









Thiazolo-Pyrimidine Analogues: Synthesis, Characterization and Docking Studies Guided Antimicrobial Activities

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Abstract: In the current study, bicyclic 1-(7-methyl-3,5-diphenyl-5H-thiazolo(3,2- α)pyrimidine-6-yl)ethanone (4a-l) derivatives have been designed and conveniently synthesized by one-pot three-component method *via* cyclocondensation of substituted 4-phenylthiazole-2-amine (1a-c), acetylacetone (2) and various aromatic aldehydes (3a-d) in the presence of *p*-toluene sulfonic acid (PTSA) under acetonitrile solvent medium. The synthesized compounds (4a-l) have been characterized by spectral analysis and subjected to docking study against protein DNA gyrase (PDB Code: 1KZN), and also, the compounds were screened for their *in vitro* antimicrobial activities. The bioassay of the synthesized compounds envisioned that the compound 4k emerged as a broad-spectrum antibacterial agent, and 4l emerged as a good antifungal agent compared to standard drug.

Keywords: thiazolo[3,2- α]pyrimidine; one-pot three-component reaction; docking studies; antimicrobial activity.

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1. Introduction

The fusion of two rings thiazole and pyrimidine containing bridgehead nitrogen atom [1-2] are attracting the attention of many medicinal chemists throughout the world to explore the framework for its potential [3]. Ritanserin and setoperone are the most common example of a biologically active compound containing thiazolopyrimidines system [4]. Thiazolo[3,2- α]pyrimidines, which is being used in various fields of therapeutic applications [5] such as antimicrobial [6-8], antiviral [9], cytotoxicity [10], insecticides [11], analgesic [12], antioxidant [13], anti-inflammatory [14] and calcium channel blocker activities [15-16]. Additionally, it was described that such a ring system could inhibit the enzyme 3'5'-cyclic AMP phosphodiesterase to have a theophylline-like activity and to be active against virulent Lewis lung tumors in mice [17]. Thiazolo[3,2- α]pyrimidine derivatives to generate enzyme inhibitors as novel therapeutical entities for severe neurodegenerative diseases and other medicinal applications [18].

The synthesis of dihydropyrimidines is a well-known three-component one-pot reaction of a substituted aldehyde, 1,3-dicarbonyl compound, and thiourea to give Biginelli compounds [19]. The Hantzsch-type condensation of dihydropyrimidines with a substituted phenacyl chlorides led to the 3-substituted-5*H*-thiazolo[3,2- α]pyrimidine derivatives [20-21]. Hussein *et al.* have been synthesized ethyl 6-methyl-4-(substituted)phenyl-2-(substituted)-phenacylthio-1,4-dihydropyrimidine-5-carboxylate hydrobromide series and screened for their antibacterial and antifungal activities [22].

Renata *et al.* [23] described the preparation of 2-iodomethyl/2-bromomethyl-2,3-dihydrothiazolo[3,2- α]pyrimidine-5-one derivatives containing alkyl groups at C-6 and/or C-7 *via* the reaction of 3-allyl-2-thiouracils with iodine chloride/bromide in methanol and Amit K Keshari *et al.* [24] have been successfully utilized *p*-toluene sulfonic acid (PTSA) catalyzed domine Knoevenagel/Michael/intramolecular cyclization for the synthesis of novel 5*H*-benzo[*h*]thiazolo[2,3-*b*]quinazoline and indeno[1,2-*d*]thiazolo[3,2- α]pyrimidine analogs bearing bridgehead nitrogen atom. The newly synthesized compounds were tested for molecular modeling and *in vitro* antitumor activity against hepatocellular carcinoma.

Recently, Asieh Khalilpour *et al.* [25] synthesized and investigated *in vitro* cytotoxicity and antioxidant activity of 2,3-dihydro-5*H*-[1,3]thiazolo[3,2- α]pyrimidine-2,3,6-tricarboxylates derivatives. The chlorine substituted compound displayed the highest cytotoxic effect in comparison with standard drug doxorubicin and effective antioxidant. Janardhan Banothu and co-workers [26] synthesized and screened for their antibacterial, antioxidant, and DNA cleavage activities of the series of thiazolo[3,2-*a*]pyrimidines in good yield by the reaction of fused 3,4-dihydropyrimidine-2(1*H*)-thiones with phenacyl bromides/3-(2-bromoacetyl)coumarins under conventional heating in acetic acid medium.

This prompted us to develop the process for the synthesis of some bicycle heterocyclic compounds containing 1,3,4-thiadiazine fused with pyrimidine moiety, i.e., 1-(7-methyl-3,5-diphenyl-5*H*-thiazolo[3,2- α]pyrimidine-6-yl)ethanone (4a-1) derivatives and study their antimicrobial studies.

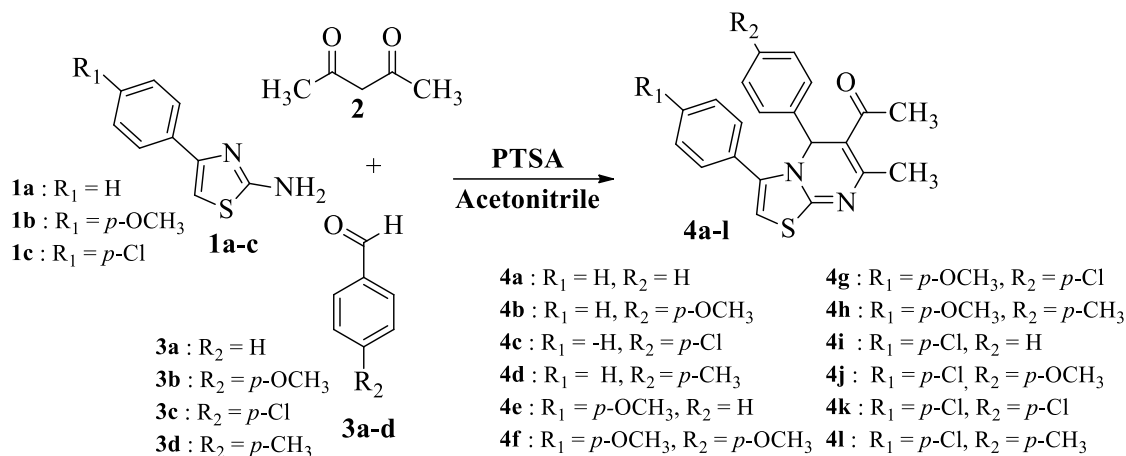
2. Materials and Methods

All chemicals/reagents were procured from Merck/Fluka Chemicals (India). The melting points were measured with micro melting point apparatus and are uncorrected. IR spectra were recorded in KBr pellets on Shimadzu 8300 spectrometer. The ¹H NMR (CDCl₃) was recorded on Agilent NMR-vnrms 400 MHz spectrometer; the chemical shifts are expressed in ppm (TMS as internal standard). ¹³C NMR (DMSO-*d*₆) spectra were obtained on Varian Gemini 100 MHz spectrometer, Mass spectra were obtained on Agilent 6330 ion trap spectrophotometer, and elemental analysis was performed on a Jusco micro-analytical data unit. TLC was achieved on pre-coated silica gel sheets (HF 254, Sd-fine), and visualization of the spots was done in iodine vapor and UV light. Chromatographic separation was carried out on silica gel (60-120) mesh using petroleum ether: acetone (9:1) as eluent.

2.1. General procedure.

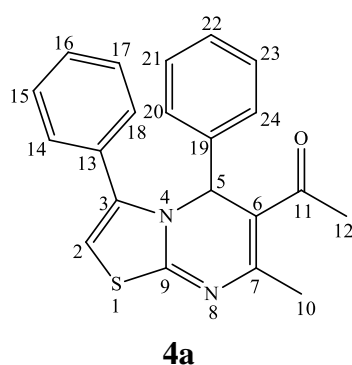
General procedure for the synthesis of 4-phenylthiazole-2-amine (1a): Solution of acetophenone (2.40g, 20.00mmole) and thiourea (1.90g, 25.00mmole) are taken in a round-bottomed flask, 10% bromine in acetic acid (25 ml) was slowly added to the flask with constant stirring. After the addition of bromine in acetic acid, the reaction mass was refluxed on a water

bath until most of the solid has gone into a solution and again refluxed the solution for 2-3 hours. The hot reaction mixture was filtered; the filtrate was cooled and alkaline with conc. ammonium hydroxide to separate solid 4-phenylthiazole-2-amine [27]. The obtained product was filtered, washed with alcohol, and dried over P₂O₅. It was recrystallized from ethanol to get colorless needles (1a). Yield (84.2%), m.p. 120-122°C.



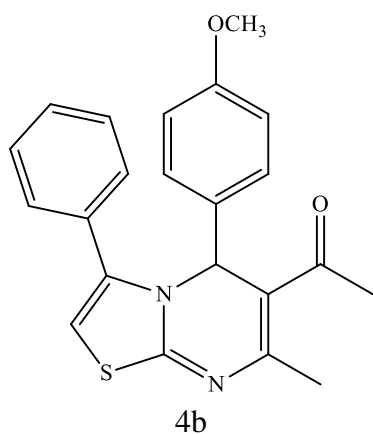
Scheme 1. Synthesis of thiazolo-pyrimidine derivatives.

The typical procedure for the synthesis of 1-(7-methyl-3,5-diphenyl-5*H*-thiazolo[3,2- α]pyrimidine-6-yl)ethanone (4a): The solution of acetylacetone (2, 2.00g, 20.00mmol) and benzaldehyde (3a, 1.06g, 10.00mmol) in acidic PTSA (2.50g, 15.00mmol) was refluxed on a water bath in the presence of acetonitrile (25 ml) for about 30 minutes. Meanwhile, the 4-phenylthiazole-2-amine (1a, 1.76g, 10.00mmol) was added to the same reaction mixture and again refluxed for about 2-3 hours. After the completion (monitored by TLC), the reaction mixture was cooled to room temperature, extracted with CHCl₃ (3 x 25 ml), washed with water (2 x 25 ml), and 2% dilute HCl solution, finally dried over anhydrous Na₂SO₄. The solvent was evaporated to give yellow viscous solid, which was subjected to chromatographic (silica gel 60-120 mesh) using petroleum ether and ethyl acetate (8:2) as eluent to get a pale yellow solid 1-(7-methyl-3,5-diphenyl-5*H*-thiazolo(3,2- α)pyrimidine-6-yl)ethanone 4a in 82% yield (2.83g); m.p. 114-116°C.



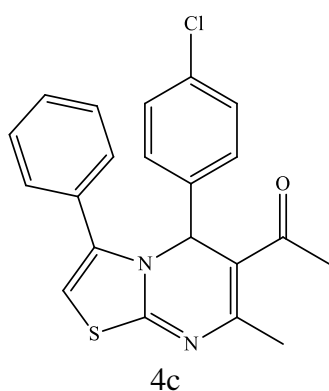
IR (KBr, cm⁻¹): γ 2970 (-CH-), 1678 (>C=O), 1566 (-C=N-) cm⁻¹; ¹H NMR (CDCl₃): δ 2.26 (s, 3H, -COCH₃), 2.94 (s, 3H, -CH₃), 5.22 (s, 1H, -CH-), 6.96 (s, 1H, >C=CH-), 7.25-7.75 (m, 10H, Ar-H); ¹³C NMR (DMSO-*d*₆): δ 21.12 (C-10), 27.21 (C-12), 65.20 (C-5), 108.42 (C-2), 126.24 (C-3), 126.80 (C-22), 127.06 (C-20 and C-24), 128.04 (C-16), 128.21 (C-14 and C-18), 128.66 (C-21 and C-23), 128.82 (C-15 and C-17), 130.80 (C-13), 131.62 (C-6), 143.44 (C-19), 151.83 (C-7), 158.64 (C-9), 196.62 (C-11); MS for C₂₁H₁₈N₂OS: 346 (MH⁺, 100%), 347 (MH⁺, 23%), 348 (MH⁺, 4.8%); Elemental Analysis (%): Calculated: C, 72.80.12; H, 5.24; N, 8.09; Found: C, 72.76; H, 5.26; N, 8.08.

1-(5-(4-methoxyphenyl)-7-methyl-3-phenyl-5*H*-thiazolo[3,2- α]pyrimidine-6-yl)ethanone (4b): Obtained from 4-phenylthiazole-2-amine (1a, 1.76g, 10.00mmol), acetylacetone (2, 2.00g, 20.00mmol) 4-methoxybenzaldehyde (3b, 1.36g, 10.00mmol) and PTSA (2.50g, 15.00mmol) as yellow solid, yield 85% (3.19g), m.p. 120-122°C.



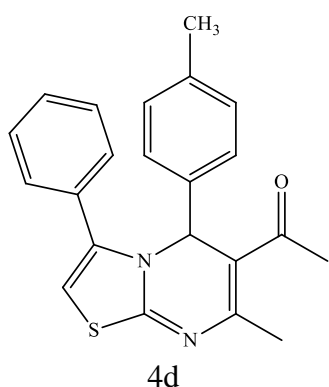
IR (KBr, cm^{-1}): γ 2972 (-CH-), 1676 ($>\text{C}=\text{O}$), 1560 (-C=N-) cm^{-1} ; ^1H NMR (CDCl_3): δ 2.28 (s, 3H, -COCH₃), 2.90 (s, 3H, -CH₃), 3.85 (s, 3H, -OCH₃), 5.26 (s, 1H, -CH-), 6.85 (s, 1H, $>\text{C}=\text{CH}-$); 6.88-7.70 (m, 9H, Ar-H), ^{13}C NMR (DMSO-*d*₆): δ 21.08 (C-10), 27.05 (C-12), 55.82 (-OCH₃), 62.14 (C-5), 108.34 (C-2), 114.26 (C-21 and C-23), 128.25 (C-16), 125.84 (C-20 and C-24), 128.54 (C-14 and C-18), 128.86 (C-15 and C-17), 132.24 (C-6), 130.74 (C-13), 135.66 (C-19), 145.14 (C-3), 151.86 (C-7), 158.60 (C-9), 158.62 (C-22), 196.84 (C-11); MS for C₂₂H₂₀N₂O₂S: 376 (MH⁺, 100%), 377 (MH⁺, 24.6%), 378 (MH²⁺, 4.7%); Elemental Analysis (%): Calculated: C, 70.19; H, 5.35; N, 7.44; Found: C, 70.18; H, 5.36; N, 7.43.

1-(5-(4-chlorophenyl)-7-methyl-3-phenyl-5H-thiazolo[3,2- α]pyrimidine-6-yl)ethanone (4c): Obtained from 4-phenylthiazole-2-amine (1a, 1.76g, 10.00mmol), acetylacetone (2, 2.00g, 20.00mmol) 4-chlorobenzaldehyde (3c, 1.40g, 10.00mmol) and PTSA (2.50g, 15.00mmol) as yellow solid, yield 80% (3.05g), m.p. 118-120°C.



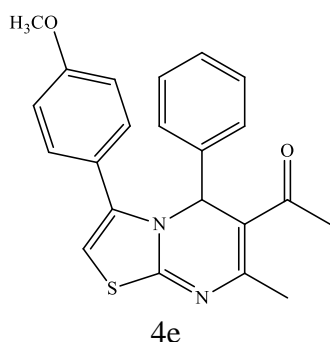
IR (KBr, cm^{-1}): γ 2968 (-CH-), 1679 ($>\text{C}=\text{O}$), 1570 (-C=N-) cm^{-1} ; ^1H NMR (CDCl_3): δ 2.27 (s, 3H, -COCH₃), 2.92 (s, 3H, -CH₃), 5.24 (s, 1H, -CH-), 6.96 (s, 1H, $>\text{C}=\text{CH}-$); 7.30-7.75 (m, 9H, Ar-H); ^{13}C NMR (DMSO-*d*₆): δ 21.32 (C-10), 27.16 (C-12), 62.30 (C-5), 108.55 (C-2), 126.04 (C-20 and C-24), 127.90 (C-16), 128.42 (C-14 and C-18), 128.60 (C-15 and C-17), 128.74 (C-21 and C-23), 130.08 (C-6), 132.26 (C-13), 132.38 (C-22), 141.50 (C-19), 145.14 (C-3), 151.64 (C-7), 158.22 (C-9), 196.46 (C-11); MS for C₂₁H₁₇ClN₂OS: 380 (MH⁺, 100%), 382 (MH²⁺, 36.5%), 381 (MH⁺, 23%); Elemental Analysis (%): Calculated: C, 66.22; H, 4.50; Cl, 9.31; N, 7.35; Found: C, 66.24; H, 4.48; Cl, 9.30; N, 7.33.

1-(7-methyl-3-phenyl-5-(*p*-tolyl)-5H-thiazolo[3,2- α]pyrimidine-6-yl)ethanone (4d): Obtained from 4-phenylthiazole-2-amine (1a, 1.76g, 10.00mmol), acetylacetone (2, 2.00g, 20.00mmol) 4-methylbenzaldehyde (3d, 1.20g, 10.00mmol) and PTSA (2.50g, 15.00mmol) as yellow solid, yield 82% (2.95g), m.p. 132-134°C.



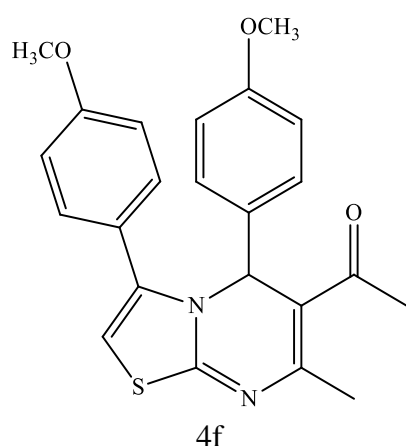
IR (KBr, cm^{-1}): γ 2970 (-CH-), 1669 ($>\text{C}=\text{O}$), 1568 (-C=N-) cm^{-1} ; ^1H NMR (CDCl_3): δ 2.23 (s, 3H, -COCH₃), 2.35 (s, 3H, Ar-CH₃), 2.94 (s, 3H, -CH₃), 5.22 (s, 1H, -CH-), 6.98 (s, 1H, $>\text{C}=\text{CH}-$), 7.10-7.74 (m, 9H, Ar-H); ^{13}C NMR (DMSO-*d*₆): δ 21.04 (-CH₃) 21.44 (C-10), 27.20 (C-12), 62.24 (C-5), 108.06 (C-2), 126.84 (C-20 and C-24), 127.80 (C-16), 128.32 (C-14 and C-18), 128.66 (C-15 and C-17), 128.72 (C-21 and C-23), 130.60 (C-13), 131.84 (C-6), 136.44 (C-22), 140.32 (C-19), 145.14 (C-3), 151.68 (C-7), 158.22 (C-9), 196.50 (C-11); MS for C₂₂H₂₀N₂O₂S: 360 (MH⁺, 100%), 361 (MH²⁺, 25.5%), 362 (MH⁺, 5.1%); Elemental Analysis (%): Calculated: C, 73.30; H, 5.59; N, 7.77; Found: C, 73.328; H, 5.60; N, 7.78.

1-(3-(4-methoxyphenyl)-7-methyl-5-phenyl-5*H*-thiazolo[3,2- α]pyrimidine-6-yl)ethanone (4e): Obtained from 4-(4-methoxyphenyl)thiazole-2-amine (1b, 2.06g, 10.00mmol), acetylacetone (2, 2.00g, 20.00mmol) benzaldehyde (3a, 1.06g, 10.00mmol) and PTSA (2.50g, 15.00mmol) as yellow solid, yield 80% (3.00g), m.p. 130-132°C.



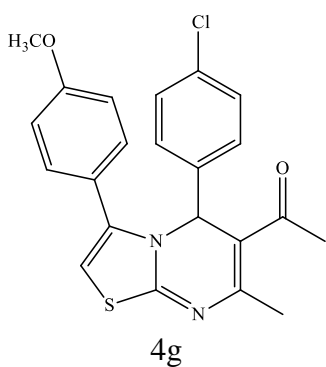
IR (KBr, cm^{-1}): γ 2969 (-CH-), 1674 (>C=O), 1574 (-C=N-) cm^{-1} ; ^1H NMR (CDCl_3): δ 2.26 (s, 3H, -COCH₃), 2.90 (s, 3H, -CH₃), 3.85 (s, 3H, -OCH₃), 5.21 (s, 1H, -CH-), 6.90 (s, 1H, >C=CH-), 6.94-7.60 (m, 9H, Ar-H); ^{13}C NMR ($\text{DMSO-}d_6$): δ 21.48 (C-10), 27.26 (C-12), 55.52 (-OCH₃), 62.08 (C-5), 108.26 (C-2), 121.24 (C-15 and C-17), 123.46 (C-13), 126.86 (C-22), 127.05 (C-20 and C-24), 128.64 (C-21 and C-23), 129.84 (C-14 and C-18), 132.00 (C-6), 143.23 (C-19), 145.08 (C-3), 151.86 (C-7), 158.44 (C-9), 159.80 (C-16), 196.54 (C-11); (C-7), 160.08 (C-9), 163.06 (C-16), MS for $\text{C}_{22}\text{H}_{20}\text{N}_2\text{O}_2\text{S}$: 376 (MH^+ , 100%), 377 (MH^{+1} , 24.2%), 378 (MH^{+2} , 4.8%); Elemental Analysis (%): Calculated: C, 70.19; H, 5.35; N, 7.44; Found: C, 70.20; H, 5.34; N, 7.42.

1-(3,5-bis(4-methoxyphenyl)-7-methyl-5*H*-thiazolo[3,2- α]pyrimidine-6-yl)ethanone (4f): Obtained from 4-(4-methoxyphenyl)thiazole-2-amine (1b, 2.07g, 10.00mmol), acetylacetone (2, 2.00g, 20.00mmol) 4-methoxybenzaldehyde (3b, 1.36g, 10.00mmol) and PTSA (2.50g, 15.00mmol) as yellow solid, yield 86% (3.49g), m.p. 140-142°C.



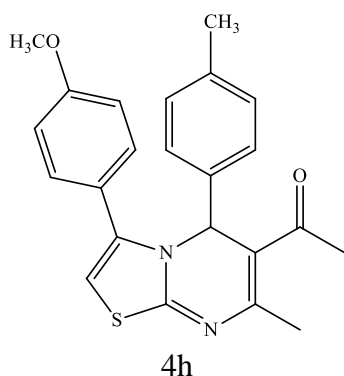
IR (KBr, cm^{-1}): γ 2967 (-CH-), 1671 (>C=O), 1573 (-C=N-) cm^{-1} ; ^1H NMR (CDCl_3): δ 2.25 (s, 3H, -COCH₃), 2.96 (s, 3H, -CH₃), 3.80-3.85 (s, 6H, -OCH₃), 5.25 (s, 1H, -CH-), 6.94 (s, 1H, >C=CH-), 6.96-7.55 (m, 8H, Ar-H); ^{13}C NMR ($\text{DMSO-}d_6$): δ 21.40 (C-10), 27.04 (C-12), 55.82 (-OCH₃), 56.04 (-OCH₃), 62.06 (C-5), 108.64 (C-2), 114.04 (C-21 and C-23), 121.28 (C-15 and C-17), 123.06 (C-13), 125.82 (C-20 and C-24), 129.86 (C-14 and C-18), 132.23 (C-6), 135.62 (C-19), 145.28 (C-3), 151.88 (C-7), 158.42 (C-9), 158.87 (C-22), 159.80 (C-16), 196.62 (C-11); MS for $\text{C}_{23}\text{H}_{22}\text{N}_2\text{O}_3\text{S}$: 406 (MH^+ , 100%), 407 (MH^{+1} , 25.2%), 408 (MH^{+2} , 4.1%); Elemental Analysis (%): Calculated: C, 67.96; H, 5.46; N, 6.89; Found: C, 67.98; H, 5.44; N, 6.85.

1-(5-(4-chlorophenyl)-3-(4-methoxyphenyl)-7-methyl-5*H*-thiazolo[3,2- α]pyrimidine-6-yl)ethanone (4g): Obtained from 4-(4-methoxyphenyl)thiazole-2-amine (1b, 2.06g, 10.00mmol), acetylacetone (2, 2.00g, 20.00mmol) 4-chlorobenzaldehyde (3c, 1.40g, 10.00mmol) and PTSA (2.50g, 15.00mmol) as yellow solid, yield 83% (3.40g), m.p. 148-150°C.



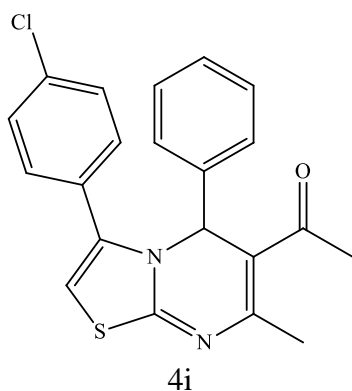
IR (KBr, cm^{-1}): γ 2971 ($-\text{CH}-$), 1672 ($>\text{C}=\text{O}$), 1571 ($-\text{C}=\text{N}-$) cm^{-1} ; ^1H NMR (CDCl_3): δ 2.28 (s, 3H, $-\text{COCH}_3$), 2.93 (s, 3H, $-\text{CH}_3$), 3.84 (s, 6H, $-\text{OCH}_3$), 5.23 (s, 1H, $-\text{CH}-$), 6.96 (s, 1H, $>\text{C}=\text{CH}-$), 6.98-7.55 (m, 8H, Ar-H); ^{13}C NMR ($\text{DMSO}-d_6$): δ 21.42 (C-10), 27.06 (C-12), 55.66 ($-\text{OCH}_3$), 62.24 (C-5), 108.26 (C-2) 121.14 (C-15 and C-17), 123.22 (C-13), 126.24 (C-20 and C-24), 128.76 (C-21 and C-23) 129.84 (C-14 and C-18), 132.04 (C-6) 132.46 (C-22), 141.40 (C-19), 144.88 (C-3), 151.80 (C-7), 158.46 (C-9), 159.90 (C-16), 196.56 (C-11); MS for $\text{C}_{22}\text{H}_{19}\text{ClN}_2\text{O}_2\text{S}$: 410 (MH^+ , 100%), 412 (MH^{+1} , 36.8%), 411 (MH^{+2} , 24%); Elemental Analysis (%): Calculated: C, 64.30; H, 4.66; Cl, 8.63; N, 6.82; Found: C, 64.28; H, 4.65; Cl, 8.62; N, 6.84.

1-(3-(4-methoxyphenyl)-7-methyl-5-(*p*-tolyl)-5H-thiazolo[3,2- α]pyrimidine-6-yl)ethanone (4h): Obtained from 4-(4-methoxyphenyl)thiazole-2-amine (1b, 2.06g, 10.00mmol), acetylacetone (2, 2.00g, 20.00mmol) 4-methylbenzaldehyde (3d, 1.20g, 10.00mmol) and PTSA (2.50g, 15.00mmol) as yellow solid, yield 81% (3.15g), m.p. 148-150°C.



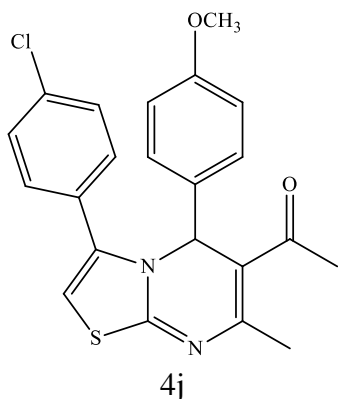
IR (KBr, cm^{-1}): γ 2970 ($-\text{CH}-$), 1675 ($>\text{C}=\text{O}$), 1576 ($-\text{C}=\text{N}-$) cm^{-1} ; ^1H NMR (CDCl_3): δ 2.26 (s, 3H, $-\text{COCH}_3$), 2.34 (s, 3H, Ar- CH_3), 2.92 (s, 3H, $-\text{CH}_3$), 3.83 (s, 3H, $-\text{OCH}_3$), 5.20 (s, 1H, $-\text{CH}-$), 6.94 (s, 1H, $>\text{C}=\text{CH}-$), 6.96-7.58 (m, 8H, Ar-H); ^{13}C NMR ($\text{DMSO}-d_6$): δ 21.22 (Ar- CH_3) 21.46 (C-10), 27.22 (C-12), 55.94 ($-\text{OCH}_3$), 62.08 (C-5), 108.40 (C-2), 126.90 (C-20 and C-24), 160.02 (C-16), 129.86 (C-14 and C-18), 121.10 (C-15 and C-17), 128.92 (C-21 and C-23), 123.24 (C-13), 132.06 (C-6), 136.40 (C-22), 140.46 (C-19), 144.92 (C-3), 151.52 (C-7), 158.20 (C-9), 196.80 (C-11); MS for $\text{C}_{23}\text{H}_{22}\text{N}_2\text{O}_2\text{S}$: 390 (MH^+ , 100%), 391 (MH^{+1} , 26.5%), 392 (MH^{+2} , 5.3%); Elemental Analysis (%): Calculated: C, 70.74; H, 5.68; N, 7.17; Found: C, 70.72; H, 5.69; N, 7.16.

1-(3-(4-chlorophenyl)-7-methyl-5-phenyl-5H-thiazolo[3,2- α]pyrimidine-6-yl)ethanone (4i): Obtained from 4-(4-chlorophenyl)thiazole-2-amine (1c, 2.10g, 10.00mmol), acetylacetone (2, 2.00g, 20.00mmol) benzaldehyde (3a, 1.06g, 10.00mmol) and PTSA (2.50g, 15.00mmol) as yellow solid, yield 80% (3.04), m.p. 144-146°C.



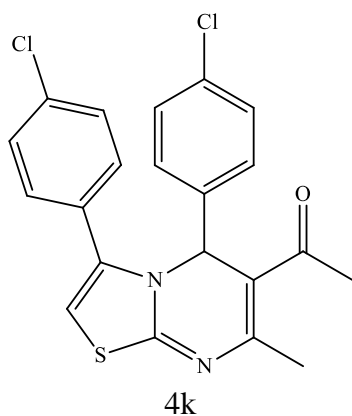
IR (KBr, cm^{-1}): γ 2972 ($-\text{CH}-$), 1673 ($>\text{C}=\text{O}$), 1577 ($-\text{C}=\text{N}-$) cm^{-1} ; ^1H NMR (CDCl_3): δ 2.28 (s, 3H, $-\text{COCH}_3$), 2.90 (s, 3H, $-\text{CH}_3$), 5.22 (s, 1H, $-\text{CH}-$), 6.95 ($>\text{C}=\text{CH}-$), 7.22-7.45 (m, 9H, Ar-H); ^{13}C NMR ($\text{DMSO}-d_6$): δ 21.30 (C-10), 27.12 (C-12), 62.04 (C-5), 108.42 (C-2), 120.46 (C-14 and C-18), 126.80 (C-22), 127.06 (C-20 and C-24), 128.64 (C-21 and C-23), 128.66 (C-15 and C-17), 128.82 (C-13), 131.94 (C-6), 133.62 (C-16), 143.32 (C-19), 145.08 (C-3), 151.92 (C-7), 158.20 (C-9), 196.40 (C-11); MS for $\text{C}_{21}\text{H}_{17}\text{ClN}_2\text{OS}$: 380 (MH^+ , 100%), 382 (MH^{+1} , 36.5%), 381 (MH^{+2} , 22.9%), 383 (7.4%); Elemental Analysis (%): Calculated: C, 66.22; H, 4.50; Cl, 9.31; N, 17.35; Found: C, 66.20; H, 4.51; Cl, 9.32; N, 17.34.

1-(3-(4-chlorophenyl)-5-(4-methoxyphenyl)-7-methyl-5*H*-thiazolo[3,2- α]pyrimidine-6-yl)ethanone (4j): Obtained from 4-(4-chlorophenyl)thiazole-2-amine (1c, 2.10g, 10.00mmol), acetylacetone (2, 2.00g, 20.00mmol) 4-methoxybenzaldehyde (3b, 1.36g, 10.00mmol) and PTSA (2.50g, 15.00mmol) as yellow solid, yield 80% (3.28g), m.p. 150-152°C.



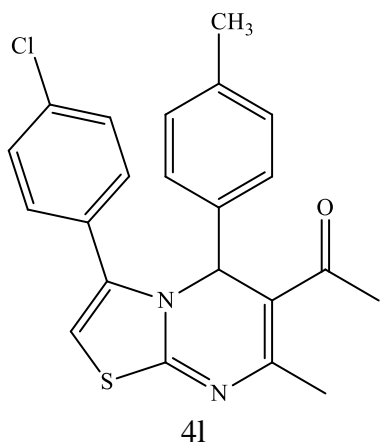
IR (KBr, cm^{-1}): γ 2967 (-CH-), 1676 ($>\text{C}=\text{O}$), 1572 (-C=N-) cm^{-1} ; ^1H NMR (CDCl_3): δ 2.24 (s, 3H, -COCH₃), 2.93 (s, 3H, -CH₃), 3.83 (s, 3H, -OCH₃), 5.23 (s, 1H, -CH-), 6.93 ($>\text{C}=\text{CH}$ -), 6.96-7.48 (m, 8H, Ar-H); ^{13}C NMR ($\text{DMSO}-d_6$): δ 21.30 (C-10), 27.04 (C-12), 55.82 (-OCH₃), 62.24 (C-5), 108.26 (C-2), 114.24 (C-21 and C-23), 120.34 (C-14 and C-18), 125.70 (C-20 and C-24), 128.50 (C-13), 128.82 (C-15 and C-17), 132.04 (C-6), 133.62 (C-16), 135.84 (C-19), 144.84 (C-3), 151.52 (C-7), 158.40 (C-9), 158.66 (C-22), 196.56 (C-11); MS for C₂₂H₁₉ClN₂O₂S: 410 (MH⁺, 100%), 412 (MH⁺, 36.80%), 411 (MH⁺, 24.0%), 413 (7.90%); Elemental Analysis (%): Calculated: C, 64.30; H, 4.66; Cl, 8.63; N, 6.82; Found: C, 64.28; H, 4.65; Cl, 8.64; N, 6.80.

1-(3,5-bis(4-chlorophenyl)-7-methyl-5*H*-thiazolo[3,2- α]pyrimidine-6-yl)ethanone (4k): Obtained from 4-(4-chlorophenyl)thiazole-2-amine (1c, 2.10g, 10.00mmol), acetylacetone (2, 2.00g, 20.00mmol) 4-chlorobenzaldehyde (3c, 1.40g, 10.00mmol) and PTSA (2.50g, 15.00mmol) as yellow solid, yield 82% (3.39g), m.p. 132-134°C.



IR (KBr, cm^{-1}): γ 2975 (-CH-), 1671 ($>\text{C}=\text{O}$), 1576 (-C=N-) cm^{-1} ; ^1H NMR (CDCl_3): δ 2.28 (s, 3H, -COCH₃), 2.91 (s, 3H, -CH₃), 5.21 (s, 1H, -CH-), 6.94 (s, 1H, $>\text{C}=\text{CH}$ -), 7.30-7.50 (m, 8H, Ar-H); ^{13}C NMR ($\text{DMSO}-d_6$): δ 21.36 (C-10), 27.09 (C-12), 62.26 (C-5), 108.28 (C-2), 120.30 (C-14 and C-18), 126.24 (C-20 and C-24), 128.68 (C-21 and C-23), 128.80 (C-15 and C-17), 129.06 (C-13), 132.02 (C-6), 132.22 (C-22), 133.64 (C-16), 141.30 (C-19), 144.80 (C-3), 151.72 (C-7), 158.42 (C-9), 196.60 (C-11); MS for C₂₁H₁₆Cl₂N₂OS: 414 (MH⁺, 100%), 416 (MH⁺, 68.4%), 415 (MH⁺, 23.7%), 417 (15.8%), 418 (13.0%); Elemental Analysis (%): Calculated: C, 60.73; H, 3.88; Cl, 17.07; N, 6.74; Found: C, 60.72; H, 3.86; Cl, 17.09; N, 6.73.

1-(3-(4-chlorophenyl)-7-methyl-5-(*p*-tolyl)-5*H*-thiazolo[3,2- α]pyrimidine-6-yl)ethanone (4l): Obtained from 4-(4-chlorophenyl)thiazole-2-amine (1c, 2.10g, 10.00mmol), acetylacetone (2, 2.00g, 20.00mmol) 4-methylbenzaldehyde (3d, 1.20g, 10.00mmol) and PTSA (2.50g, 15.00mmol) as yellow solid, yield 84% (3.30g), m.p. 130-132°C.



IR (KBr, cm^{-1}): γ 2971 (-CH-), 1677 (>C=O), 1575 (-C=N-) cm^{-1} ; ^1H NMR (CDCl_3): δ 2.27 (s, 3H, -COCH₃), 2.34 (s, 3H, Ar-CH₃), 2.92 (s, 3H, -CH₃), 5.20 (s, 1H, -CH-), 6.92 (s, 1H, >C=CH-), 7.10-7.45 (m, 8H, Ar-H); ^{13}C NMR ($\text{DMSO-}d_6$): δ 21.02 (Ar-CH₃), 21.24 (C-10), 27.14 (C-12), 62.06 (C-5), 108.40 (C-2), 120.08 (C-14 and C-18), 126.82 (C-20 and C-24), 128.70 (C-15 and C-17), 128.80 (C-13), 128.92 (C-21 and C-23), 131.94 (C-6), 133.46 (C-16), 136.56 (C-22), 140.44 (C-19), 145.08 (C-3), 151.60 (C-7), 158.36 (C-9), 196.40 (C-11); MS for $\text{C}_{22}\text{H}_{19}\text{ClN}_2\text{OS}$: 394 (MH^+ , 100%), 396 (MH^{+1} , 37.1%), 395 (MH^{+2} , 25.4%), 397 (9.1%); Elemental Analysis (%): Calculated: C, 66.91; H, 4.85; Cl, 8.98; N, 7.08; Found: C, 66.90; H, 4.83; Cl, 8.99; N, 7.09.

2.2. Biological assay.

2.2.1. Molecular docking studies.

Docking studies of the compounds 4(a-l) were carried out using Autodock-4.2 with the Lamarckian Genetic Algorithm (LGA) computational method to rationalize the plausible best-suited candidates for binding. Many of the molecules were found to have minimum binding energies ranging from -7.14 kJ/mol to -8.81 kJ/mol against protein DNA gyrase (PDB Code: 1KZN) target. Almost all the ligands showed considerable hydrogen bond interaction with active site amino acid residues. The docking study results showed a molecule that has good inhibition constant, vdW + Hbond + desolv energy with the best RMSD value against proteins targets. The details of docked score results are given in Table 1.

2.2.2. Antimicrobial activity.

The synthesized compounds 1-(7-methyl-3,5-diphenyl-5H-thiazolo(3,2- α)pyrimidine-6-yl)ethanone 4(a-l) were screened for *in vitro* antibacterial activity against *Bacillus cereus* (MTCC 8372), *Staphylococcus aureus* (MTCC 96) [gram positive bacteria] *Escherichia coli* (MTCC 724) and *Klebsiella pneumonia* (MTCC 432) [gram negative bacteria] using agar disc diffusion [28] method using Tetracycline used as standard drug. The compounds 4(a-l) were dissolved in DMF (dimethylformamide) at 50 and 100 $\mu\text{g}/\text{mL}$ concentration and placed on the inoculated plates after allowing at 4°C for 2h, they were incubated at 37°C for 24 hours, and the inhibition zone was measured in millimeters. Besides, *in vitro* antifungal screening [29] of the compounds 4(a-l) was carried out against *Aspergillus flavus* (MTCC873), *Aspergillus niger* (MTCC 281), *Fusarium oxysporum* (MTCC 284), and *Fusarium moniliform* (MTCC 156) using *Nystatin* as standard drug. The microdilution method was used to appraise the minimum inhibitory concentration (MIC) of all the obtained compounds, as summarized in Table 2. The compounds were stable in the Nutrient agar and Potato dextrose agar. The MIC for fungal strains was executed using a 96-well plate. The fungi were preserved on potato dextrose agar (PDA) medium at 28°C. Six replicate determinations were performed for all the compounds, and results were taken as a mean of at least three determinations.

3. Results and Discussion

The one-pot three-component synthesis of 1-(7-methyl-3,5-diphenyl-5*H*-thiazolo(3,2- α)pyrimidine-6-yl)ethanone 4(a-1) in good yield was achieved initially by Knoevenagel condensation reaction between active methylene acetylacetone (2) with substituted aromatic aldehydes (3a-d) using acidic PTSA [30] in the presence of acetonitrile as solvent. Meanwhile, the addition of 4-phenylthiazole-2-amine (1a-c) to the reaction mixture and refluxed for about 2-3 hours to get the target product 5*H*-thiazolo(3,2- α)pyrimidine-6-yl)ethanone derivatives 4(a-1) (Scheme 1).

The synthesized compounds were characterized by their IR, ^1H NMR, ^{13}C NMR, Mass, and elemental analysis. In the IR spectra, 4(a-1) showed the stretching vibration bands at around 2967-2975 cm^{-1} corresponding to the presence of $-\text{CH}-$ group, vibration bands 1560-1577 cm^{-1} for $-\text{C}=\text{N}-$ group and vibration band 1669-1679 cm^{-1} indicates the presence of $>\text{C}=\text{O}$ group in the compound. The ^1H NMR spectra of compounds 4(a-1) showed the signals due to $-\text{CH}_3$ proton in the region δ 2.90 to 2.96 ppm, singlet peaks of $-\text{CO}-\text{CH}_3$ group appeared in the region δ 2.23 to 2.28 ppm, while singlet peak of $-\text{CH}-$ group appeared in the region δ 5.20 to 2.26 ppm and all other aromatic protons at δ 6.88 to 7.75 ppm. The absence of $-\text{NH}_2$ singlet peak in the region δ 8.80 to 9.20 ppm confirms the formation of condensation product 4(a-1). In ^{13}C NMR spectra presence of additional peaks in the range of δ 21.08 to 21.48 ppm (C-10), δ 27.04 to 27.26 ppm (C-12), and δ 62.06 to 65.20 ppm (C-5) was observed. All synthesized compounds 4(a-1) showed MH^+ as a base peak in the mass spectra.

3.1. Structure-activity relationship.

Docking studies of compounds 4(a-1) with docked score results were portrayed in Table 1. The compounds 4k, 4l, 4j, and 4c were found to have minimum binding energy with -8.81, -8.47, -8.19, and -8.06 kJ mol^{-1} , respectively, with one hydrogen bond as shown in figure 1. Additionally, 4b and 4d possess comparable binding energy with -8.28 and -8.22 kJ mol^{-1} , respectively, without hydrogen bond. All other compounds were found to have moderate binding energy. The compound 4k, 4l, 4j, and 4c found to have the highest ligand efficiency with -0.33, -0.31, -0.29, and -0.31 respectively and all other compounds found to have moderate ligand efficiency.

3.2. Antimicrobial assay.

The series of synthesized compounds 4(a-1) contains one of the nucleoside base pyrimidine moiety, and we expected good antimicrobial activity, as shown in Table 2. The binding energy based on the docking study and antimicrobial evaluations is comparable, as shown in table 1 and table 2. The results revealed that the compounds 4k showed excellent antibacterial effect with binding energy -8.81 kJ mol^{-1} against all the tested strains; this may be due to the presence of $-\text{Cl}$ group on both para position of the phenyl rings of thiazole as well as pyrimidine moieties, 4l, 4b, 4d, and 4j and found to moderate with binding energy -8.47, -8.28, -8.22 and -8.19 kJ mol^{-1} respectively, it may be due to the presence of electron-donating group on one of the phenyl ring of the pyrimidine ring. The compounds 4i was less active against bacterial strains, but it possesses good antifungal activity with -7.25 kJ mol^{-1} binding energy; it may be the presence of $-\text{Cl}$ group at the para position of the phenyl ring of thiazole, while 4c, 4j to 4l compounds showed excellent antifungal activity and the remaining

compounds showed moderate antifungal activity with, it may be due to the presence of an electron-withdrawing group at thiazole or pyrimidine moiety.

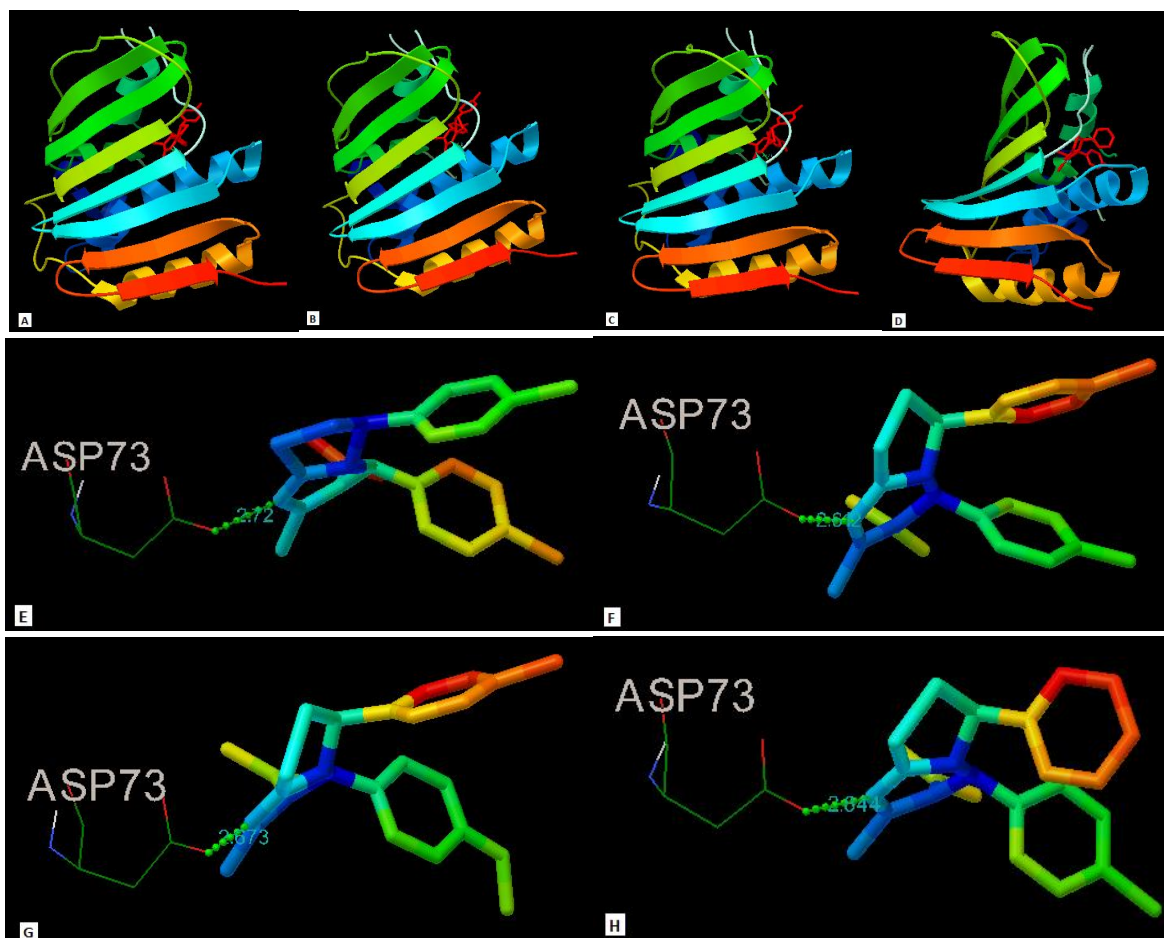


Figure 1. Molecular Docking of (A) 4k, (B) 4l, (C) 4j, (D) 4c against protein target DNA gyrase, and (E) 4k, (F) 4l, (G) 4j, (H) 4c showing hydrogen bonding.

Table 1. The docking results of the different ligand molecules (4a-l) with protein target DNA gyrase.

Compounds	Binding Energy (kJ mol ⁻¹)	Ligand Efficiency	Inhibition Constant	vdW+H-bond+desolv energy	No. of H-bonds	Bonding residues	Bond Length (Å)
4a	-7.97	-0.32	1.43	-8.85	1	1KZN:A:ASP73:OD1	2.775
4b	-8.28	-0.31	855.01	-9.47	-	-	-
4c	-8.06	-0.31	1.24	-8.93	1	1KZN:A:ASP73:OD1	2.844
4d	-8.22	-0.32	948.34	-9.17	-	-	-
4e	-7.88	-0.29	1.68	-8.98	1	1KZN:A:ASP73:OD1	2.732
4f	-7.14	-0.25	5.87	-8.57	-	-	-
4g	-7.91	-0.27	1.58	-9.35	1	1KZN:A:ASP73:OD1	2.803
4h	-7.89	-0.28	1.65	-9.05	-	-	-
4i	-7.25	-0.28	4.85	-8.11	1	1KZN:A:ASN46: HD21	1.764
4j	-8.19	-0.29	995.56	-9.31	1	1KZN:A:ASP73:OD1	2.673
4k	-8.81	-0.33	348.14	-9.68	1	1KZN:A:ASP73:OD1	2.720
4l	-8.47	-0.31	619.68	-9.34	1	1KZN:A:ASP73:OD1	2.612

Table 2. The minimal inhibitory concentration (MIC), minimal bactericidal concentration (MBC), and minimal fungicidal concentration (MFC) in µg/mL of synthesized compounds against tested strains.

Compounds	Antibacterial activity						Antifungal activity									
	Gram-positive			Gram-negative			A. flavus		A. niger		F. oxysporum		F. moniliforme			
	B. cereus MIC	B. cereus MBC	S. aureus MIC	S. aureus MBC	E. coli MIC	E. coli MBC	K pneumoniae MIC	K pneumoniae MBC	MIC	MFC	MIC	MFC	MIC	MFC		
4a	75	270	70	250	80	260	95	270	65	260	60	255	65	275	70	270
4b	18	135	25	150	25	155	20	140	60	250	50	260	50	270	60	265
4c	15	125	20	135	15	125	30	125	20	130	15	130	20	135	20	125
4d	25	135	35	150	45	145	20	130	60	250	50	220	65	270	55	230
4e	30	145	25	135	30	145	25	175	30	145	40	150	45	140	35	155
4f	50	240	45	235	50	240	45	235	80	255	50	245	55	265	55	240
4g	55	170	50	165	50	160	35	185	25	140	35	150	25	140	45	145
4h	40	160	45	150	45	155	30	180	30	210	35	200	40	195	25	200
4i	55	230	70	240	50	235	55	245	10	120	15	130	20	125	15	120
4j	20	130	30	145	20	130	15	140	15	130	18	120	20	120	15	125
4k	40	145	45	175	35	170	35	150	10	115	15	120	25	115	20	130
4l	20	150	25	165	40	210	35	135	20	130	25	135	30	135	35	145
Tetracycline	5	120	10	120	12	120	8	120	---	---	---	---	---	---	---	---
Nystatin	---	---	---	---	---	---	---	---	08	100	10	100	15	100	12	100

^a(Mean six replicate ± standard deviation).

4. Conclusions

In conclusion, we have synthesized the bicyclic thiazolo[3,2- α]pyrimidine 4(a-l) via a one-pot three-component chemical transformation of derivatives using electron-rich substituent on the phenyl ring of the thiazole and pyrimidine. This method delivers a

remarkable yield (80–86%) of the target products in 2-3 hours with low-cost PTSA. All the synthesized compounds have been investigated for their docking study and *in vitro* antimicrobial activity. Among the synthesized compounds, 4k emerged as an excellent antibacterial agent with the least binding energy against protein DNA gyrase (PDB Code: 1KZN). Also, the compounds 4k and 4l possess good antifungal activity compared with standard drugs. Hence, 4k could be a promising drug candidate for microbial infections.

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Conflicts of Interest

The authors declare no conflict of interest.

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