Bioorganic & Medicinal Chemistry Letters 25 (2015) 4393–4398

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Synthesis and structure–activity studies of the V-ATPase inhibitor saliphenylhalamide (SaliPhe) and simplified analogs

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ARTICLE INFO

Article history:
Received 30 July 2015
Revised 2 September 2015
Accepted 7 September 2015
Available online 8 September 2015

Keywords:
V-ATPase
Antiviral
Anticancer
Benzolactone
Salicylihalamide

ABSTRACT

An efficient total synthesis of the potent V-ATPase inhibitor saliphenylhalamide (SaliPhe), a synthetic variant of the natural product salicylihalamide A (SaliA), has been accomplished aimed at facilitating the development of SaliPhe as an anticancer and antiviral agent. This new approach enabled facile access to derivatives for structure–activity relationship studies, leading to simplified analogs that maintain SaliPhe's biological properties. These studies will provide a solid foundation for the continued evaluation of SaliPhe and analogs as potential anticancer and antiviral agents.

Vacuolar H^+-ATPases are emerging as important cellular targets for the identification of potential novel therapeutics. This class of enzymes plays a key role in controlling the acidity of intracellular compartments and the extracellular environment, and its role has been implicated in various diseases including cancer and antiviral infections.[1] The pharmacological modulation of the activity of these enzymes has contributed to a better understanding of their cellular function and has led to the discovery and identification of a wide variety of structurally diverse inhibitors.[2] Among them, the benzolactone enamide family emerged due to its potency and selectivity towards the mammalian V-ATPases.[3] A prominent example of this family is the macrolactone salicylihalamide A (SaliA, **1**, Fig. 1). SaliA was isolated in 1997 from an unidentified species of the marine sponge *Haliclona* sp. and showed a unique and selective cytotoxicity profile against the NCI 60 cell line human tumor panel.[4,5] This activity was subsequently ascribed to the ability of SaliA to inhibit the V-ATPase (IC_{50} <1 nM).[6,7] The promising biological properties of SaliA encouraged our research group and others to develop synthetic approaches to access SaliA and more potent derivatives to further evaluate the therapeutic potential of this natural product.[8–10] In this context, saliphenylhalamide (SaliPhe, **2**, Fig. 1)[10c] was selected from an extensive structure–activity study published by our group due to its potent inhibitory activity, selectivity, stability and synthetic access.[7,8e,10b,c]

SaliPhe has not only been useful for the study of V-ATPase function and its role in a variety of normal and pathogenic cellular processes,[7,10c,11] but also enabled in vivo modulation of V-ATPase activity as a means to inhibit the growth of human lung cancer xenografts with a $KRAS^{mut}$/$LKB1^{mut}$ oncogenotype (present in ~6% of human adenocarcinomas) in mice,[12] to attenuate wear particle-induced osteolysis in a mouse calvarial model (for the potential treatment of peri-implant osteolysis),[13] or to protect mice against a lethal challenge of a mouse-adapted influenza strain.[14] In the context of the continued promise for pharmacological V-ATPase modulation as a potential means to treat a wide host of diseases, we sought to develop a more efficient synthesis of SaliPhe that also would enable access to additional analogs that were not explored previously. The results of these studies and in vitro biological activities of new SaliPhe analogs are reported herein.

Although we already formulated a synthetic approach that supported multi-gram access to SaliPhe,[10c] one of the drawbacks was the rather lengthy 8-step sequence required to install the enamide side-chain after formation of the macrolactone was complete. As shown in Scheme 1, after ring-closing metathesis of precursor **III**, 4 steps were required to transform the primary protected alcohol into the homologated unsaturated ester **II**. An additional 4-step sequence, involving a key Curtius rearrangement/isocyanate-trapping, completed the synthesis of SaliPhe. We reasoned that a Buchwald-type Cu-catalyzed amidation of a vinyl iodide

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http://dx.doi.org/10.1016/j.bmcl.2015.09.021
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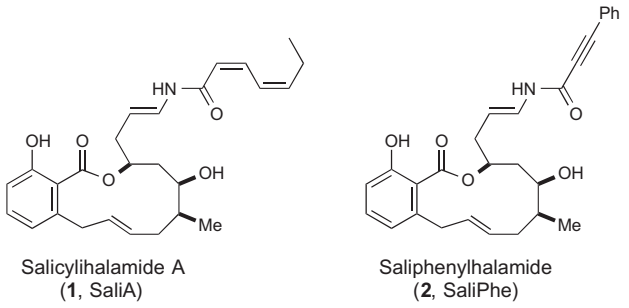


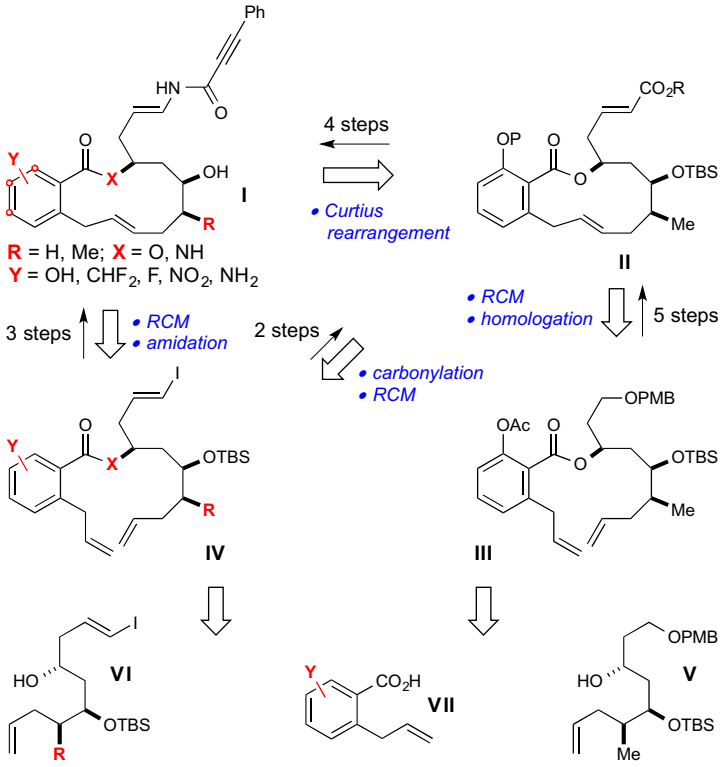
Figure 1. Structures of salicylihalamide A (**1**, SaliA) and saliphenylhalamide (**2**, SaliPhe).

macrocycle precursor such as **IV** with 3-phenylpropiolamide, followed by ring-closing metathesis, would significantly streamline the side-chain installation to a 3-step sequence (including final deprotection). Alternatively, a Pd-catalyzed carbonylation would provide a 2-step access to unsaturated ester precursor **II** instead of our previous 5-step sequence. Moreover, having the vinyl iodide functionality installed prior to esterification with a benzoic acid coupling partner **VII** (cf. alcohol **VI**) will increase convergency and facilitate analog development. The functional groups of SaliPhe selected for modification are indicated in red in Scheme 1. We did not modify the N-acyl enamide side-chain of SaliPhe because we had previously shown that it keeps the potency while being chemically more stable and easier to synthesize than the homologous side chain of SaliA. We hypothesized that the methyl group at the C6 position could be removed (R = H) without affecting the activity and aimed to explore the substituents at the phenol ring by introducing other groups (Y = F, CHF_2, NO_2, NH_2) at the *ortho*-, *meta*-, and *para*-positions (in relation to the benzoate position), and to replace the lactone by a chemically more robust lactam (X = NH).

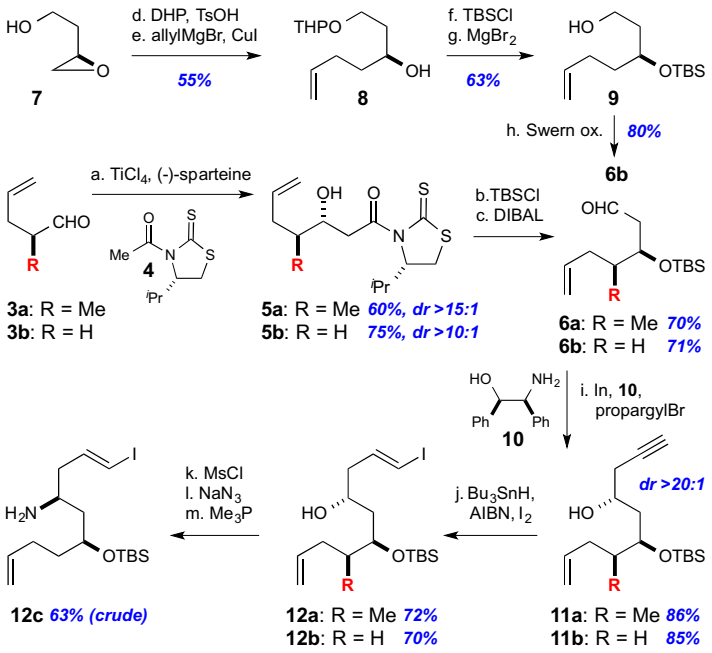
The synthesis of the alcohol fragments **VI** for SaliPhe and analogs is depicted in Scheme 2 below. Because our short and scalable synthesis of the related fragment **V** (Scheme 1) was nevertheless not fully stereoselective, we designed an improved, highly stereoselective approach that initiated with our previously synthesized aldehyde **3a** and the corresponding aldehyde **3b** lacking the C6-methyl group.[10c,15] In the event, aldol addition to **3a**,**b** followed the protocol described by Crimmins et al. using the *Z* (*O*)-titanium enolate of thiazolidinethione **4** generated with (−)-sparteine as a base.[16] The aldol products **5a**,**b** were obtained in 60–75% yield and high selectivity (dr >10–15:1). Subsequent silylation and reductive (DIBAL) cleavage of the auxiliary rendered aldehydes **6a**,**b** in ~70% yield. An alternative route to **6b** began from known epoxide **7** (readily available in three steps from inexpensive L-aspartic acid).[17] After protecting the primary alcohol of **7** as a tetrahydropyranyl ether, the epoxide was opened with allylmagnesium bromide (cat. CuI) to yield homoallylic alcohol **8** (55%, two steps). Silylation, followed by removal of the ether protecting group and oxidation afforded aldehyde **6b**.

An asymmetric Barbier-type propargylation of aldehydes **6a**,**b** was performed using indium powder, pyridine and (1*R*,2*S*)-2-amino-1,2-diphenylethanol **10** as a chiral source to provide the corresponding homopropargylic alcohols **11a**,**b** as single stereoisomers (>20:1 dr).[18–20] A subsequent radical-mediated hydrostannylation (AIBN, Bu_3SnH) was followed by a transmetallation with iodine to afford vinyl iodides **12a**,**b** in 70–72% yield. To enable the synthesis of a SaliPhe lactam analog, alcohol **12b** was treated with mesyl chloride followed by SN_2-inversion with sodium azide (63%, two steps). The corresponding azide could be reduced to the primary amine **12c** with trimethyl phosphine, but this was best done immediately prior to coupling with the benzoic acid partner (vide infra).[21]

The synthesis of SaliPhe (**2**) and the corresponding *des*-Me analog **17** via late stage Cu-mediated C–N bond formation is depicted in Scheme 3. The above prepared alcohols **12a**,**b** were esterified



Scheme 1. Retrosynthetic analysis.



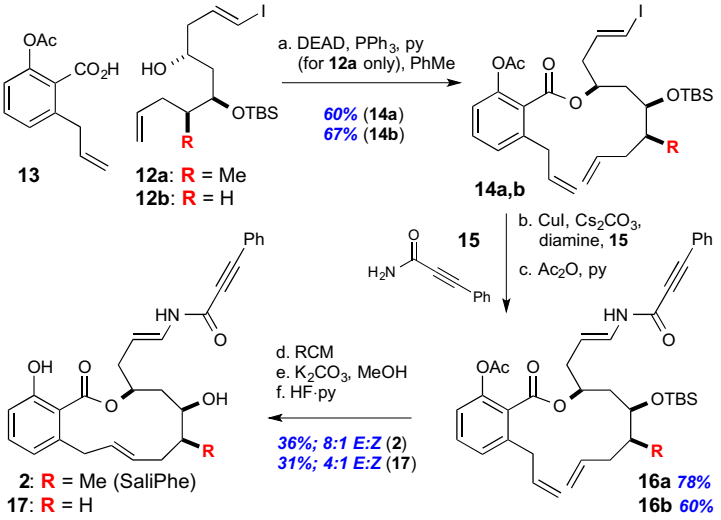
Scheme 2. Reagents and conditions: (a) $TiCl_4$ (6.0 equiv), (−)-sparteine (3.0 equiv), **4** (3.0 equiv), CH_2Cl_2, −78 °C, 30 min, (**5a**, 60%; **5b**, 75%.); (b) TBSOTf (1.2 equiv), 2,6-lutidine (1.5 equiv), CH_2Cl_2, 23 °C, 2 h; (c) DIBAL-H (1.8 equiv), toluene, −78 °C, 3 h, (**6a**, 70%; **6b**, 71%; two steps); (d) *p*-TsOH (0.05 equiv), dihydropyran (DHP, 2.0 equiv), THF, 23 °C, 4 h, 88%; (e) allylmagnesium bromide (3.0 equiv), CuI (0.1 equiv), THF, −20 °C, 1 h, 62%; (f) imidazole (5.0 equiv), TBSCl (1.2 equiv), DMF, 23 °C, 12 h, 87%; (g) $MgBr_2{\cdot}Et_2O$ (3.0 equiv), Et_2O, 23 °C, 2 h, 72%; (h) DMSO (2.0 equiv), oxalyl chloride (1.5 equiv), Et_3N (5.0 equiv), CH_2Cl_2, −78 °C, 1 h, 80%; (i) propargyl bromide (4.5 equiv), **10** (3.0 equiv), In (3.0 equiv), pyridine (3.0 equiv), THF, −78 °C to 23 °C, 10 h, (**11a**, 86%; **11b**, 85%); (j) Bu_3SnH (1.2 equiv), AIBN (0.5 equiv) toluene, 110 °C, 2 h; then I_2 (1.0 equiv), THF, −78 °C, 30 min (**12a**, 72%; **12b**, 70%); (k) MsCl (3.5 equiv), Et_3N (3.5 equiv), Et_2O, 23 °C, 1 h; (l) NaN_3 (3.0 equiv), DMF, 60 °C, 2 h, (63%, two steps); (m) Me_3P (3.0 equiv), 9:1 THF/H_2O, 60 °C, 1 h, then MW, 100 °C, 30 min (used crude).

with substituted salicylic acid **13**[22] under Mitsunobu conditions (DEAD/PPh_3).[10c] For the esterification of **12a**, a stoichiometric amount of pyridine was required to prevent competing elimination of the alcohol under the reaction conditions.[23] The *N*-acyl enamide side chain was installed by Cu-catalyzed amidation of **14a**,**b** with amide **15** under the conditions described by Buchwald et al., providing enamides **16a**,**b** in 60–78% yield after re-installation of the acetate group.[24,10f] The ring-closing metathesis of **16a**,**b** with Grubbs I catalyst provided the benzolactones **2** and **17** in 31–36% yields after global deprotection.[25,26] The *E*/*Z* isomers could be separated by flash chromatography after removing the acetate group. Of note is that the *des*-Me RCM-precursor **16b** cyclized with significant lower *E*/*Z* selectivity (4:1) compared to the parent **16a** (8:1), indicating a beneficial role for the Me-substituent in pre-organizing the substrate for an *E*-selective metathesis.[27] This lowered selectivity is observed for all *des*-methyl analogs prepared (vide supra, Chart 1). Final desilylation with buffered HF·pyridine provided SaliPhe **2** and analog **17** in ten steps from known aldehydes **3a**,**b**.

When assayed for V-ATPase inhibitory activity, it was found that *des*-Me analog **17** displayed potency comparable to SaliPhe (IC_{50} = 5 nM, **17**; 1 nM, **2**), indicating only a marginal role for the Me-group on V-ATPase inhibitory activity.[28] Based on these results, we decided to carry out the subsequent structural modifications in the *des*-Me series, and survey the influence of various aromatic substituents on biological activity. We envisioned exploring difluoromethyl, fluoro, amino, nitro and hydroxyl substituents at the *ortho*-, *meta*-, or *para*-position. Analogs **21**–**24** (see Chart 1) were readily obtained using the chemistry outlined in Scheme 3, starting from Mitsunobu reaction of alcohol **12b** with various benzoic acids.[29,30] Interestingly, the RCM-product of an intermediate with a *meta*-OH or final product with a *meta*-fluoro substituent were unstable and could not be further processed or evaluated, whereas the *o*-NO_2 substituted analog **19** (Chart 1) could not be prepared using this route due to failure of the corresponding bis-olefin intermediate to undergo the ring closing metathesis.[29]

As discussed during the retrosynthetic analysis, we also investigated whether we could intercept vinyl iodide intermediates in a Pd-catalyzed carbonylation event to produce α,β-unsaturated ester intermediates reminiscent of those utilized in our first-generation synthetic approach (cf. **IV** → **II**, Scheme 1). As shown in Scheme 4, the palladium-catalyzed carbonylation of vinyl iodides **26a**–**c** (prepared via Mitsunobu reaction of alcohol **12b** with the corresponding benzoic acids)[29] with catalytic $PdCl_2(PPh_3)_2$ (Et_3N, 1 atm of CO, MeOH, rt) proceeded smoothly to yield, after ring-closing metathesis as before, the desired unsaturated esters **27a**–**c** in good overall yields. Of note is that the ring-closing metathesis to *o*-NO_2 substituted benzolactone **27c** occurred with the best yield and *E*:*Z*-selectivity (94%, 5.6:1) of all *des*-Me analogs evaluated in this study, whereas the corresponding ring-closing metathesis with *o*-NO_2 as implemented in the route depicted in Scheme 3 did not occur at al. The remainder of the synthesis (enamide side chain formation) essentially followed our published sequence of ester hydrolysis, acyl azide formation (**28a**–**c**) followed by Curtius rearrangement and trapping of the resulting isocyanate with phenylethynyl lithium, and final deprotection.[8e,10c] In addition to providing alternate access to analog **17**, this route also furnished new analogs **18**–**20** (**20** from reduction of **19** with Fe).

We tested the ability of all new analogs for their ability to inhibit the activity of a purified reconstituted V-ATPase from bovine brain (Chart 1, IC_{50}-values shown in red).[28] Analysis of the data indicated that the *des*-Me analog **17**, and the corresponding lactam **25**[31,32] remain potent V-ATPase inhibitors with IC_{50}'s of 5 and 30 nM, respectively (versus 1 nM for SaliPhe **2**). The modifications around the phenol ring proved to be detrimental for activity. Replacement of the *o*-OH with a difluoromethyl bioisostere resulted in a 100-fold reduction in activity of analog **21** (500 nM) versus the corresponding phenol **17** (5 nM).[33] A similar loss of



Scheme 3. Reagents and conditions: (a) DEAD (2.0 equiv), **13** (1.8 equiv), PPh_3 (2.0 equiv), pyridine (1.0 equiv, for **12a** only), toluene, 0 °C (**14a**, 62%; **14b**, 68%); (b) Cs_2CO_3 (5.0 equiv), CuI (2.0 equiv), **15** (5.0 equiv), $MeHN(CH_2)_2NHMe$ (5.0 equiv), DMF, 23 °C, 2–4 h; (c) Ac_2O (1.1 equiv), pyridine (1.1 equiv), DMAP (0.1 equiv), CH_2Cl_2, 23 °C, 1 h (**16a**, 78%; **16b**, 60%); (d) Grubbs' I (0.6 equiv), CH_2Cl_2, 23 °C, 6 h; (e) K_2CO_3 (10.0 equiv), MeOH, 23 °C, 30 min (a, 45%; b, 40%); (f) HF·py, 23 °C, 2–5 days (**2**, 80%; **17**, 78%).

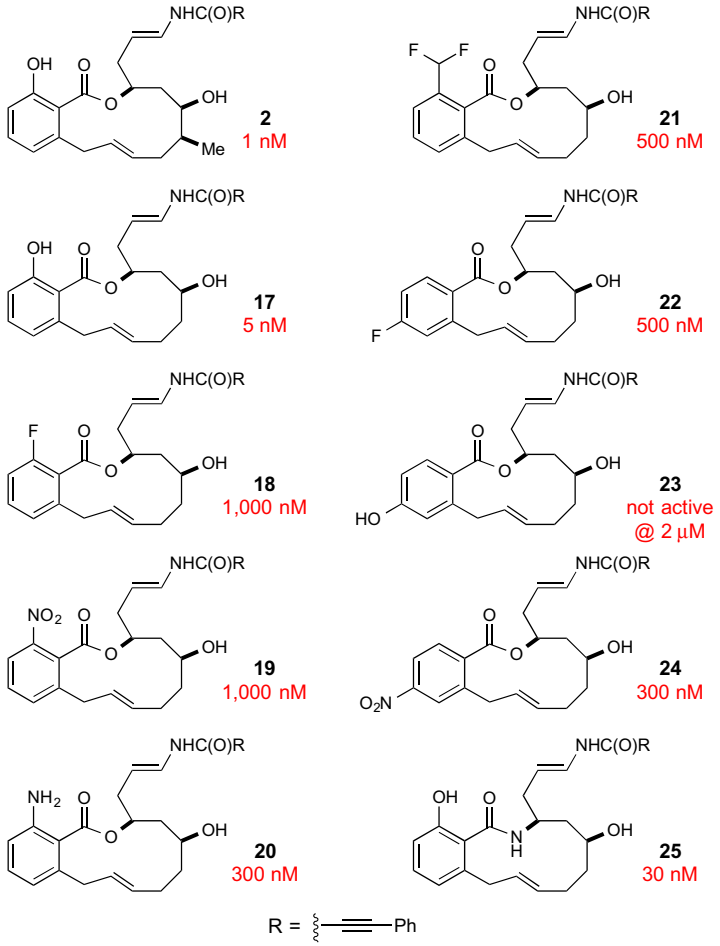
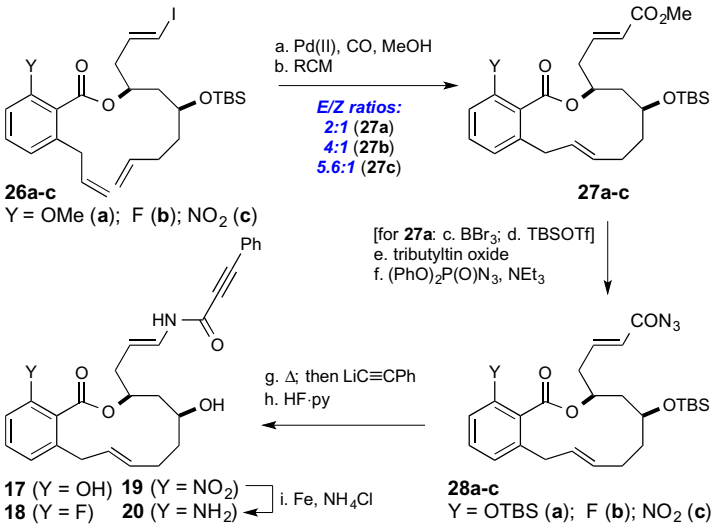


Chart 1. Structure of analogs prepared under this study. The IC_{50}'s for inhibition of V-ATPase activity are shown in red (all compounds were stable to the assay buffer).[28]

activity was noted for the *o*-amino, *p*-fluoro, and *p*-nitro analogs **20**, **22**, and **24** (IC_{50}'s 300–500 nM), and even worse for the *o*-fluoro and *o*-nitro congeners **18** and **19** (1000 nM). Finally, the *p*-phenol regioisomer of potent *des*-Me analog **17**, compound **23**, did not register any inhibitory activity up to 2 μM concentrations. In conjunction with previous SAR-studies,[8b,10a–c] we conclude that the *o*-phenol is critical for activity, whereas the alicyclic and side-chain fragments are more tolerant towards modifications.



Scheme 4. Reagents and conditions: (a) $PdCl_2(PPh_3)_2$ (10 mol %), CO, Et_3N (1.5 equiv), MeOH, 23 °C, 4 h (a, 66%; b, 77%; c, 57%); (b) Grubbs' I (10 mol %), CH_2Cl_2, 23 °C, 3 h (a, crude; b, 81%, c, 94%); (c) BBr_3 (2.0 equiv), CH_2Cl_2, −78 °C, 1 h (a, 40%, two step); (d) TBSOTf (1.2 equiv), 2,6-lutidine (1.5 equiv), CH_2Cl_2, 23 °C, 1 h (a, 98%); (e) tributyltin oxide (4.0 equiv), toluene, 120 °C, 10 h; (f) Et_3N (4.5 equiv), DPPA (4.0 equiv), benzene, 23 °C, 10 h (a, 57% two steps; b, 44% two steps; c, 50% two steps); (g) toluene, 120 °C, 3 h; evaporate solvent; then *n*-BuLi in THF (5.0 equiv), phenyl acetylene (6.0 equiv), THF, −78 °C, 1 h (a, crude; b, 51%; c, 60%); (h) HF·py, 23 °C, 2 days (**17**, 55% three steps; **18**, 75%; **193**, 83%); (i) NH_4Cl (16.0 equiv), Fe (8.0 equiv), EtOH, 23 °C, 4 h, 40%.

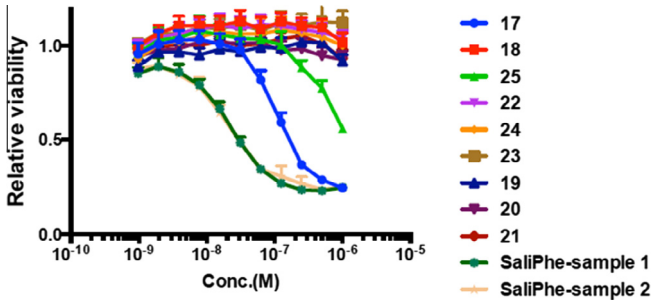


Figure 2. Dose–response curves for inhibition of the HCC-4017 lung cancer cell line with SaliPhe (**2**) and analogs **17**–**25**. Error bars indicate ± SD, $n = 3$.[34]

In previous work,[12] we found that co-occurring mutations in KRAS and LKB1, present in 6% of lung adenocarcinoma patients, are sufficient to drive addiction to the coatomer complex I (COPI)-dependent lysosome acidification, and that this vulnerability could be exploited pharmacologically by inhibition of V-ATPase activity with SaliPhe (**2**) in vitro and in vivo. We therefore evaluated the growth inhibitory effects of the SaliPhe analogs on the human HCC-4017 lung cancer cell line, which has the co-occurring $KRAS^{mut}/LKB1^{mut}$ oncogenotype (Fig. 2).[34] Whereas analog **17** only showed a reduction in growth-inhibitory activity of ~5–10 fold versus SaliPhe, lactam analog **25** was >100-fold less effective, and all other analogs tested did not register any anti-proliferative activity. These results are therefore consistent with the V-ATPase inhibitory activities observed for these compounds.

In conclusion, we have reported a short, convergent and stereoselective synthesis of the potent V-ATPase inhibitor SaliPhe. Two complementary synthetic sequences based on late stage functionalization of a common intermediate have been fully evaluated to produce and diversify SaliPhe. We have discovered potent analogs that simplify the chemical structure of SaliPhe while keeping the potent V-ATPase inhibitory and anticancer properties. Our initial structure–activity studies revealed that the *o*-phenol group is crucial for biological activity.

Acknowledgments

We thank the Robert A. Welch Foundation (I-1422) and CPRIT (RP120718) for financial support. J.K. De Brabander holds the Julie and Louis Beecherl, Jr., Chair in Medical Science; M.A. White holds the Grant A. Dove Chair for Research in Oncology and the Sherry Wigley Crow Cancer Research Endowed Chair in Honor of Robert Lewis Kirby, M.D.

Supplementary data

Supplementary data (experimental procedures and characterization data for new compounds and intermediates, copies of NMR spectra) associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2015.09.021.

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27. For discussions related to *E*/*Z*-selectivity of RCM reactions during similar benzolactone ring formations, see Refs. 8b,e,10b.
28. All analogs were assayed using purified reconstituted V-ATPase from bovine brain, see Ref. 7 for detailed experimental protocol.
29. See Supplementary Material for experimental procedures, characterization data, and copies of NMR spectra for all new analogs and intermediates.
30. *E*/*Z*-isomers en route to analog **23** could not be separated at any given stage of the synthesis, and **23** was assayed as a 1:1 mixture of *E*/*Z*-isomers.
31. The lactam was prepared via amide-formation between acid **13** and amine **12c** (Scheme 2) under peptide coupling conditions, followed by a similar sequence of reactions as depicted in Scheme 4 for the corresponding lactones (carbonylation route).[29]
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