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CCC: 0377-8282/2001

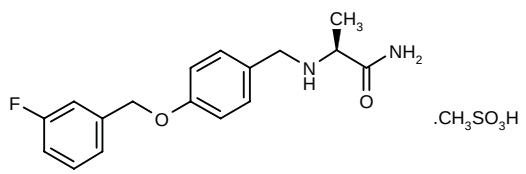
Safinamide Mesilate

Prop INNM

Antiepileptic
Antiparkinsonian

NW-1015
PNU-151774E
FCE-26743

2(*S*)-[4-(3-Fluorobenzyloxy)benzylamino]propionamide methanesulfonate



$C_{17}H_{19}FN_2O_2.CH_4O_3S$ Mol wt: 398.4527

CAS: 202825-46-5
CAS: 133865-89-1 (as free base)

EN: 169540

Synthesis

Safinamide has been obtained by reductocondensation of 4-(3-fluorobenzyloxy)benzaldehyde (I) with L-alaninamide (II) by means of sodium cyanoborohydride in methanol (1-3). Scheme 1.

Introduction

Epilepsy is a leading neurological disorder characterized by recurrent unprovoked seizures. It affects at least 50 million people worldwide and approximately 2.3 million individuals in the U.S. Epilepsy is slightly more prevalent in men than in women although race and lifestyle have no influence. Of all the newly diagnosed cases, 50-75% develop before the age of 18 and approximately 50% of all childhood cases resolve completely during early adulthood. However, prevalence increases again after the age of 65, when new cases develop and are often concomitant with neurodegenerative, cerebrovascular or neoplastic disease (4).

The seizures characteristic of epilepsy are due to transient, paroxysmal, synchronous discharges of a large population of hyperexcitable neurons in part or all of the brain. A seizure is defined as a change in sensation, awareness or behavior and it may be convulsive (*e.g.*, tonic myoclonic, tonic clonic) or nonconvulsive (*e.g.*, petit mal) in which suspension of consciousness without motor phenomenon is seen. More than 40 different types of epilepsy exist and classification of the disorder is based on etiology, idiopathic epilepsies and cryptogenic epilepsies. Complex partial, generalized tonic-clonic and simple partial seizures account for 35, 25 and 15% of all cases, respectively. No cause can be attributed to 65-75% of the cases in children and 50% of all adult cases. However, vascular disease (10-11%), hereditary (8%), trauma (5-6%), tumors (4%), degenerative diseases (3-4%) and infections (3%) are common factors that can provoke epilepsy; stroke, poisoning (*i.e.*, alcoholism), systemic illness of the mother during pregnancy and some dietary (*e.g.*, aspartame, some foods) and environmental factors can also trigger the disease (4).

Pharmacotherapy is the mainstay treatment for epilepsy and the selection of antiepileptic drugs (AEDs) for a particular patient is achieved according to seizure type. AED therapy does not cure the disease but instead can control the condition by decreasing the frequency, duration and severity of seizures. In general, the efficacy of AEDs is due to 4 main activities which include potentiation of inhibitory mechanisms (*i.e.*, GABAergic transmission), inhibition of excitatory mechanisms (*i.e.*, glutaminergic transmission), inhibition of excessive firing of neurons and inhibition of T-type calcium and

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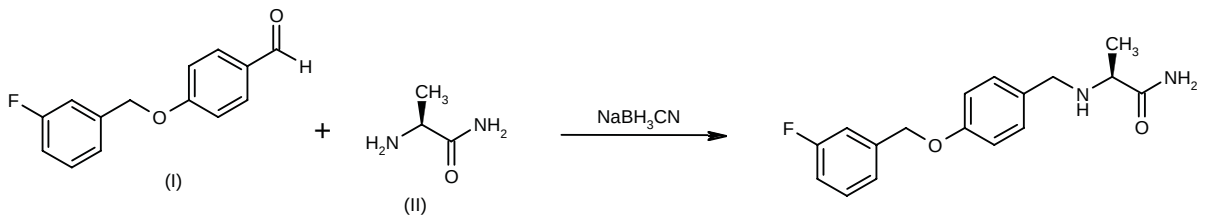
Scheme 1: Synthesis of Safinamide



voltage-gated sodium channels. The current established AEDs are phenytoin, carbamazepine, valproic acid, clobazam, phenobarbital and ethosuximide (4).

In general, since AEDs are tested on the basis of seizure type rather than etiology, novel agents are discovered through conventional screening and/or structure modification rather than through a mechanism-driven design. As a result, most available agents have multiple actions, which may account for the efficacy and the fact that the exact mechanisms of many AEDs are unknown. This is true for several standard AEDs such as clomethiazole, oxcarbazepine and felbamate in addition to several other agents (4). However, none of the currently available AEDs are ideal and most are used as add-on therapies to existing standard therapy and can be associated with chronic and acute adverse effects. Hence, the search for new AEDs continues.

In an attempt to discover new, potent and safe anticonvulsant agents, a lead compound safinamide mesilate (NW-1015; PNU-151774E) emerged. It has no affinity for GABA receptors or excitatory amino acid receptors but exhibits high affinity for sodium channels and sigma-1 binding sites. Safinamide has also been shown to be a calcium antagonist and monoamine oxidase (MAO)-B and glutamate release inhibitor. Due to its broad spectrum of action demonstrated *in vitro* and its *in vivo* anticonvulsant activity, safinamide was chosen for further development as a treatment for epilepsy and as a potential therapy for motor dysfunction associated with Parkinson's disease (1, 3-6).

Pharmacological Actions

Several *in vitro* studies have reported various mechanisms of action for safinamide that may be in part responsible for the anticonvulsive activity exhibited by the agent *in vivo*.

Results from *in vitro* studies examining the ability of safinamide to displace binding of [^{3}H]-batrachotoxin (BTX; site-2 of the Na^+ channel) or [^{3}H]-saxitoxin (STX; site-1 of the Na^+ channel) to Na^+ channels in rat membranes showed that the agent exhibited a high affinity for site-2 (IC_{50} = 8.2 ± 2 μM) and no affinity for site-1 (IC_{50} = > 300 μM). Further *in vitro* binding experiments revealed that safinamide displaced [^{3}H]-(+)-pentazocine binding to σ-1 sites in rat striatal membranes (IC_{50} = 0.0197 ± 0.7 μM), indicating a high affinity for this receptor type which was approximately 100-fold greater than that seen for σ-2 sites from whole brain preparations (IC_{50} = 1.59 μM; ligand = [^{3}H]-DTG). Safinamide had negligible affinity (IC_{50} = > 100 μM) for several other receptor types including NMDA, NMDA-gated channel, kainate, quisqualate, GABA-A, benzodiazepine and dopamine D-1 and D-2 (6, 7).

Data obtained from electrophysiological studies using rat hippocampal neuronal cultures demonstrated that the anticonvulsive mechanism of action of safinamide may, in part, be via inhibition of Na^+ and Ca^{2+} channels, stabilization of neuronal membrane excitability and/or inhibition of transmitter release. The compound (100 μM) was shown to reversibly decrease sustained repetitive firing of hippocampal neurons with no effects on the first action potential. Moreover, safinamide (10-200 μM) potently and dose-dependently inhibited tetrodotoxin-sensitive fast Na^+ channels (30% inhibition with 100 μM) and dihydropyridine-sensitive high voltage-activated calcium currents (40% inhibition with 100 μM). Although safinamide (100 μM) inhibited whole-cell peak Ca^{2+} inward currents in single cultured mouse cortical neurons, it had no effect on GABA (1 μM)- or glutamate (10 μM)-evoked currents. However, veratrine- and KCl-induced glutamate release from rat hippocampal slices was potently inhibited by the agent (IC_{50} = 56.4 and 185.5 μM, respectively (7).

The ability of safinamide to inhibit MAO-B was demonstrated both *in vitro* using rat brain and liver homogenates and *ex vivo* in rats following a single oral dose (1, 5, 10 and 60 mg/kg). Safinamide potently inhibited brain MAO-B (IC_{50} = 0.17 ± 0.1 μM) but was only weakly active against MAO-A (IC_{50} = 370 ± 0.35 μM). Similarly, safinamide inhibited liver MAO-B with IC_{50} values of 0.25 ± 0.10, 0.14 ± 0.06 and 0.13 ± 0.04 μM after 2, 60 and 120 min of incubation, respectively. Further studies using rat liver microsomes showed that the agent had no effects on regio- or stereoselective testosterone hydroxylation, indicating no induction of the cytochrome P450 system. Results from *ex vivo* experiments showed

that the agent at doses of 5 mg/kg and higher potently (86% with 5 mg/kg) and selectively inhibited brain homogenate MAO-B. Moreover, brain levels of the agent were found to be higher than plasma levels following oral dosing (10 mg/kg) with a brain/plasma concentration ratio of 20 obtained at 1 h postdosing. Brain levels decreased biexponentially thereafter with first and second half-life values of 0.9 and 9.6 h, respectively; plasma levels decreased monoexponentially with a half-life of about 0.9 h (8, 9).

The potent anticonvulsive efficacy of safinamide has been demonstrated in a number of *in vivo* electrically and chemically induced seizure models. Safinamide prevented maximal electroshock seizures (MES) in both mice (ED_{50} = 8.0 mg/kg p.o.; 4.1 mg/kg i.p.) and rats (ED_{50} = 11.8 mg/kg p.o.; 6.9 mg/kg i.p.). Repeated safinamide dosing (15.4 and 20 mg/kg p.o. for 4 days) of mice in the MES test did not alter the anticonvulsant activity of the agent, indicating a lack of tolerance for the agent. Results from chemically induced (bicuculline [0.6 mg/kg i.v.], picrotoxin [6 mg/kg s.c.], 3-mercaptopropionic acid [60 mg/kg s.c.], pentylenetetrazole [85 mg/kg s.c.] and strychnine [0.55 mg/kg i.v.]) models of grand mal seizures in mice also demonstrated the potent anticonvulsive activity of safinamide, with ED_{50} values of 26.9, 60.6, 21.5, 26.8 and 104.1 mg/kg p.o. obtained, respectively. Although safinamide (8.4 and 22 mg/kg i.p.) was not effective in altering the threshold for minimal seizure induced by i.v. infused pentylenetetrazole in mice, it showed no proconvulsant activity. Furthermore, safinamide had a low propensity to cause locomotor or cognitive side effects. The potential for safinamide to induce pscychomotor effects was low since high doses of the agent were required for ataxia to occur in the rotorod test in mice (toxic dose causing 50% of the animals to fall [TD_{50}] = 700 mg/kg p.o.). In addition, no significant changes in spontaneous horizontal or vertical locomotor activity was seen in rats after dosing with up to 400 mg/kg p.o. The agent administrated at 40 times the oral ED_{50} value (400 mg/kg) to rats did not impair passive avoidance responses (3, 6, 10).

In addition to being effective in the above models of generalized seizures, safinamide was also shown to be effective in more specific models of complex partial seizures such as an amygdala fully kindled rat model, a kainate-induced multifocal status epilepticus rat model of medically intractable complex partial seizures and neurotoxicity and a primate model of complex partial seizures. In the amygdala kindled model, treatment with the agent (1, 10 and 30 mg/kg i.p.) beginning after reproducible afterdischarge thresholds and seizures were obtained, resulted in a significant decrease in the duration of behavioral seizures (1 mg/kg i.p.) and significant reductions in seizure severity and afterdischarge duration (10 and 30 mg/kg i.p.); similar effects were observed with administration of lamotrigine and gabapentin, although higher doses of carbamazepine (30 and 60 mg/kg i.p.) and phenytoin (50 mg/kg i.p.) were required to suppress seizures and effects were not dose-dependent and were accompanied by sedation (11).

Safinamide pretreatment (10 and 30 mg/kg i.p. 15 min before kainate) was effective in the kainate-induced status epilepticus model, with significant 57 and 47% reductions, respectively, seen in the number of rats showing status epilepticus. Safinamide (30 mg/kg) pretreatment also significantly increased the latency and shortened the duration of status epilepticus. Moreover, safinamide pretreatment resulted in neuroprotective effects, with a significant increase in survival of CA4 hippocampal neurons observed as compared to a 66% loss seen at 7 days after treatment with kainic acid (12).

The efficacy of safinamide in preventing complex partial seizures (electrically induced afterdischarge in the limbic areas) was demonstrated in cynomolgus monkeys. The mean plasma levels of the agent following single oral doses (25, 50 and 75 mg/kg) peaked at 2 h postdosing and $AUC_{0\text{-}t2}$ values were found to increase with dose. Safinamide (50 and 75 mg/kg) significantly decreased EEG afterdischarge duration at 2 and 3 h postdosing, with effects sustained at 6 h postdosing. Treatment with the agent (25, 50 or 75 mg/kg) also significantly and dose-dependently attenuated electrically stimulated behavioral abnormalities. Similar effects on afterdischarges were observed at 3 and 6 h postdosing with phenytoin (50 mg/kg), although the effects on behavioral abnormalities were to a lesser extent. Moreover, results from this study also indicated a good safety profile for safinamide since doses up to 75 mg/kg did not elicit EEG or interictal behavioral alterations (*i.e.*, psychotic reactions) as do other agents acting at the σ-1 receptor (13).

Safinamide has been shown preclinically to be effective for other indications including Parkinson's disease, acute stroke and neuropathic pain.

The antiparkinsonian activity of safinamide was demonstrated from the results of 3 studies using mouse, rat and primate models of Parkinson's disease. In mice treated with MPTP (40 mg/kg s.c. x 2 separated by a 24-h interval), safinamide (10-40 mg s.c.) administered 30 min before MPTP completely prevented loss of neostriatal dopaminergic neurons. This sparing effect was not observed when safinamide was given 4 h after MPTP, although a significant sparing of thyrosine hydroxylase positive neurons in the pars compacta of the substantia nigra was observed (14). When animals were concomitantly treated with acute subthreshold (5 mg/kg) or chronic suprathreshold doses of L-dopa (20 mg/kg/day s.c. for 5 days), safinamide (1, 3 or 10 mg/kg s.c. 60 min before L-dopa and behavioral testing for acute and chronic studies, respectively) dose-dependently increased locomotion and rearing behavior in hypokinetic MPTP (administered 4-9 weeks prior to behavioral testing)-treated mice; lamotrigine and L-deprenyl but not phenytoin had comparable effects. Since neither L-dopa nor safinamide alone reinstated motor activity in MPTP-treated mice, it was concluded that the effects observed with combination treatment were synergistic (15).

The efficacy of safiamide in combination with L-dopa was further demonstrated in a study using rats with 6-OHDA-induced unilateral lesions of the medial forebrain bundle. The efficacy (*i.e.*, duration of postinjection contralateral turning) of daily L-dopa injections was found to decrease after 4 weeks of treatment. However, administration of safinamide (20 mg/kg i.p.) reinstated the efficacy of L-dopa (14).

Chronic studies in primates showed that a 12-week treatment with 10 or 20 mg/kg/day p.o. safinamide significantly increased dopamine in the putamen by 27 and 48%, respectively, as compared to controls and decreased DOPAC by 19 and 41%, respectively. The effects were accompanied by a 50 and 60% inhibition, respectively, of brain MAO-B (14).

The neuroprotective effects of safinamide were demonstrated in a study using a gerbil model of forebrain ischemia (bilateral carotid occlusion), suggesting that the agent may be effective in the treatment of acute stroke. At 7 days postocclusion, ischemia-induced hippocampal CA1 neuronal cells loss was significantly prevented (12 *vs.* 95% damage) with safinamide treatment (100 mg/kg i.p. 30 min before and after occlusion); phenytoin was only partially effective (72 *vs.* 95% damage). Moreover, safinamide but not phenytoin significantly abolished ischemia-induced passive avoidance impairment, indicating cognitive protective effects (16).

Finally, safinamide was shown to have antinociceptive properties in the formalin mouse model of tonic pain. Safinamide (15-60 mg/kg p.o.) significantly and dose-dependently decreased cumulative licking time in the acute (160.2 ± 2.6 to 63.1 ± 2.6 s) and late (74.8 ± 3.7 to 38.1 ± 3.6 s) phases of the formalin test; neither locomotor activity nor coordination were altered by treatment. In contrast, safinamide (up to 120 mg/kg p.o.) showed only limited efficacy in the hot-plate and tail-flick rodent models of acute pain (17).

Clinical Studies

The pharmacokinetics and tolerability of single-dose safinamide (2.5, 5 and 10 mg/kg p.o.) were examined in a placebo-controlled, double-blind trial conducted in 8 healthy male volunteers. The C_{max} (ng/ml), $AUC_{0-\infty}$ (ng·ml/h) and $t_{1/2}$ (h) values obtained for the 3 doses (respectively) were 1256, 32492 and 21; 2943, 77803, and 20; and 6316, 166134 and 23. All doses caused complete and sustained suppression of MAO-B activity in platelet enriched plasma fraction samples from subjects, with no effects on MAO-A; an increase in urinary PEA was observed with the 5 and 10 mg/kg doses. Treatment was well tolerated with no significant adverse events observed. Subjective sensations of light-headedness, somnolence, transient headache and a cool sensation in the distal tongue were reported. Results from this study were confirmed in other repeated-dose (1.25-5 mg/kg) trials in healthy volunteers (18, 19).

A study conducted in 6 healthy volunteers further characterized the effects of single-dose safinamide (25, 50, 75, 150, 300 and 600 mcg/kg p.o.) on MAO-B inhibition. Significant and dose-related inhibition of MAO-B in platelet enriched plasma fractions of subjects was observed starting at a dose of 75 mcg/kg (67% inhibition). Recovery was observed 24 h postdosing (19).

A phase I trial conducted in 32 healthy male volunteers showed the safety and tolerability of single-dose safinamide (0.05-10 mg/kg p.o.). Treatment was well tolerated. Dose-dependent inhibition of MAO-B was observed starting with a dose of 0.5 mg/kg; no effects on MAO-A were observed. Peak plasma levels were obtained at 1.8-2.8 h postdosing after which they decreased, with t1/2 values between 20.2 and 23.4 h (20).

Safinamide is currently undergoing phase II trials for both epilepsy and Parkinson's disease (21).

Manufacturer

Newron Pharmaceuticals SpA (IT).

References

1. Dostert, P., Pevarello, P., Heidempergher, F., Varasi, M., Bonsignori, A., Roncucci, R. (Pharmacia Corp.). *N-Phenylalkyl substd. alpha-amino carboxamide derivs. and process for their preparation.* EP 0400495, EP 0426816, JP 1992500215, US 5236957, US 5391577, US 5502079, WO 9014334.

2. Varasi, M., Bonsignori, A., Pevarello, P., Dostert, P. *Synthesis and anticonvulsant activity of new benzyloxybenzylacetamide derivatives.* 12th Int Symp Med Chem (Sept 13-17, Basel) 1992, Abst P-089.C.

3. Pevarello, P., Bonsignori, A., Dostert, P. et al. *Synthesis and anticonvulsant activity of a new class of 2-[arylalkyl)amino]alkanamide derivatives.* J Med Chem 1998, 41: 579-90.

4. Prous Science Drug R&D Backgrounders: *Epilepsy (online publication).* Updated June 26, 2001.

5. Prous Science Drug R&D Backgrounders: *Parkinson's disease (online publication).* Updated June 26, 2001.

6. Pevarello, P., Bonsignori, A., Caccia, C., Amici, R., McArthur, R.A., Fariello, R.G., Salvati, P., Varasi, M. *Sodium channel activity and sigma binding of 2-aminopropanamide anticonvulsants.* Bioorg Med Chem Lett 1999, 9: 2521-4.

7. Salvati, P., Maj, R. Caccia, C. et al. *Biochemical and electrophysiological studies on the mechanism of action of PNU-151774E, a novel antiepileptic compound.* J Pharmacol Exp Ther 1999, 288: 1151-9.

8. Strolin Benedetti, M., Marrari, P., Colombo, M., Castelli, M.G., Arand, M., Oesch, F., Dostert, P. *The anticonvulsant FCE 26743 is a selective and short-acting MAO-B inhibitor devoid of inducing properties towards cytochrome P450-dependent testosterone hydroxylation in mice and rats.* J Pharm Pharmacol 1994, 46: 814-9.

9. Strolin Benedetti, M., Tocchetti, P., Rocchetti, M., Martignoni, M., Marrari, P., Poggesi, I., Dostert, P. *Enantioselective recogni-*

tion of two anticonvulsants, FCE 26743 and FCE 28073 by MAO, and relationship between MAO-B inhibition and FCE 26743 concentrations in rat brain. Prog Brain Res 1995, 106: 123-34.

10. Fariello, R.G., McArthur, R.A., Bonsignori, A. et al. *Preclinical evaluation of PNU-151774E as a novel anticonvulsant.* J Pharmacol Exp Ther 1998, 285: 397-403.

11. Maj, R., Fariello, R.G., Pevarello, P., Varasi, M., McArthur, R.A., Salvati, P. *Anticonvulsant activity of PNU-151774E in the amygdala kindled model of complex partial seizures.* Epilepsia 1999, 40: 1523-8.

12. Maj, R., Fariello, R.G., Ukmar, G., Varasi, M., Pevarello, P., McArthur, R.A., Salvati, P. *PNU-151774E protects against kainate-induced status epilepticus and hippocampal lesions in the rat.* Eur J Pharmacol 1998, 359: 27-32.

13. Fariello, R.G., Maj, R., Marrari, P., Beard, D., Algate, C., Salvati, P. *Acute behavioral and EEG effects of NW-1015 on electrically-induced afterdischarge in conscious monkeys.* Epilepsy Res 2000, 39: 37-46.

14. Maj, R., Caccia, C., Lamberti, E., Calabresi, M., Benatti, L., Salvati, P., Fariello, R.G. *Safinamide (NW-1015): Experimental evidence of preventive and therapeutic antiparkinson properties.* 5th Int Conf Prog Alzheimer Parkinson Dis (March 31-April 5, Kyoto) 2001, Abst V-PD-50.

15. Fredriksson, A., Palomo, T., Archer, T. *Effects of co-administration of anticonvulsant and putative anticonvulsive agents and sub/suprathreshold doses of L-dopa upon motor behaviour of MPTP-treated mice.* J Neural Transm 1999, 106: 889-909.

16. Vaghi, F., Maj, R., Rosa, B., Ukmar, G., Lamberti, E., McArthur, R.A., Varasi, M., Salvati, P., Post, C. *Neuroprotective effect of PNU-151774E, a new anticonvulsant compound, in the model of global ischaemia in gerbils.* Soc Neurosci Abst 1997, 23(Part 1): Abst 212.9.

17. Salvati, P., Maj, R., McArthur, R.A., Cervini, M.A., Kozak, W., Benatti, L., Fariello, R.G. *PNU-151774E, a novel Na^+ channel blocker shows analgesic effects in some animal models.* Soc Neurosci Abst 1999, 25(Part 2): Abst 773.1.

18. Marzo, A., Dal Bo, L., Crivelli, F., Ceppi Monti, N., Ismaili, S., Fariello, R., Uhr, M.R. *Pharmacokinetics, pharmacodynamics and tolerability of NW-1015, a new anticonvulsant agent.* 5th Eilat Conf New Antiepileptic Drugs (June 25-29, Eilat) 2000, 62.

19. Caccia, C., Tocchetti, P., Dal Bo, L., Lamberti, E., Marzo, A., Salvati, P., Fariello, R.G. *Safinamide (NW-1015): Effects on platelets MAO-B activity in healthy volunteers.* 5th Int Conf Prog Alzheimer Parkinson Dis (March 31-April 5, Kyoto) 2001, Abst V-PD-51.

20. Fariello, R.G., Maj, R., Caccia, C., Benatti, L., Salvati, P. *NW-1015 a novel potential antiepileptic drug: Preclinical and early clinical development.* 5th Eilat Conf New Antiepileptic Drugs (June 25-29, Eilat) 2000, 38.

21. *Products/projects portfolio.* Newron Pharmaceuticals Web Site July 2, 2001.

Additional References

Maj, R., Breda, M., Antongiovanni, V., Bonsignori, A., Varasi, M., McArthur, R. *Anticonvulsant activity of FCE 26743 in the kindling model of epilepsy.* Pharmacol Res 1995, 31(Suppl.): 346.

Pevarello, P., Caccia, C., Fornaretto, M.G., Salvati, P., Varasi, M. *Stereoselectivity, σ binding and sodium channel blocking activity of 2-aminopropanamide anticonvulsants.* 14th Int Symp Med Chem (Sept 8-12, Maastricht) 1996, Abst P-6.49.

Savati, P., Algate, C., Beard, D. et al. *Anticonvulsant profile of FCE 26743, a novel 2-aminopropionamide derivative.* Soc Neurosci Abst 1996, 22(Part 3): Abst 825.16.

Kozak, W.A., Cervini, M.A., McArthur, R.A., Post, C., Salvati, P. *Analgesic activity of PNU-151774E, a novel anticonvulsant with sodium channel blocking properties.* Behav Pharmacol 1998, 9(Suppl. 1): S52.