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Synthesis and biological evaluation of aziridin-1-yl oxime-based vorinostat analogs as anticancer agents

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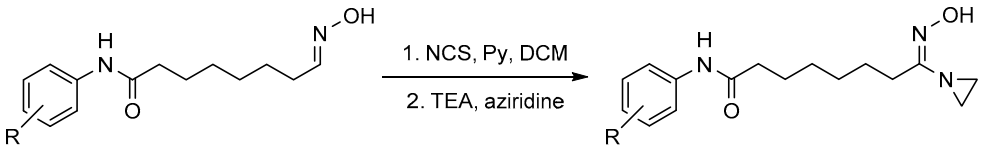
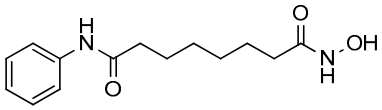


The suberoyl anilide hydroxamic acid (vorinostat) analogs with the aziridin-1-yl oxime moiety as a possible metal chelating functionality have been synthesized. Their biological activity and stability under physiological conditions have been evaluated. Although some of the synthesized compounds demonstrated high antiproliferative activity against human HT1080 fibrosarcoma (HT1080, IC_{50} 0.3–7.7 μM) comparable to vorinostat (HT1080, IC_{50} 2.4 μM), they showed only weak histone deacetylase inhibition activity in HeLa cell line extracts.

Keywords: aldoxime, aziridin-1-yl oxime, histone deacetylase, hydroximoyl chloride, suberoyl anilide hydroxamic acid, cytotoxic activity.

Vorinostat (suberoyl anilide hydroxamic acid or SAHA) (**1**) is a linear hydroxamic acid compound that is a potent enzyme inhibitor with multiple targets including I and II class histone deacetylase (HDAC). SAHA consists of a chelating group (hydroxamic acid moiety) and six-carbon spacer (connection unit) attached to a hydrophobic group (phenyl). It has been shown that hydroxamic acid moiety of SAHA binds to a zinc ion in the HDAC catalytic site allowing the rest of the molecule to lie along the protein surface with the phenyl ring oriented out of the catalytic pocket.[1]



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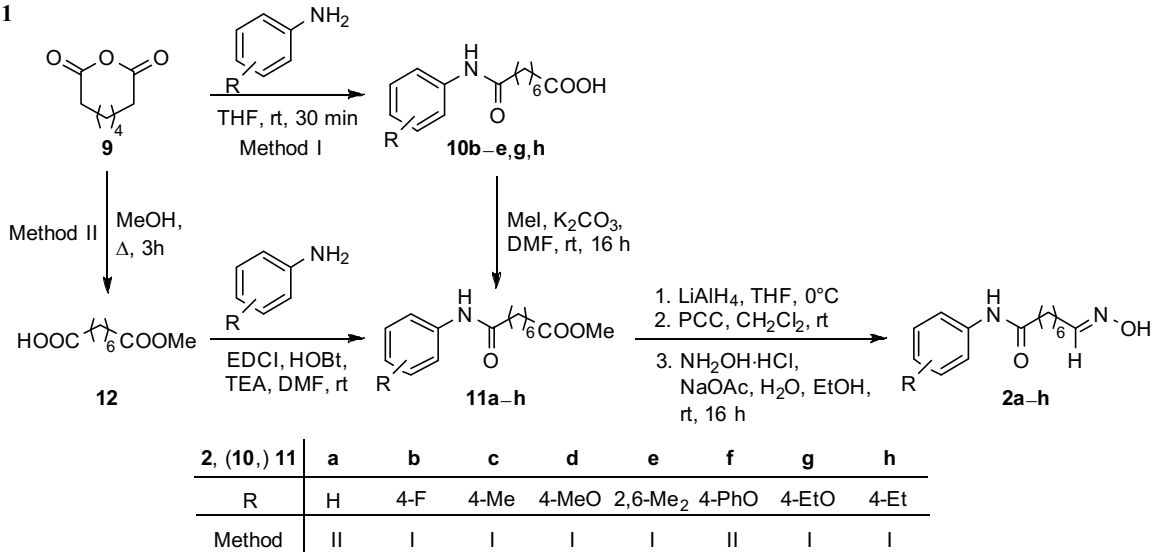
The aziridin-1-yl oxime group shows high structural similarity with hydroxamic acid. Moreover the aziridin-1-yl oxime moiety has a potential to form a covalent interaction, particularly when activated with zinc ions. Following this idea we aimed to synthesize a small molecule library of SAHA analogs and test their anticancer, as well as HDAC inhibitory activity. It has been already shown that aromatic compounds containing aziridin-1-yl oxime groups show high cytotoxic activity against different cancer cell lines.[2]

The key intermediates in the described strategy are the aliphatic aldoximes **2a**–**h**, **3**, and **4**, which were converted to the aziridin-1-yl oximes in a two-step one-pot reaction: formation of hydroximoyl chloride and addition of *N*-nucleophile (aziridine, aziridine derivative) to the nitrile oxide generated from the hydroximoyl chloride *in situ.* An alternative approach through the appropriate nitrile oxide starting from the corresponding amidoxime has been investigated by us previously.[3] Unfortunately this approach failed to provide nitrile oxides bearing an alkyl moiety.

The routes used for synthesis of SAHA analogs **5a**–**l**, **6**–**8** are depicted in Schemes 1–7. Our initial attempt to synthesize suberoyl anilide was the condensation of suberic acid with aniline at high temperatures. However, the desired compound was formed in low yield together with by-products. This prompted us to use a different synthetic route proposed by Mai.[4] Thus, suberoyl anhydride (**9**) was obtained in good yields by heating suberic acid with acetic anhydride.[4] Anhydride **9** was treated with aniline or aniline derivatives to afford monoamides **10b**–**e**,**g**,**h** which were converted into the corresponding esters **11b**–**e**,**g**,**h** by reaction with methyl iodide in the presence of potassium carbonate (Scheme 1, Method I). The second approach to prepare intermediates **11** involved the conversion of suberic acid anhydride (**9**) to its monomethyl ester **12** which was coupled with aniline or aniline derivatives using *N*-(3-dimethylaminopropyl)-*N*'-ethylcarbodiimide (EDCI) (Scheme 1, Method II).

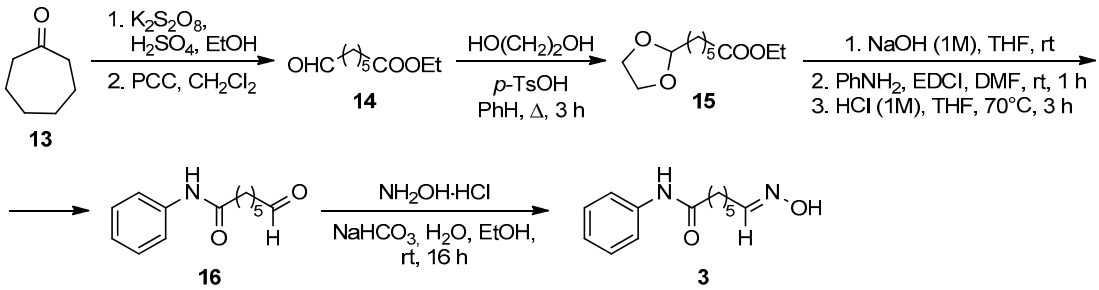
Esters **11a**–**h** were converted to aldehydes by the reduction of the ester functionality with lithium aluminum hydride (LAH) in THF followed by the oxidation with pyridinium chlorochromate (PCC). The reaction of aldehydes with hydroxylamine hydrochloride in the presence of $NaHCO_3$ or NaOAc afforded aldoximes **2a**–**h**.

Scheme 1



2, (**10**,) **11**	**a**	**b**	**c**	**d**	**e**	**f**	**g**	**h**
R	H	4-F	4-Me	4-MeO	2,6-Me_2	4-PhO	4-EtO	4-Et
Method	II	I	I	I	I	II	I	I

Scheme 2

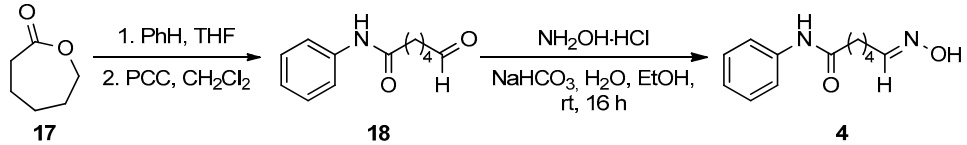


Synthesis of oxime **3** was accomplished by the reaction of cycloheptanone (**13**) with potassium persulfate in ethanol in the presence of sulfuric acid, and the resulting 6-hydroxyheptanoic acid ethyl ester was oxidized with PCC (Scheme 2).[5] The aldehyde group of the obtained intermediate **14** was protected as acetal to give compound **15**. Basic hydrolysis of the ester group and the coupling of the resulting acid with aniline was followed by acetal group cleavage. The resulting aldehyde **16** was successfully converted to aldoxime **3** in the presence of $NaHCO_3$.

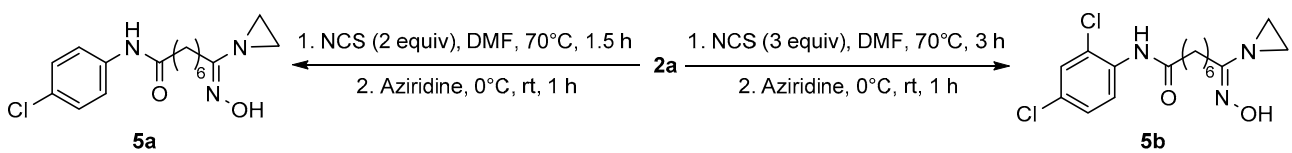
The synthetic route to the intermediate **4** starting from ε-caprolactone (**17**) is outlined in Scheme 3. The caprolactone cycle was opened in the reaction with aniline to give the anilide alcohol which was subsequently oxidized with PCC to afford aldehyde **18**[6] which was converted to the aldoxime **4**.

Aziridin-1-yl oximes were prepared by chlorination of oximes **2a**–**h**, **3**, **4**. This provided hydroximoyl chlorides[7] directly converted *in situ* to the nitrile oxide by treatment the reaction mixture with triethylamine in great excess. The following addition of aziridine allowed to obtain final compounds **5a**–**l** in low or moderate yields (Schemes 4, 5). When *N*-chlorosuccinimide (NCS) in DMF was used for the chlorination of aldoxime **2a**, hydroximoyl chlorides with chlorinated benzene ring were formed. This resulted, after the treatment with aziridine, in monochlorinated product **5a** and dichlorinated product **5b** (Scheme 4).

Scheme 3

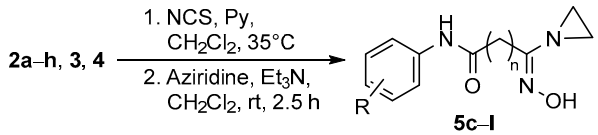


Scheme 4



The formation of hydroximoyl chloride without phenyl ring chlorination was successfully carried out with NCS and catalytic amount of pyridine in chloroform or dichloromethane (Scheme 5).[8]

Scheme 5



5	**c**	**d**	**e**	**f**	**g**	**h**	**i**	**j**	**k**	**l**
R	H	4-F	4-Me	4-MeO	2,6-Me_2	4-PhO	4-EtO	4-Et	H	H
n	6	6	6	6	6	6	6	6	5	4

To investigate the structure–activity relationship of aziridin-1-yl oxime moiety, substituted analogs **6** and **7** were prepared following a similar synthetic protocol to that shown in Scheme 4. 7-Azabicyclo[4.1.0]heptane required for the synthesis of compound **6** was obtained according to the procedure described in literature starting from cyclohexene oxide (Scheme 6).[9]

Finally, the synthesis of amidoxime **8** was realized starting from the commercially available 6-bromohexanoic acid (**19**) which was converted to the corresponding 6-cyanohexanoic acid in the reaction with sodium cyanide, followed by the reaction with aniline in the presence of EDCI.[10] Transformation of the nitrile group in compound **20** to amidoxime group by the reaction with hydroxylamine in isopropanol gave the desired product **8** in quantitative yield.

Azirindin-1-yl oxime **5a** was used as a model compound for the investigation of the configuration of oxime. The (*Z*)-configuration of the compound was confirmed by 2D NMR NOESY spectra which showed a NOE between the hydroxyl proton and the aziridine methylene protons (Fig. 1).

The stability of compound **5a** was assessed by HPLC method. These studies indicated that compound **5a** was stable at least 4 h in 0.05 M phosphate buffer solution (pH 7.4, 25°C), however after 24 h, it content in the analyzed solution was 86%, and after 120 h, only 32%.

All synthesized aziridin-1-yl oximes **5a**–**l**, **6**–**8**, structural analogs of SAHA, were tested for their cytotoxic activity on different cell lines (mouse embryonic

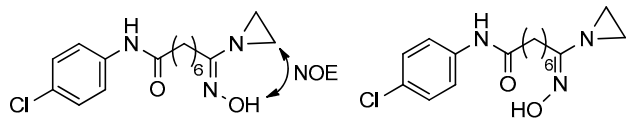


Figure 1. The *Z*- and *E*-isomers of compound **5a**.

fibroplasts 3T3, human fibrosarcoma HT1080, mouse hepatoma MG22A) using different colorimetric methods (NR – neutral red, MTT – 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, and CV – crystal violet) (Table 1).

Our results demonstrate that there is a difference in cytotoxic activity of compounds bearing diverse substituents at the phenyl moiety. Thus, compounds having doubly substituted phenyl rings **5b**,**g** are inactive, however, the activities of other SAHA analogs are comparable. A variety of substitution is tolerated at position 4 of the phenyl group when $n = 6$.

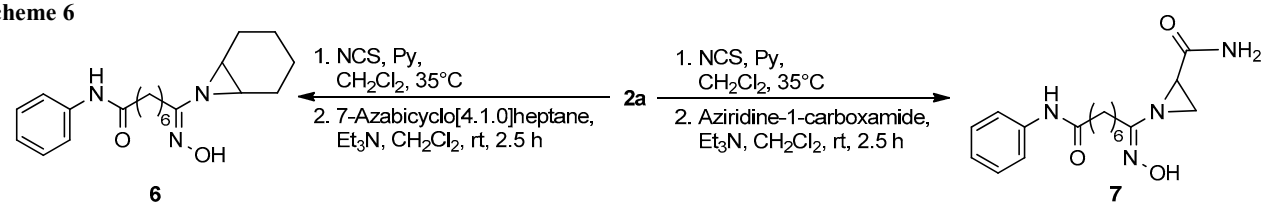
A series of homologous compounds was synthesized with a different length of the spacer unit between phenyl and aziridin-1-yl oxime groups. Thus, compounds **5k**,**l**, containing five- and four-carbon spacer, respectively, dramatically lost cytotoxic activity in comparison with compound **5c** containing a six-carbon spacer.

The influence of the substitution at the aziridine cycle on the example of compounds **6** and **7** was also examined. The introduction of azabicyclic function (compound **6**) or carboxamide substituent (compound **7**) contributes to the loss of the cytotoxic activity. The aziridine substitution with amidoxime functionality resulted in inactive compound **8**, but it might also be argued that its weak cytotoxic activity resulted from the shorter spacer unit.

The obtained final compounds can be allocated in one of the four toxicity categories based on their acute oral toxicity properties according to the cut-off criteria established by the current EU regulations.[12] Thus, most of the newly synthesized SAHA analogs **5a**,**c**–**f**,**h**,**j** can be classified as slightly toxic compounds (category 3), while compounds **5b**,**g**,**i**,**k**,**l**, **6**–**8** are practically non-toxic (category 4).

Selected SAHA analogs **5a**,**c**,**d**,**f**,**h**–**j** were tested for their ability to act as a histone deacetylase inhibitors in Hella extract using SAHA as a reference inhibitor (Table 2).

Scheme 6



Scheme 7

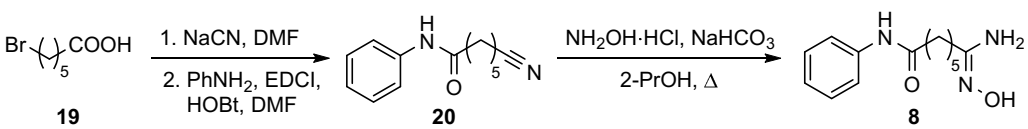
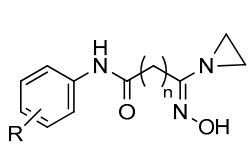
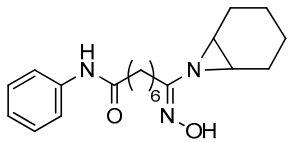


Table 1. Cytotoxic effect of SAHA analogs on different monolayer cell lines



5a–l



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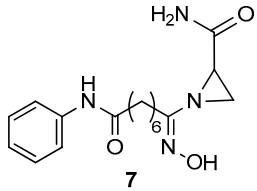

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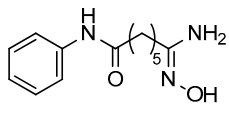

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Compound	*n*	R	3T3 LD_{50}, mg/kg (NR)	HT1080 IC_{50} μ M (CV)	HT1080 IC_{50}, μ M (MTT)	MG22A IC_{50}, μ M (CV)	MG22A IC_{50}, μ M (MTT)
SAHA	6	–	NT*	2.4**	2.4**	NT	NT
5a	6	4-Cl	97	0.6	0.6	1.2	0.9
5b	6	2,4-Cl_2	358	>10	NT	>10	NT
5c	6	H	87	0.6	0.3	1.0	1.0
5d	6	4-F	92	0.9	0.6	0.6	0.9
5e	6	4-Me	121	0.8	1.3	3.6	4.6
5f	6	4-MeO	128	1.0	1.3	2.7	2.7
5g	6	2,6-Me_2	482	>10	>10	>10	>10
5h	6	4-PhO	153	NT	1.0	NT	0.5
5i	6	4-EtO	233	NT	1.5	NT	3.0
5j	6	4-Et	146	NT	0.9	NT	0.6
5k	5	H	220	7.3	3.6	7.3	3.6
5l	4	H	261	3.8	3.8	7.6	7.7
6	6	–	275	>10	>10	>10	>10
7	6	–	2191	>10	NT	>10	NT
8	5	–	873	>10	>10	>10	>10

* NT – not tested.
** See ref.[11]

The results in Table 2 indicate that aziridin-1-yl oxime analogs were inactive in HDAC inhibition test. The synthesized SAHA analogs had significantly lower IC_{50} values than SAHA. The poor HDAC inhibition of aziridin-1-yl oximes may indicate that HDAC is not the primary pharmacological target responsible for their biological activity.

In summary, we have synthesized a series of SAHA analogs in which hydroxamic acid moiety is replaced by aziridin-1-yl oxime group. We have shown that some of the obtained compounds exhibit significant antiproliferative activity against the growth of monolayer cell lines including human HT1080 fibrosarcoma. However, all tested compounds are weak inhibitors of HDAC suggesting that aziridin-1-yl oxime analogs of SAHA do not enter covalent interaction with zinc atom in the HDAC catalytic pocket. The new enzymatic target of the obtained compound library should be established in the future.

Table 2. HDAC inhibition activity of selected aziridin-1-yl oximes

Compound	IC_{50}, μ M, 30 min incubation	IC_{50}, μ M, 3 h incubation
SAHA	0.1	0.1
5a	177	NT
5c	61	70
5d	36	37
5f	13	20
5h	32	31
5i	28	30
5j	40	24

Experimental

^{1}H NMR spectra were recorded on Varian 400 Mercury (400 MHz), Bruker Fourier 300 (300 MHz), and Varian 200 (200 MHz) spectrometers. ^{13}C NMR spectra were recorded on a Varian 400 Mercury spectrometer (100 MHz). The ^{1}H chemical shifts are given relative to residual proton signal of DMSO-d_6 signal (2.50 ppm) or $CDCl_3$ (7.26 ppm), the ^{13}C chemical shifts – relative to DMSO-d_6 signal (39.5 ppm). Ultra-performance liquid chromatography (UPLC) data were obtained on a Waters mass spectrometer (column Acquity UPLC BEH-C18) using electrospray ionization (ESI) in positive mode. Elemental analysis was performed on a Carlo Erba EA1108 elemental analyzer. Melting points were determined on a Standard Research Systems Optimelt melting point apparatus and were uncorrected. Purification of compounds was performed by flash silica gel chromatography using Merck Kieselgel (230–400 mesh), eluting with ethyl acetate and light petroleum ether in different ratios. Thin-layer chromatography was performed on silica gel and was visualized by staining with $KMnO_4$ or in UV at 210 or 254 nm. Reagents and starting materials were obtained from commercial sources and used as received.

Synthesis of target compounds 5a–c was realized starting from intermediate **12** (Method II) as follows.

8-Methoxy-8-oxooctanoic acid (12). The solution of compound **9** (2.0 g, 12.8 mmol) in methanol (6.5 ml) was stirred at reflux in pressure tube during 3 h. The reaction mixture was evaporated to dryness and treated with ether. The white precipitate formed was filtered off. Yield 1.92 g (80%). ^{1}H NMR spectrum (400 MHz, DMSO-d_6), δ, ppm (*J*, Hz): 1.15–1.28 (4H, m, $(C\underline{H}_2)_2(CH_2)_2CO_2CH_3$); 1.37–1.52 (4H, m, $HO_2C(CH_2C\underline{H}_2CH_2)_2CO_2CH_3$); 2.14 (2H, t, *J* = 7.4, $C\underline{H}_2CO_2CH_3$); 2.24 (2H, t, *J* = 7.4, $C\underline{H}_2CO_2H$); 3.54 (3H, s, CO_2CH_3, partially overlapped with water signal); 11.94 (1H, br. s, CO_2H).

Methyl 8-oxo-8-(phenylamino)octanoate (11a). Triethylamine (1.7 ml, 12 mmol, 1.5 equiv), EDCI (2.10 g, 10.4 mmol, 1.3 equiv), and HOBT (1.40 g, 10.4 mmol, 1.3 equiv) were added to a solution of the intermediate **12** (1.38 g, 8 mmol) in DMF (8 ml), followed by the addition of aniline (0.73 g, 8 mmol). The reaction mixture was stirred overnight at room temperature, treated with water (20 ml), and extracted with AcOEt. The organic phase was washed with water, dried over Na_2SO_4, filtrated, and evaporated. The residue was purified by flash chromatography (eluent AcOEt–hexane, 1:2). Yield 1.62 g (77%), white amorphous solid. ^{1}H NMR spectrum (200 MHz, DMSO-d_6), δ, ppm (*J*, Hz): 1.19–1.34 (4H, m, $(C\underline{H}_2)_2(CH_2)_2CO_2CH_3$); 1.42–1.62 (4H, m, $NH(CH_2C\underline{H}_2CH_2)_2CO_2CH_3$); 2.21–2.29 (4H, m, $NHC\underline{H}_2(CH_2)_4C\underline{H}_2CO_2CH_3$); 3.54 (3H, s, $COOCH_3$); 6.98 (1H, t, *J* = 7.4, H-4 Ph); 7.24 (2H, t, *J* = 7.9, H-3,5 Ph); 7.54 (2H, d, *J* = 7.4, H-2,6 Ph); 9.80 (1H, s, NH).

8-Hydroxy-*N*-phenyloctanamide. Lithium alumohydride (0.12 g, 3.0 mmol, 1.1 equiv) was slowly added to a solution of intermediate **11a** (0.73 g, 2.8 mmol) in dry THF (14 ml) at 0°C. After the full conversion of the starting material (TLC control), the reaction mixture was treated with methanol (1 ml), evaporated to dryness, then diluted with ethyl acetate, and extracted with water. The organic phase was dried over Na_2SO_4, filtered, and evaporated. The obtained crude product was purified by flash chromatography (eluent AcOEt–hexane, 1:2, gradient to AcOEt). Yield 0.51 g (79%), white solid. ^{1}H NMR spectrum (200 MHz, DMSO-d_6), δ, ppm (*J*, Hz): 1.21–1.27 (6H, m, $(C\underline{H}_2)_3(CH_2)_2OH$); 1.34–1.40 (2H, m, $NHC(O)CH_2C\underline{H}_2$); 1.49–1.56 (2H, m, $C\underline{H}_2CH_2OH$); 2.25 (2H, t, *J* = 7.4, $NHC(O)C\underline{H}_2$); 3.29–3.34 (2H, m, $C\underline{H}_2OH$, partially overlapped with water signal); 4.28 (1H, t, *J* = 5.1, OH); 6.95–7.00 (1H, m, H-4 Ph); 7.22–7.27 (2H, m, H-3,5 Ph); 7.52–7.57 (2H, m, H-2,6 Ph); 9.79 (1H, s, NH).

8-Oxo-*N*-phenyloctanamide. A solution of the obtained 8-hydroxy-*N*-phenyloctanamide (0.43 g, 1.8 mmol) in CH_2Cl_2 (18 ml) was added to a suspension of PCC (0.79 g, 3.7 mmol, 2 equiv) and Celite (0.79 g) in CH_2Cl_2 (18 ml). The reaction was stirred until the full conversion of the starting material (TLC control) and filtered through a short silica gel column, eluting with ether. The filtrate was evaporated. Yield 0.32 g (74%), white solid. ^{1}H NMR spectrum (200 MHz, DMSO-d_6), δ, ppm (*J*, Hz): 1.22–1.30 (4H, m, $(C\underline{H}_2)_2(CH_2)_2CHO$); 1.44–1.60 (4H, m, $NHC(O)(CH_2C\underline{H}_2CH_2)_2CHO$); 2.25 (2H, t, *J* = 7.4, $NHC(O)C\underline{H}_2$); 2.38 (2H, td, *J* = 7.4, *J* = 1.6, $C\underline{H}_2CHO$); 6.98 (1H, t, *J* = 7.4, H-4 Ph); 7.19–7.29 (2H, m, H-3,5 Ph); 7.55 (2H, d, *J* = 7.5, H-2,6 Ph); 9.63 (1H, t, *J* = 1.6, $CH_2C\underline{H}O$); 9.80 (1H, s, NH).

8-(Hydroxyimino)-*N*-phenyloctanamide (2a). The obtained 8-oxo-*N*-phenyloctanamide (0.31 g, 1.3 mmol) was dissolved in ethanol (5 ml), and sodium acetate (0.16 g, 2.0 mmol, 1.5 equiv) was added to the solution in one portion followed by the addition of hydroxylamine hydrochloride (0.14 g, 2.0 mmol, 1.5 equiv) solution in water (10 ml). The reaction mixture was stirred for 16 h at room temperature, and the precipitate that formed was filtered off and dried *in vacuo*. Yield 0.29 g (89%), amorphous solid. ^{1}H NMR spectrum (400 MHz, DMSO-d_6), δ, ppm (*J*, Hz): 1.24–1.31 (4H, m, $(C\underline{H}_2)_2(CH_2)_2CHNOH$); 1.34–1.41 (2H, m, $NHC(O)CH_2C\underline{H}_2$); 1.49–1.58 (2H, m, $C\underline{H}_2CH_2CHNOH$); 2.15–2.22 (2H, m, $NHC(O)C\underline{H}_2$); 2.25 (2H, t, *J* = 7.4, $C\underline{H}_2CHNOH$); 6.60 (1H, t, *J* = 5.4, $C\underline{H}NOH$); 6.95–7.00 (1H, m, H-4 Ph); 7.20–7.27 (2H, m, H-3,5 Ph); 7.49–7.55 (2H, m, H-2,6 Ph); 9.80 (1H, s, NH); 10.68 (1H, s, NOH).

(*Z*)-8-(Aziridin-1-yl)-*N*-(4-chlorophenyl)-8-(hydroxyimino)octanamide (5a). Aldoxime **2a** (0.30 g, 1.21 mmol) was dissolved in DMF (3 ml); NCS (0.34 g, 2.54 mmol, 2.1 equiv) was added in one portion. The reaction mixture was kept at 70°C for 1.5 h, then cooled to 0°C, and aziridine (0.78 ml, 15 mmol) was added. The reaction mixture was allowed to warm up to room temperature, stirred for an additional 1 h, diluted with AcOEt, and extracted with water. The organic phase was washed several times with AcOEt, dried over Na_2SO_4, filtrated, and evaporated at water bath temperature 30°C. The residue was purified by flash chromatography eluting with 5% Et_3N in AcOEt. Yield 165 mg (42%), white solid, mp 127.5–129°C. ^{1}H NMR spectrum (400 MHz, DMSO-d_6), δ, ppm (*J*, Hz): 1.19–1.37 (4H, m, $(C\underline{H}_2)_2(CH_2)_2C(NC_2H_4)NOH$); 1.34–1.68 (4H, m, $(CH_2C\underline{H}_2CH_2)_2C(NC_2H_4)NOH$); 1.99 (4H, s, $2CH_2$ aziridine); 2.02–2.09 (2H, m, partially overlapped with aziridine signal, $NHC(O)C\underline{H}_2$); 2.27 (2H, t, *J* = 7.4, $C\underline{H}_2C(NC_2H_4)NOH$); 7.31 (2H, d, *J* = 8.9, H-3,5 Ar); 7.59 (2H, d, *J* = 8.9, H-2,6 Ar); 9.49 (1H, s, NOH,); 9.94 (1H, s, NH). ^{13}C NMR spectrum (100 MHz, DMSO-d_6), δ, ppm: 25.4; 26.3; 26.6; 28.8; 28.9; 31.4; 36.8; 121.0; 126.9; 129.0; 138.8; 156.9 (C=NOH); 171.9 (C=O). Found, %: C 58.40; H 6.70; N 12.60. $C_{16}H_{22}ClN_3O_2$. Calculated (with 1.4% H_2O): C 58.53; H 6.91; N 12.80.

(*Z*)-8-(Aziridin-1-yl)-*N*-(2,4-dichlorophenyl)-8-(hydroxyimino)octanamide (5b). Aldoxime **2a** (0.50 g, 2 mmol) was dissolved in DMF (10 ml); NCS (0.81 g, 6 mmol, 3 equiv) was added, and the reaction mixture was kept at 70°C for 1 h. An additional amount of NCS (0.27 g, 2 mmol) was added, and reaction mixture was heated for 3 h (full conversion of starting material, TLC control), then cooled to 0°C, and aziridine (0.52 ml, 10 mmol) was added. The reaction mixture was allowed to warm up to room temperature, stirred for an additional 1 h, diluted with AcOEt, and extracted with water. The organic phase was washed several times with AcOEt, dried over Na_2SO_4, filtrated, and evaporated at water bath temperature 30°C. The residue was treated with ether, and the precipitate that formed was

filtered and dried. Yield 210 mg (29%), white solid, mp 112–114°C. ^{1}H NMR spectrum (400 MHz, DMSO-d_6), δ, ppm (*J*, Hz): 1.22–1.37 (4H, m, (C$\underline{H}_2$)$_2$(CH$_2$)$_2$C(NC$_2$H$_4$)NOH); 1.44–1.63 (4H, m, (CH$_2$C$\underline{H}_2$CH$_2$)$_2$C(NC$_2$H$_4$)NOH); 2.02 (4H, s, 2CH$_2$ aziridine); 2.08 (2H, t, *J* = 7.2, NHC(O)C$\underline{H}_2$); 2.37 (2H, t, *J* = 7.2, C$\underline{H}_2$C(NC$_2$H$_4$)NOH); 7.39 (1H, dd, *J* = 8.8, *J* = 2.3, H Ar); 7.63 (1H, d, *J* = 2.3, H Ar); 7.67–7.74 (1H, m, H Ar); 9.49 (1H, s, NOH); 9.52 (1H, s, NH). ^{13}C NMR spectrum (100 MHz, DMSO-d_6), δ, ppm: 25.4; 26.3; 26.6; 28.7; 28.8; 31.3; 36.1; 127.9; 129.3; 129.7; 134.7; 156.9 (C=NOH); 172.1 (C=O). Found, %: C 51.50; H 5.58; N 11.14. $C_{16}H_{21}Cl_2N_3O_2$. Calculated (with 4.8% HCl), %: C 51.04; H 5.76; N 11.16.

(*Z*)-8-(Aziridin-1-yl)-8-(hydroxyimino)-*N*-phenyloctanamide (5c). Pyridine (0.1 ml) was added to a solution of NCS (28 mg, 0.2 mmol) in CH_2Cl_2 (2 ml). The solution was stirred for 15 min, then aldoxime **2a** (50 mg, 0.2 mmol) was added, and the stirring was continued at 35°C until the reaction mixture became clear. After cooling to room temperature, aziridine (32 μl, 0.6 mmol, 3 equiv) and triethylamine (58 μl, 0.4 mmol, 2 equiv) were added to the reaction mixture, which was then stirred for 2.5 h at room temperature. The reaction mixture was treated with water and extracted with AcOEt. The organic phase was dried over Na_2SO_4, filtered, and evaporated at water bath temperature 30°C. The residue was purified by flash chromatography (eluent 5% Et_3N in AcOEt). Yield 26% (15 mg), white powder, mp 115–117°C. ^{1}H NMR spectrum (400 MHz, DMSO-d_6), δ, ppm (*J*, Hz): 1.23–1.32 (4H, m, (C$\underline{H}_2$)$_2$(CH$_2$)$_2$C(NC$_2$H$_4$)NOH); 1.40–1.62 (4H, m, (CH$_2$C$\underline{H}_2$CH$_2$)$_2$C(NC$_2$H$_4$)NOH); 1.98 (4H, s, 2CH$_2$ aziridine); 2.04 (2H, t, *J* = 7.4, NHC(O)C$\underline{H}_2$); 2.25 (2H, t, *J* = 7.4, C$\underline{H}_2$C(NC$_2$H$_4$)NOH); 6.97 (1H, t, *J* = 7.4, H-4 Ph); 7.24 (2H, t, *J* = 7.8, H-3,5 Ph); 7.54 (2H, d, *J* = 7.8, H-2,6 Ph); 9.46 (1H, s, NOH); 9.80 (1H, s, NH). ^{13}C NMR spectrum (100 MHz, DMSO-d_6), δ, ppm: 25.5; 26.3; 26.6; 28.8; 28.9; 31.3; 36.8; 119.4; 123.3; 129.0; 139.8; 156.9 (C=NOH); 171.6 (C=O). Found, %: C 65.34; H 8.02; N 13.78. $C_{16}H_{23}N_3O_2$. Calculated (with 7% AcOEt), %: C 65.57; H 8.09; N 13.49.

Synthesis of the target compound 5d was realized starting from intermediate **10b** (Method I) as follows.

8-[(4-Fluorophenyl)amino]-8-oxooctanoic acid (10b). 4-Fluorophenylaniline (117 μl, 1.2 mmol) was added to a stirred solution of suberic acid anhydride (**9**) (190 mg, 1.2 mmol) in anhydrous THF (12 ml). After stirring at room temperature for 30 min, the solid (bisamide) was filtered off (yield 10–15%), and the filtrate was evaporated. The residue was treated with diethyl ether, and the precipitate was filtered and dried *in vacuo* at room temperature. Yield 190 mg (63%). ^{1}H NMR spectrum (400 MHz, DMSO-d_6), δ, ppm (*J*, Hz): 1.19–1.26 (4H, m, (C$\underline{H}_2$)$_2$(CH$_2$)$_2$CO$_2$H); 1.39–1.49 (4H, m, (CH$_2$C$\underline{H}_2$CH$_2$)$_2$CO$_2$H); 2.15 (4H, t, *J* = 7.4, NHC(O)C$\underline{H}_2$(CH$_2$)$_4$C$\underline{H}_2$CO$_2$H); 6.46–6.52 (2H, m, H-3,5 Ar); 6.76–6.82 (2H, m, H-2,6 Ar); 9.90 (1H, s, NH); 11.92 (1H, br. s, COOH).

Methyl 8-[(4-fluorophenyl)amino]-8-oxooctanoate (11b). Anhydrous potassium carbonate (0.552 g, 4 mmol) was added to a stirred solution of intermediate **10b** (0.534 g, 2 mmol) in DMF (2 ml) in one portion, and the mixture was stirred for 30 min. Methyl iodide (75 μl, 1.2 mmol) was then added, and the reaction mixture was stirred for 16 h at room temperature. Then it was diluted with water (20 ml) and extracted with ethyl acetate (2×10 ml). The organic layers were combined and washed with water (2×20 ml) and brine (20 ml), dried over potassium sulfate, filtered, evaporated, and treated with Et_2O. Yield 0.36 g (64%). ^{1}H NMR spectrum (200 MHz, DMSO-d_6), δ, ppm (*J*, Hz): 1.18–1.33 (4H, m, (C$\underline{H}_2$)$_2$(CH$_2$)$_2$CO$_2$CH$_3$); 1.38–1.66 (4H, m, (CH$_2$C$\underline{H}_2$CH$_2$)$_2$CO$_2$CH$_3$); 2.19–2.33 (4H, m, NHC(O)C$\underline{H}_2$(CH$_2$)$_4$C$\underline{H}_2$CO$_2$CH$_3$); 3.56 (3H, s, CO$_2$CH$_3$); 7.04–7.16 (2H, m, H-3,5 Ar); 7.58 (2H, dd, *J* = 9.1, *J* = 5.0, H-2,6 Ar); 9.89 (1H, s, NH).

Synthesis of aldoxime 2b was carried out starting from compound **11b** analogously to the synthesis of compound **2a**.

***N*-(4-Fluorophenyl)-8-hydroxyoctanamide** was precipitated from Et_2O. Yield 68%. ^{1}H NMR spectrum (200 MHz, DMSO-d_6), δ, ppm (*J*, Hz): 1.18–1.44 (8H, m, (C$\underline{H}_2$)$_4$(CH$_2$)$_2$OH); 1.48–1.63 (2H, m, C$\underline{H}_2$CH$_2$OH); 2.26 (2H, t, *J* = 7.3, NHC(O)C$\underline{H}_2$); 3.32–3.36 (2H, m, C$\underline{H}_2$OH partially overlapped with water signal); 4.32 (1H, t, *J* = 5.2, OH); 7.03–7.17 (2H, m, H-3,5 Ar); 7.58 (2H, dd, *J* = 9.2, *J* = 5.1, H-2,6 Ar); 9.89 (1H, s, NH).

***N*-(4-Fluorophenyl)-8-oxooctanamide** was purified by flash chromatography (eluent AcOEt–hexane, 1:1). Yield 72%. ^{1}H NMR spectrum (400 MHz, DMSO-d_6), δ, ppm (*J*, Hz): 1.17–1.36 (4H, m, (C$\underline{H}_2$)$_2$(CH$_2$)$_2$CHO); 1.42–1.64 (4H, m, (CH$_2$C$\underline{H}_2$CH$_2$)$_2$CHO); 2.25 (2H, t, *J* = 7.1, NHC(O)C$\underline{H}_2$); 2.39 (2H, t, *J* = 7.1, C$\underline{H}_2$CHO, partially overlapped with DMSO signal); 7.03–7.15 (2H, m, H-3,5 Ar); 7.57 (2H, dd, *J* = 8.9, *J* = 5.2, H-2,6 Ar); 9.64 (1H, s, CHO); 9.88 (1H, s, NH).

(4-Fluorophenyl)-8-(hydroxyimino)octanamide (2b) was precipitated from Et_2O. Yield 56%. ^{1}H NMR spectrum (400 MHz, DMSO-d_6), δ, ppm (*J*, Hz): 1.20–1.45 (6H, m, (C$\underline{H}_2$)$_3$(CH$_2$)$_2$CHNOH); 1.48–1.66 (2H, m, C$\underline{H}_2$CH$_2$CHNOH); 2.12–2.32 (4H, m, NHC(O)C$\underline{H}_2$(CH$_2$)$_4$C$\underline{H}_2$CHNOH); 6.61 (1H, t, *J* = 5.0, C$\underline{H}$NOH); 7.10 (2H, m, H-3,5 Ar); 7.57 (2H, dd, *J* = 9.0, *J* = 5.0, H-2,6 Ar); 9.89 (1H, s, NH); 10.70 (1H, s, NOH).

(*Z*)-8-(Aziridin-1-yl)-*N*-(4-fluorophenyl)-8-(hydroxyimino)octanamide (5d). Pyridine (0.1 ml) was added to a solution of NCS (25 mg, 0.19 mmol) in CH_2Cl_2 (2 ml) and stirred for 15 min. Aldoxime **2b** (50 mg, 0.19 mmol) was then added, and the reaction mixture was heated at 35°C until the reaction mixture became clear. After cooling to room temperature, aziridine (29 μl, 0.57 mmol, 3 equiv) and triethylamine (53 μl, 0.38 mmol, 2 equiv) were added, the stirring was continued for 1.5 h at room temperature. The reaction mixture was treated with water and extracted with AcOEt. The organic phase was dried over Na_2SO_4, filtered, and evaporated at water bath temperature 30°C. The residue was treated with acetonitrile. Yield 24 mg (41%), white powder, mp 126–128°C (decomp.). ^{1}H NMR spectrum (400 MHz, DMSO-d_6), δ, ppm (*J*, Hz): 1.23–1.33 (4H, m, (C$\underline{H}_2$)$_2$(CH$_2$)$_2$C(NC$_2$H$_4$)NOH); 1.41–1.59 (4H, m, (CH$_2$C$\underline{H}_2$CH$_2$)$_2$C(NC$_2$H$_4$)NOH); 1.98 (4H, s, 2CH$_2$ aziridine); 2.04 (2H, t, *J* = 7.4, NHC(O)C$\underline{H}_2$); 2.24 (2H, t, *J* = 7.4,

C$\underline{H}_2$C(NC_2H_4)NOH); 7.04–7.11 (2H, m, H-3,5 Ar); 7.49–7.63 (2H, m, H-2,6 Ar); 9.46 (1H, s, NOH); 9.88 (1H, s, NH). ^{13}C NMR spectrum (100 MHz, DMSO-d_6), δ, ppm (*J*, Hz): 25.4; 26.3; 26.6; 28.8; 28.9; 31.4; 36.8; 115.0 (d, *J* = 22.1); 120.6 (d, *J* = 7.6); 135.6 (d, *J* = 2.3); 156.5; 156.9 (C=NOH); 171.9 (C=O). Found, %: C 62.49; H 7.13; N 12.96. $C_{16}H_{22}FN_3O_2$. Calculated (with 5.4% AcOEt), %: C 62.09; H 7.32; N 12.93.

Synthesis of the target compound 5e was realized starting from intermediate **10c**[1] (Method I) according to the procedure described for compound **5c**.

Methyl 8-[(4-methylphenyl)amino]-8-oxooctanoate (11c) was precipitated from Et_2O. Yield 60%. ^{1}H NMR spectrum (400 MHz, DMSO-d_6), δ, ppm (*J*, Hz): 1.16–1.32 (4H, m, ($C\underline{H}_2$)$_2$(CH_2)$_2$$CO_2CH_3$); 1.45–1.56 (4H, m, ($CH_2C\underline{H}_2CH_2$)$_2$ CO_2CH_3); 2.20 (3H, s, CH_3Ar); 2.19–2.33 (4H, m, NHC(O)C$\underline{H}_2$(CH_2)$_4$C$\underline{H}_2CO_2CH_3$); 3.54 (3H, s, $COOCH_3$); 7.04 (2H, d, *J* = 8.4, H-3,5 Ar); 7.42 (2H, d, *J* = 8.4,H-2,6 Ar); 9.71 (1H, s, NH).

8-Hydroxy-*N*-(4-methylphenyl)octanamide was purified by flash chromatography (eluent AcOEt–hexane, 1:2). Yield 67%. ^{1}H NMR spectrum (400 MHz, DMSO-d_6), δ, ppm (*J*, Hz): 1.20–1.28 (4H, m, ($C\underline{H}_2$)$_2$(CH_2)$_3$OH); 1.32–1.41 (2H, m, NHC(O)$CH_2C\underline{H}_2$); 1.48–1.57 (2H, m, C$\underline{H}_2$(CH_2)$_2$OH); 2.20 (3H, s, CH_3Ar); 2.19–2.28 (4H, m, NHC(O)C$\underline{H}_2$); 3.30–3.36 (2H, m, C$\underline{H}_2$OH, partially overlapped with water signal); 4.28 (1H, m, OH); 7.04 (2H, d, *J* = 8.2, H-3,5 Ar); 7.33–7.49 (2H, m, H-2,6 Ar); 9.70 (1H, s, NH).

***N*-(4-Methylphenyl)-8-oxooctanamide**. Yield 71%. ^{1}H NMR spectrum (400 MHz, DMSO-d_6), δ, ppm (*J*, Hz): 1.19–1.30 (4H, m, ($C\underline{H}_2$)$_2$(CH_2)$_2$CHO); 1.46–1.56 (4H, m, ($CH_2C\underline{H}_2CH_2$)$_2$CHO); 2.20 (3H, s, CH_3Ar); 2.21–2.25 (2H, m, NHC(O)C$\underline{H}_2$); 2.38 (2H, td, *J* = 7.2, *J* = 1.6, C$\underline{H}_2$CHO); 7.04 (2H, d, *J* = 8.4, H-3,5 Ar); 7.42 (2H, d, *J* = 8.4, H-2,6 Ar); 9.62 (1H, t, *J* = 1.6, CHO); 9.70 (1H, s, NH).

8-(Hydroxyimino)-*N*-(4-methylphenyl)octanamide (2c). Yield 74%. ^{1}H NMR spectrum (400 MHz, DMSO-d_6), δ, ppm (*J*, Hz): 1.20–1.35 (4H, m, ($C\underline{H}_2$)$_2$(CH_2)$_2$CHNOH); 1.42–1.61 (4H, m, ($CH_2C\underline{H}_2CH_2$)$_2$CHNOH); 2.15–2.25 (4H, m, NHC(O)C$\underline{H}_2CH_2$(CH_2)$_2$$CH_2C\underline{H}_2$CHNOH); 2.19 (3H, s, C$\underline{H}_3$Ar); 6.60 (1H, t, *J* = 5.2, $CH_2C\underline{H}$NOH); 7.04 (2H, d, *J* = 8.4, H-3,5 Ph); 7.35 (2H, d, *J* = 8.4, H-2,6 Ph); 9.70 (1H, s, NH); 10.67 (1H, s, NOH).

(*Z*)-8-(Aziridin-1-yl)-8-(hydroxyimino)-*N*-(4-methylphenyl)octanamide (5e). Yield 11%, white powder, mp 111–113°C. ^{1}H NMR spectrum (400 MHz, DMSO-d_6), δ, ppm (*J*, Hz): 1.18–1.35 (m, 4H, ($C\underline{H}_2$)$_2$(CH_2)$_2$C(NC_2H_4)NOH); 1.42–1.58 (4H, m, ($CH_2C\underline{H}_2CH_2$)$_2$C(NC_2H_4)NOH); 1.98 (4H, s, 2CH_2 aziridine); 2.01–2.05 (2H, m, NHC(O)C$\underline{H}_2$); 2.19 (3H, s, CH_3Ar); 2.23 (2H, t, *J* = 7.5, C$\underline{H}_2$C(NC_2H_4)NOH); 6.99–7.07 (2H, m, H-3,5 Ar); 7.30–7.47 (2H, m, H-2,6 Ar); 9.47 (1H, s, NOH); 9.71 (1H, s, NH). ^{13}C NMR spectrum (100 MHz, DMSO-d_6), δ, ppm: 20.8 (C$\underline{H}_3$Ar); 25.5; 26.3; 26.6; 28.8; 28.9; 31.3; 36.8; 119.5 (C-3,5 Ar); 129.4 (C-2,6 Ar); 132.2 (C-4 Ar); 137.2 (C-1 Ar); 156.9 (C=NOH); 171.4 (C=O).

Synthesis of the target compound 5f was realized starting from intermediate **10d**[1] (Method I) according to the procedure described for compound **5c**.

Methyl 8-[(4-methoxyphenyl)amino]-8-oxooctanoate (11d) was precipitated from water. Yield 65%. ^{1}H NMR spectrum (400 MHz, DMSO-d_6), δ, ppm (*J*, Hz): 1.22–1.28 (4H, m, ($C\underline{H}_2$)$_2$(CH_2)$_2$$CO_2CH_3$); 1.44–1.59 (4H, m, ($CH_2C\underline{H}_2CH_2$)$_2$$CO_2CH_3$); 2.19–2.27 (4H, m, NHC(O)C$\underline{H}_2$(CH_2)$_4$C$\underline{H}_2CO_2CH_3$); 3.54 (3H, s, CO_2CH_3); 3.67 (3H, s, CH_3OAr); 6.82 (2H, d, *J* = 9.0, H-3,5 Ar); 7.44 (2H, d, *J* = 9.0, H-2,6 Ar); 9.65 (1H, s, NH).

8-Hydroxy-*N*-(4-methoxyphenyl)octanamide was precipitated from Et_2O. Yield 59%. ^{1}H NMR spectrum (400 MHz, DMSO-d_6), δ, ppm (*J*, Hz): 1.24–1.31 (6H, m, ($C\underline{H}_2$)$_3$(CH_2)$_2$OH); 1.32–1.41 (2H, m, NHC(O)$CH_2C\underline{H}_2$); 1.45–1.62 (2H, m, C$\underline{H}_2CH_2$OH); 2.19–2.22 (2H, m, NHC(O)C$\underline{H}_2$); 3.29–3.50 (2H, m, C$\underline{H}_2$OH partially overlapped with water signal); 3.67 (3H, s, CH_3OAr); 4.28 (1H, t, *J* = 5.1, OH); 6.82 (2H, d, *J* = 9.0, H-3,5 Ar); 7.44 (2H, d, *J* = 9.0, H-2,6 Ar); 9.65 (1H, s, NH).

***N*-(4-Methoxyphenyl)-8-oxooctanamide**. Yield 77%. ^{1}H NMR spectrum (400 MHz, DMSO-d_6), δ, ppm (*J*, Hz): 1.20–1.30 (4H, m, ($C\underline{H}_2$)$_2$(CH_2)$_2$CHO); 1.44–1.56 (4H, m, ($CH_2C\underline{H}_2CH_2$)$_2$CHO); 2.18–2.25 (2H, m, NHC(O)C$\underline{H}_2$); 2.38 (2H, td, *J* = 7.2, *J* = 1.6, C$\underline{H}_2$CHO); 3.67 (3H, s, CH_3OAr); 6.82 (2H, d, *J* = 9.0, H-3,5 Ar); 7.44 (2H, d, *J* = 9.0, H-2,6 Ar); 9.62 (1H, t, *J* = 1.6, CHO); 9.65 (1H, s, NH).

8-(Hydroxyimino)-*N*-(4-methoxyphenyl)octanamide (2d). Yield 72%. ^{1}H NMR spectrum (400 MHz, DMSO-d_6), δ, ppm (*J*, Hz): 1.22–1.31 (4H, m, ($C\underline{H}_2$)$_2$(CH_2)$_2$CHNOH); 1.34–1.43 (2H, m, NHC(O)$CH_2C\underline{H}_2$); 1.49–1.58 (2H, m, C$\underline{H}_2CH_2$CHNOH); 2.16–2.25 (4H, m, NHC(O)C$\underline{H}_2$(CH_2)$_4$C$\underline{H}_2$CHNOH); 3.67 (3H, s, CH_3OAr); 6.60 (1H, t, *J* = 5.3, C$\underline{H}$NOH); 6.82 (2H, d, *J* = 9.0, H-3,5 Ar); 7.44 (2H, d, *J* = 9.0, H-2,6 Ar); 9.66 (1H, s, NH); 10.67 (1H, s, NOH).

(*Z*)-8-(Aziridin-1-yl)-8-(hydroxyimino)-*N*-(4-methoxyphenyl)octanamide (5f). Yield 70%, white powder, mp 107.5–109°C. ^{1}H NMR spectrum (400 MHz, DMSO-d_6), δ, ppm (*J*, Hz): 1.19–1.33 (4H, m, ($C\underline{H}_2$)$_2$(CH_2)$_2$C(NC_2H_4)NOH); 1.42–1.58 (4H, m, ($CH_2C\underline{H}_2CH_2$)$_2$C(NC_2H_4)NOH); 1.98 (4H, s, 2CH_2 aziridine); 2.03 (2H, t, *J* = 7.4, NHC(O)C$\underline{H}_2$); 2.21 (2H, t, *J* = 7.4, C$\underline{H}_2$C(NC_2H_4)NOH); 3.67 (3H, s, CH_3OAr); 6.81 (2H, d, *J* = 9.0, H-3,5 Ar); 7.44 (2H, d, *J* = 9.0, H-2,6 Ar); 9.48 (1H, s, NOH); 9.67 (1H, s, NH). ^{13}C NMR spectrum (100 MHz, DMSO-d_6), δ, ppm: 25.6; 26.3 26.6; 28.8; 28.9; 31.3; 36.7; 55.6 (CH_3OAr); 114.2 (C-3,5 Ar); 121.1 (C-2,6 Ar); 132.9 (C-1 Ar); 155.4 (C-4 Ar); 156.9 (C=NOH); 171.2 (C=O). Found, %: C 61.67; H 7.70; N 10.61 $C_{17}H_{25}N_3O_3$. Calculated (with 21% AcOEt), %: C 61.90; H 8.16; N 10.31.

Synthesis of the target compound 5g was realized starting from intermediate **10e**[1] (Method I) according to the procedure described for compound **5c**.

Methyl 8-[(2,6-dimethylphenyl)amino]-8-oxooctanoate (11e) was precipitated from water. Yield 85%. ^{1}H NMR spectrum (400 MHz, DMSO-d_6), δ, ppm (*J*, Hz): 1.24–1.39 (4H, m, ($C\underline{H}_2$)$_2$(CH_2)$_2$$CO_2CH_3$); 1.45–1.64 (4H, m, ($CH_2C\underline{H}_2CH_2$)$_2$$CO_2CH_3$); 2.08 (6H, br. s, 2$CH_3$Ar); 2.23–2.31 (4H, m, NHC(O)C$\underline{H}_2$(CH_2)$_4$C$\underline{H}_2CO_2CH_3$); 3.54 (3H, s, $CO_2C\underline{H}_3$); 7.01 (3H, s, H-3,4,5 Ar); 9.12 (1H, br. s, NH).

***N*-(2,6-Dimethylphenyl)-8-hydroxyoctanamide**. Yield 54%. ^{1}H NMR spectrum (400 MHz, DMSO-d_6), δ, ppm

(*J*, Hz): 1.20–1.39 (6H, m, (C$\underline{H}_2$)$_3$(CH$_2$)$_2$OH); 1.46–1.64 (4H, m, CH$_2$(CH$_2$)$_3$C$\underline{H}_2$CH$_2$OH); 2.08 (6H, br. s, 2CH$_3$Ar); 2.22 (2H, t, *J* = 7.4, NHC(O)C$\underline{H}_2$); 3.30–3.48 (2H, m, C$\underline{H}_2$OH partially overlapped with water signal); 4.29 (1H, t, *J* = 5.2, OH); 7.01 (3H, s, H-3,4,5 Ar); 9.12 (1H, br. s, NH).

***N*-(2,6-Dimethylphenyl)-8-oxooctanamide**. Yield 58%. ^{1}H NMR spectrum (400 MHz, DMSO-*d*$_6$), δ, ppm (*J*, Hz): 1.22–1.39 (4H, m, (C$\underline{H}_2$)$_2$(CH$_2$)$_2$CHO); 1.38–1.70 (4H, m, (CH$_2$C$\underline{H}_2$CH$_2$)$_2$CHO); 2.07 (6H, br. s, 2CH$_3$Ar); 2.24–2.29 (2H, m, NHC(O)C$\underline{H}_2$); 2.39 (2H, td, *J* = 7.2, *J* = 1.6, C$\underline{H}_2$CHO); 7.01 (3H, s, H-3,4,5 Ar); 9.12 (1H, br. s, NH); 9.64 (1H, t, *J* = 1.6, CHO).

***N*-(2,6-Dimethylphenyl)-8-(hydroxyimino)octanamide (2e)**. Yield 86%. ^{1}H NMR spectrum (400 MHz, DMSO-*d*$_6$), δ, ppm (*J*, Hz): 1.21–1.49 (6H, m, (C$\underline{H}_2$)$_3$(CH$_2$)$_2$CHNOH); 1.55–1.64 (2H, m, C$\underline{H}_2$CH$_2$CHNOH); 2.08 (6H, s, 2CH$_3$Ar); 2.15–2.33 (4H, m, NHC(O)C$\underline{H}_2$(CH$_2$)$_4$C$\underline{H}_2$CHNOH); 6.61 (1H, t, *J* = 5.3, C$\underline{H}$NOH); 7.01 (3H, s, H-3,4,5 Ar); 9.13 (1H, br. s, NH); 10.68 (1H, s, NOH).

(*Z*)-8-(Aziridin-1-yl)-*N*-(2,6-dimethylphenyl)-8-(hydroxyimino)octanamide (5g). Yield 16%, amorphous powder, mp 125–126.5°C. ^{1}H NMR spectrum (400 MHz, DMSO-*d*$_6$), δ, ppm (*J*, Hz): 1.22–1.40 (4H, m, (C$\underline{H}_2$)$_2$(CH$_2$)$_2$C(NC$_2$H$_4$)NOH); 1.43–1.63 (4H, m, (CH$_2$C$\underline{H}_2$CH$_2$)$_2$C(NC$_2$H$_4$)NOH); 1.98 (4H, s, 2CH$_2$ aziridine); 2.08 (6H, s, 2CH$_3$Ar); 2.03–2.10 (2H, m, NHC(O)C$\underline{H}_2$); 2.27 (2H, t, *J* = 7.3, C$\underline{H}_2$C(NC$_2$H$_4$)NOH); 7.01 (3H, s, H-3,4,5 Ar); 9.14 (1H, s, NH); 9.49 (1H, s, NOH). ^{13}C NMR spectrum (100 MHz, DMSO-*d*$_6$), δ, ppm: 18.5 (2CH$_3$Ar); 25.8; 26.5; 28.7; 28.9; 31.3; 35.8; 39.2; 126.7 (C-4 Ar); 128.0 (C-3,5 Ar); 135.6 (C-2,6 Ar); 135.7 (C-1 Ar); 156.9 (C=NOH); 171.2 (C=O).

Synthesis of the target compound 5h was realized starting from intermediate **12** (Method II) according to the procedure described for compound **5a**.

Methyl 8-oxo-8-[(4-phenoxyphenyl)amino]octanoate (11f). Yield 62%. ^{1}H NMR spectrum (400 MHz, DMSO), δ, ppm: 1.21–1.28 (4H, m, (C$\underline{H}_2$)$_2$(CH$_2$)$_2$CO$_2$CH$_3$); 1.44–1.58 (4H, m, (CH$_2$C$\underline{H}_2$CH$_2$)$_2$CO$_2$H); 2.23–2.28 (4H, m, NHC(O)C$\underline{H}_2$(CH$_2$)$_4$C$\underline{H}_2$CO$_2$H); 3.54 (3H, s, CO$_2$CH$_3$); 6.86–6.96 (4H, m, H Ph); 7.00–7.09 (1H, m, H Ph); 7.27–7.36 (2H, m, H Ar); 7.47–7.61 (2H, m, H Ar); 9.84 (1H. br. s, NH).

8-Hydroxy-*N*-(4-phenoxyphenyl)octanamide was recrystallized from Et$_2$O. Yield 42%. ^{1}H NMR spectrum (400 MHz, DMSO-*d*$_6$), δ, ppm (*J*, Hz): 1.22–1.28 (6H, br. s, (C$\underline{H}_2$)$_3$(CH$_2$)$_2$OH); 1.33–1.58 (4H, m, C$\underline{H}_2$(CH$_2$)$_3$C$\underline{H}_2$CH$_2$OH); 2.25 (2H, t, *J* = 7.4, NHC(O)C$\underline{H}_2$); 3.34 (2H, q, *J* = 6.5, C$\underline{H}_2$OH); 4.29 (1H, t, *J* = 5.2, OH); 6.89–6.95 (4H, m, H Ar); 7.03–7.08 (1H, m, H Ar); 7.29–7.35 (2H, m, H Ar); 7.54–7.58 (2H, m, H Ar); 9.84 (1H, br. s, NH).

8-Oxo-*N*-(4-phenoxyphenyl)octanamide. Yield 66%. ^{1}H NMR spectrum (400 MHz, DMSO-*d*$_6$), δ, ppm (*J*, Hz): 1.24–1.31 (4H, m, (C$\underline{H}_2$)$_2$(CH$_2$)$_2$CHO); 1.46–1.59 (4H, m, (CH$_2$C$\underline{H}_2$CH$_2$)$_2$CHO); 2.25 (2H, t, *J* = 7.4, NHC(O)C$\underline{H}_2$); 2.34 (2H, td *J* = 7.2, *J* = 1.6, C$\underline{H}_2$CHO); 6.90–6.95 (4H, m, H Ar); 7.03–7.08 (1H, m, H Ar); 7.29–7.35 (2H, m, H Ar); 7.54–7.58 (2H, m, H Ar); 9.63 (1H, t, *J* = 1.6, CHO); 9.84 (1H, br. s, NH).

8-(Hydroxyimino)-*N*-(4-phenoxyphenyl)octanamide (2f). Yield 92%. ^{1}H NMR spectrum (400 MHz, DMSO-*d*$_6$), δ, ppm (*J*, Hz): 1.24–1.31 (4H, m, (C$\underline{H}_2$)$_2$(CH$_2$)$_2$CHNOH); 1.35–1.69 (4H, m, (CH$_2$C$\underline{H}_2$CH$_2$)$_2$CHNOH); 2.16–2.27 (4H, m, NHC(O)C$\underline{H}_2$(CH$_2$)$_4$C$\underline{H}_2$CHNOH); 6.60 (1H, t, *J* = 5.3, C$\underline{H}$NOH); 6.90–6.94 (4H, m, H Ar); 7.02–7.07 (1H, m, H Ar); 7.29–7.35 (2H, m, H Ar); 7.56 (2H, d, *J* = 8.9, H Ar); 9.84 (1H, br. s, NH); 10.67 (1H, s, NOH).

(*Z*)-8-(Aziridin-1-yl)-8-(hydroxyimino)-*N*-(4-phenoxyphenyl)octanamide (5h). Yield 4%, colorless oil. ^{1}H NMR spectrum (400 MHz, CDCl$_3$), δ, ppm (*J*, Hz): 1.24–1.44 (6H, m, (C$\underline{H}_2$)$_2$(CH$_2$)$_2$C(NC$_2$H$_4$)NOH); 1.54–1.76 (4H, m, (CH$_2$C$\underline{H}_2$CH$_2$)$_2$C(NC$_2$H$_4$)NOH); 2.12–2.21 (2H, m, NHC(O)C$\underline{H}_2$); 2.17 (4H, s, 2CH$_2$ aziridine); 2.28–2.34 (2H, m, C$\underline{H}_2$C(NC$_2$H$_4$)NOH); 6.88–6.99 (4H, m, H Ar); 7.05 (1H, t, *J* = 7.4, H Ar); 7.26–7.31 (2H, m, H Ar partially overlapped with CDCl$_3$); 7.43–7.51 (2H, m, H Ar). The signals of NH and NOH protons are not observed due to exchange with traces of water.

Synthesis of the target compound 5i was realized starting from the intermediate **10g** (Method I) according to the procedure described for compound **5c**.

8-[(4-Ethoxyphenyl)amino]-8-oxooctanoic acid (10g). Yield 73%. ^{1}H NMR spectrum (400 MHz, DMSO-*d*$_6$), δ, ppm (*J*, Hz): 1.19–1.32 (7H, m, (C$\underline{H}_2$)$_2$(CH$_2$)$_2$CO$_2$H, OCH$_2$C$\underline{H}_3$); 1.40–1.60 (4H, m, (CH$_2$C$\underline{H}_2$CH$_2$)$_2$CO$_2$H); 2.11–2.26 (4H, m, NHC(O)C$\underline{H}_2$(CH$_2$)$_4$C$\underline{H}_2$CO$_2$H); 3.93 (2H, q, *J* = 6.9, OC$\underline{H}_2$CH$_3$); 6.80 (2H, dd, *J* = 9.0, *J* = 1.1, H-3,5 Ar); 7.37–7.48 (2H, m, H-2,6 Ar); 9.64 (1H, br. s, NH); 11.93 (1H, s, CO$_2$H).

Methyl 8-[(4-ethoxyphenyl)amino]-8-oxooctanoate (11g) was precipitated from water. Yield 85%. ^{1}H NMR spectrum (400 MHz, DMSO-*d*$_6$), δ, ppm (*J*, Hz): 1.23–1.32 (m, 7H, m, (C$\underline{H}_2$)$_2$(CH$_2$)$_2$CO$_2$CH$_3$, OCH$_2$C$\underline{H}_3$); 1.44–1.59 (4H, m, (CH$_2$C$\underline{H}_2$CH$_2$)$_2$CO$_2$CH$_3$); 2.08–2.35 (4H, m, NHC(O)C$\underline{H}_2$(CH$_2$)$_4$C$\underline{H}_2$CO$_2$CH$_3$); 3.54 (3H, s, CO$_2$C$\underline{H}_3$); 3.93 (2H, q, *J* = 6.9, OC$\underline{H}_2$CH$_3$); 6.80 (2H, dd, *J* = 9.0, *J* = 1.2, H-3,5 Ar); 7.22–7.66 (2H, m, H-2,6 Ar); 9.64 (1H, br. s, NH).

***N*-(4-Ethoxyphenyl)-8-hydroxyoctanamide** was precipitated from Et$_2$O. Yield 47%. ^{1}H NMR spectrum (400 MHz, DMSO-*d*$_6$), δ, ppm (*J*, Hz): 1.18–1.28 (9H, m, (C$\underline{H}_2$)$_3$(CH$_2$)$_2$OH, OCH$_2$C$\underline{H}_3$); 1.32–1.41 (2H, m, NHC(O)CH$_2$C$\underline{H}_2$); 1.46–1.64 (2H, m, C$\underline{H}_2$CH$_2$OH); 2.21 (2H, t, *J* = 7.4, NHC(O)C$\underline{H}_2$); 3.29–3.50 (2H, m, C$\underline{H}_2$OH partially overlapped with water signal); 3.93 (2H, q, *J* = 7.0, OC$\underline{H}_2$CH$_3$); 4.28 (1H, t, *J* = 5.1, OH); 6.71–6.85 (2H, m, H-3,5 Ar); 7.36–7.47 (2H, m, H-2,6 Ar); 9.64 (1H, br. s, NH).

***N*-(4-Ethoxyphenyl)-8-oxooctanamide**. Yield 43%. ^{1}H NMR spectrum (400 MHz, DMSO-*d*$_6$), δ, ppm (*J*, Hz): 1.22–1.30 (7H, m, (C$\underline{H}_2$)$_2$(CH$_2$)$_2$CHO, OCH$_2$C$\underline{H}_3$); 1.45–1.59 (4H, m, (CH$_2$C$\underline{H}_2$CH$_2$)$_2$CHO); 2.19–2.22 (2H, m, NHC(O)C$\underline{H}_2$); 2.38 (2H, td, *J* = 7.2, *J* = 1.6, C$\underline{H}_2$CHO); 3.93 (2H, q, *J* = 7.0, OC$\underline{H}_2$CH$_3$); 6.78–6.83 (2H, m, H-3,5 Ar); 7.34–7.48 (2H, m, H-2,6 Ar); 9.62 (1H, s, CHO); 9.64 (1H, br. s, NH).

***N*-(4-Ethoxyphenyl)-8-(hydroxyimino)octanamide (2g)**. Yield 90%. ^{1}H NMR spectrum (400 MHz, DMSO-*d*$_6$), δ, ppm (*J*, Hz): 1.23–1.31 (7H, m, (C$\underline{H}_2$)$_2$(CH$_2$)$_2$CHNOH, OCH$_2$C$\underline{H}_3$); 1.33–1.42 (2H, m, NHC(O)CH$_2$C$\underline{H}_2$); 1.47–1.57 (2H, m, C$\underline{H}_2$CH$_2$CHNOH); 2.15–2.25 (4H, m,

NHC(O)C$\underline{H}_2$(CH$_2$)$_4$C$\underline{H}_2$CHNOH); 3.93 (2H, q, *J* = 7.0, OC$\underline{H}_2$CH$_3$); 6.60 (1H, t, *J* = 5.3, C$\underline{H}$NOH); 6.80 (2H, d, *J* = 8.9, H-3,5 Ar); 7.43 (2H, d, *J* = 8.4, H-2,6 Ar); 9.64 (1H, s, NH); 10.67 (1H, s, OH).

(*Z*)-8-(Aziridin-1-yl)-*N*-(3-ethoxyphenyl)-8-(hydroxyimino)octanamide (5i) was precipitated from Et$_2$O. Yield 42%, oil with tendency to crystallize, mp 91°C (decomp.). ^{1}H NMR spectrum (400 MHz, DMSO-*d*$_6$), δ, ppm (*J*, Hz): 1.05 (3H, t, *J* = 7.0, OCH$_2$C$\underline{H}_3$); 1.21–1.33 (4H, m, (C$\underline{H}_2$)$_2$(CH$_2$)$_2$C(NC$_2$H$_4$)NOH); 1.35–1.59 (4H, m, (CH$_2$C$\underline{H}_2$CH$_2$)$_2$C(NC$_2$H$_4$)NOH); 1.98 (4H, s, 2CH$_2$ aziridine); 2.04 (2H, t, *J* = 7.4, NHC(O)C$\underline{H}_2$); 2.21 (2H, t, *J* = 7.4, C$\underline{H}_2$C(NC$_2$H$_4$)NOH); 3.93 (2H, q, *J* = 7.0, OC$\underline{H}_2$CH$_3$); 6.80 (2H, d, *J* = 8.9, H-3,5 Ar); 7.43 (2H, d, *J* = 8.4, H-2,6 Ar); 9.46 (1H, s, NOH); 9.65 (1H, s, NH). ^{13}C NMR spectrum (100 MHz, DMSO-*d*$_6$), δ, ppm: 14.8 (C$\underline{H}_3$CH$_2$O); 25.8; 26.3; 28.7; 28.9; 31.3; 35.8; 39.2; 58.2 (CH$_3$C$\underline{H}_2$O); 114.2 (C-3,5 Ar); 121.1 (C-2,6 Ar); 132.9 (C-1 Ar); 155.4 (C-4 Ar); 156.9 (C=NOH); 171.0 (C=O).

Synthesis of the target compound 5j was realized starting from intermediate **10h**[1] (Method I) according to the procedure described for compound **5c**.

Methyl 8-[(4-ethylphenyl)amino]-8-oxooctanoate (11h) was purified by flash chromatography (eluent AcOEt–hexane, 1:2). Yield 23%. ^{1}H NMR spectrum (400 MHz, DMSO-*d*$_6$), δ, ppm (*J*, Hz): 1.11 (3H, t, *J* = 7.6, CH$_2$C$\underline{H}_3$); 1.21–1.29 (4H, m, (C$\underline{H}_2$)$_2$(CH$_2$)$_2$CO$_2$H); 1.45–1.57 (4H, m, (CH$_2$C$\underline{H}_2$CH$_2$)$_2$CO$_2$CH$_3$); 2.21–2.27 (4H, m, NHC(O)C$\underline{H}_2$(CH$_2$)$_4$C$\underline{H}_2$CO$_2$CH$_3$); 2.50 (2H, q, *J* = 7.6, C$\underline{H}_2$CH$_3$ partially overlapped with DMSO signal); 3.54 (3H, s, COOC$\underline{H}_3$); 7.07 (2H, d, *J* = 8.5, H-3,5 Ar); 7.44 (2H, d, *J* = 8.5, H-2,6 Ar); 9.71 (1H, s, NH).

***N*-(4-Ethylphenyl)-8-hydroxyoctanamide** was precipitated from Et$_2$O. Yield 83%. ^{1}H NMR spectrum (400 MHz, DMSO-*d*$_6$), δ, ppm (*J*, Hz): 1.11 (3H, t, *J* = 7.6, CH$_2$C$\underline{H}_3$); 1.20–1.28 (6H, m, (C$\underline{H}_2$)$_3$(CH$_2$)$_2$OH); 1.33–1.39 (2H, m, NHC(O)CH$_2$C$\underline{H}_2$); 1.49–1.57 (2H, m, C$\underline{H}_2$CH$_2$OH); 2.23 (2H, t, *J* = 7.4, NHC(O)C$\underline{H}_2$); 2.50 (2H, q, *J* = 7.6, C$\underline{H}_2$CH$_3$ partially overlapped with DMSO signal); 3.34 (2H, m, C$\underline{H}_2$OH partially overlapped with water signal); 4.29 (1H, t, *J* = 5.2, OH); 7.07 (2H, d, *J* = 8.5, H-3,5 Ar); 7.44 (2H, d, *J* = 8.5, H-2,6 Ar); 9.71 (1H, s, NH).

***N*-(4-Ethylphenyl)-8-oxooctanamide** was purified by flash chromatography (eluent AcOEt–hexane, 1:4). Yield 79%. ^{1}H NMR spectrum (400 MHz, DMSO-*d*$_6$), δ, ppm (*J*, Hz): 1.11 (3H, t, *J* = 7.6, CH$_2$C$\underline{H}_3$); 1.22–1.29 (4H, m, (C$\underline{H}_2$)$_2$(CH$_2$)$_2$CHO); 1.42–1.58 (4H, m, (CH$_2$C$\underline{H}_2$CH$_2$)$_2$CHO); 2.23 (2H, t, *J* = 7.4, NHC(O)C$\underline{H}_2$); 2.38 (2H, td, *J* = 7.4, *J* = 1.6, C$\underline{H}_2$CHO); 2.50 (2H, q, *J* = 7.6, C$\underline{H}_2$CH$_3$ partially overlapped with DMSO signal); 7.07 (2H, d, *J* = 8.4, H-3,5 Ar); 7.44 (2H, d, *J* = 8.4, H-2,6 Ar); 9.62 (1H, s, CHO); 9.71 (1H, s, NH).

***N*-(4-Ethylphenyl)-8-(hydroxyimino)octanamide (2h)**. Yield 75%. ^{1}H NMR spectrum (400 MHz, DMSO-*d*$_6$), δ, ppm (*J*, Hz): 1.11 (3H, t, *J* = 7.6, CH$_2$C$\underline{H}_3$); 1.23–1.31 (4H, m, (C$\underline{H}_2$)$_2$(CH$_2$)$_2$CHNOH); 1.32–1.42 (2H, m, NHC(O)CH$_2$C$\underline{H}_2$); 1.47–1.59 (2H, m, C$\underline{H}_2$CH$_2$CHNOH); 2.15–2.27 (4H, m, NHC(O)C$\underline{H}_2$(CH$_2$)$_4$C$\underline{H}_2$CHNOH); 2.50 (2H, q, *J* = 7.6, C$\underline{H}_2$CH$_3$ partially overlapped with DMSO signal); 6.60 (1H, t, *J* = 5.4, C$\underline{H}$NOH); 7.07 (2H, d, *J* = 8.4, H-3,5 Ar); 7.44 (2H, d, *J* = 8.4, H-2,6 Ar); 9.71 (1H, s, NH); 10.67 (1H, s, NOH).

(*Z*)-8-(Aziridin-1-yl)-N-(4-ethylphenyl)-8-(hydroxyimino)octanamide (5j) was purified by flash chromatography (eluent 5% Et$_3$N in AcOEt). Yield 42%, yellowish oil. ^{1}H NMR spectrum (400 MHz, DMSO-*d*$_6$) δ, ppm (*J*, Hz): 1.11 (3H, t, *J* = 7.6, CH$_2$C$\underline{H}_3$); 1.18–1.31 (4H, m, (C$\underline{H}_2$)$_2$(CH$_2$)$_2$C(NC$_2$H$_4$)NOH); 1.42–1.58 (4H, m, (CH$_2$C$\underline{H}_2$CH$_2$)$_2$C(NC$_2$H$_4$)NOH); 1.98 (4H, s, 2CH$_2$ aziridine); 2.00–2.06 (2H, m, NHC(O)C$\underline{H}_2$); 2.23 (2H, t, *J* = 7.4, C$\underline{H}_2$C(NC$_2$H$_4$)NOH); 2.50 (2H, q, *J* = 7.6, C$\underline{H}_2$CH$_3$ partially overlapped with DMSO signal); 7.07 (2H, d, *J* = 8.2, H-3,5 Ar); 7.44 (2H, d, *J* = 8.2, H-2,6 Ar); 9.46 (1H, s, NOH); 9.72 (1H, s, NH). ^{13}C NMR spectrum (100 MHz, DMSO-*d*$_6$), δ, ppm: 14.8 (C$\underline{H}_3$CH$_2$); 25.5; 26.3; 26.6; 28.1 (CH$_3$C$\underline{H}_2$); 28.8; 28.9; 31.3; 36.8; 119.5 (C-3,5 Ar); 129.4 (C-2,6 Ar); 132.2 (C-4 Ar); 137.2 (C-1 Ar); 156.9 (C=NOH); 171.4 (C=O).

Synthesis of the target compound 5k was realized starting from the commercially available cycloheptanone (**13**) as follows.

Ethyl 6-(1,3-dioxolan-2-yl)hexanoate (15). *p*-Toluenesulfonic acid (245 mg, 1.3 mmol, 0.2 equiv) and ethylene glycol (4.26 ml, 77.6 mmol) were added to a solution of ethyl 7-oxoheptanoate (**14**)[5] (1.12 g, 6.5 mmol) in benzene (70 ml). The reaction mixture was stirred at reflux with a Dean–Stark trap for 3 h. Then the reaction mixture was evaporated to dryness. Yield 1.10 g (78%). ^{1}H NMR spectrum (200 MHz, DMSO-*d*$_6$), δ, ppm (*J*, Hz): 1.16 (3H, t, *J* = 7.3, OCH$_2$C$\underline{H}_3$); 1.23–1.60 (8H, m, (C$\underline{H}_2$)$_4$CH(OC$_2$H$_4$O)); 2.26 (2H, t, *J* = 7.3, C(O)C$\underline{H}_2$); 3.69–3.89 (4H, m, CH(OC$_2$$\underline{H}_4$O)); 4.02 (2H, q, *J* = 7.3 OC$\underline{H}_2$CH$_3$); 4.69–4.77 (1H, m, C$\underline{H}$(OC$_2$H$_4$O)).

6-(1,3-Dioxolan-2-yl)hexanoic acid. Aqueous NaOH solution (1 M, 4.61 ml) was added to a solution of intermediate **15** (1.06 g, 4.61 mmol) in THF (30 ml). The reaction mixture was stirred overnight at room temperature and evaporated to dryness to give 710 mg (73%) of white crystalline product which was submitted to the next reaction step without additional purification and characterization.

6-(1,3-Dioxolan-2-yl)-*N*-phenylhexanamide. The crude 6-(1,3-dioxolan-2-yl)hexanoic acid (325 mg, 1.5 mmol) was suspended in DMF (16 ml), and isobutyl chloroformate (223 μl, 1.7 mmol, 1.1 equiv) was added. The reaction mixture was stirred for 1 h at room temperature. Aniline (156 μl, 1.7 mmol, 1.1 equiv) was subsequently added dropwise, and the reaction mixture was stirred for 2 h at room temperature. Aqueous potassium bisulfate solution (1 M, 50 ml) was added to the reaction mixture, and the product was extracted with AcOEt, the organic phase was washed several times with water, dried over Na$_2$SO$_4$, filtrated, evaporated. The residue was purified by flash chromatography, eluent AcOEt–hexane, 1:1. Yield 385 mg (94%). ^{1}H NMR spectrum (200 MHz, DMSO-*d*$_6$), δ, ppm (*J*, Hz): 1.20–1.65 (6H, m, (C$\underline{H}_2$)$_3$CH$_2$CH(OC$_2$H$_4$O)); 2.16 (2H, t, *J* = 7.2, NHC(O)C$\underline{H}_2$); 2.26 (2H, t, *J* = 7.2, C$\underline{H}_2$CH(OC$_2$H$_4$O)); 3.63–3.91 (4H, m, CH(OC$_2$$\underline{H}_4$O)); 4.68–4.78

(1H, m, C<u>H</u>(OC_2H_4O)); 6.94–7.04 (1H, m, H-4 Ph); 7.25 (2H, t, *J* = 7.9, H-3,5 Ph); 7.52–7.59 (2H, m, H-2,6 Ph); 9.82 (1H, br. s, NH).

7-Oxo-*N*-phenylheptanamide (16). 6-(1,3-Dioxolan-2-yl)-*N*-phenylhexanamide (385 mg, 1.46 mmol) was dissolved in a mixture of THF (10 ml) and aqueous HCl (1 M, 10 ml). The reaction mixture was stirred for 3 h at 70°C, cooled to room temperature, and extracted with Et_2O. The organic phase was separated and dried over Na_2SO_4, filtered and evaporated. Yield 185 mg (54%), colorless oil. ^{1}H NMR spectrum (200 MHz, DMSO-d_6), δ, ppm (*J*, Hz): 1.17–1.62 (6H, m, ($C\underline{H}_2$)$_3$$CH_2$CHO); 2.10–2.35 (4H, m, NHC(O)$C\underline{H}_2$(CH_2)$_3$$C\underline{H}_2$CHO); 6.99 (1H, t, *J* = 7.6, H-4 Ph); 7.25 (2H, t, *J* = 7.8, H-3,5 Ph); 7.55 (2H, d, *J* = 8.2, H-2,6 Ph); 9.63 (1H, s, CHO); 9.82 (1H, br. s, NH).

7-(Hydroxyimino)-*N*-phenylheptanamide (3) was synthesized using the procedure described for the synthesis of compound **2a**, except by using Na_2CO_3 instead of NaOAc. Yield 60%. ^{1}H NMR spectrum (200 MHz, DMSO-d_6), δ, ppm (*J*, Hz): 1.12–1.74 (6H, m, ($C\underline{H}_2$)$_3$$CH_2$CHNOH); 2.09–2.32 (4H, m, NHC(O)$C\underline{H}_2$(CH_2)$_3$$C\underline{H}_2$CHNOH); 6.59–6.62 (1H, m, C<u>H</u>NOH); 6.97–7.01 (1H, m, H-4 Ph); 7.25 (2H, t, *J* = 7.6, H-3,5 Ph); 7.56 (2H, t, *J* = 7.6, H-2,6 Ph); 9.82 (1H, s, NH); 10.71 (1H, s, NOH).

(*Z*)-7-(Aziridin-1-yl)-7-(hydroxyimino)-*N*-phenylheptanamide (5k) was synthesized from compound **3** following the procedure for the synthesis of compound **5c** and purified by flash chromatography (eluent 5% Et_3N in AcOEt). Yield 6%, white powder, mp 131–132°C. ^{1}H NMR spectrum (400 MHz, DMSO-d_6), δ, ppm (*J*, Hz): 1.23–1.35 (2H, m, $C\underline{H}_2$(CH_2)$_2$$C(NC_2H_4)$NOH); 1.42–1.62 (4H, m, ($C\underline{H}_2CH_2$)$_2$$CN(C_2H_4)$NOH); 1.99 (4H, s, 2$CH_2$ aziridine); 2.05 (2H, t, *J* = 7.4, NHC(O)$C\underline{H}_2$); 2.25 (2H, t, *J* = 7.4, $C\underline{H}_2C(NC_2H_4)$NOH); 6.97 (1H, t, *J* = 7.9, H-4 Ph); 7.24 (2H, t, *J* = 7.9, H-3,5 Ph); 7.54 (2H, d, *J* = 7.6, H-2,6 Ph); 9.46 (1H, s, NOH); 9.80 (1H, s, NH).^{13}C NMR spectrum (100 MHz, DMSO-d_6), δ, ppm: 25.5; 26.4; 26.6; 28.9; 31.3; 36.6; 119.3; 123.3; 129.0; 139.8; 156.9 (C=NOH); 171.6 (C=O). Found %: C 65.46; H 7.63; N 15.60. $C_{15}H_{21}N_3O_2$. Calculated, %: C 65.43; H 7.69; N 15.26.

6-(Hydroxyimino)-*N*-phenylhexanamide (4) was synthesized from compound **18**[6] using the procedure for the synthesis of compound **2a**, except by using Na_2CO_3 instead of NaOAc. The product was isolated by filtration, dried, and used for the next step without additional purification and characterization.

(*Z*)-6-(Aziridin-1-yl)-6-(hydroxyimino)-*N*-phenylhexanamide (5l) was synthesized from compound **4** following the procedure for the synthesis of compound **5c** and purified by flash chromatography (eluent 5% Et_3N in AcOEt). Yield 6%, white powder, mp 128–131°C. ^{1}H NMR spectrum (400 MHz, DMSO-d_6), δ, ppm (*J*, Hz): 1.43–1.65 (4H, m, ($C\underline{H}_2$)$_2$$CH_2C(NC_2H_4)$NOH); 1.99 (4H, s, 2$CH_2$ aziridine); 2.08 (2H, t, *J* = 7.3, NHC(O)$C\underline{H}_2$); 2.27 (2H, t, *J* = 7.3, $C\underline{H}_2C(NC_2H_4)$NOH); 6.99 (1H, t, *J* = 7.4, H-4 Ph); 7.24 (2H, t, *J* = 7.8, H-3,5 Ph); 7.54 (2H, d, *J* = 7.8, H-2,6 Ph); 9.48 (1H, s, NOH); 9.81 (1H, s, NH). ^{13}C NMR spectrum (100 MHz, DMSO-d_6), δ, ppm: 25.5; 26.3; 26.6; 31.3; 36.8; 119.4; 123.3; 129.0; 139.8; 156.9 (C=NOH); 171.6 (C=O). Found, %: C 63.49; H 7.29; N 15.28. $C_{14}H_{19}N_3O_2$. Calculated (with 8% AcOEt), %: C 63.58; H 7.47; N 14.83.

(*Z*)-8-(7-Azabicyclo[4.1.0]heptan-7-yl)-8-(hydroxyimino)-*N*-phenyloctanamide (6) was synthesized following the procedure for the synthesis of compound **5c**. Yield 34%, oil. ^{1}H NMR spectrum (400 MHz, DMSO-d_6), δ, ppm (*J*, Hz): 1.07–1.18 (4H, m, 3,4-CH_2 azabicycle); 1.22–1.35 (4H, m, ($C\underline{H}_2$)$_2$(CH_2)$_2$$C(NC_6H_{10})$NOH); 1.43–1.59 (4H, m, ($CH_2C\underline{H}_2CH_2$)$_2$$C(NC_6H_{10})$NOH); 1.63–1.74 (2H, m) and 1.74–1.85 (2H, m, 2,5-CH_2 azabicycle); 1.97–2.04 (2H, m, NHC(O)$C\underline{H}_2$); 2.25 (2H, t, *J* = 7.4, $C\underline{H}_2C(NC_6H_{10})$NOH); 2.31–2.35 (2H, m, 1,6-CH azabicycle); 6.94–7.00 (1H, m, H-4 Ph); 7.14–7.28 (2H, m, H-3,5 Ph); 7.54 (2H, dd, *J* = 10.9, *J* = 3.4, H-2,6 Ph); 9.35 (1H, s, NOH); 9.81 (1H, s, NH). ^{13}C NMR spectrum (100 MHz, DMSO-d_6), δ, ppm: 20.8; 25.5; 26.3; 26.6; 28.8; 28.9; 31.3; 36.8; 39.2; 119.5 (C-4 Ph); 129.4 (C-3,6 Ph); 132.2 (C-2,6 Ph); 137.2 (C-1 Ph); 156.9 (C=NOH); 171.4 (C=O). Mass spectrum, *m/z*: 344 $[M+H]^+$.

1-[(1*Z*)-8-Anilino-*N*-hydroxy-8-oxooctanimidoyl]aziridine-2-carboxamide (7) was synthesized following the procedure for the synthesis of compound **5c**. Yield 14%, oil. ^{1}H NMR spectrum (400 MHz, $CDCl_3$), δ, ppm (*J*, Hz): 1.23–1.41 (4H, m, ($C\underline{H}_2$)$_2$(CH_2)$_2$$CN(C_2H_3CONH_2)$NOH); 1.44–1.51 (2H, m, NHC(O)$CH_2C\underline{H}_2$); 1.52–1.61 (2H, m, $C\underline{H}_2CH_2CN(C_2H_3CONH_2)$NOH); 1.62–1.73 (2H, m, NHC(O)$C\underline{H}_2$); 2.22–2.32 (3H, m, $C\underline{H}_2$CNOH, CH aziridine); 2.37–2.48 (1H, m, CH aziridine); 2.51–2.60 (1H, m, CH aziridine); 6.99–7.07 (1H, m, H-4 Ph); 7.21–7.35 (5H, m, H-3,5 Ph, NH, NH_2 partially overlapped with solvent signal); 7.41–7.47 (2H, m, H-2,6 Ph); 9.10 (1H, s, NOH). Mass spectrum, *m/z*: 333 $[M+H]^+$.

(*Z*)-7-Amino-7-(hydroxyimino)-*N*-phenylheptanamide (8). $NaHCO_3$ (185 mg, 2.2 mmol, 2.2 equiv) was added to a solution of hydroxylamine hydrochloride (104 mg, 1.5 mmol, 1.5 equiv) in isopropyl alcohol (2.0 ml). The resulting mixture was stirred for 15 min, and compound **20**[10] (216 mg, 1 mmol) was added. The stirring was continued at 80°C for 36 h until the consumption of the starting material. After completion of the reaction, the mixture was cooled to room temperature, precipitate was filtered off, washed with a small amount of water, and dried *in vacuo* to provide product **8** in quantitative yield. White solid, mp 78–80°C (decomp.). ^{1}H NMR spectrum (400 MHz, DMSO-d_6), δ, ppm (*J*, Hz): 1.33–1.39 (2H, m, $C\underline{H}_2$(CH_2)$_2$$C(NH_2)$NOH); 1.53–1.69 (4H, m, ($C\underline{H}_2CH_2$)$_2$$C(NH_2)$NOH); 2.02 (2H, t, *J* = 7.6, NHC(O)$C\underline{H}_2$); 2.35 (2H, t, *J* = 7.4, $C\underline{H}_2C(NH_2)$NOH); 5.04 (2H, br. s, C($N\underline{H}_2$)NOH); 7.08 (1H, t, *J* = 7.4, H-4 Ph); 7.34 (2H, t, *J* = 8.2, H-3,5 Ph); 7.65 (2H, d, *J* = 8.2, H-2,6 Ph); 8.75 (1H, s, NOH); 9.91 (1H, s, NH). ^{13}C NMR spectrum (100 MHz, DMSO-d_6), δ, ppm: 25.4; 26.6; 28.7; 31.1; 36.8; 119.4 (C-4 Ph); 123.3 (C-3,6); 129.0 (C-2,6 Ph); 139.8 (C-1 Ph); 153.3 ($C(NH_2)$NOH); 171.6(C=O). Mass spectrum, *m/z*: 250 $[M+H]^+$.

Determination of IC_{50} and LD_{50}. Evaluation of anticancer activity was performed by examining *in vitro* antiproliferative effects of the synthesized compounds in monolayer tumor cell lines HT1080 (human connective

tissue fibrosarcoma) and MG22A (mouse hepatosarcoma). The borderline concentration, relevant to the highest tolerated dose, was determined for all target compounds **5a**–**l**, **6**–**8** using the 3T3 (mouse Swiss Albino embryo fibroblasts) cell line. All cells were obtained from the ATCC collection. The basal cytotoxicity was used to predict starting doses for *in vivo* acute oral LD_{50} values in rodent. Results are summarized in Table 1.

Measurement of cell viability. HT1080 and MG22A cells were seeded in 96-well plates in Dulbecco's modified Eagle's (DMEM) medium containing 10% fetal bovine serum, 4 mM L-glutamine, without antibiotics, and cultivated for 72 h by exposure to the different concentrations of compounds. After the incubation with the test compounds, the culture medium was removed, and fresh medium with 0.2 mg/ml MTT was added in each well of the plate. After a further incubation (3 h, 37°C, 5% CO_2), the medium with MTT was removed, and 200 µl DMSO was added at once to each sample. The samples were tested at 540 nm on a Tecan multiplate reader Infinite100. The IC_{50} was calculated using the program Graph Pad Prism® 3.0.

Basal cytotoxicity test. The neutral red uptake assay was performed according to the standard protocol by Stokes et al.[13] Balb/c 3T3 cells (9000 cells/well) were placed into 96-well plates for 24 h in DMEM containing 5% fetal bovine serum and then exposed to the test compounds over a range of eight concentrations (1000, 316, 100, 31, 10, 3, 1 µg/ml) for 24 h. Untreated cells were used as a control. After 24 h, the medium was removed from all plates, and the cells were washed with 200 µl of phosphate buffered saline (PBS) for each well. Afterwards, 250 µl of neutral red solution was added (0.05 mg/ml NR in DMEM 24 h preincubated at 37°C and then filtered before use through 0.22 µm syringe filter). The plates were incubated for 3 h, and the cells were washed three times with PBS. The dye within viable cells was released by extraction with a mixture of acetic acid–ethanol–water, 1:50:49. The absorbance of neutral red was measured using a spectrophotometer multiplate reader (TECAN Infinite M1000) at 540 nm. The optical density (OD) was calculated using the formula: OD (treated cells) × 100/OD (control cells). The IC_{50} values were calculated using the program Graph Pad Prism® 3.0. The *in vivo* starting dose is the estimated LD_{50} value calculated by inserting the *in vitro* IC_{50} value into a regression formula: $\log LD_{50}$ (mmol/kg) = 0.439 $\log IC_{50}$ (mmol/l) + 0.621.[14]

HDAC inhibition activity evaluation of selected novel compounds **5a**, **6a**,**b**,**d**,**f**,**g**,**h** were performed using the HDAC Fluorimetric Assay/Drug Discovery Kit, a Fluor de Lys® Fluorescent Assay System (Enzo Life Sci.), according to the manufacturer's protocol. Aziridin-1-yl oximes **5a**,**c**,**d**,**f**,**h**–**j** were dissolved in DMSO to obtain 10 mM solution and diluted to the desired test concentration in HDAC assay buffer. An aliquot (50 µl) of each solution of the test compounds, as well as the positive control without inhibitor, were added to the wells containing HeLa extract (0.5 µl, except for the blank experiment) and HDAC fluorescent Fluor de Lys® Substrate (250 µM), employing Trichostatin A as a positive control. The HDAC reactions were allowed to proceed for 30 min at 37°C and then stopped by the addition of Fluor de Lys® Developer (50 µl). The well plate was incubated at room temperature for additional 15 min. The relative fluorescent units (RFU) were measured in a Tecan Infinite M1000 reader (excitation and emission wavelength 350 and 460 nm, respectively). The set of the RFU values for four different concentrations of each test compound were used to calculate the relative HDAC inhibitory activity. Calculation of IC_{50} was done with the GraphPad Prism 5.03 software (GraphPad Software, La Jolla, CA). All data are the mean of three independent experiments.

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