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Synthesis and pharmacological screening studies of some novel imidazo[2,1-*b*][1,3,4]thiadiazole

Madhu Kumar Dogganal Jayappa[a], Jagadeesh Prasad Dasappa[a*], Rejeesh Edavan Puthallath[b] and Prakash Anil Castelino[a]

[a]*Department of Post-Graduate Studies and Research in chemistry, Mangalore University, Mangalagangothri - 574199, Karnataka, India*
[b]*Department of Pharmacology, Government Medical College, Palakkad-678013, Kerala India*

ABSTRACT

*A series of novel heterocyclic systems 2-((6-(aryl)imidazo[2,1-b][1,3,4]thiadiazol-2-yl)thio)-N-(4-fluorophenyl)acetamide **(4a-k)** were obtained by heating 2-((5-amino-1,3,4-thiadiazol-2-yl)thio)-N-(4-fluorophenyl)acetamide **(3)** with various α-haloketones. The newly synthesized compounds were characterized by spectral techniques and evaluated for their antibacterial, antifungal, anthelmintic activity and in-vivo anti-inflammatory, analgesic activities. The pharmacological results revealed that some of the tested compounds showed good activity.*

Keywords: 5-amino-1,3,4-thiadiazole, imidazothiadiazole, antimicrobial activity, anti-inflammatory, analgesic activities

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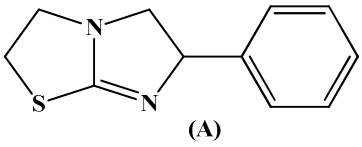
INTRODUCTION

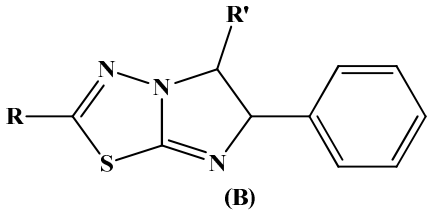
Imidazothiazole derivatives are widely used in the treatment of worm infestations in both humans and animals. For Example, Levamisol an anthelmintic, by targeting the nematode nicotinergic acetylcholine receptor [1]. Levamisole **(A)** appears to be the most effective in patients with small tumor burdens and acts by stimulating the responsiveness of the lymphocytes to tumor antigens [2]. Also, the imidazo[2,1-*b*]thiazole **(B)** derivatives of Levamisole have been reported as potential antitumor agents [3]. Later, antitumor activity of 5-formyl-6-arylimidazo-[2,1-*b*][1,3,4]thiadiazolesulfonamides **(C)** was also reported [4]. As an immunomodulator, Levamisole appears to increase the NK cells and activates T-cells in patients receiving this adjuvant along with 5FU for Stage III colon cancer. It is also found to have alkaline phosphatase inhibitory property [5]. Imidazothiazoles are heterocyclic compounds containing an imidazole ring fused to a thiazole ring. The compound has shown to have a low therapeutic index with LD_{50} = 40 mg/kg (Pigs, subcutaneous); LD_{50} = 180 mg/kg (rat, oral) [6]. Levamisole was used to treat various cancers before being withdrawn from the United States market in 2000 because of its adverse effects. It is currently approved as an anthelminthic agent in veterinary medicine [7], but is also being used illicitly as a cocaine adulterant [8]. Potential complications associated with the use of levamisole-laced cocaine include neutropenia, agranulocytosis, arthralgias, retiformpurpura, and skin necrosis [9]. The Pleiotropic effects of imidazothazole derivatives indicate vast potential for discovery of several drug candidates having various therapeutic indications. Research for the development of novel derivatives with low adverse effects is in demand nowadays. Drug discovery through functional group modification of imidazothiadiazole nucleus for another

alternatives of heterocyclic derivatives, where developing safe and efficacious derivatives seems to be necessary and promising. Hence, the design of persuasive biologically active compounds containing the annulated imidazo[2,1-*b*][1,3,4]thiadiazoles ring system which seems to be a vital move towards the goal of safer and more potent therapeutic agents we attempted.



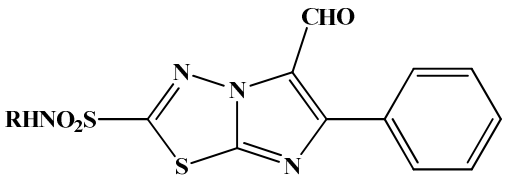
(A)

Levamisole



(B)

2,5-disubstituted-6-phenyl imidazo[2,1-b]thiadiazole



(C)

5-formyl-6-aryl imidazo[2,1-b][1,3,4]thiadiazole sulfonamide

Imidazo[2,1-*b*][1,3,4]thiadiazoles were significantly appear to be anti-inflammatory [10-16], analgesic [17-18], anthelmintic [19], antibacterial [20-26], antifungal [27,28], anticancer [29-34], cytotoxic on cancer cells [35-48], antisecretory [49], cardiotonic [50], diuretic [51], herbicidal [52], antiviral [53,54], insecticide [55], antidepressant [56], central nervous system (CNS) [57] and antitubercular activities [58-60]. Taking into consideration the above findings, a novel series of imidazo[2,1- *b*][1,3,4]thiadiazoles derivatives was designed, synthesized, characterized and has been evaluated for anti-inflammatory, analgesic, antimicrobial, and anthelmintic activities.

Pharmacology

Antibacterial Activity

The antimicrobial assays were carried out by the disc diffusion method [61] using the following bacterial strains: methicillin-sensitive gram positive *Staphylococcus aureus* (NCIM 2079) and *Bacillus subtilis* (ATCC 6633), and gram negative *Echerichia coli* (NCIM 2931) and *Klebsiella pneumonae* (NCIM 2957) procured from the National Chemical Laboratory (NCL), Pune, India. For in vitro, standard powder forms of 250mg/mL discs of streptomycin were stored at 2 to 8^{o}C until use.

Antibacterial Susceptibility Testing

The disc diffusion method was carried out for the antibacterial tests. In brief, a suspension of bacterial strains (200 µL) containing 10^6 cfu/mL of bacteria was spread on the Mueller Hinton agar (MHA) medium. The disc (6mm in diameter, purchased from HIMEDIA), impregnated with concentration of 250 µg of the test compound was placed on the inoculated agar. The negative control was prepared using the same solvent (DMSO), which was employed to dissolve the test compounds. Streptomycin 250mg/disc, 6mm in diameter was used as reference drug. The inoculated plates were incubated at 35-37 ^{o}C for 24hrs. Streptomycin was used as positive and DMSO as negative standard against bacterial strains. The diameter of the inhibitory zones in millimeter (mm) was measured**.**

Antifungal activity

Potato Dextrose agar (PDA) medium was used for the evaluation of the antifungal activity. Pure cultures of *Candida albicans, Aspergillus flavous, Aspergillus niger* and *Aspergillus fumigatus* were inoculated onto the PDA and incubated for 72hrs at 37 ^{o}C.

The antifungal property of each compound was carried out by the disc diffusion method. The test compounds were dissolved in DMSO to get a concentration of 250mg/mL. Fluconazole standard reference drug was used in the

concentration at 10mg/mL. The screening was initiated by inoculating the test fungi on PDA in the incubation temperature of 37 ^{o}C for 72 hrs. The negative control was prepared using the same solvent (DMSO), which was employed to dissolve the test compounds. Discs were made on these seeded agar plates, 0.5cm in diameter was punched with the help of sterile cork borer. The petri dishes were prepared in triplicates and maintained at 37 ^{o}C for 72hrs [62]. Antifungal activity was compared with Fluconazole as the standard. Zones of inhibition were determined for the compounds **(4a-k)**.

Anthelmintic activity

Indian adult earthworms (*eudrilus eugeniae)* collected from the Earthworm Rearing Center, Davangere (Karnataka), were washed with normal saline to remove all fecal matter and used for the anthelmintic study. Earthworms of 3-5cm length and 0.1-0.2cm width were used. The anthelmintic activity was evaluated on Indian adult earthworms due to their anatomical and physiological resemblance to the intestinal roundworm parasites found in human being. The earthworms were divided into 12 groups of 6 earthworms each of approximately equal size and released into 25mL of the desired dextrose solution. The experiment was carried out in one petriplate for each group. To study the anthelmintic property, each petriplate was treated with one of the following - (1% normal saline), albendazole (10mg/mL), or 2% of each compounds **(4a-k)** [63]. Observations were made for the time taken to cause paralysis and death time of the individual worms. Death was concluded when the worms lost their motility, followed by the fading of their body colour.

Anti-inflammatory and Analgesic activity

All the newly synthesized immidazothiadiazole analogues were evaluated for their anti-inflammatory activity against the carrageenan-induced acute paw edema method in Wistar albino rats weighing 150-200g, using the Plethysmometer following the literature method [64]. The animals were weighed and divided into different groups (control, standard and test groups) of five rats each. The first group was treated with 1mL of 1% gum acacia suspension orally (control), the second group was administered with a dose of 20 mg/kg of the Indomethacin (standard) and the third group was treated with 20 mg/kg of the suspension of test compounds. After 30 min., the animals were injected with 0.1 mL of 1% carrageenan in normal saline subcutaneously in the sub-planar region of the right hind paw. The paw volume was measured immediately (0 h) and after 30 min., 60 min. and 90 min. using the Plethysmometer. Values are expressed as mean, by one way ANOVA analysis followed by Dunnett's-t-test.

The above synthesized compounds **(4a-k)** were also tested for their analgesic activity using the Analgesiometer. Rats, of either sex, weighing between 150-200g were used for the experiment. The animals were weighed and divided into different groups (control, standard and the test groups) of five rats each. In this method heat is used as a source of pain. The animals were placed individually on an Analgesiometer, whereby the tail lies over the nichrome wire of the instrument without touching it (i.e., about 1/8 inch above the nichrome wire). The Cut-off time is 10 seconds. The end point of the sensation is when the rat lifts its tail (i.e. tail flick). The reaction time is noted at intervals of 30, 60 and 90 min. after the administration of the drug. The values are expressed as mean±SEM, by one way ANOVA analysis followed by Dunnett's -t-test.

Acute toxicity study

All the test compounds were employed for acute oral toxicity as per the Limit test (5000mg/kg/body weight/oral) OECD Guidelines [65]. With the ethical clearance certificate YUIAEC bearing number 8a/26.08.2013, the Wistar albino rats (n=6) weighing 150-200g were grouped (n=6) (both male and female in equal ratio) and each group were treated with the test compounds orally with a single 5000mg/kg dose. Several parameters as per the guidelines including morbidity and mortality were recorded before the treatment and every half an hour post dosing for 4 hours, and ther after, once in 12 hours for 48 hours.

MATERIALS AND METHODS

The melting points were determined by an open capillary method and left uncorrected. The IR spectra (in KBr pellets) were recorded on a Shimadzu FTIR 157 spectrophotometer. The ^{1}H- NMR and ^{13}C-NMR spectra were recorded ($CDCl_3$/DMSO-d_6 mixture) on the 400 MHz spectrometer using the TMS as an internal standard. Mass spectra were recorded in the Agilent Technology LC-mass spectrometer. The progress of the reaction was monitored by thin layer chromatography (TLC) on silica gel plates.

General procedure for the preparation of 2-((5-amino-1,3,4-thiadiazol-2-yl)thio)-*N*-(4-fluorophenyl) acetamide (3)

A mixture of **2** [69] (0.005 mol), anhydrous potassium carbonate (0.005 mol) and 5-amino-1,3,4-thiadiazole-4-thiol (0.005 mol) in dry acetone (20 mL) was heated under reflux for 14hrs, and then allowed to cool. The reaction mixture was filtered and the obtained precipitate was washed with water; later, the dried compound was purified by dissolving it in glacial acetic acid and re-precipitation by dil. NH_4OH.

IR (KBr, $\gamma_{max,}$ cm^{-1}): 3358 (NH), 3255 (NH_2), 3099 (Ar-H), 2933 (C-H), 1649(C=O),1544 (C=N); ^{1}H-NMR (400MHz, $CDCl_3$, δ ppm): 1.91 (s, 2H, CH_2), 2.45 (s, 2H, NH_2), 6.972-7.532 (m, 4H, 4-fluorophenyl), 9.64 (s, 1H, NH). LC MS: *m/z* = 285(M^+).

General procedure for the preparation of 2-((6-(aryl)imidazo[2,1-*b*][1,3,4]thiadiazol-2-yl)thio)-*N*-(4-fluorophenyl)acetamide (4 a-k)

Equimolar quantities of 2-((5-amino-1,3,4-thiadiazol-2-yl)thio)-*N*-(4-fluorophenyl)acetamide **(3)** (0.01 mol) and various α-bromoketones (0.01 mol) were refluxed in dry ethanol for 11 h. The excess of solvent was evaporated under pressure, and the separated solid hydrobromide was filtered, suspended in water, followed by neutralized by aqueous sodium carbonate solution to obtain free base **(4a-k)**. Followed by filteration, washed with water, dried, and recrystallized from ethanol with few drops of dimethyl formamide.

***N*-(4-fluorophenyl)-2-((6-(4-methoxyphenyl)imidazo[2,1-*b*][1,3,4]thiadiazol-2-yl)thio) acetamide (4a)**

(KBr, $\gamma_{max,}$ cm^{-1}): 3354 cm^{-1} (NH), 3062 cm^{-1} (Ar-H), 1659 (C=O), 1201 cm^{-1} (C-S), 1591 cm^{-1} (C=N); ^{1}H-NMR (400MHz, $CDCl_3$, δ ppm): 4.28 (s, 2H, CH_2), 7.14-7.25 (d, 2H, *J* = 8Hz, 4-methoxyphenyl), 7.31-7.39 (d, 2H, J = 8.2Hz, 4-methoxyphenyl), 7.57-7.62 (m, 4H, 4-fluorophenyl), 8.39 (s, 1H, imidazole), 10.42 (s, 1H, NH); ^{13}C-NMR ($CHCl_3$,DMSO-d_6) δ ppm: 145.79, 142.14, 121.36, 138.89 (imidazo[2,1-*b*][1,3,4]thiadiazole), 112.84, 115.73, 164.83, 139.84 (4-fluorophenyl), 128.52, 124.61, 126.72, 136.96 (4-methoxyphenyl), 168.31 (C=O), 37.64 (CH_2); LC MS: *m/z* = 415(M^+).

2-((6-(4-bromophenyl)imidazo[2,1-*b*][1,3,4]thiadiazol-2-yl)thio)-*N*-(4-fluorophenyl) acetamide(4b)

(KBr, $\gamma_{max,}$ cm^{-1}): 3348 cm^{-1} (NH), 3074 cm^{-1} (Ar-H), 1639 (C=O), 1221 cm^{-1} (C-S), 1589 cm^{-1} (C=N); ^{1}H-NMR (400MHz, $CDCl_3$, δ ppm): 4.27 (s, 2H, CH_2), 7.15-7.26 (d, 2H, *J* = 8.2Hz, 4-bromophenyl), 7.39-7.46 (d, 2H, J = 8.2Hz, 4-bromophenyl), 7.63-7.73 (m, 4H, 4-fluorophenyl), 8.38 (s, 1H, imidazole), 10.321 (s, 1H, NH); ^{13}C-NMR ($CHCl_3$,DMSO-d_6) δ ppm: 145.62, 142.31, 122.02, 139.72 (imidazo[2,1-*b*][1,3,4]thiadiazole), 112.79, 115.69, 163.83, 139.72 (4-fluorophenyl), 128.41, 124.59, 126.78, 136.91 (4-bromophenyl) 168.46 (C=O), 37.61 (CH_2); LC MS: *m/z* = 465(M+2).

***N*-(4-fluorophenyl)-2-((6-(p-tolyl)imidazo[2,1-*b*][1,3,4]thiadiazol-2-yl)thio)acetamide (4c)**

(KBr, $\gamma_{max,}$ cm^{-1}): 3361 cm^{-1} (NH), 3115 cm^{-1} (Ar-H), 1668 (C=O), 1215 cm^{-1} (C-S), 1598 cm^{-1} (C=N); ^{1}H-NMR (400MHz, $CDCl_3$, δ ppm): 2.30 (s, 3H, methoxy), 4.29 (s, 2H, CH_2), 7.14-7.16 (d, 2H, *J* = 8.4Hz, 4-methoxyphenyl), 7.18-7.20 (d, 2H, *J* = 8.4Hz, 4-methoxyphenyl), 7.71 -7.73 (d, 2H, *J* = 8.4Hz, 4-fluorophenyl), 7.56 -7.60 (m, 2H, 4-fluorophenyl), 8.56 (s, 1H, imidazole), 10.45 (s, 1H, NH); ^{13}C-NMR ($CHCl_3$,DMSO-d_6) δ ppm: 146.39, 143.02, 126.98, 138.93 (imidazo[2,1-*b*][1,3,4]thiadiazole), 114.35, 117.48, 163.48, 136.71 (4-fluorophenyl), 119.42, 125.13, 129.16, 136.71 (4-bromophenyl), 168.47 (C=O), 36.63 (CH_2), 22.38 (OCH_3) ; LC MS: *m/z* = 399.

***N*-(4-fluorophenyl)-2-((6-(4-nitrophenyl)imidazo[2,1-*b*][1,3,4]thiadiazol-2-yl)thio) acetamide (4d)**

(KBr, $\gamma_{max,}$ cm^{-1}): 3357 cm^{-1} (NH), 3119 cm^{-1} (Ar-H), 1658 (C=O), 1213cm^{-1} (C-S), 1561 cm^{-1} (C=N); ^{1}H-NMR (400MHz, $CDCl_3$, δ ppm): 4.27 (s, 2H, CH_2), 7.14-7.15 (d, 2H, *J* = 8.2Hz, 4-nitrophenyl), 7.19-7.24 (d, 2H, *J* = 8.2Hz, 4-nitrophenyl), 7.56 -7.67 (m, 4H, 4-fluorophenyl), 8.56 (s, 1H, imidazole), 10.43 (s, 1H, NH); ^{13}C-NMR ($CHCl_3$,DMSO-d_6) δ ppm: 148.01, 144.23, 126.76, 138.91 (imidazo[2,1-*b*][1,3,4]thiadiazole), 114.39, 117.52, 163.39, 136.61 (4-fluorophenyl), 119.31, 125.42, 128.23, 136.78 (4-bromophenyl), 168.21 (C=O), 36.29 (CH_2); LC MS: *m/z* = 330.

***N*-(4-fluorophenyl)-2-((6-phenylimidazo[2,1-*b*][1,3,4]thiadiazol-2-yl)thio)acetamide (4e)**

(KBr, $\gamma_{max,}$ cm^{-1}): 3360 cm^{-1} (NH), 3058 cm^{-1} (Ar-H), 1668 (C=O), 1217 cm^{-1} (C-S), 1595 cm^{-1} (C=N); ^{1}H-NMR (400MHz, $CDCl_3$, δ ppm): 4.30 (s, 2H, CH_2), 7.152-7.417 (m, 5H, phenyl), 7.598-7.660 (m, 4H, 4-fluorophenyl),8.425 (s,1H, imidazole), 10.546 (s, 1H, NH); ^{13}C-NMR ($CHCl_3$,DMSO-d_6) δ ppm: 144.88, 147.1,

120.11, 138.93 (imidazo[2,1-*b*][1,3,4]thiadiazole), 112.83, 116.98, 165.85, 138.93 (4-fluorophenyl), 128.63, 124.15, 127.11, 136.71 (phenyl) 169.31 (C=O), 37.74 (CH_2); LC MS: *m/z* = 385(M^+).

***N*-(4-fluorophenyl)-2-((6-(4-hydroxyphenyl)imidazo[2,1-*b*][1,3,4]thiadiazol-2-yl)thio) acetamide (4f)**
(KBr, $\gamma_{max,}$ cm^{-1}): 3361 cm^{-1} (NH), 3072 cm^{-1} (Ar-H), 1668 (C=O), 1215 cm^{-1} (C-S), 1558 cm^{-1} (C=N); ^{1}H-NMR (400MHz, $CDCl_3$, δ ppm): 4.30 (s, 2H, CH_2), 6.77-6.79 (d, 2H, *J*= 8.8Hz, 4-hydroxyphenyl), 7.38-7.40 (d, 2H, *J* = 8.8Hz, 4-hydroxyphenyl), 7.58-7.85 (m, 4H, 4-fluorophenyl),8.64 (s,1H, imidazole), 9.520 (s, 1H, OH), 10.546 (s, 1H, NH); ^{13}C-NMR ($CHCl_3$,DMSO-d_6) δ ppm: 144.88, 147.1, 120.11, 138.93 (imidazo[2,1-*b*][1,3,4]thiadiazole), 115.83, 116.98, 165.85, 138.93 (4-fluorophenyl), 128.63, 124.15, 127.11, 136.71 (phenyl) 169.31 (C=O), 37.74 (CH_2); LC MS: *m/z* = 401 (M^+).

5-(2-((2-((4-fluorophenyl)amino)-2-oxoethyl)thio)imidazo[2,1-*b*][1,3,4]thiadiazol-6-yl)-2-hydroxybenzamide (4g)
(KBr, $\gamma_{max,}$ cm^{-1}): 3359 cm^{-1} (NH), 3073 cm^{-1} (Ar-H), 1664 (C=O), 1219 cm^{-1} (C-S), 1561 cm^{-1} (C=N); ^{1}H-NMR (400MHz, $CDCl_3$, δ ppm): 4.39 (s, 2H, CH_2), 4.92 (S, 2H, NH_2), 6.67 - 6.72 (d, 2H, *J*= 8.8Hz, 4-hydroxybenzamide),7.12 (s, 1H, 4-hydroxybenzamide) 7.58-7.87 (m, 4H, 4-fluorophenyl), 8.64 (s,1H, imidazole), 9.53 (s, 1H, OH), 10.543 (s, 1H, NH); ^{13}C-NMR ($CHCl_3$,DMSO-d_6) δ ppm: 148.21, 147.13, 121.23, 138.72 (imidazo[2,1-*b*][1,3,4]thiadiazole), 115.92, 116.43, 164.33, 138.97 (4-fluorophenyl), 128.63, 124.15, 127.11, 136.71 (phenyl) 168.24 (C=O), 37.74 (CH_2); LC MS: *m/z* = 445(M^+).

***N*-(4-fluorophenyl)-2-((6-(4-fluorophenyl)imidazo[2,1-*b*][1,3,4]thiadiazol-2-yl)thio)acetamid e (4h)**
(KBr, $\gamma_{max,}$ cm^{-1}): 3351 cm^{-1} (NH), 3097 cm^{-1} (Ar-H), 1676 (C=O), 1225 cm^{-1} (C-S), 1542 cm^{-1} (C=N); ^{1}H-NMR (400MHz, $CDCl_3$, δ ppm): 4.37 (s, 2H, CH_2), 7.21– 7.35 (m, 4H, 4-fluorophenyl), 7.59-7.89 (m, 4H, 4-fluorophenyl), 8.65 (s,1H, imidazole), 10.53 (s, 1H, NH); ^{13}C-NMR ($CHCl_3$,DMSO-d_6) δ ppm: 148.21, 147.13, 121.23, 138.72 (imidazo[2,1-*b*][1,3,4]thiadiazole), 115.83, 117.21, 164.29, 138.83 (4-fluorophenyl), 168.12 (C=O), 37.69 (CH_2); LC MS: *m/z* = 402.

2-((6-(4-chlorophenyl)imidazo[2,1-*b*][1,3,4]thiadiazol-2-yl)thio)-*N*-(4-fluorophenyl)acetami de(4i)
(KBr, $\gamma_{max,}$ cm^{-1}): 3353 cm^{-1} (NH), 3081 cm^{-1} (Ar-H), 1662 (C=O), 1219 cm^{-1} (C-S), 1539 cm^{-1} (C=N); ^{1}H-NMR (400MHz, $CDCl_3$, δ ppm): 4.32 (s, 2H, CH_2), 6.93-7.12 (d, 2H, *J*= 8.4Hz, 4-chlorophenyl), 7.37-7.39 (d, 2H, *J* = 8.4Hz, 4-chlorophenyl), 7.58-7.89 (m, 4H, 4-fluorophenyl), 8.61 (s,1H, imidazole), 10.50 (s, 1H, NH); ^{13}C-NMR ($CHCl_3$,DMSO-d_6) δ ppm: 147.19, 146.46, 121.42, 138.62 (imidazo[2,1-*b*][1,3,4]thiadiazole), 115.71, 117.32, 164.48, 138.73 (4-fluorophenyl), 128.14, 124.21, 127.73, 136.62 (4-chlorophenyl), 168.32 (C=O), 37.52 (CH_2); LC MS: *m/z* = 420 (M+2).

***N*-(4-fluorophenyl)-2-((6-(4-(trifluoromethyl)phenyl)imidazo[2,1-*b*][1,3,4]thiadiazol-2-yl)thio)acetamide (4j)**
(KBr, $\gamma_{max,}$ cm^{-1}): 3342 cm^{-1} (NH), 3063 cm^{-1} (Ar-H), 1672 (C=O), 1223 cm^{-1} (C-S), 1531cm^{-1} (C=N); ^{1}H-NMR (400MHz, $CDCl_3$, δ ppm): 4.34 (s, 2H, CH_2), 7.21-7.32 (m, 4H, 4-trifluorophenyl), 7.57-7.64 (m, 4H, 4-fluorophenyl), 8.62 (s,1H, imidazole), 10.50 (s, 1H, NH); ^{13}C-NMR ($CHCl_3$,DMSO-d_6) δ ppm: 147.82, 146.73, 121.31, 138.86 (imidazo[2,1-*b*][1,3,4]thiadiazole), 115.77, 117.43, 164.47, 138.62 (4-fluorophenyl), 128.21, 124.63, 127.84, 138.70 (4-trifluorophenyl), 125.03 (trifluoro), 168.42 (C=O), 37.52 (CH_2); LC MS: *m/z* = 453 .

2-((6-(4-fluoro-3-methylphenyl)imidazo[2,1-*b*][1,3,4]thiadiazol-2-yl)thio)-*N*-(4-fluorophenyl)acetamide(4k)
(KBr, $\gamma_{max,}$ cm^{-1}): 3363 cm^{-1} (NH), 3115 cm^{-1} (Ar-H), 1668 (C=O), 1212cm^{-1} (C-S), 1595 cm^{-1} (C=N); ^{1}H-NMR (400MHz, $CDCl_3$, δ ppm): 2.22 (S,1H, CH_3), 3.97 (s, 2H, CH_2), 7.04-7.18 (m, 3H, 4-fluoro-3-methylphenyl), 7.56-7.69 (m, 4H, 4-fluorophenyl), 8.01 (S,1H, imidazole), 10.30 (s, 1H, NH); ^{13}C-NMR ($CHCl_3$,DMSO-d_6) δ ppm: 158.67, 141.62, 135.45, 122.15 (imidazo[2,1-*b*][1,3,4]thiadiazole), 164.79,135.45, 120.45, 114.42 (4-fluorophenyl), 157.62, 129.71, 126.81, 123.21, 122.15, 114.03 (4-fluoro-3-methylphenyl) 167.89 (C=O), 37.74 (CH_2); LC MS: *m/z* = 417(M^+).

RESULTS AND DISCUSSION

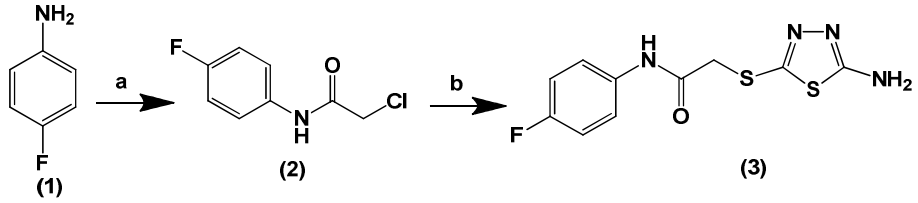
Chemistry

It is well-known that this reaction proceeds via the intermediate iminothiadiazole[66], which undergoes dehydrocyclisation to form the preferred fused heterocyclic derivatives**.** The electronic and steric factors at the 5th position of the 2-amino-5- substituted-1,3,4-thiadiazole are essential in determining the course of its reaction. Accordingly the alkylation of the thiadiazole occurs at the 3rd nitrogen with a consequent ring closer involving the

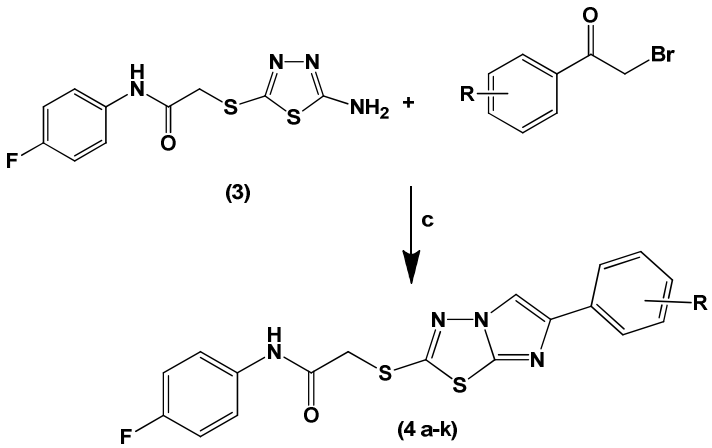
intramolecular nucleophilic addition of the 2-amino group to the carbonyl of the intermediate, followed by the elimination of water to form the desired nitrogen bridgehead heterocyclic system.

The compound 2-chloro-*N*-(4-fluorophenyl) acetamide **(2),** 2-((5-amino-1,3,4-thiadiazol-2-yl)thio)-*N*-(4-fluorophenyl)acetamide (**3)** were synthesized according to the literature [67]. The reaction has been outlined in (**scheme 1**). The synthesis of the desired novel sequence of 2-((6-(aryl)imidazo[2,1-*b*][1,3,4]thiadiazol-2-yl)thio)-*N*-(4-fluorophenyl)acetamides **(4a-k)** were afforded *via* the condensation reaction of 2-((5-amino-1,3,4-thiadiazol-2-yl)thio)-*N*-(4-fluorophenyl)acetamide **(3)** with various α-haloketones in the presence of Na_2CO_3 by following the literature [68] as depicted in (**Scheme 2**).

Reaction Schemes



Scheme-1: Reagents and Conditions: (a) DMF, 0 ^{0}C, $ClCOCH_2Cl$, stirring; (b) Acetone, 5-amino-1,3,4-thiadiazole-2-thiol, reflux14h.



Scheme-2: Reagents and Conditions: (C) Dry ethanol, Na_2CO_3, reflux for 10h

R= 4-OCH_3, 4-Br, 4-CH_3, 4-NO_2, 4-OH, 4-OH-3-$CONH_2$, 4-F, 4-Cl, 4-CF_3, 4-F-3-CF_3

The chemical structure of the compound **(3)** and its corresponding imidazo[2,1-*b*][1,3,4]thiadiazole derivatives **4c**, **4e**, **4f** and **4k** were finally ascertained spectroscopic studies. The formation of 2-((5-amino-1,3,4-thiadiazol-2-yl)thio)-*N*-(4-fluorophenyl)acetamide **(3)** was confirmed by the FT-IR spectrum, The NH_2 characteristic absorption bands appeared at 3225 cm^{-1} and 3347 cm^{-1}. The aromatic C-H stretch was observed at 3099 cm^{-1}, the C=O stretch at 1668 cm^{-1} and the C=N stretching vibrations were observed at 1544 cm^{-1}. The 400MHz ^{1}H-NMR spectrum of compound **(3)** revealed that, the two protons of NH_2 appeared as a sharp singlet at δ 3.55 ppm and the amide proton appeared as a singlet at 9.62 ppm, while the aromatic protons appeared as doublets/multiplets between δ 6.97-7.53ppm. The methyl protons appeared as a singlet at δ 4.9 ppm. The mass spectrum of **(3)** showed the protonated molecular ion peak at m/z 285.04.

Further, the FT-IR spectrum of compound (**4c)** showed characteristic absorption bands at 3361 cm^{-1} (NH), 3088 cm^{-1} (Ar H), 1668 (C=O), 1215 cm^{-1} (C-S) and 1588 cm^{-1}, which could be attributed to (C=N). The 400MHz ^{1}H-NMR spectrum of compound **(4c)** showed a singlet for the amide NH proton at δ 10.45 ppm. The signal corresponding to the two protons of the NH_2 group in 2-aminothiadiazole ring disappeared and a new sharp singlet for the aromatic imidazole proton was observed at δ 8.56 ppm. A singlet resonating at δ 4.29 ppm was due to the methylene protons, which is adjacent to the carbonyl amide group. The four protons of 4-fluorophenyl ring resonated as multiplets in the

range of 7.56 ppm -7.73 ppm. The four protons of 4-methyl phenyl ring resonated as two doublets at 7.20 ppm and 7.16 ppm with J = 8.4 Hz. The three protons of the methyl group were observed as a singlet at δ 2.30 ppm. The ^{13}C-NMR ($CHCl_3$,DMSO-d_6)spectrum of compound **(4c)** showed signals at δ 22.36, 36.63, 114.35, 117.48, 119.23, 125.13, 126.98, 129.16, 136.71, 138.93, 143.02, 146.39, 149.31, 163.48 and 168.47 which are in agreement with the actual structure. The mass spectrum showed a protonated molecular ion peak at m/z 399.00, which is consistent with the molecular formula $C_{19}H_{15}FN_4OS_2$**.**

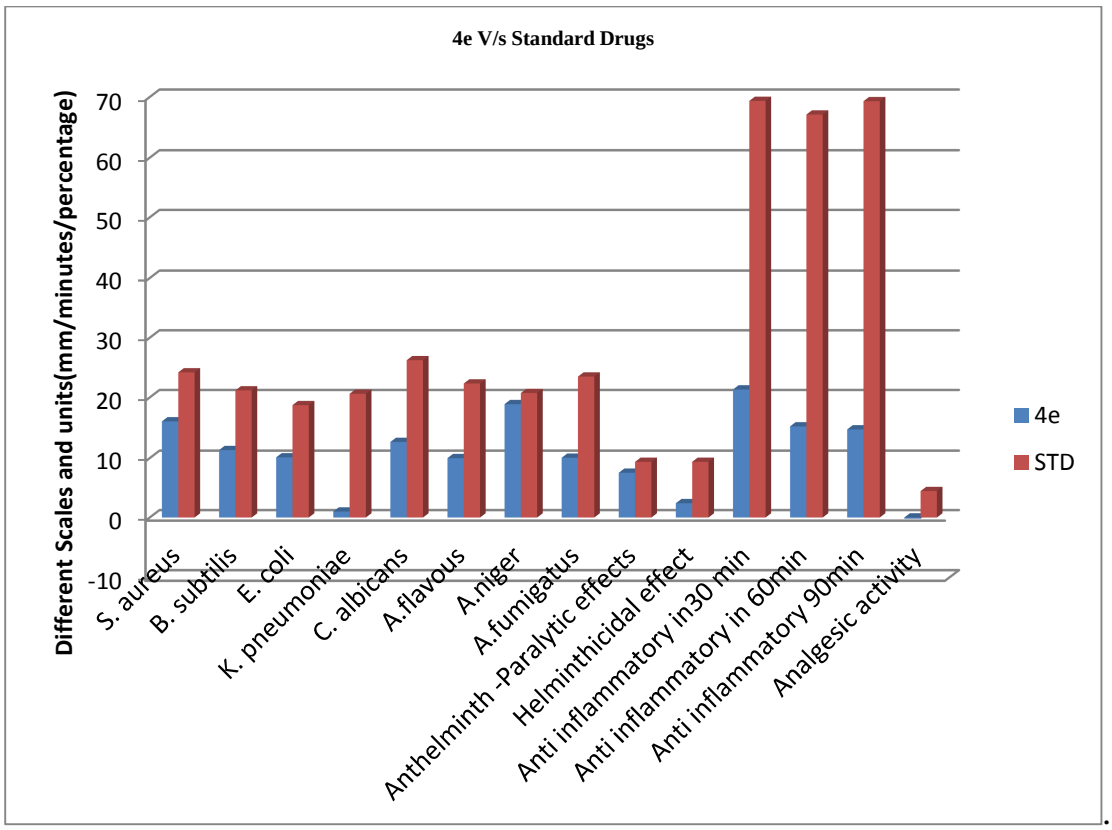
Table: 1 Characterization data of *N*-(4-fluorophenyl)-2-((6-(arylphenyl)imidazo[2,1- *b*] [1,3,4]thiadiazol-2-yl)thio)acetamide 4(a-k)

Compound	R	Mol. Formula	Mol. Wt.	M.p. (^{0}C)	Yield (%)
4a	OCH_3	$C_{19}H_{15}FN_4O_2S_2$	414	131-133	82
4b	Br	$C_{18}H_{15}BrFN_4OS_2$	463	110-112	71
4c	CH_3	$C_{18}H_{12}FN_4OS_2$	398	122-124	68
4d	NO_2	$C_{18}H_{12}FN_5O_3S_2$	430	114-116	64
4e	-	$C_{18}H_{13}FN_4OS_2$	384	127-129	73
4f	OH	$C_{18}H_{13}FN_4O_2S_2$	400	160-162	60
4g	4-OH 3-$CONH_2$	$C_{19}H_{14}FN_5O_3S_2$	444	74-76	76
4h	F	$C_{19}H_{14}F_2N_4OS_2$	402	78-80	77
4i	Cl	C18H12ClFN$_4$OS$_2$	418	111-113	67
4j	CF_3	$C_{19}H_{12}F_4N_4OS_2$	452	98-100	78
4k	4-F-3-CH_3	$C_{19}H_{11}F_5N_4OS_2$	416	92-94	84

Biological Activity

Among the synthesized compounds **(4a-k)**, **(4e)** has shown good activity against *S.aureus* and *B.subtilis*, and moderate activity against all others except against *K. pneumonia* and *E. eugeniae*. Whereas it has shown significant activity against *A.niger*. The compound exhibited anti-inflammatory activity and anthelmintic activity, but did not possess analgesic property (**Fig 1)**. Antibacterial with anti-inflammatory activity could be a promising new group since there are no such drugs licensed untill today.

Fig 1: Biological Activity profile of *N*-(4-fluorophenyl)-2-((6-phenylimidazo[2,1-*b*][1,3,4]thiadiazol-2-yl)thio)acetamide (4e)



The graph depicts comparative activity of **(4e)** and standard drugs against the control groups. The standard differs based on the tests: Streptomycin in the antibacterial test, Flucunozol in the test for antifungal activity, Albendazole in the test for anthelmintic activity, Indomethacin in the test for anti-inflammatory activity and Pentazocine in the test for analgesic activity.

Antibacterial activity

The results of the antibacterial activity reveals that, the compound **(4h)** with the fluoro group at position four showed almost equivalent potency to the standard drug streptomycin against the bacterial strain *S. aureus*. Compound **(4k)** with trifluoro at the para position showed significant activity against all the bacterial strains except *B. subtilis*. **(4b)** also exhibited good activity against the bacterial strain *S. aureus*. Whereas, compounds **(4d)** and **(4i)** showed moderate activity against *K. pneumoniae* and *S. aureus*. It should be noted that, the substitution of halogen atoms at position four on the phenyl ring enhances the antibacterial activity **(Table 2)**.

Table: 2 Antibacterial activity of *N*-(4-fluorophenyl)-2-((6(arylphenyl)imidazo[2,1- *b*] [1,3,4]thiadiazol-2-yl)thio)acetamide 4(a-k)

	Zone of inhibition(mm)			
Samples	***Staphylococus aureus***	***Bacillus subtilis***	***Escherichia coli***	***Klebsiella pneumoniae***
4a	15.16±0.28	13.1±0.36	-	10.23±0.25
4b	19.96±0.45	10.06±0.30	9.1±0.10	-
4c	-	9±0.00	10.16±0.28	9.1±0.17
4d	16.16±0.28	10.1±0.17	10.26±0.25	17.73±0.64
4e	16.06±0.11	11.33±0.30	10.13±0.23	-
4f	-	10.06±0.11	9.23±0.40	8.86±0.23
4g	16.12±0.31	10.33±0.57	-	-
4h	23.23±0.75	10.06±0.11	15.2±0.34	11.23±0.25
4i	17.66±0.57	-	13.93±0.11	15.93±0.11
4j	-	9.16±0.15	14.66±0.57	-
4k	20.66±0.57	-	17.9±0.26	18.06±0.11
Streptomycine	24.3±0.30	21.16±0.37	18.73±0.64	20.6±0.52

Antifungal activity

C. albicans : The prototype **(4e)** exhibited good activity and compound **(4c)** with CH_3 group did not alter the activity. Compounds **(4i)**, **(4d)**, and **(4k)** with Cl, 4-F-3-CH_3 and NO_2 resulted in inactive compounds. The addition of other substitutions exhibited increase in activity in the following order F, OH, OCH3, 4-OH-3-$CONH_2$, CF_3 and Br.

Table: 3 Antifungal Activity of *N*-(4-fluorophenyl)-2-((6(arylphenyl)imidazo[2,1-*b*][1,3,4] thiadiazol-2-yl)thio)acetamide 4(a-k)

Samples	***C. albicans***	***Aspergillus flavous***	***A. niger***	***A. fumigatus***
4a	14.06±0.11	12.33±0.57	-	-
4b	20.23±0.75	15.06±0.11	17.2±0.34	9.23±0.25
4c	12.66±0.57	-	15.93±0.11	11.93±0.11
4d	-	9.16±0.15	12.66±0.57	-
4e	12.66±0.57	10.00±0.20	18.9±0.26	10.06±0.11
4f	13.45±0.47	10.08±0.25	16.19±0.20	12.91±0.28
4g	15.19±0.37	18.89±0.36	14.74±0.48	11.91±0.10
4h	13.25±0.19	-	13.74±0.38	-
4i	-	14.26±0.18	-	14.30±0.39
4j	19.37±0.30	-	14.16±0.40	-
4k	-	13.19±0.50	11.11±0.10	15.29±0.19
Flucunozol	26.3±0.30	22.46±0.37	20.73±0.64	23.6±0.52

A. flavous: The prototype **(4e)** exhibited good activity. Compounds **(4f)**, **(4a)**, **(4k)** and **(4i)** with OH, OCH_3, 4-F-3-CH_3, Cl and Br substitutions showed increased activity in the respective order. Compound (**4g)** with 4-OH-3-$CONH_2$ resulted in compounds with very good activity.

A. niger: The Prototype **(4e)** exhibited excellent activity, almost equivalent to the standard [Flucunozol]. Other compounds decreased activity in the following order **(4k)**, **(4d)**, **(4h)**, **(4j)**, **(4g)**, **(4c)**, **(4f)**, **(4b)** and the remaining compounds were inactive.

A. fumigatus: The prototype **(4e)** exhibited good activity. The R-substitution of prototype **(4e)** resulted in increased potency in the following order **(4g)**, **(4c)**, **(4f)**, **(4i)**, **(4h)**, **(4c)** with the substituents 4-OH-3-$CONH_2$, 4-CH_3, 4-OH, 4-Cl, 4-F and 4-CH_3. R-substitution with Br **(4b)** decreased activity. The compounds **(4a)**, **(4h)**, **(4j)** and **(4d)** with OCH_3, F, CF_3 and NO_2 substituents were inactive (**Table 3**).

Anthelmintic activity

Paralytic effect reduced with substitution in the following order NO_2 **(4d)**, CF_3 **(4j)**, 4-F-3-CH_3 **(4k)**, OCH_3 **(4a)**, 4-OH-3-$CONH_2$ **(4g)**, CH_3 **(4c)**, OH **(4f)**, F **(4h)**, Br **(4b)** and Cl **(4i)** (**Table 4)**.

Rapidity in helminthicidal effect increased in following order OCH_3, 4-OH-3-$CONH_2$, CF_3 and 4-F-3-CF_3 and decreased in the following order Br, F, CH_3, Cl, OH and NO_2.

Table: 4 Anthelmintic Activity of *N*-(4-fluorophenyl)-2-((6(arylphenyl)imidazo[2,1-*b*] [1,3,4] thiadiazol-2-yl)thio)acetamide 4(a-k)

	Paralysis time in min	Death time in min
4a	37.1	55.56
4b	30	53.53
4c	21.5	46.9
4d	15.4	44.55
4e	13.18	42.02
4f	25.26	45.20
4g	20.13	35.28
4h	28.2	49.19
4i	40	45.38
4j	18.29	28.36
4k	19.43	23.29
Albendazole	10.65	14.52

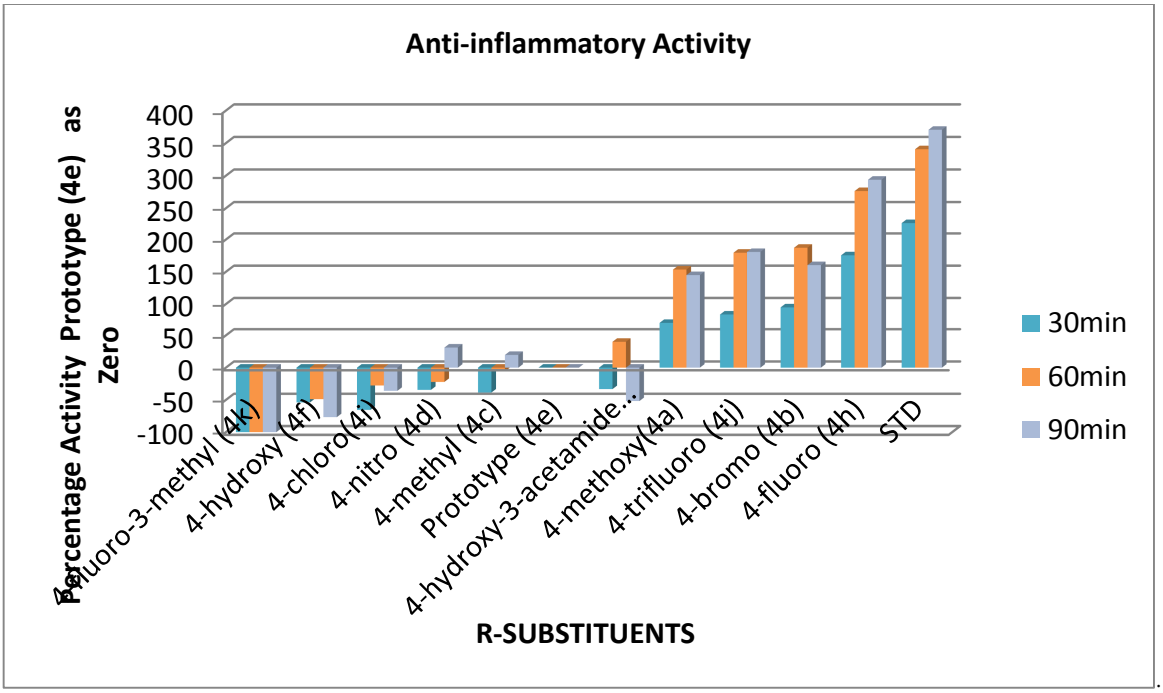
3.2.4 Anti-inflammatory activity

2-((6-(aryl)imidazo[2,1-*b*][1,3,4]thiadiazol-2-yl)thio)-*N*-(4-fluorophenyl)acetamides **(4g)**, **(4a)**, **(4j)**, **(4b)** and **(4h)** with 4-hydroxy-3-acetamide,4-methoxy,4-bromo, 4-fluoro groups showed significant anti-inflammatory activity in the increasing order. Whereas, the compounds **(4k)**, **(4f)**, **(4i)**, **(4d)** and **(4c)** with 4-fluoro-3-methyl, 4-hydroxy, 4-chloro, 4-nitro and 4-methyl substituents respectively, showed decreased in anti-inflammatory potency compared to the standard drug Indomethacin and the results are tabulated in (**Fig 2**, **Table 5**).

Table 5: Antinflammatory activity of *N*-(4-fluorophenyl)-2-((6(arylphenyl)imidazo[2,1- *b*][1,3,4]thiadiazol-2-yl)thio)acetamide 4(a-k)

Compounds	Antinflammatory activity (%) after 30 min[Mean± SD]	Antinflammatory activity (%) after 60 min[Mean± SD]	Antinflammatory activity (%) after 90 min[Mean± SD]
Control	0 ± 1.38	0 ± 1.38	0 ± 1.50
Indomethacin	70.37037 ± 0.41	70.7317 ± 0.40	71.08434 ± 0.43
4a	41.97531 ± 0.80	40.2439± 0.82	40.96386 ± 0.89
4b	51.85185 ± 0.66	54.87805 ± 0.62	54.21687 ± 0.69
4c	18.51852 ± 1.12	20.73171 ± 1.09	19.27711 ± 1.21
4d	6.17284 ± 1.29	6.097561 ± 1.29	7.228916 ± 1.39
4e	23.45679 ± 1.06	21.95122 ± 1.07	21.68675 ± 1.18
4f	8.641975 ± 1.26	8.536586 ± 1.26	12.04819 ± 1.32
4g	22.22222 ± 1.07	20.73171 ± 1.09	19.27711 ± 1.21
4h	61.72839 ± 0.53	56.09756 ± 0.60	57.83133 ± 0.63
4i	27.16049 ± 1.00	26.82927 ± 1.01	26.50602 ± 1.10
4j	37.03704 ± 0.87	31.70732 ± 0.94	31.3253 ± 1.03

Fig 2: Anti-inflammatory Activity profile of *N*-(4-fluorophenyl)-2-((6-(substituted phenyl) imidazo [2,1-*b*][1,3,4]thiadiazol-2-yl)thio)acetamide (4a-k)



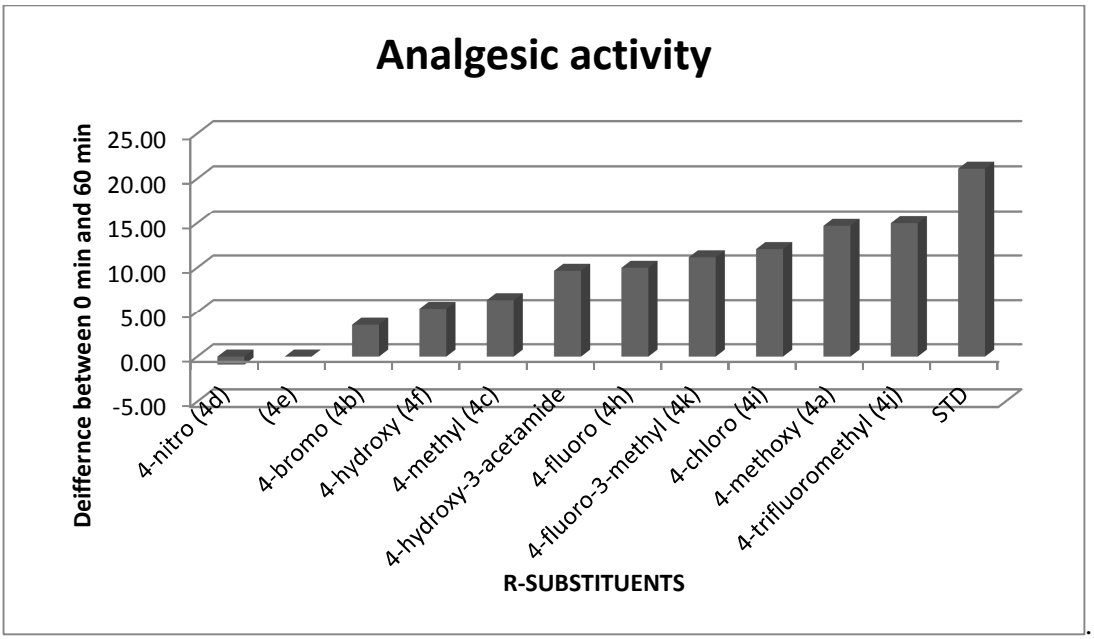
Analgesic activity

Analgesic activity data **Table 6** reveals that the compound **(4a)** with methoxy and **(4j)** trifluoro at the *p*-position exhibit excellent activity compared to the standard drug Pentazocine. Whereas the compounds **(4k)**, **(4i)** and **(4a)**, with 4-fluoro-3-methyl, chloro, methoxy substituents in the phenyl ring enhance the analgesic activity significantly in the respective order. The rest of the compounds showed moderate to least activity (**Fig 3**, **Table 6**).

Table 6: Analgesic activity of *N*-(4-fluorophenyl)-2-((6(arylphenyl)imidazo[2,1-*b*][1,3,4] thiadiazol-2-yl)thio)acetamide 4(a-k) by tail flick method

compound	Tail flick latency in sec		
	0 min	30 min	60 min
Control	3.62 ± 0.21	3.63±0.53	3.653± 0.373
Pentazocine	3.23 ± 0.048	7.322 ±0.213	7.63± 0.096
4a	3.41± .1260	6.132±0.173	6.530± 0.218
4b	3.42 ± 0.146	4.183±0.103	4.324± 0.612
4c	3.49±0.198	24.630±0.132	4.956± 0.329
4d	3.32 ± 0.156	3.136±0.219	3.432± 0.113
4e	3.43 ± 0.003	3.167±0.130	3.231±0.129
4f	3.46 ± 0.030	4.391±0.062	4.732± 0.062
4g	3.61 ± 0.034	5.293±0.068	5.731± 0.164
4h	3.45 ± 0.021	5.321±0.113	5.637±0.130
4i	3.27 ± 0.063	4.932±0.134	5.873± 0.120
4j	3.32 ± 0.048	5.300±0.082	6.500± 0.091
4k	3.31±0.132	4.565±0.124	5.731± 0.161

Fig 3: Analgesic Activity profile of *N*-(4-fluorophenyl)-2-((6-(substitutedphenyl)imidazo[2,1-*b*][1,3,4]thiadiazol-2-yl)thio)acetamide (4a-k)



3.2.6 Structure Activity Relationship Analysis

The addition of various substitutions **(R)** to the prototype **(4e)** altered the biological activity significantly. A structure activity analysis is only possible if the results obtained with individual compounds are comparable with the prototype **(4e)**. Hence, a percentage difference of the derivatives to the prototype **(4e)** in each test is depicted (**Table 7**). This structure activity analysis will offer directions for further design and synthesis of compounds of desired effect and will also aid in the selection of investigative new drugs for further development.

Table 7: Structure Activity Relationship Analysis of *N*-(4-fluorophenyl)-2-((6 (arylphenyl)imidazo[2,1- *b*][1,3,4]thiadiazol-2-yl)thio)acetamide 4(a-k)

Compound	R	*S. aureus*	*B. subtilis*	*E. coli*	*K. pneumoniae*	*C. albicans*	*A. flavous*	*A.niger*	*A. fumigatus*	Worm ifuge	Worm icidal time	Anti-inflammatory	Analgesic
4c	CH3	-100	-21	0	81.00	0	-100	-16	19	-63	-12	20	6
4f	OH	-100	-11	-9	79	6	1	-14	28	-92	-8	-77	5
4j	CF_3	-100	-19	45	-100	53	-100	-25	-100	-39	33	181	15
4a	OCH_3	-6	16	-100	92	11	23	-100	-100	-181	-32	145	15
4e	**Prototype**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
4g	4-OH-3-$CONH_2$	0	-9	-100	-100	20	89	-22	18	-53	16	-52	10
4d	NO_2	1	-11	1	167	-100	-8	-33	-100	-17	-6	31	-1
4f	Cl	10	-100	38	149	-100	43	-100	42	-203	-8	-36	12
4b	Br	24	-11	-10	-100	60	51	-9	-8	-128	-27	160	4
4k	4-F-3-CH_3	29	-100	77	171	-100	32	-41	52	-47	45	-100	11
4h	F	51	-11	50	102	5	-100	-27	-100	-114	-17	294	10
STD	-	51	87	85	196	108	125	10	135	19	65	371	21

Acute toxicity Study

None of the compounds **(4a-k)** exhibited mortality, morbidity or any adverse effects during the observation period of 48 hours at a limit dose of 5000mg/kg when given orally as a single dose.

CONCLUSION

All the novel compounds possess varying degree of biological activities which indicates that all these compounds have the potential to be developed into drugs with unique pharmacological properties. This warrants further detailed PKPD and toxicological studies with the individual compounds separately. The structure activity relationship of the series could be exploited for the development of designer drugs to meet the needs of the current healthcare systems.

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