SYNTHESIS AND ANTI-INFLAMMATORY ACTIVITY OF ETHYNYLTHIAZOLES

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A series of acetylene derivatives of thiazole using the Sonogashira cross-coupling method was synthesized and evaluated in vivo for their anti-inflammatory activity. Four compounds exhibited good anti-inflammatory activity and two inhibited soybean lipoxygenase.

Keywords: ethynylthiazoles, lipoxygenase (LOX), anti-inflammatory.

Inflammation is a complex phenomenon, involving the interrelationship between humoral and cellular reactions through a number of inflammatory mediators. A substantial body of evidence suggests the involvement of the products of the arachidonic acid metabolic pathway as mediators of a variety of cellular functions [1]. Excessive production of arachidonic acid metabolites has been shown to be important in the pathophysiology of inflammation and thrombosis [2]. Nonsteroidal anti-inflammatory drugs (NSAIDs) are of huge therapeutic benefit in the treatment of inflammatory diseases. Recently the synthesis and biological activity of substituted functional ethynylarenes and -hetarenes have been reported as a rapidly developing field of bioorganic chemistry [3]. It is also well established that the thiazolyl group is of great importance in biological systems [4-11]. Several thiazolyl derivatives were found to possess anti-inflammatory activity [4, 12]. Recently some acetylene derivatives of thiazole were reported as potent and highly selective metabotropic glutamate subtype 5 receptor antagonists with anxiolytic activity [13].

Computer analysis of structure-activity relationships and molecular modelling are widely used in developing new leads by pharmaceutical chemists [14]. The majority of currently available molecular modelling methods are designed to study the ligand-receptor interaction for one particular biological target at a time, while quantitative structure-activity relationship (QSAR) analysis is mostly applicable to the optimization of properties of the lead compounds within the same chemical series. In contrast to these methods, computer software PASS (Prediction of Activity Spectra for Substances) predicts simultaneously several hundred types of biological activity for drug-like substances from different chemical classes on the basis of their structural formulas [15-17]. It estimates probabilities not only for the desirable pharmacological effect but also for molecular mechanisms of action and different undesirable and side effects. Such analysis of heterogeneous sets increases considerably the chance to discover new chemical entities (NCEs).

The latest version of PASS 1.917 (July, 2005) predicts about 2,000 kinds of biological activity with a mean prediction accuracy of about 87%. The list of predictable biological activities includes main and side pharmacological effects (e.g., antihypertensive, hepatoprotective, sedative, etc.), mechanisms of action

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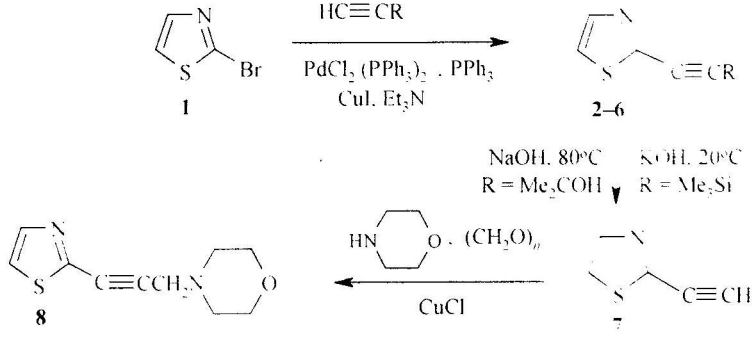
(5-hydroxytryptamine agonist, acetylcholinesterase inhibitor, adenosine uptake inhibitor, etc.), and specific toxicities (mutagenicity, carcinogenicity, teratogenicity, etc.).

The current PASS training set includes 57978 substances. It is a complex knowledge base, containing vocabularies of MNA (Multilevel Neighborhoods of Atoms), descriptors and activity names, the database for the substance structures presented by MNA descriptors, their biological activity types, and data on the structure–activity relationships.

PASS presents the biological activity spectra for each substance as a result of prediction. This is a list of biological activity types for which the calculated probability to be present (**Pa**) is greater than the calculated probability not to be present (**Pi**). Taking into account that some substances from the training set are formally considered inactive, the estimated value of **Pa** is more reliable. However, even in this case there are some other factors that significantly influence its absolute value: the number and diversity of substances revealing such activity in the training set, recall ratio, etc. In general, the higher the **Pa** value, the higher is the probability for a studied substance to be structurally similar to the known biologically active substances from the training set. The result of prediction is valuable in planning the experiment, but one should take into account some additional factors: a particular interest in some kinds of activity, desirable novelty of a substance, available facilities for experimental testing, etc. Actually, each choice is always a compromise between the desirable novelty of the studied substance and the risk to obtain a negative result in testing.

Taking into consideration the above mentioned and using PASS, we designed and synthesized compounds in which the acetylenic triple bond as well as the thiazole ring were both involved in the same molecule. Current investigation is targeted towards the development of a synthetic method for the preparation of new ethynylthiazoles and the biological evaluation and correlation of their structure with biological activities.



2 R = Ph, **3** R = CH_2OH, **4** R = Me_2COH, **5** R = Me_3Si, **6** R = α-Py

TABLE 1. Physicochemical Data and MS Spectra of Compounds **2-8**

Compound	Empirical formula	R_f*	Reaction time, h	MS, *m/z*	Yield, %
2	$C_{11}H_7NS$	0.90	4	185	82
3	C_6H_5NOS	0.50	5	139	65
4	C_8H_9NOS	0.46	5	167	62
5	$C_8H_{13}NSSi$	0.82	4	181	75
6	$C_{10}H_6N_2S$	0.57	3	186	53
7	C_5H_3NS	0.76	2	109	70
8	$C_{10}H_{12}N_2OS$	0.49	1	208	85

* Benzene–petroleum ether, 5:1 (compound **2**), chloroform–ethylacetate, 3:1 (compounds **3-5, 7**), chloroform–ethylacetate, 4:1 (compounds **6, 8**).

The Sonogashira Pd–Cu-catalyzed cross-coupling [18] of 2-bromothiazole with terminal acetylenes (Scheme 1) was used to prepare the substituted functional ethynyl derivatives of thiazoles (Table 1). The yields of the synthesized compounds are 62-85%.

Compounds **2-4** and **6-8** were characterized by IR, MS, and ^{1}H NMR spectroscopic data (Tables 1 and 2).

The *in vivo* anti-inflammatory effects of the tested compounds were assessed by using the functional model of carrageenin-induced mice paw edema and are presented in Table 3 as percentage of weight increase in the right hind paw in comparison to the uninjected left hind paw. Carrageenin-induced edema is a nonspecific inflammation resulting from a complex of diverse mediators [19]. All the investigated compounds protected against carrageenin-induced paw edema. The protection ranged from 37 to 80%, while the reference drug indomethacin induced 44% protection at an equivalent concentration. Compound **6** was the most potent followed by compounds **4** and **2**.

Lipophilicity is an important physicochemical parameter for the kinetics of biologically active compounds. In our case lipophilicity seems to affect the biological activities of the tested compounds, with the exception of compound **3**, which, although it presents the lowest clog P value, is highly potent (73%). Representative compounds **2** and **6** were tested against soybean lipoxygenase in vitro in order to delineate the

TABLE 2. ^{1}H NMR Spectra of Synthesized Compounds

Compound	δ, ppm (*J*, Hz)
2	7.54-7.55 (1H, d, *J* = 2, H-4 thiazole), 7.23-7.24 (1H, d, *J* = 2, H-5 thiazole); 7.31-7.35 (5H, m, C$_6$H$_5$)
3	7.56-7.60 (1H, d, *J* = 6, H-4 thiazole), 7.25-7.28 (1H, d, *J* = 6, H-5 thiazole); 4.5 (2H, s, CH$_2$), 2.4 (1H, s, OH)
4	7.77-7.82 (1H, d, *J* = 10, H-4 thiazole), 7.50-7.57 (1H, d, *J* = 10, H-5 thiazole); 5.4 (1H, br, OH), 1.40-1.75 (6H, m, CH$_3$)
6	8.65-8.68 (1H, d, *J* = 6, α-H pyridine), 7.89-7.93 (1H, d, *J* = 6, H-4 thiazole); 7.69-7.76 (1H, m, γ-H pyridine), 7.60-7.69 (1H, d, *J* = 7, β'-H pyridine); 7.42-7.47 (1H, d, *J* = 6, H-5 thiazole); 7.28-7.36 (1H, m, β-H pyridine)
7	7.20-7.29 (1H, d, *J* = 8, H-4 thiazole), 7.15-719 (1H, d, *J* = 8, H-5 thiazole); 2.4 (1H, s, C≡CH)
8	7.78-7.82 (1H, d, *J* = 8, H-4 thiazole), 7.33-7.38 (1H, d, *J* = 8, H-5 thiazole); 3.40-3.82 (10H, m, morpholine and CH$_2$)

TABLE 3. Calculated Lipophilicity and Biological Evaluation of Compounds **2-4, 6-8***

Compound	Clog *P*	Inhibition, %		
		CPE	LOX (0.1 mM)	LOX (1 mM)
2	3.12	67	98.7	100
3	-0.70	73	Nt	Nt
4	0.01	58	Nt	Nt
6	1.63	80	80	100
7	0.76	37	Nt	Nt
8	0.64	38	Nt	Nt

* CPE – carrageenin paw edema; LOX – lipoxygenase; Clog *P* – calculated lipophilicity.

TABLE 4. Prediction of Spectrum of Biological Activities of 2-Ethynylthiazoles

Compound	Predicted activities	Pa
2	5-Lipoxygenase inhibitor	0.561
4	5-Lipoxygenase inhibitor	0.820
6	5-Lipoxygenase inhibitor	0.661

role of the ring nature in the biological activity. Both compounds present significantly high inhibition. The phenyl ring (compound **2**) correlates with higher activity as compared with the inhibition induced by the pyridine analogue **6**. These results occur in parallel to the lipophilicity values.

For compounds **2** and **6** the values predicted for biological activity using the PASS program showed satisfactory inhibitory activity against lipoxygenase and significant antagonistic activity against leukotrienes (Table 4). The results concerning prediction of lipoxygenase inhibitory activity coincide with the experimental findings.

Thus, ethynylthiazoles could be used for the further synthesis of new biologically active compounds.

EXPERIMENTAL

Melting points were obtained with a MELTEMP II capillary apparatus (LAB Devices Holliston, MA, USA) without correction. Infrared spectra (nujol mulls) were carried out on a Perkin Elmer 597 spectrophotometer, UV-Vis Hitachi U-2001. ^{1}H NMR spectra were obtained on a Brucker AW 200 (200 MHz) apparatus and were reported downfield from TMS. Mass spectra were determined on a VG-250 instrument (VG Labs Tritech, England) with ionization energy maintained at 70 eV. The reactions were monitored by TLC on silica gel 60 F_{254} (Merck). All reagents were obtained from commercial sources.

Synthesis of 2-Ethynyl-1,3-thiazoles 2-6 (General Procedure). Compounds **2–6** were synthesized according to the scheme by refluxing a mixture of 2-bromo-1,3-thiazole (0.03 mol) and the corresponding terminal acetylene (0.035 mol) in the presence of $PdCl_2(PPh_3)_2$ (120 mg), triphenylphosphine (60 mg) and CuI (60 mg), triethylamine (0.04 mol), and benzene at 30-80°C for 3-5 h under argon with stirring.

Compound 3. Mp 119-121°C (filtration from column with Al_2O_3 and washing with benzene). IR spectrum, ν, cm^{-1}: 2235 (C≡C).

2-Methyl-4-(1,3-thiazol-2-yl)butyn-2-ol (4). Bis(triphenylphospine)palladium(II) chloride (160 mg), copper(I) iodide (80 mg), and triphenylphosphine (80 mg) were added successively to 2-bromothiazole (9.85 g, 0.06 mol) and then 2-methylbut-3-yn-2-ol (5.5g, 0.066 mol) in benzene (100 ml) and Et_3N (11 ml, 0.08 mol) under nitrogen at room temperature. The mixture was stirred under nitrogen for 4 h at 80°C. After cooling, the mixture was filtered off, and the filtrate was decolorized through silica gel (3×3 cm). Evaporation of the filtrate, addition of benzene, and recrystallization from benzene gave 6.2 g (62%) of compound **4** as sandy crystals; mp 119-120°C (petroleum ether). IR spectrum, ν, cm^{-1}: 2175 (C≡C), 3340 (OH).

Compound 6. Mp 52-54°C (benzene–petroleum ether). IR spectrum, ν, cm^{-1}: 2277 (C≡C).

Compound 7 was synthesized from 2-methyl-4-(1,3-thiazol-2-yl)-3-butyn-2-ol **4** (0.04 mol) by heating with sodium hydroxide (0.035 mol) in dry benzene under argon for 2 h. After cooling, the reaction mixture was filtered off through Al_2O_3 (3 × 3 cm) and solvent was removed to give a brown powder. The product was purified by column chromatography using SiO_2 (12 × 1.5 cm) and petroleum ether (50 ml) as eluent followed by a mixture of petroleum ether–benzene, 1:1 (50 ml). TLC of the last fraction showed one spot.

As an alternative method for preparation of compound **7** we used the treatment of 2-(trimethylsilylethynyl)-1,3-thiazole (**5**) at 20°C in the presence of KOH in methanol during 1 h. Methanol (25 ml) and

1N KOH solution (20 ml) was added to compound **5** (5.2 g, 0.37 mol). The mixture was stirred at room temperature for 1 h. MeOH was removed under vacuum, and the water layer was extracted by ether (3 times) and dried over Na_2SO_4. IR spectrum, ν, cm^{-1}: 2106 (C≡C), 3283 C≡CH).

Compound 8 was prepared from 2-(1-ethynyl)-1,3-thiazole **7** (0.25 mmol), CuCl (50 mg), paraformaldehyde (0.02 g), and morpholine (0.05 mol) in dioxane by heating at 65°C for 4 h under argon. After cooling, the mixture was filtered off through silica gel (2 × 2 cm). Compound **8** was purified by recrystallization; mp 154-156°C (from Al_2O_3 column, eluent petroleum ether), IR spectrum, ν, cm^{-1}: 2235 (C≡C).

BIOLOGICAL EVALUATION

Animals. AKR or A mice (20-30 g, groups of ten, 2-3 months old), both male and female were used. Pregnant females were excluded. The animals, bred in our laboratory, were housed under standard conditions and received a diet of commercial food pellets and water *ad libitum*. Four hours before carrageenin treatment, food and water were withdrawn from all animals.

Inhibition of Carrageenin-induced Edema [12]. Edema was induced in the right hind paw of AKR or A mice (20-30 g, 2-3 months old) by an intradermal injection of 0.05 ml 2% carrageenin in water. These studies were in accordance with recognized guidelines on animal experimentation (Guidelines for the care and use of laboratory animals published by the Greek Government 160/1991, based on EU regulations 86/609).

The tested compounds of 0.1 mmol kg body weight were suspended in water with a few drops of Tween-80 and ground in a mortar before use and were given intraperitoneally (ip) at the same time as carrageenin. The animals were euthanized 3.5 h after carrageenin injection. The experiment was repeated twice for each compound (two groups of 6 animals). The difference between the weight of the injected and uninjected paws was calculated for each animal. The change in paw weight was compared with that in control animals (injected with water) and expressed as percent inhibition of the edema (CPE % values, Table 3). Indomethacin in 0.1 mmol/kg was administered as a standard comparative drug (44% inhibition).

Inhibition of the Soybean Lipoxygenase [20]. The tested compounds dissolved in DMSO (final concentration 0.1 and 1mM) were incubated at room temperature with sodium linoleate (0.1 mM) and 0.2 ml of enzyme solution ($1/3 \times 10^{-4}$ w/v in saline). The conversion of sodium linoleate to 13-hydroperoxylinoleic acid was recorded at 234 nm and compared with nordihydroguaretic acid ((NDGA) = 94.4% at 0.1 mM), being an appropriate standard inhibitor. The results are summarized in Table 3.

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