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SYNTHESIS AND BIOLOGICAL EVALUATION OF FUSED RING PYRIDOMORPHOLINE PHENOXY DERIVATIVES.

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ABSTRACT

A series of seven novel 2-chloro-1-(2, 3-dihydro-4H-Pyrido[3, 2-b][1, 4] oxazin-4-yl) ethanone derivatives (II e - II k) were synthesized in multistep reaction from commercially available 2-amino-3-Hydroxy pyridine as a starting material. The chemical structures of the synthesized compounds were confirmed by means of ^{1}HNMR and mass spectral data. High yield and high purity indicates lack of side reaction and by product. The synthesized compounds were then examined for their antibacterial and antifungal activities. Some of them were found to possess good antibacterial and antifungal activity.

KEYWORDS: Synthesis, Fused morpholine, Phenoxy derivative, antibacterial, Antifungal activity.

INTRODUCTION

Nitrogen and oxygen containing heterocyclic compounds like morpholine[1] and fused ring morpholine[2-5] are very important building blocks in medicinal chemistry[6] field. So the morpholine derivatives are extensively very essential in the drug discovery research, which stimulate research activity in the field of the broad spectrum of biological activity [7] study. After the literature survey that many morpholine derivative molecule are shows very good biological activity in different therapeutic area such as antibacterial[8], antiviral, anticancer, antimicrobial, antidiabetic, anti-Inflammatory, antimalarial, antifungal[9], Antiemetic etc.

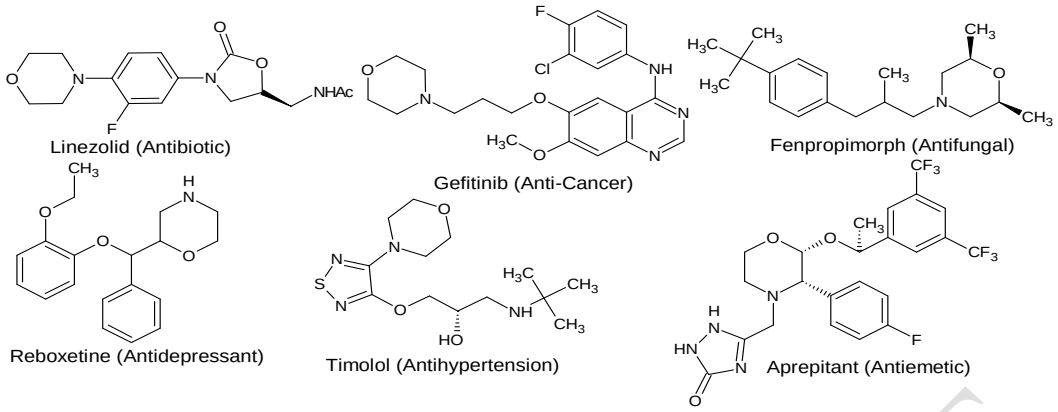


Figure 1: Marketed drugs containing a direct linked morpholine ring.

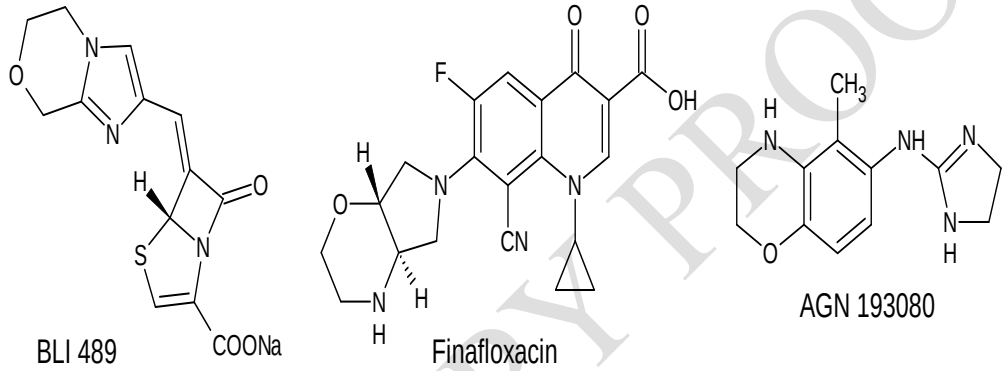


Figure 2: Clinical and preclinical drugs having a fused morpholine ring.

The synthesis of phenoxy derivatives were reported by Crowther, et al.[10] They showed that compounds bearing substituent's on the benzene nucleus, such as alkyl, alkoxy, aryloxy[11-13], were highly active. These findings prompted us to synthesize some derivatives bearing substituent's on the benzene nucleus. Hence, in the present study, some new phenoxy derivatives of 2-chloro-1-(2,3-dihydro-4H-Pyrido[3, 2-b][1, 4]oxazin-4-yl)ethanone have been synthesized. Their characterization was done by spectroscopic methods like ^{1}HNMR and mass spectral data. Further, antibacterial and antifungal activities of these derivatives have been studied.

MATERIALS AND METHODS

All reagents and solvents were used as obtained from the supplier or recrystallized/redistilled as necessary. The moiety 2-Amin-3-Hydroxy pyridine[14-15] is commercially available and is also in Sigma Aldrich. This can be also synthesized as per reported literature. Melting points were recorded on open capillary melting point apparatus and are uncorrected. Mass spectra were recorded on 'LCMS-QP2010s' instrument by direct injection method. Nuclear Magnetic

Resonance spectra (^{1}HNMR) Were recorded in DMSO-d6 on Bruker advance spectrometer at 400MHz using Tetramethylsilane (TMS) as internal standard and the chemical shift (δ) are reported in parts per million. The purity of the synthesized compounds was checked by Thin Layer Chromatography, Merck pre-coated plates (silica gel 60 F254) were visualized with UV light. Fungus Culture: Candida sp. Gram-positive microorganisms: Staphylococcus aureus, Staphylococcus albus, Streptococcus faecalis, Bacillus sp and Gram-negative microorganisms: Klebsiella pnuemoniae, Escherichia coli, Pseudomonas sp, Proteus sp were used for biological activity.

Antimicrobial Activity: The antimicrobial activity of all synthesized compounds (II e – II k) were screened against different standard organism obtained from the American type of cell culture collection, including Staphylococcus aureus, Escherichia coli and Pseudomonas sp. Agar diffusion technique at the concentration level of 5µg molar was applied. Ciprofloxacin was used as reference compounds for antibacterial activities. The antimicrobial activity of all the newly synthesized compounds were determined by well plate method in nutrient agar (Hi-media) was used for antibacterial activity. The antibacterial activity of the test compounds was assayed against gram-positive and gram-negative by Cup plate method. The compounds were tested at a concentration of a 100 µg/ml were prepared in dimethylforamide (DMF). The Petri dishes used for antibacterial screening were incubated at 37±1 for 24h; the diameters of zone of inhibition (mm) surroundings each of the wells recorded. The results were compared Ciprofloxacin of a 100µg/ml concentration (cacic, M et al., 2006).

Antifungal Activity: The antifungal activity of all synthesized compounds (II e – II k) screened against Candida sp in DMF by poisoned food technique. Fluconazole was employed as standard drug during the test procedures as references. Potato dextrose agar (PDA) Media were prepared and about 15ml of PDA was poured into each Petri plate allowed to solidify 5mm disc of seven-day-old culture of the test fungi was placed at the centre of the Petri plates and incubated at 26°C for 7 days. After incubation, the percentage inhibition was measured and three replicates were maintained for each treatment.

EXPERIMENTAL

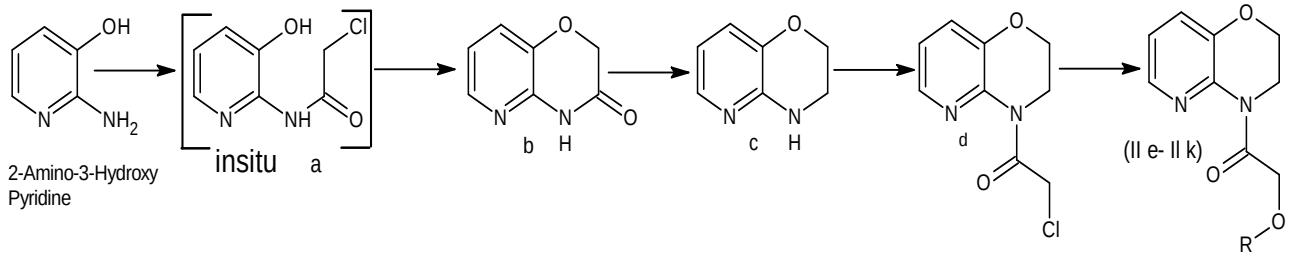
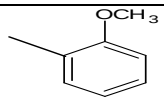
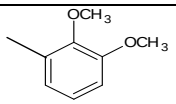
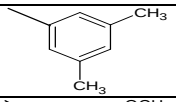
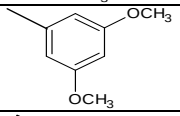
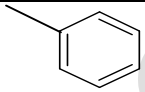
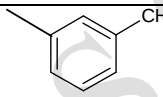
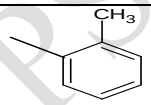


Figure 3: Reaction scheme for the synthesis of 2-chloro-1-(2,3-dihydro-4H-Pyrido[3,2-b][1,4] oxazin-4-yl)ethanone and their derivatives.

Table1: Physical data of synthesized compounds.

Code	-R	Molecular Formula	M.Wt	% Yield	M.P (°C)
II e		$C_{16}H_{16}N_2O_4$	300.30	60	155-157
II f		$C_{17}H_{18}N_2O_5$	330.33	69	164-166
II g		$C_{17}H_{18}N_2O_3$	298.33	74	151-153
II h		$C_{17}H_{18}N_2O_5$	330.33	73	172-174
II i		$C_{15}H_{14}N_2O_3$	270.28	76	141-143
II j		$C_{16}H_{16}N_2O_3$	284.30	70	149-151
II k		$C_{16}H_{16}N_2O_3$	284.30	72	147-149

Preparation of 2H-pyrido[3,2-b][1,4]oxazin-3(4H)-one (b): The chloroacetyl chloride (33.33g, 295mmol) was added drop-wise to the solution of potassium carbonate (95.5g, 692mmol) and 2-amino-3-Hydroxy pyridine (25g, 227mmol) in THF (250ml) at 0°C. The resulting suspension was stirred at room temperature for 1hr. Then the reaction mixture heated to reflux and maintained for 4h. After completion of reaction, the reaction was cooled to room temperature and the inorganic solids were removed by filtration washed with THF (25ml), the filtrate solvent was concentrated under vacuum to give a crude solid. The crude was suspended in water (250ml) and the suspension was stirred at room temperature for 1hr. Filtered and washed with water (25ml), after drying yielded the titled product (b) as off-white solid.

Preparation of 3,4-dihydro-2H-pyrido[3,2-b][1,4]oxazine (c): The compound (b) (8g, 53mmol) in tetrahydrofuran (40ml) was added slowly to the solution of lithium aluminium hydride (3g, 79mmol) in tetrahydrofuran (40ml) at 0°C and the mixture was stirred for 6 hr at room temperature. After completion of reaction, the reaction was quenched with wet sodium sulfate. The reaction mass filtered through celite bed washed with tetrahydrofuran (16ml). The filtrate was distilled out completely. Yielded the titled product (c) as off-white solid.

Preparation of 2-chloro-1-(2,3-dihydro-4H-Pyrido[3,2-b][1,4] oxazin-4-yl)ethanone (d) The chloroacetyl chloride (6.17g, 54mmol) was added to the solution of compound (c) (6.2g, 45mmol), triethylamine (6.9g, 68mmol) and 4-Dimethylaminopyridine (0.55g, 4.5mmol) in dichloromethane (60ml) at 0°C and the mixture was stirred for 4hr at 0°C. After completion of reaction, the solution was evaporated in vacuum and the residue was suspended in ethyl acetate (120ml) and washed with 2×40ml of 10% sodium bicarbonate solution. The organic layer dried with sodium sulfate and evaporated. Pure product isolated by flash column chromatography eluted with 10% ethyl acetate in hexane yielded the titled product (d) as light brown solid.

General method for the synthesis of compounds (II e - II k): The compound (d) (1mol.Eq) was added to the solution of phenol/substituted phenol (1.1mol.Eq) and potassium tert butoxide (1.5mol.Eq) in N,N-Dimethylforamide (10 volume) and the mixture was stirred for 2hr at room temperature. After completion of reaction, the reaction mass slowly poured into 40 volume of cold water and stirred for 2 hr at room temperature. Filtered and washed with water to get pure crystalline product (II e-II k).

RESULTS AND DISCUSSION

The results are obtained from various spectral data are results discussed below.

2H-pyrido[3,2-b][1,4]oxazin-3(4H)-one (b): A off-white solid; Yield 97%; M.Wt: 150.13; Mol.For: $C_7H_6N_2O_2$; LC-MS(m/z): 151.1(M+1); ^{1}HNMR (400MHz, DMSOd_6): δ 11.23 (s, 1H), 7.88-7.90 (d, 1H), 7.32-7.34 (d, 1H), 6.95-6.98 (d, 1H), 4.64 (s, 2H).

3,4-dihydro-2H-pyrido[3,2-b][1,4]oxazine (c): A off-white solid; Yield 85%; M.Wt: 136.15; Mol.For: $C_7H_8N_2O$; LC-MS(m/z): 137.0(M+1); ^{1}HNMR (400MHz, DMSOd_6): δ 7.53-7.54 (d, 1H), 6.89-6.91 (d, 1H), 6.63 (s, 1H), 6.43-6.46 (d, 1H), 4.07-4.10 (t, 2H), 3.36-3.39 (t, 2H).

2-chloro-1-(2,3-dihydro-4H-Pyrido[3,2-b][1,4] oxazin-4-yl)ethanone (d): A light brown solid; Yield 80%; M.Wt: 212.6; Mol For: $C_9H_9C1N_2O_2$; LC-MS(m/z): 213.1(M+1); ^{1}HNMR (400MHZ, $DMSOd_6$): δ 8.43-8.45 (d, 1H), 7.99-8.01 (d, 1H), 7.47-7.51 (t, 1H), 5.46 (s, 2H), 4.48-4.50 (t, 2H), 3.98-4.00 (t, 2H).

1-(2,3-dihydro-4H-Pyrido[3,2-b][1,4]oxazin-4-yl)-2-(2-methoxyphenoxy)ethanone (e): A off-white color solid; yield 60%; M.W: 300.3; Mol.For: $C_{16}H_{16}N_2O_4$; m.p: 155-157°C; LC-MS(m/z): 301.2(M+1); ^{1}HNMR (400MHz, $DMSOd_6$): δ 7.95-7.96 (d, 1H), 7.36-7.39 (d, 1H), 7.12-7.15 (q, 1H), 6.95-6.97 (d, 1H), 6.88-6.89 (m, 1H), 6.81-6.82 (d, 2H), 5.33 (s, 2H), 4.26-4.28 (t, 2H), 3.96-3.99 (t, 2H), 3.74 (s, 3H).

1-(2,3-dihydro-4H-Pyrido[3,2-b][1,4]oxazin-4-yl)-2-(2,3 dimethoxyphenoxy)ethanone (f): A off-white color solid; yield 69%; M.W: 330.3; Mol.For: $C_{17}H_{18}N_2O_5$; m.p: 164-166°C; LC-MS(m/z): 331.2(M+1); ^{1}HNMR (400MHz, $DMSOd_6$): δ 7.96-7.97 (d, 1H), 7.36-7.39 (d, 1H), 7.13-7.16 (q, 1H), 6.89-6.91 (t, 1H), 6.62-6.65 (d, 1H), 6.50-6.52 (d, 1H), 5.37 (s, 2H), 4.27-4.29 (t, 2H), 3.97-3.99 (t, 2H), 3.76 (s, 3H), 3.68 (s, 3H).

1-(2,3-dihydro-4H-Pyrido[3,2-b][1,4]oxazin-4-yl)-2-(3,5dimethylphenoxy)ethanone (g): A off-white color solid; yield 74%; M.W: 298.3; Mol.For: $C_{17}H_{18}N_2O_3$; m.p: 151-153°C; LC-MS(m/z): 299.2(M+1); ^{1}HNMR (400MHz, $DMSOd_6$): δ 7.97-7.98 (d, 1H), 7.37-7.39 (d, 1H), 7.13-7.16 (q, 1H), 6.55 (s, 1H), 6.47 (s, 2H), 5.29 (s, 2H), 4.25-4.28 (t, 2H), 3.96-3.99 (t, 2H), 2.20 (s, 6H).

1-(2,3-dihydro-4H-Pyrido[3,2-b][1,4]oxazin-4-yl)-2-(3,5dimethoxyphenoxy)ethanone (h): A off-white color solid; yield 73%; M.W: 330.3; Mol.For: $C_{17}H_{18}N_2O_5$; m.p: 172-174°C; LC-MS(m/z): 331.2(M+1); ^{1}HNMR (400MHz, $DMSOd_6$): δ 7.97-7.98 (d, 1H), 7.37-7.39 (d, 1H), 7.13-7.16 (q, 1H), 6.09-6.10 (t, 1H), 6.03 (s, 2H), 5.31 (s, 2H), 4.24-4.27 (t, 2H), 3.96-3.99 (t, 2H), 3.68 (s, 6H).

1-(2,3-dihydro-4H-Pyrido[3,2-b][1,4]oxazin-4-yl)-2-phenoxyethanone (i): A off-white color solid; yield 76%; M.W: 270.28; Mol.For: $C_{15}H_{14}N_2O_3$; m.p: 141-143°C; LC-MS(m/z): 271.2(M+1); ^{1}HNMR (400MHz, $DMSOd_6$): δ 7.97-7.98 (d, 1H), 7.37-7.39 (d, 1H), 7.24-7.28 (t, 2H), 7.14-7.17 (q, 1H), 6.86-6.92 (m, 3H), 5.35 (s, 2H), 4.26-4.29 (t, 2H), 3.97-3.99 (t, 2H).

1-(2,3-dihydro-4H-Pyrido[3,2-b][1,4]oxazin-4-yl)-2-(-3-methylphenoxy)ethanone (j): A off-white color solid; yield 70%; M.W: 284.3; Mol.For: $C_{16}H_{16}N_2O_3$; m.p: 149-151°C; LC-MS (m/z): 285.2(M+1); ^{1}HNMR (400MHz, $DMSOd_6$): δ 7.95-7.96 (d, 1H), 7.35-7.37 (d, 1H), 7.09-7.14 (m, 2H), 6.62-6.72 (m, 3H), 5.30 (s, 2H), 4.24-4.26 (t, 2H), 3.95-3.97 (t, 2H), 2.23 (s, 3H).

1-(2,3-dihydro-4H-Pyrido[3,2-b][1,4]oxazin-4-yl)-2-(-2-methylphenoxy)ethanone (k): A off-white color solid; yield 86%; M.W: 284.3; Mol.For: $C_{16}H_{16}N_2O_3$; m.p: 147-149°C; LC-MS(m/z): 285.2(M+1); ^{1}HNMR (400MHz, $DMSOd_6$): δ 7.94-7.96 (d, 1H), 7.36-7.38 (d, 1H), 7.08-7.16 (m, 3H), 6.75-6.83 (m, 2H), 5.36 (s, 2H), 4.23-4.26 (t, 2H), 3.96-3.98 (t, 2H), 2.11 (s, 3H).

BIOLOGICAL EVALUATION

Some of the synthesized compounds showed good antimicrobial activity inhibition. Antimicrobial screening results of the tested compounds are shown in Table 2. All the synthesized compounds showed moderate inhibitory activity and compound (II i) showed good antifungal activity inhibition compared to other compound. Antifungal screening results of the tested compounds are shown in Table 2.

Table 2: Antibacterial and Antifungal activity data of compounds (II e - II k).

Compound No.	Inhibition Zone Diameter (mm)								
	I	**II**	**III**	**IV**	**V**	**VI**	**VII**	**VIII**	**IX**
II e	12	14	16	12	14	18	18	17	19
II f	14	14	14	13	15	16	18	17	20
II g	13	16	15	15	14	19	21	19	16
II h	15	17	19	14	18	22	24	18	15
II i	10	15	20	16	19	23	21	14	17
II j	11	21	22	18	20	21	20	15	19
II k	14	18	19	17	22	19	18	16	18
Control(Solvent)	7	13	12	12	12	11	12	12	11
Ciprofloxacin	---	20	22	16	13	17	16	21	23
Fluconazole	14	---	---	---	---	---	---	---	---

Microbial Cultures Used to test antimicrobial Activity, *Fungus Culture:* I-Candida sp. *Gram Positive Bacteria:* II-Staphylococcus aureus, III-Staphylococcus albus, VIII-Streptococcus faecalis, IX- Bacillus sp. *Gram Negative Bacteria:* IV-Klebsiella pnuemoniae, V-Escherichia coli, VI- Pseudomonas sp, VII- Proteus s.

CONCLUSION

In this study, the synthesis of some fused ring morpholine phenoxy derivatives (II e – II k) was performed and their structures were confirmed by ^{1}HNMR, Mass spectroscopy techniques. In addition, the newly synthesized compounds were screened for their antibacterial and antifungal activities. Some of them were found to possess good antibacterial and antifungal activity.

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