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Novel Tryptophan Dioxygenase Inhibitors and combined Tryptophan Dioxygenase/5-HT Reuptake Inhibitors

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Abstract Tryptophan dioxygenase (TDO) is a liver enzyme that is responsible for the majority of the metabolism of tryptophan. A series of novel 3-(2-pyridylethenyl)indoles are shown to be potent inhibitors of TDO with selected members of the series also having 5-hydroxy tryptamine (5-HT) reuptake inhibitory activity. These compounds are shown to provide significant increases in the levels of tryptophan and 5-HT in the cerebrospinal fluid and are thus of interest for antidepressant therapy. Copyright © 1996 Elsevier Science Ltd

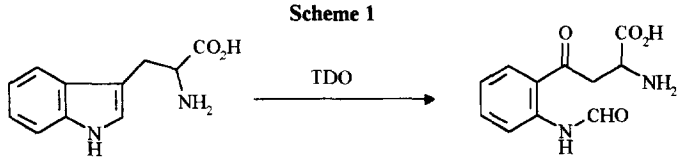
Introduction

In recent years it has become clear that monoamine neurotransmitter dysfunction and depression are closely related, and that effective treatment of depression is associated with an increased function of the serotonergic and noradrenergic pathways in the brain. The role of 5-HT in depression and antidepressant therapy has gained recent prominence with the introduction of the selective serotonin reuptake inhibitors (SSRIs) and these drugs have been shown to be clinically efficacious.[1,2] Evidence supporting a causative role for 5-HT in depression comes from a number of different areas of work and includes: (1) a lowered cerebrospinal fluid and brain level of the 5-HT metabolite, 5-hydroxyindoleacetic acid (5-HIAA), in depressives;[3,4] (2) an elevation of serotonergic function with chronic administration of various types of antidepressant therapy,[5] and (3) a decreased mood or depressive relapse when the 5-HT precursor, tryptophan, is depleted.[6,7]

A problem that remains unresolved by the introduction of the SSRIs, and is shared by other antidepressants, is a slow onset of antidepressant action (typically 2-3 weeks).[2] This delayed onset of action is possibly a result of inhibitory autoregulation (*via* activation of 5-HT_{1A} and 5-HT_{1D} receptors) of the serotonergic pathway following an initial elevation of synaptic 5-HT. Chronic administration of antidepressants would cause the desensitization of these receptors, as well as the 5-HT transporter, allowing a larger increase in synaptic 5-HT.[8] One way to avoid this slow onset of action might be to provide a greater initial elevation of 5-HT. This would result in a faster downregulation of the $5\text{-HT}_{1A/1D}$ receptors, whilst at the same time maintaining synaptic 5-HT at a higher level prior to autoreceptor downregulation. Several strands of clinical evidence support this hypothesis.[9,10]

The controlling enzyme for 5-HT synthesis in the brain, tryptophan hydroxylase, has spare capacity for the processing of its substrate, tryptophan, and it has been shown that raising brain tryptophan levels causes an increase in 5-HT levels in both whole-brain and brain extracellular fluid.[11] Indeed, tryptophan itself has been used for many years as an antidepressant, but its efficacy is limited by its rapid catabolism.[12] The major site of tryptophan catabolism is the kynurenine pathway in the liver, and the key controlling enzyme is tryptophan 2,3-dioxygenase (TDO, Scheme 1).[13] Consequently, inhibition of TDO should decrease the catabolism of systemic tryptophan, raising its concentration not only in the blood but also in the brain, and thereby increase the synthesis and subsequent release of 5-HT.

Scheme 1



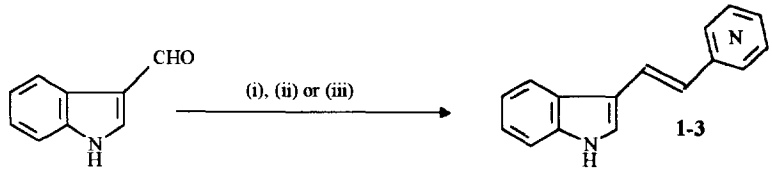
Tryptophan *N*-Formyl kynurenine

Previous *in vitro* studies have identified several inhibitors of TDO but these have been found not to have a pronounced effect on tryptophan levels in rats or humans, presumably because of bioavailability/metabolism problems.[14] In this paper we describe the effects of a series of novel TDO inhibitors, some of which are also potent 5-HT reuptake inhibitors.

Discussion

Our initial studies were carried out using a cell-free assay of TDO, measuring the rate of formation of kynurenine on HPLC.[15,16] Screening of a wide range of structures based around the indole nucleus with a side chain at the 3-position provided a set of ester and amide derivatives (particularly aryl esters and amides) of indole-3-propionic acid and indole-3-acrylic acid, which were moderately potent TDO inhibitors. These compounds suffered rapid metabolism to the parent acid when studied in whole liver cells, but they were sufficiently interesting to suggest investigation of structures based on an indole moiety linked through the 3-position to another, monocyclic, aromatic group. Investigation of a range of linking groups connecting a series of aromatic rings to the indole 3-position identified the *trans*-1,2-ethenyl connection as being optimal. For the purposes of keeping the logP of the compounds low, and generating salt-forming capability, a heterocyclic ring distal to the indole was considered essential. These observations led quickly to our identification of a series of 3-(2-pyridyl)ethenylindoles as TDO inhibitors. The general synthetic procedures employed for these compounds are depicted in Scheme 2.[17] Indole-3-carboxaldehyde underwent a clean condensation reaction with either 2-methylpyridine or 3/4-pyridylacetic acid to provide compounds **1-3** in 40-60% yield after recrystallisation.[18,19] NMR studies revealed that in each case the synthesised materials comprised only the *trans* isomer.[20]

Scheme 2



Reagents:

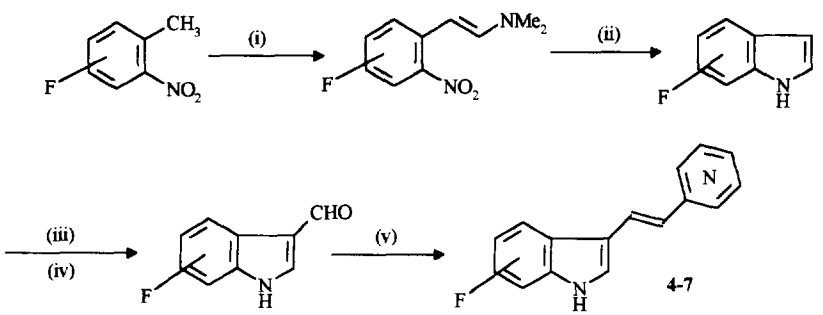
(i) 2-methylpyridine, acetic acid
(ii) 3-pyridylacetic acid, piperidine
(iii) 4-pyridylacetic acid, piperidine

Results:

		TDO Inhibition (Ki, nM)[16]
2-Pyridyl	(1)	100
3-Pyridyl	(2)	40
4-Pyridyl	(3)	30

From these initial results it is clear that the 3- and 4-pyridyl compounds are quite active as TDO inhibitors. It was found that this activity was retained in the whole cell assay and therefore these compounds warranted further investigation. Consequently an extensive SAR study was carried out, looking at the effect of additional substituents on the indole ring, pyridine ring, and the ethenyl linker. Whilst the majority of these substitutions had a detrimental effect on TDO inhibitory activity, particularly if placed on the indole-nitrogen or on the 2-position of the indole ring, it was found that fluorine substitution in the 5 or 6 position of the indole ring was well tolerated. The required substituted indoles were prepared from the corresponding fluoronitrotoluene *via* condensation with dimethylformamide dimethyacetal (DMFDMA) followed by reductive cyclisation with Raney nickel and hydrazine,[21] then formylated under Vilsmeier conditions.[22] The indole-3-aldehydes were then condensed with 3- or 4-pyridylacetic acid by heating in piperidine (Scheme 3).

Scheme 3



Reagents:

(i) DMFDMA, DMF,
(ii) Ni, MeOH, H_2NNH_2,
(iii) DMF, $POCl_3$, (iv) NaOH,
(v) piperidine/3- or 4-pyridylacetic acid

Results:

	TDO Inhibition (Ki, nM)[16]	
	3-Pyridyl	4-Pyridyl
5-Fluoro	**(4)** 50	**(5)** 40
6-Fluoro	**(6)** 30	**(7)** 30

At this point, with a range of potent TDO inhibitors in hand, we decided to look at the effects of our compounds on tryptophan and 5-HT levels *in vivo*. We felt that although whole-brain 5-HT and tryptophan can be measured, a better representation of the key, *synaptic*, levels would be achieved by observing the effect of the compounds on tryptophan and 5-HT levels measured in rat cerebrospinal fluid (CSF). This was achieved using a published procedure; briefly, 24hr-starved rats were dosed with an inhibitor and, at certain time points, CSF was removed from the cerebellomedullary cistern, under sodium pentobarbitone anaesthesia, by exposing and puncturing the atlanto-occipital membrane and removing the CSF by syringe.[23] Tryptophan and 5-HT levels were then determined by HPLC. These experiments revealed that all of the compounds described above, when administered to rats at 10mg/kg (p.o.), were capable of achieving significant levels of tryptophan elevation (around 2.5x basal levels), but a subset of compounds (those with a 4-pyridyl substituent, particularly **3** and **7**) achieved higher than expected increases in CSF 5-HT. Members of this subset of compounds typically achieved a 9-10 fold elevation in CSF 5-HT, whereas the remaining compounds gave a 1.5-1.7 fold elevation in 5-HT.[15,24] Extensive *in vitro* testing of these compounds revealed that they were acting as inhibitors of 5-HT reuptake (IC_{50}=1.5μM for **3**, 0.9μM for **7**; controls were fluoxetine, 0.35μM and fluvoxamine, 0.09μM), in addition to

their activity as TDO inhibitors.[25] Consequently these compounds were capable of elevating brain 5-HT by two separate mechanisms, resulting in the anomalous results described. Compounds **2**, **4** and **6** had negligible activity as inhibitors of 5-HT reuptake. Interestingly, when the SSRI fluoxetine (10mg/kg i.p.) was examined *in vivo* it showed a peak 5-HT elevation in the CSF of 5-fold, but when fluoxetine was co-administered with tryptophan (100mg/kg i.p.) 5-HT elevation was similar to that shown by **7**, at 9-10 fold.[23]

This study has shown that inhibition of tryptophan dioxygenase is a viable method for providing elevated levels of 5-HT and tryptophan in the cerebrospinal fluid. Furthermore, a combined inhibitor of TDO and 5-HT reuptake has been shown to provide much more significant elevation in brain 5-HT, and may therefore be useful in providing a more rapid onset of antidepressent action. This group of compounds may represent a useful new approach towards developing new treatments for depressive diseases.

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16. All measurements of TDO inhibition are an average of at least 4 recordings and are ±5nM. Briefly, rat liver was homogenised in potassium phosphate buffer (pH 7.0) containing tryptophan (1mM) and met-haemoglobin, then centrifuged and the supernatant incubated with the inhibitor at 37°C for 1hr. Perchloric acid was added to stop the reaction and the mixture centrifuged then the supernatant analysed by HPLC (ZORBAX ODS column) for L-kynurenine. See ref. 15 for full details.
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24. All quoted tryptophan and 5-HT elevation figures are a mean of 6 recordings.
25. 5-HT Reuptake was determined in chopped slices of rat cerebral cortex, using 5-[1,2-^{3}H]-HT and measuring uptake on a scintillation counter after incubation with inhibitor. See ref. 23 for full details.

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