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DESIGN AND SYNTHESIS OF SUBSTITUTED PYRROLE DERIVATIVES AS COX-2 INHIBITORS

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Docking studies into the catalytic sites of COX-2 inhibitor were used to identify potential lead COX-2 inhibitor compounds. Those derivatives with good binding energies were chosen for synthesis and biological evaluation was done to support docking results with practical data. Compound P1, P2 and P3 endowed with best binding energy (-5.72, -5.71 and -5.82 kcal/mol respectively) in docking studies with COX-2 receptor. For analgesic evaluation acetic acid induced writhing method and hot plate method were used. The results indicate that three compounds (P1, P2 and P3) possess the analgesic activity at ≥47.5 mg/kg dose level.

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Keywords: Pyrrole derivatives, COX-2 inhibitors, Docking studies, Acetic acid induced writhing, Hot plate method

1. Introduction

Non-steroidal anti-inflammatory drugs (NSAIDs) still remain among the most extensively used drugs worldwide and have been used in the treatment of inflammatory conditions like rheumatoid arthritis, osteoarthritis, orthopedic injuries, post operative pain, etc.[1, 2]. However, the use of conventional NSAIDs has been restricted due to their adverse effect especially gastrointestinal toxicity and renal insufficiency[3,4]. A major discovery in the search of novel anti-inflammatory agents without deleterious side effects exhibited by the conventional NSAIDs came from the identification of two different isoforms of the cyclo-oxygenase (COX) enzyme known as COX-1 and COX-2. COX-1 is a constitutive isoform that is involved in normal cellular functions whereas COX-2 is an inducible isoform that is expressed only after inflammatory stimulus[5, 6]. The two COX isoforms are approximately 60% homologous and their ability to inhibit one isoform selectively is attributed to the different amino acids present[3]. The COX-1 enzyme has an isoleucine residue at this position, while the corresponding residue in the COX-2 enzyme is valine. This seemingly minute difference in structure, results in a larger central channel in the COX-2 isoform. As a result, molecules, which are too large to enter the COX-1 channel, are able to enter the COX-2 channel, based solely on size discrimination at the active site. Exploitation of this phenomenon has been the key in developing selective COX-2 inhibitors. Rational drug design has led to a wide range of diarylheterocycles that have been developed as selective COX-2 inhibitors. Celecoxib[7]

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and rofecoxib[8] were among the earliest to be marketed for the treatment of acute pain, rheumatoid arthritis and osteoarthritis.

In this study we have explored COX-2 inhibitory activity of synthesized compounds against the receptor which are available from the Protein Data Bank (PDB). A small focused library with substituted pyrrole derivatives was built and screened virtually using the docking method. The results of docking were useful to investigate the global binding mode of 1CX2.

The aim of this study was to build docking model with Autodock 4.0 software and to check its reliability. During this study we went through a self-compiled 3D database to get some preferable candidates for future synthesis. On the basis of the obtained hits, we undertook an exploratory study of their *in vitro* COX-2 inhibitory effects. Those derivatives with good binding energies were chosen for synthesis and biological evaluation was done to support docking results with practical data. We believe that this procedure will be helpful in the design of novel COX-2 inhibitory compounds.

2. Results and discussion

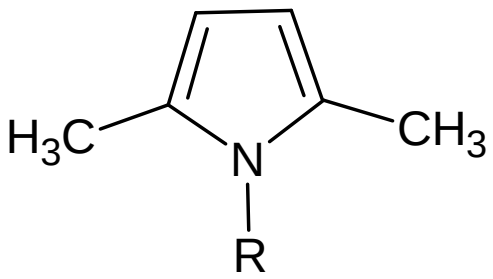
Molecular docking

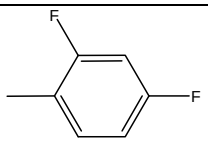
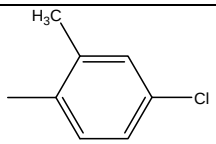
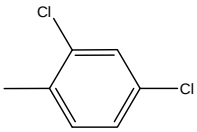
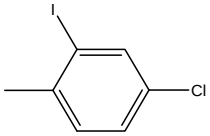
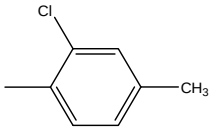
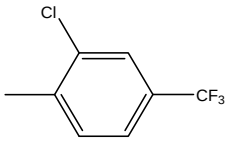
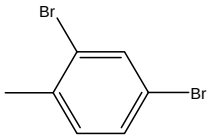
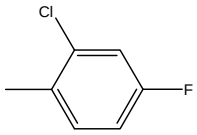
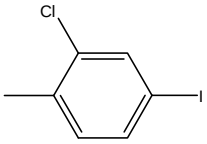
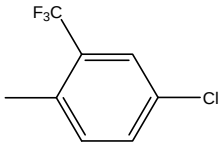
To identify potential COX-2 inhibitor compounds, docking calculations were performed using Auto Dock4.0 [9] into the 3D model of the catalytic site of COX-2 inhibitor (pdb code: 1CX2)[10].

This program starts with a ligand molecule in an arbitrary conformation, orientation, and position and finds favorable dockings in a protein-binding site using genetic algorithms. For the macromolecule, that was generated by resorting to multi body molecular dynamics, was downloading from the PDB bank server, polar hydrogens were added, and then Kollman united Atom charges and atomic solvation parameters were assigned. The grid maps of docking studies were computed using the AutoGrid4. Grid center was centered on the active site was obtained by trial and error. All parameters used in docking were default. The obtained results were evaluated in term of binding energy and docking pose into the catalytic site of COX-2 receptor. Table **1** shows the list of ligands.

The compounds were docked using Lamarckian genetic algorithm (LGA), where the number of GA=10, the population size=50, and the maximum number of energy evaluations is 250000. The result was analyzed using root mean square deviation (RMSD), estimated inhibition constant (*K*i), and estimated free energy of binding (Δ*G*). The best resulting Δ*G*=-11.5 kcal/mol was for compound 4c with RMSD 2.115 Å. The *K*i was 3.72×10-9. The binding energies of these inhibitors are listed in Table 1.

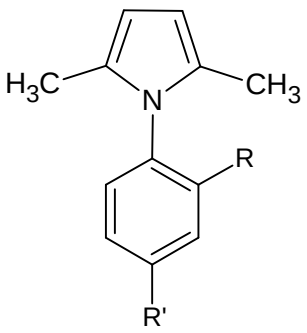
Table 1: List of substituted pyrrole derivatives (ligands) used in docking studies and mean binding energy of compounds (P1-P10) with analgesic receptor. (pdb ID: 1CX2)



Compound No.	R	Mean Binding Energy	Compound No.	R	Mean Binding Energy
P1		-5.72	**P6**		+9.38
P2		-5.71	**P7**		+6.26
P3		-5.82	**P8**		+6.26
P4		+1.05	**P9**		+6.26
P5		+6.26	**P10**		+9.64

Lamarckian genetic algorithm implemented in autodock has been successfully employed to dock inhibitors into the catalytic site of the COX-2 and to well correlate the obtained binding free energies with inhibitory activities of compounds P1-P10. Docking energy was used to identify the correct binding pose and then ranked the most best fitting target ligand complex based on their binding affinity. Tables **2** shows the energetic scores of the top solutions found during docking of compounds P1-P10. Compound P1, P2 and P3 endowed with the best binding energy among studied compounds (-5.72, -5.71 and -5.82 kcal/mol respectively) as compared to the Ketorolac (-9.64 kcal/mol). The compounds show interaction with the LYS97, GLY98, ASN101 and ILE102 residues.

Table 2: Physical data of synthesized compounds



Compund No	Amine	Molecular formula	Reaction time (hr)	Yield %	mp/bp °C
P1	2,4-difluroaniline	C12H11F2N	14	35	65-67 (mp)
P2	2,4-dichloroaniline	C12H11Cl2N	16	41	83-88
P3	2-chloro-4-methylaniline	C13H14ClN	13	52	84-90
P4	2,4-dibromoaniline	C12H11Br2N	15	37	88-92
P5	2-chloro-4-iodoaniline	C12H11ClIN	14	47	60-64
P6	2-methyl-4-chloro aniline	C13H14ClN	11	53	74-80
P7	2-iodo-4-chloroaniline	C12H11ClIN	16	59	64-69
P8	2-chloro-4-trifloromethylaniline	C13H11ClF3N	18	61	76-80
P9	2-chloro-4-fluroaniline	C12H11ClFN	12	72	88-93
P10	2-trifloromethyl-4-chloroaniline	C13H11ClF3N	16	69	169-173

Chemistry

Paal-Knorr was selected for the synthesis of substituted pyrrole derivatives (P1-P10). It was observed that the reactions are successful at room temperature and the reaction conditions are mild in comparison to other reported methods. The yield of compounds obtained was also satisfactory ranging from 35 to 72%.

Pharmacology

Compounds P1, P2 and P3 have shown analgesic effects in both methods used for screening. In writhing test these three compounds have shown <50% inhibition of writhing at the dose level of 47.50 mg/kg. At higher dose level i.e. 95 mg/kg compounds P1and P2 has shown 66.66% inhibition of writhing respectively. None of the compounds were found comparable to the standard drug Diclofenac sodium.

Similarly, in hot-plate test compound P1 and P2 has shown comparable effect at a dose level of 47.5 mg/kg at 30, 60 min duration with that of standard. When dose level was increase to 95 mg/kg compound P1 was found to possess better prolongation reaction time than that of standard drug at 30 and 60 min duration, which indicates that compounds P1 and P2 possess both central and peripheral effects.

3. Experimental

Chemistry

Paal-Knorr reaction method was selected for the synthesis of substituted pyrrole derivatives (P1-P10). The purity of the compounds was monitored by ascending thin layer chromatography (TLC) using silica gel G as stationary phase, visualized by iodine vapors. Developing solvent used in TLC was Petroleum ether: chloroform (3:2). Infrared (IR) spectra were recorded for the compounds on Shimadzu FTIR spectrophotometer 8400s (KBr). ^{1}H NMR spectra

were recorded on a Brucker Advance 300MHz spectrometer. Chemical shifts were expressed in parts per million (δ) with tetramethylsilane as an internal standard.

Synthesis

To a solution of substituted primary aromatic amine (0.024 mol) and hexane-2, 5-dione (0.02 mol) in THF (10 ml) at room temperature iodine (0.001 mol) was added. The mixture was stirred at room temperature. The reaction was monitored by TLC using silica gel G as stationary phase and mixture of organic solvents as mobile phase. An iodine vapor was used as detecting agent. Dichloromethane (50 ml) was then added to the mixture. The resulting mixture was washed successively with 5% w/v Na2S2O3 solution (20 ml), saturated NaHCO3 solution (20 ml) and brine (20 ml). The organic layer then dried with anhydrous sodium sulphate. It was concentrated under vacuum on rotary evaporator[11]. The concentrated product was purified with the help of column using silica gel as stationary phase and mixture of organic solvent as mobile phase. It was again concentrated on rotary evaporator and final product was collected.

Table 4: Analytical data of some synthesized compounds

S No.	Chemical Name	Analytical Data
01.	1-(2,4-difluorophenyl)-2,5-dimethyl-1*H*-pyrrole	IR (KBr): 3078, 2920, 2860, 1784, 1608, 1514, 1431, 1404, 1338, 1029, 873, 848, 613, 563 cm^{-1}; ^1H NMR (CDCl3) δ (ppm): 1.97 (s, 6H, $2CH_3$), 5.93 (s, 2H, CH), 7.21 (s, H, CH), 7.32-7.35 (d, H, CH), 7.55-7.58 (d, H, CH).
02.	1-(2,4-dichlorophenyl)-2,5-dimethyl-1*H*-pyrrole	IR (KBr): 3087, 3076, 2921, 2852, 1730, 1635, 1560, 1487, 1402, 1323, 867, 752cm^{-1}; ^1H NMR (CDCl3) δ (ppm): 1.99 (s, 6H, $2CH_3$), 5.92 (s, 2H, CH), 6.95-7.01 (m, 2H, CH), 7.20 (s, H, CH).
03.	1-(2-chloro-4-methylphenyl)-2,5-dimethyl-1*H*-pyrrole	IR (KBr): 3035, 2931, 2854, 1606, 1504, 1448, 1404, 1325, 871, 823, 752 cm^{-1}; ^1H NMR (CDCl3) δ (ppm): 1.87 (s, 6H, $2CH_3$), 2.33 (s, 3H, CH3), 5.84 (s, 2H, CH), 7.08-7.15 (d, 2H, CH), 7.26 (d, H, CH).
04.	2-bromo-1-(4-bromo-2-chlorophenyl)-5-methyl-1*H*-pyrrole	IR (KBr): 3080,2918,2854,1577,1554, 1479,1434,1402,1323,867,825,563 cm^{-1}; ^1H NMR (CDCl3) δ (ppm): 1.95 (s, 6H, $2CH_3$), 5.92 (s, 2H, CH), 7.14-7.17 (d, H, CH), 7.53-7.56 (d, H, CH), 7.87 (s, H, CH).
05.	2-bromo-1-(2-chloro-4-iodophenyl)-5-methyl-1*H*-pyrrole	IR (KBr): 3080,2918,2854,1610,1550,1479,1436,1400,1332,869,823,757,565 cm^{-1}. NMR (CDCl3) δ (ppm): 1.97 (s, 6H, $2CH_3$), 5.93 (s, 2H, CH), 7.21 (s, H, CH), 7.32-7.35 (d, H, CH), 7.55-7.58 (d, H, CH).
06.	2-bromo-1-(4-chloro-2-methylphenyl)-5-methyl-1*H*-pyrrole	IR (KBr): 3101,2920,2856,1631,1577,1490,1438,1409,1319,1029, 869,823,756 cm^{-1}. NMR (CDCl3) δ (ppm): 1.82 (s, 9H, $3CH_3$), 5.82 (s, 2H, CH), 6.99-7.23 (m, 3H, 3CH).
07.	2-bromo-1-(4-chloro-2-iodophenyl)-5-methyl-1*H*-pyrrole	IR (KBr): 3078,2916,2854,1629,1575,1475,1436,1402,1321,1058,869,825, 757,565cm^{-1}. NMR (CDCl3) δ (ppm): 1.87 (s, 6H, $2CH_3$), 5.85 (s, 2H, CH), 7.11-7.13 (d, H, CH), 7.35-7.36 (d, H, CH), 7.86(s, H, CH)
08.	2-bromo-1-[2-chloro-4-(trifluoromethyl)phenyl]-5-methyl-1*H*-pyrrole	IR (KBr): 3105,3053,2921,2858,1618,1506,1440,1384,837,759, 726 cm^{-1}. NMR (CDCl3) δ (ppm): 1.89 (s, 6H, $2CH_3$), 5.88 (s, 2H, CH), 7.34-7.37 (d, H, CH), 7.56-7.59 (d, H, CH), 7.75 (s, H, CH)
09.	2-bromo-1-(2-chloro-4-fluorophenyl)-5-methyl-1*H*-pyrrole	IR (KBr): 3070,2933,2862,1581,1498,1438,1406,1336,1064,823,756, 649 cm^{-1}. NMR (CDCl3) δ (ppm): 1.85 (s, 6H, $2CH_3$), 5.82 (s, 2H, CH), 6.95-7.01 (m, H, CH), 7.14-7.20 (m, 2H, 2CH)
10.	2-bromo-1-[4-chloro-2-(trifluoromethyl)phenyl]-5-methyl-1*H*-pyrrole	IR (KBr): 3078,3049,2921,2864,1602,1575,1492,1438,1417,1068,838, 756,671 cm^{-1}. NMR (CDCl3) δ (ppm): 1.91 (s, 6H, $2CH_3$), 5.89 (s, 2H, CH), 7.18-7.21 (d, H, CH), 7.61-7.63 (d, H, CH), 7.79 (s, H, CH)

The physical properties of the synthesized compounds are presented in Table **3**. The structures were characterized by both spectral and elemental analyses. The analytical data are presented in Table **4.**

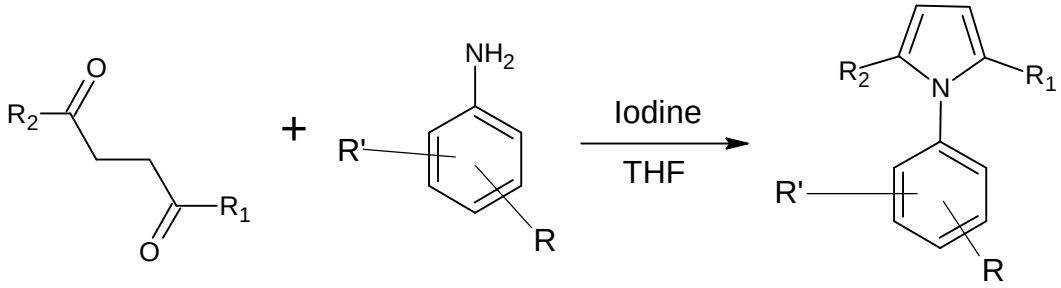


Fig. 1. Scheme of synthesis

Pharmacological screening

All the compounds were screened for analgesic activity by two methods; acetic acid induced writhing method and hot plate method. Wistar albino mice of either sex, weighing 25-30 g were used. Animals were fasted for 24 hrs before starting the experiment. The synthesized compounds were administered intraperitoneally (*i.p.*) as a suspension in 2% w/v Tween-80. The compounds were administered in doses equivalent to 1/5th, 1/10th and 1/20th of LD50[12]. Control animals received the equivalent volume of solvent. The animals were divided into different groups with six mice in each group.

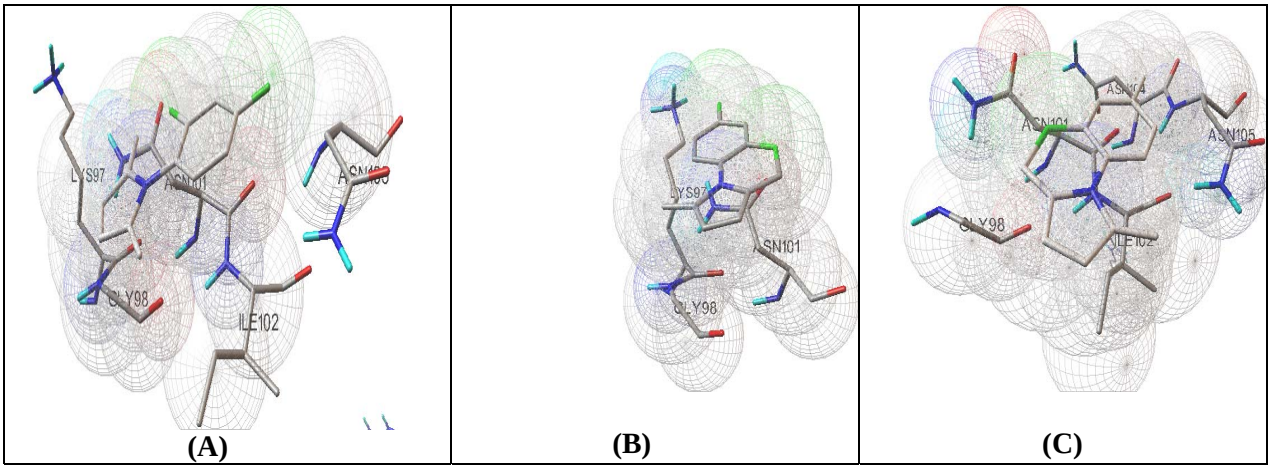


Fig. 2. (A) Docked conformation of compound P1 into binding site of COX-2 receptor. (B) Docked conformation of compound P2 into binding site of COX-2 receptor. (C) Docked conformation of compound P3 into binding site of COX-2 receptor.

Acetic Acid Induced Writhing

1% w/v Acetic acid solution (1ml/100g, *i.p.*) was administered to control group and writhing was counted for 15 min. 30 min after the administration of the test compounds and standard, Diclofenac sodium (10 mg/kg, *i.p.*)[13], 1% w/v acetic acid solution was administered and writhes were counted for 15 min.

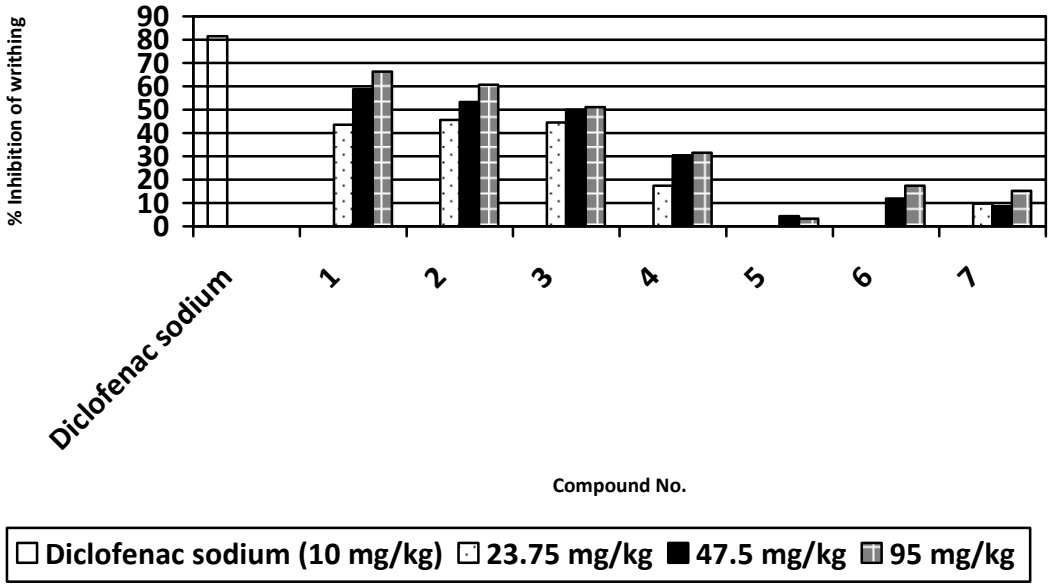


Fig. 3: % inhibition of writhing by standard and compounds (1-7) in ´writhing test' in mice.

Hot-Plate Method

Animals were placed individually on the metal plate heated to 55 ± 1 ºC. The time of appearance of the pain reactivity (licking of the forepaws, shaking or jumping) was measured as response. Experiment was performed 30 min after the treatment of test compounds and standard Pentazocine (10 mg/kg, *i.p.*)[14]. Reading was recorded after drug treatment at 30 min, 60 min, 2 h and 3 h intervals.

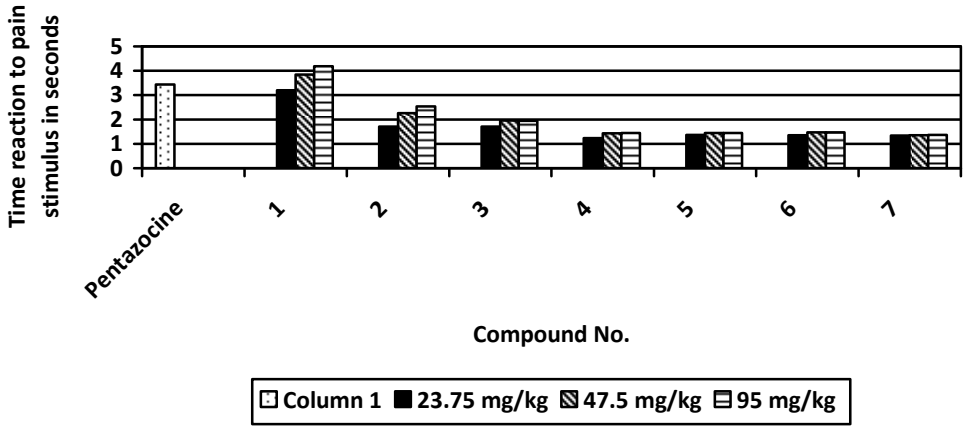


Fig. 4: Time reaction to pain stimulus (sec) in Hot plate test at 1 Hr duration.

Statistics

The data obtained from both the methods was analyzed statistically using Student's *t*-test. $p<0.05$ was considered as significant when compared to control.

Analgesic Activity

The synthesized compounds were subjected to analgesic activity evaluation at the dose level of 23.75, 47.5, 95 mg/kg (i.p.). The results were compared with standard drug Diclofenac sodium and Pentazocine by acetic acid induced writhing method and Hot plate method respectively.

4. Conclusions

During docking experiments with COX-2 compounds P1-P3 have shown average binding energy of -5.75 kcal/mol. The results shows strong potential of these substituted pyrrole derivatives to be developed as anticonvulsant drugs. For analgesic activity two different experimental models (acetic acid induced writhing and hot plate test) were used. Compounds P1, P2 and P3 have shown analgesic effect at 47.5 and 95 mg/kg dose level.

The result shows these potential substituted pyrrole derivatives helps in finding novel analgesic agent with better activity and fewer side effects. Molecules explored can be used for treatment of pain management or as lead molecules to develop new molecules.

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