MICROWAVE ASSISTED SYNTHESIS OF PYRIMIDINE LINKED DITHIADIAZINES BY SULPHUR-SULPHUR BOND FORMATION THROUGH CYCLOCONDENSATION AND STUDY OF ANTIMICROBIAL PROPERTIES

K.A.Palaspagarand P.P.Deohate*
Department of Chemistry, Shri Radhakisan Laxminarayan Toshniwal College of Science, Akola, India
kpalaspagar31@rediffmail.com, pradip222091@yahoo.co.in

ABSTRACT

The microwave assisted synthesis and characterization of series of 1-(6-aryl/alkylimino-3-phenylimino-[1,2,4,5]-dithiadiazin-4-yl)-2-(4,6-dimethyl-pyrimidin-2-yl-amino)-ethanones was initiated by treating 2-amino-4,6-dimethyl-pyrimidine with ethyl chloroacetate to give ethyl (4,6-dimethyl-pyrimidin-2-yl-amino)-acetate. It on further reaction with hydrazine hydrate afforded (4,6-dimethyl-pyrimidin-2-yl-amino)-acetic acid hydrazide. The hydrazide was reacted with N-aryl/alkyl isothiocyanates and further with N-phenyl-S-chloroisothiocarbamoyl chloride and basified to afford the title compounds exhibiting differently substituted constrained pharmacophores. The purity of compounds was checked by TLC and constituents of compounds delineated by chemical transformations, IR, ^{1}H-NMR and mass spectral studies. Title compounds were assayed for their antimicrobial properties.

Keywords: *Microwave, pyrimidine linked dithiadiazines, antimicrobial properties*

Introduction

The sulphur and nitrogen containing heterocyclic compounds were found to possess a wide variety of biological activities[1,2] and proved to be excellent versatile drugs in the field of medicinal chemistry[3]. Pyrimidine as a heterocyclic compound is an excellent core structure with diversified therapeutic applications[4]. Its fascinating use as a medicinally important compound is evidential from its varied biological properties[5]. Synthesis, structural details and biological study of substituted [1,2,4,5]-dithiadiazines was reported earlier in some scientific communications[6-10]. It was found that, *N*-aryl/alkyl-*S*-chloroisothiocarbamoyl chlorideshave enough potentiality in the synthesis of nitrogen and sulphur containing 5, 6 membered heterocyclic compounds[11,12]. It has been observed that there is scanty work on the synthesis of pyrimidine linked [1,2,4,5]-dithiadiazines.

In the present work efforts are made for microwave assisted synthesis[13-14] and characterization of series of 1-(6-aryl/alkylimino-3-phenylimino-[1,2,4,5]-dithiadiazin-4-yl)-2-(4,6-dimethyl-pyrimidin-2-yl-amino)-ethanones.

Experimental

Melting points of all the synthesized compounds were determined on a digital melting point apparatus (Veego, VMP-D) and are uncorrected. All chemicals used were of A.R. grade. The C, H and S analysis was carried out on Carlo-Erbaanalyser, N estimation was carried out on Colman-N-analyser-29. Purity of the title compounds were checked by TLC. All the reactions carried out in GMG20E-08-SLGX microwave oven at 800 W. IR spectra were recorded on Perkin-Elmer spectrophotometer using KBr disc. ^{1}H-NMR spectra were obtained on a Bruker-DRX-600 spectrophotometer in $CDCl_3$ with TMS as internal standard using $CDCl_3$ and DMSO-d_6 as solvents. Mass spectral measurements were carried out by EI method on a Jeol-JMC-300 spectrometer at 70 eV. The reagents used in the synthesis of 1-(6-aryl/alkylimino-3-phenylimino-[1,2,4,5]-dithiadiazin-4-yl)-2-(4,6-dimethyl-pyrimidin-2-yl-amino)-ethanones (7a-h) have been prepared as follows.

Synthesis of ethyl (4,6-dimethyl-pyrimidin-2-yl-amino)-acetate (2)

The parent compound ethyl (4,6-dimethyl-pyrimidin-2-yl-amino)-acetate (**2**) was prepared by irradiating the mixture of 2-amino-4,6-dimethyl-pyrimidine (**1**) (0.01 mol) and ethyl chloroacetate (0.01 mol) in 1,4-dioxane under microwave for 4 min 10 sec using anhydrous potassium carbonate as a catalyst[4]. When 1,4-dioxane was evaporated, crude solid mass was obtained, it was crystallisedfrom

absolute ethanol.

Synthesis of (4,6-dimethyl-pyrimidin-2-yl-amino)-acetic acid hydrazide (3)

The compound (4,6-dimethyl-pyrimidin-2-yl-amino)-acetic acid hydrazide (**3**) was prepared by irradiating the mixture of ethyl (4,6-dimethyl-pyrimidin-2-yl-amino)-acetate (**2**) (0.01 mol) and hydrazine hydrate (0.01 mol) in 1,4-dioxane under microwave for 1 min 30 sec, progress of the reaction was monitored by TLC. The crude solid mass obtained was crystallized from absolute ethanol in cold condition.

Synthesis of (4,6-dimethyl-pyrimidin-2-yl-amino)-acetic acid N-(N'-phenyl-thioamido)-hydrazide (5a)

The mixture of (4,6-dimethyl-pyrimidin-2-yl-amino)-acetic acid hydrazide (**3**) (0.01 mol) and N-phenyl isothiocyanate (**4a**) (0.01 mol) in chloroform was irradiated in a microwave oven for 1 min 30 sec, progress of the reaction was monitored by TLC. The crude solid mass obtained was crystallized from ethanol in cold condition and identified as (4,6-dimethyl-pyrimidin-2-yl-amino)-acetic acid N-(N'-phenyl-thioamido)-hydrazide (**5a**). This reaction was extended to synthesize other compounds (**5b-h**)using different N-aryl/alkyl isothiocyanates (**4a-h**). The reactions were monitored on silica gel-G plates by TLC.

Synthesis of 2-(4,6-dimethyl-pyrimidin-2-yl-amino)-1-(3,6-diphenylimino-[1,2,4,5]-dithiadiazin-4-yl)-ethanone (7a)

The mixture of (4,6-dimethyl-pyrimidin-2-yl-amino)-acetic acid N-(N'-phenyl-thioamido)-hydrazide (**5a**) (0.01 mol) and N-phenyl-S-chloroisothiocarbamoyl chloride (0.01 mol) in chloroform was irradiated under microwave for 2 min 30 sec, which leads to the formation of sticky mass. It was repeatedly washed with petroleum ether (60-80^{0}C). The separated solid was acidic to litmus and identified as 2-(4,6-dimethyl-pyrimidin-2-yl-amino)-1-(3,6-diphenylimino-[1,2,4,5]-dithiadiazin-4-yl)-ethanone hydrochloride (**6a**). It on basification with dilute ammonium hydroxide solution and on crystallization from ethanol afforded a free base (**7a**). This reaction was extended to synthesize other compounds (**7b-h**). The reactions were monitored on silica gel-G plates by TLC.

Results and Discussion

The parent compound 2-amino-4,6-dimethyl pyrimidine (**1**) was treated with ethyl chloroacetate in 1,4-dioxane medium using anhydrous potassium carbonate as a catalyst to yield ethyl (4,6-dimethyl-pyrimidin-2-yl-amino)-acetate (**2**), 88%, m.p. 142^{0}C (Found: C, 55.11; H, 6.98; N, 20.10. Calcd. for $C_{10}H_{15}N_3O_2$: C, 57.41; H, 7.17; N, 20.09%). The compound (**2**) was reacted with hydrazine hydrate in 1,4-dioxane to give (4,6-dimethyl-pyrimidin-2-yl-amino)-acetic acid hydrazide (**3**), 88%, m.p. 138^{0}C (Found: C, 48.17; H, 6.38; N, 35.01. Calcd. for $C_8H_{13}N_5O$: C, 49.22; H, 6.71; N, 35.87%); IR: ν_{max} 3401, 3310 (NH), 1705 (C=O), 1628 (C=N), 1336 (C-N), 1156 cm^{-1} (N-N); ^{1}H-NMR: δ ($CDCl_3$+DMSO-d_6): 7.38 (1H, s, CO-NH), 6.46 (1H, s, Pyrm-NH), 6.32 (1H, s, Pyrm-H), 3.57 (2H, s, CO-CH_2), 2.52 (2H, s, NH_2), 2.17 (6H, s, Pyrm-CH_3).

The IR spectrum of compound (**3**) showed intense absorption at 1705 cm^{-1} which suggested the presence of C=O group. The aromatic and aliphatic C-H stretching frequencies were observed in the regions 3410-3145 and 2990-2940 cm^{-1} respectively. The appearance of sharp peaks at 3401 and 3310 cm^{-1} indicated the presence of NH and NH_2 groups respectively in the molecular structure. In ^{1}H-NMR spectrum of compound (**3**) peaks at δ 7.38 and 2.52 confirmed the presence of CO-NH-NH_2 linkage which indicates the conversion of substituted ethyl acetate into acetic acid hydrazide.

The compound **(3)** on reaction with N-phenyl isothiocyanate (**4a**) afforded (4,6-dimethyl-pyrimidin-2-yl-amino)-acetic acid N-(N'-phenyl-thioamido)-hydrazide (**5a**), 79%, m.p. 134^{0}C (Found: C, 53.77; H, 5.05; N, 25.40; S, 9.08. Calcd. for $C_{15}H_{18}N_6OS$: C, 54.54; H, 5.45; N, 25.45; S, 9.69%); IR: ν_{max} 3402, 3311 (NH), 1764 (C=O), 1649 (C=N), 1311 (C-N), 1246 (C=S), 1170 cm^{-1} (N-N); ^{1}H-NMR: δ ($CDCl_3$+DMSO-d_6): 7.99 (1H, s, CO-NH), 7.75 (1H, s, Ar-NH), 7.73 (1H, s, CS-NH), 7.09-7.58 (5H, m, Ar-H), 6.41 (1H, s, Pyrm-NH), 6.29 (1H, s, Pyrm-H), 3.64 (2H, s, CO-CH_2), 2.21 (6H, s, Pyrm-CH_3).

In IR spectrum of compound (**5a**) intense absorptions at 1764 and 1246 cm^{-1} showed the presence of C=O and C=S groups respectively. The aromatic and aliphatic C-H stretching frequencies were observed in the regions 3410-3000 and 2990-2875 cm^{-1} respectively. The appearance of peaks in the region 3402-3311 cm^{-1} indicates the presence of NH groups. The ^{1}H-NMR spectrum of compound (**5a**) showed peaks at δ 7.99, 7.75, 7.73 and 6.41 which confirmed the presence of four NH groups in the molecular structure.

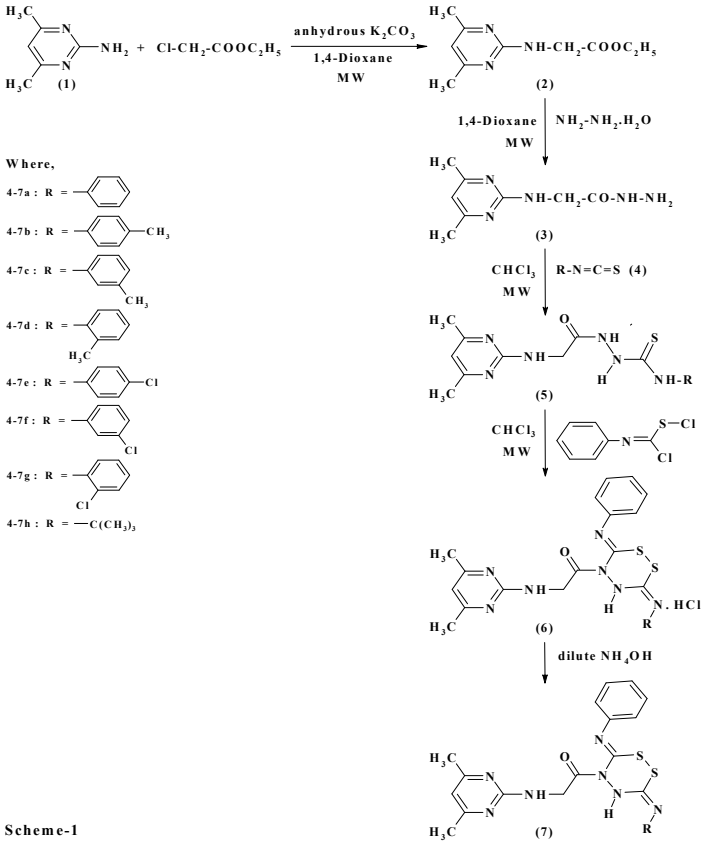
The above reaction was extended to synthesize the compounds (**5b-h**) using different N-aryl/alkyl isothiocyanates (**4a-h**): (**5b**), 84%, m.p. 118^{0}C (Found: C, 55.79; H, 5.66; N, 24.14; S, 9.18. Calcd. for $C_{16}H_{20}N_6OS$: C, 55.81; H, 5.81; N, 24.41; S, 9.30%); (**5c**), 87%, m.p. 112^{0}C (Found: C, 55.65; H, 5.77; N, 23.92; S, 9.29. Calcd. for $C_{16}H_{20}N_6OS$: C, 55.81; H, 5.81; N, 24.41; S, 9.30%); (**5d**), 88%, m.p. 111^{0}C (Found: C, 55.08; H, 5.80; N, 24.37; S, 9.21. Calcd. for $C_{16}H_{20}N_6OS$: C, 55.81; H, 5.81; N, 24.41; S, 9.30%); (**5e**), 79%, m.p. 64^{0}C (Found: C, 49.11; H, 4.61; N, 22.88; S, 8.56. Calcd. for $C_{15}H_{17}N_6OSCl$: C, 49.38; H, 4.66; N, 23.04; S, 8.77%); (**5f**), 91%, m.p. 149^{0}C (Found: C, 48.87; H, 4.44; N, 22.93; S, 8.70. Calcd. for $C_{15}H_{17}N_6OSCl$: C, 49.38; H, 4.66; N, 23.04; S, 8.77%); (**5g**), 88%, m.p. 210^{0}C (Found: C, 49.34; H, 4.69; N, 23.07; S, 8.72. Calcd. for $C_{15}H_{17}N_6OSCl$: C, 49.38; H, 4.66; N, 23.04; S, 8.77%); (**5h**), 90%, m.p. 94^{0}C (Found: C, 50.05; H, 7.12; N, 26.95; S, 10.23. Calcd. for $C_{13}H_{22}N_6OS$: C, 50.32; H, 7.09; N, 27.09; S, 10.32%).

The compound (**5a**) was then reacted with N-phenyl-S-chloroisothiocarbamoyl chloride in chloroform medium to yield 2-(4,6-dimethyl-pyrimidin-2-yl-amino)-1-(3,6-diphenylimino-[1,2,4,5]-dithiadiazin-4-yl)-ethanone hydrochloride (**6a**). It on basification with dilute ammonium hydroxide solution afforded a free base 2-(4,6-dimethyl-pyrimidin-2-yl-amino)-1-(3,6-diphenylimino-[1,2,4,5]-dithiadiazin-4-yl)-ethanone (**7a**), 91%, m.p. 112^{0}C (Found: C, 55.96; H, 4.49; N, 20.91; S, 13.51. Calcd. for $C_{22}H_{21}N_7OS_2$: C, 57.01; H, 4.53; N, 21.16; S, 13.82%); IR: ν_{max}3412, 3309 (NH), 1718 (C=O), 1653 (C=N), 1311 (C-N), 1170 (N-N), 773 (C-S), 447 cm^{-1} (S-S); ^{1}H-NMR: δ (CDCl3+DMSO-*d*6): 6.89-7.76 (10H, m, Ar-H), 6.40 (1H, s, Pyrm-NH), 6.32 (1H, s, Pyrm-H), 3.64 (2H, s, CO-CH_2), 2.43 (1H, s, Dthdz-H), 2.19 (6H, s, Pyrm-CH_3); MS: m/z 448 (M^+-CH_3), 386 (M^+-C_6H_5), 341 (M^+-$(CH_3)_2.C_4HN_2.NH$), 327 (M^+-$(CH_3)_2.C_4HN_2.NH.CH_2$), 299 (M^+-$(CH_3)_2.C_4HN_2.NH.CH_2.CO$), 164 $(CH_3)_2.C_4HN_2.NH.CH_2.CO^+$), 136 $(CH_3)_2.C_4HN_2.NH.CH_2^+$), 122 $(CH_3)_2.C_4HN_2.NH^+$), 107 $(CH_3)_2.C_4HN_2^+$).

The intense peak at 1718 cm^{-1} in IR spectrum of compound (**7a**) confirmed the presence of C=O group. Absorptions in the regions 3420-3010 and 3010-2860 indicate the aromatic and aliphatic C-H stretching frequencies respectively. The appearance of sharp peaks at 3412 and 3309 cm^{-1} indicates the presence of two NH groups in molecular structure. The ^{1}H-NMR spectrum of compound (**7a**) showed signals at δ 6.40 and 2.43 which confirmed the presence of pyrimidine-NH and dithiadiazine-NH respectively. The mass spectrum of compound (**7a**) confirmed the formation of various fragment ions. The fragment ion with m/z 299 has 100% intensity.

The above reaction was extended to synthesize 1-(6-aryl/alkylimino-3-phenylimino-[1,2,4,5]-dithiadiazin-4-yl)-2-(4,6-dimethyl-pyrimidin-2-yl-amino)-ethanones (**7b-h**): (**7b**), 89%, m.p. 110^{0}C (Found: C, 57.22; H, 4.79; N, 20.18; S, 12.95. Calcd. for $C_{23}H_{23}N_7OS_2$: C, 57.86; H, 4.82; N, 20.54; S, 13.41%); IR: ν_{max} 3404, 3309 (NH), 1751 (C=O), 1647 (C=N), 1311 (C-N), 1170 (N-N), 775 (C-S), 447 cm^{-1} (S-S); ^{1}H-NMR: δ (CDCl3+DMSO-*d*6): 6.88-7.76 (9H, m, Ar-H), 6.33 (1H, s, Pyrm-NH), 6.30 (1H, s, Pyrm-H), 3.74 (2H, s, CO-CH_2), 2.43 (1H, s, Dthdz-H), 2.25 (3H, s, Ar-CH_3), 2.19 (6H, s, Pyrm-CH_3);(**7c**)), 92%, m.p. 122^{0}C (Found: C, 57.02; H, 4.84; N, 19.97; S, 13.36. Calcd. for $C_{23}H_{23}N_7OS_2$: C, 57.86; H, 4.82; N, 20.54; S, 13.41%); (**7d**), 90%, m.p. 103^{0}C (Found: C, 57.80; H, 4.61; N, 20.51; S, 13.12. Calcd. for $C_{23}H_{23}N_7OS_2$: C, 57.86; H, 4.82; N, 20.54; S, 13.41%); (**7e**), 89%, m.p. 94^{0}C (Found: C, 52.67; H, 3.89; N, 19.61; S, 12.85. Calcd. for $C_{22}H_{20}N_7OS_2Cl$: C, 53.06; H, 4.02; N, 19.69; S, 12.86%); (**7f**), 94%, m.p. 101^{0}C (Found: C, 53.10; H, 4.06; N, 19.71; S, 12.80. Calcd. for $C_{22}H_{20}N_7OS_2Cl$: C, 53.06; H, 4.02;

N, 19.69; S, 12.86%); (**7g**), 85%, m.p. 126^{0}C (Found: C, 52.99; H, 3.97; N, 19.59; S, 12.68. Calcd. for $C_{22}H_{20}N_7OS_2Cl$: C, 53.06; H, 4.02; N, 19.69; S, 12.86%); (**7h**), 85%, m.p. 114^{0}C (Found: C, 54.11; H, 5.50; N, 22.08; S, 14.21. Calcd. for $C_{20}H_{25}N_7OS_2$: C, 54.17; H, 5.64; N, 22.12; S, 14.44%) (**Scheme-1**).



Scheme-1

All these reactions were carried out by microwave irradiation. The products obtained were characterized by spectral method[15-17]. The elemental analysis[18] satisfied the structural properties of the synthesized compounds. To conclude, the chemistry of the reactions employed together with their chemical behaviour was discussed exhibiting the importance of novel molecular templates.

Antimicrobial activity

Various methods have been proposed and adopted for the measurement of antimicrobial activity[19]. In present antimicrobial study the newly synthesized compounds were screened for their antibacterial activity using Kirby-Baeur method[20-22]. Sensitivity plates were seeded with a bacterial inoculum of 1×10^6 ml^{-1} and each well diameter 10 mm was loaded with 0.1 ml of test compound solution (1000 µg ml^{-1}). The zones of inhibition were recorded after incubation for 24 hr at 37ºC, using Vernier caliper. The bacterial organisms used included both gram-positive as well as gram-negative strains. The medium used for the study of antibacterial activity of newly synthesized compounds was Hi-media Laboratories Pvt. Ltd., India make nutrient agar. It was of bacteristatic grade and found to be suitable for the growth of all bacterial strains used in the present study.

The antibacterial activity and inhibition effect of the compounds (**7a-h**) on the growth of various bacterial organisms is summarised in table given below along with the inhibition effect of standard drug streptomycin for comparison purpose. The compound (**7e**) was found to be highly sensitive (bactericidal) against the microorganisms *E. coli* and *P. vulgaris* whereas moderately sensitive against *S. typhi* and *B. subtilis*. Majority of the compounds were found to be moderately sensitive against *S. aureus* and slightly sensitive against *S. typhi*. Compound (**7h**) was

resistant against almost all the microorganisms. To determine the minimum inhibitory concentration (MIC), the serial dilution technique was followed using nutrient broth medium. The MIC[23] values of compounds (**7e**) against *E. coli* and *P. vulgaris* were found to be 80 and 75 µg ml^{-1} respectively .

Antibacterial activity of 1-(6-aryl/alkylimino-3-phenylimino-[1,2,4,5]- dithiadiazin-4-yl)-2-(4,6-dimethyl-pyrimidin-2-yl-amino)-ethanones (7a-h)

Compounds	Microorganisms				
	E. coli	*S. aureus*	*S. typhi*	*B. subtilis*	*P. vulgaris*
7a	S 12	S 17	S 12	S 15	R
7b	S 15	S 16	R	R	S 13
7c	S 11	S 14	S 14	S 17	R
7d	S 16	S 18	S 14	S 12	S 12
7e	S 23	S 18	S 19	S 18	S 23
7f	R	S 15	S 15	S 19	S 14
7g	S 18	S 19	S 13	S 19	S 17
7h	R	R	R	S 12	R
Streptomycin	S 23	S 20	S 22	S 18	S 13

(Concentration 100 µg/ml) (Diameter of inhibition zone in mm)

R (Resistant) : (11 mm and below)
S (Sensitive) (Bactericidal) : (11 mm above)
Slightly Sensitive : (11 mm above to 15 mm)
Moderately Sensitive : (15 mm above to 20 mm)
Highly Sensitive : (20 mm above)

Conclusion

In present work microwave irradiative synthesis of 1-(6-aryl/alkylimino-3-phenylimino-[1,2,4,5]-dithiadiazin-4-yl)-2-(4,6-dimethyl-pyrimidin-2-yl-amino)-ethanones has been reported. The compounds obtained were of good quality and purity with high % yield. Microwave assisted method applied for the synthesis is quite simple, efficient, fast, clean, economic and eco-friendly.

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