Synthesis, Spectral Characterization and Antimicrobial Evaluation of Novel Thieno[2,3-d]pyrimidine-Based Chalcone Derivatives

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Abstract:

A novel series of thieno[2,3-d]pyrimidine-based chalcone derivatives (5a–5j) were successfully synthesized through a multistep synthetic protocol starting from cyclohexanone via Gewald reaction, followed by cyclization, chlorination, nucleophilic substitution, and Claisen-Schmidt condensation. The structures of the synthesized compounds were confirmed using spectroscopic techniques including ^{1}H NMR, ^{13}C NMR, and HRMS analysis. The synthesized derivatives were evaluated for their in vitro antimicrobial activity against selected Gram-positive bacteria (Staphylococcus aureus and Bacillus subtilis), Gram-negative bacteria (Escherichia coli and Pseudomonas aeruginosa), and fungal strains (Candida albicans, Saccharomyces cerevisiae, and Aspergillus brasiliensis) using the broth microdilution method. Among the tested compounds, 5a, 5g, and 5h exhibited notable antibacterial activity against both Gram-positive and Gram-negative bacteria, while 5g demonstrated consistent antifungal activity against all tested fungal strains. The presence of electron-withdrawing substituents such as chloro and nitro groups significantly enhanced antimicrobial activity. These findings suggest that thieno[2,3-d]pyrimidine-based chalcone derivatives represent promising scaffolds for the development of new antimicrobial agents.

Introduction:

Organic chemistry is important in modern research because it ties fundamental chemical principles to medical and pharmaceutical issues, allowing for the discovery and development of therapeutic molecules [1]. The separation and purification of physiologically active chemicals from plant and animal sources, as well as microbes and their fermentation products, has sparked widespread interest among researchers worldwide. Medicinal chemistry, which combines ideas from organic chemistry, biology, and some parts of physics, serves as a basis for the creation of new medicinal compounds [2]. Heterocyclic systems are a popular class of organic molecules in medicinal chemistry due to their broad range of biological activity [3].

Synthetic heterocyclic chemistry has become quite important since it is used in many different fields, such as medicines, agrochemicals, and dyeing. Pyrimidine derivatives and fused pyrimidine systems have garnered significant attention due to their varied pharmacological characteristics. Researchers have looked at pyrimidine-containing compounds a lot to make bioactive molecules and drugs, such as bronchodilators and antihistamines [4, 5]. Thienopyrimidine derivatives are an important group of heterocyclic compounds with great medicinal promise. They are made by combining thiophene and pyrimidine rings [6]. Compounds with the thienopyrimidine nucleus have been shown to have a

wide range of biological effects, including anticancer, anti-inflammatory, antibacterial, antifungal, anticonvulsant, antihyperlipidemic, and antipsychotic effects [7, 8].

Chalcones are often made by combining aryl ketones and aromatic aldehydes with the right condensing agents [9]. Chalcones are useful building blocks for making different heterocyclic compounds because they have a reactive α,β-unsaturated carbonyl system and may react in many different ways [10]. Reports in the literature show that chalcone derivatives have a wide range of biological effects, which makes them viable candidates for medicinal chemistry [11]. Chalcones are very beneficial for making new bioactive compounds since they are simple to manufacture, easy to modify, and may be used in many different ways [12]. Chalcone derivatives are still being studied, even though they have a lot of potential as scaffolds for drug development [13].

The rapid evolution and spread of antimicrobial resistance among pathogenic microbes has become a major worldwide health problem, dramatically lowering the efficacy of traditional antibiotics and antifungal medicines [14]. Misuse and abuse of antimicrobial medications have hastened the emergence of resistant strains, increasing illness, death, and healthcare expenditures globally [15]. Resistance to common bacterial and fungal infections has produced an urgent need for the development of new antimicrobial medicines with increased effectiveness and novel modes of action [16]. Heterocyclic compounds, particularly those incorporating fused pyrimidine systems and chalcone frameworks, have sparked widespread interest due to their promising antibacterial properties and structural variety [17]. As a result, the development and synthesis of novel thieno[2,3-*d*]pyrimidine-based chalcone derivatives is an essential technique for identifying effective antibacterial candidates capable of fighting resistant microbial strains.

The current study is to develop and synthesise a novel series of thieno[2,3-d]pyrimidine-based chalcone derivatives in light of the intriguing biological activities linked to both thienopyrimidine and chalcone frameworks. In order to investigate potential therapeutic uses, the synthesised compounds were characterised using suitable spectroscopic methods, and their antibacterial ability was assessed against certain bacterial and fungal species.

Result and discussion

Chemistry

The synthetic route adopted for the preparation of thieno[2,3-*d*]pyrimidine-based chalcone derivatives **(5a–5j)** is illustrated in **Scheme 1** . The target compounds were synthesized through a multistep reaction sequence starting from cyclohexanone, ethyl cyanoacetate, and sulfur powder.

Initially, 2-amino-3-ethylcarboxy-5,5-dimethyl-4,5,6,7-tetrahydrobenzo[*b*]thiophene **(1)** was synthesized via the Gewald reaction involving cyclohexanone, ethyl cyanoacetate, and elemental sulfur in the presence of diethylamine as a base catalyst. The reaction mixture was maintained at elevated temperature to facilitate cyclization, affording compound **(1)** in good yield after recrystallization from methanol. The progress of the reaction was monitored by thin-layer chromatography (TLC), confirming the formation of the desired thiophene derivative. Subsequently, compound **(1)** was converted into 6,6-dimethyl-5,6,7,8-tetrahydrobenzo [4,5]thieno[2,3-*d*]pyrimidin-4(3*H*)-one **(2)** by cyclization with formamide under reflux conditions. The intramolecular condensation resulted in the formation of the pyrimidinone ring system, yielding compound **(2)** as a crystalline solid after precipitation in ice-cold water and recrystallization.

In the next step, compound **(2)** was transformed into 4-chloro-6,6-dimethyl-5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3-*d*]pyrimidine **(3)** through chlorination using phosphorus oxychloride in the presence of catalytic phosphorus pentachloride. This reaction introduced a chloro substituent at the 4-position of the pyrimidine ring, enhancing its reactivity toward nucleophilic substitution. The excess phosphorus oxychloride was removed under reduced pressure, and the resulting product was neutralized and isolated using aqueous sodium bicarbonate. Further nucleophilic substitution of compound 3 with 4-aminoacetophenone in acetone medium afforded 1-(4-((6,6-dimethyl-5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3-*d*]pyrimidin-4-yl)amino)phenyl)ethan-1-one **(4)**. The reaction proceeded efficiently in the presence of pyridine as a base, resulting in the formation of the desired intermediate containing an acetophenone moiety attached through an amino linkage. The product was obtained in satisfactory yield after recrystallization.

Finally, the key chalcone derivatives **(5a–5j)** were synthesized through Claisen–Schmidt condensation of compound 4 with various substituted benzaldehydes in ethanol using aqueous sodium hydroxide as a base catalyst. The reaction mixture was stirred at room temperature to facilitate the formation of the α,β-unsaturated carbonyl system characteristic of chalcone derivatives. The completion of the reaction was followed by acidification with hydrochloric acid and cooling, resulting in the precipitation of the desired chalcone derivatives. The obtained products were filtered, washed, and recrystallized from ethanol to yield the final series of thieno[2,3-*d*]pyrimidine-based chalcone derivatives **(5a–5j)** in good yields.

The structures of all synthesized compounds were confirmed using appropriate spectroscopic techniques, including IR, ^{1}H NMR, ^{13}C NMR, and mass spectrometry, which

supported the successful formation of the targeted thieno[2,3-*d*]pyrimidine-based chalcone framework.

In vitro antimicrobial activity

The *in vitro* antimicrobial activities of thieno[2,3-*d*]pyrimidine based isoxazole derivatives **(5a-5j)** were evaluated by the broth dilution method. All synthesized compounds were screened for antibacterial activity against gram-positive bacteria *Staphylococcus aureus* (ATCC 6538) and *Bacillus subtilis* (ATCC 6633), gram-negative bacteria *Escherichia coli* (ATCC 25,922) and *Pseudomonas aeruginosa* (ATCC 9027). The synthesized compounds were further evaluated for their antifungal potential against yeast strains, namely *Candida albicans* (ATCC 10231) and *Saccharomyces cerevisiae* (ATCC 9763), as well as the filamentous fungus *Aspergillus brasiliensis* (ATCC 16404). The results are presented in **(Table 1)** and **(Table 2)** respectively.

Table 1 : Results of *in vitro* antibacterial activity of thieno[2,3-*d*]pyrimidine based chalcone derivatives **(5a-5j)**

Minimum Inhibitory Concentration (µg/ml)					
Compd.	**R**	**Antibacterial activity**			
		Gram-positive		**Gram-negative**	
		S. Aureus	***B. subtilis***	***E. Coli***	***P. Aeruginosa***
5a	(4-Cl)	**62.5**	200	**62.5**	500
5b	(3-Cl)	500	250	250	200
5c	(2-Cl)	125	250	200	250
5d	(2,4-Cl)	250	200	500	125
5e	(3-Br)	125	500	125	500
5f	(4-F)	500	250	250	250
5g	(2-NO_2)	200	**62.5**	200	125
5h	(4-NO_2)	250	125	**62.5**	**62.5**
5i	(3,4,5-OCH_3)	500	250	125	500
5j	(4-CH_3)	500	500	250	>500
Gentamicin		50	50	50	50
Ciprofloxacin		50	50	50	25

Table 1 shows that compound **(5a)** exhibited significant antibacterial activity against both Gram-positive and Gram-negative bacterial strains, particularly against *S. aureus* and *E. coli*. Compounds **(5g)** and **(5h)** exhibited notable activity, especially against *B. subtilis*, *E. coli*,

and *P. aeruginosa*. Compounds **(5c)** and **(5d)** showed substantial inhibitory effects against Gram-negative bacteria, while the remaining compounds demonstrated moderate to good antibacterial activity against the tested microorganisms. The enhanced activity of compounds **(5a)**, **(5g)**, and **(5h)** against Gram-positive and Gram-negative bacteria may be attributed to the presence of electron-withdrawing substituents such as chloro and nitro groups, which facilitate stronger interactions with bacterial targets. The observed activity of compounds containing nitro and di-chloro substituents may also be associated with their improved ability to penetrate bacterial cell membranes and interact with intracellular components.

Table 2 : Results of *in vitro* antifungal activity of thieno[2,3-*d*]pyrimidine based chalcone derivatives **(5a-5j)**

Minimum Inhibitory Concentration (µg/ml)				
Comp.	**R**	**Antifungal activity**		
		C. albicans	***S. cerevisiae***	***A. brasiliss***
5a	(4-Cl)	1000	1000	500
5b	(3-Cl)	500	1000	500
5c	(2-Cl)	500	500	1000
5d	(2,4-Cl)	1000	500	500
5e	(3-Br)	>1000	>1000	>1000
5f	(4-F)	1000	500	>1000
5g	(2-NO_2)	500	500	500
5h	(4-NO_2)	500	1000	500
5i	(3,4,5-OCH_3)	500	500	1000
5j	(4-CH_3)	>1000	500	500
Nystatin		100	100	100

*Bold value indicates highly active compounds against microorganism

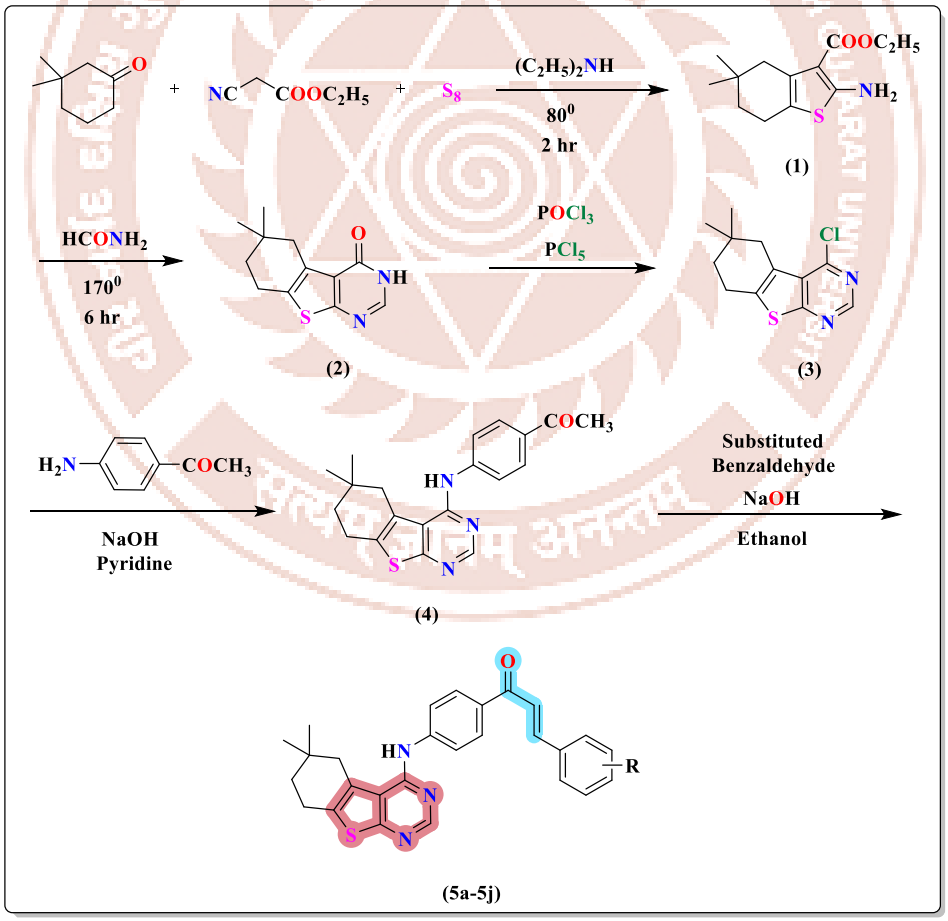
Table 2 shows that compound **(5g)** exhibited consistent antifungal activity against all tested fungal strains. Compounds **(5b)**, **(5c)**, and **(5d)** demonstrated notable antifungal activity, particularly against *Candida albicans* and *Saccharomyces cerevisiae*, while compounds **(5f)** and **(5h)** showed selective inhibitory effects against specific fungal strains. The remaining compounds displayed moderate to weak antifungal activity. The enhanced antifungal activity observed for compounds **(5g)**, **(5b)**, and **(5c)** may be attributed to the presence of electron-withdrawing groups, particularly nitro and chloro substituents, which enhance their interaction

with fungal cellular components. In contrast, compounds containing electron-donating substituents such as methyl and methoxy groups exhibited comparatively lower antifungal activity.

Experimental

Materials and methods

All chemicals, reagents, and solvents were procured from commercial suppliers such as Merck and Sigma-Aldrich and were utilized as received without any additional purification. All reactions were performed in properly dried glassware under standard laboratory conditions. Melting points were measured using the open capillary tube method. The progress of reactions was monitored by thin-layer chromatography (TLC) using silica gel 60 F254 plates (0.25 mm thickness, Merck), and the spots were visualized under short-wavelength ultraviolet light. The ^1H and ^13C nuclear magnetic resonance (NMR) spectra were recorded on a Bruker 400 MHz spectrometer using DMSO-d_6 as the solvent. Chemical shift values were expressed in parts per million (ppm).



Where, R: 4a = 4-Cl, 4b = 3-Cl, 4c = 2-Cl, 4d = 2,4-Cl, 4e = 3-Br

4f = 4-F, 4g = 2-NO_2, 4h = 4-NO_2, 4i = 3,4,5-OCH_3, 4j = 4-CH_3

Scheme 1 : synthesis of thieno[2,3-d]pyrimidine based chalcone derivatives *General procedure for the synthesis of thieno[2,3-d]pyrimidine based pyrazole derivatives (5a-5j)*

*Synthesis of 2-amino-3-ethylcarboxy-5,5-dimethyl-4,5,6,7-tetra-hydrobenzo[b] thiophene **(1)***

Sulphur powder (3.85 g, 0.12 mol) was introduced into a stirred solution of cyclohexanone (8.36 mL, 0.08 mol) and ethyl cyanoacetate (8.51 mL, 0.08 mol) at room temperature. The resulting heterogeneous reaction mixture was maintained at 80 °C for 2 h, during which diethylamine (8.27 mL, 0.08 mol) was added slowly dropwise. After completion of the addition, the reaction mixture was allowed to stand at room temperature overnight to afford 2-amino-3-ethylcarboxy-5,5-dimethyl-4,5,6,7-tetrahydrobenzo[*b*]thiophene **(1).** The reaction progress was monitored by TLC. It was washed thoroughly with cold water and recrystallized from methanol. Yield: 85 % (m.p.: 108-110 °C) [18].

*Synthesis of 6,6-dimethyl-5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3-d]pyrimidin-4(3H)-one **(2)***

2-Amino-3-ethylcarboxy-5,5-dimethyl-4,5,6,7-tetrahydrobenzo[*b*]thiophene **(1)** (18.0 g, 0.08 mol) was heated under reflux with formamide (150 mL) at 170 °C for 6 h with continuous stirring. After completion of the reaction, the hot mixture was carefully poured into ice cold water and stirred for an additional 30 min to yield 6,6-dimethyl-5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3-*d*]pyrimidin-4(3*H*)-one (2). It was collected by filtration, washed thoroughly with water and recrystallization from methanol. Yield: 82 % (m.p.: 202-204 °C) [19].

*Synthesis of 4-chloro-6,6-dimethyl-5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3-d]pyrimidine **(3)***

6,6-Dimethyl-5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3-*d*]pyrimidin-4(3*H*)-one **(2)** (1.65 g, 0.008 mol) was reacted with phosphorus oxychloride (12 mL) in the presence of a catalytic amount of phosphorus pentachloride under reflux with continuous stirring. After completion of the reaction, excess phosphorus oxychloride was removed under reduced pressure. The resulting residue was cautiously poured into an aqueous sodium bicarbonate solution to give 4-chloro-6,6-dimethyl-5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3-*d*]pyrimidine (3). It was filtered, washed thoroughly with water, dried and recrystallization from methanol. Yield: 75 % (m.p.: 106-108 °C) [20].

*Synthesis of 1-(4-((6,6-dimethyl-5,6,7,8-tetrahydrobenzo[4,5] thieno[2,3-d]pyrimidin-4-yl)amino)phenyl)ethan-1-one **(4)***

A solution of 4-aminoacetophenone (0.02 mol) in acetone (10 mL) was added dropwise over a period of 5-6 hours to a stirred solution of 4-chloro-6,6-dimethyl-5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3-*d*]pyrimidine **(3)** (0.02 mol) in acetone (30 mL) at 70-80 °C for

2 hours. After this period, pyridine (2 mL) was introduced to the reaction mixture. Upon complete addition of the 4-aminoacetophenone solution, stirring was continued for an additional 2–3 hours. The reaction mixture was then poured into ice cold water to give 1-(4-((6,6-dimethyl-5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3-*d*]pyrimidin-4-yl)phenyl) ethan-1-one **(4)**, it was filtered, washed thoroughly with water, dried and recrystallized from methanol. The final yield was in the range of 70-72 %. (m.p.: 152-154 °C) [21].

*Synthesis of 3"-(substituted phenyl)-1"-(4'-((6,6-dimethyl-5,6,7,8-tetrahydrobenzo[4,5] thieno[2,3-d]pyrimidin-4-yl)amino)phenyl) prop-2"-en-1"-one derivatives **(5a-5j)***

1-(4-((6,6-Dimethyl-5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3-*d*]pyrimidin-4-yl) phenyl)ethan-1-one **(4)** (0.02 mol) was dissolved in ethanol (50 mL) and stirred continuously. A solution of substituted benzaldehyde (0.02 mol) in ethanol was added to the reaction mixture while maintaining gentle heating in a water bath. Subsequently, a 20% aqueous NaOH (3 mL) solution was introduced dropwise. The mixture was stirred at room temperature for 24 h. Upon completion, the pH was adjusted to 2 using concentrated HCl, and the reaction mixture was stored at 0 °C overnight to give precipitates of 3"-(substituted phenyl)-1"-(4'-((6,6-dimethyl-5,6,7,8-tetrahydrobenzo- [4,5]thieno[2,3-*d*]pyrimidin-4-yl)amino)phenyl)prop-2"-en-1"-one **(5a–5j)**. It was filtered, washed thoroughly with water, dried and recrystallized from ethanol [22].

*(E)-3-(4-chlorophenyl)-1-(4-((6,6-dimethyl-5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3-d] pyrimidin-4-yl)amino)phenyl)prop-2-en-1-one **(5a)***

Yield: 82 %; ^{1}H NMR (400 MHz, DMSO-d6): 8.61 (s, 1H), 8.09-8.18 (d, 2H), 7.61 (s, 1H), 6.87-7.08 (m, 8H), 2.68-2.72 (m, 4H), 1.80-1.82 (t, 2H), 1.01-1.02 (s, 6H); ^{13}C NMR (400 MHz, DMSO-d6): 170.02, 164.02, 156.92, 152.61, 144.01, 141.91, 136.71, 134.81, 133.91, 133.61, 132.91, 131.51, 129.81, 128.21, 121.81, 119.71, 114.81, 38.60, 36.20, 31.30, 28.90, 25.90; HRMS: m/z (*M* + H)+ cacld. for $C_{27}H_{23}ClN_4OS$: 474.1364 found: 474.1366.

*(E)-3-(3-chlorophenyl)-1-(4-((6,6-dimethyl-5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3-d]pyrimidin-4-yl)amino)phenyl)prop-2-en-1-one **(5b)***

Yield: 85 %; ^{1}H NMR (400 MHz, DMSO-d6): 8.62 (s, 1H), 8.08-8.15 (d, 2H), 7.60 (s, 1H), 6.89-7.10 (m, 8H), 2.65-2.73 (m, 4H), 1.82-1.85 (t, 2H), 1.00-1.01 (s, 6H); ^{13}C NMR (400 MHz, DMSO-*d6*) δ 189.83, 163.03, 155.90, 152.19, 143.12, 143.05, 136.03, 135.76, 134.92, 133.81, 132.61, 130.56, 129.86, 128.67, 127.85, 126.97, 122.48, 118.69, 113.79, 39.83, 35.97, 30.87, 28.11, 25.81; HRMS: m/z (*M* + H)+ cacld. for $C_{27}H_{23}ClN_4OS$: 474.1329 found: 474.1332.

*(E)-3-(2-chlorophenyl)-1-(4-((6,6-dimethyl-5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3-d]pyrimidin-4-yl)amino)phenyl)prop-2-en-1-one **(5c)***

Yield: 80 %; ^{1}H NMR (400 MHz, DMSO-d6): 8.62 (s, 1H), 8.11-8.20 (d, 2H), 7.61 (s, 1H), 6.88-7.10 (m, 8H), 2.70-2.73 (m, 4H), 1.81-1.82 (t, 2H), 1.02-1.03 (s, 6H); ^{13}C NMR (400 MHz, DMSO-*d*6) δ 189.46, 163.03, 155.90, 152.19, 143.05, 139.39, 135.76, 133.86, 133.84, 133.56, 132.61, 130.79, 130.56, 129.29, 129.17, 127.85, 123.33, 118.69, 113.79, 39.83, 35.97, 30.87, 28.11, 25.81; HRMS: m/z (*M* + H)+ cacld. for $C_{27}H_{23}ClN_4OS$: 474.1329 found: 474.1331.

(E)-3-(2,4-dichlorophenyl)-1-(4-((6,6-dimethyl-5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3-d]pyrimidin-4-yl)amino)phenyl)prop-2-en-1-one ***(5d)***

Yield: 79 %; ^{1}H NMR (400 MHz, DMSO-d6): 8.61 (s, 1H), 8.10-8.16 (d, 2H), 7.60 (s, 1H), 6.89-7.12 (m, 8H), 2.65-2.71 (m, 4H), 1.79-1.80 (t, 2H), 1.01-1.02 (s, 6H); ^{13}C NMR (400 MHz, DMSO-*d*6) δ 189.46, 163.03, 155.90, 152.19, 143.05, 139.00, 135.76, 135.29, 134.83, 133.84, 132.61, 132.13, 130.56, 129.32, 129.16, 128.45, 123.87, 118.69, 113.79, 39.83, 35.97, 30.87, 28.11, 25.81; HRMS: m/z (*M* + H)+ cacld. for $C_{27}H_{23}ClN_4OS$: 508.0939 found: 508.0941.

(E)-3-(3-bromophenyl)-1-(4-((6,6-dimethyl-5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3-d]pyrimidin-4-yl)amino)phenyl)prop-2-en-1-one ***(5e)***

Yield: 81 %; ^{1}H NMR (400 MHz, DMSO-d6): 8.61 (s, 1H), 8.08-8.17 (d, 2H), 7.62 (s, 1H), 6.87-7.08 (m, 8H), 2.69-2.74 (m, 4H), 1.80-1.83 (t, 2H), 1.02-1.04 (s, 6H); ^{13}C NMR (400 MHz, DMSO-*d*6) δ 189.83, 163.03, 155.90, 152.19, 143.05, 142.64, 136.08, 135.76, 133.81, 132.61, 132.08, 131.78, 130.56, 130.26, 127.46, 124.04, 122.77, 118.69, 113.79, 39.83, 35.97, 30.87, 28.11, 25.81; HRMS: m/z (*M* + H)+ cacld. for $C_{27}H_{23}ClN_4OS$: 518.0823 found: 518.0825.

(E)-1-(4-((6,6-dimethyl-5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3-d]pyrimidin-4-yl)amino) phenyl)-3-(4-fluorophenyl)prop-2-en-1-one ***(5f)***

Yield: 82 %; ^{1}H NMR (400 MHz, DMSO-d6): 8.60 (s, 1H), 8.08-8.17 (d, 2H), 7.63 (s, 1H), 6.88-7.10 (m, 8H), 2.67-2.70 (m, 4H), 1.81-1.84 (t, 2H), 1.02-1.03 (s, 6H); ^{13}C NMR (400 MHz, DMSO-*d*6) δ 189.83, 163.72, 163.09, 163.03, 155.90, 152.19, 143.36, 143.05, 135.76, 133.81, 132.61, 130.99, 130.98, 130.56, 130.08, 130.07, 120.82, 118.69, 116.05, 116.00, 113.79, 39.83, 35.97, 30.87, 28.11, 25.81; HRMS: m/z (*M* + H)+ cacld. for $C_{27}H_{23}ClN_4OS$: 458.1624 found: 458.1626.

(E)-1-(4-((6,6-dimethyl-5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3-d]pyrimidin-4-yl)amino) phenyl)-3-(2-nitrophenyl)prop-2-en-1-one ***(5g)***

Yield: 86 %; ^{1}H NMR (400 MHz, DMSO-d6): 8.61 (s, 1H), 8.09-8.15 (d, 2H), 7.60 (s, 1H), 6.87-7.10 (m, 8H), 2.67-2.70 (m, 4H), 1.81-1.83 (t, 2H), 1.01-1.02 (s, 6H); ^{13}C NMR (400

MHz, DMSO-*d*6) δ 189.43, 163.03, 155.90, 152.19, 148.58, 143.05, 138.74, 135.76, 133.98, 132.72, 132.61, 131.45, 130.94, 130.56, 130.49, 125.09, 124.31, 118.69, 113.79, 39.83, 35.97, 30.87, 28.11, 25.81; HRMS: m/z (*M* + H)+ cacld. for $C_{27}H_{23}ClN_4OS$: 485.1569 found: 485.1571.

(E)-1-(4-((6,6-dimethyl-5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3-d]pyrimidin-4-yl)amino)phenyl)-3-(4-nitrophenyl)prop-2-en-1-one ***(5h)***

Yield: 80 %; ^{1}H NMR (400 MHz, DMSO-d6): 8.60 (s, 1H), 8.09-8.17 (d, 2H), 7.62 (s, 1H), 6.86-7.11 (m, 8H), 2.68-2.72 (m, 4H), 1.80-1.82 (t, 2H), 1.03-1.04 (s, 6H); ^{13}C NMR (400 MHz, DMSO-d6): 189.83, 163.03, 155.90, 152.19, 147.53, 143.05, 141.84, 140.88, 135.76, 133.81, 132.61, 130.56, 128.98, 124.48, 120.75, 118.69, 113.79, 39.83, 35.97, 30.87, 28.11, 25.81; HRMS: m/z (*M* + H)+ cacld. for $C_{27}H_{23}ClN_4OS$: 485.1569 found: 485.1571.

(E)-1-(4-((6,6-dimethyl-5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3-d]pyrimidin-4-yl)amino) phenyl)-3-(3,4,5-trimethoxyphenyl)prop-2-en-1-one ***(5i)***

Yield: 76 %; ^{1}H NMR (400 MHz, DMSO-d6): 8.60 (s, 1H), 8.10-8.15 (d, 2H), 7.61 (s, 1H), 6.89-7.11 (m, 8H), 2.69-2.73 (m, 4H), 1.80-1.82 (t, 2H), 1.01-1.02 (s, 6H); ^{13}C NMR (400 MHz, DMSO-d6): 13C NMR (400 MHz, DMSO-*d*6) δ 189.83, 163.03, 155.90, 153.42, 152.19, 145.11, 143.05, 141.54, 135.76, 133.81, 132.61, 130.56, 129.93, 121.42, 118.69, 113.79, 106.09, 60.66, 56.19, 39.83, 35.97, 30.87, 28.11, 25.81; HRMS: m/z (*M* + H)+ cacld. for $C_{27}H_{23}ClN_4OS$: 530.2035 found: 530.2037.

(E)-1-(4-((6,6-dimethyl-5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3-d]pyrimidin-4-yl)amino) phenyl)-3-(p-tolyl)prop-2-en-1-one ***(5j)***

Yield: 80 %; ^{1}H NMR (400 MHz, DMSO-d6): 8.61 (s, 1H), 8.10-8.18 (d, 2H), 7.62 (s, 1H), 6.87-7.08 (m, 8H), 2.68-2.73 (m, 4H), 1.81-1.82 (t, 2H), 1.01-1.02 (s, 6H); ^{13}C NMR (400 MHz, DMSO-d6): ^{13}C NMR (400 MHz, DMSO-d_6) δ 189.83, 163.03, 155.90, 152.19, 144.20, 143.05, 139.42, 135.76, 133.81, 132.61, 132.49, 130.56, 129.65, 128.94, 120.85, 118.69, 113.79, 39.83, 35.97, 30.87, 28.11, 25.81, 21.35; HRMS: m/z (*M* + H)+ cacld. for $C_{27}H_{23}ClN_4OS$: 454.1875 found: 454.1877.

Biological activity

In vitro antimicrobial assay

The antimicrobial potential of the synthesized compounds was evaluated against a variety of microorganisms. For Gram-positive bacteria, the tested strains included *Staphylococcus aureus* (ATCC 6538) and *Bacillus subtilis* (ATCC 6633). The Gram-negative bacterial strains used were *Escherichia coli* (ATCC 25,922) and *Pseudomonas aeruginosa* (ATCC 9027), In addition, the compounds were also tested against yeast strains such as

Candida albicans (ATCC 10,231) and *Saccharomyces cerevisiae* (ATCC 9763), along with the fungal strain *Aspergillus brasiliensis* (ATCC 16,404).

The antimicrobial activity was determined using the broth microdilution method recommended by the NCCLS (National Committee for Clinical Laboratory Standards). For the assay, 96-well microplates were prepared with 100 µL of Mueller-Hinton broth for bacterial cultures and Sabouraud dextrose broth for yeasts and fungi. A 100 µL aliquot of the compound stock solution (10 mg/mL in DMSO) was placed into the first row of each plate and then serially two-fold diluted. Microbial suspensions were prepared in sterile 0.9% saline, with turbidity adjusted according to the 0.5 McFarland standard. Subsequently, 10 µL of the bacterial, yeast, or spore suspension was added to each well, resulting in final inoculum concentrations of 5×10^5 CFU/mL for bacteria and 5×10^3 CFU/mL for yeasts and fungi. Gentamicin and Ciprofloxacin was used as the positive control for bacterial strain. while, Nystatin was employed as a positive control to evaluate antifungal activity. The plates were incubated for 24 h at 37 °C for bacterial strains, and 48 h at 28 °C for yeasts and fungi. Following incubation, 10 µL of a 0.6% resazurin solution was added to assess microbial growth. The minimum inhibitory concentration (MIC) was determined as the lowest concentration of the compound that prevented the resazurin colour change from blue to pink [23].

Conclusion

In the present study, a novel series of thieno[2,3-*d*]pyrimidine-based chalcone derivatives **(5a–5j)** were successfully synthesized using an efficient multistep synthetic route involving Gewald reaction, cyclization, chlorination, nucleophilic substitution, and Claisen-Schmidt condensation. The structures of all synthesized compounds were confirmed by ^{1}H NMR, ^{13}C NMR, and HRMS spectral analysis, which supported the successful formation of the targeted chalcone framework.

The antimicrobial evaluation revealed that several compounds exhibited moderate to significant antibacterial and antifungal activities against the tested microorganisms. In particular, compounds **5a**, **5g**, and **5h** demonstrated promising antibacterial activity, while compound **5g** showed consistent antifungal activity against all tested fungal strains. The enhanced activity of these derivatives may be attributed to the presence of electron-withdrawing substituents such as chloro and nitro groups, which play an important role in improving antimicrobial effectiveness.

Overall, the obtained results indicate that thieno[2,3-*d*]pyrimidine-based chalcone derivatives possess promising antimicrobial potential and may serve as valuable lead molecules for further structural modification and development of new antimicrobial agents.

Credit author statement

Tarun P. Patel: Validation, Formal analysis, Data curation, Writing - original draft, Investigation, Biological activity; **Megha A. Patel:** Conceptualization, review & editing, Biological activity; **Bhargav B. Dave:** Data curation; review & editing; Formal analysis; **Paresh S. Patel:** Methodology, Conceptualization, Resources, review, & editing, Supervision.

Declaration of competing interest

There are no potential conflicts of interest reported by the authors.

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