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Optimization of histone deacetylase inhibitor activity in non-secosteroidal vitamin D-receptor agonist hybrids

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ABSTRACT

The combination of (1α,25)-dihydroxyvitamin D_3 (1,25D) and histone deacetylase inhibitor (HDACi) trichostatin A is highly antiproliferative in numerous cancer cell lines. We have previously prepared novel non-secosteroidal hybrid molecules which simultaneously act as both vitamin D receptor (VDR) agonists and HDACi. These molecules function as cytostatic and cytotoxic agents in 1,25D-resistant SCC4 squamous carcinoma cells. Here we have extended the scope of the hybrids by making several modifications to the diarylpentane core and to the aliphatic spacer unit to develop molecules with increased potency towards HDACs while maintaining VDR agonist activity. Notably, hybrid DK-366 (**33a**), a direct analog of first-generation hybrids but lacking a methyl group on one aryl ring possesses low micromolar potency for HDAC3 and HDAC6 and is a highly effective antiproliferative agent in SCC4 cells. Chain extended hybrids such as DK-367 (**33c**) possess even greater HDAC potency and are also highly antiproliferative. These results show that we can optimize HDACi potency in hybrid molecules without sacrificing VDR agonism.

1. Introduction

(1α,25)-Dihydroxyvitamin D_3 (1,25D (**1**), Fig. 1), the bioactive metabolite of vitamin D_3, is an agonist of the vitamin D receptor (VDR), a member of the nuclear receptor family of ligand-regulated transcription factors.[1] While the central role for 1,25D is the regulation of calcium homeostasis, vitamin D response elements have been found in the regulatory regions of genes responsible for cell cycle regulation, differentiation and production of antimicrobial peptides.[1a,2] Thus, while initially characterized for its role in treating rickets, 1,25D and its analogues have shown potential for treatment of proliferative disorders, such as cancer and psoriasis, as well as enhancing innate immunity.[3]

Histone deacetylase (HDAC) inhibitors (HDACi) are a recently developed class of anticancer agents.[4] HDACs are enzymes that, along with histone acetyl transferases (HATs), control the acetylation state of histones and thus act to regulate gene transcription. In addition, HDACs and HATs regulate acetylation of non-histone proteins such as tubulin, HSP90 and p53.[5] HDACi block cell cycle progression and induce apoptosis or differentiation depending on the cell type.[6] The best known HDACi's are trichostatin A (TSA, **2**), a natural product, and suberoylanilide hydroxamic acid (SAHA, **3**), a synthetic HDACi which is used clinically for T-cell lymphoma. Many other HDACi are in clinical trials for a wide range of cancers.[4b]

Several studies have shown that the combination of 1,25D and HDACi are synergistic in cancer models, notably including those which are resistant to 1,25D alone.[7] Based on this synergy, we developed bifunctional hybrid molecules which combine agonism for the VDR with HDACi activity.[8] The first of these molecules, triciferol (**4**), was a hybrid of the structures of 1,25D and trichostatin A, wherein the dienyl hydroxamic acid of the latter was used as a side-chain replacement on the secosteroidal core of 1,25D.[8a] Triciferol possessed enhanced cytostatic and cytotoxic properties compared to 1,25D. We subsequently showed that the concept was general, as a wide-range of secosteroidal hybrids bearing different zinc-binding groups could be prepared.[8b] In addition, antagonists could be prepared by substituting the hydroxamic acid in **4** with a benzamide.[9]

A significant challenge with these first-generation hybrids was their preparation, generally requiring a lengthy 25–30 step semisynthesis from vitamin D_2 (C/D core precursor) and quinic acid (A-ring precursor). To address this issue, we examined

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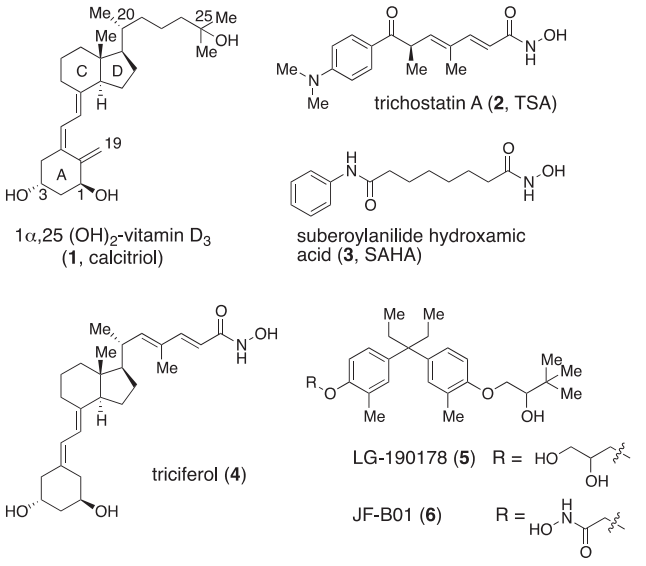


Figure 1. Structure of 1,25D, HDACi and VDR agonist/HDACi hybrids.

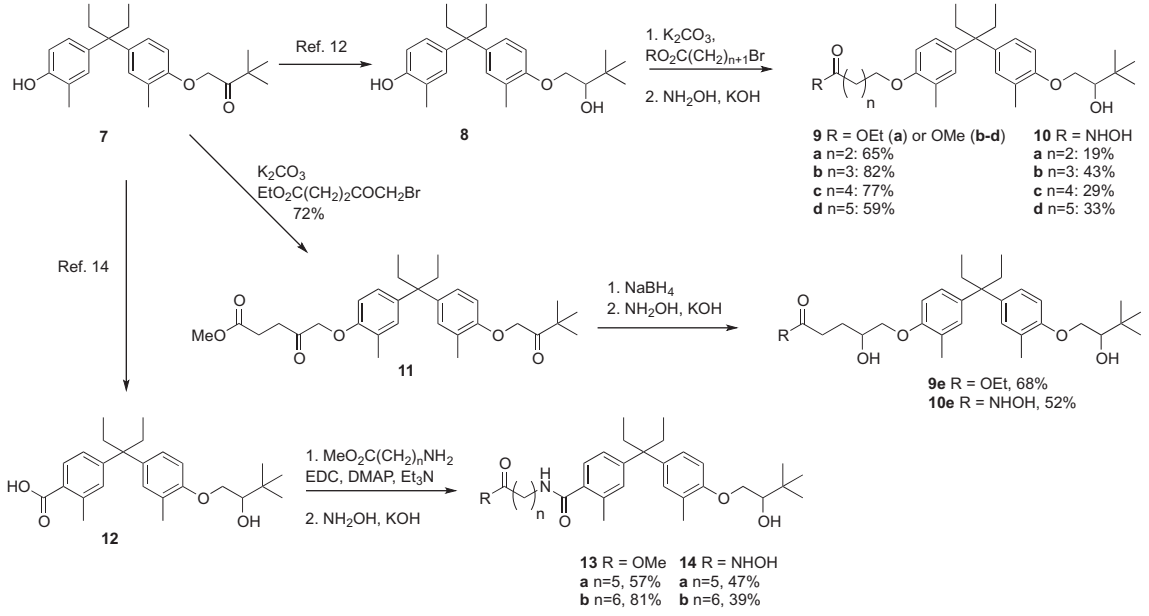
non-secosteroidal hybrids based on the skeleton of LG-190178 (**5**), a 3,3-diarylpentane analog of 1,25D.[10] These studies identified JF-B01 (**6**), wherein the A-ring mimic in **5** was replaced by a zinc-binding group to form an effective bifunctional hybrid.[8c] Notably, the non-secosteroidal hybrids required an inverted design relative to triciferol and other secosteroidal hybrids: incorporation of a zinc-binding group in the portion of LG-190178 which mimics the 1,25D side chain eliminated all VDR agonist activity. JF-B01 was active against both vitamin D-sensitive and vitamin D-resistant cancer cell lines and these studies showed the clear benefit of a bifunctional molecule: in 1,25D-resistant SCC4 cells, JF-B01 was antiproliferative at a concentration of 10 μM while analogs possessing only equivalent VDR agonist or HDACi activity were ineffective.

While JF-B01 proved to be bifunctional, its HDAC potency was reduced compared to its secosteroidal counterparts and clinical HDACi in general. In this paper, we expand on this class of compounds and show that it is possible to generate bifunctional molecules with improved HDACi potency without sacrificing VDR agonism.

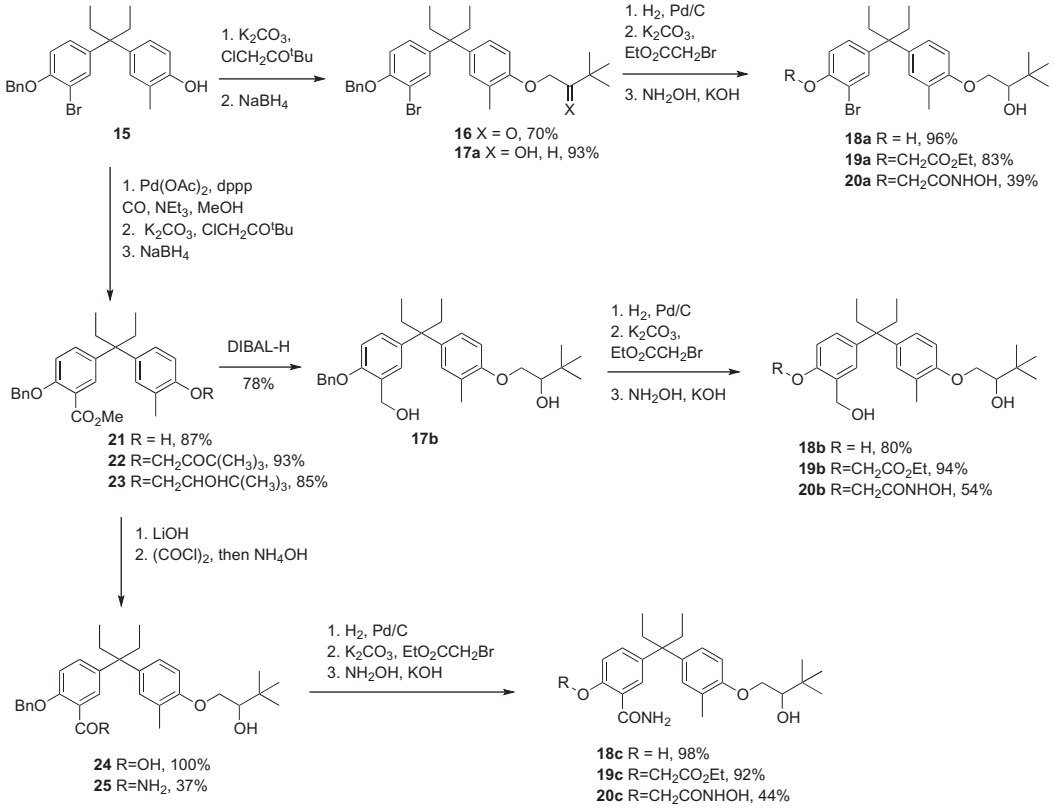
2. Hybrid design and synthesis

N-Acetylated lysine residues of histone and other proteins access the zinc-containing active site of class I, II and IV HDACs via a narrow channel.[11] The canonical structure for HDACi matches these features with a zinc binding group, most commonly a hydroxamic acid or a benzamide, connected via a linking chain that spans the channel, to a hydrophobic cap group which binds at the enzyme surface. The main structural features of 1,25D/VDR binding are hydrogen bonds to Ser237 and Arg274 (1OH), Ser278 and Tyr143 (3OH), and His 305 and His397 (25OH).[1b] LG-190178 possesses a 3,3-diarylpentane core which mimics the C/D ring of 1,25D.[10] Two phenolic groups are decorated with a 2-hydroxy-3,3-dimethylbutyl group that mimics the side-chain of 1,25D and with a 2,3-dihydroxypropyl group that mimics the A-ring diol. In JF-B01, the diol unit was replaced with a short-chain hydroxamic acid to impart HDACi activity while maintaining VDR agonist capability. While this maximized structural similarity to LG-190178, it presumably moderated the HDACi potency, as longer chain lengths are generally preferred to span the access channel.

We envisioned that lengthening the side-chain in JF-B01 would improve HDACi potency of the hybrids, but that this would have to be balanced against the potential for decreasing VDR agonist efficacy as chain length increases. Note that our prior work showed a negligible difference between enantiomers of JF-B01 for either VDR agonist or HDACi activity.[8c] Thus, in this study, we elected to prepare and assess all molecules as racemates. The hybrids were prepared from hydroxyketone **7** (Scheme 1) as a common intermediate, readily synthesized in two steps from o-cresol, 3-pentanone and 1-bromo-3,3-dimethylbutanone.[12] To prepare simple ether-linked hybrids **10a–d**, reduction of **7** with sodium borohydride afforded diol **8** which can be selectively alkylated with alkyl bromoalkanoates in the presence of potassium carbonate followed by direct installation of the hydroxamic acid using hydroxylamine and KOH. In addition to simple *n*-alkyl hydroxamates, we also prepared a hybrid with a hydroxyl group at the



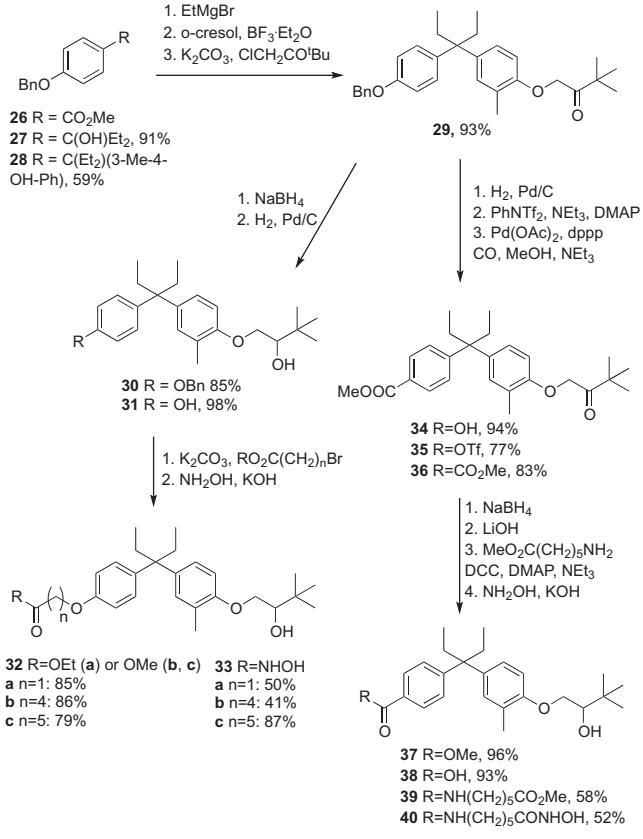
Scheme 1. Synthesis of chain extended analogs of JF-B01.



Scheme 2. Synthesis of A-ring analogs of JF-B01.

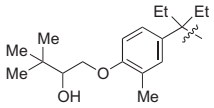
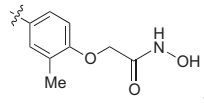
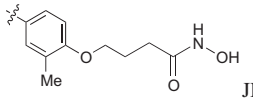
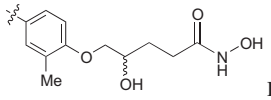
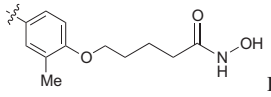
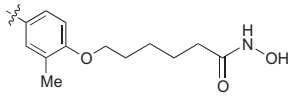
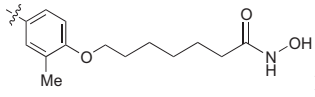
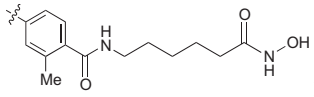
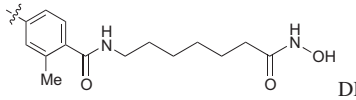
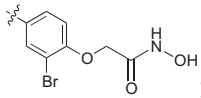
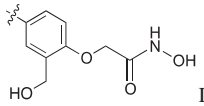
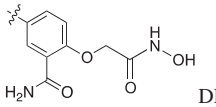
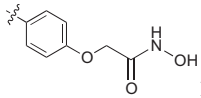
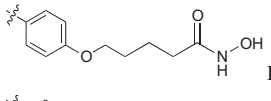
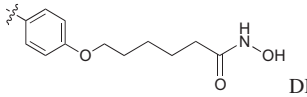
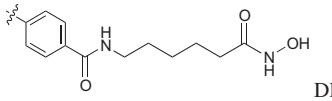
2-position of the chain (**10e**) which was expected to improve VDR affinity.[13] Direct alkylation of ketone **7** with methyl 5-bromo-4-oxopentanoate to form **11** followed by reduction of both ketones and hydroxamate formation afforded **10e** in moderate yields. Finally, the ether linkage could be replaced by a carboxamide. Conversion of the alcohol **7** to the carboxylic acid in **12** was accomplished according to an established procedure.[14] Peptide coupling of **12** with methyl 5-aminopentanoate or methyl 6-aminohexanoate followed by hydroxamate formation furnished hybrids **14a**,**b**.

The X-ray crystal structure of both LG-190178 and JF-C71 (the *2R*-enantiomer of JF-B01) showed that the A-ring methyl group occupies a pocket lined with several polar residues (Tyr175, Ser306, Cys316, Tyr323).[8c,15] Thus, we prepared several analogs to explore potential hydrogen bonding interactions in this pocket. While these would not be expected to improve HDAC potency, we postulated that increased interactions might counterbalance any loss in VDR efficacy when longer side-chains were employed. Three of these analogs were prepared via brominated biaryl **15** (Scheme 2) which was easily prepared from methyl 4-hydroxybenzoate by treatment with excess ethyl magnesium bromide followed by Fridel–Crafts alkylation with *o*-cresol. Alkylation of **15** with 1-chloropinacolone and subsequent ketone reduction afforded **17a**. Hydrogenolysis of the benzyl ester and then hydroxamate chain incorporation, as above, furnished bromo hybrid **20a**. Alternatively, carboxylation of **15** followed by alkylation/reduction afforded methyl ester **23** which could be used to make two different analogs. Reduction of the ester group and then phenol deprotection and hydroxamate side-chain introduction, as above, afforded alcohol **20b**. Alternatively, ester saponification and primary amide formation, followed by installation of the side-chain afforded hybrid **20c**.



Scheme 3. Synthesis of des-methyl analogs of JF-B01.

Table 1
VDR Agonist, HDACi and Antiproliferative Activity of VDR/HDACi hybrids

	VDR agonism	HDAC6 IC_{50} (μM)	HDAC3 IC_{50} (μM)	HDAC2 IC_{50} (μM)	Total cellular HDACi	SCC4 proliferation IC_{50} (μM)
 JF-B01 (**6**)	+++	25.0 ± 3.6[a]	21.9 ± 3.7 [a]	6.0 ± 0.4 [a]	++	12.9 ± 0.3
 JF-D15 (**10a**)	+++	34.7 ± 3.0	8.8 ± 1.4			35.0 ± 0.7
 DK305 (**10e**)	++++	28.2 ± 3.8			++	>50
 DK320 (**10b**)	+++	4.5 ± 0.3			+	16.1 ± 0.1
 DK319 (**10c**)	+++	2.7 ± 0.3	2.8 ± 0.5		++	20.9 ± 1.9
 DK347 (**10d**)	+	3.6 ± 0.1			++	>50
 DK361 (**14a**)	+	3.70 ± 0.3			+	>50
 DK362 (**14b**)	–	1.0 ± 0.2			–	>50
 DK309 (**20a**)	+++	21.0 ± 1.4			++	6.8 ± 0.2
 DK331 (**20b**)	+++	27.0 ± 3.6			+	>50
 DK341 (**20c**)	±	31.6 ± 3.1			–	>50
 DK366 (**33a**)	+++	6.6 ± 1.1	2.8 ± 0.5	1.7 ± 0.3	+++	8.1 ± 0.2
 DK381 (**33b**)	+++	3.4 ± 0.4			ND	17.0 ± 0.3
 DK367 (**33c**)	+++	1.7 ± 0.3	1.3 ± 0.3	1.4 ± 0.3	++	9.2 ± 0.1
 DK406 (**40**)	+++	0.6 ± 0.1	1.1 ± 0.3	0.5 ± 0.1	+	>50

[a] HDACi activity for (2*R*)-enantiomer (JF-C71). See Ref. 8a

In addition to the functionalized analogs above, we prepared hybrid **33a** which lacked any group at the 3-position of the A-ring as a control (Scheme 3). This hybrid was prepared in a sequence similar to **10a–d** but starting from methyl 4-benzyloxybenzoate (**26**). Based on the improved HDACi potency of **33a** (vide infra), we subsequently prepared additional analogs **33b**,**c** which incorporated longer linking chains. In addition, we prepared carboxamide linked hybrid **40** through a sequence similar to **14a**,**b**.

3. Biochemical analysis

The hybrids were assessed for both VDR agonism and HDACi activity using a series of standard assays previously established in the analysis of secosteroidal and non-secosteroidal hybrids.[8] VDR agonism was tested by RT-PCR analysis of induction of expression of the VDR target gene *CYP24*, as *CYP24* transcription is exquisitely sensitive to VDR agonists. HDACi assays were conducted in vitro using purified human HDACs using a fluorescent substrate.[16] We used class II HDAC6 due to its high activity in the in vitro assay and further assessed compounds of interest against HDAC2 and -3, both class I enzymes. Further, we assessed HDACi activity in vivo in 1,25D–resistant SCC4 cells to measure the capacity of compounds to inhibit total intracellular class I and class II HDAC activity.[17] In addition, the cytostatic (antiproliferative) and cytotoxic activities of the compounds were tested in SCC4 cells. Cytostatic activity was assessed by EdU assay, which provides a measure of DNA replication via *de novo* incorporation of the thymidine analogue EdU into DNA. Cytotoxicity was assessed by assaying for release into tissue culture media of the mitochondrial enzyme lactate dehydrogenase (LDH), which would occur under conditions of cell death. The results of all of these tests for compound function are summarized in Table 1, and specific examples are provided for compounds **33a** (DK-366), **33c** (DK-367), and **40** (DK-406) (Fig. 2 and see below).

Analysis of the chain extended analogs of JF-B01 showed that significant VDR agonism was maintained with linker lengths up to 6 atoms long (Table 1). Importantly, lengthening the chain improved HDACi potency. Although hybrids with intermediate length chains such as **10a** showed only modest improvement in HDACi compared to JF-B01 (26.1 μM), longer chains such as those found in **10b** and **10c** displayed IC_{50}s of 5.0 and 2.7 μM, respectively, against HDAC6 while maintaining VDR agonism. HDACi potency was also improved with even longer chain lengths such as **10d**, **14a**, and **14b** (3.9 μM, 3.4 μM and 1.1 μM, respectively), but these hybrids displayed significantly reduced VDR agonist activity.

Assessing A-ring variants **20a–c**, it was found that the bromide and hydroxymethyl substituted hybrids were tolerated as VDR agonists while the amide group in **20c** eliminated most agonist activity. An important observation was made with the control compound **33a** (DK-366), the des-methyl analog of JF-B01. This hybrid showed significantly improved HDAC6 potency (6.6 μM) compared to its direct analog JF-B01 while retaining excellent activity as a VDR agonist (Fig. 2, Table 1). The effect of deleting the methyl group on HDACi potency was also observed in longer chain variants of **33a**. For instance, **33b** and **40** (DK-406) displayed improved HDACi activity (1.6 μM and 560 nM, respectively) when compared to their methylated analogs **10b** and **14a** (3.9 μM and 3.4 μM, respectively), but, in the case of DK-406, with reduced potency as a VDR agonist (Fig. 2). This improved HDACi was not observed in all hybrids, as **33c** (DK367) displayed approximately equal HDACi potency and VDR agonist activity compared to its methylated analog **10c** (Table 1). The improved potencies for several of the hybrids (**10a**, **10c**, **33a**,**c** and **40**) against HDAC6 were also observed for HDAC2 and HDAC3.

We assessed the antiproliferative activities of several of the bifunctional hybrids in 1,25D-resistant SSC4 cells. In this cell line, hybrids such as JF-B01 are antiproliferative, as are combinations of VDR agonists and HDACi, while VDR agonists alone or HDACi alone are not.[8c] We found that hybrids that had similar HDACi

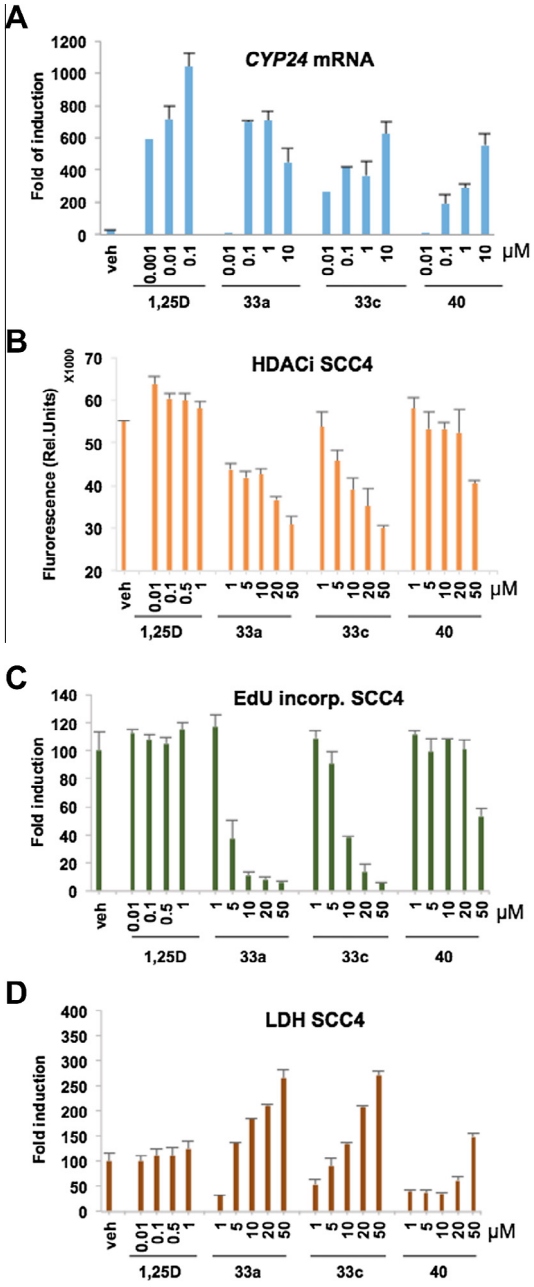


Figure 2. Biochemical analysis of hybrid function. HDACi and cytostatic activities of hybrid compounds. (A) Dose-dependent induction of VDR target gene *CYP24* induction by DK-366, -367 and -406, as indicated, along with 1,25D as a positive control. (B) HDACi activity of compounds was measured in live SCC4 cells as described in experimental procedures. HDACi activity of non-secosteroidal compounds was measured in between 1 nM (1) and 50 μM (50), whereas 1,25D was tested between 10 nM (0.01) and 1 μM (1). (C) The dose-dependent antiproliferative activities of 1,25D or non-secosteroidal compounds were assessed by measuring incorporation of fluorescent nucleoside analogue EdU in 1,25D-resistant SCC4 cells. (D) Assessment of SCC4 viability in the presence of increasing concentrations of 1,25D or hybrid molecules indicated, as measured by release of the the mitochondrial enzyme lactate dehydrogenase (LDH; see experimental procedures for details).

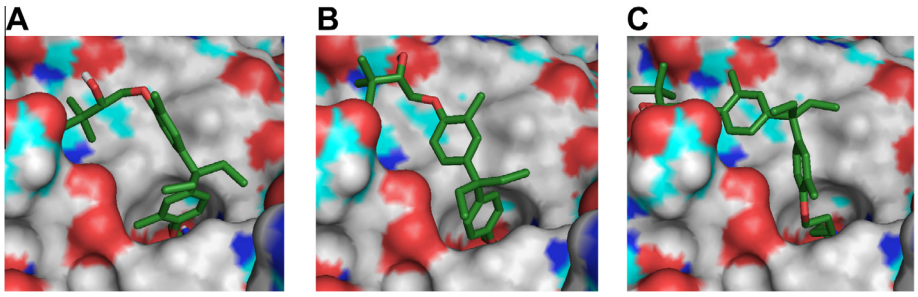


Figure 3. Docking of (A) JF-B01, (B) DK-366 and (C) DK-319 in HDAC2 (pdb 4LXZ) using FITTED. Aliphatic linker lengths in JF-B01 and DK-366 are short enough that the aryl group enters the access tube. The linker in DK-319 is sufficient to span the length of the tube, allowing the aromatic groups to bind at the enzyme surface.

potency to JF-B01 such as **10a** and **10e** had similar antiproliferative properties (Table 1). Some hybrids with more potent HDAC6i activity such as **33a** (DK-366) and **33c** (DK-367) also strongly inhibited total cellular HDAC activity and showed strong antiproliferative activity (Table 1, Fig. 2C). However, the correlation between in vitro HDACi potency with their potency as inhibitors of total HDAC activity or their antiproliferative activity was not perfect, as **40** (DK-406), the hybrid displaying the most potent HDAC6 and HDAC3 inhibitory activities in vitro, was a relatively weak inhibitor of total HDAC activity and only modestly antiproliferative in SCC4 cells (Table 1, Fig. 2C). Similarly, **40** was less potent than **33a** or **33c** in cytotoxicity assays as measured by release of LDH (Fig. 2D).

The antiproliferative activities of DK-366 (**33a**), DK-367 (**33c**) and DK-406 (**40**) in SCC4 cells were also mirrored in the 60-cell line panel of the National Cancer Institute (US). While DK-406 possessed only mild antiproliferative activity, DK-366 and -367 were active against a wide range of cancer cell lines (see Supporting information), consistent with results obtained in SCC4 cells. In particular, DK-366 displayed a median GI50 of 1 μM and a median LD50 of 10 μM across all cell lines.

4. Discussion and conclusion

The results above demonstrate that it is possible to optimize HDACi potency of VDR agonist hybrid JF-B01 through two possible modifications. Either removing the methyl group on the aromatic ring or lengthening the linking chain served to increase HDACi potency with little cost to VDR agonist activity. The effect of these modifications can be explained in light of the canonical structure for HDAC inhibitors wherein a zinc-binding group is linked to a cap group which binds at the enzyme surface. The linker spans a narrow access tube and is most commonly a straight-chain aliphatic linker. Using FITTED, a docking software that has been recently parameterized for HDACs,[18] we docked JF-B01 in HDAC2, chosen due to high homology to HDAC3 and the availability of several X-ray crystal structures.[19] As expected, the short aliphatic ether in JF-B01 is insufficient to fully span the access tube and thus one aromatic ring must be part of the linker, with the diethyl pentane and second aromatic making contacts at the surface (Fig. 3A). The branching methyl group on the aromatic ring is presumably non-ideal for the narrow channel. Either removing the methyl group, as in DK-366, to create a more compact linker (Fig 3B) or extending the aliphatic chain such that the entire diarylpentane sits on the HDAC surface (Fig. 3C), significantly improves HDACi potency in vitro. In the VDR, the aromatic methyl group points towards a shallow pocket lined by polar functional groups (Cys316, Ser306)[8c] and thus its removal does not result in significant loss of interactions with the receptor.

In general, hybrids that displayed a combination of potent VDR agonism and inhibition of total intracellular class I and class II HDAC activity possessed the strongest antiproliferative activity (see Table 1). For instance, hybrid DK-366 (**33a**) possessed excellent VDR agonist activity and intracellular HDACi activity and was one of the most potent antiproliferative hybrids assessed. In contrast, **40** which possessed excellent in vitro HDACi activity but reduced potency as a VDR agonist and weak HDACi activity in cells, was less antiproliferative. Similarly, hybrid **10d**, which possessed strong HDACi activity in vitro and moderate intracellular HDACi activity but lacked significant VDR agonist activity, was less effective against SCC4 cells. These results underscore the need for both cellular HDACi activity and VDR agonist activity for efficacious antiproliferative activity.

In conclusion, we have developed a range of bifunctional VDR agonist/HDACi hybrids and shown that improvement of HDACi potency and antiproliferative activity through straightforward modification of the hybrids is feasible. Efficacy of these hybrids in animal models of cancer will be reported in due course.

5. Experimental section

Unless otherwise stated, reactions were conducted under an argon atmosphere and glassware was oven dried prior to use. Tetrahydrofuran and diethyl ether were purified by distillation from sodium under a nitrogen atmosphere. Toluene, dichloromethane and triethylamine were purified by distillation from calcium hydride under nitrogen atmosphere. Deuterated chloroform was stored over activated 4 Å molecular sieves. All commercial reagents and solvents were used as purchased without further purification. Thin-layer chromatography (TLC) was carried out on glass plates, coated with 250 μm of 230–400 mesh silica gel that had been saturated with F-254 indicator. Flash column chromatography was carried out on 230–400 mesh silica gel (Silicycle) using reagent grade solvents. Infrared (IR) spectra were obtained using Nicolet Avatar 360 FT-IR infrared spectrophotometer. Proton and carbon nuclear magnetic resonance spectra were obtained on Varian 300, 400, and 500 or Bruker 400 and 500 MHz spectrometers. Chemical shifts (δ) were internally referenced to the residual proton resonance $CDCl_3$ (δ 7.26 ppm), CD_3OD (δ 3.31 ppm), $(CD_3)_2SO$ (δ 2.50 ppm). Coupling constants (J) are reported in Hertz (Hz). HPLC Analysis was performed using a Waters ALLIANCE instrument (e2695 with 2489 UV detector and 3100 mass spectrometer) with H_2O (0.1% formic acid) and CH_3CN, (0.1% formic acid) as a mobile phase, linear gradient from 95% H_2O and 5% CH_3CN to 100% CH_3CN in 15 min (method A) or in 10 min, then 5 min at 100% CH_3CN (method B) flow rate, 1 mL/min. HRMS were obtained by Dr. Nadim Saadeh or Dr Alexander S. Wahba at McGill University Department of Chemistry.

5.1. Ethyl 3-(4-(3-(4-(2-hydroxy-3,3-dimethylbutoxy)-3-methylphenyl)pentan-3-yl)-2-methylphenoxy)propanoate (9a)

Synthesized from **8** according to procedure similar to that for **9b** (alkylating reagent 1.5 equiv of ethyl 4-bromobutyrate) in 65%

yield as colourless oil: R_f 0.7 (20:80 EtOac/Hex); IR (thin film) 3525, 2961, 2875, 1734, 1502, 1244, 1136 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.00–6.86 (m, 4H), 6.69 (t, J = 8.0 Hz, 2H), 4.14 (q, J = 7.2 Hz, 2H), 4.09 (dd, J = 9.1, 2.7 Hz, 1H), 3.98 (t, J = 6.0 Hz, 2H), 3.86 (t, J = 8.9 Hz, 1H), 3.71 (dd, J = 8.7, 2.6 Hz, 1H), 2.54 (t, J = 7.4 Hz, 2H), 2.49 (br s, 1H), 2.18 (s, 3H), 2.16 (s, 3H), 2.15–2.08 (m, 2H), 2.03 (q, J = 7.2 Hz, 4H), 1.26 (t, J = 7.2 Hz, 3H), 1.01 (s, 9H), 0.60 (t, J = 7.2 Hz, 6H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$) d 173.51, 154.62, 154.38, 141.36, 140.69, 130.76, 130.59, 126.28, 126.16, 125.60, 125.56, 110.17, 109.80, 77.40, 69.28, 66.59, 60.53, 48.50, 33.69, 31.11, 29.41, 26.20, 24.96, 16.76, 16.64, 14.35, 8.58 ppm; HRMS (ESI) Calc. for $C_{31}H_{46}O_5Na$ $[M+Na]^+$: 521.3237, found 521.3254.

5.2. Methyl 5-(4-(3-(4-(2-hydroxy-3,3-dimethylbutoxy)-3-methylphenyl)pentan-3-yl)-2-methylphenoxy)pentanoate (9b)

Phenol **8** (45.0 mg, 0.117 mmol, 1.0 equiv) was dissolved in acetonitrile (5 mL) and potassium carbonate (32.3 mg, 0.234 mmol, 2.0 equiv) and methyl-5-bromovalerate (25.2 µl, 0.176 mmol, 1.5 equiv) was added. Reaction mixture was refluxed for 12 h, cooled to room temperature, filtered through celite and concentrated in vacuo. The crude product was purified by chromatography on silica gel eluating 5% ethyl acetate/hexanes to afford 47.9 mg transparent oil (82%). R_f 0.75 (20:80 EtOac/Hex); IR (thin film) ν 3488, 2952, 2874, 1739, 1504, 1244 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 6.96–6.87 (m, 4H), 6.69 (d, J = 8.6 Hz, 1H), 6.66 (d, J = 8.4 Hz, 1H), 4.08 (dd, J = 9.1, 2.7 Hz, 1H), 3.94 (t, J = 5.4 Hz, 2H), 3.85 (t, J = 8.9 Hz, 1H), 3.74–3.67 (m, 1H), 3.67 (s, 3H), 2.41 (t, J = 6.2 Hz, 2H), 2.17 (s, 3H), 2.15 (s, 3H), 2.01 (q, J = 7.3 Hz, 4H), 1.87–1.73 (m, 4H), 1.01 (s, 9H), 0.59 (t, J = 7.3 Hz, 6H) ppm; ^{13}C NMR (126 MHz, $CDCl_3$) δ, 174.13, 154.69, 154.20, 141.28, 140.32, 130.64, 130.40, 126.14, 125.99, 125.43, 125.39, 110.01, 109.68, 77.25, 69.12, 67.36, 51.50, 48.36, 34.04, 33.54, 29.29, 29.11, 26.05, 25.82, 24.73, 16.62, 16.52, 8.45 ppm; HRMS (ESI) Calc. for $C_{31}H_{46}O_5Na$ $[M+Na]^+$: 521.3238, found 521.3235.

5.3. Methyl 6-(4-(3-(4-(2-hydroxy-3,3-dimethylbutoxy)-3-methylphenyl)pentan-3-yl)-2-methylphenoxy)hexanoate (9c)

Synthesized from **8** according to procedure similar to that for **9b** (alkylating reagent 1.5 equiv of methyl 6-bromohexanoate) in 77% yield as colourless oil: R_f 0.75 (20:80 EtOac/Hex); IR (thin film) ν 3500, 2960, 2873, 1739, 1504, 1245 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 6.96–6.87 (m, 4H), 6.69 (d, J = 8.6 Hz, 1H), 6.67 (d, J = 8.4 Hz, 1H), 4.08 (dd, J = 9.1, 2.7 Hz, 1H), 3.95 (t, J = 5.4 Hz, 2H), 3.85 (t, J = 8.9 Hz, 1H), 3.70 (dd, J = 9.1, 2.7 Hz, 1H), 3.67 (s, 3H), 2.35 (t, J = 6.2 Hz, 2H), 2.16 (s, 3H), 2.14 (s, 3H), 2.01 (q, J = 7.3 Hz, 4H), 1.83–1.76 (m, 2H), 1.74–1.68 (m, 2H), 1.54–1.48 (m, 2H), 1.01 (s, 9H), 0.59 (t, J = 7.3 Hz, 6H) ppm; ^{13}C NMR (126 MHz, $CDCl_3$) δ 174.00, 154.61, 154.21, 141.26, 140.40, 130.64, 130.43, 126.14, 125.99, 125.45, 125.40, 110.01, 109.60, 77.21, 69.12, 67.07, 51.53, 48.36, 33.74, 33.54, 29.28, 28.85, 26.05, 21.80, 16.62, 16.53, 8.45 ppm; HRMS (ESI) Calc. for $C_{32}H_{48}O_5Na$ $[M+Na]^+$: 535.3394, found 535.3378.

5.4. Methyl 7-(4-(3-(4-(2-hydroxy-3,3-dimethylbutoxy)-3-methylphenyl)pentan-3-yl)-2-methylphenoxy)heptanoate (9d)

Synthesized from **8** according to procedure similar to that for **9b** (alkylating reagent 1.5 equiv of methyl 7-bromoheptanoate) in 59% yield as colourless oil. R_f 0.70 (20:80 EtOac/Hex); IR (thin film) ν 3320, 2952, 2937, 2865, 1739, 1504, 1220, 1137 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 6.93 (m, 3H), 6.89 (d, J = 8.5 Hz, 1H), 6.69 (d, J = 8.5 Hz, 1H), 6.66 (d, J = 8.5 Hz, 1H), 4.08 (dd, J = 9.2, 2.6 Hz, 1H), 3.91 (t, J = 6.3 Hz, 2H), 3.85 (t, J = 8.9 Hz, 1H), 3.70 (dt, J = 8.7, 2.7 Hz, 1H), 3.66 (s, 3H), 2.45 (t, J = 2.6 Hz, 1H), 2.32 (t, J = 7.5 Hz, 2H), 2.17 (s, 3H), 2.15 (s, 3H), 2.01 (q, J = 7.3 Hz, 4H), 1.85–1.73 (m, 2H), 1.72–1.61 (m, 2H), 1.53–1.45 (m, 2H), 1.43–1.33 (m, 2H), 1.01 (s, 9H), 0.59 (t, J = 7.3 Hz, 6H) ppm; ^{13}C NMR (126 MHz, $CDCl_3$) δ 174.22, 154.75, 154.21, 141.28, 140.26, 130.64, 130.39, 126.15, 125.98, 125.45, 125.39, 110.02, 109.70, 77.27, 69.13, 67.55, 51.47, 48.36, 34.00, 33.54, 29.30, 29.26, 28.90, 26.05, 25.88, 24.88, 16.62, 16.52, 8.45 ppm; HRMS (ESI) Calc. for $C_{33}H_{50}O_5Na$ $[M+Na]^+$: 549.3551, found 549.3544.

5.5. Methyl 4-hydroxy-5-(4-(3-(4-(2-hydroxy-3,3-dimethylbutoxy)-3-methylphenyl)pentan-3-yl)-2-methylphenoxy)pentanoate (9e)

Compound **11** (40.0 mg, 0.076 mmol, 1.0 equiv) was dissolved in methanol (5 mL) at 0 °C and $NaBH_4$ (8.6 mg, 0.228 mmol, 3.0 equiv) was added and reaction stirred at that temperature for 1 h. 1 M HCl solution (5 mL) was added and resulting mixture was extracted with dichloromethane (3 × 5 mL) combined organic layer were washed with saturate NaCl (5 mL), dried over anhydrous sodium sulfate, filtered, and concentrated. The crude product was purified by chromatography on silica gel eluating 10% ethyl acetate/hexanes to afford 27.2 mg transparent oil (68%). R_f 0.27 (20:80 EtOac/Hex); IR (thin film) ν 3444, 2963, 1730, 1717, 1276 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 6.94 (d, J = 9.0 Hz, 2H), 6.89 (s, 2H), 6.69 (m, 2H), 4.18–4.12 (q, J = 7.8 Hz, 2H), 4.08 (dd, J = 9.2, 2.6 Hz, 1H), 4.04 (bs, 1H), 3.96 (dd, J = 9.3, 2.6 Hz, 1H), 3.89–3.80 (m, 2H), 3.70 (d, J = 8.5 Hz, 1H), 2.59–2.48 (m, 3H), 2.44 (bs, 1H), 2.16 (s, 6H), 2.02 (q, J = 7.1 Hz, 4H), 1.97–1.79 (m, 2H), 1.26 (t, J = 7.8 Hz, 3H), 1.00 (s, 9H), 0.59 (t, J = 7.3 Hz, 6H) ppm; ^{13}C NMR (126 MHz, $CDCl_3$) δ 173.82, 154.25, 154.04, 141.21, 141.12, 130.60, 126.15, 126.11, 125.46, 125.42, 110.03, 110.00, 77.20, 71.64, 69.59, 69.13, 60.55, 48.39, 33.54, 30.46, 29.25, 28.25, 26.05, 16.63, 16.55, 14.21, 8.43 ppm; HRMS (ESI) Calc. for $C_{32}H_{46}O_6Na$ $[M+Na]^+$: 551.3343, found 551.2241.

5.6. *N*-Hydroxy-4-(4-(3-(4-(2-hydroxy-3,3-dimethylbutoxy)-3-methylphenyl)pentan-3-yl)-2-methylphenoxy)butanamide (10a)

Synthesized from **9a** according to procedure similar to that for **10b** in 19% yield as thin film. IR (thin film) 3489, 3262, 2961, 2875, 1733, 1503, 1246, 1136 cm^{-1}; ^1H NMR (500 MHz, CD_3OD) d 6.98–6.92 (m, 2H), 6.87–6.81 (m, 2H), 6.77–6.71 (m, 2H), 4.11 (dd, J = 10.0, 3.0 Hz, 1H), 3.97 (t, J = 6.0 Hz, 2H), 3.86 (dd, J = 10.0, 7.8 Hz, 1H), 3.61 (dd, J = 7.7, 2.9 Hz, 1H), 2.31 (t, J = 7.4 Hz, 2H), 2.15 (s, 3H), 2.12 (s, 3H), 2.10–2.06 (m, 2H), 2.03 (q, J = 7.2 Hz, 4H), 1.00 (s, 9H), 0.58 (t, J = 7.3 Hz, 6H) ppm; ^{13}C NMR (126 MHz, CD_3OD) d 172.45, 156.26, 156.03, 142.03 (2 coincident peaks), 131.56, 131.52, 127.17, 127.15, 126.80, 126.63, 111.20, 111.06, 78.69, 70.93, 67.99, 49.448, 35.09, 30.54, 30.20, 26.68, 26.59, 16.81, 16.67, 8.76 ppm; HRMS (ESI) Calc. for $C_{29}H_{43}O_5NNa$ $[M+Na]^+$: 508.3033, found: 508.3042;

5.7. *N*-Hydroxy-5-(4-(3-(4-(2-hydroxy-3,3-dimethylbutoxy)-3-methylphenyl)pentan-3-yl)-2-methylphenoxy)pentanamide (10b)

Ester **9b** (20.0 mg, 0.040 mmol, 1 equiv) was dissolved in methanol/tetrahydrofurane 1:1 (3 mL) and cooled to 0 °C. Solution of NH_2OH (1.32 mL of a 50 wt % in H_2O, 20.0 mmol, 500 equiv) was added, followed by 3 M aqueous KOH (93 µl, 0.28 mmol, 7.0 equiv) and the resulting reaction mixture stirred and slowly warmed to RT. After 48 h, the solvent was removed in vacuo and the residue diluted in H_2O and acidified to pH <3 with 1 M HCl before extracting with EtOAc (3 × 5 mL). The combined organic phases were dried (Na_2SO_4) and concentrated in vacuo to give a residue that was purified by octadecyl functionalized silica gel column chromatography (65:35 → 80:20 MeOH/H_2O) to afford

8.6 mg of thin film (43%). IR (thin film) ν 3238, 2962, 2933, 2873, 1655, 1609, 1503, 1275 cm^{-1}; ^1H NMR (500 MHz, CD_3OD) δ 6.97–6.91 (m, 2H), 66.85 (d, J = 2.7 Hz, 1H), 6.83 (d, J = 2.7 Hz, 1H), 6.75 (d, J = 8.6 Hz, 1H), 6.73 (d, J = 8.6 Hz, 1H), 4.11 (dd, J = 10.0, 2.9 Hz, 1H), 3.96 (t, J = 5.8 Hz, 2H), 3.86 (dd, J = 10.0, 7.7 Hz, 1H), 3.61 (dd, J = 7.7, 2.9 Hz, 1H), 2.20–2.13 (m, 5H), 2.10 (s, 3H), 2.03 (q, J = 7.4 Hz, 4H), 1.87–1.75 (m, 4H), 1.00 (s, 9H), 0.58 (t, J = 7.3 Hz, 6H) ppm; ^{13}C NMR (126 MHz, CD_3OD) δ 171.22, 154.80, 154.70, 140.61, 140.45, 130.11, 130.04, 125.73, 125.35, 125.08, 109.76, 109.62, 77.24, 69.48, 66.98, 48.00, 33.66, 32.05, 28.77, 28.56, 25.17, 22.20, 15.38, 15.23, 7.34 ppm; HRMS (ESI) Calc. for $C_{30}H_{44}O_5N$ [M-H]$^-$: 498.3225, found: 498.3232; purity >95% (LC/MS), t_r = 11.10 min (method B).

5.8. *N*-Hydroxy-6-(4-(3-(4-(2-hydroxy-3,3-dimethylbutoxy)-3-methylphenyl)pentan-3-yl)-2-methylphenoxy)hexanamide (10c)

Synthesized from **9c** according to procedure similar to that for **10b** in 29% yield as thin film. IR (thin film) ν 3238, 2961, 2940, 2873, 1654, 1503, 1220 cm^{-1}; ^1H NMR (500 MHz, CD_3OD) δ 6.97–6.90 (m, 2H), 6.85 (d, J = 2.7 Hz, 1H), 6.83 (d, J = 2.7 Hz, 1H), 6.74 (dd, J = 8.6 Hz, 2H), 4.11 (dd, J = 10.0, 3.0 Hz, 1H), 3.94 (t, J = 6.3 Hz, 2H), 3.86 (dd, J = 10.0, 7.8 Hz, 1H), 3.61 (dd, J = 7.7, 2.9 Hz, 1H), 2.15 (s, 3H), 2.12 (t, J = 6.2 Hz, 2H), 2.10 (s, 3H), 2.03 (q, J = 7.2 Hz, 4H), 1.87–1.75 (m, 2H), 1.75–1.63 (m, 2H), 1.60–1.47 (m, 2H), 0.99 (s, 9H), 0.58 (t, J = 7.3 Hz, 6H) ppm; ^{13}C NMR (125 MHz, CD_3OD) δ 171.44, 154.80, 154.77, 140.62, 140.38, 130.12, 130.03, 125.73, 125.4, 125.35, 125.10, 109.78, 109.64, 77.25, 69.50, 67.31, 33.65, 32.34, 28.79, 25.44, 25.16, 25.13, 15.36, 15.23, 7.33 ppm; HRMS (ESI) Calc. for $C_{31}H_{46}O_5N$ [M−H]$^-$: 512.3382, found: 512.3377; purity >95% LC/MS, t_r = 11.40 min (method B).

5.9. *N*-Hydroxy-7-(4-(3-(4-(2-hydroxy-3,3-dimethylbutoxy)-3-methylphenyl)pentan-3-yl)-2-methylphenoxy)heptanamide (10d)

Synthesized from **9d** according to procedure similar to that for **10b** in 33% yield as colourless film; ^1H NMR (500 MHz, CD_3OD) δ 6.97–6.91 (m, 2H), 6.85 (d, J = 2.7 Hz, 1H), 6.83 (d, J = 2.7 Hz, 1H), 6.75 (d, J = 8.6 Hz, 1H), 6.72 (d, J = 8.5 Hz, 1H), 4.11 (dd, J = 10.0, 2.9 Hz, 1H), 3.94 (t, J = 6.3 Hz, 2H), 3.86 (dd, J = 10.0, 7.8 Hz, 1H), 3.61 (dd, J = 7.8, 2.9 Hz, 1H), 2.15 (s, 3H), 2.12–2.07 (m, 5H), 2.03 (q, J = 7.3 Hz, 4H), 1.83–1.73 (m, 2H), 1.68–1.61 (m, 2H), 1.57–1.45 (m, 2H), 1.45–1.36 (m, 2H), 1.00 (s, 9H), 0.58 (t, J = 7.3 Hz, 6H). ^{13}C NMR (125 MHz, CD_3OD) δ 154.80, 140.62, 140.35, 130.11, 130.02, 125.72, 125.34, 125.06, 109.74, 109.60, 104.99, 77.2, 69.47, 67.39, 33.66, 29.98, 28.77, 28.49, 25.57, 25.34, 25.16, 15.38, 15.23, 7.33 ppm; HRMS (ESI) Calc. for $C_{32}H_{48}O_5N$ [M−H]$^-$: 526.3538, found: 526.3544; purity >95% LC/MS, t_r = 13.77 min (method A).

5.10. *N*,4-Dihydroxy-5-(4-(3-(4-(2-hydroxy-3,3-dimethylbutoxy)-3-methylphenyl)pentan-3-yl)-2-methylphenoxy)pentanamide (10e)

Synthesized from **10e** according to procedure similar to that for **10b** in 52% yield as a colourless film; IR (thin film) ν 3313,2962, 2929, 2869,1503, 1220 cm^{-1}; ^1H NMR (400 MHz, CD_3OD) δ 6.95 (d, J = 8.5 Hz, 2H), 6.84 (s, 2H), 6.75 (d, J = 8.5 Hz, 2H), 4.11 (dd, J = 10.0, 2.9 Hz, 1H), 3.96–3.82 (m, 3H), 3.61 (dd, J = 7.8, 2.8 Hz, 1H), 2.36–2.18 (m, 3H), 2.15 (s, 3H), 2.11 (s, 3H), 2.03 (q, J = 7.2 Hz, 4H), 1.89–1.74 (m, 2H), 1.00 (s, 9H), 0.58 (t, J = 7.3 Hz, 6H) ppm; ^{13}C NMR (75 MHz, CD_3OD) δ 171.34, 154.82, 154.57, 140.81, 140.57, 130.11, 125.75, 125.71, 125.36, 109.76, 77.24, 71.59, 69.48, 68.99, 48.01, 33.66, 29.30, 28.75, 25.16, 15.38, 15.25, 7.33 ppm; HRMS (ESI) Calc. for $C_{30}H_{44}O_6N$ [M-H]$^-$: 514.3174, found: 514.3182; LRMS (ESI) m/z 516.5 [M+H]$^+$. Purity >95% LC/MS, t_r = 13.00 min (method A).

5.11. Ethyl 5-(4-(3-(4-(3,3-dimethyl-2-oxobutoxy)-3-methylphenyl)-pentan-3-yl)-2-methylphenoxy)-4-oxopentanoate (11)

Synthesized from **7** according to procedure similar to that for **9b** (alkylating reagent 1.2 equiv of methyl 5-bromo-4-oxopentanoate at 40 °C for 24 h) in 72% yield as colourless oil: R_f 0.40 (20:80 EtOac/Hex); IR (thin film) ν 2966, 2958, 2877, 1727, 1502, 1220 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 6.91 (m, 2H), 6.56 (d, J = 8.3 Hz, 2H), 6.49 (d, J = 8.3 Hz, 2H), 4.83 (s, 2H), 4.55 (s, 2H), 4.13 (q, J = 7.1 Hz, 2H), 2.97 (t, J = 6.5 Hz, 2H), 2.65 (t, J = 6.5 Hz, 2H), 2.23 (s, 6H), 2.01 (q, J = 7.3 Hz, 4H), 1.41–1.04 (m, 12H), 0.58 (t, J = 7.3 Hz, 6H) ppm; ^{13}C NMR (126 MHz, $CDCl_3$) δ 210.04, 206.90, 172.55, 153.98, 153.50, 141.73, 141.34, 130.87, 130.71, 126.20, 126.01, 125.97, 125.60, 110.14, 109.74, 72.93, 69.57, 60.72, 48.42, 43.22, 33.87, 29.23, 27.46, 26.35, 16.66, 16.60, 14.16, 8.42 ppm; HRMS (ESI) Calc. for $C_{32}H_{44}O_6Na$ [M+Na]$^+$: 547.3030, found 547.3025.

5.12. Methyl 6-(4-(3-(4-(2-hydroxy-3,3-dimethylbutoxy)-3-methylphenyl)pentan-3-yl)-2-methylbenzamido)hexanoate (13a)

12 (23.0 mg, 0.56 mmol, 1.0 equiv) was dissolved in dichloromethane (3 mL) and methyl-7-aminohexanoate hydrochloride (15.3 mg, 0.084 mmol, 1.50 equiv) was added followed by EDC HCl (12.8 mmol, 0.067 mmol, 1.2 equiv), DMAP (0.70 mg, 0.0056 mmol, 0.10 equiv) and triethylamine (23.4 µl, 0.168 mmol, 3.0 equiv). Reaction was stirred at ambient temperature for 12 h. 1 M HCl solution (5 mL) was added and resulting mixture was extracted with ethylacetate (3 × 5 mL) combined organic layer were washed with saturate NaCl (5 mL), dried over anhydrous sodium sulfate, crude product was purified by chromatography on silica gel eluating 15% ethyl acetate/hexanes to afford 16.8 mg transparent oil (57%). R_f 0.30 (20:80 EtOac/Hex); IR (thin film) ν 3309, 2935, 2874, 1738, 1639, 1502, 1245 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.20 (d, J = 8.0 Hz, 1H), 7.01 (s, 1H), 6.98 (d, J = 8.0 Hz, 1H), 6.94 (d, J = 6.1 Hz, 1H), 6.86 (d, J = 2.2 Hz, 1H), 6.69 (d, J = 8.5 Hz, 1H), 5.73 (t, J = 5.7 Hz, 1H), 4.09 (dd, J = 9.2, 2.6 Hz, 1H), 3.85 (t, J = 8.9 Hz, 1H), 3.66 (dd, J = 8.7, 2.6 Hz, 1H), 3.66 (s, 3H), 3.41 (dd, J = 13.2, 6.9 Hz, 2H), 2.39 (s, 3H), 2.33 (t, J = 7.5 Hz, 2H), 2.16 (s, 3H), 2.04 (q, J = 7.3 Hz, 4H), 1.75–1.55 (m, 4H), 1.45–1.32 (m, 2H), 1.01 (s, 9H), 0.59 (t, J = 7.3 Hz, 6H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$) δ 174.02, 170.35, 154.45, 150.99, 140.35, 135.14, 133.54, 130.66, 130.64, 126.08, 125.65, 125.51, 110.06, 77.26, 69.15, 51.53, 49.04, 39.50, 33.87, 33.55, 29.41, 29.02, 26.42, 26.05, 24.51, 20.15, 16.62, 8.33 ppm; HRMS (ESI) Calc. for $C_{33}H_{50}O_5N$ [M+H]$^+$: 540.3684, found 540.3691.

5.13. Methyl 7-(4-(3-(4-(2-hydroxy-3,3-dimethylbutoxy)-3-methylphenyl)pentan-3-yl)-2-methylbenzamido)heptanoate (13b)

Synthesized from **12** according to procedure similar to that for **13a** (with 1.50 equiv of methyl-7-aminoheptanoate hydrochloride) in 81% yield as colourless oil: R_f 0.32 (20:80 EtOac/Hex); IR (thin film) ν 3309, 2926, 2857, 1738, 1639, 1608, 1536, 1461, 1246 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.21 (d, J = 8.0 Hz, 1H), 7.00 (s, 1H), 6.98 (d, J = 8.0 Hz, 1H), 6.93 (d, J = 6.1 Hz, 1H), 6.85 (d, J = 2.2 Hz, 1H), 6.69 (d, J = 8.5 Hz, 1H), 5.73 (t, J = 5.7 Hz, 1H), 4.09 (dd, J = 9.2, 2.6 Hz, 1H), 3.85 (t, J = 8.9 Hz, 1H), 3.70 (dd, J = 8.7, 2.6 Hz, 1H), 3.66 (s, 3H), 3.41 (dd, J = 13.2, 6.9 Hz, 2H), 2.39 (s, 3H), 2.31 (t, J = 7.5 Hz, 2H), 2.16 (s, 3H), 2.04 (q, J = 7.3 Hz, 4H), 1.75–1.55 (m, 4H), 1.45–1.32 (m, 4H), 1.01 (s, 9H), 0.59 (t, J = 7.3 Hz, 6H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$) δ 174.16, 170.34, 154.44, 150.97, 140.36, 135.12, 133.59, 130.64, 126.08,

125.65, 125.51, 110.06, 77.26, 69.15, 51.50, 49.04, 39.66, 33.93, 33.55, 29.53, 29.03, 28.75, 26.59, 26.05, 24.78, 20.15, 16.63, 8.34 ppm; HRMS (ESI) Calc. for $C_{34}H_{52}O_5N$ $[M+H]^+$: 554.3840, found 554.3842.

5.14. 4-(3-(4-(2-Hydroxy-3,3-dimethylbutoxy)-3-methylphenyl)-pentan-3-yl)-*N*-(6-(hydroxyamino)-6-oxohexyl)-2-methylbenzamide (14a)

Synthesized from **13a** according to procedure similar to that for **10b** in 47% yield as colourless film: IR (thin film) ν 3269, 2960, 2935, 2873, 1635, 1609, 1505, 1220 cm^{-1}; ^1H NMR (500 MHz, CD_3OD) δ 7.21 (d, J = 7.9 Hz, 1H), 7.04 (d, J = 7.9 Hz, 1H), 7.03 (s, 1H) 6.98 (d, J = 8.6 Hz, 1H), 6.80 (s, 1H), 6.76 (d, J = 8.6 Hz, 1H), 4.11 (dd, J = 10.0, 2.9 Hz, 1H), 3.86 (dd, J = 10.0, 7.8 Hz, 1H), 3.61 (dd, J = 7.7, 2.9 Hz, 1H), 3.32 (t, J = 7.0 Hz, 2H), 2.32 (s, 3H), 2.13 (s, 3H), 2.12–2.09 (m, 6H), 1.77–1.51 (m, 4H), 1.44–1.38 (m, 2H), 1.00 (s, 9H), 0.60 (t, J = 7.3 Hz, 6H) ppm; ^{13}C NMR (125 MHz, CD_3OD) δ 171.95, 171.41, 155.03, 151.04, 139.79, 134.43, 133.80, 130.10, 130.07, 126.00, 125.62, 125.11, 109.79, 77.21, 69.48, 48.68, 39.07, 33.67, 32.22, 28.63, 28.41, 26.06, 25.16, 24.99, 18.55, 15.36, 7.21 ppm; HRMS (ESI) Calc. for $C_{31}H_{45}O_5N_2$ $[M-H]^-$: 539.3491, found: 539.3504; **LRMS** (ESI) m/z 541.5 $[M+H]^+$. Purity >95% LC/MS, t_r = 12.48 min (method A).

5.15. 4-(3-(4-(2-Hydroxy-3,3-dimethylbutoxy)-3-methylphenyl)-pentan-3-yl)-*N*-(7-(hydroxyamino)-7-oxoheptyl)-2-methylbenzamide (14b)

Synthesized from **13b** according to procedure similar to that for **10b** in 39% yield as a colourless film: IR (thin film) ν 3267, 2961, 2935, 2873, 1635, 1609, 1506, 1221 cm^{-1}; ^1H NMR (500 MHz, CD_3OD) δ 7.21 (d, J = 7.9 Hz, 1H), 7.05 (d, J = 7.9 Hz, 1H), 7.03 (s, 1H), 6.98 (dd, J = 8.4, 2.4 Hz, 1H), 6.80 (s, 1H), 6.76 (d, J = 8.4 Hz, 1H), 4.11 (dd, J = 10.0, 2.9 Hz, 1H), 3.86 (dd, J = 10.0, 7.8 Hz, 1H), 3.61 (dd, J = 7.8, 2.9 Hz, 1H), 3.34 (s, 1H), 3.32 (t, J = 7.3 Hz, 2H), 2.32 (s, 3H), 2.13 (s, 3H), 2.09 (, J = 6.9 Hz, 4H), 2.07 (t, overlap, 2H), 1.78–1.50 (m, 4H), 1.48–1.29 (m, 4H), 1.00 (s, 9H), 0.60 (t, J = 7.3 Hz, 6H) ppm; ^{13}C NMR (75 MHz, CD_3OD) δ 171.89, 155.03, 151.00, 139.79, 134.39, 133.86, 130.10, 130.07, 125.97, 125.62, 125.11, 109.81, 77.21, 69.50, 48.69, 39.15, 33.66, 32.25, 28.81, 28.42, 28.33, 26.25, 25.24, 25.15, 18.52, 15.34, 7.20 ppm; HRMS (ESI) Calc. for $C_{33}H_{49}O_5N_2$ $[M-H]^-$: 553.3647, found 553.3673. LRMS (ESI) m/z 555.5 $[M+H]^+$. Purity >95% LC/MS, t_r = 12.81 min (method A).

5.16. 3-(4-(Benzyloxy)-3-bromophenyl)pentan-3-ol (15-SM)

A solution of EtMgBr in diethylether (8.3 mL, 24.8 mmol, 3.0 M, 2.5 equiv) was added dropwise to a methyl 4-(benzyloxy)-3-bromobenzoate (3.18 g, 9.90 mmol, 1.0 equiv) in dry tetrahydrofurane (40 mL) at 0 °C. Reaction mixture was warmed to rt during 5 h. Saturated NH_4Cl solution (20 mL) was added resulting mixture was extracted with ethyl acetate (3 × 15 mL). The combined organic layer was dried over anhydrous sodium sulfate, filtered, and concentrated to give 3.11 g yellow oil (90%). R_f 0.45 (20:80 EtOAc/Hex); IR (thin film) ν 3463, 3032, 2968, 1495, 1251, 1049 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.61 (d, J = 2.3 Hz, 1H), 7.51 (d, J = 7.2 Hz, 2H), 7.42 (t, J = 7.4 Hz, 2H), 7.35 (t, J = 7.3 Hz, 1H), 7.25 (dd, J = 8.5, 2.3 Hz, 1H), 6.93 (d, J = 8.6 Hz, 1H), 5.17 (s, 2H), 1.91 – 1.72 (m, 4H), 1.63 (br s, 1H), 0.79 (t, J = 7.4 Hz, 6H) ppm; ^{13}C NMR (101 MHz, $CDCl_3$) δ 153.45, 139.95, 136.67, 130.72, 128.56, 127.90, 127.03, 125.58, 113.27, 112.24,, 76.86, 70.90, 34.94, 7.80 ppm; HRMS (ESI) Calc. for $C_{18}H_{21}O_2BrNa$ $[M+Na]^+$: 371.0617, found: 371.0611.

5.17. 4-(3-(4-(Benzyloxy)-3-bromophenyl)pentan-3-yl)-2-methylphenol (15)

Tertiary alcohol (**15-SM**) (2.20 g, 6.30 mmol, 1.0 equiv) was dissolved in dichloromethane (20 mL) and *o*-cresol (2.60 mL, 25.2 mmol, 4.0 equiv) was added and reaction mixture was cooled to −78 °C. BF_3 Et_2O (4.79 mL, 37.8 mmol, 6.0 equiv) was added dropwise and reaction was stirred for 2 h at −78 °C, then warmed to rt during 4 h. Saturated aqueous sodium bicarbonate (30 mL) was added and the resulting mixture was extracted with dichloromethane (3 × 15 mL). The combined organic layer was dried over anhydrous sodium sulfate, filtered, and concentrated. The crude product was purified by chromatography on silica gel eluating 5% ethyl acetate/hexanes to afford 2.38 g of **15** (86%). R_f 0.49 (20:80 EtOAc/Hex); IR (thin film) 3437, 3032, 2967, 1493, 1253, 1051 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.50 (d, J = 7.2 Hz, 2H), 7.44–7.37 (m, 3H), 7.35 (d, J = 7.3 Hz, 1H), 7.02 (dd, J = 8.6, 2.3 Hz, 1H), 6.93–6.86 (m, 2H), 6.84 (d, J = 8.6 Hz, 1H), 6.68 (d, J = 8.0 Hz, 1H), 5.14 (s, 2H), 2.22 (s, 3H), 2.03 (q, J = 7.3 Hz, 4H), 0.63 (t, J = 7.3 Hz, 6H) ppm; ^{13}C NMR (101 MHz, $CDCl_3$) δ 152.67, 151.50, 143.38, 140.34, 136.78, 132.78, 130.56, 128.53, 128.09, 127.85, 127.03, 126.60, 122.68, 114.14, 113.00, 111.74, 70.86, 48.58, 29.24, 16.04, 8.36 ppm; HRMS (ESI) Calc. for $C_{25}H_{27}O_2BrNa$ $[M+Na]^+$: 461.1098, found: 461.1079.

5.18. 1-(4-(3-(4-(Benzyloxy)-3-bromophenyl)pentan-3-yl)-2-methylphenoxy)-3,3-dimethylbutan-2-one (16)

Synthesized from **15** according to procedure similar to that for **9b** (alkylation with 1-chloropinacolone, 1.5 equiv) in 70% yield as colourless oil: R_f 0.80 (20:80 EtOac/Hex); IR (thin film) ν 3031, 2966, 1725, 1497, 1246, 1049 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.50 (d, J = 7.3 Hz, 2H), 7.45–7.38 (m, 3H), 7.35 (d, J = 7.3 Hz, 1H), 7.02 (dd, J = 8.6, 2.3 Hz, 1H), 6.95–6.86 (m, 2H), 6.84 (d, J = 8.6 Hz, 1H), 6.53 (d, J = 9.1 Hz, 1H), 5.18 (s, 3H), 4.86 (s, 2H), 2.25 (s, 3H), 2.03 (q, J = 7.1 Hz, 4H), 1.28 (s, 9H), 0.62 (t, J = 7.4 Hz, 6H) ppm; ^{13}C NMR (101 MHz, $CDCl_3$) δ 209.99, 154.20, 152.68, 143.28, 140.66, 136.79, 132.76, 130.68, 128.53, 128.16, 127.85, 127.04, 126.24, 126.00, 113.00, 111.73, 110.31, 70.86, 69.61, 48.60, 43.24, 29.25, 26.37, 16.66, 8.38 ppm; HRMS (ESI) Calc. for $C_{31}H_{37}BrO_3Na$ $[M+Na]^+$: 559.1818, found 559.1822.

5.19. 1-(4-(3-(4-(Benzyloxy)-3-bromophenyl)pentan-3-yl)-2-methylphenoxy)-3,3-dimethylbutan-2-ol (17a)

Synthesized from **16** according to procedure similar to that for **9e** (with 1.2 equiv of $NaBH_4$) in 93% yield as colourless oil: R_f 0.69 (20:80 EtOac/Hex); IR (thin film) ν 3595, 2964, 1494, 1264 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.47 (d, J = 7.3 Hz, 2H), 7.42–7.30 (m, 3H), 6.99 (dd, J = 8.6, 2.3 Hz, 1H), 6.93 (dd, J = 8.5, 2.4 Hz, 1H), 6.91–6.85 (m, 2H), 6.81 (d, J = 8.6 Hz, 1H), 6.70 (d, J = 8.5 Hz, 1H), 5.11 (s, 2H), 4.09 (dd, J = 9.2, 2.6 Hz, 1H), 3.85 (t, J = 8.9 Hz, 1H), 3.70 (dd, J = 8.7, 2.6 Hz, 1H), 2.17 (s, 3H), 2.01 (q, J = 7.2 Hz, 4H), 0.96 (s, 9H), 0.60 (t, J = 7.3 Hz, 6H) ppm; ^{13}C NMR (125 MHz, CD_3OD) δ 154.57, 152.72, 143.37, 140.35, 136.81, 132.81, 130.58, 128.57, 128.12, 127.89, 127.06, 126.14, 125.78, 113.03, 111.80, 110.29, 77.12, 70.86, 69.29, 48.61, 33.62, 29.26, 26.12, 16.67, 8.42 ppm; HRMS (ESI) Calc. for $C_{31}H_{39}BrO_3Na$ $[M+Na]^+$: 561.1975., found 561.1978.

5.20. 1-(4-(3-(4-(Benzyloxy)-3-(hydroxymethyl)phenyl)pentan-3-yl)-2-methylphenoxy)-3,3-dimethylbutan-2-ol (17b)

23 (260 mg, 0.50 mmol, 1.0 equiv) was dissolved in dry toluene and DIBAL-H (1.1 mL, 1 M solution in toluene, 0.529 mmol, 2.2 equiv) was added at −78 °C. Reaction was stirred at this

temperature for 2 h and warmed to rt. Saturated potassium sodium tartrate solution (5 mL) was added and mixture was stirred until disappearance of solid. Resulting mixture was extracted with ethylacetate (3 × 5 mL) combined organic layer were washed with saturate NaCl (5 mL), dried over anhydrous sodium sulfate, filtered, and concentrated to give crude alcohol, that was purified by chromatography on silica gel eluating 5% ethyl acetate/hexanes to afford 192 mg transparent oil (78%). R_f 0.51 (40:60 EtOAc/Hex); IR (thin film) 3417, 2962, 2875, 1455, 1240, 1134 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.45–7.35 (m, 5H), 7.13–7.08 (m, 2H), 6.99 (dd, J = 8.6, 2.4 Hz, 1H), 6.94 (d, J = 2.0 Hz, 1H), 6.88 (d, J = 8.5 Hz, 1H), 6.73 (d, J = 8.5 Hz, 1H), 5.11 (s, 2H), 4.70 (s, 2H), 4.23–4.05 (m, 1H), 3.89 (t, J = 8.9 Hz, 1H), 3.74 (dd, J = 8.7, 2.6 Hz, 1H), 2.37 (br s, 1H), 2.20 (s, 3H), 2.12–2.04 (m, 4H), 1.04 (s, 9H), 0.64 (t, J = 7.3 Hz, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 154.45, 154.39, 141.54, 140.90, 136.92, 130.62, 129.04, 128.68, 128.34, 128.27, 128.08, 127.37, 126.16, 125.61, 110.70, 110.20, 77.31, 70.10, 69.24, 62.81, 48.56, 33.59, 29.28, 26.09, 16.64, 8.45 ppm; HRMS (ESI) Calc. for $C_{32}H_{42}O_4Na$ $[M+Na]^+$: 513.2975, found: 513.2965 ppm;

5.21. 2-Bromo-4-(3-(4-(2-hydroxy-3,3-dimethylbutoxy)-3-methylphenyl)pentan-3-yl)phenol (18a)

17a (790 mg, 1.47 mmol, 1.0 equiv) was dissolved in methanol/ethylacetate 1:1 (5 mL) at rt 10 wt % Pd/C (155 mg, 0.147 mmol, 10 mol%) was added and reaction stirred under hydrogen atmosphere for 12 h. Reactions mixture was filtered through celite and purified through column chromatography 15% EtOAC/Hexanes, to give 631 mg yellow oil (96%). R_f 0.20 (20:80 EtOac/Hex); IR (thin film) ν 3397, 2963, 1496, 1260, 1041 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.47 (d, J = 7.3 Hz, 1H), 6.99 (dd, J = 8.6, 2.3 Hz, 1H), 6.93 (dd, J = 8.5, 2.4 Hz, 1H), 6.87 (d, J = 2.0 Hz, 1H), 6.81 (d, J = 8.6 Hz, 1H), 6.70 (d, J = 8.5 Hz, 1H), 4.09 (dd, J = 9.2, 2.6 Hz, 1H), 3.85 (t, J = 8.9 Hz, 1H), 3.70 (dd, J = 8.7, 2.6 Hz, 1H), 2.17 (s, 3H), 2.01 (q, J = 7.2 Hz, 4H), 0.96 (s, 9H), 0.60 (t, J = 7.3 Hz, 6H) ppm; ^{13}C NMR (101 MHz, $CDCl_3$) δ 154.54, 149.89, 143.00, 140.33, 131.21, 130.56, 129.04, 126.10, 125.78, 115.30, 110.27, 109.64, 77.38, 69.25, 48.62, 33.61, 29.27, 26.10, 16.64, 8.38 ppm; HRMS (ESI) Calc. for $C_{24}H_{33}BrO_3Na$ $[M+Na]^+$: 471.1505., found 471.1504.

5.22. 4-(3-(4-(2-Hydroxy-3,3-dimethylbutoxy)-3-methylphenyl)-pentan-3-yl)-2-(hydroxymethyl)phenol (18b)

Synthesized from **17b** according to procedure similar to that for **18a** (reaction time 3 h) in 80% yield as a colourless oil: R_f 0.12 (20:80 EtOAc/Hex); IR (thin film) ν 3345, 1609, 1501, 1244 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.03 (dd, J = 8.5, 2.4 Hz, 1H), 6.96 (dd, J = 8.5, 2.4 Hz, 1H), 6.91 (d, J = 1.9 Hz, 1H), 6.82 (d, J = 2.3 Hz, 1H), 6.78 (d, J = 8.5 Hz, 1H), 6.71 (d, J = 8.5 Hz, 1H), 4.79 (s, 2H), 4.11 (dd, J = 9.3, 2.6 Hz, 1H), 3.87 (t, J = 8.9 Hz, 1H), 3.72 (dd, J = 8.6, 2.6 Hz, 1H), 2.45 (br s, 1H), 2.18 (s, 3H), 2.03 (dd, J = 15.4, 8.0 Hz, 4H), 1.03 (s, 9H), 0.61 (t, J = 7.3 Hz, 6H) ppm; ^{13}C NMR (101 MHz, $CDCl_3$) δ 154.34, 153.55, 141.02, 140.61, 130.63, 128.94, 127.44, 126.12, 125.57, 123.73, 115.55, 110.14, 69.19, 64.98, 48.45, 33.58, 29.26, 26.07, 16.62, 8.41 ppm; HRMS (ESI) Calc. for $C_{25}H_{36}O_4Na$ $[M+Na]^+$: 423.2506, found: 425.2503 ppm;

5.23. 2-Hydroxy-5-(3-(4-(2-hydroxy-3,3-dimethylbutoxy)-3-methylphenyl)pentan-3-yl)benzamide (18c)

Synthesized from **25** according to procedure similar to that for **18a** in 98% yield as a colourless oil: R_f 0.25 (40:60 EtOAc/Hex); IR (thin film) ν 3444, 3351, 3202, 1654, 1247 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 12.01 (s, 1H), 7.21 (dd, J = 8.8, 2.3 Hz, 1H), 7.13 (d, J = 2.3 Hz, 1H), 6.94 (dd, J = 8.4, 2.5 Hz, 1H), 6.88 (d, J = 7.0 Hz, 1H), 6.87 (s, 1H), 6.71 (d, J = 8.5 Hz, 1H), 5.84 (br s, 1H), 4.09 (dd, J = 9.2, 2.6 Hz, 1H), 3.85 (t, J = 8.9 Hz, 1H), 3.71 (dt, J = 8.7, 2.6 Hz, 1H), 2.17 (s, 3H), 2.02 (dd, J = 14.1, 6.2 Hz, 4H), 1.01 (s, 9H), 0.61 (t, J = 7.3 Hz, 6H) ppm. ^{13}C NMR (101 MHz, $CDCl_3$) δ 173.08, 159.71, 154.60, 140.17, 139.44, 135.24, 127.37, 126.18, 125.84, 125.27, 117.90, 112.29, 110.28, 69.28, 60.44, 48.60, 33.61, 29.32, 26.07, 16.62, 8.37 ppm; HRMS (ESI) Calc. for $C_{25}H_{36}NO_4$ $[M+H]^+$: 414.2639, found: 414.2631.

5.24. Ethyl 2-(2-bromo-4-(3-(4-(2-hydroxy-3,3-dimethylbutoxy)-3-methylphenyl)pentan-3-yl)phenoxy)acetate (19a)

Synthesized from **18a** according to procedure similar to that for **9b** (alkylation with 1.5 equiv of methyl bromoacetate) in 83% yield as colourless oil: R_f 0.72 (20:80 EtOac/Hex); IR (thin film) ν 3548, 2964, 1759, 1493, 1239 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.37 (d, J = 2.2 Hz, 1H), 6.99 (dd, J = 8.6, 2.3 Hz, 1H), 6.93 (dd, J = 8.4, 2.2 Hz, 1H), 6.86 (d, J = 2.1 Hz, 1H), 6.70 (d, J = 5.4 Hz, 1H), 6.68 (d, J = 5.5 Hz, 1H), 4.66 (s, 2H), 4.32–4.18 (q, J = 5.5 Hz, 2H), 4.09 (dd, J = 9.2, 2.5 Hz, 1H), 3.85 (t, J = 8.9 Hz, 1H), 3.70 (dd, J = 8.7, 2.5 Hz, 1H), 2.17 (s, 3H), 2.01 (q, J = 7.3 Hz, 4H), 1.27 (t, J = 5.5 Hz, 3H), 1.01 (s, 9H), 0.59 (t, J = 7.3 Hz, 6H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$) δ 168.55, 154.52, 152.00, 144.28, 140.14, 132.99, 130.52, 128.07, 126.05, 125.76, 112.88, 111.87, 110.19, 69.20, 68.22, 66.48, 61.39, 48.60, 33.56, 29.18, 26.05, 16.61, 14.13, 8.32 ppm. HRMS (ESI) Calc. for $C_{28}H_{39}BrO_5Na$ $[M+Na]^+$: 557.1873, found 557.1878.

5.25. Ethyl 2-(4-(3-(4-(2-hydroxy-3,3-dimethylbutoxy)-3-methylphenyl)pentan-3-yl)-2-(hydroxymethyl)phenoxy)acetate (19b)

Synthesized from **18b** according to procedure similar to that for **9b** (alkylation with 1.5 equiv of methyl bromoacetate) in 94% yield (reaction performed at room temperature for 12 h with 1.1 equiv of bromide) as a colourless oil: R_f 0.14 (20:80 EtOAc/Hex); IR (thin film) 3476, 1739, 1501, 1259, 1136 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.09 (d, J = 2.3 Hz, 1H), 7.05 (dd, J = 8.5, 2.4 Hz, 1H), 6.96 (dd, J = 8.3, 2.2 Hz, 1H), 6.90 (d, J = 1.9 Hz, 1H), 6.71 (dd, J = 8.5, 6.3 Hz, 2H), 4.70 (s, 2H), 4.69 (s, 2H), 4.29 (q, J = 7.1 Hz, 2H), 4.11 (dd, J = 8.1, 3.8 Hz, 1H), 3.88 (t, J = 8.9 Hz, 1H), 3.73 (dd, J = 8.7, 2.6 Hz, 1H), 2.36 (br s, 1H), 2.19 (s, 3H), 2.05 (q, J = 7.3 Hz, 4H), 1.31 (t, J = 7.1 Hz, 3H), 1.03 (s, 9H), 0.61 (t, J = 7.3 Hz, 6H) ppm; ^{13}C NMR (126 MHz, $CDCl_3$) δ 169.38, 154.41, 153.93, 142.61, 140.74, 130.61, 129.29, 129.06, 128.45, 126.14, 125.63, 110.98, 110.18, 77.37, 69.22, 65.67, 62.78, 61.64, 48.59, 33.58, 29.24, 26.07, 16.62, 14.12, 8.41 ppm; HRMS (ESI) Calc. for $C_{29}H_{42}O_6Na$ $[M+Na]^+$: 509.2874, found: 509.2873 ppm;

5.26. Ethyl 2-(2-carbamoyl-4-(3-(4-(2-hydroxy-3,3-dimethylbutoxy)-3-methylphenyl)pentan-3-yl)phenoxy)acetate (19c)

Synthesized from **19c** according to procedure similar to that for **9b** (alkylation with 1.5 equiv of methyl bromoacetate) in 92% yield as a colourless oil: R_f 0.15 (20:80 EtOAc/Hex); IR (thin film) ν 3444, 3353, 1751, 1667, 1589, 1497, 1215 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 8.39 (bs, 1H), 8.20 (d, J = 2.6 Hz, 1H), 7.13 (dd, J = 8.6, 2.6 Hz, 1H), 6.93 (dd, J = 8.5, 2.3 Hz, 1H), 6.85 (d, J = 1.8 Hz, 1H), 6.70 (t, J = 8.9 Hz, 2H), 5.79 (bs, 1H), 4.69 (s, 2H), 4.31 (q, J = 7.1 Hz, 2H), 4.07 (dd, J = 9.2, 2.7 Hz, 1H), 3.84 (t, J = 9.0 Hz, 1H), 3.69 (d, J = 8.7 Hz, 1H), 2.15 (s, 3H), 2.09 (q, J = 7.2 Hz, 4H), 1.32 (t, J = 7.1 Hz, 3H), 1.00 (s, 9H), 0.59 (t, J = 7.3 Hz, 6H) ppm; ^{13}C NMR (101 MHz, $CDCl_3$) δ 168.25, 154.50, 153.73, 143.15, 140.42, 133.55, 131.55, 130.58, 127.31, 126.07, 125.76, 111.85, 110.29, 69.26, 65.84, 61.89, 48.65, 33.58, 29.07, 26.24, 26.07, 16.60, 14.13, 8.32 ppm; HRMS (ESI) Calc. for $C_{29}H_{42}NO_6$ $[M+H]^+$: 500.3007, found: 500.3002.

5.27. 2-(2-Bromo-4-(3-(4-(2-hydroxy-3,3-dimethylbutoxy)-3-methylphenyl)pentan-3-yl)phenoxy)-*N*-hydroxyacetamide (20a)

Synthesized from **19a** according to procedure similar to that for **10b** in 39% yield as colourless film; IR (thin film) ν 3405, 3226, 2964, 1758,1683,1247 cm^{-1}; ^1H NMR (400 MHz, CD_3OD) δ 7.29 (d, J = 2.2 Hz, 1H), 7.12 (dd, J = 8.6, 2.2 Hz, 1H), 6.96 (dd, J = 8.5, 2.3 Hz, 1H), 6.92 (d, J = 8.7 Hz, 1H), 6.84 (d, J = 1.8 Hz, 1H), 6.78 (d, J = 8.5 Hz, 1H), 4.57 (s, 2H), 4.12 (dd, J = 10.0, 2.9 Hz, 1H), 3.87 (dd, J = 10.0, 7.9 Hz, 1H), 3.62 (dd, J = 7.8, 2.8 Hz, 1H), 2.16 (s, 3H), 2.04 (q, J = 7.3 Hz, 4H), 1.00 (s, 9H), 0.59 (t, J = 7.3 Hz, 6H). ^{13}C NMR (75 MHz, CD_3OD) δ 166.02, 155.12, 152.10, 144.76, 139.39, 132.72, 129.93, 128.97, 125.75, 125.68, 113.36, 111.30, 109.93, 77.20, 69.46, 67.38, 48.29, 33.66, 28.66, 25.16, 15.39, 7.23 ppm; HRMS (ESI) Calc. for $C_{26}H_{36}BrNO_5Na$ $[M+Na]^+$: 544.1669, found 544.1664. Purity >95% LC/MS. t_r = 13.64 min (method A).

5.28. *N*-Hydroxy-2-(4-(3-(4-(2-hydroxy-3,3-dimethylbutoxy)-3-methylphenyl)pentan-3-yl)-2-(hydroxymethyl)phenoxy)acetamide (20b)

Synthesized from **20a** according to procedure similar to that for **10b** in 54% yield colourless film; IR (thin film) ν 3333, 2963, 2933, 2873, 1748, 1675, 1501, 1220 cm^{-1}; ^1H NMR (500 MHz, CD_3OD) δ 7.13 (d, J = 2.6 Hz, 1H), 7.10 (dd, J = 8.6, 2.5 Hz, 1H), 6.97 (dd, J = 8.4, 2.6 Hz, 1H), 6.85 (d, J = 2.2 Hz, 1H), 6.83 (d, J = 8.6 Hz, 1H), 6.75 (d, J = 8.6 Hz, 1H), 4.60 (s, 2H), 4.59 (s, 2H), 4.10 (dd, J = 10.0, 2.9 Hz, 1H), 3.85 (dd, J = 10.1, 7.7 Hz, 1H), 3.61 (dd, J = 7.7, 2.9 Hz, 1H), 2.13 (s, 3H), 2.07 (q, J = 7.3 Hz, 4H), 0.99 (s, 9H), 0.59 (t, J = 7.3 Hz, 6H). ^{13}C NMR (125 MHz, CD_3OD) δ 154.92, 153.53, 142.31, 140.17, 130.07, 129.04, 128.57, 128.05, 125.69, 125.51, 110.67, 109.80, 77.22, 69.49, 66.47, 59.80, 33.66, 28.67, 25.16, 15.37, 7.28 ppm; HRMS (ESI) Calc. for $C_{27}H_{38}O_6N$ $[M-H]^-$: 472.2705, found: 472.2711. Purity >95% LC/MS. t_r = 11.91 min (method A).

5.29. 5-(3-(4-(2-Hydroxy-3,3-dimethylbutoxy)-3-methylphenyl)-pentan-3-yl)-2-(2-(hydroxyamino)-2-oxoethoxy)benzamide (20c)

Synthesized from **20b** according to procedure for **10b** in 44% yield as a colourless film; ^1H NMR (500 MHz, CD_3OD) δ 7.75 (d, J = 2.3 Hz, 1H), 7.25 (dd, J = 7.7, 1.5 Hz, 1H), 7.00 – 6.93 (m, 2H), 6.85 (s, 1H), 6.77 (d, J = 8.6 Hz, 1H), 4.66 (s, 2H), 4.11 (dd, J = 10.0, 3.0 Hz, 1H), 3.86 (dd, J = 10.0, 7.7 Hz, 1H), 3.61 (dd, J = 7.7, 2.9 Hz, 1H), 2.14 (s, 3H), 2.09 (q, J = 7.3 Hz, 4H), 0.99 (s, 9H), 0.60 (t, J = 7.3 Hz, 6H) ppm; ^{13}C NMR (125 MHz, CD_3OD) δ 169.61, 166.28, 155.20, 154.02, 143.04, 139.79, 132.77, 130.16, 125.90, 125.87, 121.73, 112.49,, 110.00, 77.37, 69.67, 66.42, 48.34, 33.80, 28.76, 25.32, 15.50, 7.39 ppm; HRMS (ESI) Calc. for $C_{27}H_{39}N_2O_6$ $[M+H]^+$: 487.2803, found: 487.2788; LRMS (ESI) m/z 487.4 $[M+H]^+$. Purity >95% LC/MS. t_r = 11.09 min (method A).

5.30. Methyl 2-(benzyloxy)-5-(3-(4-hydroxy-3-methylphenyl)-pentan-3-yl)benzoate (21)

15 (1.10 g, 2.50 mmol, 1.0 equiv) was dissolved in methanol (10 mL) and DMSO (5 mL) was added. $Pd(OAc)_2$ (56.1 mg, 0.25 mmol, 10 mol %) was added followed by 1,3-bis-(diphenylphosphino)propane (103.0 mg, 0.25 mmol, 10 mol %), and reaction stirred for 5 min at ambient temperature. Triethylamine (0.87 mL, 6.24 mmol, 2.5 equiv) was added and reaction was heated at 80 °C under CO atmosphere for 24 h. 1 M HCl solution (10 mL) was added and resulting mixture was extracted with dichloromethane (3 × 10 mL) combined organic layer were washed with saturate NaCl (5 mL), dried over anhydrous sodium sulfate, crude product was purified by chromatography on silica gel eluating 10% ethyl acetate/hexanes to afford 0.91 g transparent oil (87%). R_f 0.14 (20:80 EtOAc/Hex); IR (thin film) 3409, 2967, 1708, 1499, 1255, 1085 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.73 (d, J = 2.5 Hz, 1H), 7.51 (d, J = 7.3 Hz, 2H), 7.40 (t, J = 7.4 Hz, 2H), 7.32 (t, J = 7.3 Hz, 1H), 7.19 (dd, J = 8.7, 2.5 Hz, 1H), 6.91 (d, J = 8.8 Hz, 1H), 6.89–6.78 (m, 2H), 6.68 (d, J = 8.2 Hz, 1H), 5.16 (s, 2H), 4.90 (br s, 1H), 3.92 (s, 3H), 2.21 (s, 3H), 2.07 (q, J = 7.3 Hz, 4H), 0.63 (t, J = 7.3 Hz, 6H) ppm; ^{13}C NMR (101 MHz, $CDCl_3$) δ 167.50, 155.95, 151.62, 141.39, 140.27, 137.02, 133.56, 130.62, 128.48, 127.69, 127.32, 126.85, 126.59, 122.78, 119.55, 114.14, 113.38, 70.68, 52.04, 48.53, 29.20, 16.07, 8.37 ppm; HRMS (ESI) Calc. for $C_{27}H_{30}O_4Na$ $[M+Na]^+$: 441.2036, found: 441.2043.

5.31. Methyl 2-(benzyloxy)-5-(3-(4-(3,3-dimethyl-2-oxobutoxy)-3-methylphenyl)pentan-3-yl)benzoate (22)

Synthesized from **21** according to procedure similar to that for **9b** (alkylation with 1.5 equiv of 1-chloropinacolone) in 93% yield as a colourless oil: R_f 0.50 (20:80 EtOAc/Hex); IR (thin film) 2967, 1724, 1499, 1238, 1083 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.73 (d, J = 2.5 Hz, 1H), 7.51 (d, J = 7.4 Hz, 2H), 7.40 (t, J = 7.4 Hz, 2H), 7.32 (t, J = 7.3 Hz, 1H), 7.18 (dd, J = 8.7, 2.6 Hz, 1H), 6.99–6.86 (m, 3H), 6.53 (d, J = 9.2 Hz, 1H), 5.16 (s, 2H), 4.86 (s, 2H), 3.91 (s, 3H), 2.27 (s, 3H), 2.08 (q, J = 7.3 Hz, 4H), 1.28 (s, 9H), 0.63 (t, J = 7.3 Hz, 6H) ppm; ^{13}C NMR (101 MHz, $CDCl_3$) δ 209.93, 167.30, 155.96, 154.20, 141.24, 140.74, 137.05, 133.54, 130.75, 130.62, 128.48, 127.68, 126.84, 126.21, 126.05, 119.68, 113.41, 110.31, 70.68, 69.57, 51.95, 48.57, 43.22, 29.23, 26.37, 16.65, 8.38 ppm; HRMS (ESI) Calc. for $C_{33}H_{40}O_5Na$ $[M+Na]^+$: 539.2768, found: 539.2761.

5.32. Methyl 2-(benzyloxy)-5-(3-(4-(2-hydroxy-3,3-dimethylbutoxy)-3-methylphenyl)pentan-3-yl)benzoate (23)

Synthesized from **22** according to procedure similar to that for **11** (with 1.2 equiv of $NaBH_4$) in 85% yield as a yellow film: R_f 0.47 (20:80 EtOAc/Hex); IR (thin film) 3520, 3032, 2962, 2876, 1727, 1248, 1082 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.74 (d, J = 2.4 Hz, 1H), 7.52 (d, J = 7.3 Hz, 2H), 7.41 (t, J = 7.4 Hz, 2H), 7.33 (t, J = 7.3 Hz, 1H), 7.19 (dd, J = 8.7, 2.5 Hz, 1H), 6.97 (d, J = 8.5 Hz, 1H), 6.92 (m, 2H), 6.74 (d, J = 8.5 Hz, 1H), 5.17 (s, 2H), 4.12 (dd, J = 9.2, 2.6 Hz, 1H), 3.92 (s, 3H), 3.90–3.84 (m, 1H), 3.74 (dd, J = 8.6, 2.6 Hz, 1H), 2.44 (br s, 1H), 2.20 (s, 3H), 2.09 (q, J = 7.1 Hz, 4H), 1.05 (s, 9H), 0.64 (t, J = 7.2 Hz, 6H) ppm; ^{13}C NMR (101 MHz, $CDCl_3$) δ 167.33, 155.96, 154.52, 141.30, 140.42, 137.04, 133.48, 130.62, 130.50, 128.49, 127.69, 126.83, 126.15, 125.74, 119.69, 113.41, 110.26, 77.31, 70.69, 69.28, 51.97, 48.56, 33.60, 29.21, 26.09, 16.62, 8.38 ppm; HRMS (ESI) Calc. for $C_{33}H_{42}O_5Na$ $[M+Na]^+$: 541.2924, found: 541.2925.

5.33. 2-(Benzyloxy)-5-(3-(4-(2-hydroxy-3,3-dimethylbutoxy)-3-methylphenyl)pentan-3-yl)benzoic acid (24)

23 (190 mg, 0.38 mmol, 1.0 equiv) was dissolved in tetrahydrofurane/methanol/water (1:1:1, 6 mL) and LiOH (45.6 mg, 1.90 mmol, 5 equiv) was added. Reaction was stirred at ambient temperature until disappearance of starting material (by TLC). Reaction was acidified to pH = 1 and with ethylacetate (3 × 5 mL) combined organic layer were washed with saturate NaCl (5 mL), dried over anhydrous sodium sulfate, filtered and concentrated. The crude product was purified by chromatography on silica gel eluating 20% ethyl acetate/hexanes to afford 184 mg transparent oil in 100% yield. R_f 0.25 (40:60 EtOAc/Hex); IR (thin film) ν 3456, 3286, 3032, 2962, 1733, 1501, 1242 cm^{-1}; ^1H NMR

(400 MHz, $CDCl_3$) δ 8.19 (d, J = 2.6 Hz, 1H), 7.45–7.35 (m, 5H), 7.23 (dd, J = 8.7, 2.6 Hz, 1H), 7.00 (d, J = 8.6 Hz, 1H), 6.92 (dd, J = 8.6 and 1.8 Hz, 1H), 6.84 (s, 1H), 6.70 (d, J = 8.5 Hz, 1H), 5.25 (s, 2H), 4.09 (dd, J = 9.2, 2.7 Hz, 1H), 3.85 (t, J = 9.0 Hz, 1H), 3.70 (d, J = 9.5 Hz, 1H), 2.16 (s, 3H), 2.06 (q, J = 7.3 Hz, 4H), 1.01 (s, 9H), 0.60 (t, J = 7.3 Hz, 6H) ppm. ^{13}C NMR (101 MHz, $CDCl_3$) δ 165.61, 155.27, 154.62, 143.67, 140.00, 135.30, 134.46, 132.44, 130.51, 129.17, 129.14, 127.96, 126.05, 125.92, 116.83, 112.61, 110.37, 77.29, 72.34, 69.29, 48.67, 33.59, 29.05, 26.07, 16.63, 8.29 ppm; HRMS (ESI) Calc. for $C_{32}H_{40}O_5Na$ [M+Na]$^+$: 527.2768, found: 527.2769.

5.34. 2-(Benzyloxy)-5-(3-(4-(2-hydroxy-3,3-dimethylbutoxy)-3-methylphenyl)pentan-3-yl)benzamide (25)

24 (80 mg, 0.159 mmol, 1.0 equiv) was dissolved in dichloromethane (5 mL) and oxalyl chloride (20.4 µl, 0.238 mmol, 1.5 equiv) was added at 0 °C. Reaction mixture was stirred for 2 h at room temperature, diluted with water (5 mL). Organic layer were separated and washed with saturated NaCl solution (2 × 5 mL). Organic phase were dried (Na_2SO_4) and concentrated in vacuo to give acid chloride, that was dissolved in tetrahydrofurane (5 mL) and NH_4OH (3 mL, 28% in water) was added. Reaction was stirred vigorously for 1 h at room temperature. Reaction mixture was acidified to pH 7 by 1 M HCl solution and extracted with EtOAc (3 × 5 mL). The combined organic phases were dried (Na_2SO_4) and concentrated in vacuo to give a residue that was purified by column chromatography (20:80 EtOAc/hexanes) to afford 30.0 mg of thin film (37%). R_f 0.12 (20:80 EtOAc/Hex); IR (thin film) ν 3457, 3376, 3329, 1664, 1239 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.19 (d, J = 2.6 Hz, 1H), 7.76 (bs, 1H), 7.51–7.32 (m, 5H), 7.14 (dd, J = 8.7, 2.6 Hz, 1H), 6.98–6.90 (m, 2H), 6.88 (d, J = 1.8 Hz, 1H), 6.69 (d, J = 8.5 Hz, 1H), 5.66 (bs, 1H), 5.14 (s, 2H), 4.08 (dd, J = 9.2, 2.7 Hz, 1H), 3.85 (t, J = 9.0 Hz, 1H), 3.70 (d, J = 9.5 Hz, 1H), 2.16 (s, 3H), 2.10 (m, 4H), 1.01 (s, 9H), 0.60 (t, J = 7.3 Hz, 6H) ppm. ^{13}C NMR (101 MHz, $CDCl_3$) δ 167.47, 155.06, 154.49, 142.30, 140.54, 135.78, 133.65, 131.22, 130.59, 130.50, 128.94, 128.66, 127.89, 126.11, 125.74, 112.28, 110.31, 71.33, 69.29, 48.60, 33.61, 29.09, 26.25, 26.09, 16.63, 8.36 ppm; HRMS (ESI) Calc. for $C_{32}H_{41}NO_4Na$ [M+Na]$^+$: 526.2928, found: 526.2932.

5.35. 3-(4-(Benzyloxy)phenyl)pentan-3-ol (27)

Synthesized according to procedure **15-SM** in 91% yield as a colourless film: R_f 0.70 (20:80 EtOAc/Hex); IR (thin film) ν 3476, 3034, 2968, 1241 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.49–7.32 (m, 5H), 7.29 (d, J = 8.9 Hz, 2H), 6.95 (d, J = 8.9 Hz, 2H), 5.06 (s, 2H), 2.01–1.71 (m, 4H), 0.76 (t, J = 7.4 Hz, 6H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 157.27, 138.13, 137.11, 128.56, 127.93, 127.55, 126.64, 114.17, 77.13, 69.98, 34.87, 7.87 ppm; HRMS (ESI) Calc. for $C_{18}H_{22}O_2Na$ [M+Na]$^+$: 293.1512, found 29.1513.

5.36. 4-(3-(4-(Benzyloxy)phenyl)pentan-3-yl)-2-methylphenol (28)

Synthesized from **27** according to procedure similar to that for **15** in 59% yield as a yellow oil: R_f 0.25 (20:80 EtOAc/Hex); IR (thin film) ν 3444, 2966, 1237, 1182 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.48–7.29 (m, 5H), 7.10 (d, J = 8.9 Hz, 2H), 6.92–5.86 (m, 4H), 6.66 (d, J = 8.3 Hz, 1H), 5.04 (s, 2H), 4.61 (bs, 1H), 2.20 (s, 3H), 2.04 (q, J = 7.3 Hz, 4H), 0.62 (t, J = 7.3 Hz, 6H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$) δ 156.42, 151.32, 141.44, 141.07, 137.22, 130.64, 129.02, 128.54, 127.90, 127.60, 126.68, 122.51, 114.01, 113.78, 69.96, 48.53, 29.35, 16.07, 8.44 ppm; HRMS (ESI) Calc. for $C_{25}H_{28}O_2Na$ [M+Na]$^+$: 383.1982, found 383.1984.

5.37. 1-(4-(3-(4-(Benzyloxy)phenyl)pentan-3-yl)-2-methylphenoxy)-3,3-dimethylbutan-2-one (29)

Synthesized from **28** according to procedure similar to that for **9b** (alkylation with 1-chloropinacolone, 1.5 equiv) in 93% yield as a colourless oil: R_f 0.75 (20:80 EtOAc/hex); IR (thin film) ν 3032, 2966, 1726, 1504, 1240, 1182 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.47–7.27 (m, 5H), 7.07 (d, J = 8.8 Hz, 2H), 6.93–6.88 (m, 2H), 6.86 (d, J = 8.8 Hz, 2H), 6.49 (d, J = 8.3 Hz, 1H), 5.02 (s, 2H), 4.83 (s, 2H), 2.23 (s, 3H), 2.06–1.88 (m, 4H), 1.24 (s, 9H), 0.59 (t, J = 7.3 Hz, 6H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$) δ 210.03, 156.43, 154.00, 141.40, 141.31, 137.22, 130.78, 129.04, 128.53, 127.89, 127.59, 126.06, 126.00, 113.74, 110.14, 69.93, 69.58, 48.53, 43.23, 29.34, 26.54, 26.36, 16.67, 8.44 ppm; HRMS (ESI) Calc. for $C_{31}H_{38}O_3Na$ [M+Na]$^+$: 481.2713, found 481.2701.

5.38. 1-(4-(3-(4-(Benzyloxy)phenyl)pentan-3-yl)-2-methylphenoxy)-3,3-dimethylbutan-2-ol (30)

Synthesized from **29** according to procedure similar to that for **11** (with 1.2 equiv of $NaBH_4$) in 85% yield as a colourless oil: R_f 0.72 (20:80 EtOac/hex); IR (thin film) ν 3591, 3033, 2962, 2875, 1608, 1506, 1182 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.39 (m, 5H), 7.10 (d, J = 8.6 Hz, 1H), 6.98 (d, J = 8.4 Hz, 2H), 6.90 (m, 2H), 6.72 (d, J = 8.4 Hz, 2H), 5.04 (s, 2H), 4.10 (dd, J = 9.1, 2.3 Hz, 1H), 3.87 (t, J = 8.9 Hz, 1H), 3.72 (dd, J = 8.6, 2.2 Hz, 1H), 2.19 (s, 3H), 2.06 (q, J = 7.2 Hz, 4H), 1.03 (s, 9H), 0.63 (t, J = 7.2 Hz, 6H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$) δ 154.25, 153.18, 141.21, 141.04, 130.69, 129.15, 126.14, 125.53, 114.43, 110.10, 77.46, 69.10, 48.49, 33.58, 29.32, 26.08, 16.63, 8.42 ppm; HRMS (ESI) Calc. for $C_{31}H_{40}O_3Na$ [M+Na]$^+$: 483.2870, found 483.2857.

5.39. 4-(3-(4-(2-Hydroxy-3,3-dimethylbutoxy)-3-methylphenyl)-pentan-3-yl)phenol (31)

Synthesized from **30** according to procedure similar to that for **18a** in 98% yield as a colourless oil: R_f 0.30 (20:80 EtOac/hex); IR (thin film) ν 3373, 2964, 1243, 1178 cm^{-1} ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.02 (d, J = 8.8 Hz, 2H), 6.96 (dd, J = 8.5, 2.1 Hz, 1H), 6.90 (d, J = 1.7 Hz, 1H), 6.71 (d, J = 8.8 Hz, 2H), 6.69 (d, J = 8.5 Hz, 1H), 4.10 (dd, J = 9.2, 2.7 Hz, 1H), 3.88 (t, J = 8.9 Hz, 1H), 3.73 (dd, J = 8.6, 2.6 Hz, 1H), 2.17 (s, 3H), 2.03 (q, J = 7.3 Hz, 4H), 1.02 (s, 9H), 0.61 (t, J = 7.3 Hz, 6H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$) δ 154.25, 153.18, 141.21, 141.04, 130.69, 129.15, 126.14, 125.53, 114.43, 110.10, 77.46, 69.10, 48.49, 33.58, 29.32, 26.08, 16.63, 8.42 ppm; HRMS (ESI) Calc. for $C_{24}H_{34}O_3Na$ [M+Na]$^+$: 393.2400, found 393.2392.

5.40. Ethyl 2-(4-(3-(4-(2-hydroxy-3,3-dimethylbutoxy)-3-methylphenyl)pentan-3-yl)phenoxy)acetate (32a)

Synthesized from **31** according to procedure similar to that for **9b** (alkylation with methyl bromoacetate) in 85% yield as a colourless oil: R_f 0.60 (20:80 EtOac/hex); IR (thin film) ν 3532, 2963, 1760, 1506 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.07 (d, J = 8.7 Hz, 2H), 6.94 (dd, J = 8.5, 2.0 Hz, 1H), 6.88 (s, 1H), 6.78 (d, J = 8.6 Hz, 2H), 6.69 (d, J = 8.5 Hz, 1H), 4.58 (s, 2H), 4.26 (q, J = 6.8 Hz, 2H), 4.08 (dd, J = 9.2, 2.5 Hz, 1H), 3.85 (t, J = 8.9 Hz, 1H), 3.70 (dd, J = 8.7, 2.4 Hz, 1H), 2.16 (s, 3H), 2.02 (q, J = 7.3 Hz, 4H), 1.28 (t, J = 7.1 Hz, 3H), 1.00 (s, 9H), 0.59 (t, J = 7.3 Hz, 6H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$) δ 169.13, 155.43, 154.34, 142.20, 140.92, 130.65, 129.05, 126.13, 125.53, 113.69, 110.05, 77.21, 69.16, 68.18, 65.49, 61.26, 48.54, 33.44, 29.29, 26.15, 16.60, 14.16, 8.39 ppm; HRMS (ESI) Calc. for $C_{28}H_{40}NaO_5$ [M+Na]$^+$: 479.2768, found 479.2760.

5.41. Methyl 5-(4-(3-(4-(2-hydroxy-3,3-dimethylbutoxy)-3-methylphenyl)pentan-3-yl)phenoxy)pentanoate (32b)

Synthesized from **31** according to procedure similar to that for **9b** (alkylating reagent 1.5 equiv of methyl 5-bromovalerate)in 86% yield as a colourless oil: R_f 0.63 (20:80 EtOac/Hex); IR (thin film) ν 3528, 2959, 2875, 1736, 1608, 1508 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.05 (d, J = 8.8 Hz, 2H), 6.95 (dd, J = 8.5, 2.4 Hz, 1H), 6.90 (d, J = 2.1 Hz, 1H), 6.76 (d, J = 8.8 Hz, 2H), 6.69 (d, J = 8.5 Hz, 1H), 4.08 (dd, J = 9.2, 2.6 Hz, 1H), 3.94 (t, J = 5.7 Hz, 2H), 3.85 (t, J = 8.9 Hz, 1H), 3.70 (dd, J = 8.7, 2.6 Hz, 1H), 3.67 (s, 3H), 2.39 (t, J = 7.0 Hz, 2H), 2.17 (s, 3H), 2.03 (q, J = 7.3 Hz, 4H), 1.97–1.73 (m, 4H), 1.01 (s, 9H), 0.60 (t, J = 7.3 Hz, 6H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$) δ 173.94, 156.47, 154.29, 141.14, 140.99, 130.66, 128.95, 126.14, 125.49, 113.38, 110.03, 77.27, 69.14, 67.11, 51.55, 48.48, 33.71, 33.55, 29.31, 28.75, 26.06, 21.69, 16.62, 8.42 ppm; HRMS (ESI) Calc. for $C_{30}H_{44}O_5Na$ $[M+Na]^+$: 507.3081, found 507.3099.

5.42. Methyl 6-(4-(3-(4-(2-hydroxy-3,3-dimethylbutoxy)-3-methylphenyl)pentan-3-yl)phenoxy)hexanoate (32c)

Synthesized from **31** according to procedure similar to that for **9b** (alkylating reagent 1.5 equiv of methyl 6-bromohexanoate) in 79% yield as a colourless oil: R_f 0.65 (20:80 EtOac/Hex); IR (thin film) ν 3514, 2952, 2874, 1738, 1609, 1508, 1183 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.05 (d, J = 8.8 Hz, 2H), 6.95 (d, J = 8.4 Hz, 1H), 6.90 (s, 1H), 6.76 (d, J = 8.7 Hz, 2H), 6.69 (d, J = 8.5 Hz, 1H), 4.08 (dd, J = 9.1, 2.5 Hz, 1H), 3.92 (t, J = 6.4 Hz, 2H), 3.85 (t, J = 8.9 Hz, 1H), 3.70 (dd, J = 8.8, 2.6 Hz, 1H), 3.66 (s, 3H), 2.34 (t, J = 7.4 Hz, 2H), 2.16 (s, 3H), 2.02 (q, J = 7.2 Hz, 4H), 1.89–1.62 (m, 4H), 1.61–1.38 (m, 2H), 1.00 (s, 9H), 0.59 (t, J = 7.3 Hz, 6H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$) δ 174.11, 156.55, 154.28, 141.14, 140.92, 130.66, 128.94, 126.14, 125.48, 113.38, 110.02, 77.26, 69.13, 67.39, 51.52, 48.47, 33.99, 33.55, 29.32, 29.04, 26.06, 25.72, 24.71, 16.63, 8.42 ppm; HRMS (ESI) Calc. for $C_{31}H_{46}O_5Na$ $[M+Na]^+$: 521.3238, found 521.3230.

5.43. *N*-Hydroxy-2-(4-(3-(4-(2-hydroxy-3,3-dimethylbutoxy)-3-methylphenyl)pentan-3-yl)phenoxy)acetamide (33a)

Synthesized from **32a** according to procedure similar to that for **10b** in 50% yield as a colourless film; IR (thin film) ν 3229,2963, 2876, 1674, 1607, 1505, 1242 cm^{-1}; ^1H NMR (500 MHz, CD_3OD) δ 7.08 (d, J = 8.9 Hz, 2H), 6.95 (d, J = 8.5 Hz, 1H), 6.88–6.80 (m, 3H), 6.75 (d, J = 8.6 Hz, 1H), 4.50 (s, 2H), 4.10 (dd, J = 10.0, 2.9 Hz, 1H), 3.85 (dd, J = 10.0, 7.8 Hz, 1H), 3.61 (dd, J = 7.7, 2.9 Hz, 1H), 2.15 (s, 3H), 2.05 (q, J = 7.3 Hz, 4H), 0.99 (s, 9H), 0.58 (t, J = 7.3 Hz, 6H) ppm; ^{13}C NMR (125 MHz, CD_3OD) δ 166.56, 155.43, 154.91, 142.29, 140.20, 130.10, 128.79, 125.73, 125.47, 113.42, 109.81, 77.22, 69.48, 66.00, 48.21, 33.67, 28.83, 25.19, 15.41, 7.33 ppm; HRMS (ESI) Calc. for $C_{27}H_{34}O_5N$ $[M-H]^-$: 442.2612, found: 442.2601; LRMS (ESI) m/z 444.4 $[M+H]^+$. Purity >95% LC/MS. t_r = 12.99 min (method A).

5.44. *N*-Hydroxy-5-(4-(3-(4-(2-hydroxy-3,3-dimethylbutoxy)-3-methylphenyl)pentan-3-yl)phenoxy)pentanamide (33b)

Synthesized from **32b** according to procedure similar to that for **10b** in 41% yield as a colourless film; IR (thin film) ν 3226, 2961, 2873, 1655, 1609, 1508, 1245 cm^{-1}; ^1H NMR (500 MHz, CD_3OD) δ 7.04 (d, J = 8.6 Hz, 2H), 6.96 (d, J = 6.2 Hz, 1H), 6.84 (s, 1H), 6.76 (m, 3H), 4.10 (dd, J = 10.0, 2.9 Hz, 1H), 3.94 (t, J = 6.3 Hz, 2H), 3.86 (dd, J = 10.0, 7.8 Hz, 1H), 3.61 (dd, J = 7.8, 2.9 Hz, 1H), 2.15 (m, 5H), 2.04 (q, J = 7.3 Hz, 4H), 1.78 (d, J = 7.3 Hz, 4H), 0.99 (s, 9H), 0.58 (t, J = 7.3 Hz, 6H) ppm; ^{13}C NMR (125 MHz, CD_3OD) δ 171.59, 156.66, 154.85, 140.88, 140.43, 130.13, 128.60, 125.76, 125.40, 113.10, 109.77, 77.23, 69.47, 66.92, 48.12, 33.66, 32.02, 28.87, 28.42, 25.17, 22.09, 15.38, 7.33 ppm; HRMS (ESI) Calc. for $C_{29}H_{42}O_5N$ $[M-H]^-$: 484.3068, found: 484.3080; LRMS (ESI) m/z 486.5 $[M+H]^+$. Purity >95% LC/MS, t_r = 11.16 min (method B).

5.45. *N*-Hydroxy-6-(4-(3-(4-(2-hydroxy-3,3-dimethylbutoxy)-3-methylphenyl)pentan-3-yl)phenoxy)hexanamide (33c)

Synthesized from **32c** according to procedure similar to that for **10b** in 87% yield as a colourless film; IR (thin film) ν 3222, 2962, 2869, 1651, 1609,1508, 1247 cm^{-1}; ^1H NMR (400 MHz, CD_3OD) δ 7.02 (d, J = 8.8 Hz, 2H), 6.95 (d, J = 8.7 Hz, 1H), 6.84 (s, 1H), 6.74 (m, 3H), 4.09 (dd, J = 10.0, 2.9 Hz, 1H), 3.91 (t, J = 6.3 Hz, 2H), 3.85 (dd, J = 9.9, 7.8 Hz, 1H), 3.61 (dd, J = 7.7, 2.9 Hz, 1H), 2.14 (s, 3H), 2.11 (t, J = 7.4 Hz, 2H), 2.03 (q, J = 7.3 Hz, 4H), 1.83 – 1.61 (m, 4H), 1.50 (m, 2H), 0.99 (s, 9H), 0.58 (t, J = 7.3 Hz, 6H) ppm; ^{13}C NMR (125 MHz, CD_3OD) δ 171.46, 156.70, 154.83, 140.81, 140.44, 130.13, 128.60, 125.76, 125.38, 113.11, 109.76, 77.22, 69.45, 67.19, 48.12, 33.67, 32.31, 28.88, 28.71, 25.32, 25.21, 25.13, 15.44, 7.39 ppm; HRMS (ESI) Calc. for $C_{30}H_{44}O_5N$ $[M-H]^-$: 498.3225, found: 498.3234; LRMS (ESI) m/z 500.5 $[M+H]^+$. Purity >95% LC/MS, t_r = 11.47 min (method B).

5.46. 1-(4-(3-(4-Hydroxyphenyl)pentan-3-yl)-2-methylphenoxy)-3,3-dimethylbutan-2-one (34)

Synthesized from **29** according to procedure similar to that for **18a** in 94% yield as a yellow oil: R_f 0.35 (20:80 EtOAc/hex); IR (thin film) ν 3413, 2967, 2935, 2876, 1717, 1611, 1502, 1236, 1179 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.00 (d, J = 8.7 Hz, 2H), 6.93 – 6.87 (m, 2H), 6.70 (d, J = 8.7 Hz, 2H), 6.49 (d, J = 8.4 Hz, 1H), 5.32 (bs, 1H),4.84 (s, 2H), 2.22 (s, 3H), 2.00 (q, J = 7.3 Hz, 4H), 1.26 (s, 8H), 0.59 (t, J = 7.3 Hz, 6H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$) δ 210.52, 153.92, 153.18, 141.52, 140.96, 130.80, 129.15, 126.04, 126.01, 114.40, 110.14, 69.55, 48.49, 43.25, 29.33, 26.35, 16.65, 8.42 ppm. HRMS (ESI) Calc. for $C_{24}H_{32}NaO_3$ $[M+Na]^+$: 391.2244, found 391.2231.

5.47. 4-(3-(4-(3,3-Dimethyl-2-oxobutoxy)-3-methylphenyl)pentan-3-yl)phenyl trifluoromethanesulfonate (35)

34 (1.32 g, 3.58 mmol, 1.0 equiv) was dissolved in dichloromethane (30 mL) and cooled to 0 °C. Triethylamine (1.0 mL, 7.16 mmol, 2.0 equiv) and DMAP (218.0 mg, 1.79 mmol, 50 mol%) was added followed by *N*-phenyl-bis(trifluoromethanesulfonimide) (1.41 g, 3.94 mmol, 1.1 equiv) and reaction was stirred for 2 h at 0 °C and then gradually warmed to ambient temperature during 5 h. 1 M HCl solution (20 mL) was added and resulting mixture was extracted with dichloromethane (3 × 20 mL) combined organic layer were washed with saturate NaCl (5 mL), dried over anhydrous sodium sulfate, crude product was purified by chromatography on silica gel eluating 10% ethyl acetate/hexanes to afford 1.38 g colourless oil (77%). R_f 0.80 (20:80 EtOAc/Hex); IR (thin film) ν 3202, 2971, 2880, 1717, 1599, 1204 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.23 (d, J = 9.0 Hz, 2H), 7.13 (d, J = 8.9 Hz, 2H), 6.91 – 6.83 (m, 2H), 6.50 (d, J = 8.2 Hz, 1H), 4.85 (s, 2H), 2.24 (s, 3H), 2.05 (q, J = 7.3 Hz, 4H), 1.26 (s, 9H), 0.59 (t, J = 7.3 Hz, 6H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$) δ 210.01, 154.38, 149.76, 147.35, 140.16, 130.68, 129.97, 126.45, 126.14, 120.33, 110.33, 69.47, 49.18, 43.27, 29.36, 26.54, 26.32, 16.62, 8.31 ppm. HRMS (ESI) Calc. for $C_{25}H_{31}F_3NaO_5S$ $[M+Na]^+$: 523.1737, found 523.1721.

5.48. Methyl 4-(3-(4-(3,3-dimethyl-2-oxobutoxy)-3-methylphenyl)pentan-3-yl)benzoate (36)

35 (1.25 g, 2.50 mmol, 1.0 equiv) was dissolved in methanol (10 mL) and DMSO (5 mL) was added. $Pd(OAc)_2$ (56.1 mg, 0.25 mmol, 10 mol %) was added followed by 1,3-bis-(diphenylphosphino)propane (103.0 mg, 10 mol %), and reaction stirred for 5 minutes at ambient temperature. Triethylamine (0.87 mL, 6.24 mmol, 2.5 equiv) was added and reaction was heated at 80 °C under CO atmosphere for 24 h. 1 M HCl solution (10 mL) was added and resulting mixture was extracted with dichloromethane (3 × 10 mL) combined organic layer were washed with saturate NaCl (5 mL), dried over anhydrous sodium sulfate, crude product was purified by chromatography on silica gel eluating 10% ethyl acetate/hexanes to afford 0.85 g transparent oil (83%). R_f 0.67 (20:80 EtOAc/hex); IR (thin film) ν 2968, 2879, 1722, 1608, 1501, 1281, 1143 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.90 (d, J = 8.4 Hz, 2H), 7.24 (d, J = 8.4 Hz, 2H), 6.94 – 6.76 (m, 2H), 6.49 (d, J = 9.2 Hz, 1H), 4.83 (s, 2H), 3.89 (s, 3H), 2.21 (s, 3H), 2.07 (q, J = 7.2 Hz, 4H), 1.25 (s, 9H), 0.59 (t, J = 7.3 Hz, 6H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$) δ 210.31, 167.37, 154.74, 154.20, 140.41, 130.73, 129.56, 128.96, 128.16, 127.32, 126.29, 126.02, 123.47, 110.27, 69.50, 52.02, 49.53, 43.24, 29.08, 26.34, 16.62, 8.32 ppm. HRMS (ESI) Calc. for $C_{26}H_{34}NaO_4$ $[M+Na]^+$: 433.2349, found 433.2355.

5.49. Methyl 4-(3-(4-(2-hydroxy-3,3-dimethylbutoxy)-3-methylphenyl)-pentan-3-yl)benzoate (37)

Synthesized from **36** according to procedure similar to that for **11** (with 1.2 equiv $NaBH_4$) in 96% yield as a colourless oil: R_f 0.65 (20:80 EtOAc/Hex); IR (thin film) ν 3523, 2962, 2877, 1721, 1609, 1505, 1115 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 7.91 (d, J = 8.7 Hz, 2H), 7.24 (d, J = 8.7 Hz, 2H), 6.93 (d, J = 8.5 Hz, 1H), 6.86 (s, 1H), 6.71 (d, J = 8.5 Hz, 1H), 4.09 (dd, J = 9.2, 2.7 Hz, 1H), 3.89 (s, 3H), 3.85 (t, J = 9.0 Hz, 1H), 3.70 (dd, J = 9.2, 2.7 Hz, 1H), 2.46 (d, J = 2.9 Hz, 1H), 2.16 (s, 3H), 2.09 (q, J = 7.4 Hz, 4H), 1.01 (s, 9H), 0.60 (t, J = 7.3 Hz, 6H) ppm; ^{13}C NMR (125 MHz, $CDCl_3$) δ 167.20, 154.71, 154.58, 140.05, 130.59, 128.96, 128.11, 127.24, 126.12, 125.81, 110.20, 77.26, 69.22, 51.94, 49.52, 33.57, 29.08, 26.05, 16.60, 8.31 ppm. HRMS (ESI) Calc. for $C_{26}H_{37}O_4$ $[M+H]^+$: 413.2686, found 413.2670. HRMS (ESI) Calc. for $C_{26}H_{36}NaO_4$ $[M+Na]^+$: 435.2506, found 435.2511.

5.50. 4-(3-(4-(2-Hydroxy-3,3-dimethylbutoxy)-3-methylphenyl)-pentan-3-yl)benzoic acid (38)

Synthesized from **37** according to procedure similar to that for **24** in 93% yield as a colourless oil. R_f 0.29 (40:60 EtOAc/hex); IR (thin film) ν 3413, 2964, 2877, 2667, 2552, 1682, 1505, 1245, cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.99 (d, J = 8.3 Hz, 1H), 7.29 (d, J = 8.4 Hz, 1H), 6.94 (d, J = 8.5 Hz, 1H), 6.87 (s, 1H), 6.72 (d, J = 8.5 Hz, 1H), 4.10 (dd, J = 9.1, 2.3 Hz, 1H), 3.87 (t, J = 8.9 Hz, 1H), 3.72 (dd, J = 8.6, 2.3 Hz, 1H), 2.17 (s, 1H), 2.11 (q, J = 7.1 Hz, 2H), 1.02 (s, 4H), 0.62 (t, J = 7.1 Hz, 3H) ppm; ^{13}C NMR (75 MHz, $cdcl_3$) δ 172.12, 155.78, 154.61, 139.96, 130.57, 129.63, 128.23, 126.44, 126.13, 125.88, 110.25, 77.32, 69.18, 49.62, 33.58, 29.06, 26.07, 16.63, 8.33 ppm. HRMS (ESI) Calc. for $C_{25}H_{34}NaO_4$ $[M+Na]^+$: 421.2349, found 421.2357.

5.51. Methyl 6-(4-(3-(4-(2-hydroxy-3,3-dimethylbutoxy)-3-methylphenyl)pentan-3-yl)benzamido)hexanoate (39)

Synthesized from **38** according to procedure similar to that for **13a** (with methyl-7-aminohexanoate hydrochloride, 1.5 equiv) in 58% yield as a colourless oil: R_f 0.30 (20:80 EtOac/Hex); IR (thin film) ν 3336, 2938, 2875, 1737, 1637, 1500, 1245 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 7.63 (d, J = 8.4 Hz, 2H), 7.22 (d, J = 8.4 Hz, 2H), 6.93 (d, J = 8.3 Hz, 1H), 6.84 (s, 1H), 6.69 (d, J = 8.3 Hz, 1H), 6.12 (t, J = 5.7 Hz, 1H), 4.08 (dd, J = 10.0, 3.0 Hz, 1H), 3.85 (t, J = 9.1 Hz, 1H), 3.70 (dd, J = 9.1 and 3.0 Hz, 1H), 3,66 (s, 3H), 3.45 (q, J = 6.6 Hz, 2H), 2.33 (t, J = 6.6 Hz, 2H), 2.15 (s, 3H), 2.08 (q, J = 7.9 Hz, 4H), 1.72–1.52 (m, 4H), 1.46–1.35 (m, 2H), 1.00 (s, 9H), 0.60 (t, J = 7.9 Hz, 6H) ppm; ^{13}C NMR (125 MHz, $CDCl_3$) δ 174.07, 167.57, 154.54, 152.91, 140.17, 131.76, 130.62, 128.26, 126.21, 126.11, 125.77, 110.16, 77.20, 69.20, 51.53, 49.35, 39.65, 33.84, 33.56, 29.33, 29.11, 26.38, 26.05, 24.44, 16.61, 8.32 ppm. HRMS (ESI) Calc. for $C_{32}H_{47}NNaO_5$ $[M+Na]^+$: 548.3346, found 548.3344.

5.52. 4-(3-(4-(2-Hydroxy-3,3-dimethylbutoxy)-3-methylphenyl)-pentan-3-yl)-N-(6-(hydroxyamino)-6-oxohexyl)benzamide (40)

Synthesized from **39** according to procedure similar to that for **10b** in 52% yield as a colourless film; IR (thin film) ν 3294, 2960, 2865, 1633, 1500, 1275, 1259 cm^{-1}; ^1H NMR (300 MHz, CD_3OD) δ 7.68 (d, J = 8.4 Hz, 2H), 7.25 (d, J = 8.3 Hz, 2H), 6.97 (d, J = 8.3 Hz, 1H), 6.84 (s, 1H), 6.78 (d, J=8.3 Hz, 1H), 4.11 (dd, J = 10.0, 3.0 Hz, 1H), 3.86 (dd, J = 9.5 and 7.5 Hz, 1H), 3.61 (dd, J = 7.5 and 2.8 Hz, 1H), 3.36 (t, J = 7.5 Hz, 1H), 2,17–2.06 (m, 9H), 1.71–1.57 (m, 4H), 1.44–1.34 (m, 2H), 0.99 (s, 9H), 0.61 (t, J = 7.1 Hz, 6H) ppm; ^{13}C NMR (75 MHz, CD_3OD) δ 171.57, 155.10, 153.14, 139.50, 131.39, 130.06, 127.87, 126.16, 125.76, 125.70, 109.89, 77.20, 69.47, 49.01, 39.30, 33.66, 32.24, 28.75, 28.57, 26.06, 25.15, 25.02, 15.34, 7.24 ppm; HRMS (ESI) Calc. for $C_{31}H_{45}O_5N_2$ $[M-H]^-$: 525.3334, found: 525.3340; LRMS (ESI) m/z 527.5 $[M+H]^+$. Purity >95% (LC/MS), t_r = 12.24 min (method A).

6. Biochemical analysis of hybrid molecules

6.1. Fluorogenic HDAC inhibition assay

Purified HDAC3 and HDAC6 were purchased from Cayman Chemicals. Boc-Lys(Ac)-7-amino-4-methylcoumarin (BocLys(Ac)-AMC) was used as substrate for the HDAC assays. Substrate solution was prepared as follow: Boc(Lys-Ac)-AMC was dissolved in DMSO and diluted with HDAC buffer (15 mM tris–HCl [pH 8.1], 250 μM EDTA, 250 mM NaCl, 10% glycerol) to give 1 mM solutions containing 1.7% DMSO. Trypsin was used to stop the reaction, releasing free AMC. The trypsin solution was prepared as follow: trypsin was dissolved in HDAC buffer to give a concentration of 10 mg/mL. Release of AMC was monitored by measuring the fluorescence at 460 nm (lex = 390 nm) with a microplate reader (SpectraMax Gemini) at 37 °C. The AMC signals were recorded against a blank with buffer, substrate and trypsin but without the enzyme. All experiments were carried out at least in triplicate.

For HDAC inhibition assays, inhibitor diluted in 50 μL of HDAC buffer was mixed with 10 μL of diluted enzyme solution in HDAC buffer at room temperature. The HDAC reaction was started by adding 40 μL of substrate solution in HDAC buffer followed by 60 min of incubation with stirring at 37 °C. The reaction was stopped by adding 100 μL of trypsin solution. After a 30 min incubation with stirring at 37 °C, the release of AMC was monitored by measuring the fluorescence.

6.2. Cell culture

Human head and neck squamous carcinoma cell lines SCC4 was purchased from American Type Culture Collection (Manassas, VA) and cultured under recommended conditions. Cells were split at 60–70% confluence. For treatments, cells were split and 24 h later medium was changed to DMEM + 10% charcoal-stripped FBS. 24 h after that media was changed and cells were incubated in DMEM-F12 + 10% charcoal-stripped FBS and 1,25D (Sigma) or hybrid compounds, as indicated in the figures.

6.3. RNA isolation and RT/PCR analysis

PCR primers against *CYP24* (forward, 5′, 5′-ggcaacagttctgggtgaat, reverse, 5′-tatttgcggacaatccaaca) were designed using Primer3 software found at http://frodo.wi.mit.edu/ and have already been described in Ref. 9 RNA extraction was performed with TRIzol/chloroform (Invitrogen) as per manufacturers' instructions. RT was performed with iScript cDNA Synthesis Kit (Bio-Rad) and qPCR was performed with SsoFast Eva Green with low ROX (Bio-Rad) on an Eco qPCR cycler (Illumina), normalizing expression to GAPDH. Each experiment were performed in triplicate and repeated for three times.

6.4. EdU cell growth assay

Click-iT EdU Alexa Fluor high-throughput imaging (HCS) assay for cell proliferation. HCS assays were performed on SCC4 and SCC25 squamous carcinoma cell line (ATCC) following the manufacturer's instruction (Molecular Probes, Invitrogen). Images were analyzed for Hoechst 33342 (350/460 nm) and Alexa Fluro 647 (620/700 nm) using Image Xpress Micro (Molecular Devices, CA, USA). All samples were in triplicate.

6.5. Cell death (LDH) assay

Cytotoxicity Detection kit was purchased from Roche Diagnostics, Canada, and the assays were performed following the manufacturer's instruction. Quantitation of absorption at 500 nm was determined on a Tecan microplate reader Infinite m1000.

6.6. HDAC cell-based activity assay

The kit was purchased from Cayman Chemicals, USA. and the assays were performed following the manufacturer's instructions. Fluorescent intensity was read (excitation/emission 360/460) using a Tecan microplate reader Infinite m1000.

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Supplementary data

Supplementary data (^{1}H NMR data for all new compounds. 60-Cell line screening data from the National Cancer Institute (US)) associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmc.2015.05.011.

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