Full Paper

Synthesis and Antifungal Activity of 1-Aryl-3-phenethylamino-1-propanone Hydrochlorides and 3-Aroyl-4-aryl-1-phenethyl-4-piperidinols

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Mono-Mannich bases, 1-aryl-3-phenethylamino-1-propanone hydrochlorides, **1a**, **2a**, **3a**, **4a**, **5a**, **6a**, **7a**, **8a**, **9a**, and semi-cyclic mono-Mannich bases, 3-aroyl-4-aryl-1-phenethyl-4-piperidinols, **1b**, **2b**, **3b**, **4b**, **5b**, **6b**, **7b**, **8b**, **9b**, were synthesized by a non-classical Mannich reaction. The aryl part was: C_6H_5 for **1a**, **1b**; 4-$CH_3C_6H_4$ for **2a**, **2b**; 4-$CH_3OC_6H_4$ for **3a**, **3b**; 4-ClC_6H_4 for **4a**, **4b**; 4-FC_6H_4 for **5a**, **5b**; 4-BrC_6H_4 for **6a**, **6b**; 2,4-$(Cl)_2C_6H_3$ for **7a**, **7b**; 4-$NO_2C_6H_4$ for **8a**, **8b**; and C_4H_3S(2-yl) i.e., 2-thienyl for **9a**, **9b**. Piperidinol compounds **2b**, **3b**, **4b**, **5b**, **7b**, **8b**, and **9b** are reported here for the first time. The synthesized compounds were tested against seven types of plant pathogenic fungi and three types of human pathogenic fungi using the agar dilution assay. Itraconazole was tested against *Candida parapsilosis* as the reference compound, while Nystatin was tested as the reference compound against the other fungi. Compounds **1a**, **1b**, **2a**, **4a**, **4b**, **5a**, **5b**, **6a**, **7a**, **8a**, **9a**, and **9b** can be selected as model compounds to develop new antifungal agents against the human pathogen *Microsporum canis*. Compounds **8a** and **8b**, which had a similar antifungal activity compared with the reference compound Nystatin against the plant pathogen *Aspergillus flavus*, can serve as model compounds to develop new antifungal agents to solve agricultural problems.

Keywords: Antifungal activity / Mono-Mannich bases / Piperidinol / Synthesis

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Introduction

Mannich bases are generally formed by the reaction between a compound containing a reactive hydrogen atom, formaldehyde, and a secondary amine. On occasion, aldehydes other than formaldehyde may be employed, and the secondary amine may be replaced by ammonia and primary amines. The process whereby these compounds are formed is known as the Mannich reaction [1]. Mannich bases have several biological activities such as antimicrobial [2–11], cytotoxic [4, 12–26], anticancer [1, 27–30], analgesic [31], anti-inflammatory [4, 32–35], diuretic [36], and anticonvulsant activities [37–40]. It has been reported that they have an inhibiting effect on DNA topoisomerase I [12, 13] and II [41].

Primary and opportunistic fungal infections in humans continue to increase rapidly because of the increased number of immunocompromised persons such as patients with AIDS, cancer, and transplants [42]. In addition, the development of resistance to current antifungal therapeutics continues to drive the search for more effective new drugs.

Yet, fungal infections are also existent in plants; these pathogens cause paleness, leaf burns, and decay of plants, decrease the quality and yield of crops in agricul-

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Table 1. ^{1}H-NMR and ^{13}C-NMR spectra of the synthesized compounds.

Compound	^{1}H-NMR (DMSO)	^{13}C-NMR (DMSO)
1b	δ: 1.56 (br d, 1H, *J* = 13.2 Hz), 2.00–2.07 (m, 1H), 2.58–2.93 (m, 8H), 4.43 (dd, 1H, *J* = 11.4, 3.7 Hz), 4.93 (d, OH, *J* = 1.8 Hz), 7.02–7.58 (m, 13H), 7.82 (dd, 2H, *J* = 8.4, 1.1 Hz).	δ: 33.5, 39.9, 49.1, 50.6, 52.7, 60.2, 73.3, 125.5, 126.5, 127.1, 128.5, 128.902, 128.947, 129.3, 129.4, 134.4, 136.8, 141.1, 148.3, 204.3.
2b	δ: 1.52 (br d, 1H, *J* = 13.6 Hz), 1.91–1.97 (m, 1H), 2.14 (s, 3H), 2.32 (s, 3H), 2.59–2.91 (m, 8H), 4.39 (dd, 1H, *J* = 11.4, 3.7 Hz), 4.95 (d, OH, *J* = 2.2 Hz), 6.97 (d, 2H, *J* = 8.4 Hz), 7.14–7.27 (m, 7H), 7.39 (d, 2H, *J* = 8.1 Hz), 7.78 (d, 2H, *J* = 8.4 Hz).	δ: 21.1, 21.8, 33.5, 40.1, 49.2, 50.1, 52.8, 60.0, 73.2, 125.3, 126.5, 128.9, 129.1, 129.2, 129.4, 130.1, 134.1, 136.0, 141.1, 145.2, 145.5, 204.2.
3b	δ: 1.51 (br d, 1H, *J* = 13.6 Hz), 1.91–1.97 (m, 1H), 2.58–2.89 (m, 8H), 3.62 (s, 3H), 3.80 (s, 3H), 4.37 (dd, 1H, *J* = 11.4, 3.3 Hz), 5.04 (d, OH, *J* = 1.8 Hz), 6.72 (d, 2H, *J* = 8.8 Hz), 6.97 (d, 2H, *J* = 8.8 Hz), 7.14–7.28 (m, 5H), 7.43 (d, 2H, *J* = 8.8 Hz), 7.87 (d, 2H, *J* = 8.8 Hz).	δ: 33.5, 40.2, 49.2, 49.7, 53.0, 55.5, 56.3, 60.1, 73.1, 113.9, 114.8, 126.5, 126.6, 128.9, 129.3, 129.4, 131.5, 140.6, 141.1, 158.4, 164.5, 203.2.
4b	δ: 1.55 (br d, 1H, *J* = 13.6 Hz), 2.04–2.11 (m, 1H), 2.56–2.91 (m, 8H), 4.30 (dd, 1H, *J* = 11.0, 3.7 Hz), 4.96 (d, OH, *J* = 1.1 Hz), 7.15 (quasi d, 2H, *J* = 8.8 Hz), 7.21–7.28 (m, 5H), 7.43 (quasi d, 2H, *J* = 8.8 Hz), 7.50 (quasi d, 2H, *J* = 8.4 Hz), 7.74 (quasi d, 2H, *J* = 8.8 Hz).	δ: 33.5, 39.6, 48.9, 51.3, 52.3, 60.2, 73.1, 126.5, 127.7, 128.3, 128.9, 129.3, 129.4, 130.7, 131.8, 136.0, 138.9, 141.1, 147.2, 202.5.
5b	δ: 1.55 (br d, 1H, *J* = 13.6 Hz), 2.04–2.11 (m, 1H), 2.57–2.90 (m, 8H), 4.35 (dd, 1H, *J* = 11.0, 3.7 Hz), 4.96 (d, OH, *J* = 1.1 Hz), 6.94 (br t, 2H, *J* = 8.9 Hz), 7.14–7.28 (m, 7H), 7.52 (dd, 2H, *J* = 8.8, 5.5 Hz), 7.84 (dd, 2H, *J* = 8.9, 5.1 Hz).	δ: 33.5, 39.8, 49.0, 51.1, 52.5, 60.2, 73.1, 115.5 (d, $^{2}J_{C\text{-}F}$ = 124 Hz), 115.8 (d, $^{2}J_{C\text{-}F}$ = 126 Hz), 126.5, 127.7 (d, $^{3}J_{C\text{-}F}$ = 8 Hz), 128.9, 129.4, 131.9 (d, $^{3}J_{C\text{-}F}$ = 9 Hz), 133.9 (d, $^{4}J_{C\text{-}F}$ = 2 Hz), 141.1, 144.3 (d, $^{4}J_{C\text{-}F}$ = 3 Hz), 161.5 (d, $^{1}J_{C\text{-}F}$ = 243 Hz), 165.7 (d, $^{1}J_{C\text{-}F}$ = 252 Hz), 202.3.
6b	δ: 1.55 (br d, 1H, *J* = 13.6 Hz), 2.02–2.09 (m, 1H), 2.55–2.92 (m, 8H), 4.28 (dd, 1H, *J* = 11.2, 3.5 Hz), 4.96 (d, OH, *J* = 1.5 Hz), 7.14–7.26 (m, 5H), 7.30 (d, 2H, *J* = 8.4 Hz), 7.43 (d, 2H, *J* = 8.4 Hz), 7.56 (d, 2H, *J* = 8.4 Hz), 7.64 (d, 2H, *J* = 8.8 Hz).	δ: 33.5, 39.6, 48.9, 51.3, 52.3, 60.2, 73.1, 120.4, 126.5, 128.1, 128.2, 128.9, 129.4, 130.8, 131.3, 132.3, 136.3, 141.1, 147.6, 202.6.
7b	δ: 1.04 (t, 1H, *J* = 6.9 Hz), 1.38–1.41 (m, 1H), 2.41–2.97 (m, 8H), 4.55 (dd, 1H, *J* = 11.0, 2.9 Hz), 5.59 (d, OH, *J* = 1.5 Hz), 7.01 (d, 1H, *J* = 8.4 Hz), 7.11 (dd, 1H, *J* = 8.4, 1.8 Hz), 7.15–7.29 (m, 8H), 7.63 (d, 1H, *J* = 8.8 Hz).	δ: 33.7, 35.7, 48.7, 51.0, 52.4, 60.4, 73.7, 126.5, 127.2, 127.4, 128.9, 129.4, 129.5, 130.2, 130.4, 130.9, 131.0, 131.5, 132.9, 135.5, 139.2, 141.2, 142.7, 202.4.
8b	δ: 1.92 (br d, 1H, *J* = 14.3 Hz), 2.98–3.05 (m, 1H), 3.15–3.77 (m, 8H), 4.86 (dd, 1H, *J* = 11.3, 3.3 Hz), 6.14 (s, OH), 7.24–7.40 (m, 5H), 7.46 (quasi d, 2H, *J* = 8.8 Hz), 7.56 (quasi d, 2H, *J* = 8.8 Hz), 7.89 (quasi d, 2H, *J* = 8.8 Hz), 7.94 (quasi d, 2H, *J* = 8.8 Hz).	δ: 30.3, 36.3, 48.5, 49.8, 51.5, 57.3, 72.1, 123.67, 123.69, 127.51, 127.55, 129.3, 129.4, 129.8, 137.7, 143.4, 147.0, 149.5, 153.2, 198.2.
9b	δ: 1.77 (br d, 1H, *J* = 13.6 Hz), 2.00–2.07 (m, 1H), 2.52–2.94 (m, 8H), 4.15 (dd, 1H, *J* = 11.4, 3.7 Hz), 5.42 (d, OH, *J* = 1.1 Hz), 6.82 (dd, 1H, *J* = 4.9, 3.5 Hz), 7.05 (dd, 1H, *J* = 3.5, 1.3 Hz), 7.14–7.30 (m, 7H), 7.94–7.97 (m, 2H, overlapped 2H of thiophene).	δ: 33.5, 41.0, 48.9, 52.8, 53.2, 60.0, 72.7, 122.8, 124.5, 126.5, 127.7, 128.9, 129.4, 129.7, 135.3, 137.1, 141.1, 144.4, 154.4, 196.1.

ture, and increase the cost to produce crops, which means that plant pathogens are harmful for the agricultural economy [43].

It has been reported that Mannich bases of conjugated styryl ketones [10], isatin *N*-Mannich bases [44], acetophenone derived mono- and bis-Mannich bases, piperidinols and azine derivatives of mono-Mannich bases [2, 3, 5, 6, 10] have antifungal activity. Further, Mannich ketones possess bioactivity which may be due to the alkylating ability of α,β-unsaturated ketones that are liberated *in vivo* following deamination [15, 17, 45–49]. These reported findings directed us to synthesize mono-Mannich bases, 1-aryl-3-phenethylamino-1-propanone hydrochlorides, and semi-cyclic mono-Mannich bases, 3-aroyl-4-aryl-1-phenethyl-4-piperidinols, and to evaluate their antifungal activity.

It has also been possible to see the alterations in biological activity of the compounds depending on their chemical structures, which allowed us to find the most suitable compounds for further studies to develop new effective antifungal compounds against pathogens in humans and/or plants.

Table 2. Results of UV, IR, and MS spectra of the synthesized compounds.

Compound	Aryl	Exact Mass	UV§ λ_{max}	Log*E*	C (10^{-5} M)	MS (m/z) [M+]	IR (KBr, cm^{-1}) C=O stretching	IR (KBr, cm^{-1}) OH stretching
1b	C_6H_5	385.2	252	4.07	5.20	385.5	1679	3204
2b	4-$CH_3C_6H_4$	413.2	261	4.31	4.83	413.8	1671	3326
3b	4-$CH_3OC_6H_4$	445.2	251	4.31	4.48	445.7	1664	3324
4b	4-ClC_6H_4	453.1	260	4.30	4.40	453.8	1677	3167
5b	4-FC_6H_4	421.2	211	4.24	11.86	421.4	1676	3181
			250	3.88				
6b	4-BrC_6H_4	541.0	263	4.30	3.68	541.4	1676	3433
7b	2,4-$(Cl)_2C_6H_3$	521.1	205	4.57	9.50	521.7	1665	3462
			257	3.93				
8b	4-$NO_2C_6H_4$	475.2	265	4.43	3.15	475.8	1684	3390
9b	C_4H_3S(2-yl)	397.1	203	4.14	8.80	397.5	1642	3387
			235	3.95				
			265	3.94				
			294	3.91				

§ UV spectra of the compounds were taken in ethanol except for compound **7b**, which was taken in methanol.

Table 3. Results of the elemental analyses of the synthesized compounds.

Compound	Formula	Aryl	Elemental Analyses							
			Calculated (%)				Found (%)			
			C	H	N	S	C	H	N	S
1b	$C_{26}H_{27}NO_2$	C_6H_5	81.01	7.06	3.63		81.41	7.03	3.66	
2b	$C_{28}H_{31}NO_2$	4-$CH_3C_6H_4$	81.32	7.56	3.39		81.24	7.59	3.69	
3b	$C_{28}H_{31}NO_4$	4-$CH_3OC_6H_4$	75.48	7.01	3.14		75.66	7.04	3.29	
4b	$C_{26}H_{25}Cl_2NO_2$	4-ClC_6H_4	68.72	5.55	3.08		68.76	5.43	3.27	
5b	$C_{26}H_{25}F_2NO_2$	4-FC_6H_4	74.09	5.98	3.32		74.28	5.87	2.94	
6b	$C_{26}H_{25}Br_2NO_2$	4-BrC_6H_4	57.48	4.64	2.58		57.16	4.64	2.61	
7b	$C_{26}H_{23}Cl_4NO_2$	2,4-$(Cl)_2C_6H_3$	59.68	4.43	2.68		59.30	4.50	2.56	
8b	$C_{26}H_{26}ClN_3O_6$	4-$NO_2C_6H_4$	61.00	5.12	8.21		61.13	5.15	8.18	
9b	$C_{22}H_{23}NO_2S_2$	C_4H_3S(2-yl)	66.47	5.83	3.52	16.13	66.36	5.82	3.68	16.28

Results and discussion

Of the compounds synthesized, piperidinol compounds, **2b**, **3b**, **4b**, **5b**, **7b**, **8b**, and **9b**, will be reported for the first time in this study. The chemial structures of the compounds were confirmed by ^{1}H-NMR, ^{13}C-NMR, UV, IR, and MS spectra and the purity level of the compounds was determined by elemental analyses. The spectral data of the mono-Mannich bases were reported in our previous study [50] and the spectral data for the piperidinol compounds are shown in Tables 1 and 2. The results of the elemental analyses for the piperidinols, which are reported here for the first time, are shown in Table 3. The elemental-analyses (C, H, N, S) results of the compounds were within ± 0.4% of the calculated values.

Itraconazole was tested against *Candida parapsilosis* EA08 as the reference compound, while Nystatin was tested as the reference compound against other fungi. None of the compounds were found to be effective against *Fusarium oxysporum* CE1, *Botrytis cinerea* MFD3, and *Candida albicans* EA07 at the concentration range studied. On the other hand, Nystatin was also ineffective against *Microsporum canis* AO5 at the concentration range studied. The antifungal-activity results of the compounds are shown in Table 4.

Synthesis of the compounds

The mono-Mannich bases **1a–9a** reported in this study were synthesized as described in our previous study by the classical Mannich reaction in an acidic solution of ethanol under reflux conditions [50]. The optimal reaction conditions for the synthesis of these compounds, 1-aryl-3-phenethylamino-1-propanone hydrochlorides, were investigated by changing the molar ratios of reactants and solvent, and the acidity level of the reaction medium using compound **1a** and **9a** as representative

Table 4. Antifungal activities of the synthesized compounds as minimal inhibitory concentration (MIC in μg/mL).

Compound	Aryl	*Rhizoctonia solani* 2001	*Sclerotinia sclerotiorum* FD3	*Aspergillus flavus* FD7	*Alternaria alternata* FS2002	*Macrophamina phaseoli* CE4	*Microsporum canis* AO5	*Candida parapsilosis* EA08
1a	C_6H_5						50	
1b	C_6H_5						25	
2a	4-$CH_3C_6H_4$						25	
2b	4-$CH_3C_6H_4$							
3a	4-$CH_3OC_6H_4$					200		
3b	4-$CH_3OC_6H_4$							
4a	4-ClC_6H_4					200	200	
4b	4-ClC_6H_4				200		25	
5a	4-FC_6H_4						25	100
5b	4-FC_6H_4						12.5	
6a	4-BrC_6H_4		100				25	
6b	4-BrC_6H_4							
7a	2,4-$(Cl)_2C_6H_3$	25		100			12.5	100
7b	2,4-$(Cl)_2C_6H_3$	50			100			
8a	4-$NO_2C_6H_4$			200			12.5	100
8b	4-$NO_2C_6H_4$			200				
9a	C_4H_3S(2-yl)						25	100
9b	C_4H_3S(2-yl)						25	
Nystatin		12.5	25	200	50	50		
Itraconazole								12.5

compounds from our previous paper [50]. It has been reported that the most suitable mol ratio of the reactants was 1:1.2:1, compared with 2:2:1 suitable for ketone, paraformaldehyde, and phenethylamine hydrochloride. The most suitable reaction medium was ethanol with added concentrated hydrochloric acid (compared to the medium without solvent and only ethanol) [50]. As reported in the literature, compound **3a**, is a mono-Mannich base with methoxy substitution [31], and compound **4a**, is a mono-Mannich base with a chloride substitution [51].

Here, a brief description of the synthesis of **3a** [31]: 0.1 mol of suitable ketone and 0.12 mol amine hydrochloride were heated in ethanol for a while; 0.12 mol paraformaldehyde was added and the heating continued for 7 h. More paraformaldehyde (0.05 mol) was added and the heating continued for another 2 h. Then, the solvent was evaporated under low pressure, water was added to the reaction medium, and the mixture was washed with diethyl ether. The mixture was alkalized by 50% NaOH, washed and dried. Compound **3a** was obtained by passing HCl gas through the ether solution of the substance. In the above-mentioned reference [31], similar compounds *i.e.*, β-aminopropiophenones, were designed as open-chain analogues of the dihydroquinolones and their analgesic effects were tested. β-Aminopropiophenones had better analgesic activity than the other compounds. This can be explained by a better receptor suitability of the ß-aminopropiophenones compared with the dihydroquinolones [31].

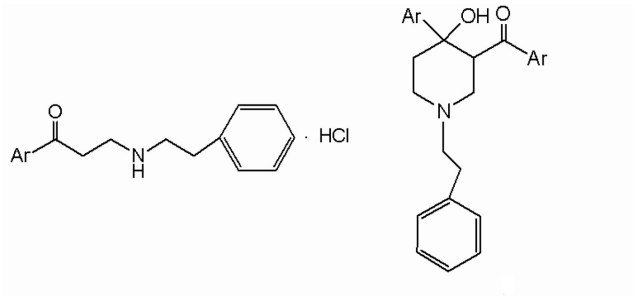
Compound **4a** was synthesized by heating the ketone, paraformaldehyde, and amine (1:1.7:1 equivalent ratio) in acidic isopropanol with a yield of 35% (as given in [51]). This compound had a muscle-relaxant activity at 300 mg/kg [51]. As described in detail above, the reported compounds **3a** and **4a** were synthesized according to the classical Mannich reaction. There is no bioactivity data related to mono-Mannich bases, except for compounds **3a** and **4a**. The mono-Mannich bases presented in this study were synthesized by an experimental procedure different from that in our previous study [50].

Of the semi-cyclic mono-Mannich bases or piperidinols, the non-substituted phenyl derivative **1b** and the 4-bromophenyl derivative **6b** are reported in the literature [52–54]: Upton *et al*. reported the potent ^{1}H-antagonistic activity of indeno[2,2-*c*]pyridines and their 4-arylpiperidinol precursors [52]. They reported to have an ^{1}H-antagonistic activity of 26 and 23 nM, respectively, at the ^{1}H-histamine receptor in guinea-pig ileum with the compounds having a piperidine structure; the aryl part in this case was phenyl, and the alkyl residues on nitrogen were methyl and ethyl. The activity level was comparable with that for the clinically-established phenindamine, which has an IC_{50} value of 36 nM. These data made Upton *et al*. screen eleven piperidine compounds *in vivo*, including compound **1b** of this study, using the 48/80 challenge

test. None of the compounds was capable of protecting mice against a lethal dose of 48/80. The calculated lipophilicity for most of the piperidines including compound **1b** was between 10 to 100 times that of the analogue, with the methyl substitution on the nitrogen of the piperidine structure. Researchers attributed this situation to the affected pharmacokinetics of the drug with respect to its site of action [52].

Compound **1b** was synthesized according to [53] by heating of 0.1 mol of phenethylamine dissolved in 20 mL of ethanol together with a solution of 0.2 mol of β-dimethylamino propiophenone hydrochloride in 50 mL water on a water bath for 2 h. The oily base, which was produced, was taken up in diethyl ether and converted to the hydrochloride salt with isopropanolic hydrochloride. The hydrochloride salt of compound **1b** was obtained in 34% yield (13 g). The compound was recrystallized from ethanol. The reported melting point was 204–205°C [55]; in [53], the melting point of compound **1b** is given as 202–203°C together with UV and IR spectral data. In both cases, compound **1b** was obtained in form of the hydrochloride salt, while, in this study, it is in base form. Yet, the synthetic methods used in the literature differed from ours used in this study. Phenethylamine and a mono-Mannich base different from its corresponding one were used for the synthesis of the piperidinol compound **1b** in the literature.

The synthesis of the bromine-containing piperidinol compound **6b** as described in [54]: 0.25 equivalent amine hydrochloride were added to the solution of one equivalent arylmethyl ketone and one equivalent paraformaldehyde in acetonitrile; then, the mixture was refluxed for 20 h in the presence of hydrochloric acid. After cooling the reaction mixture to room temperature, the solvent was removed under vacuum. The residue was dissolved in dichloromethane and washed with bicarbonate solution, water, and brine, respectively. After the organic phase was dried, the crude compound was purified by column chromatography using triethylamine/diethyl ether as eluent. The bromo compound **6b** was obtained in 74% yield. ^{1}H-NMR and ^{13}C-NMR data of this compound are reported in reference [54]. In [56], the yield of compound **6b** is also reported with 74%, but it was obtained as a syrup. This differs from our result: in our hands **6b** is solid. This compound is reported to be an inhibitor of the dopamine transporter for illnesses related to dopamine transportation. Having a phenethyl substituent on the nitrogen atom, **6b** had shown 18 times lower activity compared to the compound having an ethyl substituent on the nitrogen. In a structure-activity relationship study it was noted that the positions of the substituent on two phenyl rings play an important role for the binding and



Ar: (C_6H_5) for **1a, 1b**, (4-$CH_3C_6H_4$) for **2a**, **2b**, (4-$CH_3OC_6H_4$) for **3a, 3b**, (4-ClC_6H_4) for **4a, 4b**, (4-FC_6H_4) for **5a, 5b**, (4-BrC_6H_4) for **6a, 6b**, (2,4-$(Cl)_2C_6H_3$) for **7a**, **7b**, (4-$NO_2C_6H_4$) for **8a**, **8b**, (C_4H_3S(2-yl)) for **9a, 9b**.

Figure 1. Chemical structure of the synthesized compounds.

re-absorption of this type of compounds [54]. Additionally, with this series of compounds [54], a molecular-modeling study was also realized. As seen, compound **6b** was synthesized by a classical Mannich reaction using acetonitrile as reaction solvent, which distinguishes it from the method used in this study.

The synthetic pathway used here to synthesize all compounds included taking ketone, aldehyde, and amine hydrochloride in the mol ratio 2:2:1 and heating them in a medium without solvent. This is a non-classical method, differing from the methods described in the above mentioned literature [50, 52, 54–56]. The advantage of the method used here makes it possible to synthesize both compounds simultaneously (the mono-Mannich bases and piperidinols) in a single reaction. The synthesized mono-Mannich bases come in the form of HCl salts, the piperidinol compounds in base form, except for compound **8b**, which comes as HCl salt. The reason for this was the purification by direct crystallization of compound **8b** from the reaction medium, while the other piperidinol-type compounds were purified by passing them through a basic aluminum oxide column.

Spectral data of the compounds confirmed their chemical structures (Fig. 1). As an example, and in agreement with the chemical structure, ^{1}H- and ^{13}C-NMR data of compound **2b** (Fig. 2) are as follows: While the H15 protons on the aromatic ring neighboring the carbonyl give doublets (J = 8.4 Hz) at δ = 7.78 ppm, H20 protons on the aromatic ring connected to piperidine give doublets at δ = 7.39 ppm (J = 8.1 Hz), when the ^{1}H-NMR spectrum of the compound was analyzed. Multiplet signals at δ = 7.14–7.27 ppm belong to the H10, H11, H12, and H16 protons on the benzene rings. The H21 protons of the 4-methyl-substituted benzene ring connected directly to the piperidine ring and give a doublet at δ = 6.97 ppm (J = 8.4 Hz). The hydroxyl (OH) proton gives a doublet at δ = 4.95 ppm

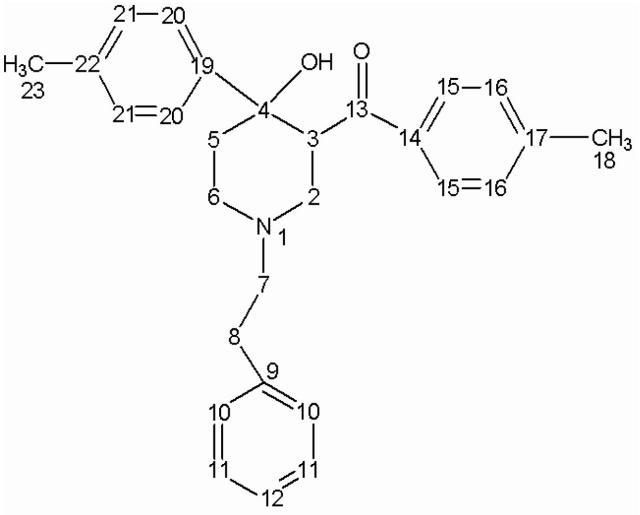


Figure 2. Representative compound 3-(4-methylbenzoyl)-4-(4-methylphenyl)-1-phenethyl-4-piperidinol numbered for the NMR studies.

(J = 2.2 Hz) by long-distance interaction with the H5b proton. The H3 proton, which is neighbor to a carbonyl, has been observed as a doublet of doublets at δ = 4.39 ppm (J = 11.4, 3.7 Hz) by interacting with the H2 protons. Methylene protons H2, H6, and H7, which are next to a nitrogen in the piperidine ring, and the benzylic proton H8 proton have been observed as multiplets at δ = 2.59–2.91 ppm. Singlet signals at δ = 2.32 ppm and δ = 2.14 ppm belong to methyl protons, which are connected to both aromatic rings. The H5b proton belonging to the piperidin ring gives a multiplet between δ = 1.91–1.97 ppm, while the H5a proton shows a wide doublet at δ = 1.52 ppm.

In the ^{13}C-NMR spectrum of the compound, carbonyl carbon at δ = 204.2 ppm, aromatic carbons at δ = 145.5, 145.2, 141.1, 136.0, 134.1, 130.1, 129.4, 129.2, 129.1, 128.9, 126.5, 125.3 ppm, methylen and methine carbons at δ = 73.2, 60.0, 52.8, 50.1, 49.2, 40.1, 33.5 ppm, methyl carbons at δ = 21.8, 21.1 ppm have been observed in accordance with the chemical structures. A strong absorption peak has been observed in the IR spectrum of compound **2b** corresponding to a carbonyl at 1671 cm^{-1} (Table 2) confirming the chemical structure. The maximum absorption band in the UV spectrum of compound **2b** was found at 261 nm in accordance with its structure (Table 2). The MS spectrum of the same compound taken by EI method provided the m/z peak at 413.8, which corresponds to the base form of compound **2b** (Table 2).

Antifungal activity of mono-Mannich bases (Table 4)

Mono-Mannich base compounds **3a**, **4a**, **6a**, **7a**, and **8a** were found to be effective against fungi, which are pathogenic in plants, while compounds **1a**, **2a**, **4a**, **5a**, **6a**, **7a**, **8a**, and **9a** were found to be effective against fungi, which are pathogenic in human. Mono-Mannich bases, which were effective against plant pathogenic fungi: the 2,4-dichloro derivative, compound **7a**, exhibited 50% of the antifungal activity the reference compound Nystatin against *Rhizoctonia solani*, while the bromo derivative **6a** was effective against *Sclerotinia sclerotiorum* (25% of the reference). Compound **7a** was twice as effective against *Aspergillus flavus* as the reference compound. Antifungal activity equal to Nystatin was found in compound **8a**, which is a nitro derivative, against *Aspergillus flavus*. The methoxy derivative **3a** and the chloro derivative **4a** were effective against *Macrophamina phaseoli* (25% of the reference compound).

Mono-Mannich bases effective against human pathogenic fungi: While reference compound Nystatin was ineffective against *Microsporum canis*, the non-substituted compound **1a** was active at 50 μg/mL, the methyl derivative **2a**, the fluoro derivative **5a**, the bromo derivative **6a**, and compound **9a**, which has thiophene ring, were active at 25 μg/mL, the 2,4-dichloro derivative **7a** and nitro derivative **8a** at 12.5 μg/mL, the chloro derivative **4a** was found effective at 200 μg/mL. Fluoro derivative **5a**, 2,4-dichloro derivative **7a**, nitro derivative **8a**, and compound **9a** (thiophene ring) had an antifungal activity of 12.5% of that of the reference compound Itraconazole.

Antifungal activity of semi-cyclic mono-Mannich bases, piperidinols (Table 4)

Semi-cyclic mono-Mannich base compounds, piperidinols, **4b**, **7b**, and **8b** were found to be effective against fungi, pathogenic to plants, while compounds **1b**, **4b**, **5b**, and **9b** were found to be effective against fungi, pathogenic in humans. Semi-cyclic mono-Mannich bases *i.e.*, piperidinols effective against plant fungi can be described as follows: the 2,4-dichloro derivative **7b** had an antifungal activity of 25% of the reference compound against *Rhizoctonia solani* and 50% activity of the reference compound against *Alternaria alternata*, while the nitro derivative, compound **8b**, had antifungal activity equal with the reference drug against *Aspergillus flavus*. The chloro derivative **4b** had 25% activity of the reference compound against *Alternaria alternata*.

The effects of semi-cyclic mono-Mannich bases *i.e.*, piperidinols, which were effective against human pathogenic fungi, can be summarized as follows: Piperidinols had antifungal activity against *Microsporum canis*, against which reference compound Nystatin was uneffective. Non-substituted phenyl **1b**, the chloro derivative **4b**, and compound **9b** in which the aromatic part is thiophene, showed antifungal activity at a concentration of 25

μg/mL, while the fluoro derivative **5b** showed activity at 12.5 μg/mL.

The effect of the replacement of the phenyl ring with a thiophene ring on the antifungal activity of the compounds could only be observed against *Microsporum canis* for compounds **1a** and **9a**, and **1b** and **9b**. Antifungal activity increased two times when the antifungal activity of compounds **1a** and **9a** was compared, while it did not change when the antifungal activity of compounds **1b** and **9b** was compared. This may suggest that the effect of ring replacement on the antifungal activity is limited. Changes in the electronic nature of the compounds did not affect the bioactivity very much.

Yet, the bioactivity was affected when the chemical structure of the compounds was changed from a mono-Mannich base to a semi-cyclic mono-Mannich base. According to this, the antifungal activity against *Rhizoctonia solani* decreased by half in piperidinol when comparing the bioactivities of **7a** and its analogue **7b**. However, the antifungal activity was not affected when the bioactivities of **8a** and its analogue **8b** were compared against *Aspergillus flavus*. The antifungal activity against *Microsporum canis* increased two times in the piperidinol compound when the bioactivities of **1a** and its analogue **1b** were compared, while antifungal activity increased eight times in piperidinol when the bioactivities of compound **4a** and its analogue **4b** were compared. Antifungal activity increased two times for the piperidinol compound **5b** compared with **5a** against *Microsporum canis*. However, the antifungal activity was not affected when the bioactivities of compounds **9a** and **9b** were compared against the same microorganism.

Although compounds **5a**, **7a**, **8a**, and **9a**, which are mono bases, were effective at 100 μg/mL against *Candida parapsilosis*, their corresponding piperidinols were ineffective at the concentration range studied. The mono-Mannich bases **3a** and **4a** were effective at 200 μg/mL against *Macrophamina phaseoli*, while their corresponding piperidinols were ineffective. Mono-Mannich bases **6a** and **7a** were effective at 100 μg/mL against *Sclerotinia sclerotiorum* and *Aspergillus flavus*, while their corresponding piperidinols were ineffective at the concentration range studied. On the other hand, piperidinol compounds **4b** and **7b** were effective against *Alternaria alternata*, while their corresponding mono derivatives were ineffective.

Mono-Mannich bases had antifungal activity especially against *Sclerotinia sclerotiorum*, *Aspergillus flavus*, *Macrophamina phaseoli*, *Candida parapsilosis*, and *Rhizoctonia solani*, while the semi-cyclic mono-Mannich bases *i. e.*, piperidinols, had antifungal activity especially against *Alternaria alternata*.

The antifungal activity was specific and intense against *Microsporum canis* as can be seen in Table 4. Furthermore, the reference compound Nystatin was not found effective against this microorganism in the concentration range studied. To conclude, compounds **1a**, **1b**, **2a**, **4a**, **4b**, **5a**, **5b**, **6a**, **7a**, **8a**, **9a**, and **9b** can be selected as model compounds to develop new antifungal agents against the human pathogen *Microsporum canis*. In addition, compounds **8a** and **8b**, which had similar antifungal activity with the reference compound Nystatin against the plant pathogen *Aspergillus flavus*, can serve as model compounds to develop new antifungal agents to solve agricultural problems.

Experimental

Materials

The following chemicals were used for the synthesis of the compounds in this study: acetophenone, 4′-methylacetophenone, 4′-nitroacetophenone, 4′-chloroacetophenone, 2-acetylthiophene (Fluka, Steinheim, Switzerland), 4′-methoxyacetophenone, 4′-fluoroacetophenone, 4′-bromoacetophenone, 2′,4′-dichloroacetophenone (Acros, Geel, Belgium), paraformaldehyde (Merck, Darmstadt, Germany), methanol, ethyl acetate (Riedel-deHaën, Seelze, Germany), diethyl ether (Fluka, Steinheim, Switzerland), and ethanol (J. T. Baker, Deventer, Holland). The ^{1}H- and ^{13}C-NMR spectra were recorded at 400/100 MHz on a Varian spectrometer (Varian, Danbury, CT, USA). EI-MS spectra were recorded on a Thermo-Finnigan mass analyzer (San Jose, CA, USA). UV spectra of compounds were recorded on a Thermo-Electron Heλios (a) (UVA 114903) spectrometer (Cambridge, UK). Infrared spectra were obtained for KBr disks on a Mattson 1000 FT-IR spectrophotometer (Cambridge, England). Elemental analyses were carried out with a Leco CHNS-932 instrument (Leco, St. Joseph, MI, USA). Melting points were determined on a Büchi 530 (Flawil, Switzerland).

*Synthesis of mono-Mannich bases and piperidinols **1a–9a** and **1b–9b** (Fig. 1)*

^{1}H-NMR, ^{13}C-NMR, UV, IR, and MS spectral data of semi-cyclic mono-Mannich bases, piperidinol compounds **1b–9b** are shown in Tables 1 and 2. The purity level of the compounds was determined by elemental analyses and the results are shown in Table 3. Elemental analyses (C, H, N, S) results of the compounds were within ± 0.4% of the calculated values. Spectral data and elemental analyses (C, H, N, S) results for mono-Mannich bases were reported in our previous study [50].

Suitable amounts of ketone, paraformaldehyde, and phenylethylamine hydrochloride in the mol ratio 2:2:1 were stirred and heated in an oil bath to synthesize the compounds.

For the compounds **1a**, **5a**, and **9a**, the solid starting compounds began to melt when the temperature reached 88, 90, and 82°C, respectively. The reaction content became clear, transparent mass at 92°C, 92°C, and 85°C, for the compounds **1a**, **5a**, and **9a**, respectively. Heating was stopped by removing the reaction flask from the oil bath. The temperature inside the balloon flask spontaneously and suddenly increased to 100, 107, and 106°C, respectively.

In the case of compounds **2a**, **7a**, and **8a**, the temperature started to increase suddenly, without the observation of a melting or clearing of the starting compounds when the temperature of the reaction medium reached 87, 93, 80°C, respectively. The reaction flask was quickly removed from the oil bath. The temperature inside the balloon spontaneously and suddenly increased to 110, 108, and 102°C, respectively.

When the temperature reached 87°C for the compound **3a**, all solid substances in the reaction medium started to melt, the reaction content turned clear, and the temperature started to increase spontaneously. Then, the reaction flask was quickly removed from the oil bath. The temperature of the reaction content reached 110°C in a short period of time for the compound **3a**.

When the temperature reached 92°C for the compound **4a**, the solid substance started to melt. When heating continued, the reaction content solidified again totally. The reaction flask was quickly removed from the oil bath. The temperature of the reaction medium spontaneously increased to 104°C.

When the temperature reached 91°C for the compound **6a**, the temperature of the reaction content suddenly started to increase without melting and becoming clear. The reaction flask was removed from the oil bath. The temperature of the reaction content reached to 103°C.

In all reactions, following the increase in temperature and removal of the flask from the oil bath, ethyl acetate (20 mL) was added to the reaction flask when the temperature had dropped to 65°C. Stirring was continued for 24 h. The formed precipitates were separated by filtration.

Compound **1a** was crystallized from ethyl acetate. Compounds **2a**, **6a**, **7a**, and **8a** were crystallized from methanol and compounds **3a**, **4a**, and **5a** were crystallized from ethanol. After filtration through the basic Al_2O_3 column, **9a** was crystallized in ethanol and dried by washing with diethyl ether. The synthetic yields of the mono-Mannich bases **1a–8a**, and **9a** were 18%, 24%, 20%, 28%, 33%, 35%, 35%, 30%, and 16%, respectively.

After isolation of compounds **1a**, **2a**, and **3a** from the reaction medium, the ethyl acetate present in the reaction flask was removed under low pressure to obtain the piperidine compounds **1b**, **2b**, and **3b**. NaOH (5%) solution was added to the obtained viscous substances of orange color. The reaction content was stirred in a water bath at 40°C. Reaction content looked like an emulsion with oil in the beginning; it became viscous and solidified by time. Stirring was continued for 24 h for the compound residues of **1a** and **2a**, while it continued for 120 h for the residue of **3a**, until it was precipitated. The precipitates were filtered and washed with water. The crude product was crystallized from methanol. Compounds **1b** and **2b** obtained yielded white solid substances with 30 and 21%, respectively. A viscous substance of yellow color, which was obtained after removal of the compound **3a**, was filtered through the basic Al_2O_3 column using ethyl acetate/hexane (60:40) solvent system. The solvent was removed under low pressure. The solid substance obtained was crystallized from methanol. The solid white compound **3b** was obtained with a yield of 24%. The melting points of the compounds **1b**, **2b**, and **3b** were 139–141°C, 129–131°C, and 112–113°C, respectively.

After isolating **8a** from the reaction medium, ethyl acetate present in the reaction flask was removed under low pressure to obtain compound **8b**. Methanol was added to the obtained viscous substance of orange color and the mixture was kept at room temperature. The crystals formed were taken by filtration and recrystallized from methanol five times. **8b** was obtained as primrose-yellow solid compound with a yield of 22%. The melting point of the compound was 213–215°C.

After isolating compounds **4a**, **5a**, **6a**, **7a**, and **9a** from the reaction medium, ethyl acetate present in the reaction flask was removed under low pressure to obtain the compounds **4b**, **5b**, **6b**, **7b**, and **9b**. The obtained viscous compounds **4b**, **5b**, **6b**, **7b** and **9b** of orange color were purified by column chromatography using basic Al_2O_3 column. The solvent systems used for chromatography of the particular compounds were as follows: ethyl acetate/hexane (10:90) for **4b** and **7b**, ethyl acetate/hexane (5:95) for **5b**, methanol/ethyl acetate (10:90) for **6b**, ethyl acetate/methanol (10:90) for **9b**. The solvent was removed under low pressure. However, compounds **4b**, **5b**, **6b**, and **9b** were recrystallized with methanol, and **7b** was recrystallized from ethanol/diethyl ether. The compounds obtained and the yields were as follows: **4b** solid white with a yield of 18%, **5b** solid white with a yield of 31%, **6b** solid white with a yield of 18%, 7**b** solid white with a yield of 28%, **9b** and solid white with a yield of 28%. The melting points of the compounds **4b**, **5b**, **6b**, **7b**, and **9b** were 132–134°C, 145–146°C, 130–132°C, 122–124°C, and 155–156°C, respectively.

Determination of the antifungal activity

Rhizoctonia solani 2001, *Fusarium oxysporum* CE1, *Sclerotinia sclerotiorum* FD3, *Aspergillus flavus* FD7, *Alternaria alternata* FS2002, *Macrophamina phaseoli* CE4, *Botrytis cinerea* MFD3 as plant pathogenic fungi and *Microsporum canis* AO5, *Candida albicans* EA07, *Candida parapsilosis* EA08 as human pathogenic fungi were used to determine the antifungal activities of the compounds. Microorganisms were provided by the Department of Genetics and Bioengineering, Faculty of Engineering and Architecture, Yeditepe University, Istanbul, Turkey. Antifungal activities of the compounds were tested using the agar dilution assay in the concentration range of 6.25 to 200 μg/mL. Minimal inhibitory concentrations (MIC) of the compounds were determined. Nystatin (Pharmatech, Denver CO, USA) and Itraconazole (Matrix Pharma) were used as reference compounds.

Microwell dilution assay

The minimal inhibitory concentration (MIC) values were determined for the yeast isolates, which were sensitive to compounds in disc diffusion assay. The inocula of the *Candida* isolates were prepared from 12-h broth cultures and suspensions were adjusted to 0.5 McFarland standard turbidity. Compounds dissolved in dimethylsulfoxide (DMSO) were first diluted to the highest concentration (200 μg/mL) to be tested, and then serial two-fold dilutions were made in order to obtain a concentration range from 6.25 to 200 μg/mL in 15 mL sterile test tubes containing Sabouraud dextrose broth (SDB) for yeast. MIC values of compounds against yeast isolates were determined based on a microwell dilution method [6]. The 96-well plates were prepared by dispensing 95 μL of nutrient broth and 5 μL of the inoculum into each well. 100 μL from the stock solutions of compounds prepared at the concentration of 200 μg/mL was added into the second wells. Then, 100 μL from their serial dilutions was transferred into five consecutive wells. The last well on each strip containing 195 μL of nutrient broth without compound and 5 μL of the inoculum was used as negative control. The final volume in each well was 200 μL. PSA and Nystatin and Itraconazole at the concentration range of 200 to 6.25 μg/mL were prepared in

nutrient broth and Sabouraud dextrose broth was used as standard drug for the positive control. 200 μL of nutrient broth was transferred into the first wells as positive control. The plate was covered. The contents of all wells were gently mixed on a plate shaker at 300 rpm for 20 s and then incubated at appropriate temperatures for 24 h. Microbial growth in each medium was determined by reading the respective absorbance at 600 nm using the ELx 800 universal microplate reader (Biotek Instrument Inc, Highland Park, VT, USA) and confirmed by plating 5 μL samples from clear wells on nutrient agar medium. The MIC was defined as the lowest concentration of the compounds to inhibit the growth of microorganisms.

Agar dilution assay – Minimal inhibitory concentration

MIC values of the fungi isolates were studied based on the agar dilution method, as described previously [6]. Compounds were added aseptically to sterile molten potato dextrose agar (PDA) medium containing compounds at the appropriate volume to produce the concentration range of 6.25 to 200 μg/mL. The resulting PDA solutions were immediately poured into Petri plates after vortexing. The plates were spot-inoculated with 5 μL (10^4 spore/mL) of each fungal isolate. Nystatin and Itraconazole were used as reference antifungal drugs. The inoculated plates were incubated at 27°C and 37°C for 72 h for plant and clinical fungi isolates, respectively. At the end of the incubation period, the plates were evaluated for presence or absence of growth. MIC values were determined as the lowest concentration of the compound where absence of growth was recorded.

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