MJBAS
PROMOTING RESEARCH & KNOWLEDGE

Mediterranean Journal of Basic and Applied Sciences (MJBAS)
Volume 7, Issue 1, Pages 133-140, January-March 2023

Synthesis of Some Novel Isoxazoline from Sec 3-Amino Pyridazine Chalcones and Their Antimicrobial Studies

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DOI: Under Assignment

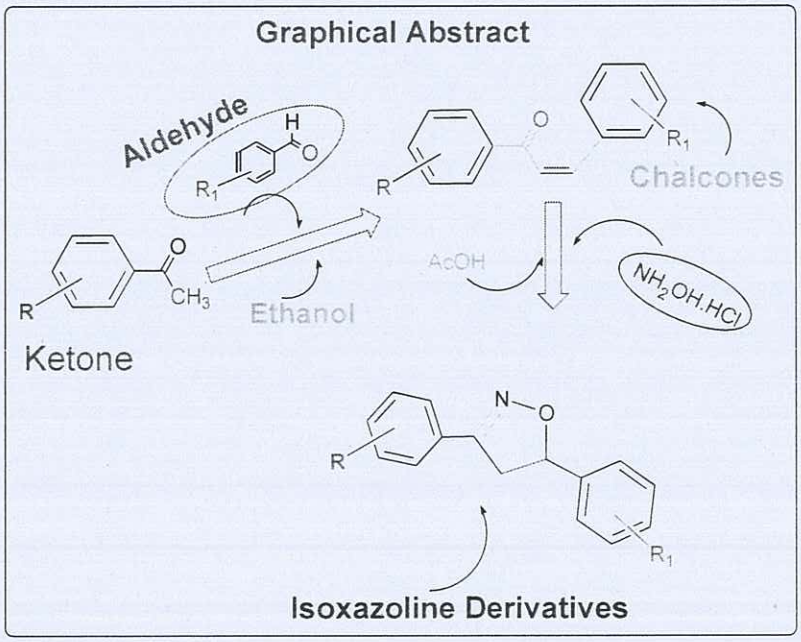
Article Received: 27 January 2023 *Article Accepted: 28 February 2023* *Article Published: 30 March 2023*

ABSTRACT

Different novel isoxazolines were synthesized by the cyclization of secondary amino derivatives with pyridazine as a substituted intermediate in the presence of hydroxylamine hydrochloride. Isoxazoline is an important class of nitrogen and oxygen-containing five-membered heterocyclic compounds having great medicinal importance. Many heterocyclic, pharmaceutically active compounds have been synthesized using it as an intermediate. Due to this activity, it is found useful in the treatment of anti-fungal, anti-bacterial, anti-cancer, anti-amoebic, and anti-inflammatory agents. The structures of the isoxazoline derivatives were confirmed by spectral analysis. The synthesized isoxazoline shows moderate activity against bacteria and fungus.

Keywords: Anti-bacterial: Antifungal chalcones; Isoxazoline.

1. Graphical Abstract



2. Introduction

The di-hydro derivatives of isoxazoles are called isoxazolines. The classical synthesis of isoxazoline compounds involves the base-catalyzed condensation of substituted aromatic ketone and various aromatic aldehydes to yield an unsaturated compound known as Chalcones, which can then be cyclized with hydroxylamine hydrochloride in an alkaline medium to yield the corresponding isoxazoline derivatives. In recent years, attention has increasingly been given to the synthesis of isoxazoline derivatives as a source of new antibacterial agents. The synthesis of novel isoxazoline derivatives remains a main focus of medicinal research. Heterocycles, like isoxazoline, have

various methods of preparation. Isoxazolines were synthesized from chalcones, representing a class of compounds of great biological importance. Isoxazoline possesses a broad spectrum of biological activity [1], [2]. It serves as an important building block for the synthesis of biologically active molecules and serves as a corrosion inhibitor for fuels and lubricants [3], [4]. Its derivatives are also effective in thrombosis animal models. Snjeev kumar and Raj K. Singh, synthesized of some 2-N-phenylamino 5-(3, 4-Dichlorophenyl)-1,3,4-Oxadiazole Derivatives Towards Antimicrobial Activity [5]. S. M. Abdulghani, Muna S. Al-Rawi and colleagues created 1,2,4- triazole Derivatives with expected biological activities [6]. A number of isoxazoline derivatives have been used as photo sensitizers and super sensitizers. Isoxazole derivatives have been known for hundreds of years, but research into their chemistry has been slow. In recent years, the synthesis of novel isoxazolines has remained a main focus of medicinal research [7]. A. P. Rajput and P. D. Girase synthesized a series of new isoxazoles by using propenones with hydroxyl amine hydrochloride. Newly synthesized compounds have been confirmed on the basis of elemental analysis and spectral studies. These compounds were screened for their in vitro antimicrobial activities. Some of the compounds exhibited encouraging results [8].

Isoxazoline scaffolds have also been discovered in diabetics [9]. It is a well-known broad-spectrum antibiotic that has been used to treat antibacterial [10], anticancer [11], anti-proliferative [12], and antifungal [13] conditions. Antimicrobial [14] and anti-inflammatory [15] agents. They are also analgesic [16], antimicrobial [17], antitumor [18], and antidepressant [19].

Mayur R. Adokar and Mangesh V. Kadu synthesized different Isoxazolines via cyclization of substituted Chalcone intermediate in the presence of hydroxylamine hydrochloride [20]. Isoxazoline derivatives have played a crucial role as intermediates in the organic synthesis of number of heterocyclic pharmacological active compounds. Shrikrishna D. Tupare et al., had synthesized 6- (3- (4, 5-di-hydro- 5- (4-Methoxyphenyl) isoxazol-3-yl) phenyl amino)pyridazin-3(2*H*)-one [21].

As a result, we were able to synthesize some isoxazoline by utilizing novel Pyridazolone amino Chalcones. The structures of these compounds have been established by spectral and elemental analysis. The melting points are uncorrected.

3. Materials and Methods

All chemicals used in this synthesis are of AR grade and used without purification.

(1) General

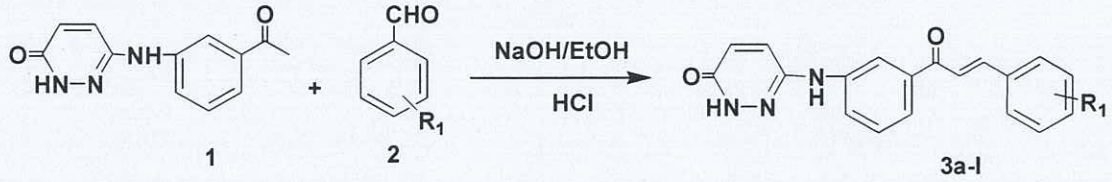
All the melting points were determined in an open capillary and are uncorrected. The purity of the compounds was determined using silica-gel-coated aluminum plates. IR spectra were recorded in KBr on a Perkin Elmer Spectrum BX series FT-IR spectrometer. ^{1}H-NMR spectra were recorded on a Bruker DRX 300 MHz NMR spectrometer using TMS as an internal standard and mass spectrum on a Joel D-300 spectrometer.

(i) Preparation of substituted Chalcones

Preparation of 6- (3- ((E)-4- (3, 4-dimethoxyphenyl) but- 3-enoyl) phenyl amino) pyridazin-3 (2H) one (3g):6-(3-acetyl phenyl amino) pyridazin-3(2H)-one (0.02 mole) and 4-chlorobenzaldehyde (0.02 mole) were

dissolved in ethanol (15 ml), and an aqueous KOH (50%, 12 ml) solution was added drop wise while stirring. The reaction mixture was stirred at room temperature and kept overnight in a bulb oven at 55-60°C. After 16–18 hrs, the reaction mixture was diluted with water and acidified with HCl (10%). The separated solid was filtered and crystallized from glacial acetic acid to give **3g**.

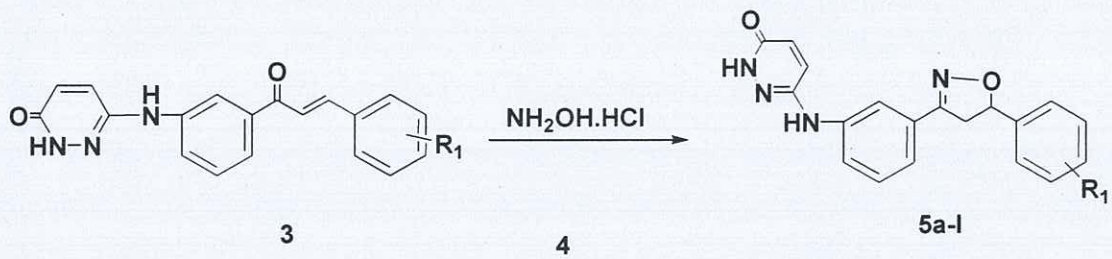
Similarly, other compounds in the series were also prepared using the same procedure. The melting points, yields, and elemental analysis of different Chalcones are listed in Table 1.



(ii) Synthesis of substituted Isoxazoline

A mixture of 6- (3- ((E) -4- (4-nitrophenyl) but- 3-enoyl) phenylamino) pyridazin -3 (2H) one(0.01mol.), hydroxylamine hydrochloride (0.01mol.) in ethanol (20ml) was refluxed. The reaction mixture was allowed to stir vigorously at 60-80^{0C}. The progress of the reaction was monitored by TLC. After 4 h of stirring, the solid obtained was separated by filtration and washed successively with water. dried and crystallized from ethanol.

Similarly, other compounds in the series were also prepared using the same procedure. The melting points, yields, and elemental analysis of different Chalcones are listed in Table 2.



4. Result and Discussion

Table 1. Physical data of synthesized Chalcones

Entry	R_1	Mol. Formula	M. Wt.	Yield(%)	Time (h)	M. p. (°C)
3a	-H	$C_{19}H_{15}N_3O_2$	317.1	65	19	192
3b	3, 4-OCH_3	$C_{21}H_{19}N_3O_4$	377.4	57	19	185
3c	4-OH	$C_{19}H_{15}N_3O_3$	333.3	56	18	155
3d	2-OH	$C_{19}H_{15}N_3O_3$	333.3	60	20	145
3e	2-Br,3,4-OCH_3	$C_{21}H_{19}BrN_3O_4$	457.3	62	17	190
3f	3-NO_2	$C_{19}H_{14}N_4O_4$	362.4	70	19	190

3g	4-Cl	$C_{19}H_{14}ClN_3O_2$	351.7	67	18	192
3h	4-NO_2	$C_{19}H_{14}N_4O_4$	362.4	60	18	182
3i	4-OCH_3	$C_{20}H_{17}N_3O_3$	347.3	56	22	138
3j	2-NO_2	$C_{19}H_{14}N_4O_4$	362.4	69	20	150
3k	3,4,5- tri- OCH_3	$C_{22}H_{21}N_3O_5$	381.3	55	18	161
3l	2, 6-di-Cl	$C_{19}H_{13}Cl_2N_3O_2$	385.7	58	19	193

Table 2. Physical data of Novel Isoxazoline Derivatives

Entry	R_1	M. Formula	Mol. Wt.	Yield(%)	Time (h)	M.p (°C)
5a	-H	**$C_{19}H_{16}N_4O_2$**	332	76	4	183
5b	3, 4 di -OCH_3	**$C_{21}H_{20}N_4O_4$**	392	80	4	155
5c	4-OH	**$C_{19}H_{16}N_4O_3$**	348	58	4	145
5d	2-OH	**$C_{19}H_{16}N_4O_3$**	348	60	4	145
5e	2-Br,3, 4-OCH_3	**$C_{21}H_{19}BrN_4O_4$**	471	62	4	190
5f	3-NO_2	**$C_{19}H_{15}N_5O_4$**	377	69	4	190
5g	4-Cl	**$C_{19}H_{15}ClN_4O_2$**	369	67	4	192
5h	4-NO_2	**$C_{19}H_{15}N_5O_4$**	377	60	4	182
5i	4-OCH_3	**$C_{20}H_{18}N_4O_3$**	362	56	4	138
5j	2-NO_2	**$C_{19}H_{15}N_5O_4$**	377	69	4	150
5k	3,4,5-tri- OCH_3	**$C_{22}H_{22}N_4O_2$**	374	55	4	161
5l	2, 6-di-Cl	**$C_{19}H_{14}Cl_2N_4O_2$**	400	58	4	193

4.1. Spectral Analysis

(a) Spectral analysis of Chalcones

i. 6-(3-((*E*)-3-(4-hydroxyphenyl) acryloyl) phenyl amino) pyridazin-3(4*H*)-one (3c): Molecular Formula = **$C_{19}H_{15}N_3O_3$**; Yield = **56** %, M.p. = **155 ^{0}C. FT-IR (KBr)**: 3226 cm^{-1} (Ar.C=C Str.), 3348 cm^{-1} (N-H Str.), 1684, 1727 cm^{-1} (2 C=O), 1581 cm^{-1} (-CH=CH-, ethenoic Str.), **^{1}HNMR(DMSO-d_6)** (δ ppm); 2.2 (d,1H), 2.7-2.8 (d,1H), 3.8 (s, 1H, NH), 7.4 (d, 2H), 7.1-7.2 (m, 4H, Ar-H), 6.83-6.90 (d, 1H), 7.4-7.5 (d,1H), 7.8-7.9(d, 2H,), 7.40-7.50 (s, 1H, NH pyridazine D_2O exchangeable), 4.8 (s, 1H pyridazine).

Mass (m/z): Calculated **333**; Found **332.5**(M^+)

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ii. 6-(3-((E)-3-(4-chlorophenyl) acryloyl) phenyl amino) pyridazin-3(2H)-one (3g): Molecular Formula = $C_{19}H_{14}ClN_3O_2$; Yield = **67** %, M.p.= **192** ^{0}**C. FT-IR (KBr)**: 3204 cm^{-1} (Ar.C=C Str.), 3347.70 cm^{-1} (N-H Str.), 1684, 1727 cm^{-1} (2 C=O), 1581 cm^{-1} (-CH=CH-, Str.), ^{1}**HNMR(DMSO-d$_6$)** (δ ppm); 2.2 (d,1H), 2.7-2.8 (d,1H), 3.8 (s, 1H, NH), 7.4 (d, 2H), 7.1-7.2 (m, 4H, Ar-H), 6.83-6.90 (d, 1H), 7.4-7.5 (d,1H), 7.8-7.9(d, 2H,), 7.40-7.50 (s, 1H, NH pyridazine D_2O exchangeable).

Mass (m/z): Calculated **351.7**; Found **352.1**(M^+)

iii. 6-(3-((E)-3-(4-methoxyphenyl) acryloyl) phenylamino) pyridazin-3 (2*H*)-one (3i): Molecular Formula = $C_{20}H_{17}N_3O_3$, Yield = **56%**, M. p. = **138^{0}C. FT-IR (KBr):** 3346.59, 3208 cm^{-1} (N-H Str.), 1676.83 cm^{-1}, 1825 cm^{-1} (2 C=O), 1560.85 cm^{-1} (-CH=CH-, str.); ^{1}**HNMR(DMSO-d$_6$),**(δppm); 2.6 (d, 1H); 2.3 (d, 1H); 3.4-3.5 (s, 3H), 4.8 (s 1H, NH) 6.88-7.95 (m, 4H, Ar-H), 6.89-7.15 (m, 4H, Ar-H), 6.83-6.90 (d, 1H J=9.8 Hz, CH pyridazine), 7.17-7.22 (d, 1H, J=9.9 Hz CH Pyridazine), 12.10 (s 1H, NH pyridazine D_2O exchangeable).

Mass;(m/z): Calculated **347.3**; Found **347.2**(M^+)

(b) Spectral analysis of isoxazolines

iv. 6-(3-(4, 5-dihydro-5-(4-hydroxy) isoxazol-3-yl) phenylamino) pyridazin-3 (2*H*)-one (3c):Molecular Formula = $C_{19}H_{16}N_4O_3$, Yield = **58%**, M. p. = **145^{0}C. FT-IR (KBr):** 3345, 3245 cm^{-1} (N-H Str.), 1665 cm^{-1} (>C=O) 1577 cm^{-1} (-CH=CH-, str.); ^{1}**HNMR(DMSO-d$_6$),**(δppm); 3.4 (d, 2H); 4.3 (t, 1H), 4.0 (s, 1H) 5.8 (d, 1H, J=9.9 Hz CH Pyridazine), 6.7 (s, 1H, J=9.8 Hz, CH pyridazine), 7.3 (s, 1H), 7.2-7.5 (m, 8H), 10.8-12.0 (s 1H, -OH D_2O exchangeable).

Mass;(m/z): Calculated **348**; Found **347.5** (M^+)

v. 6-(3-(4, 5-dihydro-5-(4-chloro) isoxazol-3-yl) phenylamino) pyridazin-3 (2*H*)-one (3g):Molecular Formula = $C_{19}H_{15}ClN_4O_2$, Yield = **67%**, M. p. = **192^{0}C. FT-IR (KBr):** 3345, 3245 cm^{-1} (N-H Str.), 1665 cm^{-1} (>C=O) 1577 cm^{-1} (-CH=CH-, str.); ^{1}**HNMR(DMSO-d$_6$),**(δppm); 3.3 (d, 2H); 3.9 (s, 1H), 4.9 (t, 1H) 5.6 (d, 1H, J=9.9 Hz CH Pyridazine), 6.6 (s, 1H, J=9.8 Hz, CH pyridazine), 7.0 (s, 1H), 7.0-7.4 (m, 8H), **Mass;(m/z)**: Calculated **366.8**; Found **367.5** (M^+).

vi. 6-(3-(4,5-dihydro-5-(4-methoxyphenyl) isoxazol-3-yl)phenylamino) pyridazin-3 (2*H*)-one(3i):Molecular Formula = $C_{20}H_{18}N_4O_3$, Yield = **56%**, M. p. = **138^{0}C. FT-IR (KBr):** 3350, 3200 cm^{-1} (N-H Str.), 1580.85 cm^{-1} (-CH=CH-, str.); ^{1}**HNMR(DMSO-d$_6$),**(δppm); 3.2-3.4 (d, 2H); 3.8 (s, 3H); 4.2 (s, 1H), 4.8 (t, 1H) 5.8 (d, 1H, J=9.9 Hz CH Pyridazine), 6.89 (s, 1H, J=9.8 Hz, CH pyridazine), 7.2 (s, 1H), 6.8-7.3 (m, 8H), **Mass;(m/z)**: Calculated **362**; Found **362.5** (M^+).

(c) Screening of Antimicrobial activity

The antibacterial activities of all the synthesized compounds were determined by the well diffusion method[21]. Two bacterial isolates, Staphylococcus aureus (ATCC No. 6538) Escherichia coli (ATCC No. 8739) used to investigate the antibacterial activities. The compounds were dissolved in DMSO at concentration of 1mg/ml. Antibacterial activity of DMSO against the test organisms was investigated, and was found to be nil. Activity of each compound

was compared with penicillin as a standard. Isoxazolines were also compared with antifungal drug "Grysofulvin". Two antifungal specimens i.e. Aspergillus Niger (ATCC 16404) and Candida Albicans (ATCC 10231) were used for antifungal activity.

Table 3. Antimicrobial Screening of novel synthesized Isoxazoline

Entry	Bacteria		Fungi	
	E. Coli ATCC 8739	**S. aureus ATCC6538**	**A. ATCC16404**	**C. albicans ATCC10231**
5a	**11mm**	**9.5mm**	-ve	**10mm**
5b	7mm	8mm	-ve	8mm
5c	4.5mm	7mm	-ve	-ve
5d	8mm	-ve	-ve	8mm
5e	10mm	6mm	-ve	9mm
5f	9mm	7mm	10mm	-ve
5g	8mm	6.5mm	5.5mm	8mm
5h	7.5mm	7mm	**11.5mm**	9mm
5i	**12.5mm**	7.5mm	-ve	8.5mm
5k	10mm	6.5mm	-ve	**13mm**
5l	-ve	**10.5mm**	8.5mm	7.5mm
Penicillin	**10.5mm**	**8.5mm**	-	-
Griseofulvin	-	-	**10.5mm**	**10mm**

On going through the results of antifungal activity (Table 3), it has been observed that isoxazolines **5a and 5i** have good activity against Escherichia coli, and 5aand **5l** have very good activity against Staphylococcus aureus when compared with the standard antibacterial drug 'penicillin'. When all these isoxazolines were compared with the anti-fungal drug "Griseofulvin." isoxazolines no. **5h** were found active against Aspergillus Niger (ATCC 16404), and **5a, & 5k were** found active against Candida albicans (ATCC 10231). Except for Aspergillus Niger, the remaining isoxazolines demonstrated significant activity against the majority of the bacteria and fungi tested.

5. Conclusion

We created new isoxazoline derivatives 5 (a–l) by combining newer chalcones with pyrazolone derivatives. All these derivatives were screened for antibacterial activity against Escherichia coli (MTCC-443), Staphylococcus aureus (MTCC-442) bacterial strains and antifungal activity against Aspergillus niger & Candida albicans All of the screened compounds exhibited good to excellent activity when compared to standard penicillin and Grysofulvin drugs.

Declarations

Source of Funding

This research work did not receive any grant from funding agencies in the public or not-for-profit sectors.

Conflict of Interests

The authors declare the total absence of conflicts of interest, both during the conduct of the experiments and during the written drafting of this work.

Consent for Publication

The authors declare that they consented to the publication of this research work.

Acknowledgements

The authors are thankful to the Principal, Konkan Education Societies, Anandibai Pradhan Science College, Nagothane, Tal. Roha Dist. Raigad and Kirti D. M. Arts, Commerce and Science College, Dadar, Mumbai for providing necessary research facilities, and IIT Bombay, Powai, Mumbai, for spectral analysis.

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