HL
Heterocyclic Letters
(www.heteroletters.org) Vol. 1, No. 1, (2011), 47-54

SYNTHESIS AND CYTOTOXICITY OF N-HYDROXY-ω-(HETARYLMETHOXY OR HETARYLTHIO)-ALKANAMIDINES

Ivars Kalvinsh , Ramona Abele, Lena Golomba, Kira Rubina, Julija Visnevska, Tatjana Beresneva, Irina Shestakova, Elina Jaschenko, Veronika Bridane, Edgars Abele

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

Abstract:
Synthesis of novel N-hydroxy-ω-(hetarylmethoxy or hetarylthio)-alkaneamidines as potential cytotoxic agents was carried out in two steps. N-hydroxy-ω-(quinolylthio)-alkaneamidines exhibit high activity *in vitro* on monolayer tumor cell lines: MG-22A (mouse hepatoma) and HT-1080 (human fibrosarcoma).

Keywords: N-hydroxy-ω-(hetarylmethoxy or hetarylthio)-alkaneamidines, phase transfer catalysis, oximes, mouse hepatoma (MG-22A) cell line, human fibrosarcoma (HT-1080) cell line, cytotoxicity.

Introduction

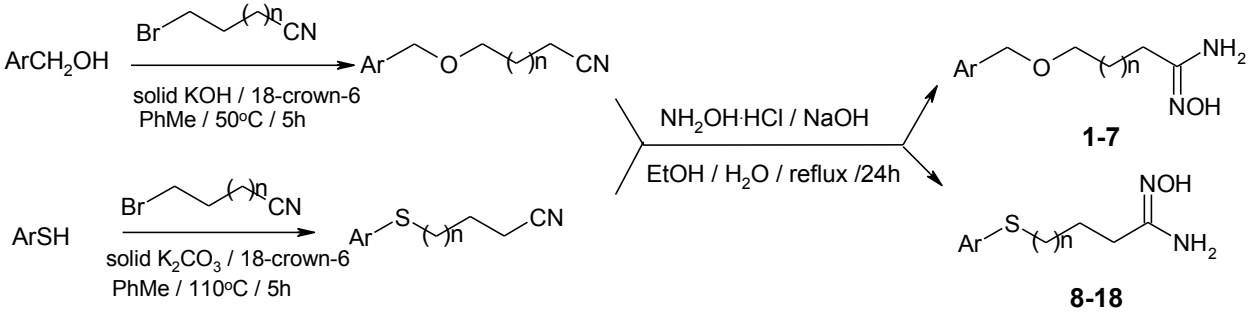
A number of reviews are devoted to the chemistry and biological activity of oximes and their derivatives [1]. Among this compounds aromatic [1], pyridine [2-3], quinoline [4-9], pyrimidine and quinazoline [10] oxime derivatives are of interest as cytotoxic and anticancer agents. For example, some silicon containing pyridine oxime O-ethers show high cytotoxicity. Thus, 2-pyridinecarbaldoxime O-(3-triethylsilylpropyl)oxime exhibit high cytotoxicity on HT-1080 (IC_{50} =3.5 μg/ml) and MG-22A (IC_{50} = 5 μg/ml) cell lines [3]. Antitumor and cytotoxic activities of pyridine [11] and quinoline [12] sulfides have also been described.

The pharmacological model of the studied type of anticancer agents consists of a aromatic [13], pyridine [14], quinoline [15] or pyrimidine [16] cap group able to interact with the rim space at the entrance of the catalytic tunnel of the enzyme linked to a hydrophobic spacer (for example, C_3-C_5-alkyl) through a polar connection unit (amide group, etc.). At the end of the hydrophobic spacer a hydroxylamine or amide group (binding group, BG) assures inhibition of enzyme (for example, protein kinases [17] or epidermal growth factor receptor tyrosine kinase [10]). However, the amidoxime moiety as BG is practically not described in literature. The aim of the research is to obtain a novel class of oximes - N-hydroxy-ω-(hetarylmethoxy or hetarylthio)-alkanamidines and to investigate their cytotoxicity.

Results and Discussion

Synthesis of N-hydroxy-ω-(hetarylmethoxy or hetarylthio)-alkaneamidines **1-18** was carried out in two steps. Alkylation of aryl- or hetarylmethanols with 1-bromo-ω-cyanoalkanes

was successfully realized in the PTC (phase transfer catalytic) system solid KOH or solid K_2CO_3 / 18-crown-6 / toluene. Products were isolated in 42-99% yields (Scheme 1, Table 1).



Scheme 1

The reaction of nitriles and hydroxylamine hydrochloride in the presence of NaOH in refluxing aqueous ethanol afforded novel amidoximes **1-18** in 29-87% yield (Table 1).

Cytotoxic activity of N-hydroxy-ω-(hetarylmethoxy or hetarylthio)-alkaneamidines **1-18** was tested *in vitro* on monolayer tumor cell lines - MG-22A and HT-1080. The activity of high effective compounds was determined also on another cell lines (Table 2). Concentrations providing 50% of tumor death effect (IC_{50}) were calculated according to the known procedure using 96 well plates.

The experimental evaluation of cytotoxicity is presented in Table 2. The preliminary analysis of the structure-activity relationship for cytotoxic activity clearly indicated a strong influence of aromatic or heteroaromatic substituent (Ar) on toxic effects *in vitro*. In the quinoline derivatives **11-13** the IC_{50} values range from 2 μg/mL (compound **13** on human fibrosarcoma HT – 1080 cell line) to 12 μg/mL for oxime **11**. It clearly indicates that the optimal length of the alkyl chain (hydrophobic spacer) between the quinoline ring and the oxime group is C_6 or C_7. Compound **13** also exhibit high activity on MG-22A cancer line (IC_{50} 2 μg/mL) (Table 2).

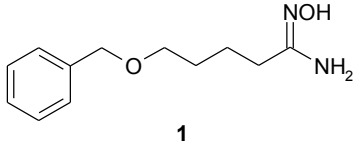
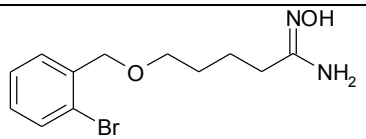
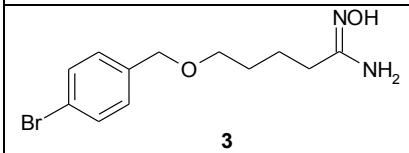
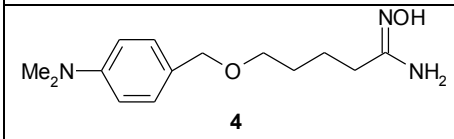
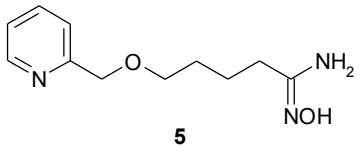
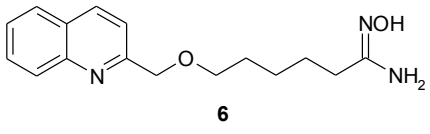
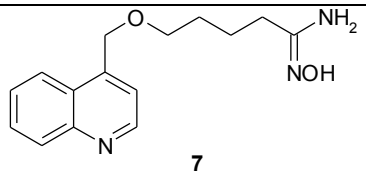
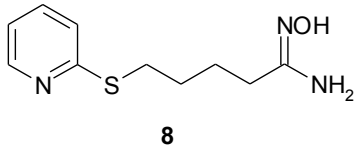
Interestingly, the substitution of sulfur for oxygen (for example, compounds **6** and **7**) in the hydrophobic spacer dramatically decreased cytotoxicity of compounds on HT-1080 and MG-22A cell lines. Aromatic **1-4**, pyridine **5**, **8-10** and pyrimidine **14, 15** derivatives were essentially inactive. Among benzothiazole amidoximes **16-18**, compound **17** exhibits high cytotoxicity on HT – 1080 cell line (IC_{50} 5 μg/mL) in comparison with compound **16** (IC_{50} 23 μg/mL) and **18** (IC_{50} 100 μg/mL).

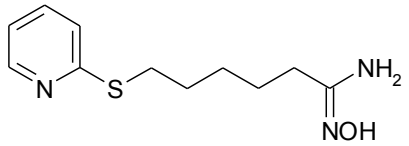
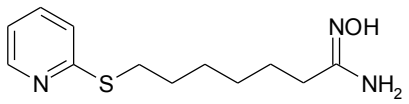
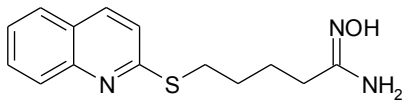
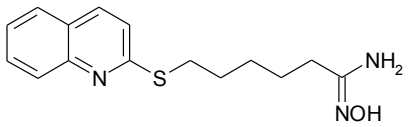
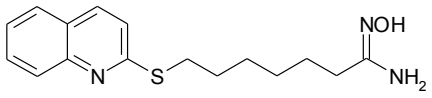
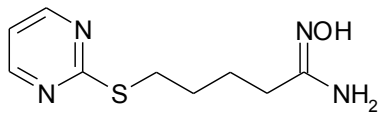
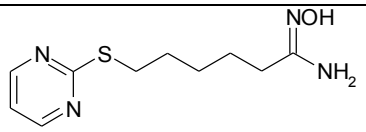
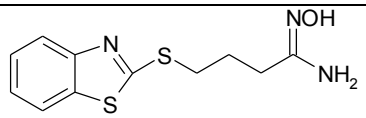
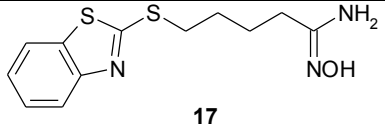
Cytotoxicity of selected compounds was studied also on H9C2 (rat cardiomyoblast), CCRF S-180 (mouse sarcoma), MIA PaCa-2 (human pancreatic carcinoma), Capan-2 (human pancreatic cancer) and PANC-1 (human pancreatic carcinoma) cell lines. It is necessary to mention the cytotoxicity of 4-bromophenyl **3** (IC_{50} 6 μg/mL), quinoline **7** (IC_{50} 7 μg/mL) and benzothiazole **16** oximes (IC_{50} 7 μg/mL) on CCRF S-180 cell line.

It was shown (Table 3) that compounds **3** (TG_{100}=300%), **11** (TG_{100}=300%), **12** (TG_{100}=300%) and **13** (TG_{100}=450%) readily increase NO concentration in the cultural medium (TG_{100}=500%) in HT-1080 cell lines. The quinoline amidoxime **12** exhibits high NO generation ability on MG-22A (TG_{100}=633%) cell line (Table 3).

Acute toxicity of synthesized compounds was tested on 3T3- Swiss Albino mice embrio fibroblasts**.** In general, compounds **1-18** exhibit middle toxicity in the range LD_{50} 394-2000 mg/kg (Table 2).

Table 1. Synthesis of N-hydroxy-ω-(hetarylmethoxy or hetarylthio)-alkanamidines (1-18)

Amidoxime	Yield, %	Melting point, °C	^{1}H NMR spectrum, δ, ppm
 **1**	30	72-74	1.60-1.75 (m, 4H, CH_2CH_2), 2.25 (m, 2H, CH_2), 3.50 (m, 2H, OCH_2), 4.49 (s, 2H, $PhCH_2$), 5.3 and 5.47 (bs and bs, 2H, NH_2), 7.32 (m, 5H, Ph)
 **2**	56	Oil	1.68-1.71 and 2.16-2.28 (both m, 6H, $(CH_2)_3$), 3.56 (t, 2H, OCH_2), 4.53 (s, 2H, CCH_2O), 5.68 (bs, 2H, NH_2), 7.08-7.53 (m, 4H, Ph)
 **3**	65	Oil	1.61-1.67 and 2.14 (both m, 6H, $(CH_2)_3$), 3.46 (t, 2H, J = 6Hz, OCH_2), 4.42 (s, 2H, CCH_2), 4.56 (bs, 2H, NH_2), 7.18 and 7.45 (both d, 4H, J = 8Hz, Ph)
 **4**	48	75-77	1.63 (m, 4H, CH_2CH_2), 2.13 and 2.50 (m and m, 2H, CCH_2 of isomeric oximes), 2.93 (s, 6H, NMe_2), 3.43 (m, 2H, OCH_2), 4.38 (s, 2H, CCH_2), 4.55 (bs, 2H, NH_2), 6.70 and 7.19 (both d, 4H, J = 8Hz, Ph)
 **5**	51	Oil	1.69 and 2.17 and 3.56 (all m, 8H, $(CH_2)_4$), 4.60 (s, 4H, NH_2 and OCH_2), 7.14-7.20 and 7.65-7.72 (both m, 2H, 4-H and 5-H), 7.41 (d, 2H, J = 8Hz, 3-H), 8.51 (d, 1H, J = 4Hz, 6-H)
 **6**	36	84-86	1.24-1.76 and 2.19-2.27 (both m, 8H, $C(CH_2)_4$), 3.58 (t, 2H, J = 7Hz, OCH_2), 4.78 (s, 2H, CCH_2O), 5.43 (bs, 2H, NH_2), 7.48-7.83 and 8.02-8.19 (all m, 6H, quinoline protons)
 **7**	87	Oil	1.71 and 2.17 (both m, 6H, $C(CH_2)_3$), 3.62 (m, 2H, OCH_2), 4.51 (bs, 2H, NH_2), 4.96 (s, 2H, CCH_2O), 7.46 (d, 1H, J = 6Hz, 3-H), 7.56 (m, 1H, 6-H), 7.71 (m, 1H, 7-H), 7.97 (m, 2H, 5-H), 8.13 (m, 1H, 8-H), 8.88 (d, 1H, J =4Hz, 2-H)
 **8**	25	53-55	1.76-1.80 (m, 4H, $(CH_2)_2$), 2.26 (m, 2H, CCH_2), 3.17 (t, 2H, J = 8Hz, SCH_2), 5.51 (bs, 2H, NH_2), 6.92-6.98 (m, 1H, 5-H), 7.15 (d, 1H, J = 8Hz, 3-H), 7.42-7.50 (m, 1H, 4-H), 8.39 (d, 1H, J = 4 Hz, 6-

			H)
 **9**	29	91	1.45-1.81 (m, 6H, $(CH_2)_3$), 2.23 (t, 2H, J = 8Hz, CCH_2), 3.15 (t, 2H, J =7Hz, SCH_2), 5.35 (bs, 2H, NH_2), 6.92-6.99 (m, 1H, 5-H), 7.15 (d, 1H, J = 8Hz, 3-H), 7.41-7.50 (m, 1H, 4-H), 8.39-8.42 (m, 1H, 6-H)
 **10**	51	45	1.24-1.75 and 2.18-2.25 (both m, 10H, $C(CH_2)_5$), 3.14 (t, 2H, J = 6Hz, SCH_2), 5.36 (bs, 2H, NH_2), 6.92-6.98 (m, 1H, 5-H), 7.15 (d, 1H, J = 8Hz, 3-H), 7.45-7.54 (m, 1H, 4-H), 8.40 (d, 1H, J = 4 Hz, 6-H)
 **11**	67	79-80	1.74-1.84, 2.22-2.34 (both m, 6H, $C(CH_2)_3$), 3.36 (t, 2H, J= 6Hz, CH_2), 4.54 and 5.48 (both bs, 2H, NH_2 of isomeric oximes), 7.16-7.21, 7.37-7.44, 7.59-7.72 and 7.84-7.93 (all m, 6H, quinoline ring protons)
 **12**	48	92-94	1.52-1.87 and 2.12-2.19 (both m, 8H, $C(CH_2)_4$), 3.33 (t, 2H, J = 8Hz, SCH_2), 4.49 (bs, 2H, NH_2), 7.16-7.21, 7.40-7.44, 7.59-7.72, 7.84-7.93 (all m, 6H, quinoline ring protons)
 **13**	47	82-84	1.41-1.75 and 2.10-2.17 (both m, 10H, $C(CH_2)_5$), 3.32 (t, 2H, J = 6Hz, SCH_2), 4.48 (bs, 2H, NH_2), 7.17-7.21, 7.37-7.44, 7.59-7.72, 7.84-7.93 (all m, 6H, quinoline ring protons)
 **14**	60	93-94	1.70-1.83 and 2.19-2.22 (both m, 6H, $(CH_2)_3$), 3.16 (t, 2H, J =8Hz, SCH_2), 4.55 (bs, 2H, NH_2), 6.95 (t, 1H, J = 5Hz, 5-H), 8.49 (d, 2H, J= 4Hz, 4-H and 6-H)
 **15**	32	71-73	1.50-1.75 and 2.11-2.19 (both m, 8H, $(CH_2)_4$), 3.13 (d, 2H, J = 8Hz, SCH_2), 4.51 (bs, 2H, NH_2), 6.94 (t, 1H, J = 7 Hz, 5-H), 8.49 (d, 2H, J = 4 Hz, 4-H and 6-H)
 **16**	47	110-113	2.09-2.20 (m, 2H, CH_2CH_2), 2.36 (t, 2H, J = 8 Hz, CCH_2), 3.40 (t, 2H, J = 7 Hz, SCH_2), 4.81 (bs, 2H, NH_2), 7.28-7.45 un 7.73-7.85 (both m, 4H, Ph)
 **17**	53	102	1.74-1.88 (m, 4H, $(CH_2)_2$), 2.19 (t, 2H, J = 6Hz, CCH_2), 3.36 (t, 2H, J = 8Hz, SCH_2), 4.59 (bs, 2H, NH_2), 7.27-7.42

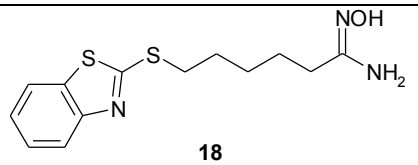
			and 7.71-7.87 (both m, 4H, Ph)
 18	23	112-114	1.53-1.86 and 2.21-2.28 (both m, 8H, $(CH_2)_4$), 3.34 (t, 2H, J = 8Hz, SCH_2), 5.31 (bs, 2H, NH_2), 7.28-7.40 and 7.73-7.87 (both m, 4H, Ph)

Table 2. ***In vitro*** **cell cytotoxicity of N-hydroxy-ω-(hetarylmethoxy or hetarylthio)-alkanamidines (1-18) IC_{50} (μg/ml).**

Nr.	3T3	HT-1080		MG-22A		H9C2		CCRF S-180		MIA PaCa-2		Capan-2		PANC-1	
	LD_{50} mg/kg	IC_{50} CV	IC_{50} MTT	IC_{50} CV	IC_{50} MTT	IC_{50} CV	IC_{50} MTT	IC_{50} CV	IC_{50} MTT	IC_{50} CV	IC_{50} MTT	IC_{50} CV	IC_{50} MTT	IC_{50} CV	IC_{50} MTT
1	>2000	>100	>100	>100	>100	-	-	-	-	-	-	-	-	-	-
2	1205	66	49	57	79	-	-	-	-	-	-	-	-	-	-
3	843	38	32	23	15	84	85	6	6	30	25	100	*	31	41
4	1486	100	100	95	*	-	-								
5	1096	*	*	100	100	-	-	-	-	-	-	-	-	-	-
6	1552	*	*	100	100	-	-	-	-	-	-	-	-	-	-
7	1203	32	27	13	23	>100	>100	7	7	100	100	>100	>100	100	100
8	1442	70	64	*	*	-	-	-	-	-	-	-	-	-	-
9	>2000	*	*	*	*	-	-	-	-	-	-	-	-	-	-
10	1013	100	*	80	*	-	-	-	-	-	-	-	-	-	-
11	578	12	15	15	14	35	45	-	-	25	25	44	60	35	38
12	637	5	4	6	10	40	60	-	-	25	25	46	62	25	25
13	394	2	2	2	2	14	14	-	-	10	8	37	43	8	8
14	1448	*	*	100	100	-	-	-	-	-	-	-	-	-	-
15	1274	100	100	29	28	-	-	-	-	-	-	-	-	-	-
16	668	23	20	22	29	70	53	6	6	35	25	>100	>100	30	30
17	507	5	9	9	12	28	40	-	-	25	25	65	80	25	25
18	2097	100	100	100	100	-	-	-	-	-	-	-	-	-	-

* No cytotoxic effect

Table 3. Intracellular NO-generation of N-hydroxy-ω-(hetarylmethoxy or hetarylthio)-alkanamidines (1-17) (nmolx10^2/200 μl), NO 100%CV, concentration 100 μg/ml.

Nr.	HT-1080	MG-22A	CCRF S-180	MIA PaCa-2	Capan-2	PANC-1
1	3	2	-	-	-	-
2	28	11	-	-	-	-
3	300	250	500	26	13	40
4	33	15	-	-	-	-
5	3	6	-	-	-	-
6	18	6	-	-	-	-
7	17	43	2	17	4	12
8	47	16	-	-	-	-
9	3	5	-	-	-	-
10	21	13	-	-	-	-
11	300	80	-	50	43	36
12	300	633	-	14	4	8
13	450	467	-	100	57	66
14	5	6	-	-	-	-
15	6	50	-	-	-	-
16	67	40	150	54	3	38
17	100	180	-	17*	7*	6*

* Concentration of compound 6,25 μg/ml.

Experimental section

^{1}H NMR spectra were recorded on a Varian 200 Mercury spectrometer and recorded in $CDCl_3$ using hexamethyldisiloxane (HMDSO) as internal standard. Thiols and hetarylmethanols and 18-crown-6 (Acros and Aldrich), 1-bromo-ω-cyanoalkanes (AlfaAesar) were used without additional purification.

General procedure for the synthesis of N-hydroxy-ω-(hetarylmethoxy or hetarylthio)-alkaneamidines (1-18). A solution of 1-(hetarylmethoxy)-ω-cyanoalkane or 1-hetarylthio-ω-cyanoalkane (16.7 mmol), hydroxylamine hydrochloride (3.46 g, 50.2 mmol) and NaOH (2.01 g, 50.2 mmol) in mixture of EtOH (15 ml) and H_2O (10 ml) was refluxed for 24 h. Reaction mixture was evaporated to dryness under reduced pressure. Product was extracted with a mixture of $CHCl_3$: EtOH 10:1 (100ml), dried over anhydrous Na_2SO_4 and the solvents were evaporated under reduced pressure. The residue was purified by column chromatography on silica gel (eluent $CHCl_3$: EtOH 10:1) to obtain desired products **1-18** (see Table 1)**.** Compounds **2, 3, 5-10, 12-18** were isolated as E-isomers. Compounds **1**, **4** and **11** were separated as ~1:1 mixture of E and Z isomers.

In vitro cytotoxicity assay

Monolayer tumor cell lines – Capan-2 (human pancreatic cancer), H9C2 (rat cardiomyoblast), HT-1080 (human connective tissue fibrosarcoma), MIA PaCa-2 (human pancreatic carcinoma), MG-22A (mouse hepatosarcoma), 3T3 (mouse Swiss Albino embryo fibroblasts), PANC-1 (human pancreatic carcinoma), CCRF S-180 (mouse sarcoma) - were cultured in standard medium (Dulbecco`s modified Eagle`s medium; DMEM) without an indicator ("Sigma") and supplemented with 10% heat-inactivated fetal bovine serum ("Sigma"). Tumor cell lines were taken 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 (2-5) x10^4 cells ml^{-1} (depending on the nature of the line) 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) [18,19]. The quantity on the control plate was taken in calculations for 100%. The concentration of NO was determined according to the Griess method (by NO_2 level in the culture medium). Sodium nitrite standard solution was used for the calibration curve. LD_{50} was tested according „Alternative Toxicological Methods" [20]. The program Graph Pad Prism® 3.0 was used for calculations ($r < 0.05$.).

Acknowledgements

This work was supported by the project of ESF Foundation of Latvia (Project N 2009/0197/1DP/1.1.1.2.0/APIA/VIAA/014).

References

1. (a) E. Abele and E. Lukevics, Chem. Heterocycl. Comp. 37, 141 (2001); (b) E. Abele, R. Abele, O. Dzenitis and E. Lukevics, Chem. Heterocycl. Comp. 39, 3 (2003); (c) E. Abele, R. Abele and E. Lukevics, Chem. Heterocycl. Comp. 39, 825 (2003); (d) E. Abele, R. Abele and E. Lukevics, Chem. Heterocycl. Comp. 40, 1 (2004); (e) E. Abele, R. Abele, K. Rubina and E. Lukevics, Chem. Heterocycl. Comp. 41, 825 (2005); (f) E. Abele, R. Abele and E. Lukevics, Chem. Heterocycl. Comp. 43, 387 (2007); (g) E. Abele, R. Abele and E. Lukevics, Chem. Heterocycl. Comp. 43, 945 (2007); (h) E. Abele, R. Abele and E. Lukevics, Chem. Heterocycl. Comp. 44, 637 (2008); (i) Abele, R. Abele and E. Lukevics, Chem. Heterocycl. Comp. 44, 769 (2008); (j) Abele, R. Abele and E. Lukevics, Chem. Heterocycl. Comp. 44, 1420 (2009);
2. I. Kalvins, V. Andrianov, I. Shestakova, I. Kanepe and I. Domracheva, I. US Pat. 7078418 (2006).
3. (a) F. Trecourt, B. Gervais, O. Mongin, C. Le Gal, F. Mongin and G. Queguiner, J. Org. Chem. 63, 2892 (1998); (b) E. Abele, R. Abele, P. Arsenyan, I. Shestakova, I. Kanepe, I. Antonenko, J. Popelis and E. Lukevics, Bioinorg. Chem. Appl. 1, 299 (2003).
4. C.-C. Tzeng, Y.-L. Chen and F.-N. Ko, US Pat. 2002 61895; Chem. Abstr. 136, 401781a (2002).
5. K.-C. Fang, Y.-L. Chen, J.-Y. Sheu, T.-C. Wang and C.-C. Tzeng, J. Med. Chem. 43, 3809 (2000).

6. J.-Y. Sheu, Y.-L. Chen, C.-C. Tzeng, S.-L. Hsu, K.-C. Fang and T.-C. Wang, Helv. Chim. Acta 86, 2481 (2003).
7. I.-L. Chen, Y.-L. Chen, C.-C. Tzeng and I.-S. Chen, Helv. Chim. Acta 85, 2214 (2002).
8. Y.-L. Chen, C.-H. Chung, I.-L. Chen, P.-H. Chen and H.-Y. Jeng, Bioorg. Med. Chem. 10, 2705 (2002).
9. S. Dallavalle, A. Ferrari, B. Biosotti, L. Merlini, S. Pence, G. Gallo, M. Marzi, M.O. Tinti, R. Martinelli, C. Pisano, P. Carminati, N. Carenini, G. Beretta, P. Perego, A. De Cesare, G. Pratesi and F. Zunino, J. Med. Chem. 44, 3264 (2001).
10. (a) G. Xu, L. Lee, P. J. Connolly, S.A. Middleton, S.L. Emanuel, T.V. Hughes, M..C. Abad, P.S. Karnachi and S.K. Wetter, PCT Int. WO Pat. 03076400 (2007); Chem. Abstr. 147, 189189m (2007); (b) M. S. Vedula, V. A. Karrubeina, J. Igbal, R. Ramanujam, S. Rajagopal, N.V.S.R. Mamidi, V.R. Josyula and M. Gutta. PCT Int Appl. WO Pat. 200469145; Chem. Abstr. 141, 207223 (2004); (c) M. D. Gaul, G. Xu, and C.A. Baumann, PCT Int Appl. WO Pat. 2006135719; Chem. Abstr. 146, 81893 (2007); (d) E. Againe Csongor, L. Czibula, F. Seboek, G. Domany and I. Greiner, GB Pat. 2449293; Chem. Abstr. 149, 576569 (2008).
11. (a) E. Lukevics, E. Abele, P. Arsenyan, R. Abele, K. Rubina, I. Shestakova, I. Domracheva and V.Vologdina, Metal Based Drugs 9, 45 (2002); (b) J.L. Greene, Jr., A.M. Williams and J.A. Montgomery, J. Med. Chem. 7, 20 (1964); (c) W.C.J. Ross, J. Med. Chem. 10, 257 (1967); (d) M.J. Gil, M.A. Manu, C. Arteaga, M. Migliaccio, I. Encio, A. Gonzalez and V. Martinez-Merino, Bioorg. Med. Chem. Lett. 9, 2321 (1999); (e) A. Gangjee, Y. Zhu and S.F. Queener, J. Med. Chem. 41, 4533 (1998); (f) A.P. Krapcho, S.N. Haydar, S. Truong-Chiott, M.P. Hacker, E. Menta and G. Beggiolin, Bioorg. Med. Chem. Lett. 10, 305 (2000).
12. (a) L. Monti, G. Franchi and C. Pellerano, Gazz. Chim. Ital. 91, 115 (1961); (b) W.O. Foye, Y.H. Kim and J.M. Kauffman, J. Pharm. Sci. 72, 1356 (1983); (b) W.O. Foye, S.H. An and T.J. Maher, J. Pharm. Sci. 73, 1168 (1984); (c) D. Bergen, R. Citarellla, M. Dutia, L. Greenberger, W. Hallett, R. Paul and D. Powell, J. Med. Chem. 42, 2145 (1999).
13. (a) J.R. Dimmock, P. Kumar, M. Chen, J.W. Quasil, J. Yang, T.M. Allen and G.Y. Kao, Pharmazie 50, 449 (1995); (b) M.R. Burns, PCT Int. WO Pat. 0172685 (2001); Chem. Abstr. 135, 272682q (2001); (c) J.-C. Madelmont and J.-M. Chezal, PCT Int. WO Pat. 200812782 (2008); Chem. Abstr. 148, 214964d (2008); (d) A.W. Cheung, S.B. Ferguson, L.H. Foley and A.J. Lovey, PCT Int. WO Pat. 0035856 (2000); Chem. Abstr. 133, 58614j (2000); (e) H. Handa, S. Kawai and N. Kusu, Jpn. Pat. 2005232101 (2005); Chem. Abstr. 143, 241994d (2005).
14. J.Y. Nagasawa, PCT Int. WO Pat. 2007146831 (2008); Chem. Abstr. 148, 93187d (2008).
15. W. Chew, S.K. Rabindran, C. Discafani-Marro, J.P. McGinnis, III, A. Wisssner and Y. Wang, PCT Int. WO Pat. 2006127203 (2006); Chem. Abstr. 146, 27723n (2007).
16. G.P. Chen, US Pat. 2004224967 (2004); Chem. Abstr. 141, 410958m (2004).
17. H. Lang, T.C. Gahman, M.R. Herbert, C. Zhao, P.L. Wash and R.L. Davis, PCT Int. WO Pat. 200776474 (2007); Chem. Abstr. 147, 143461r (2007).
18. D.J. Fast, R.C. Lynch and R.W. Leu, J. Leuckocyt. Biol. 52, 255 (1992).
19. P.J. Freshney, Culture of Animal Cells (A Manual of Basic Technique), Wiley-Liss, New York, 1994, pp. 296-297.
20. http://iccvam.niehs.nih.gov/methods/invidocs/guidance/iv_guide.htm [2004.01.10]