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Superacid and thiol-ene reactions for access to psammaplin analogues with HDAC inhibition activities

Fatima El Bahhaj [a], Jérôme Désiré [a], Christophe Blanquart [b,c], Nadine Martinet [d], Vincent Zwick [e], Claudia Simões-Pires [e], Muriel Cuendet [e], Marc Grégoire [b], Philippe Bertrand [a,c,*]

[a] Institut de Chimie des Milieux et Matériaux de Poitiers, UMR CNRS 7285, 4 rue Michel Brunet, B28, 86000 Poitiers, France
[b] Inserm, UMR 892, CNRS, UMR 6299, University of Nantes, 8 quai Moucousu, 44007 Nantes cedex 1, France
[c] Réseau Epigénétique du Cancéropôle Grand Ouest, France
[d] Institut de chimie, UMR CNRS 7272, UNSA, F-06108 Nice, France
[e] School of Pharmaceutical Sciences, University of Geneva, University of Lausanne, 30 Quai Ernest-Ansermet, 1211 Geneva, Switzerland

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Dedicated to Professor Jean Marie Coustard, a creative chemist

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ABSTRACT

An innovative synthesis of psammaplin-like structure is proposed based on original methodologies using superacid, microwaves, and S-ene chemistry. The new compounds were evaluated as histone deacetylase inhibitors. The results highlight important considerations when using disulfide prodrugs activated under reductive/oxidative conditions that must be carefully selected depending on tumor cell types.

1. Introduction

In eukaryotic cells DNA is packed in a complex structure regulating transcriptional activity. The smaller level is the nucleosome,[1] an octameric protein complex assembled from two copies of four histone proteins (H2A, H2B, H3, and H4) with about 150 base pairs wrapped around this octamer. DNA transcription is partially regulated by post translational modifications (PTM) on histones and cytosine called epigenetic marks and defining the histone code. 377 Enzymes are regulating these processes sorted in three groups: the writers, erasers, and readers, respectively, adding, removing or recognizing these marks on histones or DNA. These enzymes participate in the recruitment of transcription factors and DNA binding proteins to form multi-protein complexes, in turn regulating genes transcription. Several human diseases such as cancers, neurodegenerative diseases or metabolic disorders result from abnormal gene expression due to the disequilibrium between epigenetic enzymes activities. As PTMs are all reversible, renormalization of aberrant PTMs has been pursued using epigenetic enzymes inhibitors. Histone N^{ε}-lysine acetylation was identified as a key player, a reaction controlled by histone acetyl transferases (HAT)[2] in balance with histone deacetylases (HDAC)[3] and recognized by bromodomain-containing proteins,[4] other reactions on histones being known.[5] HDAC have been particularly studied during the last years for their over expression in several cancer cell lines, resulting in tumor suppressor gene silencing.[6]

HDAC are grouped in four classes, classes I (HDAC 1–3, 8), II (HDAC 4–7, 9, 10) and IV (HDAC 11) being zinc dependant and class III (SIRT1-7) requiring NADPH. SAHA (**1**) (suberoyl anilide hydroxy amide) and romidepsin (**2**), two HDAC inhibitors, are

* Corresponding author. Tel.: +33 549454192; fax: +33 549453702; e-mail address: philippe.bertrand@univ-poitiers.fr (P. Bertrand).

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approved for treating cutaneous T-cell lymphoma[7,8] and are currently investigated in clinical trials in single or combination therapies[9] (Fig. 1). In our search for new HDAC inhibitors, we were interested in developing libraries of new thiol compounds with innovative chemistry. Thiols are HDAC inhibitors supposed to bind the zinc atom in the active site via the free sulfhydryl group. Natural romidepsin (**2**), largazole (**4**),[10] and psammaplins (**3**) illustrate this family, with NHC-31 (**5**)[11] as a synthetic example. Romidepsin[12] is in fact considered as a natural prodrug supposed to be cleaved in cells to the free thiol, the real inhibitor. Largazole is also a prodrug, the thioester group being probably cleaved by esterases in vivo.

Psammaplins are of particular interest because of their various activities including antibacterial,[13,14] chitinase inhibition,[15] and antiproliferative activities due to HDAC inhibition. Some psammaplins, such as psammaplins A[16] and G,[17] also inhibit DNA methyltransferases (DNMT) at the nanomolar level, DNA gyrase,[18] and aminopeptidase N, another zinc-dependent enzyme playing a key role in angiogenesis.[19] Psammaplins are also natural prodrugs, as demonstrated in vitro on HDAC1 activity by incubation of psammaplin A with H_2O_2, dithiothreitol (DTT) or both.[20] In cells, the addition of butionine sulfoximine (BSO), a specific inhibitor of γ-GCS, reduced the glutathione levels and was correlated with lower histone acetylation. The disorganized microvasculature of solid tumors led to hypoxic conditions being exploited with the development of bioreductive compounds.[21] In this respect, treating hypoxic cancer cell lines such as malignant pleural mesothelioma (MPM),[22] an aggressive form of lung cancer with poor prognostic, with bioreducible disulfide compounds should promote epigenetic renormalization[23] by higher cleavage of the disulfide prodrug.

The psammaplins family consists of thioethylamide bromotyrosine metabolites forming dimers by oxidative coupling of a thiol group (Scheme 1). Psammaplin A was described for the first time in 1987, was isolated from various sponges, and has nanomolar specificity toward class I HDACs. Several crystal structures of psammaplin analogues were obtained[24,25] in order to perform SAR studies on various epigenetic targets.[26–28] These studies (Scheme 1) showed that for HDAC inhibition (1) any halogen can replace the bromine atom in the phenyl ring, (2) the phenyl ring should be close to the oxime, (3) the oxime and the disulfide groups are essential for the activity, and (4) the alkyl chain must be short. Molecular modeling using HDAC8 showed that the *o*-bromophenol group is stabilized through interactions with Tyr100 and Phe152 at the rim of the active site entrance, the oxime forming a hydrogen bonding with Asp101. Equivalent results were obtained with HDAC1 as model target. Additional syntheses or patents can be found in the literature for the synthesis of modified monomers of psammaplins or their analogues,[28–31] some of them being fluorescent.[32] Pharmacokinetics of psammaplins has also been determined.[33] Psammaplins syntheses involve in general the preparation of a protected oxime moiety from tyrosine derivatives or oxazoles and final coupling with cystamine or analogues having longer carbon chains. For psammaplin A itself, an improved synthesis was reported by Godert et al., in four steps with an overall 43% yield.[34] We describe in this article a novel and original access to psammaplin analogues and some biological investigations.

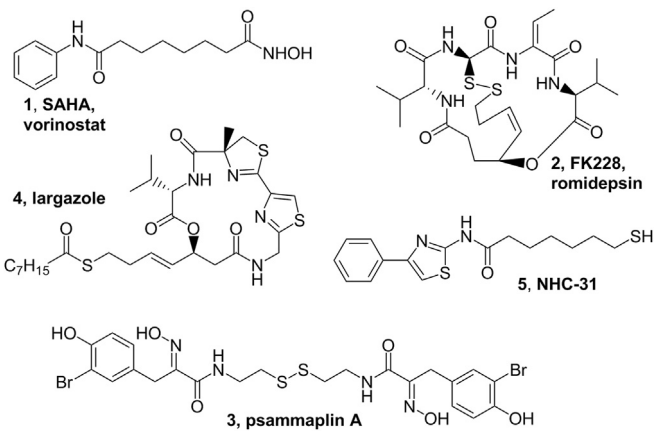
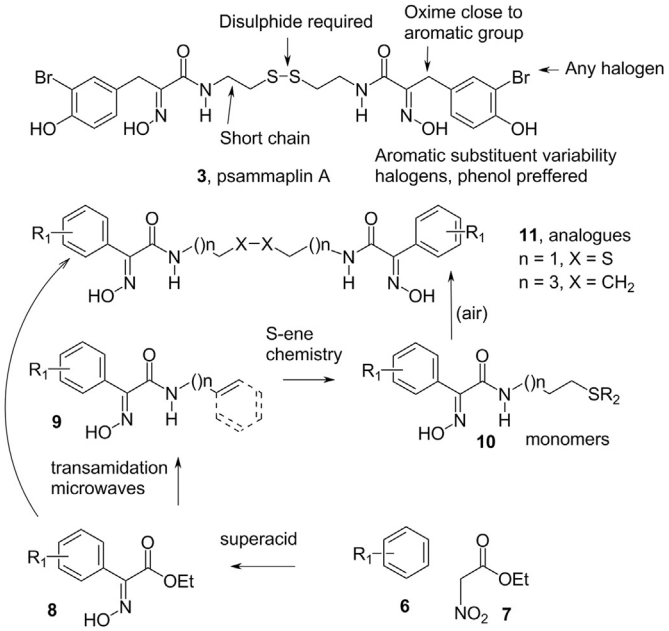


Fig. 1. SAHA and thiol-based HDAC inhibitors.



Scheme 1. Psammaplin pharmacophore and proposed new synthetic strategy toward psammaplins.

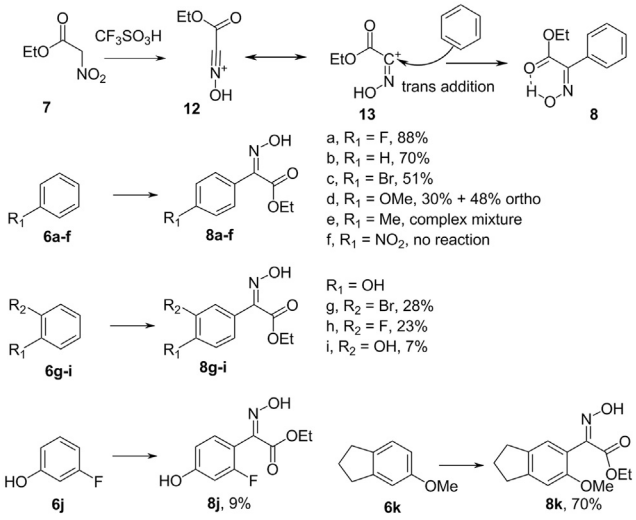
2. Results and discussion

2.1. Synthetic strategy

During our research programs on innovative syntheses in superacid media, we were able to develop an original way to obtain, in one step, an ethoxycarboxyphenyloxime scaffold **8** (Scheme 1).[35] This prompted us to investigate an innovative access to psammaplin analogues as zinc-dependent HDAC inhibitors, where the methylene group between the aromatic ring and the oxime was removed. Superacid chemistry has recently been also described for the synthesis of another family of zinc-dependent enzymes: the carbonic anhydrases.[36] The scaffold **8** was reported in a dissymmetric combinatorial synthesis of psammaplin analogues by Nicolaou' group to be used against methicillin-resistant *Staphylococcus aureus*.[37] The oxime was not tested free but as an *O*-benzyl ether. We report here the use of superacid media for the synthesis of free oxime scaffolds (Scheme 1), introducing microwaves as an efficient method to prepare an intermediate alkenylamide moiety (**9**). This moiety was then converted by thiol–ene chemistry to a thioalkylamide (**10**) as a precursor of the expected dimer analogues (**11**). Final spontaneous oxidative dimerization gave the target compounds in four steps, a competitive strategy with the one of Godert et al.[34a] Some key intermediates and the final thiol derivatives were tested as HDAC inhibitors and for their toxic activities against MPM cells.

The superacid methodology developed by some of us is based on the aromatic electrophilic substitution with a particular electrophile: the hydroxynitrilium ion. This ion is obtained by reacting ethyl nitroacetate **7** in triflic acid, a superacid (Scheme 2). The

hydroxynitrilium ion **12** is the result of water loss after several protonations of the nitro group and is in mesomeric equilibrium with the corresponding carbocation **13** that can be trapped by nucleophiles such as activated aromatic rings. The final oxime can exist as an *E*/*Z* mixture.[38] SAR studies on psammaplins showed that the aromatic ring can be substituted with no more than two groups. The scope of our strategy was determined with various activating (hydroxy, alkyloxy, alkyl) or deactivating (halogen, nitro) substituents. Compounds **8** could add novelty to the current SAR studies as such analogues were never reported in the context of HDAC inhibitors.

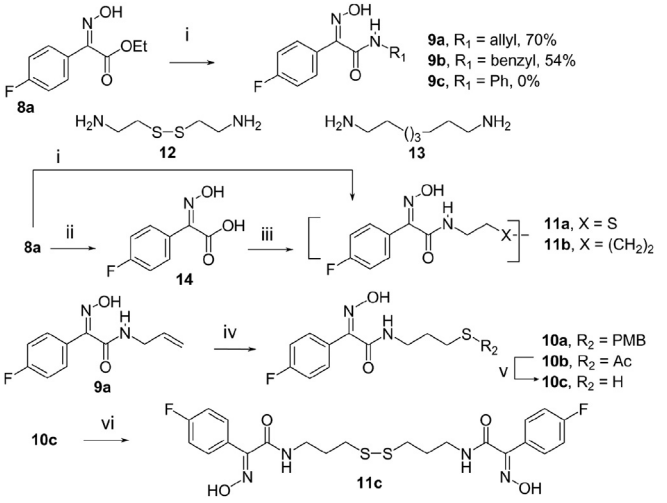


Scheme 2. Mechanism of benzaldoxime formation in superacid from ethyl nitroacetate and aromatics for the preparation of scaffold **8**. Compounds **8a**,**b**,**d**,**k** already reported in Ref. 38. For **8a** the yields have been improved with a new procedure.

2.2. Syntheses

The commercially available ethyl nitroacetate **7** was reacted with various aromatic compounds **6**, at −5 to −15 °C depending on the substrate reactivity in the presence of triflic acid (Scheme 2). Monosubstituted halogenated substrates **6a**,**c** gave the best yields and selectively provided the *para* compounds as major products. Nitrobenzene **6f** did not react and was almost fully recovered while the starting nitroacetate was not, indicating that the nitrilium ion must be formed but the aromatic ring is not reactive enough to trap it. As reported earlier, substrates **6d**,**k** gave the *ortho* isomers as the major product in good yield. In our hands, toluene did not react properly, the reaction being sometimes violent and giving a complex mixture from which the expected **8e** was not isolated. With phenols **6g**–**j** substituted with another halogen atom, the reaction was carried out after optimization at −5 °C instead of −15 °C to give moderate to good yields of compounds **8g**–**j**. The observed negative effect of the phenol group compared to monohalogenated benzene was even higher with catechol **6i**. The presence of free hydroxyl groups probably increased the apparent water solubility, making such derivatives difficult to extract from the complex mixture. This will require further optimization.

From the esters **8**, it was thought possible to prepare the psammaplin analogues **11** by transamidation of **8** with amines under classical heating or under microwave neat conditions as previously reported.[39] This methodology was developed with ester **8a** obtained with the best yields for the preparation of derivatives **11** (Scheme 3). Transamidation with basic monoamine (allyl- or benzylamine) under heating gave the expected amides **9a**,**b** in moderate to good yields and the reaction times were shortened under microwaves irradiations as expected (1 h at 100 °C under 300 W). In their reported work, Ferroud et al.[38] used ethyl acetate as a model for amidation and with benzylamine, the corresponding amide was obtained with a yield of 72%, at 200 °C and 200 W for 2.5 min. For vinylacetate, 1 h was required. Our conditions and yields appeared similar. The less basic aniline did not give the amide **9c**. Unfortunately, direct coupling of diamines such as cystamine (**12**) hydrochloride (treated or not to remove HCl) or diaminooctane (**13**) with two esters **8a** was found difficult both under heating and microwave conditions. Attempts to prepare **11a**,**b** from the ester **8a** by peptide coupling via the acid **14** (obtained in 68% yield) were not successful. This peptide coupling was described in psammaplin syntheses with protected oxime, but in our case this could have made the synthesis longer and not competitive. This suggests that the oxime protection is critical for the peptide coupling strategy.



Scheme 3. Transamidation: (i) amine, heating neat or microwave neat; (ii) LiOH 2.5 M aq THF/MeOH; (iii) **12** or **13**, EDC or DCC, NHS, CH_2Cl_2; (iv) hν, R_2–SH; (v) NaOH, MeOH; (vi) air.

Having in mind to maintain the overall synthesis as short as possible, radical S-ene chemistry was investigated with allylamide **9a** to introduce the sulfur atom in a protected form (Scheme 3). *para*-Methoxybenzylthiol and thioacetic acid gave access to the corresponding thioether and ester **10a**,**b** (UV irradiation lamp). The thioester **10b** was hydrolyzed to give the free thiol **10c** and subsequent oxidative dimerization gave the expected psammaplin analogue **11c**. Regarding the thioester **10b**, this compound can be compared to largazole (**4**) (Fig. 1). These two compounds **10b** and **4** can be considered as prodrugs that can be hydrolyzed in cells.[10] In this respect, compound **10b** was also evaluated in our biological models.

The reported configuration of natural psammaplins[13,40] is *E*, confirmed by X-ray analysis of various derivatives[24,25] or found during molecular modeling with epigenetic targets.[24,27] Calculations on isolated oximes **8** (MM2 refined by RHF/3-21G, Chembio3D tools) and psammaplin A (**2**) showed that the *Z* oxime is always more stable due to the six-membered ring formed by a hydrogen bonding between the oxime hydrogen and carbonyl oxygen atoms. Similar results were found for amides and esters. Indeed, it is possible that in the HDAC active site, the compounds could be isomerized to generate better interactions with the zinc atom and additional residues. Thus we assumed that the major isomers should be *Z*, which was also explained by the reaction conditions or mechanism in superacids, where a trans attack on the carbocation is preferred (Scheme 2) as in the Beckmann rearrangement. Fortunately, in the amide **9** series it was possible to differentiate the oxime isomers for compound **9b**, the major one being partially separated by flash

chromatography. The methylene group of the benzylamide part gave two distinct signals in proton NMR. For derivative **9b** the amide group in trans gave the more stable compound (Fig. 2). Two stable isomers with different benzyl group orientations could be found and could explain the two types of ^{1}H NMR proton signals.

2.3. Biology

Designed to be HDAC inhibitors, compounds **8**–**11** were first tested for their ability to restore histone acetylation. In order to verify if the arylcarbamoyloxime group can be isosteric to the hydroxamic acid or benzamide groups found in HDAC inhibitors, we selected intermediates **8a**,**h**,**j** and **9a**,**b** for preliminary tests. No HDAC inhibition was observed with these compounds, even at high 300 μM concentrations, indicating that the carbamoylbenzaldoxime group is not a hydroxamic acid or benzamide substitute for HDAC inhibition, possibly due to the steric hindrance of the conjugate phenyl ring.

In contrast, the final thiol derivatives **10a**–**c** and **11c** showed some HDAC inhibition in vitro (Table 1). If compound **10a**, protected as a PMB ether was expected to give no inhibition, more surprisingly compounds **10c** and **11c** gave a moderate HDAC inhibition despite their similarities to psammaplins. The percentage inhibition was determined for compound **11c** as 10% at 300 μM. Compound **11c** seems to have some selectivity for HDAC 6 compared to HDAC 2 (about 5-fold) while **10c** was only two fold more selective for HDAC 6 compared to HDAC1-2.

In order to evaluate the effect of the compounds on histone H3 acetylation in living cells, a bioluminescent resonance energy transfer (BRET)-based assay was used.[41] This assay consists in the transfection of cells with an expression vector coding for a bromodomain (BrD), which recognizes acetylated histones, fused to *Renilla* luciferase (Rluc) and with an expression vector coding for histone H3 fused to the yellow fluorescent protein (YFP). In the presence of an HDACi, the histone H3–YFP is acetylated and then recruits the BrD-Rluc. In the presence of the Rluc substrate, coelenterazine, an energy transfer from Rluc to YFP occurs due to their close proximity. This results in the emission of a fluorescent signal at 530 nm. Thus, an increase of the BRET signal reflects an increase of histone H3 acetylation. From enzymatic results, only compounds **10c** and **11c** were evaluated in the BRET assay.

Enzymatic HDAC inhibition by some psammaplins and their analogues can reach the nanomolar level but usually in cells higher concentrations are required to obtain the same effect, resulting in toxicity. For psammaplins this could be due to the necessary conversion of disulfide to free thiols,[20] limiting the amount of bioactive compound at a given time. Hypoxic or normoxic environments in tumors can also alter the formation of free thiols from disulfides. Hypoxic cancer cells such as MPM overexpress several cytochrome oxidases and various dehydrogenases[42] or reactive oxygen species (ROS) that may also modify the thiol formation from disulfides.

To take these elements into consideration we compared the BRET results for HDAC inhibition for compounds used alone or together with *N*-acetylcystein (NAC). NAC is a powerful antioxidant molecule that should favor the free thiol formation and diminish oxidative processes. This is illustrated in Fig. 3A and C. When cells were treated with compounds **10c** and **11c** a poor induction of BRET

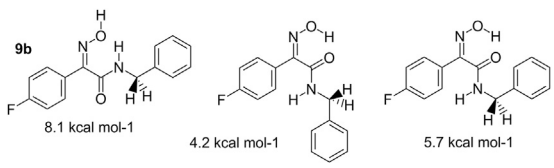


Fig. 2. Relative stability of **9b** conformers.

Table 1
HDAC inhibition (%)[a] in a nuclear extract and toward various isoforms

	% HDAC inhibition (300 μM)			
	HeLa nuclear extract	HDAC 1	HDAC 2	HDAC 6
10a	0.3	ND[b]	ND	ND
10b	5.7	ND	ND	ND
10c	13.2	19.4	13.3	29.0
11c	10.4	0	8.1	38.9

[a] Results are the means of two independent experiments.
[b] ND: not determined.

signal was observed associated with a dose dependent mild toxicity (Fig. 3B and D). This toxicity is dose dependent and could be responsible for the absence of BRET signal induction. The use of compounds **10c** and **11c** combined with NAC was then investigated. We first confirmed that the treatment with NAC alone did not change the BRET signal and cell viability as shown in Fig. 3A–D. However, the BRET signal induction by **10c** and **11c** in the presence of NAC was strongly increased (Fig. 3A and C, right): 5-fold for **11c** and 6-fold for **10c**, implying an increased histone H3 acetylation. This increase of HDAC inhibition activity was associated with a decrease of toxicity (Fig. 3B and D, right). The effect of NAC is specific to psammaplin derivatives given that it did not change HDACi activity of SAHA (Supplementary data Fig. S1A and B). These results highlight the importance of the oxidative process in cancer cells when dealing with biomolecules reacting to oxidative or reducing environment, illustrated by the activity of the disulfide prodrug **11c** in the context of HDAC inhibition.

3. Conclusion

In conclusion, an innovative synthetic strategy to access psammaplin analogues that could be used to prepare new derivatives without protecting chemistry is proposed. Interestingly, antiproliferative micromolar activities were observed in the mesothelioma cell line M56 for compounds **8a**, **9a**, and **9b** (data not shown). In contrast, other fluoro compounds including a phenol group like **8h**,**j** were inactive. Some oxime intermediates **8** showed toxic activities not correlated with HDAC inhibition. The moderate HDAC inhibition observed for final thiol derivatives when used in

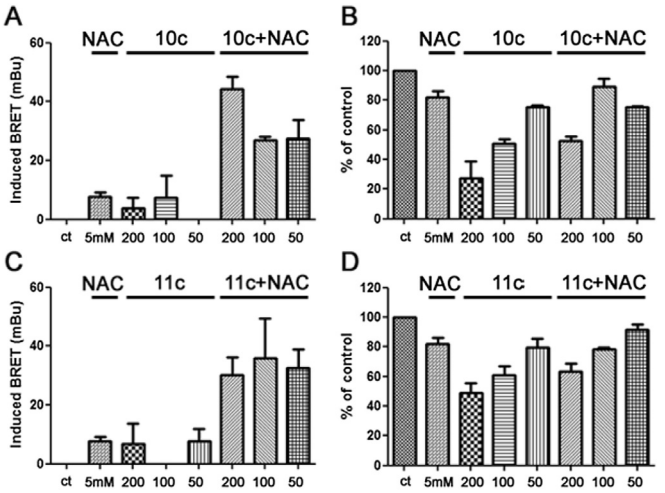


Fig. 3. Biological characterization of compounds **10c** and **11c** using BRET. MeT-5A cells were transfected with phRluc-C1 BrD and pEYFP-C1 histone H3. Then, cells were treated for 24 h with the indicated compound concentrations in the presence or not of NAC. (A, C) Induced-BRET signal obtained with the various conditions. (B, D) Viability of cells measured with the various conditions. Viability was determined by measuring luciferase activity and is expressed as the percent of luciferase activity in the control condition. Results are the means±S.E.M of three independent experiments.

combination with NAC warrant further synthetic investigations to improve the pharmacophore model for this series of analogues. The redox properties of the tumoral environment can be very important when analyzing the activities of compounds such as the final thiol derivatives **10c** and **11c**. Our work complements results described to decipher psammaplin derivatives behavior in cells with the use of an alternative ROS scavenger contributing to the improvement of histone acetylation in cells.[12,20,21] With cancer cell lines developing hypoxic conditions, the use of bioreducible/oxidable compounds should be carefully investigated.

4. Experimental section

4.1. General methods

All commercial chemicals and solvents are reagent grade and were used without further purification. Reaction were monitored by thin layer chromatography on 0.25 mm silica gel plates (60F-254, E. Merck) and visualized with UV light or 5% phosphomolybdic acid in 95% ethanol. ^{1}H NMR and ^{13}C were recorded on a Bruker 400 MHz Avance DPX. Chemical shifts are reported in parts per million (ppm, δ units). Abbreviations used: PE: petroleum ether; EtOAc: ethyl acetate; DMF: dimethylformamide; DCM: dichloromethane; THF: tetrahydrofuran.

4.2. Ethyl 2-(4-fluorophenyl)-2-hydroxyimino-acetate (8a)

In a flask was added CF_3SO_3H (5 ml, 55.8 mmol) and after cooling at −15 °C with an acetone bath a mixture of acetate **7** (0.8 ml, 7 mmol) and PhF (2 ml, 7 mmol) was added drop wise. The reaction mixture was stirred for 7 h under nitrogen atmosphere and allowed to warm to room temperature. Ice (10 g), $NaHCO_3$ (4.7 g, 55.9 mmol), and NaCl for aqueous phase saturation were then added. The resulting mixture was extracted (DCM, 4×20 ml) and the combined organic layers dried ($MgSO_4$) and concentrated under vacuum. The crude product was recrystallized from PE/DCM to give **8a** as a beige powder (1.3 g, 88%). R_f: 0.86 DCM/MeOH (10:1); ^{1}H NMR (400 MHz, acetone-d_6) δ: 1.31 (t, 3H, J=7.1 Hz), 4.29 (q, 2H, J=7.1 Hz), 7.22 (t, 2H, J=8.9 Hz), 7.59 (m, 2H), 11.47 (s, 1H); ^{19}F NMR (376 MHz, acetone-d_6) δ: −113.1, −115.1; ^{13}C NMR (100 MHz, $CDCl_3$) δ: 14.4, 62.5, 115.4/115.6, 126.2/126.3, 132.65/132.7, 149.2, 162.4, 164.6/164.9.

4.3. Ethyl 2-(4-bromophenyl)-2-hydroxyimino-acetate (8c)

In a flask was added CF_3SO_3H (4.5 ml, 51 mmol) and after cooling at −15 °C with an acetone bath a mixture of acetate **7** (0.72 ml, 6 mmol) and PhBr (2 ml, 7 mmol) was added drop wise. The reaction mixture was stirred for 7 h under nitrogen atmosphere and allowed to warm to room temperature. Ice (10 g), $NaHCO_3$ (4.7 g, 55.9 mmol), and NaCl for aqueous phase saturation were then added. The resulting mixture was extracted (DCM, 4×20 ml) and the combined organic layers dried ($MgSO_4$) during 1.5 h and concentrated under vacuum. The crude product was purified (flash chromatography, silica gel, DCM/MeOH 95:5) to give **8c** as a white solid (0.86 g, 51%). R_f: 0.84 DCM/MeOH (9:1); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.32 (t, 3H, J=8 Hz), 4.35 (q, 2H, J=7.1 Hz), 7.39 (m, 4H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 14.1, 62.4, 77.2, 128.5, 128.6, 131.0, 131.3, 132.0, 152.1.

4.4. Ethyl 2-(3-bromo-4-hydroxy-phenyl)-2-hydroxyimino-acetate (8g)

In a flask was added CF_3SO_3H (5 ml, 55.8 mmol) and after cooling at −15 °C with an acetone bath a mixture of acetate **7** (1 ml, 8.75 mmol) and 2-bromophenol (1 ml, 8.62 mmol) was added drop wise. The reaction mixture was then stirred for 6 h under nitrogen atmosphere and allowed to warm to room temperature, then poured into DCM/MeOH (4:1, 50 ml) preliminary cooled between −30 °C and −40 °C. Saturated solutions of NaCl (10 ml) and $NaHCO_3$ (55.8 mmol) were added while at −40 °C and allowed to warm to room temperature. After pH control (pH 5), the resulting mixture was extracted (DCM/MeOH 10:1, 4×20 ml), the combined organic layers dried ($MgSO_4$), and concentrated under vacuum. The crude product was recrystallized from DCM to give **8g** as a light yellow powder (695 mg, 28%). R_f: 0.5 DCM/MeOH (10:1); ^{1}H NMR (400 MHz, CD_3OD) δ: 1.33 (t, 3H, J=7.1 Hz), 4.31 (q, 2H, J=7.1 Hz), 6.93 (d, 1H, J=8.4 Hz), 7.34 (dd, 1H, J=2, 8.4 Hz), 7.70 (d, 1H, J=2 Hz); ^{13}C NMR (100 MHz, CD_3OD) δ: 14.4, 62.9, 110.0, 116.3, 123.3, 131.3, 135.5, 156.6, 148.9, 165.9.

4.5. Ethyl 2-(3-fluoro-4-hydroxy-phenyl)-2-hydroxyimino-acetate (8h)

In a flask was added CF_3SO_3H (5 ml, 55.8 mmol) and after cooling at −5 °C with an acetone bath a mixture of acetate **7** (1 ml, 8.75 mmol) and 2-fluorophenol (0.7 ml, 7.84 mmol) was added drop wise. The reaction mixture was then stirred for 6 h under nitrogen atmosphere and allowed to warm to room temperature, then poured into DCM/MeOH (4:1, 50 ml) preliminary cooled between −30 °C and −40 °C. Saturated solutions of NaCl (10 ml) and $NaHCO_3$ (55.8 mmol) were added while at −40 °C and allowed to warm to room temperature. After pH control (pH 5), the resulting mixture was extracted (DCM/MeOH 10:1, 4×20 ml), the combined organic layers dried ($MgSO_4$), and concentrated under vacuum. The crude product was recrystallized from DCM to give **8h** as a white powder (457 mg, 23%). R_f: 0.54 DCM/MeOH (10:0.5); ^{1}H NMR (400 MHz, CD_3OD) δ: 1.33 (t, 3H, J=7.1 Hz), 4.32 (q, 2H, J=7.1 Hz), 6.95 (t, 1H), 7.18 (dt, 1H, J=2, 8 Hz), 7.35 (dd, 1H, J=2, 12 Hz); ^{13}C NMR (100 MHz, CD_3OD) δ: 14.4, 62.9, 153.1, 117.9, 122.2, 127.5, 118.7, 147.4, 150.7, 165.9.

4.6. Ethyl 2-(3,4-dihydroxy-phenyl)-2-hydroxyimino-acetate (8i)

In a flask was added CF_3SO_3H (5 ml, 55.8 mmol) and after cooling at −5 °C with an acetone bath a mixture of acetate **7** (1 ml, 8.75 mmol) and catechol (700 mg, 6.36 mmol) was added drop wise. The reaction mixture was then stirred for 6 h under nitrogen atmosphere and allowed to warm to room temperature, then poured into DCM/MeOH (4:1, 50 ml) preliminary cooled between −30 °C and −40 °C. Saturated solutions of NaCl (10 ml) and $NaHCO_3$ (55.8 mmol) were added while at −40 °C and allowed to warm to room temperature. After pH control (pH 5) the resulting mixture was extracted (DCM/MeOH 10:1, 4×20 ml), the combined organic layers dried ($MgSO_4$), and concentrated under vacuum. The crude product was purified by flash chromatography (silica gel, DCM/MeOH gradient 100:0 to 90:10) to give **8i** as a brown powder (101 mg, 7%), which is a mixture of two inseparable isomers. R_f: 0.4 DCM/MeOH (10:0.5). Isomer 1 ^{1}H NMR (400 MHz, acetone-d_6) δ: 1.16 (t, 3H, J=7.1 Hz), 4.28 (q, 2H, J=7.1 Hz), 6.80 (m, 1H), 6.92 (dd, 1H, J=2, 8.3 Hz), 7.06 (d, 1H, J=2 Hz). Isomer 2 ^{1}H NMR (400 MHz, acetone-d_6) δ: 1.30 (t, 3H, J=7.1 Hz), 4.38 (q, 2H, J=7.1 Hz), 6.80 (m, 1H, H-5), 6.92 (dd, J=2, 8.3 Hz, 1H, H-6), 7.09 (d, J=2 Hz, 1H, H-2). Isomer 1 ^{13}C NMR (100 MHz, acetone-d_6) δ: 14.3, 61.6, 113.1, 115.4, 122.2, 123.0, 145.0, 147.2, 149.9, 165.0. Isomer 2 ^{13}C NMR (100 MHz, acetone-d_6) δ: 14.5, 62.1, 116.2, 117.4, 123.0, 123.9, 146.1, 148.1, 151.8, 165.4.

4.7. Ethyl 2-(2-fluoro-4-hydroxy-phenyl)-2-hydroxyimino-acetate (8j)

In a flask was added CF_3SO_3H (5 ml, 55.8 mmol) and after cooling at −25 °C with an acetone bath a mixture of acetate **7**

(0.8 ml, 21 mmol) and 3-fluorophenol (2 ml, 21 mmol) was added drop wise. The reaction mixture was then stirred for 24 h under nitrogen atmosphere and allowed to warm to room temperature, then poured into ice (10 g). Saturated NaCl (10 ml) and $NaHCO_3$ (4.7 g, 55.8 mmol) were then added. The resulting mixture was extracted (DCM/MeOH, 95:5, 4×20 ml), the combined organic layers dried ($MgSO_4$) for 1 h, and concentrated under vacuum. The crude product was purified (flash chromatography, silica gel EtOAc/PE gradient 30:70 to 90:100) to give **8j** as a beige powder (128.1 mg, 8%). ^{1}H NMR (400 MHz, acetone-d_6) δ: 1.28 (t, 3H, J=7.1 Hz), 4.26 (t, 2H, J=7.1 Hz), 6.66 (dd, 1H, J=2.3, 11.6 Hz), 6.76 (dd, 1H, J=0.4, 8.6 Hz), 7.33 (t, 1H, J=8.3 Hz), 9.11 (s, 1H), 11.55 (s, 1H); ^{13}C NMR (100 MHz, acetone-d_6) δ: 30, 100, 105, 110, 113, 130, 206.2.

4.8. 2-(4-Fluorophenyl)-2-hydroxyiminoacetic acid (14)

Ester **8a** (0.5 g, 2.37 mmol) and 2.5 M aqueous LiOH (5.4 ml) were added to a solution of THF/MeOH (27:5.4 ml (5:1)). After overnight stirring at ambient temperature, the reaction mixture was diluted with EtOAc (100 ml) and 6 N aqueous HCl was added until pH=2. The layers were separated and the organic one washed with saturated aqueous NaCl, dried ($MgSO_4$), and concentrated under vacuum. The crude compound was recrystallized from DCM/MeOH to give acid **14** as a white solid (0.41 g, 94%). R_f: 0.08 (DCM/MeOH 9:1). ^{1}H NMR (400 MHz, DMSO-D6) δ: 6.93 (d, 2H, J=J=8 Hz), 7.15 (d, 2H, J=8 Hz), 12.06 (s, 1H), 12.79 (s, 1H).

4.9. *N*-Allyl-2-(4-fluorophenyl)-2-hydroxyimino-acetamide (9a)

Fluoroester **8a** (2.82 g, 13 mmol) was dissolved in allylamine (20 ml, 268 mmol) and irradiated under microwaves at 100 °C for 1 h at 300 W (25 W consumption most of the time). After cooling, the excess allylamine was evaporated under vacuum and the resulting oil purified (flash chromatography DCM/MeOH 99:1) to give amide **9b** as a solid (2.66 g, 92%). R_f: 0.67 (DCM/MeOH 9:1); ^{1}H NMR (400 MHz, acetone-d_6) δ: (major isomer) 3.95 (m, 2H), 5.09 (qd, 1H, J=10.3, 1.5 Hz), 5.23 (qd, 1H, J=17.2, 1.8 Hz), 5.92 (m, 1H), 7.19 (m, 2H), 7.63 (m, 2H), 11.1 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 42.2, 115.1/115.3, 115.7, 126.9, 129.3, 133.1/133.2, 135.9, 150.8, 164.1.

4.10. *N*-Benzyl-2-(4-fluorophenyl)-2-hydroxyimino-acetamide (9b)

Fluoroester **8a** (100 mg, 0.47 mmol) was dissolved in benzylamine (1.04 ml, 9.48 mmol) and irradiated under microwaves at 100 °C for 1 h at 300 W. After cooling, the reaction mixture was diluted with DCM (1 ml) and washed several times with 1 M aqueous HCl until no more excess benzylamine was detected by TLC. The resulting viscous oil was recrystallized from DCM/PE to give amide **9b** as a mixture of two isomers in a 1:3.3 ratio as white solid (69.5 mg, 54%). R_f: 0.8 (DCM/MeOH 95:5); ^{1}H NMR (400 MHz, $CDCl_3$) δ: (isolated major isomer) 4.39 (d, 2H, J=6.1 Hz), 7.20 (t, 2H, J=7.0 Hz), 7.26 (t, 1H, J=7.0 Hz), 7.35 (m, 4H), 7.65 (dd, 2H, J=5.6, 8.8 Hz), 8.12 (s, 1H), 11.15 (s, 1H); (signals for minor isomer in the mixture) 4.50 (d, 2H, J=6.3 Hz), 7.32 ((t, 2H, J=8.3 Hz), 7.56 (dd, 2H, J=2.7, 4.6 Hz).

4.11. 2-(4-Fluorophenyl)-2-(hydroxyimino)-*N*-(3-((4-methoxybenzyl)thio)propyl)acetamide (10a)

A solution of **8a** (200 mg, 0.90 mmol) in acetonitrile (15 ml) was degassed under Ar for 10 min, then DPAP (46 mg, 20 mol %) and PMBSH (376 μL, 3 equiv, 2.70 mmol) were added. The mixture was stirred under UV irradiation for 1 h30, concentrated under vacuum, and purified by silica gel flash chromatography (DCM/MeOH, 98:2) to give **10a** as a white solid (255 mg, 75%). R_f=0.4 (DCM/MeOH, 98:2); ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 1.77 (m, 2H), 2.41 (t, 2H), 3.37 (q, 2H), 3.65 (s, 2H), 3.78 (s, 3H), 6.85 (d, 2H), 7.06 (t, 2H), 7.20 (d, 2H), 7.45 (m, 2H), 8.92 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 28.1, 28.5, 35.5, 38.4, 55.5, 114.1, 114.8, 115.1, 124.1, 130.0, 130.4, 131.9, 150.3, 158.5, 163.3; HRMS for $C_{18}H_{19}FN_2O_3SNa$, m/z calcd 399.1154 $[M+Na]^+$, found 399.1153.

4.12. *S*-(3-(2-(4-Fluorophenyl)-2-(hydroxyimino)acetamido)-propyl) ethanethioate (10b)

A solution of **8a** (250 mg, 1.12 mmol) in acetonitrile (15 ml) was degassed under Ar for 10 min, then DPAP (62 mg, 20 mol %) and thioacetic acid (240 μL, 3 equiv, 3.37 mmol) were added. The mixture was stirred under irradiation for 1 h30, concentrated under vacuum, and purified by silica gel flash chromatography (DCM/MeOH, 98:2) to give **10b** as a white solid (190 mg, 56%). R_f=0.4 (DCM/MeOH, 98:2); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.82 (m, 2H), 2.34 (s, 3H), 2.92 (t, 2H), 3.37 (q, 2H), 6.67 (s, 1H), 7.07 (t, 2H), 7.49 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 26.3, 29.5, 30.7, 37.8, 114.8, 115.9, 124.2, 129.2, 131.9; HRMS for $C_{12}H_{13}FN_2O_3SNa$, m/z calcd 321.0685 $[M+Na]^+$, found 321.0683.

4.13. 2-(4-Fluorophenyl)-2-(hydroxyimino)-*N*-(3-mercaptopropyl)acetamide (10c)

A solution of **10b** (190 mg, 0.64 mmol) in methanol (10 ml) was degassed under argon for 10 min, then NaSMe (45 mg, 1 equiv) was added and the mixture stirred at room temperature under argon for 45 min. The resulting solution was poured into 0.1 M aqueous HCl (20 ml) and extracted with DCM (3×15 ml). The organic layers were washed with brine (20 ml), dried ($MgSO_4$), and concentrated under vacuum. A purification by silica gel flash chromatography (DCM/MeOH, 98:2) gave **10c** (113 mg, 69%) as a white solid. R_f=0.4 (DCM/MeOH, 98:2); ^{1}H NMR (400 MHz, CD_3OD) δ: 1.75 (m, 2H), 2.44 (t, 2H), 3.20 (t, 1H), 3.31 (t, 2H), 7.03 (t, 2H), 7.44 (m, 2H); ^{13}C NMR (100 MHz, CD_3OD) δ: 22.4, 34.7, 39.0, 115.5, 116.4, 127.0, 129.3, 133.1, 151.0; HRMS for $C_{11}H_{13}FN_2O_2SNa$, m/z calcd 279.0579 $[M+Na]^+$, found 279.0578.

4.14. *N,N′*-(Disulfanediylbis(propane-3,1-diyl))bis(2-(4-fluorophenyl)-2-(hydroxyimino)acetamide) (11c)

An aqueous solution of NH_4OH (2 ml) was added to a solution of **10c** (100 mg, 0.41 mmol) in MeOH (20 ml), then the mixture was stirred at room temperature under air atmosphere for 24 h. After concentration, the crude was diluted with water (10 ml) and extracted with dichloromethane (3×20 ml). The organic layers were dried ($MgSO_4$) and concentrated under vacuum to give **11c** without further purification. R_f=0.4 (DCM/MeOH, 98:2); ^{1}H NMR (400 MHz, CD_3OD) δ (ppm): 1.87 (m, 4H), 2.66 (t, 4H), 3.31 (t, 4H), 7.03 (m, 4H), 7.44 (m, 4H); ^{13}C NMR (100 MHz, acetone-d_6) δ: 30.1, 36.4, 38.8, 115.0, 115.3, 116.1, 116.4, 127.0, 129.2, 133.1, 162.4, 164.5; HRMS for $C_{22}H_{24}F_2N_4O_4S_2Na$, m/z calcd 533.1104 $[M+Na]^+$, found 533.1108.

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Supplementary data

Material and methods for the HDAC inhibition assays and antiproliferative activities. Supplementary data associated with this article can be found in the online version, at http://dx.doi.org/10.1016/j.tet.2014.10.053.

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