BIOMIMETIC APPROACH TO POTENTIAL BENZODIAZEPINE AGONISTS AND ANTAGONISTS

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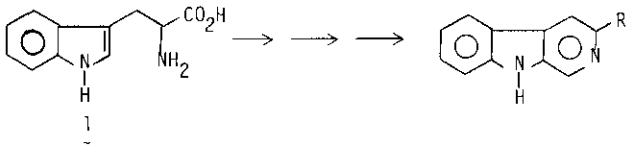
Abstract---A series of heterocycles have been prepared via the Pictet-Spengler reaction and several of these (β-carbolines 2-8, isoquinoline 11a and imidazopyridine derivative 16b) have been found to bind to the benzodiazepine receptor in vitro with moderate to high affinity.

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-carbolines 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-carbolines 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 reports[4] have suggested that endogenous peptide-like materials displace [^{3}H]-benzodiazepines from receptor sites with a relatively high affinity. Consequently, the effect of a 3-carboxamide moiety on the activity of β-carbolines has been determined. The carboxamide 8[5] 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-carbolines to the diazepam receptor(s). Pandit[6] has shown that tetrahydro-β-carbolines can be synthesized, in a

biomimetic sense, from N^5, N^{10}-methylenetetrahydrofolate 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 1 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



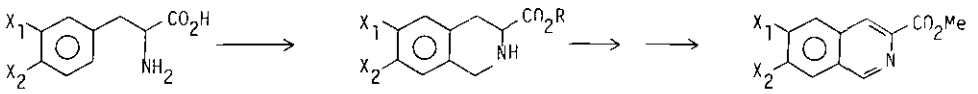
	R	K_i[19]
2,	CO_2Me	(1 nM)
3,	CH_2OH	(1470 nM)
4,	CHO	(62 nM)
5,	$COCH_3$	(58 nM)
6,	CO_2H	(24,000 nM)
7,	$CHOHCH_3$	(3160 nM)
8,	$CONH_2$	(68 nM)

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-dimethoxytetrahydroisoquinoline 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 11a[9] was obtained by heating 10a with palladium on carbon in xylene. In the histidine series the intermediate, spinacine (monohydrochloride) 13a[10] 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 16a[11] on heating for fifteen minutes with selenium dioxide in acetic acid. The 1-phenyl derivatives 14a[12] and 14b[13] were prepared by the method of Wille[14] *via* a base-catalyzed Pictet-Spengler reaction of histidine 12 with benzaldehyde, and were then converted to the corresponding esters 15a[15] 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 16b[18] was obtained by heating 15b with selenium dioxide in acetic acid (see Scheme III).

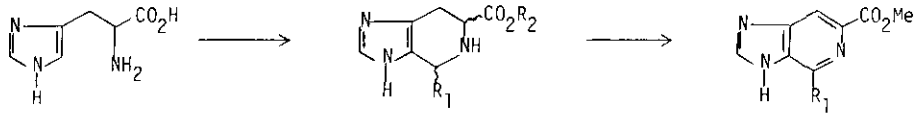
The binding affinities of the isoquinolines and imidazopyridine derivatives determined to date are shown in parentheses in Schemes II and III. While the tetrahydro derivatives 10a, 10c, 13a, 15a, and 15b were virtually inactive as expected,[3] the planar, fully aromatic isoquinoline 11a and the 4-phenylimidazopyridine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

Scheme II[19]



	X_1	X_2		
9a	H	H	Pha (1)	
9b	H	OH	Tyr	
9c	OH	OH	Dopa (d,l)	
10a	H	H	CH_3	(>100uM)
10b	H	OH	CH_3	
10c	OH	OH	H	(>100uM)
11a	H	H		(13.2 uM)
11b	H	OH		
11c	OH	OH		

Scheme III[19]



12, His (1)

	R_1	R_2		
13a	H	H		(>250 uM)
13b	H	CH_3		
14a	Ph	H	cis	
14b	Ph	H	trans	
15a	Ph	CH_3	cis	(>100 uM)
15b	Ph	CH_3	trans	(>100 uM)

	R_1	
16a	H	(>100 uM)
16b	Ph	(10 uM)

at 13.2 μM and 10 μM, 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 Pandit[6] 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).

ACKNOWLEDGEMENT. The authors wish to thank Mr. Frank Laib for the mass spectra and Ms. Linda Steward for technical assistance. We are greatly indebted to Dr. Steve Barker for sharing the results of reference 7 with us prior to publication.

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5. 8 (50% yield): mp 297-304°C; ir (KBr) 3450 (broad), 3200 (broad), 1680, 1645 cm^{-1}; nmr (Me_2SO-d_6) δ7.00-7.70 (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 (Me_2SO-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 mp[14] 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]

14. Myles Albert Wille, Ph.D. Thesis, University of Pennsylvania, 1969.

15. 15a (cis): mp 196-199°C; ir (KBr) 2400-3200 (broad), 1735 cm^{-1}; pmr (Me_2SO-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 (Me_2SO-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 (Me_2SO-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 (Me_2SO-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 (Me_2SO-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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Received, 21st December, 1981