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ISATIN OXIMES - A NOVEL SERIES OF BIOAVAILABLE NON-NMDA ANTAGONISTS

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ABSTRACT

New isatin oximes are shown to be highly selective AMPA/kainate antagonists showing no antagonism of NMDA responses. In an AMPA seizure model in mice one of the disclosed compounds has anticonvulsant effects both after i.v. and p.o. dosing.

The basic chemistry on isatins (indole-2,3-diones) was developed a century ago mainly by T. Sandmeyer.[1] A series of 6,7,8,9-tetrahydrobenz[g]-indole-2,3-dione-3-oximes **4a-e** has been found to be ligands for the AMPA/kainate glutamate receptor subtype.

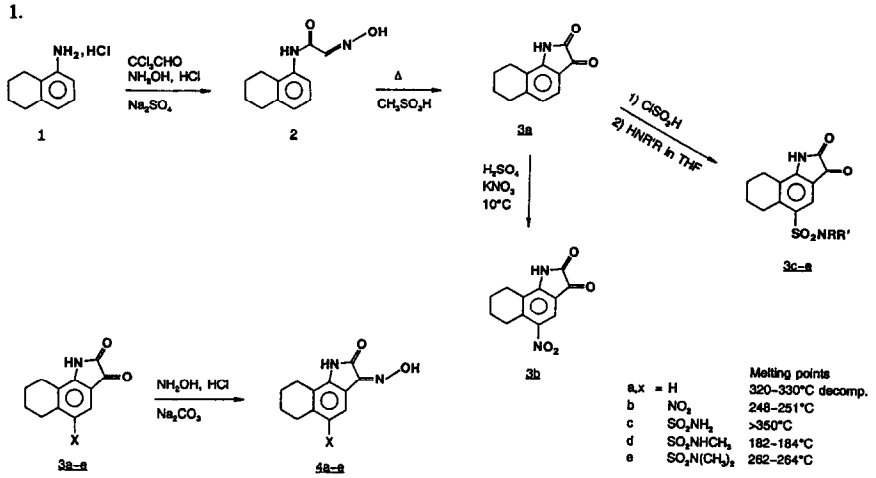
The isatin oximes presented in this work were synthesized according to well known procedures as outlined in scheme 1.

The unsubstituted oxime **4a** was found to be inactive both at the AMPA/kainate (Table 1) and the strychnine insensitive glycine binding site. However, substitution in the 5 position of **4a** with electron withdrawing groups, **4b-e**, gave compounds possessing affinity for the AMPA/kainate receptor. The affinity of the sulphonamides **4c-e** for the strychnine insensitive glycine site was in the low micromolar range (results not shown), considered too low to be of physiological relevance. In functional models in brain slices and cultured neurons (Table 2) compounds **4c-e** effectively blocked AMPA responses but showed no indication of interaction with the NMDA receptor subtype. In vitro the disclosed isatin oximes **4c-e** appeared to be somewhat less potent AMPA antagonists than the quinoxalinediones CNQX and NBQX (Table 1, 2).

The known AMPA antagonists such as the kynurenic acids and the quinoxalinediones are generally very polar compounds and often have low solubility. As a consequence these compounds have poor kinetic properties in animals. Compounds **4b-e** were tested in vivo for the ability to block AMPA induced seizures. **4e** was shown to be a potent blocker of AMPA induced seizures superior to CNQX, NBQX and the isatin oximes **4b-d** (Table 1).

Furthermore, **4e** is the first compound to show AMPA antagonism in animals after oral dosing (ED_{50} = 30 mg/kg, 30 min pretreatment). The favorable in vivo activity of **4e** may be due to the increased lipophilicity of the compound when compared to **4c-d**.

Scheme 1.



The tetrahydronaphthalene **1** was reacted with clorale and hydroxylamine to yield 54% of the isonitrosoacetanilide **2**. The ring closure forming the isatin **3a** was done in methylsulphonic acid at 40°C, isolated yield 96%. Nitration and chlorosulphonation to **3b** and **3c-e** was performed with KNO_3/H_2SO_4 and chlorosulphonic acid (neat) respectively. Finally the oximes **4a-e** were obtained from the isatins by reaction with hydroxylamine in refluxing methanol. Further experimental details are given in reference 2.

Table 1	Inhibition of ^{3}H-ligand binding to cortical membranes from rat brain[3]		Protection against AMPA induced seizures[4]
	IC_{50} (μM) ± SEM		
	^{3}H-AMPA (N = 3)	^{3}H-kainate (N = 3)	ED_{50} (mg/kg)
4a	>30	>30	>30
4b	8.1 ± 1.5	>30	>30
4c	2.8 ± 0.1	24.5 ± 5.8	>30
4d	2.8 ± 0.7	30 ± 6	30
4e	1.8 ± 0.2	27 ± 4.2	3
CNQX	0.33 ± 0.03	2.4 ± 0.9	10
NBQX	0.10 ± 0.03	4.4 ± 0.2	10

Table 2	Inhibition of AMPA and NMDA induced depolarizations in rat cortical wedge preparation[5] and elevation of $[Ca^{2+}]_i$ in cultured mouse cortical neurons[6]			
	Cortical wedge depolarization IC_{50} (μM)		$[Ca^{2+}]_i$ elevation IC_{50} (μM)	
	AMPA	NMDA	AMPA	NMDA
4b	>30	>30	>30	>30
4c	2.5	>30	3.5	>30
4d	5.2	>30	11	>30
4e	3.5	>30	5.4	>30
NBQX	0.3	>30	0.3	>30

REFERENCES AND NOTES

(1) Sumpter, W.C. *Chem. Rev.* **1944**, 393.

2) US Patent application serial number 07/727,479.

3) Honoré, T.; Nielsen, M. *Neurosci. Lett.* **1985**, *54*, 27. Honoré, T.; Drejer, J.; Nielsen, M. *Neurosci. Lett.* **1986**, *65*, 47.

4) Male or female NMRI mice (20–25 g) were used. AMPA was dissolved in distilled water. All the test compounds were prepared as ultrasonically treated suspensions in 5% cremophor and administered i.v. to 10 mice per dose 5 min before a challenging dose of 0.3 μg of AMPA administered icv in a volume of 10 μl. Number of mice having clonic seizures within the next min were recorded.
An ED_{50}-value was determined by graphical interpolation from at least three doses of the test compound as the dose protecting 50% of the mice from having clonic seizures.

5) Depolarizations induced by 2 min exposure to AMPA (5 μM) or NMDA (20 μM) in the rat cortical wedge preparation performed essentially as described by Harrison, N.L.; Simmonds, M.A. *Br. J. Pharmacol.* **1985**, *84*, 381.

6) EAA induced elevation of intracellular calcium was evaluated essentially as described by Wahl P. et al., *J. Neurochem.* **1989**, *53*, 1316. Neurons dissociated from 15–day–old mouse embryo cerebral cortex were cultured in 96–well microtiter plates for 8 days, loaded with the calcium chromophore fluo–3–AM for 1 hour and after extensive wash exposed to 5 μM AMPA or 50 μM NMDA for 4 minutes in the absence or presence of antagonists.

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