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(±)11-AMINO-2,6-DIMETHYL-1,2,3,4-TETRAHYDRO-6H-QUININDOLIN-1-ONE, A NOVEL GABA$_A$ MODULATOR WITH POTENTIAL ANXIOLYTIC ACTIVITY

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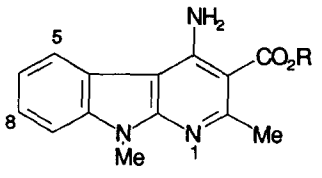
Abstract: Ketone isosteres have been investigated in a series of pyrido[2,3-b]indoles (α-carbolines). The incorporation of ring fusion into α-carbolines to produce the rigid quinindoline nucleus provides a GABA$_A$ modulator with both increased potency and a longer duration of action.

4-Amino-2,9-dimethyl-9H-pyrido[2,3-b]indole-3-carboxylic acid, ethyl ester **1** is a GABA$_A$ modulator which showed[1] good potential for the treatment of anxiety disorders. However, further biological evaluation revealed that esters such as **1** were relatively short acting *in vivo* with a duration of action of between one and two hours in the Geller-Seifter behavioural model of anxiety[2] (see Table 2). Studies undertaken[3] to determine the metabolic fate of **1** in rodents revealed that the major site of metabolism was the carboxylic ester which was cleaved to the corresponding acid **2**, which was biologically inactive. Some N-9 demethylation was also observed.

It was reasoned that replacement of the 3-carboxylic ester functionality with an appropriate alkyl ketone should provide a metabolically stable isostere.

Molecular modelling studies (see footnote in References and Notes Section) indicated that intramolecular hydrogen bonding of the 4-amino nicotinoate moiety of **1** stabilises a low energy conformation, which was confirmed by spectroscopic analysis[4]. This gives a carbonyl "in plane" hypothesis for a possible preferred active conformation. Therefore, cyclisation to produce a tetracyclic lactone or ketone should give a rigid analogue which would mimic this favoured low energy conformation.

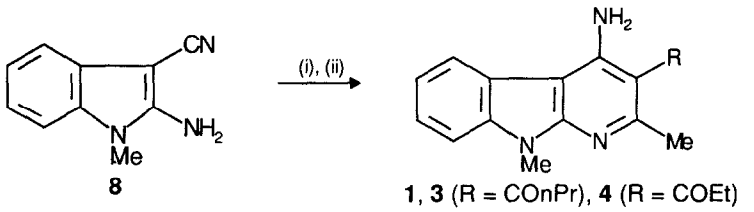


1 R = Et

2 R = H

Chemistry

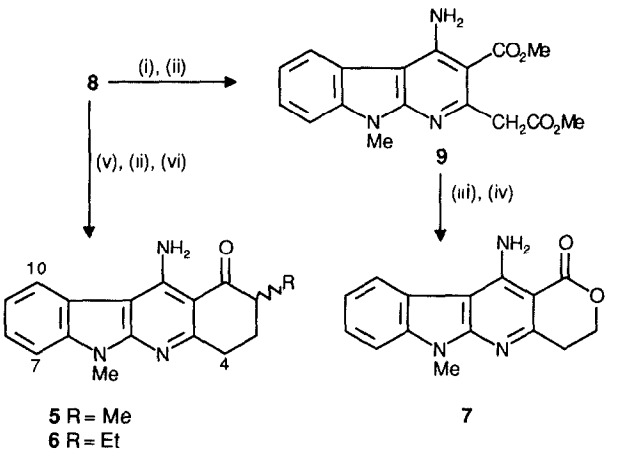
Scheme 1



Reagents:- (i) HOC(Me)=CHR, pTSA, toluene, reflux
(ii) NaOEt, EtOH, reflux or n-BuAc, reflux.

2-Amino-l-methyl-indole-3-carbonitrile **8** was converted into the desired α-carbolines **1**, **3** and **4** in around 20% overall yield using published procedures[5]. The ketones **3** and **4** were also prepared in similar yields *via* the aldehyde R = CHO, available from **1** *via* $LiAlH_4$ reduction and MnO_2 oxidation, using Grignard addition followed by oxidation with MnO_2 in chloroform at reflux.

Scheme 2



Reagents:- (i) dimethyl 1,3-acetone dicarboxylate, CSA, toluene, reflux
(ii) $SnCl_4$, n-BuAc, reflux 15 min
(iii) $LiBH_4$, THF, reflux
(iv) MnO_2, $CHCl_3$, 25°C
(v) cyclohexane-1, 3-dione, pTSA, toluene, reflux
(vi) LDA/THF/-78°C; followed by RI and warm to 25°C

The quinindolines **5** and **6** were prepared in good yield from **8**[6]. Condensation of cyclohexane-1,3-dione with **8** under Dean and Stark conditions gave an intermediate enamine which was cyclised using tin (IV)

chloride in n-butyl acetate at reflux. Alkylation under basic conditions afforded the desired targets **5** and **6**, and **5** was separated into pure enantiomers using chiral HPLC[7]. The lactone **7** was prepared using similar chemistry to obtain the diester **9** in 44% overall yield from **8**. Reduction of **9** using lithium borohydride in THF at reflux gave the corresponding diol in 86% yield which was oxidatively cyclised to the lactone **7** in 81% yield using manganese dioxide.

Biological Results and Discussion

The inhibition of [^{35}S]-t-butyl-bicyclophosphorothionate (TBPS) binding was used as a measure of a compound's ability to facilitate the opening of $GABA_A$/chloride ion channels [8].

The C-3 position carbonyl residue has been found to be important for GABA modulatory activity. Esters such as **1** were found[1] to displace [^{35}S] TBPS binding from isolated rat synaptosomes in the low micromolar region.

As can be seen in Table 1, both the n-propyl **3** and ethyl **4** ketones indeed possess similar *in vitro* potency to the ester **1**. The ethyl ketone **4** was also found to show an increased duration of action, being active at two hours post-dose in the Geller-Seifter paradigm. Unfortunately, the six membered lactone **7** was too insoluble for evaluation *in vitro* and was also found to be inactive *in vivo*. However, there were no solubility problems with the cyclic ketones **5** and **6** which were found to have a similar level of *in vitro* activity to **1**, with **5** being slightly more potent *in vivo*. It was also observed that chirality had no influence on the ability of **5** to displace [^{35}S] TBPS binding as there was no difference between *in vitro* potency of the two enantiomers of **5**[9].

Table 1: Physical and Biological Data

Cpd	mp °C[a]	[^{35}S] TBPS[b] IC_{50} μM	[^{3}H] Flu[c] IC_{50} μM	MED Geller mg/kg p.o.
1	79-80	2.0 ± 0.2	>100	10
2	230-2	>100	>100	>50
3	109-10	2.3 ± 0.3	NT	20
4	72-3	1.6 ± 0.4	>100	20
5	155-6	0.95 ± 0.1	>100	5
6	129-31	0.94 ± 0.2	NT	20
7	204-5	I	I	>20
Diazepam	-	0.01[d]	0.12[e]	2.5

a. Melting points are uncorrected; compounds analysed for C, H and N within ± 0.4% of the theoretical values; NMR spectral data are shown in ref.10.
b. The detailed procedure of this test is described in ref.8; all determinations were done in the presence of 5μM GABA. Values are a mean of three determinations.
c. Procedure as in ref.11. d. Max inhibition <50%; quoted value is an IC_{25}.
e. 100% inhibition at dose tested. I= insoluble; NT= not tested; MED = minimum effective dose.

Table 2: Biological Evaluation in the Geller-Seifter test[a]

Cpd	Dose mg/kg p.o.	1h post-dose		2h post-dose	
		FR % Change	Nr/N	FR % Change	Nr/N
1	5	2	5/6		
	10	+20**	10/15		
	20	+22**	12/16	+6	6/16
	50	+63**	15/16	+4	6/16
	100	+122**	14/16		
5	2.5	0	0/6		
	5	+19*	8/14		
	10	+43**	5/6	+22	7/14
	20	+52**	5/6	+36**	6/6
	50	+146**	5/6	+62**	6/6
	100	+137**	6/6	+141**	6/6
4	20	+41**	5/6	+28*	5/8
Diazepam	5	+57**	6/8	+38**	8/16

a. * $p<0.05$, ** $p<0.01$, Two-way ANOVA; FR is the punished responding phase; Nr/N is the number of animals responding out of those tested.

Both the ester **1** and the quinindoline **5** showed a dose related increase in the level of punished responding when compared to control animals (Table 2). However, for **1**, the degree of punished responding declined steadily over a two hour period and the compound was inactive at the higher dose of 50mg/kg p.o. at the two hour time point. Conversely, the quinindoline **5** showed a greater level of punished responding in the Geller-Seifter paradigm at both one and two hours post dose. The MED of **5** was found to be about twice that of diazepam. There were no deficits observed with **1** and **5** during the unpunished phase of responding at all doses tested, possibly indicating a good separation of anxiolytic activity from sedative side-effects[12].

From a mechanistic viewpoint, the compounds were not active at classical benzodiazepine (BDZ) sites, as measured by inhibition of [^{3}H] flunitrazepam binding (IC_{50}>100μM) to rat cerebral cortex[11]. Hence the compounds do not act as BDZ partial agonists and must modulate the $GABA_A$/chloride ion channel *via* another site.

The quinindoline **5**, with a reduced capacity for intermolecular hydrogen bonding ability as compared to the uncyclised compounds **1** and **3**[4,10], showed a log D_{oct} of 1.50 (at pH1.72 and 25°C) and a Δ log P of +1.35 suggesting[13] good brain penetration which was confirmed *in vivo*.

In conclusion, we have demonstrated that incorporation of a ring fused ketone provides a potent ester isostere with a longer duration of action.

Acknowledgements

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References and Notes

1. Bolton, D.; Forbes, I.T.; Hayward, C.J.; Piper, D.C.; Thomas, D.R.; Thompson, M.; Upton, N. *BioMed. Chem. Lett.*, paper in press.

2. Geller, I.; Seifter, J. *Pyschopharmacol.* **1960**, *1*, 482.

3. Unpublished results from Drug Metabolism and Pharmacokinetics Unit, SB.

4. In the quinindoline series, spectroscopic measurements confirm that there is a greater degree of intramolecular hydrogen bonding between the amino and carbonyl groups. For **1** the ^1H NMR (270MHz, $CDCl_3$) shows a broad 2H, s at 6.7-6.8 for the 4-NH_2 group whereas the corresponding signal for **5** is too broad to be observed. Also supported by IR (KBr disc) data for **1** 1680, 3390, 3500 and for **5** 1640, 3330, 3440cm^{-1}.

5. Forbes, I.T.; Johnson, C.N.; Thompson, M. *J. Chem. Soc. Perkin Trans.* 1 **1992**, 275.

6. Forbes, I.T.; Martin, R.T.; Thompson, M. **1992**, *US Patent* 5079246.

7. The racemic **5** obtained was separated into the two enantiomers by the use of HPLC using the following conditions. Column: Chiral-AGP 4.0x100mm; ID = 18RC. Eluent: 20/80 MeOH/0.02M aqueous phosphate buffer at pH 7.0 with a flow rate of 1.0ml/min. Detection: UV at 278nm. The retention times of the (+) and (-) enantiomers under these conditions were 34.0 and 42.2 min respectively.

8. a. Trifiletti, R.R.; Snowman, A.M.; Snyder, S.H. *Mol. Pharmacol.* **1984**, 26, 470.
   b. Maksay, G.; Simonyi, M. *Mol. Pharmacol.* **1986**, *30*, 321.

9. The IC_{50} values for the enantiomers were (+) 1.09 and (-) 1.06 μM (mean of two determinations).

10. Physical data for new compounds **3** to **7**.
    3. Recrystallisation from methanol gave off-white crystals. ^1H NMR (270 MHz, $CDCl_3$) 7.78-7.85 (d, J=8Hz, 1H) 7.40-7.49 (m, 2H), 7.24-7.34 (m, 1H), 6.17-6.31 (broad s, 2H), 3.91 (s, 3H), 2.84-2.93 (t, J=7Hz, 2H), 2.83 (s, 3H), 1.70-1.85 (m, J=7Hz, 2H), 0.91-1.02 (t, J=7Hz, 3H); analysis calculated for $C_{17}H_{19}N_3O$: C, 72.57; H, 6.81; N, 14.93; found: C, 72.59; H, 6.91; N, 14.87.
    4. Recrystallisation from ethanol gave white crystals. ^1H NMR (270 MHz, $CDCl_3$) 7.80-7.84 (d, J=8Hz, 1H), 7.41-7.46 (m, 2H), 7.26-7.33 (m, 1H), 6.23-6.34 (broad s, 2H), 3.92 (s, 3H), 2.88-

2.97 (q, J=7Hz, 2H), 2.78 (s, 3H), 1.22-1.28 (t, J=7Hz, 3H); analysis calculated for $C_{16}H_{17}N_3O$: C, 71.89; H, 6.41; N, 15.72; found: C, 71.56; H, 6.32; N, 15.89.

5. Recrystallisation from ethanol gave white crystals. ^1H NMR (270 MHz, $CDCl_3$) 7.77-7.89 (m, 1H), 7.21-7.53 (m, 3H), 3.90 (s, 3H), 3.04-3.31 (m, 2H), 2.55-2.78 (m, 1H), 2.11-2.30 (m, 1H), 1.80-2.03 (m, 1H), 1.31 (d, J=11Hz, 3H); analysis calculated for $C_{17}H_{17}N_3O$: C, 73.10; H, 6.13; N, 15.04; found: C, 73.16; H, 6.14; N, 15.02.
6. Recrystallisation from ethyl acetate-60:80 petroleum ether gave cream needles. ^1H NMR (270 MHz, $CDCl_3$) 7.71-7.88 (m, 1H), 7.21-7.50 (m, 3H), 3.89 (s, 3H), 2.98-3.33 (m, 2H), 2.38-2.57 (m, 1H), 2.16-2.35 (m, 1H), 1.80-2.13 (m, 2H), 1.50-1.75 (m, 1H), 1.06 (t, J=7Hz, 3H); analysis calculated for $C_{18}H_{19}N_3O$: C, 73.69; H, 6.53; N, 14.32; found: C, 73.87; H, 6.72; N, 14.09.
7. Recrystallisation from ethanol as off-white crystals. ^1H NMR (270 MHz, $CDCl_3$) 8.40-6.10 (broad, 2H), 7.83 (d, J=9Hz, 1H), 7.45 (m, 2H), 7.34 (dt, J=9, 2Hz, 1H), 4.56 (t, J=8Hz, 2H), 3.89 (s, 3H), 3.22 (t, J=8Hz, 2H); analysis calculated for $C_{15}H_{13}N_3O_2$: C, 67.41; H, 4.90; N, 15.72; found: C, 67.28; H, 4.90; N, 15.62.

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

12. At a dose of 40mg/kg p.o. diazepam showed a reduction in the level of unpunished responding compared to control values. **5** was examined in rat models for muscle relaxant (rotorod test), sedative (spontaneous locomotor activity test) and ethanol interaction (EtOH-induced sleeptime) properties. **5** was without significant effect at 300mg/kg p.o. In contrast, diazepam produced marked significant effects on all three parameters at doses of 5 to 20mg/kg p.o. (for methods see: *"Animal Models in Psychiatry and Neurology"*. Hannin, I.; Usdin, E. Eds; Pergamon Press, Oxford, **1977**).

13. a. Hansch, C.; Sammes, P.G.; Taylor, J.B. *"Comprehensive Medicinal Chemistry"*; Ramsden, C.A.; Ed.; Pergamon Press, Oxford, **1990**, Vol 4, p. 402.
    b. Young, R.C.; Mitchell, R.C.; Brown, T.H.; Ganellin, C.R.; Griffiths, R.; Jones, M.; Rana, K.K.; Saunders, D.; Smith, I.R.; Sore, N.E.; Wilks, T.J. *J. Med. Chem.* **1988**, *31*, 656. Partition coefficients were measured by a conventional shake-flask technique as described by:- Leo, A.; Hansch, C.; Elkins, D.; *Chem. Rev.* **1971**, *71*, 525.

14. **Footnote**:- Initial studies on the $GABA_A$ pharmacophore were carried out using Gaussian calculations with SV3 21G and STO 3G basis sets. A common volume for $GABA_A$ modulators based on the examination of active and inactive compounds was calculated. The model was refined by calculation of AM1/Gaussian potential-derived charges and 3-D electrostatic potential surfaces were produced and compared to that of **1** using SYBYL.

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