Brief Articles

Synthesis, X-ray Crystal Structure Study, and Cytostatic and Antiviral Evaluation of the Novel Cycloalkyl-*N*-aryl-hydroxamic Acids

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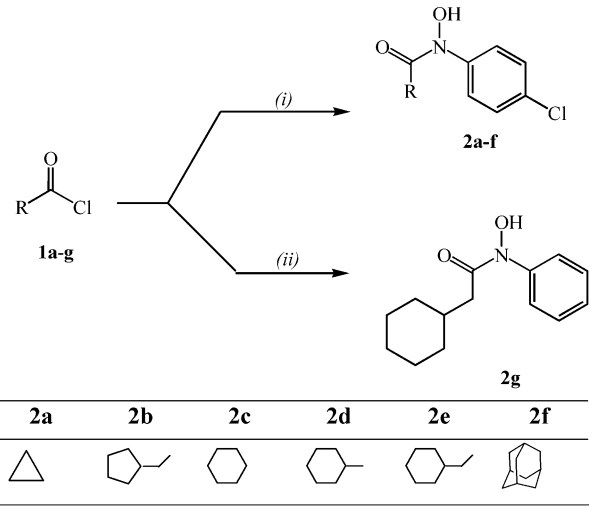
In vitro evaluation of the novel cycloalkyl-*N*-(4-chlorophenyl)-hydroxamic acids (**2a**−**g**) demonstrated that **2b**,**d**,**e** exhibited rather marked inhibitory activity ($IC_{50} = 7-10\ \mu M$) against pancreatic carcinoma, **2b**−**d** against colon carcinoma, **2d** against laryngeal carcinoma, and **2b**,**d** against breast carcinoma. **2e** showed the most pronounced anti-cytomegalovirus activity ($EC_{50} = 1.5$ and $0.8\ \mu g\ mL^{-1}$) only at ≥5-fold lower than the cytotoxic concentration. **2d** and **2f** showed modest, albeit selective, activity against cytomegalovirus (**2d**, $EC_{50} = 7.3-8.9\ \mu g\ mL^{-1}$, selectivity index 7−10; **2f**, $EC_{50} = 7-13\ \mu g\ mL^{-1}$, selectivity index 10).

Introduction

Hydroxamic acids are important iron chelators and microbial siderophores.[1,2] They are associated with diverse biological activities including antibacterial, antifungal, and antitumor profiles.[3] Succinyl, malonyl, and glutaryl hydroxamates are known to inhibit matrix metalloproteinases, a class of enzymes implicated in inflammatory, malignant, and degenerative diseases.[4] Phenoxyphenyl sulfone *N*-formylhydroxylamines (retrohydroxamates) have been recognized as potent, selective, and orally bioavailable matrix metalloproteinase inhibitors.[5] Antiinflammatory activity of hydroxamic acids is also connected with inhibition of 5-lipoxygenase, an enzyme involved in the biosynthesis of leukotrienes, mediators of inflammatory and allergic disorders.[6,7] Furthermore, hydroxamate-based compounds are effective urease,[8] ribonucleotide reductase,[9] or angiotensin converting enzyme (ACE) inhibitors.[10] Finally, some hydroxamic acids are currently accepted therapeutic agents, e.g. desferrioxamine B (treatment of iron overload), hydroxycarbamide (antineoplastic), ibuproxam, oxametacin and bufexamac (antiinflammatory and analgesic drugs), and adrafinil (α-adrenergic agonist and antidepressant).[11] Taking the pharmacological potential of this class of compounds into account, we have synthesized the new type of *N*-aryl hydroxamic acids containing various cycloalkyl groups connected to the carbonyl group with the aim to evaluate their cytostatic and antiviral activities.

Scheme 1. Synthesis of Cycloalkyl-*N*-aryl-hydroxamic Acids **2a**−**g**[a]



[a] Reagents and conditions: (i) nitrosobenzene, MeCN, catalytic amount of HCl, rt, 3−22 h; (ii) *N*-phenylhydroxylamine, *N*-methylmorpholine, anhydrous toluene, rt, 3 h.

Chemistry

Synthesis. A specific synthetic approach using acyl chlorides and nitrosobenzene as the starting reagents was applied for the preparation of hydroxamic acids **2a**−**f** (Scheme 1). This method enabled a direct introduction of the chloro-substituent in the *para* position of the benzene ring.[12] The nonhalogenated compound **2g** was synthesized from cyclohexaneacetyl chloride and *N*-phenylhydroxylamine by the usual method for hydroxamic acid synthesis.[13,14]

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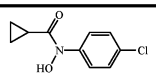
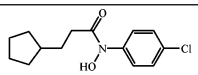
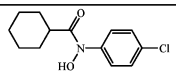
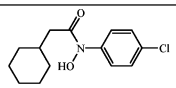
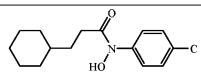
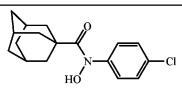
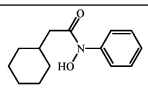
Table 1. Inhibitory Activities of Cycloalkyl-*N*-aryl-hydroxamic Acids **2a**−**g** on the Growth of Malignant Tumor Cell Lines and Normal Fibroblasts (WI38)

Compd. **2a-g**		Tumor cell growth IC_{50}[a] (μM)								
		L1210	Molt4/C8	CEM	SW620	Hep2	MiaPaCa2	MCF7	HeLa	WI38
a		22 ± 1	24 ± 3	25 ± 3	60 ± 5	> 100	> 100	> 100	80 ± 7	> 100
b		38 ± 6	28 ± 13	37 ± 1	10 ± 2	20 ± 3	7± 1	10 ± 3	30 ± 4	10 ± 1
c		16 ± 2	19 ± 5	17 ± 2	10 ± 1	40 ± 4	20 ± 3	80 ± 8	30 ± 3	100 ± 9
d		26 ± 15	33 ± 8	16 ± 4	9 ± 1	9 ± 4	8 ± 2	10 ± 3	30 ± 5	50 ± 7
e		21 ± 15	24 ± 1	36 ± 2	20 ± 7	30 ± 2	10 ± 1	20 ± 3	30 ± 4	30 ± 5
f		290 ± 46	≥ 500	> 500	> 100	> 100	> 100	> 100	>100	>100
g		27 ± 18	41 ± 1	40 ± 7	20 ± 8	> 100	40 ± 6	> 100	30 ± 7	70 ± 11

[a] 50% inhibitory concentration or compound concentration required to inhibit cell proliferation by 50%.

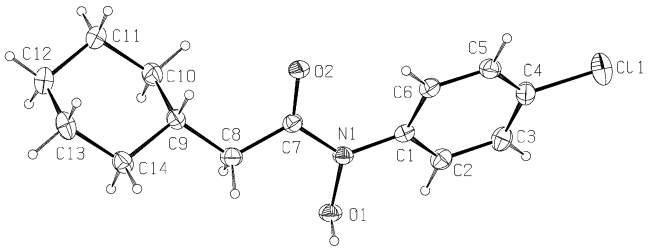


Figure 1. The molecular structure and labeling of **2d**. Displacement ellipsoids are drawn at the 20% probability level.

The structures of the novel compounds were deduced on the basis of analysis of IR spectra and chemical shifts and coupling constants in their ^1H and ^{13}C NMR spectra. The structure of **2d** was confirmed by its X-ray crystal structure analysis (Figure 1) (see Supporting Information).

Biological Results and Discussion

Cytostatic Activities. Hydroxamic acids **2a**−**g** were evaluated for their cytostatic activity against malignant tumor cell lines: murine leukemia (L1210), human T-lymphocyte cells (Molt4/C8 and CEM), colon carcinoma (SW620), laryngeal carcinoma (Hep2), pancreatic carcinoma (MiaPaCa2), breast carcinoma (MCF7), cervical carcinoma (HeLa), and human normal fibroblasts (WI38) (Table 1). **2b**,**d**,**e** exhibited rather marked inhibitory activity (IC_{50} = 7−10 μM) against pancreatic carcinoma, **2b**−**d** against colon carcinoma, **2d** against laryngeal carcinoma, and **2b**,**d** against breast carcinoma, but also against human normal fibroblasts. The best selectivity against tested tumor cell lines vs normal fibroblasts was exhibited by compounds **2a** and **2c**.

Antiviral Activities. Compounds **2b** and **2d**−**g** were evaluated against herpes simplex virus-1 (HSV-1; KOS), herpes simplex virus-2 (HSV-2; G), vaccinia virus, vesicular stomatitis virus, herpes simplex virus-1 (TK^-KOS ACV), herpes simplex virus-1 (TK^-VMW 1837), parainfluenza-3 virus, reovirus-1, Sindbis virus, Coxsackie virus B4, Punta Toro virus, and respiratory syncytial virus. **2a**−**e** and **2f** were tested against HIV-1 and HIV-2, whereas **2d**, **2e**, and **2g** were evaluated against thymidine kinase positive (TK^+) and negative (TK^-) strains of varicella-zoster virus (VZV) and cytomegalovirus (CMV, Table 2). Of all compounds examined, **2e** showed the most pronounced anti-CMV activity (EC_{50} = 1.5 and 0.8 μg mL^{-1}). On the other hand, compounds **2d** and **2f** showed selectivity, albeit modest, as antiviral agents against cytomegalovirus (**2d**, EC_{50} = 7.3 and 8.9 μg mL^{-1}, selectivity index (ratio CC_{50}/IC_{50}) 7−10; **2f**, EC_{50} = 7−13 μg mL^{-1}, selectivity index

Table 2. Activity of Hydroxamic Acids **2a**−**g** Against Cytomegalovirus in Human Embryonic Lung (HEL) Cell Cultures

	antiviral activity EC_{50}[a] (μg mL^{-1})		cytotoxicity (μg mL^{-1})	
Compd. **2a**−**g**	AD-169 strain	Davis strain	cell morphology (MCC)[b]	cell growth (CC_{50})[c]
2a	>20	>20	100	37
2b	10	8	100	20
2c	7.3	8.9	≥20	28
2d	7.9	6.2	50	129
2e	1.5	0.8	≥4	4.7
2f	13	7	100	>50
2g	8	8	100	>200
ganciclovir	0.37	0.37	≥400	nd[d]
cidofovir	0.18	0.29	≥ 400	24

[a] Effective concentration required to reduce virus plaque formation by 50%. Virus input was 100 plaque forming units (PFU). [b] Minimum cytotoxic concentration that causes a microscopically detectable alteration of cell morphology. [c] Cytotoxic concentration required to reduce cell growth by 50%. [d] Not determined.

10). These compounds therefore represent anti-CMV lead compounds for further synthetic optimization. **2b** showed some activity against HSV-2 (G) ($IC_{50} = 9.6\ \mu g\ mL^{-1}$) while **2e** showed some anti-HIV-1 activity in CEM cell cultures ($IC_{50} = 10\ \mu M$) (see Supporting Information).

Conclusions

The main purpose of this study was to evaluate new types of hydroxamic acid derivatives **2a**−**g** for their cytostatic and antiviral activities. Among the evaluated compounds, **2a** and **2c** showed the best selectivity against malignant tumor cell lines. **2e** exhibited the most pronounced anti-CMV activity ($EC_{50} = 1.5$ and 0.8 $\mu g\ mL^{-1}$) only at ≥5-fold lower than the cytotoxic concentration. **2d** and **2f** showed modest, albeit selective, activity against cytomegalovirus. These compounds are therefore anti-CMV leads for further synthetic optimization.

Experimental Section

Synthetic Procedure. (a) To a stirred solution of acyl chloride **1a**, **1d**, or **1e** (8 mmol), concentrated HCl (0.003 mL), and sodium dithionite (5 mg) in acetonitrile (40 mL) was added a solution of nitrosobenzene (7.2 mmol) in acetonitrile (40 mL)-dropwise during 1−3 h. The reaction mixture was stirred additionally for 3−22 h, and the solidified product was filtered off. Evaporation of the mother liquor under reduced pressure gave the oily residue, which was triturated with light petroleum (bp 40−80 °C). Separation of the organic phase and standing at room temperature afforded **2a**, **2d**, or **2e** as crystalline products. (b) To a solution of nitrosobenzene (3 mmol) in acetonitrile (25 mL) were added acyl chlorides **1b**, **1c**, or **1f** and concentrated HCl (0.25 mL) dropwise. The reaction mixture was stirred at room temperature for 5−22 h. Products **2b** and **2c** were isolated in the following way: The reaction mixture was neutralized with sodium hydrogen carbonate and filtered and the mother liquor evaporated under reduced pressure. The residual oil was triturated three times with light petroleum (bp 40−70 °C). Products **2b** and **2c** crystallized slowly from the separated petroleum extract. Compound **2e** was recrystallized from an acetonitrile/acetone (1:1) mixture. Yields of the reactions were in the range 1−9%, probably due to decomposition of nitrosobenzene and formation of the corresponding nonhalogenated hydroxamic acid analogues. Varying the solvents (methylene chloride, anhydrous toluene) and decreasing the reaction temperature to 0 °C did not significantly improve the yields.

***N*-Hydroxy-*N*-(4-chlorophenyl)-cyclopropylformamide (2a).** Product was recrystallized from cyclohexane. Yield: 0.075 g (5%). Mp: 114−117 °C. Mp lit.:[12,15] 118−120 °C.

***N*-Hydroxy-*N*-(4-chlorophenyl)-3-cyclopentylpropionamide (2b).** Yield: 0.05 g (6%). Mp: 123−125 °C.

***N*-Hydroxy-*N*-(4-chlorophenyl)-cyclohexylformamide (2c).** Yield: 0.02 g (3%). Mp: 137−140 °C, Mp lit.:[12] 137−140 °C.

***N*-Hydroxy-*N*-(4-chlorophenyl)-cyclohexylacetamide (2d).** Product was recrystallized from a light petroleum/cyclohexane 3:1 mixture, additionally purified by preparative thin-layer chromatography using a cyclohexane/ethyl acetate/methanol (3:1:0.3) mixture as eluent, and finally recrystallized from cyclohexane. Yield: 0.059 g (3%). Mp: 123−126 °C.

***N*-Hydroxy-*N*-(4-chlorophenyl)-3-cyclohexylpropionamide (2e).** The crude product was purified by recrystallization and preparative thin-layer chromatography using the same solvent system as for **2d.** Yield: 0.013 g (1%). Mp: 133−135 °C.

***N*-Hydroxy-*N*-(4-chlorophenyl)-adamantylformamide (2f).** Yield: 0.070 g (9%). Mp 195−198 °C.

***N*-Hydroxy-*N*-phenyl-cyclohexylacetamide (2g).** *N*-Phenylhydroxylamine (0.33 g, 3 mmol) dissolved in toluene (30 mL) and *N*-methylmorpholine (0.3049 g, 3 mmol) in toluene (15 mL) were added simultaneously to a stirred solution of cyclohexaneacetyl chloride (0.482 g, 3 mmol) in toluene (55 mL), previously cooled to 0 °C. The reaction mixture was allowed to warm gradually to room temperature, stirred for 3 h, and then extracted with water, saturated sodium hydrogen carbonate solution, 1% HCl solution, and water. The organic layer was dried over anhydrous sodium sulfate, filtered, and evaporated under reduced pressure. Triturating of the oily product with cyclohexane and cooling to 0 °C gave the crystalline product **2g**. Yield: 0.335 g (48%). Mp: 116−119 °C.

X-ray Determination. Details of the crystallographic determination are given in the Supporting Information. Crystallographic data excluding structure factors for the structure reported in this paper have been deposited with the Cambridge Crystallographic Data Center as supplementary publication number CCDC-234603. Copies of data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, U.K. [fax, +44-(0)1223−336033; e-mail, deposit@ccdc.cam.ac.uk].

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Supporting Information Available: Experimental details; $^{1}H/^{13}C$ NMR, IR, elemental analysis, and crystallographic data; cytotoxicity and antiviral activity data of **2a**−**g** in E_6SM cell cultures; protocols for the biological testing. This material is available free of charge via the Internet at http://pubs.acs.org.

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