Synthesis and Biological Activities of Some 3,6-Disubstituted Thiazolo[3,2-*b*][1,2,4]triazoles

DILEK DEMIR EROL*x, ÜNSAL ÇALIŞ*, RÜMEYSA DEMİRDAMAR‡, NURAN YULUĞ§, AND MEVLÜT ERTAN*

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Abstract □ Some new 2,3-dihydro-3-hydroxy-6-phenyl-3-(4-substituted-(phenylthiazolo[3,2-*b*][1,2,4]triazole derivatives were synthesized as antifungal agents. After their structures were confirmed by microanalysis and IR and NMR spectral analysis, their antifungal activities against *Candida albicans*, *Candida parapsilosis*, *Candida stellatoidea*, and *Candida pseudotropicalis* were investigated. Contrary to our expectations, all proved to have poor antifungal activities. Because 2,4-dihydro-3*H*-1,2,4-triazol-3-ones are a new class of anticonvulsant agents, a series of thiazolo[3,2-*b*][1,2,4]triazoles was evaluated for anticonvulsant activity and observed as potential anticonvulsant candidates. All compounds examined exhibited activity against both maximal electroshock and pentylene tetrazole-induced seizures in mice.

Most fungi are completely resistant to the action antimicrobial drugs. Only a few substances have been discovered that exert an inhibitory effect on pathogenic fungi for humans, and most of these are relatively toxic. Fungi are biochemically similar to the human host. This similarity causes a major difficulty in the development of a drug that is effective against an invading fungus but safe for the host. It is well known that there is a great need for effective and safer antifungal drugs.

The azole antifungals appear to offer the greatest scope for providing a truly effective drug. Some synthetic antifungal azole derivatives inhibit fungi by blocking the biosynthesis of certain fungal lipids, especially ergosterol in cell membranes, and by additional mechanisms. The imidazole antifungals, such as clotrimazole, miconazole, and ketoconazole, showed good topical activity, but were only of limited value for systematic administration.[1-9] Triazole derivatives are the second major chemical group of antifungal azole derivatives. They possess a broad spectrum of antifungal activity and reduced toxicity compared with the imidazole antifungals. The two leading triazole candidates under development are fluconazole and itraconazole. Both of these agents are active orally and appear to have broader spectra of activity and less toxicity than katoconazole.[10-17]

Therefore, 2,3-dihydro-3-hydroxy-6-phenyl-3-(4-substituted)phenylthiazolo[3,2-*b*][1,2,4]triazoles were synthesized and their antifungal activities were investigated by the tube dilution method.[18-20] However, antifungal activities were not so high as expected.

Because the compounds that have lipophilic aromatic rings and hydrophilic azole rings have anticonvulsant activities[21-23] and because a new class of anticonvulsant drugs has agents with a lipophilic ring and imidazole ring,[24-27] the anticonvulsant activities of the synthesized compounds were investigated by the phase I tests of the Antiepilectic Drug Development Programme (ADD).[28,29]

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Chemistry

For the synthesis of the title compounds, certain 3-substituted/or simple 5-mercapto-1,2,4-triazoles required as starting materials were prepared by the reaction of formyl chloride or benzoyl chloride with thiosemicarbazide.[30] These were converted into 2,3-dihydro-3-hydroxy-6-phenyl-3-(4-substituted phenyl)thiazolo[3,2-*b*][1,2,4]triazoles in one step by reacting 3-substituted-5-mercapto-1,2,4-triazole with *p*-substituted phenacyl bromides in dry ethanol followed by neutralization with 10% K_2CO_3 (Scheme 1).[31,32] The structures of these compounds were confirmed by elemental, IR, and ^{1}H-NMR analyses. Physical data, analytical data, and antifungal and/or anticonvulsant activity are described in Tables 1, 2, and 3 for the target molecules, respectively.

Microbiology

The antifungal activities of the compounds against fungi (*Candida albicans*, *Candida parapsilosis*, *Candida stellatoidea*, *Candida pseudotropicalis*) were determined by the tube dilution method.[18-20] The microorganisms were obtained from the collections of Hacettepe University Medical Faculty, Microbiology Department.

Pharmacology

Maximal electroshock (MES) and pentylene tetrazole-induced seizure (ScMet) tests for anticonvulsant activity and rotorod test for neurotoxicity were performed for all compounds according to the Phase I and Phase II tests of the ADD.[28,29]

Results and Discussion

p-Substituted phenacyl bromides, formyl, or benzoyl chlorides were prepared according to the methods described in literature.[22] 1,2,4-Triazole derivatives were synthesized by *N*-acylation of thiosemicarbazides with the appropriate acyl chlorides in pyridine. Bicyclic 1,2,4-triazole derivatives were synthesized by the reaction of 1,2,4-triazoles with the appropriate phenacyl halides in ethanol (Scheme 1). However, an attempted reaction between the 1,2,4-triazole derivatives and phenacyl bromide in acetic acid resulted in the elimination of water because acid catalysis led to formation of a thiazoline ring instead of the thiazole derivatives (Scheme 1).[33,34]

The assigned structures of the synthesized compounds were in accordance with their spectroscopic and chemical behavior. The IR spectral frequency characteristic of the thiazoline were assigned at 1640 cm^{-1} for C=N.[31-35] This strong band was observed at 1650−1690 cm^{-1}, which is more likely assignable to a fused ring. In addition, one hydroxyl group at the 3-position was quantitatively esterified with acetyl chloride in dry ether in the presence of triethylamine. The structures

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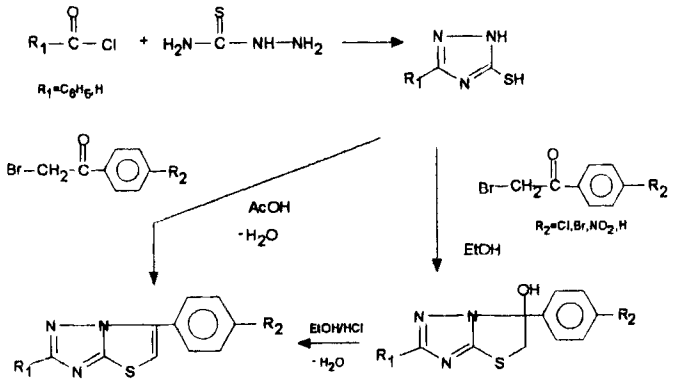
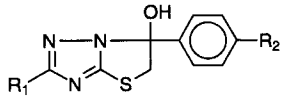


Scheme 1

Table 1—Physical and Analytical Data of the New 2,3-Dihydro-3-hydroxy-6-phenyl-3-(4-substituted phenyl)thiazolo-[3,2-*b*][1,2,4]triazoles



Compound	R_1	R_2	mp, °C[a]	Yield, %[b]	Molecular Formula[c,d]
1	H	H	228	63	$C_{10}H_9N_3OS$
2	H	4-Cl	226	60	$C_{10}H_8ClN_3OS$
3	H	4-Br	235	60	$C_{10}H_8BrN_3OS$
4	H	4-NO_2	234	62	$C_{10}H_8N_4O_3S$
5	C_6H_5-	H	135	73	$C_{16}H_{13}N_3OS$
6	C_6H_5-	4-Cl	210	74	$C_{16}H_{12}ClN_3OS$
7	C_6H_5-	4-Br	225	69	$C_{16}H_{12}BrN_3OS$
8	C_6H_5-	4-NO_2	234	67	$C_{16}H_{12}N_4O_3S$

[a] Melting points were determined on a Thomas Hoover apparatus and were uncorrected. [b] No efforts were made to optimize yields. [c] The C,H,N analysis was performed by Middle East Technical University, Department of Chemistry, Microanalysis Lab., Ankara, Turkey. The results obtained were within ±0.4% of the theoretical values. [d] All compounds were purified by recrystallization from benzene and petroleum ether.

Table 2—Spectral Data of 2,3-Dihydro-3-hydroxy-6-phenyl-3-(4-substituted phenyl)thiazolo[3,2-*b*][1,2,4]triazoles

	IR, cm^{-1} [a]					^1H NMR, ppm[b]		
		Thiazole		Triazole				
Compound	–OH	I	II	I	II	CH_2 (s)	OH (s)	Ar. Ring. (m)
1	3200–2950	1680	1450	1070	1000	4.78	14.01	8.52–7.53
2	3200–2900	1670	1450	1070	1000	4.75	13.98	8.51–7.56
3	3300–2950	1570	1450	1070	1010	4.77	14.02	8.43–7.36
4	3400–2950	1670	1450	1075	1000	4.83	14.04	8.51–8.16
5	3200–2900	1690	1470	1080	1010	4.84	14.45	8.11–7.48
6	3150–2850	1650	1460	1080	1000	4.86	14.38	8.14–7.38
7	3200–2900	1680	1450	1060	1000	4.85	14.41	8.14–7.36
8	3500–3000	1680	1455	1060	1000	4.91	14.44	8.46–7.46

[a] IR spectra were determined on a Perkin Elmer model 457 IR in KBr pellets. [b] ^1H NMR spectra were charted on a Brucker 80 MHz using tetramethylsilane as the internal standard and DMSO-d_6 as solvent; s: singlet, m: multiplet.

of the 6-acetoxy derivatives were determined by the IR spectra that showed two strong bands at 1740 and 1200 cm^{-1}. This was proved by the ^1H NMR spectra of the acetoxy derivatives, which displayed a sharp singlet signal at 2.1 ppm. All of the compounds showed a strong band in their IR spectra at 1450–1470 cm^{-1}, which was assignable to a thiazole II. The spectra of all compounds were endowed with a sharp band with medium intensity at 1080 and 1010 cm^{-1}, which was attributed to 1,2,4-triazole stretching.[31]

Methylene protons in the ^1H NMR spectra of all compounds were observed as a sharp singlet at 4.75–4.91 ppm, most likely attributable to such protons in a dihydrothiazole ring. The multiplet of the aromatic ring protons appeared at ~7.36–811 ppm, and OH protons were characterized as singlets at 13.98–14.45 ppm. All spectra were in accordance with the proposed structure of 3-,6-substitutedthiazole[3,2-*b*]-[1,2,4]triazoles.

Table 3—Minimal Inhibitory Concentration

	MIC, μg/mL			
Compound	*C. albicans*	*C. parapsilosis*	*C. stellatoides*	*C. pseudotropicalis*
1	100	100	6.25	100
2	100	100	100	100
3	100	100	100	100
4	100	100	0.375	100
5	100	100	100	100
6	100	100	100	100
7	100	100	100	100
8	3.12	3.12	1.56	0.75

The tube dilution method was used to determine the antifungal activity of the compounds. Suspensions of several fungi, such as *Candida albicans*, *Candida parapsilosis*, *Candida stellatoidea*, and *Candida pseudotropicalis*, were prepared by inoculating fresh stock cultures into separate broth tubes, each containing 5 mL of Sabouraud Dextrose Broth. The inoculated tubes were incubated at 37 °C for 24 h. This method is based on the principle of growth inhibition after inoculation of the microorganism into media containing different concentrations of the test compounds. Incubation at a suitable temperature for a specific period is a dependable method for determining the minimal inhibitory concentration (MIC).[18–20]

Compounds **1**–**7** were not active against *Candida albicans*, *Candida parapsilosis*, and *Candida pseudotropicalis*. Compounds **1** and **8** showed good activity against *Candida stellatoidea* (MIC, 6.25 and 1.56 μg/mL, respectively). Compound **4** was moderately effective against *Candida stellatoidea* (MIC, 0.375 μg/mL). Compound **8** showed good activity against all the *Candida* species (Table 3).

It is reported that the treatment of fungi by azoles caused demonstrable alterations in the cell wall and cell membrane.[1] Recent results of biomedical studies on the modes of action of the azoles demonstrated that, after contact with an azole, the fungi cells store the active substance for a prolonged period and continue to exhibit alterations in sterol synthesis even after transfer into an azole-free medium.[36]

In the initial Phase I screening for anticonvulsant activity, the compounds were suspended in polyethylene glycol 400 (30%) and administered to mice at 30-, 100-, and 300-mg/kg doses. Three animals per dose were used. Results of the Phase I screening are summarized in Table 4. At the end of 0.5 h, **1** and **5** were active at 30 mg/kg. Compounds **2** and **3** were active at 300 mg/kg in the MES test, **1** and **3** showed neurotoxicity at 300 mg/kg within 0.5 h, **5** was neurotoxic at 300 mg/kg within 4 h. However, only **2**, which was active within 0.5 h at 300 mg/kg MES, was void of any neurotoxicity. Compound **3** was active at 300 mg/kg both in the MES and ScMET, and **5** was active in both tests but at different doses.

In conclusion 3-,6-disubstitutedthiazolo[3,2-*b*][1,2,4]triazole derivatives exhibit activity against both MES and ScMET seizures in mice.

Experimental Section

Chemistry—Melting points were determined on a Thomas Hoover apparatus and are uncorrected. IR spectra were recorded on a Perkin-Elmer 457 spectrophotometer in potassium bromide pellets. The ^1H NMR spectra were determined on Brucker 80 MHz NMR spectrometer

Table 4—Anticonvulsant Screening, Phase I Test Results, and Quantification of 1, 2, 3, and 5 in the MES Test and 3 and 5 in the ScMet Test[a]

		Activity							
		MES							
		ED_{50}			ScMet			Tox	
Compound	Dose, mg/Kg	30 min	(mg/kg)	4 h	30 min	ED_{50}, mg/kg	4 h	30 min	4 h
1	30	(+)	18.8 (15.6–20.0)	(–)	(–)	(–)	(–)	NT	NT
	100	(+)		(–)	(–)	(–)	(–)	NT	NT
	300	(+)		(–)	(–)	(–)	(–)	T	NT
2	30	(–)		(–)	(–)	(–)	(–)	NT	NT
	100	(–)		(–)	(–)	(–)	(–)	NT	NT
	300	(+)	171.1 (144.8–197.4)	(–)	(–)	(–)	(–)	NT	NT
3	30	(–)		(–)	(–)	(–)	(–)	NT	NT
	100	(–)		(–)	(–)	(–)	(–)	NT	NT
	300	(+)	140.0 (120.1–159.9)[a]	(–)	(+)	223.8 (235.8–211.8)[a]	(–)	T	NT
4	30	(–)		(–)	(–)	(–)	(–)	NT	NT
	100	(–)		(–)	(–)	(–)	(–)	NT	NT
	300	(–)		(–)	(–)	(–)	(–)	NT	NT
5	30	(+)	21.25 (19.4–29.1)	(–)	(–)	(–)	(–)	NT	NT
	100	(+)		(–)	(–)	(–)	(–)	NT	NT
	300	(+)		(–)	(+)	155.2 (160–172.4)	(–)	NT	T
6	30	(–)		(–)	(–)	(–)	(–)	NT	NT
	100	(–)		(–)	(–)	(–)	(–)	NT	NT
	300	(–)		(–)	(–)	(–)	(–)	NT	T
7	30	(–)		(–)	(–)	(–)	(–)	NT	NT
	100	(–)		(–)	(–)	(–)	(–)	NT	NT
	300	(–)		(–)	(–)	(–)	(–)	NT	NT
8	30	(–)		(–)	(–)	(–)	(–)	NT	NT
	100	(–)		(–)	(–)	(–)	(–)	NT	NT
	300	(–)		(–)	(–)	(–)	(–)	NT	NT

[a] 0.25 h; (+) active; (–) inactive; Tox: neurologic toxicity (rotorod) test; NT: nontoxic; MES ED_{50} for the prevention of tonic extensor convulsions was calculated according to the method of Litchfield and Wilcoxon *J. Pharmacol. Exp. Ther.* **1949**, *96*, 99–113).

using tetramethylsilane as an internal standard. The purity of all the compounds was verified by thin-layer chromatography.

p-Substituted Phenacyl Bromides—These were prepared according to the method reported earlier.[37]

p-Substituted Benzoyl Chlorides—Formic (0.01 mol) or benzoic acid (0.01 mol) in 15 mL of thionyl chloride was refluxed for 2 h. After cooling, the reaction mixture was distilled under reduced pressure to give the corresponding acid chlorides. Formyl chloride was always kept at −65 °C for not more than 1 h; it was used freshly.

1-Formyl- or 1-Benzoyl-3-thiosemicarbazides—The solution of formyl chloride (0.01 mol) or benzoyl chloride was dissolved in 20 mL of pyridine. Thiosemicarbazide (0.01 mol) was added, and the reaction mixture was kept at 0 °C for 2 h with stirring under nitrogen. The solution was then poured into ice water. The solid was filtered and recrystallized from ethanol.

3-Substituted-5-mercapto-1,2,4-triazoles—1-Formyl- or benzoyl-3-thiosemicarbazide in 10% K_2CO_3 was refluxed for 3 h. Then, the reaction mixture was stirred at room temperature and poured into ice water that was acidified with 2 N HCl. The product was filtered and crystallized from ethanol–water.

General Procedure for Obtaining 2,3-Dihydro-3-hydroxy-6-phenyl-3-(4-substituted)-phenylthiazolo[3,2-b][1,2,4]triazoles—*p*-Substituted phenacyl bromides were added to solution of 3-substituted-5-mercapto-1,2,4-triazoles (0.01 mol) in alcohol (25 mL). The reaction mixture was heated on a steam bath under reflux for 4 h and then was cooled and filtered. After evaporation of the alcohol, the crude product obtained was purified by crystallization. Analytical data are given in Table 1.

Biological Activity—*Microbiology*—All compounds were screened for their antifungal activities against four strains of *Candida*. Sterilized Saboraud Dextrose Broth (DIFCO) was used as media. The substances dissolved in propylene glycol at 1 mg/mL gave solutions that were diluted in broth in the range 100–0.05 μg/mL. Inocula were prepared from well-growing overnight cultures of each test microorganism such that the final inoculum size was ~10^6 cells/mL. Each tube was inoculated with 0.1 mL of inoculum. Incubation was for 18 to 24 h at 37 °C. After this peak, the MIC was taken as the greatest dilution of the compound which exhibited no visible growth. The tube of culture control was seen to be macroscopically cloudy. All experiments were performed in two parallel series.

Pharmacology—A stimulator (Grass S88), constant current unit (Grass SCUIA), and corneal electrode were used. All compounds synthesized were administered intraperitoneally as 30% aqueous PEG 400 suspensions. Male mice (20 ± 3 g) local breed was used. Control animals received 30% aqueous PEG 400. Pentylenetetrazole (85 mg/kg) was administered subcutaneously at the back of neck in a volume of 10 mL/kg of body weight. The rotorod toxicity test was performed on a 1-inch diameter knurled plastic rod, at 6 rpm. Results and quantification of the compounds are given in Table 4.

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