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An Easy Procedure for Synthesis of 1,3,4-Oxadiazines: A Potential Antimicrobial Agents

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An efficient and accessible procedure for the synthesis of 1,3,4-oxadiazines was developed. Reaction involves the cyclocondensation of phenylhydrazones catalyzed by a mild base triethylamine to produce 1,3,4-oxadiazines in good yields. The synthesized new compounds were characterized by spectral studies and elemental analyses and were screened to explore *in vitro* antimicrobial activity against bacteria and fungi species. The compounds displayed good to excellent potency against tested microorganisms, in particular, compound with chloro substitution showed good antimicrobial potential.

Keywords: Antimicrobial, Hydrazone, Phosgene, Triethylamine.

INTRODUCTION

Six membered heterocyclic compounds with three hetero atoms are less scarcity in nature, but they possess good applications in most of the fields such as medical [1], agriculture as insecticides [2] and industries as adhesives [3]. Oxadiazines contains oxygen and two nitrogen atoms and were considered as interesting heterocycles for their varied biological activities. A diversity of biological effects is associated with hetero atoms at 1,2,4 or 1,3,4 positions, since they are oxa-analogues of nucleosides-6-oxadihydro uracil, among these 1,3,4-oxadiazines were the most important frameworks for a variety of bioactive molecules [4]. First representatives of 4*H*-1,3,4-oxadiazine-5,6-diones were synthesized in the first half of the 20th century by reaction of oxalyl chloride with *N*-substituted benzohydrazides and acetohydrazides [5-7].

Zen and Harada [8] reported that the synthesis of oxadiazine by the reaction of di-ketones with aliphatic nitro compounds in the presence of acetyl chloride through one-step synthesis. Oxadiazine derivatives have been demonstrated to be important scaffolds with promising therapeutic potential, which present wide activities such as antimicrobial [9], antitumor [10], anticonvulsant [11], COX-1 inhibitors [12], anti-HIV [13], *etc*. In addition, these are useful intermediates in the synthesis of prodrugs or β-lactam antibiotics, particularly the synthesis of carbapenems and penems [14]. In view of broad spectrum of applications associated with oxadiazines, we herein report the synthesis of fused 1,3,4-oxadiazines and their antimicrobial potentialities.

EXPERIMENTAL

Melting points were determined by an open capillary tube method and are uncorrected. Purity of the compounds was checked on thin layer chromatography (TLC) plates pre-coated with silica gel using solvent system ethyl acetate: *n*-hexane (1:4 v/v). The spots were visualized under UV light. The ^{1}H NMR and ^{13}C spectra were recorded on a Spect 500 MHz and 125.6 MHz spectrometer respectively, using DMSO as solvent and TMS as internal standard. The chemical shifts are expressed in δ ppm. Mass spectra were obtained on Shimadzu LCMS-2010A spectrometer (ESI). Elemental analyses were obtained on a Thermo Finnigan Flash EA 1112 CHN analyzer. Purification of compounds was done by column chromatography on silica gel (70:230 mesh Merck).

Synthesis of 2-chlorophenyl chloroformate (1): To a cold and stirred solution of phosgene (2.955 g, 0.01 mol) in dichloromethane (20 mL), a solution of 2-chlorophenol (1 g, 0.01 mol) in dichloromethane (10 mL) was added drop-wise. Then a solution of sodium hydroxide (0.4 g, 0.01 mol) in water (72 mL) was added. During addition, the temperature was maintained at 0-5 °C. Then the temperature of the reaction mixture was allowed to slowly rise to room temperature and stirred for 2 h. After completion of reaction the mixture was extracted in to ether, washed with cold water (3 × 50 mL), dried over anhydrous sodium sulphate to obtain 2-chlorophenyl chloroformate in 80 % yield.

General procedure for synthesis of hydrazones (3a-f): To a solution of 2-chlorophenyl chloroformate (0.01 mol) in

dichloromethane (15 mL), a solution of phenylhydrazine hydrochlorides (**2a-f**) (0.01 mol) in dichloromethane (10 mL) and pyridine (7-8 drops) were added. Then the mixture was stirred at room temperature for 2-3 h. The progress of the reaction was monitored by TLC. After completion, the reaction mixture was poured in to ice cold water, solids separated were filtered, washed successively with ice cold hydrochloric acid (5 %) and water and dried to obtain the hydrazones (**3a-f**).

General procedure for the synthesis of 1,3,4-oxadiazines (4a-f): To a stirred solution of hydrazones (**3a-f**, 0.01 mol) in dichloromethane (10 mL), a solution of triethylamine (2 mL) in dichloromethane (20 mL) was added. The mixture was refluxed for 4-5 h at 95-100 °C. The progress of the reaction was monitored by TLC, after completion, the reaction mass was diluted with 20 mL dichloromethane and washed with water; the organic layer was dried over anhydrous sodium sulphate. The solvent was evaporated *in vacuo* to obtain the crude products and were purified column chromatography using silica gel (60-120 mesh) and ethyl acetate:hexane (1:4 v/v) as eluent (**Scheme-I**).

1,2-Dihydro-1-phenylylbenzo[e][1,3,4]oxadiazine-3-one (4a): Yield 82 %, m.p. 141-142 °C; ^{1}H NMR: 6.263 (s, 1H, NH), 7.218-8.152 (m, 9H, Ar-H); ^{13}C NMR: 118.2 (2C), 118.7 (1C), 119.3 (2C), 119.5 (1C), 121.8 (1C), 127.3 (2C), 135.5 (1C), 140.4 (1C), 145.9 (1C), 156.6 (1C); MS *m/z*: 226 (M^+); Anal. calcd. (%) for $C_{13}H_{10}N_2O_2$: C, 69.02; H, 4.46; N, 12.38; Found (%): C, 69.23; H, 4.28; N, 12.57.

1,2-Dihydro-1-*p*-tolylbenzo[e][1,3,4]oxadiazine-3-one (4b): Yield 80 %, m.p. 126-127 °C; ^{1}H NMR: 1.274 (s, 3H, CH_3), 6.286 (s, 1H, NH), 7.243-8.189 (m, 8H, Ar-H); ^{13}C NMR: 22.4 (1C), 118.8 (2C), 119.4 (2C), 119.8 (1C), 121.8 (1C), 127.8 (1C), 130.1 (2C), 135.8 (1C), 140.3 (1C), 144.5 (1C), 156.8 (1C); MS *m/z*: 240 (M^+); Anal. calcd. (%) for $C_{14}H_{12}N_2O_2$: C, 69.99; H, 5.03; N, 11.66; Found (%): C, 69.85; H, 5.17; N, 11.81.

1-(4-Chlorophenyl)-1,2-dihydrobenzo[e][1,3,4]oxadiazine-3-one (4c): Yield 75 %, m.p. 158-159 °C; ^{1}H NMR: 6.112 (s, 1H, NH), 7.326-8.213 (m, 8H, Ar-H); ^{13}C NMR: 119.2 (2C), 120.3 (2C), 122.6 (1C), 123.8 (1C), 126.7 (1C), 129.5 (2C), 136.5 (1C), 140.1 (1C), 143.9 (1C), 157.1 (1C); MS *m/z*: 260 (M^+), 262 (M+2); Anal. calcd. (%) for $C_{13}H_9N_2O_2Cl$: C, 59.90; H, 3.48; N, 10.75; Found (%): C, 59.75; H, 3.69; N, 10.94.

1-(4-Fluorophenyl)-1,2-dihydrobenzo[e][1,3,4]oxadiazine-3-one (4d): Yield 75 %, m.p. 130-131 °C; ^{1}H NMR: 6.119 (s, 1H, NH), 7.106-8.121 (m, 8H, Ar-H); ^{13}C NMR: 117.4 (2C), 119.2 (2C), 120.2 (2C), 122.3 (1C), 125.1 (1C), 134.8 (1C), 140.6 (1C), 141.8 (1C), 148.3 (1C), 159.7 (1C); MS *m/z*: 244 (M^+); Anal. calcd. (%) for $C_{13}H_9N_2O_2F$: C, 63.93; H, 3.71; N, 11.47; Found (%): C, 63.75; H, 3.88; N, 11.63.

1,2-Dihydro-1-(4-methoxyphenyl)benzo[e][1,3,4]-oxadiazine-3-one (4e): Yield 76 %, m.p. 145-146 °C; ^{1}H NMR: 2.725 (s, 3H, OCH_3), 6.115 (s, 1H, NH), 7.112-8.065 (m, 8H, Ar-H); ^{13}C NMR: 55.6 (1C), 116.9 (2C), 119.8 (2C), 120.6 (2C), 121.8 (1C), 124.6 (1C), 135.9 (1C), 139.7 (1C), 141.6 (1C), 150.1 (1C), 159.8 (1C); MS *m/z*: 256 (M^+); Anal. calcd. (%) for $C_{14}H_{12}N_2O_3$: C, 65.62; H, 4.72; N, 10.93; Found (%): C, 65.88; H, 4.56; N, 10.71.

1,2-Dihydro-1-(2,4-dimethylphenyl)benzo[e][1,3,4]-oxadiazine-3-one (4f): Yield 78 %, m.p. 158-159 °C; ^{1}H NMR: 1.983 (s, 6H, CH_3), 6.128 (s, 1H, NH), 7.115-8.123 (m, 7H, Ar-H); ^{13}C NMR: 15.6 (1C), 23.8 (1C), 118.6 (2C), 119.2 (1C), 121.8 (1C), 124.8 (1C), 126.2 (1C), 127.1 (1C), 127.9 (1C), 128.5 (1C), 135.9 (1C), 140.3 (1C), 141.8 (1C), 160.5 (1C); MS *m/z*: 254 (M^+); Anal. calcd. (%) for $C_{15}H_{14}N_2O_2$: C, 70.85; H, 5.55; N, 11.02; Found (%): C, 71.02; H, 5.37; N, 11.24.

RESULTS AND DISCUSSION

The schematic diagram for the synthesis of target 1,3,4-oxadiazines is outlined in Fig. 1. The synthetic strategy involves the preparation of hydrazones (**3a-f**) by the reaction of substituted phenylhydrazine hydrochloride (**2a-f**) with 2-chlorophenyl chloroformate (**1**). Then, the reaction of hydrazones (**3a-f**) in the presence of triethylamine to leading to the formation of 1,3,4-oxadiazines (**4a-f**).

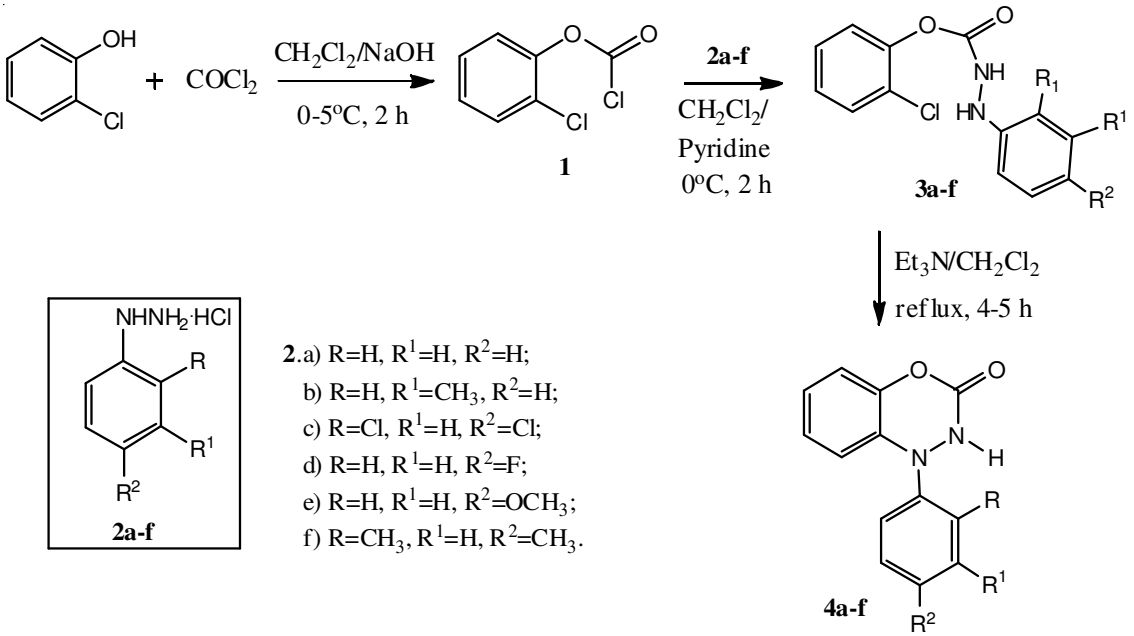

Fig. 1. Schematic diagram for the synthesis of 1,3,4-oxadiazines (**4a-f**)

TABLE-1
MIC'S OF THE TEST COMPOUNDS **4a-f** AGAINST BACTERIAL AND FUNGAL SPECIES

Compound	Minimum inhibitory concentration (MIC's) (µg/mL)*					
	S. aureus	*E. coli*	*P. aeruginosa*	*A. niger*	*A. flavus*	*C. albicans*
4a	25	25	50	25	25	50
4b	25	12.5	25	25	25	25
4c	50	25	25	25	25	50
4d	25	12.5	12.5	12.5	25	25
4e	12.5	12.5	12.5	12.5	25	25
4f	50	75	100	75	75	–
Ciprofloxacin	25	12.5	12.5	–	–	–
Nystatin	–	–	–	12.5	25	25

*The results are expressed as mean of three determinations (n = 3).

The synthesized new compounds were characterized by spectral analysis and evaluated for their *in vitro* antimicrobial activities. In ^{1}H NMR spectra, compounds **4a-f** showed the signals in the region δ 6.236-6.314 ppm, which were assigned to NH protons and signals in the region δ 7.438-8.528 ppm corresponds to aromatic protons. In ^{13}C NMR spectra, the C-O and C-N carbons of **4a-f** appeared in the region δ 136.62-138.45 and δ 141.42-143.63 ppm. While, C=O carbons of appeared in the region δ 178.64-180.92. Further, all compounds signals due to aromatic, substituent protons and carbons in the expected region. The synthesized new molecules showed M+1 ion peak as a base peak in their mass spectra. Further, the analytical data obtained for the compounds **4a-f** were in good agreement with theoretically calculated data. All these spectral and analytical results confirmed the formation of the products.

Antimicrobial activity: Microbial studies of synthesized compounds were assessed by minimum inhibitory concentration (MIC) by serial dilution method [15,16]. The compounds were screened for their antimicrobial activities against Gram-negative bacteria species *Escherichia coli, Pseudomonas aeruginosa,* Gram-positive bacteria *Staphylococcus aureus,* fungi species *Aspergillus niger, Aspergillus flavus* and *Candila albicans.* All the experiments were carried out in triplicate. The ciprofloxacin and nystatin were used as standards for antibacterial and antifungal studies respectively. The results of MIC's are depicted in Table-1.

The synthesized 1,3,4-oxadiazines exerted a wide range of modest to good *in vitro* antibacterial activity against the tested organisms. The compounds **4a** having no substitution and **4b** with methoxy substitutions showed moderate activity against the tested organisms. Compounds **4c** having CH_3 substituent showed moderate activity. It has been interesting from the results of the study that chloro and fluoro substitution in the synthesized compounds enhanced the activity to the greater extent. Compounds **4d** and **4e** demonstrated excellent activity against all organisms tested. Nitro substitution present in compound **4f** retarded the inhibitory effect against the organisms tested.

Conclusion

The simple easy accessible procedure for the synthesis of 1,3,4-oxadiazines and their *in vitro* antimicrobial activity results revealed the significance of the study. All the newly synthesized compounds exhibited moderate to good antimicrobial activity against the tested microorganisms, compounds having chloro and fluoro substituents demonstrated potent antimicrobial activities. The compound, particularly **4e** exhibited greater activity in comparison to the standard drug. The SAR study of the synthesized compounds remains the topic of interest.

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