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POTENTIAL ANXIOLYTIC AGENTS. 3. NOVEL A-RING MODIFIED PYRIDO[1,2-a]BENZIMIDAZOLES.

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Abstract: A variety of pyrido [1,2-a] benzimidazoles (PBIs) modified on the A-ring were prepared and evaluated for affinity to the benzodiazepine binding site on the GABA-A receptor and in animal models predictive of anxiolytic activity in humans. A-ring benzo-fused derivative 7 exhibited potent activity, as did the 6- and 7-pyrido compounds 3 and 4. © 1999 Elsevier Science Ltd. All rights reserved.

There is continuing medical need for drugs for the treatment of anxiety that have a greater separation of therapeutic effects from motor discoordination, sedation, and abuse liability.¹ The GABA-A receptor, which contains the well-known benzodiazepine binding site, can play a key role in the discovery of improved anxiolytic drugs. On the basis of our current understanding of the structure and function of the GABA-A receptor, it appears that subtle differences in receptor subtype selectivity can have profound effects on biological activity.² We have already described a new class of anxiolytics, termed "PBIs," as exemplified by **1**, which display potent GABA-A receptor affinity and activity in animal models predictive of anxiolytic activity in humans.³ We have varied this PBI framework and its substituents to identify compounds with the optimum properties for clinical evaluation. In this paper, we present structure-activity studies centered on the A-ring of the PBI nucleus, including structures with various substitution (**2**), with nitrogen inserted **3**–**5**, and with benzene ring fusion (**6**–**8**). Biological evaluation of these new compounds has provided useful information concerning the steric and electronic requirements for activity within the PBI series.





A-ring substituted derivatives 2 are listed in Table 1, and were prepared by chemistry we have already described.³ The routes to prepare pyridyl derivatives 3-5 were similar, starting from the three appropriate aminonitropyridines.⁴ Naphthyl derivatives 6-8 were prepared as shown in Schemes 1 and 2.



The preparation of compounds of type 7 is shown in Scheme 1. Key naphthyl diamine 9 was treated with ethyl acrylate to produce 10, which was condensed with $EtOC(O)CH_2C(OMe)=NH\cdotHCl$ to afford 11. Dieckmann condensation of 11 yielded 12, which was followed by reaction with arylamines to give 7. For the preparation of the substitution pattern found in 6 and 8, 2-amino-1-nitronaphthalene⁵ 13 was employed (Scheme 2). This compound was hydrogenated (10% Pd/C) to diamine 14, which was then treated with ethyl acrylate to afford a ca. 1:1 mixture of isomers 15 and 16 which could be separated from each other in yields of ca. 20% each.⁵ Homologation, as previously described, to 17 and 18 was followed by condensation with the appropriate aryl amines to give 6 and 8. Alternatively, 13 was reacted directly with acrylonitrile to produce 19 which was converted to the single isomer 17.

The biological activity of the A-ring modified derivatives is given in Tables 1 and 2, and along with that of diazepam in Table 1. The in vitro receptor affinity is presented along with the GABA shift (G.S.), which is the ratio of binding in the absence and presence of GABA (1 mM). A full agonist such as diazepam has a G.S. of >2.0, partial agonists range between 1.2-2.0, antagonists are at 1.0, and inverse agonists are <1.0. We sought to have the partial agonist profile to minimize side effects such as sedation and abuse liability associated with full agonists. Our in vivo evaluation has consisted of a battery of tests including the inhibition of metrazole-induced seizures in mice and an experimentally induced conflict test in rats. In both assays, the data are



presented as the dose at which efficacy is first observed (minimum effective dose, MED). The in vivo data on intraperitoneal and oral administration are shown in the tables. For the Ar portion of the amide moiety, we have restricted our disclosure here to either phenyl or fluoro-substituted phenyl to directly evaluate the effects of modification to the A-ring of the PBI structure.

A variety of A-ring substituted analogs showed good in vitro activity at the GABA-A binding site, but only a subset of these have had sufficient in vivo activity to pursue further (Table 1). Hydroxy substitution on the A ring at positions 6, 7, and 9 (viz. 19–21) provided compounds with <50 nM GABA-A receptor affinity, but these were not active in vivo. The G.S. for 19 was an unacceptably high value of 3.0, whereas the G.S. of 20 of 1.1 indicated functional antagonism. Methoxy 6- and 7-substituted compounds 22–25 displayed a similar profile, with the exception that the 2,6-difluoro derivative 23 was unexpectedly less active in vitro (156 nM IC₅₀ for 23 vs. 7.8 nM IC₅₀ for 22). The 6-methyl congeners 26 and 27 had modest in vitro affinity, but both showed activity in the metrazol seizure test when administered intraperitoneally and 26 was active at 10 mg/kg after oral administration. 7-Trifluoromethyl compound 28 exhibited much less in vitro affinity (184 nM IC₅₀) than the corresponding direct halo derivatives discussed below. The 6- and 7-chloro compounds 29–33 displayed modest to high affinity at the GABA-A receptor and also in vivo activity. For example, 2,6-difluorophenyl amide 33 had a 6.3 nM IC₅₀, a G.S. of 2.2, and activity in both animal tests when given intraperitoneally and orally. The high levels of in vitro and in vivo activity for these chloro compounds, in combination with their modest G.S. levels (1.6–2.2), prompted us to explore halo substitution in these positions in more detail. Since 8-chloro compound 34 was less active, we avoided extensive SAR modification at that position. 7-Fluoro

Table 1. Biological Data for A-Ring Substituted PBI Derivatives



					b	Metrazol Test Anticonflict				
Compd			GABAA			ED_{50}	(mg/kg)	MED	(mg/kg)	
No.	Х	Ar	Formula ^a	mp (°C)	IC ₅₀ (nM)	G.S.	ip	ро	ip	ро
19	6-HO	2-FPh	$C_{18}H_{14}FN_{3}O_{3}^{c}$	>250	10.4	3.0	-	-	-	>10
20	7-HO	Ph	$C_{18}H_{15}N_{3}O_{3}^{d}$	271-275	40.5	1.1	>30	>30	>30	>30
21	9-HO	2-FPh	C ₁₈ H ₁₄ FN ₃ O ₃	>250	14.0	2.8	>1	30	>1	>30
22	6-MeO	2-FPh	$C_{19}H_{16}FN_3O_3$	>250	7.8	1.4	-	-	-	-
23	6-MeO	2,6-F ₂ Ph	$C_{19}H_{15}F_2N_3O_3$	>250	156	3.7	-	-	-	-
24	7-MeO	Ph	C ₁₉ H ₁₆ N ₃ O ₃	215-220	39.5	2.5	3	>10	>10	>30
25	7-MeO	2-FPh	C ₁₉ H ₁₆ FN ₃ O ₃	252-254	6.1	1.2	>10	>10	>10	>30
26	6-Me	Ph	$C_{19}H_{17}N_3O_2$	224-226	10.1	2.8	ca. 1	>10	>10	>30
27	6-Me	2-FPh	$C_{19}H_{16}FN_{3}O_{2}$	269-272	96.3	1.5	1	10	10	>30
28	7-CF3	2-FPh	$C_{19}H_{13}F_4N_3O_2$	273-276	184	4.7	1	-	-	>10
29	6-Cl	2-FPh	$C_{18}H_{13}CIFN_3O_2^{e}$	243-246	7.1	1.8	1	>30	10	>10
30	6-Cl	2,6-F ₂ Ph	C ₁₈ H ₁₂ ClF ₂ N ₃ O ₂ ^f	228-231	1.1	1.7	0.3	10	10	>10
31	7-Cl	Ph	$C_{18}H_{14}CIN_3O_2$	267-269	65.6	1.6	1	20	10	>30
32	7-Cl	2-FPh	C ₁₈ H ₁₃ ClFN ₃ O ₂ ^g	265-267	5.1	1.8	0.3-1	ca. 30	10	>10
33	7-Cl	2,6-F ₂ Ph	C ₁₈ H ₁₂ ClF ₂ N ₃ O ₂ ^h	212-213	6.3	2.2	0.3-1	3-10	10	10
34	8-Cl	Ph	C ₁₈ H ₁₄ ClN ₃ O ₂	267-269	174	2.1	>10	>30	>10	30
35	7-F	2-FPh	$C_{18}H_{13}F_2N_3O_2^{i}$	266-268	1.9	1.8	<1	>30	10	>30
36	7-F	2,6-F ₂ Ph	$C_{18}H_{12}F_3N_3O_2$	214-216	6.3	2.2	0.3	3	3	3
37	6,7-F ₂	2-FPh	$C_{18}H_{12}F_3N_3O_2^{j}$	264-267	1.4	1.7	0.2	0.2	-	10
38	6,7-F ₂	2,6-F ₂ Ph	$C_{18}H_{11}F_4N_3O_2$	178-180	1.1	1.4	0.2	0.2	-	3
39	6,8-F ₂	2-FPh	$C_{18}H_{12}F_{3}N_{3}O_{2}^{k}$	272-274	19.8	1.2	>1	>1	-	>10
40	6,8-F ₂	2,6-F ₂ Ph	$C_{18}H_{11}F_4N_3O_2$	200-202	30.1	2.4	>1	>1	-	>10
41	8,9-F ₂	2-FPh	$C_{18}H_{12}F_3N_3O_2$	306-309	857	2.5	>1	>1	-	>10
42	8,9-F ₂	2,6-F ₂ Ph	$C_{18}H_{11}F_4N_3O_2$	230-232	66.2	1.7	>1	>1	-	>10
diaz	epam				4.9	2.2	0.11	0.5	5	>10

a. All compounds were characterized by 300-MHz proton NMR, MS, and elemental analysis (C, H, N; $\pm 0.4\%$ unless noted otherwise). b. Determined by competitive binding experiments with tritiated flunitrazepam in the absence of added GABA at five concentrations in tissue preparations from rat cerebral cortex. c. 0.5 mol H₂O. d. 0.5 mol CHCl₃, 0.1 mol H₂O. e. 0.5 mol MeOH, 0.75 mol H₂O; H: calcd, 4.29; found, 3.54. f. 0.75 mol H₂O. g. H: calcd, 3.66; found, 3.18. h. 0.4 mol H₂O. i. 0.25 mol H₂O. j. 0.2 mol MeOH and H₂O; H: calcd, 3.71; found, 3.22. k. C: calcd, 60.17; found, 59.29.

						Metra	zol Test	Anticonflict	
Compd				GABAA		ED ₅₀ (mg/kg)		MED	(mg/kg)
No.	Ar	Formula	mp (°C)	IC ₅₀ (nM)	G.S.	ip	ро	ip	ро
A-Ring	Pyridyl								
3a	Ph	$C_{17}H_{14}N_4O_2$	259-261	8.3	3.0	3	<30	>10	30
3b	2-FPh	$C_{17}H_{13}FN_4O_2$	271-274	3.5	2.7	1	10	10	>30
3c	2,6-F ₂ Ph	$C_{17}H_{12}F_2N_4O_2^{b}$	271-273	30.1	1.8	3	30	>10	>10
4	2,6-F ₂ Ph	$C_{17}H_{12}F_2N_4O_2$	205-207	17.7	2.4	>10	-	-	3
5a	Ph	$C_{17}H_{14}N_4O_2$	240-244	4380	2.1	>10	>10	>10	>30
5b	2-FPh	$\mathrm{C_{17}H_{13}FN_4O_2}$	263-265	291	1.3	>10	>10	>10	>30
A-Ring	Naphthyl								
6	2-FPh	$C_{22}H_{16}FN_3O_2$	278-279	154	2.8	>1	>30	-	>10
7	2-FPh	$C_{22}H_{16}FN_{3}O_{2}$	244-248	17.0	1.0	1	>30	10	>10
8	2-FPh	$C_{22}H_{16}FN_3O_2$	297-299	>1000	-	>1	>30	-	>10

Table 2. Biological Data for A-Ring Modified PBI Derivatives^a

a. Biological tests as in Table 1. b. 1 mol HCl, 1.2 mol H₂O.

compounds **35** and **36** were both active, and **36** is noteworthy because of the relatively high level of in vivo activity, including after oral administration. 6,7-Difluoro compounds **37** and **38** displayed particularly good activity, including 0.2 mg/kg MED po in the mouse metrazole and 3 mg/kg MED po in the rat conflict tests. By contrast, 6,8- and 8,9-difluoro compounds **39–42** had a much lower biological effect in the tests examined. Due to the high levels of activity seen with 6- and 7-halo substitution in the PBI series (c.f. **33** and **38**), we have focused on this modification for the bulk of our subsequent research.

Of the A-ring pyridyls examined, those with nitrogen in the 6-position of the PBI A-ring (viz. 3) proved to be the most promising. For example, 3b had a 3.5 nM IC₅₀ at the GABA-A receptor, and in vivo activity in both tests when given intraperitoneally and activity at 10 mg/kg orally in the mouse metrazol. However, the relatively high G.S. values for 3b precluded further interest. Compounds 4 and 5, in which the nitrogen is at positions 7 and 9, had higher GABA-A receptor IC₅₀s. Compound 4 had a noteworthy 3 mg/kg MED in the rat conflict when given orally. Unlike naphthyls 6–8 and compounds 19–42, the A-ring pyridyls have significant water solubility (>1%) when acid addition salts are prepared from them.⁶

Naphthyl compounds **6–8** involve dramatic spatial modifications to the PBI structure. Cook and coworkers have prepared and evaluated the three possible benzo-fused isomers of the benzodiazepines.⁷ These researchers found that the most active compounds incorporated a "linear" extension of the benzo fusion from the benzodiazepine scaffold. The SAR of our own series somewhat parallels that of the benzodiazepines described by Cook.⁷ Naphthalene **6** has a 154 nM IC₅₀ with a G.S. of 2.8, whereas **7** has a 17 nM GABA-A IC₅₀ with a G.S. of 1.0. Alternatively, the ring fusion of **8** results in much diminished activity. The high in vitro affinity of 7 was initially surprising to us, considering the lack of activity we had seen with 8-substituted derivatives (e.g., 34) earlier. Compounds 6-8 did not display any appreciable in vivo activity.

The A-ring modifications we describe here provided the basis for additional research, leading eventually to one compound being introduced into human clinical trials. The substitution of the PBI ring at positions 6 and 7 are particularly favorable, such as seen for the activity of 6,7-difluoro compound **38**. Many of the A-ring pyridyl and naphthyl derivatives are biologically active, which adds to our general understanding of the effect of structure upon function.

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References and Notes

1. (a) Jordan, A. D.; Kordik, C. P.; Reitz, A. B.; Sanfilippo, P. J. *Exp. Opin. Ther. Patents* **1996**, *6*, 1047. (b) Tulinsky, J.; Gammill, R. B. *Curr. Med. Chem.* **1994**, *3*, 226.

2. (a) McKernan, R. M.; Whiting, P. J. Trends Neurosci. **1996**, 19, 139. (b) Johnston, G. A. R. Pharmacol. Ther. **1996**, 69, 173-198. (c) Smith, G. B.; Olsen, R. W. Trends Pharacol. Sci. **1995**, 16, 162.

(a) Maryanoff, B. E.; McComsey, D. F.; Ho, W.; Shank, R. P.; Dubinsky, B. Bioorg. Med. Chem. Lett.
1996, 6, 333. (b) Maryanoff, B. E.; Ho, W.; McComsey, D. F.; Reitz, A. B.; Grous, P. P.; Nortey, S. O.; Shank, R. P.; Dubinsky, B.; Taylor, R. J., Jr.; Gardocki, J. F. J. Med. Chem. 1995, 38, 16. (d) see also: Bokanov, A. I.; Evstratova, M. I.; Turchin, K. V.; Granik, V. G.; Andreeva, N. I.; Asnina, V. V.; Golovina, S. M.; Mashkovskii, M. D. Khim.-Farm. Zh. 1997, 31, 27.

4. 3-Amino-2-nitropyridine (viz. 3): Planker, S.; Warning, K.; Herbst, G. H.; Herbst, U. M.; Herbst, B. S.; Schaeffer, G. U.S. Patent 4,952,697 (1991): *Chem. Abstr.* **1991**, *114*, 81610. 4-Amino-3-nitropyridine (viz. 4): Harris, M. G.; Stewart, R. Can. J. Chem. **1977**, 55, 3800. 2-Amino-3-nitropyridine (viz. 5) can be purchased.

5. Compound 13: Saunders, C. R.; Hamilton, C. S. *J. Amer. Chem Soc.* 1932, *54*, 636. ¹H NMR for 15 (DMSO-d₆, 360 MHz) δ 1.21 (CH₃, t, J=7.3 Hz, 3 H), 2.63 (CH₂CO, t, J=5.9 Hz, 2 H), 3.43 (NCH₂, m, 2H, nOe with H-3 at δ 7.04), 4.10 (OCH₂, q, J=7.3 Hz, 2 H), 4.65 (NH, br s, 1 H), 4.97 (NH₂, br s, 2 H, nOe with H-8 at δ 7.94), 7.04 (H-3, d, J_{AB}=9.0 Hz, 1H, nOe with NCH₂ δ 3.43), 7.14 (H-4 and H-6, m, 2H), 7.27 (H-7, m, 1 H), 7.62 (H-5, d, J=8.1 Hz, 1 H), 7.94 (H-8, d, J=8.3 Hz, 1 H, nOe with NH₂ at δ 4.97). ¹H NMR for 16 (DMSO-d₆, 360 MHz) δ 1.21 (CH₃, t, J=7.1 Hz, 3 H), 2.63 (CH₂CO, t, J=6.4 Hz, 2 H), 3.09 (NCH₂, t, J=6.4 Hz, 2H, nOe with H-8 at δ 7.95), 4.03 (NH, br s, 1H), 4.12 (OCH₂, q, J=7.1 Hz, 2 H), 5.19 (NH₂, br s, 2 H, nOe with H-3, δ 7.04), 7.04 (H-3, d, J_{AB}=8.6 Hz, 1H, nOe with NH₂ at δ 5.19), 7.11 (H-5, m, 1 H), 7.33 (H-7, m, 1 H), 7.39 (H-4, d, J=8.6 Hz, 1 H), 7.64 (H-5, d, J=8.0 Hz, 1 H), 7.95 (H-8, d, J=8.5 Hz, 1 H, nOe with NCH₂ at δ 3.09). 6. The PBIs are poorly soluble in water unless acid-addition salts can be prepared. However, PBIs are generally quite soluble in organic solvents such as methylene chloride, methanol, DMSO, or mixtures thereof.

7. Zhang, W.; Koehler, K. F.; Harris, B.; Skolnick, P.; Cook, J. M. J. Med. Chem. 1994, 37, 745.