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To the 80th Anniversary of B.I. Ionin

Synthesis, Steric Structure, and Biological Activity of 5-Methyl-2-(morpholin-4-ylamino)-5,6-dihydro-4*H*-1,3-thiazin-4-one

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Abstract—New 5,6-dihydro-1,3-thiazin-4-one derivatives have been synthesized by one-pot reaction of morpholin-4-amine and adamantan-1-amine with methacryloyl isothiocyanate. Structure of 5-methyl-2-(morpholin-4-ylamino)-5,6-dihydro-4*H*-1,3-thiazin-4-one has been determined by X-ray analysis, and its high antiradical and anti-inflammatory activity has been revealed.

Keywords: morpholin-4-amine, adamantan-1-amine, methacryloyl isothiocyanate, 5,6-dihydro-1,3-thiazin-4-one, intramolecular cyclization, antiradical activity, anti-inflammatory activity

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Number of publications on synthesis of 1,3-thiazine derivatives has recently grown due to high biological activity of these compounds, in particular antibacterial activity [1–3].

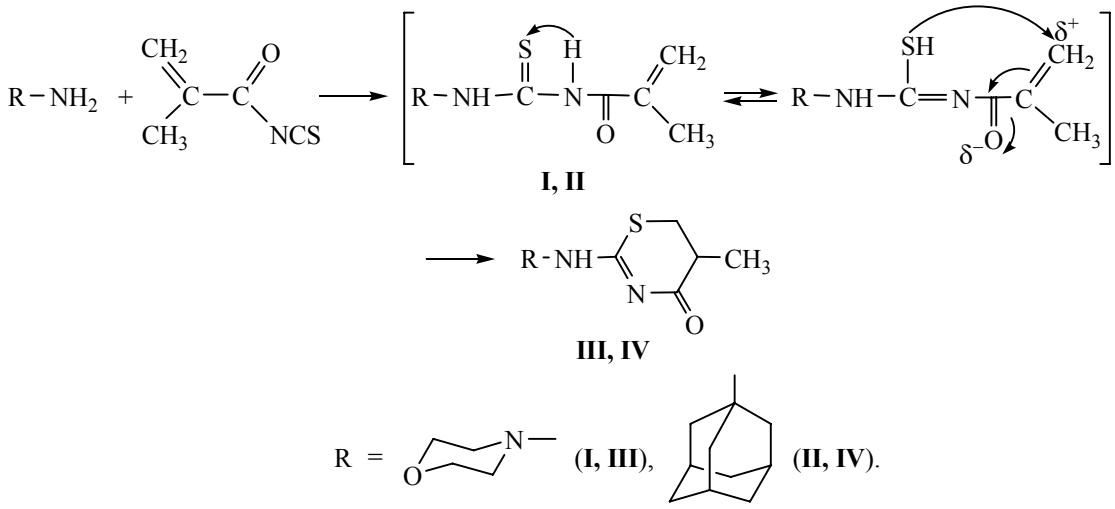
It is known that thiourea and thiosemicarbazide derivatives are convenient building blocks for preparation of sulfur-containing heterocycles [4, 5]. Reaction of isonicotinic acid hydrazide with methacryloyl isothiocyanate followed by cyclization of the intermediate thiosemicarbazide derivative has afforded substituted 5,6-dihydro-1,3-thiazin-4-one [6], a promising antitubercular drug. We have demon-strated that cyclization of intermediate *N*′-(metha-cryloylcarbamothioyl)pyridine-4-carbohydrazide into 5,6-dihydro-1,3-thiazin-4-one occurs at room temperature in propan-2-ol solution within about 1 h in the presence of certain secondary amines, such as morpholine and piperidine [7]. Another example of a related reaction is a fairly easy conversion of alkaloid anabasine into the corresponding 1,3-thiazine derivative under the action of methacryloyl isothio-cyanate [8]; preliminary assay of the product using PASS (Prediction of Activity Spectra for Substances) has revealed a high probability for its nootropic activity (P_a = 94.4%).

Extending our studies on synthesis of new 1,3-thiazine derivatives, in the present work we have examined reactions of morpholin-4-amine and adamantan-1-amine with methacryloyl isothiocyanate generated *in situ* via heating methacryloyl chloride with potassium thiocyanate in acetone. As reported earlier, the reaction involved intramolecular heterocyclization of intermediate methacrylamide derivatives **I** and **II** into the corresponding 5,6-dihydro-1,3-thiazin-4-ones **III** and **IV** [8].

The reactions were carried out under mild conditions at 25–30°C in acetone–propan-2-ol medium. The yield and purity of the products depended on the rate and order of addition of the reactants. The best yields of **III** and **IV** (60–72%) were obtained via slow addition of a freshly prepared solution of methacryloyl isothiocyanate in acetone to a solution of morpholin-4-amine or adamantan-1-amine in propan-2-ol under vigorous stirring.

If the order of addition of the reactants was reversed, a small amount of heterocyclization product **III** or **IV** was detected in the reaction mixture by thin-layer chromatography, its concentration increasing with the reaction time due to gradual intramolecular

Scheme 1.



cyclization of intermediate **I** or **II**, correspondingly. In the case of morpholin-4-amine reaction, additional amount of product **III** was separated off the mother liquor some time after isolation of the first portion of the product (40%). 1,3-Thiazine derivatives **III** and **IV** isolated via double recrystallization were white crystalline substances soluble in most organic solvents, excluding saturated hydrocarbons (Scheme 1).

Presumably, cyclization of intermediate compounds **I** and **II** into 5,6-dihydro-1,3-thiazin-4-ones **III** and **IV** involved intramolecular nucleophilic attack of the thiol tautomer sulfur atom at the electron-deficient carbon atom of the C=C bond. The fast formation of 5,6-dihydro-1,3-thiazin-4-ones **III** and **IV** upon addition of methacryloyl isothiocyanate to morpholin-4-amine and especially to adamantan-1-amine was likely promoted by excess of the amine present in the reaction mixture (it shifted the tautomeric equilibrium of **I** and **II** towards the corresponding thiol tautomer, the form undergoing the intramolecular cyclization).

The cyclic structure of **III** and **IV** was unambiguously confirmed by the absence of $=CH_2$ signals in the ^1H NMR spectra. The corresponding signals in the spectra of the methacrylic derivatives are represented by two doublets at δ 5.70 and 6.00 ppm. Furthermore, there was no amide NH proton singlet typical of the thione tautomer of **I** or **II**, whereas the methyl proton signal was split due to coupling with 5-H in the thiazine ring. The 5-H signal appeared as multiplet, and methylene protons at C^6 resonated as a pair of doublets of doublets. Those findings confirmed the formation of compounds **III** and **IV**.

The spatial structure of morpholine derivative **III** was elucidated by X-ray diffraction analysis (see figure). The bond lengths (Table 1) and bond angles (Table 2) in molecule **III** were close to the corresponding reference values [9]. According to X-ray diffraction data, the 1,3-thiazine ring ($S^1C^2N^3C^4C^5C^6$) adopted a strongly distorted *half-chair* conformation ($\Delta C_2^{5,6}$ = 4.7°). The methyl group at C^5 was oriented equatorially. Similar conformation was observed in the case of 2-phenyl-5,6-dihydro-1,3-thiazin-4-one [10]. The morpholine ring had an almost ideal *chair* conformation (ΔC_N^8 = 0.3°; the internal torsion angles are given in Table 3).

Compounds **III** and **IV** were tested for antiradical activity towards 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid radical cation $ABTS^{+\cdot}$). To do so, we compared the rates of $ABTS^+$ quenching with compounds **III** and **IV**; a semisynthetic water-soluble vitamin E analog, (±)-6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), was used as reference. The antiradical efficiency was expressed as Trolox Equivalent Antioxidant Capacity (TEAC). The TEAC value indicated the concentration of Trolox (mM) which ensures the same $ABTS^{+\cdot}$ quenching efficiency as that of 1 mmol of a tested compound. It was found the $ABTS^{+\cdot}$ scavenging activity of **III** exceeded that of Trolox by a factor of 1.54 (TEAC = 1.54±0.03). Compound **IV** showed no antiradical activity in the $ABTS^{+\cdot}$ assay.

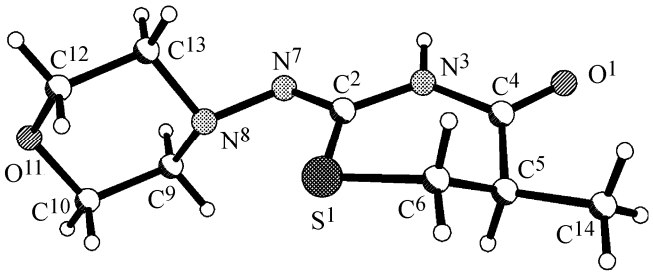
Futhermore, compound **III** was tested for anti-inflammatory effect towards acute exudative reaction (peritonitis) in rats induced by intraperitoneal injection

of 1% solution of acetic acid (1 mL per 100 g body weight). The exudate volume 3 h after injection of acetic acid in the both groups of test animals was considerably lower than that in the reference group; the effect of compound **III** was comparable with that of diclofenac sodium. The anti-inflammatory effect significantly increased as the dose of **III** was increased from 50 to 100 mg/kg (Table 4). Hence, compound **III** revealed a pronounced anti-inflammatory activity towards acute exudative reaction in rats at doses of 50 and 100 mg/kg.

EXPERIMENTAL

IR spectra of KBr pellets were recorded with a Nicolet Avatar-320 Fourier transform spectrometer. ^{1}H NMR spectra were measured with Bruker DRX500 and DRX400 instruments operating at 500 and 400 MHz, respectively, using DMSO-d_6 or $CDCl_3$ as solvent and tetramethylsilane as internal reference. Mass spectra (electron impact, 70 eV) were obtained with a Finnigan MAT Incos 50 mass spectrometer with direct sample input into the ion source. Melting points were determined using a Boetius melting point apparatus. The reactions progress was monitored with thin-layer chromatography on Sorbfil plates using propan-2-ol–benzene–aqueous ammonia (10 : 5 : 2) as eluent; spots were developed by treatment with iodine vapor.

X-ray diffraction data for compound **III** were obtained at 240 K with a Bruker Kappa APEX2 CCD diffractometer (MoK_α radiation, graphite monochromator, φ,θ-scanning, $\theta \le 28.34°$). Monoclinic crystal system with the following unit cell parameters: a = 11.092(1), b = 8.5578(9), c = 12.907(2) Å; β = 110.507(4)°; V = 1147.5(2) Å^3; Z = 4; $C_9H_{15}N_3O_2S$; space group $P2_1/n$; d_{calc} = 1.327 g/cm^3; μ = 0.268 mm^{-1}. Intensities of 2757 independent reflections were processed with a correction for absorption using SAINT [11] and SADABS [12] implemented in APEX2 software package. The structure was solved via the direct method. The positions of non-hydrogen atoms were refined in anisotropic approximation by the full-matrix least-squares procedure. Hydrogen atoms were placed in geometrically calculated positions which were refined in isotropic approximation with fixed positional and thermal parameters (*rider* model). The structure was solved and refined from 2087 reflections with $I \ge 2\sigma(I)$ (R_{int} = 0.0431) using SHELXS-97 [13] and SHELXL-97 [14]; final divergence factors R_1 = 0.0423, wR_2 = 0.1062; goodness of fit 1.025.



Structure of the molecule of 5-methyl-2-(morpholin-4-ylamino)-5,6-dihydro-4*H*-1,3-thiazin-4-one (**III**) according to the X-ray diffraction data.

Table 1. Bond lengths in molecule **III**

Bond	d, Å	Bond	d, Å
S^1–C^2	1.755(2)	N^7–N^8	1.451(2)
S^1–C^6	1.807(2)	N^8–C^9	1.463(3)
O^1–C^4	1.215(2)	N^8–C^{13}	1.468(3)
O^{11}–C^{12}	1.416(3)	C^4–C^5	1.524(3)
O^{11}–C^{10}	1.422(3)	C^5–C^6	1.515(3)
N^3–C^4	1.375(3)	C^5–C^{14}	1.530(3)
N^3–C^2	1.383(2)	C^9–C^{10}	1.513(4)
N^7–C^2	1.280(2)	C^{12}–C^{13}	1.509(3)

Table 2. Bond angles in molecule **III**

Angle	ω, deg	Angle	ω, deg
$C^2S^1C^6$	99.80(9)	$O^1C^4C^5$	123.4(2)
$C^{12}O^{11}C^{10}$	110.7(2)	$N^3C^4C^5$	117.2(2)
$C^4N^3C^2$	128.6(2)	$C^6C^5C^4$	110.1(2)
$C^2N^7N^8$	109.4(1)	$C^6C^5C^{14}$	111.4(2)
$N^7N^8C^9$	108.7(2)	$C^4C^5C^{14}$	110.2(2)
$N^7N^8C^{13}$	109.3(2)	$C^5C^6S^1$	112.7(2)
$C^9N^8C^{13}$	109.4(2)	$N^8C^9C^{10}$	108.0(2)
$N^7C^2N^3$	117.9(2)	$O^{11}C^{10}C^9$	111.7(2)
$N^7C^2S^1$	121.1(1)	$O^{11}C^{12}C^{13}$	111.9(2)
$N^3C^2S^1$	121.0(1)	$N^8C^{13}C^{12}$	107.8(2)
$O^1C^4N^3$	119.4(2)		

Table 3. Internal torsion angles in molecule **III**

Angle	τ, deg	Angle	τ, deg
Ring A		Ring B	
$C^6S^1C^2N^3$	3.9(2)	$C^13N^8C^9C^{10}$	–60.6(2)
$C^4N^3C^2S^1$	20.1(3)	$N^8C^9C^{10}O^{11}$	58.6(3)
$C^2N^3C^4C^5$	–2.3(3)	$C^{12}O^{11}C^{10}C^9$	–57.0(3)
$N^3C^4C^5C^6$	–41.5(3)	$C^{10}O^{11}C^{12}C^{13}$	57.3(3)
$C^4C^5C^6S^1$	64.6(2)	$O^{11}C^{12}C^{13}N^8$	–59.0(2)
$C^2S^1C^6C^5$	–43.8(2)	$C^9N^8C^{13}C^{12}$	60.7(2)

Table 4. Anti-inflammatory activity of compound **III** compared to diclofenac sodium

Parameter	Reference, $n = 6$	Diclofenac sodium, $n = 5$	**III**, $n = 6$	
			50 mg/kg	100 mg/kg
Animal weight, g	248±14	258±23	252±17	243±17
Amount of exudate, mL	6.4±0.3	3.8±05[a]	4.4±0.5[a]	3.5±0.6[a]

[a] $p < 0.05$ with respect to the reference group.

5-Methyl-2-(morpholin-4-ylamino)-5,6-dihydro-4*H*-1,3-thiazin-4-one (III). A solution of methacryloyl isothiocyanate was prepared by refluxing 3.35 g (32 mmol) of methacryloyl chloride and 3.1 g (32 mmol) of potassium thiocyanate in 30 mL of acetone during 2 h. The prepared solution was slowly (over 1 h) added to a solution of 3.06 g (30 mmol) of morpholin-4-amine in 10 mL of propan-2-ol under vigorous stirring at 25°C. The mixture was then stirred during 6 h at 30–40°C, the solvent was removed, and the oily residue was crystallized from propan-2-ol. Yield 4.1 g (60%), white crystals, mp 177–178° (from *i*-PrOH). Single crystals of **III** suitable for X-ray analysis were obtained by slow evaporation of the solution in propan-2-ol under normal conditions. IR spectrum (KBr), ν, cm^{-1}: 3179 (N–H), 1694 (C=O), 1599 (C=N). ^{1}H NMR spectrum (500 MHz, DMSO-d_6), δ, ppm (*J*, Hz): 1.15 d (3H, 5-CH_3, $J = 6.86$), 2.57–2.65 m [4H, N$(CH_2)_2$], 2.67–2.74 m (1H, 5-H), 2.82 d.d.d and 2.97 d.d.d (1H each, 6-H, $^3J = 4.0$, 10.3, $^2J = 13.0$), 3.65 br.t [4H, O$(CH_2)_2$, $J = 4.3$], 10.64 s (1H, NH). Mass spectrum, *m*/*z* (I_{rel}, %): 229 (82) $[M]^+$, 172 (66), 129 (89), 128 (39), 86 (34), 69 (47), 56 (31), 43 (41), 42 (100), 41 (67).

2-(1-Adamantylamino)-5-methyl-5,6-dihydro-4*H*-1,3-thiazin-4-one (IV) was synthesized similarly from 0.32 g (3.1 mmol) of methacryloyl chloride, 0.3 g (3.1 mmol) of potassium thiocyanate, and 0.56 (3.0 mmol) of adamantan-1-amine hydrochloride (preliminarily converted into the free base). Yield 0.60 g (72%), white crystals, mp 215.5–216°C (from *i*-PrOH–$CHCl_3$, 3 : 1). IR spectrum (KBr), ν, cm^{-1}: 3240 (N–H), 1672 (C=O), 1562 (C=N). ^{1}H NMR spectrum (400 MHz, $CDCl_3$), δ, ppm (*J*, Hz): 1.31 d (3H, 5-CH_3, $J = 6.9$), 1.69 br.t (6H, CH_2), 2.11 br.s (9H, CH_2, CH), 2.58–2.63 m (1H, 5-H), 2.95 d.d.d and 3.10 d.d.d (1H each, 6-H, $^3J = 4.5$, 10.0, $^2J = 12.7$), 5.19 s (1H, NH).

Antiradical activity of compounds **III** and **IV** towards 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) radical cation ($ABTS^{+\cdot}$) was assessed using Antioxidant Assay Kit (Sigma) according to the manufacturer instructions. The procedure is based on the generation of ferrylmyoglobin radical from metmyo-globin and hydrogen peroxide, which oxidized ABTS to $ABTS^{+\cdot}$. Addition of an antiradical agent leads to fast scavenging of $ABTS^{+\cdot}$, which is accompanied by characteristic changes in the spectra, so that the reaction rate can be measured [15].

Anti-inflammatory activity. Acute exudative reaction (peritonitis) in rats was induced by intraperitoneal injection of 1% solution of acetic acid at a dose of 1 mL per 100 g body weight. After 3 h, the exudate was collected, and its amount was measured [16]. The effect of compounds **III** and **IV** was studied at doses of 50 and 100 mg/kg upon oral administration as a starch suspension. The reference drug, diclofenac sodium, was administered at a dose of 25 mg/kg. Reference animals were administered an equal volume of starch mucilage. Compounds to be tested were administered at once 1 h before acetic acid injection.

The results of biological tests were processed using Statistica 6.0 software package. The data are given as mean value±standard error. Differences between groups of animals were estimated by the nonparametric Mann–Whitney U-test. Differences were considered to be reliable at a significance level *p* of lower than 0.05 [17].

REFERENCES

1. Koketsu, M., Tanaka, K., Takenaka, Y., Kwong, C.D., and Ishihara, H., *Eur. J. Pharm. Sci*., 2002, vol. 15, p. 307. DOI: 10.1016/S0928-0987(02)00014-3
2. El-Sayed Ali, T. and El-Kazak, A.M., *Eur. J. Chem*.,

2010, vol. 1, no. 1, p. 6. DOI: 10.5155/eurjchem.1.1.6-11.12.

3. Thansu, J., Kanagarajan, V., and Gopalakrishnan, M., *J. Enzyme Inhib. Med. Chem*., 2010, vol. 25, no. 6, p. 756. DOI: 10.3109/ 14756360903389898.
4. Kulakov, I.V., Nurkenov, O.A., Turdybekov, D.M., and Turdybekov, K.M., *Chem. Nat. Compd*., 2010, vol. 46, p. 257. DOI: 10.1007/s10600-010-9582-9.
5. Alimbaeva, A.S., Nurkenov, O.A., Zhakina, A.Kh., Kulakov, I.V., Turdybekov, D.M., and Turdybekov, K.M., *Russ. J. Gen. Chem*., 2009, vol. 79, no. 7, p. 1532. DOI: 10.1134/S1070363209070226.
6. Kulakov, I.V., *Chem. Heterocyclic Compd*., 2008, vol. 44, no. 7, p. 889. DOI: 10.1007/s10593-008-0126-1.
7. Kulakov, I.V., Nurkenov, O.A., Turdybekov, D.M., Isabaeva, G.M., Mahmutova, A.S., and Turdybekov, K.M., *Chem. Heterocycl. Compd*., 2009, vol. 45, no. 9, p. 1117. DOI: 10.1007/s10593-009-0398-0.
8. Kulakov, I.V. and Turdybekov, D.M., *Chem. Nat. Compd*., 2010, vol. 46, no. 4, p. 586. DOI: 10.1007/ s10600-010-9681-7.
9. Allen, F.H., Kennard, O., Watson, D.G., Brammer, L., Orpen, A.G., and Taylor, R., *J. Chem. Soc. Perkin Trans. 2*, 1987, p. S1. DOI: 10.1039/P298700000S1.
10. Tashkhodzhaev, B., Akhmedova, S., and Rozhkova, N.K., *J. Struct. Chem*., 1980, vol. 21, no. 2, p. 229.
11. *SMART V5.051 and SAINT V5.00. Area Detector Control and Integration Software*, Madison, WI-53719, USA: Bruker AXS, 1998.
12. Sheldrick, G.M., *SADABS*, Madison, WI-53719, USA: Bruker AXS, 1997.
13. Sheldrick, G.M., *Acta Crystallogr., Sect. A*, 2008, vol. 64, p. 112. DOI: 10.1107/S0108767307043930.
14. Sheldrick, G.M., *SHELXL-97. Program for the Refinement of Crystal Structures*, Gottingen, Germany: Univ. of Gottingen, 1997.
15. Miller, N.J., Rice-Evans, C., Davies, M.J., Gopinathan, V., and Milner, A., *Clin. Sci*. (London), 1993, vol. 84, p. 407.
16. Khabriev, R.U., *Rukovodstvo po eksperimental'nomu (doklinicheskomu) izucheniyu novykh farmakologicheskikh veshchestv* [Directory for Experimental (Preclinical) Trials of New Pharmacological Substances], Moscow: Meditsina, 2005.
17. Lakin, G.F., *Biometriya* (Biometrics), Moscow: Vysshaya Shkola, 1980.