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Design and synthesis of some thiazolyl and thiadiazolyl derivatives of antipyrine as potential non-acidic anti-inflammatory, analgesic and antimicrobial agents ☆

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ABSTRACT

The synthesis of two groups of structure hybrids comprising basically the antipyrine moiety attached to either polysubstituted thiazole or 2,5-disubstituted-1,3,4-thiadiazole counterparts through various linkages is described. Twelve out of the newly synthesized compounds were evaluated for their anti-inflammatory activity using two different screening protocols; namely, the formalin-induced paw edema and the turpentine oil-induced granuloma pouch bioassays, using diclofenac Na as a reference standard. The ulcerogenic effects and acute toxicity (ALD_{50}) values of these compounds were also determined. Meanwhile, the analgesic activity of the same compounds was evaluated using the rat tail withdrawal technique. Additionally, the synthesized compounds were evaluated for their in vitro antimicrobial activity. In general, compounds belonging to the thiazolylantipyrine series exhibited better biological activities than their thiadiazolyl structure variants. Collectively, compounds **6, 10, 26**, and **27** proved to display distinctive anti-inflammatory and analgesic profiles with a fast onset of action. All of the tested compounds revealed super GI safety profile and are well tolerated by the experimental animals with high safety margin ($ALD_{50} > 3.0$ g/kg). Meanwhile, compounds **7, 10, 11**, and **23** are considered to be the most active broad spectrum antimicrobial members in this study. Compound **10** could be identified as the most biologically active member within this study with an interesting dual anti-inflammatory analgesic and antibacterial profile.

1. Introduction

Non-steroidal anti-inflammatory drugs (NSAIDs) represent a heterogeneous family of pharmacologically active compounds used to alleviate acute and chronic inflammation, pain, and fever. Their clinical efficacy is closely related to their ability to inhibit both COX-1 and COX-2 isoforms of the enzyme cyclooxygenase (COX) also referred to as prostaglandin H_2 synthase since it catalyzes the conversion of arachidonic acid to prostaglandin H_2 (PGH_2).[1] The constitutive COX-1 isoform is mainly responsible for the synthesis of prostaglandins which exert cytoprotective effect in the gastrointestinal (GI) tract and control renal function in the kidneys, whereas, the inducible COX-2 is selectively activated by pro-inflammatory stimuli and facilitates the release of prostaglandins involved in the inflammatory process.[2] Consequently, their long-term clinical employment is associated with significant side effects such as gastrointestinal lesions, bleeding, and nephrotoxicity.[3]

Since the introduction of antipyrine; the first pyrazolone derivative used in the management of pain, inflammation and fever into clinical use in 1884, great attention has been focused on pyrazole derivatives as potent anti-inflammatory, analgesic and antipyretic agents.[4–11] As a result, a large number of pyrazoles have been obtained and some have gained application on the clinical level. Among the already marketed COX-2 inhibitors that comprise the pyrazole nucleus, celecoxib; 4-[5-(4-methylphenyl)-3-(trifluoromethyl)-1*H*-pyrazol-1-yl]benzenesulfonamide; proved to be a potent and GI safe anti-inflammatory and analgesic agent.[12] Furthermore, diverse chemotherapeutic activities have been ascribed to pyrazoles as antimicrobial,[13–16] antiparasitic,[17] antiviral,[18] and antineoplastic agents.[19–21] Interest in this field has been

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intensified after the discovery of the natural pyrazole C-glycoside pyrazofurin; 4-hydroxy-3-β-D-ribofuranosyl-1*H*-pyrazole-5-carboxamide. This antibiotic was reported to possess a broad spectrum of antimicrobial and antiviral activities in addition to being active against several tumor cell lines.[22] On the other hand, careful literature survey revealed that thiazole and thiadiazole ring systems have occupied a unique position in the design and synthesis of novel biologically active agents with remarkable analgesic and anti-inflammatory activities,[23–27] in addition to their well documented potential antimicrobial activities.[28–32]

Concomitant use of several drugs to treat inflammatory conditions that might be associated with some microbial infections may cause serious health problems, especially in patients with impaired liver or kidney functions. In addition, from pharmacoeconomic viewpoint, and seeking better patient compliance, the discovery of a dual anti-inflammatory–antimicrobial agent with potential activity and fewer adverse effects is a priority of the current global research, and consequently has occupied our prime interest in recent years.

Being involved in a research program aiming at finding out new structure leads that would act as potent dual anti-inflammatory–antimicrobial agents, we have reported the synthesis, anti-inflammatory and antimicrobial activities of some lead compounds comprising mainly the pyrazole counterpart substituted with various functionalities and attached to different heterocyclic ring systems through various linkages.[33–37] In particular, potential dual anti-inflammatory and antimicrobial activities were linked to the structure leads **A**[35] and **B**[37] (Fig. 1) which comprise the thiazole ring and **C**[36] (Fig. 1) which encounter the antipyrine moiety.

In view of the above-mentioned facts, we report herein the synthesis, anti-inflammatory, analgesic and antimicrobial evaluation of some novel structure hybrids incorporating both the antipyrine moiety with either the thiazole or thiadiazole ring systems through different linkages having the general formulae **D–F** (Fig. 1). This combination was suggested in an attempt to investigate the influence of such hybridization and structure variation on the anticipated biological activities, hoping to add some synergistic biological significance to the target molecules. The target compounds were rationalized so as to comprise some pharmacophores that are believed to be responsible for the biological activity of some relevant chemotherapeutic agents such as the carboxamido, ureido and thioureido functionalities. The substitution pattern of the thiazole and the thiadiazole rings was carefully selected so as to confer different electronic environment to the molecules. It should be pointed out that, in addition to the targeted anti-inflammatory, analgesic and antimicrobial activities, the ulcerogenic and acute toxicity profiles of some of the newly synthesized compounds will be also evaluated.

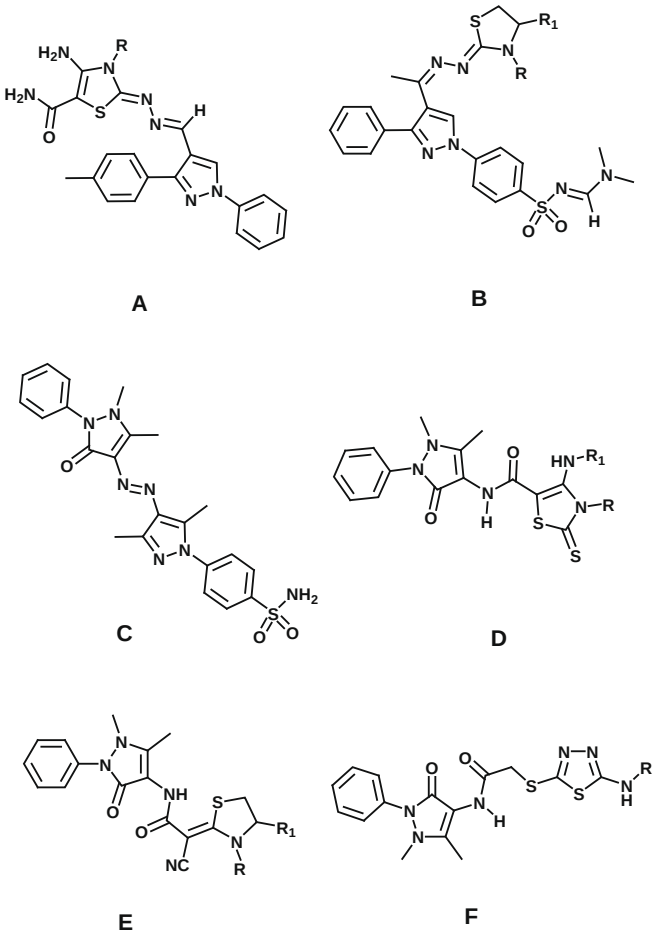


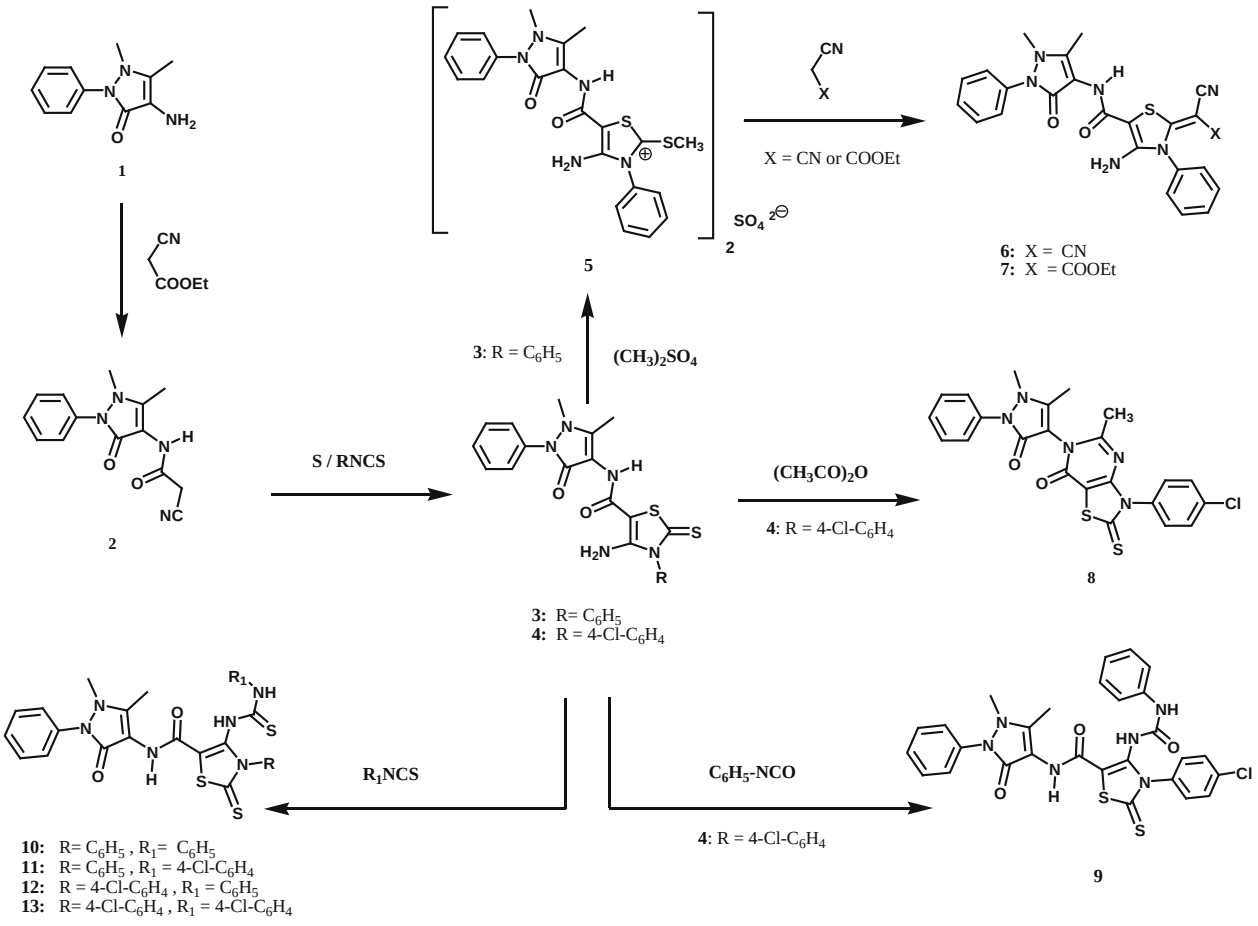
Figure 1. Structures of lead compounds **A**–**C** and general formulae of the novel series of compounds **D**–**F**.

2. Results and discussion

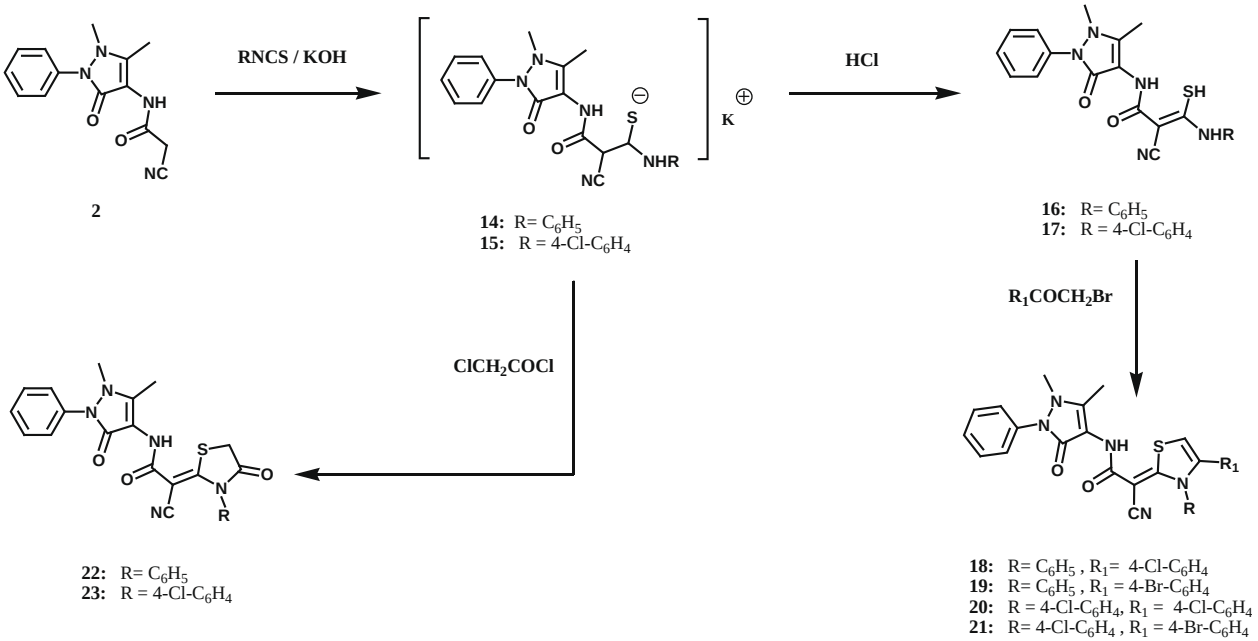
2.1. Chemistry

The synthetic strategies adopted for the synthesis of the intermediate and target compounds are depicted in Schemes 1–4. In Scheme 1, the starting compound 2-cyano-*N*-(2,5-dihydro-2,3-dimethyl-5-oxo-1-phenyl-1*H*-pyrazol-4-yl)acetamide[38] **2** was prepared by heating 4-amino-2,3-dimethyl-1-phenyl-1,2-dihydropyrazol-3-one (4-aminoantipyrine) **1** with ethyl cyanoacetate according to a literature procedure.[39] The synthesis of the key intermediate thiazolyl derivatives **3** and **4** could be achieved according to the method described by Gewald,[40] by reacting amide **2** with sulfur and the appropriate aryl isothiocyanate in the presence of triethyl amine as a basic catalyst. Refluxing **3** with dimethylsulfate in acetonitrile afforded the methyl thiothiazolium salt **5**, which in its turn was condensed with either malononitrile or ethyl cyanoacetate in presence of triethylamine to produce the methylidene derivatives **6** or **7**, respectively, according to Gewald's method.[41,42] Moreover, heating **4** with acetic anhydride resulted in the formation of the thiazolopyrimidine derivative **8** in a reasonable yield. Furthermore, the synthesis of the ureido derivative **9** and thioureido analogs **10–13** could be successfully achieved by heating a mixture of **3** or **4** with either the appropriate isocyanate or isothiocyanate derivatives, respectively, in dry dioxane containing anhydrous K_2CO_3. Shifting to Scheme 2, the intermediates **14** and **15** were prepared by adopting the same reaction conditions reported by Bukowski et al.[43] for the synthesis of similar derivatives that involves the reaction of the appropriate aryl isothiocyanate with the amide **2** in dry dimethylformamide containing two equivalents of potassium hydroxide. Upon acidification of compounds **14** and **15**, the acrylamides **16** and **17**, respectively, could be obtained. The latter acrylamides were further reacted with the selected phenacyl bromide utilizing a previously reported procedure[44] to produce the corresponding thiazoline derivatives **18**–**21**. Moreover, the thiazolidinone derivatives **22** and **23** were prepared by reacting the intermediates **14** and **15** with chloroacetyl chloride.

On the other hand, compound 2-(5-amino-1,3,4-thiadiazol-2-ylthio)-*N*-(2,5-dihydro-2,3-dimethyl-5-oxo-1-phenyl-1*H*-pyrazol-4-yl)acetamide **26** was served as a key intermediate in Scheme 3. It was prepared by reacting 2-chloro-*N*-(2,5-dihydro-2,3-dimethyl-5-oxo-1-phenyl-1*H*-pyrazol-4-yl)-acetamide **24**[45] with aminomercaptothiadiazole **25**. Formation of the *N*-acetyl derivative **27** was

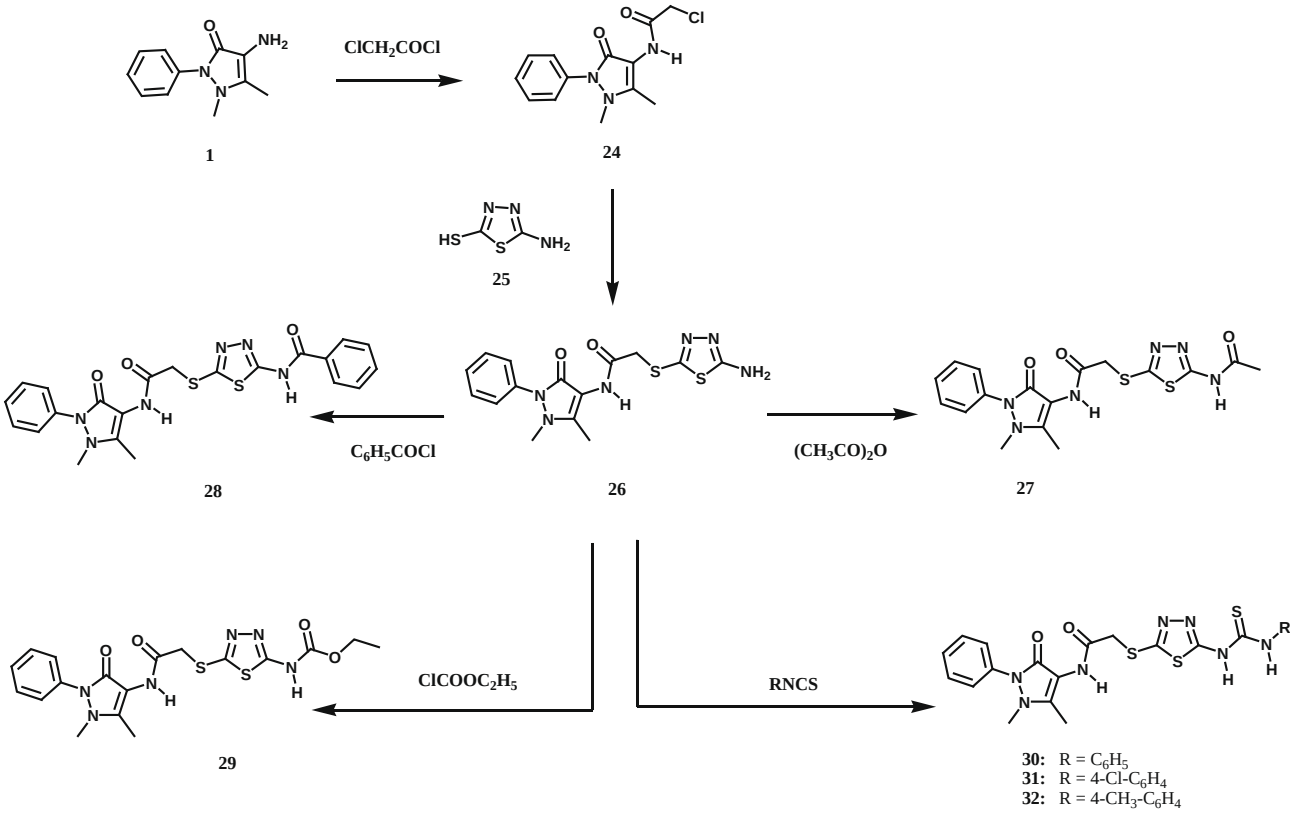


Scheme 1.

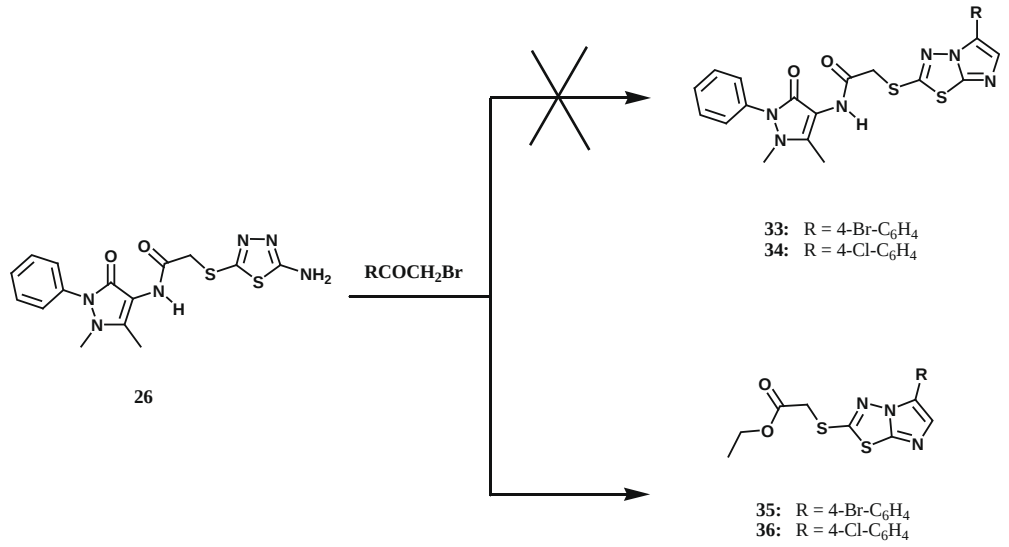


Scheme 2.

achieved in an excellent yield by heating **26** in acetic anhydride, whereas, the *N*-benzoyl derivative **28** was obtained in an excellent yield by refluxing **26** with benzoyl chloride in dry pyridine. In addition, stirring a suspension of **26** with ethyl chloroformate in dry pyridine afforded the ethoxycarbonylamino derivative **29**. Furthermore, heating **26** with the appropriate aryl isothiocyanate in dry



Scheme 3.



Scheme 4.

dioxane containing anhydrous potassium carbonate yielded the targeted thioureido derivatives **30–32**.

At this stage, it was attempted to prepare the proposed imidazothiadiazolyl derivatives of antipyrine **33** and **34**, by refluxing compound **26** with the appropriate phenacyl bromide in ethanol. Surprisingly, the (5-arylimidazo[2,1-*b*][1,3,4]thiadiazol-2-ylsulfanyl)acetic acid ethyl esters **35** and **36** were obtained instead. The structures of such unexpected compounds **35** and **36** were substantiated on basis of their IR and ^{1}H NMR spectral data. The IR spectra were characterized by the presence of C=O absorption band at 1730–1729 cm^{-1} corresponding to the ester group. Whereas, their ^{1}H NMR spectra were characterized by the complete disappearance of the signals attributed to the aminoantipyrinyl moiety and the presence of a triplet and a quartet assigned for the ester moiety.

2.2. Biological evaluation

2.2.1. Anti-inflammatory (AI) activity

To assess the AI activity of the designed compounds, selected analogs (12 compounds) from both the thiazolyl- and thiadiazolyl-antipyrine series namely; **3, 6, 7, 9, 10, 18, 20, 22, 26, 27, 29**, and

30; were evaluated by two screening protocols; namely, the formalin-induced paw edema[46] and turpentine oil-induced granuloma pouch[47] bioassays, using diclofenac Na (10 mg/kg) as a reference standard anti-inflammatory agent. The paw edema was employed as a model for acute and sub-acute inflammation, while the turpentine oil-induced granuloma pouch assay was utilized as another model for sub-acute inflammatory condition. The data obtained were presented in Tables 1–3 and expressed as means ± SE. Statistical differences of control and test groups was carried out using the Analysis of Variance (ANOVA) followed by 'Student–Newman–Keuls Multiple Comparison Test'. They were performed using computer package of the Statistical Analysis System (SAS, 1987), SAS Incorporation Institute. The difference in results was considered significant when $P < 0.05$.

2.2.1.1. Formalin-induced paw edema bioassay (acute inflammatory model). In this acute inflammatory model,[46] each test compound was dosed orally (20 mg/kg body weight) 1 h prior to induction of inflammation by formalin injection. Diclofenac Na was utilized as a reference anti-inflammatory drug at a dose of 10 mg/kg, po. the anti-inflammatory activity was then calculated 1–4 h after induction and presented in Table 1 as the mean paw volume (mL) in addition to the percentage anti-inflammatory activity (AI%).

A comparative study of the anti-inflammatory activity of test compounds relative to the reference drug at different time intervals indicated the following: after 1 h, two compounds showed distinctive pharmacokinetic profiles as revealed from their potent and rapid onset of action which was higher than diclofenac Na at a dose of 10 mg/kg, po. Concerning the thiazolylantipyrine series, outstanding AI% was recorded for compound **20** (35%), when compared with diclofenac Na (17%), whereas, other compounds were insignificantly different from the control. Among the thiadiazolylantipyrine series, the highest anti-inflammatory activity was confined to compound **27** (43%), whereas, the rest of this series showed unreliable activities. After 2 h interval, the data indicated that compounds **3, 6, 9, 10, 20, 26**, and **27** were nearly effective in inhibiting the paw edema with percentage activity of 37–43%, when compared with that of diclofenac Na (40%). Taking the anti-inflammatory activity after 3 h time interval as a criterion for comparison, it can be concluded that compound **9** from the thiazolylantipyrine series showed anti-inflammatory activity (49%) higher than diclofenac Na (44%), whereas, compounds **3, 6, 7, 10**, and **20** displayed a good anti-inflammatory activity (38–41%), however, none of them was found to be superior over the reference drug. On the contrary, the thiadiazolylantipyrines **26** and **27** proved to be more effective (49% and 46%, respectively) than diclofenac Na (44%) at the same time interval. After 4h, compounds **3, 6, 9, 10, 18, 26**, and **27** were nearly equipotent (38–43%) to the reference drug (45%).

2.2.1.2. Formalin-induced paw edema bioassay (sub-acute inflammatory model). For this sub-acute inflammatory model,[46] inflammation was induced by formalin injection in the first and third days, and test compounds were administered orally (at 20 mg/kg daily) for 7 days. Again, diclofenac Na was used as a reference anti-inflammatory agent in this assay. The anti-inflammatory activity was calculated at 1st and 8th day after induction and presented in Table 2 as the mean paw volume and the percentage anti-inflammatory activity (AI%).

The obtained data revealed that, at the 1st day, compounds **6, 10**, and **18** from the thiazolylantipyrine series displayed anti-inflammatory activity (37–40%) nearly similar to diclofenac Na (40%). Other compounds from both series showed weak to moderate anti-inflammatory activities noticeably less than the reference drug. At the 8th day, compounds **9** and **18** from thiazolylantipyrine

Table 1
Anti-inflammatory activity (AI) of some selected compounds in formalin-induced rat paw edema bioassay (acute inflammatory model)

Compound[a]	Volume of edema (mL)[b]				
	0	1 h	2 h	3 h	4 h
Control	0.30 ± 0.01	0.53 ± 0.01	0.65 ± 0.01	0.69 ± 0.01	0.72 ± 0.02
3	0.31 ± 0.01	0.46 ± 0.02 (35)[c]	0.53 ± 0.02* (37)	0.54 ± 0.03* (41)	0.55 ± 0.01* (43)
6	0.31 ± 0.01	0.48 ± 0.03 (26)	0.52 ± 0.02* (40)	0.55 ± 0.02* (38)	0.56 ± 0.04* (40)
7	0.31 ± 0.01	0.47 ± 0.02 (30)	0.57 ± 0.01 (26)	0.55 ± 0.03* (38)	0.61 ± 0.02* (29)
9	0.31 ± 0.02	0.48 ± 0.01 (26)	0.53 ± 0.02* (37)	0.51 ± 0.02* (49)	0.56 ± 0.02* (40)
10	0.32 ± 0.04	0.48 ± 0.02 (30)	0.54 ± 0.02* (37)	0.55 ± 0.03* (41)	0.57 ± 0.05* (40)
18	0.32 ± 0.01	0.46 ± 0.01 (39)	0.57 ± 0.01 (29)	0.58 ± 0.04* (33)	0.56 ± 0.02* (43)
20	0.30 ± 0.01	0.45 ± 0.01* (35)	0.51 ± 0.02* (40)	0.53 ± 0.01* (41)	0.60 ± 0.01* (29)
22	0.29 ± 0.01	0.49 ± 0.02 (13)	0.53 ± 0.03* (31)	0.59 ± 0.02 (23)	0.63 ± 0.01* (19)
26	0.31 ± 0.01	0.48 ± 0.02 (26)	0.51 ± 0.01* (43)	0.51 ± 0.02* (49)	0.57 ± 0.02* (38)
27	0.32 ± 0.01	0.45 ± 0.01* (43)	0.54 ± 0.02* (37)	0.53 ± 0.01* (46)	0.56 ± 0.03* (43)
29	0.33 ± 0.01	0.48 ± 0.04 (35)	0.62 ± 0.03 (17)	0.63 ± 0.02 (23)	0.67 ± 0.02 (19)
30	0.30 ± 0.03	0.47 ± 0.02 (26)	0.54 ± 0.03* (31)	0.61 ± 0.02 (21)	0.61 ± 0.02* (26)
Diclofenac Na	0.30 ± 0.01	0.49 ± 0.01* (17)	0.51 ± 0.02* (40)	0.52 ± 0.02* (44)	0.55 ± 0.02* (45)

* Significantly different compared to respective control values, $P < 0.05$.
[a] Dose levels, po: test compounds (20 mg/kg b.wt.), diclofenac Na (10 mg/kg b.wt.).
[b] Values are expressed as mean ± SE (number of animals $N = 5$ rats).
[c] Values between parentheses (percentage anti-inflammatory activity, AI%).

Table 2
Anti-inflammatory activity (AI) of some selected compounds in formalin-induced rat paw edema bioassay (sub-acute inflammatory model)

Compound[a]	Volume of edema (mL)[b]		
	0	1st day	8th day
Control	0.30 ± 0.01	0.65 ± 0.02	0.89 ± 0.02
3	0.31 ± 0.01	0.59 ± 0.01 (20)[c]	0.70 ± 0.01* (34)
6	0.31 ± 0.01	0.52 ± 0.03* (40)	0.71 ± 0.01* (32)
7	0.31 ± 0.01	0.56 ± 0.01* (29)	0.72 ± 0.02* (31)
9	0.31 ± 0.02	0.55 ± 0.02* (31)	0.65 ± 0.01* (42)
10	0.32 ± 0.02	0.53 ± 0.02* (40)	0.68 ± 0.02* (39)
18	0.32 ± 0.01	0.54 ± 0.02* (37)	0.65 ± 0.01* (44)
20	0.30 ± 0.01	0.55 ± 0.02* (29)	0.66 ± 0.03* (39)
22	0.29 ± 0.01	0.54 ± 0.02* (29)	0.71 ± 0.02* (17)
26	0.31 ± 0.01	0.56 ± 0.02* (29)	0.67 ± 0.02* (39)
27	0.32 ± 0.01	0.57 ± 0.02* (29)	0.68 ± 0.02* (39)
29	0.33 ± 0.01	0.58 ± 0.03 (29)	0.76 ± 0.03* (27)
30	0.30 ± 0.03	0.57 ± 0.02* (23)	0.77 ± 0.02* (20)
Diclofenac Na	0.30 ± 0.01	0.51 ± 0.01* (40)	0.62 ± 0.01* (46)

* Significantly different compared to respective control values, $P < 0.05$.
[a] Dose levels, po: test compounds (20 mg/kg b.wt.), diclofenac Na (10 mg/kg b.wt.).
[b] Values are expressed as mean ± SE (number of animals $N = 5$ rats).
[c] Values between parentheses (Percentage anti-inflammatory activity, AI%).

series were nearly effective (42% and 44%, respectively) as diclofenac Na (46%). Other compounds from both series displayed variable activities, however, none of them was found to be superior over the reference drug.

2.2.1.3. Turpentine oil-induced granuloma pouch bioassay (sub-acute inflammatory model). In this bioassay,[47] each test compound was administered orally (20 mg/kg) 1h prior to turpentine oil injection and continued for 7 days. At the 8th day, the exudates volume (mL) was measured and the percentage of granuloma inhibition was calculated. Diclofenac Na (10 mg/kg) was used as a reference drug. The results depicted in Table 3 revealed that, while most of the test compounds from both series showed anti-inflammatory activity less than the reference drug, the thiazolylantipyrine **9** was nearly equipotent (72%) with diclofenac Na (73%). Additionally, compounds **6, 10**, and **26** showed moderate anti-inflammatory activity in this bioassay with percentage of granuloma inhibition of 62%, 65%, and 62%, respectively.

Table 3
Anti-inflammatory activity of some selected compounds in turpentine oil-induced granuloma pouch in rats

Compound[a]	Volume of exudates (mL)[b]	Percentage of inhibition
Control	2.15 ± 0.06	—
3	1.07 ± 0.08*	50*
6	0.82 ± 0.02*	62*
7	1.02 ± 0.08*	53*
9	0.60 ± 0.08*	72*
10	0.75 ± 0.02*	65*
18	0.90 ± 0.09*	58*
20	0.85 ± 0.06*	60*
22	1.00 ± 0.16*	53*
26	0.82 ± 0.01*	62*
27	0.87 ± 0.10*	60*
29	1.45 ± 0.09*	33*
30	1.75 ± 0.13*	19*
Diclofenac Na	0.57 ± 0.06*	73*

* Significantly different compared to respective control values, $P < 0.05$.
[a] Dose levels, po: test compounds (20 mg/kg b.wt.), diclofenac Na (10 mg/kg b.wt.).
[b] Values are expressed as means ± SE (number of animals $N = 5$ rats).

A collective interpretation of the anti-inflammatory activity of the test compounds in pre-mentioned screens (Tables 1–3) revealed that the thiazolylantipyrines **6, 9, 10**, and **18** and the thiadiazolylantipyrine analogs **26** and **27** showed pronounced activity in the formalin paw edema screen (acute inflammatory model), nevertheless they proved to be less active in formalin paw edema and turpentine oil-induced granuloma pouch screens (sub-acute inflammatory models). These facts would suggest that such type of compounds might be effective in managing acute inflammation, while they would be less effective in controlling chronic inflammatory conditions.

2.2.1.4. Ulcerogenic activity. The twelve tested compounds that exhibited variable anti-inflammatory profiles in the pre-mentioned animal models were further evaluated for their ulcerogenic potential in rats.[48] Gross observation of the isolated rat stomachs showed a normal stomach texture for all of the tested compounds with no observable hyperemia indicating a superior GI safety profile (no ulceration) in the population of the test animals at an oral dose of 300 mg/kg, when administered twice at 2 h interval in fasted rats. It is worth-mentioning that, diclofenac Na; the reference standard anti-inflammatory drug; was found to cause 100% ulceration under the same experimental conditions.

2.2.1.5. Acute toxicity. All of the selected compounds were further evaluated for their approximate acute lethal dose ALD_{50} in male rats using a literature method.[49] The results indicated that all of the tested compounds proved to be non-toxic and are well tolerated by the experimental animals. The compounds showed a high safety margin when screened at graded doses (0.1–3.0 g/kg, po), where their ALD_{50} values were found to be >3.0 g/kg.

2.2.2. Analgesic activity

The analgesic activity of the same selected compounds was evaluated using the rat tail withdrawal technique in response to immersion in water at 55 °C,[50] using diclofenac Na as a reference drug (10 mg/kg, po). The analgesic activity was measured at 1–3 h time intervals after pain induction. The results were recorded as the average values of five administrations and the percentage increase of the reaction time in comparison with the basal values. The results were presented in Table 4 and expressed as means ± SE. Statistical differences of control and test groups was carried out as described under Section 2.2.1.

A comparative study of the analgesic activity of the test compounds relative to the reference drug at different time intervals revealed the following: after 1h, six compounds namely; **6, 7, 10, 20, 26**, and **27**, were found to exhibit fast analgesic activity similar to or even higher than (percentage increase of reaction time 27–48%) that of diclofenac Na (26%). Special high analgesic activity was displayed by the thiazolylantipyrine **10**, which exhibited potent analgesic activity almost twice (48%) as that of diclofenac Na (26%). Whereas, among the thiadiazolylantipyrine analogs, compound **26** proved to be the most potent member with percentage increase of reaction time 42%. Furthermore, the results showed that the same pattern of analgesic activity of the investigated compounds was maintained after 2 h time interval (Table 4). On the other hand, after 3 h the thiazolylantipyrines **6** and **10**, and the thiadiazolylantipyrine **26** were found to be nearly equipotent (72%, 82%,

Table 4
Analgesic activity of some selected compounds using the rat tail withdrawal technique

Compound[a]	Reaction time (s)[b]			
	0	1 h	2 h	3 h
Control	2.80 ± 0.12	2.90 ± 0.09	3.00 ± 0.40	2.97 ± 0.04
3	2.95 ± 0.06	3.50 ± 0.32 (19)[c]	4.43 ± 0.40* (50)	4.93 ± 0.31* (67)
6	2.83 ± 0.18	3.80 ± 0.29* (27)	4.12 ± 0.42* (46)	4.87 ± 0.42* (72)
7	2.87 ± 0.07	3.80 ± 0.12* (32)	4.42 ± 0.19* (50)	4.92 ± 0.12* (67)
9	2.77 ± 0.16	3.27 ± 0.18 (18)	4.13 ± 0.42* (49)	4.00 ± 0.40* (44)
10	2.57 ± 0.14	3.80 ± 0.14* (48)	4.37 ± 0.23* (70)	4.67 ± 0.26* (82)
18	2.95 ± 0.13	3.60 ± 0.22 (22)	4.23 ± 0.25* (43)	4.22 ± 0.25* (43)
20	2.92 ± 0.04	3.90 ± 0.19* (34)	4.80 ± 0.27* (64)	4.60 ± 0.53* (58)
22	2.88 ± 0.80	3.50 ± 0.16 (22)	3.47 ± 0.27 (20)	3.85 ± 0.15 (34)
26	2.60 ± 0.29	3.70 ± 0.18* (42)	4.45 ± 0.21* (71)	4.57 ± 0.25* (76)
27	2.82 ± 0.21	3.67 ± 0.34* (30)	4.50 ± 0.28* (60)	4.37 ± 0.25* (55)
29	3.00 ± 0.40	3.50 ± 0.17 (17)	3.45 ± 0.25 (15)	3.85 ± 0.25 (28)
30	2.50 ± 0.20	3.17 ± 0.20 (27)	3.57 ± 0.35 (43)	3.65 ± 0.36 (46)
Diclofenac Na	2.82 ± 0.44	3.55 ± 0.35* (26)	4.37 ± 0.23* (55)	5.07 ± 0.32* (79)

* Significantly different compared to respective control values, $P < 0.05$.
[a] Dose levels, po: test compounds (20 mg/kg b.wt.), diclophenac Na (10 mg/kg b.wt.).
[b] Values are expressed as means ± SE (number of animals $N = 5$ rats).
[c] Values between parentheses (percentage analgesic activity).

and 76%) with the reference drug (79%). However, the rest of the investigated compounds showed variable degree of analgesic activities ranging between weak to moderate (28–67%).

A deep insight in the structures of the tested compounds revealed that they represent two different main hybrids, namely the thiazolylantipyrine series (Schemes 1 and 2), and the thiadiazolylantipyrines (Scheme 3). Within the first series, the type of the thiazole ring substituent seems to modulate the anti-inflammatory and analgesic activities. Compounds comprising the 3-substituted-4-aminothiazole-2-thiones and their derivatives (Scheme 1) showed anti-inflammatory and analgesic activities better than those belonging to the 2,3-dihydro-3,4-diarylthiazoles and 3-arylthiazolidin-4-ones series presented in Scheme 2 (Tables 1–4). In this respect, it could be recognized that the key intermediate aminothiazole **3** revealed appreciable anti-inflammatory potency at both the acute and sub-acute inflammatory models together with good analgesic profile. Conversion of the 2-thioxo function at the thiazole moiety to 2-dicyanomethylene moiety (**6**; X = CN) resulted in an obvious improvement in the anti-inflammatory activity towards both models, in addition to a better analgesic activity. However, replacing the cyano moiety with a carbethoxy one (**7**; X = COOEt), did not offer any advantage in both activities over the parent compound **3**. On the other hand, derivatization of the 4-amino group at the thiazole ring into the ureido and thioureido functionalities as in **9** and **10**, resulted in a great enhancement in both the anti-inflammatory and analgesic profiles. In this view, the ureido derivative **9** exhibited potential anti-inflammatory activity at both the acute and sub-acute inflammatory models and was found to be almost equipotent with diclofenac Na, however, with weak analgesic effect. Regarding the thioureido bioisostere **10** ($R = R_1 = C_6H_5$), although it displayed significant activity at the acute inflammatory model, yet the activity at the sub-acute inflammatory model was remarkably decreased. Meanwhile, the analgesic activity of this analog was greatly enhanced to exceed that of diclofenac Na, the reference drug in this study. Shifting to the 2,3-dihydro-3,4-diarylthiazoles and 3-arylthiazolidin-4-ones members, it was found that, remarkable anti-inflammatory activity was linked with the thiazoline derivative **18** ($R = C_6H_5$, R_1 = 4-Cl–C_6H_4), where it showed recognizable activity in both the acute and sub-acute inflammatory models, however, with diminished analgesic effect. Introduction of another chlorine atom to the same structure as in **20** ($R = R_1$ = 4-Cl–C_6H_4), resulted in a marked reduction in the anti-inflammatory activity at both models, meanwhile, the analgesic activity was significantly enhanced. On the contrary, the thiazolidinone derivative **22** ($R = C_6H_5$) proved to be the least active anti-inflammatory and/or analgesic member within this series of compounds.

On the other hand, regarding the thiadiazolylantipyrine structure variants, their anti-inflammatory and analgesic activities appear to be closely related to the nature of the substituent at position-5 of the 1,3,4-thiadiazole counterpart. As can be noticed from Tables 1–4, the aminothiadiazolyl key intermediate **26** proved to be the most active member within this series with an observable anti-inflammatory potency at both the acute and sub-acute models, in addition to a potential analgesic profile. Acetylation of the amino group as in **27**, led to a significant improvement in the anti-inflammatory activity at the acute model comparable with that of diclofenac Na, nevertheless, its analgesic activity was noticeably reduced. Finally, conversion of the amino group to the carbamate or phenylthioureido functionalities as in **29** and **30**, respectively, resulted in an obvious loss of both the anti-inflammatory and analgesic activities.

Collectively, these results highlight the synergistic role of the thiazole rather than the 1,3,4-thiadiazole substituents, together with their substitution pattern, in manipulating the enhanced biological activity of such model of compounds.

2.2.3. In vitro antimicrobial activity

All of the newly synthesized target compounds were evaluated for their in vitro antibacterial activity against *Staphylococcus aureus* (ATCC 6538), *Bacillus subtilis* (NRRL B-14819) and *Micrococcus luteus* (ATCC 21881) as examples of Gram positive bacteria and *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853) and *Klebsiella pneumonia* (clinical isolate) as examples of Gram negative bacteria. They were also evaluated for their in vitro antifungal potential against *Candida albicans* (ATCC 10231) and *Aspergillus niger* (recultured) fungal strains. Agar-diffusion method was used for the determination of the preliminary antibacterial and antifungal activity. Ampicillin trihydrate and clotrimazole were used as reference drugs. The results were recorded for each tested compound as the average diameter of inhibition zones (IZ) of bacterial or fungal growth around the disks in mm. The minimum inhibitory concentration (MIC) measurement was determined for compounds that showed significant growth inhibition zones ($\geqslant$14 mm) using the twofold serial dilution method.[51] The MIC (μg/mL) values are recorded in Table 5.

The results depicted in Table 5 revealed that, 19 out of the tested 23 compounds displayed variable inhibitory effects on the growth of the tested Gram positive and Gram negative bacterial strains, while they were totally inactive against the two tested strains of fungi. In general, most of the tested compounds revealed better activity against the Gram positive rather than the Gram negative bacteria. Among the Gram positive bacteria tested, two strains namely; *S. aureus* and *B. subtilis* showed relative high sensitivity towards the tested compounds. It could also be noticed that compounds belonging to the thiazolylantipyrine series (Schemes 1

Table 5
Minimal inhibitory concentrations (MIC, μg/mL) of the active newly synthesized compounds

Compound	*S. aureus* ATCC 6538	*B. subtilis* NRRL B-14819	*M. luteus* ATCC 21881	*E. coli* ATCC 25922	*P. aeruginosa* ATCC 27853	*K. pneumonia* (clinical isolate)
3	–[a]	100	–	–	–	–
4	–	100	100	–	–	–
6	–	100	–	–	–	–
7	12.5	12.5	25	50	50	50
8	–	100	100	–	–	–
9	–	100	–	–	–	–
10	50	12.5	50	50	50	50
11	25	12.5	12.5	25	50	50
12	100	–	–	–	–	–
13	–	100	–	–	–	–
18	100	–	–	–	–	–
19	–	100	–	–	–	–
20	–	100	–	–	–	–
22	50	100	100	50	50	50
23	50	25	12.5	25	50	100
27	100	–	–	–	–	–
29	–	100	–	–	–	–
30	100	–	–	–	–	–
31	100	–	–	–	–	–
A[b]	6.25	12.5	12.5	6.25	12.5	12.5

[a] (–): totally inactive (MIC ⩾ 200 μg/mL).
[b] **A**: ampicillin trihydrate (standard broad spectrum antibiotic).

and 2) exhibited better antibacterial potentials than members of the thiadiazolylantipyrine one (Scheme 3).

Regarding the activity of the thiazolylantipyrine series (Schemes 1 and 2) against Gram positive bacteria, the results revealed that compounds **7**, **10**, **11, 22**, and **23** exhibited broad spectrum antibacterial profile against the tested three organisms. In this view, compound **7** was equipotent to ampicillin in inhibiting the growth of *B. subtilis* (MIC 12.5 μg/mL), while its activity was 50% lower than that of ampicillin against *S. aureus* and *M. luteus* species. Compound **10** showed equal activity as ampicillin against *B. subtilis* (MIC 12.5 μg/mL) together with a moderate activity against *S. aureus* and *M. luteus* (MIC 50 μg/mL). Moreover, distinctive anti-Gram positive profile was displayed by compound **11** where it proved to be equipotent as ampicillin against *B. subtilis* and *M. luteus* (MIC 12.5 μg/mL), together with a significant activity against *S. aureus* (MIC 25 μg/mL). Although the analog **22** (R = C_6H_5) exhibited moderate anti-Gram positive against the tested strains (MIC 50–100 μg/mL), yet its chloro derivative **23** (R = 4-Cl–C_6H_4) showed potential inhibitory activity against *M. luteus* equivalent to that of ampicillin (MIC 12.5 μg/mL), whereas it showed 50% of the activity of ampicillin (MIC 25 μg/mL) against *B. subtilis*. On the other hand, compounds of the same series namely; **7, 10, 11, 22**, and **23** exhibited weak to moderate growth inhibitory activity against Gram negative bacteria as revealed from their MIC values (25–100 μg/mL). Among these, compounds **11** and **23** showed relatively good growth inhibitory profiles against *E. coli* and *P. aeruginosa* (MIC 25 and 50 μg/mL, respectively) which were about 25% of the activity of ampicillin against the same organisms (MIC 6.25 and 12.5 μg/mL, respectively).

Concerning the antibacterial activity of the compounds belonging to the thiadiazolylantipyrine series (Scheme 3), compounds **27, 29, 30**, and **31**, they revealed weak growth inhibitory activity against the tested Gram positive bacteria (MIC 100 μg/mL), whereas they were totally deprived of any activity against the Gram negative bacteria strains employed in this study.

Collectively, compounds **7, 10, 11**, and **23** are considered to be the most active antimicrobial members identified in this study with a broad spectrum of antibacterial activity against both Gram positive and Gram negative bacteria.

3. Conclusion

The objective of the present study was to synthesize and investigate the anti-inflammatory, analgesic and antimicrobial activities of new pyrazole-containing compounds with the hope of discovering new structure leads serving as dual anti-inflammatory–antimicrobial agents. Our aim has been verified by the synthesis of two different groups of structure hybrids comprising basically the antipyrine moiety attached to either polysubstituted thiazole or 2,5-disubstituted-1,3,4-thiadiazole counterparts through various linkages for synergistic purpose. The obtained results clearly revealed that compounds derived from the thiazolylantipyrine series exhibited better anti-inflammatory and analgesic activities than their thiadiazolyl structure variants. Among the tested analogs, compounds **6, 9, 10, 18, 26**, and **27** showed pronounced anti-inflammatory activity comparable with diclofenac Na in the acute inflammatory model rather than the sub-acute inflammatory models, suggesting that they might be effective in managing acute inflammation rather than controlling chronic inflammatory conditions. Meanwhile, compounds **6, 7, 10, 18, 20, 22, 26**, and **27** were found to exhibit fast analgesic activity (within 2 h time interval) similar to or even higher than that of diclofenac Na. Collectively, compounds **6, 10, 26**, and **27** proved to display distinctive anti-inflammatory and analgesic profiles with a fast onset of action. Additionally, all of the tested compounds revealed super GI safety profile and are well tolerated by the experimental animals with high safety margin (ALD_{50} > 3.0 g/kg). On the other hand, some of the newly synthesized compounds were able to display variable inhibitory effects on the growth of the Gram positive and Gram negative bacteria while they were totally deprived of any antifungal activity. Compounds belonging to the thiazolylantipyrine series exhibited better antibacterial potencies than members of the thiadiazolylantipyrine one. Among these, compounds **7, 10, 11**, and **23** are considered to be the most active antimicrobial members identified in this study with a broad spectrum of antibacterial activity against

both Gram positive and Gram negative bacteria. Finally, compound **10** could be identified as the most biologically active member within this study with an interesting dual anti-inflammatory analgesic and antibacterial profile. Consequently, such type of compounds would represent a fruitful matrix for the future development of a new class of dual non-acidic anti-inflammatory–antimicrobial agents that deserves further investigation and derivatization.

4. Experimental

4.1. Chemistry

Melting points were determined in open glass capillaries on a Gallenkamp melting point apparatus and were uncorrected. The infrared (IR) spectra were recorded on Perkin-Elmer 1430 infrared spectrophotometer using the KBr plate technique. ^{1}H NMR spectra were determined on Jeol spectrometer (500 MHz) at the Microanalytical unit, Faculty of Science, Alexandria University and on a Varian spectrometer (300 MHz), Faculty of Science, Cairo University using tetramethylsilane (TMS) as the internal standard and DMSO-d_6 as the solvent (Chemical shifts in δ, ppm). Splitting patterns were designated as follows: s: singlet; d: doublet; m: multiplet. Microanalyses were performed at the Microanalytical Unit, Faculty of Science, Cairo University and at the Central lab, Faculty of Pharmacy, Alexandria University, Egypt and the found values were within ±0.4% of the theoretical values. Follow up of the reactions and checking the homogeneity of the compounds were made by TLC on silica gel-protected glass plates and the spots were detected by exposure to UV-lamp at λ 254.

4.1.1. 4-Amino-2,3-dihydro-*N*-(2,5-dihydro-2,3-dimethyl-5-oxo-1-phenyl-1*H*-pyrazol-4-yl)-3-substituted-2-thioxothiazole-5-carboxamides (3 and 4)

A mixture of compound **2** (0.23 g, 0.001 mol), finely divided sulfur (0.03 g, 0.001 mol) and triethylamine (0.1 g, 0.14 mL, 0.001 mol) in dry DMF/abs. EtOH mixture (1:1) (2 mL) was stirred at 60 °C for 30 min. The appropriate isothiocyanate (0.001 mol) was gradually added and stirring at 60 °C was continued for 6–8 h during which a yellow precipitate was partially separated out. The reaction mixture was diluted with EtOH and the obtained precipitate was filtered, washed with EtOH, dried, and crystallized from dioxane/EtOH (1:1).

4.1.1.1. 4-Amino-2,3-dihydro-*N*-(2,5-dihydro-2,3-dimethyl-5-oxo-1-phenyl-1*H*-pyrazol-4-yl)-3-phenyl-2-thioxothiazole-5-carboxamide (3). Yield: 62%, mp: 201–203 °C [reported 236–238 °C].[38] IR (cm^{-1}): 3326, 3243 (NH); 1652 (C=O); 1639, 1630 (C=N); 1248, 1045 (C–S–C). ^{1}H NMR (δ ppm): 2.19 (s, 3H, CH_3–C); 3.12 (s, 3H, CH_3–N); 6.93 (s, 2H, NH_2, D_2O exchangeable); 7.2–7.8 (m, 10H, Ar-H); 8.66 (s, 1H, NH, D_2O exchangeable). Anal. Calcd for $C_{21}H_{19}N_5O_2S_2$ (437.54): C, 57.65; H, 4.38; N, 16.01. Found: C, 58.13; H, 4.65; N, 16.38.

4.1.1.2. 4-Amino-2,3-dihydro-*N*-(2,5-dihydro-2,3-dimethyl-5-oxo-1-phenyl-1*H*-pyrazol-4-yl)-3-(4-chlorophenyl)-2-thioxothiazole-5-carboxamide (4). Yield: 53%, mp 186–188 °C. IR (cm^{-1}): 3374, 3300 (NH); 1650 (C=O); 1630, 1608 (C=N); 1245, 1073 (C–S–C). ^{1}H NMR (δ ppm): 2.12 (s, 3H, CH_3–C); 3.06 (s, 3H, CH_3–N); 6.97 (s, 2H, NH_2, D_2O exchangeable); 7.32, 7.63 (two d, J = 8.4 Hz, each 2H, C_6H_4Cl–H) 7.39 (d, J = 7.65 Hz, 2H, phenyl-$C_{2,6}$–H); 7.48 (m, 3H, phenyl-$C_{3,4,5}$–H); 8.66 (s, 1H, NH, D_2O exchangeable). Anal. Calcd for $C_{21}H_{18}ClN_5O_2S_2$ (471.98): C, 53.44; H, 3.84; N, 14.84. Found: C, 53.78; H, 4.15; N, 15.13.

4.1.2. General procedure for the synthesis of 4-amino-2-(dicyanomethylene)-2,3-dihydro-*N*-(2,5-dihydro-2,3-dimethyl-5-oxo-1-phenyl-1*H*-pyrazol-4-yl)-3-phenylthiazole-5-carboxamide (6) and ethyl 2-(5-(2,5-dihydro-2,3-dimethyl-5-oxo-1-phenyl-1*H*-pyrazol-4-ylcarbamoyl)-4-amino-3-phenylthiazol-2(3*H*)-ylidene)-2-cyanoacetate (7)

To a suspension of **3** (0.44 g, 0.001 mol) in CH_3CN (10 mL), dimethyl sulfate (0.19 g, 0.14 mL, 0.0015 mol) was added. The reaction mixture was heated under reflux for 3 h then allowed to cool. Malononitrile or ethyl cyanoacetate (0.0015 mol) and triethylamine (0.2 mL) were then added while stirring. Stirring was continued on a boiling water bath for 1 h then the reaction mixture was allowed to attain room temperature. The separated solid product was filtered, washed with cold EtOH, dried, and crystallized from the proper solvent.

4.1.2.1. 4-Amino-2-(dicyanomethylene)-2,3-dihydro-N-(2,5-dihydro-2,3-dimethyl-5-oxo-1-phenyl-1*H*-pyrazol-4-yl)-3-phenylthiazole-5-carboxamide (6). Yield: 70%, mp 238–240 °C (DMF/EtOH; 3:1). IR (cm^{-1}): 3364, 3259, 3204 (NH); 2205 (C≡N); 1650 (C=O); 1631 (C=N); 1250, 1061 (C–S–C). ^{1}H NMR (δ ppm): 2.18 (s, 3H, CH_3–C); 3.11 (s, 3H, CH_3–N); 7.00 (s, 2H, NH_2, D_2O exchangeable); 7.37 (d, J = 7.5 Hz, 2H, phenyl-$C_{2,6}$–H); 7.40 (t, J = 7.65 Hz, 2H, phenyl-$C_{3,5}$–H); 7.6–7.75 (m, 6H, Ar-H); 8.85 (s, 1H, NH, D_2O exchangeable). Anal. Calcd for $C_{24}H_{19}N_7O_2S$ (469.52): C, 61.39; H, 4.08; N, 20.88. Found: C, 61.81; H, 4.29; N, 20.76.

4.1.2.2. Ethyl 2-(5-(2,5-dihydro-2,3-dimethyl-5-oxo-1-phenyl-1*H*-pyrazol-4-ylcarbamoyl)-4-amino-3-phenylthiazol-2(3H)-ylidene)-2-cyanoacetate (7). Yield: 72%, mp 243–245 °C (EtOH). *IR (cm^{-1}):* 3374, 3269, 3208 (NH); 2199 (C≡N); 1750, 1655 (C=O); 1642 (C=N); 1285, 1061 (C–S–C); 1213, 1024 (C–O–C). ^{1}H NMR (δ ppm): 1.97 (t, J = 6.9 Hz, 3H, CH_2CH_3); 2.97 (s, 3H, CH_3); 3.90 (s, 3H, CH_3–N); 4.93 (q, J = 6.9 Hz, 2H, CH_2CH_3); 7.03 (br s, 2H, NH_2, D_2D exchangeable); 7.15 (t, J = 7.65 Hz, 1H, phenyl-C_4–H); 7.31 (d, J = 7.65 Hz, 2H, phenyl-$C_{2,6}$–H); 7.40 (t, J = 7.65 Hz, 2H, phenyl-$C_{3,5}$–H); 7.56–7.66 (m, 5H, phenyl-H); 9.62 (br s, 1H, NH D_2O exchangeable). Anal. Calcd for $C_{26}H_{24}N_6O_4S$ (516.57): C, 60.45; H, 4.68; N, 16.27. Found: C, 59.95; H, 4.43; N, 15.91.

4.1.3. 3-(4-Chlorophenyl)-6-(2,5-dihydro-2,3-dimethyl-5-oxo-1-phenyl-1*H*-pyrazol-4-yl)-5-methyl-2-thioxo-2,3-dihydro-6*H*-thiazolo[4,5-d]pyrimidin-7-one (8)

A suspension of **4** (0.47 g, 0.001 mol) in acetic anhydride (5 mL) was heated under reflux for 2 h, cooled, poured into ice-cold H_2O then set aside for an over-night. The obtained precipitate was filtered, washed with H_2O, dried, and crystallized from benzene. Yield: 70%, chars before melting. IR (cm^{-1}): 1698 (C=O), 1630 (C=N); 1248, 1064 (C–S–C). ^{1}H NMR (δ ppm): 1.90 (s, 3H, thiazolopyrimidine-C_5-CH_3); 2.13 (s, 3H, CH_3–C); 2.97 (s, 3H, CH_3–N); 7.15 (d, J = 8.4 Hz, 2H, C_6H_4Cl–$C_{2,6}$–H); 7.25 (t, J = 7.65 Hz, 1H, phenyl-C_4–H); 7.31 (d, J = 7.65 Hz, 2H, phenyl-$C_{2,6}$–H); 7.40 (t, J = 7.65 Hz, 2H, phenyl-$C_{3,5}$–H); 7.78 (d, J = 8.4 Hz, 2H, C_6H_4Cl-$C_{3,5}$–H). Anal. Calcd for $C_{23}H_{18}ClN_5O_2S_2$ (496.01): C, 55.69; H, 3.66; N, 14.12. Found: C, 55.27; H, 3.41; N, 13.91.

4.1.4. N-(2,5-Dihydro-2,3-dimethyl-5-oxo-1-phenyl-1*H*-pyrazol-4-yl)-3-(4-chlorophenyl)-4-(3-phenylureido)-2-thioxo-2,3-dihydrothiazole-5-carboxamide (9)

To a suspension of **4** (0.47g, 0.001 mol) in dry dioxane (5 mL) containing anhydrous potassium carbonate (0.14 g, 0.001 mol), phenyl isocyanate (0.12 g, 0.11 mL, 0.001 mol) was added. The reaction mixture was heated under reflux for 12 h, cooled then poured into ice-cold H_2O. The obtained precipitate was filtered, washed with H_2O, dried, and crystallized from dioxane/EtOH

(1:1); yield 71:%, mp above 300. IR (cm^{-1}): 3373 (NH); 1696, 1668 (C=O), 1630 (C=N); 1255, 1085 (C–S–C). ^1H NMR (δ ppm): 2.17 (s, 3H, CH_3–C); 3.25 (s, 3H, CH_3–N); 7.19, 7.29 (two d, each 2H, J = 8.1 Hz, C_6H_4Cl); 7.35–7.40 (m, 4H, Ar-H); 7.48–7.55 (m, 4H, Ar-H); 7.63 (d, J = 7.65 Hz, 2H, phenyl-$C_{2,6}$–H). Anal. Calcd for $C_{28}H_{23}ClN_6O_3S_2$ (591.11): C, 56.89; H, 3.92; N, 14.22. Found: C, 56.85; H, 3.86; N, 13.87.

4.1.5. *N*-(2,5-Dihydro-2,3-dimethyl-5-oxo-1-phenyl-1*H*-pyrazol-4-yl)-3-aryl-4-(3-arylthioureido)-2-thioxo-2,3-dihydrothiazole-5-carboxamides (10–13)

To a suspension of the appropriate aminothiazole derivative **3** or **4** (0.001 mol) in dry dioxane (5 mL) containing anhydrous potassium carbonate (0.14 g, 0.001 mol), the appropriate isothiocyanate (0.001 mol) was added. The reaction mixture was heated under reflux for 12 h, cooled then poured into ice-cold H_2O. The separated precipitate was filtered, washed with H_2O, dried, and crystallized from DMF/EtOH (3:1).

4.1.5.1. *N*-(2,5-Dihydro-2,3-dimethyl-5-oxo-1-phenyl-1*H*-pyrazol-4-yl)-3-phenyl-4-(3-phenylthioureido)-2-thioxo-2,3-dihydrothiazole-5-carboxamide (10). Yield: 53%, mp above 300 °C. IR (cm^{-1}): 3317–3128 (NH); 2215 (C≡N); 1665 (C=O); 1634 (C=N); 1267, 1059 (C–S–C). ^1H NMR (δ ppm): 2.21 (s, 3H, CH_3–C); 3.31 (s, 3H, CH_3–N); 7.01 (t, J = 8.1 Hz, 1H, phenyl-C_4–H); 7.13 (t, J = 8.1 Hz, 2H, phenyl-$C_{3,5}$–H); 7.30 (d, J = 8.1 Hz, 2H, phenyl-$C_{2,6}$–H); 7.40–7.60 (m, 11H, 10Ar-H and NH); 9.08 (s, 1H, NH, D_2O exchangeable). Anal. Calcd for $C_{28}H_{24}N_6O_2S_3$ (572.73): C, 58.72; H, 4.22; N, 14.67. Found: C, 59.20; H, 4.44; N, 14.38.

4.1.5.2. N-(2,5-Dihydro-2,3-dimethyl-5-oxo-1-phenyl-1*H*-pyrazol-4-yl)-3-phenyl-4-(3-(4-chlorophenyl)thioureido)-2-thioxo-2,3-dihydrothiazole-5-carboxamide (11). Yield: 58%, mp above 300 °C. IR (cm^{-1}): 3370 (NH); 2215 (C≡N); 1671 (C=O); 1615 (C=N); 1290, 1088 (C–S–C) 830 (C–Cl). ^1H NMR (δ ppm): 2.16 (s, 3H, CH_3–C); 3.27 (s, 3H, CH_3–N); 7.14 (d, J = 8.4 Hz, 2H, C_6H_4Cl–$C_{2,6}$–H); 7.31 (d, J = 8.4 Hz, 2H, phenyl-$C_{2,6}$–H); 7.34–7.58 (m, 11H, 10Ar-H and NH); 9.17 (s, 1H, NH, D_2O exchangeable). Anal. Calcd for $C_{28}H_{23}ClN_6O_2S_3$ (607.17): C, 55.39; H, 3.82; N, 13.84. Found: C, 55.01; H, 4.19; N, 13.39.

4.1.5.3. *N*-(2,5-Dihydro-2,3-dimethyl-5-oxo-1-phenyl-1*H*-pyrazol-4-yl)-3-(4-chlorophenyl)-4-(3-phenylthioureido)-2-thioxo-2,3-dihydrothiazole-5-carboxamide (12). Yield: 61%, mp above 300 °C. IR (cm^{-1}): 3250 (NH); 2250 (C≡N); 1670 (C=O); 1618 (C=N); 1292, 1055 (C–S–C) 799 (C–Cl). ^1H NMR (δ ppm): 2.16 (s, 3H, CH_3–C); 3.27 (s, 3H, CH_3–N); 7.04 (t, J = 8.1 Hz, 1H, phenyl-C_4–H); 7.14 (t, J = 7.65 Hz, 2H, phenyl-$C_{3,5}$–H); 7.27 (d, J = 8.1 Hz, 2H, phenyl-$C_{2,6}$–H); 7.36 (t, J = 8.1 Hz, 1H, phenyl-C_4–H); 7.39 (t, J = 8.1 Hz, 2H, phenyl-$C_{3,5}$–H); 7.48–7.54 (m, 4H, C_6H_4Cl–$C_{2,6}$–H & phenyl $C_{2,6}$–H); 7.60 (d, J = 8.4 Hz, 2H, C_6H_4Cl–$C_{3,5}$–H); 9.15 (s, 1H, NH, D_2O exchangeable). Anal. Calcd for $C_{28}H_{23}ClN_6O_2S_3$ (607.17): C, 55.39; H, 3.82; N, 13.84. Found: C, 55.10; H, 4.13; N, 14.10.

4.1.5.4. *N*-(2,5-Dihydro-2,3-dimethyl-5-oxo-1-phenyl-1*H*-pyrazol-4-yl)-3-(4-chlorophenyl)-4-(3-(4-chlorophenyl)thioureido)-2-thioxo-2,3-dihydrothiazole-5-carboxamide (13). Yield: 63%, mp above 300 °C. IR (cm^{-1}): 3300 (NH); 2247 (C≡N); 1690 (C=O); 1609 (C=N); 1298, 1026 (C–S–C) 883 (C–Cl). ^1H NMR (δ ppm): 2.16 (s, 3H, CH_3–C); 3.27 (s, 3H, CH_3–N); 7.19 (d, J = 8.4 Hz, 2H, C_6H_4Cl–$C_{2,6}$–H); 7.29 (d, J = 9.2 Hz, 2H, C_6H_4Cl–$C_{2,6}$–H); 7.36 (t, J = 7.65 Hz, 1H, phenyl-C_4–H); 7.38 (t, J = 7.65 Hz, 2H, phenyl-$C_{2,6}$–H); 7.48–7.54 (m, 4H, phenyl-$C_{3,5}$–H and C_6H_4Cl–$C_{3,5}$–H); 7.63 (d, J = 8.4 Hz, 2H, C_6H_4Cl–$C_{3,5}$–H); 9.22 (s, 1H, NH, D_2O exchangeable). Anal. Calcd for $C_{28}H_{22}Cl_2N_6O_2S_3$ (641.62): C, 52.41; H, 3.46; N, 13.10. Found: C, 52.20; H, 3.12; N, 13.33.

4.1.6. 2-Cyano-*N*-(2,5-dihydro-2,3-dimethyl-5-oxo-1-phenyl-1*H*-pyrazol-4-yl)-3-mercapto-3-(arylamino)acrylamides (16 and 17)

To an ice-cooled suspension of finely powdered potassium hydroxide (0.11 g, 0.002 mol) in dry DMF (5 mL), compound **2** (0.38 g, 0.001 mol) and then the appropriate isothiocyanate (0.001 mol) were added in portions with stirring. After complete addition, stirring was continued at room temperature for an over-night. The reaction mixture was then poured onto ice/cold H_2O and acidified with 0.1 N HCl to pH 3–4. The obtained precipitate was filtered, washed with H_2O, dried, and crystallized from the proper solvent.

4.1.6.1. 2-Cyano-*N*-(2,5-dihydro-2,3-dimethyl-5-oxo-1-phenyl-1*H*-pyrazol-4-yl)-3-mercapto-3-(phenylamino)acrylamide (16). Yield: 70%, mp 188–190 °C (EtOH). IR (cm^{-1}): 3340, 3114 (NH); 2630 (SH); 2197 (C≡N); 1665 (C=O, C=N); 1563, 1230, 1042, 999 (N–C=S). ^1H NMR (δ ppm): 2.93 (s, 3H, CH_3–C); 3.84 (s, 3H, CH_3–N); 7.38–7.47 (m, 2H, phenyl-$C_{2,6}$–H); 7.8–8.6 (m, 8H, Ar-H); 8.51, 10.25 (2s, each 1H, 2NH, D_2O exchangeable); 11.82 (s, 1H, SH, D_2O exchangeable). Anal. Calcd for $C_{21}H_{19}N_5O_2S$ (405.47): C, 62.20; H, 4.72; N, 17.27. Found: C, 61.91; H, 4.29; N, 17.02.

4.1.6.2. 2-Cyano-*N*-(2,5-dihydro-2,3-dimethyl-5-oxo-1-phenyl-1*H*-pyrazol-4-yl)-3-mercapto-3-(4-chlorophenylamino)acrylamide (17). Yield: 74%, mp 193–195 °C (dioxane/EtOH; 1:1). IR (cm^{-1}): 3334 (NH); 2693 (SH); 2184 (C≡N); 1660 (C=O); 1642, 1625 (C=N); 1578, 1207, 1087, 972 (N–C=S). ^1H NMR (δ ppm): 2.16 (s, 3H, CH_3–C); 3.0 (s, 3H, CH_3–N); 7.2–7.3 (m, 2H, C_6H_4Cl–$C_{2,6}$–H); 7.33–7.37 (m, 3H, phenyl-$C_{2,4,6}$–H); 7.49 (t, J = 7.8 Hz, 2H, phenyl-$C_{3,5}$–H); 7.7–7.8 (m, 2H, chlorophenyl-$C_{3,5}$–H). Anal. Calcd for $C_{21}H_{18}ClN_5O_2S$ (439.92): C, 57.33; H, 4.12; N, 15.92. Found: C, 57.63; H, 4.43; N, 15.51.

4.1.7. 2-Cyano-*N*-(2,5-dihydro-2,3-dimethyl-5-oxo-1-phenyl-1*H*-pyrazol-4-yl)-2-(3,4-diarylthiazol-2(3*H*)-ylidene)acetamides (18–21)

To a suspension of the appropriate acrylamide **16** or **17** (0.001 mol) in abs. EtOH (10 mL), the appropriate phenacyl bromide (0.001 mol) was added. The reaction mixture was heated under reflux for 2–3 h then allowed to cool. The obtained precipitate was filtered, washed with EtOH, dried, and crystallized from DMF/EtOH (3:1).

4.1.7.1. 2-[4-(4-Chlorophenyl)-3-phenyl-3*H*-thiazol-2-ylidene]-2-cyano-*N*-(2,5-dihydro-2,3-dimethyl-5-oxo-1-phenyl-1*H*-pyrazol-4-yl)acetamide (18). Yield: 70%, mp 278–280 °C. IR (cm^{-1}): 3125 (NH); 2215 (C≡N); 1657 (C=O); 1625 (C=N); 1226, 1082 (C–S–C) 857 (C–Cl). ^1H NMR (δ ppm): 2.13 (s, 3H, CH_3–C); 3.04 (s, 3H, CH_3–N); 7.19–7.50 (m, 15H, Ar-H and thiazoline-C_5–H); 7.66 (s, 1H, NH, D_2O exchangeable). Anal. Calcd for $C_{29}H_{22}ClN_5O_2S$ (540.04): C, 64.50; H, 4.11; N, 12.97. Found: C, 64.17; H, 3.96; N, 12.88.

4.1.7.2. 2-[4-(4-Bromophenyl)-3-phenyl-3*H*-thiazol-2-ylidene]-2-cyano-N-(2,5-dihydro-2,3-dimethyl-5-oxo-1*H*-pyrazol-4-yl) (19). Yield: 72%, mp 278–280 °C. IR (cm^{-1}): 3357 (NH); 2192 (C≡N); 1674 (C=O); 1637 (C=N); 1272, 1068 (C–S–C); 694 (C–Br). ^1H NMR (δ ppm): 2.13 (s, 3H, CH_3–C); 3.04 (s, 3H, CH_3–N); 7.10 (d, J = 8.4 Hz, 2H, phenyl-$C_{2,6}$–H); 7.17 (s, 1H, thiazoline-C_5–H); 7.25–7.49 (m, 12H, Ar-H) 7.70 (s, 1H, NH, D_2O exchangeable). Anal. Calcd for $C_{29}H_{22}BrN_5O_2S$ (584.49): C, 59.59; H, 3.79; N, 11.98. Found: C, 59.59; H, 3.95; N, 12.06.

4.1.7.3. 2-[3,4-Bis-(4-chlorophenyl)-3*H*-thiazol-2-ylidene]-2-cyano-N-(2,5-dihydro-2,3-dimethyl-5-oxo-1-phenyl-1*H*-pyrazol-4-yl)acetamide (20). Yield: 75%, mp 272–74 °C. IR (cm^{-1}): 3307 (NH); 2200 (C≡N); 1655 (C=O); 1612 (C=N); 1204, 1089 (C–S–C) 857 (C–Cl). ^1H NMR (δ ppm): 2.13 (s, 3H, CH_3–C); 3.04 (s, 3H, CH_3–N); 7.10 (d, J = 8.4 Hz, 2H, C_6H_4Cl–$C_{2,6}$–H); 7.20 (s, 1H, thiazoline-C_5–H); 7.27 (t, J = 7.65 Hz, phenyl-C_4–H); 7.30–7.49 (m, 10H, Ar-H) 7.70 (s, 1H, NH, D_2O exchangeable). Anal. Calcd for $C_{29}H_{21}Cl_2N_5O_2S$ (574.48): C, 60.63; H, 3.68; N, 12.19. Found: C, 60.44; H, 3.71; N, 12.11.

4.1.7.4. 2-[4-(4-Bromophenyl)-3-(4-chlorophenyl)-3*H*-thiazol-2-ylidene]-2-cyano-*N*-(2,5-dihydro-2,3-dimethyl-5-oxo-1-phenyl-1*H*-pyrazol-4-yl)-acetamide (21). Yield: 80%, mp 290–2 °C. *IR (cm^{-1}).* 3373 (NH); 2176 (C≡N); 1668 (C=O); 1624 (C=N); 1269, 1092 (C–S–C); 877 (C–Cl); 681 (C–Br). ^1H NMR (δ ppm): 2.08 (s, 3H, CH_3–C); 3.01 (s, 3H, CH_3–N); 7.12 (d, J = 7.65 Hz, 2H, C_6H_4Cl–$C_{2,6}$–H); 7.17 (s, 1H, thiazoline-C_5–H); 7.28 (t, J = 7.65 Hz, phenyl-C_4–H); 7.31 (d, J = 7.65 Hz, 2H, phenyl-$C_{2,6}$–H); 7.42–7.49 (m, 6H, C_6H_4Cl–$C_{3,5}$–1H–H, C_6H_4Br–$C_{2,6}$–H and phenyl-$C_{3,5}$–H); 7.52 (d, J = 7.65 Hz, 2H, C_6H_4Br–$C_{3,5}$–H); 7.77 (s, 1H, NH, D_2O exchangeable). Anal. Calcd for $C_{29}H_{21}BrClN_5O_2S$ (618.93): C, 56.28; H, 3.42; N, 11.32. Found: C, 56.47; H, 3.50; N, 11.32.

4.1.8. 2-Cyano-*N*-(2,5-dihydro-2,3-dimethyl-5-oxo-1-phenyl-1*H*-pyrazol-4-yl)-2-(3-aryl-4-oxothiazolidin-2-ylidene)-acetamides (22 and 23)

To an ice-cooled suspension of finely powdered potassium hydroxide (0.11 g, 0.002 mol) in dry DMF (5 mL), compound **2** (0.38 g, 0.001 mol) and then the appropriate isothiocyanate (0.001 mol) were added in portions with stirring. After complete addition, stirring was continued at room temperature for an over-night. Chloroacetyl chloride (0.11 g, 0.08 mL, 0.001 mol) was then added to the ice-cooled reaction mixture and stirring was continued at room temperature for an over-night. The reaction mixture was then poured onto ice cold H_2O and the separated solid product was filtered, washed with H_2O, dried, and crystallized from the proper solvent.

4.1.8.1. 2-Cyano-*N*-(2,5-dihydro-2,3-dimethyl-5-oxo-1-phenyl-1*H*-pyrazol-4-yl)-2-(4-oxo-3-phenylthiazolidin-2-ylidene)acetamide (22). Yield: 82%, chars before melting (EtOH). IR (cm^{-1}): 3310, 3226 (NH); 1660 (C=O); 1642, 1625 (C=N); 1236, 1046 (C–S–C). ^1H NMR (δ ppm): 2.11 (s, 3H, CH_3–C); 3.05 (s, 3H, CH_3–N); 3.97 (s, 2H, thiazolidinone-C_5-H_2); 7.32–7.54 (m, 10H, Ar-H); 8.39 (s, 1H, NH, D_2O exchangeable). Anal. Calcd for $C_{23}H_{19}N_5O_3S$ (445.49): C, 62.01; H, 4.30; N, 15.72. Found: C, 62.43; H, 3.97; N, 15.84.

4.1.8.2. 2-Cyano-*N*-(2,5-dihydro-2,3-dimethyl-5-oxo-1-phenyl-1*H*-pyrazol-4-yl)-2-(3-(4-chlorophenyl)-4-oxothiazolidin-2-ylidene)acetamide (23). Yield: 85%, chars before melting (ethyl acetate/pet. ether; 3:1). IR (cm^{-1}): 3310, 3226 (NH); 1660 (C=O); 1642, 1625 (C=N); 1236, 1046 (C–S–C). ^1H NMR (δ ppm): 2.04 (s, 3H, CH_3–C); 3.00 (s, 3H, CH_3–N); 3.91 (s, 2H, thiazolidinone-C_5–H_2); 7.26–7.33 (m, 4H, phenyl-$C_{2,6}$–H and C_6H_4Cl–$C_{2,6}$–H); 7.43–7.50 (m, 3H, phenyl-$C_{3,4,5}$–H); 7.55 (d, J = 8.4 Hz, 2H, C_6H_4Cl-$C_{3,5}$–H), 8.47 (s, 1H, NH, D_2O exchangeable). Anal. Calcd for $C_{23}H_{18}ClN_5O_3S$ (479.94): C, 57.56; H, 3.78; N, 14.59. Found: C, 57.63; H, 4.03; N, 14.29.

4.1.9. 2-(5-Amino-1,3,4-thiadiazol-2-ylthio)-*N*-(2,5-dihydro-2,3-dimethyl-5-oxo-1-phenyl-1*H*-pyrazol-4-yl)acetamide (26)

A mixture of **24**[45] (1.08 g, 0.004 mol), anhydrous potassium carbonate (0.56 g, 0.004 mol) and 5-amino-1,3,4-thiadiazole-4-thiol **25** (0.54 g, 0.004 mol) in dry acetone (20 mL) was heated under reflux for 6 h then allowed to cool. The reaction mixture was filtered and the obtained precipitate was washed with H_2O, dried and purified by dissolving in glacial acetic acid and re-precipitation by dil. NH_4OH. Yield: 66%, mp 229–31 °C. IR (cm^{-1}): 3407, 3267, 3167 (NH); 1655 (C=O); 1645, 1608 (C=N); 1217, 1062 (C–S–C). ^1H NMR (δ ppm): 2.90 (s, 3H, CH_3–C); 3.84 (s, 3H, CH_3–N); 4.75 (s, 2H, CH_2); 8.08 (br s, 2H, NH_2, D_2O exchangeable); 8.12–8.16 (m, 3H, phenyl-$C_{2,4,6}$–H); 7.95 (t, J = 8.1 Hz, 2H, phenyl-$C_{3,5}$–H); 10.17 (s, 1H, NH, D_2O exchangeable). Anal. Calcd for $C_{15}H_{16}N_6O_2S_2$ (376.46): C, 47.86; H, 4.28; N, 22.32. Found: C, 47.81; H, 4.12; N, 22.32.

4.1.10. 2-(5-Acetamido-1,3,4-thiadiazol-2-ylthio)-*N*-(2,5-dihydro-2,3-dimethyl-5-oxo-1-phen-yl-1*H*-pyrazol-4-yl)-acetamide (27)

A suspension of **26** (0.38 g, 0.001 mol) in acetic anhydride (5 mL) was heated under reflux for 10 min then allowed to cool. The obtained solid was filtered, washed with EtOH, dried, and crystallized from DMF. Yield: 72%, mp 244–6 °C. IR (cm^{-1}): 3245, 3203, 3147 (NH); 1674, 1652 (C=O), 1627 (C=N); 1257, 1050 (C–S–C). ^1H NMR (δ ppm): 2.10 (s, 3H, CH_3–C); 2.18 (s, 3H, CH_3CO); 3.04 (s, 3H, CH_3–N); 4.14 (s, 2H, CH_2); 7.29–7.36 (m, 3H, phenyl-$C_{2,4,6}$–H); 7.85 (t, J = 7.8 Hz, 2H, phenyl-$C_{3,5}$–H); 9.44, 12.57 (two s, each 1H, 2NH, D_2O exchangeable). Anal. Calcd for $C_{17}H_{18}N_6O_3S_2$ (418.49): C, 48.79; H, 4.34; N, 20.08. Found: C, 49.17; H, 4.26; N, 20.24.

4.1.11. *N*-(5-((2,5-Dihydro-2,3-dimethyl-5-oxo-1-phenyl-1*H*-pyrazol-4-ylcarbamoyl)methylthio)-1,3,4-thiadiazol-2-yl)-benzamide (28)

To a stirred ice-cooled suspension of **26** (0.38 g, 0.001 mol) in dry pyridine (5 mL), benzoyl chloride (0.14 g, 0.12 mL, 0.001 mol) was added drop-wise. The reaction mixture was heated under reflux for 6h then allowed to cool. The obtained precipitate was filtered, washed with EtOH, dried, and crystallized from dioxane/EtOH (3:1). Yield: 52%, mp 258–260 °C. IR (cm^{-1}): 3240, 3204 (NH); 1651 (C=O); 1626 (C=N); 1250, 1061 (C–S–C). ^1H NMR (δ ppm): 2.08 (s, 3H, CH_3–C); 3.01 (s, 3H, CH_3–N); 4.16 (s, 2H, CH_2); 7.25–7.33 (m, 3H, phenyl-$C_{2,4,6}$–H); 7.46 (t, J = 7.65 Hz, 2H, phenyl-$C_{3,5}$–H); 7.53 (t, J = 7.65 Hz, 2H, benzoyl-$C_{3,5}$–H); 7.64 (t, J = 7.65 Hz, 1H, benzoyl-C_4–H) 8.07 (d, J = 7.65 Hz, 2H, benzoyl-$C_{2,6}$–H) 9.50, 13.12 (two s, each 1H, 2NH, D_2O exchangeable). Anal. Calcd for $C_{22}H_{20}N_6O_3S_2$ (480.56): C, 54.98; H, 4.19; N, 17.49. Found: C, 55.21; H, 4.29; N, 17.17.

4.1.12. Ethyl 5-((2,5-dihydro-2,3-dimethyl-5-oxo-1-phenyl-1*H*-pyrazol-4-yl-carbamoyl)methylthio)-1,3,4-thiadiazol-2-ylcarbamate (29)

To a stirred ice-cooled suspension of **26** (0.38 g, 0.001 mol) in dry pyridine (5 mL), ethyl chloroformate (0.16 g, 0.14 mL, 0.0015 mol) was added dropwise. Stirring was maintained at room temperature for an over-night then poured into ice-cold H_2O. The obtained precipitate was filtered, washed with H_2O, dried and crystallized from dioxane/EtOH (3:1). Yield: 66%, mp 234–6 °C. IR (cm^{-1}): 3254, 3209 (NH); 1718, 1678 (C=O); 1639, 1618 (C=N); 1247, 1061 (C–S–C); 1207, 1038 (C–O–C). ^1H NMR (δ ppm): 2.05 (t, J = 6.9 Hz, 3H, CH_2CH_3); 2.90 (s, 3H, CH_3–C); 3.84 (s, 3H, CH_3–N); 4.15 (s, 2H, SCH_2); 4.91 (q, J = 6.9 Hz, 2H, CH_2CH_3); 8.12–8.16 (m, 3H, phenyl-$C_{2,4,6}$–H); 8.29 (t, J = 8.1 Hz, 2H, phenyl-$C_{3,5}$–H); 10.23, 13.01 (two s, each 1H, 2NH, D_2O exchangeable). Anal. Calcd for $C_{18}H_{20}N_6O_4S_2$ (448.52): C, 48.20; H, 4.49; N, 18.74. Found: C, 48.41; H, 4.16; N, 18.68.

4.1.13. 1-(5-((2,5-Dihydro-2,3-dimethyl-5-oxo-1-phenyl-1*H*-pyrazol-4-yl-carbamoyl)methylthio)-1,3,4-thiadiazol-2-yl)-3-arylthioureas (30–32)

To a suspension of **26** (0.38 g, 0.001 mol) in dry dioxane (5 mL) containing anhydrous potassium carbonate (0.14 g, 0.001 mol), the appropriate isothiocyanate (0.001 mol) was added. The reaction mixture was heated under reflux for 12 h, cooled then poured into ice-cold H_2O. The solid product formed was filtered, washed with H_2O, dried, and crystallized from DMF/EtOH (3:1).

4.1.13.1. 1-(5-((2,5-Dihydro-2,3-dimethyl-5-oxo-1-phenyl-1*H*-pyrazol-4-yl-carbamoyl)methyl-thio)-1,3,4-thiadiazol-2-yl)-3-phenylthiourea (30). Yield: 40%, mp 230–2 °C. IR (cm^{-1}): 3325, 3225 (NH); 1661 (C=O); 1625 (C=N); 1258, 1091 (C–S–C). Anal. Calcd for $C_{22}H_{21}N_7O_2S_3$ (511.64): C, 51.64; H, 4.14; N, 19.16. Found: C, 51.35; H, 4.19; N, 18.68.

4.1.13.2. 1-(5-((2,5-Dihydro-2,3-dimethyl-5-oxo-1-phenyl-1*H*-pyrazol-4-yl-carbamoyl)methyl-thio)-1,3,4-thiadiazol-2-yl)-3-(4-chlorophenyl)thiourea (31). Yield: 43%, mp 235–6 °C. IR (cm^{-1}): 3320, 3237, 3193 (NH); 1671 (C=O); 1630 (C=N); 1234, 1095 (C–S–C) 819 (C–Cl). ^{1}H NMR (δ ppm): 2.90 (s, 3H, CH_3–C); 3.84 (s, 3H, CH_3–N); 4.92 (s, 2H, CH_2); 8.08–8.19 (m, 4H, Ar-H); 8.27–8.32 (m, 3H, Ar-H); 8.59 (d, J = 9 Hz, 3H, p-C_6H_4Cl–$C_{3,5}$–H); 10.16, 10.26, 11.34 (3s, each 1H, 3NH, D_2O exchangable). Anal. Calcd for $C_{22}H_{20}ClN_7O_2S_3$ (546.09): C, 48.39; H, 3.69; N, 17.95. Found: C, 48.43; H, 4.01; N, 17.90.

4.1.13.3. 1-(5-((2,5-Dihydro-2,3-dimethyl-5-oxo-1-phenyl-1*H*-pyrazol-4-yl-carbamoyl)methyl-thio)-1,3,4-thiadiazol-2-yl)-3-(4-tolyl)thiourea (32). Yield: 39%, mp 233–4 °C. IR (cm^{-1}): 3320–3109 (NH); 1690 (C=O); 1620 (C=N); 1260, 1090 (C–S–C). ^{1}H NMR (δ ppm): 2.07 (s, 3H, CH_3–C); 2.20 (s, 3H, tolyl-CH_3); 3.01 (s, 3H, CH_3-N); 4.08 (s, 2H, CH_2); 7.10 (d, J = 7.65 Hz, 2H, p-$C_6H_4CH_3$–$C_{2,6}$–H); 7.27–7.33 (m, 3H, phenyl-$C_{2,4,6}$–H); 7.43–7.50 (m, 4H, phenyl-$C_{3,5}$–H and p-$C_6H_4CH_3$–$C_{3,5}$–H); 9.46, 10.35, 12.80 (3s, each 1H, 3NH, D_2O exchangable). Anal. Calcd for $C_{23}H_{23}N_7O_2S_3$ (525.67): C, 52.55; H, 4.41; N, 18.65. Found: C, 52.26; H, 4.23; N, 18.35.

4.1.14. Ethyl 2-(5-arylimidazo[2,1-b][1,3,4]thiadiazol-2-ylsulfanyl)acetates (35 and 36)

A mixture of **26** (0.38 g, 0.001 mol) and the appropriate phenacyl bromide (0.0011 mol) in absolute EtOH (10 mL) was heated under reflux for 24 h then allowed to cool. The separated precipitate was filtered, washed with EtOH, dried, and crystallized from EtOH.

4.1.14.1. Ethyl 2-(5-(4-bromophenyl)imidazo [2,1-b][1,3,4]thiadiazol-2-ylsulfanyl)acetate (35). Yield: 45%, mp 154–6 °C. IR (cm^{-1}): 1730 (C=O), 1650 (C=N); 1250, 1069 (C–S–C); 1032 (C-O-C); 732 (C-Br). ^{1}H NMR (δ ppm): 1.17 (t, J = 6.85 Hz, 3H, CH_2CH_3); 4.13 (q, J = 6.85 Hz, 2H, CH_2CH_3); 4.24 (s, 2H, CH_2); 7.55, 7.76 (two d, J = 8.4 Hz, each 2H, C_6H_4–Br); 8.67 (s, 1H, imidazothiadiazole-C_6–H). Anal. Calcd for $C_{14}H_{12}BrN_3O_2S_2$ (398.30): C, 42.22; H, 3.04; N, 10.55. Found: C, 42.53; H, 3.25; N, 10.56.

4.1.14.2. E(5-(4-chlorophenyl)imidazo [2,1-b][1,3,4]thiadiazol-2-ylsulfanyl)acetate (36). Yield: 40%, mp 155–157 °C. IR (cm^{-1}): 1729 (C=O), 1650 (C=N); 1299, 1089 (C–S–C); 1032 (C–O–C); 835 (C–Cl). ^{1}H NMR (δ ppm): 1.16 (t, J = 6.85 Hz, 3H, CH_2CH_3); 4.13 (q, J = 6.85 Hz, 2H, CH_2CH_3); 4.24 (s, 2H, CH_2); 7.42, 7.82 (two d, J = 8.4 Hz, each 2H, C_6H_4–Cl); 8.66 (s, 1H, imidazothiadiazole-C_6–H). Anal. Calcd for $C_{14}H_{12}ClN_3O_2S_2$ (353.85): C, 47.52; H, 3.42; N, 11.88. Found: C, 47.98; H, 3.73; N, 11.38.

4.2. Biological evaluation

4.2.1. Anti-inflammatory (AI) activity

4.2.1.1. Formalin-induced paw edema bioassay (acute inflammatory model). Male albino rats weighing 120–150 g were used throughout the assay. They were kept in the animal house under standard condition of light and temperature with free access to food and water. The animals were randomly divided into groups each of five rats. One group of five rats was kept as a control and another group received the standard drug diclofenac Na (at a dose of 10 mg/kg body weight po). A solution of formalin (2%, 0.1 mL) was injected into the subplanter region of the left hind paw under light ether anesthesia 1h after oral administration (po) of the test compound (at a dose level of 20 mg/kg body weight). The paw volume (ml) was measured by means of water plethysmometer and re-measured again 1, 2, 3, and 4 h after administration of formalin. The edema was expressed as an increase in the volume of paw, and the percentage of edema inhibition for each rat and each group was obtained as follows:

$$\%\ \text{Inhibition} = (\text{Vt} - \text{Vo})\ \text{control} - (\text{Vt} - \text{Vo})\ \text{tested compound}/(\text{Vt} - \text{Vo})\ \text{control} \times 100$$

where Vt = volume of edema at specific time interval and Vo = volume of edema at zero time interval.

4.2.1.2. Formalin-induced paw edema bioassay (sub-acute inflammatory model). Rats in the first experiment were given the same test compounds at a dose level of 20 mg/kg body weight daily for 7 consecutive days. A solution of formalin (2%, 0.1 mL) was injected into the subplanter region of the left hind paw under light ether anesthesia 1 h after oral administration (po) of the test compound. A second injection of formalin (2%, 0.1 mL) was given on the third day. The changes in the volume of paw were measured plethymographically at the first and eighth days.

4.2.1.3. Turpentine oil-induced granuloma pouch bioassay (sub-acute inflammatory model). Male albino rats weighing 120–150 g were used throughout this assay. They were kept in the animal house under standard condition of light and temperature with free access to food and water. One group of five rats was kept as a control and another group received the standard drug diclofenac Na (at a dose of 10 mg/kg body weight po). Subcutaneous dorsal granuloma pouch was made in ether-anesthetized rats by injecting 2 mL of air, followed by injection of 0.5 ml of turpentine oil into it. All of the test compounds were administered orally (at a dose level of 20 mg/kg body weight) one hour prior to turpentine oil injection and continued for seven consecutive days. On the eighth day, the paw was opened under anesthesia and the exudates were taken out with a syringe. The volume (mL) of the exudates was measured and the percentage inhibition of inflammation relative to the reference drug (diclofenac Na) was determined as follows:

$$\%\ \text{Inhibition} = \text{V control} - \text{V treated}/\text{V control} \times 100$$

4.2.1.4. Ulcerogenic activity. Male albino rats (180–200 g) were divided into groups each of five animals and were fasted for 12 h prior to the administration of the test compounds. Water was given ad libitum. Control group received 1% gum acacia orally. Other groups received diclofenac Na or the test compounds orally in two equal doses at 0 and 12 h for three successive days at a dose of 300 mg/kg per day. Animals were sacrificed by diethyl ether 6 h after the last dose and their stomachs were removed. An opening at the greater curvature was made and the stomach was cleaned by

washing with cold saline and inspected with a 3× magnifying lens for any evidence of hyperemia, haemorrhage, definite hemorrhagic erosion or ulcer.

4.2.1.5. Acute toxicity. Twelve groups of rats (180–200 g) each consists of five animals, were used in this test. The animals were fasted for 24 h prior to administration of the test compounds. The compounds were given orally in graded doses of 0.1–3.0 g/kg body weight, po. The compounds were screened at graded doses for their acute lethal doses (ALD_{50}) and the mortalities were recorded at each dose level after 24 h.

4.2.2. Analgesic activity

Analgesic activity was determined using tail withdrawal response to immersion of rat tail in water at 55 °C according to the procedure described by Janssen et al.[50] Male albino rats weighing 120–150 g were used throughout this assay. They were kept in the animal house under standard condition of light and temperature with free access to food and water. The animals were randomly divided into groups each of five rats. One group of five rats was kept as a control and another group received the standard drug diclofenac Na (at a dose of 10 mg/kg body weight po). The tested compounds were administrated orally at a dose of 20 mg/kg and diclofenac Na was used as a reference drug (10 mg/kg). The recorded values were the average of five administrations ±SE and the percentage increase of the reaction time (after 1–3 h time intervals) was calculated in comparison with the basal values.

4.2.3. In vitro antimicrobial activity

Standard sterilized filter paper disks (5 mm diameter) impregnated with a solution of the test compound in DMSO (1 mg/mL) were placed on an agar plate seeded with the appropriate test organism in triplicates. The utilized test organisms were: *S. aureus* (ATCC 6538), *B. subtilis* (NRRL B-14819) and *M. luteus* (ATCC 21881) as examples of Gram positive bacteria and *E. coli* (ATCC 25922), *P. aeruginosa* (ATCC 27853) and *K. pneumonia* (clinical isolate) as examples of Gram negative bacteria. They were also evaluated for their in vitro antifungal potential against *C. albicans* (ATCC 10231) and *A. niger* (recultured) fungal strains were utilized as representatives for fungi. Ampicillin trihydrate and clotrimazole were used as standard antibacterial and antifungal agents, respectively. DMSO alone was used as control at the same above-mentioned concentration. The plates were incubated at 37 °C for 24 h for bacteria and for 7 days for fungi. Compounds that showed significant growth inhibition zones ($\geqslant$14 mm) using the twofold serial dilution technique, were further evaluated for their minimal inhibitory concentrations (MICs).

5. Minimal inhibitory concentration (MIC) measurement

The microdilution susceptibility test in Müller–Hinton Broth (Oxoid) and Sabouraud Liquid Medium (Oxoid) was used for the determination of antibacterial and antifungal activity, respectively. Stock solutions of the tested compounds, ampicillin trihydrate and clotrimazole were prepared in DMSO at concentration of 800 μg/mL followed by twofold dilution at concentrations of (400, 200, ... 6.25 μg/ml). The microorganism suspensions at 10^6 CFU/ml (Colony Forming U/mL) concentration were inoculated to the corresponding wells. Plates were incubated at 36 °C for 24–48 h and the minimal inhibitory concentrations (MIC) were determined. Control experiments were also done.

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