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UNUSUAL SYNTHESIS AND CYTOTOXICITY OF N-[2-(BENZOTHIAZOL-2-SULFONYL)-1-ETHOXYETHOXY]-5-(BENZOTHIAZOL-2-YLSULFANYL)PENTANAMIDINE

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Addition of N-hydroxy-5-(benzothiazolylthio)pentanamidine to *E*-2-(2-chlorovinylsulfonyl)benzothiazole in the presence of NaH was studied. The main product of reaction – N-[2-(benzothiazol-2-sulfonyl)-1-ethoxy-ethoxy]-5-(benzothiazol-2-ylsulfanyl)pentanamidine exhibits high cytotoxicity.

Key words: *N-hydroxy-5-(benzothiazolylthio)pentanamidine, addition, N-[2-(benzothiazol-2-sulfonyl)-1-ethoxyethoxy]-5-(benzothiazol-2-ylsulfanyl)-pentanamidine, cytotoxicity.*

INTRODUCTION

Thiazole derivatives are widely investigated as cytotoxic and antitumor agents [1–8]. Substituted imidazo[2,1-b]thiazoles exhibit high antitumor and cytotoxic activities [9]. It is necessary to mention that many publications are devoted to thiazole oximes, which feature in the composition of cephalosporin antibiotics [10]. Beside this, thiazole oximes [11] or their platinum complexes are tested as cytotoxic and antitumor agents [12]. Recently high cytotoxic activity of N-hydroxy-ω-(benzothiazolylthio)alkanamidines on a wide range of cancer cell lines has been evaluated [13].

Synthesis and reactions of 2-chlorovinyl sulfones have been recently reviewed [14]. Some works are dedicated to conjugate addition of O-nucleophiles to 2-chlorovinyl sulfones. Interaction of 2-chlorovinyl sulfones with sodium methylate is strongly influenced by amounts of this base. Generally, interaction of compounds 2-chlorovinyl sulfones with NaOMe in MeOH leads to formation of mixture of mono- and double addition products [15]. Interaction of 2-chlorovinyl sulfone with MeOH, and then with BuLi / MeI, affords (*E*)-2-methoxy-1-methyl-1-(phenylsulfonyl)ethylene [16].

The main aim of this work is investigation of addition of N-hydroxy-5-(benzothiazolylsulfanyl)pentanamidine [13] to *E*-2-(2-chlorovinyl sulfonyl)benzothiazole.

EXPERIMENTAL

^{1}H NMR spectra were recorded on a *Varian 200 Mercury* instrument using $CDCl_3$ as a solvent. Mass spectra were registered on a *GC-MS HP 6890* (70 eV) apparatus. 2-Mercaptobenzothiazole, 1,1,2-trichloroethane, *m*-CPBA (*m*-chloroperoxybenzoic acid), sodium hydride (60% suspension in oil) and 18-crown-6 (*Acros*) were used without additional purification.

Synthesis of 2-(2,2-dichloroethanesulfanyl)benzothiazole (**2**). 1,1,2-Trichloroethane (4.1 ml, 44 mmol) was added under stirring to the mixture of 2-mercaptobenzothiazole (**1**) (20 mmol), K_2CO_3 (8.28 g, 60 mmol), KI (6.64 g, 40 mmol) and 18-crown-6 (528 mg, 2 mmol) in 25 ml of xylene. Reaction mixture was refluxed for 2 hours, cooled and filtered. The solvent was evaporated under reduced pressure to obtain crude 2-(2,2-dichloroethanesulfanyl)benzothiazole (**2**) as yellow liquid. ^{1}H NMR spectrum (200 MHz, δ): 4.07 (d, 2H, *J* = 7 Hz, CH_2); 6.19 (t, 1H, *J* = 7 Hz, $CHCl_2$); 7.31 and 7.46 (both t, 2H, *J* = 8 Hz, 5-H and 6-H); 7.76 (d, 1H, *J* = 8 Hz, 4-H); 7.87 (m, 1H, *J* = 8 Hz, 7-H). Mass spectrum, *m/z* (relative intensity): 263 (M^+, 13), 228 (21), 192 (9), 180 (4), 167 (100), 108 (18).

Synthesis of *E*-2-(2-chlorovinylsulfanyl)benzothiazole (**3**). Finely powdered KOH (2.24 g, 20 mmol) in 25 ml of toluene was added to reaction mixture containing 2-(2,2-dichloroethanesulfanyl)benzothiazole (**2**) from the previous step. Reaction mixture was stirred for 45 minutes (GC-MS control) at room temperature, filtered and evaporated. The residue was purified by column chromatography using toluene as eluent to obtain intermediate *E*-2-(2-chlorovinylsulfanyl)benzothiazole (**3**) as yellow liquid. ^{1}H NMR spectrum (200 MHz, δ): 6.71 and 6.97 (both d, 1H, *J* = 13 Hz, CH=CH); 7.33 and 7.45 (both t, 2H, *J* = 8 Hz, 5-H and 6-H); 7.78 and 7.92 (both d, 2H, *J* = 8 Hz, 4-H and 7-H). Mass spectrum, *m/z* (relative intensity): 227 (M^+, 2), 192 (100), 148 (10), 108 (10), 96 (10), 69 (9).

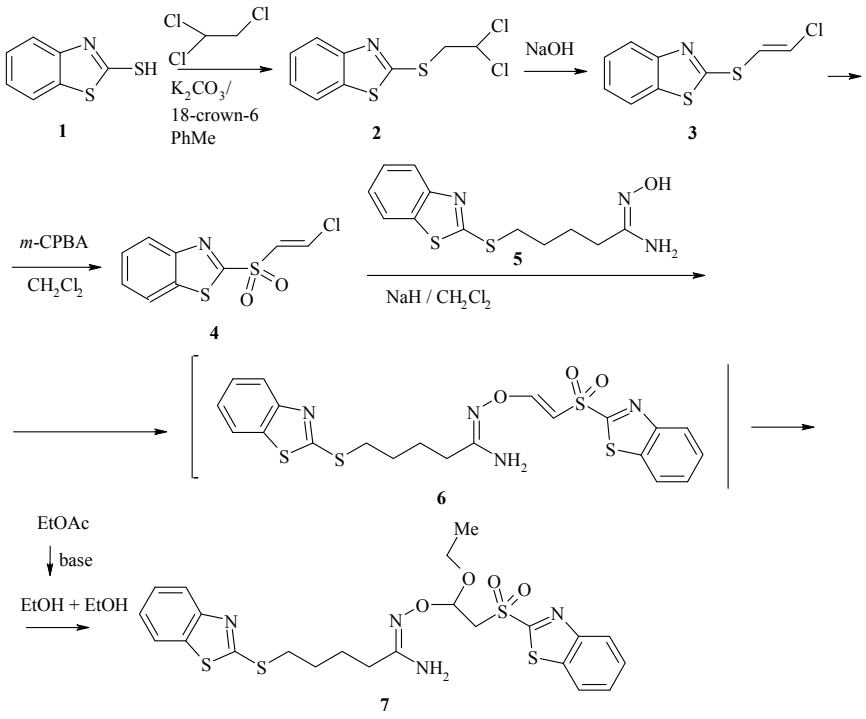
Synthesis of *E*-2-(2-chlorovinylsulfonyl)benzothiazole (**4**). *m*-Chloroperoxybenzoic acid (15.35 g, 88.9 mmol) was added portionwise under stirring to the mixture of *E*-2-(2-chlorovinylsulfanyl)benzothiazole (**3**) from the previous step in 50 ml of dry dichloromethane. Reaction mixture was stirred overnight at room temperature and filtered. The filtrate was concentrated at reduced pressure. The residue was purified by column chromatography using hexane : ethyl acetate (2 : 1) as eluent to obtain *E*-2-(2-chlorovinylsulfonyl)benzothiazole (**4**) (m.p. 117°C) in 17% overall yield from compound **1**. ^{1}H NMR spectrum (200 MHz, δ): 7.05 and 7.69 (both d, 2H, *J* = 13 Hz, CH=CH); 7.58–7.68 (m, 2H, 5-H and 6-H in benzothiazole); 8.01 and 8.22 (both d, 2H, *J* = 8 Hz, 4-H and 7-H in benzothiazole). Mass spectrum, *m/z* (relative intensity): 259 (M^+, 11), 224 (20), 194 (8), 178 (5), 160 (100), 134 (26), 108 (11), 90 (20), 63 (21), 50 (5).

Synthesis of N-[2-(benzothiazol-2-sulfonyl)-1-ethoxyethoxy]-5-(benzothiazol-2-ylsulfanyl)pentanamidine (7). Solution of N-hydroxy-5-(benzothiazolylsulfanyl)pentanamidine (**5**) (0.281 g, 1 mmol) in 3 ml of dry dichloromethane was slowly added to the mixture of NaH (60% dispersion in oil) (30 mg, 1.2 mmol) in 2 ml of dry CH_2Cl_2. Reaction mixture was stirred for 1 h at room temperature and *E*-2-(2-chlorovinylsulfonyl)benzothiazole (**4**) (0.26 g, 1 mmol) was added. Reaction mixture was stirred at room temperature for 2 h. The solvent was evaporated under reduced pressure and product was extracted with wet ethyl acetate. The residue was purified by column chromatography using hexane : ethyl acetate in different proportions as eluent. Yield 6 % of compound **7** with m.p. 127°C. ^{1}H NMR spectrum (200 MHz, δ): 0.92 (t, 2H, *J* = 8.0 Hz, CH_3); 1.16–1.25, 1.59–1.67 and 2.01–2.05 (all m, 6H, $(CH_2)_2$ and $CHC\underline{H}_2$); 3.33 (t, 2H, *J* = 7.6 Hz, SCH_2); 3.47–3.56 and 3.70–3.79 (both m, 2H, $C\underline{H}CH_2$); 3.97–4.08 (m, 2H, $C\underline{H}_2CH_3$); 4.33 (s, 2H, NH_2); 5.49 (t, 1H,

J = 5.6 Hz, CH); 7.15–7.61, 7.74–7.98 and 8.18–8.21 (all m, 8H, both C_6H_4). LC-MS: 551 (M^++1, 100).

***In vitro* cytotoxicity assay**. Monolayer tumor cell lines – HT-1080 (human fibrosarcoma), MG-22A (mouse hepatoma), 3T3 (mouse *Swiss Albino* embryo fibroblasts) were cultured in standard medium (Dulbecco's modified Eagle's medium; DMEM, *Sigma*) and supplemented with 10% fetal bovine serum (*Sigma*). Tumor cell lines were obtained from the ATCC. After the ampoule had thawed, cells from one to four passages were used in three concentrations of test compound: 1; 10 and 100 μg ml^{-1}. About 10×10^4 cells·ml^{-1} were placed in 96-well plates immediately after compounds were added to the wells; the volume of each plate was 200 μl. The control cells without test compounds were cultured on separate plate. The plates were incubated for 72 h, at 37 °C, in 5% CO_2 atmosphere. The number of surviving cells was determined using tri(4-dimethylaminophenyl)methyl chloride (crystal violet: CV) or 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolinium bromide (MTT) [17, 18]. The quantity on the control plate was taken in calculations for 100%. LD_{50} was tested according the „Alternative Toxicological Methods". The program *Graph Pad Prism® 3.0* was used for calculations (r < 0.05.).

RESULTS AND DISCUSSION



We have obtained *E*-2-(2-chlorovinylsulfonyl)benzothiazole (**4**) by oxidation of an intermediate 2-chlorovinyl sulfide **3**, prepared from the corresponding 2-mercaptobenzothiazole (**1**) in the system *$ClCH_2CHCl_2$ / K_2CO_3 (then KOH) / KI / 18-crown-6 / PhMe*. Reaction of the compound **4** with sodium salt of N-hydroxy-5-(benzothiazolylsulfanyl)pentanamidine (**5**), prepared *in situ* from the compound **5** [13] and NaH in CH_2Cl_2 at room temperature, afforded after the separation by column chromatography (eluent EtOAc: hexane) N-[2-(benzothiazol-2-sulfonyl)-1-ethoxyethoxy]-5-(benzothiazol-2-ylsulfanyl)pentanamidi-

ne (**7**) as the main product in 6% yield. The formation of the desired addition product **6** was not observed in this case. The mechanism of formation of compound **7** included base-promoted addition of ethanol, generated from ethyl acetate and base (NaOH from hydrolysis of NaH), to an intermediate **6** during the both reaction mixture work-up and column chromatography.

Table 1. **Cytotoxicity of N-[2-(benzothiazol-2-sulfonyl)-1-ethoxyethoxy]-5-(benzothiazolyl-2-ylsulfanyl)pentanamidine (7) IC_{50}** (μg/ml)

Compound	HT-1080, IC_{50}	MG-22A, IC_{50}	3T3, LD_{50}, mg/kg
7	3	4	275

Cytotoxic activity of compound **7** was tested *in vitro* on two monolayer tumor cell lines: MG-22A and HT-1080 (Table 1). Compound **7** exhibits high activity on above cancer cell lines. Basic toxicity of this compound is high (LD_{50} 275 mg/kg) and was detected on mouse normal fibroblasts.

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REFERENCES

1. Moody, C.J., Roffey, J.R., Stephens, M.A., Stratford, I.J. (1997). Synthesis and cytotoxic activity of indolyl thiazoles. *Antitumor Drugs, 8* (5), 489–499.
2. Kumar, R., Lown, J.W. (2003). Synthesis and antitumor cytotoxicity evaluation of novel thiazole-containing glycosylated polyamides. *Eur J. Org. Chem.,* (24), 4842-4851.
3. Lu, Y., Li, C.-M., Wang, Z., Ross, C.R., Chen, J., Dalton, J., Li, W., Miller, D.D. (2009). Discovery of 4-substituted methoxybenzoyl-arylthiazole as novel anticancer agents: synthesis, biological evaluation and structure–activity relationships. *J. Med.. Chem., 52* (6), 1701–1711.
4. Sengupta, S., Smitha, S.L., Thomas, N.E., Santhoshkumar, T.R., Devi, S.K.C., Sreejalekshmi, K.G., Rajasekharan, K.N. (2005). 4-Amino-5-benzoyl-2-(4-methoxyphenylamino)-thiazole (DAT1): a cytotoxic agent towards cancer cells and a probe for tubulin–microtubule system. *Brit. J. Pharmacol., 145* (8), 1076–1083.
5. Lu, Y., Li, C.-M., Wang, Z., Chen, J., Mohler, M.L., Li, W., Dalton, J.T., Miller, D.D. (2011). Design, synthesis, and SAR studies of 4-substituted methoxybenzoyl-arylthiazoles annalogues as potent and orally bioavailable anticancer agents. *J. Med. Chem., 54* (13), 4678–4693.
6. Oanh, D.T., Hai, H.V., Park, S.H., Kim, H.-J., Han, B.-W., Kim, H.-S., Hong, J.-T., Han, S.-B., Hue, V.T., Nam, N.-H. (2011). Benzothiazole-containing hydroxamic acids as histone deacetylase inhibitors and antitumor agents. *Bioorg. Med. Chem. Lett., 21* (24), 7509–7512.
7. Liu, W., Zhou, J., Qi, F., Bensdorf, K., Li, Z., Zhang, H., Qian, H., Huang, W., Cai, X., Cao, P., Wellner, A., Gust, R. (2011). Synthesis and biological activities of 2-aminothiazole-5-carboxylic acid phenylamide derivatives. *Arch. Pharm., 344* (7), 451–458.
8. Kumbhare, R.M., Kumar, K.V., Ramaiah, M.J., Dadmal, T., Pashpavalli, S.N., Muchopadhyay, D., Divya, B., Devi, T.A., Kosurkar, U., Pal-Bhadra, M. (2011). Synthesis and biological evaluation of novel Mannich bases of 2-arylimidazo[2,1-b]benzothiazoles as potential anti-cancer agents. *Eur. J. Med. Chem., 46* (9), 4258–4266.
9. Park, J.-H., El-Gamal, M.I., Lee, Y.S., Oh, C.H. (2011). New imidazo[2,1-b]thiazole derivatives: synthesis, *in vitro* anticancer evaluation, and *in silico* studies. *Eur. J. Med. Chem., 46* (12), 5769–5777.
10. Abele, E., Abele, R., Lukevics, E. (2007). Oximes of five-membered heterocyclic compounds with two heteroatoms. 2. Reactions and biological activity. *Chem. Heterocycl. Comp., 43* (8), 945–977.

11. Harmsen, S., Meijerman, I., Beijnen, J.H., Schellens, J.H.M. (2007). The role of nuclear receptors in pharmacokinetic drug–drug interactions in oncology. *Cancer Treatment Reviews, 33* (4), 369–380.
12. Komeda, S., Lutz, M., Spek, A.L., Chikuma, M., Reedijk, J. (2000). New antitumor-active azole-bridged dinuclear platinum (II) complexes: synthesis, characterization, crystal structures, and cytotoxic studies. *Inorg. Chem., 39* (19), 4230–4236.
13. Kalvinsh, I., Abele, R., Golomba, L., Rubina, K., Visnevska, J., Beresneva, T., Shestakova, I., Jaschenko, E., Bridane, V., Abele, E. (2011). Synthesis and cytotoxicity of N-hydroxy-ω-(hetarylmethoxy or hetarylthio)-alkanamidines. *Heterocycl. Lett., 1* (1), 47–54.
14. Višņevska, J., Ābele, E. (2011). Synthesis and reactions of 2-chlorovinyl sulfones. *Latvian J. Chem.*, *50* (1/2), 22–31.
15. Maioli, L., Modena, G. (1959). Nucleophilic reactions in ethylene derivatives. 2. Mechanism of substitution of chlorine in 1-arylsulfonyl-2-chloro-ethylenes. Reaction with alcoholates *Gazz. Chim. Ital*., *89* (7), 854–865.
16. Padwa, A., Bullock, W.H., Dyszlewski, A.D., McCombie, S.W., Shankar, B.B., Ganguly, A.K. (1991). Alkylation of 2-oxy-substituted 1-sulfonylallyl and 1-sulfonylvinyl anions. New routes to functionalized carbocycles and dihydrofurans. *J. Org. Chem., 56* (11), 3556–3564.
17. Fast, D.J., Lynch, R.C., Leu, R.W. (1992). Nitric oxide production by tumor targets in response to TNF: paradoxical correlation with susceptibility to TNF-mediated cytotoxicity without direct involvement in the cytotoxic mechanism. *J. Leuckocyt. Biol., 52* (3), 255–261.
18. Freshney, P.J. Culture of Animal Cells (A Manual of Basic Technique), Wiley-Liss, New York, 1994, pp. 296-297.

N-[2-(BENZTIAZOL-2-SULFONIL)-1-ETOKSIETOKSI]-5-(BENZTIAZOL-2-ILSULFANIL)PENTĀNAMIDĪNA NEGAIDĪTA SINTĒZE UN CITOTOKSICITĀTE

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K O P S A V I L K U M S

Izpētīta N-hidroksi-5-(benztiazoliltio)pentānamidīna pievienošanās reakcija *E*-2-(2-hlorvinilsulfonil)benztiazolam nātrija hidrīda klātbūtnē. Galvenais reakcijas produkts – N-[2-(benztiazol-2-sulfonil)-1-etoksietoksi]-5-(benztiazol-2-ilsulfanil)pentānamidīns uzrādīja augstu citotoksisko aktivitāti. Produktu atdalīšanai un analīzei lietota kolonnu hromatogrāfijas metode (eluents EtOAc : heksāns).

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