

BIOMIMETIC APPROACH TO POTENTIAL BENZODIAZEPINE AGONISTS AND ANTAGONISTS

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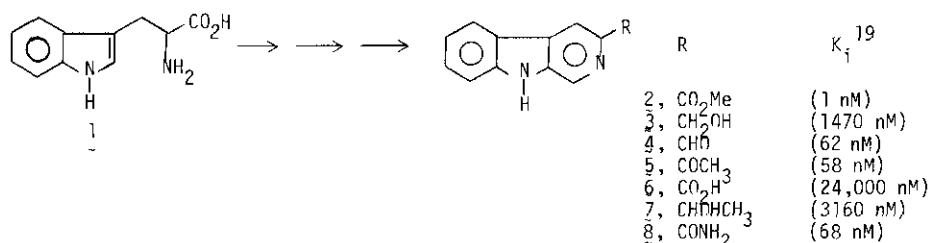
Abstract--A series of heterocycles have been prepared via the Pictet-Spengler reaction and several of these (β -carboline 2-8, isoquinoline 11a and imidazopyridine derivative 16b) have been found to bind to the benzodiazepine receptor in vitro with moderate to high affinity.

The discovery of high affinity, saturable and stereospecific binding sites for the benzodiazepines has prompted an intensive search for endogenous ligands for this receptor.^{1,2} Since 3-substituted β -carboline such as 3-carboethoxy- β -carboline or a closely related derivative^{3a} have been proposed to be endogenous ligands of the benzodiazepine receptor, we have prepared a series of β -carboline and tested their abilities to bind to brain benzodiazepine receptors in vitro. These studies suggest that in the β -carboline series, a carbonyl moiety markedly augments affinity for the receptor. The syntheses of compounds 2-7 employed for this study have been described elsewhere.^{3b}

Recently, several reports⁴ have suggested that endogenous peptide-like materials displace [³H]-benzodiazepines from receptor sites with a relatively high affinity. Consequently, the effect of a 3-carboxamide moiety on the activity of β -carboline has been determined. The carboxamide 8⁵ was prepared by treating a methanol solution of 2 with dry ammonia, moreover, this amide 8 did indeed bind tightly to the receptor ($K_i=68$ nM). The related alcohols 3 and 7 both demonstrated weaker binding with K_i 's of 1470 and 3160 nM, respectively, which again indicates that the carbonyl is extremely important for binding of β -carboline to the diazepam receptor(s). Pandit⁶ has shown that tetrahydro- β -carboline can be synthesized, in a

biomimetic sense, from N⁵, N¹⁰-methylene tetrahydrofolate models which suggests the biosynthetic machinery for synthesis of the C-1 unit of β -carbolines may be present *in vivo*. One can envisage the postulated origin of 3-carboxy- β -carbolines *in vivo* to be derived from tryptophan and a C-1 unit, moreover] need not be considered the only amino acid involved in such a process. Other acids such as phenylalanine, tyrosine, dopa and histidine might well con-

Scheme I

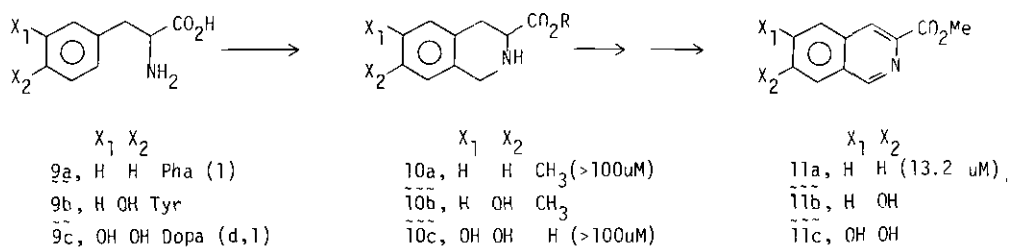


dense *in vivo* with a C-1 unit to provide planar, 3-carboxy-substituted systems similar to β -carbolines.³ In fact, Barker *et al.* have recently reported the isolation of 6,7-dimethoxy-tetrahydroisoquinoline and 6,7-dihydroxytetrahydroisoquinoline from mammalian systems;⁷ a result which adds impetus to the present investigation. Our studies in this area are illustrated in Schemes II and III.

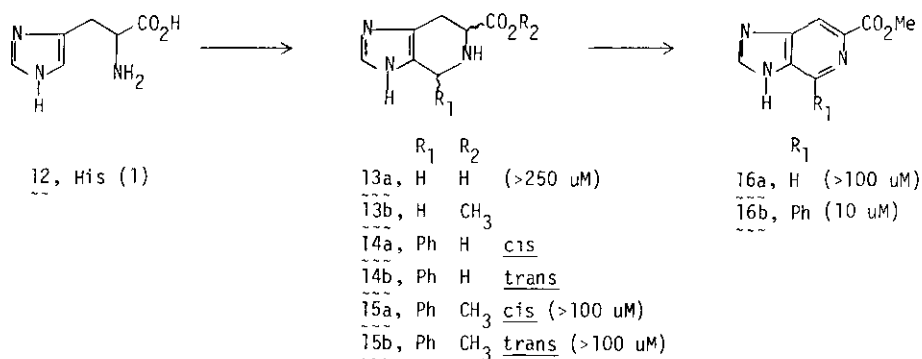
Phenylalanine 9a was converted to the tetrahydroisoquinoline 10a by way of a Pictet-Spengler reaction with formaldehyde,⁸ followed by esterification of the resulting acid with methanolic hydrogen chloride. The desired 3-methoxycarbonylisoquinoline 11a⁹ was obtained by heating 10a with palladium on carbon in xylene. In the histidine series the intermediate, spinacine (monohydrochloride) 13a¹⁰ was synthesized in 90% yield by heating an aqueous solution of the monohydrochloride salt of 12 with formaldehyde. Treatment of 13a with methanolic hydrogen chloride provided the desired ester 13b, which was converted to 16a¹¹ on heating for fifteen minutes with selenium dioxide in acetic acid. The l-phenyl derivatives 14a¹² and 14b¹³ were prepared by the method of Wille¹⁴ via a base-catalyzed Pictet-Spengler reaction of histidine 12 with benzaldehyde, and were then converted to the corresponding esters 15a¹⁵ and 15b,¹⁶ respectively on heating in methanolic hydrogen chloride solution. The stereochemistry of the two diastereomers 15a and 15b was assigned by C-13 spectroscopy; a method previously employed in our laboratory under similar circumstances in the tetrahydro- β -carboline series.¹⁷ The signals for carbon-1 and carbon-3 in the *trans* diastereomer 15b (53.83 and 51.98 ppm) were upfield from those of the *cis* isomer 15a (57.37 and 56.06 ppm) as expected.¹⁷ The fully aromatic target 16b¹⁸ was obtained by heating 15b with selenium dioxide in acetic acid (see Scheme III).

The binding affinities of the isoquinolines and imidazopyridine derivatives determined to date are shown in parentheses in Schemes II and III. While the tetrahydro derivatives 10a, 10c, 13a, 15a, and 15b were virtually inactive as expected,³ the planar, fully aromatic isoquinoline 11a and the 4-phenylimidazopyridine derivative 16b demonstrated moderate activity

Scheme II¹⁹



Scheme III¹⁹



at 13.2 μ M and 10 μ M, respectively. It is noteworthy that the binding affinities of 11a and 16b are quite similar to that of the β -carboline, harmane; a molecule proposed as a putative endogenous ligand for the benzodiazepine receptor by Rommelspacher *et al.*²⁰ However, neither the potency, concentration, nor neuroanatomic localization of harmane in mammalian brain support this hypothesis (see reference 3 for details), nonetheless β -carbolines did in general bind with a higher affinity, as shown in Scheme I

The *in vitro* activity of the isoquinoline 11a and the 4-phenylimidazopyridine derivative 16b is interesting from a structure-activity standpoint, as well as in a biomimetic sense. The fact that amino acids other than tryptophan 1 can be condensed with a C-1 unit and later converted to 3-carboxy-substituted heterocycles which bind to the receptor provides chemical proof that such a process is possible *in vitro*. Moreover, the recent work of Pandit⁶ and Barker *et al.*⁷ (isoquinoline area) provides tangible evidence that similar transformations are also possible *in vivo*. In addition, successful extrapolation of structure-activity relationships in the β -carboline area^{3,21} to other heterocycles such as 11a and 16b has been

accomplished and the activities of such compounds *in vitro* and *in vivo* may help to shed light on the structure of the endogenous ligand for the benzodiazapine receptor(s).

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5. 8 (50% yield): mp 297-304°C; ir (KBr) 3450 (broad), 3200 (broad), 1680, 1645 cm^{-1} ; nmr ($\text{Me}_2\text{SO}-d_6$) δ 7.00-7.70 (M, 5H), 7.80-8.00 (m, 1H), 8.20 (doublet of doublets, J=7Hz, 1Hz, 1H), 8.72 (s, 1H), 8.75 (s, 1H); Mass spectrum (C.I., CH_4) 212 (M+1, 100).
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9. Preparation of compounds 10a, 10c and 11a have been described in reference 3.
10. 13a: mp 285°C, lit. mp 276°C; ir (KBr) 2200-3600, 1620, 1370 cm^{-1} ; nmr (D_2O) δ 3.20-3.40 (m, 2H), 4.35 (m, 2H), 8.3 (s, 1H); mass spectrum (70 ev) m/e 167 (M^+ , 30), 122 (69), 94 (100). Literature reference, J. Wellisch, *J. Biochemische Zeitschrift*, 1913, 49, 173.
11. 16a: mp 248°C; ir (KBr) 3150 (broad) and 1700 cm^{-1} ; nmr ($\text{Me}_2\text{SO}-d_6$) δ 3.90 (s, 3H), 8.38 (s, 1H), 8.55 (s, 1H), 9.05 (s, 1H); mass spectrum (C.I., CH_4) 178 (M + 1, 100).
12. 14a: mp 212-213°C, lit mp¹⁴ 212-213°C. The stereochemistry of 14a was determined to be cis from the carbon-13 nmr spectrum of the corresponding ester 15a.¹⁷
13. 14b: mp 255-257°C, lit mp 267-268°C.¹⁴ The infra red spectrum was superimposable with that of an authentic sample prepared by the method of Wille (see reference 4). The stereochemistry of 14b was assigned trans based on the carbon-13 nmr spectrum of the corresponding ester 15b.¹⁷
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15. 15a (cis): mp 196-199°C; ir (KBr) 2400-3200 (broad), 1735 cm^{-1} ; pmr ($\text{Me}_2\text{SO-d}_6$) δ 2.75 (m, 1H), 2.90 (m, 1H), 3.70 (s, 3H, OCH_3), 3.90 (m, 1H), 5.00 (s, 1H), 7.30 (s, 5H), 7.35 (s, 1H); cmr ($\text{Me}_2\text{SO-d}_6$) δ 26.87 (t), 51.68 (q), 56.06 (d), 57.35 (d), 126.83, 127.15, 127.86, 128.24, 131.73, 133.92, 142.60, 172.47; mass spectrum (C.I., CH_4) 258 (M + 1, 100);
16. 15b (trans): mp 217-218°C; ir (KBr) 3350, 3200-2400 (broad), 1735 cm^{-1} ; pmr ($\text{Me}_2\text{SO-d}_6$) δ 2.85 (M, 1H), 2.90 (m, 1H), 3.70 (s, 3H), 3.75 (m, 1H), 5.10 (s, 1H), 7.30 (s, 5H), 7.45 (s, 1H); cmr ($\text{Me}_2\text{SO-d}_6$) δ 26.18 (d), 51.59 (q), 51.98 (d), 53.83 (d), 126.39, 126.70, 127.85, 128.25, 130.78, 133.93, 143.29, 173.28; mass spectrum (C.I., CH_4) 258 (M + 1, 100).
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18. 16b: mp 227-229°C; ir (KBr) 3150 (broad) 1705 cm^{-1} ; nmr ($\text{Me}_2\text{SO-d}_6$) δ 3.90 (s, 3H), 7.40-7.60 (m, 5H), 8.25 (s, 1H), 8.40 (s, 1H), 8.70-8.9 (m, 1H); mass spectrum (C.I., CH_4) 254 (M + 1, 100).
19. Numbers in parentheses are in vitro biological activities. The procedures for testing are described in ref. 3; for comparison purposes, the K_i of diazepam is ~5 nM.
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