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FULL PAPER

Facile green one-pot synthesis of novel thiazolo[3,2-a]pyrimidine derivatives using Fe_3O_4@L-arginine and their biological investigation as potent antimicrobial agents

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Thiazolopyrimidine derivatives are well known because of their excellent therapeutic properties. In this investigation, an effective one-pot three-component method is described for the synthesis of novel 2-[(*Z*)-1-(substituted phenyl)methylidine]-7-methyl-3-oxo-5-(substituted phenyl)-2,3-dihydro-5*H*-thiazolo[3,2-a]pyrimidine-6-carboxilic acid *tert*-butyl ester derivatives by condensation reaction of 3,4-dihydropyrimidine-2(1*H*)-thiones, various aromatic aldehydes and chloroacetyl chloride under reflux conditions in the presence of Fe_3O_4@L-arginine nanoparticles as a magnetically reusable and eco-friendly catalyst with short reaction times and moderate yields. The chemical structures of all synthesized compounds were determined using infrared, ^{1}H NMR and ^{13}C NMR spectroscopies. *In vitro* antimicrobial activities of 3,4-dihydropyrimidine-2(1*H*)-thiones and newly fused thiazolo[3,2-a]pyrimidine derivatives were examined using the well diffusion method against diverse pathogenic strains, namely *Staphylococcus aureus* ATCC 6538, *S. epidermidis* ATCC 12228, *Escherichia coli* ATCC 8739 and *Pseudomonas aeruginosa* ATCC 9027 (bacteria), *Candida albicans* ATCC 10231 (yeast) and *Aspergillus niger* ATCC 16404 (fungus). The compounds having 2-hydroxy, 4-hydroxy, 2-chloro and 4-chloro groups attached to the phenyl ring on the pyrimidine and 4-CH_3, 4-OCH_3 and 3-NO_2 groups attached to benzylidine on the thiazolo moiety showed significant antibacterial activity.

KEYWORDS

amino acid, antimicrobial activity, multi-component reaction, thiazolo[3,2-a]pyrimidine

1 | INTRODUCTION

Antimicrobial agents used to treat infectious diseases caused by various pathogenic strains (bacteria, fungi, parasites and viruses) are essential medicines for human and animal health.[1] The treatment of infection still is a significant and challenging subject because of resistance to current therapeutic agents and emergence of infectious diseases.[2] Pyrimidines as constituents of DNA and RNA represent diverse pharmacological agents as beneficial bactericides and fungicides.[3–5] Thiazole ring unit as a common structural feature is employed in various pharmaceutically significant molecules.[6] This heterocyclic moiety in condensed fused systems has exhibited a wide range of biological activity.[2,7] Diverse literature reports demonstrate many medicinal applications of abundant fused pyrimidine ring systems, such as anticancer,[8–10] antibacterial,[11,12] antiviral,[13] antifungal[14,15] and anti-inflammatory[16] agents. As both heterocyclic moieties, thiazolo and pyrimidine, have shown medicinal activities, a combination of the two is expected to have excellent biological features. The Biginelli reaction is one of the important multi-component methods for the synthesis of dihydropyrimidines by the one-pot cyclocondensation of aldehydes, β-ketoesters and urea or thiourea. Because of the various pharmacological and medicinal properties of 3,4-dihydropyrimidine-2(1*H*)-thione derivatives, diverse

techniques have been used for the preparation of these compounds using a variety of catalysts such as Lewis and Brønsted acids, heterogeneous acid catalysts and ionic liquids.[17–22]

Recently, we have reported the green synthesis of 3,4-dihydropyrimidine-2(1*H*)-thione derivatives in good yield by the reactions of thiourea, *tert*-butyl acetoacetate and aromatic aldehydes under solvent-free conditions in the presence of L-proline-*N*-sulfonic acid-functionalized magnetic nanoparticles.[23] Pursuant to the above considerations, in the research presented here, we concentrated on developing an innovative, easy and effective synthetic methodology for the quick synthesis and transformation of pyrimidinthione derivatives to new analogues of 2-[(*Z*)-1-(substituted phenyl)methylidine]-7-methyl-3-oxo-5-(substituted phenyl)-2,3-dihydro-5*H*-thiazolo[3,2-a]pyrimidine-6-carboxilic acid *tert*-butyl acetoacetate derivatives through the one-pot three-component reaction of 3,4-dihydropyrimidine-2(1*H*)-thiones with chloroacetyl chloride and various aromatic aldehydes using Fe_3O_4@L-arginine as a retrievable, non-toxic and environmentally compatible catalyst in ethanol under reflux conditions with moderate yields. All the novel compounds were characterized using spectroscopic analyses and investigated for their antifungal and antibacterial activities.

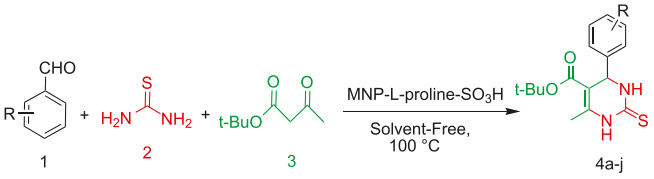
2 | RESULTS AND DISCUSSION

2.1 | Synthesis and characterization of thiazolo[3,2-a] pyrimidine derivatives

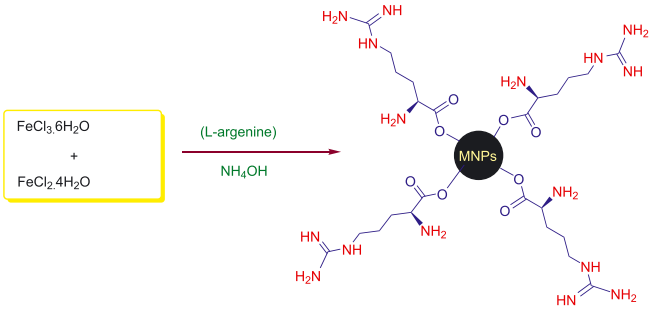
Firstly, 3,4-dihydropyrimidin-2(1*H*)-thione derivatives (**4a–j**) as starting materials were synthesized by one-pot three-component condensation reaction (Biginelli reaction) of thiourea, aromatic aldehydes and *tert*-butyl acetoacetate in the presence of Fe_3O_4@L-proline-SO_3H magnetite nanoparticles under solvent-free conditions using a facile procedure (Scheme 1).[23]

L-Arginine-coated magnetic nanoparticles (Fe_3O_4@L-arginine) were prepared by chemical co-precipitation of $FeCl_3{\cdot}6H_2O$ and $FeCl_2{\cdot}4H_2O$ salt solutions in the presence of L-arginine according to a reported procedure (Scheme 2).[24]

Next, novel *tert*-butyl-2-[(*Z*)-1-(substituted phenyl)methylidine]-7-methyl-3-oxo-5-(substituted phenyl)-2,3-dihydro-5*H*-thiazolo[3,2-a]pyrimidine-6-carboxylate derivatives (**7a–j**) were synthesized by condensation of **4a–j** with chloroacetyl chloride and aromatic aldehydes (Scheme 3).

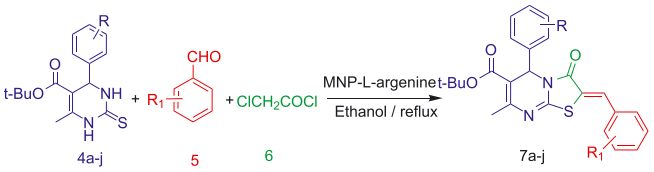


SCHEME 1 Synthesis of 3,4-dihydropyrimidine-2-(1*H*)-thiones



SCHEME 2 Preparation of Fe_3O_4@L-arginine

In order to optimize the reaction conditions and obtain the best catalytic activity, the reaction of *tert*-butyl-6-methyl-4-phenyl-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (**4a**; 2 mmol), benzaldehyde (2 mmol) and chloroacetyl chloride (3 mmol) was used as a model, and was conducted using various reaction parameters such as solvent and amount of catalyst. First, the model reaction was performed using several solvents and bases. According to the results, the best effect was obtained using ethanol as an effective and green solvent. In the second step, we examined the amount of catalyst. In the absence of Fe_3O_4@L-arginine, no product was found, but desired product was observed on applying Fe_3O_4@L-arginine. It is evident from Table 1 (entry 8) that applying more than the specified quantity of catalyst did not have a positive effect on the yield of product



SCHEME 3 Synthesis of thiazolo[3,2-a]pyrimidine thiones

TABLE 1 One-pot three-component reaction of **1a** (2 mmol), benzaldehyde (2 mmol) and chloroacetyl chloride (3 mmol) under various conditions

Entry	Catalyst (g)	Solvent	Time (h)	Yield (%)[a]
1	—	EtOH	24	None
2	Fe_3O_4@arginine (0.1)	DMSO	10	10
3	Fe_3O_4@arginine (0.1)	DMF	10	13
4	Fe_3O_4@arginine (0.1)	Water	24	—
5	Fe_3O_4@arginine (0.1)	MeOH	5	39
6	Fe_3O_4@arginine (0.1)	*n*-Hexane	10	28
7	Fe_3O_4@arginine (0.1)	EtOH	4	67
8	Fe_3O_4@arginine (0.12)	EtOH	4	67
9	Fe_3O_4@arginine (0.08)	EtOH	10	51
10	NaOAc (0.1)	EtOH	12	29
11	NaOH (0.1)	EtOH	24	20
12	KOH (0.1)	EtOH	24	23
13	Pyridine (0.1)	EtOH	12	35
14	NaH (0.1)	EtOH	10	58

[a]Isolated yield.

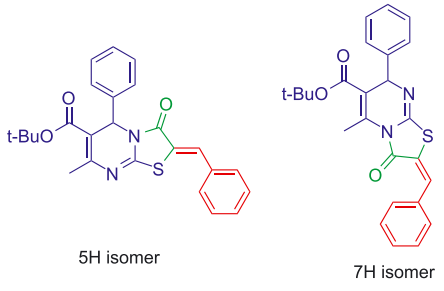
and 100 mg of catalyst represented the best result in terms of yield. The optimization was continued by investigating the effect of various bases on the progress of the reaction. As evident from Table 1, Fe_3O_4@L-arginine was chosen as the most desirable base, giving the best yield of 67%. Eventually, as evident from Table 1, the best result for the preparation of thiazolo[3,2-a]pyrimidine derivatives was obtained by applying 100 mg of Fe_3O_4@L-arginine under reflux in the presence of ethanol as solvent.

With the optimized conditions in hand, several substituted aromatic aldehydes were reacted with **4a–j** and chloroacetyl chloride in the presence of Fe_3O_4@L-arginine under reflux condition and in ethanol as a benign solvent (Table 2). Aromatic aldehydes with both electron-donating and electron-withdrawing groups participate in the one-pot multi-component reaction to give acceptable yields of products. The structures of all fused thiazolo[3,2-a]pyrimidine derivatives were assigned using infrared (IR), ^{1}H NMR and ^{13}C NMR spectroscopies. As for the IR spectra, the absence of absorption bands at about 3184–3423 and 1028–1181 cm^{-1} related to NH and C═S bond stretching in 3,4-dihydropyrimidine-2(1*H*)-thiones and the appearance of absorption band at approximately 1574–1611 cm^{-1} corresponding to imine (─C═N─) group confirm the formation of the fused thiazolo[3,2-a]pyrimidines.

Also, a strong stretching band at 1691–1701 cm^{-1} confirms the presence of carbonyl group in the thiazole pyrimidine derivatives. There are two isomeric cyclization products (5*H* or 7*H* isomers) in this reaction (Scheme 4).

TABLE 2 Preparation of thiazolo[3,2-a]pyrimidine derivatives catalysed by Fe_3O_4@L-arginine

Entry	R	R_1	Product	Yield (%)[a]	M.p. (°C)
1	2-Cl	4-OCH_3	**7a**	69	189–191
2	4-Cl	3-NO_2	**7b**	69	174–176
3	2-OH	4-CH_3	**7c**	67	217–219
4	4-OH	3-NO_2	**7d**	71	228–230
5	2-CH_3	4-OH	**7e**	67	221–223
6	4-CH_3	4-Cl	**7f**	66	183–185
7	3-NO_2	4-CH_3	**7g**	67	201–203
8	2-NO_2	4-Cl	**7h**	68	195–197
9	4-OCH_3	2-NO_2	**7i**	69	208–210
10	H	3-NO_2	**7j**	67	178–180



SCHEME 4 Possible isomeric thiazolo[3,2-a]pyrimidine derivatives

According to the ^{1}H NMR spectra of compounds **7a–j**, the absence of singlet at 8.51–9.32 ppm for thioamide (─N─H) in the starting material and appearance of singlet signal at 7.68 to 8.05 ppm represent support for the formation of compounds **7a–j**. Additional confirmation was obtained from ^{13}C NMR spectra. Downfield shift of the pyrimidine H-5 in **7a–j** compared to the pyrimidine H-4 in **4a–j** because of the electronegative effect of the C═O group in position 3 demonstrates that the 5*H* condensation product was obtained (Scheme 4).[25]

Two isomeric compounds can be anticipated for 5*H*–thiazolo[3,2-a]pyrimidine derivatives. Probably due to the lower steric interaction between the carbonyl group of the thiazolo ring and the phenyl ring, only the *Z*-isomer product rather than the *E*-isomer was obtained.[26]

2.2 | Antimicrobial activity

Pharmacological assessment is one of the most important methods for characterizing the activity of compounds. In recent years, concerns about infectious diseases have been raised to some extent.[27,28] The most commonly used antimicrobial agents are used to treat diverse types of infectious diseases. The antimicrobial effects of compound **4a–j**, **7a–j** and standard drugs were determined *in vitro* from inhibition zones and zone diameters using two Gram-positive bacteria (*Staphylococcus aureus* ATCC 6538 and *S. epidermidis* ATCC 12228), two Gram-negative bacteria (*Escherichia coli* ATCC 8739 and *Pseudomonas aeruginosa* ATCC9027). Antifungal activity was determined using *Aspergillus niger* ATCC 16404 and *Candida albicans* ATCC 10231.

The antimicrobial activities of all compounds were compared with those of the standard antibacterial drug ciprofloxacin and antifungal drug clotrimazole. The evaluation of antimicrobial effect was conducted by measuring the diameter of growth inhibition (Table 3), and the minimum inhibitory concentration (MIC) was determined (Table 4). The investigation found that the potential antibacterial activity is ascribed to the presence of pharmacologically active moieties such as 2-hydroxy, 4-hydroxy, 2-chloro and 4-chloro groups attached to the phenyl ring on the pyrimidine moiety and 4-methyl, 4-methoxy and 3-nitro groups attached to benzylidine on the thiazolo ring. It is worth nothing that, among the compounds, **4a–d** and **7a–d** displayed excellent antibacterial activity against all the tested bacterial strains with zone inhibition in the range 18–27 mm. The other compounds showed moderate to poor inhibition against both Gram-positive and Gram-negative bacteria.

The MIC of all compounds was also appraised. All tested compounds exhibited a variety of MICs, ranging from 6 to 200 μg ml^{-1} against Gram-positive and Gram-negative bacterial strains, compared to the standard drug ciprofloxacin with MIC value of 25 μg ml^{-1}. It is notable that the tested

TABLE 3 Antimicrobial activity as inhibition zone diameters (mm) of novel thiazolo[3,2-a]pyrimidines against pathogenic strains based on well diffusion assay method

	Gram-positive bacteria		Gram-negative bacteria		Fungus	Yeast
Entry	*S. aureus* ATCC 6538	*S. epidermidis* ATCC 12228	*E. coli* ATCC 8739	*P. aeruginosa* ATCC 9027	*A. niger* ATCC 16404	*C. albicans* ATCC 10231
4a	20	22	20	25	14	NA
4b	19	23	21	21	16	NA
4c	18	21	21	23	15	11
4d	21	20	20	25	15	14
4e	14	NA	13	15	17	13
4f	14	17	11	14	15	NA
4 g	16	17	15	12	13	15
4 h	17	13	NA	13	15	12
4i	14	15	10	12	14	12
4j	14	11	14	NA	16	NA
7a	23	25	22	27	14	13
7b	22	26	22	24	18	NA
7c	22	24	25	25	15	12
7d	25	26	23	26	17	14
7e	16	13	13	17	19	15
7f	15	17	14	17	17	NA
7g	16	18	15	14	16	16
7h	17	13	12	15	19	12
7i	15	18	13	16	17	14
7j	16	14	14	12	17	NA
Ciprofloxacin	20	24	23	25	NA	NA
Clotrimazole	NA	NA	NA	NA	33	30

TABLE 4 Minimum inhibitory concentrations (μg ml^{-1}) against pathogenic strains based on two-fold serial dilution method

Entry	*S. aureus* ATCC 6538	*S. epidermidis* ATCC 12228	*E. coli* ATCC 8739	*P. aeruginosa* ATCC 9027	*A. niger* ATCC 16404	*C. albicans* ATCC 10231
4a	25	25	25	25	200	200
4b	25	50	25	25	NA	NA
4c	12.5	25	25	25	200	200
4d	25	25	50	50	200	NA
4e	100	NA	200	200	200	NA
4f	50	100	NA	100	N.A	NA
4 g	100	100	200	NA	200	200
4 h	100	200	NA	100	200	NA
4i	100	100	NA	NA	200	NA
4j	100	200	200	NA	NA	NA
7a	6.25	6.25	12.5	12.5	200	200
7b	12.5	50	25	25	200	NA
7c	12.5	12.5	12.5	25	100	200
7d	6.25	6.25	25	25	200	NA
7e	100	200	100	100	100	NA
7f	50	50	200	100	NA	NA
7g	50	100	100	200	200	200
7h	100	100	NA	50	100	100
7i	100	50	100	200	100	NA
7j	100	100	200	NA	200	NA
Ciprofloxacin	25	25	25	25	—	—
Clotrimazole	—	—	—	—	25	25

compounds were not found to be effective against yeast and fungus compared to the standard drug clotrimazole as evident from Tables 3 and 4.

3 | EXPERIMENTAL

3.1 | General and instrumentation

All reagents and solvents in this investigation were procured from the Sigma Aldrich and applied without further purification. IR spectra of all compounds in our work were obtained over the region 400–4000 cm^{-1} with a PerkinElmer 550 spectrometer in potassium bromide pellets. Melting points were estimated with an Electrothermal 9200 apparatus and are not corrected. ^{1}H NMR and ^{13}C NMR spectra were obtained using a Bruker Advance spectrometer with DMSO-d_6 as a solvent using Me_4Si as an internal reference. TLC was performed on silica gel poly gram SILG/UV 254 nm plates.

3.2 | Synthesis of Fe_3O_4@L-arginine magnetic nanoparticles

Fe_3O_4@L-arginine was prepared by chemical co-precipitation according to a literature method.[24] Briefly, a mixture of $FeCl_3{\cdot}6H_2O$ (5 mmol) and $FeCl_2{\cdot}4H_2O$ (2.5 mmol) salts was dissolved in 100 ml of deionized water to afford an orange solution under vigorous mixing. Then, 2 mmol of L-arginine and 30 ml of ammonia solution (25%) were added to the solution to form a black suspension with pH of approximately 11. This suspension was refluxed under vigorous stirring at 100 °C for 6 h. Fe_3O_4@L-arginine nanoparticles were isolated from the aqueous solution using an external magnet, washed with water several times and finally dried in an oven overnight.

3.3 | General procedure for synthesis of compounds 7a–j

In a clean round-bottomed flask, to a mixture of 3,4-dihydropyrimidine-2(1*H*)-thione (2 mmol), aromatic aldehyde (2 mmol) and chloroacetyl chloride (3 mmol) were added ethanol and a catalytic amount of Fe_3O_4@L-arginine (0.1 g). The mixture was reacted under reflux condition for 4 h. After completion of the reaction, the catalyst was removed from the resulting solution with aid of an external magnet. The solvent was evaporated to afford the crude solid. Finally, the resulting solid was filtered, recrystallized from ethanol and dried at 60 °C under vacuum for 3 h.

All products were characterized using ^{1}H NMR, ^{13}CNMR and IR spectroscopies. The results related to spectral data for all samples are described below.

3.3.1 | *tert*-Butyl-7-methyl-2-(4-methoxybenzylidine)-5-(2-chlorophenyl)-3-oxo-2,3-dihydro-5*H*-thiazolo[3,2-a]pyrimidine-6-carboxylate (7a)

IR (KBr, ν_{max}, cm^{-1}): 3044, 2914, 1714, 1691, 1611, 1599, 1201, 1091, 832, 754, 701. ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 1.30 (t, 9H, t-Bu), 2.15 (s, 3H, CH_3), 3.61 (s, 3H, OCH_3), 5.51 (s, 1H, H-5), 6.83–7.71 (m, 8H, Ar-H), 7.89 (s, 1H, exocyclic CH). ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 166.7 (C═O), 164.3 (N─C═O), 160.1 (C═N), 157.6, 154.5, 140.5, 139.7, 133.6, 130.8, 130.0, 128.8, 128.0, 126.9, 125.3, 121.7, 116.8, 113.5, 79.3, 56.7, 43.9, 29.5, 19.8.

3.3.2 | *tert*-Butyl-7-methyl-2-(3-nitrobenzylidine)-5-(4-chlorophenyl)-3-oxo-2,3-dihydro-5*H*-thiazolo[3,2-a]pyrimidine-6-carboxylate (7b)

IR (KBr, ν_{max}, cm^{-1}): 3093, 2997, 1727, 1700, 1601, 1599, 1218, 1192, 814, 691. ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 1.22 (t, 9H, t-Bu), 2.23 (s, 3H, CH_3), 5.58 (s, 1H, H-5), 7.08–8.16 (m, 8H, Ar-H), 7.71 (s, 1H, exocyclic CH). ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 166.5 (C═O), 164.2 (N─C═O), 159.9 (C═N), 154.8, 146.6, 140.6, 139.5, 137.8, 133.1, 132.6, 131.4, 129.5, 129.1, 121.6, 119.4, 118.7, 116.6, 80.4, 56.4, 29.8, 20.4.

3.3.3 | *tert*-Butyl-7-methyl-2-(4-methylbenzylidine)-5-(2-hydroxyphenyl)-3-oxo-2,3-dihydro-5*H*-thiazolo[3,2-a]pyrimidine-6-carboxylate (7c)

IR (KBr, ν_{max}, cm^{-1}): 3329, 3107, 2894, 1729, 1696, 1598, 1576, 1238, 1201, 839, 703. ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 1.40 (t, 9H, t-Bu), 2.09 (s, 3H, CH_3), 2.32 (s, 3H, CH_3), 5.04 (s, 1H, OH), 5.90 (s, 1H, H-5), 6.94–7.82 (m, 8H, Ar-H), 7.94 (s, 1H, exocyclic CH). ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 167.4 (C═O), 164.1 (N─C═O), 159.5 (C═N), 154.5, 153.9, 139.4, 135.6, 130.3, 127.3, 126.4, 126.0, 124.7, 121.9, 121.6, 119.7, 116.4, 113.6, 79.8, 47.9, 29.4, 26.1, 19.7.

3.3.4 | *tert*-Butyl-7-methyl-2-(3-nitrobenzylidine)-5-(4-hydroxyphenyl)-3-oxo-2,3-dihydro-5*H*-thiazolo[3,2-a]pyrimidine-6-carboxylate (7d)

IR (KBr, ν_{max}, cm^{-1}): 3350, 3049, 2878, 1707, 1699, 1588, 1547, 1259, 1205, 854, 690. ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 1.40 (t, 9H, t-Bu), 2.25 (s, 3H, CH_3), 4.96 (s, 1H, OH), 5.91 (s, 1H, H-5), 6.77–8.19 (m, 8H, Ar-H), 7.75 (s, 1H, exocyclic CH). ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 167.0 (C═O), 165.1 (N─C═O), 160.4 (C═N), 154.3, 152.6, 149.1, 141.1, 134.8, 133.6, 131.4, 128.1, 127.3, 121.4, 120.1, 118.9, 117.1, 116.8, 80.6, 56.8, 28.4, 20.1.

3.3.5 | *tert*-Butyl-7-methyl-2-(4-hydroxybenzylidine)-5-(2-methylphenyl)-3-oxo-2,3-dihydro-5*H*-thiazolo[3,2-a]pyrimidine-6-carboxylate (7e)

IR (KBr, ν_{max}, cm^{-1}): 3314, 3064, 2904, 1708, 1693, 1607, 1561, 1253, 1199, 823, 705. ^{1}H NMR (400 MHz, DMSO-d_6,

δ, ppm): 1.36 (t, 9H, t-Bu), 2.03 (s, 3H, CH_3), 2.44 (s, 3H, CH_3), 5.28 (s, 1H, OH), 5.71 (s, 1H, H-5), 6.79–7.67 (m, 8H, Ar-H), 7.79 (s, 1H, exocyclic CH). ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 166.8 (C═O), 163.7 (N─C═O), 159.7 (C═N), 158.5, 153.7, 140.9, 140.3, 133.8, 127.5, 127.0, 126.4, 125.8, 125.1, 123.8, 120.7, 115.7, 114.0, 80.0, 50.6, 28.8, 19.9, 17.1.

3.3.6 | *tert*-Butyl-7-methyl-2-(4-chlorobenzylidine)-5-(4-methylphenyl)-3-oxo-2,3-dihydro-5*H*-thiazolo[3,2-a]pyrimidine-6-carboxylate (7f)

IR (KBr, ν_{max}, cm^{-1}): 3079, 2909, 1699, 1694, 1596, 1553, 1217, 1193, 848, 741, 711. ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 1.38 (t, 9H, t-Bu), 1.94 (s, 3H, CH_3), 2.32 (S, 3H, CH_3), 5.90 (s, 1H, H-5), 7.18–7.48 (m, 8H, Ar-H), 7.68 (s, 1H, exocyclic CH). ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 167.5 (C═O), 164.7 (N─C═O), 159.8 (C═N), 154, 139.6, 137.7, 135.1, 131.7, 131.0, 128.4, 128, 126.9, 125.7, 121.7, 116.6, 80.4, 56.3, 24.8, 29.3, 20.3.

3.3.7 | *tert*-Butyl-7-methyl-2-(4-methylbenzylidine)-5-(3-nitrophenyl)-3-oxo-2,3-dihydro-5*H*-thiazolo[3,2-a]pyrimidine-6-carboxylate (7g)

IR (KBr, ν_{max}, cm^{-1}): 3078, 2843, 1725, 1698, 1587, 1551, 1244, 1201, 831, 687. ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 1.19 (t, 9H, t-Bu), 1.86 (s, 3H, CH_3), 2.18 (s, 1H, CH_3), 5.75 (s, 1H, H-5), 7.09–8.14 (m, 8H, Ar-H), 7.82 (s, 1H, exocyclic CH). ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 166.3 (C═O), 165.1 (N─C═O), 160.0 (C═N), 153.7, 147.6, 142.8, 141.0, 138.9, 134.0, 130.7, 130.0, 128.4, 127.5, 121.8, 120.4, 117.8, 116.0, 81.0, 55.4, 29.3, 25.4, 20.3.

3.3.8 | *tert*-Butyl-7-methyl-2-(4-chlorobenzylidine)-5-(2-nitrophenyl)-3-oxo-2,3-dihydro-5*H*-thiazolo[3,2-a]pyrimidine-6-carboxylate (7h)

IR (KBr, ν_{max}, cm^{-1}): 3075, 2879, 1720, 1693, 1590, 1541, 1266, 1210, 841, 723, 695. ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 1.40 (t, 9H, t-Bu), 2.25 (s, 3H, CH_3), 5.43 (s, 1H, H-5), 7.39–8.14 (m, 8H, Ar-H), 8.05 (s, 1H, exocyclic CH). ^{13}C NMR (75 MHz, DMSO-d_6, δ, ppm): 166.8 (C═O), 163.9 (N─C═O), 159.6 (C═N), 153.9, 145.9, 140.3, 135.8, 133.1, 131.7, 131.1, 129.9, 128.3, 127.9, 127.0, 120.9, 118.4, 116.6, 79.9, 51.4, 28.7, 19.9.

3.3.9 | *tert*-Butyl-7-methyl-2-(2-nitrobenzylidine)-5-(4-methoxyphenyl)-3-oxo-2,3-dihydro-5*H*-thiazolo[3,2-a]pyrimidine-6-carboxylate (7i)

IR (KBr, ν_{max}, cm^{-1}): 3057, 2878, 1704, 1697, 1574, 1546, 1286, 1224, ,811, 697. ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 1.37 (t, 9H, t-Bu), 2.26 (s, 3H, CH_3), 3.72 (s, 3H, OCH_3), 5.72 (s, 1H, H-5), 7.06–8.15 (m, 8H, Ar-H), 8.31 (s, 1H, exocyclic CH). ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 166.4 (C═O), 164.7 (N─C═O), 159.8 (C═N), 156.4, 153.9, 144.8, 140.1, 135.3, 133.7, 130.8, 129.3, 126.8, 126.0, 121.0, 119.6, 116.0, 112.9, 80.4, 56.5, 56.3, 29.1, 19.4.

3.3.10 | *tert*-Butyl-7-methyl-2-(3-nitrobenzylidine)-5-phenyl-3-oxo-2,3-dihydro-5*H*-thiazolo[3,2-a]pyrimidine-6-carboxylate (7j)

IR (KBr, ν_{max}, cm^{-1}): 3031, 2861, 1714, 1701, 1593, 1576, 1210, 1196, 817, 700. ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 1.39 (t, 9H, t-Bu), 2.35 (s, 3H, CH_3), 5.72 (s, 1H, H-5), 7.12–8.14 (m, 9H, Ar-H), 7.75 (s, 1H, exocyclic CH). ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 166.3 (C═O), 165.2 (N─C═O), 159.7 (C═N), 154.2, 147.8, 142.7, 140.9, 134.1, 129.8, 127.3, 126.1, 125.4, 125.0, 121.7, 120.8, 119.1, 116.0, 79.6, 54.8, 29.1, 20.1.

3.4 | Biological assay

3.4.1 | *In vitro* antimicrobial assay

All synthesized compounds (**4a–j** and **7a–j**) were tested for antimicrobial activity against pathogenic strains by applying the well diffusion assay method and MIC technique.

3.4.2 | Primary screening

The *in vitro* antimicrobial activities of **4a–j** and **7a–j** were determined using the agar well diffusion method[1,29] against a panel of microorganisms, namely *S. aureus* (ATCC 6538) and *S. epidermidis* (ATCC 12228) as Gram-positive bacteria, *E. coli* (ATCC 8739) and *P. aeruginosa* (ATCC 9027) as Gram-negative bacteria, and *A. niger* (ATCC 16404) and *C. albicans* (ATCC 10231) as fungus and yeast, respectively. Microbial colonies were prepared in 5 ml of tripticase soya broth from a fresh isolation plate. The broth was incubated for 6 h at 37 °C to observe obvious growth. A 0.5 McFarland standard was prepared by mixing 0.05 ml of 0.1% (*w*/*v*) $BaCl_2{\cdot}2H_2O$ in phosphate buffer saline (PBS) with 9.95 ml of 1% (*v*/v) sulfuric acid in PBS. The turbidity of all six cultures using sterile PBS was controlled to 0.5 McFarland turbidity standard to obtain a 10^8 cfu ml^{-1} suspension. To prepare incolums of yeast and fungi including 10^6 cfu ml^{-1} suspension, the 10^8 cfu ml^{-1} suspension was diluted 10^2 times in trypticase soya broth. All the examined compounds and ciprofloxacin as antibacterial standard and clotrimazole as antifungal standard were prepared by dissolving 50 mg of each compound in 1 ml of DMSO. So DMSO was applied as a negative control for all the experiment samples.

An amount of 100 μl of suspension containing 1×10^8 cfu ml^{-1} of each examined bacterial strain and 1×10^6 cfu ml^{-1} of yeast and fungus was mixed with 20 ml of Mueller–Hinton agar and Sabouraud dextrose agar, respectively, and transferred into sterilized Petri plates. Wells of 8 mm in diameter were punched in the solidified agar plates and 100 μl of test solution was charged to individual wells. The loaded plates were preserved for incubation at 37 °C for 24 h for bacteria and 48 h at 28 °C for fungi. After the incubation time, the antimicrobial activity of each sample

was determined by measuring the inhibition zone around each well and comparing with standard drug. The results of measurement of zone of inhibition are presented in Table 3. Every test in the antibacterial and antifungal assay was repeated twice.

3.4.3 | MIC determination

Antimicrobial activities of all the compounds and standards were evaluated using the serial dilution method.[30] Each test compound and standard drug individually was prepared in DMSO to obtain 0.4 mg ml^{-1} concentration (stock solution). The purpose of the serial dilution method is the determination of the lowest concentration of the examined antimicrobial agent to inhibit the visible growth of the organisms being investigated. Mueller–Hinton and Sabouraud dextrose broths were used as bacterial and fungal nutrients. The inoculum size of all strains was adjusted to 10^8 cfu ml^{-1} using 0.5 McFarland standard solution for each antimicrobial compound and standard drug (ciprofloxacin for bacteria and clotrimazole for fungi), and 78 tubes of 5 ml volume were ordered in six rows such that each row contained 13 tubes. Afterwards, 1 ml of Mueller–Hinton broth (for rows 1–4) and 1 ml of Sabouraud dextrose broth (for rows 5 and 6) were added in the tubes 1–13 in each row. Then 1000 µl of antimicrobial suspension (stock solution) was charged to the first tube in each row and mixed. After mixing, 1 ml of the first tube in each row was serially carried over to the second tube in the same row, mixed, and the content of the second tube was transferred to the third tube in each row. This serial dilution was repeated to the twelfth tube in each of the rows and 1000 µl of the twelfth tube was disregarded. The twelfth tube without inoculum was used as a negative control and the last tube without antimicrobial compound was used as a positive control. This dilution provided antimicrobial concentrations of 200, 100, 50, 25, 12.5, 6.25, 3.12, 1.56, 0.78, 0.39, 0.2 and 0.1 mg ml^{-1}. Finally, 100 µl of inoculums of *S. aureus*, *S. epidermidis*, *E. coli*, *P. aeruginosa*, *C. albicans* and *A. niger* were added respectively to tubes 1–11 and 13 in the rows 1–6 and were incubated for 24 h at 37 °C and for 48 h at 28 °C for bacteria and fungi, respectively. The highest dilution of active sample to prevent evident growth of the organism was supposed as the MIC.

4 | CONCLUSIONS

In this research, we have synthesized a series of 2-[(*Z*)-1-(substituted phenyl)methylidine]-7-methyl-3-oxo-5-(substituted phenyl)-2,3-dihydro-5*H*-thiazolo[3,2-a]pyrimidine-6-carboxilic acid *tert*-butyl ester derivatives (**7a–j**) in reasonable yield in the presence of Fe_3O_4@L-arginine as a recoverable and environmentally benign catalyst and ethanol as solvent. All the novel synthesized samples were characterized using ^{1}H NMR, ^{13}C NMR and IR spectroscopies. Antibacterial and antifungal activities of compounds **4a–j** and **7a–j** were studied and the results demonstrated that the presence of 2-chloro, 4-chloro, 2-hydroxy and 4-hydroxy substituents on the phenyl ring of the pyrimidine core and 4-methyl, 4-methoxy and 3-nitro groups attached to benzylidine on the thiazol core enhanced the antibacterial activity. Compounds **4a–d** and **7a–d** were found to have potent antibacterial activity against bacterial strains compared to the standard drug ciprofloxacin. Other compounds revealed moderate to weak antibacterial activity. It is notable that antifungal activity was not observed for our study compounds. According to relationships between the structure of the heterocyclic scaffold and the detected antimicrobial property, various biological activities were shown. Perhaps the nature of the heterocyclic ring as well as the presence of different substituents caused a specific change of activity. From the antimicrobial investigation results, one can deduce that a combination of two different heterocyclic systems, namely pyrimidine and thiazol, enhances the antibacterial activity.

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