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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Synthesis of novel 1, 2, 4-triazolopyrimidines and their evaluation as antimicrobial agents

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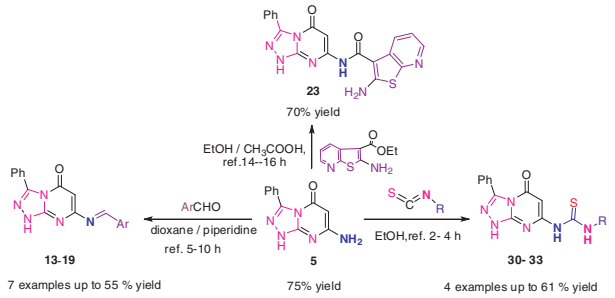
Abstract 7-aminopyrimidin-5(1*H*)-one (5) was utilized as a starting material for the preparation of new, Schiff bases (**13–19**), amides **23**, **24**, imide **25**, as well as thiourea derivatives **30–33** incorporating the triazolopyrimidines nucleus in their molecules. Structural elucidations for the new compounds were based upon compatible micro-analytical and spectroscopic measurement. Biological evaluation indicated that compounds **23**, **31**, **33**, **32**, and **30** possess promising antimicrobial activities.

Graphical Abstract



Antimicrobial activity of compounds **23, 31, 33, 32** and **30** have found higher than commonly used antibiotics like [Penicillin G or Streptomycin] and [Itraconazole or Clotrimazole]

Keywords Triazolopyrimidines · Benzohydrazide · Isothiocyocyanate · Antimicrobial activity

Introduction

Pyrimidines and purines are the main bases constituents of nucleic acids. In addition of other rings to the pyrimidines core is expected to result in novel biological potentialities (Aly et al. 2012; Mohamed et al. 2010; Abdel-Latif et al. 2007). Consequently, the aza analogs of purines, mainly the triazolopyrimidines, also are important (Mohamed et al. 2010). The study of compounds incorporating the triazolopyrimidine developed due to their varied effects in diverse domains. Triazolopyrimidines (TPs), a subtype of purine analogs, have been the subject of chemical and biological studies due to their interesting pharmacology including antibacterial (Broom et al. 1976; Grivsky et al. 1980; Nargund et al. 1991), antifungal (Chen et al. 2008), antimicrobial (Donkor et al. 1995), antihypertensive (Degraw et al. 1974), antileishmanial (Ellingboe and Princeton 1996; Pandey et al. 2004) anticonvulsant (Deyanov et al. 1991), antitumor (Navarro et al. 1998), cytotoxicity (Magan et al. 2004), therapeutic potentiality (Magan et al. 2005), potent and selective ATP site directed inhibition of the EGF-receptor protein tyrosine kinase (Traxler et al. 1996) and cardiovascular (Sato et al. 1980) activities. In addition, triazolopyrimidines are versatile ligands and their derived coordination compounds can be considered as model systems for metal-ligand interactions observed in biological systems (Fischer 2008; Salas et al. 1999). Trapidil, the well-known TP derivative behaves as a phosphodiesterase inhibitor and as a platelet-derived growth factor antagonist

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(Fischer 2008). Triazolopyrimidine antibiotic was isolated from nature Essramycin and was proved to be potent antibiotic agents (El-Gendy et al. 2008). Bearing these facts in mind, coupled with our growing interest in the realm of biologically active heterocycles (Abu-Hashem et al. 2011; Abu-Hashem and Aly 2012; Hussein et al. 2010; Abu-Hashem et al. 2011; Khidre et al. 2011), we report now on the synthesis and antimicrobial evaluation of novel triazolepyrimidines. Hybird presence of the trizole and pyrimidine moieties in the same compound is hoped to boost the bioactivity.

Results and discussion

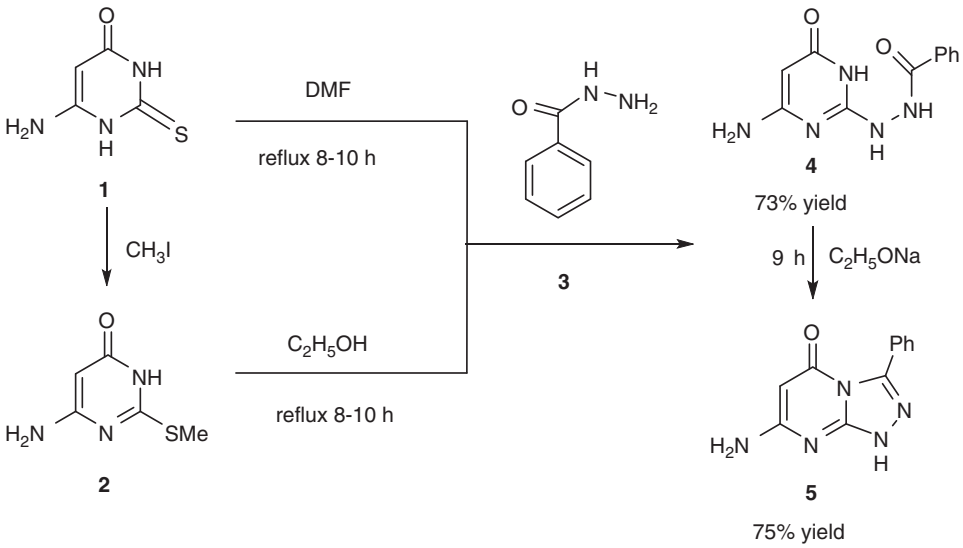
Chemistry

In this investigation, the starting 7-Aminopyrimidin-5(1*H*)-one key derivative (**5**) was prepared via heating under reflux of 6-aminothiouracil (**1**) with benzohydrazide (**3**) in dimethyl formamide, followed by stirring under reflux of the formed pyrimdine (**4**) in sodium ethoxide. In another route compound (**4**) was prepared by alkylation of 6-aminothiouracil (**1**) with methyl-iodide in ethanolic potassium hydroxide solution afforded 6-amino-2-(methylthio) pyrimidin-4(3*H*)-one (**2**) (Guiney et al. 2003).The last compound (**2**) reacted with benzohydrazide (**3**) in absolute ethanol to give the corresponding (**4**) (Scheme 1).

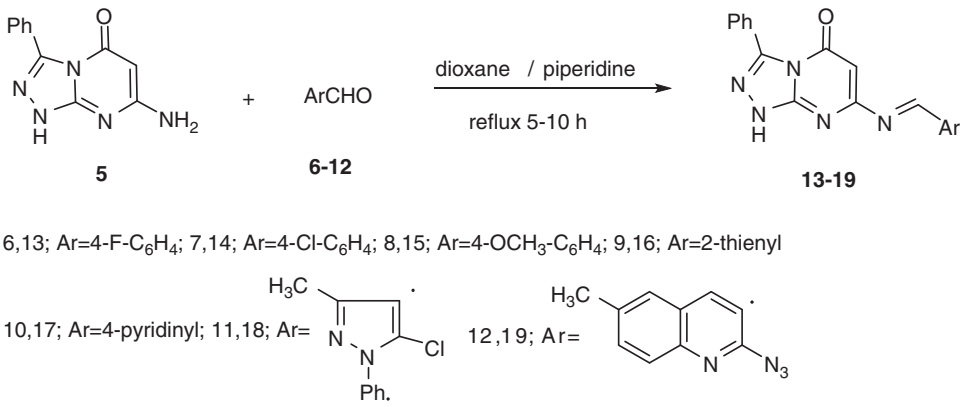
Reaction of 7-amino-3-phenyl-[1,2,4]triazolo [4,3-a] pyrimidin-5(1*H*)-one (**5**) with some aromatic aldehydes namely: 4-fluorobenzaldehyde (**6**), 4-chlorobenzaldehyde (7), 4-methoxybenzaldehyde (**8**), thiophene-2-carbaldehyde (**9**), isonicotinaldehyde (**10**) in dioxane containing catalytic amount of piperidine or with 5-chloro-3-methyl-1-phenyl-1*H*-pyrazole-4-carbaldehyde (**11**) or 2-azido-6-methylquinoline-3-carbaldehyde (**12**) in ethanol containing catalytic amount of acetic acid afforded the corresponding Schiff bases (**13–19**), (Scheme 2).

Furthermore, reaction of compound **5** with ethyl 3-aminothieno [2,3-b] pyridine-2-carboxylate (**20**), ethyl

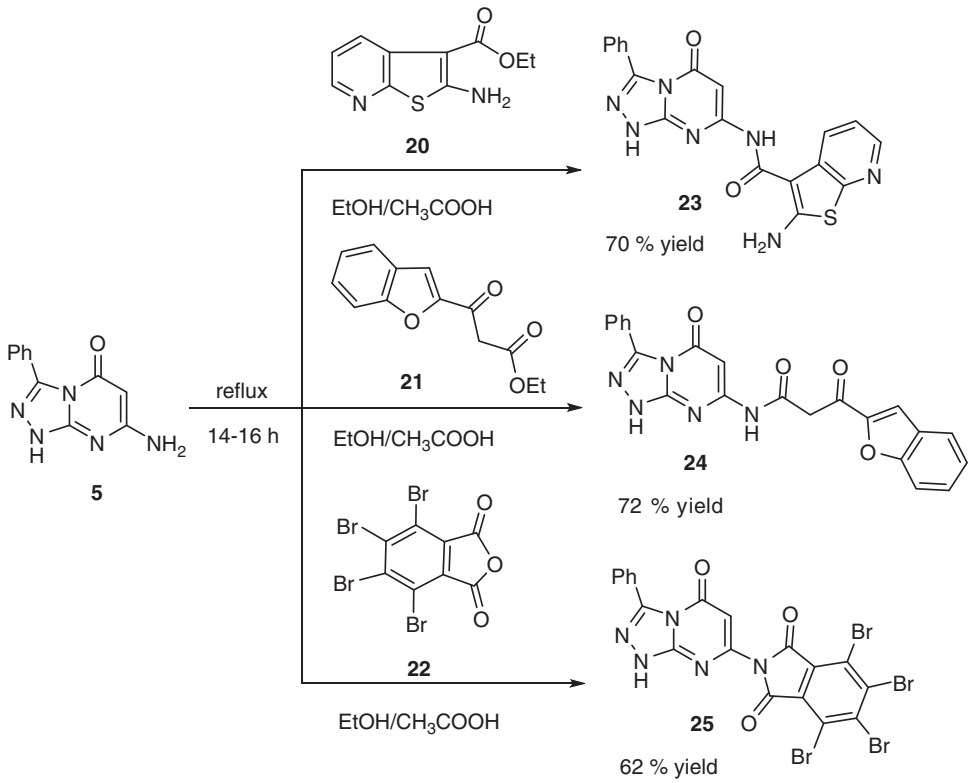
Scheme 1 Synthesis of 7-amino-3-phenyl-[1, 2, 4]triazolo [4,3-a] pyrimidin-5(1*H*)-one (**5**)



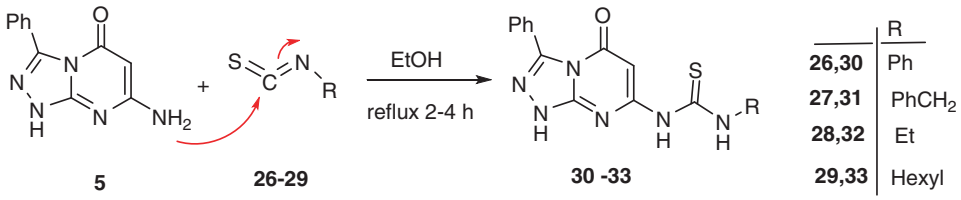
Scheme 2 Synthesis of (*E*)-3-phenyl-7-(substitutedmethyleneamino)-[1, 2, 4]triazolo [4,3-a] pyrimidin -5 (1*H*)-one (**13-19**)



Scheme 3 Synthesis of amides **23, 24**, and imide **25**



Scheme 4 Synthesis of thiourea derivatives **30–33**



4-(2,3-dihydro benzofuran-2-yl)-2,4-dioxobutanoate (**21**) or 4,5,6,7-tetrabromoiso-benzofuran-1,3-dione (**22**) in a mixture of absolute ethanol and few drops of glacial acetic acid as catalyst afforded the corresponding amides (**23**, **24**) and imide (**25**), respectively (Scheme 3).

Moreover, compound **5** was treated with isothiocyocyanate derivatives namely: phenyl- isothiocyanate (**26**), benzylisothiocyanate (**27**), ethylisothiocyanate (**28**) and cyclohexyl- isothiocyanate (**29**), respectively, under refluxing in ethanol to afford the corresponding thiourea derivatives (**30–33**), (Scheme 4). The structures of new compounds confirmed by elemental analyses and spectral data are presented in experimental Section.

Biological activity

In vitro Antimicrobial screening (Cruickshank et al. 1975) of the new product (Tables 1 and 2) indicated that compounds **23** and (**30–33**) were the most potent against *S. aureus*. While compounds (**23–25**) and (**30–33**) were the most active against *Bacillus subtilis*; showing moderate influence on *Pseudomonas aeruginosa*. Compounds (**30–33**) were active against *Escherichia coli* and also exhibited marked antifungal properties.

Structure–activity relationships (SAR)

All compounds were tested in vitro of antimicrobial activity, comparing the results obtained for the antimicrobial properties of the compounds reported in this study with their structures, the following SAR are postulated (Fig. 1): (i) It seems that most of the Schiff's bases, amids, imide and thiourea derivatives are more potent than the starting TP **5** which may be due to the presence of the N=CH–R, NHCOR and NHCSNHR moieties, respectively. (ii) The antimicrobial activities of Schiff's bases **13–15** follow the order **13** < **14** < **15**, which may be attributed to the electronic effect of the *p*-substituents, i.e, OCH_3>Cl>F by +M.

Table 1 Antibacterial activity of compounds [a, b, c]

Compounds	Gram positive bacteria		Gram negative bacteria	
	Staphylococcus aureus (RCMB 000106)[d]	*Bacillus subtilis* (RCMB 000107)	*Pseudomonas aeruginosa* (RCMB 000102)	*Escherichia coli* (RCMB 000103)
1	10.30 ± 0.52	8.85 ± 0.45	NA	NA
2	11.10 ± 0.32	9.10 ± 0.60	NA	NA
3	NA	NA	NA	NA
4	12.90 ± 0.10	10.40 ± 0.20	NA	7.10 ± 0.52
5	14.40 ± 0.28	12.80 ± 0.50	NA	7.80 ± 0.74
13	15.50 ± 0.62	14.50 ± 0.56	NA	8.20 ± 0.60
14	17.60 ± 0.81	15.10 ± 0.34	NA	9.50 ± 0.12
15	19.40 ± 0.27	16.60 ± 0.25	8.55 ± 0.28	10.22 ± 0.08
16	22.34 ± 0.10	20.70 ± 0.33	11.20 ± 0.42	13.60 ± 0.85
17	20.80 ± 0.05	18.80 ± 0.48	10.30 ± 0.82	12.45 ± 0.07
18	23.50 ± 0.50	22.40 ± 0.75	12.90 ± 0.35	14.20 ± 0.15
19	24.70 ± 0.35	24.10 ± 0.52	13.80 ± 0.55	15.30 ± 0.70
23	30.20 ± 0.52	33.80 ± 0.66	22.40 ± 0.25	24.50 ± 0.09
24	25.80 ± 0.15	26.90 ± 0.50	15.30 ± 0.54	18.40 ± 0.35
25	25.40 ± 0.28	25.50 ± 0.32	14.60 ± 0.70	16.70 ± 0.80
30	26.60 ± 0.08	28.60 ± 0.80	16.70 ± 0.40	19.10 ± 0.20
31	29.10 ± 0.35	32.50 ± 0.40	20.10 ± 0.65	23.60 ± 0.10
32	27.30 ± 0.24	29.40 ± 0.70	18.50 ± 0.50	20.80 ± 0.40
33	28.50 ± 0.40	30.10 ± 0.30	19.20 ± 0.80	21.50 ± 0.50
Penicillin G	30.50 ± 0.62	34.25 ± 0.85	29.45 ± 0.20	35.40 ± 0.55
Streptomycin	28 ± 0.30	30 ± 0.50	26 ± 0.30	27 ± 0.46

[a] Mean zone of inhibition in mm±standard deviation beyond well diameter (6 mm) produced on a range of environmental and clinically pathogenic microorganisms using (10 mg/mL) concentration of tested samples. The concentration used for the standard antibiotic was (30 µg/mL)

[b] the test was done using the diffusion agar technique. Well diameter: 6.0 mm (100 µL was tested)

[c] Data are expressed in the form of mean ± SD

[d] *RCMB* Regional center for mycology and biotechnology culture collection

[e] *NA* No activity

(iii) Compounds **23** and **30–33** have good antimicrobial activities, which may be attributed to presence of thiophene and thiourea moieties respectively. (iv) The antimicrobial activities of thiourea **30–33** follow the order **31 > 33 > 32 > 30**, which may be attributed to the withdrawing effect of phenyl moiety (vi) Compound 16 has good antimicrobial activities than the other Schiff 's bases, which may be attributed to presence of thiophene (Fig. 1).

Conclusions

7-amino-3-phenyl-[1, 2, 4] triazolo [4, 3-a] pyrimidin-5 (1*H*)-one **(5)** have potential to act as antimicrobial agent by modification of their structure. Introduction of C=N, CONH, CSNH group to these position vanished the antimicrobial activity as well as affecting the solubility. Further studies on the evaluation of relationship between bioactivities and structure are suggested.

Experimental

Materials and methods

All melting points are in degree centigrade (uncorrected) and were determined on Gallenkamp electric melting point apparatus. Thin layer chromatography (TLC) analysis was carried out on silica gel 60 F254 precoated aluminum sheets. The Infrared (IR) spectra were recorded (KBr) on a Perkin–Elmer 1430 spectrometer (λ, cm-1) in National Research Center, Egypt. ^{1}H-NMR,\$^{13}$C-NMR Spectra were measured on JEOL-ECA 500 and JEOL JNM-LA-400 FT NMR Spectrometers at 500, 125 MHz, respectively, using tetramethylsilane as an internal reference and DMSO-d6 as solvent at the Microanalytical Center in National Research

Table 2 Antifungal activity of compounds [a, b, c]

Compounds	Fungi			
	Aspergillus fumigatus (RCMB 002003)[d]	*Geotrichum candidum* (RCMB 052006)	*Candida albicans* (RCMB 005002)	*Syncephalastrum racemosum* (RCMB 005003)
1	NA	NA	NA	NA
2	NA	7.70 ± 0.60	NA	NA
3	NA	NA	NA	NA
4	8.10 ± 0.05	8.50 ± 0.80	7.10 ± 0.40	NA
5	9.50 ± 0.60	9.80 ± 0.50	8.60 ± 0.50	NA
13	10.10 ± 0.75	10.50 ± 0.85	9.80 ± 0.44	NA
14	11.30 ± 0.20	11.10 ± 0.10	10.20 ± 0.30	NA
15	12.80 ± 0.45	12.20 ± 0.25	11.90 ± 0.06	NA
16	15.20 ± 0.50	14.80 ± 0.60	13.70 ± 0.05	NA
17	14.60 ± 0.08	13.40 ± 0.09	12.30 ± 0.15	NA
18	16.82 ± 0.20	15.50 ± 0.40	14.80 ± 0.33	8.20 ± 0.60
19	17.40 ± 0.61	16.30 ± 0.81	15.60 ± 0.40	9.30 ± 0.40
23	25.10 ± 0.45	24.50 ± 0.60	23.20 ± 0.80	18.50 ± 0.10
24	19.70 ± 0.10	18.60 ± 0.30	18.20 ± 0.70	11.10 ± 0.50
25	18.20 ± 0.40	17.20 ± 0.55	16.10 ± 0.82	10.40 ± 0.70
30	20.10 ± 0.09	19.70 ± 0.10	19.10 ± 0.40	12.50 ± 0.60
31	24.20 ± 0.55	23.10 ± 0.85	22.40 ± 0.60	16.40 ± 0.30
32	21.50 ± 0.30	20.40 ± 0.20	20.20 ± 0.50	14.60 ± 0.80
33	22.80 ± 0.60	21.90 ± 0.50	21.50 ± 0.30	15.80 ± 0.20
Itraconazole	30 ± 0.08	29 ± 0.40	28 ± 0.05	24 ± 0.10
Clotrimazole	28 ± 0.20	25 ± 0.50	20 ± 0.30	22 ± 0.08

[a] Mean zone of inhibition in mm ± standard deviation beyond well diameter (6 mm) produced on a range of environmental and clinically pathogenic microorganisms using (10 mg/mL) concentration of tested samples. The concentration used for the standard antibiotic was (30 g/mL)

[b] the test was done using the diffusion agar technique. Well diameter: 6.0 mm (100 µL was tested)

[c] Data are expressed in the form of mean ± SD

[d] *RCMB* Regional center for mycology and biotechnology culture collection

[e] *NA* No activity

Center, Egypt. The mass spectra (EI) were recorded on GCMS-QP 1000 EX (Shimadzu) at National Research Center, Egypt. Elemental analyses (C, H and N) were carried out at the Microanalytical Center in National Research Center, Egypt. The elemental analyses were found to agree favorably with the calculated values. Biological activities were carried in Regional Center for Mycology and Biotechnology, Al-Azhar University, Nasr City, Cairo, Egypt.

Synthesis of N′*-(6-amino-1, 2, 3, 4-tetrahydro-4-oxopyrimidin-2-yl) benzo-hydrazide (**4**)*

To a solution of compound **1**, (1.43 g, 10 mmol) or **2** (1.57 g, 10 mmol) in DMF (30 ml) and ethanol (25 mL) respectively, benzohydrazide **3** (1.36 g, 10 mmol) was added. The reaction mixture was heated under reflux for 8–10 h, and then allowed to cool, poured into ice/water and the formed solid product was collected by filtration and crystallized from ethanol/benzene to give compound **4**; Yellow crystals, yield 73 % and m.p. >325 °C; IR (KBr): (ν/cm^{-1}) = 3360 (br, NH, NH_2), 3035 (CH–Ar), 1690, 1680 (2CO), 1622 (C=N); 1555 (C=C); ^{1}H NMR (DMSO-d_6): δ (ppm) = 4.66 (s, 1H, C_5-H), 6. 35 (br, NH_2, D_2O exchangeable) 7.48–7.90 (m, 5H, ArH): 11.51, 11.61 (br, 2NH, D_2O exchangeable), 12.10 (br, NH, D_2O exchangeable); ^{13}C–NMR (DMSO-d6): δ ppm) = 82.5 (C_5, pyrimidine), 127.5, 128.7, 132.1, 132.4 (6C, Ar–C), 158.6 (C_6, pyrimidine), 161.2 (C_4, pyrimidine), 164.5 (C_2 pyrimidine), 165.1(CONH); MS (EI, 70 eV): m/z (%) = 247 (M^++2, 2.1), 245 (M^+, 6.3), 242 (100), 241 (18.5), 229 (12.6), 196 (34.4), 184 (25.3), 156 (24.2), 125 (17.9), 110 (15.7), 97 (7.4), 68 (15.7), 67 (45.3), 59(11.6). Chemical Formula: $C_{11}H_{11}N_5O_2$ (245.24); Calcd. C, 53.87; H, 4.52; N, 28.56 %. Found: C, 53.80; H, 4.52; N, 28.56 %.

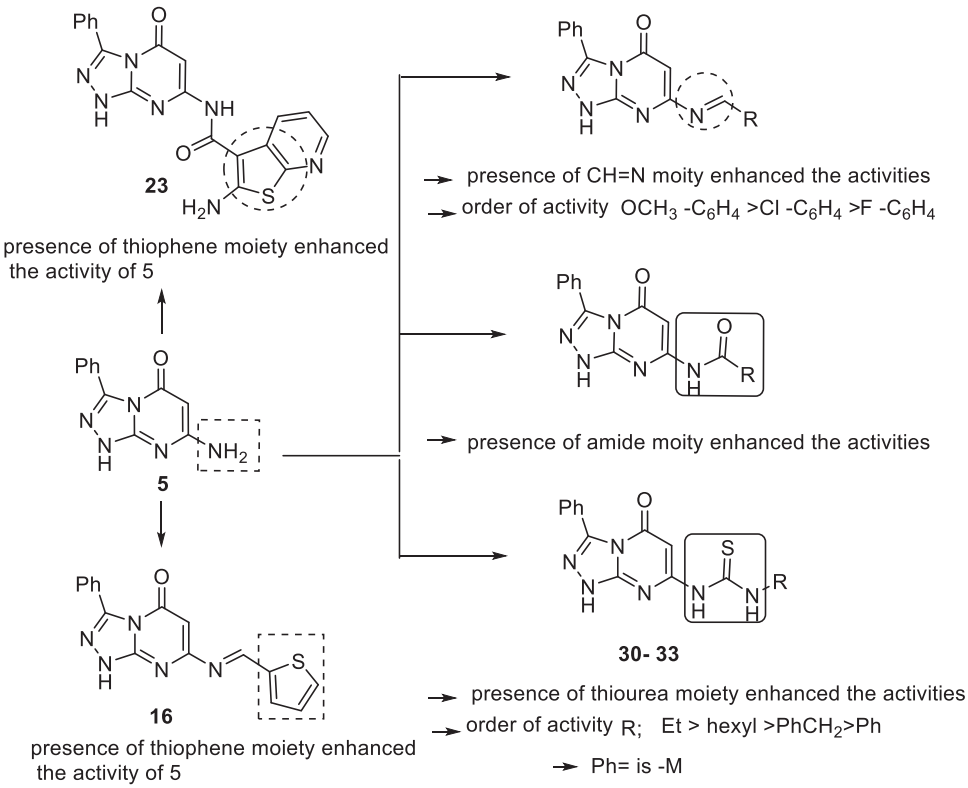


Fig. 1 SAR of the more potent antimicrobial triazolopyrimidines

*Synthesis of 7-amino-3-phenyl-[1, 2, 4]triazolo[4,3-a] pyrimidin-5 (1*H*)-one (**5**)*

A solution of compound **4** (2.45 g, 10 mmol) in ethanol (50 ml) containing sodium ethoxide (prepared by dissolving sodium metal (0.23 g, 10 mmol in ethanol) was heated under reflux for 9 h, the reaction mixture was cooled and the deposited precipitate was filtered off, washed with ethanol. The formed salt was dissolve in water (50 ml) and acidified with 10 % HCl, the formed precipitate was filtered, dried and crystallized from methanol to afford **5**; Yellowish crystals, 75 % and m.p. >325 °C; IR (KBr): (ν/ cm^{-1}) = 3312, 3352 (br, 2NH, NH_2), 3038 (CH aryl), 1675 (CO), 1620 (C=N), 1558(C=C); ^{1}H NMR (DMSO-d_6): δ (ppm) = 4.66 (s,1H,C_6-H), 6.34 (br, 2H, NH_2, D_2O exchangeable), 7.49–7.91 (m, 5H, phenyl) and 11.65 (br, 1H, NH, D_2O exchangeable); ^{13}C–NMR (DMSO-d_6): δ (ppm) = 83.1 (C_6, pyrimidine), 128.1, 128.6, 128.8, 131.2 (6C, Ar–C), 150.8 (C_3, triazole ring); 158.5 (C_7, pyrimidine), 160.4 (C_{8a}, pyrimidine); 162.5 (C_5, pyrimidine); MS (EI, 70 eV): m/z (%) = 229 (M^++2, 11.6) 227 (M^+, 20.2), 203 (27.9), 125 (100), 103 (18.5), 91 (51.1), 77 (37.0), 63 (74.4); Chemical Formula: $C_{11}H_9N_5O$ (227.22); Calcd.: C, 58.14; H, 3.99; N, 30.82 %; Found: C, 58.10; H, 3.95; N, 30.75 %.

*General procedure for synthesis of 7-(substituted-arylideneamino)-3-phenyl-[1, 2, 4] triazolo-[4,3-a] pyrimidin-5 (1*H*)-one (**13–17**)*

A mixture of compound (**5**) (2.27 g, 10 mmol) and 4-fluorobenzaldehyde (**6**) (1.24 g, 10 mmol), 4-chlorobenzaldehyde (**7**) (1.40 g, 10 mmol), 4-methoxybenzaldehyde (**8**) (1.36 g, 10 mmol), isonicotinaldehyde (**9**) (1.07 g, 10 mmol) or thiophene-2-carbaldehyde (**10**) (1.2 mL, 10 mmol) in dioxane (40 ml) containing a catalyst amount of piperidine (0.5 mL), was stirred and heated under reflux for 5–10 h (TLC control).The reaction mixture was cooled, the formed precipitate filtered off, dried and recrystallized from the appropriate solvent to afford (**13–17**).

*7-(4-Fluorobenzylideneamino)-3-phenyl-[1, 2, 4]triazolo [4,3-a]pyrimidin-5(1*H*)-one (**13**)*

Yellow crystals, yield 65 %, m.p. 240–242 °C, reaction time 5 h and crystallized from EtOH / benzene; IR (KBr): (ν/ cm^{-1}) = 3310 (br, NH), 3030 (CH aryl), 1670 (CO), 1615 (C=N), 1550 (C=C);^{1}H NMR (DMSO-d_6): δ (ppm) = 4.66 (s, 1H, C_6-H), 7.57–7.65 (m, 5H, phenyl), 7.82–7.84 (dd, 2H, J = 8.40 Hz, ArH), 7.93–7.95 (dd, 2H, J = 8.44 Hz, Ar–H), 8.23 (s, 1H, CH=N), 11.64 (br, NH, D_2O

exchangeable); ^{13}C-NMR (DMSO-d_6): δ (ppm) = 112.1 (C_6, pyrimidine), 128.3, 128.7, 128.9, 129.4, 130.5, 131.4, 132.8, 146.2 (12C, Ar–C), 151.3 (C_3, triazole), 158.1 (C_7, pyrimidine), 162.2 (C_{8a}, pyrimidine), 163.5 (CH=N) 165.8 (C_5, pyrimidine); Chemical Formula: $C_{18}H_{12}FN_5O$ (333.32); Calcd.: C, 64.86; H, 3.63; N, 21.01 %; Found: C, 64.80; H, 3.60; N, 21.08 %.

*7-(4-Chlorobenzylideneamino)-3-phenyl-[1, 2, 4]triazolo [4,3-a]pyrimidin-5(1*H*)-one (**14**)*

Brown powder, yield 70 %, m.p. 290–292 °C, reaction time 7 h and crystallized from MeOH; IR (KBr): (ν/cm^{-1}) = 3312 (br, NH), 3025 (CH aryl), 1672 (CO), 1618 (C=N), 1552 (C=C); ^{1}H NMR (DMSO-d_6): δ (ppm) = 4.67 (s, 1H, C_6-H), 7.56–7.64 (m, 5H, phenyl), 7.83–7.85 (dd, 2H, J = 8.30 Hz, ArH), 7.94–7.96 (dd, 2H, J = 8.33 Hz, ArH), 8.23 (s, 1H, CH=N) and 11.65 (br, NH, D_2O exchangeable); ^{13}C-NMR (DMSO-d_6): δ (ppm) = 112.1 (C_6, pyrimidine), 128.2, 128.6, 128.8, 129.1, 130.1, 130.8, 132.1, 136.8 (12C, Ar–C), 151.6 (C_3, triazole), 157.8 (C_7, pyrimidine), 161.1(C_{8a}, pyrimidine), 162.7 (CH=N), 163.1 (C_5, pyrimidine); Chemical Formula: $C_{18}H_{12}ClN_5O$ (349.77); Calcd.: C, 61.81; H, 3.46; N, 20.02 %; Found: C, 61.85; H, 3.40; N, 20.10 %.

*7-(4-Methoxybenzylideneamino)-3-phenyl-[1,2,4]triazolo [4,3-a]pyrimidin-5(1*H*)-one(**15**)*

White crystals, yield 64 %, m. P. 260–262 °C, reaction time 6 h and crystallized from EtOH / benzene; IR (KBr): (ν/cm^{-1}) = 3315 (br, NH), 3032 (CH aryl), 1671 (CO), 1619 (C=N), 1551(C=C); ^{1}H NMR (DMSO-d_6): δ (ppm) = 3.85 (s, 3H, OCH_3), 4.67 (s, 1H, C_6-H), 7.55–7.63 (m, 5H, phenyl), 7.81–7.83 (dd, 2H, J = 8.36 Hz, Ar–H), 7.92–7.94 (dd, 2H, J = 8.39 Hz, Ar–H), 8.24 (s, 1H, CH=N), 11.66 (br, NH, D_2O exchangeable); ^{13}C-NMR (DMSO-d_6): δ (ppm) = 55.9 (OCH_3), 112.2 (C_6, pyrimidine), 124.5, 126.5, 128.4, 128.8, 129.5, 130.8, 131.6, 149.1 (12C, Ar–C), 151.5 (C_3, triazole), 158.4 (C_7, pyrimidine), 162.8 (C_{8a}, pyrimidine), 163.7 (CH=N). 165.1 (C_5, pyrimidine); MS (EI, 70 eV): m/z (%) = 345(M^+, 44.4), 270 (22.1), 230 (33.2), 195(100), 139 (66.4), 59(88.8); Chemical Formula: $C_{19}H_{15}N_5O_2$ (345.35); Calcd.; C, 66.08; H, 4.38; N, 20.28 Found: C, 66.10; H, 4.38; N, 20.32 %.

*3-Phenyl-7-(thiophen-2-ylmethyleneamino)-[1,2,4]triazolo [4,3-a]pyrimidin-5(1*H*)-one (**16**)*

Yellowish crystals, yield 85 %; m. P. 226–228 °C, reaction time 10 h and crystallized from MeOH; IR (KBr): (ν/cm^{-1}) = 3320 (br, NH), 3030 (CH aryl), 1680 (CO), 1618 (C=N),1555 (C=C); ^{1}H NMR (DMSO-d_6): δ (ppm) = 4.67 (C_6-H, pyrimidine), 7.25 (t, 1H, J = 2.20 Hz, thiophene ring), 7.34–7.42 (dd, 2H, J = 2.24 Hz, thiophene ring), 7.52–7.88 (m, 5H, phenyl), 8.24 (s, 1H, CH=N) and 11.68 (br, NH, D_2O exchangeable); ^{13}C-NMR (DMSO-d_6): δ (ppm) = 112.5 (C_6, pyrimidine), 127.4, 128.2, 128.6, 128.7, 128.8, 130.1, 130.3, 144.5 (10 C, aromatic carbon atoms), 150.8 (C_3, triazole), 158.8 (C_7, pyrimidine), 162.5 (C_{8a}, pyrimidine),163.6 (CH=N) and 165.2 (C_5, pyrimidine); Chemical formula: $C_{16}H_{11}N_5OS$ (321.36)); Calcd.: C, 59.80; H, 3.45; N, 21.79 %; Found: C, 59.75; H, 3.40; N, 21.70 %.

*3-Phenyl-7-(pyridin-4-ylmethyleneamino)-[1, 2, 4]triazolo [4,3-a]pyrimidin-5(1*H*)-one (**17**)*

White powder in 75 % yield, m.p. 302–304 °C, reaction time 8 h and crystallized from MeOH; IR (KBr): (ν/cm^{-1}) = 3317 (br, NH), 3028 (CH aryl), 1677 (CO), 1615 (C=N) and 1550 (C=C); ^{1}H NMR (DMSO-d_6): δ (ppm) = 4.68 (C_6-H, pyrimidine), 7.04–7.12 (dd, 2*H*, J = 8.10 Hz, pyridine ring), 7.25–7.33 (dd, 2*H*, J = 8.14 Hz, pyridine ring), 7.50–7.85 (m, 5H, phenyl), 8.25 (s, 1H, CH=N); 11.65 (br, NH, D_2O exchangeable); ^{13}C-NMR (DMSO-d_6): δ (ppm) = 111.9 (C_6, pyrimidine), 114.5, 116.8, 118.9, 124.4, 127.2, 131.5, 137.7 (11C, Ar–C), 149.6 (C_3, triazole), 158.7 (C_7, pyrimidine), 159.1(C_{8a}, pyrimidine), 159.3 (CH=N) 159.9 (C_5, pyrimidine); Chemical formula: $C_{17}H_{12}N_6O$ (316.32); Calcd.: C, 64.55; H, 3.82; N, 26.57 %; Found: C, 64.50; H, 3.78; N, 26.50 %.

*General procedure for synthesis of 7-((5-chloro-3-methyl-1-phenyl-1*H*-pyrazol-4-yl)methyleneamino)-3-phenyl-[1,2,4] triazolo [4,3-a]pyrimidin-5(1*H*)-one (**18**) and 7-((2-Azido-6-methylquinolin-3-yl)methyleneamino)-3-phenyl[1,2,4] triazolo[4,3-a] pyrimidin-5(1*H*)-one (**19**)*

To a suspension of compound **5** (2.27 g, 10 mmol) in glacial acetic acid (50 mL) or in mixture of absolute ethanol (35 mL) containing glacial acetic acid (1 mL), 5-chloro-3-methyl-1-phenyl-1*H*-pyrazole-4-carbaldehyde (**16**) (2.20 g, 10 mmol) or ethyl 2-azido-6-methylquinoline-3-carbaldehyde (**17**) (2.12 g, 10 mmol) was added, respectively. The reaction mixture was stirred and heated under reflux for 10 and 15 h, respectively. The reaction mixture was cooled, the formed precipitate filtered off, dried and recrystallized from the appropriate solvent to afford **18** and **19**.

*7-((5-Chloro-3-methyl-1-phenyl-1*H*-pyrazol-4-yl) methyleneamino)-3-phenyl-[1, 2, 4] triazolo [4, 3-a] pyrimidin-5(1*H*)-one (**18**)*

Brown crystals, yield 60 %, m. P. 340–342 °C, reaction time 6 h and crystallized from EtOH; IR (KBr): (ν/cm^{-1}) = 3322

(br, NH), 3035(CH aryl), 2995(CH aliph.), 1685 (CO), 1615 (C=N), 1552 (C=C); ^{1}H NMR (DMSO-d_6): δ (ppm) = 2.35 (s, 3H, CH_3), 4.66 (s, 1H, C_6-H, pyrimidine), 7.21–7.92 (m,10H, Ar–H), 8.23 (s, 1H, CH=N), 11.69 (br, NH, D_2O exchangeable); ^{13}C-NMR (DMSO-d_6): δ (ppm) = 15.2 (CH_3), 112.4 (C_6, pyrimidine), 116.8, 121.2, 128.3, 128.5, 128.9, 129.8, 130.3, 143.6 (12C, Ar–C), 144.5, 146.1, 149 (3C, pyrazole ring), 151.1 (C_3, triazole), 158.4 (C_7, pyrimidine), 162.5 (C_{8a}, pyrimidine), 163.7(CH=N), 165.9 (C_5, pyrimidine); MS (EI, 70 eV): m/z (%) = 430 (M^+, 35.7), 384 (100), 342 (21.4), 125 (35.7), 60 (42.8); Chemical Formula: $C_{22}H_{16}ClN_7O$ (429.87)); Calcd.: C, 61.47; H, 3.75; N, 22.81 %; Found: C, 61.55; H, 3.70; N, 22.76 %.

*7-((2-Azido-6-methylquinolin-3-yl)methyleneamino)-3-phenyl[1,2,4]triazolo[4,3-a] pyrimidin-5(1*H*)-one (**19**)*

White crystals, 55 % yield, m.p. >350 °C (melted) and crystallized from benzene; IR (KBr): (ν/cm^{-1}) = 3320 (br, NH), 3030(CH aryl), 2992 (CH aliph.), 1682 (CO), 1617 (C=N), 1551 (C=C); ^{1}H NMR (DMSO-d_6): δ (ppm) = 2.35 (s, 3*H*, CH_3), 4.68 (C_6-H, pyrimidine), 7.45–7.52 (dd,2*H*, *J* = 8.55 Hz, quinoline ring), 7.60 (s,1*H*, quinoline ring), 7.64–7.94 (m,5*H*, phenyl), 8.25 (s, 1*H*, CH=N); 11.64 (br, NH, D_2O exchangeable); ^{13}C-NMR (DMSO-d_6): δ (ppm) = 38.5 (1C, CH_3), 81.9 (C_6, pyrimidine), 121.1, 124.8, 126.2, 128.5, 128.6, 128.7, 129.2, 130.2, 131.5, 135.6, 136.5, 146.5, 147.1 (15C, Ar–C), 151.5 (C_3, triazole), 161.1 (C_7, pyrimidine), 162.4 (C_{8a}, pyrimidine), 163.5 (CH=N) 169.1 (C_5, pyrimidine); MS (EI, 70 eV): *m/z* (%) = 423 (M^++2, 98.0), 421 (M^+, 100), 379 (20.0), 353 (27.3), 325 (15.5), 307(19.1), 274 (11.8), 213 (20.1), 121 (19.0), 77 (22.7); Chemical Formula: $C_{22}H_{15}N_9O$ (421.42); Calcd.: C, 62.70; H, 3.59; N, 29.91 %; Found: C, 62.65; H, 3.50; N, 29.85 %.

*General procedure for synthesis of 2-amino-*N*-(1,5-dihydro-5-oxo-3-phenyl-[1,2,4] triazolo [4,3-a]pyrimidin-7-yl)benzo[b]thiophene-3-carboxamide (**23**) 3-(Benzofuran-2-yl)-3-oxo-*N*-(5-Oxo-3-phenyl-1,5-dihydro-[1,2,4]triazolo [4,3-a]pyrimidin-7-yl)propanamide (**24**) and 4,5, 6,7-tetrabromo-2-(1,5-dihydro-5-oxo-3-phenyl-[1,2,4]triazolo [4,3-a]pyrimidin-7-yl)isoindoli ne-1,3-dione(**25**)*

A mixture of **5** (2.27 g, 10 mmol) and ethyl 3-aminothieno [2, 3-b] pyridine-2-carboxylate (**20**), (2.22 g, 10 mmol), ethyl 4-(2,3-dihydrobenzofuran-2-yl)-2,4-dioxobutanoate (**21**) (2.62 g,10 mmol) or 4,5,6,7-tetrabromoisobenzofuran-1,3-dione (**22**), (4.63 g,10 mmol) in mixture of absolute ethanol (40 mL) containing glacial acetic acid (1 mL) was refluxed for 14–16 h. The reaction mixture was allowed to cool to room temperature, filtered off, washed with ethanol to give **23–25.**

*2-Amino-*N*-(1,5-dihydro-5-oxo-3-phenyl-[1,2,4]triazolo [4,3-a]pyrimidin-7-yl)benzo[b] thiophene-3-carboxamide (**23**)*

Yellowish crystals, yield 70 %, m.p. >350 °C, reaction time 6 h and crystallized from dioxane; IR (KBr): (ν/cm^{-1}) = 3380 (br, 2NH, NH_2), 3040 (CH aryl), 1695, 1684 (2CO), 1625 (C=N); 1556 (C=C); ^{1}H NMR (DMSO-d_6): δ (ppm) = 4.68 (s, 1H, C_6-H, pyrimidine), 6. 35 (br, NH_2, D_2O exchangeable), 7.12 (t,1H, *J* = 8.20 Hz, pyridine ring), 7.15 (d,1H, *J* = 8.22 Hz, pyridine ring), 7.18 (d,1H, *J* = 8.23 Hz, pyridine ring), 7.48–7.91 (m,5H, phenyl), 11.51 (br, NH, D_2O exchangeable); 11.61 (br, NH, D_2O exchangeable); ^{13}C-NMR (DMSO-d_6): δ (ppm) = 90.4 (C_6, pyrimidine), 121.4, 128.2, 128.4, 128.8, 129.1, 129.6, 130.1, 131.4, 137.5, 145.5, 152.4 (14C, Arc), 151.5 (C_3, triazole), 157.1 (C_7, pyrimidine), 161.3 (C_{8a}, pyrimidine), 164.5 (C_5, pyrimidine), 165.8 (CONH); MS (EI, 70 eV): *m/z* (%) = 405 (M^++2, 29.3), 403 (M^+, 33.5), 366 (26.8), 342 (19.5), 302 (21.9), 279 (9.7), 220(36.6), 153(9.7), 125 (100), 115 (9.7), 63 (4.8); Chemical formula: $C_{19}H_{13}N_7O_2S$ (403.42)); Calcd.: C, 56.50; H, 3.20; N, 24.26 %; Found: C, 56.55; H, 3.16; N, 24.20 %.

*3-(Benzofuran-2-yl)-3-oxo-N-(5-oxo-3-phenyl-1,5-dihydro-[1,2,4]triazolo[4,3-a]pyrimidin-7-yl)propanamide (**24**)*

Yellow crystals, yield 72 %, m.P.162–164 °C, reaction time 10 h and crystallized from MeOH; IR (KBr): (ν/cm^{-1}) = 3395 (br, 2NH), 3045 (CH aryl), 2994 (CH aliph.), 1740, 1730, 1694, 1685 (4CO), 1624 (C=N),1552 (C=C); ^{1}H NMR (DMSO-d_6): δ (ppm) = 4.15 (s, 2H, CH_2), 4.67 (s, 1*H*, C_5-H, pyrimidine), 5.15 (s, 1H, benzofuran),7.01–7.25 (m,4*H*, benzofuran), 7.50–7.90 (m, 5H, phenyl), 11.52 (br, NH, D_2O exchangeable); 11.62 (br, NH, D_2O exchangeable); ^{13}C-NMR (DMSO-d_6): δ (ppm) = 62.8 (CH_2), 90.4 (C_6, pyrimidine), 114.2, 121.1, 125.2, 127.3, 128.4, 128.6, 128.8,129.9,130.3, 151.2, 152.1 (13C, Ar–C), 154.2 (C_3, triazole), 158.5 (C_7, pyrimidine), 160.5 (C_{8a}, pyrimidine), 163.6 (C_5, pyrimidine), 165.8 (CONH) 190.1 (COCO), 198.8 (CH_2CO); MS (EI, 70 eV): *m/z* (%) = 413 (M^+, 45.5), 414 (12.7), 415 (9.1), Chemical formula: $C_{22}H_{15}N_5O_4$ (413.39); Calcd.: C, 63.92; H, 3.66; N, 16.94 %; Found: C, 63.85; H, 3.75; N, 16.82 %.

*4,5,6,7-Tetrabromo-2-(1,5-dihydro-5-oxo-3-phenyl-[1,2,4] triazolo[4,3-a]pyrimidin-7-yl) isoindoline-1,3-dione (**25**)*

Yellowish crystals in 62 % yield, m.p. 232–234 °C, reaction time 14 h and crystallized from DMF; IR (KBr):

(ν/cm^{-1}) = 3332 (br, NH), 3042 (CH aryl), 1720, 1702, 1682 (3CO), 1622 (C=N), 1554(C=C); ^{1}H NMR (DMSO-d_6): δ (ppm) = 4.68 (s, 1*H*, C_5-H, pyrimidine), 7.48–7.90 (m, 5H, phenyl), 11.66 (br, NH, D_2O exchangeable); ^{13}C-NMR (DMSO-d_6): δ (ppm) = 90.2 (C_6, pyrimidine), 125.1, 128.2, 128.5, 128.9, 129.8, 130.2, 139.4 (12C, Ar–C) 150.5 (C_3, triazole), 153.4 (C_7, pyrimidine), 156.8 (C_{8a}, pyrimidine), 162.9 (C_5, pyrimidine), 166.2 (2CON); Chemical formula: $C_{19}H_7Br_4N_5O_3$ (672.91)); Calcd.: C, 33.91; H, 1.05; N, 10.41 %; Found: C, 33.85; H, 1.10; N, 10.50 %.

General procedure for synthesis of 1-(5-oxo-3-phenyl-1,5-dihydro-[1,2,4] triazolo [4,3-a] pyrimidin-7-yl)-3-phenyl thiourea (**30**), 1-benzyl-3-(5-oxo-3-phenyl-1,5-dihydro-[1,2,4] tria zolo[4,3-a]pyrimidin-7-yl)thiourea (**31**), 1-ethyl-3-(5-oxo-3-phenyl-1,5-dihydro-[1,2,4] tria zolo[4,3-a] pyrimidin-7-yl)thiourea (**32**), 1-cyclohexyl-3-(5-oxo-3-phenyl-1, 5-dihydro-[1, 2,4] triazolo[4,3-a]pyrimidin-7-yl)thiourea (**33**); A mixture of **5** (2.27 g, 10 mmol) and the appropriate phenylisothiocyanate **(26),** (1.35 g, 10 mmol), benzylisothiocyanate **(27)**, (1.49 g, 10 mmol), ethylisothoicyanate **(28),** (0.87 g, 10 mmol) or cyclohexylisothiocyanate **(29)** (1.41 g, 10 mmol) in absolute ethanol (30 ml) was refluxed for 2–4 h (TLC) and then left to cool. The precipitate which formed was filtered off, dried and crystallized from the appropriate solvent to give (**30–33**).

*1-(5-Oxo-3-phenyl-1,5-dihydro-[1,2,4] triazolo [4,3-a] pyrimidin-7-yl)-3-phenyl thiourea (**30**)*

White crystals, yield 66 %; m.p. 206–208 °C, reaction time 2 h and crystallized from MeOH; IR (KBr): (ν/cm^{-1}) = 3335, 3255, 3220 (3NH), 3040 (CH aryl), 1680 (CO), 1355 (C=S), 1621 (C=N), 1552 (C=C); ^{1}H NMR (DMSO-d_6): δ (ppm) = 4.67 (s, 1H, C_5-H, pyrimidine), 7.01–7.90 (m,10H, Ar–H), 11.51 (br, NH, D_2O exchangeable), 11.62 (br, NH, D_2O exchangeable), 12.10 (br, NH, D_2O exchangeable); ^{13}C-NMR (DMSO-d_6): δ (ppm) = 92.5 (C_6, pyrimidine), 126.4, 128.1, 128.2, 128.5,128.9, 129.2, 130.5,138.8 (12 C, Ar–C), 150.2 (C_3, triazole), 153.2 (C_7, pyrimidine), 157.4 (C_{8a}, pyrimidine), 163.5 (C_5, pyrimidine), 176.5 (C=S); Its MS: [M^+], m/z 362 (90 %); Chemical formula: $C_{18}H_{14}N_6OS$ (362.41); Calcd.: C, 59.65; H, 3.89; N, 23.19 %; Found: C, 59.60; H, 3.80; N, 23.14 %.

*1-Benzyl-3-(5-oxo-3-phenyl-1, 5-dihydro-[1, 2, 4]triazolo [4,3-a]pyrimidin-7-yl)thiourea (**31**)*

Yellowish crystals, yield 60 %, m.p. 174–176 °C, reaction time 4 h and crystallized from acetone; IR (KBr): (ν/cm^{-1}) = 3334, 3254, 3221 (3NH), 3035 (CH aryl), 2985 (CH aliph), 1682 (CO), 1352 (C=S), 1622 (C=N), 1551 (C=C); ^{1}H NMR (DMSO-d_6): δ (ppm) = 4.66 (s, 1*H*, C_5-H, pyrimidine), 4.82 (d, 2*H*, CH_2-Ph), 7.02–7.95 (m, 10*H*, ArH), 11.50 (br, NH, D_2O exchangeable), 11.60 (br, NH, D_2O exchangeable), 12.12 (br, NH, D_2O exchangeable); ^{13}C-NMR (DMSO-d_6): δ (ppm) = 60.1 (1C, CH_2), 95.1 (C_6, pyrimidine), 126.6,126.9,128.2, 128.4, 128.6,128.8, 130.2,138.1 (12C, Ar–C), 151.5 (C_3, triazole), 154.4 (C_7, pyrimidine), 158.2 (C_{8a}, pyrimidine), 164.1 (C_5, pyrimidine), 180.8 (C=S); MS (EI, 70 eV): *m/z* (%) = 378 (M^++2, 1.3) 366 (M^+, 10.5), 253 (3.9), 239 (3.4), 112 (13.6), 57 (100); Chemical formula: $C_{19}H_{16}N_6OS$ (376.43); Calcd.: C, 60.62; H, 4.28; N, 22.33 %; Found: C, 60.55; H, 4.20; N, 22.27 %.

*1-Ethyl-3-(5-oxo-3-phenyl-1, 5-dihydro-[1, 2, 4]triazolo [4,3-a] pyrimidin-7-yl)thiourea (**32**)*

White crystals in 70 % yield, m.p. 190–192 °C, reaction time 2 h and crystallized from dioxane; IR (KBr): (ν/cm^{-1}) = 3330, 3250, 3220 (3NH), 3040 (CH aryl), 2975 (CH aliph), 1684 (CO),1355 (C=S), 1618 (C=N), 1550 (C=C); ^{1}H NMR (DMSO-d_6): δ (ppm) = 1.32 (t, 3*H*, CH_3), 4.45 (q, 2*H*, CH_2), 4.67 (s, 1*H*, C_5-H, pyrimidine), 7.52–7.92 (m, 5*H*, phenyl), 11.52 (br, NH, D_2O exchangeable), 11.61 (br, NH, D_2O exchangeable), 12.11 (br, NH, D_2O exchangeable); ^{13}C-NMR (DMSO-d_6): δ (ppm) = 18.5 (CH_3), 55.5 (CH_2), 91.2(C_6, pyrimidine), 128.3, 128.5, 128.9, 130.4 (6C, Ar–C), 150.2 (C_3, triazole), 158.5 (C_7, pyrimidine), 159.5 (C_{8a}, pyrimidine), 165.5 (C_5, pyrimidine), 185.5 (C=S); MS (EI, 70 eV): *m/z* (%) = 314 (M^+, 30.1), 239 (45.7), 165 (40.0), 125 (85.1), 55(100); Chemical formula: $C_{14}H_{14}N_6OS$ (314.37); Calcd.: C, 53.49; H, 4.49; N, 26.73 %; Found: C, 53.40; H, 4.40; N, 26.65 %.

*1-Cyclohexyl-3-(5-oxo-3-phenyl-1,5-dihydro-[1,2,4] triazolo[4,3-a]pyrimidin-7-yl)thiourea (**33**)*

Yellowish crystals, yield 61 %, m.p. 217–219 °C, reaction time 4 h and crystallized from methanol; IR (KBr): (ν/cm^{-1}) = 3340, 3258, 3230 (3NH), 3035 (CH aryl), 2970 (CH aliph.), 1682 (CO), 1358(C=S), 1615 (C=N), 1554 (C=C);^{1}H NMR (DMSO-d_6): δ (ppm) = 1.21–1.75 (m, 10*H*, cyclohexyl), 2.65 (m, 1*H*, cyclohexyl), 4.68 (s, 1*H*, C_5-H, pyrimidine),7.50–7.90 (m, 5H, phenyl), 11.53 (br, NH, D_2O exchangeable), 11.64 (br, NH, D_2O exchangeable), 12.13 (br, NH, D_2O exchangeable); ^{13}C-NMR (DMSO-d6,δ,ppm); 25.5, 25.8, 32.8, 55.1(6C, cyclohexyl), 94.1(C_6, pyrimidine), 128.2, 128.6, 128.8, 130.2 (6C, Ar–C), 149.8 (C_3, triazole), 155.2 (C_7, pyrimidine), 161.5 (C_{8a}, pyrimidine), 166.2 (C_5, pyrimidine), 184.2 (C=S); Chemical formula: $C_{18}H_{20}N_6OS$ (368.46); Calcd.: C, 58.68; H, 5.47; N, 22.81 %; Found: C, 58.60; H, 5.41; N, 22.75 %.

Microbiology

Evaluation of in vitro antibacterial and antifungal activity

The tested compounds were evaluated by the agar diffusion technique (Cruickshank et al. 1975) using a 2 mg mL^{-1} solution in DMSO. The test organisms were four bacterial strains: *Staphylococcus aureus* and *Bacillus subtilis* (as Gram positive bacteria) and *Pseudomonas aeruginosa, Escherichia coli* and *Salmonella typhi* (as Gram negative bacteria). And two fungi: *Aspergillus fumigatus*, *Geotrichum candidum*, *Candida albicans*, *Syncephalastrum racemosum*. A control using DMSO without the test compound was included for each organism. Penicillin G, and Streptomycin were purchased form the Egyptian market and used in a concentration 10 mg/mL as reference drugs for antibacterial activity, whereas, Itraconazole, Clotrimazole were used as reference drugs for antifungal activity. The bacteria and fungi were tested on nutrient agar and potato dextrose agar media, respectively. Three plates were used for each compound as replicates. The plates were incubated for 24 h and seven days for bacteria and fungi, respectively. After the incubation period, the diameter of inhibition
zone was measured as an indicator for the activity of the compounds.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no competing interests.

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