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Synthesis, Characterization of Microwave assisted Novel functionalized Pyrimido pyrimidine– tethered to benzothiazole Derivative and their Pharmacological Screening

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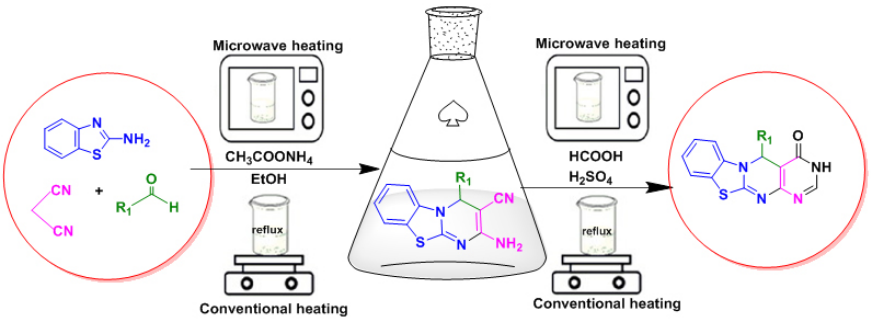
ABSTRACT

There is a constant need to synthesise new and desired compounds. The current study focusses on the two-step, microwave-assisted three-component synthesis of new lead compounds, specifically 5-phenyl-3,5-dihydro-4H-benzothiazolo[3,2-a] pyrimido[4,5-d] pyrimidinone. Target molecules were verified using a variety of spectral techniques for analysis, such as Mass, IR, 1H and 13C NMR spectrometry. The synthetic derivatives' antimicrobial abilities were measured. The Broth Dilution method was utilised to measure their minimum inhibitory concentration activity against both Gram-negative as well as Gram-positive bacteria and fungi. There are several advantages to this protocol. outstanding yields, a quick reaction, simple workup, and no need for column chromatography. Both traditional methods and microwave-assisted irradiation were used to establish the mentioned reactions.

Graphical Abstract



Keywords: Amino benzothiazole, Benzothiazolo-pyridopyrimidine, Biological exertion, Fused Pyrimido-benzothiazole, Microwave-assisted technique.

INTRODUCTION

Heterocyclic compounds are the cyclic compounds which contain one or more different elements (such as nitrogen, oxygen, sulphur, phosphorus, silicon and selenium) other than carbon in their ring structure. Heterocyclic elements have an important role in our everyday existence. Heterocycles include the vast majority of biologically active agrochemicals and pharmaceuticals. In nature, they are widely dispersed. Heterocyclic ring systems are found in a wide range of substances, including alkaloids, antibiotics, vitamins, essential amino acids, haemoglobin, hormones, chlorophyll, and many synthetic medications and dyes. Because of their unique chemical reactivity and ability to act as easy convenient structures for adding physiologically active substituents, heterocyclic compounds are highly suitable for use as pharmaceuticals. Because of their many pharmacophoric qualities, including antimicrobial, antifungal, anti-malarial, anti-inflammatory, antiviral, and anticancer effects, among many others, heterocyclic compounds with nitrogen and sulphur atoms are crucial in the field of pharmaceutical chemistry. The advent of combinatorial or "library" techniques transformed the process of developing new leads for drug design and structure function relationship studies. "The developer of green chemistry covers the need for innovations in chemical manufacturing to advance a sustainable future"[1]. According to the literature, a lot of researchers have recently employed the MCR strategy to synthesise various benzothiazole[3,2-a] pyrimidines. In light of these attributes, the design and synthesis of novel hybrid molecules incorporating both pyrimido[4,5-d]pyrimidine and benzothiazole cores offer a promising strategy for the development of potent therapeutic agents. The fusion of these two pharmacophores is expected to result in synergistic effects, potentially leading to enhanced efficacy and target specificity. This study focuses on the synthesis and characterization of a novel functionalized pyrimido[4,5-d]pyrimidine tethered to a benzothiazole derivative, aiming to explore its structural features and evaluate its potential for further biological investigations. There have been studies on multicomponent reactions that involve aldehydes, 2-amino-benzothiazole, and βketo ester using several kinds of catalysts, including $PdCl_2$, nano-Fe_3O_4@SiO_2-$TiCl_3$, Fe_3O_4/cellulose/Cu (II), Fe_3O_4/nano-kaolin/Ti4+, Fe_3O_4/nano cellulose/BF_3, $FeFe_3$ and $AlCl_3$[2-8]. thiazolo [3,2-a] pyrimidine was synthesised using a microwave method that was reported under clean conditions. The method of column chromatography was used to purify the resultant compounds, but less than 90 percent of the desired products were obtained[9]. Even so, appropriate sustainable and environmentally friendly procedures are still needed for pyrimido[4,5-d] pyrimidinone tethered benzothiazole derivatives under green conditions. This could be the cause of the lower number of side products that are frequently produced by reactions carried out using microwave irradiation at an optimal reaction temperature as opposed to reaction when heated conventionally, during which the reaction temperature is frequently suboptimal[10-13].

In pursuit of green methodologies, Inspired by the results of the previously published study[14-15]]. Here, we describe the application of a microwave-assisted method to create new derivatives of several functionalised benzothiazole[3,2-a] pyrimidines through a reaction between malononitrile and 2-amino-benzothiazole with various aldehyde using ammonium acetate as an efficient catalyst in methanol solvent under microwave assisted and This paper presents a rapid and effective response of various benzothiazole[3,2-a] pyrimidines derivative with formic acid for the synthesis of 5-phenyl-3,5-dihydro-4H-benzothiazolo[3,2-a] pyrimido[4,5-d] pyrimidinone derivatives with sulphuric acid catalysed significantly improved output and a quick reaction time is presented in order to create novel structural motifs with encouraging bioactivity.

RESULT AND DISCUSSION

In Step 1 of our study, we screened for the presence and absence of various catalysts in the designs reaction that involved benzaldehyde(1a), malononitrile(2), and 2-aminobenzothaizole(3). Spectral analysis using 1H NMR and 13C NMR validated each screening procedures. We found that 36% of the end result was within 60 minutes during the reflux temperature without the use of any catalyst, based on examining results (Entry 1 in Table 1). The above-described reaction is carried via one step.i.e. In the reaction of 1a and 2, benzylidene malononitrile was not separated.

However, the addition of 3 resulted in the creation of an intermediary at room temperature that was subsequently microwave-exposed, producing product 4a. It indicated how 2-amino benzothiazole its own behaved as a catalyst in the Knoevenagel condensation of malononitrile and benzaldehyde. Therefore, we experimented with various catalysts to boost the yield, and the outcomes are shown in Table 1 and observed that a 53% yield was generated when Et3N was used for a catalyst output at 10 and 20 mol% (Entry 2 and 3 in Table 1). improving the catalyst's amount had no impact on the output of 4a. After that, using various salts of ammonium as a catalyst (they are listed entry 4-8 in Table 1), we were able to produce 59% yield of ammonium acetate in 30 minutes at irradiated microwave. The use of ammonium acetate 30 mol% generated the most effective outcomes achieved (number of 11 in Table 1), yielding an amazing 89% of the end result within only eight minutes we looked into various solvent for the chemical process (Entries 1–7 in Table 2) and discovered overall, Ethanol was the best fluid for solvents because the end result residue split while heated and there was no need for further purifying beyond washing it with hot ethanol. While the reaction did not proceed with water (Entries 1 in Table 2), After cooling, under reaction with methanol, n-propanol, n-butanol and DCM (dichloro methane) (Entries 2,4,5 and 6 in Table 2), the end result residue became separated, with very low yields of 27%, 26% ,16%, and 53% respectively Table 2 shows the findings of our additional investigation into the range and versatility of aromatic aldehydes compounds in this multicomponent reaction. TLC showed that the reaction was finished while the solution of THF and DMSO were employed as solvents, however even after cooling, the result remained undifferentiated. This might be because, under ideal reaction conditions, such as the reaction of Compounds 1 and 2 at room temperature with ethanol as the solvent and 30 mol% catalyst were very soluble in these solvents. Compound 3 was then added, and the mixture was refluxed or microwave-irradiated

Table 1: Screening of the catalyst for the model reactiona. (step-1)

Entry	(MW irradiation) Conditions				
	Catalyst	Amount mol% of Catalyst	Solvent	Time (min)	Yield (%)
1	-	-	Ethanol	60	36
2	Et_3N	10	Ethanol	60	53
3	Et_3N	20	Ethanol	60	53
4	$(NH_4)_2CO_3$	20	Ethanol	30	38
5	NH_4Cl	20	Ethanol	120	no reaction
6	$(NH_4)_2SO_4$	20	Ethanol	120	no reaction
7	AcOH	20	Ethanol	120	no reaction
8	NH_4OAc	10	Ethanol	30	59
9	NH_4OAc	20	Ethanol	25	68
10	NH_4OAc	25	Ethanol	20	76
11	NH_4OAc	30	Ethanol	8	89
12	NH_4OAc	35	Ethanol	8	89

Table 2: Solvent screening for the model reactiona. (Step-1)

Entry	Conditions		Yield (%)
	Solvent	Time(min)	
1	Water	35	no reaction
2	Methanol	30	27
3	Ethanol	4	89
4	n-Propanol	38	26
5	n-Butanol	35	16
6	DCM	12	53
7	Ethanol: Water (1:1)	30	68

a Reaction conditions: Catalyst (30 mol% NH4OAc), 1a-l (5 mmol), 2 (5 mmol), 5 mL of solvent swirled at room temperature, following the addition of 3 (5 mmol) dissolved in five millilitres of solvent and microwave-irradiated.

Table 3: Evaluation of the Efficiency of Conventional and Microwave (MW) Processes of the Production of Pyrimidine and Pyrimidine-Pyrimidine Derivatives 4a-l, 5a-l

Compound	MW irradiation Method A		Conventional Condition Method B	
	% of Yield	Time (min)	% of Yield	Time (hour)
4a	89	4	73	2
4b	89	4	75	2.5
4c	90	4	75	3
4d	88	5	70	3
4e	89	5	70	3
4f	90	5	70	3
4g	89	4	71	3
4h	87	5	70	3
4i	88	4	78	3
4j	89	5	77	4
4k	87	5	72	3
4l	86	5	75	3.5
5a	87	9	71	8
5b	82	11	79	9
5c	81	10	70	8
5d	80	12	67	9
5e	79	13	61	9
5f	83	14	69	9
5g	85	12	65	8
5h	86	10	69	9
5i	83	13	68	10
5j	79	11	71	9
5k	78	14	64	11
5l	71	15	61	12

during the necessary amount of time, the products were highly soluble in these solvents.

Lately we have focused on synthesising specific heterocyclic compounds with expected biological activity under microwave-assisted conditions that are both environmentally friendly and time-efficient[16]. The originally characterised compounds 4a–l, 5a–l were re-synthesised using microwave stimulation in order to improve reaction yields and shorten reaction times. Reaction yields were enhanced by 15–25% as opposed to the traditional approaches, and reaction durations significantly shortened, according to the findings for both MW-assisted and Conventional processes, which appear in Table 3.

EXPERIMENTAL

Every chemical and solvents used was bought from an industrial supplier then utilised with no further purification.The final derivatives' melting temperatures, which appeared uncorrected, were estimated using a Veego melting point device. Using n-hexane: ethyl acetate as a developing solvent, TLC aluminium plates covered with silica gel were utilised to check the purity of the synthesised compounds. The spots were then exposed to a UV light chamber. Bruker FT-IR alpha-t was used to obtain the IR spectra for each derivative (ATR). Bruker spectrometers (400 MHz and 100 MHz, respectively) were used to obtain 1H and 13C NMR data (DMSO-d6 (1H NMR; 2.49 ppm and 13C NMR; 39.30 ppm) was used as solvent and

Table 4: MIC values (in *μ*g/ml) for the antimicrobial activity of the synthesised compounds 5a–l

	Bacillus subtilis **MTCC No. 441 (Gram-positive bacteria)**	***Escherichia coli*** **MTCC No. 443 (Gram-positive bacteria)**	***Candida albicans*** **MTCC No. 227 (Fungi)**
(H) 5a	150	100	600
(2-Cl) 5b	125	125	250
(4-NO_2)5c	120	140	500
(3-OH)5d	65.5	100	300
(3-Cl) 5e	80	60.5	600
(4-Cl) 5f	100	62.5	500
(2-OH)5g	80.5	120	500
(3-OCH3,4-OH)5h	100	62.5	1000
(4-N(CH_3)$_2$)5i	62.5	125	500
(2-Cl,4-NO_2)5j	60.5	65.5	250
Furfural 5k	100	200	1000
Indole-3-carbaldehyde 5l	62.5	200	500
Chloramphenicol	50	50	--
Griseofulvin	--	--	500

TMS as reference). The value of chemical shift are expressed in βppm as a unit. A Schmindzu mass spectrophotometer was used to determine the mass spectra data for each derivative. Synthesis was carried out using an MS Black Microwave Synthesiser with A Millstone in recent years Organic biosynthesis Unit (Micro SYNTH with touch control terminal) and An ongoing concentrated microwave radiation delivery method were used to conduct microwave reactions under magnetic stirring in a glass pressure jar (10 mL) that was covered with a membrane. A calibrated thermal temperature controller was used to keep an eye on The ambient temperature of the component mixtures underneath the reaction jar, and the resulting pressure was managed by a pressure detector attached to the jar's membrane.

General Procedure

Step-1: Synthesis of benzothiazolo[3,2-a] pyrimidine derivatives (4a-l)

Method A (Conventional method)

A mixture of NH_4OCOCH_3 (0.116 g, 30 mol%) and malononitrile (0.33 g, 0.005 mol) in ethanol was added, and then benzaldehyde (0.005 mol) was added and agitated for 5–10 min at room temperature. After being solidified, Intermediate benzylidene malononitrile was heated to 70β which aided in its dissolution in ethanol. Once the reaction mixture had a clear solution, 2-amino benzothiazole compound (0.751g, 0.005 mole) was mixed with 10 mL of Ethanol and allowed to reflux for one to two hours. Progress on the reaction tracked with TLC (Ethyl acetate : Hexane; 3:7). The resultant mixture was washed with water following the reaction. The precipitate of the final product was filtered. Additionally, the product is recrystallised using heated alcohol to yield the pure product. Compounds 4b–l were prepared similarly, although the reflux time varied slightly.

Method B (Microwave assisted method)

A Mixture of malononitrile (0.33g, 0.005 mole) and NH_4OCOCH_3 (0.116g, 30 mol%) in Ethanol, benzaldehyde (0.005 mole) was added and agitated for 5–10 min at room temperature. After being solidified, Intermediate benzylidene malononitrile was irradiated in a microwave oven at 500 W for about 30 sec. which aided in its dissolution in ethanol. Once the reaction mixture had a clear solution, 2-amino benzothiazole compound (0.751g, 0.005 mole) was added to 10 mL of ethanol The mixture was then exposed to 500 W of microwave radiation for 4 min. at periods of around 30 seconds. Progress on the reaction tracked with TLC (Ethyl acetate : Hexane; 3:7). The resultant mixture was washed with water following the reaction. The precipitate of the final product was filtered.

Additionally, the product is recrystallised using heated alcohol to yield the pure product. Compounds 4b–l were prepared similarly, although the reflux time varied slightly.

Step-2: Synthesis of final compounds(5a-l)

Method A (Conventional method)

Each of the compounds 4a–l (1.9 mmol), 5–10 mL of 80% formic acid and taken a catalytic quantity of H2SO4 was heated on reflux at 1500C for 8 hours. After being allowed to cool to a normal temperature, the resultant combination was added to cold ice water. The filtered residue was gathered, evaporated, and recrystallised using the alcohol.

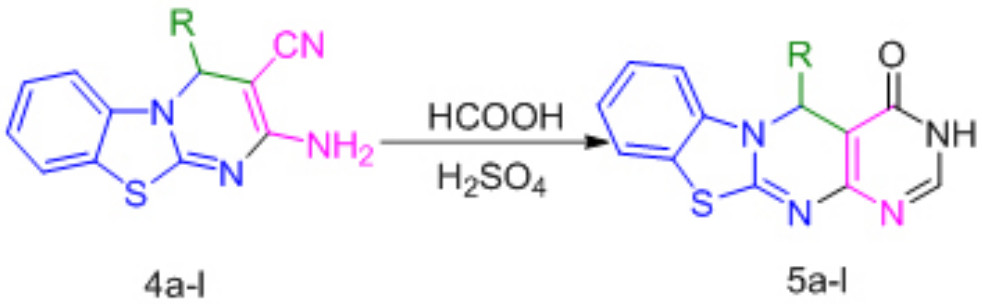
Method B (Microwave assisted method)

Each of compounds 4a-l (1.9 mmol) , 5-10 mL of formic acid (80%) and taken a sufficient quantity of concentrated sulphuric acid were irradiated in microwave oven set to 600 W for 15 min. Similar to method A, the reaction mixture was treated to yield compounds 5a-l.

Proposed mechanism

Based on the experimental findings and earlier research[15], Scheme 2 presented a suggested mechanism for the reaction. The final product of treating 4a-l with formic acid was 5-phenyl-3,5-dihydro-4H-benzothiazolo[3,2-a] pyrimido[4,5-d] pyrimidinone 5a-l, which was obtained by heating it for several hours or by irradiating it in a microwave oven. (Scheme 1, step 2). The elemental and spectral data from compounds 5a–l were compatible ("Experimental"). It is believed that the formation of 5a-l occurs thought that two moles of water molecules are eventually eliminated by a condensation reaction, and a partial hydrolysis reaction (Scheme 2).

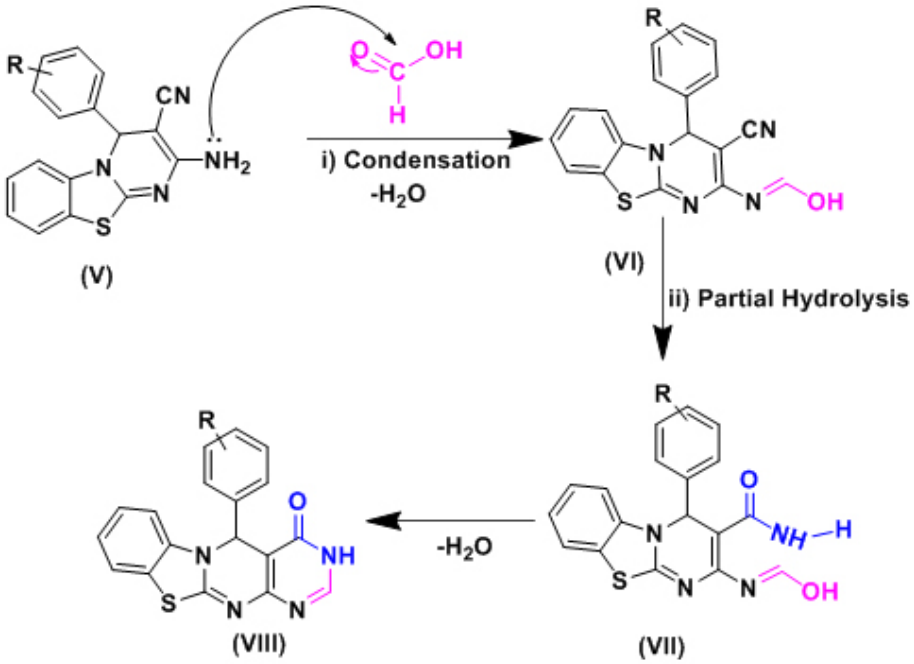
Synthesis of Benzothiazolo[3,2-a] pyrimidine derivative 4a–l. (Step-1



Synthesis of 5-phenyl-3,5-dihydro-4H-benzothiazolo[3,2-a] pyrimido[4,5-d] pyrimidinone 5a-l. (Step- 2)

a, R = C_6H_5
b, R = C_6H_4Cl-o
c, R = C_6H_4 NO2-p
d, R = C_6H_4OH-m
e, R = C_6H_4Cl-m
f, R = C_6H_4Cl-p
g, R = C_6H_4OH-o
h, R = C_6H_3OH-p, OCH_3-m
i, R = $C_6H_4N(CH3)_2$-p
j, R = C_6H_3Cl-o, NO_2-p
k, R = Furfural
l, R = Indole-3-carbaldehyde

Scheme 1:



Scheme 2: The possible mechanism for the formation of step-2

Analytical Discussion

Synthesis of 5-phenyl-3,5-dihydro-4H-benzothiazolo[3,2-a] pyrimido[4,5-d] pyrimidin-4-one (5a)

M.P.: 244 °C, Yellow solid, Yield 87%.

IR spectrum (KBr, cm^{-1}): 3246,3109,2922,1680,1476 cm-1. ;1H NMR spectrum (400 MHz, DMSO-d6,β δ, ppm): δ=4.56 (s, H, *C-H),7.20-8.70(m,10H, Ar-H),12.47(d,1H, NH).,13CNMR spectrum (100MHz, DMSO-d6, δ, ppm)β=126.0,117.4,112.8,122.3,139 .0,117.2,157.2,148.6,148.3,162.2,123. 8,69.7,137 .2,126.9,128.6,126.7., MS spectrum (m/z): 333.4 $[M+H]^+$. $C_{18}H_{12}N_4OS$.

Synthesis of 5-(2-chlorophenyl)-3,5-dihydro-4H-benzo [4,5] thiazolo [3,2-a] pyrimido [4,5-d] pyrimidin-4-one (5b)

M.P.:270°C, Dark Yellow solid, Yield 82%.

IR spectrum (KBr, cm^{-1}):3285,3119,2892,1691,1501, 756 cm-1.;1H NMR spectrum (400 MHz, DMSO-d6, δ , ppm): β=4.59 (s, H, *C-H), 7.10-8.71 (m,9H, Ar-H), 11.79(d,1H, NH)., 13CNMR spectrum (100MHz, DMSO-d6, δ, ppm): δ =126.3, 117.2, 117.4, 122.9, 122.0, 139.3, 158.3, 150.1, 150.3,162.2,123.2,65 .5,142.8,126.6,128.6,128.1,132.2., MS spectrum (m/z):368 $[M+H]^+$.$C_{18}H_{11}ClN_4OS$.

Synthesis of 5-(4-nitrophenyl)-3,5-dihydro-4H-benzo [4,5] thiazolo[3,2-a] pyrimido [4,5-d] pyrimidin-4-one(5c)

M.P.: 241 °C, Yellow solid, Yield 81%.

IR spectrum (KBr, cm^{-1}): 3245, 3099,2912,1688,1487 cm-1.;1H NMR spectrum (400 MHz, DMSO-d6, δ, ppm): β δ =4.57(s, H, *C-H),7.38-8.70(m,9H, Ar-H),12.11(d,1H, NH).,13CNMR spectrum (100MHz, DMSO-d6,βppm): β δ =126.3, 117.2, 117.4, 122.9, 122, 139.3, 158.3, 150.1, 150.3, 162.2,123.2, 65.5, 142.8,126.6,128.6,128.1,132.2., MS spectrum (m/z): 379 M_{+2}. $C_{18}H_{11}N_5O_3S$.

Synthesis of 5-(3-hydroxyphenyl)-3,5-dihydro-4H-benzo [4,5] thiazolo[3,2-a] pyrimido [4,5-d] pyrimidin-4-one(5d)

M.P.: 256°C, Yellow solid, Yield 80%.

IR spectrum (KBr, cm^{-1}): 3574, 3312, 3255, 3119, 2916,1 698,1477cm-1.;1H NMR spectrum (400MHz, DMSO-d6, δ, ppm): δ =4.57(s,1H, *C-H),6.82-8.70(m,9H, Ar-H),12.13(d,1H, OH),11.18(s,1H, NH).,13CNMRspectrum(100MHz,DMSO-d6,βppm):β δ =126.3,117.4,117.2,122.9,122.0,139.3,158.8, 150.4 ,150.2,162,123,70.9,144.8,119.5,112.6,15 6.8,113.9. 129.9., MS spectrum(m/z):349$[M+H]^+$. $C_{18}H_{12}N_4O_2S$.

Synthesis of 5-(3-chlorophenyl)-3,5-dihydro-4H-benzo [4,5] thiazolo[3,2-a] pyrimido[4,5-d] pyrimidin-4-one(5e)

M.P.:236°C, Yellow solid, Yield 79%.

IR spectrum (KBr, cm^{-1}):3292, 3129, 2902, 1688, 1499, 786 cm^{-1}.;^{1}H NMR spectrum (400 MHz, DMSO-d6,βδ,ppm): δ=4.59 (s, H, *C-H),7.08-8.70(m,9H,Ar-H),11.74(d,1H,NH).,13CNMR spectrum (100MHz,DMSO-d6,βδ,ppm)βδ 126.2,117.4,117.3,122.9,122.1,139.4,158.0,150.0,150.8,162.7,123.7,70.1,144.8,126.7,125.0,134.1,129.9,126.8., MS spectrum (m/z): 368 [M+H] +.$C_{18}H_{11}ClN_4OS$.

Synthesis of 5-(4-chlorophenyl)-3,5-dihydro-4H-benzo [4,5] thiazolo[3,2-a] pyrimido [4,5-d] pyrimidin-4-one(5f)

M.P.:233°C, Light-yellow solid, Yield 83%.

IR spectrum (KBr, cm^{-1}): 3298, 3124, 2892, 1681, 1489, 784cm^{-1}.;^{1}H NMR spectrum (400MHz, DMSO-d6, δ, ppm): δ=4.58(s,H,*C-H),7.10-8.71(m,9H, Ar-H),11.78(d,1H, NH).,13CNMR spectrum (100MHz, DMSO-d6, δ, ppm): β=126.3,117.4,117.2,122.9,122,139.2,158.3,150.2,150.6, 162.2,123.2,70.6,135.8,126.1,128.6, 132.3., MS spectrum (m/z):369 M^{+2}. $C_{18}H^{11}ClN^4OS$.

Synthesis of 5-(2-hydroxyphenyl)-3,5-dihydro-4H-benzo [4,5] thiazolo[3,2-a] pyrimido [4,5-d] pyrimidin-4-one(5g)

M.P.:258°C, Yellow solid, Yield 85%.

IRspectrum (KBr, cm^{-1}): 3562, 3322, 3258, 3121, 2919, 1684,1468cm-1.;1HNMR spectrum (400MHz, DMSO-d6, δ, ppm): δ=4.57(s,1H, *C-H),6.83-8.70(m,9H, Ar-H),10.21(s,1H, OH),11.89(d,1H, NH).,13CNMR spectrum (100MHz, DMSO-d6, δ, ppm): β=126.2, 117.1,117.2,122.7,122.0,139.4,158.3,150.1,150.2,162.2,123.2,64.4,154.0,117.5,115.6, 121.1,128.3, 128.3., MS spectrum (m/z): 349 [M+H] +. $C_{18}H_{12}N_4O_2S$.

Synthesis of 5-(4-hydroxy-3-methoxyphenyl)-3,5-dihydro-4H-benzo [4,5] thiazolo[3,2-a] pyrimido[4,5-d] pyrimidin-4-one(5h)

M.P.289oC, Brown solid, Yield 86%.

IR spectrum (KBr, cm^{-1}): 3575, 3322, 3260, 3121, 2919, 1684, 1470cm^{-1}.;1HNMR spectrum (400MHz, DMSO-d6,βδ,ppm): β=4.59 (s,1H,*C-H), 3.79(s,3H, CH3), 6.81-8.70(m, 9H,ArH),10.92(d,1H,OH),11.18(s,1H,NH).,13CNMRspectrum(100MHz,DMSO-d6,βδ,ppm):βδ.3,117. 4,117.2, 122.9, 122.0, 139.3, 158.3, 150.0, 150.1, 162.4, 123.7, 70.9, 136.9,118.5,115.4,146.7,147.3,112.4,56.1.,MSspectrum(m/z): 379.4 [M+H] +. $C_{19}H_{14}N_4O_3S$.

Synthesis of 5-(4-(dimethyl amino) phenyl)-3,5-dihydro-4H-benzo [4,5] thiazolo[3,2-a] pyrimido[4,5-d] pyrimidin-4-one(5i)

M.P.269°C, Dark Brown solid, Yield 83%.

IR spectrum (KBr, cm^{-1}): 3181,3041,2842,1650,1520 cm^{-1}.;1H NMR spectrum (400 MHz, DMSO-d6, δ, ppm): δ =3.09(s,2H, CH2),4.58(s,H,*C-H),6.65-8.74(m,9H,Ar-H),11.80(d,1H,NH).,13CNMR spectrum (100MHz,DMSO-d6,δ,ppm)β=41.3, 126.3, 117.4, 117.2,122.9,139.3,122.0,158.2, 150.1,15 0.2,162.2,123.2,70.7,137.2, 126.8,126.1, 112.7,149.1.,MS spectrum (m/z):376[M+H] +. $C_{20}H_{17}N_5OS$.

Synthesis of 5-(2-chloro-4-nitrophenyl)-3,5-dihydro-4H-benzo [4,5] thiazolo[3,2-a] pyrimido[4,5-d] pyrimidin-4-one(5j)

M.P.260°C, Yellow solid, Yield 79%.

IR spectrum (KBr, cm^{-1}): 3252, 3089,2902,1690, 1477,761cm^{-1}.;1H NMR spectrum (400 MHz, DMSO-d6, δ, ppm): δ=4.60 (s, H, *C-H),7.11-8.73(m,9H, Ar-H),12.16(d,1H, -NH).,13CNMR spectrum (100MHz, DMSO-d6, δ, ppm): β=126.1, 117.0,117.3,122.7,122.1,139.3,158.2,150.4,150.5, 162.6,123.3,65.5,148.9,133.1,124.0,121.8,147.2, 129.2., MS spectrum(m/z):414M+2.$C_{18}H_{10}ClN_5O_3S$.

Synthesis of 5-(furan-2-yl)-3,5-dihydro-4H-benzo [4,5] thiazolo[3,2-a] pyrimido[4,5-d] pyrimidin-4-one(5k)

M.P.288°C, Black solid, Yield 78%.

IR spectrum (KBr, cm^{-1}): 3399, 3158, 1679, 1442, 1190cm^{-1}.;1H NMR spectrum (400 MHz, DMSO-d6, δ, ppm):βδ =4.87(s, H, *C-H),6.85-8.68(m,8H, Ar-H),10.17(d,1H, -NH).,13C-NMR spectrum (100MHz,DMSO-d6,βδ,ppm): β=131.6,116.2,130.6, 129.3,132.0,116.1,162.4,137.2,149.1,163.3,130.8, 60.8,151.9,105.9,107.3,136.8., MS spectrum (m/z): 323[M+H] +. $C_{16}H_{10}N_4O_2S$.

Synthesis of 5-(1H-indol-3-yl)-3,5-dihydro-4H-benzo [4,5] thiazolo[3,2-a] pyrimido[4,5-d] pyrimidin-4-one (5l)

M.P.280°C, Dark chocolate solid, Yield 71%.

IR spectrum (KBr, cm^1):3399,3158,2205,1679, 1442,1190 cm^{-1}.;1H NMR spectrum (400 MHz,

DMSO-d6,β δ,ppm):β=4.77(s, 1H, *C-H),6.97-8.78(m,10H, Ar-H),10.79,11.67(d,2H,-NH).,13CNMR spectrum(100MHz,DMSO-d6,βppm):βδ =126.3, 117 .2,118.6,122.9,139.0,122.0.1,158.4,150.1,150.2, 16 2.3,123.8,70.3,112.1,105.9,117.3,119.8,121.7,111. 1,136.5,123.0,127.4, MS spectrum (m/z): 373[M+H] +. $C_{20}H_{13}N_5OS$.

Pharmacological Screening[17-19] Material and Methods

Selection of bacterial strains for present investigation

Pathogenic bacteria used for antibacterial activity were subculture and characterized by standard methods of identification. Following strains of bacteria were used for antibacterial screening:

Escherichia coli (Gram -ve): Causes cholecystitis, urinary tract infections, bacteraemia, gastroenteritis, pneumonia, gastroenteritis and neonatal meningitis

Bacillus Subtilis (Gram +ve): Causes pneumonia, an infection of the lungs, infection of the membranes surrounding the brain and spinal cord.

Chloramphenicol has been used as the standard medication the present research.

Selection of fungal strains for present investigation

Pathogenic fungi were sub cultured on potato dextrose agar (PDA) and characterized by standard methods of identification. Following strains of fungi were used for antifungal screening:

Candida albicans: agent responsible for human vaginal and oral opportunistic infections as well as nail plate infections caused by candidal onychomycosis.

Griseofulvin has been used as the standard medication the present research.

The least inhibitory concentration (MIC) of the new Pyrimido pyrimidine-tethered to benzothiazole derivatives has been determined utilising the Broth Dilution Method in order to evaluate its in vitro antibacterial activity. The antibacterial activity of the Pyrimido pyrimidine-tethered benzothiazole derivatives was assessed against a variety of Gram-positive (*Bacillus subtilis* MTCC No. 441) and Gram-negative (*Escherichia coli* MTCC No. 443) microorganisms. Additionally, the synthetic compounds' antifungal efficacy against Candida albicans MTCC No. 227 was examined. The antibiotics chloramphenicol was selected as antibacterial agents as references in order to assess and compare the potency of the compounds that were tested under identical conditions. Griseofulvin served as a reference antifungal. The measurement obtained has compared with standard to determine activity index and to predict whether the bacterial and fungal species tested is resistant or sensitive to the antibiotic. The results of this investigation are presented in Table 4. The majority of the tested compounds had MIC values between 60 and 150 µg/mL and exhibit varying antibacterial activity against Gram-positive bacteria, ranging from poor to outstanding. As indicated in Table 4, For Bacillus subtilis compounds 5d, 5i, 5j, and 5l showed the greatest activity (MIC) in comparison to the reference antibiotics chloramphenicol (MIC = 50 µg/mL). The tested Gram-negative bacteria had MIC values ranging from 60 to 200 µg/mL, and all of these compounds were shown to be more active against them than the common medication chloramphenicol (MIC = 50 µg/mL). Notably, compounds 5e, 5f, 5h, and 5j have outstanding activity (MIC) against Bacillus subtilis with MIC values ranging from 60.5 to 65.5 µg/mL, in contrast to the reference antibiotics, chloramphenicol (MIC = 50 µg/mL). According to the antifungal screening results (Table 4), compounds 5c, 5f, 5g, 5i, and 5l were showing activity against the tested fungi that was similar to that of the reference antifungal Griseofulvin (MIC = 500 µg/mL).

CONCLUSION

In summary, we were able to successfully create benzothiazolo[3,2-a] pyrimidine derivatives (4a-l) using a one-pot, three-component reaction in 86 % to 90% yield and novel 5-phenyl-3,5-dihydro-4H benzothiazolo[3,2-a] pyrimido[4,5-d] pyrimidinone derivatives (5a-l) convenient one-pot reaction in 71% to 87% yield. via reaction using two methods (MW irradiation and conventional methods). The reaction goes through cyclisation, rearrangements, Michael addition, and Knoevenagel condensation. It is worth mentioning that this protocol proceeded without the use of toxic solvents, excellent yields in a shorter reaction time with highly pure products are another benefit of this method. This one-pot atom economic method furnished an excellent

yield of corresponding products with a wide range of substituted aldehydes and thus provided high substrate scope under mild reaction conditions. Additionally, FTIR, 1H-NMR, 13C-NMR, and MS were among the techniques used to characterise all synthesised compounds and confirm their synthesis and the anti-fungal and anti-microbial properties of these prepared compounds were further evaluated. Given the presence of an electron withdrawing group on the benzene ring, the synthesized derivatives 4-Cl, (2-Cl,4-NO2), 4-N(CH3)2, (3-OCH3, 4-OH) and Indole-3-carbaldehyde have fairly promising action as effective therapeutic agents, as demonstrated by a SAR investigation.

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Conflict of interest

The authors declare that they have no conflict of interest.

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