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Original Research Article

MICROWAVE ENHANCED SYNTHESIS AND ANTIMICROBIAL ACTIVITY OF SOME NOVEL BENZOFURAN LINKED ISOXAZOLE DERIVATIVES

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ABSTRACT

The synthesis and biological evaluation of some novel benzofuran linked isoxazole derivatives **2a-j** was aimed at creating a compact new structures with a hope to get much more potent compounds with less side effects. A simple, facile microwave mediated synthesis of ten new compounds were synthesized by reacting 1-(benzofuran-2-yl)-3-(substituted phenyl)-prop-2-en-1-ones with hydroxylamine hydrochloride to yield various isoxazoles **2a-j**. All the compounds were characterized by physical and spectral data. The compounds were screened for anti-microbial activities. Compounds **2d & 2j** were found to possess significant anti-bacterial activity against both gram positive and gram negative bacteria at the tested concentrations when compared with that of standard drug ampicillin. In anti-fungal study, compounds **2j, 2e** and **2d** have exhibited almost similar anti fungal activity when compared with standard drug fluconazole. These compounds can be further exploited to get the potent lead compound.

Key words: Microwave mediated synthesis, Benzofuran, Isoxazoles, antimicrobial activity.

INTRODUCTION

The need for structurally diverse compound libraries for screening in lead discovery has driven the development of new strategies for the preparation of organic molecules in neat conditions. One of those high-speed techniques is microwave mediated synthesis, which has emerged as a new tool in organic synthesis. Important advantages of this technique include highly accelerated rate of the reaction, reduction in reaction time with improvement in the yield and quality of product.[1] Nitrogen-oxygen containing heterocycles have been exclusively used as important pharmacophores in drug design. Isoxazoles are important group of heterocycles possessing a wide spectrum of pharmacological activities and the Isoxazole ring can be considered as a bioisostere for both carboxyl and amide functionality.

They display versatile biological activities such as

- antibacterial[2]
- antifungal[3]
- antimycobacterial[4]
- antiviral[5]
- anti-HIV[6]
- analgesic[7]
- antistress[8]
- antithrombolic[9]
- anticonvulsant[10]
- antihyperlipidemic[11]
- anticancer activity[12].

Isoxazole ring is also present in antimicrobial drugs like Sulfisoxazole, Oxacillin and Cloxacillin. Besides, benzofurans, being among most important key building blocks for a variety of biologically important molecules and explored extensively due to their broad pharmacological activities such as

- antibacterial [13]
- antifungal[14]
- analgesic [15]
- antidepressant[16]
- antidiabetic[17]
- and anticancer activities [18].

Therefore, due to easy accessibility and diverse chemical reactivity of these two useful synthons and stimulated by above mentioned considerations prompted us to couple these two synthons to get a compact structure and explore the antimicrobial activity.

EXPERIMENTAL

All the chemicals and solvents were analytical grade and used without further purification. Melting points were determined on open capillaries, using Boitus melting point apparatus, expressed in °C and are uncorrected. ^{1}H NMR spectra of the compounds were on Bruker ALPHA-T FTIR spectrophotometer. The mass spectrums of the compounds were recorded on Bruker AMX-400 MHz NMR spectrophotometer using TMS as an internal standard and the values are expressed in δ ppm. Infrared spectra were recorded in KBr disc recorded either on Agilent-1100 ESI-Mass (Turbo Spray)

Spectro photometer. Microanalyses were carried out with a Perkin-Elmer model-2400 series II apparatus and were within ± 0.4% of the theoretical values. Column chromatography was performed on silica gel (230-400 mesh).

General procedure

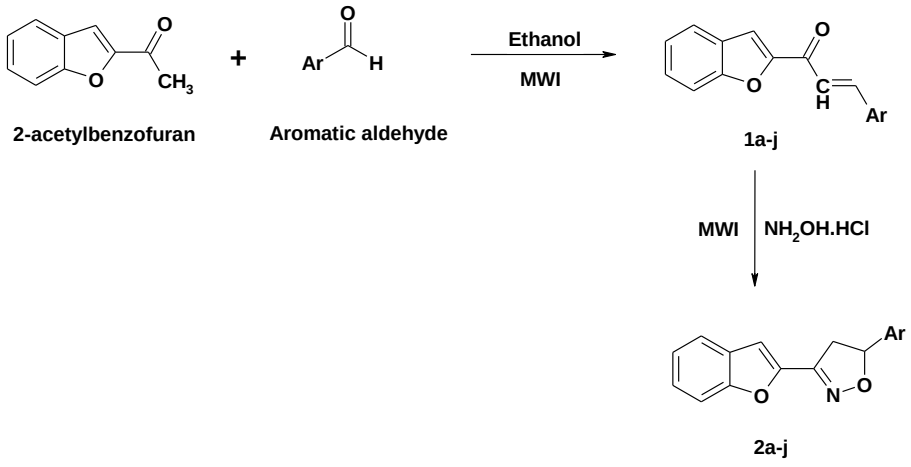
Synthesis of 1-(benzofuran-2-yl)-3-(substitutedphenyl)-prop-2-en-1-ones (1a-j)

To a mixture of 2-acetyl benzofuran (0.01 mol) and various substituted aromatic aldehydes (0.01 mol) were dissolved in ethanol (50 mL). To this reaction mixture aqueous sodium hydroxide (70 %, 5 mL) was added drop wise with constant stirring. The reaction mixture was then irradiated in microwave for 3 min with 100% intensity. The progress of reaction was monitored by TLC using a mixture of hexane and ethyl acetate as a mobile phase. After completion of reaction the reaction mixture cooled to room temperature and neutralized with concentrated hydrochloric acid, and then the solid separated was collected and crystallized from suitable solvent. The purity of all compounds was established by TLC using a mixture of hexane and ethyl acetate as a mobile phase.

Synthesis of 3-(benzofuran-2-yl)-5-(substituted phenyl)-4, 5-dihydroisoxazoles (2a-j)

To a mixture of 1-(benzofuran-2-yl)-3-(substituted phenyl)-prop-2-en-1-one **1a-j** (0.01mol) and hydroxylamine hydrochloride (0.01 mol) were dissolved in anhydrous ethanol (50 mL). To this reaction mixture aqueous sodium hydroxide (10 %, 6 mL) was added and then irradiated in microwave oven for 5 min at 80% intensity. The progress of reaction was monitored by TLC using a mixture of hexane and ethyl acetate as a mobile phase. After completion reaction as indicated in TLC the reaction mixture was poured into ice cold water, a fine product was obtained then filtered, washed with water and crystallized from suitable solvent. The purity of the compound was checked by TLC using a mixture of hexane and ethyl acetate as a mobile phase.

General Scheme:



Antimicrobial activity:

All the synthesized compounds **2a-j** were screened for their antibacterial activity against *Staphylococcus aureus* (NCIM-2079), *Bacillus subtilis* (NCIM-2063), *Escherichia coli* (NCIM-2068) and *Proteus vulgaris* (NCIM-2027) by serial tube dilution technique [19-20] using ampicillin as reference standard, and antifungal activity against *Aspergillus* niger (ATCC-6275) and *Candida tropicalis* (ATCC-1369) by using fluconazole as reference standard. The observed minimum inhibitory concentrations (MIC) values for all the synthesized compounds are presented in **Table 4.**

RESULTS AND DISCUSSION

i) *Chemistry:*

Synthesis of 1-(benzofuran-2-yl)-3-phenylprop-2-en-1-one (1a)

To a mixture of 2-acetyl benzofuran **2** (0.01 mol) and benzaldehyde (0.01 mol) were dissolved in ethanol (50 mL). To this reaction mixture aqueous sodium hydroxide (70 %, 5 mL) was added drop wise with constant stirring. The reaction mixture was then irradiated in microwave for 3 min with 100% intensity. The progress of reaction was monitored by TLC using a mixture of hexane and ethyl acetate as a mobile phase. After completion of reaction the reaction mixture cooled to room temperature and neutralized with concentrated hydrochloric acid, and then the solid separated was collected and crystallized from ethanol. The purity of the compound was established by TLC using ethyl acetate and hexane mixture (20:80) as mobile phase.

Compound **1a** analyzed for $C_{17}H_{12}O_2$, m.p 128-130^{o}C, well supported by its $[M+H]^+$ ion at *m/z* 249.99 in its positive mode electrospray ionization mass spectrum.

The IR (cm^{-1}) spectrum showed the characteristic intense bands at 1662 (C=O) and 1442 (C=C).

The ^1H NMR (δ ppm) spectrum of compound **1a** showed characteristic signals of CO-CH= and =CH-Ar at 7.5 and 7.7 as doublets respectively, a multiplet in between 7.6–8.6 integrating for the ten aromatic protons.

The results of elemental analysis were also in close agreement with those of the calculated values. Based on the above spectral data, the structure of the compound **1a** was confirmed as 1-(benzofuran-2-yl)-3-phenylprop-2-en-1-one.

Synthesis of 3-(benzofuran-2-yl)-4, 5-dihydro-5-phenylisoxazole (2a)

To a mixture of 1-(benzofuran-2-yl)-3-phenylprop-2-en-1-one **(1a)** (0.01 mol) and hydroxylamine hydrochloride (0.01 mol) were dissolved in anhydrous ethanol (50 mL). To this reaction mixture aqueous sodium hydroxide (10 %, 6 mL) was added and then irradiated in microwave oven for 5 min at 80% intensity. The progress of reaction was monitored by TLC using a

mixture of hexane and ethyl acetate as a mobile phase. After completion reaction as indicated in TLC the reaction mixture was poured into ice cold water, a fine product was obtained then filtered, washed with water and crystallized from ethanol. The purity of the compound was checked by TLC using a mixture of ethyl acetate and hexane mixture (30:70) as mobile phase.

Compound **2a** analyzed for $C_{17}H_{13}NO_2$, m.p 148-151^{o}C, well supported by its $[M+H]^+$ ion at *m/z* 264.21 in its positive mode electrospray ionization mass spectrum.

The IR (cm^{-1}) spectrum showed the characteristic intense bands at 1548 (C=N) and 1446 (C=C). The ^{1}H NMR (δ ppm) spectrum of compound **2a** revealed doublets at 3.7, 4.3 and 6.5 due to two protons of C-4 of isoxazoles and one proton of C-5 of isoxazole respectively, a multiplet in the region between 7.5– 8.4 for the ten aromatic protons.

The results of elemental analysis were also in close agreement with those of the calculated values. Based on the above spectral data, the structure of the compound **2a** was confirmed as 3-(benzofuran-2-yl)-4,5-dihydro-5-phenylisoxazole.

By adopting the above synthetic procedures, compounds **2a-j** were synthesized. All these compounds are new and the characteristic physical and spectral data were presented separately in the table form.

Table.1. Physical data of synthesized compounds (1a-j)

Compound	Ar	Formula	Melting Point (^{o}C)	Yield (%)
1a	phenyl	$C_{17}H_{12}O_2$	128	72
1b	2-chlorophenyl	$C_{17}H_{11}O_2Cl$	210	66
1c	4-pyridyl	$C_{16}H_{11}NO_2$	186	58
1d	2,4-dichlorophenyl	$C_{17}H_{10}O_2Cl_2$	225	64
1e	4-nitrophenyl	$C_{17}H_{11}NO_4$	258	54
1f	2-thienyl	$C_{15}H_{10}O_2S$	245	55
1g	3-methoxyphenyl	$C_{18}H_{14}O_3$	213	63
1h	2-pyridyl	$C_{16}H_{11}NO_2$	235	66
1i	4-hydroxyphenyl	$C_{17}H_{12}O_3$	204	66
1j	4-fluorophenyl	$C_{17}H_{11}O_2F$	218	74

Table.2. Physical data of synthesized compounds (2a-j)

Compound	Ar	Formula	Melting Point (°C)	Yield (%)
2a	phenyl	$C_{17}H_{13}NO_2$	148	82
2b	2-chlorophenyl	$C_{17}H_{12}NO_2Cl$	161	72
2c	4-pyridyl	$C_{16}H_{12}N_2O_2$	330	68
2d	2,4-dichlorophenyl	$C_{17}H_{11}NO_2Cl_2$	238	66
2e	4-nitrophenyl	$C_{17}H_{12}N_2O_4$	266	73
2f	2-thienyl	$C_{15}H_{11}NO_2S$	203	45
2g	3-methoxyphenyl	$C_{18}H_{15}NO_3$	217	63
2h	2-pyridyl	$C_{16}H_{12}N_2O_2$	212	68
2i	4-hydroxyphenyl	$C_{17}H_{13}NO_3$	222	57
2j	4-fluorophenyl	$C_{17}H_{12}NO_2F$	230	73

Table.3.^{1}H NMR data of selected compounds (2a-j)

Compound	Chemical shift (δ) in ppm
2a	3.7 & 4.3 (2H, d, C-4, isoxazole), 6.55 (1H, d, C-5, isoxazole), 7.5-8.4 (10H, m, Ar-H).
2c	3.8 & 4.20 (2H, d, C-4, isoxazole), 6.45 (1H, d, C-5, isoxazole), 7.6-8.8 (9H, m, Ar-H).
2d	3.7 & 4.3 (2H, d, C-4, isoxazole), 6.3 (1H, d, C-5, isoxazole), 7.6-8.4 (8H, m, Ar-H).
2e	3.75 & 4.3 (2H, d, C-4, isoxazole), 6.4 (1H, d, C-5, isoxazole), 7.8-8.4 (9H, m, Ar-H).
2f	3.7 & 4.25 (2H, d, C-4, isoxazole), 6.3 (1H, d, C-5, isoxazole), 7.6-8.3 (8H, m, Ar-H).
2j	3.7 & 4.2 (2H, d, C-4, isoxazole), 6.5 (1H, d, C-5, isoxazole), 7.4-8.4 (9H, m, Ar-H).

Table.4.Antimicrobial activity of 2-aminopyrimidine derivatives (2a-j). (Expressed as MIC in µg/mL)

	Antimicrobial activity					
Compound	***B.subtilis***	***S.aureus***	***E.coli***	***P.vulgaris***	***A.* niger**	***C. tropicalis***
2a	256	256	256	128	256	256
2b	128	64	128	128	64	128
2c	256	256	256	128	128	128
2d	128	64	64	64	64	128
2e	256	256	128	128	64	128
2f	256	256	256	128	128	128
2g	256	256	256	256	256	256
2h	256	256	256	128	128	256
2i	256	128	256	256	256	128
2j	128	64	128	64	128	128
Ampicillin	<1	<1	<1	<1	-	-
Fluconazole	-	-	-	-	<2	<2

ii) *Antibacterial activity:*

All synthesized pyrazoles **(2a-j)** have been evaluated for their antibacterial activity against *E.coli, P.vulgaris* (Gram-negative) and *S.aureus* and *B.subtilis* (Gram-positive) using serial tube dilution method.

The results of this evaluation compared with ampicillin as reference standard.

From the above results it is evident that all the isoxazoles showed antibacterial activity with different MIC values against the tested

organisms, but not comparable with that of the standard. Among the tested compounds **2d** (2,4-dichlorophenyl) and **2j** (4-fluorophenyl) was found to be potent against *B. subtilis* with a MIC value of 128 µg/mL. The compound **2b** (2-chlorophenyl), **2d** (2,4-dichlorophenyl) and **2j** (4-fluorophenyl) was potent against *S. aureus* with a MIC value of 64 µg/mL. The compound **2d** was active against *E. coli* with a MIC value of 64 µg/mL. The compounds **2d** & **2j** were active against *P. vulgaris* with a MIC value of 64 µg/mL. The other compounds also exhibited activity with a MIC values ranging from 128-256 µg/mL. Among all the compounds tested, compounds **2d** and **2j** possessed maximum activity which may be due to electron withdrawing substituents such as 2,4-dichlorophenyl and 4-fluorophenyl moieties at C-5 position of isoxazole and thus reveals the importance of such groups for favorable antibacterial activity. This also suggested that isoxazoles having number of these substituents at different positions of the aromatic or heteroaromatic rings when synthesized may demonstrate promising antibacterial activity. Infact, it was observed in the present study that 2-acetylbenzofuran ring contributed favorably to the antibacterial activity.

iii) *Antifungal activity*:

The antifungal activity of isoxazoles **(2a-j)** have been evaluated against *A.niger* and *C.tropicalis* and fluconazole employed as reference standard by using serial tube dilution method.

A close examination of the antifungal data of isoxazole revealed that some of the compounds in this series have been found effective against both fungi at 64 µg/mL concentration level when compared with reference standard fluconazole. Among the compounds tested for antifungal activity, compounds **2b** (2-chlorophenyl) and **2d** (2,4-dichlorophenyl) and **2e** (4-nitrophenyl) found to be potent against *A. niger* with a MIC value of 64 µg/mL. All the other compounds showed activity with a MIC values ranging from 128-256 µg/mL which was less when compared to the activity of other compound tested.

Compounds **2b, 2d, 2e** and **2j** possessed maximum activity which may be due to the presence of 2-chlorophenyl, 2,4-dichlorophenyl, 4-nitrophenyl pharmacophore at C-5 position of isoxazole structure. This reveals the importance of the electronic effects of the substituents present on the aromatic ring in enhancing the antifungal activity. Moreover it has been found that compounds **2c, 2i, 2g,** and **2h** also exhibited moderate activity against both the fungi.

CONCLUSION

In the present study we have demonstrated a simple, efficient and cleaner strategy for the synthesis of benzofuran linked isoxazoles by reacting of different chalcones with hydroxylamine hydrochloride in alcoholic NaOH under microwave

irradiation conditions. With encouraging antimicrobial activity results, all the synthesized compounds need to be evaluated in terms of active concentration and also examine the mechanism of compounds responsible for antimicrobial activity. All the synthesized compounds can be further explored for structural modifications and studies concerning the structure-activity relationships are in progress in our laboratory.

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