats ("Vogel conflict" test),[15] the latter of which affords anxiolytic activity in terms of a minimum effective dose (MED). In Table 2, we also tested selected compounds for their interaction with ethanol by their ability to potentiate the effects of ethanol on sleep[16] and as skeletal muscle relaxant and/or sedative activity via the horizontal screen assay.[17] Intraperitoneal (i.p.) or oral (p.o.) administration was as indicated.
7. The monohydrochloride salt of **3a** is highly soluble in water, whereas prototype **1** (free base) is very insoluble in water. For **3a** the logP (octanol-water partition coefficient) is 3.02 and the pK_a is 6.12, whereas the logP for **1** is 33 and the pK_a is 8.05 (all values experimentally measured).

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