European Journal of Medicinal Chemistry 45 (2010) 2994–3004

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Original article

Synthesis and biological evaluation of some thio containing pyrrolo [2,3-d] Pyrimidine derivatives for their anti-inflammatory and anti-microbial activities

Mosaad S. Mohamed [a,*], Rehab Kamel [b], Samar S. Fatahala [a]

[a] Pharmaceutical Organic Chemistry Department, Faculty of Pharmacy, Helwan University, Helwan, Egypt
[b] Toxicology and Pharmacology Department, Faculty of Pharmacy, Helwan University, Helwan, Egypt

ARTICLE INFO

Article history:
Received 28 January 2010
Received in revised form
15 March 2010
Accepted 17 March 2010
Available online 25 March 2010

Keywords:
Pyrrolo [2,3-*d*]pyrimidine
Anti-inflammatory activity
Anti-microbial activity

ABSTRACT

The pyrroles **Ia–c** were used as precursor for the preparation of pyrrolo [2, 3-*d*] pyrimidine-2 and/or 4 thione derivatives **IIa–f**. A series of 8-Aryl-pyrrolo [2, 3-*d*] thiazolo[3,2-a]pyrimidine **VI and VII** were prepared. Alkylation of the thione compounds in basic medium afforded the pyrrolo [2, 3-*d*] pyrimidine **IV**. Also, some 2-amino pyrrolo [2, 3-*d*]pyrimidines **V** were obtained. Some newly synthesized compounds were examined for their in vitro anti-inflammatory. Also, all new compounds were examined for their in vivo anti-microbial activity. Several derivatives, showed a promising anti-inflammatory activity in compared to ibuprofen. In this paper, we examine and discuss the structure–activity relationships and anti-inflammatory activities of these compounds.

1. Introduction

The key roles purines and pyrimidines play in cellular processes have made them valuable leads for drug discovery. One important class of pyrimidine is 2-thiopyrimidine (2-TP) and its derivatives, which are also well known as 2-mercaptopyrimidine compounds [1]. In 2-TP ring sulfur atom serves as an interesting replacement for the existing oxygen atom bonded to C-2 in uridine base [2]. Considering this assumption 2-TPs have attracted substantial interest of synthetic-biochemists [3]. The European patent [4] revealed the application of 2-TP derivatives in preparation of cardiotonic drugs. Studies by Pathak et al. evaluated primary activity of 2-TP derivatives against Mycobacterium tuberculosis (Mtb) [5].

6-Thiopurine (6 TP) [6] is a thio analog of hypoxanthine the natural product of purine metabolism (Fig. 3).

Since the discovery of this antimetabolite more than half a century ago, several thousand 6 TP derivatives have been synthesized and characterized in biological experiments. Which proved to be very effective for the treatment of leukemias [7] autoimmune and rheumatic disorders, [7–10] and for immunosuppressant during organ transplanta tion [7,8]. Pyrrolo[3,2-*d*]pyrimidines, a class of 7-deazapurine analogues, exhibit for instance interesting biological activity in part due to their resemblance to pyrimidines and purines. For example Tolmetin (Rumatol®) and ketorolac (ketolac®) (Fig. 1), a well known non steroidal anti-inflammatory drugs (NSAIDs) [11] which act by inhibition of prostaglandin synthesis constitutes the primary mechanism of the anti-inflammatory action of these drugs. PNU-142731A [12] (Fig. 1) is an anti-inflammatory, pyrrolopyrimidine that inhibits the production of cytokines in vivo. Tubercidin, Toyocamycin and Sangivamycin (Fig. 3) are naturally occurring pyrrolo [2,3-*d*]pyrimidine nucleoside antibiotics [13,14]. These compounds (Fig. 2) have been observed to be active against the growth of some micro-organisms (Fig. 4).

In our previous work; we have found [15–17] that the addition of sulfur and/or ring-containing nitrogen allowed the activity to increase in the direction of anti-microbial and anti-inflammatory. Motivated by the abovementioned findings and in continuation of our investigations in this field [15–17], we aimed to synthesize novel derivatives of pyrrolopyrimidines and their thio related compounds for study of the structure activity relationships. The heterocyclic system pyrrole was selected on the basis of previous reports [15–17].

2. Results and discussion

2.1. Chemistry

The synthetic strategy to synthesize the target sulfur compounds **II, III, IV–VII** is depicted in Schemes 1–3. Compounds [15] **Ia–c** were used as key compound for this study and for further syntheses due to the presence of the enaminonitrile moiety, which is well known as a highly reactive and convenient reagent for the

* Corresponding author.
E-mail address: samarradwan1@yahoo.com (M.S. Mohamed).

0223-5234/$ – see front matter © 2010 Elsevier Masson SAS. All rights reserved.
doi:10.1016/j.ejmech.2010.03.028

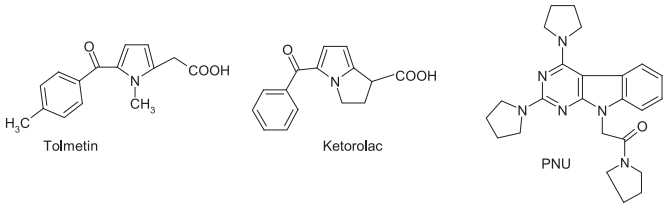


Fig. 1. Anti-inflammatory drugs (NSAIDs).

synthesis of nitrogen heterocycles [18,19]. Thus, the reaction of **Ia–c** with thiourea [20], and carbon disulfide [23,25,26] in DMF/pyridine and/or NaOH/DMSO, afforded the corresponding pyrimidine derivatives **IIa–f**. Refluxing [20,22,27] of compounds **Ia–c** with phenyl isothiocyanate, in pyridine or DMF to give compound **IIg–h** directly. Yet in stirring **Ia–c** with phenyl isothiocyanate without reflux, gave compounds **IIIa–c**, which on heated at 80 °C afforded the expected compound **IIg–h**.

Alkylation [21–25] of Pyrrolopyrimidin-2-thiones **IIa–i** with a series of α-halocarbonyl compounds using 3 different methods, gave the corresponding S-alkylated pyrimidine derivatives **IVa–l**. The reaction [25,26] of S-alkylated compounds **IVa–f** with certain aromatic amine and/or hydrazine, independently, yielded the 2-amino derivative **Va–f**.

Using the same procedures as reported [23,25,26,28–30], that heating under reflux a mixture of thio compounds **IId–f**, chloroacetic acid, aromatic aldehyde in acetic acid, and acetic anhydride compounds **VII** were obtained in a good yield. When the reaction was performed without aromatic aldehyde, the product was identified as thiazolopyrimidine derivative **VI**. Compound **VII** had been independently synthesized through the interaction of **VI** with aromatic aldehyde in the presence of a catalytic amount of piperidine or triethylamine.

2.2. Biological results and discussion

2.2.1. In vivo anti-inflammatory assay (Table 1)

The newly obtained compounds were tested for their anti-inflammatory activity using the carrageenan-induced rat paw oedema assay. The potency and duration of action was compared with those of the reference compound ibuprofen. The vivo anti-inflammatory activity is shown in Table 1.

2.2.2. Anti-microbial assay

All of the newly obtained compounds were tested in vitro against a number of bacteria (including Gram-positive and Gram-negative) and *Candida albicans* as fungi. As shown in the result all tested compound show very poor activity as anti-microbial agent. Compound **IIa** (2-thio-pyrimidine) has shown the highest activity against two types of bacteria (*Staphylococcus aureus* and *Bacillus subtilis*). Compounds **Ic** and **Va** have shown the activity against *B. subtilis* only. Only one of the tested compounds was more potent

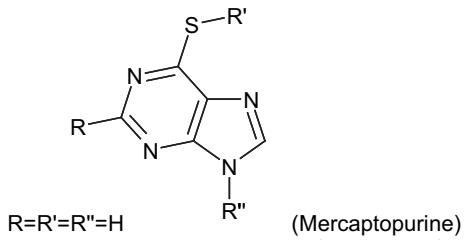


Fig. 2. 6-Thiopurine (6 TP).

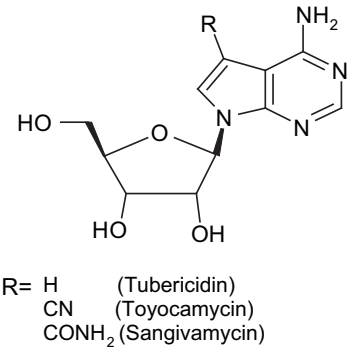


Fig. 3. Pyrrolopyrimidine nucleoside antibiotics.

than the reference drug. All the tested compounds have shown no activity against fungi. All the other tested compounds showed no anti-microbial activity. Test results of activity for compounds are summarized in Tables 2, 3.

2.2.3. Biological discussion

The potential therapeutic activity of these compounds was assessed both for their anti-inflammatory activity. Ibuprofen was used as reference compound for the in vivo assays.

The anti-inflammatory activity of these synthesized compounds in vivo was evaluated using the carrageenan-induced rat paw oedema assay. The potency and duration of action was compared with those of the reference compound ibuprofen (Table 1).

Compounds **IIb** and **Va** have a stronger anti-inflammatory effect in vivo than ibuprofen. In addition, the in vivo anti-inflammatory activity was rapidly observed (2 h and 3 h) and sustained after 4 h, whereas ibuprofen activity decreased quickly. Likewise, compounds **IIc, IIh** and **Vb**, showed a higher inhibitory action at 2 h and 3 h for the carrageenan-induced oedema than ibuprofen. Compound **Ic**, showed the same behavior as the ibuprofen (increased by time) and had same potency as it.

To analyze structure–activity relationships, three structural components were considered, the nature of the heterocycle nucleus, the position of the side chain on the heterocycle system, and the function of the side chain.

First, regarding the side chain function, thino group confers greater activity than the dithieno or alkyl thieno: while **IIb** showed % inh ≈ 95 (2 h and 3 h post-carrageenan) the dithieno **IId–f** presented % inh ≈ 3–4.8 (2 h and 3 h post-carrageenan) were completely lack of anti-inflammatory activity. Hydrazino group in **Va** showed % inh ≈ 79 (4 h post-carrageenan) and in **Vb** showed % inh ≈ 95 (3 h post-carrageenan), over the thiazolo ring in **VII** which was completely in active.

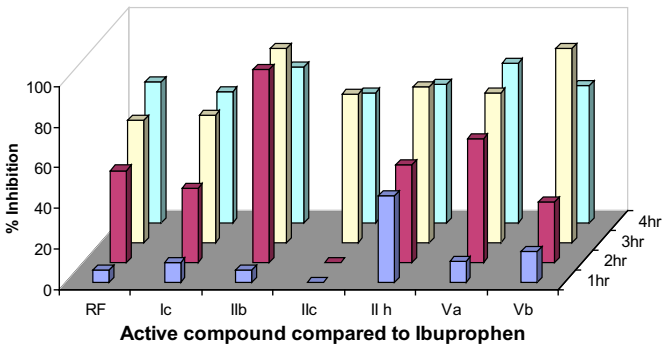
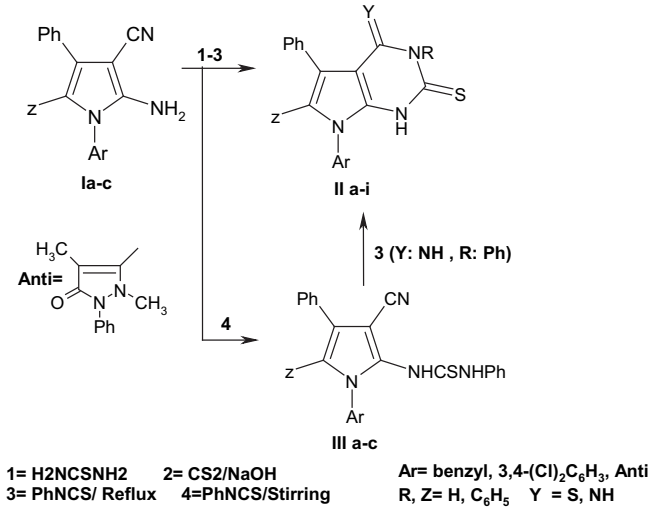
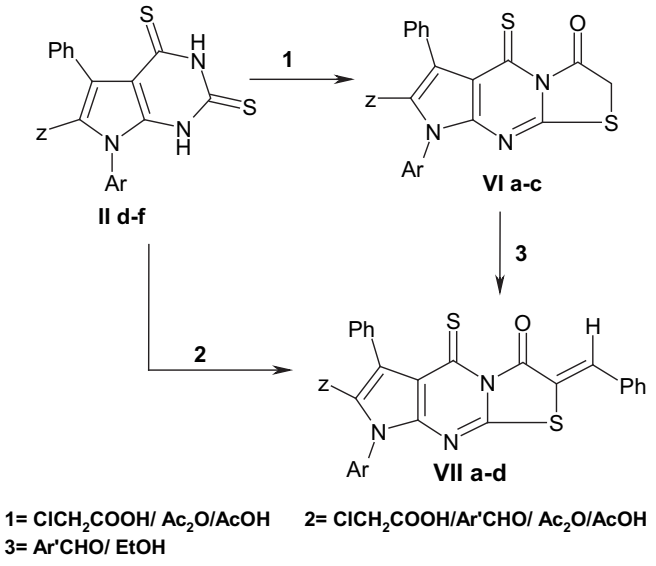


Fig. 4. Anti-inflammatory effect of tested compound, show that **IIb, IIh, Va,** and **Vb** have highest activity (more potent than the reference drug (RF)).



Scheme 1.



Scheme 3.

The influence of the nature of the aromatic heterocyclic system easily observed as pyrrolopyrimidine (**IIb, IIc, IIh, Va, Vb**) condensed ring showed the highest activity over open pyrrole **Ia**–**c** and thiazolo fused ring **VI**–**VII**. Also the 3, 4-dichlorophenyl (**IIb, IIh Vb**) showed highest activity than benzyl (**IIa, Va**) and antipyrine in **IIc**. Where antipyrine **Ic** increase the activity over the other 2 aromatic ring in open pyrrole form as shown in **Ia, Ib**.

The introduction of thino group at position 2 rather than 2 and 4 increase the activity (**IIb** over **IId**–**f**). Also N^3-phenyl in compound **IIh** showed the activity (% inh ≈ 42 (1 h post-carrageenan)) from first ably over all tested compounds.

2.2.4. Conclusion

We have synthesized and evaluated a series of heterocyclic compounds as potential anti-inflammatory agents. Based on their structure, we conclude that the best aromatic nucleus is the pyrrole with an 3,4-dichlorophenyl ring substituent and thino or hydrazine subunit on the C-2 such as in compound **IIb** and **Va,b**. From the biological tests, this group of pyrrole was a promising as anti-inflammatory agent while can say that they deprived from their anti-microbial activity.

3. Experimental

3.1. General methods

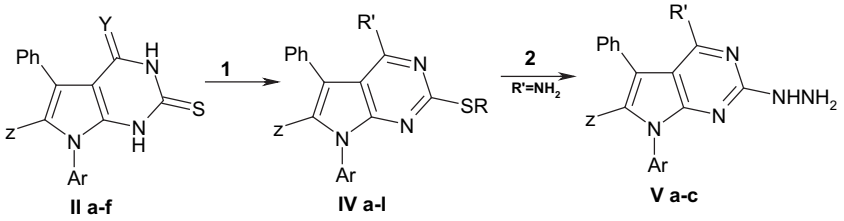
All melting points are uncorrected and measured using Electrothermal IA 9100 apparatus (Shimadzu, Japan). IR spectra were recorded as potassium bromide pellets on a Perkin–Elmer 1650 spectrophotometer (USA), Faculty of Science, Cairo University, Cairo, Egypt. ^{1}H NMR spectra were determined on a Varian Mercury (300 MHz) spectrometer (Varian UK) and chemical shifts were expressed as ppm against TMS as internal reference (Faculty of Science, Cairo University, Cairo, Egypt). Mass spectra were recorded on 70 eV EI Ms-QP 1000 EX (Shimadzu, Japan), Faculty of Science, Cairo University, Cairo, Egypt. Microanalyses were operated using Vario, Elmentar apparatus (Shimadzu, Japan), Organic Microanalysis Unit, Faculty of Science, Cairo University, Cairo, Egypt and the results were within the accepted range (±0.40) of the calculated values. Column Chromatography was performed on (Merck) Silica gel 60 (particle size 0.06–0.20 mm).

3.2. 4-Amino-7-(Aryl)-5,6-disubstitututed-1H-pyrrolo[2,3-d] pyrim-idine-2(7H)-thione (IIa–c)

A mixture of amino cyano **I** (0.01 mol) and thiourea (0.02 mol) was refluxed in dry ethanol (20 mL) for 6 h. Then evaporated under reduced pressure and the residues were recrystallized from methanol to give **IIa**–**c**.

3.2.1. 4-Amino-7-benzyl-5, 6-diphenyl-1H-pyrrolo [2, 3-d] pyrimidine-2(7H)-thione (Ia)

Yield 76%, m.p. 188–190 °C. IR (KBr) υ (cm^{-1}): 3430, 3330 (NH_2), 1620 (C=S), absence of (C≡N). *m/z*: 408 (M^+, 30.0%), 409 ($M^+ + 1$,



Scheme 2.

9.3%), 410 ($M^+ + 2$, 1.2%). ^{1}H NMR (DMSO, 500 MHz) δ (ppm): 5.22 (s, 2H, CH_2), 6.0 (br.s, 2H, NH_2, D_2O exchangeable), 7.0–7.8 (m, 15H, Ar-H), 9.20 (s, 1H, NH, D_2O exchangeable). Anal. Calcd for $C_{25}H_{20}N_4S$ (408.52): C, 73.50; H, 4.93; N, 13.71; S, 7.85. Found: C, 73.86; H, 4.99; N, 13.98; S, 8.05

3.2.2. 4-Amino-7-(3,4-dichlorophenyl)-5-phenyl-1H-pyrrolo[2,3-d] pyram-idine-2(7H)-thione (IIb)

Yield 74%, m.p. 280–282 °C. IR (KBr) υ (cm^{-1}): 3420, 3350 (NH_2), 1610 (C=S), absence of (C≡N). *m/z*: 386 (M^+, ^{35}Cl, 38.2%), 387 ($M^+ + 1$, 20.4%), 388 ($M^+ + 2$, ^{37}Cl, 12.8%), 390 ($M^+ + 4$, $2 \times (^{37}Cl)$,4.28%). ^{1}H NMR (DMSO, 500 MHz) δ (ppm): 6.1 (br.s, 2H, NH_2, D_2O exchangeable), 7.0–7.8 (m, 8H, Ar-H), 8.1 (s, 1H, C_6–H), 9.20 (s, 1H, NH, D_2O exchangeable). Anal. Calcd for $C_{18}H_{12}Cl_2N_4S$ (386.29): C, 55.82; H, 3.12; Cl, 18.31; N, 14.47; S, 8.28. Found: C, 56.03; H, 3.42; Cl, 18.62; N, 14.73; S, 8.42.

3.2.3. 5-(4-Amino-5,6-diphenyl-2-thioxo-1H-pyrrolo[2,3-d] pyrimidin-7(2H)-yl)-1,4-dimethyl-2-phenyl-1H-pyrazol-3(2H)-one (IIc)

Yield 74%, m.p. 170–172 °C. IR (KBr) υ (cm^{-1}): 3410, 3330 (NH_2), 1630 (C=S), absence of (C≡N). *m/z*: 504 (M^+, 70.0%), 505 ($M^+ + 1$, 22.17%), 506 ($M^+ + 2$, 3.73%). ^{1}H NMR (DMSO, 500 MHz) δ (ppm): 2.43 (s, 3H, CH_3), 3.12 (s, 3H, N–CH_3), 6.3 (br.s, 2H, NH_2, D_2O exchangeable), 7.0–7.8 (m, 15H, Ar-H), 9.35 (s, 1H, NH, D_2O exchangeable). Anal. Calcd for $C_{29}H_{24}N_6OS$ (504.61): C, 69.03; H, 4.79; N, 16.65; O, 3.17; S, 6.35, Found: C, 69.40; H, 4.92; N, 16.81; O, 3.25; S, 6.59.

3.3. General method for preparation of IId–f

Method A: A mixture of **I** (0.02 mol) and carbon disulfide (5 mL) in dry pyridine (15 mL) was heated under reflux on a steam bath for 24 h. The solid product that separated after cooling was collected and recrystallized from ethanol/H_2O to give **IId–f** as orange yellow needles.

Method B: A mixture of **I** (0.001 mol), carbon disulfide (6 mL), in dimethyl sulphoxide (25 mL) and 10% aqueous KOH (5.6 mL, 0.01 mol) was added over period of time not exceed 30 min with stirring at ice bath, was stirred at r.t. for 24 h and then diluted with water. The precipitate formed was filtered off, was washed with water,acidified with HCl, dried, and recrystallized from ethanol/H_2O to give **IId–f**.

Method C: A mixture of **I** (0.01 mol) and excess carbon disulfide (7 mL) in ethanolic sodium hydroxide solution (0.01 mol in 20 mL of ethanol) was heated at 80 °C with stirring for 12 h. The reaction mixture was allowed to cool to r.t., stand allover the night, poured into water, and neutralized by diluted HCl. The solid product obtained was filtered off, dried and crystallized from ethanol/H_2O to give **IId–f**.

Compounds **IId–f** prepared by Methods A, B and C have the identical m.p. and mixed m.p.

3.3.1. 7-Benzyl-5,6-diphenyl-1H-pyrrolo [2, 3-d] pyrimidine-2,4 (3H, 7H)-dithione (IId)

Yield (A) 70**%**, (B) 75%, and (C) 70%, m.p. 98–102 °C. IR (KBr) υ (cm^{-1}): 3380 (NH), 1620(C=S), absence of (C≡N). *m/z*: 425 (M^+, 12%), 426 ($M^+ + 1$, 3.5%), 427 ($M^+ + 2$, 2.1%).^{1}H NMR (DMSO, 500 MHz) δ (ppm): 5.90 (s, 2H, CH_2), 7.1–7.7 (m, 15H, Ar-H), 9.2 (s, 1H, NH, N^1, D2O exchangeable), 10.85 (s, 1H, NH, N^3, D_2O exchangeable). Anal. Calcd for $C_{25}H_{19}N_3S_2$ (425.57): C, 70.56; H, 4.50; N, 9.87; S, 15.07. Found: C, 70.64; H, 4.82; N, 9.98; S, 15.35.

3.3.2. 7-(3, 4-dichlorophenyl)-5-phenyl-1H-pyrrolo [2,3-d] pyrimidine-2,4 (3H, 7H)-dithione (IIe)

Yield (A) 70%, (B) 80%, and (C) 74%, m.p. 280–282 °C. IR (KBr) υ (cm^{-1}): 3390 (NH), 1630(C=S), absence of (C≡N). *m/z*: 402 (M^+, ^{35}Cl, 100.0%), 403 ($M^+ + 1$, 21.2%), 404 ($M^+ + 2$, ^{37}Cl, 73.0%). ^{1}H NMR (DMSO, 500 MHz) δ (ppm): 7.1–7.8 (m, 8H, Ar-H), 8.0 (s, 1H, C_6–H), 9.11 (s, 1H, NH, N^1, D_2O exchangeable), 10.86 (s, 1H, NH, N^3, D_2O exchangeable). Anal. Calcd for $C_{18}H_{11}Cl_2N_3S_2$ (402.98): C, 53.47; H, 2.74; Cl, 17.54; N, 10.39; S, 15.86. Found: C, 53.58; H, 3.01; Cl, 17.68; N, 10.45; S, 15.01.

3.3.3. (5, 6-diphenyl-2, 4-dithioxo-3, 4-dihydro-1H-pyrrolo[2,3-d] pyrimi-din-7(2H)-yl)-1,4-dimethyl-2-phenyl-1H-pyrazol-3(2H)-one (IIf)

Yield (A) 75%, (B) 85%, and (C) 76%, m.p. 135–138 °C. IR (KBr) υ (cm^{-1}): 3420, 3380 (NH), 1680 (C=O), 1620 (C=C), absence of (C≡N). *m/z*: 521 (M^+, 15%), 522 ($M^+ + 1$, 4.7%), 523 ($M^+ + 2$, 1.51%). ^{1}H NMR (DMSO, 500 MHz) δ (ppm): ^{1}H NMR (DMSO, 500 MHz) δ (ppm): 2.43 (s, 3H, CH_3), 3.24 (s, 3H, N–CH_3), 6.7–7.8 (m, 15H, Ar-H), 9.52 (s, 1H, NH, N^1, D_2O exchangeable), 11.80 (s, 1H, NH, N^3, D_2O exchangeable). Anal. Calcd for $C_{29}H_{23}N_5OS_2$ (521.66): C, 66.77; H, 4.44; N, 13.43; O, 3.07; S, 12.29. Found: C, 66.59; H, 4.28; N, 13.58; O, 2.98; S, 12.00.

3.4. General method for preparation of II g–i

Method A: A mixture of **I** (0.01 mol) and phenyl isothiocyanate (0.01 mol) was dissolved in pyridine. The reaction mixture was refluxed in an oil bath for 12 h, then cooled and poured into ice bath. The product was precipitated, filtered, washed with acidified water, dried and recrystallized from ethanol/H_2O to give **II g–i**.

Method B: A mixture of **I** or **III** (0.01 mol) and phenyl isothiocyanate (0.01 mol) was dissolved in dimethyl formamide (25 mL) and 10% aqueous KOH (5.6 mL, 0.01 mol) was added over period of time not exceed 30 min with stirring at 90 °C for 12 h and then diluted with water. The precipitate formed was filtered off, was washed with acidified water, dried, and recrystallized from ethanol/H_2O to give **II g–i**. Compound **II h–i** prepared by Methods A and B have the identical m.p. and mixed m.p.

3.4.1. 7-Benzyl-4-imino-3,5,6-triphenyl-3, 4-dihydro-1H-pyrrolo [2,3-d]pyrimidine-2(7H)-thione (II g)

Yield (A) 70% and (B) 75%, m.p. 112–115 °C. IR (KBr) υ (cm^{-1}): 3380 (NH), 1620(C=NH), absence of (C≡N). *m/z*: 484 (M^+, 21%), 485 ($M^+ + 1$, 7.8%), 486 ($M^+ + 2$, 2.5%), 1H NMR (DMSO, 500 MHz) δ (ppm): 5.92 (s, 2H, CH_2), 6.8–7.7 (m, 20H, Ar-H), 9.2 (s, 1H, NH (pyrimidine), D_2O exchangeable), 10.85 (s, 1H, NH, C=NH, D_2O exchangeable). Anal. Calcd for $C_{31}H_{24}N_4S$ (484.61): C, 76.83; H, 4.99; N, 11.56; S, 6.62. Found: C, 77.04; H, 5.12; N, 11.98; S, 6.82

3.4.2. 7-(3,4-dichlorophenyl)-4-imino-3,5-diphenyl-3,4-dihydro-1H-pyrrolo [2,3-d]pyrimidine-2(7H)-thione (II h)

Yield (A) 71% and (B) 74%, m.p. 260–262 °C. IR (KBr) υ (cm^{-1}): 3390 (NH), 1630(C=N), absence of (C≡N). *m/z*: 462.05 (M^+, ^{35}Cl, 100.0%), 463 ($M^+ + 1$, 26.9%), 464 ($M^+ + 2$, ^{37}Cl, 68.5%), 466 ($M^+ + 4$, $2(^{37}Cl)$, 13.6%). ^{1}H NMR (DMSO, 500 MHz) δ (ppm): 6.8–7.8 (m, 13H, Ar-H), 8.0 (s, 1H, C_6–H), 9.21 (s, 1H, NH (pyrimidine), D_2O exchangeable), 10.80 (s, 1H, NH, C=NH, D_2O exchangeable). Anal. Calcd for $C_{24}H_{16}Cl_2N_4S$ (462.38): C, 62.21; H, 3.48; Cl, 15.30; N, 12.09; S, 6.92. Found: C, 62.52; H, 3.59; Cl, 15.68; N, 12.28; S, 7.01.

3.4.3. 5-(4-Imino-3,5,6-triphenyl-2-thioxo-3,4-dihydro-1H-pyrrolo [2,3-d] pyr-imidin-7(2H)-yl)-1,4-dimethyl-2-phenyl-1H-pyrazol-3 (2H)-one (II i)

Yield (A) 71%, (B) 81%, m.p. 92–94 °C. IR (KBr) υ (cm^{-1}): 3420, 3380 (NH_2), 3360 (NH), 1680 (C=O), 1620(C=C), absence of (C≡N).

Table 1
In vivo anti-inflammatory activity

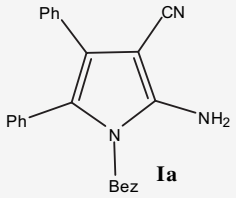
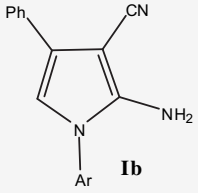
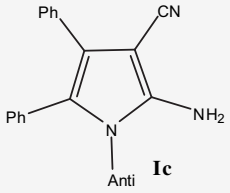
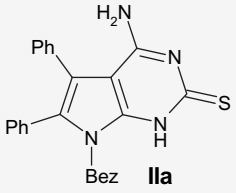
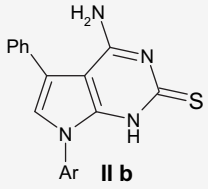
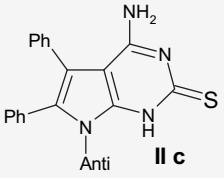
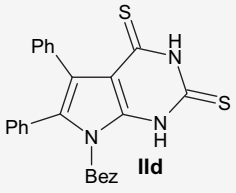
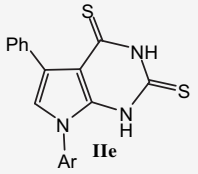
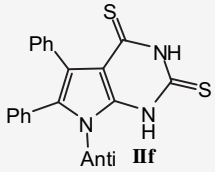
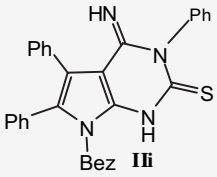
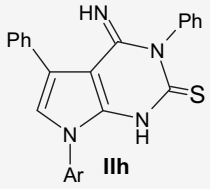
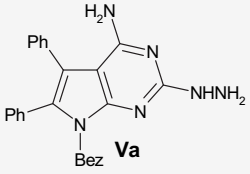
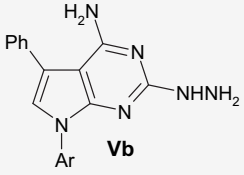
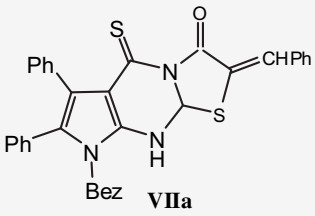
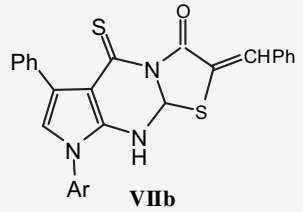
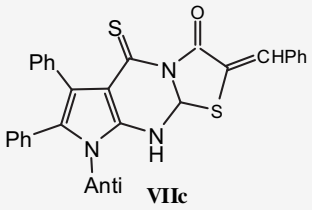
Compounds	Oedema induced by carrageenan (% oedema inh. relative to control)							
	1 h		2 h		3 h		4 h	
	Swel	% inh	Swel	% inh	Swel	% inh	Swel	% inh
 **Ia**	0.223	3.04	0.249	4.23	0.472	12.59	0.48	23.8
 **Ib**	0.225	2.17	0.228	12.3	0.465	13.88	0.51	19.04
 **Ic**	0.208	9.5	0.038*	36.53	0.122**	62.62	0.178*	64.68
 **IIa**	0.226	1.73	0.25	3.84	0.468	13.97	0.474	24.76
 **II b**	0.216	6.08	0.012**	95.38	0.024***	95.95	0.202*	76.82
 **II c**	0.32	0	0.32	0	0.116***	73.34	0.18*	64.28
 **IId**	0.224	2.608	0.246	5.38	0.472	13.23	0.456	27.61

Table 1 (*continued*)

Compounds	Oedema induced by carrageenan (% oedema inh. relative to control)							
	1 h		2 h		3 h		4 h	
	Swel	% inh	Swel	% inh	Swel	% inh	Swel	% inh
 **IIe**	0.226	1.73	0.26	4.48	0.5	12.53	0.52	26
 **IIf**	0.23	0	0.28	3.94	0.451	14.1	0.501	28.8
 **IIi**	0.224	1.98	0.249	5.08	0.470	13.03	0.441	27.98
 **IIh**	0.172	42	0.135	48.07	0.022**	76.83	0.198*	68.57
 **Va**	0.206	10.43	0.101	61.15	0.142***	73.9	0.132**	79.04
 **Vb**	0.196	14.78	0.182	30	0.022***	95.58	0.282	67.43
 **VIIa**	0.222	3.498	0.241	7.31	0.49	9.92	0.55	12.69

(*continued on next page*)

Table 1 (*continued*)

Compounds	Oedema induced by carrageenan (% oedema inh. relative to control)							
	1 h		2 h		3 h		4 h	
	Swel	% inh	Swel	% inh	Swel	% inh	Swel	% inh
 **VIIb**	0.229	0	0.239	8.07	0.451	17.1	0.501	20.47
 **VIIc**	0.226	1.74	0.249	4.23	0.49	9.92	0.441	28.57
Ibuprophen	0.216	6.08	0.14	45	0.214**	60.66	0.192*	69.52
Control	0.23		0.26		0.544		0.63	

Swel = the mean difference of tested compound and control.
% inhibition = (1 − rt/rc) × 100 [rt = result of tested group; rc = result of control group].
* indicate compound is significantly difference compared to the tested drug. As indicated: $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.
Swel = swelling; % inh = % inhibition; Bez = CH_2Ph; Ar = 3,4-$Cl_2C_6H_3$; Anti = Antipyrine.

m/*z*: 580 (M^+, 15%), 581 ($M^+ + 1$, 5.2%), 582 ($M^+ + 2$, 1.1%). ^{1}H NMR (DMSO, 500 MHz) δ (ppm): 2.43 (s, 3H, CH_3), 3.24 (s, 3H, N–CH_3), 6.7–7.8 (m, 20H, Ar-H), 9.21 (s, 1H, NH (pyrimidine), D_2O exchangeable), 10.80 (s, 1H, NH, C=NH, D_2O exchangeable). Anal. Calcd for $C_{35}H_{28}N_6OS$ (580.70): C, 72.39; H, 4.86; N, 14.47; O, 2.76; S, 5.52. Found: C, 72.65; H, 5.08; N, 14.78; O, 2.98; S, 5.78.

3.5. General method for preparation of III a–c

A mixture of **I** (0.01 mol) and phenyl isothiocyanate (0.01 mol) was dissolved in dimethyl formamide (25 mL) and 10% aqueous KOH (5.6 mL, 0.01 mol) was added over period of time not exceed 30 min with stirring at 50 °C for 24 h and then diluted with water. The precipitate formed was filtered off, was washed with acidified water, dried, and recrystallized from ethanol to give **III a–c**.

Table 2
Antibacterial and antifungal in vitro activity expressed as diameter of growth inhibitory area.

Strain	Disc diffusion test (mm)				
	Compounds and reference drugs				
	Ic	IIa	IId	A	B
C. albicans ATCC 10231	–	–	–	–	20
P. aeruginosa ATCC 278533	–	–	–	12	–
M. phlei ATCC 10142	–	–	–	–	–
E. col ATCC 25922	–	–	–	13	–
S. aureus ATCC 29213		16	–	13	–
B. subtilis ATCC 6633	10	25	8	12	–

All tested compound = 20 μg/disc × 30 μg/disc.
A (amoxicillin) = 25 μg mL^{-1}; B (fluconaze) = 4 μg mL^{-1} – inactive compound.
N.B: Ia,b, IIa–c, IIe–j*, II, IV*, V*, and VI*, were inactive towards all tested organisms in both used concentration.

3.5.1. 1-(1-Benzyl-3-cyano-4,5-diphenyl-1H-pyrrol-2-yl)-3-phenylthiourea (IIIa)

Yield 60%, m.p. 96–100 °C. IR (KBr) υ (cm^{-1}): 3390 (NH), 3350 (NH), 2250 (C≡N), 1620(C=N). *m*/*z*: 484 (M^+, 10%), 485 ($M^+ + 1$, 3.8%), 486 ($M^+ + 2$, 1.2%). ^{1}H NMR (DMSO, 500 MHz) δ (ppm): 5.92 (s, 2H, CH_2), 6.8–7.7 (m, 20H, Ar-H), 11.56 (s, 1H, NH, D_2O exchangeable), 12.25 (s, 1H, NH, S=C–NH, D_2O exchangeable).Anal. Calcd for $C_{31}H_{24}N_4S$ (484.61): C, 76.83; H, 4.99; N, 11.56; S, 6.62. Found: C, 77.06; H, 5.22; N, 11.78; S, 6.80.

3.5.2. 1-(3-Cyano-1-(3,4-dichlorophenyl)-4-phenyl-1H-pyrrol-2-yl)-3-phenyl thiourea (IIIb)

Yield 60%, m.p. 92–96 °C. IR (KBr) υ (cm^{-1}): 3390 (NH), 3350 (NH), 2230 (C≡N), 1620(C=N). *m*/*z*: 463 (M^+, ^{35}Cl, 100.0%), 463 ($M^+ + 1$, 26.9%), 465 ($M^+ + 2$, ^{37}Cl, 68.5%). ^{1}H NMR (DMSO, 500 MHz) δ (ppm): 6.8–7.8 (m, 13H, Ar-H), 8.02 (s, 1H, C_6–H), 11.52 (s, 1H, NH, D_2O exchangeable), 12.45 (s, 1H, NH, S=C–NH, D_2O exchangeable). Anal. Calcd for $C_{24}H_{16}Cl_2N_4S$ (463.38): C, 62.21; H, 3.48; Cl, 15.30; N, 12.09; S, 6.92. Found: C, 62.58; H, 3.69; Cl, 15.55; N, 12.42; S, 7.0.

Table 3
Anti-microbial activity results (MIC μg/ml) of newly synthesized compounds with the standard drugs.

Compds & Stander	MIC of the tested compounds (μg/ml) against				
	B. subtilis ATCC 6633	*S. aureus* ATCC 29213	*E. coli* ATCC 25922	*P. aeruginosa* ATCC 278533	*C. albicans* ATCC 10231
Ic	512	NT	NT	NT	NT
IIa	512	256	NT	NT	NT
IId	512	NT	NT	NT	NT
Amoxacillin	64	16	256	64	NT
Fluconazol	NT	NT	NT	NT	512

NT = Not tested.

3.5.3. 1-(3-Cyano-1-(2,4-dimethyl-5-oxo-1-phenyl-2,5-dihydro-1H-pyrazol-3-yl)-4,5-diphenyl-1H-pyrrol-2-yl)-3-phenylthiourea (IIIc)

Yield 81%, m.p. 75–78 °C. IR (KBr) υ (cm^{-1}): 3380 (NH), 2235 (C≡N), 1690 (C=O), 1620(C=N). *m/z*: 580 (M^+, 20%), 581 ($M^+ + 1$, 7.64%), 582 ($M^+ + 2$, 1.8%). ^1H NMR (DMSO, 500 MHz) δ (ppm): ^1H NMR (DMSO, 500 MHz) δ (ppm): 2.43 (s, 3H, CH_3), 3.24 (s, 3H, N–CH_3), 6.7–7.8 (m, 20H, Ar-H), 11.21 (s, 1H, NH, D_2O exchangeable), 12.28 (s, 1H, NH, S=C–NH, D_2O exchangeable). Anal. Calcd for $C_{35}H_{28}N_6OS$ (580.70): C, 72.39; H, 4.86; N, 14.47; O, 2.76; S, 5.52. Found: C, 72.65; H, 5.08; N, 14.78; O, 2.98; S, 5.78.

3.6. 4-Amino-7-(Aryl)-5,6-disubstitututed-2-(alkylthio)-1Hpyrrolo [2,3-d]pyrimidine-2(7H)-thione (IV)

Method A: To a warmed ethanolic potassium hydroxide solution (prepared by dissolving 0.56 g, 0.01 mol of potassium hydroxide in 50 mL of ethanol) compound **IIa–f** (0.01 mol) was added, and heating was continued for 30 min. The mixture was allowed to cool to r.t., and alkyl halide (0.02 mol) was added. The mixture was stirred under reflux for 5 h, allowed to cool to r.t., and finally poured into cold water (100 mL). The solid product precipitated was filtered off and washed with 100 mL water, residue was dried off and recrystallized from methanol

Method B: A mixture of **IIa–f** (0.01 mol), alkyl halide (0.05 mol), and anhydrous sodium acetate (2 g) in ethanol (30 mL) was heated under reflux for 6 h, and then left to cool. The product that formed was filtered off and recrystallized from methanol

Method C: A mixture of **IIa–f** (0.01 mol) and anhydrous potassium carbonate (2.07 g, 0.015 mol) were dissolved in dry acetone (30 ml) and heated at 80 °C under for 30 min. Methyl iodide (0.61 ml, 0.01 mol) was added to the refluxing solution and the mixture heated for 5 h on the water bath. The excess solvent was removed and the residue was recrystallized ethanol. Compound **IV** prepared by Methods A, B and C has the identical m.p. and mixed m.p.

3.6.1. 7-Benzyl-2-(methylthio)-5, 6-diphenyl-7H-pyrrolo [2,3-d] pyrimidin-4-amine (IVa)

Yield 80%, m.p. 140–142 °C. IR (KBr) υ (cm^{-1}): 3350 (NH), 1625 (C=C). *m/z*: 422 (M^+, 60%), 423 ($M^+ + 1$, 17.52%), 424 ($M^+ + 2$, 2.7%). ^1H NMR (DMSO, 500 MHz) δ (ppm): 2.43 (s, 3H, CH_3), 6.02 (s, 2H, CH_2), 6.58 (s, 2H, NH_2, D_2O exchangeable), 6.9–7.8 (m, 15H, Ar-H). Anal. Calcd for $C_{26}H_{22}N_4S$ (422.54): C, 73.90; H, 5.25; N, 13.26; S, 7.59; Found: C, 74.01; H, 5.48; N, 13.50; S, 7.86.

3.6.2. 7-(3,4-dichlorophenyl)-2-(methylthio)-5-phenyl-7H-pyrrolo [2,3-d] pyrimidin-4-amine (IVb)

Yield 80%, m.p. 140–145 °C. IR (KBr) υ (cm^{-1}): 3420, 3350 (NH_2), 1620 (C=C), absence of (C≡N). *m/z*: 401 (M^+, ^{35}Cl, 71.3%), 401 ($M^+ + 1$, 16.8%), 402 ($M^+ + 2$, ^{37}Cl, 46.87%). ^1H NMR (DMSO, 500 MHz) δ (ppm): 2.23 (s, 3H, CH_3), 6.1 (br.s, 2H, NH_2, D_2O exchangeable), 7.0–7.8 (m, 8H, Ar-H), 8.1 (s, 1H, C_6–H). Anal. Calcd for $C_{19}H_{14}Cl_2N_4S$ (401.31): C, 56.86; H, 3.52; Cl, 17.67; N, 13.96; S, 7.99. Found: C, 57.03; H, 3.68; Cl, 17.00; N, 14.13; S, 6.89.

3.6.3. 5-(4-Amino-2-(methylthio)-5, 6-diphenyl-7H-pyrrolo[2,3-d] pyrimidin-7-yl)-1,4-dimethyl-2-phenyl-1H-pyrazol-3(2H)-one (IVc)

Yield 84%, m.p. 135–137 °C. IR (KBr) υ (cm^{-1}): 3410, 3330 (NH_2), 1698 (C=O), 1640 (C=C). *m/z*: 518 (M^+, 52%), 519 ($M^+ + 1$, 18.5%), 520 ($M^+ + 2$, 2.82%). ^1H NMR (DMSO, 500 MHz) δ (ppm): 2.43 (s, 3H, CH_3), 2.58 (s, 3H, S–CH_3), 3.21 (s, 3H, N–CH_3), 6.41 (br.s, 2H, NH_2, D_2O exchangeable), 7.0–7.8 (m, 15H, Ar-H). Anal. Calcd for $C_{30}H_{26}N_6OS$ (518.63): C, 69.48; H, 5.05; N, 16.20; O, 3.08; S, 6.18, Found: C, 69.59; H, 5.32; N, 16.57; O, 3.27; S, 6.58.

3.6.4. 7-Benzyl-2-(ethylthio)-5, 6-diphenyl-7H-pyrrolo [2, 3-d] pyrimidin-4-amine (IVd)

Yield 80%, m.p. 172–176 °C. IR (KBr) υ (cm^{-1}): 3350 (NH), 1625 (C=C). *m/z*: 436 (M^+, 44%), 437 ($M^+ + 1$, 12.98%), 438 ($M^+ + 2$, 2.28%).^1H NMR (DMSO, 500 MHz) δ (ppm): 1.53 (t, 3H, $J = 7.2$ Hz, CH_3), 2.43 (q, 2H, $J = 7.2$ Hz, CH_2), 5.98 (s, 2H, CH2), 6.58 (s, 2H, NH_2, D2O exchangeable), 6.9–7.8 (m, 15H, Ar-H). Anal. Calcd for $C_{27}H_{24}N_4S$ (436.57): C, 74.28; H, 5.54; N, 12.83; S, 7.3; Found: C, 74.51; H, 5.68; N, 13.10; S, 7.56.

3.6.5. 7-(3,4-dichlorophenyl)-2-(ethylthio)-5-phenyl-7H-pyrrolo [2,3-d] pyr- -imidin-4-amine (IVe)

Yield 80%, m.p. 128–130 °C. IR (KBr) υ (cm^{-1}): 3420, 3350 (NH_2), 1620 (C=C), absence of (C≡N). *m/z*: 414 (M^+, ^{35}Cl, 43.2%), 415 ($M^+ + 1$, 8.9%), 416 ($M^+ + 2$, ^{37}Cl, 28.9%). ^1H NMR (DMSO, 500 MHz) δ (ppm): 1.43 (t, 3H, $J = 6.8$,CH_3), 2.23 (q, 3H, $J = 6.8$, CH_3), 6.1 (br.s, 2H, NH_2, D_2O exchangeable), 7.0–7.8 (m, 8H, Ar-H), 8.1 (s, 1H, C_6–H). Anal. Calcd for $C_{20}H_{16}Cl_2N_4S$ (414.34): C, 57.84; H, 3.88; Cl, 17.07; N, 13.49; S, 7.72. Found: C, 58.03; H, 3.98; Cl, 17.41; N, 13.65; S, 7.89.

3.6.6. 5-(4-Amino-2-(ethylthio)-5,6-diphenyl-7H-pyrrolo[2,3-d] pyrimidin-7-yl)-1,4-dimethyl-2-phenyl-1H-pyrazol-3(2H)-one (IVf)

Yield 84%, m.p. 145–148 °C. IR (KBr) υ (cm^{-1}): 3450, 3330 (NH_2), 1710 (C=O), 1630 (C=C). *m/z*: 532 (M^+, 35.5%), 533 ($M^+ + 1$, 12.12%), 534.21 ($M^+ + 2$, 2.35%). ^1H NMR (DMSO, 500 MHz) δ (ppm): ^1H NMR (DMSO, 500 MHz) δ (ppm): 1.43 (t, 3H, $J = 7.5$, CH_3), 2.43 (s, 3H, CH_3), 3.01 (q, 3H, $J = 7.5$, CH_3), 3.21 (s, 3H, N–CH_3), 6.41 (br.s, 2H, NH_2, D_2O exchangeable), 7.0–7.8 (m, 15H, Ar-H). Anal. Calcd for $C_{31}H_{28}N_6OS$ (532.66): C, 69.90; H, 5.30; N, 15.78; O, 3.00; S, 6.02, Found: C, 70.01; H, 5.38; N, 16.01; O, 3.25; S, 6.35.

3.6.7. 7-Benzyl-2, 4-bis(methylthio)-5,6-diphenyl-7H-pyrrolo[2,3-d]pyrimidine (IVg)

Yield 75%, m.p. 125–128 °C. IR (KBr) υ (cm^{-1}): 2925 (CH aliph.), 1620(C=S), absence of (NH). *m/z*: 453 (M^+, 25%), 454 ($M^+ + 1$, 7.5%), 455 ($M^+ + 2$, 2.4%), ^1H NMR (DMSO, 500 MHz) δ (ppm): 2.6 (s, 6H, CH_3), 5.92 (s, 2H, CH_2), 7.1–7.7 (m, 15H, Ar-H). Anal. Calcd for $C_{27}H_{23}N_3S_2$ (453.62): C, 71.49; H, 5.11; N, 9.26; S, 14.14. Found: C, 70.98; H, 4.89; N, 9.48; S, 14.25.

3.6.8. 7-(3,4-dichlorophenyl)-2,4-bis(methylthio)-5-phenyl-7H-pyrrolo[2,3-d]pyrimidine (IVh)

Yield 80%, m.p. 185–188 °C. IR (KBr) υ (cm^{-1}): 2930 (CH aliph.), 1630(C=S), absence of (NH). *m/z*: 431 (M^+, ^{35}Cl, 100%), 432 ($M^+ + 1$, 23.5%), 433 ($M^+ + 2$, ^{37}Cl, 64.5%). ^1H NMR (DMSO, 500 MHz) δ (ppm): 2.45 (s, 6H, CH_3), 7.1–7.8 (m, 8H, Ar-H), 8.0 (s, 1H, C_6–H). Anal. Calcd for $C_{20}H_{15}Cl_2N_3S_2$ (432.39): C, 55.56; H, 3.50; Cl, 16.40; N, 9.72; S, 14.83. Found: C, 55.58; H, 3.42; Cl, 16.69; N, 10.15; S, 15.0.

3.6.9. 5-(2,4-bis(methylthio)-5,6-diphenyl-7H-pyrrolo[2,3-d] pyrimidin-7-yl)–1,4-dimethyl-2-phenyl-1H-pyrazol-3(2H)-one (IVi)

Yield 76%, m.p. 158–160 °C. IR (KBr) υ (cm^{-1}): 2920 (CH aliph.), 1690 (C=O), 1630(C=S), absence of (NH). *m/z*: 549 (M^+, 42%), 550 ($M^+ + 1$, 15%), 551 ($M^+ + 2$, 4.1%). ^1H NMR (DMSO, 500 MHz) δ (ppm): ^1H NMR (DMSO, 500 MHz) δ (ppm): 2.43 (s, 3H, CH_3), 2.58 (s, 6H, CH_3), 3.24 (s, 3H, N–CH_3), 6.7–7.8 (m, 15H, Ar-H). Anal. Calcd for $C_{31}H_{27}N_5OS_2$ (549.71): C, 67.73; H, 4.95; N, 12.74; O, 2.91; S, 11.67. Found: C, 67.59; H, 4.68; N, 13.0; O, 2.98; S, 11.60.

3.6.10. 7-Benzyl-2,4-bis(ethylthio)-5,6-diphenyl-7H-pyrrolo[2,3-d] pyrimidine (IVj)

Yield 78%, m.p. 115–120 °C. IR (KBr) υ (cm^{-1}): 2925 (CH aliph.), 1640(C=N), absence of (NH). *m/z*: 481 (M^+, 36%), 482 ($M^+ + 1$,

11.7%), 483 ($M^+ + 2$, 3.51%). ^{1}H NMR (DMSO, 500 MHz) δ (ppm): 1.6–2.0 (t, 6H, CH_3), 3.1–3.2 (q, 4H, CH_2), 5.91 (s, 2H, CH_2), 7.0–7.7 (m, 15H, Ar-H). Anal. Calcd for $C_{29}H_{27}N_3S_2$ (481.67): C, 72.31; H, 5.65; N, 8.72; S, 13.31. Found: C, 72.58; H, 5.59; N, 8.49; S, 13.25.

3.6.11. 7-(3,4-dichlorophenyl)-2,4-bis(ethylthio)-5-phenyl-7H-pyrrolo[2,3-d]pyrimidine (IVk)

Yield 85%, m.p. 170–174 °C. IR (KBr) υ (cm^{-1}): 2930 (CH aliph.), 1625(C=N), absence of (NH). *m/z*: 460 (M^+, ^{35}Cl, 100%), 461 ($M^+ + 1$, 25.4%), 462 ($M^+ + 2$, ^{37}Cl, 73.6%). ^{1}H NMR (DMSO, 500 MHz) δ (ppm): 1.7–2.0 (t, 6H, CH_3), 3.0–3.1 (q, 4H, CH_2), 6.9–7.8 (m, 8H, Ar-H), 8.1 (s, 1H, C_6–H). Anal. Calcd for $C_{22}H_{19}Cl_2N_3S_2$ (460.44): C, 57.39; H, 4.16; Cl, 15.40; N, 9.13; S, 13.93. Found: C, 57.58; H, 4.42; Cl, 15.69; N, 9.25; S, 14.01.

3.6.12. 5-(2,4-bis(ethylthio)-5,6-diphenyl-7H-pyrrolo[2,3-d] pyrimidin-7-yl) –1,4-dimethyl-2-phenyl-1H-pyrazol-3(2H)-one (IVl)

Yield 74%, m.p. 162–166 °C. IR (KBr) υ (cm^{-1}): 2920 (CH aliph.), 1690 (C=O), 1630(C=N), absence of (NH). *m/z*: 577 (M^+, 22%), 578 ($M^+ + 1$, 8.37%), 579 ($M^+ + 2$, 2.1%). ^{1}H NMR (DMSO, 500 MHz) δ (ppm): ^{1}H NMR (DMSO, 500 MHz) δ (ppm): 1.6–2.0 (t, 6H, CH_3), 2.43 (s, 3H, CH_3), 3.0–3.1 (q, 4H, CH_2), 3.24 (s, 3H, N–CH_3), 6.7–7.8 (m, 15H, Ar-H). Anal. Calcd for $C_{33}H_{31}N_5OS_2$ (577.76): C, 68.60; H, 5.41; N, 12.12; O, 2.77; S, 11.10. Found: C, 68.79; H, 5.58; N, 12.01; O, 2.98; S, 11.35.

3.7. General method to prepare V

Method A: A mixture of **IV a**–**f** (0.01 mol) and hydrazine hydrate (99%, 0.03 mol) was heated under reflux in pyridine (30 mL) for 6 h and then left to cool. Pour to ice water/acidified with HCl. The solid product was filtered off, and recrystallized from ethanol to give **V.**

Method B. A mixture of **IV a**–**f** (0.01 mol) and hydrazine hydrate (99%, 0.03 mol) in absolute ethanol (20 mL) was refluxed for 10 h. The reaction mixture was poured onto ice. The product was isolated and recrystallized from methanol to give **V** as buff colored crystals. Compound **V** prepared by Methods A and B have the identical m.p. and mixed m.p.

3.7.1. 7-Benzyl-2-hydrazinyl-5, 6-diphenyl-7H-pyrrolo [2,3-d] pyrimidin-4-amine (Va)

Yield (A) 60**%** and (B) 85%, m.p. 100–102 °C. IR (KBr) υ (cm^{-1}): 3460–3380 (NH2), 3350 (NH), 1630(C=C). *m/z*: 406 (M^+, 10%), 407 ($M^+ + 1$, 3.1%). ^{1}H NMR (DMSO, 500 MHz) δ (ppm): 4.85 (s, 2H, NH_2, D_2O exchangeable), 5.92 (s, 2H, CH_2), 6.75 (s, 2H, NH_2, D_2O exchangeable), 6.9–7.8 (m, 15H, Ar-H), 9.2 (s, 1H, NH, D_2O exchangeable). Anal. Calcd for $C_{25}H_{22}N_6$ (406.48): C, 73.87; H, 5.46; N, 20.67**7**. Found: C, 74.01; H, 5.49; N, 21.00.

3.7.2. 7-(3,4-dichlorophenyl)-2-hydrazinyl-5-phenyl-7H-pyrrolo [2,3-d] pyrimidin-4-amine (Vb)

Yield (A) 52**%** and (B) 80%, m.p. 166–168 °C. IR (KBr) υ (cm^{-1}): 3480–3400 (NH2), 3380 (NH), 1640(C=C). *m/z*: 384 (M^+, ^{35}Cl, 100%), 385 ($M^+ + 1$, 19.6%), 386 ($M^+ + 2$, ^{37}Cl, 63.9%), 388 ($M^+ + 4$, 2 (^{37}Cl), 9.6%). ^{1}H NMR (DMSO, 500 MHz) δ (ppm): 4.68 (s, 2H, NH_2, D_2O exchangeable), 6.70 (s, 2H, NH_2, D_2O exchangeable), 7.0–7.8 (m, 8H, Ar-H), 8.0 (s, 1H, C_6–H). 9.36 (s, 1H, NH, D_2O exchangeable). Anal. Calcd for $C_{18}H_{14}Cl_2N_6$ (385.25): C, 56.12; H, 3.66; Cl, 18.41; N, 21.81. Found: C, 56.48; H, 3.98; Cl, 18.78; N, 22.01.

3.7.3. 5-(4-Amino-2-hydrazinyl-5,6-diphenyl-7H-pyrrolo[2,3-d] pyrimidin-7-yl)-1,4-dimethyl-2-phenyl-1H-pyrazol-3(2H)-one (Vc)

Yield (A) 56**%** and (B) 84%, m.p. 165–168 °C. IR (KBr) υ (cm^{-1}): 3420, 3390 (NH_2), 3340 (NH), 1710 (C=O), 1640(C=C). *m/z*: 502 (M^+, 65%), 503 ($M^+ + 1$, 20.1%), ^{1}H NMR (DMSO, 500 MHz) δ (ppm): ^{1}H NMR (DMSO, 500 MHz) δ (ppm): 2.43 (s, 3H, CH_3), 3.21 (s, 3H, N–CH_3), 4.78 (s, 2H, NH_2, D_2O exchangeable), 6.70 (s, 2H, NH_2, D_2O exchangeable), 7.0–7.8 (m, 15H, Ar-H), 9.30 (s, 1H, NH, D_2O exchangeable). Anal. Calcd for $C_{29}H_{26}N_8O$ (502.57): C, 69.31; H, 5.21; N, 22.30; O, 3.18, Found: C, 69.61; H, 5.39; N, 22.78; O, 3.28.

3.8. General method to prepare VI

A mixture of **IId**–**f** (0.01 mol), chloroacetic acid (0.03 mol), and fused sodium acetate (2 g) in a mixture of acetic acid/acetic anhydride (30 mL, 1:1) was heated under reflux for 9 h and left to cool. The reaction mixture was then diluted with water, shaken well, and allowed to stand 6 h. The residue was triturate with ethanol; solid product was filtered off and recrystallized from the ethanol/H_2O to give **VI**.

3.8.1. 8-Benzyl-6,7-diphenyl-5-thioxo-5,8-dihydropyrrolo[2,3-d] thiazolo[3,2-a]pyrimidin-3(2H)-one (VIa)

Yield 55%, m.p. 114–117 °C. IR (KBr) υ (cm^{-1}): 1720 (C=O), 1620 (C=S). *m/z*: 465 (M^+, 10%), 466 ($M^+ + 1$, 3.52%). ^{1}H NMR (DMSO, 500 MHz) δ (ppm): 3.8 (s, 2H, CO–CH_2–S), 5.70 (s, 2H, CH_2), 7.0–7.7 (m, 15H, Ar-H). Anal. Calcd for $C_{27}H_{19}N_3OS_2$ (465.59): C, 69.65; H, 4.11; N, 9.03; O, 3.44; S, 13.77. Found: C, 70.01; H, 4.02; N, 9.38; O, 3.24; S, 14.05.

3.8.2. 8-(3,4-dichlorophenyl)-6-phenyl-5-thioxo-5,8-dihydropyrrolo[2,3-d]thia-zolo[3,2-a]pyrimidin-3(2H)-one (VIb)

Yield 54%, m.p. 210–215 °C. IR (KBr) υ (cm^{-1}): 1710 (C=O), 1620 (C=S). *m/z*: 444 (M^+, ^{35}Cl, 73.6%), 443 ($M^+ + 1$,21.8%), 446 ($M^+ + 2$, ^{37}Cl, 46.0%). ^{1}H NMR (DMSO, 500 MHz) δ (ppm): 3.9 (s, 2H, CO–CH_2–S), 7.2–7.8 (m, 8H, Ar-H), 8.0 (s, 1H, C_6–H). Anal. Calcd for $C_{20}H_{11}Cl_2N_3OS_2$ (444.36): C, 54.06; H, 2.50; Cl, 15.96; N, 9.46; O, 3.60; S, 14.43. Found: C, 54.38; H, 2.42; Cl, 15.69; N, 9.15; O, 3.40; S, 14.21.

3.8.3. 8-(2,4-Dimethyl-5-oxo-1-phenyl-2,5-dihydro-1H-pyrazol-3-yl)-6,7-di phenyl-5-thioxo-5,8-dihydropyrrolo[2,3-d]thiazolo[3,2-a] pyrimidin-3(2H)-one (VIc)

Yield 54%, m.p. 180–183 °C. IR (KBr) υ (cm^{-1}): 1710–1690 (C=O), 1630 (C=S). *m/z*: 561 (M^+, 25.0%), 562 ($M^+ + 1$, 9.21%), 563 ($M^+ + 2$, 2.68%). ^{1}H NMR (DMSO, 500 MHz) δ (ppm): ^{1}H NMR (DMSO, 500 MHz) δ (ppm): 2.43 (s, 3H, CH_3), 3.24 (s, 3H, N–CH_3), 3.9 (s, 2H, CO–CH_2–S), 6.7–7.8 (m, 15H, Ar-H). Anal. Calcd for $C_{31}H_{23}N_5O_2S_2$ (561.68): C, 66.29; H, 4.13; N, 12.47; O, 5.70; S, 11.42. Found: C, 66.49; H, 4.48; N, 12.12; O, 5.98; S, 11.25.

3.9. General method to prepare VII

Method A: A mixture of **IId**–**f** (0.01 mol), chloroacetic acid (0.08 mol), appropriate aldehyde (0.05 mol), and fused sodium acetate (2.0 g) in a mixture of acetic acid/acetic anhydride (30 mL, 1:1) was heated under reflux for 8 h and left to cool. The reaction mixture was diluted with water. The residue was triturate with ethanol; solid product was filtered off and recrystallized from the ethanol/H_2O to give **VII**.

Method B: To a mixture of **VI** (0.01 mol) and appropriate aldehyde (0.01 mol) in absolute ethanol (30 mL) was added piperidine (5 drops). The mixture was heated under reflux for 8 h. The crystalline product thus obtained after cooling was collected and recrystallized from ethanol to give **VII** as orange crystals. Compound **VII** prepared by methods A and B have the same m.p. and mixed m.p.

3.9.1. 8-Benzyl-2-benzylidene-6,7-diphenyl-5-thioxo-5,8-dihydropyrrolo[2, 3-d] thiazolo[3,2-a]pyrimidin-3(2H)-one(VII a)

Yield (A) 65% and (B) 85%, m.p. 140–143 °C. IR (KBr) υ (cm^{-1}): 1720 (C=O), 1620(C=S). *m/z*: 553 (M^+, 11.89%), 554 ($M^+ + 1$, 4.81%), 555 ($M^+ + 2$, 1.12%). ^{1}H NMR (DMSO, 500 MHz) δ (ppm): 4.9 (s, 1H, C=CH), 5.75 (s, 2H, CH_2), 6.8–7.7 (m, 20H, Ar-H). Anal. Calcd for $C_{34}H_{23}N_3OS_2$ (553.69): C, 73.75; H, 4.19; N, 7.59; O, 2.89; S, 11.58. Found: C, 73.91; H, 4.35; N, 7.92; O, 3.04; S, 11.85.

3.9.2. 2-Benzylidene-8-(3,4-dichlorophenyl)-6-phenyl-5-thioxo-5,8-dihydro pyrrolo[2,3-d] thiazolo[3,2-a]pyrimidin-3(2H)-one (VII b)

Yield (A) 55% and (B) 86%, m.p. 260–265 °C. IR (KBr) υ (cm^{-1}): 1710 (C=O), 1620 (C=S). *m/z*: 531 (M^+, ^{35}Cl, 33.0%),532 ($M^+ + 1$, 9.1%) 533 ($M^+ + 2$, ^{37}Cl, 23.4%). ^{1}H NMR (DMSO, 500 MHz) δ (ppm): 2.3 (s, 1H, C=CH), 6.8–7.8 (m, 13H, Ar-H), 8.0 (s, 1H, C_6–H). Anal. Calcd for $C_{27}H_{15}Cl_2N_3OS_2$ (532.464): C, 60.90; H, 2.84; Cl, 13.32; N, 7.89; O, 3.00; S, 12.04. Found: C, 61.18; H, 3.02; Cl, 12.79; N, 7.93; O, 3.20; S, 11.86.

3.9.3. 2-Benzylidene-8-(2,4-dimethyl-5-oxo-1-phenyl-2,5-dihydro-1H-pyrazol-3-yl)-6,7-diphenyl-5-thioxo-5,8-dihydropyrrolo[2,3-d] thiazolo[3,2-a]py- -rimidin-3(2H)-one (VII c)

Yield (A) 52% and (B) 80%, m.p. 150–155 °C. IR (KBr) υ (cm^{-1}): 1710–1680 (C=O), 1620 (C=S). *m/z*: 649 (M^+,12.40%), 650 ($M^+ + 1$,5.6%), 651 ($M^+ + 2$, 1.25%). ^{1}H NMR (DMSO, 500 MHz) δ (ppm): ^{1}H NMR (DMSO, 500 MHz) δ (ppm): 2.3 (s, 1H, C=CH), 2.43 (s, 3H, CH_3), 3.24 (s, 3H, N–CH_3), 6.9–7.8 (m, 20H, Ar-H). Anal. Calcd for $C_{38}H_{27}N_5O_2S_2$ (649.783): C, 70.24; H, 4.19; N, 10.78; O, 4.92; S, 9.87. Found: C, 70.49; H, 4.28; N, 10.52; O, 4.98; S, 9.55.

3.10. Biological assay

3.10.1. Anti-inflammatory activity [31]

In vivo assay: The carrageenan-induced rat paw oedema assay was carried out using a modified winter's method as a preliminary screening test [32]. The rats (in groups of five animals weighing 120–170 g, young adult male Sprague–Dawley) were housed in a controlled environment and provided with standard rodent chow and water for 24 h before a dose of the test compounds (50 and 70 mg/kg sc) was administered. One hour later, the volume of the right hind paw was measured, and 0.05 mL of a 1% suspension of carrageenan in sterile pyrogen-free 0.9% NaCl solution was injected subcutaneously into planter aponeurosis of the hind paw. One hour after the injection of carrageenan, the paw volume was again measured by a water pletysmometer. The mean increase of paw volume at each time interval was compared with that of control group (five rats treated with carrageenan, but without test compounds) at the same time intervals. The percentage inhibition values were calculated according to the formula: % Anti-inflammatory activity = $(1 - R_t/R_c) \times 100$ (R_t = result of tested group; Rc = result of control group). All experiments involving animals were carried out using protocols approved by the Animal Committee, University of Barcelona (Spain). Animal care was in compliance with Generalitat of Catalunya regulations on protection of animals used for experimental and other scientific purposes.

3.10.2. Anti-microbial activity

3.10.2.1. Materials and methods. Antibacterial activity was examined by the disc-diffusion method and the MIC method under standard conditions of the National Committee for Clinical Laboratory Standards [33]. The in vitro anti-microbial activity of the synthesized compounds was investigated against several pathogenic representative Gram-positive bacteria (*S. aureus* ATCC 29213, *B. subtilis* ATCC 6633, *Mycobacterium phlei* ATCC 10142) and Gram-negative bacteria (*Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 278533), and (*C. albicans* ATCC 10231) as a representative for fungi. All micro-organisms used were obtained from the culture collection of the Department of Microbiology and Immunology, Faculty of Pharmacy, Helwan University, Cairo, Egypt.

Media for disc sensitivity tests were the nutrient agar and Muller–Hinton agar (MHA) purchased from Difco (USA). Nonsterile powder of the tested compound was dissolved in sterile DMSO to yield 20 and 30 μg mL^{-1}, and passed through 0.2 μm membrane filters (Millipore Corp, USA). The filtrates were dispensed as 2 mL samples into sterile, small screw-capped vials, frozen and kept stored at −15 °C. The vials were refrozen after thawing. Disc diffusion sensitivity test was done in the manner identical to that of Bauer et al. [34]. DMSO showed no inhibition zones. Amoxicillin (Bioanalyse, Turkey) and Fluconazol (Sigma–Aldrich, USA) were used as reference substances.

MIC (minimum inhibitory concentration) for each tested compound was determined on Muller-Hinton agar (MHA), by micro dilution technique according to NCCLS guidelines 1997 [35]. All bacterial isolates were sub cultured in MHA plates and incubated overnight at 37 °C and all Candida isolates were sub cultured in SDA plates at 35 °C for 24–48 h. The micro-organisms were passage at least twice to ensure purity and viability. The solution of the newly synthesized compounds and standard drugs were prepared at 1024, 512, 256, 128, 64, 32 and 16 μg/ml concentrations using serial two-folds dilutions in DMSO (dimethyl sulphoxide), each concentration was mixed with sterile nutrient agar (Sigma–Aldrich) in sterile plate, bacteria inoculum was added to each well of the micro dilution trays. The trays were incubated at 37 °C in a humid chamber and MIC end points were read after 24 h of incubation.

The lowest concentration of the compound that completely inhibits macroscopic growth was determined and minimum inhibitory concentrations (MICs) were reported. DMSO (Sigma–Aldrich, 80%), pure micro-organisms, and pure media were used as control wells.

References

[1] S.M. Sondhi, R.N. Goyal, A.M. Lahoti, Bioorg. Med. Chem. 13 (2005) 3185.
[2] (a) H. Sierzputowska-Gracz, E. Sochacka, A. Malkiewicz, K. Kuo, C. Gehrke, P.F. Agris, J. Am. Chem. Soc. 109 (1987) 7171;
(b) E. Sochacka, I. Fratczak, Tetrahedron Lett. 45 (2004) 6729.
[3] (a) S. Stoyanov, I. Petkov, L. Antonov, T. Stoyanova, P. Karagiannides, P. Aslanidis, Can. J. Chem. 68 (1990) 1482;
(b) J.C. Hazelton, B. Iddon, H. Suschitzky, L.H.J. Woolley, Chem. Soc. Perkin Trans. 1 6 (1992) 685.
[4] Z.G. Hajos, R.M. Kanojia, Chem. Abstr. 116 (1991) 83701.
[5] A.K. Pathak, V. Pathak, L.E. Seit, W.J. Sulng, R.C.J. Reynolds, Med. Chem. 41 (2004) 273.
[6] E.V. Aleksandrova1, Pharm. Chem. J. 37 (2003) 645.
[7] M.D. Mashkovskii, Drugs. Moscow 1–2 (2000) (in Russian).
[8] G.N. Pershin, Z.h. Vses, o-va im, Mendeleev Communications 15 (2) (1970) 216.
[9] E. Berger, L. Rumbach, La Revue de médecine interne 20 (1999) 3465.
[10] (a) T. Munster, D.E. Furst, Clin. Exp. Rheumatol. 17 (1999) 529;
(b) R.P. Baughman, E.E. Lower, Clin. Immunother. 6 (1996) 431;
(c) C.G. Groth, L. Backman, I.M. Morales, Transplantation 67 (1999) 1036;
(d) N. Perico, G. Remuzzi, Drugs 54 (4) (1999) 533.
[11] J.L.S. Banitt, G.L. Bundy, I.M. Richards, J. Pharmacol. Exp. Ther. 290 (1999) 188.
[12] B. Howard Cottam, Z. Kazimierczuk, S. Geary, P. McKernan, J. Med. Chem. 28 (1985) 1461.
[13] M.J. Motsuoka, Antibiotics (Tokyo) 13A (1960) 121.
[14] K. Anzai, S.J. Marumo, Antibiotics (Tokyo) 10A (1957) 20.
[15] M.A. Mohamed, A.E. Rashad, M.E.A. Zaki, S.S. Fatahala, Acta Pharm. 55 (2005) 237.
[16] M.A. Mohamed, A.E. Rashad, M.E.A. Zaki, S.S. Fatahala, Arch. Pharm. Chem. Life Sci. (2006) 339.
[17] M.A. Mohamed, A.E. Rashad, M.E.A. Zaki, S.S. Fatahala, M.Z. Monem, Naturforsch C (2007) 62.
[18] M.T. Cocco, C. Congiu, A. Maccioni, V. Onnis, J. Heterocycl. 30 (1993) 253.
[19] A.Z.A. Hassanien, I.S. Abdel Hafiz, M.H. Elnagdi, J. Chem. Res. (S) (1999) 8.
[20] A.A. Aly, Phosphorus, Sulfur, and Silicon 181 (2006) 2395.

[21] Z.H. Ismail, S.M. Abdel-Gawad, M.M. Ghorab, Phosphorus, Sulfur, and Silicon 178 (2003) 1795.
[22] A.M.K. El-Dean, M.E. Abdel-Moneam, Phosphorus, Sulfur, and Silicon 177 (2002) 2745.
[23] A.E. Abdel-Rahman, I.M.A. Awad, E.A. Bakhete, Phosphorus, Sulfur, and Silicon 60 (1991) 189.
[24] M.I.A. Moneam, Phosphorus, Sulfur, and Silicon 180 (2005) 633.
[25] A.B.A. El-Gazzar, H.A.R. Hussein, A.S. Aly, Phosphorus, Sulfur, and Silicon 181 (2006) 2771.
[26] T.I. El-Emary, Sh.A. Abdel-Mohsen, Phosphorus, Sulfur, and Silicon 181 (2006) 2459.
[27] K.M. Hassan, M.S.K. Youssef, M.F. El-zohry, R. Abo El-wafa, Phosphorus, Sulfur, and Silicon 40 (1988) 237.
[28] F. Abou-El, G. Hamman', M.I. All, J. Chem. Eng. Data 26 (1981) 99.
[29] M.B. Devani, C.J. Shishoo, U.S. Pathak, S.H. Parikh, G.F. Shah, A.C. Padhya, J. Pharm. Sci. 65 (1976) 660.
[30] S.M. Hussain, A.M.H. El-Reedy, A.M. Rezk, Kh.A Sife-Eldien, J. Heterocyclic. Chem. 24 (1987) 1605.
[31] Y. Harrak, G. Rosell, G. Daidone, Bioorg. Med. Chem. 15 (2007) 4876.
[32] C.A. Winter, E.A. Risley, G.W.J. Nuss, Pharmacol. Exp. Ther. 141 (1963) 369.
[33] National Committee for Clinical Laboratory Standards, Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically Approved Standard NCCLS Document M7-A4, fourth ed., vol. 17, no. 2. NCCLS, 1997;
Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts; Approved Standard. NCCLS Document M27-A2, second ed., vol. 22, no. 15; National Committee for Clinical Laboratory Standards: 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087-1898 USA, (2002).
[34] A.W. Bauer, M.M. Kirby, J.C. Sherris, Am. J. Clin. Pathol. 45 (1996) 493.
[35] A.C. Gales, H.S. Sader, R.N. Jones, Urinary tract infection trends in Latin American hospitals: report from the SENTRY antimicrobial surveillance program. Diagn. Microbial. Infect. Dis. 44 (1997–2000) 289.