

One-Pot Synthesis of Novel Substituted Phenyl-1,5-dihydro-2H-benzo[4,5]thiazolo[3,2-a]pyrimido[4,5-d]pyrimidine Derivatives as Potent Antimicrobial Agents

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Abstract

In this paper, we have reported the synthesis of novel substituted phenyl-1,5-dihydro-2H-benzo[4,5]thiazolo[3,2-a]pyrimido[4,5-d]pyrimidine derivatives (**4a-m**). The title compounds were synthesized by the reaction of substituted 2-aminobenzothiazole, barbituric/thiobarbituric acid and substituted benzaldehydes using 2-3 drops of HCl in ethanol. The synthesized compounds were evaluated for their antimicrobial efficiency against various microbial strains at different concentrations. Among the tested compounds, the compounds **4k** and **4j** were found to be more active against all the tested pathogens. Additionally, selected compounds were screened for *in silico* molecular docking studies.

Keywords: Condensation; Pyrimidine; Antimicrobial activity; Molecular docking study

Introduction

Multicomponent reactions (MCR) play an important role in organic, combinatorial and medicinal chemistry [1] as it furnishes products with a high degree of chemical and structural variability. Their productivity and the simplicity of reaction techniques make MCRs inexpensive, less time-consuming and ecofriendly in comparison to conventional multistep synthesis [2,3]. The exploitation of a simple molecule with various functionalities for the synthesis of bio heterocycles is a useful contribution in the heterocyclic chemistry [4]. Recently, the chemistry and biological outlines of several pharmacophores of 2-substituted benzothiazole products have been addressed [5,6]. The effect of substituents on the benzothiazole ring displayed accompanying structure-activity relationship [7]. Furthermore, benzothiazole derivatives are essential scaffolds in drug design associated with broad verities of medicinal uses [8]. On the other hand, pyrimidine is also a familiar class of heterocyclic compound possessing a wide range of biological activities and their importance in medicine is very much recognized [9]. Several pyrimidine derivatives have exhibited efficiency in fighting various diseases and observed to use as good beneficial agents such as antibacterial [10], antifungal [11], anti-tumor [12] and anti-HIV agents [13].

Encouraged by the above results, in this paper we have reported the synthesis of some novel substituted phenyl-1,5-dihydro-2H-benzo[4,5]thiazolo[3,2-a]pyrimido[4,5-d]pyrimidine derivatives. All the newly synthesized compounds were screened for the *in vitro* antimicrobial activities. In addition, we described the analysis of potentially active target compounds against DNA Gyrase and CYP51 ligands by comparing the various docked orientations of the molecules.

Experimental Section

Materials and methods

All reactions were performed at reflux temperature with stirring, the chemicals were purchased from Merck and solvents were used without further purification. Analytical thin layer chromatography was performed with E. Merck silica gel GF254 glass plates and melting point was determined using thermal analyzer (Shimadzu DS-50). The FTIR spectra were obtained using KBr pellets on Shimadzu spectrometer,

the ¹H-NMR and ¹³C-NMR spectrum were recorded on Bruker 400 MHz and 100 MHz respectively in DMSO-*d*₆ as a solvent using tetramethylsilane (TMS) as internal standard. LCMS were obtained using C-18 column on Shimadzu, LCMS 2010A, Japan.

General procedure for synthesis of substituted phenyl-1,5-dihydro-2H-benzo[4,5]thiazolo[3,2-a]pyrimido[4,5-d]pyrimidine derivatives (**4a-m**)

The mixture of substituted 2-aminobenzothiazoles (1 mmol), barbituric/thiobarbituric acid (1 mmol), substituted benzaldehydes (1 mmol) and 2-3 drops of HCl in ethanol was refluxed with constant stirring for about 8 h. After completion of the reaction, the reaction mixture was poured into the crushed ice with vigorous stirring and the solid residue separated was filtered, dried and recrystallized using ethanol.

5-(4-Hydroxyphenyl)-1,5-dihydro-2H-benzo[4,5]thiazolo[3,2-a]pyrimido[4,5-d]pyrimidine-2,4(3H)-dione (4a**):** Pale yellow; yield 91%; mp 308-310°C; IR (cm⁻¹): 1593, 1666, 2916, 3266, 3431; ¹H-NMR (DMSO-*d*₆) δ ppm: 5.89 (s, 1H, N-C(H)), 6.83-8.28 (m, 8H, Ar-H), 9.44 (s, 1H, Ar-OH), 11.33 (s, 1H, NH), 11.41 (s, 1H, NH); ¹³C-NMR (DMSO-*d*₆) δ ppm: 61.85, 119.41, 121.78, 123.47, 125.25, 126.06, 126.94, 134.33, 136.54, 147.25, 149.60, 155.05, 157.91, 161.51, 168.52; LCMS: m/z [M+1]: 365.00.

5-(3-Fluorophenyl)-1,5-dihydro-2H-benzo[4,5]thiazolo[3,2-a]pyrimido[4,5-d]pyrimidine-2,4(3H)-dione (4b**):** Light yellow;

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yield 88%; mp 262-264°C; IR (cm⁻¹): 752, 1597, 1643, 3249; ¹H-NMR (DMSO-d₆) δppm: 5.99 (s, 1H, N-CH), 6.15-8.22 (m, 8H, Ar-H), 11.52 (s, 1H, NH), 11.61 (s, 1H, NH); ¹³C-NMR (DMSO-d₆) δppm: 61.87, 113.10, 118.77, 121.47, 122.94, 124.26, 125.51, 126.02, 126.17, 129.25, 134.01, 136.21, 146.66, 148.92, 157.31, 161.87, 164.92, 166.13; LCMS: m/z [M+1]: 367.00.

5-(3-Nitrophenyl)-1,5-dihydro-2H-benzo[4,5]thiazolo[3,2-a]pyrimido[4,5-d]pyrimidine-2,4(3H)-dione (4c): Dark yellow; yield 90%; mp 300-302°C; IR (cm⁻¹): 1600, 1655, 3215; ¹H-NMR (DMSO-d₆) δppm: 5.98 (s, 1H, N-CH), 7.79-6.69 (m, 8H, Ar-H), 11.45 (s, 1H, NH), 11.57 (s, 1H, NH); ¹³C-NMR (DMSO-d₆) δppm: 60.05, 100.12, 119.15, 124.47, 124.56, 126.51, 12.02, 128.17, 129.25, 133.08, 137.01, 147.08, 148.88, 155.34, 157.33, 163.22, 167.12; LCMS: m/z [M+1]: 395.03.

9-Chloro-5-(3-nitrophenyl)-2-thioxo-1,2,3,5-tetrahydro-4H-benzo[4,5]thiazolo[3,2-a]pyrimido[4,5-d]pyrimidin-4-one (4d): Light brown; yield 88%; mp 293-295°C; IR (cm⁻¹): 782, 1365, 1610, 1640, 3255; ¹H-NMR (DMSO-d₆) δppm: 5.58 (s, 1H, N-CH), 6.29-7.82 (m, 7H, Ar-H), 11.62 (s, 1H, NH), 11.71 (s, 1H, NH); ¹³C-NMR (DMSO-d₆) δppm: 59.55, 102.10, 118.10, 124.40, 124.65, 126.50, 127.82, 128.37, 129.45, 133.18, 137.51, 147.58, 148.62, 154.30, 157.20, 163.02, 167.18; LCMS: m/z [M+1] and [M+2]: 443 and 445.

9-Chloro-5-(3-fluorophenyl)-2-thioxo-1,2,3,5-tetrahydro-4H-benzo[4,5]thiazolo[3,2-a]pyrimido[4,5-d]pyrimidin-4-one (4e): Light brown; yield 85%; mp 293-295°C; IR (cm⁻¹): 756, 788, 1610, 1667, 3205; ¹H-NMR (DMSO-d₆) δppm: 5.89 (s, 1H, N-CH), 6.15-8.28 (m, 7H, Ar-H), 11.50 (s, 1H, NH), 11.61 (s, 1H, NH); ¹³C-NMR (DMSO-d₆) δppm: 55.85, 98.07, 112.18, 122.47, 123.24, 124.36, 125.21, 126.22, 126.47, 129.22, 135.01, 136.26, 147.18, 148.82, 157.21, 160.82, 164.32, 167.24; LCMS: m/z [M+1] and [M+2]: 416 and 418.

9-Chloro-5-(2,6-difluorophenyl)-2-thioxo-1,2,3,5-tetrahydro-4H-benzo[4,5]thiazolo[3,2-a]pyrimido[4,5-d]pyrimidin-4-one (4f): Yellow; yield 83%; mp 256-258°C; IR (cm⁻¹): 756, 788, 1610, 1647, 3205; ¹H-NMR (DMSO-d₆) δppm: 5.89 (s, 1H, N-CH), 6.15-8.28 (m, 6H, Ar-H), 11.33 (s, 1H, NH), 11.41 (s, 1H, NH); ¹³C-NMR (DMSO-d₆) δppm: 54.83, 100.04, 111.16, 123.24, 124.36, 125.71, 126.42, 126.36, 132.22, 135.81, 136.86, 147.08, 148.62, 157.31, 161.12, 164.32, 167.24; LCMS: m/z [M+1] and [M+2]: 434.80 and 436.10.

9-Chloro-5-(4-hydroxyphenyl)-2-thioxo-1,2,3,5-tetrahydro-4H-benzo[4,5]thiazolo[3,2-a]pyrimido[4,5-d]pyrimidin-4-one (4g): Dark orange; yield 90%; mp 310-312°C; IR (cm⁻¹): 785, 1335, 1610, 1641, 3260, 3448; ¹H-NMR (DMSO-d₆) δppm: 5.30 (s, 1H, N-CH), 6.82-8.27 (m, 7H, Ar-H), 9.53 (s, 1H, OH), 11.33 (s, 1H, NH), 11.41 (s, 1H, NH); ¹³C-NMR (DMSO-d₆) δppm: 60.05, 96.29, 119.01, 121.88, 123.07, 125.26, 126.16 (2C), 126.96, 134.13, 138.04, 147.35, 149.20, 155.55, 162.23, 157.82, 162.01, 169.02; LCMS: m/z [M+1] and [M+2]: 415.10 and 417.00.

5-(4-Chlorophenyl)-1,5-dihydro-2H-benzo[4,5]thiazolo[3,2-a]pyrimido[4,5-d]pyrimidine-2,4(3H)-dione (4h): Light gray; yield 86%; mp 290-292°C; IR (cm⁻¹): 784, 1605, 1658, 1678, 3218; ¹H-NMR (DMSO-d₆) δppm: 5.96 (s, 1H, N-CH), 6.84-8.30 (m, 8H, Ar-H), 11.02 (s, 1H, NH), 11.11 (s, 1H, NH); ¹³C-NMR (DMSO-d₆) δppm: 65.24, 96.00, 113.11, 123.28, 124.10, 127.82, 128.37, 129.45, 134.12, 138.21, 148.18, 149.60, 155.20, 157.25, 162.23, 168.10; LCMS: m/z [M+1] and [M+2]: 383.02 and 385.03.

5-(P-tolyl)-1,5-dihydro-2H-benzo[4,5]thiazolo[3,2-a]pyrimido[4,5-d]pyrimidine-2,4(3H)-dione (4i): Light white; yield 90%; mp 285-287°C; IR (cm⁻¹): 1610, 1652, 3220; ¹H-NMR (DMSO-d₆) δppm: 2.43 (s, 3H, CH₃), 5.98 (s, 1H, N-CH), 6.80-8.32 (m, 8H, Ar-

H), 11.10 (s, 1H, NH), 11.22 (s, 1H, NH); ¹³C-NMR (DMSO-d₆) δppm: 21.18, 63.28, 95.02, 114.10, 123.15, 124.36, 126.23, 127.30, 128.32, 129.46, 135.10, 138.01, 147.14, 148.90, 155.30, 157.05, 167.18; LCMS: m/z [M+1]: 363.06.

5-Phenyl-1,5-dihydro-2H-benzo[4,5]thiazolo[3,2-a]pyrimido[4,5-d]pyrimidine-2,4(3H)-dione (4j): Light gray; yield 85%; mp 248-250°C; IR (cm⁻¹): 1615, 1720, 1678, 3240; ¹H-NMR (DMSO-d₆) δppm: 5.93 (s, 1H, N-CH), 6.76-8.30 (m, 9H, Ar-H), 11.12 (s, 1H, NH), 11.20 (s, 1H, NH); ¹³C-NMR (DMSO-d₆) δppm: 61.20, 94.08, 113.18, 122.10, 123.66, 126.22, 128.62, 130.16, 136.12, 139.09, 148.10, 149.50, 156.35, 158.08, 162.23, 168.10; LCMS: m/z [M+1]: 349.06.

5-Phenyl-2-thioxo-1,2,3,5-tetrahydro-4H-benzo[4,5]thiazolo[3,2-a]pyrimido[4,5-d]pyrimidin-4-one (4k): Light yellow; yield 88%; mp 276-278°C; IR (cm⁻¹): 1618, 1542, 1644, 3245(NH); ¹H-NMR (DMSO-d₆) δppm: 5.96 (s, 1H, N-CH), 6.78-8.32 (m, 9H, Ar-H), 11.24 (s, 1H, NH), 11.33 (s, 1H, NH); ¹³C-NMR (DMSO-d₆) δppm: 65.28, 98.48, 113.25, 123.10, 124.60, 126.23, 128.65, 129.12, 135.13, 138.29, 147.20, 148.20, 157.33, 158.56, 162.22, 168.20; LCMS: m/z [M+1]: 365.40.

2-Thioxo-5-(p-tolyl)-1,2,3,5-tetrahydro-4H-benzo[4,5]thiazolo[3,2-a]pyrimido[4,5-d]pyrimidin-4-one (4l): Light yellow; yield 90%; mp 326-328°C; IR (cm⁻¹): 1545, 1620, 1671, 3255; ¹H-NMR (DMSO-d₆) δppm: 2.43 (s, 3H, CH₃), 5.96 (s, 1H, N-CH), 6.78-8.32 (m, 9H, Ar-H), 11.03 (s, 1H, NH), 11.11 (s, 1H, NH); ¹³C-NMR (DMSO-d₆) δppm: 22.24, 64.25, 95.43, 113.05, 123.36, 124.62, 125.26, 128.86, 130.54, 135.16, 138.23, 147.00, 148.28, 156.96, 158.88, 163.45, 169.24; LCMS: m/z [M+1]: 379.06.

5-(4-Chlorophenyl)-2-thioxo-1,2,3,5-tetrahydro-4H-benzo[4,5]thiazolo[3,2-a]pyrimido[4,5-d]pyrimidin-4-one (4m): Pale yellow; yield 88%; mp 288-290°C; IR (cm⁻¹): 785, 1343, 1614, 1658, 3240; ¹H-NMR (DMSO-d₆) δppm: 5.99 (s, 1H, N-CH), 6.75-8.35 (m, 8H, Ar-H), 11.10 (s, 1H, NH), 11.22 (s, 1H, NH); ¹³C-NMR (DMSO-d₆) δppm: 66.32, 108.23, 112.03, 124.06, 125.02, 126.36, 128.23, 129.04, 133.56, 137.07, 146.05, 147.88, 155.16, 157.54, 163.43, 169.14; LCMS: m/z [M+1] and [M+2]: 398 and 400.01.

Antimicrobial activity

The synthesized compounds were screened for *in vitro* antimicrobial activity against five pathogenic bacterial strains viz., *Escherichia coli* (MTCC 1559), *Pseudomonas syringae* (MTCC-1604), *Salmonella typhi* (MTCC-734), *Staphylococcus aureus* (MTCC-902) and *Xanthomonas campestris* (MTCC-2286) and five fungal strains, *Alternaria solani* (MTCC-2101), *Aspergillus flavus* (MTCC-277), *Fusarium oxysporum* (MTCC-284), *Candida albicans* (MTCC-1637) and *Chrysosporium keratinophilum* (MTCC-1367).

The *in vitro* antimicrobial activity was determined by agar well diffusion method [14]. The 10% DMSO was used as a negative control whereas ciprofloxacin was used as standard for antibacterial activity and bavistin for antifungal activity. The Minimum Inhibitory Concentration (MIC) measurements were achieved by using serial broth-dilution method [15].

In silico molecular docking studies

The structure of synthesized molecules and standards were drawn in Chem Bio Draw tool (Chem Bio Office Ultra 14.0 suite) assigned with proper 2D orientation and structure of each was checked for structural drawing error. Energy of each molecule was minimized using Chem Bio 3D (Chem Bio Office Ultra 14.0 suite). The energy

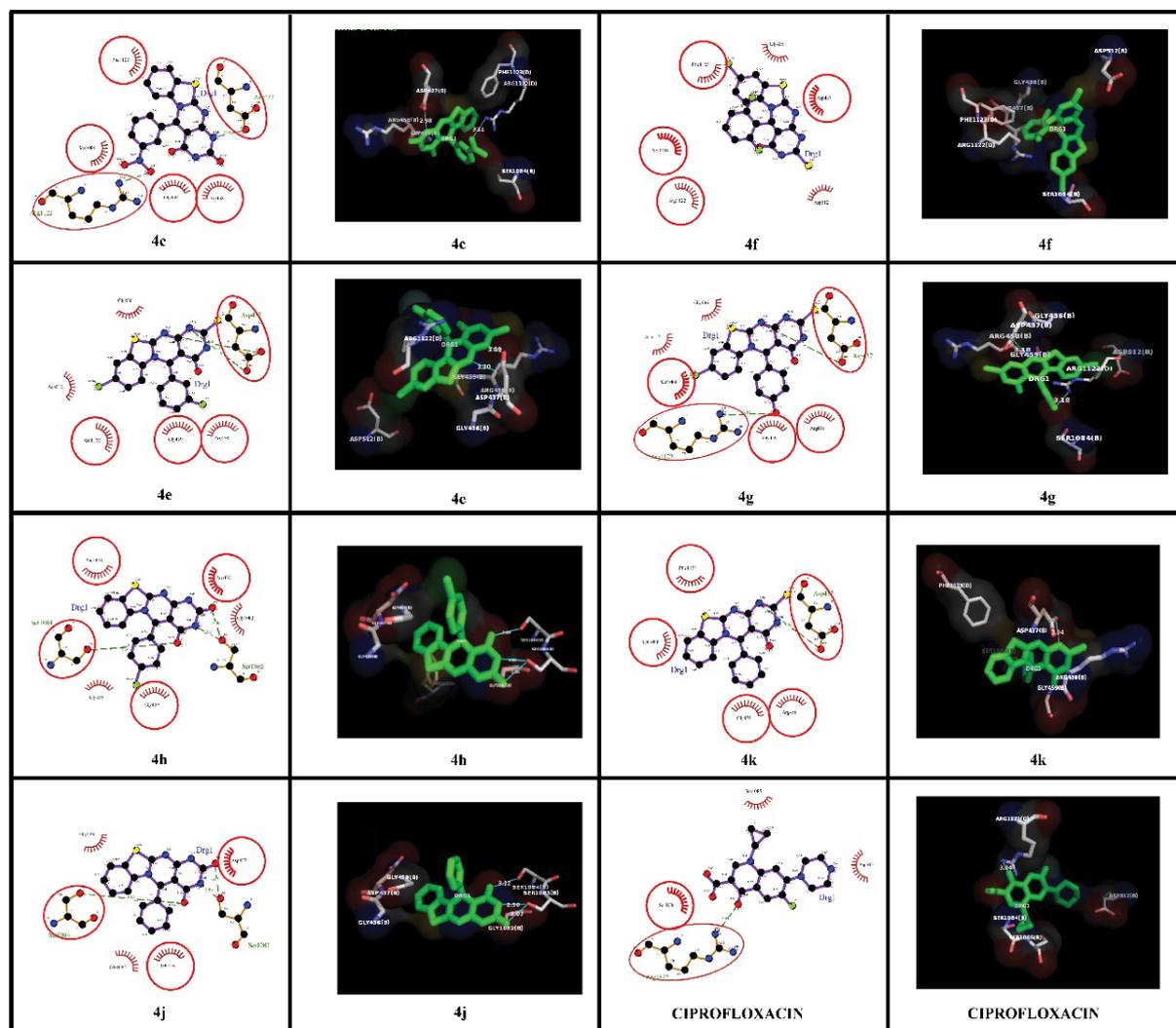


Figure 1: Interaction of synthesized molecules 4c, 4f, 4e, 4g, 4h, 4k, 4j and Ciprofloxacin with DNA Gyrase.

minimized ligand molecules were then used as input for Auto Dock Vina, in order to carry out the docking simulation. The protein data bank (PDB) coordinate file with the name DNA Gyrase and CYP51 was used as receptor molecule in antibacterial and antifungal activity respectively [16,17].

Results and Discussion

Chemistry

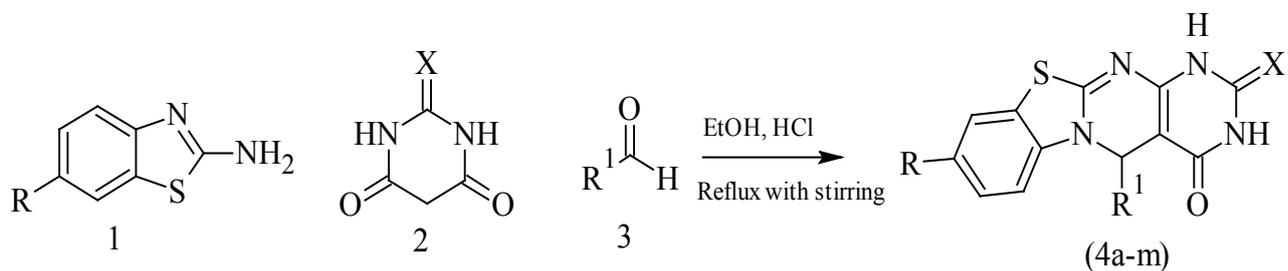
In the present study, the synthesis of target compounds was achieved according to the reaction illustrated in the Scheme 1. The novel substituted phenyl-1,5-dihydro-2H-benzo[4,5]thiazolo[3,2-a]pyrimido[4,5-d]pyrimidine derivatives (**4a-m**) were synthesized via the one pot three component condensation reaction between 2-amino benzothiazole, barbituric/thiobarbituric acid and substituted benzaldehyde in ethanol using hydrochloric acid as a catalyst at reflux temperature with constant stirring.

The structure of the desired substituted phenyl-1,5-dihydro-2H-benzo[4,5]thiazolo[3,2-a]pyrimido[4,5-d]pyrimidine derivatives (**4a-m**) were confirmed by IR, NMR and Mass spectral data. The

IR spectrum of compound **4a** showed broad peak at 3431 cm^{-1} due to hydroxyl group and another band in the region 3266 cm^{-1} that corresponded to NH functionality. The absorption bands at 1666 and 1593 cm^{-1} corresponded C=O and C=N group respectively. The $^1\text{H-NMR}$ spectrum of compound **4a** exhibited two singlets at δ 11.33 and 11.41 ppm corresponded for two NH protons and another singlet at δ 9.44 ppm which confirmed the presence of OH proton. The multiplet between δ 8.28-6.84 ppm is due to aromatic protons and a singlet at δ 5.89 ppm corresponds to pyrimidine CH proton. Further, $^{13}\text{C-NMR}$ spectrum of compound **4a** confirmed the proposed structure by appearance of signal at δ 168.52 ppm due to the C=O carbon and another signal at δ 161.51 ppm correspond to C=N carbon of barbituric acid ring. Another signal at δ 157.91 ppm attributed to C-OH carbon. The mass spectrum of compound **4a** showed molecular ion peak [M^+] at m/z 365.00 which corresponds to the molecular weight of the compound **4a**.

In vitro antimicrobial activity

All the tested samples showed appreciable antibacterial activity against the tested pathogens at 1, 2.5 and 5 mg/mL concentrations. The highest zone of inhibition was observed by compound **4j** followed by



R- H, Cl

X- O, S

R¹- C₆H₅, C₆H₄OH(p), C₆H₄F(m), C₆H₄NO₂(m), C₆H₄F₂(o), C₆H₄Cl(p), C₆H₄CH₃(p)

Scheme 1: Synthesis of novel phenyl-1,5-dihydro-2H-benzo[4,5]thiazolo[3,2-a]pyrimido[4,5-d]pyrimidine derivatives (4a-m).

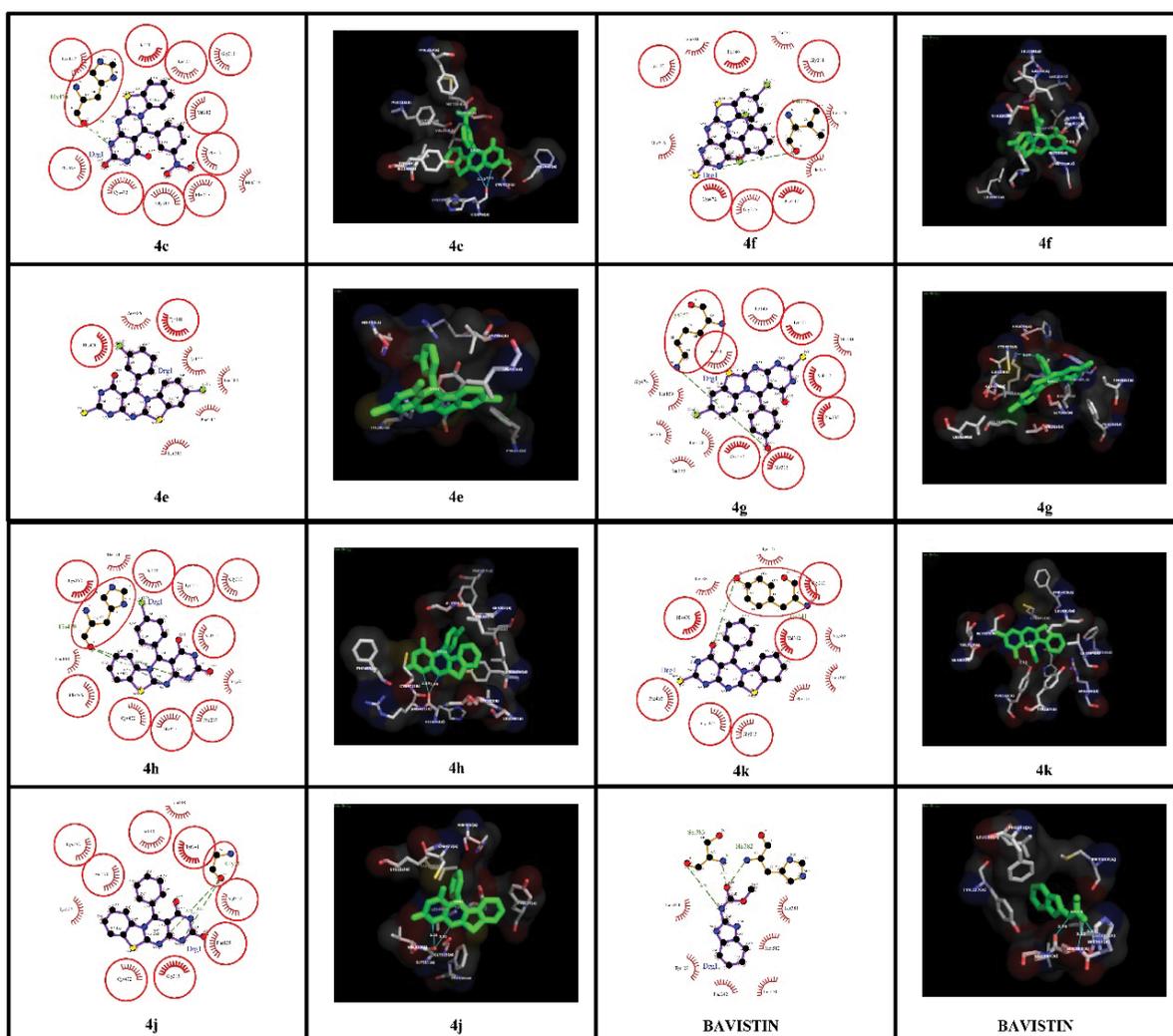


Figure 2: Interaction of synthesized molecules 4c, 4f, 4e, 4g, 4h, 4k, 4j and Bavistin with CYP51.

Compound Conc in mg/mL	Zone of inhibition in mm														
	S.a			S.t			P.s			X.c			E.c		
	1.0	2.5	5.0	1.0	2.5	5.0	1.0	2.5	5.0	1.0	2.5	5.0	1.0	2.5	5.0
4a	14	18	23	0	10	18	7	13	18	7	13	19	0	0	0
4b	14	18	23	11	16	22	0	8	14	8	15	17	7	18	23
4c	14	20	25	15	21	24	7	13	20	9	12	14	17	20	24
4d	14	21	28	16	20	24	7	14	21	9	13	15	18	21	25
4e	9	14	21	18	21	27	9	15	23	7	11	16	12	17	22
4f	18	20	24	9	12	20	8	13	17	8	14	18	18	19	24
4g	17	21	28	11	17	21	7	12	16	8	15	19	14	18	25
4h	11	18	25	0	10	16	0	6	13	9	16	20	14	18	23
4i	9	14	21	10	15	20	0	7	14	7	13	15	15	24	28
4j	19	24	30	11	18	24	7	15	19	8	12	17	11	15	24
4k	11	18	24	12	19	25	6	14	24	9	15	20	11	18	21
4l	11	14	20	14	19	22	0	8	14	8	13	18	16	20	25
4m	11	15	21	10	18	24	0	7	13	7	12	17	18	20	26
Ciproflaxin	14	18	32	16	23	30	15	20	29	14	21	32	18	25	35

S.a- *Staphylococcus aureus*; S.t- *Salmonella typhi*; P.s- *Pseudomonas syringae*; X.c- *Xanthomonas campestris*; E.c- *Escherichia coli*

Table 1: Antibacterial activity results of synthesized compounds (4a-m).

Compound Conc in mg/mL	Zone of inhibition in mm														
	A.s			A.f			F.o			C.a			C.k		
	1.0	2.5	5.0	1.0	2.5	5.0	1.0	2.5	5.0	1.0	2.5	5.0	1.0	2.5	5.0
4a	7	11	14	7	14	21	0	7	14	7	13	19	7	10	15
4b	7	13	15	7	13	22	7	13	20	8	15	17	0	7	13
4c	7	11	14	7	11	22	5	7	11	9	12	14	7	12	15
4d	7	12	14	7	12	24	0	6	12	9	13	15	7	11	16
4e	8	11	16	8	11	21	7	12	23	7	11	16	9	13	17
4f	9	14	13	9	12	20	0	7	13	8	14	18	8	12	17
4g	8	10	15	8	15	24	7	13	24	8	15	19	7	12	16
4h	9	12	14	9	14	25	0	6	13	9	16	20	0	7	14
4i	9	11	15	9	15	20	0	7	14	7	13	15	0	7	13
4j	7	12	16	7	14	24	7	13	21	8	12	17	7	14	18
4k	6	9	14	6	12	23	0	7	14	9	15	20	6	11	19
4l	7	11	15	7	13	24	9	15	26	8	13	18	0	7	14
4m	7	12	17	7	13	25	0	7	13	7	12	17	9	14	20
Bavistin	14	19	25	11	16	29	9	15	26	11	18	29	10	18	29

Table 2: Antifungal activity results of synthesized compounds (4a-m).

A.s- *Alternaria solani*; A.f- *Aspergillus flavus*; F.o- *Fusarium oxysporum*; C.a- *Candida albicans*; C.k- *Cryzosporium keratinophilum*

Compound	Minimum inhibitory concentration (µg/mL)									
	S.a	S.t	P.s	X.c	E.c	A.s	A.f	F.o	C.a	C.k
4a	200	250	350	350	250	300	400	500	500	1000
4b	250	200	200	200	300	300	300	500	500	500
4c	150	100	200	250	300	400	500	500	500	1000
4d	100	200	250	250	100	250	300	400	400	500
4e	150	100	100	100	75	300	400	500	500	1000
4f	150	150	200	250	200	400	400	500	500	1000
4g	100	150	200	250	200	400	200	400	500	500
4h	100	200	150	200	100	300	150	300	250	400
4i	150	100	200	200	150	300	500	500	400	500
4j	50	100	200	200	50	150	150	150	400	400
4k	150	150	50	100	50	200	300	300	250	500
4l	200	250	200	250	150	250	250	150	300	400
4m	150	250	300	300	200	150	250	250	400	350
Ciproflaxin	25	75	25	75	50	---	---	---	---	---
Bavistin	---	---	---	---	---	100	100	150	200	300

Table 3: Minimum antimicrobial concentration of synthesized compounds (4a-m).

S.a- *Staphylococcus aureus*; S.t- *Salmonella typhi*; P.s- *Pseudomonas syringae*; X.c- *Xanthomonas campestris*; E.c- *Escherichia coli*; A.s- *Alternaria solani*; A.f- *Aspergillus flavus*; F.o- *Fusarium oxysporum*; C.a- *Candida albicans*; C.k- *Cryzosporium keratinophilum*

Compound	Affinity (kcal/mol)	H-bonds	H-bond length (Å)	H-bond with	Hydrophobic Interactions
4c	-5.9	2	2.98 3.11	2XCT:Asp437 ::4c:NAD 2XCT:Arg1122 ::4c:	Asp437, Arg458, Gly459, Ser1084, Phe1123
4e	-5.3	2	3.09 3.20	2XCT:Asp437 ::4e:NAD 2XCT:Asp437 ::4e:NAJ	Gly436, Arg458, Gly459, Asp512, Arg1122
4f	-5.1	0	-	-	Gly436, Asp437, Asp512, Ser1084, Arg1122, Phe1123
4g	-5.5	2	3.18 3.18	2XCT:Asp437 ::4g:NAJ 2XCT:Arg1122 ::4g:OBA	Gly436, Arg458, Gly459, Asp512, Ser1084
4h	-5.6	3	2.82 3.09 3.09	2XCT:Ser1085 ::4h:NAE 2XCT:Ser1085 ::4h:OAY 2XCT:Ser1084 ::4h:OAX	Gly436, Asp437, Gly459, Gly1082, Phe1123
4j	-5.4	3	2.90 3.07 3.12	2XCT:Ser1085 ::4j:NAE 2XCT:Ser1085 ::4j:OAY 2XCT:Ser1084 ::4j:OAX	Gly436, Asp437, Gly459, Gly1082
4k	-5.1	1	3.04	2XCT:Asp437 ::4k:NAD	Arg458, gly459, Ser1084, Phe1123
Ciprofloxacin	-4.4	1	3.04	2XCT:Arg1122 :: CIPROFLOXACIN:OAQ	Asp512, Ser1084, Ser1085

Table 4: Docking results for antibacterial activity of synthesized compounds (4a-m).

Compound	Affinity (kcal/mol)	H-bonds	H-bond length (Å)	H-bond with	Hydrophobic interactions
4c	-9.6	2	3.01 3.10	5JLC:His470::4c:NAD 5JLC:His470::4c:NAJ	Phe135, Ile140, Tyr141, Lys152, Phe237, Gly311, Val312, Met314, Gly315, Phe465, Cys472
4e	-9.6	0	-	-	Tyr127, Tyr141, Phe242, Leu381, Leu384, Arg386, His 470
4f	-10.0	1	3.03	5JLC:Val312::4f:NAE	Thr131, Ile140, Leu148, Lys152, Phe237, Gly311, Gly315, Gly316, Leu381, Ile472, Cys472
4g	-9.6	1	3.17	5JLC:Lys152::4g:OBA	Thr131, Phe135, Ile140, Tyr141, Leu148, Val155, Leu159, Val312, Gly315, His470, Cys472, Ile473, Gly474
4h	-9.3	2	2.93 2.96	5JLC:His470::4h:NAD 5JLC:His470::4h:NAJ	Thr131, Ile140, Tyr141, Leu148, Lys152, Phe237, Gly311, Val312, Gly315, Phe465, Arg471, Cys472
4j	-9.0	2	3.31 3.35	5JLC:Gly311::4j:NAJ 5JLC:Gly311::4j:NAD	Tyr127, Phe135, Ile140, Tyr141, Leu148, Lys 152, Val312, Gly315, His470, Cys472
4k	-9.0	1	2.91	5JLC:Tyr141::4k:OAX	Phe114, Tyr127, Gly311, Val312, Gly315, Leu381, Leu384, Arg386, Phe465, His470, Cys472
Bavistin	-7.1	3	2.78 3.12 3.21	5JLC:Ser383::BAVISTIN:NAJ 5JLC:Ser383::BAVISTIN:OAM 5JLC:His382::BAVISTIN:OAM	Tyr127, Leu130, Phe242, Leu381, Leu384, Met512

Table 5: Docking results for antifungal activity of synthesized compounds (4a-m).

4d, 4e and 4k (Table 1).

Similarly, the tested samples were screened for their antifungal activity and they showed appreciable inhibitory activity against the tested pathogens. The compounds **4i, 4e** and **4m** exhibited remarkable antifungal activity compared to other compounds (Table 2).

The synthesized compounds were also subjected for the MIC study and the results are displayed in Table 3. Among all the compounds, compound **4k** and **4j** were found to be more effective against *E. coli*, *P. syringae* and *S. aureus* with MIC value of 50 µg/mL. The compounds **4d, 4e, 4f, 4g, 4h, 4i** and **4l** showed good potencies with MIC value in the range of 75-250 µg/mL against all the bacterial strains.

Among all the compounds screened for antifungal efficacy, compounds **4j, 4h** and **4m** were found to have good MIC value of 150 µg/mL against *F. oxysporum*, *A. flavus* and *A. solani* respectively. The compounds **4d, 4e, 4f, 4g, 4i** and **4l** displayed considerable activity with MIC value in the range of 200-500 µg/mL against all the fungal strains.

The obtained results revealed that, human pathogenic bacteria *E. coli*, *S. aureus* and *S. typhi* were more susceptible to all the tested compounds and these results were in agreement with the findings of Sahu et al. [18]. The plant pathogens viz, *A. flavus* and *F. oxysporum* were more susceptible to tested compounds and *X. campestris*, *P. syringae* were found to be less susceptible [19]. All the compounds showed appreciable antimicrobial activity and the activity was comparable with the standard drugs ciprofloxacin and bavistin.

In silico molecular docking studies

The docking of receptors DNA Gyrase and CYP51 with synthesized compounds **4c, 4e, 4f, 4g, 4h, 4j** and **4k** exhibited well-established bonds with amino acids (Asp437, Arg1122, Ser1085, His470, Val312, Lys152, Gly311 and Tyr141 respectively) in the receptor active pocket. The synthesized molecules having 2D structure were converted to energy minimized 3D structures and were further used for *in silico* protein-ligand docking. Figures 1 and 2 (2D and 3D image) showed the docked images of synthesized molecules including standard drugs ciprofloxacin and bavistin. All the compounds showed appreciating results with encouraging binding energy and exhibited the bonding with one or other amino acids in the active pockets. *In silico* studies revealed that all the synthesized molecules showed good binding energy with strong affinity towards the target protein DNA Gyrase and CYP51 ranging from -5.1 to -5.9 kcal/mol and -9.0 and -9.6 respectively (Tables 4 and 5).

Conclusion

In the present investigation, we have reported the synthesis of series of novel substituted phenyl-1,5-dihydro-2H-benzo[4,5]thiazolo[3,2-a]pyrimido[4,5-d]pyrimidine derivatives (**4a-m**). The desired compounds were prepared in one pot using three different components with high yields and all the synthesized compounds were screened for the antimicrobial activity. The results showed that most of the derivatives inhibited the growth with higher inhibition zones and it may be due to the structural orientation and different substituents. Hence, can be used as effective antimicrobial drugs in future.

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