Synthesis, Characterization, Crystal Structures, Analgesic and Antioxidant Activities of Thiourea Derivatives

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Summary: 1-(2-chlorobenzoyl)-3-(2,3-dimethyl-5-oxo-1-phenyl-2,5-dihydro-1H-pyrazol-4-yl)thiourea (**Cl2AAP)** and 1-(2-chlorobenzoyl)-3-(pyridine-2-yl)thiourea (**Cl2AP**) were synthesized and characterized by spectroscopic methods (FT-IR, ^{1}H NMR) and single crystal X-ray diffraction. Both compounds crystallized in monoclinic system and solved in $P2_1/n$, Z = 4 and $P2_1/c$, Z = 12 space groups respectively. Pharmacological screenings of the synthesized compounds have shown comparable analgesic activity to that of the standard diclofenac sodium at 30 mg/kg body weight dose. The DPPH and ABTS free radical scavenging activity of Cl2AAP are more significant than Cl2AP as compared to standard (IC50= 10, 60; 45, 75; and <10 µg/ml for standard Ascorbic acid respectively).

Key Words: Thiourea derivatives, Analgesic activity, Writhing, Antioxident activity, DPPH, ABTS, XRD.

Introduction

Pain is treated with opoid analgesics and non steroidal anti-inflammatory drugs (NSAID's). NSAIDs diminish pain sensation by inhibiting the enzyme cyclooxygenase (COX) and thereby the production of prostaglandins that are the metabolic products of arachidonic acid (AA) [1]. However, prolong use of NSAIDs is accompanied by many side effects, including gastrointestinal bleeding, lesions, as well as nephro and hepato-toxicities [2, 3]. Developing safer analgesics and designing new drug molecules without such side effects has gained more attention of the researchers [4].

Free radicals are atomic/ molecular chemicals having unpaired electrons. These free radicals and reactive oxygen species (ROS) like superoxide anion (O_2), hydroxyl (OH), peroxyl (RO_2) and nitric oxide (NO) are highly reactive species produced continuously in the body during normal physiological processes [5]. Antioxidant defense mechanisms of the body normally inactivate these free radicals/ ROS; however, in pathological conditions these radicals and ROS are overproduced and unfavorably react with macromolecules, DNA, lipids and proteins leading to degenerative diseases like atherosclerosis, heart attack, cancer, asthma, hepatitis, dermatitis and liver cirrhosis [6]. In old age, degeneration of neurons results in increased production of ROS causing proteins, lipids, cell membrane, nucleic acids damage and apoptotic cell death [7].

Antioxidants provide first-line defense mechanism against all types of free radicals. So they are very important for optimum health maintenance. Presently, the need of antioxidants consumption gained much importance because of more chances of getting exposed to free radicals. To maintain a balanced and healthy lifestyle, diet supplemented with antioxidants has got good recognition for protection against free radicals [8]. Synthetic antioxidants are used widely these days because of their effectiveness and cheapness as compared to natural antioxidants [9].

Synthesis of heterocyclic compounds having biological and commercial importance is focused these days [10]. Antipyrine compounds are of paramount importance in modern organic synthesis because of their huge biological importance [11]. 4-amino antipyrine is a derivative of antipyrine, used as a precursor for synthesis of polyfunctionally substituted heterocyclic compounds, which are well known for biological activities like anti-inflammatory, analgesic, antioxidant, antimicrobial and anticancer [12].

Similarly 2-aminopyridine derivatives have also been reported to possess various pharmacological activities like antioxidant [13], antibacterial [14], antifungal [15], antidiabetic [16], antiviral [17] and analgesic activity [18].

Based on the reported chemical and biological importance of 4-aminoantipyrine and 2-aminopyridine we report herein the synthesis, analgesic and antioxidant activities of 4-amino antipyrine and 2-aminopyridine thiourea derivatives.

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Experimental

Chemistry

2-chlorobenzoyl chloride (sigma Aldrich), 4-aminoantipyrine (sigma Aldrich), 2-aminopyridine (sigma Aldrich), potassium thiocyanate (sigma Aldrich) were purchased from local supplier. Reaction progress was assessed by thin-layer chromatography (TLC). The spots were visualized on silica gel-G coated aluminum plates (Merck 60F-254, 0.5 mm), using UV light at 254 nm wavelength and subsequent iodine vaporization.

Characterization of synthesized compounds was done with BRUKER (Varian Mercury 300 MHz) FT spectrometer, to obtained ^{1}H-NMR spectra using DMSO-d6. The IR spectra was obtained with Perkin Elmer FTIR system BX LR64912C. The x-ray crystallography was done on KAPPA Bruker APEX-*II* Diffractometer. Barnstead Electrothermal Thermo Scientific 9100 apparatus was used to determine melting point of the synthesized compounds.

General Procedure for Synthesis of Cl2AAP and Cl2AP

The synthesis was carried out according to the reported protocol [19]. To 0.485 g of Potassium thiocyanate (5 mmol) in 10 ml acetone was added drop wise 0.633 ml of 2-chlorobenzoyl chloride solution (5 mmol) in 10 ml acetone and mixture was refluxed for three hours at 50-55 °C. The reaction mixture was then filtered to remove potassium chloride. Then 1.01 g of 4-aminoantipyrine (5 mmol) in acetone (10 ml) were added drop wise to filtrate at 50-55 °C and refluxed for 5-6 hours. Precipitation started after 45 min. The precipitate was filtered and washed with excess acetone and finally re-crystallized in a mixture of DMSO-ethanol (1:1) to give 1-(2-chlorobenzoyl)-3-(2,3-dimethyl-5-oxo-1-phenyl-2,5-dihydro-1H-pyrazol-4-yl)thiourea.

Similar procedure was followed for the synthesis of 1-(2-chlorobenzoyl)-3-(pyridine-2-yl)thiourea except vacuum evaporation of the final reaction mixture, the resulted solids in the rotary flask was washed with water, n-hexane and re-crystallized in chloroform/methanol mixture (2:1).

1-(2-chlorobenzoyl)-3-(2,3-dimethyl-5-oxo-1-phenyl-2,5-dihydro-1H-pyrazol-4-yl)thiourea **(Cl2AAP)**

Yield: 90% m.p: 235 °C. ^{1}H NMR (DMSO-d_6, 300 MHz) δ 12.11 (s, 1H), 11.30 (s, 1H), 7.75 – 7.22 (m, 9H), 3.13 (s, 3H), 2.22 (s, 3H) IR (KBr, cm^{-1}): 3130 (N–H), 2910 (C–H aromatic), 1690-1650 (C=O), 1545-1485 (C–H aliphatic), 1280 (C=S), 1175 (C–N), 755 (C-Cl).

1-(2-chlorobenzoyl)-3-(pyridine-2-yl)thiourea **(Cl2AP)**

Yeild: 90% m.p: 116 °C. ^{1}H NMR (DMSO-d_6, 300 MHz) δ 13.01 (s, 1H), 12.28 (d, J = 17.6 Hz, 1H), 8.77 (s, 1H), 8.44 (d, J = 5.0 Hz, 1H), 7.93 (td, J = 7.8, 1.9 Hz, 1H), 7.78 – 7.25 (m, 5H) IR (KBr, cm^{-1}): 3130 (N–H), 2910 (C–H aromatic), 1690-1650 (C=O), 1545-1485 (C–H aliphatic), 1280 (C=S), 1175 (C–N), 755 (C-Cl).

X-Ray Crystallography

The structures of the synthesized compounds were solved by direct methods using SHELXS97 and refinement was performed with SHELXL97. Full-matrix least-squares refinement was based on F^2. All non-hydrogen atom parameters were refined anisotropically. Although the hydrogen atoms appeared in different Fourier maps but were positioned geometrically to their idealized positions, NH= 0.86, CH= 0.93(aromatic) and 0.96(CH3) Å, and refined with a "riding model" with isotropic displacement parameters Uiso(H) = xUeq(C, N), whereas x = 1.5 for methyl and x = 1.2 for all other H-atoms. Crystal's data and details of the structure are listed in following Table-1.

Central Analgesic Activity (Tail Immersion Method)

Following previous reported protocol, tail immersion method was used to evaluate the central analgesic activity of the compounds [21]. Swiss albino mice having weight between 20–25 g were selected for this study. The animals were divided into four groups of six animals each. Group I, received 0.5% w/v of tween 80 (2 ml/kg) in normal saline intra-peritoneally, which was kept as a control group. Group II, received diclofenac sodium (25 mg/kg), and was used as standard. Group III and Group IV (test groups) received 25 mg/kg body weight dose of synthetic compounds. After the drug administration, time taken by mice to withdraw its tail from hot water was noted. The withdrawal time for each

mouse was noted before drug administration. Time taken in seconds to withdraw its tail clearly from hot water by mice was noted. The reaction time readings were recorded at 0 (soon after *ip* injection of the compounds), 30, 60, 120 and 180 min, respectively. The mean time of test and standard groups was noted and compared then using the following formula, percent analgesic activity was measured

$$\% \text{ Antinociceptive activity} = \{(A2 - A1)/A2\} \times 100$$

where A1 is the reaction time of control and A2 is reaction time of test group.

Antioxidant Activity

DPPH Free Radical Scavenging Activity

Antioxidant activity of the synthesized compounds was evaluated by using a stable free radical, i.e. scavenging influence of the 1, 1-Diphenyl-2-picrylhydrazyl (DPPH). Ascorbic acid was used as a standard (positive control) [22]. Briefly, dilute solutions of the synthetic compounds and positive control in methanol were prepared ranging from 62.5-1000 µg/ml. Then 0.002% solution of DPPH was prepared in methanol by taking 2 mg of DPPH in 100 ml methanol. DPPH solution (1 ml) was added to different prepared concentrations of standard and samples (1 ml). These solutions were then kept in a dark area for about 30 min. By using spectronic spectrophotometer, the absorption of each sample was measured against methanol and DPPH solution as a blank at wavelength of 517 nm. Using the following formula percent radical scavenging activity was calculated.

$$\% \text{ scavenging activity} = \frac{A\text{-}A^{o}}{A} \times 100$$

"A" = absorption of control, "Aº" = absorption of sample

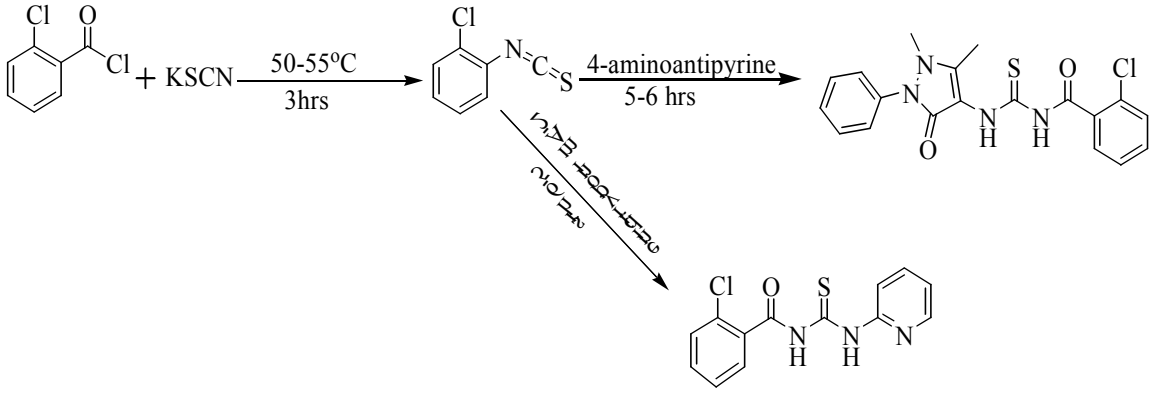
The IC50 values were calculated for test samples and standard, which is the minimum concentration of test sample required to reduce 50% of the free radical concentration.

ABTS Free Radical Scavenging Antioxidant Activity

Based on the reported protocol, ABTS free radical scavenging activity was carried out [23]. The activity is based on the measurement of absorbance at 734 nm, obtained as a result of antioxidant ability of sample to scavenge ABTS radical cation. 7 mM ABTS and 2.45 mM potassium persulphate solution were mixed in order to prepare ABTS solution. The ABTS solution was then incubated for 16 hours at room temperature in the dark. Before addition to sample solutions, methanol was added to ABTS solution to get an absorbance of 0.706 ± 0.001 at 734 nm. Sample solutions of synthesized compounds were prepared by adding methanol, to obtain different concentrations (62.5-1000 µg/ml) of the synthesized compounds. To each sample and standard's (Ascorbic acid) solutions, 3 ml of ABTS solution was added and after incubation of one-minute absorbance was measured. Experiment was performed in triplicate manner. Using the formula, the percent scavenging activity of the tested samples was obtained.

$$\% \text{ scavenging activity} = \frac{A\text{-}A^{o}}{A} \times 100$$

where A is absorbance of ABTS and Aº is the combine absorbance of tested samples and ABTS solution mixture.



Scheme-1: Synthetic pathway towards thiourea derivatives.

Table-1: Summary of crystallographic data and structural parameters of synthesized compounds.

	Cl2AAP	Cl2AP
Formula	$C_{19}H_{17}ClN_4O_2S$	$C_{13}H_{10}ClN_3OS$
M_r	400.88	291.75
Crystal Size (mm)	0.45 × 0.38 × 0.28	0.40 × 0.34 × 0.30
Crystal system	MonoClinic	MonoClinic
Space group	$P2_1/n$	$P2_1/c$
a, b, c (Å)	9.2398 (5), 10.1831(6), 19.7907 (13)	14.1955(8), 10.4596(5), 27.7797(13)
α, β, γ (°)	90, 92.120(3), 90	90, 99.513(2), 90
V (Å)^3	1860.83(19)	4068.0(4)
Z	4	12
$\Delta\rho$max., min (e.Å^{-3})	0.853, -0.569	0.677, −0.650
Mo $K\alpha$ radiation, λ (Å)	0.71073	0.71073
D_{calcs}[g cm^{-1}]	1.431	1.429
F(000)	832	1800
μ (mm^{-1})	0.34	0.43
Diffractometer	Bruker KAPPA APEX II	Bruker KAPPA APEX II
Data collection range	h = −11→7, k = −9→12, l = −24→24	h = −18→18, k = −12→13, l = −35→33
No. of reflections	3654	8887
No of parameters	220	514
Temperature (K)	296(2)	296(2)
R_{int}	0.027	0.036
No. of measured, independent and observed [I>2 σ(I)] reflections	13515, 3654, 2871	32857, 8887, 5624
GOF	1.049	1.017
$R[F^2 > 2\sigma(F^2)]$, $wR(F^2)$	0.0585, 0.1514	0.0490, 0.1197

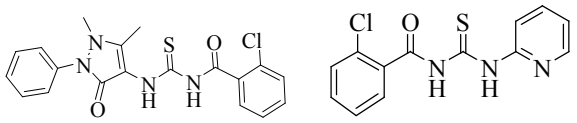
Results and Discussion

Chemistry

Synthetic route for the synthesis of thiourea derivative is shown in the Scheme-1. ^{1}H NMR of synthesized compound (Cl2AAP) shows respective peaks for methyl protons as singlets at 2.22 and 3.13 ppm. Peaks for NH of the thiourea group are observed at 11.30 and 12.11 ppm respectively as singlets. The aromatic protons give a multiplet peak from 7.22-7.75 ppm. The spectra show 17 protons that are in accordance with the structure of the synthesized compound. The IR spectrum of Cl2AAP shows characteristic absorption peaks at 3130 for N–H stretching, at 2910 for C–H aromatic, at 1690-1650 for C=O, at 1545-1485 for C–H aliphatic, at 1280 for C=S, at 1175 for C–N and at 755 for C-Cl stretching. Similarly ^{1}H NMR of compound (Cl2AP) shows two singlets for NH protons at 13.01 and 8.77 ppm. At 12.28 ppm doublet is seen for proton attached to C adjacent to N of pyridine ring. All other peaks correspond to their relative aromatic protons. The IR spectrum of compound (Cl2AP) shows characteristic absorption peaks at 3125 for N-H stretching, at 3040 for C–H, at 1690 for C=O, at 1540 for C=N, at 1120-1100 for C=S, at 1260 for N-C=S and at 755 for C–Cl stretching.

Crystal Structure and Conformations

The molecular structures of compounds Cl2AAP and Cl2AP are presented in Fig. 1 and 2 respectively. Selected bond lengths, bond angles and torsion angles are listed in Table-2 while H-bonding interactions are given in Table-3.



Cl2AAP Cl2AP

Fig. 1: Structures of the synthesized compounds.

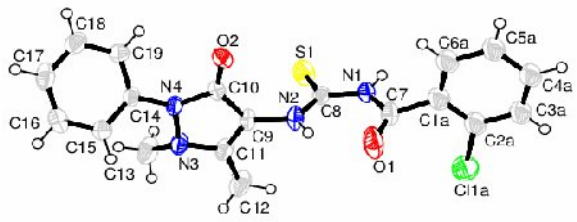


Fig. 2: ORTEP Diagram of Cl2AAP at 50% probability level. The minor portion is omitted for clarity.

In Cl2AAP (Fig. 2), 2-chlorobenzene is disordered on opposite sides with two sets of sites and have an occupancy ratio of 0.760(2):0.240(2). The disordered benzene is treated as regular hexagon and all C-atoms are treated as having same anisotropic thermal parameters. The dihedral angle between the planes of the disordered rings is 18.214 (460)°. The dihedral angle between (C9/C10/C11/N3/N4) and (C14—C19) is 41.755 (128)°. The molecules are stabilized due to various types of H-interactions.

Table 2: Selected bond angles, bond lengths and torsion angles (A˚, ◦).

Cl2AAP		Cl2AP	
C1A-C7	1.525(4)	Cl1-C2	1.728(3)
C2A-Cl1A	1.710(3)	S1-C8	1.649(2)
C7-N1	1.357(3)	O1-C7	1.217(3)
C7-O1	1.215(4)	N1-C7	1.370(3)
C8-N1	1.398(3)	N1-C8	1.399(3)
C8-N2	1.333(3)	N2-C8	1.339(3)
C8-S1	1.652(3)	N2-C9	1.411(4)
C9-N2	1.417(3)	C1-C7	1.501(3)
C1A-C7	1.525(4)	N2-C9-N3	110.6(2)
C2A-C1A-C7-N1	-107.2(3)	N2-C9-C10	126.3(2)
C6A-C1A-C7-N1	79.7(3)	C8-N1-C7-O1	2.8(4)
C6A-C1A-C7-O1	-95.9(3)	C8-N1-C7-C1	-179.4(2)
S1-C8-N1-C7	-170.8(2)	C7-N1-C8-S1	177.9(2)
S1-C8-N2-C9	-9.4(3)	C7-N1-C8-N2	-1.8(4)
C10-C9-N2-C8	-89.7(3)	C9-N2-C8-S1	-2.7(4)
C11-C9-N2-C8	95.4(3)	C9-N2-C8-N1	176.9(2)
C15-C14-N4-C10	-126.0(3)	C8-N2-C9-N3	176.7(2)
C15-C14-N4-N3	26.2(3)	C8-N2-C9-C10	-5.1(5)
C19-C14-N4-C10	54.6(3)	C2-C1-C7-O1	-98.2(3)
C19-C14-N4-N3	-153.1(2)	C6-C1-C7-O1	81.0(4)
C2A-C1A-C7-N1	-107.2(3)		

Table-3: Bonding geometry of Cl2AAP and Cl2AP (A˚, ◦).

Compound	D—H···A	D—H	H···A	D···A		D—H···A
Cl2AAP	C12—H15B···Cl1Bi	0.96	2.72	3.676	(6)	173.5
	C13—H13C···Cl1Aii	0.96	2.93	3.745	(3)	143.2
	N1—H1···O2iii	0.86	2.01	2.823	(3)	157.7
	N2—H2···O1	0.86	1.99	2.663	(3)	134.5
	N1—H1···N6	0.86	2.16	2.995	(3)	164.7
	N2—H2···O1	0.86	1.93	2.662	(3)	142.6
Cl2AP	C10—H10···S1	0.93	2.57	3.219	(3)	127.0
	N4—H4A···N9i	0.86	2.23	3.080	(3)	170.8
	N5—H5A···O2	0.86	1.94	2.668	(3)	142.3
	C16—H16···O1ii	0.93	2.59	3.478	(4)	159.9
	C23—H23···S2	0.93	2.53	3.186	(3)	127.6
	N7—H7···N3iii	0.86	2.13	2.966	(3)	165.5
	N8—H8···O3	0.86	1.94	2.667	(3)	141.7
	C36—H36···S3	0.93	2.54	3.192	(3)	127.3

Symmetry codes for Cl2AAP: (i) x, y+1, z; (ii) x+1/2, −y+1/2, z+1/2; (iii) −x+2, −y, −z.
Symmetry codes for Cl2AP: (i) −x, y+1/2, −z+1/2; (ii) −x, −y+1, −z; (iii) x, −y+1/2, z+1/2.

As shown in Fig. 3, there are 3 symmetrically independent molecules in the asymmetric unit for Cl2AP. In first molecule the dihedral angle between the benzene ring A (C1—C6) and the pyridine ring B (C9—C13/N3) is 76.264 (103)˚, in second molecule the dihedral angle between the benzene ring C (C14—C19) and the pyridine ring D (C22—C26/N6) is 62.395 (082)˚ and in third molecule the dihedral angle between the benzene ring E (C27—C32) and the pyridine ring F (C35—C39/N9) is 72.491 (98)˚. The molecules are stabilized due to various types of H-interactions.

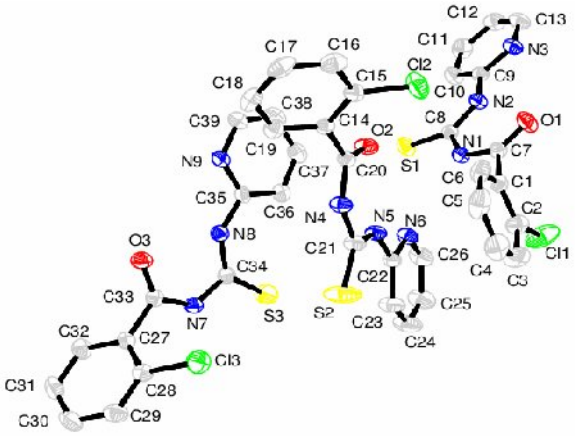


Fig. 3: ORTEP Diagram of Cl2AP at 50% probability level. The H-atoms are omitted for clarity.

Tail Immersion Method

Both synthetic compounds were found active showing enhanced percent analgesic activity. The percent analgesic activity of the tested compounds was recorded at different time intervals, i.e. 30, 60, 120 and 180 min. Both compounds and standard diclofenac showed highest activity at 120-minute intervals. At this time interval compound Cl2AP showed 77.05 percent, Cl2AAP showed 75.69 percent and diclofenac sodium showed 85.01 percent analgesic effect as shown in Table-5.

Table-4: Analgesic activity (writhing data) of the synthesized thiourea derivatives.

Samples	Dose (mg/kg)	Mean writhes (SEM)	% Analgesic activity
Tween 80	0.5%	52.33±0.42	0.00
Diclofenac Sodium	15	6.40±0.00***	87.76
	30	3.67±0.49***	92.98
Cl2AP	15	8.66±0.33***	83.45
	30	6.33±1.45***	86.90
Cl2AAP	15	12.00±0.57***	77.06
	30	7.66±0.88***	85.36

Data expressed as Mean ± SEM (n=6), all vs control at 95% confidence of interval. *P*>0.05 (non- significant) and *P*<0.001 *** (highly significant)

Antioxidant Activity

DPPH Scavenging Effect

Test samples were assayed against DPPH free radicals which showed noticeable free radical scavenging potentials. The antioxidant activity of the synthesized thiourea derivatives was compared with Ascorbic acid (positive control). Antioxidant activity was measured in terms of IC50 values. The Cl2AP demonstrated percent scavenging activity of 79.48 ± 0.85, 70.14 ± 1.46, 61.91 ± 1.70, 58.16 ± 2.02 and 55.06±1.00 at different concentrations (1000-62.5 µg/ml) respectively with IC50 value of 45µg/ml. Similarly, the Cl2AAP showed 75.81 ± 1.60, 70.15 ± 2.02, 65.88 ± 1.48, 62.96 ± 2.62 and 61.04 ± 1.00% antioxidant activity at different concentrations (1000-62.5 µg/ml) respectively with IC50 value of 10 µg/ml as shown in Table-6.

ABTS Free Radical Scavenging Activity

The ABTS free radical scavenging potentials of the tested compounds are summarized in Table-7. Compound Cl2AAP was found most active against ABTS free radical. It caused 88.10 ± 1.56. 77.81 ± 2.25, 69.87±0.94, 60.92±1.36 and 50.33±1.76 percent ABTS free scavenging activity at 1000, 500, 250, 125 and 62.5 µg/ml concentrations respectively with IC50 value of 60 µg/ml. The percent ABTS free radical scavenging activity of compound Cl2AP was also prominent at higher concentrations. It showed 83.91 ± 0.95, 74.33 ± 0.88, 68.09 ± 0.62, 59.24 ± 0.62 and 47.82 ± 0.86 percent ABTS free radical scavenging activity at concentrations of 1000, 500, 250, 125 and 62.5 µg/ml respectively with IC50 value of 75 µg/ml as compared to the standard used in the experiment.

Table-5: Analgesic activity (tail immersion test) of the synthesized compounds.

Mean reaction time in seconds (MEAN±SEM)					% Activity		
Time (min)	**Control**	**Standard**	**Cl2AP**	**Cl2AAP**	**Standard**	**Cl2AP**	**Cl2AAP**
30	**1.03±0.14**	**5.46±0.26*** **	**3.20±0.20*** **	**2.41±0.17** **	**81.13**	**67.81**	**57.26**
60	**1.16±0.38**	**7.56±0.28*** **	**4.76±0.40*** **	**4.50±0.17*** **	**84.65**	**75.63**	**74.22**
120	**1.23±0.13**	**8.21±0.17*** **	**5.36±0.28*** **	**5.06±0.17*** **	**85.01**	**77.05**	**75.69**
180	**1.00±0.30**	**6.20±0.11*** **	**3.25±0.21*** **	**2.40±0.15** **	**83.87**	**69.23**	**58.33**

Data expressed as Mean ± SEM (n=6), all vs control at 95% confidence of interval. *P*>0.05 (non- significant) and *P*<0.001 *** (highly significant)

Table-6: DPPH free radical scavenging activity of synthesized compounds.

Sample	Concentration(µg/ml)	% scavenging effect Mean ± SEM	IC50(µg/ml)
Cl2AP	**1000**	**79.48±0.85*** **	**45**
	500	**70.14±1.46*** **	
	250	**61.91±1.70*** **	
	125	**58.16±2.02*** **	
	62.5	**55.06±1.00*** **	
Cl2AAP	**1000**	**75.81±1.60*** **	**10**
	500	**70.15±2.02*** **	
	250	**65.88±1.48*** **	
	125	**62.96±2.62*** **	
	62.5	**61.04±1.00*** **	
Ascorbic acid	**1000**	**92.55±0.35**	**<10**
	500	**87.84±0.26**	
	250	**81.33±0.88**	
	125	**76.54±0.54**	
	62.5	**72.67±0.19**	

Data is represented as mean ± SEM, where n=3

The results of this experiment are presented as mean ± SEM. The level of significance was calculated by two way ANOVA followed by Bonferroni test. *P*<0.05 were considered statistically significant and *P*<0.001 as highly significant.

Table-7: ABTS free radical scavenging activity of compounds.

Sample	Concentration(µg/ml)	Percent scavenging Mean ± SEM	IC50(µg/ml)
Cl2AP	**1000**	**83.91±0.95*** **	**75**
	500	**74.33±0.88*** **	
	250	**68.09±0.62*** **	
	125	**59.24±0.62*** **	
	62.5	**47.82±0.86*** **	
Cl2AAP	**1000**	**88.10±1.56***	**60**
	500	**77.81±2.25*** **	
	250	**69.87±0.94*** **	
	125	**60.92±1.36*** **	
	62.5	**50.33±1.76*** **	
Ascorbic acid	**1000**	**92.55±0.35**	**<10**
	500	**87.84±0.26**	
	250	**81.33±0.88**	
	125	**76.54±0.54**	
	62.5	**72.67±0.19**	

Data is represented as mean ± SEM, where n=3

The results of this experiment are presented as mean ± SEM. Two way ANOVA followed by Bonferroni test used for level of significance. *P*<0.05 were considered statistically significant and *P*<0.001 as highly significant.

Conclusion

1-(2-chlorobenzoyl)-3-(2,3-dimethyl-5-oxo-1-phenyl-2,5-dihydro-1H-pyrazol-4-yl)thiourea and 1-(2-chlorobenzoyl)-3-(pyridine-2-yl)thiourea were successfully synthesized, characterized and subjected to analgesic and antioxidant screenings. Peripheral analgesic activity of Cl2AP is slightly greater than CL2AAP (86.90, 85.37 %) as compared to Diclofenac (92.98 %) at 30 mg/kg dose (P<0.001), while central analgesic activity of both compounds at the 120th minute is 77.05 and 75.69 % respectively as compared to the standard analgesic (P<0.001). The DPPH and ABTS free radical scavenging activity of Cl2AAP are more significant than Cl2AP as compared to standard (IC_{50}= 10, 60; 45, 75; and <10 µg/ml for standard Ascorbic acid respectively) (P<0.001). Results of the preliminary biological screening of these compounds show that these compounds could offer a hopeful scaffold in discovery of a novel drug having analgesic and antioxidant potentials.

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Supplementary Material

The Cif files and crystal data of these compounds have been submitted to the Cambridge Crystallographic Data Center as supplementary publication numbers CCDC No 1033252 for Cl2AP and CCDC No 1033257 for Cl2AAP and can be obtained on request free of charge at deposit@ccdc.cam.ac.uk.

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