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Synthesis and Biological Evaluation of Pseudosaccharin Amines as Antifungal agents

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Abstract Fungal is a deadly infection and kills many people annually. There is a need for new antifungal agents due to the emergence of drug resistant fungal infections. In this report we describe the pseudosaccharin amine derivatives with antifungal activities against *Candida albicans* and *A. fumigatus*. Compound 4g showed MIC of 7 and 25 µg/mL against *Candida albicans* and *A. fumigatus* respectively.

Keywords Antifungal, Azole, Minimum Inhibitory Concentration, Pseudosaccharin amine

Introduction

Fungal is a deadly infection and kills many people annually. In the first meeting of experts which was organized by World Health Organization (WHO) deliberated on the development of priority list of fungal infections [1]. The fungal pathogens which commonly seen in human infections are *Aspergillus fumigates* [2], *Candida albicans* [2, 14] and *Cryptococcus neoformans* [3]. Topical infection to life-threatening diseases are caused by fungi [4,5,12]. In recent times, the deaths due to invasive fungal infections are increasing in immune compromised or immune supressed individuals [6,7]. The treatment available to treat the fungal infections includes flucanazole and variconazole which are azole class of compounds [8,11,13]. The other agents include nystatin and amphotericin B while caspofungin and micafungin are echinocandins [8]. Important to note that, due to frequent use of fluconazole, the resistance to azole class of compound is on rise [9, 16]. Hence, there is an urgent need to develop new antifungal agents. In order to synthesize the compounds with potential antifungal activity, we selected the pseudosaccharin amine class of compound. Pseudosaccharin amines with various biological activities are reported. These include KV1.5 channel blocking activity [10]. In this paper, we describe the antifungal activity of pseudosaccharin amine derivatives against *Candida albicans* and *A. fumigatus*.

Results and Discussion

Various pseudosaccharin amine derivatives were synthesized as shown in scheme 1. Initially, the sodium saccharin was treated with dilute HCl to obtain saccharin [2]. The chlorination of saccharin was carried out accoutring to the literature procedure using thionyl chloride [10]. The required derivatives were prepared by treating 3 with various amines in 1,4-dioxane to afford 4a-4k as solid substances. The analytical data of these compounds is shown in experimental part.

Scheme 1: Synthesis of compounds

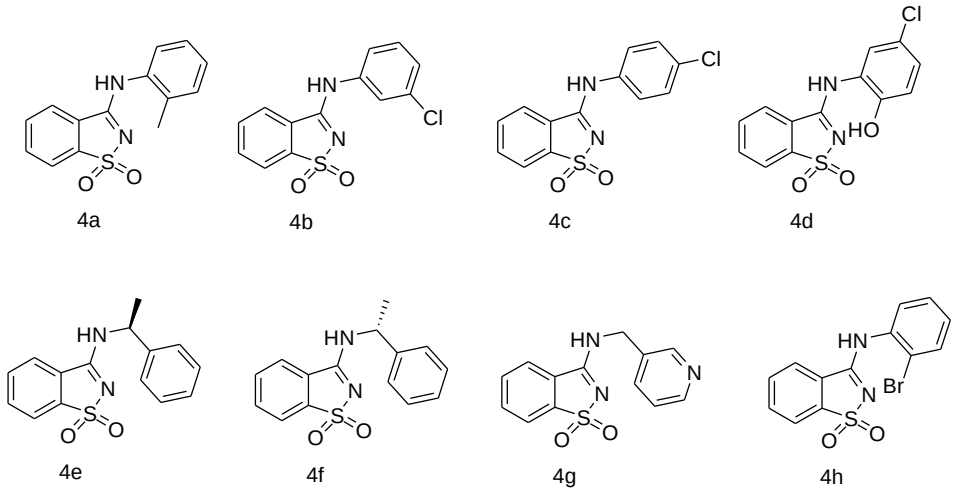

Figure 1: Structures of synthesized derivatives

The synthesized compounds were tested against *C. albicans* and *A. fumigatus*. The inhibitory data for the compounds is shown in table 1. Most of the compounds showed moderate activity against *C. albicans*. In particular, compound 4g showed MIC value of 7 µg/mL against *C. albicans*. Compound 4e and 4f showed MIC of 12 µg/mL against *C. albicans* indicating there is no preference for any particular stereochemistry for the activity. Most of the compounds (4a, 4b, 4c, 4d, 4e) showed weak inhibition of *A. fumigatus*. The compound 4f showed moderate inhibition with MIC of 38 µg/mL against *A. fumigatus*. Compound 4g was the potent inhibitor of *A. fumigatus*. The inhibitory data indicates that the derivatives showed relatively better activity against *C. albicans* as compared to *A. fumigatus* and compound 4h was the most active against both the fungi.

Table 1: Minimum inhibitory concentration (MIC in µg/mL) of compounds *against C. albicans* and *A. fumigatus*

Compound	***C. albicans***	***A. fumigatus***
4a	48	87
4b	28	63
4c	14	68
4d	39	96
4e	12	45
4f	12	38
4g	7	25
4h	22	61

Experimental

General procedure for the synthesis of compounds 4a-4h

Pseudosaccharin chloride 3 (500 mg) and respective amine (1 equivalent) was added in 1,4-dioxan (15 mL). The reaction mixture was refluxed for 6 hrs. After completion of the reaction, ice was added into the mixture. The precipitate was formed which was separated by filtration and dried. The compounds were analysed by using IR and NMR spectroscopy.

Analytical data for compounds

Compound 4a: ^{1}H NMR (300 MHz, DMSO-d6): ppm 10.27 (bs, 1H), 8.25-8.17 (m, 1H), 7.83-7.77 (m, 1H), 7.70-7.63 (m, 2H), 7.39-7.33 (m, 1H), 7.26-7.16 (m, 3H), 2.27 (s, 3H); IR $_{vmax}$ cm^{-1} (KBr) 3786.38, 3456.26, 3269.44, 2409.93, 1612.73, 1564.97, 1521.38, 1492.23, 1459.86, 1340.89, 1283.01, 1149.81, 1049.24, 967.86, 790.80, 772.09, 753.94, 702.96.

Compound 4b: ^{1}H NMR (300 MHz, DMSO-d6): ppm 10.37 (bs, 1H), 8.33-8.25 (m, 1H), 7.92 (t, *J* = 1.9 Hz, 1H), 7.86-7.75 (m, 2H), 7.70-7.60 (m, 2H), 7.27 (t, *J* = 8.1 Hz, 1H), 7.14-7.06 (m,1H); IR $_{vmax}$ cm^{-1} (KBr)3787.83, 3699.21, 3662.02, 3577.37, 3319.61, 2423.98, 2233.66, 1615.75, 1593.04, 1539.66, 1463.18, 1436.01, 1299.79, 1261.34, 1155.03, 997.65, 957.24, 880.87, 774.15, 746.92, 710.08.

Compound 4c: ^{1}H NMR (300 MHz, DMSO-d6): ppm 10.41 (bs,1H), 8.32-8.24 (m, 1H), 7.85-7.79 (m, 3H), 7.70-7.62 (m, 2H), 7.33-7.29 (m, 2H); IR $_{vmax}$ cm^{-1} (KBr) 3679.71, 2969.77, 2967.02, 2075.63, 1612.49, 1346.99, 1286.34, 1156.36, 1054.84, 1032.46, 818.66, 769.99, 737.52.

Compound 4d: ^{1}H NMR (300 MHz, DMSO-d6): ppm 9.92 (bs, 1H), 9.32 (bs, 1H), 8.05-7.96 (m, 2H), 7.88-7.80 (m, 1H), 7.73-7.63 (m, 2H), 6.97 (dd, *J* = 6.2, 2.5 Hz, 1H), 6.84 (d, *J* = 14.3 Hz, 1H); IR $_{vmax}$ cm^{-1} (KBr) 2918.95, 1620.61, 1564.95, 1299.30, 1158.68, 956.51, 874.57, 811.41, 770.44, 737.62.

Compound 4e: ^{1}H NMR (300 MHz, DMSO-d6): ppm 9.01 (bs, 1H), 8.12 (d, *J* = 7.5 Hz, 1H), 7.74 (d, *J* = 7.1 Hz, 1H), 7.66-7.50 (m, 2H), 7.41-7.32 (m, 2H), 7.32-7.15 (m, 3H), 5.36 (q, *J* = 7.2 Hz, 1H), 1.60 (d, *J* = 7.0 Hz, 1H); IR $_{vmax}$ cm^{-1} (KBr) 3275.85, 3098.59, 2978.72, 2929.15, 1613.58, 1527.50, 1458.90, 1282.09, 1121.04, 947.34, 767.96, 703.87, 603.98, 564.15.

Compound 4f: ^{1}H NMR (300 MHz, DMSO-d6): 9.04 (d, *J* = 7.7 Hz, 1H), 8.14 (d, *J* = 6.1 Hz, 1H), 7.80-7.71 (m, 1H), 7.65-7.51 (m, 2H), 7.36-7.33 (m, 2H), 7.32-7.17 (m, 3H), 5.36 (q, *J* = 7.2 Hz, 1H), 1.60 (d, *J* = 6.9 Hz, 1H); IR $_{vmax}$ cm^{-1} (KBr) 3276.57, 3098.72, 2980.27, 2936.02, 1723.17, 1613.28, 1528.06, 1460.53, 1350.08, 1286.65, 1154.25, 1053.29, 948.14, 704.10, 603.71, 551.62, 405.01.

Compound 4g: ^{1}H NMR (300 MHz, DMSO-d6): 8.67 (bs, 1H), 8.58 (d, *J* = 4.8 Hz, 1H), 7.85 (d, *J* = 14.4 Hz, 1H), 7.81-7.75 (m, 1H), 7.27-7.60 (m, 3H), 7.39 (d, *J* = 7.8 Hz, 1H), 7.34-7.28 (m, 1H), 4.90 (d, *J* = 4.6 Hz, 1H); IR $_{vmax}$ cm^{-1} (KBr) 3290.84, 3061.24, 2924.05, 1921.19, 1632.02, 1590.77, 1502.09, 1433.81, 1366.48, 1297.02, 1155.14, 1070.68, 999.25, 959.01, 826.78, 750.72, 617.57, 583.09, 530.83, 459.34.

Compound 4h: ^{1}H NMR (300 MHz, DMSO-d6): 10.26 (bs,1H), 8.25 (d, *J* = 6.3 Hz, 1H), 7.86-7.79 (m, 1H), 7.73-7.66 (m, 3H), 7.65-7.56 (m, 1H), 7.42-7.30 (m, 1H), 7.20-7.11 (m, 1H);); IR $_{vmax}$ cm^{-1} (KBr) 3449.51, 3355.35, 1597.03, 1560.56, 1468.33, 1310.48, 1156.27, 1023.51, 949.60, 772.68, 744.38, 705.87, 651.06, 531.13, 403.34.

MIC determination

Determination of MIC: Minimum inhibition concentration (MIC) is the lowest concentration of the compound that inhibited the visible growth of fungi. The tube dilution method was used to determine the MIC. The DMSO stock of the compound was used in the assay. The serial dilution of stock solution was carried out to generate various concentrations of compounds. One ml of respective cultures having an OD of 0.2 (~ McFarland standard) along with the test sample in the tubes were incubated at 37 ºC for 16 h. The turbidity of each tube was then measured and compared with the control tube [15].

Conclusion

In summary, we demonstrated that pseudosaccharin amine derivatives have antifungal activity. Compound 4g showed MIC of 7 and 25 µg/mL against *Candida albicans* and *A. fumigatus*, respectively.

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