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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Substituted 2-[2-(pyridin-3-yl) phenyl] acetamides and ureas: design, synthesis, and anticonvulsant screening in mice

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Abstract Five novel 2-[2[(pyridin-3-yl) phenyl] acetamides and ureas were designed based on a two-point pharmacophore, synthesized by improved methods, and screened for anticonvulsant activity in maximal electroshock seizure (MES) and ScPTZ-induced seizure models in Swiss mice at doses of 30, 100, and 300 mg/kg body weight. Compound **15** displayed potent anticonvulsant activity in MES test with ED_{50} value of 8.28 mg/kg and higher protective index ($TD_{50}/ED_{50} = 26$) compared with Phenytoin, which can be considered as a promising lead.

Keywords Phenylacetamides · Phenylacetylureas · Pyridinylphenylacetamides · MES test · ScPTZ test

Introduction

Epilepsy is one of the most frequent neurological afflictions in man characterized by excessive temporal neuronal discharges resulting in uncontrolled convulsions. It is estimated that there are ~50 million people with epilepsy worldwide (Duron *et al*., 2005; Loscher, 1998). Marketed anticonvulsants suffer from a broad range of adverse effects such as ataxia, gingival hyperplasia (Phenytoin), vertigo, rash (Carbamazepine), and Stevens–Johnson syndrome (Lamotrigine) (Linn and Kadaba, 1997; Perucca, 1996). A significant group of patients (20–30%) are resistant to the currently used therapeutic agents (Kwan *et al*., 2000). Thus, the need for more efficacious drugs is imperative.

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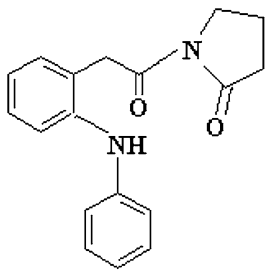
Inhibition of neuronal cation conductance is a proven mechanism wherein drugs like Phenytoin and Carbamazepine act to control seizures (Taylor, 1996). In our previous study, a series of derivatives of anilinophenylacetamides were found to have anticonvulsant activities among which 1-{[2-(Phenylamino) phenyl] acetyl}-2-pyrrolidinone (**14**) showed the strongest activity with a median effective dose (ED_{50}) value of 8.0 mg/kg in maximal electroshock seizure (MES) test and protective index (PI) (ED_{50}/TD_{50}) value of 87.5, which was much greater than the PI value of the prototype drug Phenytoin (Shindikar *et al*., 2006). These findings prompted us to find more molecules possessing better therapeutic action.



(**14**)

Phenylacetamides and related compounds are known to possess wide range of biological activities (Almasirad *et al*., 2006; Afantitis *et al*., 2006; Ertan *et al*., 2007; Lee *et al*., 1994; Rineh *et al*., 2007). In order to search for more active compounds which would fit the derived pharmacophore, we designed and prepared a series of 2-[2-(pyridin-3-yl) phenyl] acetamides. The new compounds were evaluated as anticonvulsant agents in experimental epilepsy models, i.e., MES test and pentylenetetrazole-induced seizure (ScPTZ) test. The rotarod test was performed to evaluate neurotoxicity.

Results and discussion

Design

Molecular Operating Environment software version 2003 on a Pentium IV 1.6 GHz workstation was utilized for designing test compounds. The training set comprised anticonvulsants-Phenytoin, Carbamazepine, Lamotrigine, Remacemide, Rufinamide, and Zonisamide. Superposition of the minimum energy conformers of each of the molecules of the training set led to identification of common structural features like presence of at least one aromatic ring (Aro.) and a hydrogen bond acceptor/donor (H.A/D) unit (Fig. 1). This query was subjected to 3D database search wherein molecules obeying the distance constraints were retrieved. Among these were 2-aryl-substituted phenylacetamides. Out of these, 2-heteroaryl substituent was chosen based on lipophilicity. Modifications in the amide functionality led to the generation of the novel set of molecules.

Chemistry

Synthesis of test compounds was accomplished using 2-[2-(pyridin-3-yl) phenyl] acetamide (**15**) as shown in Scheme 1. Condensation of compound **15** with semicarbazide hydrochloride/thiosemicarbazide at higher temperatures (180°C) led to synthesis of **16** and **17,** respectively. Similarly, reaction of **15** with cyclohexylamine/*tert*-butyl amine in the presence of lead tetra-acetate at room temperature gave high yields of compounds **18** and **19,** respectively.

In the present study, synthesis of substituted amides (**16** and **17**) was achieved more efficiently by a novel process, which involved condensation of compound **15** with various amines in acidic environment. Under these conditions the amide i.e., **15** was converted into a good acylating agent which caused the reaction taking place in a short period of time. The above reaction when carried out without the addition of acid required longer period of time (Sowa and Nieuwland, 1937). The conventional method of synthesizing amides involves converting the corresponding acid to the acid chloride followed by treatment with amine, which is accompanied by the formation of side products. It is also difficult to handle acid chlorides because of their unstable nature. The present method has overcome all these difficulties.

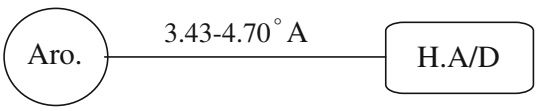


Fig. 1 Pharmacophore for anticonvulsant activity

Synthesis of compounds **18** and **19** has also been achieved by a novel method i.e., lead-tetraacetate-catalyzed oxidative rearrangement of amides to ureas (Scheme 2). It involves formation of a complex with lead tetraacetate to give isocyanate, which on treatment with amine offers the substituted urea. This method offered many advantages such as (i) one pot synthesis and (ii) cost effectiveness.

Pharmacology

Results of anticonvulsant screening (Table 1) showed that Compound **15** had potent anticonvulsant activity in MES test. It was, therefore, further tested at doses ranging from 23 to 2.0 mg/kg, and an ED_{50} value determined was 8.28 mg/kg (Table 2). Compounds **16** and **17** were, however, not effective. Compounds **18** and **19** reduced the duration of seizure (to 8.0–10.0 s) and prevented death. In chemically induced seizures, test compounds did not completely abolish seizures.

Compound **15** was subjected to neurotoxicity testing so as to determine its protective index.

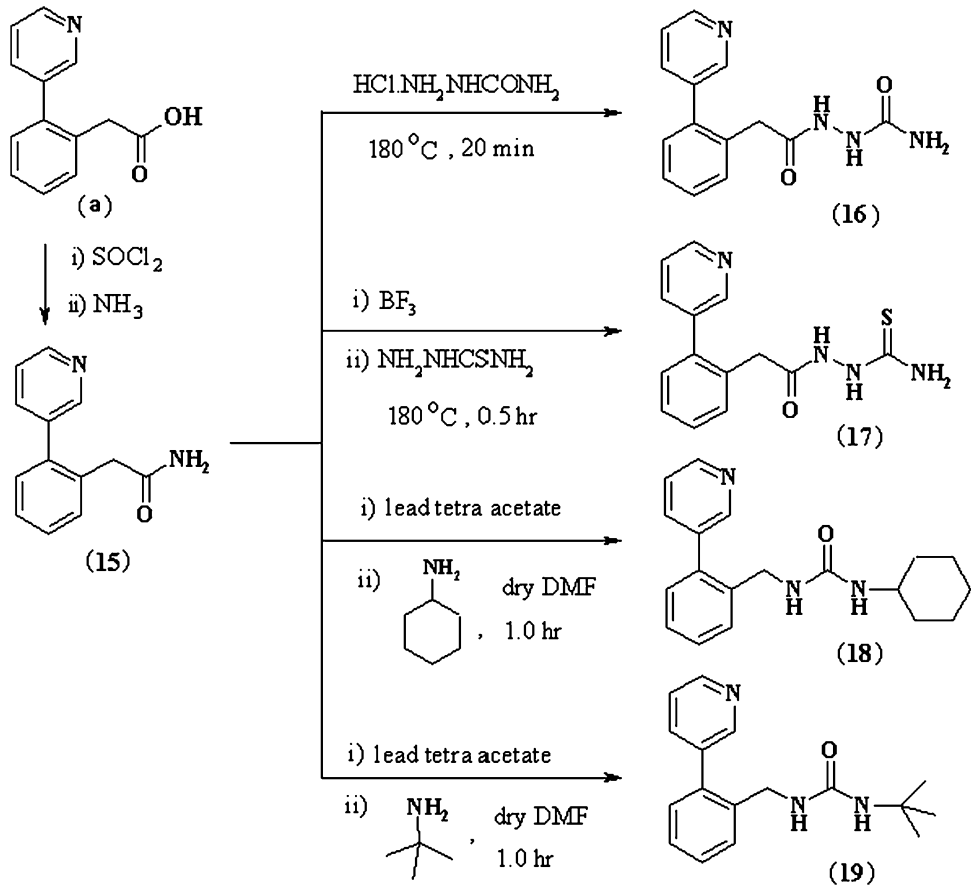
In conclusion, we were able to obtain in this study a new lead compound **15** with anticonvulsant activity comparable with standard drugs like Phenytoin and/or Carbamazepine. **15** also exhibited higher protective index compared to Phenytoin. The present study indicates that derivatization of the parent amide (**15**) into more substituted amides or into ureas has led to loss of activity. In biphenyl systems, there is restricted rotation between the two aromatic rings. When either of the rings is substituted with a side chain, the molecule cannot reach the desired minimum energy conformation essential for binding to the receptor. This results in higher energy of the system. Another factor responsible for unfavorable binding to the receptor may have some bearing on the steric effect of large amide functionality or on the size of the Na channel pores.

Experimental

Synthesis

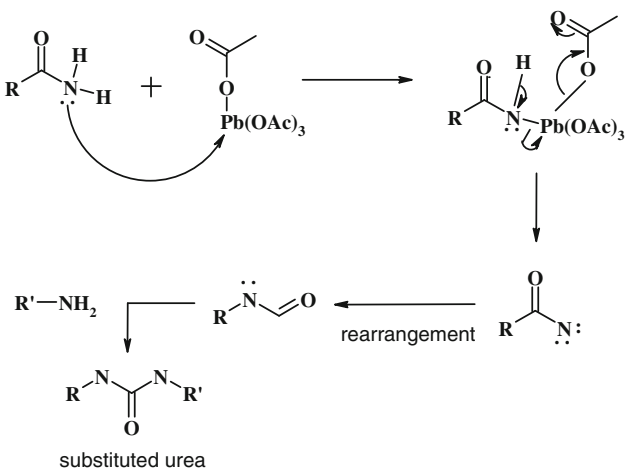
Progress of all the reactions was monitored by thin-layer chromatography using Merck precoated silica gel GF 254. Melting points were recorded on an electrically heated melting point apparatus. Infrared (IR) spectra were recorded on JASCO FT-IR 5300. Nuclear magnetic resonance (^{1}H-NMR) spectra were recorded on VARIAN-VXR-300 S at 300 MHz instrument and chemical shifts were expressed in ppm relative to tetramethylsilane as an internal standard.

Scheme 1 Synthesis route of compounds **15–19**



Synthesis of 2-[2-(pyridin-3-yl) phenyl] acetamide (**15**)

2-[2-(pyridin-3-yl) phenyl] acetic acid (**a**) (35 g, 0.16 mol) was reacted with redistilled thionyl chloride (24.4 g, 0.20 mol) for an hour. The 2-[2-(pyridin-3-yl) phenyl] acetyl chloride thus obtained was cooled to 10°C. To this was added ammonia solution in a dropwise manner with stirring. After addition was complete, the reaction mixture was stirred for 2 h and the solid precipitated out was filtered off. The solid was then washed with water, dried, and recrystallized from aqueous ethanol.



Scheme 2 Mechanism of lead-tetraacetate-catalyzed synthesis of substituted ureas

Table 1 Anticonvulsant screening data of test compounds **15–19**

Compound administered	Protection against maximal electroshock induced seizures		Protection against subcutaneous Pentylenetetrazole induced seizures	
	0.5 h	4.0 h	0.5 h	4.0 h
15	+++	+++	–	–
16	–	–	–	–
17	–	–	–	–
18	–	–	–	–
19	–	–	–	–
Phenytoin	+++	+++		
Carbamazepine	+++	+++		
Diazepam			+++	+++
Phenobarbitone			+++	+++
Vehicle treated	–	–	–	–

Statistical analysis was conducted by the method of Litchfield and Wilcoxon (1949), $P < 0.025$ compared to vehicle treated control groups

+++ activity at 30 mg/kg, ++ activity at 100 mg/kg, + activity at 300 mg/kg, – no activity even at 300 mg/kg

Table 2 Effective dose and protective index of Compound **15**

Compound	MES ED_{50}^{a}	TD_{50}^{a}	Protective index (PI)[b]
15	8.28 [0.5]	215 [0.5]	26
Phenytoin	9.5 [2.0]	66 [2.0]	6.9

[a] ED_{50} and TD_{50} values are in mg/kg. Numbers in parentheses are 95% confidence intervals. The dose data was obtained at the 'time of peak effect' (indicated in hours in square brackets)

[b] PI calculated as (TD_{50}/ED_{50})

Yield $= 81\%$. Melting point $= 124-125^{\circ}C$

^{1}H-NMR (DMSO-d_6): 8.5 (m, 1H, Ar); 7.8 (m, 2H, Ar); 7.4–7.1 (m, 5H, Ar); 5.2 (s, 2H, NH_2); 3.4 (s, 2H, –CH_2–). IR (KBr) cm^{-1}**:** 3393 (N–H), 1685 (C=O); MS: m/z 213 (M + 1); Anal. Calcd. for $C_{13}H_{12}N_2O$**:** C 73.57; H 5.70; N 13.20; O 7.54. Found**:** C 73.52; H 5.60; N 13.25; O 7.49.

Synthesis of *N*-(carbamoylamino)-2-[2-(pyridin-3-yl) phenyl] acetamide (**16**)

A mixture of (**15**) (0.057 mol, 12.0 g) and semicarbazide hydrochloride (0.05 mol, 5.5 g) was stirred in a 250-ml round-bottomed flask equipped with a reflux condensor. The reaction mixture was heated to 180°C for 20 min. After cooling to room temperature, 100 ml of water was added and the mixture stirred for 20 min. The solid obtained was filtered off, washed with sodium bicarbonate solution followed by washing with water, and dried. The product was recrystallized from aqueous ethanol.

Yield $= 78\%$, Melting point $= 248-250^{\circ}C$.

^{1}H-NMR (DMSO-d_6)**:** 8.5 (m, 1H, Ar); 7.8 (m, 2H, Ar); 7.4–7.1 (m, 5H, Ar); 5.2 (s, 2H, NH_2); 3.4 (s, 2H, –CH_2–). IR (KBr) cm^{-1}**:** 3240 (N–H), 1682 (C=O). MS: m/z 271 (M + 1); Anal. Calcd. for $C_{14}H_{14}N_4O_2$**:** C 62.21; H 5.22; N 20.73; O 11.84. Found**:** C 62.29; H 5.15; N 20.68; O 11.79.

Synthesis of *N*-(carbamothioylamino)-2-[2-(pyridin-3-yl) phenyl] acetamide (**17**)

A mixture of (**15**) (0.057 mol, 12.0 g) and thiosemicarbazide (0.05 mol, 4.5 g) was taken in a 250-ml round-bottomed flask equipped with a reflux condensor. To the above mixture boron trifluoride etherate was added, and the reaction mixture was heated to 180°C for 20 min. After cooling to room temperature, 100-ml of ice-water was added and the mixture stirred for 20 min. The solid obtained was filtered off, washed with dilute HCl followed by washing with water, and dried. The product was recrystallized from absolute ethanol.

Yield $= 60\%$, Melting point $= 180-181^{\circ}C$.

^{1}H-NMR (DMSO-d_6)**:** 8.5 (m, 1H, Ar); 7.8 (m, 2H, Ar); 7.4–7.1 (m, 5H, Ar); 5.2 (s, 2H, NH_2); 3.4 (s, 2H, –CH_2–). IR (KBr) cm^{-1}**:** 3240 (N–H), 1682 (C=O). MS: m/z 287 (M + 1); Anal. Calcd. for $C_{14}H_{14}N_4OS$**:** C 58.72; H 4.93; N 19.57; O 5.59; S 11.20. Found**:** C 58.0; H 4.95; N 19.49; O 5.63; S 11.11.

Synthesis of 1-cyclohexyl-3-{[2-(pyridin-3-yl) phenyl] methyl}-urea (**18**)

A 250-ml round-bottomed flask was charged with (**15**) (12.0 g, 0.057 mol) in 40 ml of dry *N,N*-dimethylformamide. To this was added lead tetraacetate (25.2 g, 0.057 mol), and the reaction mixture stirred for 20 min after which the corresponding isocyanate was formed. Cyclohexylamine (10 g, 0.1 mol) was added dropwise and the reaction mixture stirred for 1 h. The reaction mixture was then quenched in cold water, and the precipitate obtained was filtered off. The solid was washed with dilute HCl followed by washing with water and then dried. The product was purified by column chromatography (petroleum ether 70: chloroform 30).

Yield $= 84\%$. Melting point $= 245-246^{\circ}C$.

^{1}H-NMR (DMSO-d_6)**:** 8.5 (m, 1H, Ar); 7.8 (m, 2H, Ar); 7.4–7.1 (m, 5H, Ar); 5.4 (s, 2H, NH_2); 3.8 (s, 2H, –CH_2–); 3.5 (m, 1H, –CH–); 2.5–2.4 (m, 5H, cyclohexyl). IR (KBr) cm^{-1}**:** 3389 (N–H), 1653 (C=O). MS: m/z 310 (M + 1); Anal. Calcd. for $C_{19}H_{23}N_3O$**:** C 73.78; H 7.44; N 13.58; O 5.17. Found**:** C 73.69; H 7.42; N 13.62; O 5.12.

Synthesis of 3-tert-butyl-1-{[2-(pyridin-3-yl) phenyl] methyl}-urea (**19**)

A 250-ml round-bottomed flask was charged with (**15**) (12.0 g, 0.057 mol) in 40 ml of dry *N,N*-dimethylformamide. To this was added lead tetraacetate (25.2 g, 0.057 mol) and the reaction mixture stirred for 20 min after which the corresponding isocyanate was formed. *tert*-butylamine (7.3 g, 0.1 mol) was added dropwise, and the reaction mixture was stirred for 1 h. The reaction mixture was then quenched in cold water, and the precipitate obtained was filtered off. The solid was washed with dilute HCl followed by washing with water, and then dried. The product was purified by column chromatography (petroleum ether 70: chloroform 30).

Yield $= 81\%$. Melting point $= 232-233^{\circ}C$.

^{1}H-NMR (DMSO-d_6)**:** 8.5 (m, 1H, Ar); 7.8 (m, 2H, Ar); 7.4–7.1 (m, 5H, Ar); 5.4 (s, 2H, NH_2); 3.8 (m, 2H, –CH_2–); 2.0 (m, 9H, *t*-butyl). IR (KBr) cm^{-1}**:** 3389 (N–H), 1653 (C=O). MS: m/z 284 (M + 1); Anal. Calcd. for $C_{17}H_{21}N_3O$**:**

C 72.08; H 7.42; N 14.84; O5.65. Found**:** C 71.98; H 7.37; N 14.91; O 5.61.

Pharmacology

Experimental protocol was approved by Institutional Ethics Committee (Regd.No. 242/CPCSEA). Swiss albino male mice in the weight range of 25–30 g were used. Mice were fed on standard laboratory diet and supplied with drinking water. Acute toxicity studies were carried out according to OECD guidelines (1998, 2001) to ascertain the safety of the compounds (**15–19**) before performing anticonvulsant activity testing. Test compounds (**15–19**) were suspended in 0.5% tween80 and administered intraperitoneally at three dose levels (30, 100, and 300 mg kg^{-1}) and were screened for anticonvulsant activity using MES and ScPTZ-induced seizure models (OECD, 1998, 2001; Clark *et al*., 1984; Krall *et al*., 1978).

MES test

The mice were subjected to electrical current delivered through corneal electrodes (40 mA, 50 Hz for 0.2 s) at 0.5 and 4.0 h after administration of test/standard compounds. Failure to extend the hind limbs to an angle with the trunk greater than 90° was defined as protection.

ScPTZ test

Pentylenetetrazole at a dose of 85 mg/kg was administered subcutaneously at 0.5 and 4.0 h after administration of test/standard compounds. Protection was defined as a failure to observe a single episode of clonic spasms of at least 5 s duration during a 30-min period following administration of test/standard compound.

Determination of ED_{50} of compound **15**

ED_{50} is a the dosage that makes half of the animals react positively to dugs. Karber's method is commonly used to determine ED_{50}. In this method, the drug dosage is arranged by geometric progression growth. First, the dose range of 100 and 0% positive responses to drug is determined according to experience or the literature data. As per our observations during anticonvulsant screening, the mice responded positively till 30 mg/kg for compound **15** (Table 1). Hence, the dose was further lowered, wherein 23.0 and 2.0 mg/kg were found as doses having 100 and 0% positive responses, respectively. Nine groups of mice were administered the dosage which was calculated from the following formula

$$r = \log^{-1}\left(\frac{\log D_k - \log D_i}{n-1}\right)$$

where r is the ratio of dosage of adjacent group, D_k is the maximum dosage, D_i is the minimum dosage, and n is the number of groups

$$r = \log^{-1}\left(\frac{\log 23 - \log 2}{9-1}\right)$$

$$r = 1.355$$

ED_{50} value was then determined by the following formula:

$$\mathrm{ED}_{50} = \log^{-1}\left[X_k - \frac{i}{2}\sum_{n=1}^{k-1}(P_n + P_{n+1})\right]$$

where X_k is the logarithm of maximum dosage, and i is the logarithm of ratio of adjacent groups, i.e., $\log(r)$

$$\sum_{n=1}^{k-1}(P_n + P_{n+1}) = 80$$

Cumulative total of number of mice showing positive response in each group was 80.

$$\mathrm{ED}_{50} = \log^{-1}\left[\log(23) - \frac{\log(1.355)}{2} \times (80)\right]$$

$$\mathrm{ED}_{50} = \log^{-1}(1.3617 - 5.280)$$

$$\mathrm{ED}_{50} = \log^{-1}(\overline{3}.9183)$$

$$\mathrm{ED}_{50} = 8.285$$

Determination of Median Neurotoxic dose (TD_{50}) of Compound **15**

Rotarod Test

The median minimal neurotoxic dose (TD_{50}) in mice was determined by the rotarod procedure. Suspension of compound **15** in 0.5% tween80 in saline was administered intraperitoneally with dose levels in increasing order (100–600 mg/kg). The mice were placed on a 1-inch-diameter knurled plastic rod rotating at 6 rpm. Unimpaired mice can easily remain on a rod rotating at this speed. The inability of the mice to maintain their balance for at least 1 min in three successive trials was interpreted as demonstration of motor impairment or toxicity. Results are shown in Table 2.

TD_{50} is a the dosage that produces neurotoxicity in 50% of animal population. The drug dosage is arranged by geometric progression growth. First the dose range producing 0 and 100% negative response i.e., inability of the mouse to easily remain on a rod 1-inch diameter knurled

plastic rod rotating at 6 rpm for at least 1 min on administration of drug is determined. As per our observations, the mice responded positively i.e., remained on rod till 100 mg/kg. Hence, the dose was progressively increased till 600 mg/kg, when all the mice fell off the rod. Eight groups of mice were administered the dosage that was calculated from the following formula:

$$r = \log^{-1}\left(\frac{\log D_k - \log D_i}{n-1}\right)$$

where r is the ratio of dosage of adjacent group, D_k is the maximum dosage, D_i is the minimum dosage, and n is the number of groups

$$r = \log^{-1}\left(\frac{\log(600) - \log(100)}{8-1}\right)$$

$$r = 1.29$$

TD_{50} value was determined by the following formula:

$$TD_{50} = \log^{-1}\left[X_k - \frac{i}{2}\sum_{n=1}^{k-1}(P_n + P_{n+1})\right]$$

where X_k is the logarithm of maximum dosage, and i is the logarithm of ratio of adjacent groups, i.e., log (r)

$$\sum_{n=1}^{k-1}(P_n + P_{n+1}) = 74$$

Cumulative total of number of mice falling off the rod in each group was 74.

$$TD_{50} = \log^{-1}\left[\log(600) - \frac{\log(1.290)}{2} \times (74)\right]$$

$$TD_{50} = \log^{-1}(2.7782 - 4.1107)$$

$$TD_{50} = \log^{-1}(\overline{1}.3325)$$

$$TD_{50} = 215.0$$

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References

Afantitis A, Melagraki G, Sarimvies H, Koktentis PA, Markopoulos J, Markopoulov OI (2006) Investigation of substituent effect of 1-(3, 3-diphenyl propyl)-piperidinyl phenyl acetamides on CCR5 binding affinity using QSAR and virtual screening techniques. J Comp-Aided Mol Des 20:93–95

Almasirad A, Hosseini R, Jalalizadeh H, Moghaddam ZR, Abaeian N, Janafrooz M, Abbaspour M, Ziaee V, Dalvandi A, Shafiee A (2006) Synthesis and analgesic activity of 2-phenoxy benzoic acid and *N*-phenyl anthranilic acid hydrazides. Biol Pharm Bull 29(6):1180–1185

Clark CR, Wells MJM, Sansom RT, Norris GN, Dockens RC, Ravis WR (1984) Anticonvulsant activity of some 4-aminobenzamides. J Med Chem 27:779–782

Duron RM, Medina MT, Martinez-Juarez IE, Bailey JN, Katerina TP, Ramirej RR, Castro-Ortega RH, Castronieja IP, Hachado-Salas J, Mifa L, Delgado-Escuta AV (2005) Seizures of idiopathic generalized epiplepsies. Epilepsia 46(9):34–47

Ertan T, Yildiz I, Sozhan, Teniz-Arpaki O, Kaynak F, Yalchin I, Aki-Server F, Abbasoglu U (2007) Synthesis and biological evaluation of new *N*-(2-hydroxy-4- (or5) nitro/amino phenyl) benzamides and phenylacetamides as antimicrobial agents. Bioorg Med Chem 15(5):2032–2044

Krall RL, Penry JK, White BG, Kupferberg HJ, Swinyard EA (1978) Antiepileptic drug development: II. Anticonvulsant drug screening. Epilepsia 19:409–428

Kwan P, Brodie MJ (2000) Early identification of refractory epilepsy. N Eng J Med 342(5):314–319

Lee B, Kim JH, Park NS, Kong JY (1994) KR-25018—a novel orally active analgesic with non-narcotic properties. Phenylacetamides, one of capsicin derivatives. Arch Pharmacol Res 17(5):304–308

Linn Z, Kadaba PK (1997) Molecular targets for the rational design of antiepileptic drugs and related neuroprotective agents. Med Res Rev 17:537–572

Litchfield JT, Wilcoxon FA (1949) A simplified method of evaluating dose-effect experiments. J Pharmacol Expt Ther 96:99–113

Loscher W (1998) New visions in the pharmacology of anticonvulsion. Eur J Pharmacol 342:1–13

OECD (Organization for Economic Cooperation and Development, 1998, 2001 USA) guidelines

Perucca E (1996) The new generation of antiepileptic drugs: advantages and disadvantages. Br J Clin Pharmacol 42:531–543

Rineh A, Mahmoodi N, Abdollahi M, Fouramadi A, Sorkhi M, Shafiee A (2007) Synthesis, analgesic and anti-inflammatory activity of 4-(2-phenoxyphenyl) semicarbazones. Arch Pharm Chem Life Sci 340(8):409–415

Shindikar AV, Khan F, Viswanathan CL (2006) Design, synthesis and invivo anticonvulsant screening in mice of novel phenylacetamides. Eur J Med Chem 41(6):786–792

Sowa FJ, Nieuwland JA (1937) Organic reactions with boron fluoride. XIV. the reaction of amides with acids and amines. J Am Chem Soc 59:1202–1203

Taylor CP (1996) Voltage gated Na^+ channels as targets for anticonvulsant, analgesic and neuroprotective drugs. Curr Pharm Des 2:375–381