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SYNTHESIS AND EVALUATION OF 2-BENZOTHIAZOLE FORMAMIDOXIMES AS NOVEL CLASS OF CYTOTOXIC AGENTS

Lena Golomba, Elina Jaschenko, Anita Gulbe, Irina Shestakova, Edgars Abele

Latvian Institute of Organic Synthesis, 21 Aizkraukles Street, Riga, LV-1006, Latvia,
E-mail: abele@osi.lv

ABSTRACT:
Synthesis of 2-pyridine, 2-thiazole and 2-benzothiazole substituted formamidoximes from corresponding amines in the system DMF-DMA / $NH_2OH \cdot HCl$ / i-PrOH were described. The cytotoxicity of studied compounds towards HT-1080 (human fibrosarcoma), MG-22A (mouse hepatoma) and 3T3 (mouse embryonic fibroblasts) cancer cell lines was presented. 2-Benzothiazole formamidoxime exhibit high activity against HT-1080 and MG-22A cancer cell lines.

Keywords: 2-Pyridine formamidoxime, 2-thiazole formamidoxime, 2-benzothiazole formamidoximes, heteroaromatic amines, cytotoxicity

INTRODUCTION

Benzothiazole amino derivatives are of interest as anticancer and cytotoxic agents [I-IV]. Beside this, three recent reviews were dedicated to antitumoral activity of benzothiazole derivatives [V-VII]. Biological activity of benzothiazole oximes was reviewed too [VIII]. Recently cytotoxic activity of benzothiazole amidoximes [IXa] and antitubercular activity of N'-hydroxy-N-(4H,5H-naphtho[1,2-d]thiazol-2-yl)methanimidamides were presented [IXb]. Synthesis of unknown benzothiazole formamidoximes is one of aim of the present work. 2-Pyridine formamidoxime (**1**), according literature data, was prepared by treatment of 2-aminopyridine with DMF-DMA (then with $NH_2OH \cdot HCl$) [X] or by interaction of N-pyridyl-2-ylthioformamide with NH_2OH in MeOH [XIa,b]. Similarly was prepared thiazole formamidoxime (**2**) [XIc]. The second aim is investigation of cytotoxicity of obtained formamidoximes.

RESULTS AND DISCUSSION

Herein we report a detailed synthesis of novel benzothiazole **3a-9a** formamidoximes from corresponding amino derivatives **3-9** by two step method**.** The first reaction step include treatment of compounds **1-9** with dimethylformamide dimethylacetal (DMF-DMA) leading to imine intermediates ($HetN{=}CHNMe_2$). Treatment of these intermediates with $NH_2OH \cdot HCl$ in i-PrOH afforded desired formamidoximes **1a-9a** in 20-77% yields (Table 1).

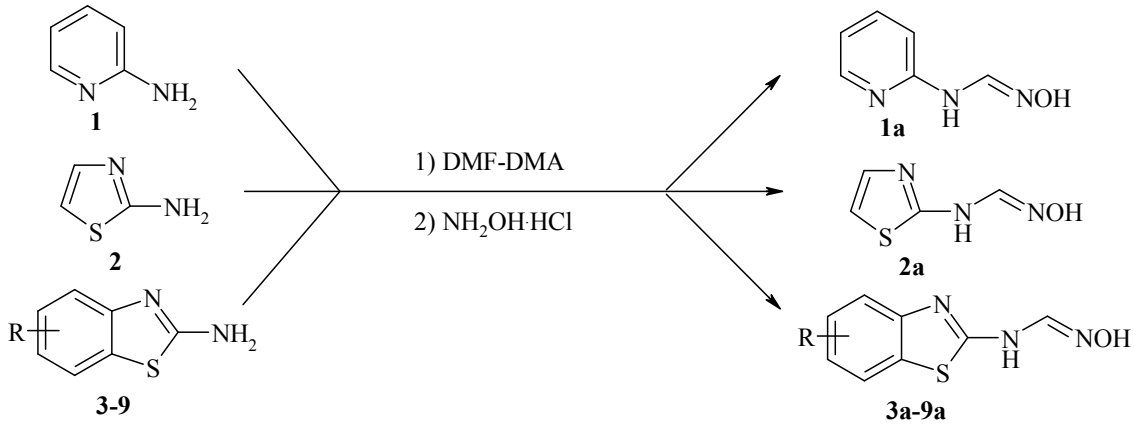
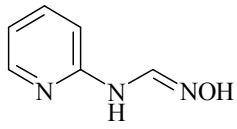
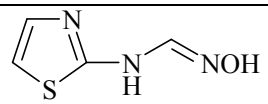
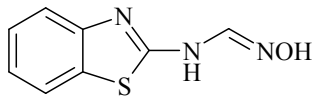
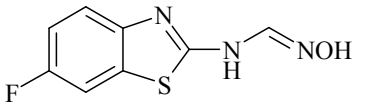
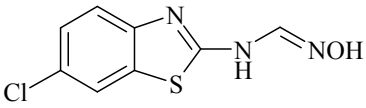
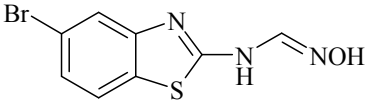
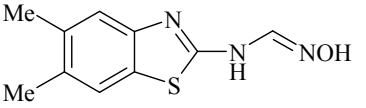
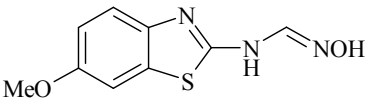
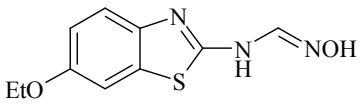


Table 1. Synthesis of formamidoximes **1a-9a** from amines **1-9** in the system DMF-DMA / NH_2OH / i-PrOH.

Product	Yield, %	Melting point, °C	^{1}H NMR, δ, ppm	^{13}C NMR, δ, ppm	LC-MS
 **1a**	77	89	6.80-6.86 and 7.55-7.64 (both m, 2H, 4-H and 5-H), 7.04 (d, 1H, J = 8Hz, 3-H), 7.85 (d, 1H, CH), 8.11-8.14 (m, 1H, 6-H), 9.34 (d, 1H, J = 10Hz, NH), 10.07 (s, 1H, OH)	110.29, 116.17, 135.54, 137.97, 147.37, 152.50	138 (M^++1, 100), 123 (25), 120 (41), 111 (18)
 **2a**	20	161	6.95 and 7.21 (both bs, 2H, H-4 and H-5), 7.56 (d, 1H, J = 8.8 Hz, CH), 10.24 (s, 1H, OH), 10.38 (d, 1H, J = 8.8 Hz, NH)	110.77, 135.75, 138.39, 162.33	144 (M^++1, 50), 126 (100)
 **3a**	21	152	7.13-7.21, 7.30-7.37, 7.57-7.61, 7.70-7.74, 7.81-7.85 (all m, 5H, C_6H_4 and CH), 10.52 (s, 1H, OH), 10.75 (d, 1H, J = 10Hz, NH).	119.43, 121.42, 122.53, 125.84, 131.15, 135.07, 150.83, 161.05	194 (M^++1, 100), 151 (17)

 **4a**	46	183	7.17 and 7.58 (both m, 2H, H-4 and H-5), 7.69 (s, 1H, CH), 7.76 (d, 1H, J = 2.0 Hz, H-7), 10.53 (s, 1H, NH), 10.74 (s, 1H, OH)	108.06, 113.46, 120.20, 132.31, 134.96, 147.59, 156.77, 160.99	212 (M^++1, 50), 194 (90), 256 (100), 142 (35)
 **5a**	52	172	7.34 and 7.56 (both d, 2H, J = 7.2 Hz, H-4 and H-5), 7.70 (d, 1H, J = 8.8 Hz, CH), 7.97 (s, 1H, H-7), 10.57 (s, 1H, OH), 10.84 (d, 1H, J = 8.8 Hz, NH)	120.45, 121.22, 126.09, 126.45, 132.87, 134.88, 149.73, 161.86	228 (M^++1, 50), 210 (80), 184 (100), 158 (35)
 **6a**	77	204	7.33 and 7.80 (both d, 2H, J = 8.4 Hz, H-6 and H-7), 7.69 (s, 1H, CH), 7.76 (d, 1H, J = 2.0 Hz, H-4), 10.60 (s, 1H, OH), 10.89 (d, 1H, J = 10Hz, NH)	118.60, 121.78, 123.20, 125.06, 130.46, 134.78, 132.32, 162.69	274 (M^++1, 90), 256 (100), 230 (80), 165 (50)
 **7a**	45	195	2.25 and 2.26 (both s, 3H, Me), 7.38 and 7.54 (both s, 2H, both benzothiazole protons), 7.67 (d, 1H, J = 9.2 Hz, CH), 10.44 (s, 1H, OH), 10.62 (d, 1H, J = 9.2 Hz, NH)	19.31, 19.59, 120.10, 121.31, 128.29, 131.09, 134.24, 135.21, 149.30, 160.20	222 (M^++1, 70), 204 (100), 170 (50).
 **8a**	27	161	3.76 (s, 3H, Me), 6.93 and 7.65 (both d, J =10Hz, 4-H and 5-H), 7.46 (s, 1H, 7-H), 7.50 (d, 1H, J = 8Hz, NH), 10.44 (s, 1H, OH), 10.58 (d, 1H, J = 10Hz, NH)	55.50, 105.42, 113.76, 119.90, 132.32, 135.19, 144.91, 155.34, 159.14	224 (M^++1, 90), 206 (100), 181 (80)

 **9a**	39	148	1.32 (t, 3H, J = 8Hz, Me), 4.01 (q, 2H, CH_2), 6.92 and 7.67 (both d, J =10Hz, 4-H and 5-H), 7.44 (s, 1H, 7-H), 7.47 (d, 1H, J = 8Hz, NH), 10.45 (s, 1H, OH), 10.58 (d, 1H, J = 10Hz, NH)	14.62, 63.48, 106.04, 114.17, 119.87, 132.28, 135.18, 144.83, 154.55, 159.09	238 (M^++1, 90), 220 (100), 195 (60)

Cytotoxic activity of formamidoximes **1a-9a** was tested *in vitro* on the monolayer tumor cell lines: MG-22A (mouse hepatoma) and HT-1080 (human fibrosarcoma) (Table 2). Concentrations providing 50% of tumor death effect (IC_{50}) were calculated according to the known procedure using 96 well plates. A preliminary analysis of the structure-activity relationship for the cytotoxic action clearly indicates the strong influence of substituent in 2-benzothiazole ring on toxic effects *in vitro*. Among 2-benzothiazole formamidoximes **3a-9a** unsubstituted compound **3a** exhibit the high cytotoxicity on the MG-22A and HT-1080 cell lines (IC_{50} 3 μg/mL). Among substituted products **4a-9a** dimethyl substituted product **7a** exhibit high cytotoxicity on the HT–1080 (IC_{50} 6 μg/mL) and methoxy substituted product **8a** on the MG-22A (IC_{50} 7 μg/mL) cell lines. Halogen substituted benzothiazole **4a-6a** and thiazole **1a** formamidoximes exhibit middle cytotoxicity on the HT–1080 and MG-22A cancer cell lines. Pyridine formamidoxime **1a** was essentially inactive on the both above cancer cells lines.

Acute toxicity of synthesized compounds was tested on 3T3- Swiss Albino mice embrio fibroblasts**.** In general, the compounds **2a-9a** exhibit middle toxicity in the range LD_{50} 451-985 mg/kg (Table 2).

Table 2. Cytotoxicity of formamidoximes 1-9a IC_{50} (μg/ml)

Compound	HT-1080, IC_{50}	MG-22A, IC_{50}	3T3, LD_{50}
1a	[x]	*	>2000
2a	27	18	458
3a	3	3	985
4a	24	19	591
5a	25	18	615
6a	17	35	653
7a	6	14	531
8a	15	7	647
9a	20	12	451

* No cytotoxic activity

EXPERIMENTAL SECTION

^1H and ^{13}C NMR spectra were recorded on a spectrometer Varian 400MR (400 MHz) in DMSO-D_6 using TMS as internal standard. LC-MS spectra were recorded on Alliance Waters 2695 instrument and Waters 3100 mass detector. 2-Aminothiazole and 2-aminobenzothiazoles,

dimethylformamide dimethylacetal (DMF-DMA) and hydroxylamine hydrochloride (Acros and AlfaAesar) were used without additional purification.

Typical procedure for the preparation of formamidoximes 1a-9a. A mixture of 2-aminopyridine **1** or aminothiazoles **2-9** (5 mmol), DMF-DMA (0.87 ml, 6.5 mmol) in isopropanol (2 ml) was refluxed for 3h. Reaction mixture was cooled to 50^{o}C, hydroxylamine hydrochloride (0.45g, 6.5 mmol) was added and reaction mixture was stirred at 50^{o}C for 12 h. Reaction mixture was evaporated to dryness, recrystallized from EtOH or purified by column chromatography (eluent CH_2Cl_2 : EtOH 10:1). The properties of obtained compounds **1a-9a** see Table 1.

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; "Sigma") 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 test compound: 1, 10 and 100 μg ml $^{-1}$. About 10 x10^4 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 72h, 37^{o}C, 5% CO_2. 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) [XII, XIII]. The quantity on the control plate was taken in calculations for 100%. LD_{50} was tested according „Alternative Toxicological Methods" [XIV]. The program Graph Pad Prism® 3.0 was used for calculations (r□< 0.05.).

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