Bioorganic & Medicinal Chemistry Letters, Vol.3, No.10, pp. 1941-1946, 1993
Printed in Great Britain

0960-894X/93 $6.00 + .00

SYNTHESIS AND POTENTIAL ANXIOLYTIC ACTIVITY OF 4-AMINO-PYRIDO[2,3-b]INDOLES

David Bolton, Ian. T. Forbes, Clare. J. Hayward, David. C. Piper, David. R. Thomas, Mervyn Thompson* and Neil Upton

SmithKline Beecham Pharmaceuticals, Coldharbour Road, The Pinnacles, Harlow, Essex CM19 5AD.

(*Received in Belgium* 8 *June* 1993; *accepted* 19 *July* 1993)

Abstract: A novel series of 4-amino-pyrido[2,3-b]indoles is presented as $GABA_A$ modulators with good potential as therapeutic agents for the treatment of anxiety disorders.

Benzodiazepines exhibit a wide range of pharmacological actions, including anxiolytic activity, which are mediated by specific high affinity receptor sites in the central nervous system (CNS)[1]. The benzodiazepine (BDZ) receptor is one constituent of a supramolecular complex which also contains separate, but allosterically coupled, recognition sites for γ-aminobutyric acid (GABA) and barbiturates. The oligomeric units of this $GABA_A$/BDZ/Cl^- channel complex form a drug and transmitter controlled chloride channel[2,3]. Despite advances made in terms of molecular characterization, the search continues for selective anxiolytics which are devoid of the unwanted side-effects typical of BDZs such as ataxia, sedation and interaction with ethanol.

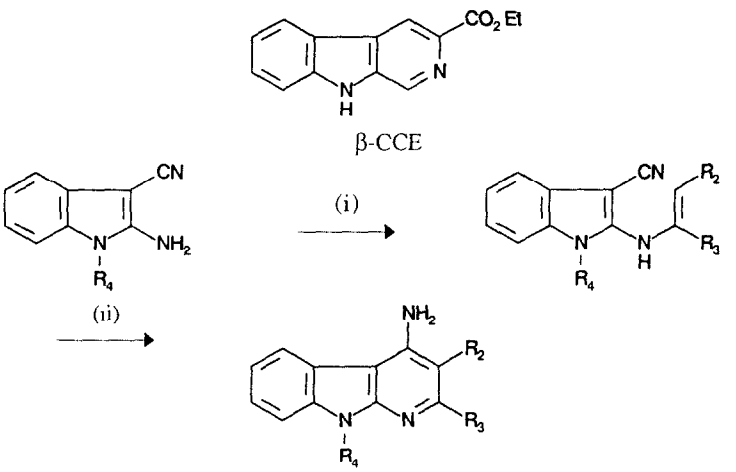
Literature studies[4] indicate that [^{35}S]-t-butyl-bicyclophosphorothionate (TBPS) binds with high affinity to the $GABA_A$ complex when the chloride channel is in the closed state but with low affinity when in the open state. Compounds can, therefore, inhibit high affinity binding either by competitive displacement (e.g. picrotoxin) or by shifting the channel to the open state, which appears to be the case for the currently described series of pyrido[2,3-b]indoles (α-carbolines). In contrast, potentiation of binding can occur by a shift towards the closed state of the channel. For example, it has been demonstrated[5] that both foot-shock stress and anxiogenic β-carbolines increase [^{35}S]TBPS binding.

The effect of compounds on [^{35}S] TBPS binding appears to parallel chloride conductance and gives a measure of drug efficacy in terms of permeability of the chloride channel[6]. Since anxiolytics, which act at the $GABA_A$ complex, are thought to act by promoting GABA-induced chloride conductance, the effects on TBPS binding have been used to predict potential anxiolytic activity of our compounds. In addition, direct interaction at the BDZ site of the complex was investigated using [^{3}H]-flunitrazepam binding[7].

The challenge was to modify the structure of the anxiogenic β-carboline β-CCE, to produce compounds which would stabilise the open conformation of the receptor complex and so possess anxiolytic properties. With this aim in mind, a series of α-carbolines was prepared and evaluated and the results are shown in the Table. *In vivo* anxiolytic activity was assessed by the degree of anticonflict activity, measured by significant increases in punished levels, in the Geller-Seifter behavioural paradigm[8].

Chemistry and SAR

Scheme



Reagents:-(i) $HOC(R_3)=CHR_2$, pTSA, toluene, reflux;
(ii) Appropriate Na alkoxide in alcohol, reflux or $SnCl_4$, n-BuOAc, reflux.

Compounds were synthesised as outlined in the Scheme by cyclisation of enamine intermediates using methods described in our earlier publications[9]. The acid **13** was prepared from the corresponding ester by hydrolysis with aqueous sodium hydroxide in ethanol. The amide **14** can also be made *via* the acid chloride by treatment with ammonia[9]. Ester and 9-position variations (R_4) were carried out by appropriate alkylation of the acid **13** and tricyclic **1** respectively. The alcohol **16** was prepared in 95% yield by reduction of ester **2** using lithium aluminium hydride in THF at 25°.

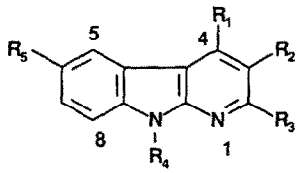


Table:- Physical and Biological Data for Pyrido[2,3-b]indoles

Cpd	m.p. °C[a]	R_1	R_2	R_3	R_4	R_5	[^{35}S]TBPS[b] $IC_{50}\mu M$	[^{3}H] Flu $IC_{50}\mu M$	MED Geller mg/kg p.o.[c]
(1)	260-6	NH_2	CO_2Me	Me	H	H	I	I	5
(2)	98-100	NH_2	CO_2Me	Me	Me	H	14.6±2.8	>100	10
(3)	90-1	NHMe	CO_2Me	Me	Me	H	17.1±1.8	82.3±4.8	20
(4)	157-8	NH_2	CO_2Me	Me	CO_2Me	H	9.5±2.5	>30	20
(5)	96-7	NH_2	CO_2Me	Me	C_5H_{11}	H	80±7.5	>100	>50
(6)	168-70	NH_2	CO_2Me	Ph	Me	H	11.2±0.6	>30	50
(7)	108-10	NH_2	CO_2Me	Me	Et	H	5.9±1.7	>100	5
(8)	79-80	NH_2	CO_2Et	Me	Me	H	4.6±0.8	>100	10
(9)	139-40	NH_2	CO_2Et	Me	CH_2Ph	H	I	I	>50
(10)	82-3	NH_2	$CO_2{}^nPr$	Me	Me	H	2.2±0.3	>100	10
(11)	94-5	NH_2	CO_2⌒	Me	Me	H	3.8±0.4	>100	20
(12)	126-7	NH_2	CO_2CH_2Ph	Me	Me	H	>30	>30	>50
(13)	230-2	NH_2	CO_2H	Me	Me	H	>100	>100	>50
(14)	322-7	NH_2	$CONH_2$	Me	H	H	>100	>100	>50
(15)	179-8	NH_2	$PO(OMe)_2$	Me	Me	H	>30	>30	>20
(16)	207-9	NH_2	CH_2OH	Me	Me	H	>100	>100	>50
(17)	203-8	NH_2	CO_2Me	Me	Me	Cl	I	I	50
Diazepam							74	0.012	2.5

I= insoluble

[a]Melting points are uncorrected; compounds analyzed for C, H and N within ±0.4% of the theoretical values.

[b]All TBPS data were obtained in the absence of GABA using procedures described in refs. 4 & 6. When the IC_{50} of **8** was measured in the presence of 5μM GABA, a 3-fold increase in potency was observed (IC_{50} 1.3 ± 0.34 μM). Values are a mean of three determinations.

[c]Compounds were administered orally to groups of 8 CFY rats 30min before testing; MED level was obtained using Two Way Analysis of Variance (ANOVA) with $p<0.05$.

Biological Results and Discussion

It can be seen from the results presented in the Table that, in general, 3-carboxylic esters are potent chloride ion channel openers and displace [^{35}S] TBPS at the low micromolar level. The propyl **10** and allyl **11** esters show the best *in vitro* potency (2.2 and 3.8μM respectively). This indicates that certain α-carbolines possess good potential as anti-anxiety agents and this is further evidenced by favourable anti-conflict activity for **1** and **7** when compared to the response of diazepam. Replacement of the C-3 alkyl ester function by acid **13**, amide **14**, phosphonate **15**, alcohol **16** or large lipophilic ester, such as benzyl **12** abolishes all potential anxiolytic activity as the compounds were inactive *in vitro*. The lack of effect of the amide **14** is in marked contrast to the β-carbolines where replacement of an ester function by amide maintained the BDZ partial inverse agonist profile of the series, with potentiation of TBPS binding[5]. Introduction of bulky lipophilic groups at the C-2 and N-9 position of the nucleus resulted in a reduction of *in vivo* activity with both compounds **5** and **6** but the 2-phenyl analogue **6** maintained a reasonable *in vitro* potency. Attempts to improve the CNS bioavailability by introduction of a halogen into the 6-position, as in the 6-chloro compound **17**, proved detrimental.

From these data, it can be seen that compounds in this series do not compete for the BDZ receptor site (IC_{50}>30μM). Other studies[10] have shown that **8**, when administered at doses of 30-300mg/kg p.o. does not displace [^{3}H]-flunitrazepam binding from various mouse brain regions (cortex, cerebellum and hippocampus) *in vivo*.

Yang and Olsen[11] described an assay of GABA channel function by monitoring tracer [$^{36}Cl^{-}$]flux in rat brain slices which allows *in vitro* study of transmembrane Cl^{-} ion movement in response to GABA stimulation. The Cl^{-} flux response to the $GABA_A$ agonist muscimol is inhibited by $GABA_A$ antagonists and enhanced by BDZs as well as barbiturates (which possess intrinsic activity in their own right). The effect of **8** on [$^{36}Cl^{-}$] uptake into rat brain cortical synaptoneurosomes was investigated to measure the ability of α-carbolines to promote Cl^{-} channel opening. Compound **8** was found to potentiate GABA agonist stimulated [$^{36}Cl^{-}$] uptake in a manner that differs from barbiturates since the compound did not possess intrinsic activity in the absence of the GABA agonist muscimol[12]. Hence α-carbolines act at a novel site that appears to be distinct from the BDZ and barbiturate sites.

Some measure of the propensity of compounds to cause motor incoordination can also be obtained from the Geller-Seifter model. No decrements were observed on unpunished responding levels at the doses tested in the Geller-Seifter model (up to 100mg/kg p.o. in the case of **1**, **7**, and **8**), giving some indication of lack of side-effects like sedation and muscle relaxation[13]. Such selectivity, indicative of a good therapeutic ratio, is an advantage over classical BDZ's[14].

However, with the N-benzyl **9** and amide **14** analogues, deficits were noticed on punished responding (-13% and -21% respectively at 50mg/kg p.o.; 2-way ANOVA, $p<0.05$).

Conclusion

In summary, the initial profile of a series of α-carbolines as $GABA_A$ modulators has been presented as potential therapeutic agents for the treatment of anxiety disorders and structure activity relationships have been discussed. The compounds have been found to act as anxioselective agents since they appear to lack the unwanted side-effect profile of the classical BDZs such as diazepam.

A number of compounds was selected for further evaluation and full details of therapeutic profile compared with that of BDZ's will appear elsewhere.

Acknowledgement

We wish to thank Miss Kellie Shrimpton for her assistance in preparing this manuscript.

References And Notes

1. Greenblatt, D.J.; Shader, R.I. *"Benzodiazepines in Clinical Practice"*; John Wiley and Sons, New York, **1974**

2. Skolnick, P.; Paul, S. "ISI Atlas of Science"; *Pharmacol.* **1988**, *2*, 19.

3. Pritchett, D; Luddens, H.; Seeburg, P.H. *Science* **1989**, *245*, 1389.

4. Gee, K.W.; Lawrence, L.J.; Yamamura, H.I. *Mol. Pharmacol.* **1986**, *30*, 218.

5. Concas, A.; Serra, M.; Atsoggiu, T.; Biggio, G. *J. Neurochem.* **1988**, *51*, 1868.

6. Maksay, G.; Simonyi, M. *Mol. Pharmacol.* **1986**, *30*, 321.

7. Martin, I.L.; Candy, J.M. *Neuropharmacology* **1978**, *17*, 993.

8. Anxiolytic properties were evaluated using the Geller-Seifter procedure based on that originally described in *Psychopharmacologia* **1960**, *1*, 482. This procedure has been shown to be selective for anti-anxiety agents (Cook and Sepinwall, "Mechanism of Action of Benzodiazepines". Costa, E.; Greengard, P. Eds; Raven Press, New York, **1975**,1.).

9. (a)Thompson, M.; Forbes, I.T. **1990**, US *Patent* 4952584
(b)Forbes, I.T.; Johnson, C.N.; Thompson, M. *J. Chem. Soc. Perkin Trans.* 1 **1992**, 275.

10. Data not presented. For methods see: Mennini, T.; Garattini, S. in *"Benzodiazepine Recognition Site Ligands: Biochemistry and Pharmacology"*. Biggio, G.; Costa, E. Eds; Raven Press, New York, **1983**, 189-99.

11. Yang, J.S.-J; Olsen, R.W. *J. Pharmacol. Exp. Ther.* **1987**, 241, 677.

12. Taylor, D.J.; Thomas, D.R.; Unpublished results, method as in ref. 11. A non-competitive (allosteric) inhibition of [^{35}S] TBPS binding was supported by the fact that inhibitory potency was increased in the presence of GABA for **8**. (i.e. the inhibition is GABA dependent). In contrast, inhibition by the competitive antagonist picrotoxin was independent of GABA concentration (IC_{50}'s were 0.2 ± 0.01 and 0.2 ± 0.02 μM in the absence and presence of 5μM GABA respectively). Hence the profile of inhibition by this series of GABA modulators clearly differs from that of a competitive antagonist.

13. Stean, T.; Upton, N.; Data not presented.
Diazepam, **7** and **8** were further evaluated in rat models for muscle relaxant (rotorod test), sedative (spontaneous locomotor activity test, SLA) and ethanol interaction (EtOH-induced sleeptime) properties. **7** and **8** were without effect at 100mg/kg p.o. and **8** was without significant effect on SLA and EtOH at 300mg/kg p.o. In contrast, diazepam produced marked significant effects on all three parameters at 10 to 20mg/kg p.o. [Compounds were administered to rats (n = 12) and observations recorded, at intervals of 30min., up to 2h post-dose; for methods see: "Animal Models in Psychiatry and Neurology". Hannin, I.; Usdin, E. Eds; Pergamon Press, Oxford, **1977**].

14. Diazepam at a dose of 40mg/kg p.o. (16 times MED) shows a very large decrement (- 19%; 2-way ANOVA, $p<0.01$) in the level of unpunished responding in all animals tested, (n=16) when compared to control animals.