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Stereodefined and polyunsaturated inhibitors of histone deacetylase based on (2*E*,4*E*)-5-arylpenta-2,4-dienoic acid hydroxyamides

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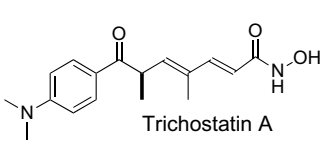
Abstract—Syntheses of (2*E*,4*E*)-5-arylpenta-2,4-dienoic acid hydroxyamides are described, some of which are potent inhibitors of histone deacetylase, a double bond conferring more than a 10-fold increase in potency compared with the triple bond analogue oxamflatin. Variation of substituents on the aromatic ring has a marked effect on potency, in vitro IC_{50} values down to 50 nM being obtained.

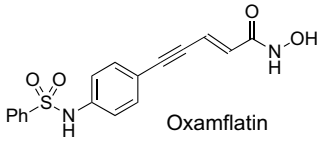
1. Introduction

In 1964 was described the first isolation of histone deacetylase from crude nuclear extracts of cells,[1] but only recently has molecular characterisation of isoforms of the enzyme been achieved.[2] Inhibitors of histone deacetylase (HDAC) enzymes are a new and promising class of anticancer agents, able to regulate transcription and inhibit cancer cell proliferation by induction of cell cycle arrest, differentiation and/or apoptosis.[3] Reversible post-translational acetylation of ε-amino groups of highly conserved lysine residues in nucleosome core histones is important in the modification of chromatin topology and gene transcription.[4–6] Acetylation status is controlled by the opposing activities of histone acetyltransferase (HAT) and HDAC enzymes. HDAC inhibitors induce histone hyperacetylation associated with transcriptional activation of certain genes but repression of an equal or larger number of other genes. Non-histone proteins including transcription factors, nuclear hormone receptors and α-tubulin are also targets for acetylation and there is increasing evidence that acetylation has an important regulatory role in diverse cellular processes. In the HDAC enzyme pocket, the chain portion of the natural product trichostatin A can take the place of an acetylated lysine residue of histone protein, the potent inhibition of HDAC by trichostatin A being consistent with the binding of its hydroxamic acid unit to a zinc atom in the catalytic pocket of the enzyme, as shown in a crystal structure of a zinc complex of trichostatin A bound to the histone deacetylase-like protein HDLP.[7]

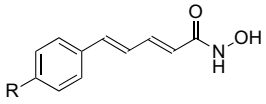
As part of our anticancer programme centred on therapy using small molecules, we have identified novel antileukaemic agents.[8a,b] By using structural features



Trichostatin A



Oxamflatin



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present in the natural product trichostatin A, a potent inhibitor of HDAC, we sought to design and synthesise novel small-molecule therapeutic agents.[9] It was of interest to ascertain whether HDAC activity could still be obtained by formal excision of the propionyl unit present in trichostatin A, and whether improved metabolic stability might also result. We were encouraged by the moderate HDAC inhibitory activity reported for oxamflatin, a mammalian antitumour agent that causes morphological reversion of cancer cells[10] and induces activation of the transcription factor Jun D.[11] Oxamflatin contains an enyne group linking the aromatic portion and the hydroxamic acid terminus. Here, we report that a *trans*,*trans*-1,3-butadiene linkage is indeed compatible with potent HDAC inhibition, a 10-fold improvement on oxamflatin ($IC_{50} = 2\,\mu M$) being readily achieved, and we describe the synthesis and activities of some arylpenta-2,4-dienoic acid hydroxyamides with IC_{50} values extending down to 50 nM.

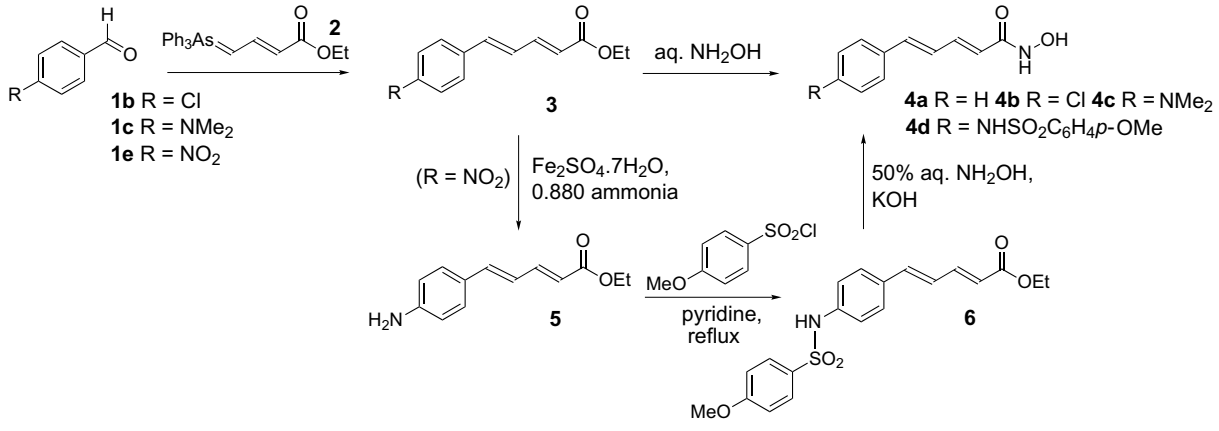
2. Chemistry

Few 5-arylpenta-2,4-diene acid hydroxyamides are known, and the most general method suffers from very low yields in the Wittig olefination of aromatic aldehydes.[12] (2*E*,4*E*)-5-Arylpenta-2,4-diene acid hydroxyamides that lack further substitution at the 2-, 3-, 4- and 5-positions, and their corresponding carboxylic acid and ester precursors present challenges since (a) conventional Wittig olefinations are often not efficient, (b) the diene configuration must be controlled and (c) appropriately substituted cinnamaldehdyes may be inaccessible. We sought to overcome all those problems by reaction of an aromatic aldehyde with a stabilised arsenic ylid (Scheme 1), the latter being known for achieving high (*E*)-selectivity.[13] Reaction of aromatic aldehydes **1b**, **1c** and **1e** in THF with the ylid derived from 3-ethoxycarbonylallylidenetriphenylarsonium bromide, using 2.0 equiv K_2CO_3 for **1b** (7 days at 0 °C then 10 min at 20 °C) and 1.1 equiv *n*-BuLi for **1c** and **1e** (20 min at 0 °C then 10 min at 20 °C) afforded the corresponding dienic esters **3b** (83%), **3c** (51%) and **3e** (81%). Treatment of esters **3b** and **3c** in methanol with a mixture of 50% aqueous hydroxylamine (10 equiv) and KOH (3.0 equiv) in methanol (addition at 0 °C over 30 min followed by a further period at 0 °C for 30 min, then stirring for 16 h at 20 °C; procedure A) afforded the hydroxamic acids **4b** (59%) and **4c** (66%) after recrystallisation from ethyl acetate.

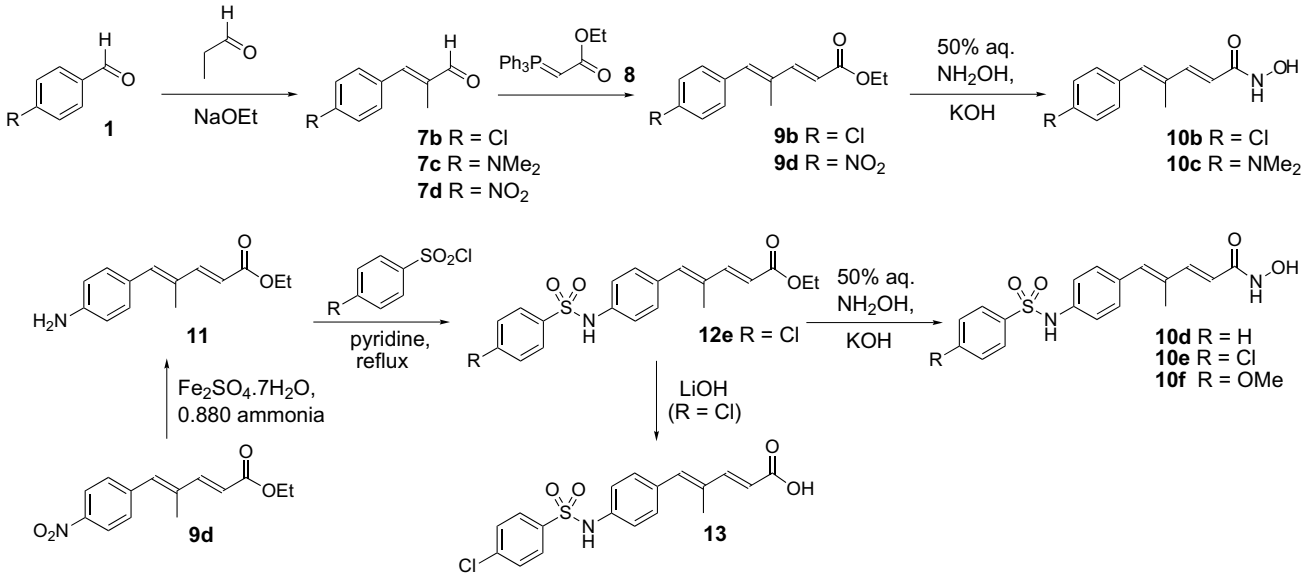
The parent compound **4a** was prepared in 83% yield by reaction of cinnamaldehyde with (triphenyl-γ^5-phosphanylidene)acetic acid ethyl ester (1.2 equiv) in toluene at 40 °C for 6 days to give the ester **3a**, which was converted into hydroxamic acid **4a** (45%) using 50% aqueous hydroxylamine and KOH as above (procedure A) but with a final period of 48 h at 20 °C.

A diene hydroxamic acid containing a sulfonamide linker capable of extending into the 'cap' region of histone deacetylase was secured by the reduction of the nitro compound **3e** to the arylamine **5** (83%) by stirring with a mixture of $FeSO_4{\cdot}7H_2O$ (12 equiv), 0.880 aqueous ammonia (6 mL/mmol arylamine) and ethanol (8 mL/mmol arylamine) at 60 °C for 10 min (procedure B), over-reduction being avoided under those conditions. Reaction of **5** with *p*-methoxybenzenesulfonyl chloride (1.5 equiv) in pyridine at reflux for 24 h afforded the sulfonamide **6** (90%), which was converted into the hydroxamic acid **4d** (62%) using a mixture of 50% aqueous hydroxylamine and KOH as above (procedure A, but with a final period of 48 h at 20 °C).

Syntheses of (2*E*,4*E*)-4-methyl-5-arylpenta-2,4-dienoic acid hydroxyamides with (*E*,*E*)-selectivity were conveniently achieved by means of an aldol condensation with concomitant dehydration, followed by a Wittig olefination (Scheme 2). The known[14] (*E*)-aldehydes **7b**, **7c** and **7d** were reacted with (triphenyl-γ^5-phosphanylidene)acetic acid ethyl ester (**8**) to give the (2*E*,4*E*)-dienic esters **9b**,[15] **9c** and **9d** in respective yields of 40%, 63% and 92%. Hydrolysis of **9b** with NaOH (2.0 equiv) in ethanol (portionwise addition of water until turbid, at intervals over the reflux period of 2 h) gave after neutralisation the corresponding dienic acid (96%), which was treated with oxalyl chloride (1.1 equiv) and the acid chloride reacted with 50% aqueous hydroxylamine in



Scheme 1. Synthesis of (2*E*,4*E*)-5-arylpenta-2,4-dienoic acid hydroxyamides.



Scheme 2. Synthesis of (2*E*,4*E*)-4-methyl-5-arylpenta-2,4-dienoic acid hydroxyamides.

THF to give the hydroxamic acid **10b** (35%). The direct conversion of ester **7c** into hydroxamic acid **10c** was more satisfactory, a 47% yield being achieved using a mixture of 50% aqueous hydroxylamine (10 equiv) and KOH (3.0 equiv) in methanol (procedure A).

Reduction of the nitro ester **9d** by stirring with a mixture of $FeSO_4{\cdot}7H_2O$ (12 equiv), 0.880 aqueous ammonia (6 mL/mmol arylamine) and ethanol (8 mL/mmol arylamine) at 60 °C for 10 min afforded the aromatic amine **11** (92%) that proved to be a versatile intermediate in the synthesis of a variety of novel sulfonamide-containing hydroxamic acids **10d–f**. Reaction of **11** with an arenesulfonyl chloride (2.0 equiv) in pyridine at reflux for 2 h gave the corresponding sulfonamides **12d** (73%), **12e** (44%) and **12f** (83%); those were converted into the respective hydroxamic acids **10d** (69%), **10e** (44%) and **10f** (59%) using a mixture of 50% aqueous hydroxylamine (10 equiv) and KOH (3.0 equiv) in methanol. Hydrolysis of ester **12e** with LiOH (5.0 equiv) in 1:1 THF–water at 70 °C for 16 h gave the acid **13** (91%). All intermediates and products were isolated in exclusively the desired (*E*,*E*)-configuration.

Catalytic hydrogenation of the esters **3b** and **9d** in methanol at 20 °C over 10% Pd–C (up to 2 h) afforded the corresponding saturated esters (91% and 95%, respectively), which were converted into the respective hydroxamic acids **14** (60%) and **15** (54%) using a mixture of 50% aqueous hydroxylamine (10 equiv) and KOH (3.0 equiv) in methanol.

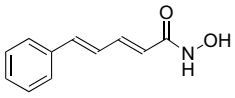
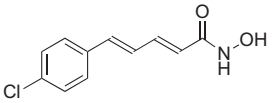
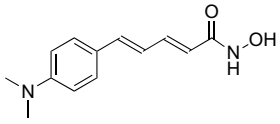
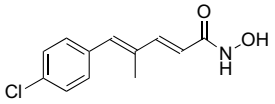
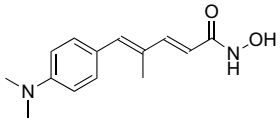
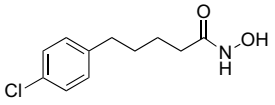
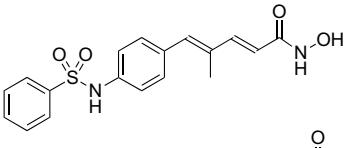
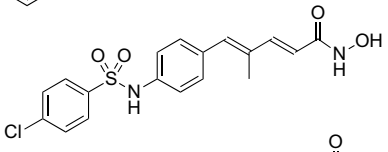
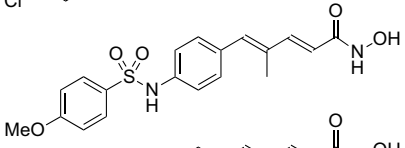
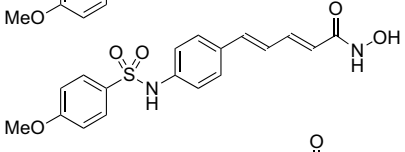
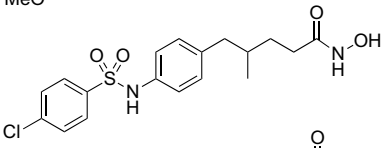
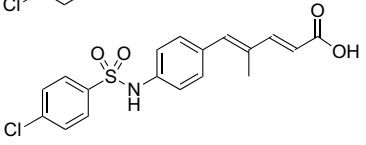
3. Biological results and discussion

Table 1 shows the IC_{50} values for the inhibition of histone deacetylase[16] of various compounds described above. The parent diene hydroxamic acid **4a** shows appreciable potency ($IC_{50} = 279$ nM) that is little altered by a *p*-chloro group (**4b**); surprisingly, a *p*-dimethylamino group as part of the simple aryldiene structure is much less potent (1.7 μM). Evidently, this *p*-dimethylamino group does not occupy the same region of the enzyme as does trichostatin A, presumably because **4b** possesses a shorter chain length. In view of the 4-methyl group present in trichostatin A, the *p*-chloro and *p*-dimethylamino derivatives **10b** and **10c** were tested; while **10c** is appreciably more active than **4c** the desired potency was not achieved.

The moderate potency of the simple arylpentadiene series was ascribed to a combination of a short chain length and the lack of a group that could extend into the 'cap' region. At this point, the convenience of the amine intermediates **5** and **11** was exploited by sulfonylation, with the choice of a relatively lipophilic terminus, in view of the predominantly hydrophobic region of histone deacetylase near the periphery of the catalytic 'tunnel'. The parent sulfonamide **10d** showed promising potency ($IC_{50} = 172$ nM); moreover the corresponding *p*-chloro and *p*-methoxy derivatives **10e** and **10f** showed excellent potency ($IC_{50} = 49$ and 74 nM, respectively). Comparison of **10f** with **4d** ($IC_{50} = 10.4$ mM) shows that a 4-methyl group can increase in vitro potency by over two orders of magnitude, a remarkable observation that has some parallel with the potency of trichostatin A ($IC_{50} = 5$ nM), which also contains substitution at both the positions C-4 and C-6. Further investigation of this observation is in progress. The unsaturated chain compounds **4b** and **10e** were found to be, respectively, 10 and 24 times more potent than their saturated counterparts **14** and **15**. Lastly, the carboxylic acid **13** was far less potent than **10e**, in keeping with the much weaker binding of zinc to carboxylate compared with hydroxamate.

In conclusion, the efficacy of arylpenta-2,4-dienoic acid hydroxyamides indicates that neither *the keto group nor the full seven-carbon chain in trichostatin A is essential for*

Table 1. In vitro inhibition of histone deacetylase[16]

Compd	Structure	HDAC IC_{50} (nM)
4a		279±46
4b		252±83
4c		1700±200
10b		302±204
10c		788±334
14		2580±520
10d		172±38
10e		49±4
10f		74±16
4d		10,400±1300
15		1170±50
13		>100,000

potent enzyme inhibition to be achieved. Also confirmed is that *trans*,*trans*-stereochemical rigidity is desirable, presumably providing a locking of conformation similar to that conferred by the *trans*,*trans*-configuration and zig-zag backbone of trichostatin A. The novel inhibitors of histone deacetylase herein described are notable for their nonpeptidic nature, which may confer improved in vivo stability over the more common amidic inhibitors that can be cleaved by peptidases. Additionally, **10e** was shown to inhibit proliferation of breast cancer cell lines at low micromolar concentrations.

Acknowledgements

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enzymes) prepared according to: Dignam, J. D.; Lebovitz, R. M.; Roeder, R. G. *Nucl. Acids Res.* **1983**, *11*, 1475, with a [^{3}H]acetate-radiolabelled peptide substrate followed by extraction of the released product ([^{3}H]acetic acid) with ethyl acetate and quantification by liquid scintillation counting. The substrate was a synthetic peptide corresponding to the N-terminal residues **14**–**21** of histone H4 that had been chemically acetylated in vitro with [^{3}H]acetic acid sodium salt (3.7 GBq/mmol; New England Nuclear Boston, MA) as described in Ref. 5 above. The concentration of compound that inhibited histone deacetylase enzymatic activity by 50% (IC_{50}) was determined graphically in each case using nonlinear regression analysis to fit inhibition data to the appropriate dose–response curve (GraphPad Prism Version 3.0; GraphPad Software Inc. San Diego, CA). Each test compound was assayed in duplicate whilst positive control (trichostatin A) and negative control samples were assayed in triplicate.