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Synthesis And Antifungal Activity Of Terbinafine Analogues

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Abstract: A series of 1-(substitutedphenoxy methyl) naphthalene was synthesized and evaluated for antifungal activity using terbinafine as a standard by agar streak dilution method. These were prepared by condensation of 1- chloromethyl naphthalene with different substituted aryl alcohols Terbinafine is a therapeutically used inhibitor of fungal squalene epoxidase that has prompted extensive derivatisation programs for structure-activity relationship studies. In the present study, functional analogues of terbinafine were synthesized that lack the central tertiary amino group but have polar substituents at the tert-butyl residue of the side chain. Evaluation of the antifungal potential revealed that representatives of this novel structural type can also exhibit broad antifungal activity, indicating that the central amino function of allylamine antimycotics is not essential for inhibition of fungal growth. Potency appears to correlate with the polarity of the introduced functional groups, while broad antifungal activity seems to be restricted to compounds with basic substituents.
Keywords: Terbinafine, 1- chloromethyl naphthalene, Squalene epoxidase, Antimycotics, Allylamine.

Introduction

The treatment of fungal infection is still challenging due to an increase in mycotic infection and emergence of drug resistance has necessitated development of new antifungal agents[1-2]. Squalene epoxidase (SE; squalene monooxygenase; EC 1.14.99.7) is a membrane-bound enzyme in fungi that is responsible for conversion of squalene to squalene-2,3-epoxide which is further converted into steroidal molecules and ultimately to ergosterol. This step of epoxidation is important in ergosterol biosynthesis since it may lead to cascade of biosynthetic pathways. Allylamine antifungals such as naftifine and terbinafine act as squalene-2, 3-epoxidase inhibitors[3-4]. The allylamine naftifine was fortuitously discovered during the search for drugs to treat central nervous system (CNS) disorders. Naftifine was the first representative of the allylamine antimycotic, known to act by selective inhibition of the fungal squalene epoxidase. Further exploration of Structure activity relationship study led to discovery of terbinafine which is more potent[5-6]. Nussebaumer[7-9] reported antimycotic allylamine terbinafine in which naphthalene and the tert-butyl acetylene moieties were retained but the spacer between these two groups was varied.

Further, considering terbinafine as standard 1-chloromethyl naphthalene was modified by reacting with various aryl alcohols to contain more polar centre (-O-) than (-N-) and it was found that they still retain and exhibited potent antifungal potency against *C.albicans* and *A.niger* species of fungi. Hence, we planned to synthesize 1-(substitutedphenoxy methyl) naphthalene analogues which have potent antifungal potency.

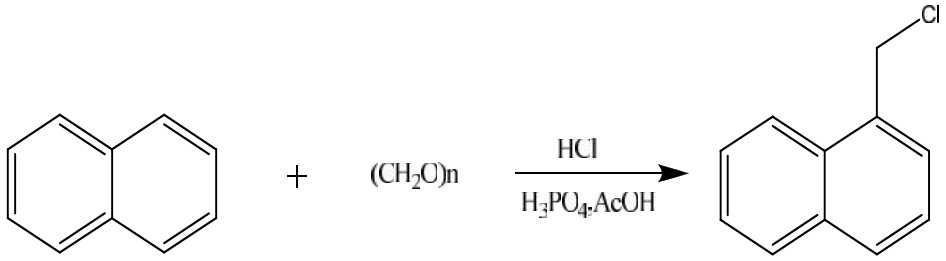
Materials And Methods

Chemistry

Melting points were determined in open capillary melting point apparatus. All chemicals used in this study were purchased from Aldrich Co. (Milwaukee, WL, USA). Purity and homogeneity of synthesized compounds was checked using thin layer chromatography on silica gel plates with a fluorescent indicator and the R_f values were calculated. IR spectra were recorded using KBr pellets on JASCO FT-IR 5300 and *SHIMADZU – FTIR3100* spectrophotometer. ^{1}H-NMR spectra were obtained with "VARIAN-NMR 300 *MERCURY*" spectrometer with TMS as an internal standard. Chemical shifts were reported as δ (ppm). Sabouraud's Dextrose agar for antifungal activity was purchased from Hi media. The microorganisms were incubated in an incubator bath.

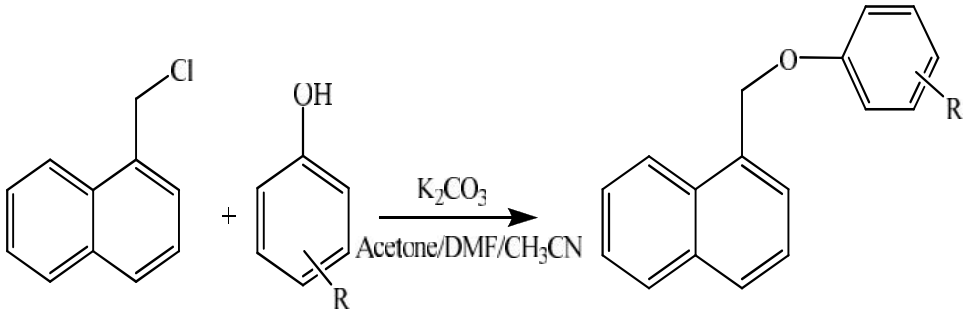
Scheme of Synthesis

Step 1: Synthesis of 1-chloromethyl naphthalene



Naphthalene Paraformaldehyde 1-Chloromethyl Naphthalene

Step 2: Synthesis of substituted naphthalene ethers



1-Chloromethyl naphthalene R = Substituted phenols 1-naphthylmethoxy derivatives

Synthesis

Preparation of 1-chloromethyl naphthalene

In a three-necked round bottom flask (RBF), fitted with a reflux condenser and mechanical stirrer were placed 25g (0.19 mole) of naphthalene, 9g (0.27 mole) of *p*-formaldehyde, 26 mL (0.1 mole) of glacial acetic acid, 30 mL of conc. hydrochloric acid and 14 ml of o-phosphoric acid was added to this mixture and heated in a water bath at 80-85°C. The reaction mixture was stirred for 9-10 hrs and cooled to 15-20°C, poured in to cold water (200mL). Water was decanted from oily layer and washed with portions of cold water (3×200 mL). The organic layer was dried over anhydrous sodium sulphate, filtered and extracted with ether[11]. Upon recovery of solvent 1-chloromethyl naphthalene was obtained.

Yield: 55%, m.p.: 155-158°C, R_f: 0.55 (2:0.5 Hexane: ethyl acetate, v/v).

IR: 3020 (Ar-CH), 2900 (-CH), 780 (-CCl)

^{1}H-NMR ($CDCl_3$): 7.5-8.038 (s, d, m, 7H, Ar-H), 5.01 (s, 2H, -CH_2)

Preparation of substituted naphthalene ethers (1-14)

General procedure: In a 250 mL RBF, 1.49 mL (0.01 mole) of 1-chloromethyl naphthalene (1.49 mL, 0.01 mole) was dissolved in 30 mL of dry acetone. To this solution, 0.01 mole of aryl alcohols and 0.01 mol of anhydrous potassium carbonate were added. The resulting reaction mixture was then refluxed for 24 hrs. After completion of reaction, the reaction mixture was cooled and poured into 60 mL of cold water. The residue was filtered, washed well with water, dried to afford final compound (1-14) and was recrystallized from methanol.

Antifungal activity

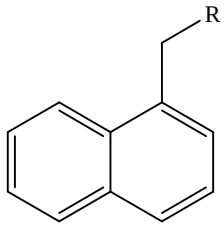
Minimum inhibitory concentration of the synthesized compounds was evaluated out using agar streak dilution method (Hawkey and Lewis, 1994). *Candida albicans* NCIM 3557 was used for antifungal activity test. Stock solutions of the synthesized compounds (50 µg/ml) were prepared in dimethyl sulfoxide (DMSO) and graded quantities of the test compounds were incorporated in specified quantity of molten sterile Sabouraud dextrose agar medium. A specified quantity of the medium (40– 45ºC) containing the test compound was poured into a petri dish to a depth of 3–4 mm and allowed to solidify. Suspension of the fungi containing approximately 105 colony-forming units (cfu)/ml was applied to these plates and incubated at 37ºC for 48 h. The MIC was considered to be the lowest concentration of the test substance exhibiting no visible growth of fungi on the plate[10]. The antifungal activity data of the synthesized compounds is given in Table 2 and 3.

Results And Discussion

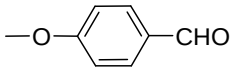
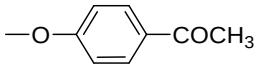
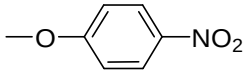
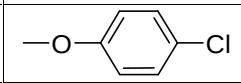
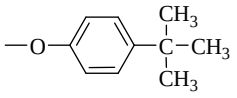
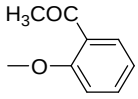
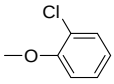
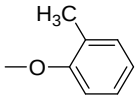
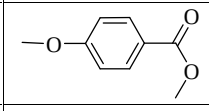
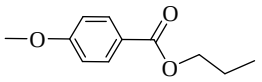
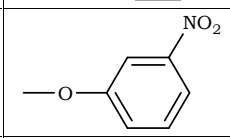
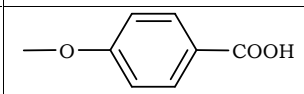
Chemistry

Different 1-(substituted phenoxy methyl) naphthalene have been synthesized as per scheme 1. In the first step, synthesis of 1-chloromethyl naphthalene (III) was carried out using naphthalene (II) and *p*-formaldehyde in the presence of hydrochloric acid and phosphoric acid. 1-Chloromethyl naphthalene was condensed with different substituted aryl alcohols to get finally substituted 1-(substituted phenoxy methyl) naphthalene (1-14). The structures of synthesized compounds were confirmed by chromatographic and spectral analysis. The physicochemical characteristics of synthesized compounds are summarized in Table 1.

Table 1 Physicochemical characterization and structures of 1-14



Where R is substituted phenols

Compound No.	R	Molecular Formula	% Yield	m.p.* (°C)	R_f
1		$C_{18}H_{14}O_2$	54	110-112	0.61***
2		$C_{19}H_{16}O_2$	57	134-126	0.58***
3		$C_{17}H_{13}NO_3$	61	120-122	0.73***
4		$C_{17}H_{13}ClO$	58	116-118	0.74***
5		$C_{21}H_{22}O$	56	138-140	0.53***
6		$C_{19}H_{16}O_2$	53	130-132	0.64**
7		$C_{17}H_{13}ClO$	54	121-123	0.72**
8		$C_{18}H_{16}O$	60	102-104	0.74***
9		$C_{18}H_{16}O$	62	108-111	0.58***
10		$C_{19}H_{16}O_3$	61	86-89	0.67***
11		$C_{21}H_{20}O_3$	59	92-94	0.59***
12		$C_{18}H_{16}O_2$	62	88-90	0.58**
13		$C_{17}H_{13}NO_3$	59	113-115	0.61***
14		$C_{18}H_{14}O_3$	60	94-96	0.61***

*****Melting point uncorrected
****** Benzene: Ethyl acetate (4:1)
*******n-Hexane: Ethyl acetate (4:1)

1-(4-Formylphenoxy methyl) naphthalene (1)

IR: 3069, 2929 cm^{-1}(Ar-CH), 2873 cm^{-1} (-CH), 1686 cm^{-1} (-CO), 1598 cm^{-1} (Ar-C=C), 1212 cm^{-1} (C-O-C)

^{1}H-NMR ($CDCl_3$): 7.1-8.1 (m, 11H, Ar-H), 5.64 (s, 2H, -CH_2), 9.87 (s, H,-CHO)

1-(4-Acetylphenoxy methyl) naphthalene (2)

IR: 3060, 2936 cm^{-1}(Ar-CH), 2880 cm^{-1} (-CH), 1673 cm^{-1} (-CO), 1593 cm^{-1} (Ar-C=C), 1244 cm^{-1} (C-O-C)

1-(4-Nitrophenoxy methyl) naphthalene (3)

IR: 3070, 2936 cm^{-1}(Ar-CH), 2883 cm^{-1} (-CH), 1508 cm^{-1} (-N=O), 1591 cm^{-1} (Ar-C=C), 1254 cm^{-1} (C-O-C)

^{1}H-NMR ($CDCl_3$): 7.03-8.18 (m, 11H, Ar-H), 5.60 (s, 2H, -CH_2)

1-(4-Chlorophenoxy methyl) naphthalene (4)

IR: 3063, 2923 cm^{-1}(Ar-CH), 2872 cm^{-1} (-CH), 1589 cm^{-1} (Ar-C=C), 1237 cm^{-1} (C-O-C), 798 cm^{-1} (C-Cl)

1-(4-tert-Butylphenoxy methyl) naphthalene (5)

IR: 3054, 2965 cm^{-1}(Ar-CH), 2863 cm^{-1} (-CH), 1364 cm^{-1} (*ter*-butyl unsymmetrical doublet), 1591 cm^{-1} (Ar-C=C), 1254 cm^{-1} (C-O-C)

^{1}H-NMR ($CDCl_3$): 6.69-7.18 (m, 11H, Ar-H), 5.45 (s, 2H, -CH_2), 1.34(s, 9H, –tert-butyl group)

1-(2-Acetylphenoxy methyl) naphthalene (6)

IR: 3071, 2930 cm^{-1}(Ar-CH), 2879 cm^{-1} (-CH), 1662 cm^{-1} (-C=O), 1592 cm^{-1} (Ar-C=C), 1238 cm^{-1} (C-O-C)

1-(2-Chlorophenoxy methyl) naphthalene (7)

IR: 3065, 2945 cm^{-1}(Ar-CH), 2888 cm^{-1} (-CH), 1585 cm^{-1} (Ar-C=C), 1241 cm^{-1} (C-O-C), 781cm^{-1}(-C-Cl)

^{1}H-NMR ($CDCl_3$): 6.90-7.77 (m, 11H, Ar-H), 5.593 (s, 2H, -CH_2)

1-(2-Methylphenoxy methyl) naphthalene (8)

IR: 3073, 2955 cm^{-1}(Ar-CH), 2877 cm^{-1} (-CH), 1595.81 cm^{-1} (Ar-C=C), 1240 cm^{-1} (C-O-C), 885 cm^{-1} (Aromatic –CH_3 out of plane stretching)

1-(4-Methylphenoxy methyl) naphthalene (9)

IR: 3043, 2918 cm^{-1}(Ar-CH), 2864 cm^{-1} (-CH), 1508 cm^{-1} (Ar-C=C), 1234 cm^{-1} (C-O-C), 886cm^{-1}(Aromatic –CH_3 out of plane stretching)

^{1}H-NMR ($CDCl_3$): 6.95-7.78 (m, 11H, Ar-H), 5.425 (s, 2H, -CH_2), 2.35cm^{-1} (C-H protons of –CH_3 group)

Methyl 4-((naphthalen-5-yl) methoxy) benzoate (10)

IR: 3060, 2961 cm-1(Ar-CH), 2864 cm-1 (-CH), 1508 cm-1 (Ar-C=C), 1234 cm-1 (C-O-C), 886cm-1(Aromatic –CH_3 out of plane stretching)

^{1}H-NMR ($CDCl_3$): 6.95-7.78 (m, 11H, Ar-H), 5.425 (s, 2H, -CH_2), 2.35cm-1 (C-H protons of –CH_3 group)

Propyl 4-((naphthalen-5-yl) methoxy) benzoate (11)

IR: 3048, 2966 cm-1(Ar-CH), 2878 cm-1 (-CH), 1507.1 cm-1 (Ar-C=C), 1274 cm-1 (C-O-C), 1166cm-1(-C=O)

^{1}H-NMR ($CDCl_3$): 7.10-7.77 (m, 11H, Ar-H), 5.56 (s, 2H, -CH_2), 1.82(t,2H,-CH_2), 4.25(q, 2H, -CH_2), 0.96(s, 3H,-CH_3)

1-(4-Methoxyphenoxy methyl) naphthalene (12)

IR: 3051, 2945 cm-1(Ar-CH), 2894 cm-1 (-CH), 1507.1 cm-1 (Ar-C=C), 1228 cm-1 (C-O-C)

^{1}H-NMR ($CDCl_3$): 6.90-7.82 (m, 11H, Ar-H), 5.45 (s, 2H, -CH_2), 1.82(t, 2H,-CH_2), 4.25(q, 2H, -CH_2), 0.96(s, 3H,-CH_3)

1-(3-Nitrophenoxy methyl) naphthalene (13)

IR: 3051, 2903 cm-1(Ar-CH), 2866 cm-1 (-CH), 1528.31 cm-1 (-NO_2), 1242 cm-1 (C-O-C)

^{1}H-NMR ($CDCl_3$): 6.90-7.82 (m, 11H, Ar-H), 5.45 (s, 2H, -CH_2), 1.82(t, 2H,-CH_2), 4.25(q, 2H, -CH_2), 0.96(s, 3H,-CH_3)

1-(4-Carboxylphenoxy methyl) naphthalene (14)

IR: 3060, 2936 cm^{-1}(Ar-CH), 2880 cm^{-1} (-CH), 1673 cm^{-1} (-CO), 1593 cm^{-1} (Ar-C=C), 1244 cm^{-1} (C-O-C).

Antifungal activity

The synthesized 1-(substitutedphenoxy methyl) naphthalene compounds were tested in vitro for their growth inhibitory activities against pathogenic fungi by comparison with terbinafine as a fungicidal standard agent. As indicated in the Table II, among the 1-(substituted phenoxy methyl)naphthalene compounds 3, 4 and 14 showed generally good activities against *C. albicans* and *A. Niger* at the MIC level ranging from 12.5 to 150 µg/ml. Compound 3 and 4 exhibited maximum activity, while 12 and 13 exhibited moderate activity compared to standard terbinafine. These compounds have substituents such as nitro and chloro substituents respectively in these structures.

The compounds were evaluated for *in vitro* antifungal activity against *Candida albicans* ATCC-10231 (NCIM-3471), *Penicillium notatum* NCIM-745, *Aspergillus Niger* NCIM-575, *Aspergillus fumigatus* NCIM-630. Terbinafine was used as standard. The zone of inhibition and MIC of the synthesized compounds are reported in Table 2 and 3. The photographic representation of antifungal activity of Terbinafine and compound 3 is presented in Figure 1 and 2.

Table 2. *In vitro* antifungal activity of 1-(substitutedphenoxy methyl)naphthalene Derivatives (Zone of Inhibition)

Compound No.	Zone of Inhibition in mm at 25 µg/ml against			
	Aspergillus Niger	***Aspergillus Fumigates***	***Penicillium Notatum***	***Candida Albicans***
1	11	13	NA	14
2	13	14	10	12
3	29	21	23	28
4	26	23	28	23
5	14	10	8	12
6	12	NA	6	8
7	11	8	9	14
8	8	7	10	18
9	NA	10	8	12
10	7	12	14	13
11	10	NA	NA	8
12	16	14	18	22
13	15	17	14	18
14	24	22	27	29
Terbinafine	31	27	31	34

NA- Not active

Table 3. *In vitro* antifungal activity of 1-(substitutedphenoxy methyl)naphthalene (MIC) against *C.albicans*

Compound No.	MIC(µg/ml)
1	150
2	125
3	**25**
4	**25**
5	150
6	150
7	100
8	125
9	100
10	150
11	200
12	50
13	50
14	**25**
Terbinafine	**12.5**

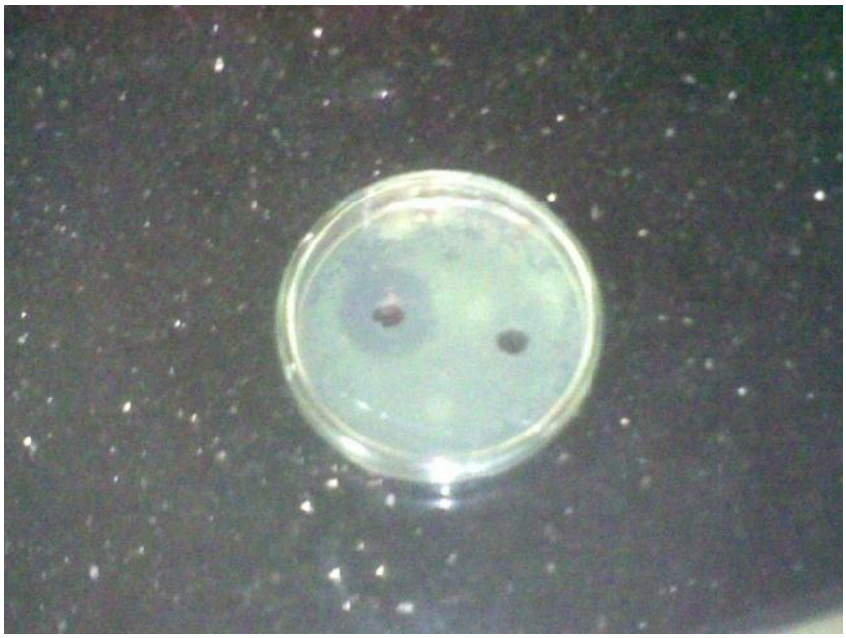


Figure 1 Antifungal activity of standard Terbinafine

VMKS-3 **VMKS-3**

(*Candida albicans*) **(*Aspergillus niger*)**

Figure 2 Antifungal activity of compound 3 compared with Terbinafine

Conclusion

Several 1-(substitutedphenoxy methyl) naphthalene derivatives have been synthesized and tested for antifungal activity using agar streak dilution method. Activity was expressed in terms of minimum inhibitory concentration (MIC). Most of the compounds showed the good antifungal activity.

Acknowledgement

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