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An Efficient Synthesis and Reactions of 5-Acetyl-6-Methyl-4-(1,3-Diphenyl-1*H*-Pyrazol-4-yl)-3,4-Dihydropyrmidin-2(1*H*)-Thione as Potential Antimicrobial and Anti-Inflammatory Agents

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Abstract–The synthesis of 5-acetyl-6-methyl-4-(1,3-diphenyl-1*H*-pyrazol-4-yl)-3,4-dihydropyrmidin-2(1*H*)-thione was achieved by one-pot three-component synthesis using $CaCl_2$ in refluxing EtOH. The starting compound was utilized to synthesize a new series of 5-pyrazolyl; isoxazolyl; pyrimidinyl derivatives *via* the synthesized chalcone. Also, fused isoxazolo [5,4-*d*]pyrimidine and pyrazolo[3,4-*d*]pyrimidine were obtained by the treatment of 5-acetyl derivative with hydroxyl amine and/ or hydrazine hydrate. Also, the thiosemicarbazide derivative was prepared and utilized to synthesize other new thiazole derivatives. The structures of all compounds have been established on the basis of their analytical and spectral data. All compounds was also evaluated for their antibacterial and antifungal activity against various strains of bacteria and fungi. Also, the anti-inflammatory activity of some of synthesized compounds was evaluated using the carrageenan induced paw oedema test in rats using indomethacin as the reference drug.

Keywords: one pot reaction, DHPMs, chalcone, azole, thiazole, biological activity

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INTRODUCTION

Several derivatives of dihydropyrimidine (DHPMs) with diverse substitution patterns have been synthesized on the basis of reactions established by Biginelli [1–4]. Among them, the Biginelli reaction involving a multicomponent condensation of aldehyde, β-dicarbonyl compounds, urea or thiourea provides an easy access to the preparation of DHPMs as these reactions (MCRs) are known to be facile, efficient and economically viable [5–8]. The 3,4-dihydropyrimidine derivatives represents a heterocylic system of remarkable pharmacological efficiency. In the past decades, abroad range of biological effects, including antiviral, antitumor, antibacterial, and anti-inflammatory activities has been ascribed to these partly reduced pyrimidine derivatives [9–11]. More recently, appropriately functionalized DHPMs have emerged as, e.g., orally active antihypertensive agents [12]. Furthermore, dihydropyrimidine derivatives are also reported to have showed different pharmacological activities like antimalarial, antitubercular, antidiabetic, antiepileptic, antileishmanial, antiproliferative activities. [13]. A number of functionalized DHPMs have been found to be potent calcium channel blockers agents [14], analgesic [15], DHPMs have also been exploited as design elements in the development of functional materials such as polymers [16], adhesive [17], dyes [18]. In continuation of our interest in developing novel methodologies and synthesis [19–21]. The aim of this work was to synthesize 5-acetyl-6-methyl-4-(1,3-diphenyl-1*H*-pyrazol-4-yl)-3,4-dihydropyrmidin-2(1*H*)-thione using $CaCl_2$ as catalyst [22] and its derived heterocycles to be evaluated for their antimicrobial activities.

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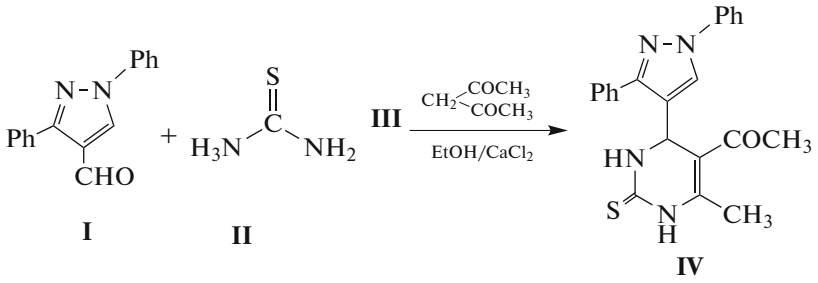
RESULTS AND DISCUSSIONS

Chemistry

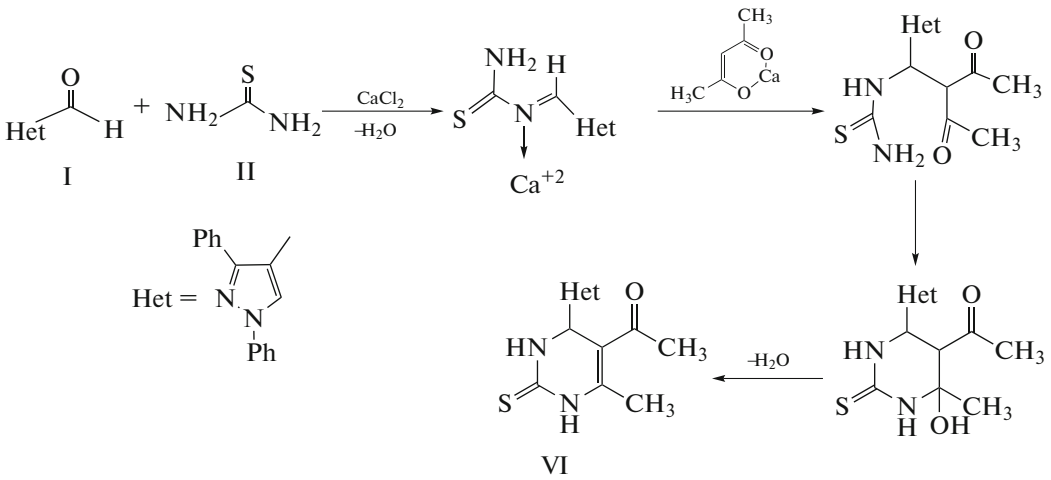
The title compounds, 5-acetyl-6-methyl-4-(1,3-diphenyl-1*H*-pyrazol-4-yl)-3,4-dihydro pyrmidin-2(1*H*)-thione (**IV**) were synthesized *via* three step reactions from biginelli reaction, using 1,3-diphenyl-1H-pyrazole-4-carbaldehyde (**I**), thiourea (**II**), acetylacetone (**III**) in ethanol under reflux in the presence of catalytic amount of $CaCl_2$ as prompt catalyst [22] (Scheme 1). The mechanism may proceed similar to that of Kappe [23], for the calcium catalyzed Beginelli reactions. The imine intermediate formed from the aldehyde (**I**) and thiourea (**II**), stabilized by calcium ions. Next, the addition of β-diketone enolate followed by cyclodehydration of open-chain thioureide, would afford target dihydropyrimidine thione derivative) (**IV**) (Scheme 2). IR spectrum of compound (**IV**) showed bands at 1704 and 3242–3424 cm^{-1} assigned for carbonyl and NH groups, respectively. The mass

spectrum revealed a molecular ion peak at m/z = 388.42 (M^+, 93.15%) corresponding to a molecular formula $C_{22}H_{20}N_4OS$. Also, the ^1H NMR spectrum showed six charactrestic singlet signals at δ 2.15, 2.25, 5.55, 8.55, 8.78 and 9.49 ppm assigned for two methyl, CH-pyrimidine, CH-pyrazole and two NH groups (D_2O exchangeable), respectively, and multiplets at δ 7.32–8.04 ppm due to aromatic protons. In ^{13}C NMR spectrum, the appearance of the carbon signals at δ 16.78, 26.45 and 46.22 ppm for CH_3, $COCH_3$ and pyrimdine-C4, as well as the carbonyl (C=O) at δ 174.37 and thiocarbonyl (C=S) at δ 197.44 ppm. Claisen–Schmidt reaction of the acetyl derivative (**IV**) with different *p*-substituted aromatic aldehydes in ethanol and in presence of aqueous sodium hydroxide (25%), afforded the corresponding chalcones (**Va–c**). These compounds were characterized by IR, ^1H NMR, ^{13}C NMR and MS. The IR spectrum showed the carbonyl peaks (C=O) at 1655–1674 cm^{-1}. The ^1H NMR spectrum of (**Vb**) exhibited five singlet signals at δ 2.35, 5,47, 6.75, 9.75 and 10.26 ppm due to CH_3, CH-pyrimidine, vinylic CH (COCH=), two NH protons, respectively. Also, aryl hydrogen shift in the range span from 7.25–7.44 ppm resulted in the specific double doublet separation signal which indicates the benzene ring is *p*-substituted. The MS spectrum of compound (**Vc**), displayed the appearance of the M^+ peak at m/z 506.76 (M^+, 46.71%) corresponding to the molecular formula ($C_{30}H_{26}N_4O_2S$).

Presence of α-β-unsaturated keto function makes chalcones very prone to undergo reaction with bidentate nucleophiles to give five and six membered heterocyclic moieties. We intended to utilized this procedure to explore the formation of compounds (**VI–XI**) containing the pyrazole, isoxazole, pyrimidine and pyrane rings linked on 5-position of dihydropyrimidine nucleus. Thus, cyclocondensation of chalcones (**Vb**) (R = Cl) with hydrazine hydrate or phenyl hydrazine in dry ethanol gave the corresponding pyrazole and N-phenylpyrazole derivatives (**VIa**) and (**VIb**), respectively. Also, the reaction of (**Vb**) with hydroxylamine hydrochloride in the presence of anhydrous sodium acetate led to the formation of 5-(5-(4-chlorophenyl)-4,5-dihydroisoxazol-3-yl)-3,4-dihydro-6-methyl-4-(1,3-diphenyl-1*H*-pyrazol-4-yl)pyrimidine-2(1*H*)-thione (**VII**). Similarly, the reaction of (**Vb**) with urea and/or thiourea in presence of aqueous potassium hydroxide (5%) gave the corresponding pyrimidinone and pyrimdinethione derivatives (**VIIIa**, **b**) respectively.

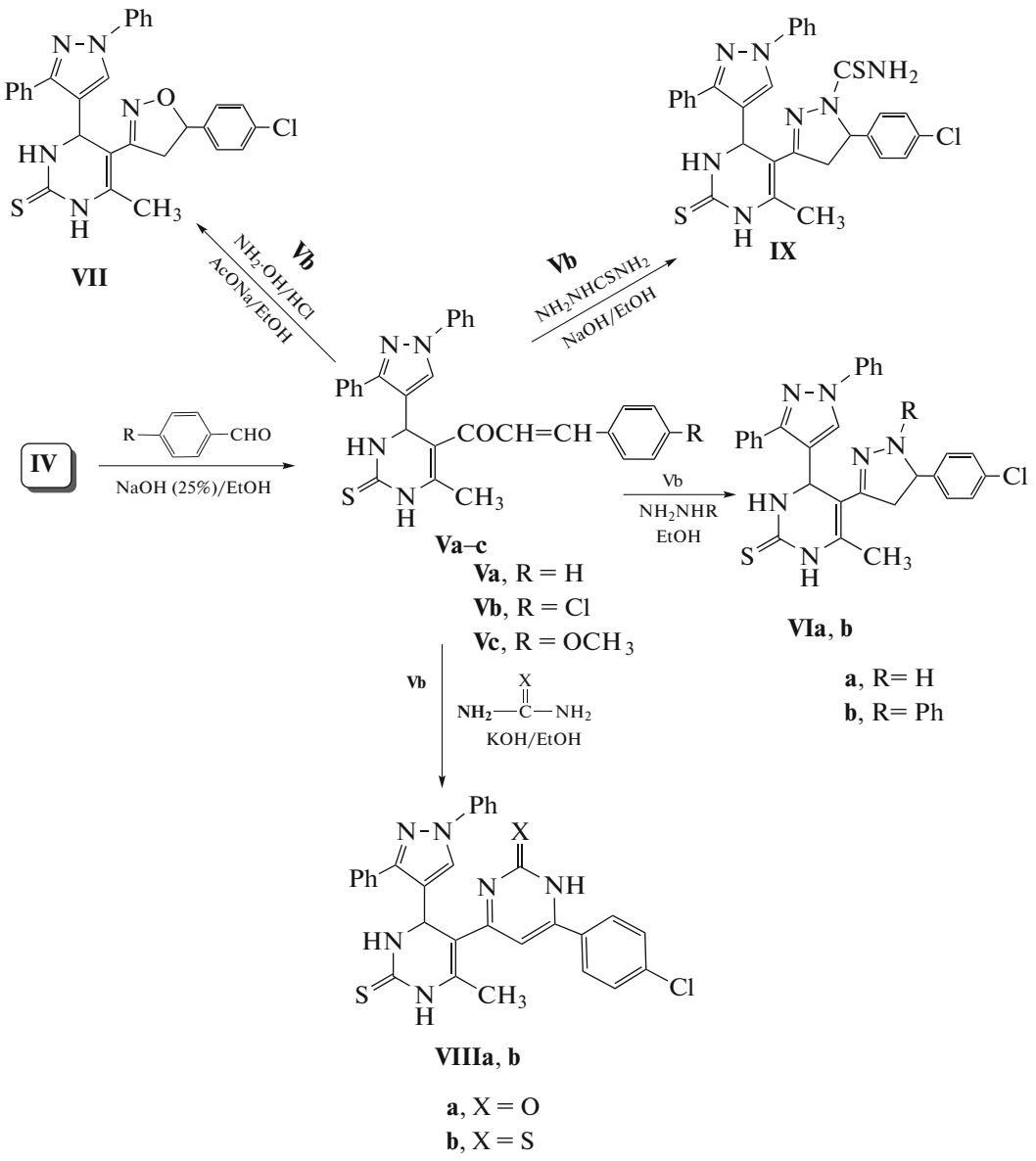


Scheme 1. Synthesis of 4-pyrazoloyl dihydropyrimidinethione (**IV**).



Scheme 2. Mechanistic pathway for the synthesis of (**IV**).

The ^{1}H NMR spectrum of (**VIa**, **b**), showed doublet of doublets of $-CH_2$ near about δ 3.12–4.15 ppm and doublet of doublets of –CH at δ 4.85–5.27 ppm, confirmed the cyclisation in pyrazoline. The IR spectra of (**VII**) clearly showed the formation of isoxazoline ring by the appearance of band at 1509 cm^{-1} for (=C=N–O). ^{1}H NMR data also confirmed the synthesis by showing a doublet at δ 3.75 ppm for 2H and a triplet at δ 4.40 ppm for 1H of isoxazoline ring. The structure of compounds (**VIIIa**, **b**) was established by spectroscopic methods. The IR spectra of (**VIIIa**), showed a strong absorption bands at 1686, 3270, 3298, 3415 cm^{-1} for the C=O and N–H bond stretching. While, with compound (**VIIIb**), it showed appearance of new band at 1249 cm^{-1} characteristic of C=S and disappearance of band characteristic of C=O in the starting material. The MS spectrum of compound (**VIIIb**), displayed the appearance of the M^+ peak at m/z 567.18 (M^+, 38.44%) corresponding to the molecular formula ($C_{30}H_{23}ClN_6S_2$). Condensation of chalcone (**Vb**) with thiosemicarbazide has become an extremely popular method for making pyrazoline derivatives. Thus, treatment of (**Vb**) with thiosemicarbazide in an ethanolic sodium hydroxide solution, yielded the pyrazolecarbothioamide (**IX**).The IR spectra of compound (**IX**) showed intense bands at 3425, 3250, 3177, 1580 and 1370 cm^{-1} due to NH, NH_2, C=N and C=S groups, respectively. In the ^{1}H NMR spectra, pyrazoline protons CH_2 appears at 3.46 and 4.0 ppm as doublet of doublets. The pyrazoline CH proton also appeared as doublet of doublets at 5.28 ppm, reinforcing the ring formation in compound (**IX**) (Scheme 3). On the other hand, treatment of the acetyl derivative (**IV**) with hydroxyl amine hydrochloride in the presence of sodium acetate gave 4,5,7,7a-tetrahydro-3,7a-dimethyl-4-(1,3-diphenyl-1*H*-pyrazol-4-yl)isoxazolo[5,4-*d*]pyrimidine-6(3a*H*)-thione (**X**). Similarly, upon refluxing of (**IV**) with hydrazine hydrate in dioxane, 4,5,7,7a-tetrahydro-3,7a-dimethyl-4-(1,3-diphenyl-1*H*-pyrazol-4-yl)-1*H*-pyrazolo[3,4-*d*]pyrimidine-6(3a*H*)-thione (**XI**). Cyclization was formed by a Michael-type addition of the oxime (or hydrazone) proton to C(5) with linking of the oxygen (or nitrogen) atom to C(6) of the pyrimidine ring [24]. Both compounds were confirmed *via* elemental and spectral data. Thus, ir specrum, of (**X**) showed the absence of absorption band of the OH oxime, which reinforced the cyclization. Also, ^{1}H NMR spectrum of isoxazolo and pyrazolo derivatives (**X**), (**XI**), revealed a significant additional singlet signals at 3.80 and 4.25 ppm due to C(5)–H of the condensed isoxazole and pyrazole rings with the tetrahydropyrimidine. In ^{13}C NMR spectrum of compound (**X**), the appearance of the carbon signals at δ 71.45, and 76.11 ppm for the condensed pyrimidine-C(5) and C(6) as saturated carbons, reinforced the cyclization mechanism. The MS spectrum of compound (**XI**), displayed the appearance of the M^+ peak at m/z 402.39 (M^+, 68.12%) corresponding to the molecular formula ($C_{22}H_{22}N_6S$). Condensation of compound (**IV**) with thiosemicarbazide under reflux in acetic acid, produced the 1-(1-(1,2,3,4-tetrahydro-6-methyl-4-(1,3-diphenyl-1*H*-pyrazol-4-yl)-2-thioxopyrimidin-5-yl)ethylidene)thiosemicarbazide (**XII**). The chemical structure of (**XII**) was confirmed by its elemental and spectral data. Thus, IR spectrum showed the disappearance of an absorption band at 1704 cm^{-1} of C=O group in the starting compound (**IV**), and revealed characteristic bands at 3450, 3375 and at 3278 cm^{-1} due to NH and NH_2 groups. ^{1}HNMR of (**XII**), displayed an additional two singlet signals at 8.75, 9.11, 9.65 and 11.15 ppm attributed to NH_2 and NH, respectively. Treatment of thiourea derivatives (**XII**) with ethyl chloroacetate in presence of fused sodium acetate in ethanol under reflux, yielded the corresponding 2-{2-[1-(6-methyl-4-(1,3-diphenyl-1*H*-pyrazol-4-yl-2-thioxo-1,2,3,4-tetrahydropyrimidin-5-yl)ethylidene]-hydrazinyl}-1,3-thiazol-4(5*H*)-one (**XIII**). The IR spectrum of the thiazolidinone (**XIII**) was characterized by the presence of the characteristic bands at 3390, 3317, 3150 and 1720 cm^{-1} due to NH and C=O functions, respectively.^{1}HNMR showed four characteristic singlet signals at 13.56, 9.20, 7.95 and 4.66 ppm attributed to NH and CH_2(thiazolidine) protons, respectively. The latter compound (**XIII**) was condensed with 4-chlorobenzaldehyde in presence of piperidine led to the formation of the corresponding 4-chlorobenzylidine derivative (**XIV**). The IR spectrum of (**XIV**) revealed the presence of conjugated C=O (thiazolidine) at 1688 cm^{-1}. Whereas, its ^{1}HNMR showed the absence of singlet peak at 4.66 due to CH_2 (thiazolidine) and gave instead a singlet peak at 8.15 ppm due to CH (arylidine) proton. The MS spectrum of compound (**XIV**) displayed the appearance of the M^+ peak at m/z 624.29 (M^+, 68.13%) corresponding to the molecular formula ($C_{32}H_{26}ClN_7OS_2$). Finally, the thiosemicarbazide derivative (**XII**), was allowed to react with α-haloketones to yield the corresponding thiazolidines derivatives (**XVa**, **b**) (Scheme 4). ^{1}H NMR spectrum of (**XVa**), exhibited three singlet signals at δ 2.27, 2.35 and 2.43 ppm for three CH_3 protons. In addition, a singlet at 6.15 ppm attributable to thiazoles H-5. Mass spectrum of (**XVb**), showed a peak analogous to the molecular ion at m/z 561.37 (M^+, 48%) (Scheme 4).



Scheme 3. Synthetic route for target compounds (**V**–**IX**).

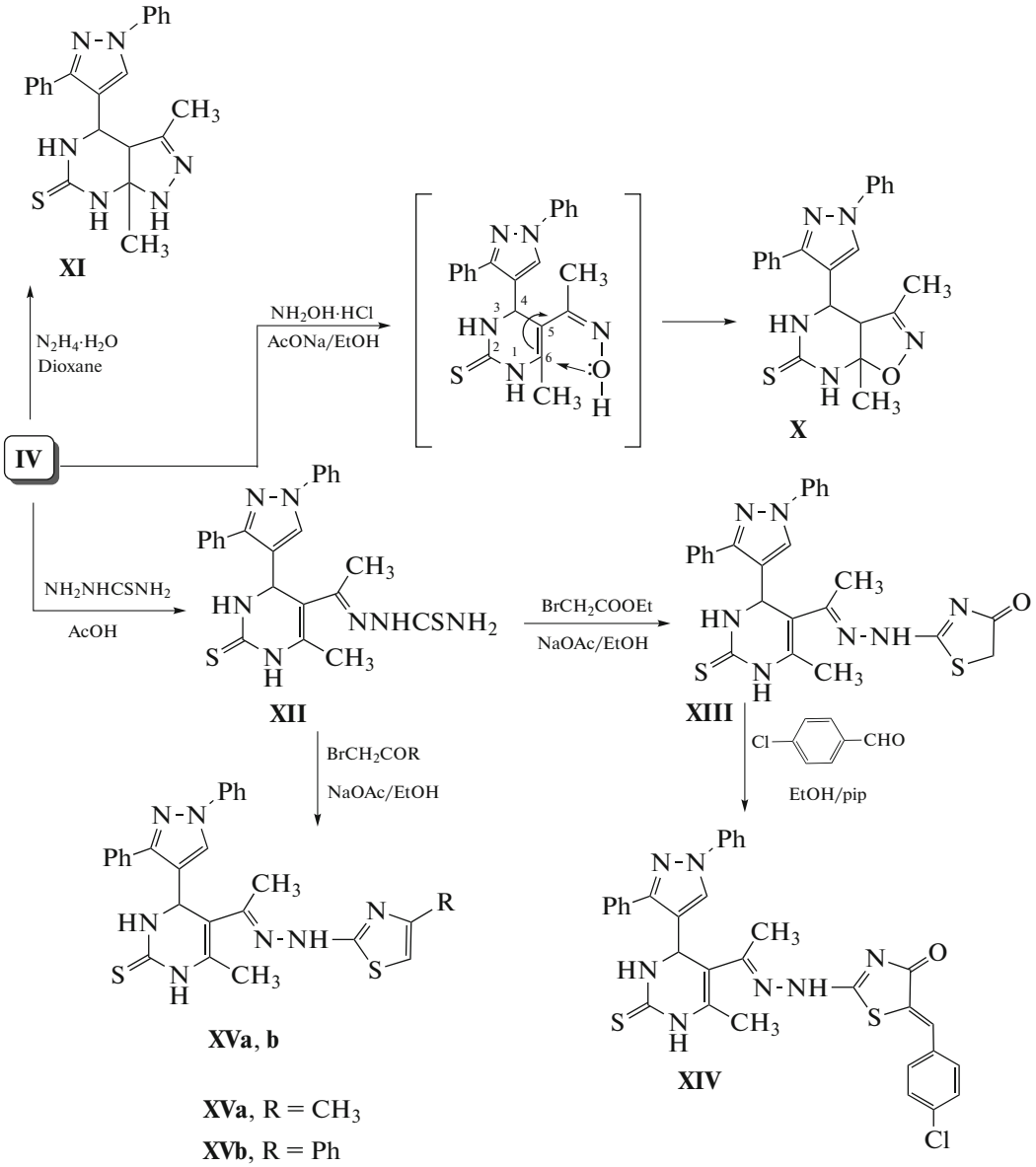
Biological Activity

All of the synthesized compounds (**IV**–**XV**) were screened against bacterial strains *Staphylococcus aureus* (AUMC B.54), *Bacillus cereus* (AUMC B.52) as gram-positive bacteria and *Escherichia coli* (AUMC B.53), *Pseudomonas aeruginosa* (AUMC B.73) as gram-negative bacteria and fungal strains *Candida albicans* (AUMC No. 214), *Aspergillus flavus* (AUMC no. 1276) using the agar well-diffusion method [25]. The screening tests were carried out in triplicate and the results were expressed as a mean of three determinations. Chloramphenicol and Clotrimazole were used as standards. Data are represented as % inhibition with reference to standards in Table1.

Antibacterial Activity

The starting material 5-acetyl-6-methyl derivative (**IV**) have a considerable activity against the tested bacteria (46–82%). Conversion of (**IV**) to the corresponding chalcones (**Va**–**c**) derivatives. Only, the 4-chloro derivative (**Vb**) exihibited a promosing activity towards the gram-negative bacteria (*E. coli*, *P. aeruginosa*, >100). Among the pyrazolo, isoxazolo and pyrimido derivatives (**VIa**, **b**), (**VII**), (**VIIIa**, **b**), the pyrazolo derivative (**VIa**) did not exihibit activity against tested bacteria. While, its *N*-phenyl pyrazole derivative, (**VIb**), showed a strong antifungal activity against both fungi species (75, 90%). Whereas, the isoxazole one (**VII**) was the most potent against *E. coli* (96%). Also, the thiopyrimidine derivative (**VIIIb**), showed strong activity towards all the bacteria species

(>100). Building up a new fused isoxazolo and pyrazolopyrimidine ring systems (**X**), (**XI**), showed a weak to mild antibacterial activity (31–79%). Conversion of the acetyl derivative **IV** to its thiosemicarbazide one (**XII**) found to be a good antifungal against *S. aureus* (61%) as well as more potent against *B. cereus*. (>100). On the other hand, among the thiazolidine derivatives (**XIII**), (**XIV**), (**XVa**), (**XVb**), only the 4-phenyl thiazole (**XVb**), exihihbited a promosing antibacterial activity against the bacteria organisms (>100).



Scheme 4. Synthetic route for target compounds (**X–XV**).

Antifungal Activity

With respect to the antifungal activity, it has been observed from Table1, that mainly four compounds 5-*N*-phenylpyrazole (**VIb**), 5-thiopyrmidine (**VIIIb**), 4-chlorobenzylidine thiazolidine (**XIV**) and 4-phenylthiazole (**XVb**) derivatives, only showed strongest activity against *A. flavus* (86...>100%). Other compounds possessed weak to moderate antifungal activity against the tested fungi (39–68%).

Anti-Inflammatory Activity

Five compounds were selected to evaluate for their anti-inflammatory activity (**IV**), (**Vb**), (**VIb**), (**VIIIb**), (**XI**), using the carrageenan induced rat paw oedema model using indomethacin as the reference drug [26]. Mean changes in paw oedema thickness of animals pretreated with the tested compounds after 1, 2, 3 and 4 h from induction of inflammation was measured and the inhibition percent of oedema by the tested compounds was calculated. The relative potencies % of the

Table 1. Anti-microbial activity of compounds (**IV**–**XV**)

Compd. no.	Diameter of zone of inhibition (mm)/% inhibition with reference to standard*					
	Gram-positive bacteria		Gram-nagative bacteria		Fungi	
	S. aureus	*B. cereus*	*E. coli*	*P. aeruginosa*	*C. albicans*	*A. flavus*
(IV)	15 (46)	19 (79)	22 (81)	14 (82)	–	–
(Va)	9 (50)	–	–	–	–	–
(Vb)	13 (72)	16 (66)	31 (>100)	23 (>100)	11 (39)	27 (64)
(Vc)	10 (55)	13 (54)	15 (56)	–	–	–
(VIa)	–	–	–	–	–	–
(VIb)	–	–	–	–	21 (75)	38 (90)
(VII)	–	–	26 (96)	–	–	–
(VIIIa)	12 (66)	9 (37)	13 (48)	–	–	21 (50)
(VIIIb)	19 (>100)	29 (>100)	33 (>100)	26 (>100)	10 (36)	36 (86)
(IX)	14 (78)	18 (75)	17 (63)	–	15 (54)	–
(X)	10 (55)	19 (79)	11 (41)	9 (31)	17 (61)	–
(XI)	10 (55)	17 (71)	20 (74)	13 (76)	–	–
(XII)	11 (61)	35 (>100)	11 (41)	8 (47)	–	–
(XIII)	10 (55)	16 (66)	–	–	–	25 (60)
(XIV)	9 (20)	14 (58)	–	–	–	39 (93)
(XVa)	12 (66)	10 (42)	13 (48)	8 (47)	19 (68)	26 (62)
(XVb)	22 (>100)	29 (>100)	37 (>100)	24 (>100)	–	44 (>100)
Chloramphenicol	18	24	27	17	–	–
Clotrimazole	–	–	–	–	28	42

* The percentage zone of inhibition was calculated against the bacterial and fungal strains. Solvent and negative control: DMSO (dimethylsulfoxide). "–" No inhibition observed.

tested compounds compared to indomethacin at the fourth hour was also calculated (Table 2). Compounds in the present series had weak to strong anti-flammatory effects ranging from 24.0 to 50.6% edema reduction. The results of the anti-inflammatory activity revealed that, the acetyl derivative (**IV**), possessed a moderate activity (30.3%), in comparison to around 52.8% seen with the standard, indomethacin. Conversion of acetyl derivative (**IV**) to its chalcone (**Vb**), with 4-chloro group, strongly enhanced the activity (42.7%). Building up of *N*-phenyl pyrazole (**VIb**) moiety from (**Vb**), reduced strongly the activity to almost half (24%). Interestingly, a promosing result was found with the construction of 5-thiopyrimido moiety, compound (**VIIIb**), which gave the highest activity (50.6%). However, the fused pyrazolopyrimidine derivative (**XI**) had little anti-inflammatory activity (26.9%) in comparison to the edema inhibition of the standard, indomethacin (52.8%). These findings indicate that the 4-chloro benzylidene and thioxopyrimidin (**Vb**), (**VIIIb**) derivatives could represent a highly promising group for the development of anti-inflammatory drugs.

CONCLUSION

New derivatrives of DHPM, were synthesized and evaluated for their antibacterial, antifungal, and anti-inflammatrory activities. A significant level of activity was illustrated. Compounds (**Vb**), (**VIb**), (**VII**), (**VIIIb**), (**XII**), (**XIV**) and (**XVb**) were the most potent against the tested microorganism. Also, compounds (**Vb**) and (**VIIIb**) were the most anti-inflammatory activity comparable to indomethacin. Such compounds appear to offer a suitable template for the design of more powerful antibacterial agents and further studies are under way to this end in our laboratory

EXPERIMENTAL

General Procedures

The time required for completion of each reaction was monitored by TLC. All melting points are uncorrected and were measured on a Gallenkamp apparatus. The IR spectra were recorded on a Shimadzu 470. IR spectrometer (KBr, ν_{max} cm^{-1}). The ^{1}H and ^{13}C NMR spectra in deuterated dimethylsulfoxide (DMSO-d_6) were measured on a Varian EM-200 (^{1}H:

Table 2. Anti-inflammatory activity of tested compounds using acute carrageenan-induced Paw Oedema in rats

Compd. no	Mean swelling volume ± S.E.M[a] (% inhibition of oedema)				
	1 h	2 h	3 h	4 h	Potency[b]
Control	0.76 ± 0.02	0.77 ± 0.02	0.80 ± 0.04	0.89 ± 0.04	–
Indomethacine	0.57 ± 0.06 (25.3)	0.45 ± .04 (41.5)	0.43 ± 0.04(46.2)	0.42 ± 0.03 (52.8)	1.0
(IV)	0.69 ± 0.02 (9.2)	0.66 ± 0.04 (14.3)	0.64 ± 0.02 (20.0)	0.62 ± 0.04 (30.3)	0.57
(Vb)	0.65 ± 0.04 (14.4)	0.61 ± 0.03 (20.7)	0.56 ± 0.04 (30.0)	0.51 ± 0.05 (42.7)	0.81
(VIb)	0.72 ± 0.05 (5.3)	0.70 ± 0.03 (9.3)	0.69 ± 0.04 (13.7)	0.68 ± 0.04 (24)	0.45
(VIIIb)	0.68 ± 0.03 (10.5)	0.55 ± 0.04 (28.5)	0.46 ± 0.07 (42.5)	0.44 ± 0.03 (50.6)	0.96
(XI)	0.73 ± 0.02 (3.9)	0.68 ± 0.04 (11.6)	0.66 ± 0.04 (17.5)	0.65 ± 0.03 (26.9)	0.51

[a]S.E.M. = Standard error mean and all showed at least significant difference at $p < 0.05$ in comparison with control group.

[b]Potency was expressed as percentage oedema inhibition of the tested compounds relative to percentage oedema inhibition of indomethacin "reference standard" at 4 h effect.

400 MHz, ^{13}C: 100 MHz) spectrometer with TMS as internal standard. Mass spectra were determined on a JEOL JMS-600 spectrometer. Elemental analyses (C, H, N, and S) were performed on an elemental analysis system GmbH Vario EL V2.3. Compound (**IV**) was prepared as reported in the literature [27] with melting point 202–204°C.

Synthesis of 5-acetyl-6-methyl-4-(1,3-diphenyl-1*H*-pyrazol-4-yl)-3,4-dihydropyrmidin-2(1*H*)-thione (IV). A solution of 1,3-diphenyl-1*H*-pyrazole-4-carbaldehyde (**I**) (1 g, 4 mmole), thiourea (**II**) (0.46 g, 6 mmole) and acetyl acetone (0.4 mL, 4 mmole) and in ethanol (20 mL) was refluxed in the presence of calcium chloride (0.168 g, 20 mol %) for 2 h (TLC). The reaction mixture was poured onto crushed ice and the solid product was separated by filtering and recrystallizing from ethanol to afford the desired product (**IV**) as buff crystals in 91% yield; mp 205–207°C. IR: 1240 (C=S), 1704 (C=O), 3242, 3424 (NH); ^{1}H NMR: 2.15 (3, 3H, CH_3), 2.25 (s, 3H, $COCH_3$), 5.55 (s, 1H, pyrimdine-H4), 7.32–8.04 (m, 10H, Ar–H), 8.55 (s, 1H, pyrazole-H), 8.78 (s, 1H, NH, D_2O exchangeable)), 9.49 (s, 1H, NH, D_2O exchangeable). ^{13}C NMR: 16.78 (CH_3), 26.45 ($CO\underline{CH_3}$), 46.22 (pyrimdine-C4), 119.79, 124.93, 126.78, 127.48, 128.42, 128.66, 129.06, 129.39, 129.95, 130.11, 139.27, 139.75, 141.46, 144.28, 148.11, 150.82, 155.66 (Ph, C3-C5-pyrazole, C5, C6-pyrimidine,), 174.37 (C=O), 197.44 (C=S); EI-MS, *m/z* (%): 388.42 (M^+, 93.15%). Anal. calcd. for $C_{22}H_{20}N_4OS$: C, 68.02; H, 5.19; N, 14.42; S, 8.25%. Found: C, 68.36; H, 5.22; N, 14.19; S, 8.38%.

General procedure for the synthesis of 1-(1,2,3,4-tetrahydro-6-methyl-4-(1,3-diphenyl-1*H*-pyrazol-4-yl)-2-thioxopyrimidin-5-yl)-3-substituted phenylprop-2-en-1-one (Va–c). A mixture of acetyl derivative (**IV**) (6 mmol) and aromatic aldehydes (6 mmol) was dissolved in ethanol (30 mL), then NaOH 25% solution (6 mL) was added drop wise, while the temperature was kept under 10°C, the reaction mixture was stirred under this condition for 1 hour, then the stirring was continued at room temperature for 8 hours. Thereafter the reaction mixture was poured into ice-water; the precipitated solid was filtered off, and recrystallized from aqueous ethanol to give the target products (**Va–c**).

1-(1,2,3,4-Tetrahydro-6-methyl-4-(1,3-diphenyl-1*H*-pyrazol-4-yl)-2-thioxopyrimidin-5-yl)-3-phenylprop-2-en-1-one (Va). Yellow crystals; yield: 86%, mp 135–137°C; IR: 1660 (CO), 3255, 3480 (br, NH); ^{1}H NMR: 2.33 (s, 3H, CH_3), 5.50 (s, 1H, CH-pyrimdine), 6.68 (s, 1H, CO–CH=), 7.45–8.35 (m, 16H, Ar–H, ph-CH=), 8.48 (s, 1H, pyrazole-H), 9.56 (s, 1H, NH, D_2O exchangeable), 10.23 (s, 1H, NH, D_2O exchangeable). EI-MS, *m/z* (%): 476.15 (M^+, 56%). Anal. calcd. for $C_{29}H_{24}N_4OS$ (476.59): C, 73.08; H, 5.08; N, 11.76; S, 6.73%. Found: C, 73.33; H, 5.22; N, 11.82; S, 6.71%.

3-(4-Chlorophenyl)-1-(1,2,3,4-tetrahydro-6-methyl-4-(1,3-diphenyl-1*H*-pyrazol-4-yl)-2-thioxopyrimidin-5-yl)prop-2-en-1-one (Vb). Yellow crystals; yield: 64%, mp 223–225°C; IR: 1655 (CO), 3270, 3420 (NH); ^{1}H NMR: 2.35 (s, 3H, CH_3), 5.47 (s, 1H, CH-pyrimdine), 6.75 (s, 1H, CO–CH=), 7.25 (d, J = 7.80 Hz, 2H, C-2 and C6, Ar–H, *p*-Cl), 7.44 (d, J = 7.70 Hz, 2H, C-3 and C5, Ar–H, *p*-Cl), 7.47–8.40 (m, 11H, Ar–H, ph–CH=), 8.55 (s, 1H, pyrazole-H), 9.75 (s, 1H, NH, D_2O exchangeable), 10.26 (s, 1H, NH, D_2O exchangeable). Anal. calcd. for $C_{29}H_{23}ClN_4OS$ (511.04): C, 68.16; H, 4.54; Cl, 6.94; N, 10.96; S, 6.27%. Found: C, 68.27; H, 4.48; Cl, 7.02; N, 10.79; S, 6.31%.

1-(1,2,3,4-Tetrahydro-6-methyl-4-(1,3-diphenyl-1*H*-pyrazol-4-yl)-2-thioxopyrimidin-5-yl)-3-(4-methoxyphenyl)prop-2-en-1-one (Vc). Yellow crystals; yield: 77%, mp 235–237°C; IR: 1674 (CO), 3180, 3460 (NH); ^{1}H NMR: 2.28 (s, 3H, CH_3), 3.70 (s, 3H, OCH_3), 5.51 (s, 1H, CH-pyrimdine), 6.61 (s, 1H, CO–CH=), 7.15–8.28 (m, 15H, Ar–H, ph-CH=), 8.48 (s, 1H, pyrazole-H), 8.95 (s, 1H, NH), 9.37 (s, 1H, NH). ^{13}C NMR: 15.66 (CH_3), 44.29 (pyrimdine-

C4), 53.40 (OCH_3), 116.11, 119.50, 122.11, 122.39, 124.50, 124.98, 128.16, 128.47, 129.17, 129.48, 132.18, 134.11, 135.67, 136.22, 138.45, 138.72, 139,70, 140.24, 140.66, 141.15, 143.19, 147.76, 150.45, 150.82, 155.66 (Ph, vinylic CH=CH, C3–C5-pyrazole, C5, C6-pyrimidine), 178.37 (C=S), 199.71 (C=O); EI-MS, *m/z* (%): 506.76 (M^+, 46.71%). Anal. calcd. for $C_{30}H_{26}N_4O_2S$ (506.62): C, 71.12; H, 5.17; N, 11.06; S, 6.33%. Found: C, 71.31; H, 5.13; N, 10.98; S, 6.16%.

General procedure for the synthesis of 5-(5-(4-chlorophenyl)-4,5-dihydro-1*H*(phenyl)-pyrazol-3-yl)-3,4-dihydro-6-methyl-4-(1,3-diphenyl-1*H*-pyrazol-4-yl)-pyrimidine-2(1*H*)-thione (VIa, b). A mixture of chalcone (**Vb**) (0.001 mol), hydrazine hydrate or phenyl hydrazine (0.001 mol) was heated under reflux for 4 h, in 20 mL ethanol then cooled and the residual material was filtered off and recrystallized from ethanol.

5-(5-(4-Chlorophenyl)-4,5-dihydro-1*H*-pyrazol-3-yl)-3,4-dihydro-6-methyl-4-(1,3-diphenyl-1*H*-pyrazol-4-yl)pyrimidine-2(1*H*)-thione (VIa). Yellow crystals; yield: 65%, mp 188–190°C; IR: 3189, 3222, 3478 (NH); ^{1}H NMR: 2.30 (s, 3H, CH_3), 3.12 (dd, 1H, CH_2-pyrazoline), 4.15 (dd, 1H, CH_2-pyrazoline), 4.85 (dd, 1H, CH-pyrazoline), 5.25 (s, 1H, CH-pyrimdine), 7.20 (d, J = 8.0 Hz, 2H, C-2 and C6, Ar–H), 7.48 (d, J = 8.50 Hz, 2H, C-3 and C5, Ar–H), 7.51–8.31 (m, 10H, Ar–H), 8.53 (s, 1H, pyrazole-H), 8.60 (s, 1H, NH, D_2O exchangeable), 9.09 (s, 1H, NH, D_2O exchangeable), 11.12 (s, 1H, NH, D_2O exchangeable). Anal. calcd. for $C_{29}H_{25}ClN_6S$ (525.07): C, 66.34; H, 4.80; Cl, 6.75; N, 16.01; S, 6.11%. Found: C, 66.60; H, 4.93; Cl, 6.63; N, 16.15; S, 6.37%.

5-(5-(4-Chlorophenyl)-4,5-dihydro-1-phenyl-pyrazol-3-yl)-3,4-dihydro-6-methyl-4-(1,3-diphenyl-1*H*-pyrazol-4-yl)pyrimidine-2(1*H*)-thione (VIb). Yellow crystals; yield: 71%, mp 212–214°C; IR: 3189, 3222, 3478 (NH); ^{1}H NMR: 2.27 (s, 3H, CH_3), 3.23 (dd, 1H, CH_2-pyrazoline), 4.15 (dd, 1H, CH_2-pyrazoline), 5.27 (dd, 1H, CH-pyrazoline), 5.54 (s, 1H, CH-pyrimdine), 7.20 (d, J = 8.04 Hz, 2H, C-2 and C6, Ar–H), 7.39 (d, J = 8.80 *Hz*, 2H, C-3 and C5, Ar–H), 8.48 (m, 15H, Ar–H), 8.68 (s, 1H, pyrazole-H), 9.11 (s, 1H, NH), 10.70 (s, 1H, NH) ; EI-MS, *m/z* (%): 601.06 (M^+, 70.23%); Anal. calcd. for $C_{35}H_{29}ClN_6S$ (601.16): C, 69.93; H, 4.86; Cl, 5.90; N, 13.98; S, 5.33%. Found: C, 67.11; H, 4.91; Cl, 5.84; N, 14.27; S, 6.35%.

Synthesis of 5-(5-(4-chlorophenyl)-4,5-dihydroisoxazol-3-yl)-3,4-dihydro-6-methyl-4-(1,3-diphenyl-1*H*-pyrazol-4-yl)pyrimidine-2(1*H*)-thione (VII). A mixture of chalcone (**Vb**) (0.51 g, 0.001 mol), hydroxylamine hydrochloride (0.07 g, 0.001 mol) and anhydrous sodium acetate (1 g) was refluxed in ethanol (20 mL) for 7 h, then allowed to cool and poured into cold water. The precipitated product thus formed was collected by filtration, dried and recrystallized from methanol to give compound (**VII**) as white crystals; yield: 59%, mp 248–250°C; IR: 3277, 3455 (NH), 1579 (C=N), 860 (N–O): ^{1}H NMR: 2.31(s, 3H, CH_3), 3.36 (dd, 1H, CH_2-isoxazole), 3.78 (dd, 1H, CH_2-isoxazole), 4.95 (dd, 1H, CH-isoxazole), 5.70 (s, 1H, CH-pyrimdine), 7.24 (d, J = 7.70 Hz, 2H, C-2 and C6, Ar–H, *p*-Cl), 7.41 (d, J = 8.04 Hz, 2H, C-3 and C5, Ar–H, *p*-Cl), 8.40 (m, 10H, Ar–H), 8.57 (s, 1H, pyrazole-H), 8.70 (s, 1H, NH), 9.85 (s, 1H, NH); Anal. calcd. for $C_{29}H_{24}ClN_5OS$ (526.05): C, 66.21; H, 4.60; Cl, 6.74; N, 13.31; S, 6.10%. Found: C, 66.36; H, 4.75; Cl, 6.77; N, 13.30; S, 6.18%.

General procedure for the synthesis of 6-(4-chlorophenyl)-4-(1,2,3,4-tetrahydro-6-methyl-4-(1,3-diphenyl-1*H*-pyrazol-4-yl)-2-thioxopyrimidin-5-yl)pyrimidin-2(1*H*)-one(thione) (VIIIa, b). A mixture of chalcone (**Vb**) (0.02 mol), urea or thiourea (0.02 mol) were dissolved in KOH (5%, 10 mL) was stirred about 2–3 hours with a magnetic stirrer. This was then poured into 100 mL of cold water with continuous stirring for an hour and then kept in refrigerator for 24 hours. The precipitate obtained was filtered, washed and recrystallized ethanol.

6-(4-Chlorophenyl)-4-(1,2,3,4-tetrahydro-6-methyl-4-(1,3-diphenyl-1*H*-pyrazol-4-yl)-2-thioxopyrimidin-5-yl)pyrimidin-2(1*H*)-one (VIIIa). Pale yellow crystals; yield: 62%, mp 238–240°C; IR: 3270, 3298, 3415 (NH), 1686 (C=O) ; ^{1}H NMR: 2.35 (s, 3H, CH_3), 5.68 (s, 1H, CH-pyrimdine), 7.25 (d, J = 7.65 Hz, 2H, C-2 and C6, Ar–H, *p*-Cl), 7.38 (d, J = 8.20 Hz, 2H, C-3 and C5, Ar–H, *p*-Cl), 8.54 (m, 11H, Ar–H), 8.65 (s, 1H, pyrazole-H), 9.15 (s, 1H, NH), 9.66 (s, 1H, NH), 13.15 (s, 1H, NH); Anal. calcd. for $C_{30}H_{23}ClN_6OS$ (551.06): C, 65.39; H, 4.21; Cl, 6.43; N, 15.25; S, 5.82%. Found: C, 65.63; H, 4.19; Cl, 6.52; N, 15.48; S, 5.81%.

6-(4-Chlorophenyl)-4-(1,2,3,4-tetrahydro-6-methyl-4-(1,3-diphenyl-1*H*-pyrazol-4-yl)-2-thioxopyrimidin-5-yl)pyrimidin-2(1*H*)-thione (VIIIb). Pale brown crystals; yield: 69%, mp 282–284°C; IR: 3290, 3310, 3439 (NH), 1249 (C=S) ; ^{1}H NMR: 2.30 (s, 3H, CH_3), 5.55(s, 1H, CH-pyrimdine), 7.27 (d, J = 8.0 Hz, 2H, C-2 and C6, Ar–H, *p*-Cl), 7.40 (d, J = 8.20 Hz, 2H, C-3 and C5, Ar–H, *p*-Cl), 8.59 (m, 11H, Ar–H), 8.72 (s, 1H, pyrazole-H), 10.20 (s, 1H, NH), 11.17 (s, 1H, NH), 11.27 (s, 1H, NH); EI-MS, *m/z* (%): 567.18 (M^+, 38.44%); Anal. calcd. for $C_{30}H_{23}ClN_6S_2$ (567.13): C, 63.53; H, 4.09; Cl, 6.25; N, 14.82; S, 11.31%. Found: C, 63.72; H, 4.15; Cl, 6.29; N, 14.91; S, 11.27%.

Synthesis of 5-(4-chlorophenyl)-4,5-dihydro-3-(1,2,3,4-tetrahydro-6-methyl-4-(1,3-diphenyl-1*H*-pyrazol-4-yl)-2-thioxopyrimidin-5-yl)pyrazole-1-carbothioamide (IX). A mixture chalcone (**Vb**) (0.51 g, 0.001 mol) and thiosemicarbazide (0.18 g, 0.001 mol) in and ethanolic sodium hydroxide (25%, 10 mL) was refluxed for 3 h. The reaction mixture was allowed to cool to room temperature, whereby a solid formed that was filtered off and crystallized from dioxane to give

compound (**IX**) as yellow crystals, yield: 70%, mp 217–219°C; IR: 3177, 3250, 3425 (NH), 1580 (C=N), 1370 (C=S): ^{1}H NMR: 2.36 (s, 3H, CH_3), 3.46 (dd, 1H, CH_2-pyrazoline), 4.00 (dd, 1H, CH_2-pyrazoline), 5.28 (dd, 1H, CH-pyrazoline), 5.65 (s, 1H, CH-pyrimdine), 6.72 (s, 1H, NH_2), 7.20 (d, J = 9.20 Hz, 2H, C-2 and C6, Ar–H, *p*-Cl), 7.43 (d, J = 8.90 Hz, 2H, C-3 and C5, Ar–H, *p*-Cl), 8.50 (m, 10H, Ar–H), 8.61 (s, 1H, pyrazole-H), 9.15 (s, 1H, NH), 10.48 (s, 1H, NH); Anal. calcd. for $C_{30}H_{26}ClN_7S_2$ (584.16): C, 61.68; H, 4.49; Cl, 6.07; N, 16.78; S, 10.98%. Found: C, 61.77; H, 4.62; Cl, 6.29; N, 16.84; S, 11.12%.

Synthesis of 4,5,7,7a-tetrahydro-3,7a-dimethyl-4-(1,3-diphenyl-1*H*-pyrazol-4-yl) isoxazolo [5,4-*d*]pyrimidine-6(3a*H*)-thione (X). A mixture of acetyl derivative (**IV**) (0.77 g, 2 mmol), hydroxyl amine hydrochloride (0.14 g, 2 mmol) and fused sodium acetate (1 g) was heated under reflux in ethanol (30 mL), The reaction mixture was allowed to cool to room temperature, thereafter the reaction mixture was poured into ice-water, acidified with dilute HCl. the precipitated solid was filtered off, and recrystallized from aqueous ethanol to give compound (**X**) as yellow crystals, yield: 62%, mp 252–254°C; IR: 3270, 3440 (NH), 1560 (C=N), 1285 (C=S): ^{1}H NMR: 2.33 (s, 3H, CH_3), 2.41 (s, 3H, CH_3), 3.80 (s, 1H, C5-H)), 5.50 (s, 1H, CH-pyrimdine), 7.25–8.41 (m, 10H, Ar–H), 8.65 (s, 1H, pyrazole-H), 9.22 (s, 1H, NH), 11.30 (s, 1H, NH); ^{13}C NMR: 15.44 (CH_3), 18.33 (CH_3), 44.45 (pyrimdine-C4), 71.45 (pyrimidine-C5), 76.11 (pyrimidine-C6), 116.44, 118.34, 123.27, 124.84, 127.11, 128.67, 133.22, 136.01, 139.60, 145.30, 148.19, 150.55, 151.44, 154.07, 155.16, 176.73 (C=S), 199.46 (C=O); Anal. calcd. for $C_{22}H_{21}N_5OS$ (403.51): C, 65.49; H, 5.25; N, 17.36; S, 7.95%. Found: C, 65.41; H, 5.38; N, 17.60; S, 8.02%.

Synthesis of 4,5,7,7a-tetrahydro-3,7a-dimethyl-4-(1,3-diphenyl-1*H*-pyrazol-4-yl)-1*H*-pyrazolo[3,4-*d*]-pyrimidine-6(3a*H*)-thione (XI). A mixture of acetyl derivative (**IV**) (0.77 g, 2 mmol) and hydrazine hydrate (0.14 g, 2 mmol) in dioxane (20 mL) was heated under reflux. The reaction mixture was allowed to cool to room temperature, thereafter the reaction mixture was poured into ice-water, acidified with dilute HCl. the precipitated solid was filtered off, and recrystallized from aqueous ethanol to give compound (**XI**) as pale yellow crystals, yield: 60%, mp 189–191°C; IR: 3317, 3260, 3415 (3 NH), 1575 (C=N), 1291 (C=S): ^{1}H NMR: 2.37 (s, 3H, CH_3), 2.46 (s, 3H, CH_3), 4.25 (s, 1H, C5-H), 7.23–8.37 (m, 10H, Ar–H), 5.36 (s, 1H, CH-pyrimdine), 8.55 (s, 1H, pyrazole-H), 9..15 (s, 1H, NH), 10.22 (s, 1H, NH), 13.20 (s, 1H, NH); Anal. calcd. for $C_{22}H_{22}N_6S$ (402.52): C, 65.65; H, 5.51; N, 20.88; S, 7.97%. Found: C, 65.72; H, 5.49; N, 20.93; S, 8.17%.

Synthesis of 1-(1-(1,2,3,4-tetrahydro-6-methyl-4-(1,3-diphenyl-1*H*-pyrazol-4-yl)-2-thioxopyrimidin-5-yl)ethylidene)thiosemicarbazide (XII). To a solution of acetyl derivative (**IV**) (0.77 g, 2 mmol) in acetic acid (25 mL), thiosemicarbazide (0.18 g, 2 mmol) was added. The reaction mixture was heated under reflux for 2 h, and then poured onto ice/cold water. The precipitated solid was filtered off, and recrystallized from aqueous ethanol to give compound (**XII**) as pale yellow crystals, yield: 71%, mp 173–175°C; IR: 3450, 3375, 3278 (NH, NH_2), 1385 (C=S): ^{1}H NMR: 2.26 (s, 3H, CH_3), 2.31 (s, 3H, CH_3), 5.42 (s, 1H, CH-pyrimdine), 7.19–8.40 (m, 10H, Ar–H), 8.48 (s, 1H, pyrazole-H), 8.75 (s, 1H, NH), 9.11 (s, 2H, NH_2), 9.65 (s, 1H, NH), 11.15 (s, 1H, NH); Anal. calcd. for $C_{23}H_{23}N_7S_2$ (461.61): C, 59.84; H, 5.02; N, 21.24; S, 13.89%. Found: C, 60.06; H, 5.27; N, 21.39; S, 13.72%.

Synthesis of 2-{2-[1-(6-methyl-4-(1,3-diphenyl-1*H*-pyrazol-4-yl-2-thioxo-1,2,3,4-tetrahydropyrimidin-5-yl)ethylidene]hydrazinyl}-1,3-thiazol-4(5*H*)-one (XIII). A mixture of thiosemicarazide (**XII**) (0.92 g, 2 mmol), ethyl chloroacetate (0.24 g, 2 mmol) and fused sodium acetate (1 g) was heated under reflux in ethanol (30 mL) for 6 h. The reaction mixture was allowed to cool to room temperature, thereafter the reaction mixture was poured into ice-water, acidified with dilute HCl. the precipitated solid was filtered off, and recrystallized from ethanol to give compound (**XIII**) as yellow crystals, yield: 68%, mp 202–204°C; IR: 3390, 3317, 3150 (NH), 1720 (C=O), 1290 (C=S): ^{1}H NMR: 2.30 (s, 3H, CH_3), 2.41 (s, 3H, CH_3), 4.66 (s, 2H, CH_2-thiazolidine), 5.25 (s, 1H, CH-pyrimdine), 7.15–8.39 (m, 10H, Ar–H), 8.60 (s, 1H, pyrazole-H), 9.20 (s, 1H, NH), 13.56 (s, H, NH); Anal. calcd. for $C_{25}H_{23}N_7OS_2$ (501.63): C, 59.86; H, 4.62; N, 19.55; S, 12.78%. Found: C, 59.91; H, 4.88; N, 19.67; S, 12.73%.

Synthesis of 5-(4-chloro-benzylidene)-2-(*N*'-{1-[4-(1,3-diphenyl-1*H*-pyrazol-4-yl)-6-methyl-2-thioxo-1,2,3,4-tetrahydro-pyrimidin-5-yl]-ethylidene}-hydrazino)-thiazol-4-one (XIV). A mixture of (**XIII**) (0.5 g, 1 mmol), 4-chlorobenzaldehyde (0.14 g, 1 mmol) was heated under reflux in ethanol (20 mL) in the presence of drops of piperidine for 4 h. The reaction mixture was allowed to cool to room temperature, thereafter the reaction mixture was poured into ice-water. The precipitated solid was filtered off, and recrystallized from dioxane to give compound (**XIV**) as pale yellow crystals, yield: 64%, mp 266–268°C; IR: 3420, 3377(NH), 1688 (C=O), 1310 (C=S): ^{1}H NMR: 2.33 (s, 3H, CH_3), 2.46 (s, 3H, CH_3), 5.29 (s, 1H, CH-pyrimdine), 7.45 (d, J = 7.0 Hz, 2H, C-2 and C6, Ar–H, *p*-Cl), 7.63(d, J = 8.20 Hz, 2H, C-3 and C5, Ar–H, *p*-Cl), 8.39 (m, 11H, Ar–H, CH-arylidene), 8.59 (s, 1H, pyrazole-H), 9.38 (s, 1H, NH), 11.56 (s, H, NH); EI-MS, m/z (%): 624.29 (M^+, 68.30%) Anal. calcd. for $C_{32}H_{26}ClN_7OS_2$ (624.18): C, 61.58; H, 4.20; Cl, 5.68; N, 15.75; S, 10.27%. Found: C, 61.73; H, 4.43; Cl, 5.81; N, 16.09; S, 10.19%.

Synthesis of 4-(1,3-diphenyl-1*H*-pyrazol-4-yl)-6-methyl-5-{1-[(4-methyl(phenyl)-thiazol-2-yl)-hydrazono]-ethyl}-3,4-dihydro-1*H*-pyrimidine-2-thione (XVa, b). A mixture of thiosemicarbazone (**XII**) (2 mmol), chloroacetone or phenacyl bromide (2 mmol) was refluxed in the presence of anhydrous sodium acetate (0.9 g) in ethanol (30 mL) for 4 h. The solid product separated from the hot mixture was filtered off, washed with water and recrystallized from the proper solvent.

4-(1,3-Diphenyl-1*H*-pyrazol-4-yl)-6-methyl-5-{1-[(4-methyl-thiazol-2-yl)-hydrazono]-ethyl}-3,4-dihydro-1*H*-pyrimidine-2-thione (XVa). Yellow crystals; yield: 82%, mp 261–263°C; IR: 3373, 3345, 3219 (NH), 1260 (C=S) ; ^{1}H NMR: 2.27 (s, 3H, CH_3), 2.35 (s, 3H, CH_3), 2.43 (s, 3H, CH_3), 5.48(s, 1H, CH-pyrimdine), 6.15 (s, 1H, thiazole-H5), 7.17–8.15 (m, 15H, Ar–H), 8.56 (s, 1H, pyrazole-H), 9.65 (s, 1H, NH), 10.33 (s, 1H, NH), 12.09 (s, 1H, NH); Anal. calcd. for $C_{26}H_{25}N_7S_2$ (499.65): C, 62.50; H, 5.04; N, 19.62; S, 12.83%. Found: C, 62.81; H, 4.96; N, 19.84; S, 12.87%.

4-(1,3-diphenyl-1*H*-pyrazol-4-yl)-6-methyl-5-{1-[(4-phenyl-thiazol-2-yl)-hydrazono]-ethyl}-3,4-dihydro-1*H*-pyrimidine-2-thione (XVb). Yellow crystals; yield: 72%, mp 277–279°C; IR: 3398, 3310 (NH), 1275 (C=S) ; ^{1}H NMR: 2.38 (s, 3H, CH_3), 2.49 (s, 3H, CH_3), 5.28 (s, 1H, CH-pyrimdine), 6.27 (s, 1H, thiazole-H5), 6.95–8.40 (m, 15H, Ar–H), 8.65 (s, 1H, pyrazole-H), 9.36 (s, 1H, NH), 9.82 (s, 1H, NH), 11.15 (s, 1H, NH);); EI-MS, *m/z* (%): 561.37 (M^+, 48.0%); Anal. calcd. for $C_{31}H_{27}N_7S_2$ (561.72): C, 66.28; H, 4.48; N, 17.45; S, 11.42%. Found: C, 66.41; H, 4.77; N, 17.64; S, 11.69%.

Antimicrobial Activity

The antimicrobial activity of the synthesized compounds was screened against bacterial strains *Staphylococcus aureus* (AUMC B.54), *Bacillus cereus* (AUMC B.52) as gram-positive bacteria and *Escherichia coli* (AUMC B.53), *Pseudomonas aeruginosa* (AUMC B.73) as gram-negative bacteria and fungal strains *Candida albicans* (AUMC no. 214), *Aspergillus flavus* (AUMC no. 1276) using the agar well-diffusion method. all microbial strains were kindly provided by the Assiut University Mycological Centre (AUMC). To prepare inocula for bioassay, bacterial strains were individually cultured for 48 h in 100 mL conical flasks containing 30 mL nutrient broth medium. Fungi were grown for 7 days in 100 mL conicals containing 30 mL Sabouraud's dextrose broth. Bioassay was done in 10 cm sterile plastic Petri plates in which microbial suspension (1 mL/plate) and 15 mL appropriate agar medium (15 mL/plate) were poured. Nutrient agar and Sabouraud's dextrose agar were respectively used for bacteria and fungi. After solidification of the media, 5 mm diameter cavities were cut in the solidified agar (4 cavities/plate) using sterile cork borer. Chemical compounds dissolved in DMSO at 2% w/v (=20 mg/mL) were pipetted in the cavities. The screening tests were carried out in triplicate and the results were expressed as a mean of three determinations. Chloramphenicol and Clotrimazole were used as standards. Data are represented as % inhibition with reference to standards in (Table1).

Anti-Inflammatory Activity Screening

The anti-inflammatory activity screening for the prepared compounds (**IV**), (**Vb**), (**VIb**), (**VIIIb**) and (**XI**) was performmed in vivo by the acute carrageenan-induced paw oedema standard method in comparison to indomethacin as reference drug [26]. The test is based on the pedal inflammation in rat paws induced by sub-plantar injection of 0.2 mL carrageenan (0.2%) suspension (5% NaCMC) into the right hind paw of the rats. Male adult albino rats (120–150 g) were grouped, each of five animals. The thickness of rat paw was measured by a Veriner caliper (SMIEC, China) before and after 1 h of carrageenan injection to detect the inflammation induced by carrageenan. Test compounds at doses of 10 mg/kg (body weight) were injected i.p. to seven groups of rats. Control group received the vehicle (5% NaCMC); reference group received indomethacin at 10 mg/kg (body weight). The difference between the thicknesses of the two paws was taken as a measure of oedema. The measurement was carried out at 1, 2, 3 and 4 h, after injection of the tested compounds, the reference drug, and the vehicle. The anti-inflammatory activity was expressed as percentage inhibition of oedema volume in treated animals in comparison with the control group according to the equation below.

$$\%\ \text{Inhibition} = (\text{Vc} - \text{Vt})/\text{Vc} \times 100.$$

Where Vt is the mean increase in paw volume in rats treated with test compounds and Vc is the mean increase in paw volume in control group of rats.

Potency of the tested compounds was calculated relative to indomethacin ''reference standard'' treated group according to the following equation:

Potency = Percentage edema inhibition of tested compound treated group/Percentage edema inhibition of indomethacin treated group (Table 2).

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COMPLIANCE WITH ETHICAL STANDARDS

This article does not contain any studies involving human participants performed by any of the authors and

does not contain any studies involving animals performed by any of the author.

Conflict of Interests

The authors report no conflicts of interest.

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