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ARTICLE

A facile access to novel heterocyclic analogues of chalcone from newly synthesized ketone containing isoxazole and benzoxazinone ring

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A series of novel heterocyclic analogs of chalcone containing isoxazole and benzoxazinone ring as a prime motif was rationally designed and synthesized. There is nothing in the literature about such α, β-unsaturated ketones. These compounds were synthesized by Claisen–Schmidt condensation reaction of its ketone precursor with different aromatic aldehyde using ethanol as solvent and piperidine as a base. Our procedure offer easy access to isoxazole and benzoxazinone based chalcone derivatives providing yields in the range of 81 – 93% within 16 min under mild condition. Out of 21, 11 compounds were screened for their antibacterial activities. Among the screened compounds, compounds **5aj, 5ak** and **5bj** showed excellent antibacterial activities with MIC: 0.34, 0.59 and 0.74 mg.mL^{-1} respectively as compared to standard drug.

1. Introduction

Chalcones are important naturally occurring plant constituents and common structures and precursor in biologically active heterocycles, in particular, of benzothiazepines, pyrazolines, pyrimidines, isoxazolines flavonoids and isoflavonoids. These display a wide range of pharmaceutical, extensive agricultural and synthetic applications such as, anti-inflammatory,[1a] antifungal,[1b] antibacterial,[1c] antiviral,[1d] antiprotozoal,[1e] anticancerous,[1f] anti-tubercular,[1g] hyperglycemic agents,[1h] agrochemicals,[1i] scavenging,[1j] and PET scan imaging probes,[1k]. From synthetic prospect, chalcone derivatives are gaining more importance due to their critical role in the organic synthesis. Chalcone moiety containing nitrogen heterocycles have been reported as active compounds against herpes simplex virus-1(HSV-1)[2a] and human immunodeficiency virus -1(HIV-1)[2b]. These compounds also exhibit antiproliferative activity towards leukemia cell lines[2c].

Isoxazole derivatives exhibit a unique and interesting class of five-membered heterocycle because of their synthetic versatility and effective biological activities. Heterocyclic compounds with isoxazole moiety were found to be valuable intermediates for medicinal drugs. They are related with a broad spectrum of biological and pharmaceutical activities such as anti-inflammatory[3a], anticancer,[3b] analgesic,[3c] antiviral,[3d] antimicrobial,[3e] anthelmintic,[3f] anticonvulsant,[3g] antidepressant,[3h] potent selective agonists of the human cloned dopamine D4 receptor,[3i] antagonist activity[3j] and antinociceptive activity[3k].

The benzoxazinone moiety has been extensively studied due to prevalence of these moieties in the cores of natural products and pharmaceuticals. The benzoxazinones produced by a wide range of plants including commercially important cereal crops such as rye, maize and wheat[4]. Benzoxazinones are well-known to show a broad range of biological properties including antifeedant[5a], antimicrobial,[5b] antiphlogistic[5c] and insecticidal effects[5d]. These are known for their unique chemical motif with potential pharmaceutical activity and have been recognised as “privileged scaffold” in terms of drugs design[6].

Chalcones on integration with different biologically active frameworks have always shown comparative more potent activities; like benzfuran chalcones have exhibited both antioxidant and antibacterial activity. Benzimidazole-‘‘a heterocyclic scaffold’’ is a widely used medicinal component due to its broad spectrum of biological activities. For example, there are some recent reports on the efficient ultrasound-assisted or solvent-free Claisen–Schmidt synthesis of heterocycle-chalcone hybrids, such as quinoline chalcones,[7] pyrazole chalcones[8] and quinoline-appended ferrocenyl chalcones[9]. Heterocyclic rings especially the benzoxazinone and isoxazole rings, represent an expedient choice for the synthesis of pharmaceutical compounds with different activities and noble safety profiles. As a consequence, the remarkable bioactivity along with the unique structural features displayed by these heteroaryl-based chalcones makes them a particularly attractive target for the synthetic efforts.

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On account of these aspects and in order to expand the structure diversity for current medicinal chemistry needs, we would like to report here an efficient, and the detailed synthetic approach for the synthesis of novel isoxazole and benzoxazinone-based chalcone derivatives, namely 6-bromo-4-(3-(3-(4-substitutedphenyl)acryloyl)-4,5-dihydroisoxazol-5-yl)-4-methyl-1H-benzo[d][1,3]oxazin-2(4H)-one through Claisen–Schmidt condensation reaction of 4-((3-acetyl-4,5-dihydroisoxazol-5-yl)methyl)-6-bromo-4-methyl-1H-benzo[d][1,3]oxazin-2(4H)-one and various aromatic aldehydes with piperidine as base and ethanol as a solvent for 10-16 min under microwave irradiation providing 81–93% yield.

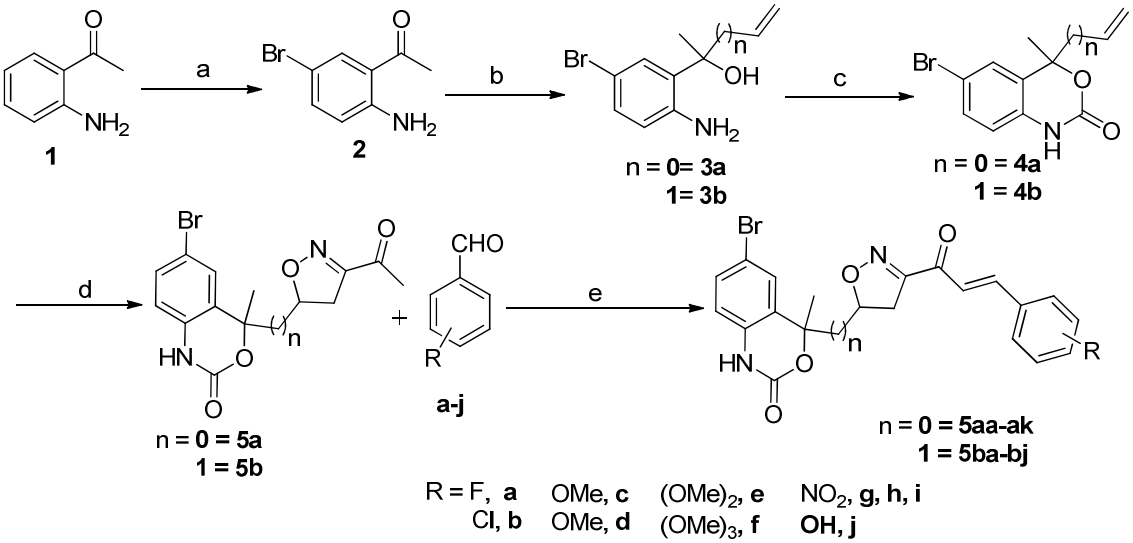
Many methods have been developed for the synthesis of chalcone derivatives, to the best of our knowledge, the synthesis of isoxazole and benzoxazinone-based chalcones under microwave irradiation has not been reported. To contribute to the development of environmentally benign organic chemistry, and in the context of our ongoing work on the 'green' synthesis of potential biologically active heterocycles,[10] we have explored the above Claisen– Schmidt condensation reaction with the aim of providing a simple method for the synthesis of novel isoxazole and benzoxazinone-based chalcone derivatives.

2. Results and discussion

2.1 Chemistry

Chalcones **5aa**-**ak** and **5ba**-**bj** were synthesized via Claisen–Schmidt condensation reaction with substituted benzaldehyde and **5a** or **5b**, piperidine in ethanol, at 80 ^{0}C for 15 min under microwave irradiation (Scheme 1). After completion of the reaction, the mixture was filtered to collect the precipitates and purification by recrystallization affords the pure chalcones **5aa**-**ak** and **5ba**-**bj** in 81–93% yield. The compounds **5aa**-**ak** and **5ba**-**bj** were obtained from corresponding ketones (**5a** and **5b**). The intermediate ketones were produced in a four-step synthesis (Scheme 1).

Scheme 1 Synthesis of Benzoxazinone-isoxazole endowed chalcone



Reagents and condition: (a) NBS, acetonitrile, 3h (b) allyl or vinylmagnesium bromide, THF, -78 ^{0}C, inert, 5h (c) carbonyldiimidazole, THF, 60 ^{0}C, 12h (d) Ceric ammonium nitrate, acetone, 60 ^{0}C, overnight (e) ethanol, piperidine, MW 80 ^{0}C, 10-16 min.

In the first step, the commercially available starting material 2-aminoacetophenone (**1**) was transformed into 5-bromo-2-aminoacetophenone (**2**) by using N-bromosucciniimide in acetonitrile at 0 ^{0}C in high yields (92%)[11]. Then compound **2** was treated with vinyl or allylmagnesium bromide to furnish corresponding products **3a** or **3b** respectively via Grignard reaction in excellent yield (95%). Next step of the synthesis was the conversion of compound **3a** or **3b** to their corresponding products **4a** or **4b** respectively using carbonyldiimidazole in THF in 96% yield[12]. They were then treated with ceric ammonium nitrate and acetone to obtain compounds **5a** or **5b** which were treated with various substituted aldehydes in ethanol/piperidine at 80 ^{0}C to obtain the final compounds **5aa**-**ak** and **5ba**-**bj** in good to excellent yields (81–93%)[13]. To find optimum protocol for the transformation various conditions for a prototypical reaction were explored as shown in Table 1.

Initially, the reaction was carried out in methanol and ethanol in the presence of NaOH, KOH at 60 ^{0}C and in *t*-BuOK at 80 ^{0}C for 6h (Table 1, entries 1, 2, 3). The reaction did not proceed, instead some decomposed products were obtained. Then different bases were examined and it was observed that pyridine promoted the reaction in low yields (45%, Table 1, entry 4). Among the bases screened, piperidine was found to be the most effective catalyst. The

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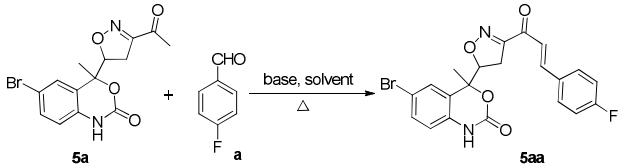
influence of solvent system was then evaluated. *tert*-Butanol furnished the desired product in low yield (55%, Table 1, entry 5).

Table 1. Optimization of base and solvent.[a]



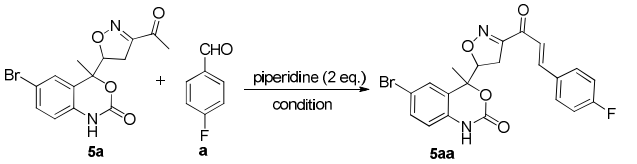
Entry	Solvent	Base	Base equiv.	Temp. (^{0}C)	Time (h)	Yield[b] (%)
1	MeOH	NaOH	1	60	6	-
2	EtOH	KOH	1	60	6	-
3	t-BuOH	t-BuOK	1	80	5	-
4	EtOH	Pyridine	2	reflux	6	45
5	t-BuOH	Piperidine	2	reflux	7	55
6	EtOH	Piperidine	1	reflux	8	67
7	EtOH	Piperidine	1.5	reflux	8	72
8	**EtOH**	**Piperidine**	**2**	**reflux**	**6**	**85**
9	EtOH	Piperidine	3	reflux	8	85

[a]General condition: 4-(3-acetyl-4,5-dihydroisoxazol-5-yl)-6-bromo-4-methyl-1H-benzo[d][1,3]oxazin-2(4H)-one **5a** (1 mmol), 4-fluro benzaldehyde **a** (1 mmol).
[b]Isolated yield.

To our delight, by using ethanol as solvent, moderate yield was achieved (67%, Table 1, entry 6). Regarding the influence of the solvent in this reaction, EtOH was achieved as optimal solvent (Table 1, entry 8). It can be easily rationalized that use of ethanol enhances the solubility of reagents and thus increased the product yield. It is important to note that when the amount of piperidine is increased from 1 to 1.5 eq., an increase in the yield was observed to 72% (Table 1, entry 7). Further increase in the amount of piperidine to 2 eq. the desired product **5aa** was delivered in 85% yield (Table 1, entry 8). However, when 3 eq. of piperidine was taken in the reaction system, the product yield remained unchanged (85%, Table 1, entry 9).

With the optimal reaction condition regarding base and solvent in hands, we thought to explore different reaction conditions and temperature for the reaction (Table 2). The physical grinding of substrates with piperidine in a mortar and pestle for 1 h (monitored by TLC) without any solvent at room temperature did not result in appreciable yield of the product (Table 2, entry 1). The same reaction took around 6 h under conventional heating (80 ^{0}C) to afford **5aa** in 85% yield as discussed above (Table 2, entry 2). However, increase or decrease in temperature of the reaction system to 70 or 90 ^{0}C, a decrease in the yield was observed (Table 2, entry 3, 4). On the other hand, when the same reaction is done under solvent free thermal heating condition, the desired product was furnished in moderate yield (67% Table 2, entry 5).

Table 2. Optimization of reaction temperature and conditions.[a-e]



Entry	Condition	Temp. (^{0}C)	Time (min)	Yield(%)[e]
1	grinding	Rt	1h	-[d]
2	CH[b]	80	6h	85
3	CH[b]	70	8h	71
4	CH[b]	90	6h	80
5	CH[b]	60	8h	67[d]
6	MW[c]	70	20	69
7	MW[c]	80	15	90
8	MW[c]	85	15	88
9	MW[c]	90	15	84
10	**MW[c]**	**80**	**12**	**92**
11	MW[c]	80	10	79
12	MW[c]	80	12	73[d]

[a]General condition: 4-(3-acetyl-4,5-dihydroisoxazol-5-yl)-6-bromo-4-methyl-1H-benzo[d][1,3]oxazin-2(4H)-one **5a** (1 mmol), benzaldehyde **a** (1 mmol); [b]Conventional heating; [c]Anton Paar Monowave 300 reactor; [d]Reaction carried out in neat condition; [e]Isolated yield.

For comparison, the same reaction was subjected to microwave irradiation and effect of irradiation time and temperature on the efficiency and yield were briefly investigated. First, the effect of irradiation temperature was briefly examined. It was observed that yield of **5aa** at 70 ^{0}C (69%) was abruptly increased to 90% by increasing the irradiation temperature up to 80 ^{0}C, but it became lower when the microwave temperature exceeded 80 ^{0}C (Table 2, entries 6, 7, 8, 9). Thus, 80 ^{0}C was chosen as the optimum irradiation temperature for **5aa**. Then the effect of microwave irradiation time was studied, and 12 min was found to be the optimized reaction time for the formation of **5aa**. So, we led to conclude that microwave irradiation at 80 ^{0}C for 12 minutes was the optimal condition for the reaction to get best yield of the product. Using microwave irradiation, an improvement in terms of yields and reaction time was achieved,

On the basis of these optimized conditions, various combinations of novel ketone **4a** and **4b** and substituted benzaldehydes were employed for the Claisen–Schmidt condensation reaction, allowing the reaction to be completed within 16 min in good yields of 81–93% and also high purities (Table 3).

The purified products were characterized by FTIR, ^{1}H NMR, ^{13}C NMR, and HRMS. The IR spectra of compounds showed the characteristic band: the ν(C=O) peak observed at 1702 cm^{-1} shifts to a lower frequency of 1636–1655 cm^{-1} in chalcones. This is due to the conjugation of the π-electrons of the benzene moiety with those of the ethylene moiety in the enone linkage.

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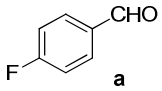
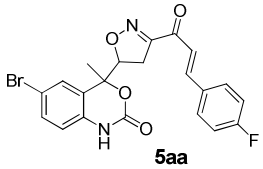
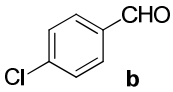
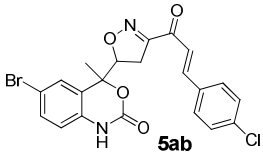
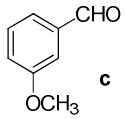
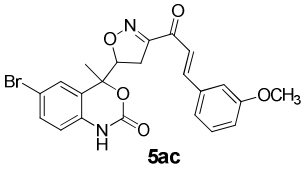
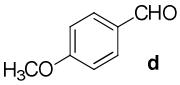
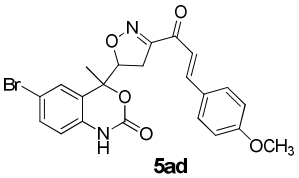
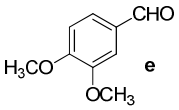
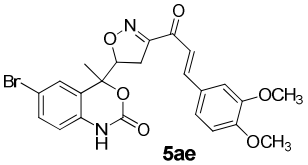
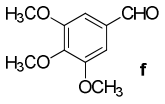
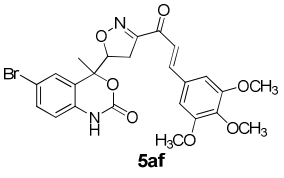
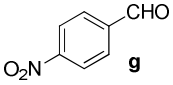
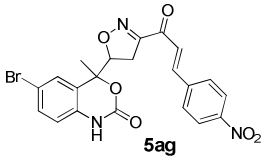
Table 3. List of the synthesized benzoxazinone-isoxazole endowed chalcone derivatives.[a,*]

Entry	ketone	aldehyde	product	Time (min)	Yield (%)[b]
1	**5a**	 **a**	 **5aa**	11	92
2	**5a**	 **b**	 **5ab**	12	89
3	**5a**	 **c**	 **5ac**	12	87
4	**5a**	 **d**	 **5ad**	15	85
5	**5a**	 **e**	 **5ae**	15	85
6	**5a**	 **f**	 **5af**	16	83
7	**5a**	 **g**	 **5ag**	10	93

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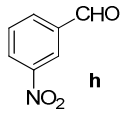
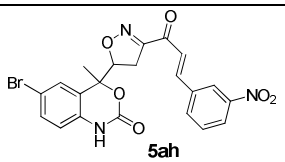
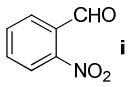
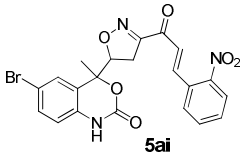
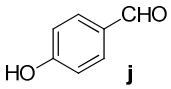
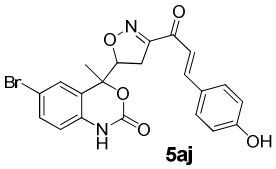
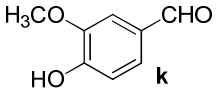
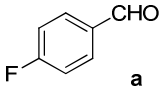
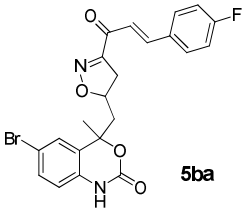
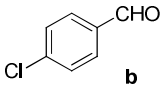
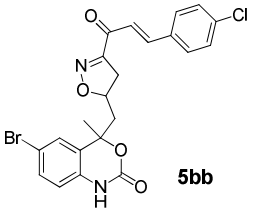
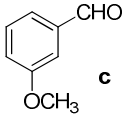
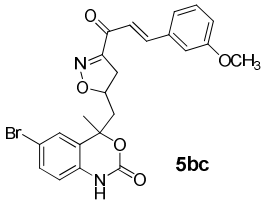
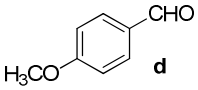
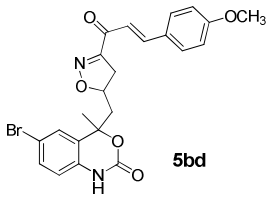
8	**5a**	 **h**	 **5ah**	10	88
9	**5a**	 **i**	 **5ai**	10	84
10	**5a**	 **j**	 **5aj**	14	85
11	**5a**	 **k**	 **5ak**	14	87
12	**5b**	 **a**	 **5ba**	11	86
13	**5b**	 **b**	 **5bb**	12	84
14	**5b**	 **c**	 **5bc**	15	86
15	**5b**	 **d**	 **5bd**	15	82

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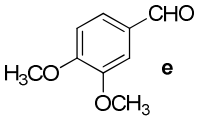
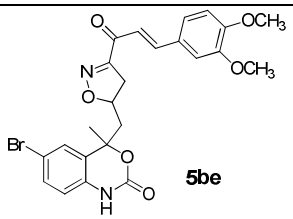
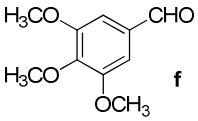
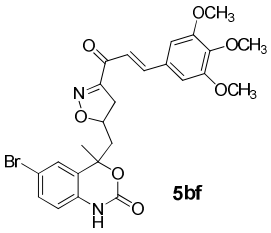
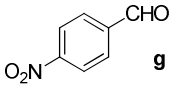
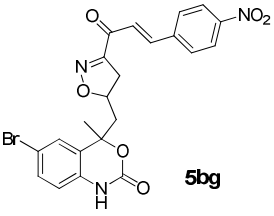
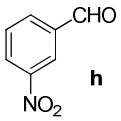
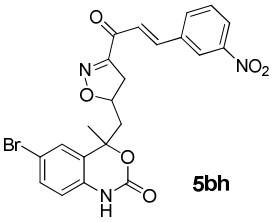
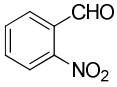
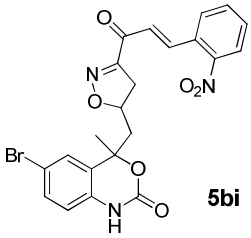
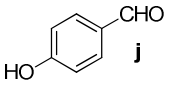
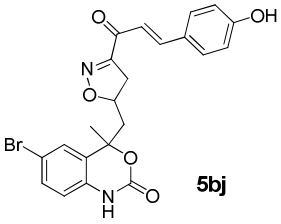
16	**5b**	 **e**	 **5be**	18	80
17	**5b**	 **f**	 **5bf**	18	75
18	**5b**	 **g**	 **5bg**	10	87
19	**5b**	 **h**	 **5bh**	10	89
20	**5b**	 **i**	 **5bi**	10	88
21	**5b**	 **j**	 **5bj**	14	86

[a]General condition: 4-(3-acetyl-4,5-dihydroisoxazol-5-yl)-6-bromo-4-methyl-1H-benzo[d][1,3]oxazin-2(4H)-one **5a** (1 mmol), benzaldehyde (**a-k)** (1 mmol), piperidine (2 mmol), ethanol; *Anton Paar Monowave 300 reactor. Irradiation Power: 850 W; Ramp time: 1 min. 70 °C; holding temp: 80 ^{0}C; [b]Isolated yield.

2.2. Biological activity

The newly synthesized compounds were evaluated for their *in vitro* antibacterial activity against *S. aureus* and *B. subtilis* as examples of Gram-positive bacteria and *E. coli* and *S. flexneri* as examples of Gram-negative bacteria. Out of these (Table 4) showing only 11 most potent antibacterial active compound.

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2.2.1. Antibacterial activity. As seen in the Table 4, compound **5aj** (MIC: 0.34 mg.ml^{-1}) and compounds **5af, 5ag, 5ak, 5bg, 5bj** (MIC: 1.78, 1.42, 0.59, 1.36, 0.74 mg.ml^{-1}) have shown better antibacterial activity than positive controls against *S. aureus*. In case of *B. subtilis*, all compounds have shown lower activity than ampicillin. Moreover, compounds **5af, 5aj, 5ak, 5bg, 5bj** (MIC: 3.10 - 3.12 mg.ml^{-1}) were found to be equally potent as cefadroxil against *B. subtilis*. Compounds **5af**, **5ag**, **5aj**, **5ak**, **5bg**, **5bj** (MIC: 0.34 – 1.45 mg.ml^{-1}) have shown better activity than positive controls against *E. coli* while compounds **5af** and **5ag** (MIC: 1.45 and 1.53 mg.ml^{-1}) have shown approx. equal activity to the reference drugs. Compounds **5aj** and **5bj** have shown admirable antibacterial activity (MIC: 0.95 and 1.55 mg.ml^{-1}) against *Shigella flexneri* compared to ampicillin and cefadroxil. Thus, the hydroxy group on ring D carrying a compounds **5aj** and **5bj** have shown excellent (MIC: 0.34 mg.ml^{-1}) to good (MIC: 1.78 mg.ml^{-1}) activity against *S. aureus*. Against *B. subtilis* also, the methoxy group carrying compounds **5af** and **5bf** have shown (MIC: 3.10 - 3.75 mg.ml^{-1}) antibacterial activity to the standard drugs. Against *E. coli*, compounds **5af**, **5ag**, **5aj**, **5ak**, **5bg** and **5bj** have shown excellent activity compared to ampicillin and cefadroxil depending on the methoxy and hydroxyl group. Against *Shigella flexneri*, all compounds have shown low activity compared to the standard drugs without depending on functional groups on aromatic ring except **5aj** and **5bj**.

Table 4. Minimum inhibitory concentration (MIC, mg.mL^{-1}) of some newly synthesized compounds against bacterial strains

Compound	Gram-positive		Gram-negative	
	S.aureus	*B.subtilis*	*E.coli*	*S.flexneri*
5af	**1.78**	**3.10**	**1.45**	2.23
5ag	**1.42**	3.42	**1.53**	2.25
5ah	6.2	6.45	7.8	6.5
5ai	7.1	7.5	7.	7.5
5aj	**0.34**	**3.12**	**0.34**	**0.95**
5ak	**0.59**	**3.12**	**0.59**	1.75
5bf	2.12	3.75	2.25	2.15
5bg	**1.36**	**3.12**	**1.32**	2.26
5bh	6.8	7.1	7.2	7.5
5bi	7.7	8.2	7.9	8.5
5bj	**0.74**	**3.10**	**0.72**	**1.55**
Ampicillin	0.78	0.39	1.56	0.78
Cefadroxil	1.56	3.12	0.78	1.56

3. Conclusions

In conclusion, we have developed a convenient and simple method for the preparation of novel bioactive benzoxazinone-isoxazole-clubbed chalcone derivatives via the reaction of new ketone derivatives with substituted aldehydes in the presence of piperidine. The rapid conversion, excellent yield, and operational simplicity are great advantages of the proposed method. Furthermore, the reaction conditions employed include microwave irradiation as a source of energy, in short reaction time, contemplating the principles of green chemistry. Compounds **5aj**, **5ak** and **5bj** exhibited the highest biological activity toward Gram-positive *S. aureus* and Gram-negative *E.coli* bacteria with MIC: 0.34, 0.59 and 0.74 mg.mL^{-1} respectively as compare to standard drug.

4. Experimental

All the required chemicals were purchased from Merck and Aldrich Chemical Company. Pre-coated aluminium sheets (silica gel 60 F254, Merck) were used for thin-layer chromatography (TLC) and spots were visualized under UV light.IR spectra were recorded with KBr on Thermo Nicolet FT-IR spectrophotometer. ^{1}H NMR and ^{13}C NMR spectra were recorded respectively on Bruker Spectrospin DPX 500 MHz and Bruker Spectrospin DPX 125 MHz spectrometer using $CDCl_3$ as a solvent and trimethylsilane (TMS) as an internal standard. Splitting patterns are designated as follows; s = singlet, d = doublet, m = multiplet, br = broad. Chemical shift (δ) values are given in ppm. High-resolution mass spectra (HRMS) were obtained on a Brüker micrOTOF™-Q II mass spectrometer (ESIMS).

4.1. Microwave Irradiation Experiment.

All microwave experiments were carried out in a dedicated Anton Paar Monowave-300 reactor®, operating at a frequency of 2.455 GHz with continuous irradiation power of 0 to 850 W. The reactions were performed in a G-10 Borosilicate glass vial sealed with Teflon septum and placed in a microwave cavity. Initially, microwave of required power was used and temperature was being ramped from room temperature to a desired temperature. Once this temperature was attained, the process vial was held at this temperature for required time. The reactions were continuously stirred. Temperature was measured by an IR sensor. After the experiments a cooling jet cooled the reaction vessel to ambient temperature.

4.2. General procedure for synthesis of compounds

4.2.1. Preparation of compound 5a and 5b: A mixture of 6-bromo-4-methyl-4-vinyl-1H-benzo[d][1,3]oxazin-2(4H)-one (**4a**) or 4-allyl-6-bromo-4-methyl-1H-benzo[d][1,3]oxazin-2(4H)-one (**4b**) (5 mmol) and ammonium cerium(IV) nitrate (2.74 g, 5 mmol) in acetone (20 ml) was stirred under reflux for 12 h. The reaction mixture was extracted with EtOAc (50 ml) and washed with aq. $NaHCO_3$ solution (2 ×30 ml), saturated aq. NaCl (2 ×30 ml), and water (2 ×30 ml). The ethereal solution was dried over Na_2SO_4 and concentrated in a vacuum. The resulting solid was chromatographed on silica gel. Elution with hexane– EtOAc (8:2) gave 4-(3-acetyl-4,5-dihydroisoxazol-5-yl)-6-bromo-4-methyl-1H-benzo[d][1,3]oxazin-2(4H)-one (**5a**) and 4-((3-acetyl-4,5-dihydroisoxazol-5-yl)methyl)-6-bromo-4-methyl-1H-benzo[d][1,3]oxazin-2(4H)-one (**5b)** as white solid (70%).

4.2.2. Preparation of Chalcones 5aa-ak and 5ba-bj: Dissolve 1 mmol of the substituted aldehyde and 1 mmol of the **5a** or **5b** in 10 mL of ethanol in a G-10 process vial capped with Teflon septum. 2 mmol of piperidine then added to the reaction vial using a micropipette. After a pre-stirring for one minute, the vial was subjected to microwave irradiation with the initial ramp time of 1 minute at 70 °C. The temperature was then raised to 80 ^{0}C with the holding time of 10-16 min. After completion of the reaction, cool the mixture in an ice-water bath until crystal formation is complete. Add 10 mL of ice-cold water to the flask and vacuum filter. Wash the crystals with water followed by ice-cold ethanol. Allow to air-dry. Recrystallize from 95% ethanol if necessary to afford the pure chalcones **5aa**-**ak** and **5ba**-**bj** in 81–93% yield.

4.3. Characterization data

4.3.1. (*E*)-6-bromo-4-(3-(3-(4-fluorophenyl)acryloyl)-4,5-dihydroisoxazol-5-yl)-4-methyl-1H-benzo[d][1,3]oxazin-2(4H)-one (5aa)

Yield: 0.42g (92%) as yellow solid; MP 212-214 °C; ^{1}H NMR (500 MHz, $CDCl_3$, ppm): δ 1.78 (s, 3H), 2.87 – 2.94 (m, 1H), 3.0 – 3.06 (m, 1H), 3.89 – 3.97 (m, 1H), 6.78 (d, *J* = 14.0 Hz, 1H), 7.24 (s, 1H), 7.34 (d, *J* = 9.5 Hz, 1H), 7.40 (d, *J* = 9.5 Hz, 2H), 7.48 (d, *J* = 6.5 Hz, 2H), 7.56 (d, *J* = 8.5 Hz, 1H), 7.94 (d, *J* = 14.0 Hz, 1H), 9.67 (s, br, D_2O exchangeable, 1H); ^{13}C NMR (125 MHz, $CDCl_3$, ppm): δ 22.1, 26.7, 84.9, 86.1, 117.0, 120.9, 126.9, 127.5, 129.1, 130.3, 130.9, 131.5, 132.4, 134.0, 138.0, 138.3, 138.8, 153.3, 189.3; FTIR (KBr, ν = cm^{-1}): 1638, 1716, 3331; HRMS (ESI+): m/z calcd. for $C_{21}H_{16}BrFN_2NaO_4$ $[M+Na]^+$: 481.0175 found : 481.0171.

4.4. Biological activity

4.4.1. Antibacterial assay. Minimum inhibitory concentration (MIC) is the lowest concentration of an antimicrobial compound that inhibits the visible growth of a microorganism. MIC values of the compounds against bacterial isolates were determined on the basis of the micro-well dilution method following National committee for clinical laboratory standards (NCCLS) recommendations.[14] In this method we made stock of chemically synthesized compounds at a concentration of 10 mg.mL^{-1} in DMSO, which was further converted to working solution of concentration 1 mg.mL^{-1}. Using a micropipette, 100 µl of media was dispensed into all wells of a pre-sterilized micro titre plate (experiment was done in triplicate). Two fold serial dilutions (100, 50, 25, 0.78125 mg.mL^{-1}) were carried out from the well #1 to the well #10 and excess media (100µl) was discarded from the last well (#10). A liquid broth culture of the test organism was grown to log phase in Luria Bertani broth (LB broth) for 24 h at 37 ^{0}C. The optical density of the liquid culture was determined at 600 nm and diluted in such a way that each well received 107 cfu.ml^{-1} of bacterial culture. Appropriate positive and negative controls were also included in the study. The positive control contained only microbial cells whereas the negative control contained only standard drug solution (ampicillin and cefadroxil). All experimental procedures were performed under sterile conditions using bio-safety hood and microtitre plates were incubated at 37 ^{0}C for 24 h.

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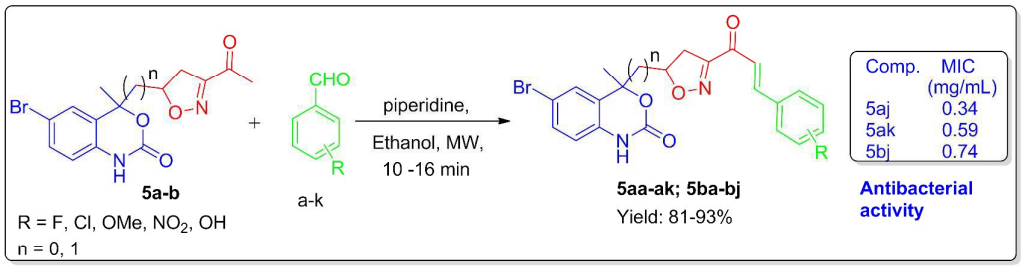

A series of novel heterocyclic analogs of chalcone containing isoxazole and benzoxazinone ring as a prime motif was rationally designed and synthesized. There is nothing in the literature about such α, β-unsaturated ketones. These compounds were synthesized by Claisen–Schmidt condensation reaction of its ketone precursor with different aromatic aldehyde using ethanol as solvent and piperidine as a base. Our procedure offer easy access to isoxazole and benzoxazinone based chalcone derivatives providing yields in the range of 81 – 93% within 16 min under mild condition. Out of 21, 11 compounds were screened for their antibacterial activities. Among the screened compounds, compounds **5aj, 5ak** and **5bj** showed excellent antibacterial activities with MIC: 0.34, 0.59 and 0.74 mg.mL^{-1} respectively as compared to standard drug.

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