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Synthesis and Biological activities of 1,2-Benzisoxazoles and their *N*-Glucosides

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Abstract

2-oximinoacetyl-4-acetyl phenol 2 was prepared by the interaction of 2,4-diacetyl phenol 1 with hydroxylamine hydrochloride using suitable solvent. Cyclization of product 2 with acetic anhydride using N,N-Dimethylformamide afforded 3-methyl-5-acetyl-1,2-benzisoxazole 3. Nitration of compound 3 with nitrating mixture produces 3-methyl-5-acetyl-7-nitro-1,2-benzisoxazole 4. 3-Methyl-5-acetyl-7-amine-1,2-benzisoxazole 5 was prepared by the reduction of product 4 using tin and hydrochloric acid. Different 3-methyl-5-(3'-aryl prop-2'-enoyl)-7-amine-1,2-benzisoxazoles 6a-j have been synthesized by the interaction of appropriate 3-methyl-5-acetyl-7-amine-1,2-benzisoxazole 5 with different aromatic aldehydes using piperidine. The reaction of 3-methyl-5-(3'-aryl prop-2'-enoyl)-7-amine-1,2-benzisoxazoles 6a-j with hydrazine hydrate in alcoholic KOH to obtained 3-methyl-5-(3'-aryl-1H- pyrazol-5'-yl)-7-amine-1,2-benzisoxazoles 7a-j. Condensation of tetra-O-acetyl-α-D-glucopyranosyl bromide with compounds 7a-j furnishes 3-methyl-5-(3'-aryl-1H-pyrazol-5'-yl)-7-amine-(2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl)-1,2-benzisoxazoles 8a-j which on deprotection to get 3-methyl-5-(3'-aryl-1H- pyrazol-5'-yl)-7-amine-(β-D-glucopyranosyl)-1,2-benzisoxazoles 9a-j. The synthesized compounds were characterized on the basis of IR, ^{1}H NMR, ^{13}C NMR and Mass Spectroscopy, Elemental analysis, TLC, chemical properties and Polarographic studies. All compounds have been screened for antimicrobial activities and some compounds show potent activities.

Keywords: 1,2-Benzisoxazole, Amino compounds, Pyrazoles, *N*-Glucosides and Polarography.

Introduction

Heterocyclic chemistry is a vast and expanding area of chemistry because of the obvious applications of compounds derived from heterocyclic rings in pharmacy, medicine, agriculture and other fields. The chemistry of heterocyclic compounds is as relevant as that of alicyclic or aromatic compounds. Heterocyclic compounds used as pharmaceuticals, agrochemicals and veterinary products, used as optical brightening agents, as antioxidants, as corrosion inhibitors and as additives with a variety of other functions[1]. Also, many dyestuffs and pigments have heterocyclic structures[2]. A heterocyclic compounds such as isoxazole, pyrazoles, furans, pyrroles, thiazines, oxazines etc. exhibit diverse pharmacological activities such as potential anti-fungal agents, anti-bacterial agents, antiviral, anti-inflammatory, herbicidal, anticancer, cytotoxic, anaesthetics, insecticidal[3-11] etc. The term pyrazole refers to the class of simple aromatic ring organic compounds of the heterocyclic series. The first natural pyrazole, 1-pyrazolyl-alanine, was isolated from seeds of watermelons[12] in 1959. Pyrazole derivatives show application in agrochemicals as herbicides and in pharmaceutical industry as active pharmaceuticals; the COX-2 inhibitor has further highlighted the importance of these heterocyclic rings in medicinal chemistry. Many pyrazoles are used for the treatment of thyroid and leukaemia having possessed wide range of pharmacological activities like antioxidant, antipyretic, anti-invasive, antiviral, anti-inflammatory, anti-depressant, agrochemicals, and dyestuffs in sunscreen materials etc[13]. The derivatives of 1,3,4-oxidiazole possess antibacterial, fungitoxic, insecticidal, herbicidal and anticancer[14-18]. Cocconcelli, et al, reported aryl azoles shows neuroprotective activity[19].

Benzisoxazoles have recently attracted attention as an important class of heterocyclic compounds in the field of drugs and pharmaceuticals. These compounds are widely used as analgesic, anticonvulsant**,** antipsychotic and antimicrobial[20-21] agents. They are present in large number of pharmaceutical important products with antitumor, antithrombotic and cholinesterase-inhibiting properties[22-23]. 1,2-Benzisoxazoles derivatives have been found to possess antidepressant and hypotensive activity[24], as potent and selective inhibitors of the enzyme acetyl cholinesterase[25], evaluated as a potential antipsychotic D2/5-HT2 antagonists[26]. Two patents have claimed that substituted 3-(aminoalkylamino)-1,2-benzisoxazoles are useful for the treatment of various memory dysfunctions and as antidepressants by inhibiting monoamine oxidase[27]. 6-fluro-4-piperidinyl-1,2-benzisoxazole-amides were synthesized and tested against antimicrobial agents and reported potent inhibition against antimicrobial stains[28].

Heterocyclic substituted chalcones were prepared by Bombardeli and Valenti reported some of them were introduced for the treatment of breast cancer, menopausal disorders and osteoporosis[29].

Chalcone derivatives have found a wide range of application in the pharmacological activities such as potential cytotoxic properties are reported by Bhatt, et al.[30], antiviral, anaesthetics, mydriatics, antimicrobial, anti-mitotic, antitumor, cytotoxicity, antipyretic, antifungal, anti-inflammatory, insecticidal and anti-HIV properties[31-35]. Venkatachalam et al. reported anti-oxidant activity of substituted chalcone[36].

Glucosylation improves the solubility of various drugs without affecting their activities and attaching of the glucosidic moiety into the molecules increases its hydrophilicity than the respective aglycon. β-Glucosylation can improve the drug targeting to the cells due to their solubility in the membrane components[37]. The carbohydrate moiety enhances the water and lipid solubility of the pharmacophoric group and the major active molecule is the aglycone which is responsible for its biological activities[38]. The screening results indicate that glucosides showed moderate to excellent antibacterial activity against *E. coli* and *S. aureus* organisms as compared to aglycon[39-40].

In the view of pronounced biological and pharmacological applications of Chalcones, 1,2-Benzisoxazoles, Nitro, Amine derivatives, Pyrazoles and *N*-Glucosides. It was planned to synthesize new chemical entities having active pharmacological functions namely chalcones, benzisoxazoles, nitro, amine, pyrazoles and their *N*-glucosides moiety in a single molecular framework as a new biological active compounds.

Materials and Methods

Melting points were determined on a melting point apparatus in open capillaries and are uncorrected. IR spectra were recorded on Bruker infrared spectrometer, ^{1}H NMR, ^{13}C NMR spectra on Bruker Avance II 400 NMR spectrometer and MS spectra were recorded and polarograms were recorded on Elico CL-362 polarograph.

General Synthesis: Synthesis of 2,4-diacetyl phenol (1). It is prepared by Fries rearrangement reaction as reported in literature. The crude product 2,4-diacetyl phenol was crystallized from aqueous alcohol, decolourised with activated charcoal powder and pure colourless crystalline solid product was obtained. Yield 14.5g, 81.4%, MP 97ºC and its alcoholic solution gave violet colour with neutral $FeCl_3$ solution.

IR: υ_{max}cm^{-1}:3353(br. OH peak), 2868-2972 (C-H str. In benzene), 1641 & 1670 (C=O two), 1489 (C=C str. in benzene); ^{1}H-NMR: δ12.69 (s, 1H, OH), δ8.44 (d, *J*=2.04 Hz, 1H, Ar-H), δ8.05-8.08 (m, 1H, Ar-H), δ7.04 (d, *J*=8.76 Hz, Ar-H), δ2.72 (s, 3H, CH), δ2.59 (s, 3H, CH); ^{13}C-NMR: δ204.85 (C=O), δ195.84 (C=O), δ166.07 (C-1), δ136.37 (C-5), δ131.87 (C-4), δ130.86 (C-3), δ119.22 (C-2), δ115.37 (C-1), δ26.76 (CH_3), δ26.32 (CH_3); MS: m/z 179, 164, 161, 137.

Synthesis of 2-oximinoacetyl-4-acetyl phenol (2): A mixture of 2,4-diacetyl phenol (17.8g, 0.1M), hydroxylamine hydrochloride (6.9g, 0.1M), sodium acetate (8.2g, 0.1M) and 50mL of EtOH/H_2O (7:3) was refluxed for 2hrs. After cooling colourless solid were filtered, washed with water, dried and crystallised by aq. alcohol. (Yield 16.2g, 83.93%), MP 174ºC and its alcoholic solution gave violet colour with neutral $FeCl_3$ solution.

Synthesis of 3-methyl-5-acetyl-1,2-Benzisoxazole (3): To a solution of 2-oximinoacetyl-4-acetyl phenol (19.3g, 0.1M) and *N,N*-dimethylformamide (8.0mL), sodium acetate (18.0g, 0.22M) and acetic anhydride (21.8mL, 0.23M) were added, the reaction mixture was refluxed for 4hr at which state all the starting material was consumed as indicated by TLC. After cooling the reaction mixture was poured into ice cold water. A brownish solid was filtered and dried. (Yield 13.1g, 74.81%), MP 112ºC and its alcoholic solution gave no violet colour with neutral $FeCl_3$ solution.

IR: υ_{max}cm^{-1}:2862-3057 (C-H str. In benzene), 1633 (C=O), 1467 (C=C str. in benzene). ^{1}H-NMR: δ8.03 (d, *J*=1.08 Hz, 1H, Ar-H), δ7.96-7.99 (m, 1H, Ar-H), δ7.57 (d, *J*=8.84 Hz, 1H, Ar-H), δ2.69 (s, 3H, CH_3), δ2.47 (s, 3H, CH_3); ^{13}C-NMR: δ194.15 (C=O), δ168.66 (C-8), δ155.50 (C-3), δ130.56 (C-5), δ128.83 (C-6), δ122.70 (C-4), δ120.83 (C-9), δ110.10 (C-7), δ24.79 (CH_3), δ14.77 (CH_3).

Synthesis of 3-methyl-5-acetyl-7-nitro-1,2-benzisoxazole (4): Nitration of 3-methyl-5-acetyl-1,2-benzisoxazole (17.5g, 0.1M) in nitrating mixture [conc. H_2SO_4 (30mL) and conc. HNO_3 (7mL)]. After addition was complete, the reaction mixture was warmed to room temperature and stirred. After the starting material was consumed as indicated by TLC the reaction mixture was poured slowly on ice with constant shaking. The resulting yellow solid was filtered and dried. Yield 20.40g, 86.44%, MP 66ºC.

Synthesis of 3-methyl-5-acetyl-7-amine-1,2-benzisoxazole (5): The appropriate 3-methyl-5-acetyl-7-nitro-1,2-benzisoxazole (23.6g, 0.1M) and tin granules (35.6g, 0.3M) were thoroughly ground together and then concentrated HCl (100 mL) was added slowly with vigorous stirring. After the addition of acid the reaction mixture was boiled for 30 min. and then allowed to cool to room temperature. The solution was diluted with water (50mL) cooled in an ice bath and made alkaline with 20 % NaOH solution added over 5-10 min. The resulting precipitate was filtered, washed with 2M NaOH and then water. (Yield 12.3g, 64.73%), MP 136ºC and functional group test i.e. Dye test was positive.

IR: υ_{max}cm^{-1}: 3323 (-NH_2), 3261 (-NH_2), 2885-3054 (C-H str. In

benzene), 1637 (C=O), 1568 (C=N), 1615 (bend N-H); ^{1}H-NMR: δ8.36-8.39 (t, 1H, Ar-H), δ8.11-8.18 (t, 1H, Ar-H), δ4.24 (s, 2H, NH_2), δ2.67 (s, 3H, CH_3), δ2.56 (s, 3H, CH_3); MS: m/z 190, 178 , 173, 162, 160, 134, 131.

Synthesis of 3-methyl-5-(3′-phenyl prop-2′-enoyl)-7-amine-1,2-benzisoxazole (6a). Condensation of 3-methyl-5-acetyl-7-amine-1,2-benzisoxazole (1.90g, 0.01M) with benzaldehyde (1.0 mL, 0.01M) in ethyl alcohol (25 mL) using a few drops of piperidine for 40 min. The reaction mixture was cooled to 0ºC, yellow solid compound formed was washed with water. (Yield 2.10g, 75.50%), MP 92ºC and its alcoholic solution turned red with alkali and decolourised with bromine water and it gave dark red colour with conc. H_2SO_4.

IR: υ_{max}cm^{-1}: 3359 (-NH_2), 3253 (-NH_2), 2840-3067 (C-H str. In benzene), 1734 (C=O). ^{1}H-NMR: δ8.79, (d, *J*=5.96, 1H, C=O-C-H=C-H), δ8.47, (t, 1H, C=O-C-H=C-H), δ8.21 (d, *J*=8.08 Hz, 1H, Ar-H), δ8.07 (d, *J*=9.84 Hz, 1H, Ar-H), δ7.45-7.82 (m, 4H, Ar-H), δ7.03 (d, *J*=11.12 Hz,1H, Ar-H), δ4.22 (s, 2H, NH_2), δ2.69 (s, 3H, CH_3); ^{13}C-NMR: δ198.31 (C=O), δ160.61 (C-8), δ150.69 (C-9), δ141.79 (ethylene CH), δ139.32 (C-1′), δ136.06(C-5), δ133.96 (C-7), δ131.80 (C-3′), δ129.27 (C-5′), δ127.39 (C-4′), δ126.97 (C-2′), δ125.98(C-6′), δ125.18 (C-9), δ123.09 (ethylene CH), δ119.03 (C-6), δ114.43 (C-4), δ14.78 (CH_3); MS: m/z 279, 262, 247, 184, 179, 164, 160, 136.

In the same way, other chalcones 3-methyl-5-(3′-aryl prop-2′-enoyl)-7-amine-1,2-benzisoxazoles **(6b-j)** were prepared.

Synthesis of 3-methyl-5-(3′-phenyl-1*H*-pyrazol-5-yl)-7-amine-1,2-benzisoxazole (7a). A mixture of 3-methyl-5-(3′-phenyl prop-2′-enoyl)-7-amine-1,2-benzisoxazole (2.78g, 0.01M), hydrazine hydrate (0.5g), ethyl alcohol (15mL) and KOH (0.4g) was refluxed on water bath for 4hours. It was cooled and acidified with glacial acetic acid (1.5mL) and was poured on ice-cold water (50mL), dried and crystallised with aqueous alcohol. Yield 63%, MP 126ºC. It did not give dark red colour with conc. H_2SO_4.

IR: υ_{max}cm^{-1}: 3243 (-NH_2), 3198 (-NH_2), 3064-2839 (C-H str. In benzene), 1693 (C=N). ^{1}H-NMR: δ8.36-6.87(7 H, Ar-H), δ6.98 (s, 1H, Pyrazole), δ11.69 (s, 1H, NH-pyrazole), δ4.89 (s, 2H, NH_2), δ2.55 (s, 3H, CH_3); ^{13}C-NMR: δ160.61(Pyrazole), δ159.0(Pyrazole), δ160.5 (C-3), δ147.3 (C-8), δ136.0 (C-5), δ132.4 (C-1′), δ131.8 (C-3′), δ130.2 (C-5′), δ129.4 (C-4′), δ125.6 (C-7), δ 125.9 (C-2′), δ123.6 (C-6′), δ121.1 (C-9), δ115.3 (C-6), δ110.8 (C-4), δ98.3 (Pyrazole), δ 20.5 (CH_3); MS: m/z 291, 274, 147, 143.

Following the above procedure, other 3-methyl-5-(3′-aryl-1*H*-pyrazol-5′-yl)-7-amine-1,2-benzisoxazoles (7b-j) were prepared. The characterization data of these compounds are summarised in Table-1.

Table-1
Characterization data of 3-methyl-5-(3′-aryl-1*H*- pyrazol-5-yl)-7-amine-1,2-benzisoxazoles (7a-j)

Comp	R	Molecular formula	Mol. Wt.	MP C	Yield (%)	Found (Calculated) %		
						C	H	N
7a	C_6H_5	$C_{17}H_{14}N_4O$	290.3	126	63	70.33 (72.12)	4.86 (4.90)	19.30 (19.05)
7b	4-ClC_6H_4	$C_{17}H_{13}ClN_4O$	334.7	110	68	62.87 (63.40)	4.03 (4.21)	17.25 (18.30)
7c	2-$NO_2C_6H_4$	$C_{17}H_{13}N_5O_3$	335.3	122	65	60.89 (61.80)	3.91 (4.10)	20.89 (22.30)
7d	4-$NO_2C_6H_4$	$C_{17}H_{13}N_5O_3$	335.3	112	70	60.89 (63.05)	3.91 (4.00)	20.89 (19.69)
7e	4-OHC_6H_4	$C_{17}H_{14}N_4O_2$	306.3	128	67	66.66 (69.78)	4.61 (4.65)	18.29 (19.57)
7f	2-OHC_6H_4	$C_{17}H_{14}N_4O_2$	306.3	133	55	66.66 (67.21)	4.61 (4.60)	18.29 (17.98)
7g	2-C_4H_3O	$C_{15}H_{12}N_4O_2$	280.2	166	58	64.28 (63.13)	4.32 (4.56)	19.99 (19.20)
7h	2-$CH_3OC_6H_4$	$C_{18}H_{16}N_4O_2$	320.3	101	56	67.49 (69.56)	5.03 (5.32)	17.49 (17.50)
7i	4-$CH_3OC_6H_4$	$C_{18}H_{16}N_4O_2$	320.3	98	48	67.49 (66.98)	5.03 (4.90)	17.49 (19.82)
7j	6-$CH_3OC_6H_4$	$C_{18}H_{16}N_4O_2$	320.3	109	62	67.49 (70.26)	5.03 (5.10)	17.49 (16.36)

Synthesis of 3-methyl-5-(3'-phenyl-1*H*-pyrazol-5'-yl)-7-amine-(β-D-glucopyranosyl)-1,2-benzisoxazole (9a). It was prepared from 3-methyl-5-(3'-phenyl-1*H*-pyrazol-5'-yl)-7-amine-1,2-benzisoxazole (2.90g, 0.01M) refluxed with tetra-*O*-acetyl glucuropyranosyl bromide (TAGBr) (3.0g, 0.01M) in presence of tetra butyl ammonium bromide (PTC) using dichloromethane as a solvent. The deprotection of above obtained compound 3-methyl-5-(3'-phenyl-1*H*-pyrazol-5'-yl)-7-amine-(β-D-2,3,4,6-tetra – *O* - acetyl glucopyranosyl)-1,2-benzisoxazole was done by sodium methoxide in methanol and filtered from ion exchange resin (Amberlite IR 120, H^+, cation exchanger) to get target molecules.

IR: $\upsilon_{max}cm^{-1}$: 3366 (str.OH), 3143 (N-H), 2929 (Ar-H str), 1634(C=N)[41]. ^{1}H-NMR: δ8.38-7.03 (m, 7H, Ar-H), δ6.93 (s, 1H, Pyrazole), δ4.51-4.47 (m, 1H in glucose), δ4.65 (s, 1H, NH), δ3.81-2.70 (6H, glucose), δ2.55 (s, 3H, CH_3); ^{13}C-NMR: δ152.0 (Pyrazole), δ151.3 (Pyrazole), δ156.7 (C-3), δ149.8 (C-8), δ131.3 (C-5), δ130.2 (C-1′), δ129.1 (C-3′), δ127.9 (C-5′), δ125.6 (C-4′), δ126.2 (C-7), δ 124.7 (C-2′), δ124.9 (C-6′), δ123.7 (C-9), δ112.2 (C-6), δ110.5 (C-4), δ99.5 (Pyrazole), δ81.3 (glucose C-1), δ73.9 (glucose C-5), δ72.3 (glucose C-3), δ71.6 (glucose C-4), δ69.4 (glucose C-2), δ63.6 (glucose C-6), δ18.4 (CH_3)[41] MS: m/z 453, 289, 274, 248, 180, 164, 143, 131[43].

Following the above procedure, other *N*-glucosides 3-methyl-5-(3'-aryl-1*H*-pyrazol-5'-yl)-7-amine-(β-D-glucopyranosyl)-1,2-benzisoxazoles (9b-j) were prepared. The characterization data of these compounds are summarized in Table-2.

Polarographic studies: Polarographic studies of 3-methyl-5-(3'-phenyl-1*H*-pyrazol-5'-yl)-7-amine-1,2-benzisoxazole and 3-methyl-5-(3'-phenyl-1*H*-pyrazol-5'-yl)-7-amine-(β-D-glucopyranosyl) -1,2-benzisoxazole were carried out using Elico CL-362 polarograph based on microprocessor operation. The electrode system consisted of dropping mercury electrode as working electrode, platinum wire as auxiliary electrode and saturated calomel electrode as reference electrode. The supporting electrolyte used was 0.1 M KCl solution.

The supporting electrolyte solution was deareated with nitrogen for 15 minutes and polarograms were recorded in DC and DPP modes. To this solution, various concentrations of ethanolic solutions of 3-methyl-5-(3'-phenyl-1*H*-pyrazol-5'-yl)-7-amine-1,2-benzisoxazole were added and polarograms were recorded for each addition.

The DC polarogram shows a distinct polarographic wave with half wave potential ($E_{1/2}$) -1.700V which matches with the literature value for heterocyclic compounds such as pyrazole group[42]. The differential pulse polarogram shows a distinct peak with peak potential -1.650V.

The supporting electrolyte solution was deareated with nitrogen for 15 minutes and polarograms were recorded in DC and DPP modes. To this solution, various concentrations of ethanolic solutions of 3-methyl-5-(3'-phenyl-1*H*-pyrazol-5'-yl)-7-amine-(β-D-glucopyranosyl)-1,2-benzisoxazole were added and polarograms were recorded for each addition.

Table-2
Characterization data of 3-methyl-5-(3'-aryl-1*H*- pyrazol-5'-yl)-7-amine-(β-D- glucopyranosyl)-1,2-benzisoxazoles (9a-j)

Comp	R	Molecular formula	Mol. Wt.	Found (Calculated) %		
				C	H	N
9a	C_6H_5	$C_{23}H_{24}N_4O_6$	452.4	61.05 (61.68)	5.35 (5.56)	12.38 (13.00)
9b	4-ClC_6H_4	$C_{23}H_{23}ClN_4O_6$	486.9	56.74 (58.32)	4.76 (4.66)	11.51 (12.30)
9c	2-$NO_2C_6H_4$	$C_{23}H_{23}N_5O_8$	497.4	55.53 (56.80)	4.66 (4.45)	14.08 (14.96)
9d	4-$NO_2C_6H_4$	$C_{23}H_{23}N_5O_8$	497.4	55.53 (54.20)	4.66 (4.40)	14.08 (13.90)
9e	4-OHC_6H_4	$C_{23}H_{24}N_4O_7$	468.4	58.97 (59.30)	5.16 (5.33)	11.96 (12.60)
9f	2-OHC_6H_4	$C_{23}H_{24}N_4O_7$	468.4	58.97 (60.20)	5.16 (4.99)	11.96 (11.48)
9g	2-C_4H_3O	$C_{21}H_{22}N_4O_7$	442.1	57.01 (56.85)	5.01 (5.00)	12.06 (12.82)
9h	2-$CH_3OC_6H_4$	$C_{24}H_{26}N_4O_7$	482.4	59.74 (60.58)	5.43 (5.95)	11.61 (11.56)
9i	4-$CH_3OC_6H_4$	$C_{24}H_{26}N_4O_7$	482.4	59.74 (62.10)	5.43 (5.48)	11.61 (10.90)
9j	6-$CH_3OC_6H_4$	$C_{24}H_{26}N_4O_7$	482.4	59.74 (60.25)	5.43 (6.36)	11.61 (12.10)

The DC polarogram shows a distinct polarographic wave with half wave potential ($E_{1/2}$) -1.600V which matches with the literature value for sugar group. The differential pulse polarogram shows a distinct peak with peak potential -1.550V[42].

Results and Discussion

2,4-Diacetyl phenol (1) was synthesised as per reported work in the literature and the structure was confirmed by IR spectrum shows absorption band at 3353 cm^{-1}, which indicates the presence of phenolic –OH group and two peaks shown in the range of 1670 cm^{-1} and 1641 cm^{-1}, it proven two acetyl group in aforesaid compound. The ^{1}HNMR spectra showed phenolic proton at δ12.69 ppm and the molecular mass of compound was confirmed by ion peak at m/z 179.2. The oximinoacetyl-4-acetyl phenol (2) was prepared by the reaction with hydroxylamine hydrochloride and reflux for 1hr in ethanol and water[43]. The obtained product (2) are refluxed with DMF in presence of acetic anhydride afforded 3-methyl-5-acetyl-1,2-benzisoxazole (3). In IR and ^{1}HNMR studies it is observed that the disappearance of phenolic –OH group also in ^{13}CNMR spectra observed the peak at δ168.66 ppm and δ110.10 ppm it confirmed the cyclization and formation of compound (3). Nitration of (3) with nitrating mixture gives 3-methyl-5-acetyl-7-nitro-1,2-benzisoxazole[44] (4) which on refluxed with reducing mixture tin metal granules and conc. hydrochloric acid to yield reduced product 3-methyl-5-acetyl-7-amine-1,2-benzisoxazole[45] (5). The obtained compound 5 was confirmed by IR spectra and shown two peaks at 3359 cm^{-1} and 3253 cm^{-1}, ^{1}HNMR spectra indicates the peak of two protons at δ4.24 ppm and MS shows molecular ion peak at m/z 190.

The compounds 3-methyl-5-(3'-aryl prop-2'-enoyl)-7-amine-1,2-benzisoxazoles (6a-j) are prepared by the interaction of 5 with different aromatic and heterocyclic aldehydes using suitable solvent[46]. In IR spectrum the absorption band observed at 1734 $cm^{-1}$for >C=O group in chalcone. In ^{13}CNMR spectra two peaks observed at δ141ppm and δ123 ppm for ethylenic (-CH) and for >C=O the peak appears at δ198 ppm. The reaction of 3-methyl-5-(3'-aryl prop-2'-enoyl)-7-amine-1,2-benzisoxazoles (6a-j) with hydrazine hydrate in alcoholic KOH obtained 3-methyl-5-(3'-aryl-1*H*-pyrazol-5'-yl)-7-amine-1,2-benzisoxazoles[46] (7a-j). Mass spectra show the molecular ion peak at m/z 291. The 3-methyl-5-(3'-aryl-1*H*-pyrazol-5'-yl)-7-amine-(β-D-2, 3, 4, 6-tetra-*O*-acetyl glucopyranosyl)-1,2-benzisoxazoles (8a-j) have been prepared by Glucosylation of 3-methyl-5-(3'-aryl-1*H*-pyrazol-5'-yl)-7-amine-1,2-benzisoxazoles with tetra-*O*-acetyl glucuropyranosyl bromide using PTC and dichloromethane as a solvent. All the synthesized compounds were deprotected by Sodium methoxide in methanol to obtained target molecules 3-methyl-5-(3'-aryl-1*H*-pyrazol-5'-yl)-7-amine-(β-D-glucopyranosyl)-1,2-benzisoxazoles[47] (9a-j). The IR spectra of compound show strong band in the range of 3366 cm^{-1} due to glucosyl –OH. The ^{1}HNMR spectra show a multiplet due to the glucosyl ring protons in the ranges of δ3.81-2.70 ppm and the doublet of anomeric proton of the glucose moiety within the region of δ4.51-4.47 ppm. The ^{13}CNMR spectra show the signal for β-anomeric carbon is observed at δ81.3 ppm.

Antimicrobial activity: The antifungal screening of compounds 9a-9j were carried out against two fungi viz., *Candida albicans* and *Aspergillus niger* adopting the disc diffusion method. The comparison of results was done by using clotrimazole as a standard. The compounds 9b, 9c, 9g and 9h were active and 9a, 9d, 9e, 9f, 9i and 9j were moderately active against *A. niger.* The compounds 9a-9e, 9h and 9i were active and 9f, 9g and 9j was less active against fungi *C. albicans* at 800µg/mL concentration. Similarly, the compounds 9a-9j was screened for their antibacterial activity against *Escherichia coli* and *Staphylococcus aureus* by disc diffusion method. The standard Ciprofloxacin was used for the comparison of results. The screening result showed the entire compound active against both the bacteria tested at 800µg/mL concentration. Compounds 9b, 9e, 9h and 9j were active and 9a, 9c, 9d, 9f and 9i showed moderately active against bacteria *E. coli* and 9a, 9b, 9d, 9e, 9i and 9j were active and 9c, 9f, 9g and 9h showed less activity against bacteria *S. aureus*.

Conclusion

In this article, we have synthesized a new series of 1,2-benzisoxazoles and their derivatives like chalcones, pyrazoles, amines and their *N*-glucosides and evaluated for their antimicrobial activities. The compounds 9a-j was screened for anti-bacterial and anti-fungal activities. Compounds 9a-e, 9g, 9h and 9i were active against fungi while 9a, 9b, 9d, 9e, 9i and 9j was active against bacteria.

Thus novel benzisoxazoles derivatives can be incorporated to the family of bioactive heterocyclic compounds.

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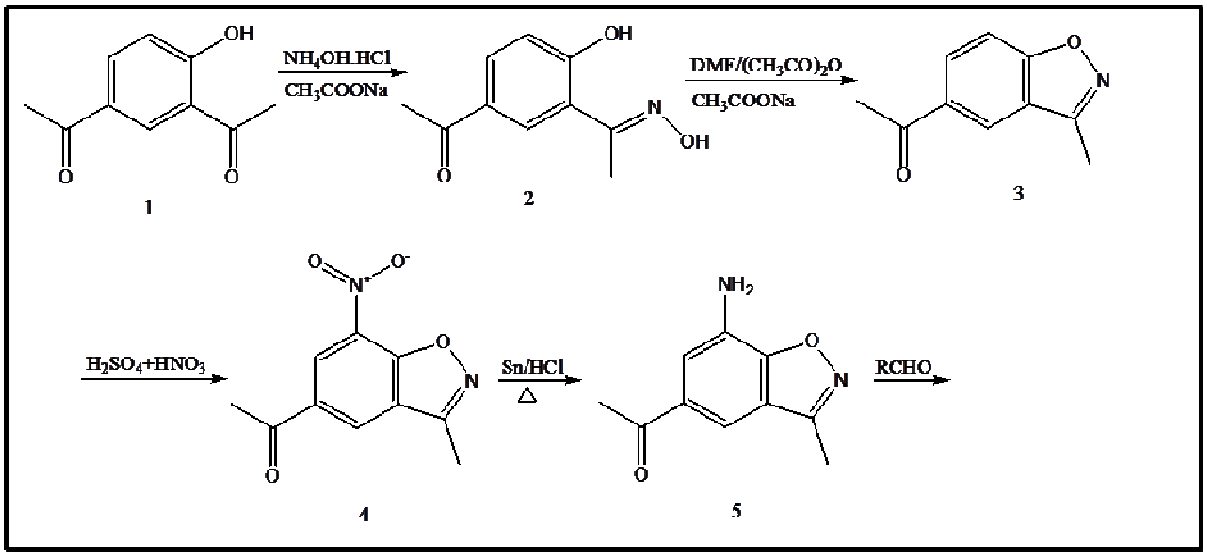
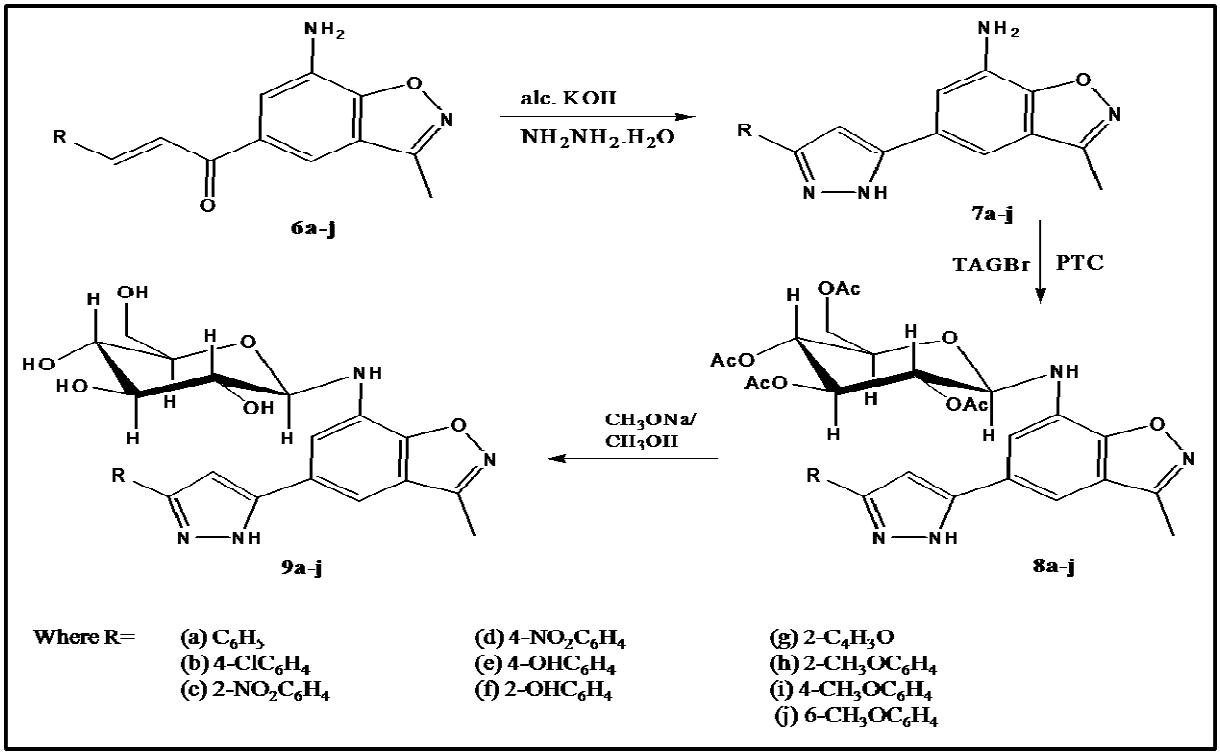


Scheme-1
Synthesis of 3-Methyl-5-acetyl-1,2-benzisoxazole



Scheme-2
Synthesis of 3-methyl-5-(3'aryl-1*H*- pyrazol-5-yl)-7-amine-(β-D-glucopyranosyl)- 1,2-Benzisoxazoles

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