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Original Article

Synthesis, Characterization and *in vivo* Biological Activity of n-(2-chlorophenyl)-2-(2-methyl-5-nitro-1h-imidazol-1-yl) Acetamide and its Derivatives

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ABSTRACT

Background: A heterocyclic hydrocarbon with distinct fundamental structural features in its molecular structure is imidazole. In Generalized seizure, patient brain has abnormal electrical activity on both sides of the brain but in partial seizures, it happens when electrical activity surges in one part of brain. In the present work, molecular docking analysis was conducted on proposed protein derivatives from the protein data bank using the Auto Dock for Docking programme. Study on protein binding affinity using N-(2-chlorophenyl)-2-(2-methyl-5-nitro-1H-imidazol-1-yl) acetamide derivatives and the GABA receptor. Even if a number of innovative anticonvulsants have been created in India, some types of seizures are still not adequately controlled by smaller side effects even after the use of modern and revolutionary treatments. The World Health Organisation (WHO) estimates that 65 million people worldwide suffer from epilepsy. Nevertheless, epilepsy is an uncontrollable, neurological seizure. **Materials and Methods:** 2-cholro aniline, 2-nitro aniline, 3-nitro aniline, formic acid, acetamide, benzamide and ethanamide have been employed in this study. **Results:** Our research led us to the conclusion that a variety of compounds have strong anticonvulsant properties. 2-(2-methyl-5-nitro-1H-imidazol-1-yl)-N-{2-[(3-nitrophenyl)amino]phenyl} acetamide OL4;2-{[(2-methyl-5-nitro-1H-imidazol-1-yl) acetyl]amino}phenyl acetate OL5; 2-{[(2-methyl-5-nitro-1H-imidazol-1-yl)acetyl]amino}phenyl propanoate OL6; N-(2-{[(2-methyl-5-nitro-1H-imidazol-1-yl)acetyl]amino}phenyl)propanamide OL7 gives strong anti-convulsant effects against phenytoin drug. **Conclusion:** The title compounds and its derivatives were examined for their ability to treat convulsions. Studies of the relationship between structure and activity revealed that compounds containing imidazole derivatives that have an electron-withdrawing group have higher activity than those that have an electron-donating group.

Keywords: 2-Cholro aniline, 2-Nitro aniline, 3-Nitro aniline, Formic acid, Acetamide, Anti-convulsant activity.

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INTRODUCTION

Scientific study on the epidemiology shares that 60 million beings around the world are impacted by epilepsy, a common neurological condition affecting the brain.[1,2] Only 30% of those experiencing uncontrollable seizures have recovered, despite the fact that there are already over 40 distinct anti-epileptic medications available on the market.[3,4] consequently, quite a lot of exploration is going on antiepileptic compounds currently. Its key objective is to discover creative anticonvulsant medications. Based on seizure type, *in vivo* testing strategies were used to recognise Imidazole derivatives.[5] Many heterocyclic components were familiar with biological significance since they share structural similarities with purine nucleobases and Imidazole derivatives, which selectively inhibit tissue cell growth and eventually suppress angiogenesis *in vitro* and *in vivo* through their biological activity. Seizures are nothing more than unacceptable electrical activity in the brain and are signified by physical modifications in a patient's execute that take place during treatment.[6] Going through a patient's brain seizure condition can be rather concerning. When a patient experiences a seizure, there is something happening with disorganised brain

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activity. In most cases, an excess of patient brain cells becoming activated at the same point triggers a seizure. Seizures can be assigned to two categories: partial and broad. Though partial seizures develop when electrical activity spikes in one particular area of the brain, widespread seizures engage irregular electrical activity on both sides of the patient's brain.[7,8] There are multiple root causes of seizures, including enhanced blood sugar or salt levels, brain harm after a stroke, or issues related to head trauma. Patients might acquire brain tumours from birth. Epidemiologic research indicate that sixty million people worldwide suffer from epilepsy, a major neurological illness.[9,10] A total of 40,000 new convulsion cases have been detected throughout the world. In excess of forty unique convulsion therapies are available on the Indian market; however, about 39% of patients with spontaneous seizures have found relief.[11] As a result, many researchers have spent time working on derivatives of antiepileptic drugs subsequently. The main aim of the research is to summarise imidazole derivatives by employing a biodegradable catalyst and several kinds of solvents and chemicals.[12,13] The presence of aryl/alkyl creates including -CH_3, C_2H_5, C_6H_5 and so on, together with electron donor companies so as -NO_2, -CHO, C=OR, -CN, -COOH and -COOR, might be essential for the activity of an anticonvulsant. In 1944, the imidazole nucleus has been discovered. It has an imidazole ring in it. Purine and its structure are analogous. Because imidazole had a variety of medication functions, it has an important heterocyclic nucleus. Scientist Hoebrecker created the first imidazole in 1872. A hydrogen atom that bonds with nitrogen at the 1-position appears in imidazole (see Figure 1). These days, imidazole is the predominant moiety simply due to its extensive pharmacological qualities.

MATERIALS AND METHODS

Materials

Chemicals such as 2-cholro aniline, 2-nitro aniline, 3-nitro aniline, formic acid, acetamide, benzamide and ethanamide have been employed in this study. The synthetic process of imidazole derivatives was carried out using 2-methyl-5-nitro-1H-imidazole and chloroacetic acid.

Methods

Every imidazole derivative has been produced using a traditional technique. Utilising a capillary open tube approach, the point at which it melts has been confirmed. TLC and FTIR have been used to examine the purity of the compounds and a Perkin Elmer Spectrum has been used to generate IR spectra (KBr pellets). Bruker AVANCE III 500 MHz (AV 500) spectroscopy instruments have recorded H-NMR spectra (Nuclear Magnetic Resonance Spectroscopy). The test compound is at in derivative in a dose of (100 mg/Kg, 200 mg/kg and 400 mg/kg) and suspended in 1% Tween 80. The control animals received the vehicle. An ear electrode was used to generate a greater electric current; the increment was 0.1 mA/0.2 s at 50 Hz, with a starting value of 1 mA and 100mg/kg in 10% DMSO, standard drug Phenytoin (SD fine chemicals) (100 mg/Kg, 200 mg/kg and 400 mg/kg) were given by oral route. Maintenance of Animals.

Experimental Work

Synthesis of (2-methyl-5-nitro-1*H*-imidazol-1-yl) acetic acid: (Scheme A) [mentioned in Figure 2]

40 mL of dimethyl sulphoxide dissolve with 10 g of 2-methyl-5-nitro-1H-imidazole and in 40 mL of ethanol is mix with 10 g of chloroacetic acid, in a flask with a round bottom. The mixture was heated in a heating mantel to 80º for 1.30 hr. After the liquid cooled, 10% NAOH solution was added little by little and resulting in yellowish ppt forms. Ice-cold water was used to formation of crystals in the reaction flask. The crude product was cleaned with around 250 mL of cold water after being recrystallized with ethanol.

Synthesis of *N*-(2-chlorophenyl)-2-(2-methyl-5-nitro-1*H*-imidazol-1-yl) acetamide: (Scheme A) [mentioned in Figure 2]

2 g of the stated above product and 12 mL of 2-chloroaniline were combined in a flask with a round bottom. The mixture was treated with the help of heating mantel at 75º for 2 hr, after which a reddish hue appeared. The reaction flask was then rinsed and filtered with ice-cold water. After washing the crude result with roughly 25 mL of cold water, alcohol was used to recrystallize it. Dry naturally to get a finished product that is pink.

Synthesis of2-(2-methyl-5-nitro-1*H*-imidazol-1-yl)-*N*-[2-(phenylamino)phenyl]acetamide: (Scheme A) [mentioned in Figure 2]

2 g of the product mentioned above should be dissolved in ethanol in a round-bottomed flask. The reaction mixture should then be treated for 1.25 hr at 70ºC in a heating mantel, along with 12 mL of 2-chloro aniline. When the RBF cools, a dark pinkish tint appears. It is then added to cold water and filtered using water. The finished product then dries naturally. After filtering the reaction mixture, ethanol was used to recrystallize it. After being cleaned with ice-cold water, the product was filtered.

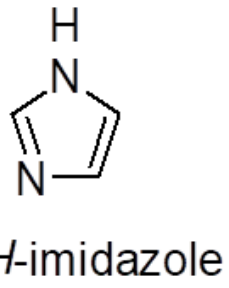

Figure 1: Imidazole heterocyclic nucleus.

Synthesis of *N*-(2-{[(2-methyl-5-nitro-1 *H*-imidazol-1-yl)acetyl]amino}phenyl) propanamide: OL1(Scheme B): [mentioned in Figure 3]

2 g of the product mentioned above should be dissolved in 6 mL of ethanol, added to which should be 5 mL of aniline. The reaction mixture should then be heated for 1.35 hr at 75°C in a heating mantel. Crystals and a dark reddish tint develop after cooling. Add some sulfuric acid after that and ppt will form. Then let the RBF to cool, add it to cold water and use water to filter it. After then, the finished item dries naturally. After filtering, ethanol was used to recrystallize the reaction mixture. After being filtered, the product was cleaned in ice-cold water.

Synthesis of *N*-[2-(acetylamino)phenyl]-2-(2-methyl-5-nitro-1*H*-imidazol-1-yl) propanoate: OL2 (Scheme B): [mentioned in Figure 3]

One gram of the product mentioned previously should be dissolved in 9 mL of methanol and 10 g of benzoic acid are mixed with 6 mL of benzene. The reaction mixture should then be heated for 50 min at 72°C in a heating mantel. White crystals start to form around the RBF after cooling. The RBF is then allowed to cool before being added to cold water and filtered using water. The finished product then dries naturally. After filtering the reaction mixture, alcohol was used to recrystallize it. After being cleaned with ice-cold water, the product was filtered.

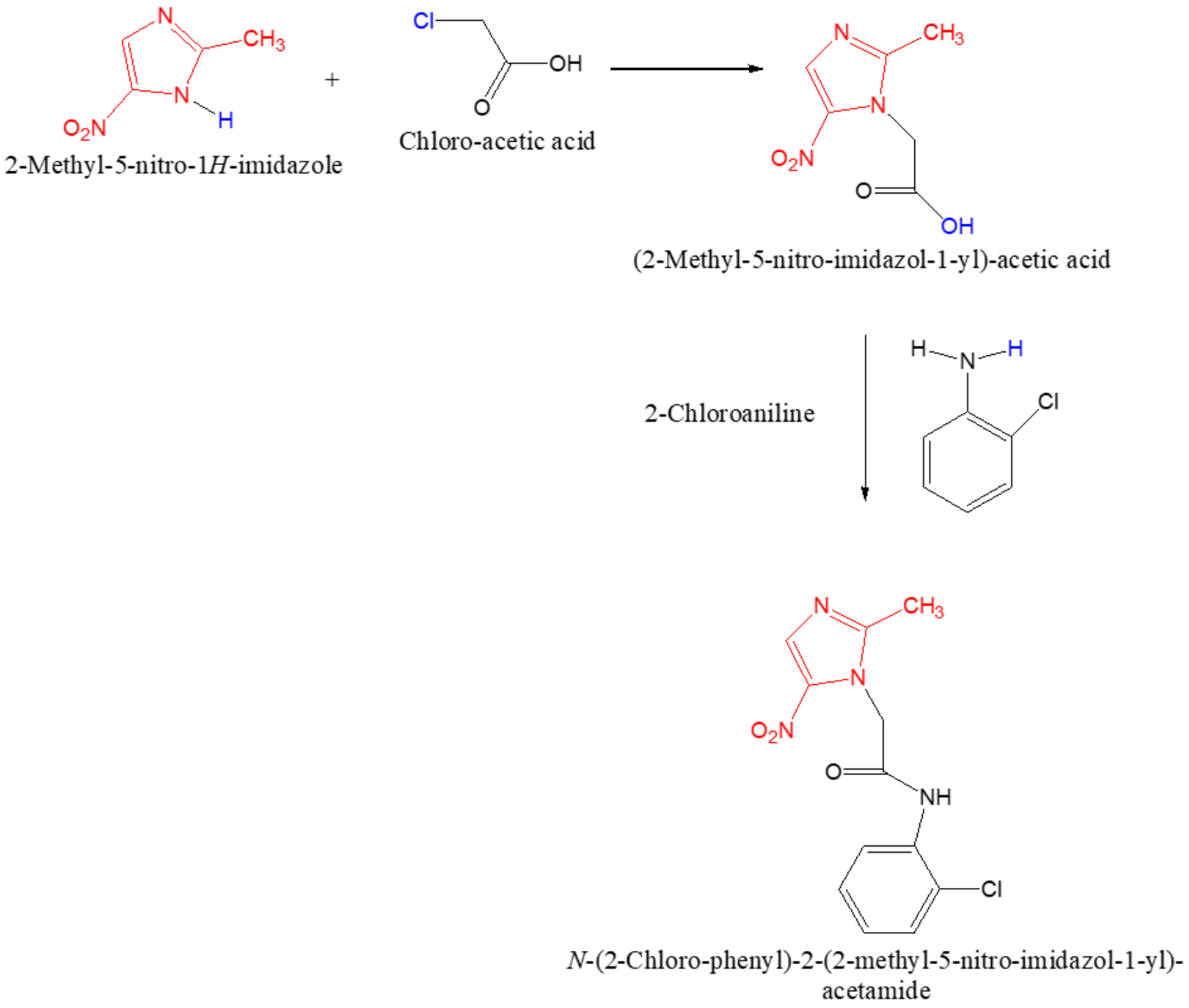

Figure 2: Scheme A for synthesis of *N*-(2-chlorophenyl)-2-(2-methyl-5-nitro-1*H*-imidazol-1-yl) acetamide.

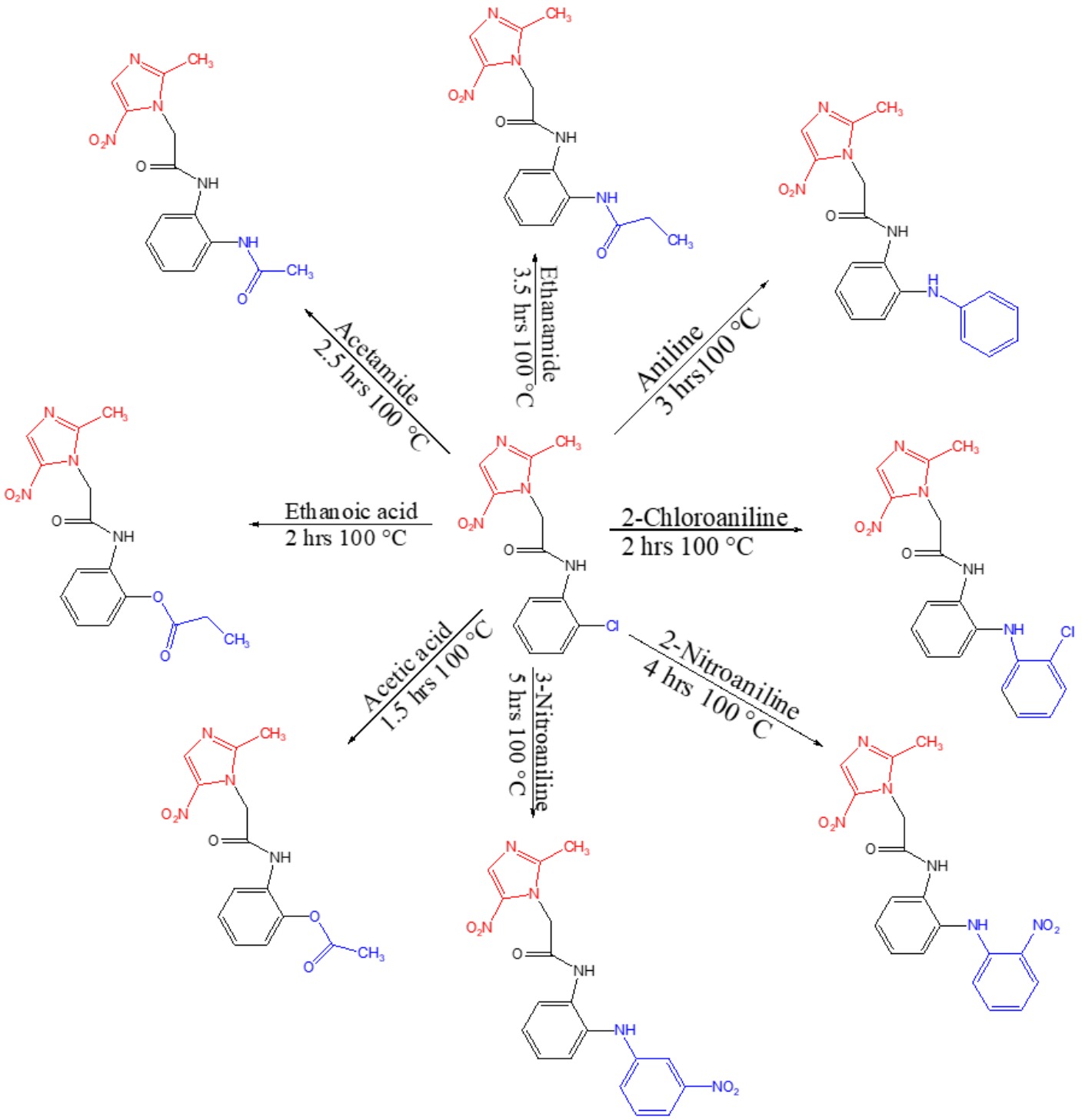

Figure 3: Scheme B for synthesis of *N*-(2-chlorophenyl)-2-(2-methyl-5-nitro-1*H*-imidazol-1-yl) acetamide derivatives.

Synthesis of 2-{[(2-methyl-5-nitro-1*H*-imidazol-1-yl) acetyl]amino}phenyl acetamide : OL3 (Scheme B) [mentioned in Figure 3]

One gram of the product mentioned previously should be dissolved in 6 mL of ethanol and 6 mL of glacial acetic acid should be added. The reaction mixture should then be heated for 1.25 hr at 72°C in a heating mantel. White crystals start to form around the RBF after cooling. The RBF is then allowed to cool before being added to cold water and filtered using water. The finished product then dries naturally. After filtering the reaction mixture, alcohol was used to recrystallize it. After being cleaned with ice-cold water, the product was filtered.

Synthesis of 2-{[(2-methyl-5-nitro-1*H*-imidazol-1-yl) acetyl]amino}phenyl acetate: OL4 (Scheme B): [mentioned in Figure 3]

One gram of the product mentioned previously should be dissolved in 10 mL of ethanol and 1 g of meta-nitroaniline is mixed with 15 mL of Methanol. The reaction mixture should then be heated for 1.25 hr at 72°C in a heating mantel. Yellowish colour liquid at the bottom and White crystals start to form around the RBF after cooling. The RBF is then allowed to cool before being added to cold water and filtered using water. The finished product then dries naturally. After filtering the reaction mixture, alcohol was used to recrystallize it. After being cleaned with ice-cold water, the product was filtered.

Synthesis of 2-(2-methyl-5-nitro-1*H*-imidazol-1-yl)-*N*-{2-[(3-nitrophenyl)amino]phenyl} acetamide : OL5 (Scheme B): [mentioned in Figure 3]

One gram of the product mentioned previously should be dissolved in 5 mL of ethanol and 5 mL of 2-nitroaniline are mixed with above product. The reaction mixture should then be heated for 1.15 hr at 72°C in a heating mantel. Dark pink colour liquid at the bottom and add into HCl obtain ppt after cooling. The RBF is then allowed to cool before being added to cold water and filtered using water. The finished product then dries naturally. After filtering the reaction mixture, alcohol was used to recrystallize it. After being cleaned with ice-cold water, the product was filtered.

Synthesis of 2-(2-methyl-5-nitro-1*H*-imidazol-1-yl)-*N*-{2-[(2-nitrophenyl)amino]phenyl} acetamide: OL6 (Scheme B) [mentioned in Figure 3]

One gram of the product mentioned previously should be dissolved in 10 mL of ethanol and 1 g of 2,4-dinitroaniline are mixed with 10 mL of Methanol. The reaction mixture should then be heated for 1.35 hr at 80°C in a heating mantel. Pinkish colour liquid at the bottom and White crystals start to form around the RBF after cooling. The RBF is then allowed to cool before being added to cold water and filtered using water. The finished product then dries naturally. After filtering the reaction mixture, alcohol was used to recrystallize it. After being cleaned with ice-cold water, the product was filtered.

Table 1: Physical Data of imidazole and its Derivatives.

Derivatives code	Yield %	M.P. (°C)	Molecular Formula
OL1	94	130-132	$C_{20}H_{16}N_2$
OL2	98	302-304	$C_{22}H_{20}N_2O_2$
OL3	94	170-173	$C_{22}H_{20}N_2O_4$
OL4	90	132-134	$C_{20}H_{16}N_2O_2$
OL5	94	124-126	$C_{20}H_{14}N_4O_2$
OL6	98	124-126	$C_{20}H_{14}Cl_2N_2$
OL7	94	184-186	$C_{20}H_{14}N_4O_4$
OL8	92	166-168	$C_{24}H_{24}N_2O_4$

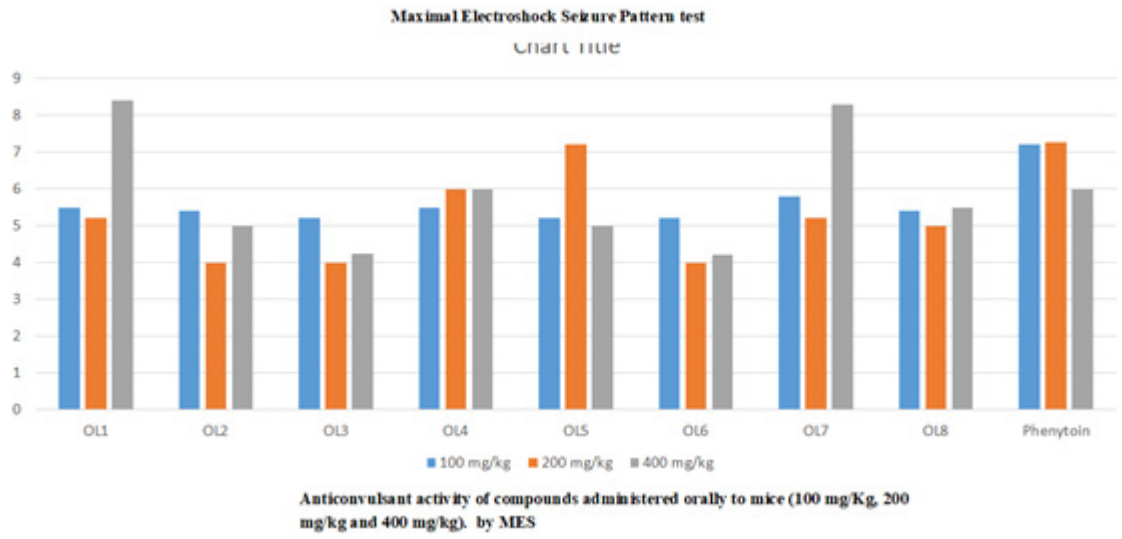

Figure 4: Anticonvulsant activity of compounds administered orally to mice (100 mg/Kg, 200 mg/kg and 400 mg/kg) by MES.

Table 2: Anticonvulsant activity of compounds administered orally to mice (100 mg/Kg, 200 mg/kg and 400 mg/kg) by MES.

Compound	Dose	Mean convulsion threshold
OL1	100	5.5±0.2887
	200	5.2±0.3742
	400	8.4±0.5099
OL2	100	5.8±0.3742
	200	4.0±0.3162
	400	5.0±0.3162
OL3	100	5.2±0.3742
	200	4.0±0.3162
	400	4.25±0.25
OL4	100	5.8±0.3742
	200	6.6±0.400
	400	6.6±0.5099
OL5	100	6.2±0.5831
	200	7.25±0.2500
	400	5.0±0.3162
OL6	100	5.2±0.3742
	200	4.0±0.3162
	400	4.25±0.25
OL7	100	5.8±0.3742
	200	5.2±0.3742
	400	8.4±0.5099
OL8	100	5.8±0.3742
	200	4.0±0.3162
	400	5.0±0.3162
Phenytoin	100	7.2±0.5831
	200	7.25±0.2500
	400	6.0±0.3162

Synthesis of 2-(2-methyl-5-nitro-1*H*-imidazol-1-yl)-*N*-{2-[(2-nitrophenyl)amino]phenyl} acetamide: OL7 (Scheme B) [mention in Figure 3]

One gram of the product mentioned previously should be dissolved in 10 mL of ethanol and 1 g of propionic acid is mixed with 10 mL of Methanol. The reaction mixture should then be heated for 1.35 hr at 80°C in a heating mantel. Pinkish colour liquid at the bottom and White crystals start to form around the RBF after cooling. The RBF is then allowed to cool before being added to cold water and filtered using water. The finished product then dries naturally. After filtering the reaction mixture, alcohol was used to recrystallize it. After being cleaned with ice-cold water, the product was filtered.

Synthesis of 2-(2-methyl-5-nitro-1*H*-imidazol-1-yl)-*N*-{2-[(2-nitrophenyl)amino]phenyl} acetamide: OL8 (Scheme B) [mentioned in Figure 3]

One gramme of the product mentioned previously should be dissolved in 10 mL of ethanol and 1 g of ethanamide is mixed with 10 mL of Methanol. The reaction mixture should then be heated for 1.35 hr at 80°C in a heating mantel. Pinkish colour liquid at the bottom and White crystals start to form around the RBF after cooling. The RBF is then allowed to cool before being added to cold water and filtered using water. The finished product then dries naturally. After filtering the reaction mixture, alcohol was used to recrystallize it. After being cleaned with ice-cold water, the product was filtered.

RESULTS

Spectral Data

Synthesis of 2-(2-methyl-5-nitro-1H-imidazol-1-yl)-N-[2-(phenylamino)phenyl]acetamide: OL1 (Scheme B)

FTIR (KBr) ν cm^{-1}: 1538.17 C=C Stretch(Aromatic), 3055.00 C-H Stretch(Aromatic), 2853.21 C-N bend (Aromatic), 3537.45 N-H Stretch (Aromatic), 1486.83 N-H bend (Aromatic) 1443.86 amide functional group; 1H NMR (400 MHz, DMSO): δ 2.4 CH_3(s, 3H, J=11.4), δ 4.5 and 6.8 CH_2 Group (s, 2H, J=13.1), δ 7.3-7.9 Ar C-H (m, 10H, J=14.3)

Synthesis of N-{2-[(2-chlorophenyl)amino]phenyl}-2-(2-methyl-5-nitro-1H-imidazol-1-yl)acetamide: OL2 (Scheme B)

FTIR (KBr) ν cm^{-1}: 1415.88 C=C Stretch(Aromatic), 1177.11 C-C Stretch(Aromatic), 2550.66 C-N Stretch(Aromatic), 3313 N-H Stretch(Aromatic), 3396.50 N-H Stretch (Aliphatic), 1719.41 C=O Stretch (amide), 2822.50 C-H(Aromatic); 1H NMR (400 MHz, DMSO): δ 3.63 CH_3(s, 3H, J=11.7), δ 6.36 CH_2 Group (s, 2H, J=13.1), δ 7.18–7.25 Ar C-H (m, 9H, J=14.3); δ 12.98-12.56 N-H (2H, s, J=17.4).

Synthesis of 2-(2-methyl-5-nitro-1H-imidazol-1-yl)-N-{2-[(2-nitrophenyl)amino]phenyl} acetamide: OL3 (Scheme B)

FTIR (KBr) ν cm^{-1}: 1510.56.6 C=C Stretch(Aromatic), 1299.01 C-C Stretch(Aromatic), 2798.14 C-N bend (Aromatic), 1466.36 N-H bend (Aromatic), 3381.39 N-H Stretch(Aromatic), 1623.56 C=O (amides); 1H NMR (400 MHz, DMSO): δ 2.5 CH_3(s, 3H, J=11.7), δ 13.3-12.9 N-H (s, 2H, J=11.4), δ 7.5-7.98 Ar C-H (m, 9H, J=14.3), δ 6.6 CH_2 Group (s, 2H, J=12.1).

Synthesis of 2-(2-methyl-5-nitro-1H-imidazol-1-yl)-N-{2-[(3-nitrophenyl)amino]phenyl} acetamide : OL4 (Scheme B)

FTIR (KBr) ν cm^{-1}: 1506.10 C=C Stretch(Aromatic), 1076.22 C-O Stretch (Aromatic), 3083.65 C-N stretch (Aromatic), 3324.95 N-H Strech (Aliphatic), 1615.97 C=O Stretch (amide), 1257.73 C-C Stretch(Aromatic);1H NMR (400 MHz, DMSO): δ 2.5 CH_3(s, 3H, J=11.7), δ 6.3 CH_2 (s, 2H, J=11.4), δ 7.7-7.98 Ar C-H (m, 9H, J=14.3), δ 11.5-11.2 N-H (s, 2H, J=10.4).

Synthesis of 2-{[(2-methyl-5-nitro-1H-imidazol-1-yl) acetyl]amino}phenyl acetate: OL5 (Scheme B)

FTIR (KBr) ν cm^{-1}: 1507.26 C=C Stretch(Aromatic), 1617.35 C=O Stretch (amide), 2702.76 C-N bend (Aromatic), 946.82 N-H Bend (Aromatic), 3372.76 N-H Stretch (Aliphatic), 1576.01 N-O (Aromatic), 804.36 C-H bend (Aliphatic); 1H NMR (400 MHz, DMSO): δ 3.3-2.9 CH_3 (s, 6H, J=11.4), 6.4 CH_2 Group (s, 2H, J=13.1), 7.0-7.9 Ar C-H (m, 6H, J=14.3), δ 11.7 N-H (s, 1H, J=10.1).

Synthesis of 2-{[(2-methyl-5-nitro-1H-imidazol-1-yl) acetyl]amino}phenyl propanoate : OL6 (Scheme B)

FTIR (KBr) ν cm^{-1}: 1613.76 C=C Stretch(Aromatic), 1152.16 C-C Stretch(Aromatic), 1201.45 C-N Stretch (Aromatic), 3378.73 N-H Stretch(Aromatic), 1613.76 C=O Stretch (amide), 1573.07 N-O (Aromatic); 1H NMR (400 MHz, DMSO): δ 2.2 CH_3(s, 3H, J=11.7), 3.3-3.1CH_3 (s, 6H, J=11.4), 7.0-7.8 Ar C-H (m, 6H, J=14.3), 6.4-6.1 CH_2 Group (s, 4H, J=12.1).

Synthesis of N-(2-{[(2-methyl-5-nitro-1H-imidazol-1-yl)acetyl]amino}phenyl)propanamide: OL7 (Scheme B)

FTIR (KBr) ν cm^{-1}: 1498.45 C=C Stretch(Aromatic), 1160.01 C-C Stretch(Aromatic), 1301.72 C-N Stretch(Aromatic), 3313 N-H Stretch(Aromatic), 1699.5 C=O Stretch (Carboxylic Acid), 2096.24 O-H bend (Aliphatic), 1670.10 C=O (amide), 2844 C-H(Aromatic); 1H NMR (400 MHz, DMSO): δ 2.5 CH3(s, 3H, J=11.7), 3.3 CH_3 (s, 3H, J=11.4), δ 6.6 CH_2 Group (s, 2H, J=13.1), 6.4 CH_2 Group (s, 2H, J=11.1), 7.0-7.8 Ar C-H (m, 5H, J=14.3), 10.4-10.2 N-H (s, 2H, J=14.7).

Synthesis of N-[2-(acetylamino)phenyl]-2-(2-methyl-5-nitro-1H-imidazol-1-yl)acetamide: OL8 (Scheme B)

FTIR (KBr) ν cm^{-1}: 1586 C=C Stretch (Aromatic), 2844 C-H Stretch (Aromatic),1132.01 C-C Stretch (Aromatic), 1332.83 C-N Stretch (Aromatic), 3359 N-H Stretch (Aromatic), 1295.5 C=O (amide), 1566.07 N-O (Aromatic); 1H NMR (400 MHz, DMSO): δ 2.5 CH_3(s, 3H, J=10.5), 3.3 CH_3 (s, 3H, J=11.4), 7.1-7.9 Ar C-H (m, 6H, J=14.3), 6.4 CH_2 Group (s, 2H, J=11.1), 10.1-10.4 N-H (s, 2H, J=11.7).

Biological evaluation

Maximal Electroshock Seizure Pattern test

Kitano *et al.* and Krall *et al.* utilized the following methods in carrying out the procedure: six mice were given the reference drug and the chemical under investigation orally 60 min earlier the test (100 mg/Kg, 200 mg/kg and 400 mg/kg) and suspended in 1% Tween 80. The control animals received the vehicle. An ear electrode was used to generate a greater electric current; the increment was 0.1 mA/0.2 s at 50 Hz, with a starting value of 1 mA. The outcome was estimated by assessing the tonic extension of the hind limbs and the maximum electric current the animal could withstand at the end of the trial was measured to establish the mean convulsion threshold. The mean convulsion threshold of each chemical was matched by the mean MES test conclusions, which were measured mA. The mean maximum current for beneficial hind limb extension seizures formed by electroshock was calculated for each drug Table 1 and Figure 4. The expected variance and maximum seizures threshold of the drugs under investigation were evaluated using the chi-squared test. This compound was tested at a dose 100, 200 and 400 mg/Kg in the MES screen and the mean convulsion threshold was found to be 7.25±0.2500.

DISCUSSION

The syntheses of Novel substituted imidazole derivatives and Physical characteristic of imidazole derivatives (Table 1) from OL1 to OL8 were undertaken as per the scheme B See Figure 3. The solvents had been redistilled and dehydrated proceeding to be hired trendy process for the training of catalyst procedure. The response mixture was in addition stirred at room temperature for particular time. Later than the achievement of made of the reaction (as specify by way of TLC), a strong was separated by means of filtration on Buchner funnel using whatman paper. The strong product became then washed with distilled water and dried to achieve smooth products (OL1-OL8) in admirable yields. FTIR spectra were obtained on a Perkin Elmer Spectrum1 FT-IR instrument (KBr pellets). There is comparison Chart (Figure 4) of Anticonvulsant activity of Novel substituted imidazole derivatives at different concentrations like Low dose; Middle Dose and High Dose Method for their ability to reduce seizure spread (Table 2). Phenytoin was used as standard drugs. Each animal will be observed individually for convulsive behavior for next 30 min. The anticonvulsant data of titled compounds (OL1-OL8) was summarized in Table 2 and graphical representation was summarized in Figure 4, compounds 2-(2-methyl-5-nitro-1H-imidazol-1-yl)-N-[2-(phenylamino) phenyl]acetamide OL1 (Scheme B) and 2-{[(2-methyl-5-nitro-1H-imidazol-1-yl)acetyl]amino}phenyl propanoate OL6 (Scheme B) showed protection at maximum dose level of 200 mg/kg and each animal will be observed individually for convulsive behavior for next 30 min. Compound N-(2-{[(2-methyl-5-nitro-

1H-imidazol-1-yl)acetyl]amino}phenyl)propanamideOL7 (Scheme B) and N-[2-(acetylamino)phenyl]-2-(2-methyl-5-nitro-1H-imidazol-1-yl)acetamide OL8 (Scheme B) displayed protection at maximum dose level (100 mg/kg) and each animal will be observed individually for convulsive behavior for next 30 min. Compound 2-(2-methyl-5-nitro-1H-imidazol-1-yl)-N-{2-[(2-nitrophenyl)amino]phenyl} acetamide OL3 (Scheme B) and 2-(2-methyl-5-nitro-1H-imidazol-1-yl)-N-{2-[(3-nitrophenyl) amino]phenyl} acetamide OL4 (Scheme B) containing aromatic group, showed less protection against induced seizures at lower dose level (400 mg/kg). The results of Anticonvulsant Activity testing of the prepared compounds were shown in Table 2 and Figure 4. For the past eight decades, reliable animal models of seizures and epilepsy have been used to guide the quest for antiepileptic medications with improved effectiveness and tolerance. Animal models can provide a precise understanding of the pathophysiology of a dysfunction or dysfunctions and can be used to forecast a compound's therapeutic efficacy.[14] Clinical observations of patients using the earliest anti-seizures, such as bromides, phenobarbital and mephobarbital, led to their development. Merritt and Putnam started looking for medications in an animal model back in 1937.[15] The choice of an appropriate animal model depends on the goals of the experiment, including evaluating a large number of chemicals in a short amount of time, the animal's mortality rate, its capacity to regulate seizures, its resemblance to clinical settings and its cost. Nevertheless, a pure seizure that is triggered in a normal animal cannot be employed as a model of epilepsy since epilepsy is characterised by recurring seizures. The distinction between animal models of epilepsy and animal models of epileptic seizures is crucial for researchers to comprehend. The current study's findings suggest that an isatin derivative significantly exhibited anticonvulsant activity and effectively protected wistar rats against MES seizures.[16] When compared to the control, the effect of N-(2-chlorophenyl)-2-(2-methyl-5-nitro-1H-imidazol-1-yl) acetamide derivatives at a dose of 50 mg/kg was very significant. However, at a dose of 100 mg/kg, there was significant anticonvulsant activity that was comparable to the reference drug in terms of reducing the duration and extension as well as the percent mortality.[17,18] The most likely validated preclinical test to determine whether medications are helpful against tonic-clonic (grandmal) type generalised seizures is the Maximal Electroshock-induced Seizure test (MES). This concept is based on the fact that a defining hallmark of epileptic activity is activation by repetitive electrical pulses created in various neural regions. Anti-epileptic medications that inhibit the MES-induced tonic extension phase have frequently been claimed to work by preventing the spread of seizures.[19] All currently prescribed medications that are clinically effective in treating generalised tonic-clonic convulsions (such as valproic acid, carbamazepine and phenytoin) work by either blocking volt-age-dependent Na+ channels or by blocking glutamatergic excitation mediated by the NMDA receptor, as demonstrated by their efficacy in the MES test. The presence of anticonvulsant properties is suggested by the significant inhibition of edge neralized tonic-clonic seizures in the MES test by N-(2-chlorophenyl)-2-(2-methyl-5-nitro-1H-imidazol-1-yl) acetamide derivatives. This could be due to either the inhibition of voltage-dependent Na+ channels or the derivative's role as an NMDA antagonist. The N-(2-chlorophenyl)-2-(2-methyl-5-nitro-1H-imidazol-1-yl) acetamide derivatives may be selected for additional biological activity modification in light of the encouraging results.

CONCLUSION

Convectional technique was used to synthesised various imidazole derivatives. It was possible to synthesised all eight imidazole derivatives. Good yields of every chemical were produced. NMR spectroscopy was used at Pune University's Central Instrumentation Facility, Savitribai Phule, to confirm the structure of the synthesised chemicals. Research on the biological effects of anti-convulsants was conducted at the university level using 150-200 g Wistar rats. Imidazole compounds shown greater anticonvulsant efficacy against various convulsion types in this study. It became apparent that most of the synthesised chemicals exhibited strong anti-convulsant properties. Considering synthesised compounds to other imidazole, greater activity was shown. Consequently, the combination was created. 2-(2-methyl-5-nitro-1H-imidazol-1-yl)-N-{2-[(3-nitrophenyl) amino]phenyl} acetamide OL4; 2-{[(2-methyl-5-nitro-1H-imidazol-1-yl)acetyl]amino}phenyl acetate OL5; 2-{[(2-methyl-5-nitro-1H-imidazol-1-yl)acetyl]amino}phenyl propanoate OL6; N-(2-{[(2-methyl-5-nitro-1H-imidazol-1-yl)acetyl]amino} phenyl)propanamide OL7 whenever used in the presence of phenytoin,-2-(2-methyl-5-nitro-1H-imidazol-1-yl)acetamide OL8 showed efficient anticonvulsant efficacy.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

FTIR: Fourier transform infrared spectroscopy; **NMR spectroscopy:** Nuclear magnetic spectroscopy; **MS:** Mass spectroscopy; **KBr:** Potassium Bromide; **% yield:** Percentage yields; **M.P.:** Melting point; **mg/kg:** Milligram/ kilograms; **sec:** seconds; **δ:** Chemical shift; **Mol. Wt:** Molecular Weight; **gm:** Gram.

REFERENCES

1. Anger T, Madge DJ, Mulla M, Riddall D. Medicinal Chemistry of Neuronal Voltage-Gated Sodium Channel Blockers. Journal of Medicinal Chemistry. 2001;44:115-37.
2. Ragavendran JV, Sriram D, Kotapati S, Stables J, Yogeeswari P. Newer GABA Derivatives for the Treatment of Epilepsy Including Febrile Seizures: A Bioisosteric Approach. European Journal of Medicinal Chemistry. 2008;43:2650-5.
3. Wu T, Ohgoh M, Hanada T. Mode of Seizure Inhibition by Sodium Channel Blockers, an SV2A Ligand and an AMPA Receptor Antagonist in a Rat Amygdala Kindling Model. Epilepsy Research. 2019;154:42-9.
4. Alexander SM, Harkless J, Butcher JR, Scott KR, Jackson-Ayotunde LP. An Examination of Some Ethyl Ester Enaminone Deriva tives as Anticonvulsant Agents. Bioorganic and Medicinal Chemistry. 2013;21:3272-9.
5. Heinbockel T, Wang Z, Jackson-Ayotunde PL. Allosteric Modulation of GABAA Receptors by an Anilino Enaminone in an Olfactory Center of the Mouse Brain. Pharmaceuticals. 2014;7:1069-90.
6. Wang Z, Sun L, Jackson PL, Scott RK, Heinbockel T. A Substituted Anilino Enaminone Acts as a Novel Positive Allosteric Modulator of GABAA Receptors in the Mouse Brain. Journal of Pharmacology and Experimental Therapeutics. 2011;336:916-24.
7. Jackson PL, Hanson CD, Farrell AK, Raymond BJ, Stables JP, Eddington ND, Scott KR. An Explanation of Anticonvulsant Activity and Toxicity Per Linus Pauling's Clathrate Hypothesis. *European* Journal of Medicinal Chemistry. 2012;51;42-51.
8. Anderson AJ, Nicholson JM, Bakare O, Butcher RJ, Wilson TL, Scott KR. Further Studies on the Anticonvulsant Activity and Potential Type IV Phosphodiesterase Inhibitory Activity of Substituted Vinylic Benzamides. Bioorganic & Medicinal Chemistry. 2006;14:997-1006.
9. Jackson PL, Scott KR, Southerland WM, Fang Y. CoMFA and CoMSIA Studies on Some Anticonvulsant Enaminones. Bioorganic & Medicinal Chemistry. 2009;17:133-40.
10. Edafiogho IO, Kombian SB, Anathalaskshmi KVV, Salama NN, Eddington ND, Wilson TL, Alexander MS, Jackson PL, Hanson CD, Scott KR. Exploring Additional Therapeutic Activities. Journal of Pharmaceutical Sciences. 2007;96:2509-31.
11. Amaye IJ, Heinbockel T, Woods J, Wang Z, Martin-Caraballo M, Jack- son-Ayotunde P. 6 Hz Active Anticonvulsant Fluorinated N-Benzamide Enaminones and Their Inhibitory Neuronal Activity. International Journal of Environmental Research and Public Health. 2018;15:1784.
12. Sula A, Booker J, Ng LC, Naylor CE, DeCaen PG, Wallace BA. The Complete Structure of an Activated Open Sodium Channel. Nature Communications. 2012;8:14205-14.
13. Temme L, Boergel F, Schepmann D, Robaa D, Sippl W, Daniliuc C, *et al.* Impact of hydroxy moieties at the benzo annulene ring system of GluN2B ligands: Design, synthesis and biological evaluation.Bioorganic and Medicinal Chemistry. 2019;27(23):115146.
14. Yadagiri B, Holagunda UD, Bantu R, Nagarapu L, Guguloth V, Polepally S, *et al.* Rational design, synthesis and anti-proliferative evaluation of novel benzosuberone tethered with hydrazide-hydrazones. Bioorganic and medicinal chemistry letters. 2014;24(21):5041-4.
15. Althagafi I, Farghaly TA, Abbas EM, Harras MF. Benzosuberone as Precursor for Synthesis of Antimicrobial Agents: Synthesis, Antimicrobial Activity and Molecular Docking. Polycyclic Aromatic Compounds. 2021;41(8):1646-66.
16. Grimaldi TB, Back DF, Zeni G. Potassium tert-butoxide promoted annulation of 2-alkynylphenyl propargyl ethers: Selective synthesis of benzofuran and 12 H-benzoannulene derivatives. The Journal of organic chemistry. 2013;78(21):11017-31.
17. Ahmed SK, Haese NN, Cowan JT, Pathak V, Moukha-Chafiq O, Smith VJ, *et al.* Targeting chikungunya virus replication by benzoannulene inhibitors. Journal of Medicinal Chemistry. 2021;64(8):4762-86.
18. Oh CH, Kim JH, Oh BK, Park JR, Lee JH. Gold-Catalyzed Sequential Activation of Propargylic Carboxylates: A Facile Route to Benzoannulenes. Chemistry-A European Journal. 2013;19(8):2592-6.
19. Lazzari P, Distinto R, Manca I, Baillie G, Murineddu G, Pira M, *et al.* A critical review of both the synthesis approach and the receptor profile of the 8-chloro-1-(2', 4'-dichlorophenyl)-N-piperidin-1-yl-1, 4, 5, 6-tetra hydro benzo [6, 7] cyclohepta [1, 2-c] pyrazole-3-carboxamide and analogue derivatives. European Journal of Medicinal Chemistry. 2016;121:194-208.

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