Synthesis of Novel 2-Phenyl-2*H*-pyrazolo[4,3-*c*]isoquinolin-3-ols: Topological Comparisons with Analogues of 2-Phenyl-2,5-dihydropyrazolo[4,3-*c*]quinolin-3(3*H*)-ones at Benzodiazepine Receptors

Michael S. Allen,† Phil Skolnick,‡ and James M. Cook*,†

Department of Chemistry, University of Wisconsin-Milwaukee, Milwaukee, Wisconsin 53201, and Laboratory of Neuroscience, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, Maryland 20892.
Received June 17, 1991

Based on the topology of pyrazoloquinolinones **10–12**, a series of 2-phenyl-2*H*-pyrazolo[4,3-*c*]isoquinolines **6a–d**, **7a–d**, **8**, and **9** have been synthesized and evaluated for their ability to inhibit radioligand binding to benzodiazepine receptors (BzR). Modification of the hydrogen bonding donor and acceptor characteristics of the NH and C=O functionalities of the pyrazoloquinolinones **10–12** resulted in ligands with dramatically reduced affinities ($IC_{50} \gg 2\ \mu M$) for BzR. The low affinities of **6a–d**, **7a–d**, **8**, and **9** are consistent with the involvement of the NH function present on diverse classes of inverse agonists (β-carbolines, diindoles, and pyrazoloquinolinones) with a hydrogen bond acceptor site (A_2) on the binding protein. Moreover, it supports the involvement of the carbonyl function of the pyrazoloquinolinones and the pyridine nitrogen atom of β-carbolines and diindoles with a hydrogen bond donor site (H_1). Finally, the results from this work indicate that a simultaneous interaction at both hydrogen bond donor (H_1) and acceptor sites (A_2) at BzR is required for high affinity binding of inverse agonists.

Introduction

Benzodiazepine receptor ligands effect a wide range of pharmacological actions[1] ranging from muscle relaxant, sedative, anxiolytic and anticonvulsant produced by full agonists ("GABA-positive" ligands) to the convulsant and anxiogenic effects of inverse agonists ("GABA-negative" ligands).[2] During the past several years, we[3-8] have attempted to model the BzR for agonist and inverse agonist/antagonist sites. In a recent paper, the most complete model to date of the pharmacophore for inverse agonists/antagonists at the BzR was reported.[6] This model was based on the in vitro and in vivo structure–activity relationships (SAR) of pyridodiindoles, β-carbolines, and pyrazoloquinolines. This model contains three major structural components: a hydrogen bond acceptor site (A_2), a hydrogen bond donor site (H_1), and a lipophilic pocket in the binding cleft that readily accommodates substituents at position 3 of β-carbolines with chain lengths of five atoms or less. Results from a 3D-QSAR electrostatic map are consistent with the existence of the hydrogen-bonding sites designated H_1 and A_2. The steric map supports the existence of a lipophilic binding pocket as described.[6]

Recently, synthetic efforts have led to the design and successful synthesis of a series of pyrazoloisoquinoline derivatives as potential ligands at BzR. One of the principal aims of this investigation was to determine the importance of the quinoline NH functionality of the 2-phenyl-2,5-dihydropyrazolo[4,3-*c*]quinolin-3(3*H*)-ones **10–12** and was based on the topology of the pyrazoloquinolinone series reported by Yokoyama et al. in 1982.[9] The affinities of these new agents at BzR were measured in order to evaluate the proposed model[6] of the receptor pharmacophore.

Chemistry

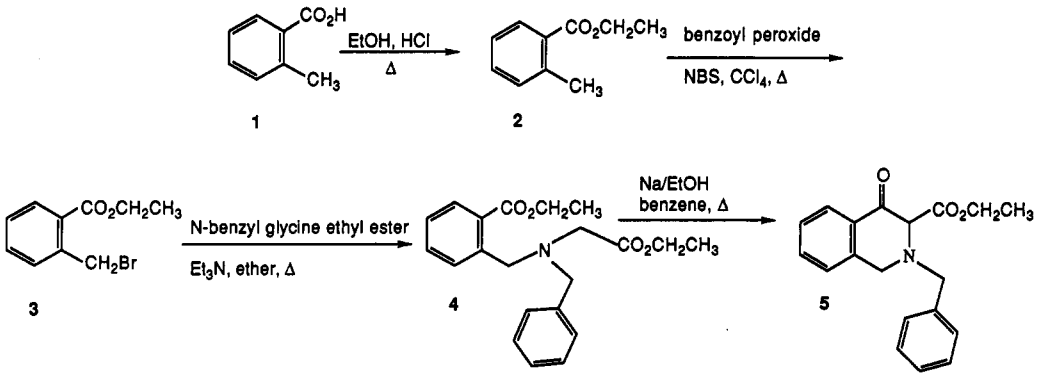
The synthesis of pyrazoloisoquinoline ligands **7a–d**, **8**, **9**, centered on the preparation of isoquinoline β-keto ester **5**,[10] analogous to the work of Hinton and Mann[11] as well as Grethe et al.[12] (Scheme I). In brief, *o*-toluic acid **1** was esterified under classical Fischer esterification conditions. The methyl function of ester **2** was converted into the bromide via a radical process (NBS, benzoyl peroxide, CCl_4), and this procedure furnished bromomethyl benzoate **3**. Alkylation of *N*-benzylglycine ethyl ester with **3** pro-

* To whom correspondence should be addressed.
† University of Wisconsin—Milwaukee.
‡ National Institute of Diabetes and Digestive and Kidney Diseases.

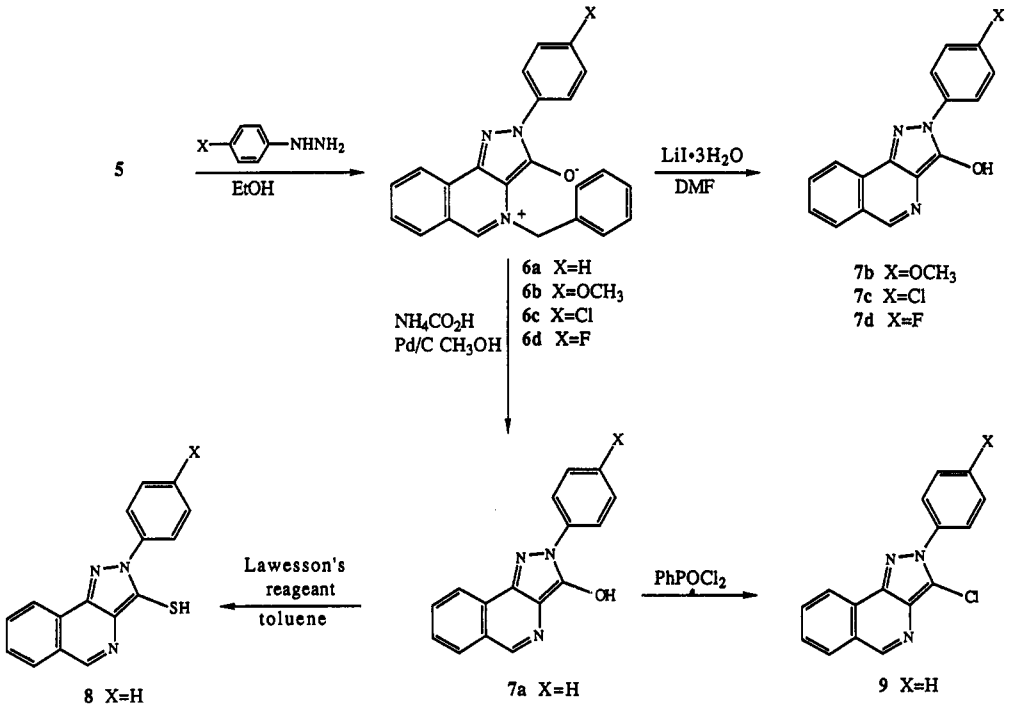
(1) Skolnick, P.; Paul, S. *GABA and Benzodiazepine Receptors*; Squires, R., Ed.; CRC Press: Boca Raton, FL, 1988; Vol. II, pp 91–102.
(2) Squires, R. F., Ed. *GABA and Benzodiazepine Receptors*; CRC Press: Boca Raton, FL, 1988; Vols. I and II.
(3) Allen, M. S.; Hagen, T. J.; Trudell, M. L.; Codding, P. W.; Skolnick, P.; Cook, J. M. Synthesis of Novel 3-Substituted β-Carbolines as Benzodiazepine Receptor Ligands: Probing the Benzodiazepine Receptor Pharmacophore. *J. Med. Chem.* **1988**, *31*, 1854–1861.
(4) Hollinshead, S. P.; Trudell, M. L.; Skolnick, P.; Cook, J. M. Structural Requirements for Agonist Actions at the Benzodiazepine Receptor: Studies with Analogues of 6-(Benzyloxy)-4-(methoxymethyl)-β-carboxylic Acid Ethyl Ester. *J. Med. Chem.* **1990**, *33*, 1062–1069.
(5) Trudell, M. L.; Lifer, S. L.; Tan, Y. C.; Martin, M. J.; Deng, L.; Skolnick, P.; Cook, J. M. Synthesis of Substituted 7,12-Dihydropyrido[3,2-b:5,4-b']diindoles: Receptor Ligands with Inverse Agonist/Antagonist Properties. *J. Med. Chem.* **1990**, *33*, 2412–2420.
(6) 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.; Skolnick, P.; Cook, J. M. Synthesis and Computer-Assisted Analyses of the Pharmacophore for the Benzodiazepine Receptor Inverse Agonist Site. *J. Med. Chem.* **1990**, *33*, 2343–2357.
(7) Diaz-Arauzo, H.; Evoniuk, G. E.; Skolnick, P.; Cook, J. M. The Agonist Pharmacophore of the Benzodiazepine Receptor. Synthesis of a Selective Anticonvulsant/Anxiolytic. *J. Med. Chem.* **1991**, *34*, 1754–1756.
(8) Diaz-Arauzo, H.; Koehler, K. F.; Hagen, T. J.; Evoniuk, G. E.; Skolnick, P.; Cook, J. M. Synthetic and Computer-Assisted Analyses of the Pharmacophore for Agonists at Benzodiazepine Receptors. *Life Sci.* **1991**, *49*, 207–216.
(9) Yokoyama, N.; Ritter, B.; Newbert, A. D. 2-Arylpyrazolo[4,3-*c*]quinolin-3-ones: Novel Agonists, Partial Agonists and Antagonists of Benzodiazepines. *J. Med. Chem.* **1982**, *25*, 337–339.
(10) Deng, L. M.S. Thesis, I. The Synthesis of 7,12-Dihydropyrido[3,2-b:5,4-b']diindole and Indolo[3,2-b]isoquinoline Ligands with Which to Study the Pharmacophore of the Benzodiazepine Receptor Inverse Agonist Site. II. The Study of *Trans* Diastereoselectivity in the Pictet-Spengler Reaction. University of Wisconsin—Milwaukee, 1990.
(11) Hinton, I. G.; Mann, F. G. Cyclic Keto-amines. Part IV. The Synthesis and Reactions of 1,2,3,4-Tetrahydro-2-methyl-4-oxoisoquinoline. *J. Chem. Soc.* **1959**, 599–608.
(12) Grethe, G.; Lee, H. L.; Uskokovic, M.; Brossi, A. Syntheses in the Isoquinoline Series. Synthesis and Chemical Transformation of 2,3-Dihydro-4(1H)-isoquinolones. *J. Org. Chem.* **1968**, *33*, 494–503.

0022-2623/92/1835-0368$03.00/0

Scheme I



Scheme II



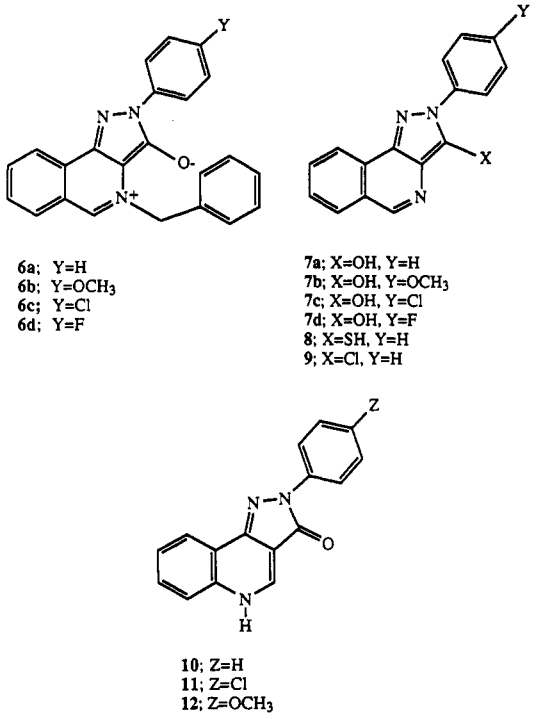
vided diester **4**, which was then subjected to Dieckmann condensation (Na/EtOH, benzene) to provide β-keto ester **5**. Formation of the *N*-benzylpyrazoloisoquinoline ligands **6a–d** was accomplished by reaction of β-keto ester **5** with the appropriately substituted *p*-phenylhydrazines (Scheme II). Examination of ligands **6a–d** by spectroscopy indicated that these materials were fully aromatic and existed in a zwitterionic form (see the Experimental Section for details). Removal of the *N*-benzyl function of the zwitterionic pyrazoloisoquinolines **6a–d** to furnish **7a–d** was achieved by three different methods including classical catalytic hydrogenation (Pd/C, H_2, methanol). The yields, however, from this process were generally poor. The method of catalytic transfer hydrogenation (ammonium formate, Pd/C, methanol) reported by Spatola et al.[13] was chosen to convert **6a** into **7a**. The yields of this procedure were shown to be superior, and reaction times were reduced in comparison to those of traditional catalytic hydrogenation. Since it is known that ammonium formate assisted catalytic transfer hydrogenolysis is an efficient method for the dehalogenation of aromatic halides,[14] an alternate route to **7c–d** was needed. During the course of this search a new synthetic method for the removal of quaternary *N*-benzyl functions was developed. This process involves the use of iodide anion in the form of lithium iodide to displace nucleophilically the benzyl group from the quaternary amine function. This generated the *p*-chloro and *p*-fluoro pyrazoloisoquinolines **7c–d**, respectively, and benzyl iodide as the byproduct. This iodide-mediated displacement/debenzylation reaction was executed in DMF and provided workable yields of **7c** (62%) and **7d** (64%). This procedure was also found to be superior for the debenzylation of the *p*-methoxy *N*-benzylpyrazoloisoquinoline **6b** to furnish **7b** (66%). In the latter case, however, catalytic hydrogenation and catalytic transfer hydrogenation were also effective.

The importance of the quinoline NH functionality of the 2-phenyl-2,5-dihydropyrazolo[4,3-c]quinolin-3(3*H*)-ones **10–12** (see Chart I) for high affinity binding to BzR was questioned. Conversion of the quinoline NH functionality into a hydrogen bond acceptor (N:) atom and simultaneous displacement of the nitrogen functionality one bond via the isoquinoline nucleus achieved this purpose. This

(13) Anwer, M. K.; Spatola, A. F. An Advantageous Method for the Rapid Removal of Hydrogenolysable Protecting Groups Under Ambient Conditions: Synthesis of Leucine-Enkephalin. *Synthesis* **1980**, 929–932.

(14) Anwer, M. K.; Spatola, A. F. Applications of Ammonium Formate Catalytic Transfer Hydrogenolysis: IV. A Facile Method for Dehalogenation of Aromatic Chlorocarbons. *Tetrahedron Lett.* **1985**, *26*, 1381–1384.

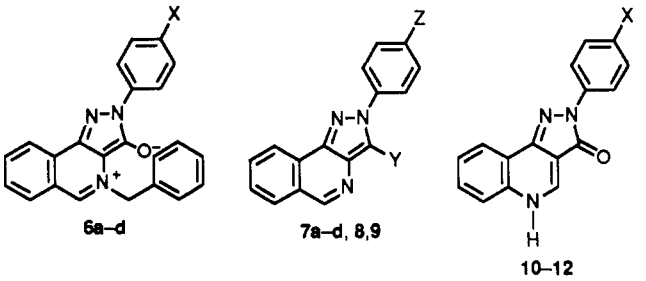
Chart I



provided the new class of pyrazoloisoquinoline ligands **7a–d**. Infrared spectroscopy of these ligands revealed that they existed in the enolic form. According to the proposed pharmacophore,[6] the electron density on the oxygen function of the pyrazoloquinoline nucleus provides an important hydrogen bond acceptor interaction with the receptor protein. Therefore, we sought to alter the hydrogen bond donor characteristics of **7a–d** by the conversion of enols **7a–d** into the corresponding chloro and sulfur analogues. This transformed the hydrogen bond donor characteristics of enol **7a** into a hydrogen bond acceptor unit as required. Evidently, the repulsive interaction between the two acidic hydrogens is greater than any attraction between the hydrogen bond donor site (H_1) and the oxygen atom of the hydroxyl group. However, treatment of enol **7a** with Lawesson's reagent[15] in toluene gave the thiol **8** rather than the desired thioketone tautomer (45% yield). On the other hand, reaction of enol **7a** with phenylphosphonic dichloride, according to the method of Chang et al.[16] furnished the desired chloro compound **9** (64%). The thioketone exists predominantly in the enethiol form[17] via a 5-centered hydrogen bond between the thiol hydrogen atom and the isoquinoline nitrogen function. Comparison of the infrared spectrum of **8** with that of 6-methoxy-8-mercaptoquinoline[18] supported the existence of this intramolecular association (H bond).[18] Synthesis of the chloropyrazoloisoquinoline **9** was invoked in order to replace the hydrogen bond donor (OH) functionality of the pyrazoloisoquinolin-3-ols with hydrogen bond acceptor functionality (:C̈l:) similar to that (C=Ö:) found in the original pyrazoloquinolinone series.[9]

(15) Pedersen, B. S.; Scheibye, S.; Nilsson, N. H.; Lawesson, S. O. Studies on Organophosphorus Compounds. XX. Syntheses of Thioketones. *Bull. Soc. Chim. Belg.* **1978**, *8*, 223–228.
(16) Chang, J. C.; El-Sheikh, M.; Harmon, A.; Avasthi, K.; Cook, J. M. Synthesis of 1,6-Diazaphenalene. *J. Org. Chem.* **1981**, *46*, 4188–4193.
(17) Duus, F.; Pedersen, B. S.; Lawesson, S. O. Studies on Enethiols. V. Syntheses of α-Thioacyllactones and α-Thioacylthiollactones. Structure Determination by NMR Spectroscopy. *Tetrahedron* **1969**, *25*, 5703–5720.
(18) Crampton, M. R. *The Chemistry of the Thiol Group, Part 1*; Patai, S., Ed.; John Wiley and Sons: New York, 1974; pp 379–415.

Table I. In Vitro Binding of 2-Phenyl-2*H*-pyrazolo[4,3-c]isoquinolines



no.	X	IC_{50}, nM	no.	Y	Z	IC_{50}, nM
6a	H	>2000	**7a**	OH	H	>2000
6b	OCH_3	>2000	**7b**	OH	OCH_3	>2000
6c	Cl	>2000	**7c**	OH	Cl	>2000
6d	F	>2000	**7d**	OH	F	>2000
10	H	0.4[a]	**8**	SH	H	>2000
11	Cl	0.6[a]	**9**	Cl	H	>2000
12	OCH_3	0.9[a]				

[a] See reference 9 for details.

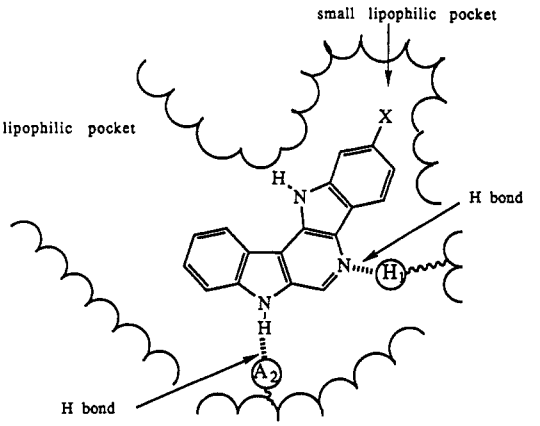


Figure 1. Proposed model of the benzodiazepine receptor inverse agonist/antagonist active site shown with the diindole **14** interacting at the hydrogen bond donor site (H_1) and hydrogen bond acceptor site (A_2) on the binding protein.

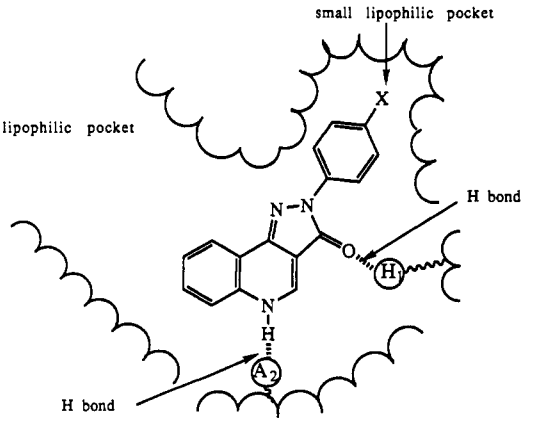


Figure 2. Interactions at the proposed inverse agonist/antagonist pharmacophore for the pyrazoloquinolinone analogues **10**, X = H; **11**, X = Cl; **12**, X = OCH_3.

Results and Discussion

The potencies of the substituted pyrazoloisoquinolines **6a–d**, **7a–d**, **8**, and **9** to inhibit [^{3}H]flunitrazepam binding to BzR are summarized in Table I. The isoquinoline analogues synthesized all exhibited low affinities, with IC_{50} values > 2 μM. These affinities are in marked contrast

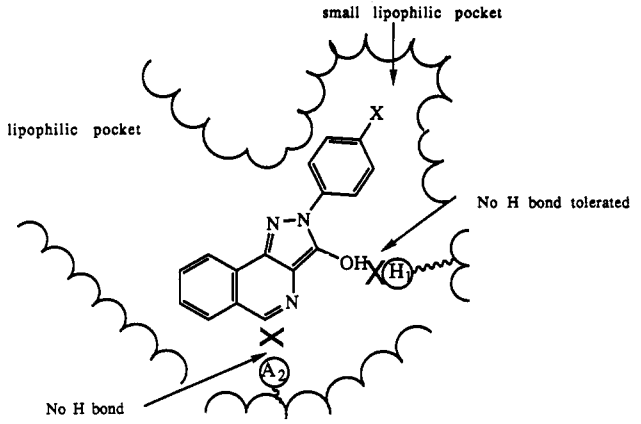


Figure 3. Interactions at the proposed inverse agonist/antagonist pharmacophore for the pyrazoloisoquinolin-3-ols **7a–d**.

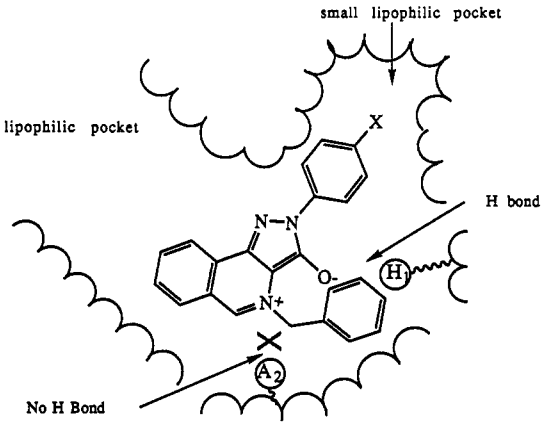


Figure 4. Interactions at the proposed inverse agonist/antagonist pharmacophore for the *N*-benzylpyrazoloisoquinolines **6a–d**.

to the high pM–low nM affinities of **10–12**. The dramatic reduction in affinity of the pyrazoloisoquinolines can be attributed to either the functional changes or the positional changes (quinoline → isoquinoline) inherent in these molecules and is in complete agreement with the recently proposed model for high affinity binding to BzR of inverse agonists/antagonists.[6] According to the model, a hydrogen bond acceptor site (A_2) on the receptor is proposed to interact with the N(7) hydrogen nuclei of the diindoles and the quinoline NH functionality of the pyrazoloquinolinones.[6] Furthermore, a proposed hydrogen bond donor site (H_1) on the BzR interacts with the N(5) nitrogen atom of the diindoles and the carbonyl oxygen atom of the pyrazoloquinolinones (see Figures 1 and 2), respectively. More importantly, it appears that ligand interaction with both sites is a prerequisite for high affinity binding of inverse agonists to BzR.[6] The present findings indicate that the topological dissimilarities are such that interaction with the two hydrogen bond donor and acceptor sites on the receptor are highly unlikely (see Figure 3). Thus, the *N*-benzyl derivatives **6a–d** all exhibit extremely low affinity for BzR ($IC_{50} > 2$ μM). The pyrazolo oxygen atom in this series **6a–d** could interact at H_1 of the receptor site; however, the benzyl group of **6a–d** presents a steric hindrance to any interaction at A_2 in the plane of the pyrazoloisoquinoline ring system, even if an NH function were present (see Figure 4). Consistent with a previous proposal,[3,6] the descriptors A_2 and H_1 are essential for high-affinity binding, since ligands **7a–d** also exhibited low affinity for BzR ($IC_{50} > 2$ μM). The isoquinoline nitrogen atom of pyrazoloisoquinolines **7a–d** represents a hydrogen bond acceptor site and would be incapable of hydrogen bonding to A_2 on the receptor protein. Moreover, the enol OH

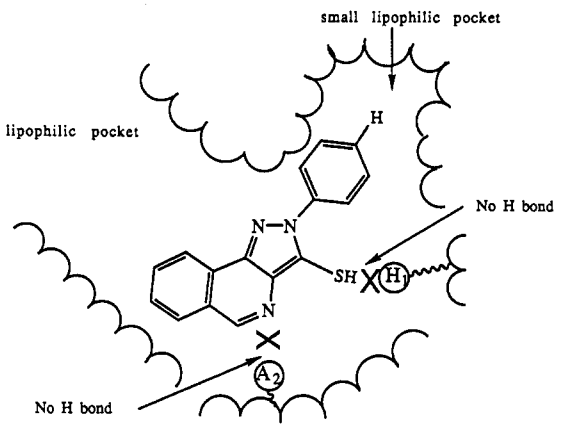


Figure 5. Interactions at the proposed inverse agonist/antagonist pharmacophore for the pyrazoloisoquinoline-3-thiol **8**.

function is now a hydrogen bond donor unit. According to the model (see Figure 1), a hydrogen bond acceptor atom is required at that location in order to hydrogen bond at site H_1 on the binding protein. Taken either together or separately, these two modifications resulted in the dramatic reduction in affinity associated with pyrazoloisoquinolinols **7a–d** compared with **10–12** (see Figure 3).

In contrast, it has been proposed that the enol tautomeric form of 2-(4-chlorophenyl)-2,5-dihydropyrazolo[4,3-*c*]quinolin-3(3*H*)-one (CGS-9896, **11**, IC_{50} = 0.6 nM) is bioactive.[19] This proposal was based solely upon spectroscopic and computational data and is inconsistent with the more recently formulated model.[3,6] This proposed enol tautomer[19] of **11** would have a topology very similar to pyrazoloisoquinolinol **7c**, which binds to BzR with an affinity more than 3 orders of magnitude lower than **11**. This suggests that the keto rather than the proposed enol form is the bioactive form.[4] In the keto form, the quinoline **11** is able to interact at both hydrogen bonding interaction sites A_2, H_1 of the pharmacophore,[3,6] whereas the enol tautomer cannot interact at either site, regardless of the direction of approach of the ligand to the binding site.

In order to alter the structural components of **7a** to accommodate the proposed hydrogen bond donating site (H_1) of the pharmacophore, the synthesis of the thioketone derivative **8** was carried out. Consequently, **8** was found to exist in the enethiol form (IR: SH, 2520 cm^{-1}) rather than as the desired thioketone tautomer. The low affinity of **8** ($IC_{50} > 2$ μM) is consistent with that of the pyrazoloisoquinolinol **7a** (Figure 5). The parent **7a** was then converted into the chloro derivative **9** ($IC_{50} > 2$ μM). The lone pair of electrons on the chlorine atom (hydrogen bond acceptor) is in the proper geometry to interact with the hydrogen bond donor site (H_1) on the binding protein. In spite of this interaction, a hydrogen bond interaction at A_2 is not possible with these isoquinolines, and high affinity binding of this ligand at BzR was not observed (see Figure 6). This result is not unexpected, since whenever one of the hydrogen bonds to A_2 or H_1 cannot be formed, a ligand with reduced affinity to the BzR was produced.[3,6] Recent examples of this include the [1]benzothieno[2,3-*c*]-pyridine-3-carboxylic acid esters,[20] the 9-substituted β-

(19) Villar, H. O.; Uyeno, E. T.; Toll, L.; Polgar, W.; Davies, M. F.; Loew, G. H. Molecular Determinants of Benzodiazepine Receptor Affinities and Anticonvulsant Activities. *Mol. Pharmacol.* **1989**, *36*, 589–600.

(20) Kawakubo, H.; Okazaki, K.; Nagatani, T.; Takao, K.; Hasimoto, S.; Sugihara, T. Potent Anticonflict Activity and Lessening of Memory Impairment with a Series of Novel [1] Benzothieno[2,3-c]pyridines and 1,2,3,4-Tetrahydro [1] Benzothieno[2,3-c]pyridines. *J. Med. Chem.* **1990**, *33*, 3110–3116.

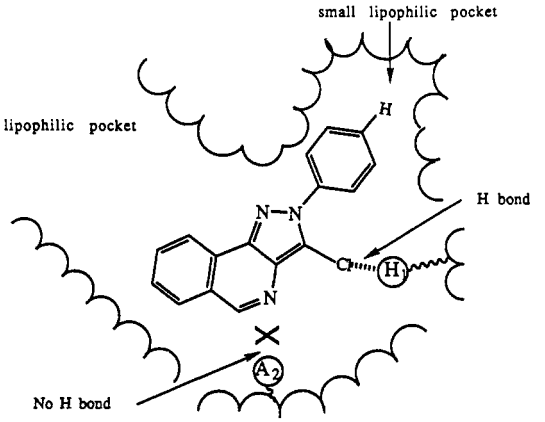
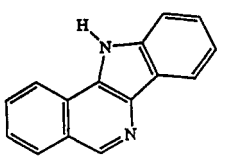
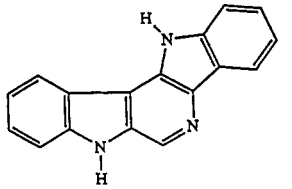


Figure 6. Interactions at the proposed inverse agonist/antagonist pharmacophore for the 3-chloropyrazoloisoquinoline **9**.

carbolines,[21] and various carbazoles and indolocarbazoles.[3] In addition, the indolo[3,2-*b*]isoquinoline **13** has been recently prepared and shown to be inactive ($IC_{50} \gg 2\ \mu M$) at BzR.[10] As reported,[3] the diindole **14** exhibits an IC_{50} of 4 nM, but simple deletion of the N(7)-H function has completely eliminated the affinity of this diindole to provide the inactive indolo[3,2-*b*]isoquinoline.[10]



13 (>>2,000 nM)



14 (4 nM)

Examination of these data clearly demonstrate that modification of the hydrogen bond donor or acceptor characteristics of the NH and carbonyl functionalities of the BzR active CGS-pyrazoloquinolinones **10–12** results in ligands (**7a–d**, **8**, **9**) with affinities more than 3 orders of magnitude lower than the original pyrazoloquinolinones (**10–12**). In addition, the low affinity of the chloropyrazolo derivative **9** ($IC_{50} > 2\ \mu M$) demonstrates that both hydrogen bond interactions are necessary for high affinity at BzR.[3,6,21] Even if the character of the isoquinoline nitrogen atom (N:, hydrogen bond acceptor) could be transformed into a hydrogen bond donor (NH) unit, it is unlikely that a high affinity BzR ligand would result. The newly proposed NH function would now be one bond length distant from that in **10–12** and 1.4 Å further removed from the hydrogen bond acceptor site (A_2). In summary, the low affinities of the pyrazoloisoquinolines **6a–d**, **7a–d**, **8**, and **9** are completely consistent with the previously proposed model[3,6] of the inverse agonist pharmacophore and support the involvement of the NH function of β-carbolines, diindoles, and pyrazoloquinolinones with a hydrogen bond acceptor site (A_2) on the receptor for inverse agonist activity. Moreover, this work confirms the involvement of the carbonyl function of the pyrazoloquinolinones or the pyridine nitrogen atom of β-carbolines [N(2)] and diindoles [N(7)] with a hydrogen bond donor site (H_1) at the binding site. This is also consistent with ligands that possess a carbonyl group at position 3 of β-carbolines, wherein the carbonyl group has been suggested to participate in a 3-centered hydrogen bond that involves the pyridine nitrogen atom.[3,22–24]

Experimental Section

Receptor Binding. [^{3}H]Flunitrazepam binding to rat cerebral cortical membranes was accomplished by using a modification of the previously described method.[25] In brief, rats were killed by decapitation and the cerebral cortex was removed. Tissue was disrupted in 100 volumes of Tris-HCl buffer (50 mM, pH 7.4) with a Polytron homogenizer (15 s, setting 6-7, Brinkmann Instruments, Westbury, NY) and centrifuged (4 °C) for 20 min at 20000*g*. Tissue was resuspended in an equal volume of buffer and recentrifuged. This procedure was repeated a total of three times, and the tissue was resuspended in 50 volumes of buffer. Triplicate incubations (1 mL) consisted of tissue (0.3 mL), drug solution (0.1 mL), buffer (0.5 mL), and radioligand (0.1 mL). Incubations (4 °C) were initiated by addition of [^{3}H]flunitrazepam (final concentration, ~1 nM; specific activity 81.8 Ci/mmol, DuPont-NEN, Boston, MA) and terminated after 120 min by rapid filtration through GF/B filters and washing with two 5-mL aliquots of ice-cold buffer using a Brandel M-24R filtering manifold. Nonspecific binding was determined by substituting nonradioactive flunitrazepam (final concentration, 10 μM) for the drug solution and represented <10% of the total binding. Specific binding was defined as the difference in binding obtained in the presence and absence of 10 μM flunitrazepam. Potencies were estimated using at least six concentrations (generally 1–10 000 nM) of inhibitor.

Melting points were taken on a Thomas-Hoover melting point apparatus or an Electrothermal Model IA 8100 digital melting point apparatus and are uncorrected. Proton NMR spectra were recorded on a Bruker 250-MHz NMR spectrometer or on a GE 500-MHz instrument. Infrared spectra were recorded with a Mattson Polaris IR-10400 or a Nicolet FTIR DX V5.07 spectrometer. Mass spectral data (EI/CI) were obtained on a Hewlett-Packard 5985 B GC–mass spectrometer, while high-resolution mass spectral data were obtained from a Finnigan HR mass spectrometer. Microanalyses were performed on a Perkin-Elmer 240C elemental analyzer. Analytical TLC plates employed were E. Merck Brinkman UV active silica gel (Kieselgel 60 F254) on plastic. The synthesis of **13**[10] will be reported elsewhere.[26] To prove that compounds **7a–d** do not exist in the zwitterionic form, additional ^{1}H NMR, FTIR, and TLC studies were carried out.

In the ^{1}H NMR (250 MHz), comparison of the proton absorption of the hydrogen singlet adjacent to the isoquinoline nitrogen atom in **6a** and **7a** (both free bases in deuterated dimethyl sulfoxide) showed a difference of 0.88 ppm (**7a**, 8.07 ppm vs **6a**, 8.95 ppm). As one might expect, the proton absorption of the hydrogen singlet in **6a** would be more deshielded adjacent to a quaternary nitrogen function than the proton absorption of the hydrogen singlet in **7a** adjacent to a tertiary nitrogen atom. Therefore, if compounds **7a–d** existed in the zwitterionic form, the proton absorption of the hydrogen singlet would be expected to be more in the range of 8.9–9.0 ppm. This is not the case. In addition, infrared spectra of **7a** (free base) in varying concentrations of tetrahydrofuran showed no shift in hydroxyl absorption

(21) Huth, A.; Schmiechen, R.; Motoc, I.; Beetz, I.; Brietkopf, A.; Frost, E.; Schumann, I.; Thielert, K. The 9-NH Group, an Essential Structural Fragment for High Affinity of β-Carboline Esters to the Benzodiazepine Receptor. *Arch. Pharm. (Weinheim)* **1988**, *321*, 297–301.

(22) Loew, G. H.; Nienow, J. R.; Paulson, M. Theoretical Structure Activity Studies of Benzodiazepine Analogues: Requirements for Receptor Affinity and Activity. *Mol. Pharmacol.* **1984**, *26*, 19–34.

(23) Loew, G. H.; Nienow, J.; Lawson, J. A.; Toll, L.; Uyeno, E. T. Theoretical Structure–Activity Studies of β-Carboline Analogs: Requirements for Benzodiazepine Receptor Affinity and Antagonist Activity. *Mol. Pharmacol.* **1985**, *28*, 17–31.

(24) Codding, P. W.; Roszak, A. W.; Szkaradzinska, M. B.; Cook, J. M.; Aha, L. J. Modeling the Benzodiazepine Receptor Using Structural and Theoretical Characterization of Novel β-Carbolines. *Trends in Medicinal Chemistry '88*; Van der Goot, H., Domany, G., Pallos, L., Timmerman, H., Eds.; Elsevier Science: Amsterdam, 1989; pp 109–120.

(25) Trudell, M. L.; Basile, A. S.; Shannon, H. E.; Skolnick, P.; Cook, J. M. Synthesis of 7,12-dihydropyrido[3,4-b:5,4-b']diindoles. A Novel Class of Rigid, Planar Benzodiazepine Receptor Ligands. *J. Med. Chem.* **1987**, *30*, 456–458.

(26) Allen, M. S.; Deng, L.; Dorn, L. J.; Laloggia, A. J.; Skolnick, P.; Cook, J. M., manuscript in preparation.

(3564 cm^{-1}). This information is consistent with the presence of an intramolecular hydrogen bond and not the existence of a zwitterionic molecule. Moreover, from thin-layer chromatography, the R_f value of **6a** (0.2 ethyl acetate) is lower than that of **7a** (0.4 ethyl acetate). This clearly attests to the zwitterionic polar nature of **6a**, but not of **7a**.

2-Benzyl-3-carbethoxy-2,3-dihydro-4(1*H*)-isoquinolone Hydrochloride (5). The diester **4** (133.8 g, 0.38 mol) was dissolved in anhydrous benzene (500 mL). The solution which resulted was added dropwise to a solution of sodium ethoxide (9.4 g Na, 0.41 mol, anhydrous ethanol 75 mL) in anhydrous benzene (300 mL). The addition was carried out under nitrogen. After the addition of **4** was completed, the clear solution which formed was heated to 100 °C, and a benzene–ethanol azeotrope (100 mL) was distilled off. An additional portion of anhydrous benzene (100 mL) was added to the solution, and heating was continued for 6 h. The mixture was cooled to room temperature, and water (800 mL) was added to the solution, after which concentrated hydrochloric acid was added dropwise until the aqueous layer was brought to pH 4. The organic layer which remained was separated from the medium, and the aqueous layer was extracted with diethyl ether (3 × 400 mL). The combined ethereal solution was washed with water (1 × 800 mL) and dried (Na_2SO_4), and the solvent was removed under reduced pressure. The residual oil was dissolved in a saturated solution of ethanol–hydrogen chloride. Upon addition of diethyl ether, the β-keto ester **5** crystallized. The product was filtered, washed with ether (3 × 25 mL), and dried to provide pure **5** (110 g, 84%): mp 126–127 °C; IR (KBr) 1754, 1692, 1602, 1397, 1276 cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 1.01 (t, 3 H, J = 7.1 Hz), 4.15 (q, 2 H, J = 7.1 Hz), 5.98 (s, 2 H), 7.26–7.40 (m, 5 H), 8.11 (t, 1 H, J = 8.1 Hz), 8.23 (t, 1 H, J = 8.3 Hz), 8.46 (d, 1 H, J = 7.9 Hz), 8.63 (d, 1 H, J = 8.3 Hz), 9.83 (s, 1 H); MS (CI, CH_4) 310 (M + 1). Anal. ($C_{19}H_{19}NO_3$·HCl) C, H, N.

4-Benzyl-3-hydroxy-2-phenyl-2*H*-pyrazolo[4,3-*c*]isoquinolinium Hydroxide, Inner Salt (6a). To a stirred solution of the isoquinoline β-keto ester **5** (2.0 g, 5.8 mmol) in anhydrous ethanol (20 mL) was added phenylhydrazine (5.7 mL, 58 mmol). The mixture which resulted was stirred at reflux for 12 h, after which ether (20 mL) was added. The precipitate which formed was filtered, washed with ether (3 × 10 mL), and dried to yield **6a** (1.74 g, 85%): mp 235–238 °C; IR (KBr) 3400, 1615, 1555, 1460, 1420, 1375 cm^{-1}; ^1H NMR (Me_2SO-d_6, D_2O) δ 6.12 (s, 2 H), 7.20 (t, 1 H, J = 7.4 Hz), 7.32–7.47 (m, 5 H), 7.62 (d, 2 H, J = 7.6 Hz), 7.74 (t, 1 H, J = 7.3 Hz), 7.95 (t, 1 H, J = 7.3 Hz), 8.12 (d, 1 H, J = 8.1 Hz), 8.17 (d, 2 H, J = 8.3 Hz), 8.33 (d, 1 H, J = 8.1 Hz), 8.87 (s, 1 H); MS (CI, CH_4) 352 (M + 1); high-resolution MS m/e 351.1385 ($C_{23}H_{17}N_3O$ requires 351.1372). Anal. ($C_{23}H_{17}N_3O$) C, H, N.

4-Benzyl-3-hydroxy-2-(*p*-methoxyphenyl)-2*H*-pyrazolo[4,3-*c*]isoquinolinium Hydroxide, Inner Salt (6b). The 4-methoxyphenylhydrazine hydrochloride (3.0 g, 17.2 mmol) was added to a saturated solution of sodium carbonate (25 mL), stirred for 5 min, and then extracted with chloroform (3 × 10 mL). The combined organic extracts were dried (Na_2SO_4), and the solvent was removed in vacuo. To the oil which formed were added the β-keto ester **5** (1.0 g, 2.9 mmol) and anhydrous ethanol (12 mL). The mixture which resulted was stirred at reflux for 12 h, after which time ether (15 mL) was added. The precipitate which resulted was filtered, washed with ether (3 × 5 mL), and dried to provide **6b** (605 mg, 55%): mp 216–217 °C; IR (KBr) 1611, 1506, 1453, 1409, 1241, 826, 740, 702 cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 3.78 (s, 3 H), 6.18 (s, 2 H), 7.01 (d, 2 H, J = 9.2 Hz), 7.38 (m, 3 H), 7.72 (m, 3 H), 7.92 (t, 1 H, J = 7.2 Hz), 8.12 (d, 1 H, J = 7.9 Hz), 8.20 (d, 2 H, J = 9.1 Hz), 8.32 (d, 1 H, J = 7.9 Hz), 8.88 (s, 1 H); MS (CI, CH_4) 382 (M + 1); high-resolution MS m/e 381.1465 ($C_{24}H_{19}N_3O_2$ requires 381.1477). Anal. ($C_{24}H_{19}N_3O_2$) C, H, N.

4-Benzyl-2-(*p*-chlorophenyl)-3-hydroxy-2*H*-pyrazolo[4,3-*c*]isoquinolinium Hydroxide, Inner Salt (6c). The 4-chlorophenylhydrazine hydrochloride (5.0 g, 28 mmol) was added to a saturated solution of sodium carbonate (30 mL) and stirred for 5 min. The aqueous solution was extracted with chloroform (3 × 100 mL). The combined organic layers were dried over sodium sulfate, and the solvent was removed in vacuo. To the oil which resulted were added the isoquinoline β-keto ester **5** (1.0 g, 2.9 mmol) and anhydrous ethanol (15 mL). The mixture which formed was then stirred at reflux for 24 h, after which ether (20 mL) was added. The precipitate which resulted was filtered, washed with ether (3 × 5 mL), and dried to yield **6c** (799 mg, 72%): mp 252–257 °C; IR (KBr) 1611, 1528, 1487, 1454, 1403, 1325, 1086, 829, 740, 701 cm^{-1}; ^1H NMR (Me_2SO-d_6, D_2O) δ 6.11 (s, 2 H), 7.33–7.40 (m, 3 H), 7.46 (d, 2 H, J = 8.9 Hz), 7.61 (d, 2 H, J = 6.2 Hz), 7.75 (t, 1 H, J = 7.4 Hz), 7.96 (t, 1 H, J = 7.3 Hz), 8.12 (d, 1 H, J = 8.0 Hz), 8.24 (d, 2 H, J = 8.2 Hz), 8.32 (d, 1 H, J = 8.0 Hz), 8.88 (s, 1 H); MS (CI, CH_4), 386 (M + 1); high-resolution MS m/e 385.0967 ($C_{23}H_{16}N_3OCl$ requires 385.0982). Anal. ($C_{23}H_{16}N_3OCl$) C, H, N.

4-Benzyl-2-(*p*-fluorophenyl)-3-hydroxy-2*H*-pyrazolo[4,3-*c*]isoquinolinium Hydroxide, Inner Salt (6d). The 4-fluorophenylhydrazine hydrochloride (4.71 g, 28.5 mmol) was added to a saturated solution of sodium carbonate (30 mL) and stirred for 5 min. The aqueous solution was extracted with chloroform (3 × 100 mL). The combined organic layers were dried (Na_2SO_4), and the solvent was removed in vacuo. To the oil which resulted was added the isoquinoline β-keto ester **5** (700 mg, 2.03 mmol) and anhydrous ethanol (15 mL). The mixture which resulted was then stirred at reflux for 8 h, after which ether (20 mL) was added. The precipitate which formed was filtered, washed with ether (3 × 5 mL), and dried to yield **6d** (615 mg, 82%): mp 218–219 °C; IR (KBr) 1613, 1528, 1503, 1453, 1403, 1328, 1206, 828, 703 cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 6.17 (s, 2 H), 7.24–7.43 (m, 5 H), 7.67 (d, 2 H, J = 7.6 Hz), 7.75 (t, 1 H, J = 8.2 Hz), 7.95 (t, 1 H, J = 8.2 Hz), 8.13 (d, 1 H, J = 8.1 Hz), 8.31–8.37 (m, 3 H), 8.93 (s, 1 H); MS (CI, CH_4) 370 (M + 1); high-resolution MS m/e 369.1276 ($C_{23}H_{16}N_3OF$ requires 369.1277). Anal. ($C_{23}H_{16}N_3OF$) C, H, N.

2-Phenyl-2*H*-pyrazolo[4,3-*c*]isoquinolin-3-ol Hydrochloride (7a). To a stirred solution of the isoquinolinium hydroxide inner salt **6a** (100 mg, 0.285 mmol) and ammonium formate (400 mg, 6.34 mmol) in anhydrous methanol (100 mL) was added 10% Pd/C (112 mg). The solution which resulted was stirred at room temperature for 8 h, after which the reaction solution was filtered through Celite. The filtrate was evaporated to dryness. Excess ammonium formate was removed when the residue was taken up in ethyl acetate (100 mL) and washed with a saturated solution of sodium chloride (2 × 50 mL). The organic layer was dried over sodium sulfate and evaporated under reduced pressure to yield the free base **7a** (60 mg, 81%). Upon the addition of a cold saturated solution of methanol–hydrogen chloride to the free base **7a** in methanol, a precipitate formed which was filtered and washed with cold ether (3 × 10 mL) to provide **7a** as the hydrochloride salt: mp 200–203 °C; IR (KBr) 3600–3300 (broad), 1684, 1633, 1488, 1417, 1382 cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 7.32 (t, 1 H, J = 7.4 Hz), 7.55 (t, 2 H, J = 7.5 Hz), 7.85–8.05 (m, 4 H), 8.27 (d, 1 H, J = 7.9 Hz), 8.35 (d, 1 H, J = 7.9 Hz), 9.05 (s, 1 H); MS (CI, CH_4) 262 (M + 1); high-resolution MS m/e 261.0909 ($C_{16}H_{11}N_3O$ requires 261.0902). Anal. ($C_{16}H_{11}N_3O$·HCl) C, H, N.

2-(*p*-Methoxyphenyl)-2*H*-pyrazolo[4,3-*c*]isoquinolin-3-ol Hydrochloride (7b). (*p*-Methoxyphenyl)isoquinolinium hydroxide **6b** (100 mg, 0.262 mmol) and lithium iodide trihydrate (175 mg, 0.93 mmol, 3.55 equiv) were added to dimethylformamide (10 mL). The mixture which resulted was brought to reflux and stirred for 72 h, after which the dimethylformamide was removed by Kugelrohr distillation. The residue which remained was dissolved in chloroform (150 mL), washed with 5% $Na_2S_2O_3$ (3 × 100 mL), and dried (Na_2SO_4). The solvent was then evaporated under reduced pressure. Upon addition of ether (15 mL), a precipitate formed which was filtered, washed with ether (3 × 5 mL), and dried to provide free base **7b** (50 mg, 66%). Formation of the hydrochloride salt was accomplished by addition of a cold saturated solution of methanol–hydrogen chloride to the free base **7b** dissolved in methanol: mp 230 °C dec; IR (KBr) 1692, 1652, 1635, 1511, 1432, 1251, 935 cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 3.81 (s, 3 H), 7.12 (d, 2 H, J = 9.1 Hz), 7.86 (m, 3 H), 7.97 (t, 1 H, J = 7.5 Hz), 8.28 (d, 1 H, J = 7.8 Hz), 8.35 (d, 1 H, J = 7.8 Hz), 9.04 (s, 1 H); MS (CI, CH_4) 292 (M + 1); high-resolution MS m/e 291.0995 ($C_{17}H_{13}N_3O_2$) requires 291.1008. Anal. ($C_{17}H_{13}N_3O_2$) C, H, N.

2-(*p*-Chlorophenyl)-2*H*-pyrazolo[4,3-*c*]isoquinolin-3-ol Hydrochloride (7c). (*p*-Chlorophenyl)isoquinolinium hydroxide

6c (567 mg, 1.47 mmol) and lithium iodide trihydrate (8.62 g, 46 mmol) were added to dimethylformamide (15 mL). The mixture which resulted was brought to reflux and stirred for 72 h, after which the DMF was removed by Kugelrohr distillation. The residue which remained was dissolved in chloroform (250 mL), washed with 5% $Na_2S_2O_3$ (7 × 100 mL), and dried (Na_2SO_4). The solvent was then evaporated under reduced pressure, and ether (25 mL) was added. The precipitate which resulted was filtered, washed with ether (3 × 5 mL), and dried to yield the free base of **7c** (270 mg, 62%). Formation of the hydrochloride salt was accomplished with the addition of a cold saturated solution of methanol–hydrogen chloride to the free base in methanol: mp 250–252 °C; IR (KBr) 1651, 1644, 1633, 1488, 1424, 1383, 1318, 832, 756 cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 7.62 (d, 2 H, J = 9.0 Hz), 7.88 (t, 1 H, J = 8.0 Hz), 7.98 (t, 1 H, J = 8.2 Hz), 8.06 (d, 2 H, J = 9.0 Hz), 8.28 (d, 1 H, J = 7.9 Hz), 8.36 (d, 1 H, J = 7.7 Hz), 9.03 (s, 1 H); MS (CI, CH_4) 296 (M + 1); high-resolution MS m/e 295.0495 ($C_{16}H_{10}N_3OCl$ requires 295.0512). Anal. ($C_{16}H_{10}N_3OCl$) C, H, N.

2-(*p*-Fluorophenyl)-2*H*-pyrazolo[4,3-*c*]isoquinolin-3-ol Hydrochloride (7d). (*p*-Fluorophenyl)isoquinolinium hydroxide **6d** (100 mg, 0.271 mmol) and lithium iodide trihydrate (3.5 g, 18.7 mmol) were added to dimethylformamide (10 mL). The mixture which resulted was brought to reflux and stirred for 48 h, after which the DMF was removed by Kugelrohr distillation. The residue which remained was taken up in chloroform (50 mL), washed with 5% $Na_2S_2O_3$ (4 × 100 mL), and dried over sodium sulfate. The solvent was then removed under reduced pressure to yield an oil which was solidified upon the addition of ether. The solid was filtered, washed with ether (3 × 5 mL), and dried to provide the isoquinoline free base **7d** (49 mg, 64%). Upon the addition of a cold saturated solution of methanol–hydrogen chloride to the free base **7d** in methanol, the hydrochloride salt **7d** was isolated: mp 255–259 °C; IR (KBr) free base 1617, 1583, 1502, 1432, 1382, 1209, 1080, 836 cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 7.40 (t, 2 H, J = 8.9 Hz), 7.87 (t, 1 H, J = 7.5 Hz), 7.98 (t, 1 H, J = 8.1 Hz), 8.03 (dd, 2 H, J = 9.1 Hz, 4.9 Hz), 8.28 (d, 1 H, J = 7.6 Hz), 8.35 (d, 1 H, J = 7.8 Hz), 9.04 (s, 1 H); MS (CI, CH_4) 280 (M + 1); high-resolution MS m/e 279.0818 ($C_{16}H_{10}N_3OF$ requires 279.0808). Anal. ($C_{16}H_{10}N_3OF$·HCl) C, H, N.

2-Phenyl-2*H*-pyrazolo[4,3-*c*]isoquinoline-3-thiol (8). To a stirred solution of anhydrous toluene (10 mL) and Lawesson's reagent (41 mg, 0.10 mmol) was added pyrazoloisoquinolin-3-ol hydrochloride **7a** (50 mg, 0.17 mmol). The mixture which resulted was brought to 110 °C under nitrogen with stirring. After 4 h the solution was cooled to room temperature, and the toluene was removed under reduced pressure. The residue was then purified by flash chromatography (SiO_2) with chloroform as the eluant to provide pure **8** (21 mg, 45%): mp 220 °C dec; IR (KBr) 2520, 1620, 1592, 1494, 1388, 1260, 1096, 1020, 801 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.55 (s, 1 H), 7.14 (m, 3 H), 7.32 (d, 2 H, J = 8.1 Hz), 7.68 (t, 1 H, J = 8.6 Hz), 7.83 (t, 1 H, J = 8.0 Hz), 7.91 (d, 1 H, J = 7.9 Hz), 8.51 (d, 1 H, J = 8.1 Hz), 8.78 (s, 1 H); EIMS m/z 277 (M^+); high-resolution MS m/e 277.0674 ($C_{16}H_{11}N_3S$ requires 277.0674). The title compound **8** was shown to be homogeneous by TLC on silica gel (R_f = 0.11; ethyl acetate).

3-Chloro-2-phenyl-2*H*-pyrazolo[4,3-*c*]isoquinoline (9). 2-Phenyl-2*H*-pyrazolo[4,3-*c*]isoquinolin-3-ol hydrochloride **7a** (60 mg, 0.200 mmol) was dissolved in phenylphosphonic dichloride (7 mL). The resulting solution was stirred and warmed to 90 °C. After 30 min the temperature was increased to 125 °C for 2 h, after which the cooled reaction mixture was poured over ice water (50 mL). The resulting solution was basified to pH 8.5 with saturated aqueous Na_2CO_3, followed by extraction with chloroform (3 × 50 mL). The combined organic extracts were dried (Na_2SO_4), and the solvent was removed under reduced pressure to yield the free base **9** (36 mg, 64%): mp 185–186 °C; IR (KBr) 1596, 1560, 1499, 1475, 1387, 754, 690, 574 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.55 (m, 3 H), 7.73 (m, 3 H), 7.83 (t, 1 H, J = 8.0 Hz), 8.03 (d, 1 H, J = 7.7 Hz), 8.55 (d, 1 H, J = 7.4 Hz), 8.99 (s, 1 H); EIMS m/z 279 (M^+), 244 (M^+ - Cl); high-resolution MS m/e 279.0563 ($C_{16}H_{10}N_3Cl$ requires 279.0563). The title compound **9** was shown to be homogeneous by TLC on silica gel [R_f 0.22; $CHCl_3$ (55%), hexane (45%)].

Acknowledgment. We wish to thank Jim Laloggia for excellent technical assistance and the NIMH (MH 36644) for generous financial support.

Registry No. **4**, 137695-87-5; **5**, 53726-69-5; **6a**, 137695-77-3; **6b**, 137695-78-4; **6c**, 137695-79-5; **6d**, 137695-80-8; **97a**, 137695-81-9; **7b**, 137695-82-0; **7c**, 137695-83-1; **7d**, 137695-84-2; **8**, 137695-86-4; **9**, 137695-85-3; **10**, 77779-60-3; **11**, 77779-36-3; **12**, 77779-50-1; $PhNHNH_2$, 100-63-0; p-$MeOC_6H_4NHNH_2$, 3471-32-7; p-$ClC_6H_4NHNH_2$, 1073-69-4; p-$FC_6H_4NHNH_2$, 371-14-2; LiI, 10377-51-2; $PhPOCl_2$, 824-72-6; flunitrazepam-*t*, 80573-68-8; flunitrazepam, 1622-62-4.

Synthesis and Substance P Receptor Binding Activity of Androstano[3,2-*b*]pyrimido[1,2-*a*]benzimidazoles

Bhaskar R. Venepalli,* Lisa D. Aimone,† Kenneth C. Appell, Malcolm R. Bell,† John A. Dority,† Ramanuj Goswami, Patricia L. Hall, Virendra Kumar,† Kristine B. Lawrence, Margaret E. Logan, Patricia M. Scensny, Judith A. Seelye, Bruce E. Tomczuk, and John M. Yanni

Life Sciences Research Laboratories, Eastman Kodak Company, Rochester, New York 14650-2158, and Sterling Research Group, Rensselaer, New York 12144. *Received July 11, 1991*

Several heterosteroids containing a dihydroethisterone skeleton were prepared and shown to displace substance P in a receptor binding assay. Further biochemical (kinetic and Scatchard analyses) and pharmacological evaluation (substance P-induced plasma extravasation and salivation in the rat) of a representative example in this series (**5a**) established that these compounds are competitive antagonists at the substance P receptor.

Introduction

Substance P (Figure 1) is an undecapeptide that belongs to a family of neurotransmitters known as neurokinins that includes the structurally related neurokinin A (NKA) and neurokinin B (NKB).[1] Based on the relative potencies of these agonists, three neurokinin receptors, generally referred as NK-1, NK-2, and NK-3, have been proposed. Recently three NK receptors have been cloned and sequenced,[2,3] validating this classification. Substance P (SP),

* To whom correspondence should be addressed at Eastman Fine Chemicals, Eastman Kodak Company, 1001 Lee Road, Rochester, NY, 14652-3512.

† Sterling Research Group.

(1) Burcher, E. The Study of Tachykinin Receptors. *Clin. Exp. Pharmacol. Physiol.* **1989**, *16*, 539–543.

(2) Shigemoto, R.; Yokota, Y.; Tsuchida, K.; Nakanishi, S. Cloning and Expression of a Rat Neuromedin K Receptor cDNA. *J. Biol. Chem.* **1990**, *265*, 623–628.

(3) Hershey, A. D.; Krause, J. E. Molecular Characterization of a Functional cDNA Encoding the Rat Substance P Receptor. *Science* **1990**, *247*, 958–962.

0022-2623/92/1835-0374$03.00/0