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Potential Anxiolytic Agents. Part 4: Novel Orally-Active N^5-Substituted Pyrido[1,2-*a*]benzimidazoles with High GABA-A Receptor Affinity

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Abstract—A series of pyrido[1,2-*a*]benzimidazoles (PBIs) with substitution on the N^5-nitrogen has been synthesized and found to possess high affinity for the benzodiazepine (BZD) site on the GABA-A receptor. The compounds evaluated include those bearing a heteroalkyl group and heterocyclic rings. The most promising of these compounds is ethoxymethyl analogue **24**, which has an IC_{50} of 0.1 nM for the BZD site on the GABA-A receptor and has been advanced to human clinical trials.

Our continuing investigations into anxiolytics with an improved margin of safety as compared to marketed drugs have prompted us and others to explore the PBI chemical series (viz. **1**).[1–7] During the course of these studies, we found that substitution of N^5 on the PBI nucleus produced compounds with very high GABA-A receptor affinities and favorable in vivo therapeutic indices. Substitution at N^5 was conducted originally in an attempt to improve the physical properties of the series such as aqueous solubility. The PBIs are generally water insoluble which can complicate drug development, even though their therapeutically effective dose is expected to be quite low (ca. 1–10 mg/day). Therefore, we focused our efforts on N^5 substitution bearing polar functionality (viz. **2**) including those groups such as amino or basic heterocycles which could form acid-addition salts. In this paper we describe the synthesis and structure–activity relationships of a series of N^5-substituted PBI derivatives as potential anxiolytics.

Chemistry

N^5-Substituted PBI analogues were prepared in two steps from carboxylic acid ester **3**[4] as shown in Scheme 1. Conversion of **3** to various amides **4** was accomplished by heating an appropriately substituted aniline in a suitable solvent such as xylene or dimethylformamide. Treatment of **4** with a strong base (NaH), 15-crown-5 and a reactive electrophile such as a halo alkyl ether in dimethylformamide (DMF) promoted direct alkylation. Alternatively, the sodium salt of **4** was prepared separately using freshly formed EtONa and stored as a solid and then added to the reaction mixture with the crown ether. In addition, the alkylation reaction of **4** with dialkylaminoethyl halides proceeded under phase transfer catalysis conditions utilizing aqueous sodium hydroxide and benzyltrialkylammonium halide in chloroform. These chloroethylamines demonstrated enhanced reactivity as compared to the corresponding chloropropylamines under the same reaction conditions, as the former reacts presumably through an aziridinium intermediate.

Since only reactive electrophiles worked well in the direct alkylation procedure, we developed Mitsunobu reaction conditions for reactions of **4** with functionalized alcohols, which proved to be fairly general except for those substrates which bear an electron-withdrawing group or a basic free NH group. Nonetheless, coupling of **4** with the appropriate alcohols was conducted with diethyl (DEAD) or diisopropylazodicarboxylate (DIAD) or 1,1′-(azodicarbonyl) dipiperidine (ADDP) and either triphenyl or tributylphosphine in tetrahydrofuran or

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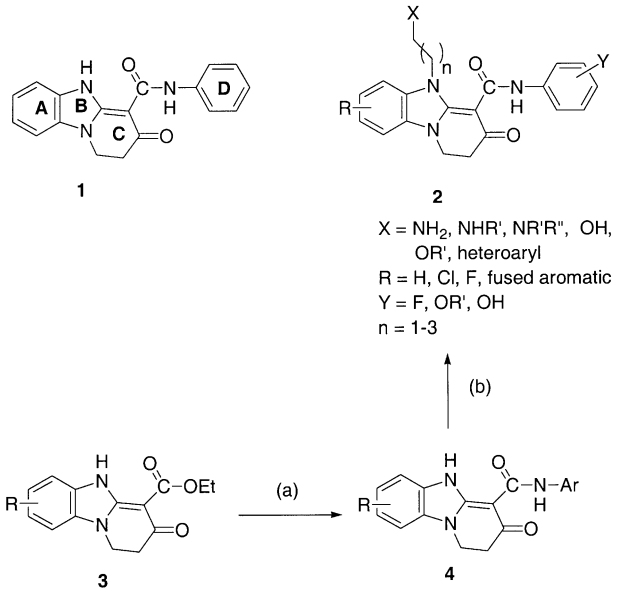
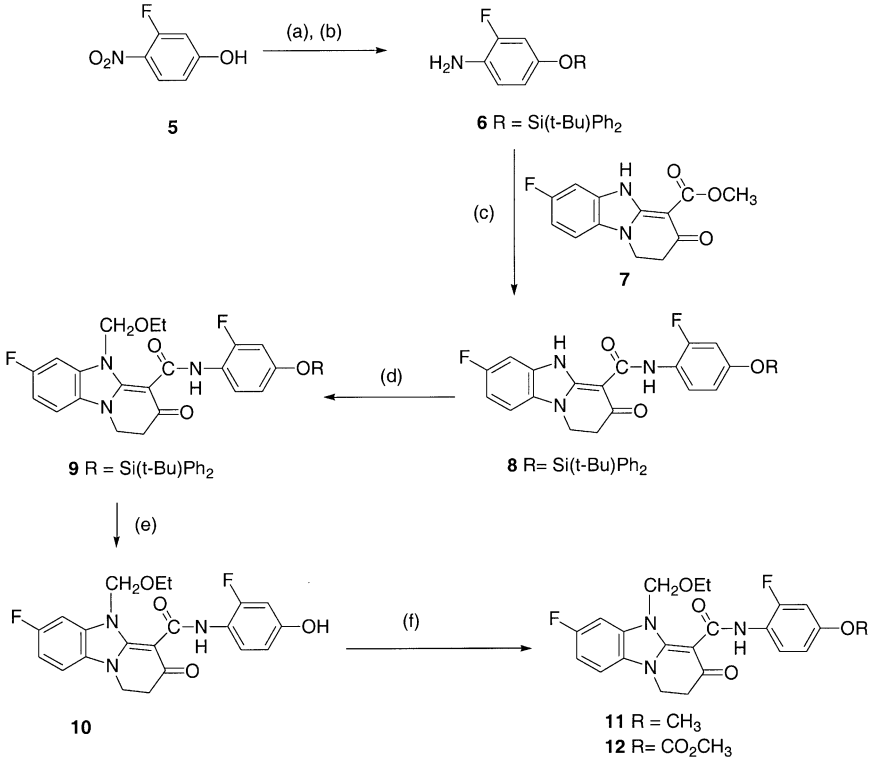


Scheme 1. Reagents and conditions: (a) $ArNH_2$, xylene, 140 °C; (b) NaH, 15-crown-5, RX, DMF or RCH_2OH, DEAD, Ph_3P, THF, rt or RX, $R_4N^+ X^-$, aq NaOH, $CHCl_3$, rt.

dichloromethane to give the desired compound directly.[8–10] Certain ring modified compounds required the synthesis of particular anilines prior to condensation with **3**. For example as shown in Scheme 2, phenol **5** was protected by silylation such as with reaction of *t*-$BuPh_2SiCl$, followed by reduction of the nitro group with hydrogen gas at 55 psi and Pd/C in 3:1 mixture of MeOH/EtOAc to give **6**, and then condensation with ester **7** to afford **8**. Incorporation of the ethoxymethyl group onto N^5 as described previously led to **9**, which produced **10** upon removal of the silyl group with nBu_4NF. The phenolic hydroxyl of **10** was further reacted with electrophiles MeI and $ClCO_2Me$ to furnish compounds **11** and **12**.

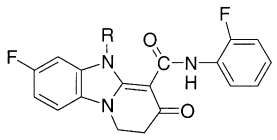
Results and Discussion

The biological activity of the PBI derivatives is shown in Tables 1–4, along with that of the reference benzodiazepine diazepam. Specifically, these compounds were evaluated for their in vitro affinities (IC_{50} values) for the BZD site on the GABA-A receptor by competition experiments with the radioligand [^{3}H]-flunitrazepam;[11] compounds were tested at five concentrations in a tissue preparation from rat cerebral cortex. In addition, the GABA shift (G.S.), which is the ratio of the binding in the absence and presence of GABA, was also determined. Our goal was to identify compounds with high affinity for the GABA-A receptor and which have a partial agonist profile (G.S. = 1.2–1.8).[4] These compounds would be expected to have minimal side effects such as daytime sedation and abuse liabilities which are characteristic of full agonists such as diazepam (G.S. > 2.0). Our in vivo evaluation has consisted of a series of tests including inhibition of pentylenetetrazole (PTZ)-induced seizures in mice and experimentally induced conflict in rats. In the rat conflict assay, the data are presented as the dose at which efficacy is first observed (minimum effective dose, MED) whereas in the PTZ assay, the data are expressed as the dose that



Scheme 2. Reagents and conditions: (a) *t*$BuPh_2SiCl$, imidazole, DMF; (b) H_2, 10% Pd/C, MeOH/EtOAc; (c) xylene, 140 °C; (d) NaH, $ClCH_2OEt$, 15-crown-5, DMF; (e) *n*Bu_4NF, THF; (f) MeI, K_2CO_3 18-crown-6, DMF; or CH_3OCOCl, pyridine, $CHCl_3$.

Table 1. Biological data for ring B substituted PBI derivatives



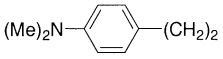
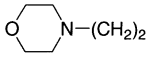
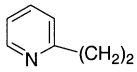
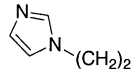
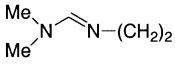
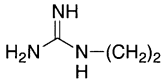
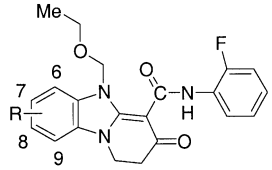
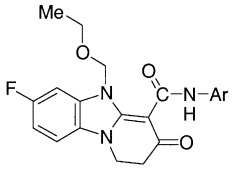
No.	R	$GABA_A$ IC_{50} (nM)	G.S.	PTZ (mouse) ED_{50} (mg/kg)		Anticonflict (rat) MED (mg/kg)	
				ip	po	ip	po
13	H	1.9	1.8	≤1	>30	10	>30
14	$(Me)_2N(CH_2)_2$	49.9	2.7	0.5	≤3	<10	3
15	$(Me)_2N(CH_2)_3$	3.3	1.4	3	30	10	>10
16	$H_2N(CH_2)_2$	5.1	2.7	0.3	10	10	10
17	$MeO(CH_2)_2NH(CH_2)_2$	7.4	1.8	1	1	10	10
18		8.5	1.3	1	<30	>10	—
19		6.1	2.2	1	30	>10	—
20		0.9	2.0	0.3	10	10	>10
21		1.6	2.6	1	10	—	>10
22		35.0	2.1	≤1	30	—	30
23		5.6	1.7	>1	>30	—	>10
24	$EtOCH_2$	0.1	1.2	0.01	0.04	10	0.4
25	$EtO(CH_2)_2$	0.5	3.2	≤1	0.3	≤10	1
26	$EtO(CH_2)_3$	0.3	1.6	≤1	0.3	—	1
27		2.3	2.2	≤1	3	—	3
28	$MeO(CH_2)_2OCH_2$	0.8	2.2	0.003	0.1	≤10	3
29	$HO(CH_2)_2$	1.2	2.7	≤1	<30	10	>10
30	$HO(CH_2)_3$	1.1	1.8	0.1	0.5	10	10
31	$MeOCO_2(CH_2)_2$	0.3	2.2	≤1	3	—	3
32	$MeCO_2(CH_2)_2$	0.2	1.2	≤1	3	—	3
	Diazepam	4.9	2.2	0.11	0.5	5	>10

Table 2. Biological data for ring A substituted PBI derivatives



No.	R	$GABA_A$ IC_{50} (nM)	G.S.	PTZ (mouse) ED_{50} (mg/kg)		Anticonflict (rat) MED (mg/kg)	
				ip	po	ip	po
33	H	151.0	2.0	>1	3	—	>10
34	7-Cl	0.5	2.8	<1	2	—	>10
35	8,9-F_2	126.0	1.7	1	>30	—	>10
36	6,8-F_2	13.3	2.9	—	—	—	>10
37	6,7-F_2	0.5	1.7	<1	<3	—	1
38	7,8-CH=CH-CH=CH—	2.2	1.4	<1	>30	—	>10

Table 3. Biological data for ring D modified PBI derivatives



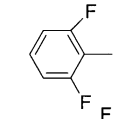
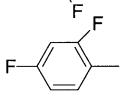
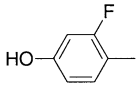
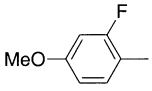
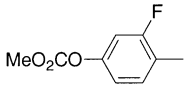
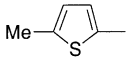
No.	Ar	$GABA_A$ IC_{50} (nM)	G.S.	PTZ (mouse) ED_{50} (mg/kg)		Anticonflict (rat) MED (mg/kg)	
				ip	po	ip	po
39		0.6	1.6	1	3	>10	>10
40		1.2	2.0	≤1	<3	0.1	0.1
10		1.4	2.0	—	0.3–3	1	1–3
11		0.4	2.2	—	0.3	0.1	0.03
12		4.3	3.2	1	3–30	—	1
41		1.0	1.7	≤1	<0.2	—	0.3
42		1.0	2.1	≤1	0.2	—	3
43		0.7	2.3	≤1	0.5	—	0.3
44		0.7	1.6	≤1	10	—	>10

Table 4. In vivo biological data for selected compounds

No.	PTZ (mouse) ED_{50} (mg/kg)		Anticonflict (AC, rat) MED (mg/kg)		EtOH[a] sleep (rat) MED	Ratio EtOH/AC	HS[b] ED_{50} (mouse)		Ratio HS/PTZ
	ip	po	ip	po	po	po	ip	po	po
24	0.01	0.04	10	0.4	0.3	1	0.3	27	675
28	0.003	0.1	≤10	3	3	1	0.07	30	300
40	<1	<3	0.1	0.1	0.1	1	<1	10	3
Diazepam	0.3	0.5	5	5	5	1	0.1	6.3	13

[a]Potentiation of EtOH-induced sleep time.
[b]Horizontal screen.

antagonizes seizures induced in 50% of the mice (ED_{50}). The in vivo data upon intraperitoneal (ip) and oral administration (po) are shown in the tables. The data in Table 1 illustrate the replacement of the N^5 hydrogen in **13** with various heteroatom groups and heterocyclic rings, and allowing ring C to be unsubstituted, the 7-fluoro substitution on ring A, and 2-fluoro substitution on ring D. This pattern of fluoro substitution was selected because it was found previously to impart high levels of GABA-A receptor affinity.

In general, compounds with alkylamino substitution on the N^5 position (viz, **14**–**17**) displayed lower affinity for the GABA-A receptor than the direct unsubstituted

analogue **13** but were more orally active in the in vivo assays. However, aromatic amine **18** and cyclic amine **19** were less potent in the binding assay and the PTZ in vivo assay. The 2-pyridylethyl derivative **20** demonstrated a 2-fold increase in potency (IC_{50} of 0.9 nM) at the GABA-A receptor as compared to **13** and was found to be more orally active in the pentylenetetrazole and anticonflict biological tests. Imidazolylethyl compound **21** showed comparable in vitro activity and improved in vivo activity. Highly basic amidine **22** exhibited less activity in the binding assay with larger G.S. value of 2.1. Similiarly, guanidine **23** revealed diminished in vitro activity but a lower G.S. value of 1.7. Compounds **22** and **23** showed no improvement in oral activity in the in vivo tests. Although formation of the acid addition salts of compounds **14**–**21** did result in an increase in their water solubility, the oral activities of these compounds were not improved enough to warrant further consideration.

We then decided to incorporate oxygen-containing groups at the N^5 position of the PBI nucleus. We were delighted to find that the ethoxymethyl derivative **24** displayed a superior IC_{50} of 0.1 nM to the GABA-A receptor with an acceptable G.S. = 1.2 as compared to compound **13**. In addition, compound **24** displayed particularly excellent in vivo activity, including 0.04 mg/kg MED po in the mouse PTZ and 0.4 mg/kg ED po in the rat conflict test. Increasing the chain length by one carbon produced ethoxyethyl compound **25**, which was 5-fold less potent in vitro than **24** with an unexpectedly large G.S. of 3.2. Compound **25** possessed 7-fold less oral activity in the PTZ test and 3-fold less oral activity in the conflict test. Ethoxypropyl homologue **26** displayed only 3-fold less potency in the binding assay but 7-fold less activity po in the mouse PTZ and 3-fold less activity in the rat conflict tests. Cyclic ether compounds, such as tetrahydrofuranyl methyl analogue **27** were generally less active than acyclic ether **24**. Diether **28**, with an IC_{50} = 0.8 nM and a rather high G.S. of 2.2, did show 3-fold less po activity in the PTZ test and an 8-fold reduction in the rat conflict test. Alcohols **29** and **30** showed an 8-fold reduction in in vitro activity and exhibited diminished po in vivo activity. Methoxycarbonyloxy analogue **31** and acetate **32**, designed as prodrugs of **29**, showed comparable in vivo potency of 3 mg/kg po in the Vogel conflict and PTZ tests, but were generally less active than **24**.

Based on the biological data discussed thus far, incorporation of the ethoxymethyl group on the N^5 position of the PBI nucleus was found to impart favorable anxiolytic activity. The best compound for continued structure–activity relationship (SAR) studies was **24**. The next region for SAR development in this series centered on ring A and particularly halogen atom substitution, with the placement of halogen atoms at various positions on the phenyl ring (Table 2). Absence of a fluoro group at position 7 (**33**) resulted in a ca. 1000-fold decrease in vitro affinity versus compound **24**. The 7-chlorophenyl analogue **34** exhibited in vitro potency but a higher G.S. than **24**. The 8,9-difluorophenyl compound **35** was unexpectedly less active in vitro (IC_{50} = 126.0 nM) than **24**, whereas 6,8-, and 6,7-difluorophenyl compounds **36** and **37** had 13.3 and 0.5 nM IC_{50}s in vitro. In addition, **37** displayed 3-fold less activity po in the anticonflict assay. These results suggest that halogen substitution of the 6 and/or 7 positions are favorable for GABA-A receptor binding. In a previous report, the A ring naphthyl derivative with 7,8 fusion was found to be the most potent of the three possible benzo ring fused isomers.[3] Incorporation of the naphthyl ring as a phenyl replacement afforded compound **38**, which demonstrated weaker binding affinity (IC_{50} = 2.2 nM) for the GABA-A receptor than **24** and diminished in vivo activity in the rat anticonflict test.

Lastly, our SAR studies focused on modifications of ring D (Table 3). The 2,6- and 2,4-difluorophenyl compounds **39** and **40** showed slightly less in vitro activity, but only **40** exhibited appreciable activity in the anticonflict assay. In vitro metabolism of **24** in rat and human hepatic S9 fractions revealed that para hydroxylation on the D ring benzene ring was the major site for metabolism (viz. **10**). In fact, **10** was formed to the extent of 60 and 30% upon incubation of **24** with rat and human microsomes, respectively, after 1 h, and all other metabolites only accounted for 10 and 20% of the original drug sample. Therefore, 2-fluoro-4-hydroxy phenyl compound **10** was prepared as shown earlier (Scheme 2), and was found to be 14-fold less active in vitro than **24**, and 30-fold less active po in the rat conflict assay. The 2-fluoro-4-methoxy and 2-fluoro-4-methoxycarbonyloxy compounds **11** and **12** were synthesized as potential prodrugs of **10**. The biological activity of **11** was 3-fold better in the binding assay and 30-fold more active po in the rat conflict assay. Thiophene was utilized as a bioisosteric replacement for the D ring phenyl. Specifically, *N*-(3-thienyl) and (2-thienyl)carboxamides **41** and **42** were synthesized and each was found to be significantly less active in vitro than **24** and less active po in in vivo efficacy tests. Incorporation of a 5-methyl substituent (viz. **43**) and a 3-chloro group (viz. **44**) on the 2-thienyl ring slightly enhanced binding potency relative to **42** but nonetheless each analogue was less active in the binding assay and po in the PTZ in vivo assay than **24**.

The in vivo biological data highlighting efficacy and side effect testing for PBI compounds **24**, **28**, **40**, and diazepam are shown in Table 4. Several of these compounds had pharmacological attributes that warranted further investigation. In fact, compound **24** has excellent oral efficacy, in both the PTZ (mice) and conflict assays (rats), while possessing a beneficial separation from the side effects of motor impairment in the horizontal screen (HS) assay (mice; see Table 4). Compound **28**, shows appreciable separation between efficacy in the PTZ assay and the horizontal screen side effect test. Lastly, compound **40** was found to display the least separation in the (HS/PTZ) side-effect profile (Table 4). Among these compounds which were evaluated in detail, there was no difference in the relative degree of ethanol sleep time. Given that the results for **24** are quite favorable relative to close analogue **28** and diazepam, this compound is presently being investigated more extensively.[12]

Conclusions

We describe here a systematic series of modifications on the PBI nucleus with the goal of improving biological activity and physical properties. We examined substitutions and modifications at the N-5 position and on rings A, B, and D to produce potent, orally active anxiolytics which modulate the BZD site of the GABA-A receptor with a reasonably acceptable side effect profile. Elaboration of initial lead **13** by incorporation of various heteroalkyl and heterocyclic alkyl groups at the N-5 position led to identification of the ethoxymethyl group as preferred. Further investigations led to selection of the 7-fluoro group as the best substituent on phenyl ring A and the 2-fluoro group as the optimal group on phenyl ring D. As a result, compound **24** was identified during the course of these SAR studies as a potential anxiolytic agent for human clinical evaluation.

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