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Research Article

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Synthesis and Biological Evaluation of Some Novel Isoxazoles and Benzodiazepines

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ABSTRACT

A new series of Isoxazole and Benzodizepine derivatives were synthesized from chalcones and evaluated for their Antimicrobial activities. First Chalcones were prepared by treatment of Furan-2-Carbaldehyde with different acetophenones by Claisen-Schimidt Condensation. Various Isoxazole derivatives were prepared by reaction of Chalcone with Hydroxylamine Hydrochloride and Sodium Acetate in ethanol and Benzodizepine derivatives were prepared by reaction of Chalcone in ethanol with o-phenylenediamine in presence of piperidine. The structures of the newly synthesized Isoxazole and Benzodiazepine derivatives have been established on the basis of their spectral data. The synthesized selected compounds were evaluated for their antimicrobial activities.

Keywords: Isoxazole, Benzodiazepine, Chalcones, Antibacterial activity, Antifungal activity.

INTRODUCTION

Heterocyclic chemistry is a branch which is inseparable from mankind because human are totally dependent on the drugs derives from heterocyclic rings. Much attention has paid to the synthesis of nitrogen containing heterocyclic compounds like isoxazole and benzodiazepine mainly due to their broad spectrum of biological and pharmacological activities.

Derivatives of Isoxazole [1-3] have played a crucial role in the history of heterocyclic chemistry and been used extensively important pharmacophores and synthons in the field of organic chemistry. Owing to their versatile chemotherapeutic importance, a significant amount of research effort has been focused on these nuclei.

Isoxazole derivatives exhibit various biological activities such as, Antibacterial [4-6], Anticonvulsant [7-8], Anticholestermic [9], Anticancer [10], Anthelmintics [11]. Antiinflammatory [12-15], Adenosine antagonist [16], Fungicidal [17-19], Herbicidal [20-21], Hypoglycemic [22], Muscle relaxant [23-24], Nematocidal [25], Insecticidal [26], Antiviral [27] and Antimicrobial [28].

1,5-benzodiazepines have recently attracted attention as an important class of heterocyclic compounds in the field of drugs and pharmaceuticals. These compounds are widely used as anticonvulsant, antianxiety, analgesic, sedative, antidepressive, hypnotic agents [29] as well as anti-inflammatory agents [30] Other than their biological importance, benzodiazepine derivatives are also commercially used as dyes for acrylic fibres [31]. Moreover, 1,5-benzodiazepine derivatives are valuable synthons that can be used in the preparation of other fused ring compounds such as triazolo, oxadiazolo, oxazino or furanobenzodiazepines [32] As a result, research in this area is still very active and is directed towards the synthesis of compounds with enhanced pharmacological activity.

Led by these considerations, it appeared of interest to synthesize novel Isoxazole and Benzodiazepine derivatives and to investigate for their antimicrobial activities.

EXPERIMENTAL SECTION

Chemicals and reagent

Furan-2-Carbaldehyde, Various Acetophenone, Hydroxylamine Hydrochloride, Sodium Acetate, Ethanol, Piperidine, o-phenylene diamine, Acetic Acid, Ethanol, Ampicillin and Griseofulvin.

Experimental procedures

STEP.1

Chalcones By Claisen-Schimidt Condensation By Reaction Of Aldehyde With Various Acetophenone

Place a solution of 22g of sodium hydroxide in 200ml of water and 122.5ml of rectified spirit in a 500ml bolt-head flask provided with a mechanical Stirrer. Immerse the flask in a bath of crushed ice, pour in 0.43mol of freshly distilled Acetophenone, start the stirrer and then add 0.43mol of pure Furfuraldehyde. Keep the temperature of the mixture at about 25^0C (the limits are 15- 30^0C) and stir vigorously until the mixture is so thick that stirring is no longer effective (2-3 hr). Remove the stirrer and leave the reaction mixture in an ice chest or refrigerator overnight. Filter the product with suction on a buchner funnel or a sintered glass funnel, wash with cold water until the washings are neutral to litmus and then with 20ml of icecold rectified spirit. The crude chalcone after drying in the air weigh 88g and melts at 50-54^0C. Recrystallized from rectified spirit warmed to 50^0C (about 5ml per gm). The yield of pure benzylideneacetophenone is 77 gm. [a pale yellow solid, mp 56-57^0C, 85%]. This substance should be handled with great care since it acts as a skin irritant**.**

STEP 2(a):

Various Isoxazoles From Chalcones

Chalcone 0.02 mol, Hydroxylamine hydrochloride 0.02 mol and sodium acetate in ethanol 25 ml was refluxed for 6hr. The mixture was concentrated by distilling out the solvent under reduced pressure and poured into ice water. The precipitate obtained was filtered, washed and recrystallized.

A-6 IR (ν_{max}): 3377.9(-NH_2), 3126.6(Ar-H), 1654.6(Ar-C=C), 1602.7(C=N), 1435.8(CH_2 of Isoxazole), 1234.1(C-O-C). ^{1}H NMR (DMSO): 7.33-7.53(4H,m,Ar-H), 6.60-6.67(3H,m,Furan) 5.04(1H,d,CH of Isoxazole), 3.57(2H,s, NH_2), 2.51(2H,d,CH_2 of Isoxazole). Mass: m/z 228.1 (M^+)

B-6 IR (ν_{max}): 3149.7(Ar-H), 1655.8 (Ar-C=C), 1601.5 (C=N), 1441.6 (CH_2 of Isoxazole), 1217.2(C-O-C), 529.4(C-Br). ^{1}H NMR (DMSO): 7.33-7.53(4H,m,Ar-H), 6.60-6.67(3H,m,Furan) 5.04(1H,d,CH of Isoxazole), 2.51(2H,d,CH_2 of Isoxazole). Mass: m/z 292.13 (M^+)

C-6 IR (ν_{max}): 3196.2 (Ar-H), 1679.6 (Ar-C=C), 1654.7 (C=N), 1543.4(-NO_2), 1415.8 (CH_2 of Isoxazole), 1233.3 (C-O-C). ^{1}H NMR (DMSO): 7.34-7.68(4H, m, Ar-H), 6.61-6.78 (3H, m, Furan), 5.22(1H, d, CH of Isoxazole), 2.72 (2H, d, CH_2 of Isoxazole). Mass: m/z 258.1 (M^+)

F-6 IR (ν_{max}): 3105.5 (Ar-H), 1654.1 (Ar-C=C), 1622.9 (C=N), 1432.2 (CH_2 of Isoxazole), 1211.3 (C-O-C). ^{1}H NMR (DMSO): 7.42-7.54(4H, m, Ar-H), 6.97-7.02 (3H, m, Furan), 4.89(1H, s, CH of Isoxazole), 3.01(3H, s, CH_3), 2.61 (2H, m, CH_2 of Isoxazole). Mass: m/z 227.0 (M^+).

STEP 2(b):

Various Benzodiazepines From Chalcones

To a solution of Chalcone (0.602 g, 2 mmol) in ethanol (30 ml) a few drops of piperidine and o-phenylenediamine(0.21 g, 2 mmol) were added. The mixture was heated under reflux for 3-4 hour and then Acetic Acid (1 ml) was added. Refluxing was continued for another 3-4 hour. About half of the solvent was distilled off under reduced pressure and the oily residue was allowed to stand at room temperature overnight. The crystalline solid product obtained thus separated was filtered, washed with cold aqueous ethanol (2-3 ml, 50: 50 by v/v) and dried.

The reaction of o-phenylenediamine and chalcones afford 1,5-benzodiazepine.

A-7 IR (ν_{max}): 3477.9 (-NH), 3357.7 (-NH_2), 3138.6 (Ar-H), 1651.6 (Ar-C=C), 1623.6 (C=N), 1228.7 (C-O-C). ^{1}H NMR (DMSO): 7.82-8.15(4H, m, Ar-H), 7.12(1H, s, CH of Benzodiazepine [aldimine]) 6.60-6.63 (3H, m, Furan), 5.24(2H, s, NH_2), 4.51(1H, s, CH of Benzodiazepine [methine]), 4.12 (1H, s, NH) 3.57(1H, s, CH of Benzodiazepine [methine]).Mass: m/z 227.1 (M^+).

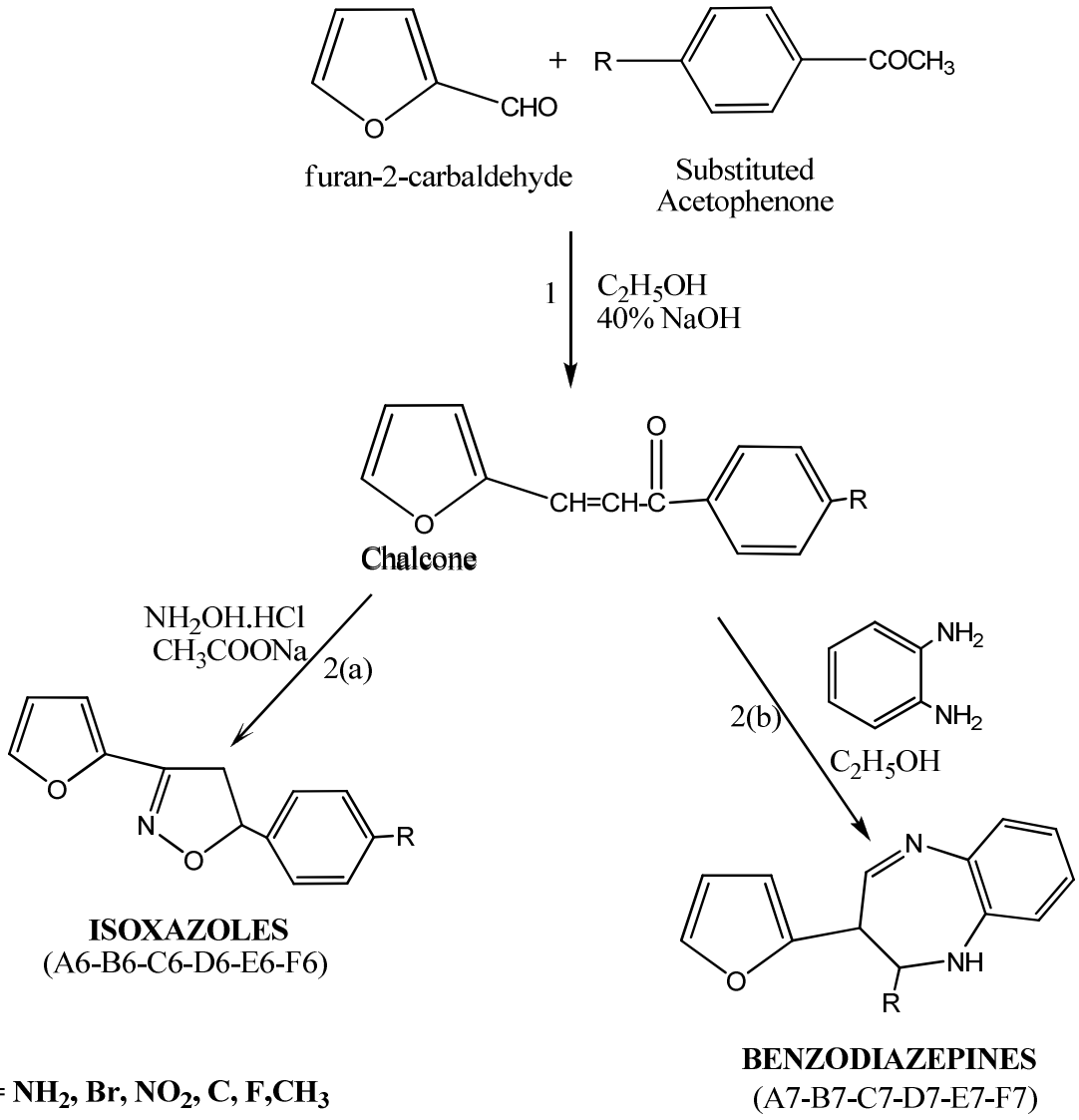
B-7 IR (ν_{max}): 3478.6 (-NH), 3089.6 (Ar-H), 1656.8 (Ar-C=C), 1624.1 (C=N), 1225.9 (C-O-C), 527.8(C-Br). ^{1}H NMR (DMSO): 7.94-8.07(4H, m, Ar-H), 6.57(1H, s, CH of Benzodiazepine [aldimine]), 6.00-6.03 (3H, m, Furan),

4.12(1H, s, CH of Benzodiazepine [methine]), 3.81 (1H, s, NH) 3.03(1H, s, CH of Benzodiazepine [methine]). Mass: m/z 291.1 (M^+).

C-7 IR (ν_{max}): 3498.9 (-NH), 3177.6 (Ar-H), 1698.5 (Ar-C=C), 1638.7 (C=N), 1547.9 (-NO_2), 1215.2 (C-O-C). ^{1}H NMR (DMSO): 7.84-8.19(4H, m, Ar-H), 7.04(1H, s, CH of Benzodiazepine [aldimine]), 6.30-6.33 (3H, m, Furan), 4.38(1H, s, CH of Benzodiazepine [methine]), 4.01 (1H, s, NH) 3.34(1H, s, CH of Benzodiazepine [methine]). Mass: m/z 258.2 (M+1).

F-7 IR (ν_{max}): 3414.0 (-NH), 3159.0 (Ar-H), 1653.0 (Ar-C=C), 1620.0 (C=N), 1218.1 (C-O-C). ^{1}H NMR (DMSO): 8.34-8.48(4H, m, Ar-H), 7.31(1H, s, CH of Benzodiazepine [aldimine]), 6.57-6.59 (3H, m, Furan), 4.61(1H, s, CH of Benzodiazepine [methine]), 4.31 (1H, s, NH) 3.36(1H, s, CH of Benzodiazepine [methine]), 2.84(3H, s, CH_3). Mass: m/z 226.2 (M^+)

SCHEME



General procedures

Melting points were determined in open capillaries and are uncorrected. IR spectra were recorded using a Perkin-Elmer 237 spectrophotometer. ^{1}H NMR spectra were recorded on Bruker AM 400 instrument (at 400 MHz) using tetramethylsilane (TMS) as an internal standard and DMSO-d6 as a solvent. Chemical shifts are given in parts per million (ppm). Splitting patterns are designated as follows: s- singlet, d- doublet, t- triplet, q-quartet and m-multiplet. Mass spectra (MS) were recorded on MSroute JMS 600-H. All the synthesized compounds were purified by recrystallization. The reactions were followed up and the purity of compounds was monitored on pre-coated TLC plates and visualizing the spots in ultraviolet light.

In vitro anti–microbial screening [33]
The synthesized compounds were subjected to antimicrobial screening by Cup plate method for zone of inhibition. The Antibacterial activity was tested against various gram positive and Gram negative bacteria and anti fungal activity against various fungal strains compared with standard drug (Ampicillin and Griseofulvin).

Antibacterial Activity
Each Petri dish containing Multer-Hinton agar medium was inoculated with one bacterial culture by spreading the suspension of the organism with a sterile glass rod with a bended tip. In each plate cups of 6mm diameter were made at equal distances using sterile cork borer. One cup was filled wih 0.1 ml of standard drug ie., ampicillin, one was filled with 0.1 ml of DMF, others were filled with 0.1 ml of synthesized compound's solution in sterile DMF.

All plates were kept in the refrigerator for 30 minutes to allow the diffusion of sample to the surrounding agar medium. The Petri dishes were incubated at 37^0C for 24 hrs. Diameter of the zone of inhibition was measured and the average diameter for each sample was calculated. The diameter obtained for the test samples were compared with that produced by standard ampicillin.
The results were described in TABLE 2

Antifungal Activity
Each Petri dish containing nutrient agar medium was inoculated with one fungal culture by spreading the suspension of the organism with a sterile glass rod with a bended tip. In each plate cups of 6mm diameter were made at equal distances using sterile cork borer. One cup was filled with 0.1 ml of standard drug ie., griseofulvin, one was filled with 0.1 ml of DMF, others were filled with 0.1 ml of synthesized compound's solution in sterile DMF.

All plates were kept in the refrigerator for 30 minutes to allow the diffusion of sample to the surrounding agar medium. The Petri dishes were incubated at 25^0C for 48 hours. Diameter of the zone of inhibition was measured and the average diameter for each sample was calculated. The diameter obtained for the test samples were compared with that produced by standard griseofulvin.
The results were described in the TABLE 2

RESULTS AND DISCUSSION

In our study, new series of compounds B-6, C-7 and F-7 showed moderate to significant antibacterial and antifungal activity when compared with standard drugs. However it is less than standard drugs like Ampicillin and Griseofulvin but compounds B-7 and D-7 Showed significant antibacterial activity and A-6, B-6, C-6 and F-7 Showed significant antifungal activity when compared to standard drug.

The effect of synthesized Isoxazoles and Benzodizepines on bacterial and fungal strains are summarized in TABLE 2.
Results of present study demonstrate that, a new class of different Isoxazoles and Benzodiazepines synthesized from chalcones and evaluated for antibacterial and antifungal activities. Among the tested B-7 and D-7 compound showed better antibacterial activity while A-6, B-6, C-6 and F-7 compound showed better antifungal activity. It can be concluded that Isoxazoles and Benzodiazepines synthesized from chalcones certainly holds great promise towards good active leads in medicinal chemistry.

TABLE 1 PHYSICAL DATA OF THE SYNTHESIZED COMPOUNDS

Compound No.	Physical State	Melting point(°)	Yield (%)	Molecular Formula
A-6	Yellow Crystals	120-125°C	35.40%	$C_{13}H_{12}N_2O_2$
B-6	Dark Yellow Crystals	141-145°C	42.30%	$C_{13}H_{10}BrNO_2$
C-6	Black Semi-Solid	115-120°C	38.40%	$C_{13}H_{10}N_2O_4$
D-6	Yellow Semi-Solid	148-150°C	40.10%	$C_{13}H_{10}ClNO_2$
E-6	Black Semi-Solid	125-128°C	32.20%	$C_{13}H_{10}FNO_2$
F-6	Light Yellow Semi-Solid	135-138°C	38.50%	$C_{14}H_{13}NO_2$
A-7	Black Solid	138-140°C	48.88%	$C_{13}H_{13}N_3O$
B-7	Yellow Solid	200-202°C	60.15%	$C_{13}H_{11}BrN_2O$
C-7	Dark Yellow Solid	145-149°C	65.50%	$C_{13}H_{11}N_3O_3$
D-7	Pale Yellow Solid	172-174°C	52.80%	$C_{13}H_{11}ClN_2O$
E-7	Black Solid	152-154°C	45.20%	$C_{13}H_{11}FN_2O$
F-7	Yellow Solid	160-162°C	52.75%	$C_{14}H_{14}N_2O$

TABLE 2 ANTIMICROBIAL ACTIVITY OF THE SYNTHESIZED COMPOUNDS

Sl. no.	Compound number	Diameter of zone of inhibition (mm)					
		S.aureus	***B.subtitis***	***E.coli***	***P.aeruginosa***	***A.Niger***	***C.albicans***
1	A-6	13	-	-	11	11	12
2	B-6	14	9	-	12	13	12
3	C-6	12	-	-	11	12	11
4	D-6	13	-	09	12	11	10
5	E-6	11	-	-	12	10	10
6	F-6	12	-	11	13	10	11
7	A-7	11	-	12	13	09	09
8	B-7	13	12	14	12	10	11
9	C-7	10	12	10	11	12	09
10	D-7	12	13	11	13	09	10
11	E-7	10	-	10	11	09	10
12	F-7	11	12	12	13	12	11
13	Ampicillin	15	15	16	16	-	-
14	Griseofulvin	-	-	-	-	15	15

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