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Antifungal Activity of Some Monoazaphenoxazine Carboxamide Derivatives

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Abstract: The antifungal activity of some monoazaphenoxazine carboxamide derivatives was studied against *Absidia corymbifera, Epidermophyton floccosum, Fusarium solani, Trichophyton rubrum, Mucor mucedo* and *Penicillum specie* using sabouraud's dextrose agar. Compound **2a**, **2b**, and **2c** showed good sensitivity against *Absidia corymbifera, Epidermophyton floccosum, Fusarium solani, and Trichophyton rubrum*. Compounds **2d** and **2e** reveal low sensitivity against *Mucor mucedo* and *Penicillum specie*. The presence of chloro-and-nitro substituents on the azaphenoxazine carboxamide ring played a significant role towards its antifungal activities

Keywords: azaphenoxazine, carboxamide, sensitivity, Fungal, Pathogens

1. Introduction

Diseases caused by fungal infections are a serious source of concern and often trigger connections to some other infections. The most important and challenging problem to the medicinal chemist is the tenacity to fight against the drug resistant fungi. Thus, developing antimicrobial drugs and maintaining their potency, in opposition to resistance by different classes of microorganisms as well as possessing a broad spectrum of antimicrobial activity are some of the major concern of research in this area. In recent years, considerable interest has been devoted to finding a new methodology for the synthesis of azaphenoxazine building blocks. Specifically, phenoxazine based chemical probe with aryl substituents have been documented as potent microbial agents [1- 4]. They are a veritable group of heterocycle in the field of medicinal chemistry due to their biological activities including anticancer [8, 14], anti-inflammatory [13], antitubercular [10] and antimycobacterial [12] properties. Several substituted phenoxazines display important biological properties like antiviral activity [16], antitumor effects [14], antibacterial [7, 9, 10] and multidrug resistance reversal activity [5, 8]. Beside the general antibacterial activity, phenoxazines were shown to have a significant antifungal ability [2, 11]. This aspect is of interest to the present work, since some antimicrobial activities were found to be sensitive to azaphenoxazine derivatives [4]. In this connection, it was reported that azaphenoxazine carboxamide (1), a monoazaphenoxazine product, presented considerable inhibitory activity against various Gram-positive

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and Gram-negative bacteria [4]. The synthetic approach adopted in this study was drawn upon the methodology used in our earlier paper [16].

All the compounds **2a** to **2e** contain unique phenoxazine moiety having structural resemblance to the generic azaphenoxazine (1). This structural activity relationship instigated us to further evaluate the antifungal activities of these five synthesized compounds against *Absidia corymbifera, Epidermophyton floccosum, Fusarium solani, Trichophyton rubrum, Mucor mucedo* and *Penicillum specie.*

2. Materials and Methods

2.1. Materials

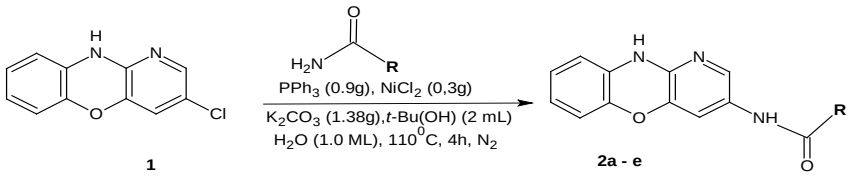
Nutrient broth, Muller-Hinton broth and agar powder were purchased from Hi-Media. Dimethylsulphoxide (DMSO) was purchased from E. Merck. Reference antibiotic disks were purchased from Hi-Media. The other materials were purchased from E. Merck. Compounds **2a** to **2e** used in this work were synthesized in our laboratory.

2.2. Fungal Culture

Fungal cultures of *Absidia corymbifera, Epidermophyton floccosum, Fusarium solani, Trichophyton rubrum, Mucor mucedo* and *Penicillum specie* were obtained from the Microbial Type Culture Collection (MTCC), Institute of Microbial Technology (IMTECH), Chandigarh, India. These strains were maintained on dextrose agar slants, subcultured regularly (every 30 days) and stored at 4^{o}C as well as – 80^{o}C by preparing suspensions in 10% glycerol.

2.3. Synthesis of Monoazaphenoxazine Carboxamide Derivatives

A Buchwald-Hartwig coupling of 3-chloro-1-azaphenoxazine (1) with various amides (Benzamide, formamide, urea, trichloroacetamide and nitrobenzamide) was 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. The method is summarized in scheme (I).



R = C_6H_5, H, NH_2, CCl_3 and $C_6H_5NO_2$

Scheme 1: Synthesis of azaphenoxazine carboxamide derivatives

2.3.1 Synthesis of compound 2a

Dark brown, yield 85%, m.p.210 – 212 ^{0}C, IR (KBr, cm^{-1}): 3369 (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.

2.3.2 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.

2.3.3 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-NH2); MS (m/z): 198(3), 209(7), 225(10), 242(M+, 100) exact mass Calcd for $C_{11}H_7N_2$ $ONHCONH_2$ 242.0256. Found 242.1562.

2.3.4 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 $C_{13}H_7N_2ONHCOCI_3$ 344.5001. Found 344.5212.

2.3.5 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 (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 $C_{11}H_7N_2ONHCOC_6H_5NO_2$ 364.1000. Found 364.1012.

2.4. Inoculum Preparation

Inoculums were prepared by transferring three to five well-isolated colonies of identical morphology to 5mL sterile nutrient broth from the respective dextrose agar plates. The broth cultures were then incubated for 24 h at 37 ^{0}C. The procedure was performed in accordance with the guidelines in [15] using a RPMI 1640 medium.

2.5. Antifungal activity Assay

The synthesized compounds were screened for their antifungal activity against *Absidia corymbifera, Epidermophyton floccosum, Fusarium solani, Trichophyton rubrum, Mucor mucedo,* and *Penicillum specie*. Sabouraud's dextrose agar (SDA) medium was used for the growth of fungi and testing was done in Sabouraud's dextrose broth (SDB) medium. The subculture and the viable count were carried out in accordance with the guidelines in [15]. The incubation temperature was maintained at 28 ± 1°C for about 72 h. The concentration of the test compound (50 µg/mL), solvent (DMSO - 200 mg/mL) and *Ketoconazole* (10 µg/disc) was used as positive reference standard. After the incubation period the zone of inhibition around the paper disc were measured in millimeters.

The sensitivity of each derivative was classified by the diameter of the inhibition zone as per the procedure documented in [19]. For weakly sensitive strain (+), the total diameter lie between 1.00 – 1.25 mm; mildly sensitive strain (++), the total diameter lie between 2.00 – 2.50 mm; very sensitive strain (+++), total diameter lie between 4.00 – 5.00 mm; and extremely sensitive strain (++++), the total diameter larger than 6.00 mm was obtained.

2.6. Minimum Inhibitory Concentration (MIC) Determination

Minimum inhibitory concentration (MIC) of compound is defined as the lowest concentration that will inhibit the visible growth of a microorganism after overnight incubation. The lowest concentration of the test compounds which caused apparently the inhibition of growth of organism, was taken as the minimum inhibitory concentration (MIC). The minimum inhibitory concentration was recorded by visual observation after 72 h of incubation. The sterile distilled water and DMSO did not show any inhibition.

3. Results and Discussion

In the present work the mean value of the antifungal activity of monoazaphenoxazine carboxamide derivatives are presented in Table 1.

Table 1: Antifungal activity of azaphenoxazine carboxamide derivatives on fungal pathogens

Compound	**Zone of inhibition in diameter (mm)**					
	Ac	Ef	Fs	Tr	Mm	Ps
2a	1.25	5.00	1.25	1.25	5.00	15.0
2b	15.0	15.0	5.00	2.50	15.0	15.0
2c	2.50	15.0	5.00	1.25	15.0	15.0
2d	15.0	15.0	15.0	15.0	5.00	15.0
2e	15.0	15.0	15.0	15.0	5.00	5.00
Ketoconazole	15.0	15.0	15.0	15.0	5.00	5.00

The results obtained in this study indicated that the fungal pathogens tested against the derivative **2a** showed that *p. specie* was found to be highly sensitive to its action followed by *E. floccosum*, *M. mucedo, A. corymbifera, F. solani* and *T. rubrum.* The minimum inhibitory concentration of **2a** among the fungal pathogens (shown in **Table 2**) reveal that *A. corymbifera, F. solani* and *T. rubrum* were found to be most sensitive, followed by *E. floccosum* and *M. mucedo* being mildly sensitive. *P. specie* was comparatively

least sensitive to **2a**. The antifungal activity as well as the microbial inhibitory action of **2a** could be attributed to phenyl ring substituent attached to the parent compound.

Among the fungal pathogens tested against **2b**, the result indicated that *A. corymbifera, E. floccosum, M. mucedo* and *P. specie* were found to be highly sensitive to their action followed by *F. solani* and *T. rubrum***.** The minimum inhibitory concentration of **2b** among the fungal pathogens reveals that *T. rubrum* and *M. mucedo* were most sensitive, followed by *F. solani. A. corymbifera, E. floccosum* and *P. specie* were least sensitive. The microbial inhibitory action of **2b** may be due to the formyl substituent attached to the ring that enhanced its antimicrobial activity.

Among the fungal pathogens tested against **2c**, the result indicated that *E. floccosum, M. mucedo* and *P. specie* were found to be highly sensitive to their action, followed by *F. solani, A. corymbifera* and *T. rubrum*. The minimum inhibitory control of **2c** reveals that *T. rubrum* was found to be most sensitive, followed by *A. corymbifera, F. solani and M. mucedo. E. floccosum* and *P. specie* were comparatively less sensitive to the action of derivative **2c.** The antifungal activity of **2c** may be due the action of amino substituent attached to the parent compound.

Table 2: Minimum inhibitory concentration of azaphenoxazine carboxamide derivatives on the growth of fungal pathogens

Fungal pathogens	**Azaphenoxazine carboxamide derivatives**				
	2a	**2b**	**2c**	**2d**	**2e**
A. corymbifera	++++	+	+++	+	+
E. floccosum	++	+	+	+	+
F. solani	++++	++	++	+	+
T. rubrum	++++	+++	++++	+	+
M. mucedo	++	++	++	++	++
P. specie	+	+	+	+	+

+: Weakly Sensitive, ++: Mildly Sensitive, +++: Very Sensitive, ++++: Extremely Sensitive

The results obtained for **2d** and **2e** indicated that *A. corymbifera, E. floccosum, F. solani, T. rubrum* and *P. specie* were found to be highly sensitive to their action, followed by *M. mucedo* and *P. specie* as with **2e**. The minimum inhibitory control of **2d** and **2e** show that *M. mucedo* was the most sensitive fungal pathogen, followed by *A. corymbifera, E. floccosum, F. solani, T. rubrum* and *P. specie*. The antifungal activity of **2d** and **2e** may be due to the presence of the chloro-and-nitro substituents on the phenoxazine moiety.

From the SAR investigation, compounds **2d** and **2e** showed greatest antifungal activity compared to compounds **2a**, **2c** and **2b**. Due to the changing nature of substituents on the synthesized phenoxazine ring reveals that the carboxamide functionalized derivatives show relatively significant antifungal activity.

4. Conclusion

This report presents the pioneering findings on the potent antifungal activity of compounds **2a** to **2e** against *Absidia corymbifera, Epidermophyton floccosum, Fusarium solani, Trichophyton rubrum, Mucor mucedo* and *Penicillum specie*. The novelty of the synthesized compounds with highly efficient synthetic protocol largely supports them as potential antifungal candidates. The presence of azaphenoxazine carboxamide moiety and the attached substituent's on the phenyl ring played a significant role towards its antifungal activities.

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Reference

[1]. Balaban, A. T., Constantinescu, T., Baratoic, R. D., Spataru, N., Spataru, T., Ionita, G., Beteringhe, A., Draghici, C., Caprioc, M. T., Baciu, I., Radutiu,C. R., 2009. Wurster aza crown ethers with N-para-phenylphenothiazine or phenoxazine groups. Arkivoc. xiii, 342 - 362

[2]. Balaban, A. T., Constantinescu, T., Baratoic, R. D., Spataru, N., Spataru, T., Ionita, G., Beteringhe, A., Draghici, C., Caprioc, M. T., Baciu, I., Radutiu, C. R., 2008. 3, 5-Dinitro-N-(41- benzo-15-crown)-benzamide derivatives-Synthesis and Properties. Arkivoc. xi, 307-321.

[3]. Gatti, R., Gioia, M. G., Dipietra, A. M., 2002. Fluorescent labelling of biomolecules with organic probes. Anal. Chim. Acta. 11, 474-479.

[4]. Agbo, S. A., Anoh, V. A., Okoro, U. C., 2014. Nickel catalyzed amidation reaction in the synthesis of azaphenoxazine carboxamides. J. Applicable. Chem. 3 (6), 2526-2532.

[5]. Wesolowska, O., Wolhar, J., Westman, G., Samuelsson, K., Kawase, M., Ocsovszki, I., Motohashi, N., Michalak, K., 2006. Benzo[a]phenoxazines: A new group of potent p-glycoprotein inhibitors. In vivo. 20, 109-114.

[6]. Jorgensen, J. H., Ferraro, M. J., 2009. Antibiotic susceptibility testing: A review of general principles and contemporary practices. Clin. Infect. Dis. 49, 1749-1755.

[7]. Frade, V. H. J., Sousa, M. J., Moura, J. C. V. P., Goncalves, M. S. T., 2009. Synthesis, characterization and antibacterial activity of new benzo[a]phenoxazine based fluorophores. Tetrahedron Letters. 48 (47), 8347-8352.

[8]. Eregowda, G. B., Kalpana, H. N., Thimmaiah, K. N., 2000. Synthesis and analysis of structural features of phenoxazine analogues needed to reverse vinblastine resistance in multidrug resistant (MDR) cancer cells. Indian J. Med. Chem.39B, 243-245.

[9]. Shimizu, S,, Suzuki, M., Tomoda A., Arai, S., Taguchi, H., Hanawa, T; Kamiya, S., 2004. Phenoxazine compounds produced by the reactions with bovine hemoglobin show antimicrobial activity against non-tuberculosis mycobacteria.Tohoku J. Exp. Med. 203, 47-52.

[10]. Poonam, K., Santosh, K. S., Savitri, D. S., 2014. Synthesis, Characterization and Evaluation of Some Novel 5-(2-aryl-4-oxo-1, 3- thiazolidine)-2-(phenoxazinyl methyl)-1,3,4-thiadiazole Derivatives as Antimicrobial and Antitubercular Agents J. applicable Chem. 3 (2): 525-534.

[11]. Liu H-L., Li, Z., Anthosen, T., 2000. Synthesis and fungicidal activity of 2-imino-3-(4arylthiazol-2-yl)-thiazolidin-4-ones and their 5-arylidene derivatives. Molecules. 5, 1055-1061.

[12]. Srivastava, T., Gaikwad, A. K., Haq, W., Sinha, S. 2005. Synthesis and biological evaluation of 4 thiazolidinone derivatives as potential antimycobacterial agents. Arkivoc, 20, 120-130

[13]. Yandav, R., Srivastava, S. D, Srivastava, K. S., 2005. Synthesis, antimicrobial and anti-inflammatory activities of 4-oxothiazolidines and their 5-arylidenes. Indian J. Chem. 44, 1262-1266.

[14]. Fumika, S., Ken, H., Mariko, I., Gunnar, W., Kristin, S., Masami, K., Noboru, M., Hiroshi, S., 2007. Tumor-specificity and type of cell death induced by Phenoxazines. Anticancer Res. 27, 4233-4238.

[15]. Clinical and Laboratory Standards Institute: Performance standards for antimicrobial Susceptibility testing; Sixteen International supplements. CLSI document M100 S16. Vol 26-3; M7-A7, Vol.26-2; M2-A9, Vol. 26-1. Wayne PA. USA 2006

[16]. Anoh, V .A., Agbo, S. A., and Swande, P. I., 2014. Antimicrobial evaluation of some monoazaphenoxazine carboxamides: Structural activity relationship (SAR). In Proceedings of the 18th International Electronic Conference on Synthetic Organic Chemistry, 1-30 November; Sciforum Electronic Conference Series, 18, 2014, b008; doi:10.3390/ecsoc-18-b008.