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Synthesis and *In Vitro* Antimicrobial Activity of Some Novel Azaphenoxazine Carboxamide Derivatives

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Abstract: *A series of some novel azaphenoxazine carboxamide derivatives **2a – e** were synthesized by coupling of 3-chloro-1-azaphenoxazine with different amides via nickel catalyzed reaction. The synthesized compounds were characterized by FT-IR, [1]H NMR, [13]C NMR and MS spectral studies. All compounds were evaluated for in vitro antibacterial and antifungal activities. The compounds **2d** and **2e** exhibited good antibacterial activity while compounds **2a** and **2c** showed good antifungal activity against tested pathogenic bacterial and fungal strains.*

Keywords: *Azaphenoxazine, Carboxamide, Nickel(II)Chloride, Antibacterial, Antifungal*

I. Introduction

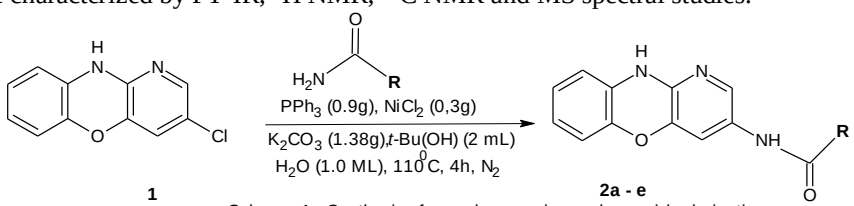
Infectious diseases caused as a result of pathogenic bacterial and fungal infections are a serious concern to the existence of mankind and often have intricate connection to other diverse diseases when the body system fails. Thus, developing antimicrobial therapies in this regard does require a major source of research in this area. Recently, considerable focus has been deployed to developing new methodologies for the synthesis of phenoxazine building blocks. Azaphenoxazine fragment was substituted with varied synthetic reactions to develop more improved moieties with enhanced activities [4]. Specifically, phenoxazine based chemical probe with aryl substituents have been documented as potent microbial agents [4, 8, 9]. It is a veritable group of heterocycle in the field of medicinal chemistry due to their biological activities including anticancer, anti-inflammatory [3] and cytotoxic properties [10]. Several substituted phenoxazines display important biological properties like antiviral activity [10], antitumor effects [1], antibacterial [9], antifungal [2, 10] and multidrug resistance reversal activity [6].

3-chloro-1-azaphenoxazine is an intermediate used in the preparation of azaphenoxazine carboxamide. Azaphenoxazine carboxamide contains the functional groups of phenoxazine and amide as part of its molecular scaffold. Antibacterial activity of compounds containing nature of functional fragment and substituted amides [2, 4, 9] has been reported. In the present study, some azaphenoxazine carboxamides derivatives **2a-e** has been synthesized and their antimicrobial activity was determined against tested pathogenic microbial strains.

II. Experimental

All solvents and reagents were purchased from Sigma-Zayo Chemicals in sure-seal bottles and were used as received. Melting points were determined on a Fisher-Johns apparatus. The FT-IR spectra were recorded using KBr discs on a Shimadzu model 8400S infrared spectrophotometer. The NMR spectra were recorded on a Bruker DRX 400 spectrophotometer in DMSO at 400 MHz and 100 MHz ^{1}H NMR and ^{13}C NMR with tetramethylsilane as the internal standard. Mass spectral data were recorded on a Shimadzu LK-9000 Mass spectrophotometer. Silica gel column chromatography was performed using Merck 7734 Silica Gel (60 - 230 Mesh) and Merck-made TLC plates.

Azaphenoxazine carboxamide derivatives **2a-e** were prepared by the method summarized in scheme 1. The reaction of 3-chloro-1-azaphenoxazine **(1)** with various amides (Benzamide, formamide, urea, trichloroacetamide and nitrobenzamide) were carried out in the presence of nickel (II) chloride, triphenylphosphine ligand , potassium trioxocarbonate (iv), water and ter-butanol was heated under nitrogen atmosphere with a good yield ranging from 62 – 98 % with light yellow to brown solids. These synthesized compounds were characterized by FT-IR, ^{1}H NMR, ^{13}C NMR and MS spectral studies.



Scheme 1: Synthesis of azaphenoxazine carboxamide derivatives

2.1 General Procedure for the synthesis of Azaphenoxazine carboxamides derivatives 2a – e: A mixture of nickel (II) chloride (0.3 g, 0.001 mol), triphenylphosphine ligand (0.9 g, 0.001 mol), potassium trioxocarbonate (iv) (1.38 g, 0.01 mol), 3-chloro-1-azaphenoxazine (7.2 g, 0.03 mol), amide (4.0 g, 0.03 mol), water (1 mL) and ter-butanol (2 mL) was heated to 110 ^{o}C for 4h under nitrogen atmosphere. The progress of the reaction was monitored by TLC. Upon completion, the solvent was removed under reduced pressure and residue was taken in water and extracted with ethyl acetate. The solvent was further evaporated to get the desired crude product which was purified by column chromatography over silica gel (60 - 230 mesh) using hexane: ethyl acetate (8:2) as an eluent.

2.1.1Synthesis of compound 2a: Dark brown, yield 85%, m.p.210 – 212 ^{0}C, IR (KBr, cm^{-1}): 3369 cm^{-1} (C-H , Ar-H), 3191 and 3071 (N-H), 1655 (C=O, amide, C=N and C=C), 1403 and 1303 (C-C str), 1030 (C-N str), and 707 (C-O-C); ^{1}H NMR (400 MHz, DMSO) δ ppm: 7.24 (td, J= (3.72, 6.41, 5.35)Hz, 2H, 2N-H), 7.46 (m, 2H, H (2), H (4), 7.88 (t, 3H, H6, H7, H8); ^{13}C NMR (100 MHz, DMSO) ppm: 129.3 (C of aromatic ring), 129.6 (C=N, C-2), 131.8 (C=C, C-8), 133.7 (C=O, amide). MS (m/z) 105(5), 245(3), 296(2), 303 (M^{+}, 100); exact mass calcd for $C_{11}H_7N_2ONCOC_6H_5$ 303.0000. Found 303.1023

2.1.2 Synthesis of compound 2b: Light brown, yield 82%. m.p.208 - 209 ^{0}C, IR (KBr, cm^{-1}): 3352 (C-H str. of the aromatic system), 3053 (N-H), 1596 (C=O of the carbonyl (amide), C=N and C=C), 692 (C-O-C); ^{1}H NMR (400 MHz, DMSO) δ ppm: 7.24 (td, j=(2.99, 7.40)Hz, 2H, 2NH), 7.40 (m, 4H, H2, H4, H8, H9); ^{13}C NMR (100 MHz, DMSO) ppm:129.2 (C of aromatic ring), 129.3 (C=N, C-2), 129.4 (C=C, C-8), 133.7 (C=O, amide), 133.9 (C-H) , 137.3 (C=C, C-4); MS (m/z): 137(1), 178(15), 210(3), 219(7), 227(M^{+}, 100) exact mass calcd for $C_{11}H_7NHONNHCHO$ 227.0012. Found 227.1520

2.1.3 Synthesis of compound 2c: Light brown, yield 62 %; m.p. 248 - 250 ^{0}C, IR (KBr, cm^{-1}): 3419 (C-H, Ar-H), 1650 (C=O, C=N and C=C), 1083 (C-N), 673 (C-O-C). ^{1}H NMR (400 MHz, DMSO) δ ppm: 7.24, (ddt, J (1.83, 4.56, 7.55)Hz, 2H, 2NH), 7.39 (m, 3H, H7, H8, H9); ^{13}C NMR (100 MHz, DMSO) ppm: 129.3 (Ar-C), 129.4 (C=N, C-2), 129.6 (C=C, C-8), 133.7 (>C=O, amide), 137.1 (C-NH_2); MS (m/z): 198(3), 209(7), 225(10). 242 (M^{+}, 100) exact mass Calcd for $C_{11}H_7N_2ONHCONH_2$ 242.0256. Found 242.1562

2.1.4 Synthesis of compound 2d: Light yellow, yield 98%, m.p 288 – 290 ^{0} C, IR (KBr, cm^{-1}): 3414 (C-H str. of the aromatic system), 3073 (N-H), 1676 (C=O str. of the amide, C=N and C=C), 1022 (C-N), 823(C-Cl), 823 (C-O-C); ^{1}H NMR (400 MHz, DMSO) δ ppm: 7.24 (ddt (J=2.42, 6.04, 7.56)Hz, 2H, 2NH), 7.39 (dd, J=(2.06, 3.86)Hz, 3H, H6, H7, H8); ^{13}C NMR (100 MHz, DMSO) ppm: 129.3 (C of aromatic ring), 129.4 (C=N, C-2), 129.6 (C=C-8), 132.0 (C-Cl), 132.1 (C=C, C-3)132.6 (C=C, C-7), 133.8 (C=O, amide), 137.2 (C=C, C-4); MS (m/z): 198(7), 215(6), 298(10), 344 (M^{+}, 100) exact mass Calcd for $C_{11}H_7N_2ONHCOCI_3$ 344.5001. Found 344.5212

2.1.5 Synthesis of compound 2e: Dark brown, yield 84%, m.p 287 – 289 ^{0}C, IR (KBr, cm^{-1}): 3442 (C-H str. of the aromatic system), 3182 and 3078 cm^{-1} (N-H), 1672 (>C=O str of the carbonyl, >C=N and C=C str), 1529 (C-C), 1365 (C-NO_2), 1122 (C-N), 713 (C-O-C). ^{1}H NMR (400 MHz, DMSO) δ ppm: 7.20 (S, 2H, 2NH), δ 7.34(S, 2H, H6, H7); ^{13}C NMR (100 MHz, DMSO) ppm: 126.3 (C of aromatic ring), 129.5 (C=N, C-2), 130.5 (C=C, C-8), 133.7 (C=O, amide), 139.4 (C=C, C-9),166.3 (C-NO_2); MS (m/z): 221(3), 285(7), 312(3), 364(M^{+}, 100) exact mass Calcd for $C_{11}N_7N_2ONHCOC_6H_5NO_2$ 364.1000. Found 364.1012

2.2 Antibacterial activity: The synthesized compounds were screened against Gram-positive bacteria (Staphylococcus aureus, Salmonella typhii, Klebsiella pneumonia) and Gram-negative bacteria (Escherichia coli, Pseudomonas aeruginosa, Streptococcus pyogene) in ethanol by disc diffusion method on nutrient agar medium [7, 10]. The sterile medium (Nutrient Agar Medium, 15mL) in each periplates was uniformly smeared with culture of Gram-positive and Gram-negative bacteria. Sterile discs of 10 mm diameter (Hi-Media) were made in each of the petriplates, to which 50 µL (1 mg/mL i.e., 50 µL/disc) of the different synthesized compounds were added. The treatments also include 50 µL of ethanol as negative control, ampicillin as positive reference standard. For each treatment three replicates were maintained. The plates were incubated at 25 ± 2 ^{0}C for 24 h and the size of the minimum inhibition concentration, if any, was determined.

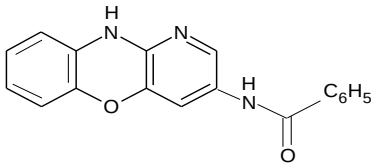
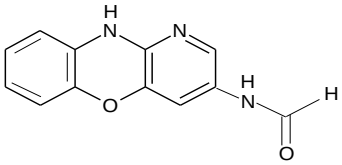
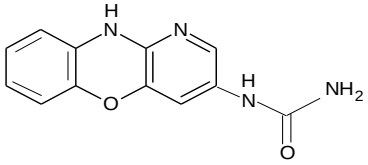
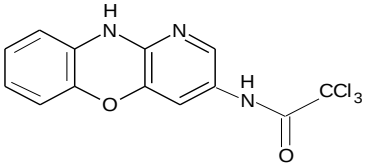
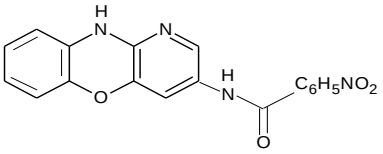
2.3 Antifungal activity: The synthesized compounds were screened for their antifungal activity against Absidia corymbifera, Epidermophyton floccosum, Fusarium solani, Trichophyton rubrum, Mucor mucedo, Candida albicans in ethanol by poisoned food technique [7]. Potato Dextrose Agar (PDA) media was prepared and about 15 mL of PDA was poured into each petriplates and was allowed to solidify. 5 mm disc of seven days old culture of the test fungi was placed at the centre of the petriplates and incubated at 26 ^{0}C for 7 days. After

incubation, the minimum inhibitory concentration was measured and three replicates were maintained for each treatment. Ketoconazole was used as standard. All the synthesized compounds were tested (at the dosage of 500 µL of the novel compounds/petriplate, where concentration was 0.1 mg/mL) by poisoned food technique.

III. Result and Discussion

In the present study, some five new compounds were synthesized. Structure of the synthesized compounds was established on the basis of FT-IR, ^{1}H NMR, ^{13}C NMR and mass spectral data. The chemical structure and physical data of novel compounds are given in Table 1.

Table 1. Chemical structure and physical data of azaphenoxazine carboxamide derivatives **2a – e**

Compound	R	Structure	Yield	m.p. (^{0}C)
2a	C_6H_5		85.00	210 - 212
2b	H		85.00	208 - 209
2c	NH_2		62.00	248 - 250
2d	CCl_3		98.00	288 - 290
2e	$C_6H_5NO_2$		84.00	287 - 289

The FT-IR spectra of **2a - e** were recorded using KBr pellets in the range of 4000 - 400 cm^{-1}. The absorption bands at 1655-1672 cm^{-1} are due to the presence of C=O stretch. The absorption band at 2360 cm^{-1} is due to the N-H stretch in compound **1**. The N-H absorption bands at 3071 -3078 cm^{-1} in **2a - e** confirmed the synthesized compounds. The strong bands at 1596 – 1676 cm^{-1} are attributed to CO stretch of the amide.

The characteristic resonance peaks in ^{1}H NMR for the new compounds were reported using DMSO. The expected resonances were assigned by their peak multiplicity and integration. The resonance appearing in the range of δ 7.41 – 7.39 ppm as multiplets, doublets and singlets is attributed to the aromatic protons. The proton spectral data agree with respect to the number of protons and their chemical shifts with the proposed structures.

The carbon-13 NMR spectral data of CCl in **1** show single resonance at δ 131.8 ppm which is absent in the spectra of **2a – e**. The resonance appearing in the range of δ 133.8 – 133.3 ppm is attributed to the carbonyl and amide series. The integration of spectra shows good agreement with the synthesized compounds. The synthesized compounds were further confirmed by the appearance of molecular ion peak in mass spectra. Mass spectra of all the newly synthesized compounds showed M^{+} fragmentation peak in agreement with their molecular formula.

The antimicrobial activities of the azaphenoxazine carboxamide derivatives **2a – e** were determined against various microbial types. The experimental result of antimicrobial activity showed varied degree of

efficacy of the compounds against different microbial strains (Table 2). Compounds **2d** and **2e** showed very good antibacterial activity against **Sa**, **Ec**, **Kp** and **Sp**. Compounds 2b and **2c** were moderately active against **Sp** and **Ed**. Compounds **2a - e** were weakly active against **St**, **Ed**, **Sp**, **Pa**, **Sa**, and **Kp**. The drug ampicillin used as standard drug exerted good antibacterial activity against **Sa**, **Kp** and **Sp**. It was also moderately active against **Pa**.

Compound **2a** showed good inhibitory activity against fungi **Ac**, **Fs** and **Tr** and compound **2c** exhibit good activity against fungi **Ac** and **Tr**. Compounds **2a – e** were moderately active against **Ef**, **Fs** and **Mm**. Weakly antifungal activity was also expressed by compounds **2a – e**. Compounds **2d** and **2e** show weak activity in comparison to the standard drug against fungi **Ac**, **Ef**, **Fs** and **Tr**.

Table 2. *In vitro* Antibacterial and Antifungal Activities of **2a – e**

Compound	Antibacterial activity (MIC) µg/mL						Antifungal activity (MIC) µg/mL					
	St	Sa	Ed	Kp	Sp	Pa	Ac	Ef	Fs	Tr	Mm	Ps
2a	15.0	2.50	15.0	2.50	15.0	15.0	1.25	5.00	1.25	1.25	5.00	15.0
2b	15.0	2.50	15.0	2.50	5.00	15.0	15.0	15.0	5.00	2.50	15.0	15.0
2c	2.50	15.0	5.00	15.0	5.00	1.25	2.50	15.0	5.00	1.25	15.0	15.0
2d	1.25	15.0	1.25	1.25	15.0	15.0	15.0	15.0	15.0	15.0	5.00	15.0
2e	15.0	2.50	2.50	2.50	2.50	15.0	15.0	15.0	15.0	15.0	5.00	5.00
Ampicillin	15.0	2.50	15.0	1.25	1.25	5.00	NA	NA	NA	NA	NA	NA
Ketoconazole	NA	NA	NA	NA	NA	NA	15.0	15.0	15.0	15.0	5.00	5.00

Abbreviation: St – Salmonella typhii; Sa – Staphylococcus aureus; Ec – Escherichia coli; Kp – Klebsiella pneumonia; Sp – Streptococcus pyogene; Pa – Pseudomonas aeruginosa; Ac – Absidia corymbifera; Ef – Epidermophyton floccosum; Fs – Fusarium solani; Tr – Trichophyton rubrum; Mm – Mucor mucedo; Ps – Penicillium specie; NA – not applicable

With respect to the SAR drawn for compounds **2a – e**. The chloro-and-nitro groups attached as substituents on the phenoxazine ring **2d** and **2e** showed remarkable activity in the antibacterial test. With the nitro substituent exhibiting highest activity followed by the chloro substituent due to it's less electron withdrawing ability. Compounds **2d** and **2e** showed least antifungal activity compared to compounds **2a**, **2c** and **2b**. This is attributed to the strong electron withdrawing ability of the chloro and nitro substituents on **2d** and **2e** against the less electron withdrawing species of **2a**, **2c** and **2b** as well as the standard drug used. Due to the changing substituents on the synthesized phenoxazine ring, it can be revealed that the presence of electron withdrawing group on azaphenoxazine carboxamide functionalized ring increases antibacterial activity and decreases antifungal activity.

IV. Conclusion

Some new azaphenoxazine carboxamide derivatives **2a - e** were synthesized and their antimicrobial activity have been evaluated. The chloro-and-nitro groups in **2d** and **2e** produced significant changes in activity against Gram-postive and Gram-negative bacteria while the benzamido, methanamido, and aminomethanamido groups in **2a** and **2c** produced significant changes in activity against absidia corymbifera, fusarium solani, trichophyton rubrum.The SAR studies revealed that the substituents on the azaphenoxazine carboxamide ring are responsible for the antimicrobial activity.

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