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

Bruce E. Maryanoff, Samuel O. Nortey, James J. McNally, Pauline J. Sanfilippo, David F. McComsey, Barry Dubinsky, Richard P. Shank, and Allen B. Reitz*

*Drug Discovery, R. W. Johnson Pharmaceutical Research Institute*

*Spring House, PA 19477, U.S.A.*

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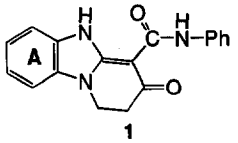
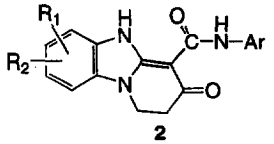
**Abstract:** A variety of pyrido[1,2-*a*]benzimidazoles (PBIs) modified on the A-ring were prepared and evaluated for affinity to the benzodiazepine binding site on the GABA-A receptor and in animal models predictive of anxiolytic activity in humans. A-ring benzo-fused derivative **7** exhibited potent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

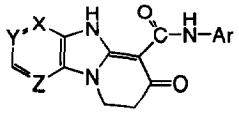
There is continuing medical need for drugs for the treatment of anxiety that have a greater separation of therapeutic effects from motor discoordination, sedation, and abuse liability.[1] The GABA-A receptor, which contains the well-known benzodiazepine binding site, can play a key role in the discovery 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.




**1**

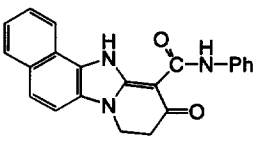



**2**




**3** X = N, Y = Z = CH
**4** Y = N, X = Z = CH
**5** Z = N, X = Y = CH


*PII:* S0960-894X(99)00240-1

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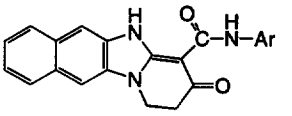
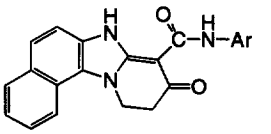
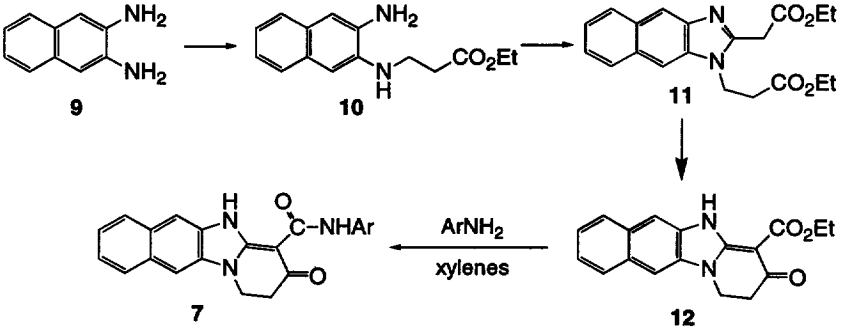



7




8

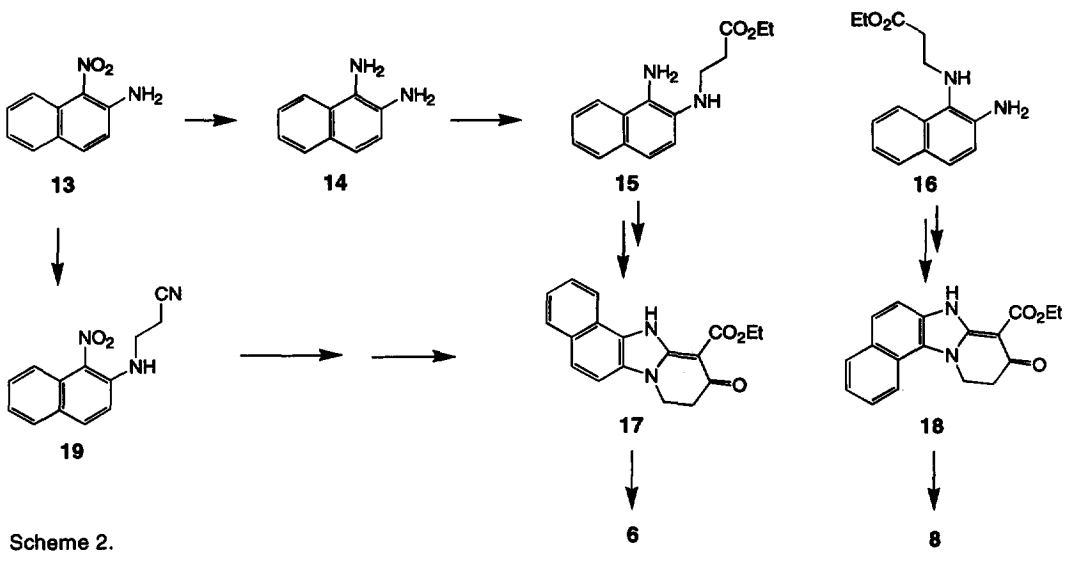
A-ring substituted derivatives **2** are listed in Table 1, and were prepared by chemistry we have already described.[3] The routes to prepare pyridyl derivatives **3–5** were similar, starting from the three appropriate aminonitropyridines.[4] Naphthyl derivatives **6–8** were prepared as shown in Schemes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




Scheme 1.

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 $EtOC(O)CH_2C(OMe)=NH\cdot HCl$ 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-nitronaphthalene[5] **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

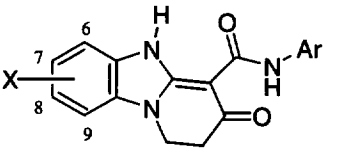



Scheme 2.

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 $IC_{50}$ for **23** vs. 7.8 nM $IC_{50}$ 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 $IC_{50}$) 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 $IC_{50}$, 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




| Compd No. | X | Ar | Formula[a] | mp (°C) | $GABA_A$[b] $IC_{50}$ (nM) | G.S. | Metrazol Test $ED_{50}$ (mg/kg) ip | Metrazol Test $ED_{50}$ (mg/kg) po | Anticonflict MED (mg/kg) ip | Anticonflict MED (mg/kg) po |
|---|---|---|---|---|---|---|---|---|---|---|
| **19** | 6-HO | 2-FPh | $C_{18}H_{14}FN_3O_3$[c] | >250 | 10.4 | 3.0 | - | - | - | >10 |
| **20** | 7-HO | Ph | $C_{18}H_{15}N_3O_3$[d] | 271-275 | 40.5 | 1.1 | >30 | >30 | >30 | >30 |
| **21** | 9-HO | 2-FPh | $C_{18}H_{14}FN_3O_3$ | >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 | $C_{19}H_{16}N_3O_3$ | 215-220 | 39.5 | 2.5 | 3 | >10 | >10 | >30 |
| **25** | 7-MeO | 2-FPh | $C_{19}H_{16}FN_3O_3$ | 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 | $C_{19}H_{16}FN_3O_2$ | 269-272 | 96.3 | 1.5 | 1 | 10 | 10 | >30 |
| **28** | 7-$CF_3$ | 2-FPh | $C_{19}H_{13}F_4N_3O_2$ | 273-276 | 184 | 4.7 | 1 | - | - | >10 |
| **29** | 6-Cl | 2-FPh | $C_{18}H_{13}ClFN_3O_2$[e] | 243-246 | 7.1 | 1.8 | 1 | >30 | 10 | >10 |
| **30** | 6-Cl | 2,6-$F_2$Ph | $C_{18}H_{12}ClF_2N_3O_2$[f] | 228-231 | 1.1 | 1.7 | 0.3 | 10 | 10 | >10 |
| **31** | 7-Cl | Ph | $C_{18}H_{14}ClN_3O_2$ | 267-269 | 65.6 | 1.6 | 1 | 20 | 10 | >30 |
| **32** | 7-Cl | 2-FPh | $C_{18}H_{13}ClFN_3O_2$[g] | 265-267 | 5.1 | 1.8 | 0.3-1 | ca. 30 | 10 | >10 |
| **33** | 7-Cl | 2,6-$F_2$Ph | $C_{18}H_{12}ClF_2N_3O_2$[h] | 212-213 | 6.3 | 2.2 | 0.3-1 | 3-10 | 10 | 10 |
| **34** | 8-Cl | Ph | $C_{18}H_{14}ClN_3O_2$ | 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 | $C_{18}H_{12}F_3N_3O_2$[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 |
| diazepam | | | | | 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; ±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 $H_2O$. d. 0.5 mol $CHCl_3$, 0.1 mol $H_2O$. e. 0.5 mol MeOH, 0.75 mol $H_2O$; H: calcd, 4.29; found, 3.54. f. 0.75 mol $H_2O$. g. H: calcd, 3.66; found, 3.18. h. 0.4 mol $H_2O$. i. 0.25 mol $H_2O$. j. 0.2 mol MeOH and $H_2O$; H: calcd, 3.71; found, 3.22. k. C: calcd, 60.17; found, 59.29.

**Table 2.** Biological Data for A-Ring Modified PBI Derivatives[a]

| Compd No. | Ar | Formula | mp (°C) | $GABA_A$ $IC_{50}$ (nM) | G.S. | Metrazol Test $ED_{50}$ (mg/kg) ip | Metrazol Test $ED_{50}$ (mg/kg) po | Anticonflict MED (mg/kg) ip | Anticonflict MED (mg/kg) po |
|---|---|---|---|---|---|---|---|---|---|
| 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 | $C_{17}H_{13}FN_4O_2$ | 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 | $C_{22}H_{16}FN_3O_2$ | 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 |

a. Biological tests as in Table 1. b. 1 mol HCl, 1.2 mol $H_2O$.

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 $IC_{50}$ 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 $IC_{50}$s. 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 co-workers 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 $IC_{50}$ with a G.S. of 2.8, whereas **7** has a 17 nM GABA-A $IC_{50}$ 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.

**Acknowledgment**: We thank the following for their help and advice: James Cook (Univ. of Wisconsin), Coralie Davis, David Demeter, Samantha Deluca, Diane Gauthier, Phil Grous, Winston Ho, and Anil Vaidya.

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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. $^1$H NMR for **15** (DMSO-$d_6$, 360 MHz) δ 1.21 ($CH_3$, t, J=7.3 Hz, 3 H), 2.63 ($CH_2CO$, t, J=5.9 Hz, 2 H), 3.43 ($NCH_2$, m, 2H, nOe with H-3 at δ 7.04), 4.10 ($OCH_2$, q, J=7.3 Hz, 2 H), 4.65 (NH, br s, 1 H), 4.97 ($NH_2$, 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 $NCH_2$ 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 $NH_2$ at δ 4.97). $^1$H NMR for **16** (DMSO-$d_6$, 360 MHz) δ 1.21 ($CH_3$, t, J=7.1 Hz, 3 H), 2.63 ($CH_2CO$, t, J=6.4 Hz, 2 H), 3.09 ($NCH_2$, t, J=6.4 Hz, 2H, nOe with H-8 at δ 7.95), 4.03 (NH, br s, 1H), 4.12 ($OCH_2$, q, J=7.1 Hz, 2 H), 5.19 ($NH_2$, br s, 2 H, nOe with H-3, δ 7.04), 7.04 (H-3, d, $J_{AB}$=8.6 Hz, 1H, nOe with $NH_2$ 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 $NCH_2$ 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.