# Anxiolytic Properties of Certain Annelated [1,2,4]Triazolo[1,5-*c*]pyrimidin-5(6*H*)-ones

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Modification of the benzodiazepine (BZ) receptor binding template 2-aryl[1,2,4]triazolo[1,5-*c*]quinazolin-5(6*H*)-one by replacement of the annelated benzene ring with various alicyclic and heterocyclic moieties led to novel structures with potent BZ receptor binding affinity. High affinity was found in some cycloalkyl-annelated [1,2,4]triazolo-[1,5-*c*]pyrimidin-5(6*H*)-ones and in some 7,8,9,10-tetrahydropyrido[3,4-*e*][1,2,4]triazolo[1,5-*c*]pyrimidin-5(6*H*)-ones, in which the degree of activity was strongly dependent on the N-substituent in the 9-position. Nine compounds with BZ receptor $IC_{50}$ binding affinity values equal or superior to diazepam were evaluated in secondary screening. One of these, 9-benzyl-2-phenyl-7,8,9,10-tetrahydropyrido[3,4-*e*][1,2,4]triazolo[1,5-*c*]pyrimidin-5(6*H*)-one, showed good activity in rats as a potential anxiolytic agent without sedative liability. However, it increased the rotorod deficit produced by ethanol at anxiolytic doses, an indication of alcohol interaction. Thus, none of the compounds showed an advantage over CGS 9896 (Yokoyama et al. *J. Med. Chem.* **1982**, *25*, 337–339), which is free of sedative and alcohol interaction potential as measured by the test procedures described.

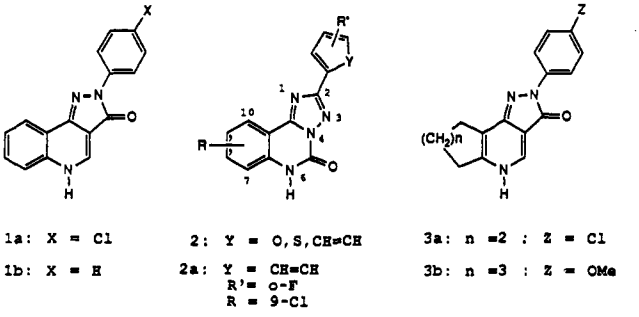
## Introduction

The discovery of the anxiomodulators **1a** and **1b**[1,2] (Chart I) pioneered an important departure from the benzodiazepine structure found in the most prominent anxiolytics known in 1982. Other tricyclic templates were examined for benzodiazepine (BZ) binding activity and certain 2-aryl- and 2-heteroaryl[1,2,4]triazolo[1,5-*c*]-quinazolin-5(6*H*)-ones[3] (**2**) were found to have potent BZ binding activity. The most interesting of these was **2** wherein Y is an ethylenic linkage, R′ is *o*-fluoro, and R is 9-chloro (**2a**). In this series only examples of BZ antagonists (or inverse agonists[4]) were found. Since modification of **1a** led to **3a**[5] and **3b**,[6] both of which are BZ agonists, it seemed likely that some agonists could be found among similar variants of **2**, namely, *e*-annelated [1,2,4]triazolo-[1,5-*c*]pyrimidin-5(6*H*)-ones.

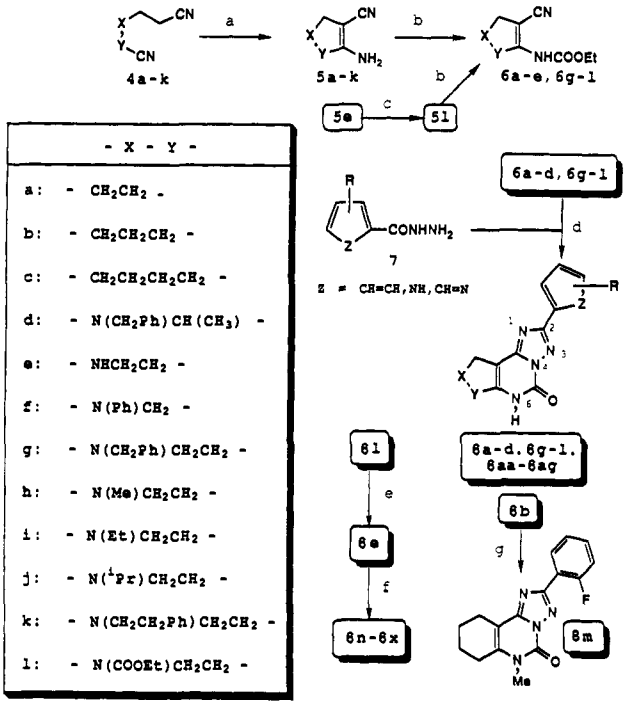
## Synthesis of the Target Compounds

Synthetic routes to these novel tricyclic structures were based on the most favored method for preparing [1,2,4]-triazolo[1,5-*c*]quinazolin-5(6*H*)-ones,[3] derived from an anthranilonitrile. As illustrated in Scheme I, examples of heterocyclic substitutes for the anthranilonitrile were synthesized by preparing enamino nitriles **5a–k** through the Thorpe–Ziegler cyclization[7] of the required dinitriles

Chart I




Scheme I. Synthetic Routes to Key Targets[a]

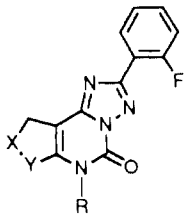



[a] Reagents: (a) NaH, KO$^t$Bu or NaN(SiMe$_3$)$_2$; (b) 2NaOEt, $(EtO)_2CO$; (c) ClCOOEt, $NaHCO_3$, MeCOEt; (d) $MeOCH_2CH_2OH$, $Pr_3N$ or *N*-methylpyrrolidinone; (e) 4 N NaOH, $MeOCH_2CH_2OH$, then HCl; (f) See the text; (g) NaOMe, DMSO, MeI.

(1) Yokoyama, N.; Ritter, B.; Neubert, A. 2-Arylpyrazolo[4,3-*c*]-quinolin-3-ones: Novel Agonist, Partial Agonist, and Antagonist of Benzodiazepines. *J. Med. Chem.* **1982**, *25*, 337–339.
(2) Czernik, A. J.; Petrack, B.; Kalinsky, H. J.; Psychoyos, S.; Cash, W. D.; Tsai, C.; Rinehart, R. K.; Granat, F. R.; Lovell, R. A.; Brundish, D. E.; Wade, R. CGS 8216: Receptor Binding Characteristics of a Potent Benzodiazepine Antagonist. *Life Sci.* **1982**, *30*, 363–372.
(3) Francis, J. E.; Cash, W. D.; Barbaz, B. S.; Bernard, P. S.; Lovell, R. A.; Mazzenga, G. C.; Friedmann, R. C.; Hyun, J. L.; Braunwalder, A. F.; Loo, P. S.; Bennett, D. A. Synthesis and Benzodiazepine Binding Activity of a Series of Novel [1,2,4]-Triazolo[1,5-*c*]quinazolin-5(6*H*)-ones. *J. Med. Chem.* **1991**, *34*, 281–290.
(4) (a) Braestrup, C.; Schmiechen, R.; Neff, G.; Neilsen, M.; Petersen, E. N. Interaction of Convulsive Ligands with Benzodiazepine Receptors. *Science* **1982**, *216*, 1241–1243. (b) Wood, P. L.; Loo, P.; Braunwalder, A.; Yokoyama, N.; Cheney, D. L. *In vitro* Characterization of Benzodiazepine Receptor Agonists, Antagonists, Inverse Agonists and Agonist/Antagonists. *J. Pharmacol. Exptl. Ther.* **1984**, *231*, 572–576.
(5) Bennett, D. A.; Amrick, C. L.; Wilson, D. E.; Boast, C. A.; Loo, P.; Bernard, P. S.; Schmutz, M.; Gerhardt, S. C.; Braunwalder, A.; Klebs, K.; Yokoyama, N.; Liebman, J. M. Pharmacological Characterization of CGS 17867A as a Benzodiazepine Receptor Agonist Devoid of Limiting Behavioral Effects. *Drug Dev. Res.* **1987**, *11*, 219–233.
(6) Williams, M.; Bennett, D. A.; Loo, P. S.; Braunwalder, A. F.; Amrick, C. L.; Wilson, D. E.; Thompson, T. A.; Schmutz, M.; Yokoyama, N.; Wasley, J. W. F. CGS 20625, a Novel Pyrazolopyridine Anxiolytic. *J. Pharmacol. Exptl. Ther.* **1989**, *248*, 89–96.

0022-2623/91/1834-2899$02.50/0 

Table I. Ring Modifications

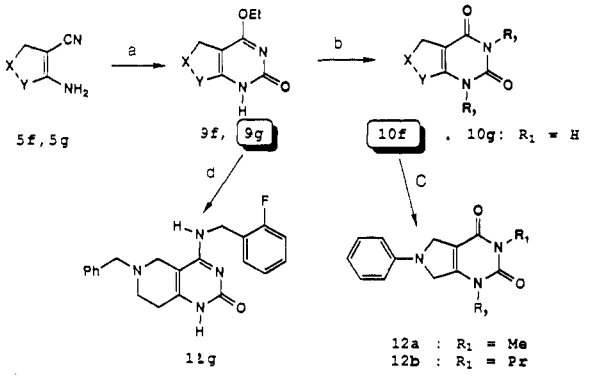


| no. | mp, °C | % yield | solvent | FNZB $IC_{50}$,[c] nM | GABA ratio |
|---|---|---|---|---|---|
| 8a | 251–253 | 19 | EtOH | 4.7 | ND[d] |
| 8b | 266–268 | 55 | $MeOCH_2CH_2OH$ | 1.7 | ND |
| 8c | 257–259 | 51 | $MeOCH(CH_3)CH_2OH$ | 7.2[h] | 1.18 |
| 8d | 290–293 | 9 | $MeOCH_2CH_2OH$ | >1000[h] | ND |
| 8e | 254–257 | 75 | $MeOCH_2CH_2OH$ | 442 | ND |
| 8g | 306–309[e] | 16 | DMAC/MeOH | 0.44 | 1.06 |
| 8h | 285–287[e] | 34 | MeOH | 180 | ND |
| 8i | 284–286[e] | 38 | MeOH | 150 | ND |
| 8j | 300–303[e] | 11 | MeOH | 105[h] | ND |
| 8k | 167–171[e] | 6 | MeOH | 28[h] | ND |
| 8l | 307–311 | 26 | $MeOCH_2CH_2OH$ | 12.1 | 1.1 |
| 8m | 236–239 | 54 | $MeOCH_2CH_2OH$ | 57[h] | ND |
| 1b | | | | 0.45 ± 0.05[f] | 0.82 |
| 2a | | | | 0.5[h] | 0.79 |
| diazepam | | | | 4.4 ± 0.2[g] | ND |

[a] Yields of purified products are reported. [b] Solvent used for recrystallization in last step. [c] Flunitrazepam binding. [d] Not determined. [e] Methanesulfonate salt. [f] $n = 3$. [g] $n = 13$. [h] Modified binding test (ref 5).

**4a–k**. Though several of the known dinitriles were cyclized with potassium hydride in tetrahydrofuran,[7d] or potassium *tert*-butoxide in toluene,[7e,f] the preferred method of cyclization was through the use of sodium hexamethyldisilazane in ether.[7g] Intermediate **5l** was prepared by acylation of **5e** with ethyl chloroformate and sodium bicarbonate in 2-butanone. The enamino nitriles were converted to cyanourethanes **6** with diethyl carbonate in the presence of 2 mol of sodium ethoxide in ethanol at reflux. When the urethane was reacted with an aryl or heteroaryl carbohydrazide **7** in a mixture of 2-methoxyethanol and tri-*n*-propylamine at reflux or with 1-methyl-2-pyrrolidinone, the desired heterocycle **8** was formed in one step, with the exception of **8e**, which was prepared by deacylation of **8l**. From the triazoloquinazolinone series,[3] we learned that the *o*-fluorophenyl substituent at the 2-position, as in **2a**, produced the optimal binding potency. Consequently the first examples in the new series were prepared from *o*-fluorobenzhydrazide as **7**. In example **5f**, the major product during attempted urethane formation was annelated pyrimidone **9f** (Scheme II) and **6f** was not isolated from the mother liquor. Byproduct **9f** was further characterized by hydrolysis to annelated pyrimidinedione **10f** (R′ = H) and alkylated to form derivatives (**12**) wherein R′ was methyl or *n*-propyl.[8] Conversions of type **5** to **9** have been documented.[9] During a resynthesis of **6g**, we isolated **9g** from the mother liquors of the desired urethane and this was further characterized by saponification to dione **10g** and conversion to **11g**. It is likely that alkoxypyrimidinones were formed to some extent as byproducts in other examples. The urethanes were generally identified by IR/NMR and used without purification because the targets produced in the next step crystallized readily, though overall yields were sometimes low. Compound **8m** was prepared by alkylation of **8b** (Experimental Section). These target

**Scheme II**. Condensed Pyrimidinones[a]



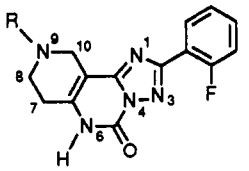

[a] Reagents: (a) 2NaOEt, $(EtO)_2CO$, EtOH; (b) 3 N HCl; (c) $R_1$ halogen, 10% NaOH, $Bu_4NBr$, toluene; (d) *o*-fluorobenzylamine.

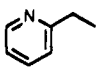
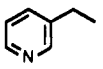
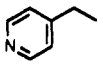
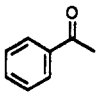
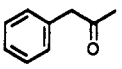
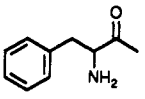
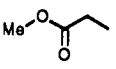
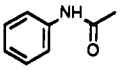
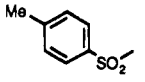
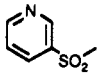
(7) (a) Thorpe, J. F. The Formation and Reactions of Imino Compounds Part XI. The Formation of 1-Imino-2-cyanocyclopentane from Adiponitrile. *J. Chem. Soc.* **1909**, *95*, 1901–1903. (b) Ziegler, K.; Eberle, H.; Ohlinger, H. *Liebigs Ann. Chem.* **1933**, *504*, 94–130. (c) The Chemistry of Cyclic Enaminonitriles and o-Aminonitriles. *Advances in Organic Chemistry: Methods and Results*; Taylor, E. C., Ed.; McKillop, A. Interscience Publishers: New York, 1970; Vol. 7, pp 1–56. (d) Brown, C. A. Rapid, High Yield Condensations of Esters and Nitriles via Kaliation. *Synthesis* **1975**, 326–327. (e) Thompson, Q. E. Adiponitrile—A Novel Self-condensation Sequence. *J. Am. Chem. Soc.* **1958**, *80*, 5483–5487. (f) Cavalla, J. F. 3-Amino-4-cyano-3-pyrrolines: Their Hydolysis to 3-Pyrrolidones and Their Reaction with Hydrogen Sulphide. *J. Chem. Soc.* **1962**, 4664–4672. (g) Rodriguez-Hahn, L.; Parra, M. M.; Martinez, M. A Study of the Thorpe–Ziegler Reaction in Very Mild Conditions. *Synth. Commun.* **1984**, *14*(10), 967–972. (h) Taub, D.; Kuo, C. H.; Wendler, N. L. Transformations in the 1,2,5,6-Tetrahydropyridine Series. *J. Chem. Soc. (C)* **1967**, 1558–1564.

(8) These derivatives were interesting per se as they are structural mimics of adenosine antagonists derived from 8-phenyltheophylline. Unfortunately, they showed no significant affinity for either $A_1$ or $A_2$ receptors[21] (G. A. Stone, CIBA-GEIGY, personal communication).

(9) Breukink, K. W.; Verkade, P. E. Preparation of Quinazoline Derivatives through Ring Closure of Aromatic Ortho-cyanoacylamino Compounds in Alkaline Alcoholic or Phenolic Medium. II. *Recl. Trav. Chim.* **1960**, *79*, 443–453.

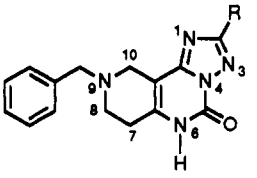
**Table II.** Modification of the 9-Position



| no. | R | mp, °C | % yield from 8e | solvent | FNZB $IC_{50}$,[g] nm | GABA ratio |
|---|---|---|---|---|---|---|
| **8n** |  | 267–269[a] | 48 | MeOH | 0.64 | 1.65 |
| **8o** |  | 274–276[a] | 24 | MeOH | 0.74 | 1.4 |
| **8p** |  | 281–283[a] | 38 | MeOH | 1.89 | 1.14 |
| **8q** |  | 342–344 | 39 | *b* | 50 | ND[c] |
| **8r** |  | 310–313 | 79 | *b* | 140 | ND |
| **8s** |  | 305–307[d] | 70 | *b* | >100 | ND |
| **8t** |  | 265–266 | 28 | EtOH | 27 | 1.06 |
| **8u** |  | 291–293 | 87 | *b* | 67 | ND |
| **8v** |  | 300–303 | 67 | DMAC/MeCOMe | 67 | 0.74 |
| **8w** |  | 307–309 | 68 | $CH_2Cl_2$/MeOH | 1.77 | 0.99 |
| **8x** |  | 230–234[f] | 10 | $MeOCH_2CH_2OH$ | 5.34 | 1.3 |
| **1b** | | | | | 0.45 ± 0.05[h] | 0.82 |

[a] Dimethanesulfonate salt. [b] Obtained pure from reaction. [c] Not determined. [d] Hydrochloride salt. [e] Methanesulfonate salt. [f] $n = 3$. [g] Modified test.

**Table III.** Modification of the 2-Substituent



| no. | R | mp, °C | % yield | solvent[b] | FNZB $IC_{50}$,[a] nM | GABA ratio |
|---|---|---|---|---|---|---|
| **8aa** | phenyl | 257–259 | 35 | $MeOCH_2CH_2OH$ | 2.19 | 1.4 |
| **8ab** | 3-$FC_6H_4$ | 299–302[c] | 41 | MeOH | 2.4 | 1.6 |
| **8ac** | 4-$FC_6H_4$ | 252–256 | 14 | DMAC/MeOH | 5.35 | 1.7 |
| **8ad** | 2-$ClC_6H_4$ | 295–296[c] | 18 | MeOH | 1.7 | 1.4 |
| **8ae** | 4-$ClC_6H_4$ | 250–256 | 17 | DMAC | 22.4 | 2.45 |
| **8af** | 2-pyrrolyl | 302–304 | 41 | MeOH/$CH_2Cl_2$ | 7.6 | 1.65 |
| **8ag** | 2-pyridyl | 290–292[c] | 31 | MeOH | 20.6 | 1.6 |
| **1b** | | | | | 0.45 ± 0.05[d] | 0.82 |

[a] Yield in cyclization step including salt forming step. [b] Solvent for recrystallization in final step. [c] Methanesulfonate salt. [d] $n = 3$. [e] Modified binding test (ref 5).

compounds are summarized in Table I.

Further examples (Table II) were prepared by reaction of **8e** with either an alkylating agent (**8n–p**), an acylating agent (**8q–t**), an isocyanate (**8u,v**), or a sulfonyl chloride (**8w,x**). Modifications of the *o*-fluorophenyl substituent at the 2-position of **8g** was synthesized from **6g** and the

appropriate carbohydrazide. These are listed in Table III as **8aa–ag**.

## Screening Methods

Relative affinities of our target compounds were defined by testing each compound for its ability to displace [$^3$H]flunitrazepam (FNZ) from rat forebrain as described by Braestrup and Squires.[10] During the course of our work, the BZ receptor was defined as a complex involving a GABA (γ-aminobutyric acid) receptor[11a] and a chloride channel.[11b] Consequently, a modified binding procedure was used for those examples noted, and the results are summarized in Tables I–III along with the GABA ratio for some of the compounds. This ratio, the $IC_{50}$ in the absence of GABA compared to the $IC_{50}$ in the presence of GABA, is believed to be a useful predictor of the type of activity observed in whole animal models. A ratio significantly less than 1 is thought to indicate BZ antagonist or inverse agonist activity whereas a ratio of 2 or higher indicates a partial or full agonist.[5,6] Intermediate ratios are less distinct as to their a priori biological significance.

Compounds with an $IC_{50}$ of 20 nM or better in FNZ binding were tested in vivo in rat models. Antagonism of pentylenetetrazole (PTZ) discriminative stimuli, indicative of an anxiolytic response,[12] was first determined.[5] Antagonism of diazepam-induced rotorod deficit was used to determine BZ antagonist/inverse agonist activity. Selected compounds were tested for potential anxiolytic activity in the Cook–Davidson behavioral conflict paradigm[5] (CD) and for their ability to reduce nonconflict responding (VI), an indication of potential sedation and/or muscle relaxation.[5] Finally, selected compounds were evaluated for their ability to increase the rotorod deficit produced by ethanol[5] as an indication of potential ethanol interaction with the anxiolytic agent.

## Structure–Activity Relationships (SAR)

Compounds **8a–c** were potent in binding affinity (less than 10 nM) and the two examples structurally related to **3a** and **3b** (**8b,c**) were examined further. Methylation at the 6-position of **8b** (Example **8m**) appeared to reduce binding affinity severely and therefore further examples bearing substituents at this position were not pursued. Tetrahydropyrido-annelated structures (**8e,g–l,n–x**) varied widely in binding affinity. Compound **8e** had poor affinity, but marked improvement was achieved by substitution of the basic nitrogen (position 9). Compounds **8l** and **8w**, though potent in binding affinity, were inactive at 30 mg/kg po as agonists or antagonists. These compounds were poorly soluble and poor absorption may have been responsible for the lack of activity. Analogue **8x**, with a GABA ratio of 1.3, was inactive as an agonist at 10 mg/kg po. Since we were interested only in potent, orally active compounds **8l**, **8w**, and **8x** were not further pursued. *N*-Benzyl analogue **8g** (9-benzyl-2-(2-fluorophenyl)-7,8,9,10-tetrahydropyrido[3,4-*e*][1,2,4]triazolo[1,5-*c*]pyrimidin-5(6*H*)-one, CGS 18791)[13] showed excellent binding affinity and was also readily solubilized in aqueous acid. Structural isomer **8d**, having a five-membered ring bearing a methyl group, showed no significant BZ receptor binding. Compounds **10g** and **11g**, containing some of the structural elements of **8g**, were inactive.[14] Best results in binding affinity were observed with compounds having a methylene attached to the basic nitrogen of **8e** appended with a phenyl (**8g**) or pyridyl (**8n–p**) moiety. Since **8g** showed the best binding affinity results within this set of compounds, this target was modified by replacement of the *o*-fluorophenyl substituent with other aryl and heteroaryl moieties (**8aa–ag**). In this new group, binding $IC_{50}$ values were all in the low nanomolar range, although no result was superior to the value determined for **8g**. The GABA ratio values were somewhat higher than that for **8g** and the highest (2.45) was associated with *p*-chlorophenyl

Table IV. Profiles of Selected Anxiomodulating Agents

| no. | FNZB $IC_{50}$[h] | GABA ratio | antagonism of PTZ stimuli | conflict[a] (FR, po) | VI[b] | EtOH[c] |
|---|---|---|---|---|---|---|
| **8b** | 1.7 | ND[f] | 2.4 (0.8–8.8)[d] | 30 | ia[e] | |
| 8c | 7.2* | 1.18 | 4.4 (2.5–7.6) | 10 | 10 | |
| **8g** | 0.44* | 1.06 | 0.66 (0.43–1.14) | 10 | 30 | |
| **8n** | 0.64* | 1.65 | 50% at 10 mg/kg | | | |
| 8o | 0.74* | 1.40 | 3.65 (1.4–10) | 10 | 10 | |
| 8p | 1.89* | 1.14 | 50% at 10 mg/kg | | | |
| 8t | 27* | 1.06 | 12% at 30 mg/kg | | | |
| **8aa** | 2.19* | 1.40 | 2.6 (1.2–5.2) | 3 | ia | 1.0 |
| **8ab** | 2.4* | 1.57 | 20% at 30 mg/kg | | | |
| **8ac** | 5.35* | 1.71 | 2.55 (1.1–5.3) | 30 | 30 | |
| **8ad** | 1.7* | 1.42 | 0.5 (0.2–1.1) | 3 | 3 | |
| **8ae** | 22.4* | 2.45 | 6.43 (3.13–13.27) | ia | 30 | |
| **8af** | 7.6* | 1.65 | 17.3 (ND) | | | |
| **8ag** | 20.6* | 1.59 | 26 (12.7–63.3) | | | |
| 1a | 0.5* | 1.10 | 2.3 (1.76–3.48) | 10 | ia | 30 |
| **DZ**[g] | 23 | 3.10 | 1.23 (0.59–2.76) | 10 | 10 | 5 |

[a] Minimally effective oral dose causing increase in conflict responding. [b] Minimally effective oral dose causing VI response decrement. [c] Minimally effective oral dose causing ethanol interaction. [d] 95% confidence limits in parentheses. [e] No significant response at highest dose tested. [f] Not determined. [g] Diazepam. [h] Modified FNZ binding test.

Table V. Effects of Selected Compounds on Nonconflict and Conflict Operant Responding of Rats in the Cook–Davidson Model[a]

| compound | dose, mg/kg po | % change in response rate: nonconflict (VI) | conflict (FR) |
|---|---|---|---|
| **8b** | 30.0 | 1 ± 3 | 132 ± 32* |
| 8c | 3.0 | 11 ± 7 | 79 ± 42 |
| | 10.0 | 53 ± 7* | 232 ± 46* |
| | 30.0 | 49 ± 10* | 236 ± 36* |
| **8g** | 1.0 | 0 ± 7 | 19 ± 3 |
| | 3.0 | −13 ± 15 | 173 ± 55 |
| | 10.0 | −44 ± 7* | 206 ± 63 |
| | 30.0 | −52 ± 12* | 282 ± 126* |
| 8o | 3.0 | −8 ± 3 | 129 ± 39 |
| | 10.0 | −21 ± 5* | 322 ± 29* |
| **8aa** | 1.0 | 0 | 35 ± 13 |
| | 3.0 | −4 ± 4 | 167 ± 16* |
| | 10.0 | 12 ± 6 | 367 ± 44* |
| **8ac** | 10.0 | −17 ± 16 | 30 ± 21 |
| | 30.0 | −38 ± 7* | 372 ± 77* |
| **8ad** | 1.0 | 1 ± 2 | 18 ± 7 |
| | 3.0 | −40 ± 8* | 58 ± 12* |
| **8ae** | 10.0 | 4 ± 4 | 104 ± 42 |
| | 30.0 | −45 ± 9* | 188 ± 144 |
| diazepam | 3.0 | 4 ± 7 | 159 ± 50 |
| | 10.0 | 30 ± 29 | 425 ± 96* |
| | 30.0 | −2 ± 18 | 257 ± 86* |

[a] *Significance: $P < 0.05$.

(10) (a) Braestrup, C.; Squires, R. F. Brain Specific Benzodiazepine Receptors. *Br. J. Psychiatry* **1978**, *133*, 249–260. (b) Pharmacological Characterization of Benzodiazepine Receptors in the Brain. *Eur. J. Pharmacol.* **1978**, *48*, 263–270.

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(12) Lal, H.; Sherman, G. T. Interoceptive Discriminative Stimuli in the Development of CNS Drugs and a Case of an Animal Model of Anxiety. *Annu. Rep. Med. Chem.* **1980**, *15*, 51–58.

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(14) Flunitrazepam* binding results: **10f**, 3% inhibition at 1 μM; 11f, 4% inhibition at 1 μM.

Table VI. Interaction of 8aa with Ethanol in Rotorod Performance[a]

| dose, mg/kg | ethanol $ED_{50}$, mg/kg (confidence limits) | dose, mg/kg | ethanol $ED_{50}$, mg/kg (confidence limits) |
|---|---|---|---|
| 0 | 1315 (1141–1516) | 3 | 832 (728–943)* |
| 1 | 984 (854–1132)* | 10 | 563 (410–728)* |

[a] *Significance: $P < 0.05$.

derivative **8ae**, structurally related to **1a** and **3a**.

A total of 14 compounds now remained to be further profiled and these are shown in Table IV. Six compounds, **8n**, **8p**, **8t**, **8ab**, **8af**, and **8ag**, were only weakly active in the PTZ antagonist screen and were not further pursued. Five of the remaining eight compounds produced $ED_{50}$ values in the PTZ discrimination test of less than 10 mg/kg. Compounds **8ac** and **8ae** appeared to have weak profiles and were not further pursued. Of the six remaining candidates (**8b,c,g,o,aa,ad**) only one compound, **8aa** (CGS 19985), emerged as a lead. This compound produced an increase in conflict responding greater than 300% above baseline (Table V) and showed no response-suppressing effects in the nonconflict component. With all of the other compounds, either shallow or minimal increases in conflict responding were noted or significant decreases in nonconflict responding occurred, suggesting sedation.

Since **8aa** showed good efficacy in the conflict test and no obvious sedative potential, we expected that this compound would also show no interaction with alcohol at those doses producing the preclinical antianxiety profile. However, at each of the doses assessed (1.0, 3.0, and 10 mg/kg), significant interactions were noted in the alcohol-induced rotorod deficit test procedure (Table VI). Since these doses were those required for anxiolytic efficacy, this suggests that **8aa** would be no better than the classical BZs currently on the market.

## Summary

Examples of 2-substituted [1,2,4]triazolo[1,5-c]-quinazolines were investigated for anxiomodulating effects, but only examples of antagonists and/or inverse agonists were found.[3] In contrast, spacial replacement of the benzene ring of the [1,2,4]triazolo[1,5-c]quinazolin-5-(6H)-one moiety led to several BZ agonists superior to diazepam both in binding affinity to the BZ receptor and in potency in animal models. The 9-(arylalkyl)- or heterocyclic-alkyl-substituted 7,8,9,10-tetrahydropyrido[3,4-e][1,2,4]triazolo[1,5-c]pyrimidin-5(6H)-ones were particularly attractive as they were readily solubilized in acidic media and as a whole appeared orally active. The lead compound **8aa** was effective in the Cook–Davidson conflict test as an anxiolytic agent without sedative potential, but it showed a potential for alcohol interaction.

Urea **8v**, in which the basicity of the ring nitrogen was neutralized, showed a GABA ratio well below unity. This suggests that the compound is either an antagonist or inverse agonist. It was not further investigated.

Although **8aa** fell short of our goal for a clinical candidate, the ease of synthesis of this compound makes it an attractive tool for further biological investigation and a lead for further drug targets. The SAR findings for this chemical series should be of interest to investigators who are mapping BZ receptor sites.[15]

(15) For example: Allen, M. S.; Tan, Y.-C.; Trudell, M. L.; Narayanan, K.; Schindler, L. R.; Martin, M. J.; Schultz, C.; Hagen, T. J.; Koehler, K. F.; Codding, P. W.; Skolnik, P.; Cook, J. M. Synthetic and Computor-Assisted Analyses of the Pharmacophore for the Benzodiazepine Receptor Inverse Agonist Site. *J. Med. Chem.* **1990**, *33*, 2343–2357.

## Experimental Section

**Chemistry.** All compounds indicated by "C, H, N" had microanalytical values within 0.4 units and exhibited IR and NMR spectra consistent with their structures. Details of the spectra are illustrated for key examples. Proton NMR were determined on a Varian EM-390 instrument in deuterated dimethyl sulfoxide with tetramethylsilane as internal standard, unless otherwise stated. IR spectra taken in Nujol mulls unless otherwise stated were recorded on a Perkin-Elmer Model 457 spectrometer. All reactions were carried out under nitrogen. Melting points are uncorrected and were determined on a Thomas-Hoover capillary melting point apparatus or, if above 250 °C, on a Reichart hot stage apparatus. UV spectra were determined on a Perkin-Elmer Lambda 7 UV–vis spectrophotometer. Mass spectra were taken with a Hewlett-Packard 5985 mass spectrometer.

**Dinitriles 4a–k.** Adiponitrile (**4a**), pimelonitrile (**4b**), suberonitrile (**4c**), and 3,3′-iminodipropionitrile (90%) (**4e**) were purchased from Aldrich Chemical Co. *N*-(1-Cyanoethyl)-*N*-(2-cyanoethyl)benzylamine (**4d**) was prepared in 91% yield by treatment of N-(2-cyanoethyl)benzylamine with lactonitrile in toluene by the procedure of Cavalla.[7f] *N*-(2-Cyanoethyl)-*N*-(cyanomethyl)aniline (**4f**) was prepared by treatment of *N*-(2-cyanoethyl)aniline[16] with paraformaldehyde and potassium cyanide in glacial acetic acid as described by Dimroth and Aurich.[17] Dinitriles **4g**, **4h**, and **4i** were prepared by alkylation of **4e** with benzyl bromide, methyl iodide, and ethyl iodide, respectively, at room temperature overnight in ethanol in the presence of sodium bicarbonate. Compounds **4j** and **4k** were prepared from isopropylamine and β-phenethylamine, respectively, in water by dropwise addition of 3 mol of acrylonitrile followed by overnight stirring. Those dinitriles synthesized were purified by normal-phase flash chromatography over silica gel and their structures ascertained by NMR and IR analysis.

**Amino Nitriles 5a–l.** Compounds **5a–c** were prepared by cyclization of **4a–c**, respectively, with potassium hydride in tetrahydrofuran as described by Brown.[7d] **5d**, **5e**,[7h], **5f**, **5g**,[7c] **5h**, and **5i**[7c] were prepared as described by Cavalla[7f] using potassium *tert*-butoxide in *tert*-butyl alcohol.

**Ethyl 4-Amino-3-cyano-1,2,5,6-tetrahydropyridine-1-carboxylate (5l).** To a stirring mixture of **5e** (1.59 g, 12.9 mmol) and sodium bicarbonate (2.44 g, 29 mmol) in 2-butanone (40 mL) was added gradually ethyl chloroformate (2.47 mL, 25.8 mmol) and the mixture was stirred for 2 h. The white precipitate that formed was filtered off and the filtrate concentrated to dryness at reduced pressure to afford a white solid, mp 139–141 °C (2.0 g, 79%), pure by TLC. IR 3380, 3300, 3180, 2135, 1680, 1575 $cm^{-1}$; NMR δ 1.3 (t, $CH_3$), 2.33 (dd, $CH_2$), 3.6 (dd, $CH_2$), 4.05 (s, $CH_2$), 4.2 (q, $CH_2CH_3$), 6.35 (s, exchanges with $D_2O$, $NH_2$); MS *m/e* 195 ($M^+$), 166 ($M^+ - C_2H_5$), 122 ($M^+ - COOC_2H_5$). Anal. ($C_9H_{13}N_3O_2$) C, H, N.

**Hydrazides 7.** Benzhydrazide, 2-chlorobenzhydrazide, and 4-chlorobenzhydrazide were purchased from Aldrich Chemical Co., Milwaukee, WI. 2-, 3-, and 4-fluorobenzhydrazide were obtained from Trans World Chemicals, Chevy Chase, MD. 2-Pyridinecarbohydrazide was bought from American Tokyo Kasei, Portland, OR. 2-Pyrrolecarbohydrazide was prepared as described by Yale and coworkers.[18]

**2-(2-Fluorophenyl)-6,7,8,9-tetrahydro-5*H*-cyclopenta-[*e*][1,2,4]triazolo[1,5-*c*]pyrimidin-5-one (8a).** Sodium (38.5 g, 1.67 mol) was dissolved in absolute ethanol (500 mL) in an apparatus connected to a still head and condenser. Compound **5a** (22.5 g, 0.21 mol) and diethyl carbonate (250 mL) were added. The mixture was stirred in an oil bath at 110 °C for 2 h as ethanol distilled from the solution. The residue was cooled in ice, treated cautiously with acetic acid (125 mL) and water (500 mL), and

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(17) Dimroth, K.; Aurich, H. G. *Chem. Ber.* **1965**, *98*, 3902–3906.

(18) Yale, H. L.; Losee, K.; Martins, J.; Holsing, M.; Perry, F. M.; Bernstein, J. Chemotherapy of Tuberculosis. VIII. The Synthesis of Acid Hydrazides, Their Derivatives and Related Compounds. *J. Am. Chem. Soc.* **1953**, *75*, 1933–1942.

extracted with ether (3 × 250 mL). The organic layer was dried over magnesium sulfate and concentrated at reduced pressure to a brown oil. This was triturated with a mixture of ether and petroleum ether to induce crystallization. The off-white solid was collected and dried to afford 27.6 g (73%) of **6a**: mp 60–66 °C (lit.[19] mp 68.7–69.1 °C); NMR ($CDCl_3$) δ 1.33 (t, $CH_3$), 1.9–2.35 (m, $CH_2$), 2.42–2.8 (m, $CH_2$), 2.95–3.3 (m, $CH_2$), 4.3 (q, $CH_2CH_3$), 7.7 (s, NH, exchangeable with $D_2O$); IR 3460, 3360, 3230, 2208, 2190, 1750, 1640 $cm^{-1}$. A mixture of **6a** (10.0 g, 55.5 mmol), 2-fluorobenzhydrazide (8.56 g, 55.5 mmol), 2-methoxyethanol (185 mL), and tri-*n*-propylamine (7.4 mL) was heated at reflux over 16 h, cooled, and treated with water gradually to initiate crystallization. After overnight refrigeration, the solid was collected, recrystallized from ethanol, and dried to afford 2.85 g (19%) of **8a** as a white solid: mp 251–253 °C; NMR δ 2.1–2.25 (m, $CH_2$), 2.8–2.95 (m, 2 $CH_2$), 7.3–7.45 (m, 2 H), 7.5–7.6 (m, 1 H), 8.1–8.2 (m, 1 H), 12.35 (NHCO); IR 3125, 1722, 1640, 1590, 1333, 755 $cm^{-1}$; MS *m/e* 270 ($M^+$), 242 ($M^+$ - CO), 121 (2-fluorobenzonitrile). Anal. ($C_{14}H_{11}FN_4O$) C, H, N. Similar conditions were used to prepare **8h–l** and **8b**.

**2-(2-Fluorophenyl)-6,7,8,9,10,11-hexahydro-5*H*-cyclohepta[*e*][1,2,4]triazolo[1,5-*c*]pyrimidin-5-one (8c).** To a freshly prepared solution of sodium ethoxide in ethanol (from 11.88 g, 517 mmol, of sodium in 150 mL of ethanol) was added **5c** (8.8 g, 65 mmol) and the mixture heated at 110 °C for 1 h. Diethyl carbonate (75 mL) was added and the mixture heated for 2 h at reflux. It was ice-cooled and treated cautiously with acetic acid (60 mL) and water (400 mL). This was extracted with ether (4 × 150 mL), dried over magnesium sulfate, concentrated at reduced pressure to an oil, taken up in ethyl acetate (300 mL), washed with water (2 × 100 mL), and dried over magnesium sulfate. Removal of the solvent at reduced pressure and then under oil-pump vacuum gave **6c** (11.8 g, 88%) as a thick oil showing one spot by TLC: NMR ($CDCl_3$) δ 1.3 (t, $CH_3$), 1.4–1.8 (m, $CH_2CH_2$), 2.2–2.5 (m, $CH_2$), 2.7–3.0 (m, $CH_2$), 4.2 (q, $CH_2CH_3$), 7.3 (s, NHCO); IR (neat) 3260, 2175, 1725, 1615, 1380, 1210 $cm^{-1}$. A mixture of **6c** (11.8 g, 57 mmol) and 2-fluorobenzhydrazide (8.74 g, 57 mmol) in 1-methyl-2-pyrrolidinone (140 mL) was stirred at 170 °C for 16 h, cooled, and treated gradually with water to cause crystallization. The solid was recrystallized from *dl*-1-methoxy-2-propanol to afford a first crop of **8c** (8.6 g, 51%), pure by TLC: mp 257–259 °C; NMR δ 1.75–1.95 (m, 3 $CH_2$), 2.75 (dd, $CH_2$), 2.9 (dd, $CH_2$), 7.3–7.5 (m, 2 aryl H), 7.5–7.6 (m, aryl H), 8.1–8.2 (m, aryl H), 11.9 (s, NH); IR 3080, 1716, 1627, 1553, 1498, 1327, 1220, 765 $cm^{-1}$; MS *m/e* 298 ($M^+$). Anal. ($C_{16}H_{15}FN_4O$) C, H, N. Similar conditions were used to prepare **8d**.

**2-(2-Fluorophenyl)-7,8,9,10-tetrahydropyrido[3,4-*e*][1,2,4]triazolo[1,5-*c*]pyrimidin-5(6*H*)-one (8e).** A mixture of 4 N sodium hydroxide (73 mL), 2-methoxyethanol (145 mL), and **8l** (15.4 g, 43 mmol) was stirred at 120 °C overnight. The mixture was cooled and adjusted to pH 6 with dilute hydrochloric acid, whereupon gas evolution occurred and solid material separated. The solid was collected, taken up in hot 2-methoxyethanol, filtered free of inorganic material, and cooled to form white crystals (9.2 g, 75%, mp 247–248 °C). It was dried under high vacuum to give the pure sample, mp 254–257 °C. The zwitterionic material dissolved in water at pH 3.4 or lower and at pH above 10.5: IR 2800–2400 (br), 1610, 1569, 1536 $cm^{-1}$; UV (MeOH, λ, ε) (neutral) 273 (12 440), 241 (26 260); (acidic) 269 (sh) (11 990), 242 (26 940); (alkaline) 284 (13 530), 232 (25 740); NMR δ 2.5 (m, $CH_2$), 3.0 (dd, $CH_2$), 3.8 (s, $CH_2$), 7.3–7.45 (m, 2 H), 7.55–7.65 (m, H), 8.05–8.15 (m, 1 H), exchangeable hydrogens spread over baseline. Anal. ($C_{14}H_{12}FN_5O$) C, H, N.

**2-(2-Fluorophenyl)-7,8,9,10-tetrahydro-9-(phenylmethyl)pyrido[3,4-*e*][1,2,4]triazolo[1,5-*c*]pyrimidin-5-(6*H*)-one (8g).** To sodium (21.3 g, 926 mmol) dissolved in absolute ethanol (275 mL) were added **5g** (24.7 g, 116 mmol) and diethyl carbonate (137 mL). It was stirred 4 h at reflux and at ambient temperature over 16 h. The ice-cooled reaction mixture was treated with a mixture of acetic acid (13 mL) and water (1 L) and extracted with ether (3 × 300 mL), which caused the precipitation of a white solid, insoluble in ether. The solid was collected and air-dried (9.1 g, 27%, mp 224–225 °C). Recrystallization of a sample from ethanol raised the melting point to 238–241 °C, but the IR spectrum was unchanged. This product was **4-ethoxy-5,6,7,8-tetrahydro-6-(phenylmethyl)-pyrido[4,3-*d*]pyrimidin-2(1*H*)-one (9g)** [IR 2850–2500 (br), 1665, 1650 (split peak), 1577, 1427, 1333, 1244, 1072, 1028 $cm^{-1}$; NMR δ 1.2 (t, $CH_3CH_2$), 2.4–2.6 (m, $CH_2$), 3.15 (s, $CH_2$), 3.65 (s, $CH_2$), 4.25 (q, $CH_3CH_2$), 7.2–7.35 (m, 5 aryl H), 11.25 (s, NH). Anal. ($C_{16}H_{19}N_3O_2$) C, H, N.] The ether extract was dried ($Mg_2SO_4$) and concentrated at reduced pressure to a thick oil. It was chromatographed over silica with 1:1 ethyl acetate/hexane to afford two fractions, the first (4.73 g) containing a major product and two minor impurities and the second (5.3 g) containing only the major product, which formed a low-melting solid. This material (10.3 g, 31%), **6g**, was used without further purification: IR 3390, 3280, 2940–2700 (br), 2200, 1745, 1635, 1490, 1200, 1030, 740, 695 $cm^{-1}$; NMR δ 1.15 (t, $CH_3CH_2$), 2.55 (br s, $CH_2$), 3.1 (br s, $CH_2$), 3.6 (s, $CH_2$), 4.15 (q, $CH_3CH_2$), 7.5 (s, aryl H), 9.7 (br s, NH). A mixture of **6g** (24.8 g, 87 mmol), 2-fluorobenzhydrazide (13.4 g, 87 mmol), and 1-methyl-2-pyrrolidinone (200 mL) was stirred overnight at 170 °C. Addition of water to the cooled mixture caused the crude product to settle out as a thick oil. The aqueous layer was removed by decantation and the oil taken up in 2-propanol (100 mL). A solid (1.7 g) was obtained and water (300 mL) was then added to the mother liquor to form a second crop of product (5.7 g). After three days under refrigeration, the mother liquor yielded a third crop of solid (10.8 g). The first two crops were converted in methanol to the methanesulfonate salt and then reconverted to the free base with aqueous ammonia to afford the free base **8g** (5.1 g): mp 242–245 °C; MS 375 ($M^+$), 374 (M - 1), 284 (M - benzyl). Anal. ($C_{21}H_{18}FN_5O$) C, H, N. This was combined with the third crop of **8g** and converted to the mesylate salt (13.9 g). Dissolution in dimethylacetamide (400 mL) followed by methanol (400 mL) gave the pure salt as a white solid (5.3 g, 16%, mp 306–309 °C): IR 2840–2400 (br), 1730, 1668, 1618, 1575, 1225, 1150, 1035, 770, 700 $cm^{-1}$; NMR δ 2.3 (s, $CH_3S$), 2.9–3.1 (m, $CH_2$), 3.5–3.8 (m, $CH_2$), 4.35 (m, $CH_2$), 4.6 (s, $CH_2$), 7.3–8.2 (9 aryl H), 10.35 (acidic H), 12.4 (NH). Anal. ($C_{22}H_{22}FN_5O_4S$) C, H, N. Concentration of the mother liquor and trituration with acetone produced 7.15 g of less pure salt.

**2-(2-Fluorophenyl)-6-methyl-7,8,9,10-tetrahydro[1,2,4]triazolo[1,5-*c*]quinazolin-5(6*H*)-one (8m).** A mixture of **8b** (1.4 g, 5 mmol) and sodium methoxide (0.3 g) was dissolved under stirring in dimethyl sulfoxide (50 mL) at 85 °C. It was allowed to cool to room temperature and methyl iodide (0.4 mL) in dimethyl sulfoxide (10 mL) was added. After heating for 0.5 h at 50 °C, some starting material was detected by TLC. Additional base (0.1 g) and methyl iodide (0.3 mL) in dimethyl sulfoxide (10 mL) were added, and the mixture was stirred at 50–80 °C over 0.5 h. It was quenched in ice-water and the solid collected, washed with water, and vacuum-oven-dried to afford white crystals (mp 228–232 °C) free of starting material. The solid was recrystallized from 2-methoxyethanol to afford pure **8m** (800 mg, 54%, mp 236–239 °C): IR 1705, 1710 (split), 1625, 1565, 755 $cm^{-1}$; NMR δ 1.55–1.95 (m, 2 $CH_2$), 2.55–2.8 (m, 2 $CH_2$), 3.5 (s, $CH_3$), 7.25–8.25 (m, 4 aryl H); UV (MeOH, λ, ε) 240 (22 670), 277 (11 410), compared to **8b**, 240 (19 870), 272 (9790); (MeOH + KOH) 239 (23 660), 277 (11 220) compared to **8b**, 233 (20 030), 283 (10 530); MS *m/e* 298 ($M^+$), 283 ($M^+$ - $CH_3$). Anal. ($C_{16}H_{15}FN_4O$) C, H, N.

**2-(2-Fluorophenyl)-7,8,9,10-tetrahydro-9-(2-pyridylmethyl)pyrido[3,4-*e*][1,2,4]triazolo[1,5-*c*]pyrimidin-5-(6*H*)-one (8n).** To a slurry of **8e** (0.20 g, 0.7 mmol) in dry dimethylformamide (10 mL) was added triethylamine (0.22 mL, 1.5 mmol) followed by 2-picolyl chloride hydrochloride (0.22 g, 1.34 mmol) in portions. After overnight stirring at room temperature, water (30 mL) was added dropwise and the clear solution extracted with methylene chloride (3 × 10 mL). The extracts were dried ($Na_2SO_4$) and concentrated to a salmon-pink solid (160 mg). A portion (100 mg) in methanol (5 mL) was treated with methanesulfonic acid (0.05 mL) to form a clear solution which formed crystals on cooling to −10 °C. The solid was washed with cold methanol and dried at 100 °C/0.15 mm over 16 h to afford the pure dimesylate salt (120 mg, 48%, mp 267–269 °C): NMR (TFA) δ 2.5 (s, 2 $CH_3$), 3.6 (m, $CH_2$), 4.2 (m, $CH_2$), 5.15 (s, $CH_2$), 5.4 (s, $CH_2$), 7.25–9.05 (m, 8 aromatic H). Anal. ($C_{22}H_{25}FN_6O_7S_2$) C,

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H, N. In a similar way, 8o was prepared from 3-picolyl chloride hydrochloride, 8p from 4-picolyl chloride hydrochloride, 8t from methyl bromoacetate, 8w from *p*-toluenesulfonyl chloride, and 8x from 3-pyridinesulfonyl chloride.

**9-Benzoyl-2-(2-fluorophenyl)-7,8,9,10-tetrahydropyrido[3,4-*e*][1,2,4]triazolo[1,5-*c*]pyrimidin-5(6*H*)-one (8q).** To a stirring mixture of **8e** (1.03 g, 3.6 mmol) and triethylamine (0.9 mL) in 1-methyl-2-pyrrolidinone (18 mL) in an ice bath was added benzoyl chloride (0.83 g, 7.2 mmol) and the whole was stirred for 44 h at room temperature. It was diluted with water (100 mL) and extracted with ethyl acetate. Solid material which was insoluble in both layers was collected, washed with water and ethyl acetate, and dried at 100 °C under vacuum to afford pure **8q** (0.54 g, 39%, mp 342–344 °C): IR, 3080, 1736, 1650, 1600, 1573, 1257, 765, 712 $cm^{-1}$; NMR $\delta$ 3.75 (s, $CH_2$), 3.6–4.0 (d, $CH_2$), 4.45–4.75 (d, $CH_2$), 7.3–8.3 (m, 9 aryl *H*), 12.2 (s, N*H*); MS 389 ($M^+$), 284 (M – PhCO), 105 ($PhCO^+$), 77 ($Ph^+$). Anal. ($C_{21}H_{16}FN_5O_2$) C, H, N.

**2-(2-Fluorophenyl)-7,8,9,10-tetrahydro-9-(phenylacetyl)pyrido[3,4-*e*][1,2,4]triazolo[1,5-*c*]pyrimidin-5(6*H*)-one (8r).** A mixture of **8e** (0.57 g, 2 mmol) and phenacyl chloride (3 mL) was stirred at 120 °C for 4 h. Solid began to form within a few minutes. The mixture was cooled and triturated with ether (30 mL). The white solid was collected, washed with ether, and dried under high vacuum to afford pure **8r** (0.62 g, 79%, mp 310–313 °C): IR 3120, 1740, 1645, 1562, 1444, 1231, 752 $cm^{-1}$. Anal. ($C_{22}H_{18}FN_5O_2$) C, H, N. Under similar conditions and workup, **8e** was reacted with phenyl isocyanate to afford pure **8u** (87% yield, mp 291–293 °C): IR 3320, 3080, 1725, 1700, 1675, 1650, 1517, 1438, 1221, 762 $cm^{-1}$; NMR $\delta$ 2.7 (m, $CH_2$), 3.8 (m, $CH_2$), 4.6 (s, $CH_2$), 6.9 (m, aryl *H*), 7.2–7.6 (m, 7 aryl *H*), 8.2 (m, aryl *H*), 8.85 (N*H*), 12.1 (s, N*H*). Anal. ($C_{21}H_{17}FN_6O_2$) C, H, N.

**9-[(*S*)-2-Amino-3-phenylpropionyl]-2-(2-fluorophenyl)-7,8,9,10-tetrahydropyrido[3,4-*e*][1,2,4]triazolo[1,5-*c*]pyrimidin-5(6*H*)-one Hydrochloride (8s).** To a solution of *N*-*t*-BOC-L-phenylalanine (Sigma Chemical Co., 0.93 g, 3.5 mmol) in dimethylformamide (20 mL) was added *N*,*N*′-carbonyldiimidazole (0.62 g, 3.8 mmol) and after 30 min of stirring, **8e** (1.0 g, 3.5 mmol) was added. The mixture was stirred for 18 h at room temperature and the clear solution was treated dropwise with water (60 mL) to precipitate an off-white solid. This was washed with water and air-dried to afford the *N*-*tert*-butoxy derivative of **8s** as the free base (1.6 g, 90%, mp 240–243 °C). This derivative (1.0 g, 2 mmol) was dissolved in a mixture of methanol (40 mL)/methylene chloride (40 mL) at 35 °C and 3 N HCl in ether (5 mL) was added dropwise. The clear solution was allowed to stand overnight and then concentrated to dryness at 55 °C (15 mm). The resulting foam was redissolved in water (10 mL) and filtered and the filtrate concentrated to incipient dryness at reduced pressure. The resulting oil was triturated with acetone (30 mL) to form light tan crystals. The material was dried at 110 °C (1 mm) for 16 h to afford **8s** as the hemihydrate (0.75 g, 78%, mp 305–307 °C): $[\alpha]^D$ = –72.1° (*c* = 1, $H_2O$); IR 3500–3300 (br), 2800–2400 (br), 1732, 1650, 1568, 1324, 1226, 758 $cm^{-1}$. Anal. ($C_{23}H_{21}FN_6O_2 \cdot {}^1/_2H_2O$) C, H, N.

**2-(2-Fluorophenyl)-6,7,8,10-tetrahydro-5-oxopyrido[3,4-*e*][1,2,4]triazolo[1,5-*c*]pyrimidine-9(5*H*)-carboxamide (8v).** To a solution of 8e (570 mg, 2 mmol) in 50% aqueous acetic acid (5 mL) was added dropwise a solution of potassium cyanate (324 mg) in water (3 mL). A precipitate began to appear. After 1 h, the mixture was gradually heated to 80 °C, then cooled and diluted with water (10 mL). The precipitate was washed with water, vacuum-oven-dried, and dissolved in dimethylacetamide (10 mL). A precipitate began to form. After 2 h, acetone (10 mL) was added and the precipitate was collected, washed with acetone, and dried at 100 °C (1 mm) overnight to afford pure **8v** (440 mg, 67%, mp 300–303 °C): IR 3350, 3180, 3070, 1728, 1652, 1588, 1325, 760, 715 $cm^{-1}$; NMR ($CF_3COOH$) $\delta$ 3.25 (m, $CH_2$), 4.1 (m, $CH_2$), 5.05 (s, $CH_2$), 7.3–7.5 (m, 2 aryl *H*), 7.8 (m, aryl *H*), 8.2 (m, aryl *H*). Anal. ($C_{15}H_{13}FN_6O_2$) C, H, N.

**2-(3-Fluorophenyl)-7,8,9,10-tetrahydro-9-(phenylmethyl)pyrido[3,4-*e*][1,2,4]triazolo[1,5-*c*]pyrimidin-5(6*H*)-one (8ab).** A mixture of **6c** (10.0 g, 35 mmol), 3-fluorobenzhydrazide (5.41 g, 35 mmol), and 1-methyl-2-pyrrolidinone (80 mL) was stirred 18 h at 180 °C. The reaction mixture was worked up as described for **8g**. The solid was converted to its methanesulfonate salt and recrystallized from dimethylacetamide and methanol to afford the pure salt of **8ab** (6.77 g, 41%, mp 299–302 °C): IR 3050, 1740, 1665, 1580, 1425, 1040, 765 $cm^{-1}$; NMR $\delta$ 2.4 (s, $CH_3S$), 3.05–3.25 (m, $CH_2$), 3.5–3.8 (m, $CH_2$), 4.35–4.5 (m, $CH_2$), 4.7 (s, $CH_2$), 7.2–8.2 (m, 9 aryl *H*), 10.6 (br s, O*H*), 12.6 (N*H*). Anal. ($C_{22}H_{22}FN_5O_4S$) C, H, N. Similarly, **6c** was reacted with 4-fluorobenzhydrazide to form **8ac**, with 2-chlorobenzhydrazide to form **8ad** (characterized as the methanesulfonate), with 2-pyrrolecarbohydrazide to form **8af**, and with 2-pyridinecarbohydrazide to form **8ag** (purified through the methanesulfonate salt).

**7,8,9,10-Tetrahydro-2-phenyl-9-(phenylmethyl)pyrido[3,4-*e*][1,2,4]triazolo[1,5-*c*]quinazolin-5(6*H*)-one (8aa).** A mixture of **6c** (5.3 g, 18.6 mmol), benzhydrazide (2.53 g, 18.6 mmol), dimethylacetamide (70 mL), and ethyldiisopropylamine (0.5 mL) was heated at 150 °C for 16 h. It was concentrated to dryness at reduced pressure. The residue was stirred with 2-propanol (200 mL) for 30 min and the solid which formed was recrystallized from 2-methoxyethanol to afford pure **8aa** (2.3 g, 35%, mp 257–259 °C): IR 3060, 1733, 1650, 1565, 1524, 1335, 1209, 756, 725 $cm^{-1}$; NMR $\delta$ 2.7 (m, $CH_2$), 2.8 (m, $CH_2$), 3.3 (m, $CH_2$), 3.5 (s, $CH_2$), 3.75 (s, $CH_2$), 7.25–7.55 (8 aryl *H*), 8.1 (m, 2 aryl *H*), 12.0 (N*H*); MS *m*/*e* 357 ($M^+$), 266 (M – $C_6H_5CH_2$). Anal. ($C_{21}H_{19}N_5O$) C, H, N. In a similar way, **6c** was reacted with 4-chlorobenzhydrazide to form **8ae**, characterized and purified through its methanesulfonate salt.

**4-Ethoxy-5,7-dihydro-6-phenylpyrrolo[3,4-*d*]pyrimidin-2(1*H*)-one (9f).** To a solution of sodium (15.2 g, 0.66 mol) in absolute ethanol (180 mL) was added **5f** (15.3 g, 83 mmol) and the mixture heated for 1 h at reflux. Diethyl carbonate (90 mL) was added and the whole refluxed for 2 h. The mixture was ice cooled and treated with acetic acid (8 mL) and water (600 mL) to form a solution. Extraction with ethyl acetate (4 × 200 mL) caused precipitation of white solid (8.0 g, 38%) identified by IR and NMR as **9f**. The organic extract was dried over $Mg_2SO_4$ and concentrated at reduced pressure to produce a second crop of solid (5.4 g) identified as a mixture of **9f** with some **6f** (TLC, NMR; IR 2210 $cm^{-1}$). The first crop was recrystallized from *dl*-2-methoxy-1-propanol, washed with water, and dried to afford pure **9f** (6.0 g, mp 249–252 °C): IR 3055, 1650, 1610, 1590, 1500, 1430, 1390, 1335, 1100, 740 $cm^{-1}$; NMR $\delta$ 1.35 (t, $CH_3$), 4.2–4.5 (m, 3 $CH_2$), 6.5–6.85 (m, 3 aryl *H*), 7.15–7.4 (2 aryl *H*), 10.5 (br s, N*H*); MS *m*/*e* 257 ($M^+$), 256 ($M^+$ – 1), 185 ($M^+$ – $C_3H_6NO$), 77 ($C_6H_5^+$). Anal. ($C_{14}H_{15}N_3O_2$) C, H, N.

**5,7-Dihydro-6-phenylpyrrolo[3,4-*d*]pyrimidine-2,4-(1*H*,3*H*)-dione (10f).** A mixture of **9f** (3.9 g, 15.2 mmol) and 3 N hydrochloric acid (80 mL) was heated at reflux for 4 h. The mixture was cooled and the product collected, washed with water, and vacuum-oven-dried. The white solid (3.3 g, 95%, mp >300 °C) was analytically pure: IR 3180, 1720, 1675, 1610, 1510, 1200, 1000, 855, 755 $cm^{-1}$; NMR $\delta$ 4.15–4.4 (m, 2 $CH_2$), 6.5–6.85 (m, 3 aryl *H*), 7.15–7.4 (m, 2 aryl *H*), 11.2 (N*H*), 11.6 (N*H*); MS *m*/*e* 229 ($M^+$), 228 ($M^+$ – 1), 185 ($M^+$ – $CONH_2$), 77 ($C_6H_5^+$). Anal. ($C_{12}H_{11}N_3O_2$) C, H, N.

**5,7-Dihydro-6-phenyl-1,3-di-*n*-propylpyrrolo[3,4-*d*]pyrimidine-2,4(1*H*,3*H*)-dione (12b).** To a suspension of **10f** (1.0 g, 4.4 mmol) in 10% sodium hydroxide (3.5 mL, 8.8 mmol) was added tetrabutylammonium bromide (0.56 g, 1.76 mmol), *n*-propyl bromide (1.2 mL, 13.2 mmol), and toluene (13 mL). The mixture was stirred at 60 °C for 2 days and then separated between water (20 mL) and ethyl acetate (20 mL). The aqueous layer was extracted with ethyl acetate, and the organic extracts were washed with water, dried over $Mg_2SO_4$, and concentrated to dryness at reduced pressure to a thick oil. This was crystallized from ethanol–water to afford an off-white solid (400 mg, 29%, mp 133–137 °C): IR 1710, 1680, 1650, 1605, 1510, 750 $cm^{-1}$; NMR $\delta$ 0.8–1.1 (2 t, 2 $CH_3$), 1.45–1.9 (m, 2 $CH_2$), 3.65–4.0 (2 q, 2 $CH_2$), 4.3–4.7 (m, 2 $CH_2$), 6.65–6.85 (m, 3 aryl *H*), 7.2–7.4 (m, 2 aryl *H*); MS *m*/*e* 313 ($M^+$), 312 ($M^+$ – 1), 270 ($M^+$ – propyl), 227 ($M^+$ – 2 propyl), 185. Anal. ($C_{18}H_{23}N_3O_2$) C, H, N.

When n-propyl bromide was replaced by methyl iodide and the reaction temperature was lowered to 40 °C, 1,3-dimethyl derivative **12a** was obtained as a white solid (mp 248–252 °C) in 67% yield: IR 1705, 1670, 1645, 1600, 1540, 1510, 1350, 1180, 745 $cm^{-1}$; NMR $\delta$ 3.0–3.3 (2 t, 2 $CH_3$), 4.2–4.6 (m, 2 $CH_2$), 6.5–6.7 (m, 3 aryl *H*), 7.0–7.2 (m, 2 aryl *H*); MS *m*/*e* 257 ($M^+$), 256 ($M^+$ – 1),

199, 171, 104, 77 ($C_6H_5^+$). Anal. ($C_{14}H_{15}N_3O_2$) C, H, N.

**5,6,7,8-Tetrahydro-6-(phenylmethyl)pyrido[4,3-*d*]pyrimidine-2,4(1*H*,3*H*)-dione (10g).** A mixture of **9g** (2.2 g, 7.7 mmol) and 3 N hydrochloric acid (40 mL) was stirred at reflux for 4 h, cooled, and gradually made basic to pH 8 with ammonium hydroxide. Solid began to appear at ca. pH 4. The solid was washed with water and dried under high vacuum to afford white crystals (1.95 g, 98%, mp 290-292 °C): IR 3160, 1735, 1700, 1655, 1520, 1150, 750 $cm^{-1}$; NMR $\delta$ 2.35-2.75 (m, 2 $CH_2$), 3.05 (s, $CH_2$), 3.65 (s, $CH_2$), 7.4 (s, 5 aryl *H*); MS *m/e* 257 ($M^+$), 256 ($M^+ - 1$), 166 ($M^+ - C_6H_5CH_2$). Anal. ($C_{14}H_{15}N_3O_2$) C, H, N.

**4-[[(2-Fluorophenyl)methyl]amino]-5,6,7,8-tetrahydro-6-(phenylmethyl)pyrido[4,3-*d*]pyrimidin-2(1*H*)-one (11g).** A mixture of **9g** (1.0 g, 3.5 mmol) and 2-fluorobenzylamine (5 mL) was heated at 150 °C for 2 h, cooled, and filtered. The solid was washed thoroughly with ether and dried at 100 °C under high vacuum. The pale yellow solid (320 mg, 25%, mp 241-247 °C) was analytically pure: IR 3210, 1668, 1640, 1580, 1540, 1350, 1230, 755 $cm^{-1}$; NMR $\delta$ 2.3-2.75 (m, 2 $CH_2$), 3.3 (m, $CH_2$), 3.75 (s, $CH_2$), 4.6 (d, $CH_2$), 7.15-7.55 (m, 9 aryl *H*), 10.5 (s, N*H*); MS *m/e* 364 ($M^+$), 363 ($M^+ - 1$), 320 ($M^+ - CONH_2$), 255 ($M^+ - CH_2C_6H_4F$), 109 ($CH_2C_6H_4F^+$). Anal. ($C_{21}H_{21}FN_4O$) C, H, N.

**Biology. Radioligand Binding Assays.** The determination of [$^3$H]flunitrazepam binding in rat forebrain (FNZ text) was carried out essentially as described previously.[10,3] In this procedure, the geometrical mean of diazepam $IC_{50}$s was 4.4 ± 0.2 nM ($n$ = 13). [$^3$H]Flunitrazepam binding to the BZ receptor complex (designated FNZ*) was determined as described in detail in our earlier publication.[3] In this procedure, $IC_{50}$ values for 1b ($n$ = 3) were 0.37 ± 0.04 nM without GABA and 0.45 ± 0.05 with GABA to give a GABA ratio of 0.82.[3] Similarly, **1a** showed an $IC_{50}$ value of 0.5 nM without GABA and a GABA ratio of 1.10.[4b]

**Behavioral Tests. PTZ Drug Discrimination.** Rats were trained to discriminate the anxiogenic effects of a subconvulsant dose of pentylenetetrazole (PTZ), according to the procedure of Bennett et al.[5] Animals then were administered the test compound 30 min prior to the anxiogenic dose of PTZ. Antagonism of the PTZ stimulus was indicative of an anxiolytic response.[12] $ED_{50}$ values were calculated by probit analysis.[20]

**Antagonism of Diazepam-Induced Rotorod Deficit.** Compounds were tested for their ability to antagonize the rotorod deficit induced by diazepam (30 mg/kg ip) as described in an earlier publication.[3] The test compound was administered po 30 min prior to diazepam. After an additional 30 min, rats were tested for their ability to remain on a rotating rod. If the test agent antagonized the rotorod deficit typically noted with diazepam, it was identified as a potential BZ antagonist/inverse agonist.

**Cook-Davidson Behavioral Conflict Paradigm.** Potential anxiolytic activity was determined by the Cook-Davidson conflict procedure in rats as described by Bennett et al.[5] The VI (nonconflict) portion of this test also provided information as to the potential sedation/muscle relaxation. Rats were trained to press a lever for milk reward both in the presence (conflict) and absence (nonconflict) of shock. Compounds with anxiolytic potential increase responding during the shock component. Rats press for milk reward even if it means experiencing a mild shock. If a compound decreases nonconflict responding (no shock), it has the potential for producing sedation and/or muscle relaxation.

**Ethanol Interaction.** Drug interaction with ethanol was evaluated by using the rotorod procedure. Vehicle or test compounds were orally administered to male Wistar (Crl:(WI)BR) rats (130-160 g) 30 min prior to ethanol. Rats were then administered various doses of ethanol ip 30 min before testing on the rotorod. Rotorod performance was assessed with a conventional rotorod apparatus modified by placing a section of polyethylene material over the drum. The rat was placed on the drum, which rotated at a speed of 16 rpm, and was required to remain on the drum for 30 s. Each animal was allowed up to three trials to reach the criterion. Any animal not meeting the criterion was considered to have neurological deficit. The $ED_{50}$ values for ethanol alone, or in combination with various test drug doses, were estimated. The $ED_{50}$ values were calculated by probit analysis.[20]

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# 1,3-Dihydro-2*H*-imidazo[4,5-*b*]quinolin-2-ones—Inhibitors of Blood Platelet cAMP Phosphodiesterase and Induced Aggregation[1]

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A series of 1,3-dihydro-2*H*-imidazo[4,5-*b*]quinolin-2-one derivatives was synthesized and evaluated as inhibitors of cAMP hydrolysis by a crude human platelet phosphodiesterase preparation and as inhibitors of ADP- and collagen-induced aggregation of rabbit blood platelets. The parent structure **7a**, demonstrated potent inhibitory activity that was enhanced by the introduction of alkyl, alkoxy, or halogen substituents at the 5-, 6-, 7-, and 8-positions. Methylation at N-1 or N-3 produced weaker inhibitors of cAMP PDE and platelet aggregation. 1,3,9,9a-Tetrahydro-2*H*-imidazo[4,5-*b*]quinolin-2-ones (**6**) were found to be equipotent with their fully oxidized congeners (**7**). On the basis of platelet inhibitory properties in vitro, efficacy at preventing thrombus formation in animal models of thrombosis, and a favorable hemodynamic profile, 1,3-dihydro-7,8-dimethyl-2*H*-imidazo[4,5-*b*]quinolin-2-one (**7o**, BMY 20844) was selected for advancement into toxicological evaluation and clinical trial. An efficient synthesis of **7o** is described.

Blood platelets normally circulate as quiescent disk-shaped cells found in the outermost layer of blood where they are poised to fulfill their role in hemostasis. Platelet involvement has been implicated in a number of disease states including migraine,[2] asthma,[3] atherosclerosis,[4] and tumor-cell metastasis,[5] while inadequately controlled platelet activation and aggregation may lead to vascular

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