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# THE SYNTHESIS OF PYRAZOLO[4,3-*c*]- AND IMIDAZO[4,5-*c*]- ARYL[*e*]FUSED PYRIDINES AS STRUCTURAL ANALOGUES OF 4-AMINONICOTINOATES.

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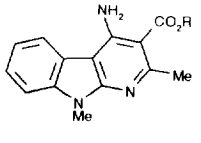
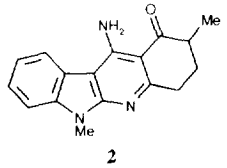
**Abstract**: A series of 4-aminobenzothienopyridine-3-carboxylates **4** to **10** has been prepared and the SAR of these $GABA_A$ modulators discussed. Replacement of the 4-aminonicotinoate moiety by imidazo [4,5-*c*] ring fusion provides potent bioisosteres **12** to **14**.

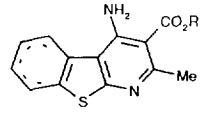
As part of a programme directed towards the search for anxioselective agents, that do not possess the unwanted side-effects associated with classical benzodiazepines (BDZ's) such as diazepam, we identified a series of pyrido[2,3-*b*]indoles (*e.g.* **1** and **2**) which showed a different profile to the BDZ's.[1,2,3] The potential of these compounds as $GABA_A$ modulators was assessed by measurement of their ability to inhibit [$^{35}$S]-t-butyl-phosphorothionate (TBPS) binding *in vitro*. The use of [$^{35}$S]TBPS in such studies has been shown to correlate well with the opening of the BDZ/$GABA_A$/chloride ion channel and hence provides an indication of potential anxiolytic activity.[4] The simple alkyl esters such as **1** (R=Me, Et, Pr) were all of similar potency *in vitro* with $IC_{50}$ values in the low micromolar range ($IC_{50}$ *ca* 2μM).




**1**




**2**




**3**

A chemical programme designed to exploit this key finding led to the discovery that replacement of the indole nucleus with either a saturated cycloalkyl fused thiophene or an aromatised benzothiophene (*e.g.* **3**) at the left hand portion of the structure provided good bioisosteres.[3] The amino esters **A** (Table) do not appear to act as classical BDZ partial agonists.[5]

Earlier work, directed towards the construction of a pharmacophore for this series of novel $GABA_A$ modulators, suggested that the active conformation of the molecules was planar with an intramolecular hydrogen bond between the 4-amino group and the carbonyl of the 3-carboxylate group. This hypothesis was supported by the synthesis of the constrained quinindoline **2**, which was slightly more potent than the corresponding ester (**1** R=Et).[2]

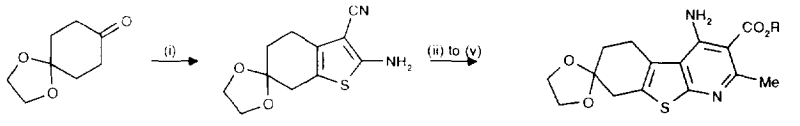
Further molecular modelling studies using SYBYL revealed that the imidazo fused tetracyclic stucture **B** (Table) should be a reasonable mimic of the proposed active "in plane" conformation of the nicotinoate moiety of the $GABA_A$ modulators.[6] Accordingly, we prepared analogues of the most potent esters and the assembly of these is shown in Scheme 2.

An alternative low energy conformation involving bonding of the 4-$NH_2$ group to the ether, rather than the carbonyl, oxygen of the 3-carboxylate group was also examined using SYBYL. The pyrazolo[4,3-*c*]-pyridine **15** was designed to mimic this alternative possibility. Although **15** is a rigid structure the carbonyl and benzyl groups can access a similar region of space to that occupied by such a conformation.

## Chemistry

In order to further explore structure activity relationships (SAR) in the benzothienopyridine series we prepared a number of functionalised 7-ketals. The approach and methodology for the assembly of the esters **A** (**4** to **10**), from 1,4-cyclohexanedione monoethylene ketal **16**, is outlined[7] in Scheme 1.

**Scheme 1**




**16** **17** **(4-10)**

*Reagents and Conditions*

(i) sulfur, malononitrile, $Et_2NH$, MeOH, 5°C; 50% yield

(ii) ethyl 3-ethoxycrotonate, pTSA, toluene, reflux

(iii) NaOEt, EtOH, reflux

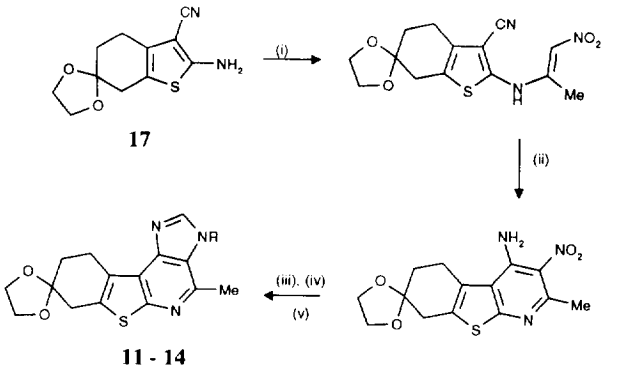
(iv) KOH, 10% aqueous MeOH, reflux

(v) RBr, $K_2CO_3$, DMF, 25°C

The modified route towards imidazo compounds **11** to **14** is described in Scheme 2. A crucial reaction in this sequence was formation of the key nitroenamine intermediate from **17** using nitroacetone dicyclohexylamine salt.[8] Cyclization with copper (I) acetate,[2] followed by reduction and ring closure with triethyl orthoformate at reflux[9] gave the desired imidazo target **11** which on alkylation, under basic conditions, furnished compounds **12-14**. Proof of regiochemistry of the alkylation products of (**11**, R=H) was obtained by 2-D nmr assignment.[10]

The synthesis of the pyrazolone **15** is shown in Scheme 3. Attempts to assemble the pyrazolone ring *via* the 4-chloronicotinoate ester, prepared from the corresponding pyridone with phosphorus oxychloride at reflux as published,[11] were unsuccessful. Hence, the more reactive 4-triflate was prepared and displacement by benzyl hydrazine/triethylamine at reflux afforded the cyclised pyrazolone **15**.

**Scheme 2** **Synthesis of Imidazo[4,5-*c*]benzo[*b*]thieno[2,3-*b*]pyridines**




*Reagents and Conditions*

(i) nitroacetone dicyclohexylamine salt,[8] CSA, $CH_2Cl_2$; followed by powdered 4Å sieves, RT, 24h.

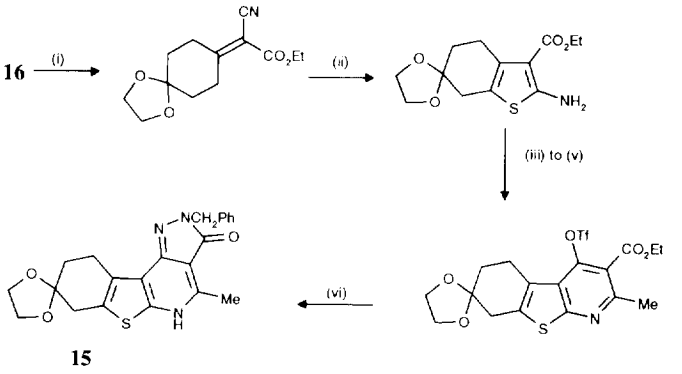
(ii) Cu (I) acetate, n-butyl acetate, reflux; 30% yield overall

(iii) $Na_2S_2O_4$, EtOH, $H_2O$, reflux;[9] 89% yield

(iv) $HC(OEt)_3$, reflux; 90% yield.

(v) RBr, NaH, DMF, 25°C; 61-79% yields.

**Scheme 3** **Synthesis of Pyrazolo[4,3-*c*]benzo[*b*]thieno[2,3-*b*]pyridines**




*Reagents and Conditions*

(i) ethyl cyanoacetate, $Et_2NH$, AcOH, toluene; 57% yield

(ii) sulfur, $Et_2NH$, EtOH; 95% yield

(iii) ethyl 3-ethoxycrotonate, CSA, reflux

(iv) NaOEt, EtOH, reflux; 71% yield overall for steps (iii) and (iv)

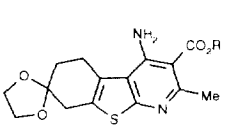
(v) triflic anhydride, pyridine; 64% yield

(vi) benzyl hydrazine, $Et_3N$, reflux; 75% yield

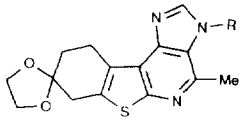
**Biological Results and SAR**

Examination of the amino esters **4-10** *in vitro* revealed that incorporation of a degree of unsaturation or $sp^2$ character into the carboxy side-chain increased the level of potency when compared to simple alkyl esters (see Table). For example, the propargyl **5** (0.78μM), butynyl **6** (0.32μM) and cyclopropylmethyl **7** (0.87μM) esters were much more potent than the ethyl ester **4** which had an $IC_{50}$ of 16μM. This is similar to observations noted for a series of N-substituted pyrazolo[3,4-*b*]pyridine-5-carboxamides which were BDZ partial agonists.[12] These authors showed that unsaturation in the amide side-chain was one of the features necessary for optimal interaction with brain BDZ receptors where N-allyl and N-cyclopropylmethyl amides provided the most potent compounds. In our series, the benzyl **9** and 4-chlorobenzyl **10** esters were also potent compounds. This is in contrast to earlier work with the pyrido[2,3-*b*]indole series where the benzyl ester (**1**, R=$CH_2Ph$) was much less potent than simple alkyl esters.[1]

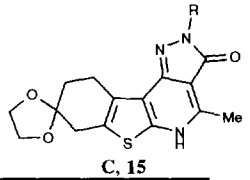
**Table : Physical and Biological Data for Compounds**




A, 4 - 10




B, 11 - 14




C, 15

| Cpd | mpt °C[a] | Structure | R | [$^{35}$S]TBPS[b] $IC_{50}$(μM) |
|---|---|---|---|---|
| **4** | 121-2 | A | Et | 16.4 |
| **5** | 128-9 | A | $CH_2C{\equiv}CH$ | 0.78 |
| **6** | 154-5 | A | $(CH_2)_2C{\equiv}CH$ | 0.32 |
| **7** | 115-6 | A | $CH_2$–cyclopropyl | 0.87 |
| **8** | 97-8 | A | $(CH_2)_2$–cyclopropyl | 0.24[c] |
| **9** | 142-3 | A | $CH_2Ph$ | 1.4 |
| **10** | 148-9 | A | $CH_2$-4-ClPh | 0.31 |
| **11** | 160-3 | B | H | >100 |
| **12** | 195-7 | B | $CH_2$–cyclopropyl | 8.9 |
| **13** | 176-7 | B | $(CH_2)_2$–cyclopropyl | 6.3[c] |
| **14** | 173-4 | B | $(CH_2)_2$ -4 -ClPh | 2.7 |
| **15** | 265-7 | C | $CH_2Ph$ | >100 |

[a] Melting points are uncorrected; compounds analysed for C, H, and N within ± 0.4% of theoretical values; satisfactory 250MHz $^1$H nmr data were obtained. [b] The detailed procedure of this test is described in ref. 13 and all determinations were done in the presence of 1μM GABA. Values represent a mean of at least two determinations. [c] Single determination done in duplicate.

Biological evaluation of the imidazo[4,5-*c*]pyridines **12** to **14** revealed that while the compounds were active *in vitro* they possessed only a tenth of the potency of their ester counterparts (see Table). For example, although the N-cyclopropylmethyl **12** and cyclopropylethyl **13** compounds ($IC_{50}$ 8.9 and 6.3μM respectively) showed potency at a level similar to that of a simple alkyl ester (**4**, $IC_{50}$ 16μM) this was much

lower than the value for the corresponding cyclopropylalkyl esters **7** and **8** ($IC_{50}$ 0.87 and 0.24μM respectively). Similarly, the N-4-chlorophenylethylimidazole **14** was less potent than the ester **10**.

The inactivity of the NH imidazole **11** ($IC_{50}$>100μM) parallels the lack of activity found with carboxylic acids in both the benzothienopyridine (*e.g.* **A** or **3**, R=H)[14] and pyrido[2,3-*b*]indole series (*e.g.* **1**, R=H).[1] This observation supports the requirement for lipophilic binding at this region of the molecule. Interestingly, in addition, the lack of activity with acids in general is in agreement with the literature report that carboxylic acids are also inactive in the pyrazolo[4,3-*b*]pyridine series of BDZ partial agonists.[12]

Biological evaluation of the pyrazolo[4,3-*c*]pyridine **15** *in vitro* revealed that the compound was inactive ($IC_{50}$ >100μM), suggesting that this rigid structure does not fix a receptor binding conformation of the esters.

## Conclusion

In an attempt to further elucidate the active binding conformation of amino esters **A**, the imidazo[4,5-*c*]-pyridines, designed to mimic a conformation of the esters in which there is a hydrogen bond between the carbonyl oxygen and the amino group, were synthesised. Activity was retained at about one tenth of the level of that for amino esters lending further support to this being the receptor active conformation. The lower potency may be a consequence of electronic differences and/or a less favoured spatial arrangement of the side-chain. The inactivity of the N-benzyl pyrazolo[4,3-*c*]pyridine **15**, which fixes an alternative conformation of the amino esters, namely that in which there is a hydrogen bond between the ether oxygen of the ester and the amino group, is also consistent with the hypothesis. Further details of pharmacophore mapping studies to help delineate the structural and electronic requirements for modulation of the GABA/BDZ/chloride ion channel will be published elsewhere.

## Acknowledgements

We wish to thank Analytical Sciences, SB Harlow, for analytical and spectroscopic data; Claire Duffy for skilled technical assistance and Kellie Shrimpton for assistance in the preparation of this manuscript. Dave Bolton is also thanked for his early work towards the synthesis of 3-nitropyridines.

## 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.*, **1993**, *3*, 1941.

2. Blackburn, T.P.; Bolton, D.; Forbes, I.T.; Johnson, C.N.; Martin, R.T.; Thomas, D.R.; Thompson, M.; Upton, N. *BioMed. Chem. Lett.*, **1994**, *4*, 279.

3. Blackburn, T.P.; Davies, D.T.; Forbes, I.T.; Hayward, C.J.; Johnson C.N.; Martin, R.T.; Piper, D.C.; Thomas, D.R.; Thompson, M.; Upton, N.; Ward, R.W. *BioMed. Chem. Lett.*, paper in press.

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

5. As seen with previous $GABA_A$ modulators from this series (see refs. 1 to 3 above) the compounds do not appear to act *via* direct modulation of the BDZ site. Compounds **4** to **7** and **9** did not displace [$^3$H] flunitrazepam binding *in vitro* ($EC_{50}$ >100μM). Electrophysiology studies with compound **7** in bovine adrenomedullary chromaffin cells have shown that these compounds modulate GABA-induced membrane current in a different way to BDZ's and barbiturates. Details are published in Benham, C.D.; Meadows, H.J.; Thomas, D.R.; Wood, M.D. **1994**, *PCT*, WO94/25027.

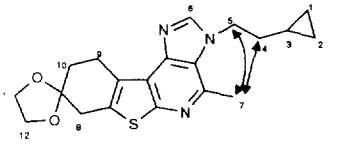
6. Initial studies on the $GABA_A$ pharmacophore were carried out using Gaussian calculations with SV3 21G and STO 3G basis sets. A common volume for $GABA_A$ modulators based on the examination of active and inactive compounds was calculated. The model was refined by calculation of AM1/Gaussian potential-derived charges and 3-D electrostatic potential surfaces were produced and compared to those of **1** and **2** using SYBYL.

7. Martin, R.T.; Thompson, M.; Ward, R.W. **1994**, *PCT*, WO94/18205 and *Chem. Abs.*, **1995**, *122*, 31498k.

8. Wade, P.A.; Price, D.T.; Carroll, P.J.; Dailey, W.P.; *J. Org. Chem.*, **1990**, *55*, 3051.

9. Kuroda, T.; Suzuki, F. *J. Het. Chem.*, **1991**, *28*, 2029.

10. *e.g.* for **13**. White solid, m.p. 176-7° (recrystallisation from EtOAc).$\nu_{max/cm}{}^{-1}$ 2954, 2925, 2854, 1463, 1377, 1063; $\delta_H$ (270MHz) 8.00 (1H, s, H-6), 4.55 (2H, t, J=6.8Hz, H-5), 4.10 (4H, s, H-11, H-12), 3.45 (2H, m, H-9), 3.14 (2H, s, H-8), 2.95 (3H, s, Me), 2.12 (2H, t, J=6.2Hz, H-10), 1.75 (2H, m, H-4), 0.60 (1H, m, H-3), 0.48 (2H, m, H-2), 0.20 (2H, m, H-1); $^m/_z$ 369 ($M^+$); Found: C, 64.96; H, 6.10; N, 11.27. $C_{20}H_{23}N_3O_2S$ requires C, 65.01; H, 6.27; N, 11.37%. The regiochemistry for the alkylation reaction was determined by N.O.E. experiments, to be the above structure. Strong N.O.E. between H-5 & Me-7. Strong N.O.E. between H-4 & Me-7. This was similar for **12** and **14**.



11. Forbes, I.T.; Johnson, C.N.; Jones, G.E.; Loudon, J.; Nicholass, J.; Thompson, M.; Upton, N. *J. Med. Chem.*, 1990, **30**, 2641.

12. Bare, T.M.; McLaren, C.D.; Campbell, J.B.; Firor, J.W.; Resch, J.F.; Walters, C.P.; Salama, A.I.; Meiners, B.A.; Patel, J.B. *J. Med. Chem.*, **1989**, *32*, 2561.

13. 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.

14. Duffy, C.; Thomas, D.R. unpublished work, personal communication.

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