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Comparison of Conventional and Microwave Assisted Synthesis, Structural study and Antimicrobial screening of Substituted [1,2,4,5]-Tetrazines

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Abstract

A series of 5,6-diaryl-[1,2,3,4]-tetrazines **4** have been obtained by microwave irradiation followed by the basification of their dihydroiodides **3** with dilute ammonium hydroxide solution. These compounds were synthesized also by conventional heating for comparison. The latter were synthesized by the oxidative cyclization of 1,2-diaryl-2-hydrazino-ethylidine amines **2** using ethanolic iodine. The parent compounds **2** were prepared by refluxing the mixtures of substituted benzils **1** and hydrazine hydrate in *n*-propanol for 60 hr. Compounds **4** on acylation with acetic anhydride in 1:2 ratio afforded 2,3-diacetyl derivatives **5** and on reaction with sodium nitrite in 1:2 ratio in acidic medium afforded 2,5-dinitroso derivatives **6**. The structures of synthesized compounds were established on the basis of chemical transformation, elemental analysis, IR, ^{1}H-NMR and Mass spectral studies. The title compounds have been assayed for their antimicrobial activity against gram-positive as well as gram-negative microorganisms.

Keywords : [1,2,4,5]-Tetrazines, synthesis, structural study, antimicrobial screening

Introduction

Microwave-assisted synthesis is a branch of green chemistry. Microwave-assisted synthesis has gained much attention in recent years. Microwave irradiation-assisted chemical transformations are pollution free, eco-friendly and offer high yields together with simplicity in processing and handling.

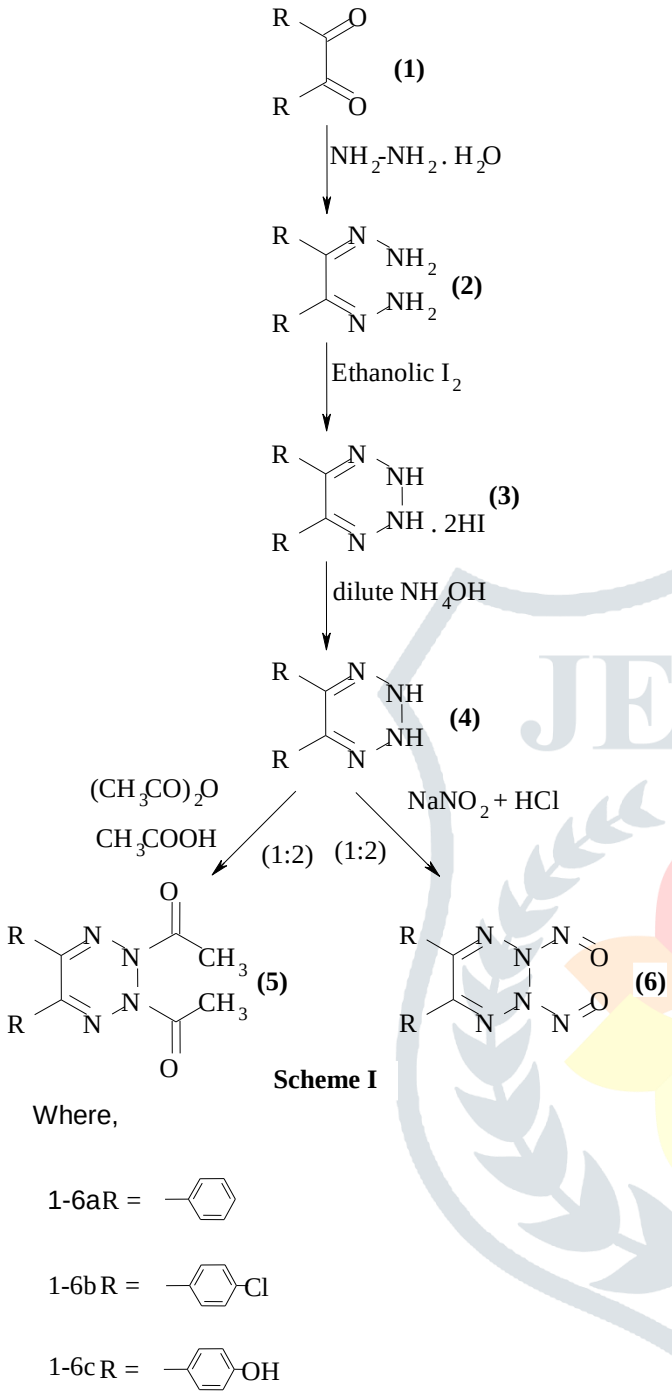
Literature survey made on [1,2,4,5]- tetrazines showed that 3,6-symetrically distributed [1,2,4,5]-tetrazines are obtained from the treatment of nitrile derivatives with hydrazine or aldehyde with substituted hydrazines[1-2]. Besides this various other routes[3-9] have been reported. Synthesis of [1,2,4,5]-tetrazines with interaction of substituted phenacyl-pyridinium bromide and aromatic diazonium salt solution have been reported earlier[10]. In present work synthesis of 5,6-diaryl-[1,2,3,4]-tetrazines using substituted benzils and iodine is reported.

Results and Discussion

The parent compounds, 1,2-diaryl-2-hydrazino-ethylidine amines **2a-c** were prepared by refluxing the mixture of substituted benzils **1a-c**, (0.01 mole) and hydrazine hydrate (0.02 mole) in *n*-propanol (20 mL) on a water bath for 60 hr. Separately, pastes of compounds **2a-c** were prepared in ethanol and ethanolic iodine solution was added drop by drop to these paste with constant stirring. The colour of iodine was initially disappeared. The addition was continued till violet colour of iodine persisted. The mixtures were left overnight at room temperature. The separated solids were found to be acidic to litmus and on determination of equivalent weights, identified as 5,6-diaryl-[1,2,3,4]-tetrazine dihydroiodides **3a-c**. These compounds were basified with dilute ammonium hydroxide solution to yield free bases **4a-c**. Free bases **4a-c** on acylation with acetic anhydride in 1:2 ratio using glacial acetic acid as a solvent afforded 2,3-diacetyl derivatives **5a-c** and on reaction with sodium nitrite in 1:2 ratio in acidic medium afforded 2,5-dinitroso derivatives **6a-c** (Scheme I).

Antimicrobial activity

The synthesized compounds **4a-c** were screened for their antibacterial activity using cup plate diffusion method.[11,12] The bacterial organisms used included both gram-positive as well as gram-negative strains like *E. coli*, *S. aureus*, *S. typhi*, *B. subtilis* and *A. aerogenes*. Sensitivity plates were seeded with a bacterial innoculum of $1x10^6$ CIU/mL and each well (diameter 10 mm) was loaded with 0.1 mL of test compound solution (1000 µg/mL) in DMF, so that concentration of each test compound was 100 µg/mL. The zones of inhibition were recorded after incubation for 24 hr at 37^0C, using Vernier caliper. Inhibition zone record of the compounds clearly indicated that **4b** and **4c** were highly active against *B. subtilis* and *S. typhi* and moderately active against *E. coli*. Majority of the compounds were found inactive against *S. aureus* and *A. aerogenes*.



Scheme I

Where,

1-6a R =

1-6b R =

1-6c R =

TABLE1: YIELDS, M**ELTING** POINTS AND TOTAL REACTION TIME FOR SYNTHESISED [1,2,4,5]-Tetrazines (For all synthesis 180 w, MW was used)

Compound	**% Yield**		**MP (O c)**		**Total Reaction Time**	
	Conventional	**MW**	**Conventional**	**MW**	**Conventional**	**MW**
1-6a	78	80	240	239	2h.00 min.	4min. 30 s.

1-6b	70	75	246	247	2h.15 min.	5min. 20 s.
1-6c	65	70	248	246	2h.10 min.	4min. 40 s.

Experimental Section

The melting points of all synthesized compounds were recorded using hot paraffin-bath and are uncorrected. Chemicals used were of AR grade. ^{1}H NMR spectra were recorded on VARIAN, USA Mercury Plus 300 MHz spectrometer with TMS as internal standard using $CDCl_3$ and DMSO-d_6 as solvents. IR spectra were recorded on MAGNA, USA 550 spectrophotometer in the frequency range 4000-50 cm^{-1}. Mass spectra were recorded on VARIAN, USA 410 Prostar Binary LC with 500 MS IT PDA Detector. Purity of the compounds was checked on silica gel-G plates by TLC.

Synthesis of 1,2-diphenyl-2-hydrazino-ethylidine amine 2a.

The compound 1,2-diphenyl-2-hydrazino-ethylidine amine **2a** was prepared by refluxing the mixture of benzil **1,** (0.01 mole) and hydrazine hydrate (0.02 mole) in *n*-propanol (20 mL) on a water bath for 60 hr. The reaction mixture was cooled. The resulting solid was washed with cold ethanol and crystallized from chloroform, **2a** (80%), m.p.150^{0}C.

Synthesis of 5,6-diphenyl-[1,2,3,4]-tetrazine 4a.

Paste of compound **2a** was prepared in ethanol and ethanolic iodine solution was added drop by drop to this paste with constant stirring. The colour of iodine was initially disappeared. The addition was continued till violet colour of iodine persisted. The mixture was left overnight at room temperature. The separated solid was found to be acidic to litmus and on determination of equivalent weight, identified as 5,6-diphenyl-[1,2,3,4]-tetrazine dihydroiodide **3a**. Similarly, other compounds, **3b-c** were prepared.

On basification of **3a** with dilute ammonia solution a free base **4a** was obtained, it was crystallised from aqueous ethanol, m.p. 186^{0}C (Found: C, 70.80; H, 4.80; N, 23.19. Calcd. for $C_{14}H_{12}N_4$: C, 71.18; H, 5.08; N, 23.727%); IR: 3353 (NH),1566 (C=N), 1228 cm^{-1} (N-N)[13,14]; ^{1}H NMR ($CDCl_3$+DMSO-d_6): 7.24-7.57 (10H, m,

Ar-H), 5.74 (2H, s, NH); MS: m/z 236 (M^+), 234 (M^+-2H). Similarly, free base **4b** was prepared from **3b**: **4b**, m.p. 240^{0}C (Found: C, 54.80; H, 3.05; N, 18.19. Calcd. for $C_{14}H_{10}N_4Cl_2$: C, 55.08; H, 3.27; N, 18.36%); IR: 3353 (NH), 1587 (C=N), 1220 cm^{-1} (N-N); ^{1}H NMR ($CDCl_3$+DMSO-d_6): 7.24-7.57 (10H, m, Ar-H), 5.74 (2H, s, NH); MS: m/z 305 (M^+), 234 (M^+-2Cl), 193 (M^+-C_6H_4Cl). This reaction was extended to synthesize the free base, **4c**: m.p. 224^{0}C (Found: C, 61.99; H, 4.18; N, 20.70. Calcd. for $C_{14}H_{12}N_4O_2$: C, 62.68; H, 4.47; N, 20.89%).

Synthesis of 2,3-diacetyl-5,6-diphenyl-[1,2,3,4]-tetrazine 5a.

A mixture of 5,6-diphenyl-[1,2,3,4]- tetrazine **4a,** (0.01 mole) and acetic anhydride (0.02 mole) in glacial acetic acid (10 mL) was refluxed for 2 hr. The reaction mixture was cooled and poured on a little crushed ice with water to give **5a**. It was crystallized from aqueous ethanol, m.p. 249^{0}C (Found: C, 67.10; H, 4.89; N, 17.32. Calcd. for $C_{18}H_{16}N_4O_2$: C, 67.50; H, 5.00; N, 17.50%); IR: 1683 (C=O), 1527 (C=N), 1305 (C-N), 1220 cm^{-1} (N-N); ^{1}H NMR ($CDCl_3$+DMSO-d_6): 7.24-7.64 (10H, m, Ar-H), 2.45 (6H, s, CO-CH_3); MS: m/z 277 (M^+-$COCH_3$), 234 (M^+-2$COCH_3$), 206 (M^+-2$NCOCH_3$). This reaction was extended to synthesize other diacetyl derivatives, **5b-c**: **5b**, m.p. 251^{0}C (Found: C, 55.10; H, 3.05; N, 14.19. Calcd. for $C_{16}H_{14}N_4O_2Cl_2$: C, 55.52; H, 3.59; N, 14.39%); **5c**, m.p. 242^{0}C (Found: C, 61.10; H, 4.18; N, 15.80. Calcd. for $C_{16}H_{16}N_4O_4$: C, 61.36; H, 4.54; N, 15.90%) .

Synthesis of 2,3-dinitroso-5,6-diphenyl-[1,2,3,4]-tetrazine 6a.

A solution of 5,6-diphenyl-[1,2,3,4]-tetrazine **4a**, (0.01 mole) in 10 mL of ethanol was cooled below 5°C and a solution of sodium nitrite (0.02 mole) in 2.5 mL of concentrated hydrochloric acid and 2.5 mL of water was added to it and allowed to stand for 30 minutes. A greenish yellow solid was precipitated out **6a** and crystallized from ethanol-acetone mixture, m.p. 240°C (Found: C, 56.96; H, 3.23; N, 28.32. Calcd. for $C_{14}H_{10}N_6O_2$: C, 57.14; H, 3.40; N, 28.57%); IR: 1590 (N=O), 1588 (C=N), 1205 cm^{-1} (N-N); ^{1}H NMR ($CDCl_3$+DMSO-d_6): 7.24-7.62 (10H, m, Ar-H); MS: m/z 294 (M^+), 264 (M^+-NO), 234 (M^+-2NO). This reaction was extended to synthesize other dinitroso derivatives, **6b-c**: **6b**, m.p. 246^{0}C (Found: C, 46.10; H, 2.12; N, 22.96. Calcd. for $C_{14}H_8N_6O_2Cl_2$: C, 46.28; H, 2.20; N, 23.14%); **5c**, m.p. 241^{0}C (Found: C, 50.99; H, 2.98; N, 25.11. Calcd. for $C_{14}H_{10}N_6O_4$: C, 51.53; H, 3.06; N, 25.76%) .

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