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Research on antibacterial and antifungal agents. X. Synthesis and antimicrobial activities of 1-phenyl-2-(1*H*-azol-1-yl) ethane derivatives. Anticonvulsant activity of 1-(4-methylphenyl)-2-(1*H*-imidazol-1-yl) ethanol

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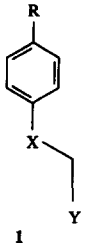
Summary — The synthesis and antimicrobial activity of new phenylazolylethane derivatives are reported. Antimicrobial data in comparison with those obtained with miconazole, fluconazole, enilconazole, imazalil sulfate, pipemidic acid and minocycline showed that tested compounds generally possessed poor or weak activity. Toxicity and anticonvulsant activity of 1-(4-methylphenyl)-2-(1*H*-imidazol-1-yl)ethanol and structural likeness to Denzimol were also tested. The obtained results showed interesting anticonvulsant activity but only on the supraspinal region.

azoles / antifungal activity / anticonvulsant agent

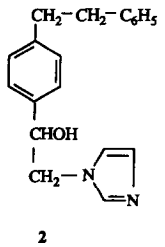
Introduction

Ogata [1] has recently pointed out that the tertiary 1-phenyl-ethanol structure could be the pharmacophore responsible for the oral antifungal activity showed by many azolylethanols. On the other hand, many antifungal active compounds possess the arylazolylethane as part of their common structure [2] and many azole compounds containing an oxime group have been reported in the literature [3–6] as fungicides. With this in mind, we have synthesized the derivatives **1** as a further extension of our previous research [7–9] on compounds containing an imidazole or triazole ring.



1

R = H; Br; Cl; F; CH_3
Y = 1-Imidazolyl; 1-Triazolyl
a) X = C = O; b) X = CH–OH; c) X = C = NOH



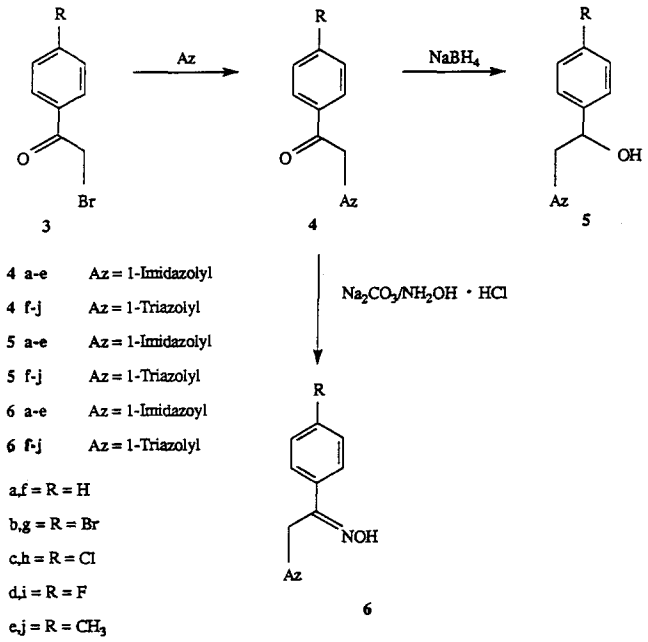
2

Denzymol

In the present communication we report the synthesis and *in vitro* antimicrobial activity against *Candida albicans*, *Candida* sp, Gram-positive or Gram-negative bacteria and isolates of pathogenic plant fungi. Moreover, we describe part of the anticonvulsant activity spectrum displayed by **1b** (R = CH_3; Y = imidazolyl). This research was suggested by the structural likeness of **1b** to Denzimol **2** [10–13].

Chemistry

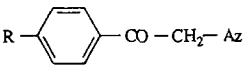
Synthetic pathways for the synthesis of the title compounds have been illustrated in scheme 1. Bromo derivatives **3**, obtained by bromination of the corresponding acetophenone precursors, were treated with imidazole in DMF to provide the 2-(1*H*-imidazol-1-yl)-1-phenylethanones **4a–e** (table I). It was confirmed that the obtained products were a mixture of **4a–e** and the corresponding 1,3-bis(phenacyl) imidazolium halides which were separated by the different solubilities of the components, as reported by Nardi [10]. *Vice versa*, derivatives **4f–j** were prepared from **3** by reaction on an ice-bath with 1,2,4-triazole in the presence of triethylamine (table I). In this case the bis(phenacyl)triazolium halides were not found. 2-(1*H*-1,2,4-Triazol-1-yl)-1-phenylethanones



Scheme 1.

4f–j yield is closely dependent on the temperature and molar ratio of the reagents but is not dependent on the reaction time. In fact, at a temperature of > 0°C the yield decreases and when 1,2,4-triazole is added in a ratio of 2:1 or 3:1 in comparison to bromo derivatives **3**, it proves difficult to remove residual 1,2,4-triazole from the reaction mixture and thus reduces the yield of ethanones **4f–j**. The sodium borohydride reduction of **4a–j** afforded the enantiomer mixture of the alcohols **5a–j** (table II), which were analyzed by high-performance liquid chromatography (HPLC) on the chiral stationary phase, as reported in the *Experimental procedures*. The oxime derivatives **6a–j** (table III) were prepared in fairly good yield by reaction from **4a–j** and hydroxylamine hydrochloride in the presence of dry sodium carbonate. Oximes **6a–j** were obtained and isolated as *syn* and *anti* mixtures. We selected this synthetic route to prepare oximes **6a–j** because the reaction, as reported by Mixich and Thiele [14], between 1-phenyl-2-bromo ethanoxime and imidazole or 1,2,4-triazole afforded a mixture of different products and the isolation of **6a–j** from these mixtures was very difficult. This is in agreement with what was reported by Gilchrist [15] regarding the possibility that the reaction between haloximes and azoles could give different products. The structure of the compounds **5a–j** and **6a–j** was confirmed by elemental analyses and spectral data. **4a–e**, **5a–e** and **5h** were in agreement with the literature data [16, 17].

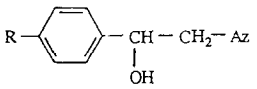
Table I. Physicochemical data for compounds **4a–j**.



Compound	*R*	*Az*	*mp (°C)*	*Yield (%)*	*Crystn solvent*	*Formula (MW)*
4a*	H	Im	113-5	65	a	$C_{11}H_{10}N_2O$ (186.10)
4b*	Br	Im	165-7	80	a	$C_{11}H_9BrN_2O$ (265.10)
4c*	Cl	Im	160-2	70	b	$C_{11}H_9ClN_2O$ (220.70)
4d*	F	Im	143-5	65	b	$C_{11}H_9FN_2O$ (204.22)
4e*	CH_3	Im	132-4	75	b	$C_{12}H_{12}N_2O$ (200.24)
4f	H	Tr	120-2	50	c	$C_{10}H_9N_3O$ (187.00)
4g	Br	Tr	185-8	45	c	$C_{10}H_8BrN_3O$ (265.90)
4h	Cl	Tr	152-5	40	c	$C_{10}H_8ClN_3O$ (221.40)
4i	F	Tr	135-6	45	c	$C_{10}H_8FN_3O$ (205.20)
4j	CH_3	Tr	122-4	30	c	$C_{11}H_{11}N_3O$ (201.00)

a: ethylacetate/ethanol (2:1); b: ethanol; c: benzene; * lit [16].

Table II. Physicochemical data for compounds **5a–j**.



Compound	*R*	*Az*	*mp (°C)*	*Yield (%)*	*Crystn solvent*	*Formula (MW)*	*Analyses*
5a*	H	Im	155-7	60	a	$C_{11}H_{12}N_2O$ (188.20)	C, H, N
5b*	Br	Im	198-200	75	a	$C_{11}H_{11}BrN_2O$ (266.90)	C, H, Br, N
5c*	Cl	Im	188-90	70	b	$C_{11}H_{11}ClN_2O$ (222.70)	C, H, Cl, N
5d*	F	Im	147-9	65	b	$C_{11}H_{11}FN_2O$ (206.22)	C, H, F, N
5e*	CH_3	Im	162-5	70	a	$C_{12}H_{14}N_2O$ (202.26)	C, H, N
5f	H	Tr	121-3	40	c	$C_{10}H_{11}N_3O$ (189.00)	C, H, N
5g	Br	Tr	127-30	50	c	$C_{10}H_{10}BrN_3O$ (267.90)	C, H, Br, N
5h**	Cl	Tr	124-5	50	c	$C_{10}H_{10}ClN_3O$ (223.45)	C, H, Cl, N
5i	F	Tr	96-8	40	c	$C_{10}H_{10}FN_3O$ (207.00)	C, H, F, N
5j	CH_3	Tr	135-7	45	c	$C_{11}H_{13}N_3O$ (203.00)	C, H, N

a: ethylacetate/ethanol (2:1); b: ethanol; c: benzene; * lit [16]; ** lit [17].

Results and discussion

Microbiology

The *in vitro* test results have been summarized in tables V–IX. Compounds **4a**, **4f–g**, **4i–j**, **5f**, **5i–j** and **6f–j** are inactive against *Candida albicans* and *Candida* sp. Compound **5a** is inactive against *Candida albicans* but shows weak activity against *Candida* sp. Derivatives **4b**, **4d**, **4h**, **5d**, **6a** and **6e** show poor antifungal activity against *Candida albicans* and are completely inactive against *Candida* sp. Compound **5c** was weakly active against *Candida albicans* but inactive against *Candida* sp.

Derivatives **4c**, **4e**, **5b**, **5e** and **5g** showed weak activity against both *Candida albicans* and *Candida* sp. **5h** showed moderate activity against *Candida albicans* only.

Derivative **6b** exhibited poor activity against *Candida albicans* but interesting activity against 2 strains of *Candida* sp. The most antifungal active compounds were **6c** and **6d**.

Antibacterial screening was carried out only for the moderate active compounds **6a–e**. Among the synthesized compounds the most antibacterially active was **6c**, which showed better activity than fluconazole or miconazole, and which was sometimes similar to that of pipemidic acid and minocycline.

Finally, **4a–j**, **5a–j** and **6a–j** were also tested for their inhibitory effect on pseudomycelium formation by 4 strains of *Candida albicans* cultured in Eagle's minimal essential medium (EMEM). All tested compounds were inactive at the concentrations used and we therefore decided not to screen *in vivo* activity.

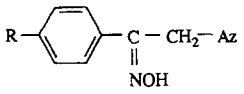
From the microbiological data on *Candida albicans* and *Candida* sp, the following conclusions can be drawn:

– The presence of the imidazole nucleus seems more important to the activity than that of the triazole nucleus. In fact, all triazole derivatives are inactive.

– As regards the imidazole derivatives, the presence of the oxime group is important for activity and the introduction of chlorine or fluorine atoms markedly affects antimicrobial activity. Indeed, the most active compounds were **6c** and **6d**.

– It is worth pointing out the anomalous result regarding our products in comparison to what was

Table III. Physicochemical data for compounds **6a–j**.



Compound	*R*	*Az*	*mp (°C)*	*Yield (%)*	*Crystn solvent*	*Formula (MW)*	*Analyses*
6a	H	Im	175-8	60	a	$C_{11}H_{11}N_3O$ (201.23)	C, H, N
6b	Br	Im	206-8	70	a	$C_{11}H_{10}BrN_3O$ (280.13)	C, H, Br, N
6c	Cl	Im	200-2	65	b	$C_{11}H_{10}ClN_3O$ (235.70)	C, H, Cl, N
6d	F	Im	188-90	60	a	$C_{11}H_{10}FN_3O$ (219.22)	C, H, F, N
6e	CH_3	Im	172-5	60	b	$C_{12}H_{13}N_3O$ (215.30)	C, H, N
6f	H	Tr	163-5	55	c	$C_{10}H_{10}N_4O$ (202.00)	C, H, N
6g	Br	Tr	197-200	55	d	$C_{10}H_9BrN_4O$ (208.90)	C, H, Br, N
6h	Cl	Tr	199-202	50	c	$C_{10}H_9ClN_4O$ (236.45)	C, H, Cl, N
6i	F	Tr	173-5	60	c	$C_{10}H_9FN_4O$ (220.00)	C, H, F, N
6j	CH_3	Tr	147-50	60	c	$C_{11}H_{12}N_4O$ (216.00)	C, H, N

a: ethylacetate/ethanol (2:1); b: ethanol; c: chloroform; d: ethylacetate.

reported by Ogata [1] for analogue derivatives. We found that compounds with some microbiological activity were not inhibitors of *Candida albicans* pseudomycelium formation, while the opposite was the case for those tested by Ogata. In fact compounds inactive *in vitro* showed a good inhibitory effect on pseudomycelium formation. It may be possible that Ogata's compounds were strongly influenced by the presence of a further azole nucleus in the molecule and greater molecular lipophilia.

Moreover, compounds **5a–j** and **6a–j** were tested against isolates of pathogenic plant fungi to study the effect on mycelial radial growth. Among the tested compounds, the most active against *Phomopsis* sp isolates were triazole derivatives **5f–j**; their activity was not enhanced by concentration. In fact, these compounds showed the same activity at 6.25 μg/ml and at 100 μg/ml. This result is surprising but also interesting, so we aim to carry out a further study in order to elucidate the matter. The other derivatives **5a–e** and **6a–j** were found to be practically inactive.

As regards activity against the *Drechslera graminea* isolates, all compounds showed weak or poor activity.

In conclusion, all the obtained data do not allow us to make a strict correlation of structure activity–relationships. Moreover, it was not possible to compare the activity of our most active derivatives to that of the parent oxime compounds reported in the literature [3–6] because all experimental data have been published in patent form.

Table IV. HPLC* analysis of enantiomer mixtures of **5a–j**.

Compound	K'_1	α
5a	3.03	1.00
5b	3.09	1.15
5c	2.43	1.20
5d	1.78	1.22
5e	4.22	1.00
5f	2.47	1.08
5g	2.49	1.19
5h	1.97	1.14
5i	1.51	1.00
5j	3.09	1.22

*Chromatographic conditions: chiralcel OD column, 250 x 4.6 (id) mm; eluent *n*-hexane/*i*-propanol 75:25 v/v; flow rate, 2 ml/min; temperature, 25°C; UV detection, 254 nm.

Table V. Antimycotic activity of miconazole and fluconazole compared with activity of the compounds **4a–j**, **5a–j** and **6f–j** against 38 strains of *Candida albicans* and 6 strains of *Candida* sp*.

Compound	*Candida albicans*			*Candida sp*			*C a AD6*	*C a FE40*	*C a USL*	*C a A31*
	R%	$n\bar{X}$	*Range (μg/ml)*	*R%*	$n\bar{X}$	*Range (μg/ml)*	*MEC (μg/ml)*	*MEC (μg/ml)*	*MEC (μg/ml)*	*MEC (μg/ml)*
Miconazole	0	0.53	< 0.2-1.56	0	4.98	< 0.2-25	1.25	0.62	0.31	1.25
Fluconazole	10.5	19	< 0.2-200	0	100.9	0.8-200	> 20	> 20	> 20	> 20
4a	100	–	> 200	100	–	> 200	> 20	> 20	> 20	> 20
4b	97.3	200	200-> 200	100	–	> 200	> 20	> 20	> 20	> 20
4c	78.9	175	100-> 200	83.3	200	200-> 200	> 20	> 20	> 20	> 20
4d	97.3	100	100-> 200	100	–	> 200	> 20	> 20	> 20	> 20
4e	97.3	50	50-> 200	83.3	100	100-> 200	> 20	> 20	> 20	> 20
4f	100	–	> 200	100	–	> 200	> 20	> 20	> 20	> 20
4g	100	–	> 200	100	–	> 200	> 20	> 20	> 20	> 20
4h	97	200	200-> 200	100	–	> 200	> 20	> 20	> 20	> 20
4i	100	–	> 200	100	–	> 200	> 20	> 20	> 20	> 20
4j	100	–	> 200	100	–	> 200	> 20	> 20	> 20	> 20
5a	100	–	> 200	16.6	200	200-> 200	> 20	> 20	> 20	> 20
5b	56.2	188.8	200-> 200	50	200	200-> 200	> 20	> 20	> 20	> 20
5c	26.3	200	200-> 200	100	–	> 200	> 20	> 20	> 20	> 20
5d	97.3	200	200-> 200	100	–	> 200	> 20	> 20	> 20	> 20
5e	84.6	162.5	50-> 200	66.6	200	200-> 200	> 20	> 20	> 20	> 20
5f	100	–	> 200	100	–	> 200	> 20	> 20	> 20	> 20
5g	59	112	100-> 200	86	100	100-> 200	> 20	> 20	> 20	> 20
5h	18	177	50-> 200	86	100	100-> 200	> 20	> 20	> 20	> 20
5i	100	–	> 200	100	–	> 200	> 20	> 20	> 20	> 20
5j	100	–	> 200	100	–	> 200	> 20	> 20	> 20	> 20
6f	100	–	> 200	100	–	> 200	> 20	> 20	> 20	> 20
6g	97	200	200-> 200	100	–	> 200	> 20	> 20	> 20	> 20
6h	100	–	> 200	100	–	> 200	> 20	> 20	> 20	> 20
6i	100	–	> 200	100	–	> 200	> 20	> 20	> 20	> 20
6j	100	–	> 200	100	–	> 200	> 20	> 20	> 20	> 20

R%: percentage of resistant strains; $n\bar{X}$: MIC mean values of sensitive strains; *1 *C glabrata*, 1 *C tropicalis*, 1 *C guillermondii*, 1 *C krusei*, 1 *C parapsilosis*, 1 *C lipolytica*, at pH = 7.2 and minimal effective concentration (MEC) which prevented typical pseudomycelium formation of 4 strains of *Candida albicans*.

We can only point out that imidazole derivatives are generally more active against *Candida albicans* and *Candida* sp than the corresponding triazole derivatives. On the other hand, triazole derivatives are at times more active against isolates of pathogenic plant fungi than the corresponding imidazole derivatives.

Anticonvulsive activity

Acute toxicity

No lethal effect or immediate effect on CNS and ANS was recorded after single administration of **5e** up to doses of 100 mg/kg ip or 300 mg/kg *po*. Moreover, no macroscopic alteration in the main target organs was observed at the end of the observation period. Toxic symptoms in the CNS such as tremors and twitches were present at doses from 300 mg/kg ip and 500 mg/kg *po*. Lethality data have been reported in table X.

Effects on MES seizures

The results observed in this test (tables XI, XII), clearly indicate that **5e** exerted a protective effect against MES (supramaximal electroshock seizures). The compound reduced the duration of the extensor tonic phase of seizures, and after *po* administration at dose of 300 mg/kg, completely abolished the extensor tonic phase. Phenobarbital sodium (50 mg/kg ip) selected as reference drug, exerted complete protection against MES seizures.

Effects on 'psychomotor' electroshock

5e had no effect in this test, whereas phenobarbital sodium (50 mg/kg ip) completely abolished the effect of the electrical stimulus (table XIII).

Effect on maximal PTZ seizures

Intraperitoneally administered **5e** was inactive against pentylenetetrazole (PTZ); when administered orally, it

Table VI. Antimycotic activity of miconazole and fluconazole compared to the activity of compounds **6a–e** against 38 strains of *Candida albicans* at pH = 7.2, MIC values of miconazole, fluconazole and compounds **6a–e** against 6 strains of *Candida* sp*.

Compound	*C albicans*			*Candida sp*						*C a AD6*	*C a FE40*	*C a USL*	*C a A31*
	R%	$n\bar{X}$	*Range (μg/ml)*	*C gl*	*C tr*	*C gui*	*C kr*	*C p*	*C l*	*MEC (μg/ml)*	*MEC (μg/ml)*	*MEC (μg/ml)*	*MEC (μg/ml)*
Miconazole	0	0.53	< 0.2-1.56	0.8	0.8	< 0.2	3.12	25	< 0.2	1.25	0.62	0.31	1.25
Fluconazole	10.5	19	< 0.2-200	200	200	3.12	200	1.56	0.8	> 20	> 20	> 20	> 20
6a	42.1	186.3	100-> 200	> 200	> 200	200	> 200	200	200	> 20	> 20	> 20	> 20
6b	89	50	50-> 200	> 200	> 200	50	> 200	50	> 200	> 20	> 20	> 20	> 20
6c	11.7	47	25-> 200	200	200	100	200	50	25	> 20	> 20	> 20	> 20
6d	2.6	95.2	25-> 200	100	100	100	100	100	100	> 20	> 20	> 20	> 20
6e	92.1	166.6	100-> 200	> 200	> 200	200	> 200	> 200	200	> 20	> 20	> 20	>20

R% = percentage of resistant strains; $n\bar{X}$ = MIC mean values of sensitive strains; *1 *C glabrata*, 1 *C tropicalis*, 1 *C guillermondii*, 1 *C krusei*, 1 *C parapsilosis*, 1 *C lipolytica*, at pH = 7.2 and minimal effective concentration (MEC) which prevented typical pseudomycelium formation of 4 strains of *Candida albicans*.

Table VII. MIC (μg/ml) values of pipemidic acid, minocycline, fluconazole, miconazole and compounds **6a–e** against 7 strains of Gram-positive bacteria and 14 strains of Gram-negative bacteria at pH = 7.2.

Microorganism	*Pipemidic acid*	*Minocycline*	*Fluconazole*	*Miconazole*	***6a***	***6b***	***6c***	***6d***	***6e***
Staph aureus MI 2	50	6.25	> 200	> 200	> 200	> 200	3.12	200	> 200
Staph mitis MI	100	3.12	> 200	3.12	> 200	> 200	12.5	200	> 200
Staph haemolyticus 1M	100	6.25	> 200	3.12	> 200	200	200	> 200	> 200
Staph piag MI	50	3.12	> 200	25	200	200	200	200	> 200
Strep 72	50	6.25	> 200	12.5	> 200	> 200	12.5	200	> 200
Strep faecalis MI	50	3.12	> 200	25	> 200	> 200	> 200	> 200	> 200
Staph haemolit MI	3.12	3.12	> 200	> 200	200	> 200	> 200	> 200	> 200
E coli haemolit 1 MI	3.12	6.25	> 200	> 200	> 200	> 200	> 200	> 200	> 200
E coli 1M	100	100	> 200	> 200	> 200	> 200	> 200	> 200	> 200
E coli 3	100	6.25	> 200	> 200	> 200	200	100	200	> 200
E coli 4	100	6.25	> 200	> 200	200	200	100	200	> 200
E coli EC 1156	6.25	25	> 200	> 200	> 200	> 200	> 200	> 200	> 200
E coli haem 2 MI	6.25	25	> 200	25	> 200	200	50	> 200	> 200
Kb 1489	6.25	6.25	> 200	> 200	> 200	> 200	100	> 200	> 200
Pseudomonas 884	6.25	50	> 200	> 200	> 200	> 200	> 200	> 200	> 200
Pseudomonas 1333	6.25	50	> 200	> 200	> 200	> 200	> 200	> 200	> 200
Pseudomonas 990	6.25	50	> 200	> 200	> 200	> 200	> 200	> 200	> 200
Serratia odoriphera	100	6.25	> 200	> 200	200	200	100	> 200	> 200
Yersinia enterocol	> 200	6.25	> 200	> 200	200	100	> 200	200	200
Proteus mirabilis MI	3.12	25	> 200	> 200	> 200	> 200	> 200	> 200	> 200
Proteus mirabilis	3.12	25	> 200	> 200	> 200	> 200	12.5	> 200	> 200

Kb: Klebsiella.

Table VIII. Effect of compounds **5a–j** and **6a–j** on radial growth of *Drechslera graminea* (Rabenh ex Schlecht) Shoem isolates at pH = 5.6.

Compound	*Concentration*									
	6.25 μg/ml		*12.5 μg/ml*		*25 μg/ml*		*50 μg/ml*		*100 μg/ml*	
	rg (mm)	*% inhib*	*rg (mm)*	*% inhib*	*rg (mm)*	*% inhib*	*rg (mm)*	*% inhib*	*rg (mm)*	*% inhib*
5a	16	11.1	15	16.7	14	22.2	13	27.8	12	33.3
5b	17	0.05	16	11.1	14	22.2	13	27.8	10	44.4
5c	17	0.05	17	0.05	16	11.1	16	11.1	15	16.7
5d	16	11.1	16	11.1	15	16.7	14	22.2	14	22.2
5e	17	0.05	17	0.05	16	11.1	16	11.1	15	16.7
5f	15	16.7	15	16.7	15	16.7	15	16.7	10	44.4
5g	15	16.7	15	16.7	15	16.7	15	16.7	10	44.4
5h	18	0	18	0	10	44.4	10	44.4	10	44.4
5i	15	16.7	15	16.7	15	16.7	15	16.7	12	33.3
5j	10	44.4	10	44.4	10	44.4	10	44.4	10	44.4
6a	15	16.7	15	16.7	14	22.2	13	27.8	12	33.3
6b	18	0	16	11.1	16	11.1	15	16.7	15	16.7
6c	17	0.05	17	0.05	16	11.1	15	16.7	15	16.7
6d	16	11.1	14	22.2	14	22.2	13	27.8	12	33.3
6e	16	11.1	14	22.2	14	22.22	13	27.8	12	33.3
6f	16	11.1	14	22.2	14	22.2	12	33.3	10	44.4
6g	18	0	17	0.05	17	0.05	16	11.1	14	22.2
6h	18	0	16	11.1	15	16.7	14	22.2	12	33.3
6i	14	22.2	13	27.8	12	33.3	12	33.3	10	44.4
6j	18	0	18	0	18	0	17	0.05	14	22.2
Imazalil sulfate	18	0	18	0	14	22.2	10	44.4	8	55.5
Enilconazole	18	0	18	0	12	33.3	0	100	0	100

rg: Radial growth on potato dextrose agar (Oxoid); diameter of colonies in control treatments with DMSO was 18 mm; diameter of colonies in control treatments was 22 mm.

slightly potentiated PTZ lethality. Phenobarbital sodium induced complete protection (table XIV), in agreement with the results obtained in the 'psychomotor' electroshock test.

Effects on strychnine lethality

Pretreatment with **5e** considerably increased lethality in mice receiving a fixed dose of strychnine nitrate (1 mg/kg) (table XV).

In conclusion, the results obtained under our experimental conditions suggest that **5e** should be regarded as an anticonvulsant acting only on the supraspinal region, since it almost exclusively blocks the tonic component of seizures [18, 19]. The lack of effect on 'psychomotor' electroshock and maximal PTZ seizures indicates that **5e** does not raise the seizure threshold. The enhancement of strychnine toxicity may be due to an excitatory action of the drug on primary afferents and motoneurons, as reported in the case of phenytoin [18]. Moreover, anti-strychnine activity is considered to reflect muscle relaxant rather than antiepileptic properties [19]. Further studies are required to better evaluate the pharmacological activities of **5e**, also because this compound is a racemate and this may involve a lower activity compared with L or D isomers. Moreover, the water insolubility of **5e** may result in erratic absorption from the administration sites.

Experimental protocols

Chemistry

Melting points were uncorrected and taken on a Fisher–Johns apparatus. Infrared spectra (nujol mulls) were run on a Perkin–Elmer spectrophotometer 297. NMR spectra were recorded on a Varian EM 390 (90 MHz) spectrometer using DMSO or chloroform as solvent and TMS as internal standard. Analytical liquid chromatography was performed using a Waters HPLC apparatus (Water Assoc, Milford, MA, USA) consisting of 2 M510 Mod solvent delivery systems, a U6K Mod injector and a Mod 490 programmable multi-wavelength detector. Chromatographic data were collected and processed on a Waters Assoc Mod 840 data and chromatography control station. Compounds **5** and **6** were analyzed for C, H, N and, when present, Cl, Br, F. The analyzed values were within ± 0.4% of the calculated values. Microanalyses were performed by A Pietrogrande, Padua, Italy. Merck aluminium oxide (II–III according to Brockmann) was used for chromatographic purification and chloroform was used as solvent. Carlo–Erba

Table IX. Effect of compounds **5a–j** and **6a–j** on radial growth of *Phomopsis* sp isolates at pH = 5.6.

Compound	*6.25 μg/ml*		*12.5 μg/ml*		*25 μg/ml*		*50 μg/ml*		*100 μg/ml*	
	rg (mm)	*% inhib*	*rg (mm)*	*% inhib*	*rg (mm)*	*% inhib*	*rg (mm)*	*% inhib*	*rg (mm)*	*% inhib*
5a	41	0	40	0.02	40	0.02	39	0.05	30	26.8
5b	41	0	41	0	40	0.02	38	0.07	35	14.6
5c	41	0	41	0	41	0	41	0	36	12.2
5d	41	0	41	0	41	0	41	0	38	0.07
5e	41	0	41	0	41	0	41	0	37	0.1
5f	30	26.8	30	26.8	30	26.8	30	26.8	30	26.8
5g	25	39.0	25	39.0	25	39.0	25	39.0	25	39.0
5h	30	26.8	30	26.8	30	26.8	30	26.8	30	26.8
5i	25	39.0	25	39.0	25	39.0	25	39.0	25	39.0
5j	30	26.8	25	39.0	25	39.0	25	39.0	25	39.0
6a	41	0	41	0	41	0	41	0	40	0.02
6b	41	0	41	0	41	0	41	0	36	12.2
6c	41	0	41	0	41	0	41	0	38	0.07
6d	41	0	41	0	41	0	41	0	40	0.02
6e	41	0	41	0	41	0	41	0	40	0.02
6f	41	0	40	0.02	39	0.05	39	0.05	37	0.1
6g	41	0	40	0.02	39	0.05	39	0.05	38	0.07
6h	41	0	41	0	40	0.02	40	0.02	38	0.07
6i	41	0	40	0.02	39	0.05	38	0.07	37	0.1
6j	41	0	41	0	40	0.02	40	0.02	39	0.05
Imazalil sulfate	35	14.6	30	26.8	20	51.2	0	100	0	100
Enilconazole	24	41.5	15	63.4	12	70.7	0	100	0	100

rg: Radial growth on potato dextrose agar (Oxoid); diameter of colonies in control treatments with DMSO was 41 mm; diameter of colonies in control treatments was 46 mm.

Table X. Acute toxicity after administration of a single dose of **5e** in mice.

Dose (mg/kg)	*Administration route*	*Lethality (%)*
100	ip	0
300	ip	50
400	ip	100
300	*po*	0
500	*po*	30
750	*po*	37.5
1000	*po*	42.5

stratocrom aluminium oxide plates with fluorescent indicator were used for thin–layer chromatography (TLC) to check the purity of all compounds.

1-Phenyl-2-(1H-imidazol-1-yl)ethan-1-ones ***4a–e***

A mixture of the appropriate phenacyl bromide **3** (0.01 mol), imidazole (Fluka) (0.05 mol) and 3 ml DMF (*N,N*-dimethylformamide) (Carlo–Erba) was stirred at 5–10°C for 3 h. It was then poured into water and the precipitate filtered, washed with water and dried. The crude product was a mixture of **4** and the corresponding 1,3-bis(phenacyl) imidazolium halide which were separated by crystallization. Yields, melting points, crystallization solvents and elements analyzed have been reported in table I.

Compound **4a**: IR: 1680 (C=O) cm^{-1}. ^{1}H-NMR: δ (ppm) = 5.33 (s, 2H, CH_2); 6.95–8.10 (unr m, 8H, C_6H_5 and imidazole).

1-Phenyl-2-(1H-1,2,4-triazol-1-yl)ethan-1-ones ***4f–j***

To a stirred cooled solution of 100 ml chloroform and triethylamine (Carlo–Erba) (0.01 mol) were simultaneously added dropwise a solution of 1,2,4-triazole (Fluka) (0.01 mol) in chloroform and a solution of the appropriate phenacyl bromide (0.01 mol) in chloroform. The reaction mixture was controlled by TLC and stirred on an ice-bath for 4 h, then evaporated under reduced pressure. The residue was dissolved in chloroform and passed through an aluminium oxide column. The eluates were collected after TLC and the solvent removed to afford **4f–j** which were recrystallized from benzene. Yields, melting points, solvents crystallization and analyzed elements have been reported in table I.

Compound **4f**: IR: 1680 (C=O) cm^{-1}. ^{1}H-NMR: δ (ppm) = 5.70 (s, 2H, CH_2); 7.63–8.30 (unr m, 7H, C_6H_5 and triazole).

1-Phenyl-2-(azol-1-yl)ethan-1-ols ***5a–j***

A mixture of the appropriate **4** (0.01 mol), 0.756 g (0.02 mol) $NaBH_4$ (Carlo–Erba) and 30 ml dry ethanol was refluxed for 3 h (**5a–e**) or 5 h (**5f–j**). The reaction was controlled by TLC. After solvent evaporation, the mixture was neutralized with dilute HCl and then refluxed for 30 min. After the mixture had cooled, the solution was alkalinized with NaOH and the precipitate collected and crystallized. Yields, melting points, crystallization solvents and elements analyzed have been reported in table II.

Table XI. Effect of **5e** (50 and 100 mg/kg ip) and phenobarbital sodium (50 mg/kg ip) on MES (supramaximal electroshock seizure) tonic seizures in mice.

Treatment	*Tonic seizure duration (s) Minutes after treatment*					*Lethality (%)*
	0	*+ 25'*	*+ 60'*	*+ 90'*	*+ 150'*	
Vehicle	20.44 ± 1.56	21.67 ± 0.96	21.67 ± 0.90	22.44 ± 0.90	22.90 ± 1.19	10
5e (50 mg/kg)	19.12 ± 1.76	16.75 ± 2.76	18.25 ± 0.84	18.87 ± 0.95	21.37 ± 1.82	20
5e (100 mg/kg)	18.80 ± 0.96	12.60 ± 2.84*,**	11.50 ± 3.16*,**	16.20 ± 2.87	19.70 ± 2.70	0
Phenobarbital	19.40 ± 1.91	0.00 ± 0.00*,**	0.11 ± 0.11*,**	0.00 ± 0.00*,**	0.00** ± 0.00*	0

*$p < 0.01$ *vs* t_0; **$p < 0.01$ *vs* vehicle.

Table XII. Effect of **5e** (100, 200, 300 mg/kg *po*) on MES (supramaximal electroshock seizure) tonic seizures in mice.

Treatment	*Tonic seizure duration (s) Minutes after treatment*				*Lethality (%)*
	0	*+ 30'*	*+ 60'*	*+ 120'*	
Vehicle	18.25 ± 0.65	19.81 ± 0.65	20.87 ± 0.75	21.37 ± 0.76	20
5e (100 mg/kg)	17.60 ± 0.56	14.90 ± 3.39*	20.20 ± 1.11	21.90 ± 0.98	0
5e (200 mg/kg)	17.11 ± 0.90	5.56 ± 2.84**,***	5.22 ± 2.70**,***	15.44 ± 2.01	10
5e (300 mg/kg)	17.90 ± 0.77	6.00 ± 3.18**,***	0.00 ± 0.00**,***	9.60 ± 3.26**,**	0

*$p < 0.05$ *vs* t_0; **$p < 0.01$ *vs* t_0; ***$p < 0.01$ *vs* control.

Table XIII. Effect of **5e** (100 mg/kg ip and 200 mg/kg *po*) and phenobarbital sodium (50 mg/kg ip) on 'psycomotor' electroshock in mice.

Treatment		*Administration route*	*Duration of 'stunning' behaviour Minutes after treatment*		
			0	*+ 30'*	*+ 60'*
Vehicle		ip	21.60 ± 3.44	24.20 ± 2.80	24.30 ± 2.60
5e	(100 mg/kg)	ip	21.80 ± 4.34	20.00 ± 2.03	18.60 ± 1.67
	(200 mg/kg)	*po*	19.10 ± 2.36	14.60 ± 1.75*	17.50 ± 1.38
Phenobarbital	(50 mg/kg)	ip	24.90 ± 3.27	0.80 ± 0.80**,***	0.00 ± 0.00**,***

*$p < 0.05$ *vs* vehicle; **$p < 0.01$ *vs* vehicle; ***$p < 0.01$ *vs* t_0.

Table XIV. Effect of **5e** and phenobarbital sodium treatment on pentylenetetrazol (PTZ) lethality in mice.

Treatment	*Dose (mg/kg)*	*Administration route*	*% Lethality after PTZ*		
			120 mg/kg	*100 mg/kg*	*80 mg/kg*
Vehicle		*po*	100	100	25
5e	100	*po*	100	(*)	50
5e	200	*po*	100	100	50
Vehicle		ip	100	100	50
5e	50	ip	100	(*)	60
5e	100	ip	100	100	60
Phenobarbital	50	ip	0	0	0

*Non-tested doses.

Table XV. Effect of **5e** and phenobarbital sodium treatment on strychnine lethality (1 mg/kg ip) in mice.

Treatment	*Dose (mg/kg)*	*Administration route*	*% Lethality after strychnine*
Vehicle		*po*	30
5e	100	*po*	100
5e	200	*po*	100
Vehicle		ip	40
5e	50	ip	100
5e	100	ip	100
Phenobarbital	50	ip	0

Compound **5a**: IR: 3280 (OH) cm^{-1}. ^{1}H-NMR: δ (ppm) = 4.10 (unr m, 2H, CH_2); 4.83 (br, 1H, CH); 5.76 (br, 1H, OH); 6.83–7.56 (m, 8H, C_6H_5 and imidazole).

Compound **5f**: IR: 3280 (OH) cm^{-1}. ^{1}H-NMR: δ (ppm) = 4.63 (d, 2H, CH_2, J = 6 cps); 5.27 (br, 1H, CH); 6.03 (br, 1H, OH); 7.66–8.73 (unr m, 7H, C_6H_5 and triazole).

1-Phenyl-2-(azol-1-yl)ethan-1-oximes ***6a–j***

To a hot solution of the appropriate **5** (0.01 mol) in 50 ml ethanol was added dropwise a hot solution of 1.0 g (0.015 mol) hydroxylamine hydrochloride and 1.6 g (0.015 mol) dry Na_2CO_3 in 20 ml water. The mixture was refluxed for 4 h. After the mixture had cooled the precipitate obtained was collected, washed with water, dried and crystallized from a suitable solvent. Yields, melting points, crystallization solvents and elements analyzed have been reported in table III.

Compound **6a**: ^{1}H-NMR: δ (ppm) = 5.40 (s, 2H, CH_2); 6.90–7.73 (unr m, 8H, C_6H_5 and imidazole); 12.22 (br, 1H, OH).

Compound **6f**: ^{1}H-NMR: δ (ppm) = 5.66 (m, 2H, CH_2); 7.66–8.76 (unr m, 7H, C_6H_5 and triazole); 12.18 (br, 1H, OH).

The enantiomer mixtures of **5a–j** were analyzed by chiral HPLC as reported by Okamoto [20]; data have been summarized in table IV. Chiracel OD was used as chiral stationary phase based on cellulose tris (3,5-dimethylphenylcarbamate) coated on silica gel. Hexane/IPA (isopropylalcohol) (Carlo–Erba) (75:25% v/v) were used as eluents.

K_1' is the capacity factor of the first eluted enantiomer, defined as $(t_1-t_0)/t_0$ where t_1 and t_0 are the retention times of the first eluted enantiomer and of a non-retained solute, respectively. K_2' is the capacity factor of the second eluted enantiomer, defined as $(t_2-t_0)/t_0$ where t_2 and t_0 are the retention times of the second eluted enantiomer and of a non-retained solute, respectively. α is the enantioselectivity factor, defined as the ratio of the capacity factors of the 2 enantiomers (K_2'/K_1').

Pharmacological section

Microbiology

Derivatives **4a–j**, **5a–j**, **6a–j** were tested for *in vitro* antifungal activity against *Candida albicans* and various strains of *Candida* sp. Antibacterial activity against Gram-positive and Gram-negative bacteria was also investigated. Miconazole, fluconazole, pipemidic acid and minocycline were used as positive controls for antifungal and antibacterial activities.

Minimum inhibitory concentration (MIC) was determined using the method of progressive double dilution in solid media [21]. Data were recorded after 36 h (fungi) or 18 h (bacteria); incubation was performed at 37°C. The substances were dissolved in DMSO (10 mg/ml); further dilution in the test medium furnished the required concentration, generally in the range of 0.2–200 μg/ml. The cultures were obtained on Sabouraud (BBL) for fungi and BHI (BBL) for bacteria after 18 h incubation at 37°C. Tests were carried out using Sabouraud agar (BBL) with 70 μg/ml sodium dodecyl sulfate (SDS) and Muller–Hinton agar (BBL) with 70 μg/ml SDS; inocula were 10^7 for bacteria and 10^8 for *Candida*. Media MIC value ($n\bar{X}$) and R% were calculated as previously reported [7]. The following species of fungi and bacteria isolated from various clinical specimens were tested: 38 *Candida albicans*, 6 *Candida* sp (1 *C glabrata*, 1 *C tropicalis*, 1 *C guillermondii*, 1 *C krusei*, 1 *C parapsilosis*, 1 *C lipolytica*); 2 strains of *Staphylococcus haemoliticus*, 1 of *S aureus*, 1 of *S mitis*, 1 of *S pyogenes*, 2 of *Streptococcus* sp, 6 of *Escherichia coli*, 1 of *Klebsiella* sp, 3 of *Pseudomonas* sp, 1 of *Yersinia enterocolitica*, 1 of *Serratia odoriphera*, 2 of *Proteus mirabilis*.

The compounds **4a–j**, **5a–j** and **6a–j** were also tested, as reported by Simonetti [22], for their inhibitory effect on pseudomycelium formation by *Candida albicans* cultured in Eagle's minimal essential medium (EMEM, Flow Laboratories). The compounds were tested in the range 0.030–20 μg/ml and minimal effective concentration (MEC, μg/ml) was defined as the lowest concentration of compound that prevented typical pseudomycelium formation. Inocula were 10^5 and fluconazole was used as positive control. The evaluation of the inhibitory activity one mycelial radial growth

of pathogenic plant fungi isolates was carried out as reported by Massolini [23]. *Drechslera graminea* (Rabenh ex Schlecht) Shoem and *Phomopsis* sp isolates were used for this assay. The isolates used were supplied by A Porta-Puglia of the Istituto Sperimentale per la Patologia Vegetale, Rome. Imazalil sulfate (Janssen code N 009934), enilconazole (Janssen code N 024336), **5a–j** and **6a–j** were dissolved in DMSO (5 mg/ml); further dilution in the test medium furnished the required concentration in the range 6.25–100 μg/ml.

The cultures were obtained on potato dextrose agar (Oxoid) at pH = 5.6. Data were recorded after 72 h at 22°C. The activity of the compounds was estimated on the basis of percentage of growth inhibition by comparing the diameter of the zone of mycelial growth with that on the reference control with DMSO.

Anticonvulsive activity

Male albino Swiss mice (CD-1, Charles River) weighing from 18 to 25 g received food and water *ad libitum* except during the experiments and in the case of oral drug administration when food was withdrawn 12 h beforehand. All drugs were dissolved in saline, except for **5e** which was suspended in methylcellulose 0.5% in saline.

Acute toxicity

The acute toxicity of a single administration of **5e** *via* different routes (oral and ip) was studied in adult mice [24]. Group number was 8 animals/dose. Mortality was observed for 7 d. Behaviour and acute toxicity symptoms were recorded 30 min after drug administration according to the procedure of Irwin. The tests employed for evaluation of anticonvulsant activity were: A) the supramaximal electroshock (MES) seizure pattern test; B) the 'psychomotor' electroshock test; C) the maximal pentylenetetrazol (PTZ) seizure pattern test; and D) the strychnine lethality test.

Supramaximal electroshock

Mice (10 for each dose level) were stimulated through corneal electrodes with 60 Hz alternating current, 100 V amplitude, 25 mA, for 0.2 s prior to and at different times after ip or *po* administration of drugs. In control animals, the resulting seizure showed a tonic phase of limb flexion of ≈ 2.5 s, followed by full tonic extension of ≈ 15 s followed by a few clonic jerks. For testing purposes, only the duration of tonic extensor seizures and the incidence of post-tonic asphyxial deaths were considered. Abolition of the tonic phase of seizures indicated protective activity [25–27]. Drugs with a good protective index in this test are considered effective against tonic-clonic seizures.

'Psychomotor' electroshock

Mice (10 for each dose level) were exposed to shock at a low frequency of 6 Hz, with 1-ms pulses at 60 V for a total 5 s [26, 27]. The resulting seizure took the form of a severe clonus, involving loss of righting reflex, or clonus with maintenance of upright posture, or 'stunned' behaviour, in which the animal remained upright but stationary for some s before renewing its normal locomotion and exploratory behaviour. The end-point for drug protection was the ability of the animal to walk away within 10 s after delivery of the shock. Drugs with a good protective index in this test are effective against psychomotor epilepsy as well as against *petit mal*.

Maximal PTZ seizures

Mice (10 for each dose level) were administered sc 80, 100 and 120 mg/kg PTZ. Within 30 min, drug administration resulted in myoclonic jerks, clonic seizures, tonic seizures and death in a dose-dependent percentage. Drugs with a good protective index in this test are effective against *petit mal* [26, 27].

Strychnine lethality

In the strychnine test, ip doses of strychnine nitrate were administered to produce 100% lethality (1.5 mg/kg) or 30% lethality (1 mg/kg) in order to evaluate the capacity of **5e** to prolong life beyond the usual time of death or to increase mortality from a low dose of strychnine [26, 27]. Strychnine seizures are notably resistant to typical anticonvulsants. In contrast, typical sedative barbiturates provide a good protection. Obtained results have been presented as mean ± SE or as percentages with a split-plot design.

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