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Microwave assisted synthesis and antimicrobial activity of some novel pyrimidine derivatives

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Abstract: The synthesis of thiazolo[5,4-*d*]pyrimidines can be achieved from different 5-thiazolidinones, 2-butyl-1*H*-imidazole-5-carbaldehyde and thiourea using microwave irradiation within 5 min. The structures of the products were supported by FTIR, PMR and mass spectral data. The *in vitro* antimicrobial activity of the synthesized thiazolo[5,4-*d*]pyrimidines **1a-j**, having substituents at the 1- and 3-positions, were determined by the cup-plate method against several standard strains chosen to define the spectrum and potency of the new compounds. The antimicrobial activities of the thiazolo[5,4-*d*]pyrimidines **1a-j** are compared with those of known chosen standard drugs, *viz*. ampicillin, chloramphenicol, ciprofloxacin, norfloxacin and griseofulvin.

Keywords: microwave irradiation, thiazolo[5,4-*d*]pyrimidines, antimicrobial activity.

INTRODUCTION

In the family of heterocyclic compounds, nitrogen-containing heterocycles with a sulfur atom are an important class of compounds in medicinal chemistry. There has been considerable interest in the development of preparative methods for the production of pyrimidines. This seems to be because pyrimidines represent one of the most active classes of compounds, possessing a wide spectrum of biological activities, *viz*. significant *in vitro* activity against unrelated DNA and RNA viruses, including polio and herpes viruses, diuretic,[1] antitumor,[2] anti-HIV,[3] cardiovascular,[4] etc., as well as to understand the mechanism of the reaction for pyrimidine synthesis, which is commonly known as the Biginelli condensation.[5]

To overcome the drawbacks of the Biginelli reaction, several protocols, such as the use of polyphosphoric acid (PPA), aluminium trichloride ($AlCl_3$), boron trifuloride (BF_3), conc. HCl, *etc.* and microwave procedures were introduced to improve the yield and conditions of the Biginelli reaction. In this way, it was found that phosphorus pentoxide is a highly efficient protocol for the Biginelli reaction,

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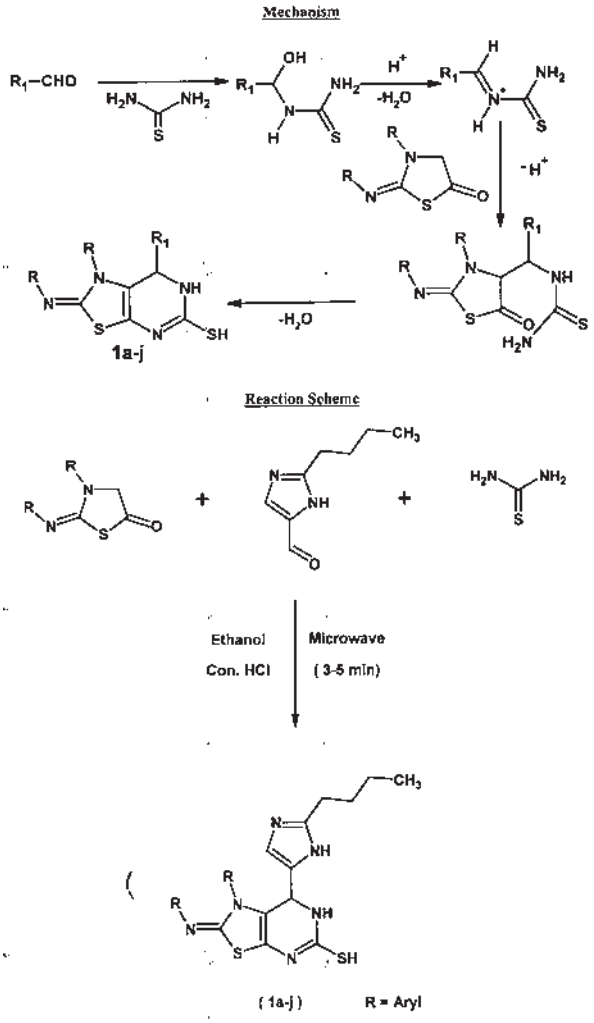
which is used in a catalytic amount (200 mg). The method for the synthesis of Biginelli compounds using microwave irradiation is an easy one. The reaction was performed in alcoholic medium; the progress was controlled by TLC.

The present research manuscript reports a basic study of the Biginelli condensation reaction, in which the condensation between an aldehyde and urea has some similarities to the Mannich condensation (Scheme 1). The generated iminium intermediate acts as an electrophile for condensation with the amino group of urea in acidic medium and they were exposed to microwave irradiation (Table I) for completion of the reaction, which was indicated by TLC data. After the completion of the reaction, the cyclized thiazolo[5,4-*d*]pyrimidines **1a-j** separated and were crystallized from ethanol.



Scheme 1.

All the new compounds gave satisfactory elemental analyses (C, H, N, S) within ± 0.5 % of the theoretical values (Table II) and the structures were in accordance with their spectroscopic data.

TABLE I. Power and time of microwave irradiation

Compd. No.	Power / W	*t* / min
1a	100	3
1b	200	5
1c	200	5
1d	200	5
1e	300	4
1f	300	4
1g	300	3
1h	400	3
1i	400	4
1j	300	4

Microwave irradiation is a non-conventional energy source, the popularity and synthetic utility in synthetic organic chemistry of which has increased considerably in recent years.[6] In the present research, synthetic approaches for the synthesis of thiazolo[5,4-*d*]pyrimidine derivatives were investigated and then all the components were screened for their *in vitro* biological activity, such as antimicrobial activity towards gram positive and gram negative bacterial strains and antifungal activity at different concentrations. The biological activities of the synthesized components are compared with standard drugs, such as ampicillin, chloramphenicol, ciprofloxacin, norfloxacin and griseofulvin.

The structures of the synthesized compounds are supported by elemental analysis, as well as FTIR, PMR and mass spectral data.

EXPERIMENTAL

Melting points were determined routinely in an open capillary tube and are uncorrected. Formation of compounds was routinely checked by TLC on silica gel-G plates of 0.5 mm thickness and the spots were located by iodine. A scientific Qpro-M microwave oven was used and the irradiation time varied by 1–2 min. The PMR spectra were recorded in $CDCl_3$ on a Brucker DRX-300 at 300 MHz. The IR spectra were recorded on a Shimadzu-8400 FT-IR spectrometer in KBr (λ in cm^{-1}). Elemental analyses of the newly synthesized compounds were carried out on a Carlo Erba 1108 analyzer and results within the range of the theoretical value were found. Mass spectra were scanned on a GCMS-QP 200 instrument. A scientific Qpro-M microwave oven was used for the rapid and eco-friendly synthesis.

*Preparation of 1-aryl-2-(arylimino)-7-(2-butyl-1*H*-imidazol-5-yl)-5-mercapto-1,2,6,7-tetrahydrothiazolo*[*5,4-*d]*pyrimidines (**1a-j**)*

A mixture of the required 3-aryl-2-(arylimino)-thiazolidin-5-ones (0.01 M), 2-butyl-1*H*-imidazole-5-carbaldehyde (0.001 M) and thiourea (0.001 M) in ethanol (10.0 ml) and conc. hydrochloric acid (2.0 ml) were exposed to microwave irradiation for the indicated time (See Table I). After completion of the reaction, a solid material separated (compd. **1f**) which was recrystallized from ethanol.

The other compounds **1a-j** were prepared in a similar manner.

Antimicrobial activity

Antimicrobial activity testing was carried out using the cup-plate method,[7] which is described below.

Antibacterial activity

Streptococcus pyogenes MTCC-442*, Streptococcus aureus* MTCC-96 and *Bacillus subtilis* MTCC-441 (Gram positive bacteria) were grown in nutrient broth and *E. coli* MTCC-443 (Gram negative bacterium) in Peptone water (PW, 1 % bacteriological peptone and 0.5 % NaCl) for 24 h; this gave the optimum growth of the test bacteria. Each purified compound was dissolved in dimethylformamide (DMF), which had been sterilized by filtration through a sintered glass filter, and stored at 4 °C. Each agent was then added to molten nutrient agar in the following concentrations (μg/ml): 0 (control), 5, 10, 25, 50, 100, 200,500 and poured into a sterile petri dish. The pH of the media was maintained at 7.2 to 7.4. The inoculum consisted of an overnight growth broth culture of a bacterium diluted in such a manner that a 2 mm (internal diameter) loopful of the culture contained 105 colony-forming units (CFU). These were then spot inoculated onto nutrient agar plates containing increasing amounts of a compound, incubated at 37 °C for up to 24 h to determine the minimum inhibitory concentration (MIC),[8,9] which were recorded as zones of inhibition in mm for the bacteria.

Antifungal activity

Candida albicans MTCC-227 and *Aspergillus niger* MTCC-282 were employed for the testing of the antifungal activity using the cup-plate method. The culture was maintained on Sabouraud's agar for 72 h; this gave the optimum growth of the test fungal spores. Each purified compound was dissolved in dimethylformamide, sterilized by filtration using a sintered glass filter and stored. Each agent was then added to Sabouraud's agar in the following concentrations (μg/ml): 0 (control), 5, 10, 25, 50, 100, 200, 500 and poured into a sterile petri dish. The inoculum consisted of an overnight-grown broth culture of a fungus diluted in such a manner that a 2 mm (internal diameter) loopful of the culture contain 105 colony-forming units (CFU). These were then spot inoculated onto Sabouraud's agar plates containing increasing amounts of the compound and then incubated at 37 °C for up to 48 h to determine the minimum inhibitory concentration (MIC).[8,9]

RESULTS AND DISCUSSION

The physical data for compound **1f** are as follows: Yield 92 %, m.p. 90 °C. Anal. Calcd. for $C_{26}H_{28}N_6O_2S_2$; Required: C, 60.00; H, 5.38; N, 16.15 % Found: C, 59.92; H, 5.35; N, 16.18 %. IR (KBr) ν_{max} cm^{-1}: 3400 (N–H str.), 3040 (C–H str.), 2920 (C–H str., asym.), 2858 (C–H str., sym.), 1620 (N–N def.), 1596 (N–H def.), 1550 (C=N), 1455 (C–H def., asym.), 1434 (–CH_2 bending), 1380 (–CH_3 bending), 1365 (C–H def., sym.), 1323 (C–N str.), 1170 (C–H i.p. def.), 1130 (C–N str.), 692 (C–H o.o.p.def.). PMR δ /ppm (TFA): 0.901 (*t*, 3H, – CH_3), 1.364 (*q*, 2H, –CH_2), 1.673 (*m*, 2H, –CH_2), 2.676 (*t*, 2H, –CH_2), 3.802 (*s*, 3H, –OCH_3), 3.841 (*s*, 3H, –OCH_3), 6.526–7.356 (*m*, 13H, Ar-H + – NH + –SH). Mass spectrum of the compound exhibited a molecular ion peak at *m/z* 521 (M^+).

The physical data of the other prepared compounds are given in Table II.

Antimicrobial activity

The MIC values of the test solutions are recorded in Tables III–V which are recorded in zones of inhibition in mm for the bacteria and fungi.

TABLE II. Physical and analytical data

Compd. No.	R	Molecular formula	M.p. °C	Yield %	R_f value[a]	% of nitrogen	
						Calcd.	Found
1a	C_6H_5	$C_{24}H_{24}N_6S_2$	129	92	0.45	18.25	18.27
1b	2-CH_3-C_6H_4	$C_{26}H_{28}N_6S_2$	125	88	0.49	17.20	17.23
1c	3-CH_3-C_6H_4	$C_{26}H_{28}N_6S_2$	35	90	0.46	17.20	17.23
1d	4-CH_3-C_6H_4	$C_{26}H_{28}N_6S_2$	152	94	0.52	17.20	17.23
1e	2-OCH_3-C_6H_4	$C_{26}H_{26}N_6O_2S_2$	155	85	0.44	16.15	16.18
1f	4-OCH_3-C_6H_4	$C_{26}H_{28}N_6O_2S_2$	90	92	0.45	16.15	16.18
1g	2-NO_2-C_6H_4	$C_{24}H_{22}N_8O_4S_2$	150	85	0.45	20.35	20.37
1h	4-NO_2-C_6H_4	$C_{24}H_{22}N_8O_4S_2$	118	90	0.51	20.35	20.37
1i	2-Cl-C_6H_4	$C_{24}H_{22}N_6S_2Cl_2$	138	85	0.46	15.87	15.86
1j	4-Cl-C_6H_4	$C_{24}H_{22}N_6S_2Cl_2$	110	90	0.53	15.87	15.86

[a]Solvent system: hexane–ethyl acetate (6.5:3.5)

TABLE III. Comparative antimicrobial activity of 1-aryl-2-(arylimino)-7-(2-butyl-1*H*-imidazol-5-yl)-5-mercapto-1,2,6,7-tetrahydrothiazolo[5,4-*d*]pyrimidines (**1a-j**) (concentration in µg/ml)

Compd.No	R	Antibacterial activity (Zones of inhibition in mm)													
		S. pyogenes MTCC-442							*S. aureus* MTCC-96						
		5	10	25	50	100	250	500	5	10	25	50	100	250	500
1a	C_6H_5	–	–	12	12	13	13	14	–	–	13	14	16	16	16
1b	2-CH_3-C_6H_4	–	–	10	11	12	13	14	–	–	12	12	14	15	17
1c	3-CH_3-C_6H_4	–	–	11	11	12	14	14	–	–	13	13	14	15	16
1d	4-CH_3-C_6H_4	–	–	12	16	14	14	14	–	–	14	14	15	16	17
1e	2-OCH_3-C_6H_4	–	–	11	11	13	13	14	–	–	12	13	15	15	16
1f	4-OCH_3-C_6H_4	–	–	10	10	12	12	13	–	–	12	12	14	15	17
1g	2-NO_2-C_6H_4	–	–	12	16	18	14	14	–	–	14	14	15	18	16
1h	4-NO_2-C_6H_4	–	–	11	16	13	13	14	–	–	13	12	16	18	16
1i	2-Cl-C_6H_4	–	–	12	12	12	13	13	–	–	13	13	14	15	15
1j	4-Cl-C_6H_4	–	–	10	10	12	13	14	–	–	12	12	14	15	15
Ampicillin		11	13	14	16	18	19	20	10	12	13	14	16	18	21
Chloramphenicol		10	12	13	19	20	20	22	12	13	14	19	20	21	22
Ciprofloxacin		16	18	19	21	21	22	22	17	18	19	21	22	22	23
Norfloxacin		18	18	19	20	21	21	23	19	20	22	25	26	28	28

N.B. (–): No activity

TABLE IV. Comparative antimicrobial activity of 1-aryl-2-(arylimino)-7-(2-butyl-1*H*-imidazol-5-yl)-5-mercapto-1,2,6,7-tetrahydrothiazolo[5,4-*d*]pyrimidines (**1a-j**) (concentration in μg/ml)

Compd. No.	R	Antibacterial activity (Zones of inhibition in mm)													
		E. coli MTCC-443							*B. subtilis* MTCC-441						
		5	10	25	50	100	200	500	5	10	25	50	100	200	500
1a	C_6H_5	–	–	14	15	17	17	18	–	–	16	17	18	19	19
1b	2-CH_3-C_6H_4	–	–	12	14	14	16	17	–	–	14	15	16	18	19
1c	3-CH_3-C_6H_4	–	–	15	16	16	18	18	–	–	16	17	17	18	19
1d	4-CH_3-C_6H_4	–	–	15	16	17	17	17	–	–	17	17	17	18	19
1e	2-OCH_3-C_6H_4	–	–	14	14	16	17	18	–	–	16	16	17	18	19
1f	4-OCH_3-C_6H_4	–	–	13	14	15	17	18	–	–	15	15	17	18	18
1g	2-NO_2-C_6H_4	–	–	15	16	16	17	18	–	–	17	17	18	19	19
1h	4-NO_2-C_6H_4	–	–	14	15	15	16	17	–	–	15	15	16	18	19
1i	2-Cl-C_6H_4	–	–	15	16	16	18	18	–	–	16	16	17	17	19
1j	4-Cl-C_6H_4	–	–	13	14	15	17	17	–	–	15	15	17	17	18
Ampicillin		14	14	15	16	19	20	22	12	16	18	19	20	21	23
Chloramphenicol		14	15	17	23	23	23	23	12	14	16	19	22	23	23
Ciprofloxacin		20	21	23	28	28	28	28	16	17	19	22	22	23	23
Norfloxacin		22	23	25	26	27	29	29	19	20	22	23	24	25	28

N.B.(–): No activity

TABLE V. Comparative antimicrobial activity of 1-aryl-2-(arylimino)-7-(2-butyl-1*H*-imidazol-5-yl)-5-mercapto-1,2,6,7-tetrahydrothiazolo[5,4-*d*]pyrimidines (**1a-j**) concentration in μg/ml

Compd No.	R	Antibacterial activity (Zones of inhibition in mm)													
		C. albicans MTCC-227							*A. niger* MTCC-282						
		5	10	25	50	100	200	500	5	10	25	50	100	200	500
1a	C_6H_5	–	–	18	19	20	20	21	–	–	19	20	21	22	24
1b	2-CH_3-C_6H_4	–	–	16	17	19	20	20	–	–	18	18	20	21	22
1c	3-CH_3-C_6H_4	–	–	17	18	19	20	22	–	–	19	19	21	21	24
1d	4-CH_3-C_6H_4	–	–	18	18	19	21	21	–	–	19	19	20	22	23
1e	2-OCH_3-C_6H_4	–	–	17	18	20	20	21	–	–	19	19	21	22	24
1f	4-OCH_3-C_6H_4	–	–	17	18	20	20	21	–	–	18	18	21	22	23
1g	2-NO_2-C_6H_4	–	–	23	19	20	20	21	–	–	21	20	21	21	23
1h	4-NO_2-C_6H_4	–	–	23	17	19	20	21	–	–	18	19	20	22	24
1i	2-Cl-C_6H_4	–	–	18	18	19	20	21	–	–	21	19	21	22	23
1j	4-Cl-C_6H_4	–	–	17	18	19	20	20	–	–	18	19	20	21	22
Griseofulvin		19	22	23	25	25	28	28	18	19	21	22	22	24	26

N.B. (–): No Activity

Antimicrobial activities of all the compounds (**1a-j**) are compared with known chosen standard drugs, *i.e.*, ampicillin, chloramphenicol, ciprofloxacin, norfloxacin and griseofulvin, in Tables III–V.

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ИЗВОД

МИКРОТАЛАСНА СИНТЕЗА И АНТИМИКРОБНА АКТИВНОСТ НЕКИХ НОВИХ ДЕРИВАТА ПИРИМИДИНА

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Синтеза тиазоло[5,4-*d*]пиримидина може да се оствари из различитих 5-тиазолидина, 2-бутил-1*H*-имидазол-5-карбалдехида и тиоурее коришћењем микроталасног зрачења током 5 min. Структура производа потврђена је коришћењем FTIR, PMR и подацима добијеним масеном спектроскопијом. *In vitro* антимикробна активност, према неколико стандардних сојева, синтетизованих тиазоло[5,4-*d*]пиримидина **1a-j**, који имају супституенте у 1- и 3-положајима, испитивана је методом бунарчића у агару да би се одредиле лепеза и могућности нових једињења. Антимикробна активност тиазоло[5,4-*d*]пиримидина **1a-j** упоређена је са активношћу познатих стандардних лекова као што су ампицилин, хлорамфеникол, ципрофлоксацин, норфлоксацин и гризеофулвин.

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