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Sodium Channel Activity and Sigma Binding of 2-Aminopropanamide Anticonvulsants

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Abstract: Sodium channel blocking, anticonvulsant activity, and sigma (σ) binding of selected leads in a series of α-amino amide anticonvulsants were examined. While anticonvulsant compounds were always endowed with low micromolar sodium (Na^+) channel site-2 binding, compounds with low site-2 Na^+ channel affinity failed to control seizures. No correlation could be drawn with σ_1 binding. Both anticonvulsant and Na^+ channel blocking activities were independent of stereochemistry, while σ_1 binding seems to be favoured by an S-configuration on the aminoamide moiety.

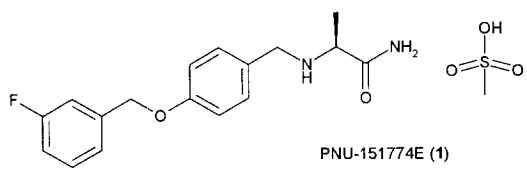
Keywords: *Anticonvulsants; Neurologically active compds; Sodium channel; Sigma receptors*

Epilepsy is a leading neurological disorder in man, currently affecting 50 milion people world-wide. Classical antiepileptic drugs, such as phenobarbital, phenytoin carbamazepine and valproic acid do not produce complete or satisfactory seizure control in roughly 25-30% of the treated patients.[1]

Some novel drugs for epilepsy have recently been marketed (e.g., vigabatrin, lamotrigine, gabapentin, oxcarbazepine, felbamate) and it appears that some of them may be better tolerated than established anticonvulsant drugs.[2]

Despite the variety of drugs now available in the market that permit control of the seizures in the majority of cases, there is still a need for new antiepileptic drugs with more potent and broader spectrum anticonvulsant effect and less toxicity.

In a program aimed at the discovery of new, potent and safe anticonvulsant agents, various 2-aminopropanamides were evaluated and found highly active in physically- and chemically-induced animal seizure models.[3] PNU-151774E (**1**) was the lead compound of this new class of anticonvulsants.[4] Its receptorial profile demonstrated no affinity for GABA receptors or excitatory amino acid (EAA) receptors, but a high affinity for sodium channels and sigma-1 (σ_1) binding sites.[5]



PNU-151774E (**1**)

Although the role of σ binding sites in the CNS remains unclear, there is a considerable evidence supporting their biological relevance.[6,7] In particular, a link between σ activity and inhibition of glutamate (Glu) release has been recently proposed[8-11] and σ binding sites have also been

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involved in the potentiation of kainic acid (KA), an EAA with potent excitotoxic and pro-convulsant properties.[12]

1 was found to inhibit at low micromolar concentrations the binding of ^{3}H-batrachotoxin α-20-benzoate (BTX-B) at site 2 of rat brain sodium channels. Several lines of evidence converge towards an important role of sodium channel blockade in neurologic disorders such as epilepsy and cerebral ischemia.[13-15] Many compounds endowed with anticonvulsant activity (e.g. phenytoin, carbamazepine, lamotrigine, dextromethorphan, riluzole) are thought to display their effect mainly through blockade of the voltage-gated neuronal sodium channels.[16]

In the present work possible correlations between rat brain sodium channel site-2 affinity, σ binding, and anticonvulsant activity in the MES test were examined for four selected leads in the 2-aminopropanamide series (**1,3,5,7**) and their R-enantiomers (**2,4,6,8**) and compounds bearing different spacers between the two phenyl moieties or in the aminoamide part of **1**.

Compounds (**2-12**) were synthesized by reductive alkylation of the suitable optically pure α-amino acid derivatives and aldehyde following a previously described protocol.[3,17]

The affinity of (**1-12**) to σ binding sites (Table 1) did not correlate with their anticonvulsant activity as measured in the MES test. Some of the compounds (i.e., **1** or **5**) displayed a high affinity for the σ_1-site and were potent anticonvulsants. Others (i.e. **4, 10** and **11**) were ineffective σ_1 ligands, while retaining a similar anticonvulsant activity. Since a lack of correlation emerged in this study between σ_1 affinity and anticonvulsant activity of **1-12**, no attempt was made to establish a functional role (agonism or antagonism) for these products at σ receptors. It is interesting to note, however, that σ_1-binding does not affect the safety of the compounds, as judged by a comparison of the rotorod values for **1, 5** and **7**, and for **4, 10** and **11** (Table 1). The lack of effect of **1** at NMDA receptors[5] combined with its nanomolar affinity to σ_1 sites is in agreement with the current view that there is no direct interaction between σ site ligands and NMDA receptors.[18]

All compounds displayed only micromolar affinity for σ_2 binding site.[19] This affinity seems hardly related with their anticonvulsant effect since there is no evidence in the literature of a relationship between micromolar σ_2 binding, a property shared by many different classes of compounds, and neuroprotective effect.[6,20]

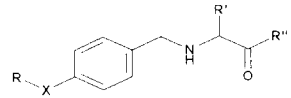
Compounds **1-12** were devoid of affinity to the site 1 of rat brain sodium channels, labeled by ^{3}H-saxitoxin (TTX-site)(Table 1). A broad correlation between the binding of **1-12** to the BTX-site at the α-subunit of rat brain sodium channel and their anticonvulsant activity (Table 1) could be observed. All compounds blocked this channel at micromolar concentrations and exhibited similar anticonvulsant action. Of course different specific bioavailabilities of different compounds makes it difficult to quantitatively compare sodium channel binding and anticonvulsant activity.

It is worthwhile to note that anticonvulsants that are classified among sodium channel blockers (e.g., lamotrigine (LMT) and dextromethorphan (DXM), Table 1) are seemingly acting on the channels in a different manner: DXM is a potent site-2 sodium channel blocker while LMT is much weaker and thus must exert its activity through a different mechanism of blockade. Interestingly compounds **1-10** displayed protective indeces (PIs) consistently higher than the two reference compounds or other proven anticonvulsants.[3,4]

There was no stereoselectivity both in the anti-MES effect and in the sodium channel blocking activity for all compounds. However, a certain degree of stereoselectivity was detectable in the σ_1 binding affinity: the S enantiomers were generally better ligands than the R counterparts.

Table 1

Structure, receptor binding and anticonvulsant activity of compounds **1-12** and reference standards



Cpd	R	X	R'	R''	Cfg	Na^+-Channel Binding[a] IC_{50} (μM)[b]±SEM		σ_1 IC_{50} (nM)[b]±SEM	MES test[d] ED_{50} (mg/Kg)	Rotorod Test[e] (TD_{50})	PI[e] (TD_{50}/ED_{50})
						[^{3}H]-BTX	[^{3}H]-STX	[^{3}H]-PTZ			
1	3-F-Ph	CH_2O	CH_3	NH_2	S	8.2±2.0	>300	19.7±0.7	8.0 (7.0-9.1)	626(557-703)	78.2
2	3-F-Ph	CH_2O	CH_3	NH_2	R	8.5±2.0	>300	280±15	7.2 (5.9-8.9)	584(410-831)	87.8
3	3-F-Ph	CH_2O	CH_2OH	$NHCH_3$	S	26.3±2.9	>300	588±32	13.0(8.8-18.8)	915(725-1358)	70.4
4	3-F-Ph	CH_2O	CH_2OH	$NHCH_3$	R	17.9±2.1	>300	6,600±400	6.4(4.7-8.7)	593(468-652)	92.6
5	Ph	$(CH_2)_3O$	CH_3	NH_2	S	13.2±4.2	>300	19.3±0.9	6.7(5.1-8.8)	584(410-831)	87.2
6	Ph	$(CH_2)_3O$	CH_3	NH_2	R	7.8±1.6	>300	280±21	12.6(7.1-32.4)	1284(912-1808)	101.9
7	3-F-Ph	CH_2NH	CH_3	NH_2	S	13.9±2.2	>300	34.4±2.9	6.6(4.3-10.0)	583(523-651)	88.3
8	3-F-Ph	CH_2NH	CH_3	NH_2	R	11.3±2.2	>300	280±18	1.7(1.0-2.8)	272(221-333)	160.0
9	Ph	$(CH_2)_4O$	CH_3	NH_2	S	1.1±0.2	>300	550±44	12.4 (8.4-18.2)	1005 (783-2050)	81.0
10	Ph	$(CH_2)_5O$	CH_3	NH_2	S	1.4±0.3	>300	>10,000	12.5(0.34-40.6)	1011 (784-3184)	80.8
11	Me	O	CH_3	NH_2	S	145.8±16.9	>300	>10,000	>100	nd	nd
12	Ph	CH_2O	CH_3	OH	S	150.8±18.3	>300	nd	>100	nd	nd
Lamotrigine						185.9±23.7	>100	>10,000	2.2(1.3-3.8)	84(75-95)	38.2
Dextromethorphan						1.3±0.2	>100	nd	12.9 (8.4-16.5)	nc[f]	nc[f]

a)[^{3}H]-Batrachotoxin ([^{3}H]-BTX) and [^{3}H]-Saxitoxin ([^{3}H]-STX) binding to rat brain sodium channels were carried out according to Catterall.[21] b) Rat membranes were incubated with different radioligands in the presence of 9-10 concentrations of the molecules to be tested (10^{-9} - $3x10^{-4}$) c) [^{3}H]-(+)-Pentazocine ([^{3}H]-PTZ) binding to σ_1-sites was performed as described by De Costa *et al*[22] with slight modifications. d) Maximal electroshock seizures (MES) were done using the procedure described by White *et al.*[23] An Ugo Basile electroconvulsometer (Model ECT UNIT 7801) was used to deliver an electrical stimulus sufficient to produce a hindlimb tonic extensor response in at least 97% of control animals. The stimulus was delivered intra-aurally through clip electrodes in mice (0.7 s of a 28 mA shock, with a pulse train of 80 Hz having a pulse duration of 0.4 ms). The acute effects of compounds administered 60 min orally (po) before MES induction were examined. Complete suppression of the hindlimb tonic extensor component of seizures was taken as evidence of anticonvulsant activity. e) Psychomotor side-effects of the compounds were examined by rotorod. Mice trained to the rotorod (Ugo Basile; 10 rpm) were used. The compounds were administered 60 min before the test and the number of animals falling over 1-2 min test period were monitored.[24] The toxic dose causing 50% of animals treated to fall from the rotorod (TD_{50}) of each compound was calculated. This dose was combined with the effective dose of each anticonvulsant in the MES test necessary to protect 50% of the animals treated (ED_{50}) in order to calculate protective indices (PI) of each compound. The PI is defined as the ratio of the TD_{50} in the rotorod test over the ED_{50} in the anticonvulsant test. d) Rotorod IC_{50} and TI could not be calculated for dextromethorphan because of significant mortality (LD_{50}: 234 mg/kg) already at doses that did not cause rotorod impairment of 50% of the animals.

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