



SYNTHESIS AND ANTIBACTERIAL ACTIVITY OF SOME NOVEL ISOXAZOLYL SCHIFF'S BASES

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ABSTRACT

A series of isoxazolyl schiff's bases (3a-h) were synthesized from *N*-(5-methyl-3-isoxazolyl)-3-oxobutanamide (1). The compound (1) upon heating with different primary amines in methanol furnished (*E*)-3-(methylimino)-*N*-(5-methylisoxazol-3-yl)butanamides (3a-h) in excellent yield. The structure of all the synthesized compounds has been established by IR, ¹H NMR, ¹³C NMR and mass spectral data. All the synthesized compounds (3a-h) were screened for antibacterial activity against two Gram-positive (*Bacillus subtilis* MTCC 121 and *Staphylococcus aureus* MTCC 96) and two Gram-negative (*Escherichia coli* MTCC 43 and *Klebsiella pneumoniae* MTCC 530) bacterial strains. The Schiff's bases (*E*)-*N*-(5-methylisoxazol-3-yl)-3-(phenylimino)butanamide 3c (MIC values 11±0.28, 14±0.10, 12±0.31, 14±0.31, at 40µg/µL,) and (*E*)-3-(benzylimino)-*N*-(5-methylisoxazol-3-yl)butanamide 3f (MIC values 12±0.38, 14±0.10, 13±0.21, 14±0.32, at 40µg/µL,) showed significant activity against all the bacteria tested compared to other compounds in this series. All other compounds except compounds 3a and 3b have shown considerable antibacterial activity against different bacterial stains. Compound 3a and 3b could show activity only at the high concentration. Furthermore, substitutions at phenyl nucleus have not much affected the antibacterial activity.

KEYWORDS: Amino isoxazole, primary amines, Schiff's base, antibacterial activity.



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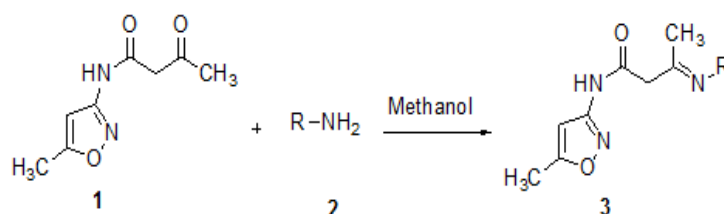
INTRODUCTION

Heterocycles are abundant in nature and are of great significance to life; hence they have attracted considerable attention towards the design of biologically active molecules and advanced organic materials. Among wide variety of heterocycles that have been explored for developing pharmacologically important molecules, isoxazole unit, constitutes an important group due to wide variety of biological activity such as CNS – active,¹ antitumor,² chemotherapeutic agents,³ and found to possess vasodilating effect,⁴ similar to that of nifedipine. Schiff's bases and their amine analogues derived from various aromatic aldehydes and ketones were reported to possess fungicidal,⁵ bacteriicidal,⁶ antiviral,⁷ antioxidant⁸ and antimicrobial activity⁹ Due to diverse structural aspects of Schiff bases and their analogues, a wide range of these compounds have been synthesized. Keeping the diverse therapeutic activities of Schiff's base analogues derived from aldehydes in view, it was contemplated to synthesize a series of isoxazolyl Schiff's base derivatives and screen

them for their *in vitro* antibacterial activity. Herein we report the synthesis and antibacterial evaluation of isoxazolyl Schiff's bases.

MATERIALS AND METHODS

All the chemicals were purchased from Sigma-Aldrich; all the reagents were analytically pure. Bacterial strains are procured from microbial type culture collection (MTCC), Institute of Microbial Technology (IMTECH), Chandigarh., India. Melting points were determined on a Cintex melting point apparatus and are uncorrected. The purity of the compounds checked by TLC (Merck precoted) and visualized under U.V. Light. IR Spectra were recorded as a KBr pellet with a Perkin Elmer BX series FT-IR spectrophotometer; ¹H NMR spectra were recorded in CDCl₃ with a Bruker 300 MHz instrument. Chemical Shift values were reported in δ (ppm) using TMS as an internal standard. ESI mass spectra were recorded on an Agilent LC-MSD mass Spectrometer. Elemental analyses were performed on a Carlo Erba 106 and Perkin-Elmer model 240 analysers.



3a: R = CH₃; **3b:** R = CH₃-CH₂; **3c:** R = C₆H₅; **3d:** R = 4-ClC₆H₄; **3e:** R = 4-OCH₃C₆H₄;
3f: R = C₆H₅-CH₂; **3g:** R = 4-ClC₆H₄-CH₂; **3h:** R = 4-OCH₃C₆H₄-CH₂

Scheme-1

Synthesis of compounds 3a-g

General procedure for synthesis of (E)-3-(methylimino)-N-(5-methylisoxazol-3-yl)butanamide 3a-h

A mixture of *N*-(5-methyl-3-isoxazolyl)-3-oxobutanamide (**1**) (0.01 mol) and methyl amine (**2a**) (0.01 mol) was taken in methanol, 4-5 drops of glacial acetic acid was added to it, and the contents were refluxed for 4-6 h. After completion of the reaction (monitored by TLC), the reaction mixture was cooled, the separated solid was filtered, washed with cold methanol and dried under vacuum.

(E)-3-(Methylimino)-N-(5-methylisoxazol-3-yl)butanamide 3a

IR (KBr): 3201 (NH), 1680 (NHCO), 1595 (-C=N) cm⁻¹; ¹H NMR (300 MHz, CDCl₃) δ: 1.95 (s, 3H, CH₃), 2.35 (s, 3H, isoxazole-CH₃), 2.99 (s, 3H, N-CH₃), 3.45 (s, 2H, CH₂), 6.60 (s, 1H, isoxazole-H), 8.05 (bs, 1H, NH, D₂O exchangeable); ¹³C NMR (75 MHz, CDCl₃) δ: 12.1, 18.8, 33.9, 44.5, 95.2, 158.8, 162.0, 167.6, 178.4; ESI-MS: m/z [M + 1]⁺ 196.

(E)-3-(Ethylimino)-N-(5-methylisoxazol-3-yl)butanamide 3b

IR (KBr) : 3204 (NH), 1685 (NHCO), 1590 (-C=N) cm⁻¹; ¹H NMR (300 MHz, CDCl₃) δ : 1.09 (s, 3H, N-CH₂-CH₃, J = 7.4 Hz), 1.95 (s, 3H, CH₃), 2.40 (s, 3H, isoxazole-CH₃), 3.19 (s, 2H, CH₂-CH₃, J = 7.4 Hz), 3.42 (s, 2H,

CH₂), 6.58 (s, 1H, isoxazole-H), 8.15 (bs, 1H, NH, D₂O exchangeable); ESI-MS: m/z [M+1]⁺ 210.

(E)-N-(5-Methylisoxazol-3-yl)-3-(phenylimino)butanamide 3c

IR (KBr): 3197 (NH), 1688 (NHCO), 1585 (-C=N) cm⁻¹; ¹H NMR (300 MHz, CDCl₃) δ: 2.18 (s, 3H, CH₃), 2.39 (s, 3H, isoxazole-CH₃), 3.49 (s, 2H, CH₂), 6.70 (s, 1H, isoxazole-H), 7.10-7.36 (m, 5H, Ar-H), 8.05 (bs, 1H, NH, D₂O exchangeable); ¹³C NMR (75 MHz, CDCl₃) δ: 12.7, 20.5, 35.0, 96.4, 124.8, 129.0, 129.3, 134.0, 159.0, 163.0, 167.7, 178.8; ESI-MS: m/z [M+1]⁺ 258.

(E)-3-(4-Chlorophenylimino)-N-(5-methylisoxazol-3-yl)butanamide 3d

IR (KBr): 3207 (NH), 1692 (NHCO), 1580 (-C=N) cm⁻¹; ¹H NMR (300 MHz, CDCl₃) δ: 2.05 (s, 3H, CH₃), 2.40 (s, 3H, isoxazole-CH₃), 3.56 (s, 2H, CH₂), 6.65 (s, 1H, isoxazole-H), 7.05 (d, 2H, Ar-H, J = 7.9 Hz), 7.30 (d, 2H, Ar-H, J = 7.9 Hz), 8.20 (bs, 1H, NH, D₂O exchangeable); ESI-MS: m/z [M+1]⁺ 292.

(E)-3-(4-Methoxyphenylimino)-N-(5-methylisoxazol-3-yl)butanamide 3e

IR (KBr): 3152 (NH), 1665 (NHCO), 1552 (-C=N) cm⁻¹; ¹H NMR (300 MHz, CDCl₃) δ: 1.97 (s, 3H, CH₃), 2.36 (s, 3H, isoxazole-CH₃), 3.52 (s, 2H, CH₂), 3.85 (s, 3H, OCH₃), 6.61 (s, 1H, isoxazole-H), 6.86 (d, 2H, Ar-H, J = 8 Hz), 7.19 (d, 2H, Ar-H, J = 8 Hz), 7.58 (bs, 1H, NH, D₂O exchangeable); ESI-MS: m/z [M+1]⁺ 288.

(E)-3-(Benzylimino)-N-(5-methylisoxazol-3-yl)butanamide 3f

IR (KBr): 3218 (NH), 1685 (NHCO), 1587 (-C=N) cm^{-1} ; ^1H NMR (300 MHz, CDCl_3) δ : 1.95 (s, 3H, CH_3), 2.38 (s, 3H, isoxazole- CH_3), 3.54 (s, 2H, CH_2), 4.32 (s, 2H, Ph- CH_2), 6.65 (s, 1H, isoxazole-H), 7.22-7.39 (m, 5H, Ar-H), 7.95 (bs, 1H, NH, D_2O exchangeable); ^{13}C NMR (75 MHz, CDCl_3) δ : 12.6, 19.5, 34.0, 56.7, 96.3, 126.3, 127.7, 128.71, 138.6, 158.0, 161.8, 168.2, 180.2; ESI-MS: m/z $[\text{M}+1]^+$ 272.

(E)-3-(4-Chlorobenzylimino)-N-(5-methylisoxazol-3-yl)butanamide 3g

IR (KBr): 3200 (NH), 1690 (NHCO), 1588 (-C=N) cm^{-1} ; ^1H NMR (300 MHz, CDCl_3) δ : 2.00 (s, 3H, CH_3), 2.40 (s, 3H, isoxazole- CH_3), 3.52 (s, 2H, CH_2), 4.32 (s, 2H, Ph-

CH_2), 6.62 (s, 1H, isoxazole-H), 7.09 (d, 2H, Ar-H, $J = 7.9$ Hz), 7.31 (d, 2H, Ar-H, $J = 7.9$ Hz), 8.18 (bs, 1H, NH, D_2O exchangeable); ESI-MS: m/z $[\text{M}+1]^+$ 306.

(E)-3-(4-Methoxybenzylimino)-N-(5-methylisoxazol-3-yl)butanamide 3h

IR (KBr): 3190 (NH), 1689 (NHCO), 1582 (-C=N) cm^{-1} ; ^1H NMR (300 MHz, CDCl_3) δ : 1.94 (s, 3H, CH_3), 2.35 (s, 3H, isoxazole- CH_3), 3.55 (s, 2H, CH_2), 3.89 (s, 3H, OCH_3), 4.37 (s, 2H, Ph- CH_2), 6.64 (s, 1H, isoxazole-H), 6.88 (d, 2H, Ar-H, $J = 8$ Hz), 7.18 (d, 2H, Ar-H, $J = 8$ Hz), 7.68 (bs, 1H, NH, D_2O exchangeable); ^{13}C NMR (75 MHz, CDCl_3) δ : 12.4, 19.3, 34.1, 55.1, 56.9, 96.1, 113.9, 127.9, 130.5, 156.2, 158.9, 161.6, 167.7, 168.8, 180.8; ESI-MS: m/z $[\text{M}+1]^+$ 302.

Table I
The physicochemical characteristics of the newly synthesized compounds 3a-h

Comd.	R	Mol. Formula (Mol. Wt.)	Yield (%)	M.P ($^{\circ}\text{C}$)	Found (%) (Calcd)		
					C	H	N
3a	CH_3	$\text{C}_9\text{H}_{13}\text{N}_3\text{O}_2$ (195)	83	139-140	55.39 (55.37)	6.72 (6.71)	21.54 (21.52)
3b	C_2H_5	$\text{C}_{10}\text{H}_{15}\text{N}_3\text{O}_2$ (209)	84	146-148	57.38 (57.40)	7.22 (7.23)	20.07 (20.08)
3c	C_6H_5	$\text{C}_{14}\text{H}_{15}\text{N}_3\text{O}_2$ (257)	88	161-162	65.33 (65.35)	5.89 (5.88)	16.31 (16.33)
3d	4- ClC_6H_4	$\text{C}_{14}\text{H}_{14}\text{ClN}_3\text{O}_2$ (291)	90	173-175	57.62 (57.64)	4.84 (4.84)	14.42 (14.40)
3e	4- $\text{OCH}_3\text{C}_6\text{H}_4$	$\text{C}_{15}\text{H}_{17}\text{N}_3\text{O}_3$ (287)	89	168-170	62.73 (62.71)	5.98 (5.96)	14.62 (14.63)
3f	$\text{C}_6\text{H}_5\text{-CH}_2$	$\text{C}_{15}\text{H}_{17}\text{N}_3\text{O}_2$ (271)	88	180-182	66.42 (66.40)	6.30 (6.32)	15.50 (15.49)
3g	4- $\text{ClC}_6\text{H}_4\text{-CH}_2$	$\text{C}_{15}\text{H}_{16}\text{ClN}_3\text{O}_2$ (305)	91	189-191	58.90 (58.92)	5.29 (5.27)	13.72 (13.74)
3h	4- $\text{OCH}_3\text{C}_6\text{H}_4\text{-CH}_2$	$\text{C}_{16}\text{H}_{19}\text{N}_3\text{O}_3$ (301)	90	187-188	63.75 (63.77)	6.38 (6.36)	13.96 (13.94)

Antibacterial activity

The newly synthesized compounds (3a-h) were screened for antibacterial activity against two Gram-positive (*Bacillus subtilis* MTCC 121 and *Staphylococcus aureus* MTCC 96) and two Gram-negative (*Escherichia coli* MTCC 43 and *Klebsiella pneumoniae* MTCC 530) bacterial strains using Ampicillin as a standard and agar well diffusion method.¹⁰ With aid of sterile 1 mL pipette, about 0.2 mL of the broth culture of the test organism was added to an 18 mL sterile molten diagnostic sensitivity test agar. This was well mixed and poured into previously sterilized Petri dishes, which was properly labeled

according to the test organisms. The required numbers of wells (holes) bored into the medium using sterile cork borer. The wells (diameter 10 mm) were then filled up aseptically with solution of the test compounds in DMSO using Pasteur pipettes. Ampicillin was used as a standard antibacterial agent at concentration 20 $\mu\text{g}/20$ μl . The plates were allowed to stand for about 1 hr on the bench for proper diffusion of the antibacterial agents in to the medium and then incubated upright at 37 $^{\circ}\text{C}$ for 24 h. The inhibition zone were recorded in mm using antibiotic zone scale indicated the relative susceptibility of the bacteria for the test compounds 3a-h and ampicillin standard (Table-2).

Table 2
In vitro antibacterial activity of the newly synthesized compounds 3a-h.
Diameter of inhibition Zone in (mm \pm SD)^a

Compound	<i>Escherichia coli</i>	<i>Klebsiella pneumoniae</i>	<i>Staphylococcus aureus</i>	<i>Bacillus subtilis</i>
3a (20 $\mu\text{g}/20$ μl)	0	0	0	2 \pm 0.21
3a (40 $\mu\text{g}/20$ μl)	3 \pm 0.19	2 \pm 0.39	2 \pm 0.19	4 \pm 0.21
3b (20 $\mu\text{g}/20$ μl)	0	0	0	3 \pm 0.39
3b (40 $\mu\text{g}/20$ μl)	2 \pm 0.38	2 \pm 0.19	2 \pm 0.41	4 \pm 0.41
3c (20 $\mu\text{g}/20$ μl)	10 \pm 0.39	10 \pm 0.29	10 \pm 0.29	12 \pm 0.29
3c (40 $\mu\text{g}/20$ μl)	11 \pm 0.28	14 \pm 0.10	12 \pm 0.31	14 \pm 0.31
3d (20 $\mu\text{g}/20$ μl)	6 \pm 0.29	5 \pm 0.18	7 \pm 0.32	7 \pm 0.40
3d (40 $\mu\text{g}/20$ μl)	8 \pm 0.29	7 \pm 0.18	8 \pm 0.41	8 \pm 0.21
3e (20 $\mu\text{g}/20$ μl)	6 \pm 0.28	4 \pm 0.29	6 \pm 0.29	8 \pm 0.19
3e (40 $\mu\text{g}/20$ μl)	8 \pm 0.39	5 \pm 0.21	9 \pm 0.29	10 \pm 0.21
3f (20 $\mu\text{g}/20$ μl)	10 \pm 0.19	11 \pm 0.22	11 \pm 0.21	13 \pm 0.41

3f (40µg/20µl)	12 ± 0.38	14 ± 0.1	13 ± 0.21	14 ± 0.32
3g (20µg/20µl)	7 ± 0.19	5 ± 0.11	8 ± 0.21	9 ± 0.31
3g (40µg/20µl)	8 ± 0.19	8 ± 0.19	9 ± 0.39	7 ± 0.21
3h (20µg/20µl)	6 ± 0.29	5 ± 0.41	6 ± 0.31	10 ± 0.19
3h (40µg/20µl)	8 ± 0.28	7 ± 0.42	8 ± 0.29	10 ± 0.29
Ampicillin (20µg/20µl)	14	16	14	15

^a Mean value of 3 trials

RESULTS AND DISCUSSIONS

Chemistry

In continuation of our work related to synthesis of isoxazole derivatives¹¹⁻¹⁴, the starting compound *N*-(5-methyl-3-isoxazolyl)-3-oxobutanamide (1) has been synthesized from 3-amino-5-methyl isoxazole and ethyl acetoacetate.¹⁵ The condensation of compound (1) with different primary amines (2a-h) in methanol, in the presence of catalytic amount glacial acetic acid to give corresponding Schiff's bases (3a-h) in excellent yields (Table-1). The structure of all the newly synthesized compounds were confirmed by their spectroscopic data (IR, ¹H NMR, ¹³C NMR and mass) and elemental analyses. Disappearance of the carbonyl group absorption at 1720 cm⁻¹ in the IR spectrum of compound (1) and the presence of new band at 1595 cm⁻¹ due to -C=N functional group confirmed the structure of compound (3a), further in ¹H NMR four independent signals are appeared at δ 1.95, 2.35, 2.99, 3.45 and 6.60 due to -C=CH₃, isoxazole-CH₃, N-CH₃, -N=C-CH₂ and isoxazole-H respectively and the NH proton was observed as a broad singlet at δ 8.05, it is D₂O exchangeable. The mass spectra of the compounds (3a-h) showed [M + 1] peaks which were agreement with their molecular formula.

Antibacterial activity

Antibacterial activity has been evaluated against two Gram-positive (*Bacillus subtilis* MTCC 121 and *Staphylococcus aureus* MTCC 96) and two Gram-negative (*Escherichia coli* MTCC 43 and *Klebsiella pneumoniae* MTCC 530) bacteria. The Schiff's bases (*E*)-*N*-(5-methylisoxazol-3-yl)-3-(phenylimino)butanamide 3c (MIC values 11±0.28, 14±0.10, 12±0.31, 14±0.31, at 40µg/µL,) and (*E*)-3-(benzylimino)-*N*-(5-methylisoxazol-3-yl)butanamide 3f (MIC values 12±0.38, 14±0.10, 13±0.21, 14±0.32, at 40µg/µL) showed significant activity against all the bacteria tested compared to other compounds in this series. All the synthesized compounds (3a-h), except 3a and 3b have shown considerable antibacterial activity against different bacterial strains. Compound 3a and 3b

could show activity only at the high concentration. Furthermore, substitutions at phenyl nucleus have not much affected the antibacterial activity. The outstanding properties of this new class of antibacterial reagents (compounds 3a and 3f) deserve further investigation in order to clarify the mode of action at molecular level, responsible for the activity observed. More extensive study is also warranted to determine additional physicochemical and biological parameter to have a deeper insight into structure activity relationship and to optimize the effectiveness of this series of derivatives. Conclusion: A series of new isoxazolyl schiff's bases were successfully synthesized in high yield (Table 1). The structures of the new compounds were confirmed by their IR, ¹H NMR, ¹³C NMR and mass spectral data. All the synthesized compounds (3a-h) were screened for antibacterial activity against two Gram-positive (*Bacillus subtilis* MTCC 121 and *Staphylococcus aureus* MTCC 96) and two Gram-negative (*Escherichia coli* MTCC 43 and *Klebsiella pneumoniae* MTCC 530) bacterial strains. The Schiff's bases (*E*)-*N*-(5-methylisoxazol-3-yl)-3-(phenylimino)butanamide 3c and (*E*)-3-(benzylimino)-*N*-(5-methylisoxazol-3-yl)butanamide 3f showed significant activity against all the bacteria tested compared to other compounds in this series. Furthermore, substitutions at phenyl nucleus have not much affected the antibacterial activity.

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CONFLICT OF INTEREST

Conflict of interest declared none.

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