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ON THE BIOACTIVE CONFORMATION OF THE GABA UPTAKE INHIBITOR SK&F 89976-A

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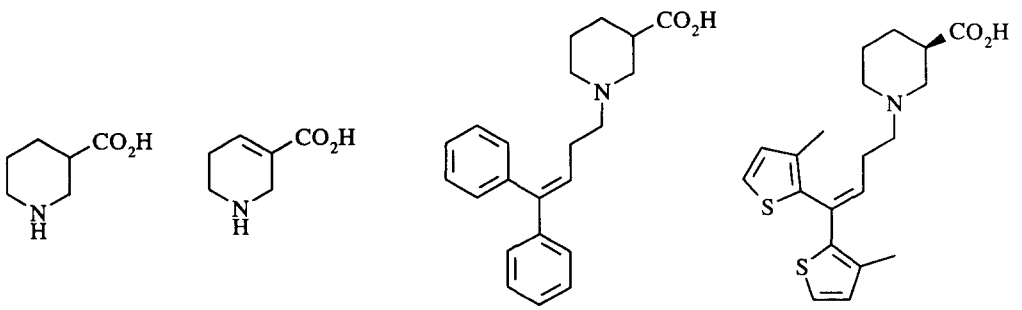
Abstract: SK&F 89976-A (**3**) is a potent GABA uptake inhibitor, with a flexible diphenylbutenyl side chain. Conformationally restrained analogues of this compound, **14, 16, 18** and **20** were designed and synthesized to study the conformational preferences for binding to cloned GABA transporters. We conclude from this study that SK&F 89976-A probably binds to the GABA transporter in its fully extended conformation.

Introduction: γ-Aminobutyric acid (GABA) is the major inhibitory neurotransmitter in the mammalian central nervous system. GABA is removed from the synaptic cleft by specific high-affinity, sodium dependent transporters located on both the neurons and surrounding glial cells. Such transporters aid in the termination of synaptic transmission, prevent the spread of GABA to neighboring synapses, and allow GABA to be reutilized. Inhibition of GABA uptake represents a novel approach to increasing the synaptic levels of GABA, thereby enhancing inhibitory neurotransmission.[4] Such agents may be useful in the treatment of a number of neurological and psychological disorders, in particular epilepsy and anxiety.[5] The therapeutic potential of classical GABA uptake transport inhibitors such as nipecotic acid (**1**) and guvacine (**2**) is limited by their inability to penetrate the blood-brain-barrier, presumably attributable to their hydrophilic nature. To avoid this problem, Ali *et al.*[6] attached a diarylbutenyl side chain to the nitrogen of nipecotic acid. The resulting compound SK&F 89976-A (**3**),[6] as well as related compounds,[7] display anti-convulsant activity in laboratory animals, and one derivative, Tiagabine (**4**) (Figure 1), is currently in phase III clinical trials for the treatment of complex partial seizures.[8]

Figure 1.



1 2 3 4

Molecular biologists have cloned four high affinity GABA transporters called GAT-1, GAT-2, GAT-3 and BGT-1. We recently reported that these transporters differ in their cellular distribution in the brain, as well as

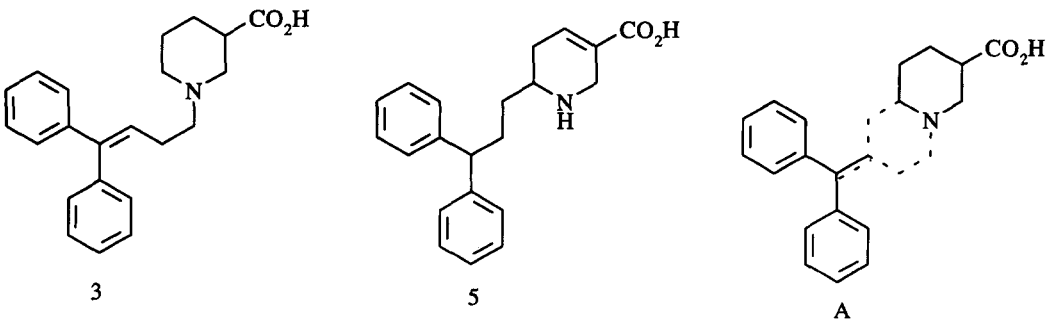
their sensitivity to pharmacological agents.[9] We have also shown that lipophilic GABA uptake inhibitors like SK&F 89976-A (**3**),[6] Tiagabine (**4**)[8] and CI-966 [10] are selective for the cloned GABA transporter GAT-1.[11a,b] suggesting that inhibition of GABA transport at this site underlies their anticonvulsant activity.

The increased affinity of compounds like SK&F 89976-A and Tiagabine for the GABA transport system has been attributed to the presence of the diarylbutenyl side chain.[6] Elucidating the molecular mechanisms by which the lipophilic side chains enhance the potency of these compounds is complicated by the lack of knowledge concerning the tertiary structure of the transporter as well as by the flexible nature of the diarylbutenyl side chain, which can adopt a variety of conformations. As a first step to understand the bioactive conformation of the diphenylbutenyl side chain, we describe the design, synthesis and biological activity of several conformationally restrained analogues of SK&F 89976-A.

Discussion: Goka *et al.* recently described a general pharmacophore model for GABA uptake inhibitors.[12] Based on this model they synthesized compound (**5**) (Figure 2) which is a guvacine derivative having a lipophilic 3,3-diphenylpropyl side chain. This compound was found to be equipotent with SK&F 89976-A (0.1μM) in brain synaptosomes indicating that a lipophilic group at position 6 of the guvacine ring is tolerated by the GABA transporter.

Of the many possible conformations for **3**, the Goka model predicts the diphenylbutenyl side chain to be oriented as shown in Figure 2, in order for the aromatic rings to be placed at an optimum distance between the amino group and the acidic center of the piperidine ring.[13] The same model predicts the side chain of compound (**5**) to be oriented as shown in Figure 2. Overlapping structures **3** and **5** led us to design several conformationally restricted quinolizidine analogues with the general structure A.

Figure 2.



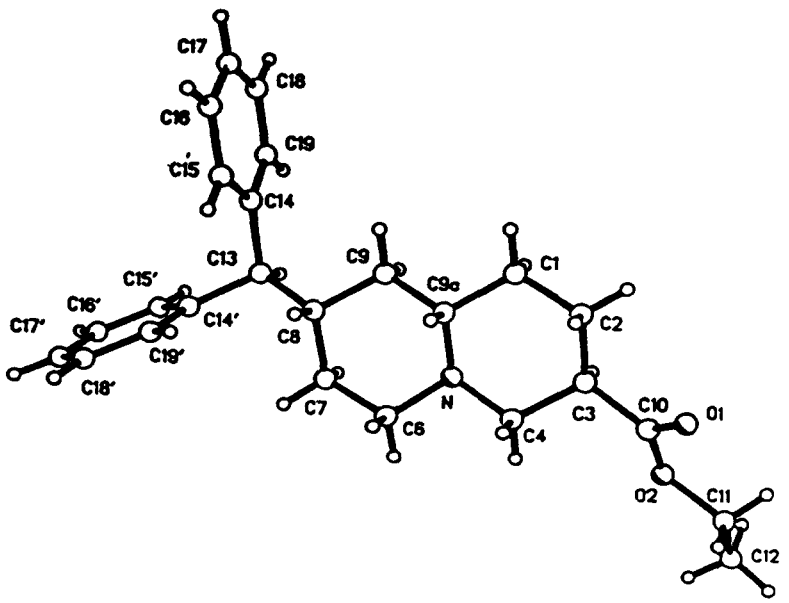
3 5 A

This design locks the conformation of the diphenylbutenyl side chain of SK&F 89976-A. The synthesis of these new quinolizidine analogues based on structure **A** is outlined in Scheme 1.

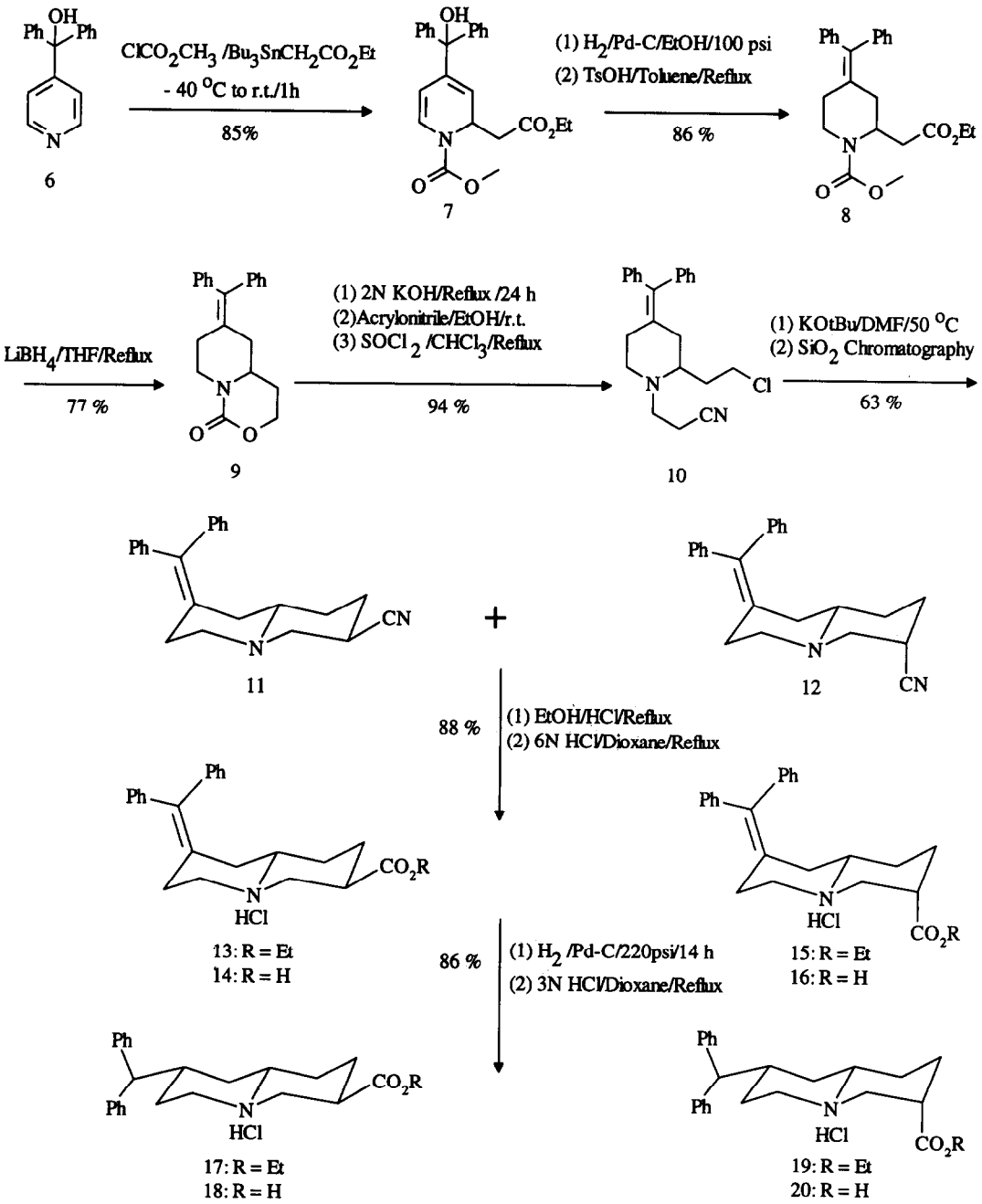
The synthesis starts from commercially available α-(4-pyridyl)benzhydrol (**6**). An important step in the synthesis, is the addition of an acetate group at position 2 of the pyridine ring of **6** (Scheme 1, step 1) to give **7**. This was accomplished using our recently developed method for carboethoxymethylation, employing

ethyl(tributylstannyl)acetate.[14] Hydrogenation followed by elimination of the tertiary hydroxy group of the 1,2-dihydropyridine **7**, gave the diphenylpiperidine **8**. Reduction of the ester group of **8** gave the tetrahydro-oxazinone **9** *via* intramolecular cyclization of the intermediate alcohol onto the carbamate carbonyl. Hydrolysis of the oxazinone with aq. KOH, followed by chlorination of the resulting alcohol with $SOCl_2$ and Michael addition of the γ-aminochloride with acrylonitrile gave **10** in 94% overall yield. Cyclization using potassium *tert*-butoxide in DMF gave good yields of the cyanoquinolizidine's **11** and **12**, which could easily be separated by silica gel flash chromatography. The equatorial orientation of the cyano group on the quinolizidine **11** was evident from the NMR spectrum in which the axial proton on the carbon bearing the cyano group, resonated as a triplet of triplets whereas the same proton in **12** resonated as a broad singlet. This was further confirmed by the X-ray crystal structure of the hydrogenated derivative **17** (*vide infra*). Compound **11** was converted to the ester **13** and hydrolyzed to give the acid **14**. The other diasteromer **12** was converted to the acid **16** in an identical fashion. Diastereomers **13** and **15** were also individually transformed into the amino acids **18** and **20** via hydrogenation of the double bond followed by hydrolysis of the ester. Hydrogenation was expected to be "product development controlled" to give the more stable isomers **17** or **19** in which the orientation of the diphenylmethyl group is equatorial. This was further confirmed by the X-ray crystal structure of **17** (Figure 3).[15]

Figure 3.



Scheme 1.


ClCO2CH3 /Bu3SnCH2CO2Et
- 40 °C to r.t./1h
85%
(1) H2/Pd-C/EtOH/100 psi
(2) TsOH/Toluene/Reflux
86 %
LiBH4/THF/Reflux
77 %
(1) 2N KOH/Reflux /24 h
(2)Acrylonitrile/EtOH/r.t.
(3) SOCl2 /CHCl3/Reflux
94 %
(1) KOtBu/DMF/50 °C
(2) SiO2 Chromatography
63 %
88 %
(1) EtOH/HCl/Reflux
(2) 6N HCl/Dioxane/Reflux
86 %
(1) H2 /Pd-C/220psi/14 h
(2) 3N HCl/Dioxane/Reflux
6
7
8
9
10
11
12
13: R = Et
14: R = H
15: R = Et
16: R = H
17: R = Et
18: R = H
19: R = Et
20: R = H

The inhibitory activity of compounds **14**, **16**, **18** and **20** at cloned GABA transporters is outlined in Table 1.

Table 1.

	IC_{50} (μM)[a]			
Compound	hGAT-1	rGAT-2	hGAT-3	hBGT-1
3	0.13 ± 0.03	550 ± 85	944 ± 110	7210 ± 2500
14	270 ± 25	810 ± 59	3233 ± 459	436 ± 48
16	809 ± 74	757 ± 85	876 ± 81	335 ± 40
18	270 ± 42	669 ± 61	782 ± 100	809 ± 91
20	632 ± 74	477 ± 51	2043 ± 740	2900 ± 1200

The compounds shown were examined for their ability to inhibit uptake of [^{3}H] GABA by each of the cloned GABA transporters as described in ref 11a. In the table, "h" refers to human and "r" refers to rat clone.

As indicated earlier, SK&F 89976-A and compound **5** are potent GABA uptake inhibitors and SK&F 89976-A is specific for the cloned GABA transporter GAT-1. However, Table 1 indicates that the four conformationally restrained analogues **14**, **16**, **18**, and **20** have negligible affinity for GAT-1 and for the other the cloned GABA transporters. It is known from SAR studies, that nipecotic acid derivatives in which the acid group is equatorial are more potent than their axial counterparts.[16] The lack of activity of **16** and **20** can therefore be explained based on this observation. The low potency of **18** is probably because of saturation of the double bond, since derivatives of SK&F 89976-A in which the double bond is hydrogenated are known to be very poor GABA uptake inhibitors. However, the very low potency of **14** is somewhat surprising, suggesting that the lipophilic moiety of this compound is unable to bind to those regions of the transporter with which the lipophilic group of SK&F 89976-A interacts. Based on this finding we speculate that the lipophilic side chain of GABA uptake inhibitors like SK&F 89976-A, Tiagabine and CI-966 bind to the GABA transporter in their fully extended conformation. It is also possible that the side chains of compounds like SK&F 89976-A and compound **5** bind to two different regions of the transporter.

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References and Notes:

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3. Present Address: Trophix Pharmaceuticals Inc., 40 Cragwood Road, South Plainfield, NJ 07080.

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15. X-Ray crystallographic data was obtained from Crystalytics Company, Lincoln, Nebraska. Single crystals of $C_{25}H_{31}NO_2$ are, at 20 ± 1^{0}C, triclinic, space group $P1^- - C^1_i$ (No.2) with $\underline{a}$ = 8.715(2)Å, $\underline{b}$ = 9.883(4)Å, $\underline{c}$ = 13.715(5)Å, α = 107.14(3),^{0} β = 94.63(3),^{0} γ= 99.78(3),^{0} V = 1101.7(6)Å^3, and Z = 2 {d_{calcd} = 1.138gcm^{-3}; μa(MoKα^-) = 0.07 mm^{-1}}. A total of 3027 independent reflections having 2θ (MoKα^-) < 45.8^0 (the equivalent of 0.6 limiting CuKα^- spheres) were collected on a computer - controlled Nicolet autodiffractometer using full (1.30^0 - wide) ω scans and graphite-monochromated MoK$_\alpha$- radiation. The structure was solved using "Direct Methods" techniques with the Siemens SHELXTL-PC software package as modified at Crystalytics Company. The refined fractional atomic coordinates, the bond lengths, bond angles, the hydrogen atom coordinates and thermal parameters have been deposited at the Cambridge Crystallographic Data Center (CCDC).

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