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SHORT COMMUNICATION

Microwave synthesis and antibacterial activities of some 2-amino-6-(phenylthio)pyridine-3,5-dicarbonitrile derivatives containing 1,3,4-oxadiazole moiety

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ABSTRACT

5-(4-Aminophenyl)-2-thiol-1,3,4-oxadiazole **(3)** was synthesized *via* the reaction of carbon disulfide with 4-aminobenzoyl hydrazide in presence of potassium hydroxide in absolute ethanol. Compound **3** was converted to the corresponding diazonium salt which was introduced in coupling reaction with alkaline solution of 2-hydroxybenzaldehyde as coupling reagent to give azooxadiazole derivative (**4)** containing aldehyde group. The resulting aldehyde (**4)** was then introduced in condensation reactions with the aromatic Thiophenol derivatives including using microwave irradiation technique in absolute ethanol to produce ten 2-amino-4-(2-hydroxy-5-((4-(5-mercapto-1,3,4-oxadiazol-2-yl)phenyl)diazenyl)phenyl)-6-(phenylthio)pyridine-3,5-dicarbonitrile derivatives (**5a-j**), respectively *in vitro* Antibacterial activity of the target compounds were investigated using two types of bacteria, *Staphylococcus aureus* (Gram-positive) and *Escherichia coli* (Gram-negative). The results indicated that the newly synthesized 2-amino-4-(2-hydroxy-5-((4-(5-mercapto-1,3,4-oxadiazol-2-yl)phenyl) diazenyl)phenyl)-6-(phenylthio)pyridine-3,5-dicarbonitrile derivatives (compounds **5d** and **5f**) showed enhanced activity against Gram-negative bacteria when compared with that of the control drug (gentamycin).

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***Keywords*:** oxadiazole, pyridine, antibacterial

1. INTRODUCTION

MCRs constitute an especially attractive synthetic strategy since they provide easy and rapid access to large libraries of organic compounds with diverse substitution patterns[1-3]. As MCRs are one-pot reactions, they are easier to carry out than multi step syntheses. The developing of new MCRs and improving known multi-component reactions are an area of considerable current interest. One such reaction is the synthesis of pyridine. Coupled with high throughput library screening, this strategy was an important development in the drug discovery in the context of rapid identification and optimization of biologically active lead compounds. Among them, 2-amino-3,5-dicarbonitrile-6-sulfanylpyridines exhibit various pharmacological activities and are useful as anti-hepatitis B virus[4], antiprion[5], antibacterial[6], anti-cancer agents[7] and as potassium channel openers for treatment of urinary incontinence[8]. Moreover, some of these compounds were found to be highly selective ligands for adenosine receptors[9], implicated Parkinson's disease, hypoxia/ischemia, asthma, kidney disease, and epilepsy [10].

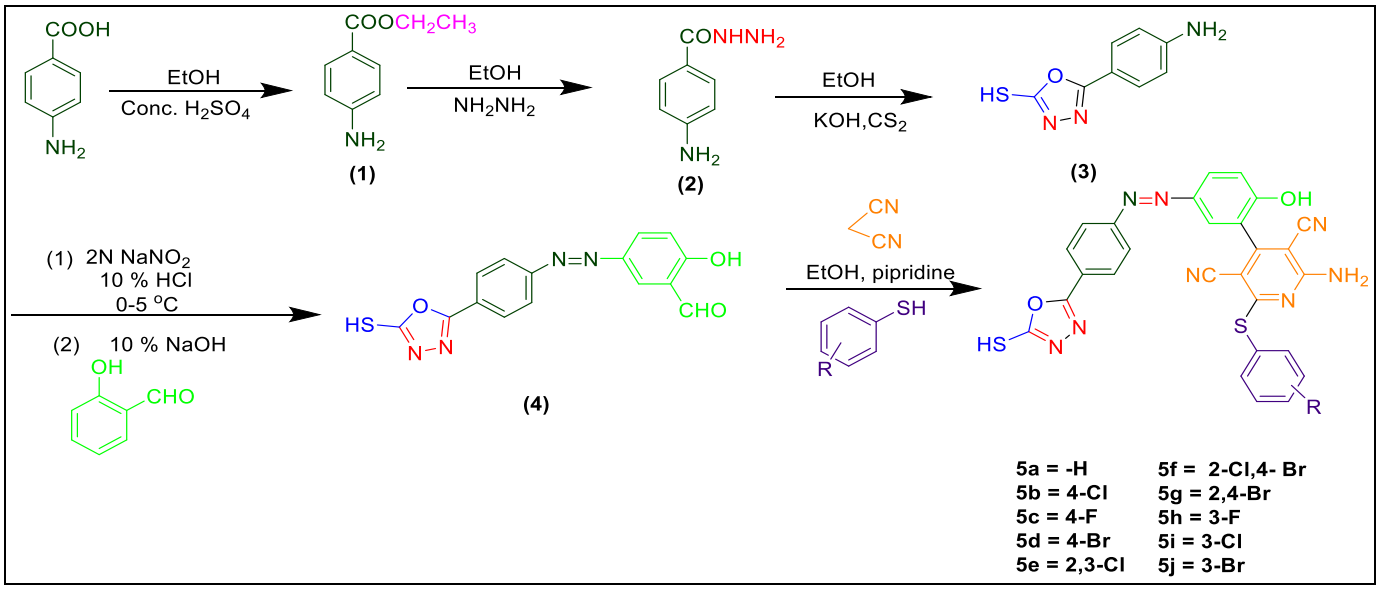
A three-component condensation of aldehyde **(4)**, malononitrile, and thiophenol is one of the most prominent existing procedures used for the synthesis of 2-amino-3,5-dicarbonitrile-6-thiopyridines. Generally, this condensation has been carried out under basic conditions using various bases such as, Et_3N, DABCO, piperidine[11], morpholine, thiomorpholine, pyrrolidine, N,N-DIPEA, pyridine, 2,4,6-collidine, DMAPand DBU[12,13]. Moreover, basic ionic liquid 1-methyl-3-butylimidazolium Lewis hydroxide, that is [bmim] OH[14] and using a variety of Lewis acids such $ZnCl_2$, $AlCl_3$, $FeCl_3$, I_2, Cu $(OTf)_3$, $InCl_3$, and $BF_3{\cdot}Et_2O$[15].

However, most of these methods suffer by the formation of inevitable side products, which results in lower yield of desired product with long reaction time. Keeping the medicinal values of pyridine-3, 5-dicarbonitriles in mind, we considered it necessary to develop an efficient high yielding synthetic protocol for the synthesis of this class of compounds.

2. EXPERIMENTAL

4-Aminobenzoic hydrazide was converted to 5-(4-aminophenyl)-2-thiol-1,3,4-oxadiazole (**3**) by treating it with carbon disulfide in presence of potassium hydroxide as catalyst in absolute ethanol. Diazotization of amino group in compound **3** using sodium nitrite and hydrochloric acid generated the corresponding diazonium salt which was directly introduced in coupling reaction with 2-hydroxybenzaldehyde dissolved in sodium hydroxide solution to give azo-oxadiazole derivative **4** containing aldehyde group[28]. Aldehyde group of 2-hydroxy-5-((4-(5-mercapto-1,3,4-oxadiazol-2-yl)phenyl)diazenyl)benzaldehyde **4** was condensed with the thiophenol including using microwave irradiation in absolute ethanol to produce ten 2-amino-6-(phenylthio) pyridine-3,5-dicarbonitrile derivatives of 1,3,4-oxadiazole **5a-j** respectively, as the platforms for this work (**Scheme 1).**

The chemical structures of the target compounds synthesized were confirmed from IR, ^1H NMR, ^{13}C NMR and MS spectra analysis and were in good agreement with the proposed structures.



Scheme 1.

2. 1. Biological evaluation

Antifungal activity: all synthesize compound of 2-amino-4-(2-hydroxy-5-((4-(5-mercapto-1,3,4-oxadiazol-2-yl)phenyl)diazenyl)phenyl)-6-(phenylthio)pyridine-3,5-dicarbonitrile derivatives (5a-j) were evaluation for their antifungal activity by measuring minimum inhibitory concentration (µg/mL).the results of antifungal activities (Table 1) indicated that most of the target compounds 5a, 5b, 5c, 5d, 5f, 5h, 5i and 5j display very potent antifungal activity against *C.* albicans and *A. niger*, while Compound 5a,5i and 5j show more potent activity better than Nystatin, Greseofulvin, Ciprofloxacin, Chloramphenicol.

Antibacterial activity: Based on the antimicrobial results for all the synthesized 2-amino-4-(2-hydroxy-5-((4-(5-mercapto-1,3,4-oxadiazol-2-yl)phenyl)diazenyl)phenyl)-6-(phenylthio) pyridine-3,5-dicarbonitrile derivatives 5a-j only compound 5a, 5b, 5h and 5i exhibited good antibacterial activity (Table 1) againstthe various gram positive (*S. aureus*, *S. pyogenes*) and gram negative (*E. coli*, *P. aeruginosa*) bacterial strains and MIC values were comparable to those observed against the standard drugs Nystatin, Greseofulvin, Ciprofloxacin, Chloramphenicol.

2. 2. Experimental section

Thin-layer chromatography was accomplished on 0.2-mm precoated plates of silica gel G60 F254 (Merck). Visualization was made with UV light (254 and 365 nm) or with an iodine vapor. IR spectra were recorded on a FTIR-8400 spectrophotometer using DRS prob. ^{1}H (400 MHz), ^{13}C (100 MHz) NMR spectra were recorded on a Bruker AVANCE II spectrometer in $CDCl_3$ and DMSO. Chemical shifts are expressed in δ ppm downfield from TMS as an internal standard. Mass spectra were determined using direct inlet probe on a GCMS-QP 2010 mass

spectrometer (Shimadzu). Solvents were evaporated with a BUCHI rotary evaporator. Melting points were measured in open capillaries and are uncorrected.

Table 1. Antibacterial/fungal activity table [microgramme/ml]

Compounds and standard drugs	Antibacterial activity				Antifungal acitivity	
	Minimum inhibitory concentration µg/ml				Minimum inhibitory concentration µg/ml	
	Gram +Ve Bacteria		Gram -Ve Bacteria			
	S. aureus	***S. pyogenes***	***E. coli***	***P. aeruginosa***	***C. albicans***	***A. niger***
(5a)	15.62	62.5	**15.625**	**15.625**	**15.625**	**31.25**
(5b)	31.25	15.625	**15.625**	**7.82**	62.5	**31.25**
(5c)	62.5	31.25	31.25	31.25	**15.625**	62.5
(5d)	15.62	62.5	31.25	62.5	**15.625**	62.5
(5e)	31.25	**7.82**	15.625	62.5	62.5	62.5
(5f)	7.81	31.25	**7.82**	62.5	**31.25**	62.5
(5g)	15.625	15.625	**15.625**	62.5	**15.625**	62.5
(5h)	**7.82**	62.5	31.25	31.25	**31.25**	62.5
(5i)	31.25	31.25	**15.625**	**15.625**	**15.625**	**15.625**
(5j)	15.62	31.25	31.25	**15.625**	**15.625**	**15.625**
Nystatin	-	-	-	-	31.25	31.25
Greseofulvin					15.625	15.625
Ciprofloxacin	7.8	7.8	15.625	15.625	-	-
Chloramphenicol	7.8	7.8	7.8	7.8	-	-

Synthetic procedure of ethyl 4-aminobenzoate (1)

A mixture of p-amino benzoic acid (1 mmol) and ethanol (3 mmol) were refluxed in 60 ml for 4 hours.the resultant mixture was concentrated, cool and poured into ice cold water.

The solid mass thus separated out was dried.

Synthetic procedure of 4-aminobenzohydrazide (2)

A mixture of ethyl 4-aminobenzoate (1 mmol) and hydrazine hydrate (2 mmol) were refluxed in ethanol for 7 hours. The resultant mixture was concentrated, cooled and poured into crushed ice. The solid mass thus seperated out was dried and recrystallized from ethanol.

Synthetic procedure of 5-(4-aminophenyl)-1,3,4-oxadiazole-2-thiol (3)

A mixture of 4-aminobenzohydrazide **(2)** (1 mmol) of KOH and 10ml of CS_2 were refluxed in 50ml of 95% ethanol for 7-8 hours. The resultant mixture was concentrated and cooled to room temperature, acidified with dil. HCl. and the crude product was filtered and recrystallized from ethanol.

Synthetic procedure of 2-hydroxy-5-((4-(5-mercapto-1,3,4-oxadiazol-2-yl)phenyl) diazenyl) benzaldehyde (4)

A mixture of 5-(4-aminophenyl)-1,3,4-oxadiazole-2-thiol **(3) (**1 mmol) and sodium nitrite (1.5 mmol)) were mixed in acidic media to for diazonium chloride salt in situ. Which was immediately treated with 10 % NaOH solution along with salysaldehyde (1 mmol) to give intermediate 4.

Synthetic procedure of 2-amino-4-(2-hydroxy-5-((4-(5-mercapto-1,3,4-oxadiazol-2-yl)phenyl)diazenyl)phenyl)-6-(phenylthio)pyridine-3,5-dicarbonitrile (5a-j)

2-amino-4-(2-hydroxy-5-((4-(5-mercapto-1,3,4-oxadiazol-2-yl)phenyl)diazenyl)phenyl)-6-(phenylthio)pyridine-3,5-dicarbonitrile **(5a-j)** (1 mmol) were prepared form Intermediate 4, malanonitrile (2 mmol) and thiophenol dereivative (1.2 mmol) in ethanol using catalytic amount of piperidine as a base.

2-amino-4-(2-hydroxy-5-((4-(5-mercapto-1,3,4-oxadiazol-2-yl)phenyl)diazenyl)phenyl)-6-(phenylthio)pyridine-3,5-dicarbonitrile (5a)

White coloured ,Yield: 77% compound, m.p: 173-175 ºC , ^{1}H NMR (400 MHz, Chloroform) δ 8.11 – 7.96 (m, 3H), 7.84 (s, 1H), 7.77 – 7.64 (m, 2H), 7.32 – 7.20 (m, 2H), 7.10 (t, *J* = 1.5 Hz, 3H), 7.02 (s, 1H), 3.46 (s, 1H), 2.31 – 2.27 (m, 2H), 2.06 (s, 1H).^{13}C NMR (100 MHz) δ 173(s), 165 (s), 164 (s), 163 (s), 158 (s), 157 (s), 144 (s), 144 (s), 136(s), 131(m), 130 (m), 129 (s), 127 (d, *J* = 7.3 Hz), 125 (m), 124 (s), 124 (s), 118(m), 116(s), 115 (s), 115(s), 86 (s), 84 (s). LC mass m/z: 548, ($C_{27}H_{16}N_8O_2S_2$).

2-amino-6-((4-chlorophenyl)thio)-4-(2-hydroxy-5-((4-(5-mercapto-1,3,4-oxadiazol-2-yl)phenyl)diazenyl)phenyl)pyridine-3,5-dicarbonitrile (5b)

White coloured ,Yield: 81% compound, m.p: 193-195 ºC , ^{1}H NMR (400 MHz, Chloroform) δ 8.09 – 7.96 (m, 3H), 7.82 (s, 1H), 7.75 – 7.65 (m, 2H), 7.30 – 7.17 (m, 2H), 7.17 – 7.07 (m, 2H), 7.07 (s, 1H), 3.96 (s, 1H), 2.09 (s, 1H), 0.85 – 0.81 (m, 2H). ^{13}C NMR (100 MHz) δ 173 (s), 165(s), 164 (s), 163 (s), 158(s), 157(s), 144(s), 144(s), 136 (d, J = 10.4 Hz), 132(m), 129.33 (m), 127 (d, J = 7.3 Hz), 125 (m), 124 (s), 124 (s), 118 (m), 116 (s), 115 (s), 115 (s), 86 (s), 84 (s). LC mass m/z: 582, ($C_{27}H_{15}ClN_8O_2S_2$).

2-amino-6-((4-fluorophenyl)thio)-4-(2-hydroxy-5-((4-(5-mercapto-1,3,4-oxadiazol-2-yl)phenyl)diazenyl)phenyl)pyridine-3,5-dicarbonitrile (5c)

White coloured ,Yield: 77% compound, m.p: 188-199 ºC , ^{1}H NMR (400 MHz, Chloroform) δ 8.09 (s, 1H), 8.07 – 7.96 (m, 2H), 7.84 (s, 1H), 7.77 – 7.64 (m, 2H), 7.27 – 7.23 (m, 2H), 7.10

(s, 1H), 6.90 – 6.83 (m, 2H), 3.57 (s, 1H), 2.15 – 2.04 (m, 3H). ^{13}C NMR (125 MHz δ 173 (s), 165 (s), 164(s), 163 (d, J = 6.6 Hz), 158 (s), 157 (s), 144 (s), 144(s), 131 (m), 131 (s), 127 (d, J = 7.3 Hz), 125 (m), 124 (s), 124 (s), 118(m), 116 (s), 115(s), 115(s), 86 (s), 85 (s). LC mass m/z: 566, ($C_{27}H_{15}FN_8O_2S_2$).

2-amino-6-((4-bromophenyl)thio)-4-(2-hydroxy-5-((4-(5-mercapto-1,3,4-oxadiazol-2-yl)phenyl)diazenyl)phenyl)pyridine-3,5-dicarbonitrile (5d)

White coloured ,Yield: 77% compound, m.p: 188-199 ºC, ^{1}H NMR (400 MHz) δ 8.09 – 7.96 (m, 3H), 7.82 (s, 1H), 7.74 – 7.65 (m, 2H), 7.38 – 7.24 (m, 2H), 7.24 – 7.15 (m, 2H), 7.07 (s, 1H), 3.96 (s, 1H), 2.09 (s, 1H), 0.84 – 0.81 (m, 2H). ^{13}C NMR (100 MHz) δ 173 (s), 165 (s), 165(s), 164 (s), 158(s), 157(s), 145(s), 144 (s), 135 (s), 132(m), 130(m), 127 (d, J = 7.3 Hz), 125(m), 125 (s), 124 (s), 122(s), 118(m), 116 (s), 115(s), 115(s), 86 (s), 85 (s). LC mass m/z: 626, ($C_{27}H_{15}BrN_8O_2S_2$).

2-amino-6-((2,3-dichlorophenyl)thio)-4-(2-hydroxy-5-((4-(5-mercapto-1,3,4-oxadiazol-2-yl)phenyl)diazenyl)phenyl)pyridine-3,5-dicarbonitrile (5e)

White coloured ,Yield: 77% compound, m.p: 188-199 ºC, ^{1}H NMR (400 MHz) δ 8.11 – 7.96 (m, 3H), 7.84 (s, 1H), 7.78 – 7.64 (m, 2H), 7.13 (d, *J* = 31.2 Hz, 2H), 6.97 (d, *J* = 26.4 Hz, 2H), 3.49 (s, 1H), 2.30 – 2.26 (m, 2H), 2.06 (s, 1H). ^{13}C NMR (100 MHz) δ 173 (s), 165(s), 164 (s), 164(s), 158 (s), 157(s), 145 (s), 145 (s), 138 (s), 137(s), 135(s), 134(s), 132 (s), 128 (s), 127(d, J = 7.3 Hz), 125 (m), 124(s), 124 (s), 118(m), 116(s), 115(s), 115 (s), 86 (s), 85 (s). LC mass m/z: 617, ($C_{27}H_{14}Cl_2N_8O_2S_2$).

3. CONCLUSIONS

All the final compounds 5a-j successfully synthesized. purified by Column chromatography and characterized by different spectroscopical techniques like ^{1}H NMR, ^{13}C NMR and MS analysis, all the compounds were carried out for their antibacterial and anti-fungal activity using 2 gram positive and 2 gram negative bacterias as well as two fungal stain. From this study we came to know that all the compounds emerged out as potent antibacterial and anti-fungal agents expect 5a, 5b, 5i and 5j.

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