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Original paper

Synthesis and antimycotic activity of new (1*H*-1,2,4-triazol-1-ylmethyl)benzeneamine derivatives*

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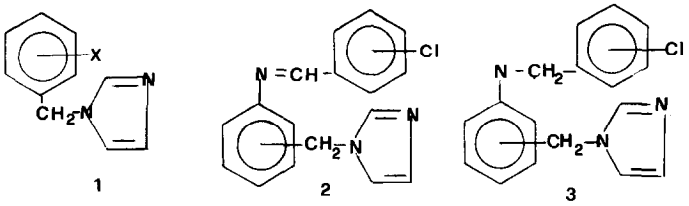
Summary — The synthesis and antimycotic activity of new triazole derivatives are reported. Microbiological assays show a good effectiveness against *Aspergillus flavus, A. parassiticus* and *Fusarium solani.* The same structures are completely inactive against *Microsporum gypseum, Penicillum* sp. and *Candida albicans.*

Résumé — **Synthèse et activité antifongique de nouveaux dérivés du triazole.** *Les résultats microbiologiques indiquent une excellente activité contre* Aspergillus flavus, A. parassiticus *et* Fusarium solani *mais une complète inactivité contre* Microsporum gypseum, Penicillum *sp. et* Candida albicans.

1*H*-1,2,4-triazole derivatives / antimycotic activity / plants fungi antigerminate activity

Introduction

In our previous work with compounds containing an imidazole ring, we have reported the synthesis and antimicrobial activity of compounds with the general structure **1** (Fig. 1).



X = N=CH−Ph_s; NH−CH_2−Ph_s; NH−CO−Ph_s

The biological data from structures **2** and **3** (1, 2) showed an interesting activity *in vitro* against *Candida albicans* and *C. sp.* (Fig. 1). Moreover from *in vivo* results the same activity was determined for Miconazole and (*N*-chlorobenzyl)-3-(1*H*-imidazol-1-ylmethyl)benzeneamines (2). Finally the (*N*-4-chloro-benzyl)-2-(1*H*-imidazol-1-ylmethyl)benzeneamine exhibited better activity than bifonazole, ketoconazole and miconazole *in vitro* and *in vivo* (3, 4).

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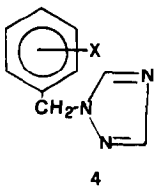
Encouraged by these results we decided to extend our synthetic program to compounds with general structure **4** (Fig. 2) to study:

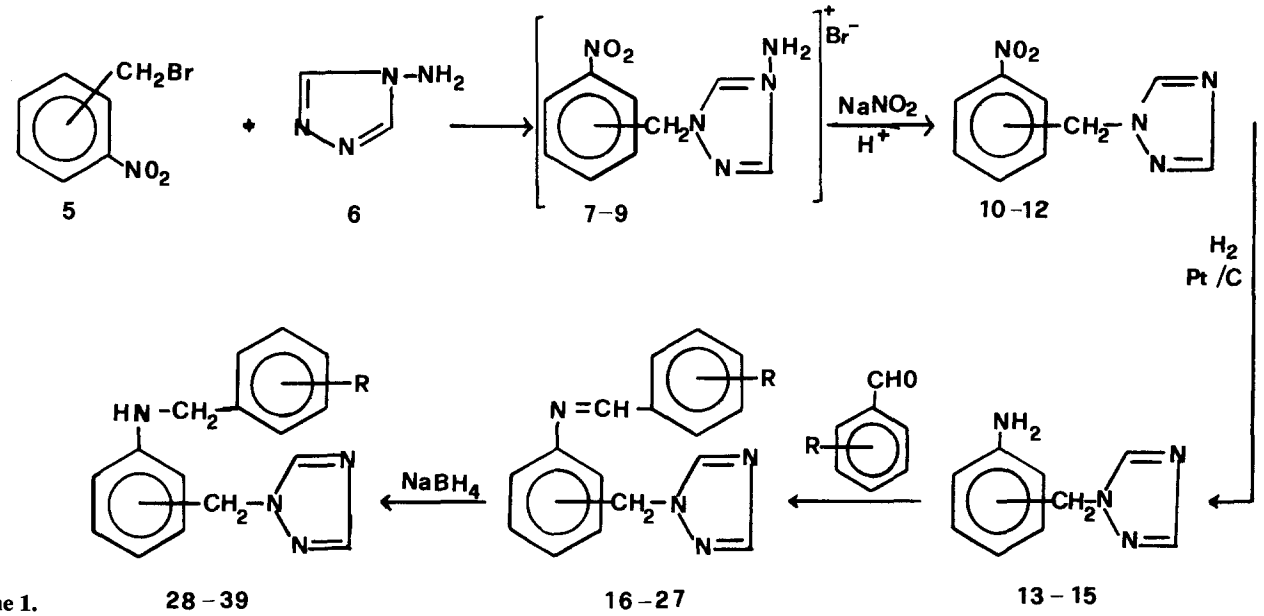


X = N=CH−Ph_s; NH−CH_2−Ph_s

a) effectiveness of triazole nucleus antimycotic activity against *Candida* or *Dermatophytes* in agreement with biological results exhibited by numerous triazole analogues as reported by Zirngibl (5) and, more recently, for fluconazole and itraconazole (6–8). b) Effectiveness of structures **4** against *Aspergillus flavus, A. parassiticus* and *Fusarium solani,* in agreement with the main characteristic of triazole derivatives which are strongly active against plant fungi.

Chemistry

Synthetic pathways for the title compounds are illustrated in Scheme 1. 2,3 or 4-(1*H*-triazol-1-ylmethyl)benzeneamines **13**–**15** were used as starting materials. The prepa-



Scheme 1.

ration of **13–15** was accomplished by catalytic hydrogenation (on Pd/C 10%) of **10–12** which was obtained from the reaction between a suitable nitrobenzylbromide and aminotriazole (9). From the benzeneamines **13–15** and appropriate benzaldehydes the Schiff bases **16–27** were obtained; they were further reduced with sodium borohydride in dry ethanol to the corresponding amines **28–30**, **32–39**. The sodium borohydride reduction of **19** did afford the required amine **31** which was easily obtained from 4-nitrobenzylbromide and **13**. The structure of all compounds was confirmed by elemental analyses and IR spectra. The NMR spectra were performed only for derivatives **28–39** and are in agreement with the proposed structures.

Results and Discussion

Candida albicans, Penicillum sp. *and* Microsporum gypseum
All compounds are inactive at concentration ≥200 μg/ml against these species (miconazole inhibition area diameter = 25 mm).

Aspergillus flavus *and* Aspergillus parassiticus
At 0.5 μg/ml concentration, derivatives **20**, **23–25** and **9**, **11**, **17**, **26**, **29**, **30** inhibit 100%, 80–90% and 40–50% germination respectively. The other derivatives were inactive.

Fusarium solani
All compounds tested were active (100% inhibition) at 0.5 μg/ml. At 0.1 μg/ml derivatives **20**, **24**, **29** and **23** inhibit 100%, 75% and 55% germination respectively. The remainders are scarcely active.

From the microbiological data, the triazole derivatives have excellent plant antifungal activity **20**, **23**, **24**, **25**, **29** as well as specific activity at very low concentration against *Aspergillus flavus*, *A. parassiticus* and *Fusarium solani*. These are widely responsible for food damage during both picking and stockage.

In comparison with our previous imidazole analogues the introduction of triazole nucleus eliminates activity against *Candida*. Nevertheless from these first data the activity would seem to depend on the presence of 4–Cl or 2,4–Cl_2 substituents in analogous structures. In contrast to imidazole derivatives, the *meta* and *para* triazole derivatives would seem to be more active than corresponding *ortho*.

Experimental protocols

Chemistry

Melting points were taken on a Fisher-Johns apparatus and were not corrected. IR spectra were run (nujol mulls) on a Perkin-Elmer spectrophotometer model 297. NMR spectra were recorded on a Varian EM 390 spectrometer using deuterochloroform as solvent and TMS as internal standard. Satisfactory analytical data (±0.3%) were obtained for all compounds. Microanalyses were performed by A. Pietrogrande, Padova (Italy).

4-Amino-1-nitrobenzyl-1,2,4-triazolium bromides ***(7–9)***
A well powdered suitable nitrobenzylbromide **5** (0.1 mol) was added to a solution of 4-amino-4-*H*-1,2,4-triazole **6** (0.1 mol) in 250 ml of dry ethanol. The solution was refluxed for 4 h and cooled at room temperature. The precipitate was filtered off and washed with petroleum ether. Yields and physical data are reported in Table I.

(1H-1,2,4-Triazol-1-ylmethyl)nitrobenzenes ***(10–12)***
A 10% $NaNO_2$ aqueous solution was added dropwise to a solution of **7–9** (0.01 mol) in 250 ml of HCl 5%, stirred and cooled to 0°C until it gave a positive test with iodine-starch paper. The solution was neutralized with conc. KOH and the precipitate filtered off. Yields and physical data are reported in Table I.

(1H-1,2,4-Triazol-1-ylmethyl)benzeneamines ***(13–15)***
A solution of **10–12** (10 mmol) in 150 ml of ethylacetate was hydrogenated at 60°C (1 atm) in the presence of 200 mg of 10% palladium on char-

coal. After the adsorption of hydrogen had stopped, the mixture was filtered in order to remove the catalyst and the filtrate, evaporated under reduced pressure, to give a solid residue which was crystallized from suitable solvent. Yields, physical and spectroscopical data are reported in Tables I and II.

N-*(Benzal)-(1*H-*1,2,4-triazol-1-ylmethyl)benzeneamines* **(16–27)**
A solution of the appropriate benzaldehyde (5 mmol) and (1*H*-1,2,4-triazol-1-ylmethyl)benzeneamines **13–15** (5 mmol) in 100 ml of dry ethanol were added to 50 ml of dry benzene. The mixture was heated at reflux for 24 h and the water formed during the reaction was eliminated by a Dean-Stark apparatus containing anhydrous sodium sulphate. The solvent was removed under reduced pressure and the residue was washed with petroleum ether to remove the aldehyde excess until a solid was formed. Yields and physical data are reported in Table I.

N-*(Benzyl)-(1*H-*1,2,4-triazol-1-ylmethyl)benzeneamines* **(28–30, 32–39)**
A stirred solution of appropriate Schiff bases **16–18**, **20–27** (5 mmol) in 20 ml of dry ethanol was added dropwise at room temperature to a solution of $NaBH_4$ (15 mmol) in 20 ml of dry ethanol. The reaction was heated at reflux for 2 h and subsequently evaporated. The residue was dissolved in water and extracted with ethylacetate. The evaporation of the organic layer gave a solid which was crystallized from a suitable solvent. Yields, physical and spectroscopical data are reported in Tables I and II.

N-*(4-Nitro-benzyl)-2-(1-*H-*1,2,4-triazol-1-ylmethyl)benzeneamine* **(31)**
A solution of 4-nitrobenzylbromide (0.01 mol), triethylamine (0.01) and **13** (0.01 mol) in 100 ml of anhydrous toluene was refluxed for 4 h. The solvent was removed under reduced pressure and the residue was dissolved in ethylacetate. The organic layer, washed with water and dried on anhydrous sodium sulfate, was evaporated and the oil obtained was purified on column of aluminium oxide (Merck II–III) eluted with $CHCl_3$ and subsequently with ethylacetate. The ethylacetate fraction gave the amine **31**. Yield, physical and spectroscopical data are reported in Tables I and II.

Microbiology

Samples were solubilized in DMSO (1 mg / ml) and subsequently water diluted to the required concentration.

Derivatives **7–39** were tested *in vitro* for antifungal activity against 2 strains of *Candida albicans*, 1 strain of *Microsporum gypseum* and 1 strain of *Penicillum sp.* (all strains were obtained from clinical specimens).

Tests, repeated 5 times, were carried out on buffered Shadomy agar (A / S Rosco).

A suspension (10^6 cells / ml in distilled and sterile water) of *Candida albicans,* obtained on Sabouraud dextrose agar (B.B.L.) after 24 h of incubation at 27°C was employed as test. A fragment (0.5×0.5 mm) of *micelium* of suitable dermatofita, obtained on Sabouraud dextrose agar (B.B.L.) after 14 d incubation at 27°C, was suspended in 5 ml of distilled and sterile water and homogenized (Rotamixer, Hook and Tucker LTD). 0.01 ml of each suspension was blended with 20 ml of the test medium on Petri disks (12 cm i.d.) and tested with blank paper disc (B.B.L.) soaked with 25 μl of suitable derivative solution. Data were recorded after 2 and 4 d *(Candida)* or 7 and 10 d *(Dermatofites)* incubated at 27°C and expressed in mm of inhibition area diameter (i.a.d.). Miconazole was used as reference compound.

Derivatives **7–39** were tested for *in vitro* activities against germination of conidia of *Aspergillus flavus, A. parassiticus* (aflatoxine producers) and *Fusarium solani* (potato pathogenic).

Table I.

Comp.	Pos.	R	Y(%)	mp(°C)	Solv.	Formula
7	2		45	150–5	c	$C_9H_{10}BrN_5O_2$
8	3		75	130–1	c	$C_9H_{10}BrN_5O_2$
9	4		80	198–9	c	$C_9H_{10}BrN_5O_2$
10	2		71	66–7	b	$C_9H_8N_4O_2$
11	3		40	114–5	b	$C_9H_8N_4O_2$
12	4		65	118–9	b	$C_9H_8N_4O_2$
13	2		70	62–3	a	$C_9H_{10}N_4$
14	3		75	87–8	a	$C_9H_{10}N_4$
15	4		80	121–2	a	$C_9H_{10}N_4$
16	2	$2,4Cl_2$	50	120–3	a	$C_{16}H_{12}Cl_2N_4$
17	2	4Cl	52	123–7	a	$C_{16}H_{13}ClN_4$
18	2	4F	40	92–3	a	$C_{16}H_{13}FN_4$
19	2	$4NO_2$	45	123–4	a	$C_{16}H_{13}N_5O_2$
20	3	$2,4Cl_2$	55	98–9	a	$C_{16}H_{12}Cl_2N_4$
21	3	4Cl	42	75–8	a	$C_{16}H_{13}ClN_4$
22	3	4F	43	101–3	a	$C_{16}H_{13}FN_4$
23	3	$4NO_2$	51	88–9	a	$C_{16}H_{13}N_5O_2$
24	4	$2,4Cl_2$	52	111–2	a	$C_{16}H_{12}Cl_2N_4$
25	4	4Cl	59	138–9	a	$C_{16}H_{13}ClN_4$
26	4	4F	50	84–5	a	$C_{16}H_{13}FN_4$
27	4	$4NO_2$	58	208–9	a	$C_{16}H_{13}N_5O_2$
28	2	$2,4Cl_2$	90	130–1	a	$C_{16}H_{14}Cl_2N_4$
29	2	4Cl	85	75–7	a	$C_{16}H_{15}ClN_4$
30	2	4F	80	81–3	a	$C_{16}H_{15}FN_4$
31	2	$4NO_2$	80	88–9	a	$C_{16}H_{15}N_5O_2$
32	3	$2,4Cl_2$	80	88–9	a	$C_{16}H_{14}Cl_2N_4$
33	3	4Cl	78	110–1	a	$C_{16}H_{15}ClN_4$
34	3	4F	80	126–7	a	$C_{16}H_{15}FN_4$
35	3	$4NO_2$	80	113–4	a	$C_{16}H_{15}N_5O_2$
36	4	$2,4Cl_2$	80	137–9	a	$C_{16}H_{14}Cl_2N_4$
37	4	4Cl	75	121–3	a	$C_{16}H_{15}ClN_4$
38	4	4F	90	125–7	a	$C_{16}H_{15}FN_4$
39	4	$4NO_2$	75	158–9	a	$C_{16}H_{15}N_5O_2$

a=Ethyl acetate; b=Chloroform; c=EtOH; pos=position of rest of alkyl triazole nucleus.

Table II.

Comp.	NMR	δ ppm			
29, 32, 33, 36, 37		4.2	1H	unr. m	*NH*−CH_2
		4.3	2H	s	*CH_2*−NH
		5.2	2H	s	*CH_2*−Tr
		6.2−7.5		unr. m	Ar. protons
		7.9−8.1	2H	ss	Tr. protons
28		4.5	2H	d J=6 cps	*CH_2*−NH
		5.3	2H	s	*CH_2*−Tr
		5.8	1H	t J=6 cps	*NH*−Ch_2
		6.3−7.5	7H	unr. m	Ar. protons
		7.9−8.1	2H	ss	Tr. protons
34, 38		3.3	1H	unr. m	*NH*−CH_2
		4.2	2H	s	*CH_2*−NH
		5.3	2H	s	*CH_2*−Tr
		6.5−7.5	8H	unr. m	Ar. protons
		7.9−8.1	2H	ss	Tr. protons
30		4.3	2H	s	*CH_2*−NH
		5.2	3H	unr. m	*CH_2*−Tr and *NH*−CH_2
		6.6−7.5	8H	unr. m	Ar. protons
		7.9−8.1	2H	ss	Tr. protons
31, 39		4.4	2H	d J=6 cps	*CH_2*−NH
		5.3	2H	s	*CH_2*−Tr
		5.8	1H	t J=6 cps	*NH*−CH_2
		6.4−8.5	10H	unr. m	Ar. and Tr. protons
35		4.5	2H	s	*CH_2*−NH
		5.3	2H	s	*CH_2*−Tr
		6.0	1H	unr. m	*NH*−CH_2
		6.3−8.4	10H	unr. m	Ar. and Tr. protons
	IR	cm^{-1}			
7−9		3220−3080	*NH_2*−Tr (broad and unresolved band)		
13−15		3350−3300	*NH_2*−Ar		
28−39		3300	*NH*−CH_2		
7−12		1520, 1340	NO_2		

Tests, repeated 5 times, were carried out using Czapek agar (0.2% w/v) with yeast extract (Difco) (0.2% w/v). 10^6 *conidia* were added to the *test medium* (5% ml of media in 20 ml test-tube (5 cm i.d.)) and data were recorded after 12 h incubation at 30°C.

The value of germination (100%) in the *test medium* alone was used as reference control. The range concentration (0.1−0.5 μg/ml) of usual germination inhibitors as BHT and BHA was employed to estimate microbiological activity.

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