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7-AMINOALKYL-2-AMINO-1,2,3,4-TETRAHYDRO-2-NAPHTHOIC ACIDS AS INHIBITORS OF TRYPTOPHAN UPTAKE

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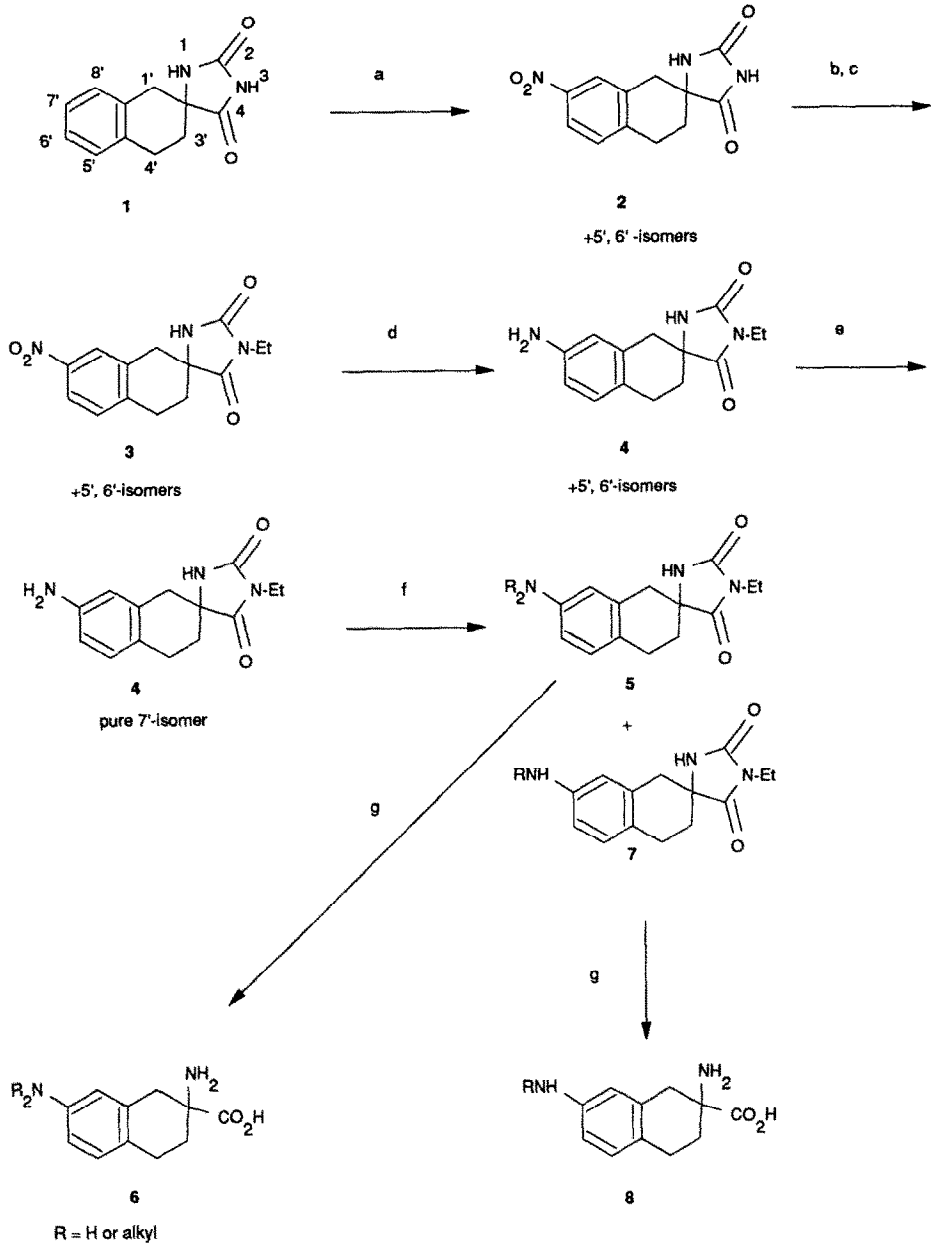
Abstract

A series of non-alkylating DL 7-amino-substituted 2-amino-1,2,3,4-tetrahydro-2-naphthoic acids (**6a-c**, **8a-b**, Table) have been prepared and shown to be potent inhibitors (K_i~μM) of tryptophan uptake in W_iD_f cells.

There are several transport systems involved in the uptake of tryptophan into eukaryotic cells.[1] Amongst these, the L-system is important in several cell types.[1] The L-system is the predominant system responsible for tryptophan transport into tumour cells and may play an important role in the antiproliferative effects of interferon-gamma on tumour cells, mediated by induction of the tryptophan-degrading enzyme indolamine 2,3-dioxygenase.[2] Inhibitors of tryptophan transport would therefore permit the study of the importance of this process in anti-tumour therapy with interferon-gamma. To date the L-system inhibitor DL-2-amino-7-bis [(2-chloroethyl) amino]-1,2,3,4-tetrahydro-2-naphthoic acid **9** has been synthesised in less than 0.7% overall yield.[3] Both this compound and its hydroxyethyl analogue **10** were prepared by us and found to be potent inhibitors of tryptophan uptake in W_iD_f adenocarcinoma cells (Table). Although these two compounds are good inhibitors, they suffer from the disadvantage that their uptake inhibitory properties could in part be due to alkylation of the transporter itself or other cellular proteins.[4] In an attempt to circumvent this drawback, we decided to prepare a series of DL-7-alkylamino-2-amino-1,2,3,4-tetrahydro-2-naphthoic acids which would not be expected to alkylate any such transporter and evaluate their effect on tryptophan uptake.

β-Tetralone-hydantoin **1** (Scheme) was nitrated to give the 7'-nitro-compound **2** and isomers, mp 239-240°C; tlc, 1 spot R_f 0.60, silica gel (MeOH:CH_2Cl_2,1:9)[3,5]. The low solubility of the nitro-hydantoin **2** proved problematical for the next series of reactions and it was therefore alkylated[6] to give the *N*3-ethyl derivative **3** and its isomers, mp 139-142°C; tlc, 2 spots R_f 0.40 and 0.49, alumina (CH_2Cl_2:EtOAc, 2:1). The nitro-compound **3** was reduced with H_2/Pd-C to the amino-compound **4** and isomers, mp 104-106°C; tlc, 2 spots R_f 0.50 (5'-isomer) and 0.37 (6'- and 7'-isomers), silica gel (CH_2Cl_2:EtOAc, 2:1) in approximately equal proportions. Fractional crystallisation of the mixture of the corresponding hydrochlorides (prepared from the bases in CH_2Cl_2-ethereal HCl) from propanol-ether (twice) removed the 5'-isomer and increased the 7'-to 6'-isomer ratio to 2:1. Two further recrystallisations on the free bases using hot EtOH gave the pure 7'-isomer **4**, mp 177-9°C; tlc, 1 spot R_f 0.37, silica gel (CH_2Cl_2: EtOAc, 2:1). The structure of the 7'-isomer was confirmed by ^{1}H NMR (in $CDCl_3$; 360MHz) measurements including a nuclear Overhauser enhancement.[7] The pure 7'-amino-*N*3-ethylhydantoin **4** was next reductively alkylated at the 7'-amino group with the appropriate carbonyl compound and sodium cyanoborohydride[8]. This was carried out in acetonitrile and the carbonyl compounds were formaldehyde, propionaldehyde and acetone, to give the alkylamino-hydantoins **5** and **7** (**5** R=Me, $MeCH_2CH_2$; **7** R=$MeCH_2cH_2$, Me_2CH) respectively. These and the unalkylated hydantoin **4** were hydrolysed either with Ba $(OH)_2$ or with conc. hydrochloric acid (130°C, sealed tube). [3,6,9]. The latter is the preferred mode of hydrolysis. The compounds (Table) were isolated either as hydrochlorides or the zwitterionic form; the latter were produced and purified by precipitation from aqueous solution at neutral pH. All were homogeneous by tlc on silica gel (n-BuOH:AcOH: H_2O, 4:1:1, or $CHCl_3$: MeOH:17% NH_3, 2:1:1), and characterised by ^{1}H NMR and MS/analytical data.

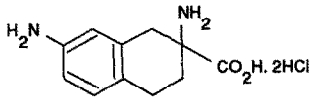
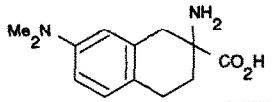
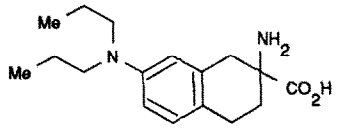
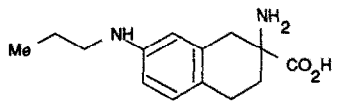
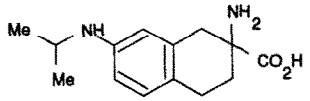
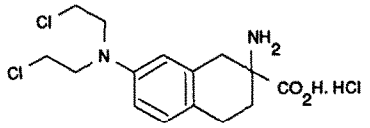
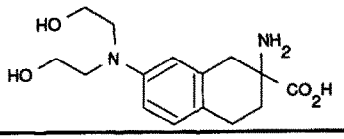
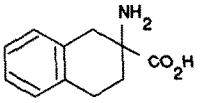


Reagents: (a) HNO_3, rt (91%) (b) NaOH, H_2O-EtOH (91%)
(c) EtBr, KI, EtOH (53%) (d) H_2, Pd-C (61%)
(e) HCl, fractional crystallisation (11%)
(f) $NaBH_3CN$, RCHO or $R_2C{=}O$, CH_3CN (29-51%)
(g) $Ba(OH)_2$ or conc.HCl, Δ (11-78%)

SCHEME

TABLE

Inhibitors of tryptophan uptake, based on 2-amino-1,2,3,4-tetrahydro-2-naphthoic acid.

		K_i Mean ±SEM(μM)
6a		34 ± 4.5
6b		1.4 ± 0.01
6c		7.4 ±1.4
8a		1.7 ± 0.3
8b		0.6 ± 0.1
9		0.8 ± 0.4
10		1.4 ± 0.2
11		37 ± 6.1

The effect of the substituted 2-amino-1,2,3,4-tetrahydro-2-naphthoic acids on tryptophan uptake is summarised in the Table; the result for the unsubstituted example **11** is included for comparison. All the compounds in the Table were competitive inhibitors of the uptake [10] and the K_i values for compounds **6b, 6c, 8a** and **8b** show that these compounds constitute a set of potent inhibitors of tryptophan uptake in this cell system. The activity of compound **8b**[11] is particularly noteworthy; it shows that a compound which is not an alkylating agent, **8b,** can be as potent as compounds that are known to be alkylators, e.g. **9** or that could be hypothesised to be alkylators, e.g. **10.** The inhibition is not, therefore, dependent on alkylation of the transporter protein. These compounds are all acting as inhibitors of L-system transport, since they inhibit tryptophan uptake to the same extent (ca 95%) as the prototype inhibitor of L-systems, BCH,[12] and the potent L-system inhibitor compound **9**[3] in our cell system.

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References and Notes

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4 Zimmerman, J. J.; Feldman, S. *Principles of Medicinal Chemistry*; Foye, W.O., Ed., Lea and Febiger: Philadelphia, 1974; p.15.
5. According to ref. 3, nitration of β-tetralone-hydantoin gives the 5'-and 7'-nitro-derivatives. Our work confirms that the 6'-nitro-derivative is also formed and that the 7':6':5' isomer ratio is 1:0 7:1.
6. Mauger, A. B.; Ross, W. C. J. *Biochem. Pharmacol,*. 1962, **11**, 847.
7. Nuclear Overhauser difference spectra confirmed the 7'-amino substitution pattern of the aromatic ring. Aromatic H resonances were observed at δ 6.34 (d,1H, J_1 = 2Hz, meta-coupling, H-8'), 6.50 (dd, 1H, J_1 = 2Hz, meta-coupling; J_2 = 8.5Hz, ortho-coupling, H-6') and 6.91 (d,1H, J_2 = 8.5 Hz, ortho-coupling, H-5'). Irradiation of the equatorial I'-H signal (δ2.57) showed positive enhancements to its axial geminal partner (δ3.25), the hydantoin NH(δ5.74) and to H-8' (δ6.34). Since the latter shows only meta-coupling, it must be ortho to the NH_2 and hence the amino-group is in the 7'-position. The 7'-isomer 4 was 99.5 ± 0.3% pure by integration of the aromatic H resonances in the ^{1}H NMR spectrum.
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9 Goodson, L. H.; Honigberg, I.L.; Lehman, J. J.; Burton, W. H. *J. Org. Chem.* 1960, **25**, 1920.
10. The uptake of ^{14}C-tryptophan was studied in W_iD_f human adenocarcinoma cells by standard procedures (see refs. 3 and 13) using 5 second incubations ([^{14}C-tryptophan] = 10μM, 100μM) and the effect of the compounds (Table 1) determined at 0-100μM. Dixon plots (see ref.14) were constructed and these demonstrated that the compounds were competitive inhibitors. From the Dixon plots, K_i values were derived and each of the values in the Table is the mean ± SEM of three separate determinations.
11. Compound **8b** had mp >199°C (decomp); tlc, one spot R_f 0.I5 silica gel (n-BuOH:AcOH:H_2O, 4:1:1) Analysis calculated for $C_{14}H_{20}N_2O_2$ I.25H_2O: C,62.08;H,8.37; N,I0.35. Found: C,62.14; H,8 18:N,10.29.
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