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Isosteric replacement of the *Z*-enone with haloethyl ketone and *E*-enone in a resorcylic acid lactone series and biological evaluation

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ABSTRACT

The synthesis of a small library of resorcylic acid lactones and evaluation of their biological properties as kinase inhibitors is described. Within the series *E*-enones were found more active than corresponding *Z*-enones as inhibitors of a subset of kinases containing a conserved cysteine. Replacement of the enone moiety with a β-haloketone group led to compounds with an interesting kinase selectivity profile and also antiproliferative activity against Jurkat cells. An *E*-enone derivative also showed activity against capillary tube formation based on a co-culture of primary human umbilical cord endothelial cells (HUVECs) and vascular smooth muscle cells (vSMCs).

Protein kinases have become one of the most intensively pursued classes of drug targets. As virtually every signal transduction process is dependant on phosphate transfer, inhibition of kinase activity can elicit a physiological response.[1] Initial concerns raised about kinases as suitable drug targets have given way to much research in this area[2] and some kinase inhibitors have now been approved for clinical use.[3–5]

Macrocyclic compounds are attractive for the bioactive molecule discovery because their rigid scaffolds can decrease the entropic cost of target binding and limit access to non binding conformations, resulting in higher affinity and greater binding specificity than corresponding linear counterparts.[6,7] Resorcylic acid lactones (RALs) constitute a family of polyketide mycotoxins with a variety of biological activities.[8] Representative examples of this family are the heat shock protein 90 (Hsp90) inhibitor radicicol[9] and the estrogen agonist zearalenone.[10] Hypothemycin,[11] a RAL containing a *Z*-enone moiety that initially did not reveal any particularly interesting activity, was later shown to inhibit *ras*-transformation and T cell activation.[12,13] Hypothemycin and other closely related RALs (LL-Z1640-2[14] and L-783,277[15]) gained attention as compounds that irreversibly and selectively inhibit a subset of protein kinases such as mitogen-activated protein (MAP) kinase (MAPK) kinase (MEK), extracellular signal-regulated kinase (ERK) and TAK1, and platelet-derived growth factor receptor (PDGFR).[14,15] These findings have been recently corroborated by Santi and co-workers, who reported that hypothemycin irreversibly inactivates ERK2 by forming a covalent Michael adduct with the cys166 positioned in the ATP-binding pocket of this kinase.[11b]

A structure-bioinformatics analysis of the kinome revealed that 46 out of the 518 putative kinases contain a cysteine residue adequately positioned to participate in the Michael addition to the *Z*-enone of hypothemycin. As many of the kinases from this group contribute to the development, progression, and aggressiveness of cancer, RALs are currently considered a highly promising class of compounds for oncology drug discovery. Herein, we report a preliminary biological evaluation of the library of RAL derivatives shown in Figure 2 in both enzymatic and cellular assays.

Structure–activity relationship (SAR) studies[16] related to the RALs (Fig. 1) indicate that the *Z*-enone and that the homoallylic diol are important in order to achieve highly potent kinase inhibition.[17] When the homoallylic diol is present then *E*-enones were significantly less active than the corresponding *Z*-enones.[15] Methylation of either of the hydroxyl groups of the diol led to reduction in potency.[16g] Removal of the conjugated ketone as in aigialomycin D[18] or pochonin-based[19] resorcylides led to compounds where kinase inhibitory activity was conserved, albeit the compounds were less potent. We were interested in evaluation of analogues **1–6** where the *Z*-enone group found in RALs is replaced with a haloethyl ketone isostere. We believed that the β-haloketo derivatives would also have the potential to undergo nucleophilic displacement reaction with the same conserved cysteine residue in kinases that bind RALs. We thus developed the synthesis of such

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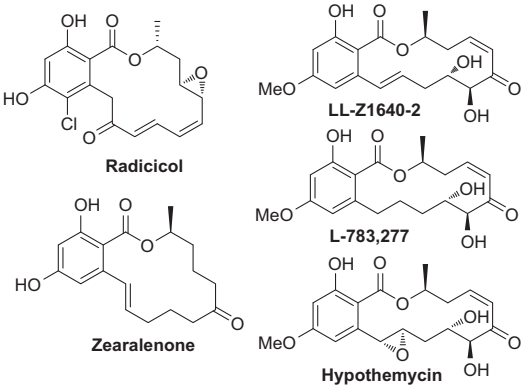


Figure 1. Selected naturally occurring RALs.

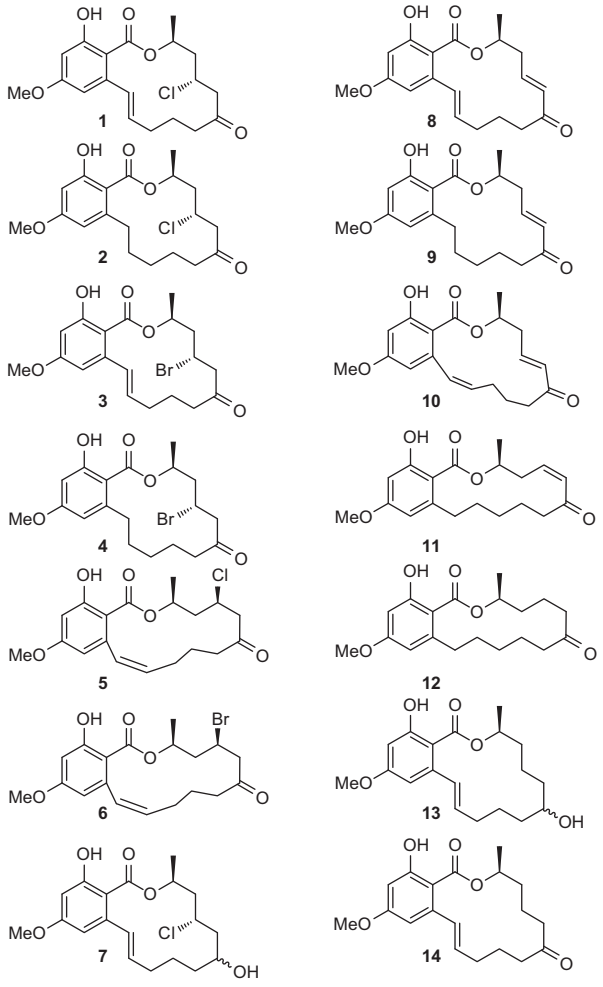


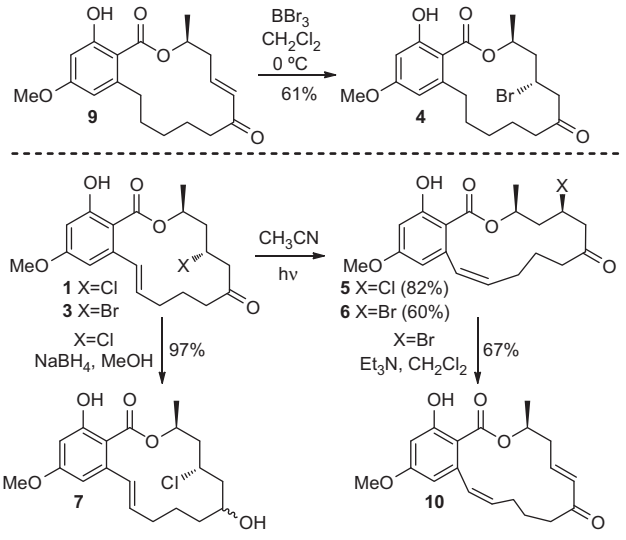
Figure 2. RALs evaluated herein.

halogenated compounds **1–7**, and also prepared analogous *E*-enones **8–10**, *Z*-enone **11** as well as the reduced congeners **12–14**. The nature of the group adjacent to the aromatic ring (C1′–C2′) influences the macrocycle conformation and consequently the selectivity of RALs.[17] Thus **5**, **6**, and **10** which have a *Z*-configuration at the styryl double bond were prepared; interestingly this stereochemical feature has being recently found in naturally occurring aigialomycin E.[20]

The details of the synthetic route to all compounds in Figure 2 with the exception of **4**–**7**, **10**, and **13** have been recently disclosed.[21] β-Bromoketone **4**[22] was prepared by treatment of **9** with boron tribromide. The preparation of **7** was completed with a view to investigating the effect of removing the ketone at C6′ on the biological properties; this was achieved by reduction of **1** using sodium borohydride in methanol. An 87:13 mixture of diastereoisomers was obtained after chromatographic purification (stereochemistry not assigned). To generate analogues bearing a *Z*-alkene at the C1′–C2′ position, halides **1** and **3** were recognized as valuable intermediates and were subjected to a photoisomerization reaction. Hence, upon exposure to light (350 nm) **1** and **3** were readily isomerised, affording *Z*-olefins **5** and **6** in 82% and 60% yield respectively.[23] Under basic conditions (Et_3N, CH_2Cl_2) **5** was transformed to *E*-enone **10**[24] to complete the series (Scheme 1).

A subset of the library members shown in Figure 2 was next screened against a panel of 13 kinases,[25] which, with the exception of ERK5, were all putative cysteine containing kinases with potential for irreversible inhibition by RALs (Table 1). Thus, a commercial sample of the potent kinase inhibitor LL-Z1640-2[26] and staurosporine were tested together with the synthetic library as controls. The IC_{50} values of LL-Z1640-2 are considerably lower than the those reported previously;[17] it is believed that the differences are due to the different assay conditions.[27] All the compounds selectively inhibited kinases which had the conserved cysteine residue in the ATP-binding site (i.e., PDGFRα vs ERK5). However, they did not indiscriminately inhibit all kinases with the same potency demonstrating that selectivity may be achieved within this subset of the kinome. PDGFRα was the most potently inhibited kinase in the panel for all RAL library members followed by FLT3, VEGFR2, VEGFR3, and c-kit. The absence of the C4′,C5′-diol generally results in a significant reduction in inhibitory activity compared to the highly potent LL-Z1640-2 (0.24 nM, PDGFRα) and deletion of ability of the RALs to inhibit a number of the kinases (ERK1, ERK2, GSK3β, GSK3α). However, *E*-enones **8** (27 nM) and **9** (89 nM) were considerably more active as inhibitors of PDGFRα than *Z*-enone **11** (440 nM). All the β-haloketones are less potent than the corresponding *E*-enones but bromide **3** (87 nM, PDGFRα) is also more potent than the *Z*-enone. The β-chloroketones **1** (1.2 μM) and **2** (1.4 μM), although generally less active than enones as inhibitors of kinases, do show an interesting selective inhibition of PDGFRα.

The lower inhibitory activity observed for the chlorides **1**, **2**, and **5** compared to the enones **9**–**11** could be due to a mechanism where elimination needs to occur first of all. This could be slow in some kinase assays. The lower activity could also be due to a slower rate of reaction of the chlorides with the cysteine in the



Scheme 1. Synthesis of RAL analogues.

Table 1
IC_{50} (μM) of selected library members against a panel of 13 kinases[a]

RAL	PDGFRα	VEGFR2	VEGFR3	MEK1	FLT3	c-kit	VEGFR1	PDGFRβ	ERK2	ERK1	GSK3β	GSK3α	ERK5
LL-Z1640-2	0.00024	0.00049	0.0005	0.002	0.0049	0.0096	0.012	0.041	1.3	1.4	1.9	18	>100
1	1.2	nc	>100	nc	34	>100	nc	nc	nc	nc	nc	nc	nc
2	1.4	93	17	nc	16	47	nc	nc	nc	nc	nc	nc	nc
3	0.087	>100	5.5	nc	2.5	2.8	nc	>100	nc	nc	nc	nc	nc
4	2.3	4.3	5.8	nc	2.4	11	>100	nc	nc	nc	nc	nc	nc
5	14	82	>100	nc	12	100	nc	nc	nc	nc	nc	nc	nc
6	8.6	24	65	nc	7.1	81	nc	nc	nc	nc	nc	nc	nc
8	0.027	1.0	1.4	22	0.33	1.3	51	9.2	>100	>100	>100	>100	nc
9	0.089	0.31	0.72	12	0.33	1.3	33	21	>100	>100	>100	nc	nc
10	0.45	2.3	1.6	>100	0.59	1.4	15	>100	nc	nc	nc	nc	nc
11	0.44	0.54	1.2	17	0.85	4.1	>100	42	nc	nc	nc	nc	nc

>concn IC_{50} value above the highest test concentration. Concentration–response curve showed less than 50% effect at the highest tested concentration.
[a] nc: IC_{50} value not calculable. Concentration–response curve showed less than 25% effect at the highest tested concentration.

kinase active site or to reduced affinity of the halide for the kinase compared to enone.[28] We thus investigated susceptibility of the chlorides to elimination in the kinase assay conditions. The representative chloride **1** was stable at pH 7.0–7.5 in HEPES buffer at 22 °C over a 24 h period according to mass spectrometric analysis of the reaction mixture. This would suggest that if elimination was to occur it would require the presence of the kinase. In the presence of the kinase we could not detect the formation of the enone or any other RAL derivative by mass spectrometry at the concentrations at which the assay was carried out. When an N-protected cysteine was exposed to the halide in the presence of triethylamine in $CDCl_3$ then elimination occurred over 24 h and this was followed by conjugation of the cysteine. The halide was stable to the cysteine in the absence of the base but it is difficult to rule out the possibility that a substitution reaction could take place in the enzyme active site. Overall bromides show higher potency than the corresponding chlorides. This could be due to a more rapid substitution reaction in the kinase active site or a higher rate of elimination in the presence of the kinase. The inhibitory activities and selectivity profiles of the halides are not identical with the enones. It is not possible based on these experiments and observations to confirm if elimination occurred before reaction with the kinase.

Compounds **1**–**14** were next assayed against human T leukemia (Jurkat) cells using the CellTiter-Glo™ luminescent cell viability assay.[29] Table 2 shows the antiproliferative activities measured after 24 and 48 h exposure of cells to test compounds. The activity of analogues containing either the enone or β-haloketone moieties is within a fourfold range of the activity of LL-Z1640-2. The β-haloketones are slightly more potent than the corresponding enones and the activity of **6** is comparable to that of LL-Z1640-2. The *Z*-enone **11** is almost twofold less active than the corresponding *E*-enone **9** after 24 h exposure. However, its activity appears to be highly time-dependent, as shown by the data measured after 48 h of exposure. The time-response correlation is less pronounced for other members of the library. Compounds **7** and **12**–**14** are inactive at concentrations up to 100 μM. The data suggests that proliferation of this cell line is not highly dependant on kinases in Table 1 given that LL-Z1640-2 is not significantly more potent than weaker kinase inhibitors.

In a preliminary screening, selected analogues **7**, **9**, and **12** were evaluated for their ability to inhibit capillary like tube formation in vitro. A co-culture of primary human umbilical cord endothelial cells (HUVECs) and vascular smooth muscle cells (vSMC) results in formation of an organotypic capillary network (Fig. 3a) recapitulating many facets of in vivo angiogenesis and would be more highly dependant on some kinases in Table 1, especially those which are angiogenic promoters. The co-culture assay entails live image analysis of fluorescent protein-expressing primary human endothelial cells that form tubular networks within 72 h postseeding.[30] Compounds **9** (11 μM) and **7** (33 μM) show a moderate inhibitory effect, while the full reduced congener **12** is inactive at concentrations up to 100 μM. Evaluation of the other members of the RAL series in the angiogenic assay is currently under investigation.

Table 2
Antiproliferative activity of selected library members against a Jurkat cell line[a]

RAL	IC_{50} (μM)	
	24 h	48 h
LL-Z1640-2	7.0 ± 1.4	5.5 ± 2.8
1	10.9 ± 0.7	9.2 ± 1.4
2	10.6 ± 0.8	8.4 ± 2.2
3	11.7 ± 0.4	9.7 ± 0.7
4	11.4 ± 0.7	9.7 ± 0.2
5	9.5 ± 2.7	8.8 ± 1.3
6	6.1 ± 0.03	7.5 ± 3.5
7	nc[b]	nc[b]
8	11.0 ± 1.4	8.9 ± 1.5
9	13.0 ± 1.4	9.7 ± 1.6
10	19.5 ± 0.7	15.4 ± 0.8
11	26.0 ± 4.2	11.0 ± 0.3

[a] IC_{50} values calculated using percent of growth versus untreated control. Values expressed as mean ± SD of two experiments.
[b] nc: IC_{50} value not calculable.

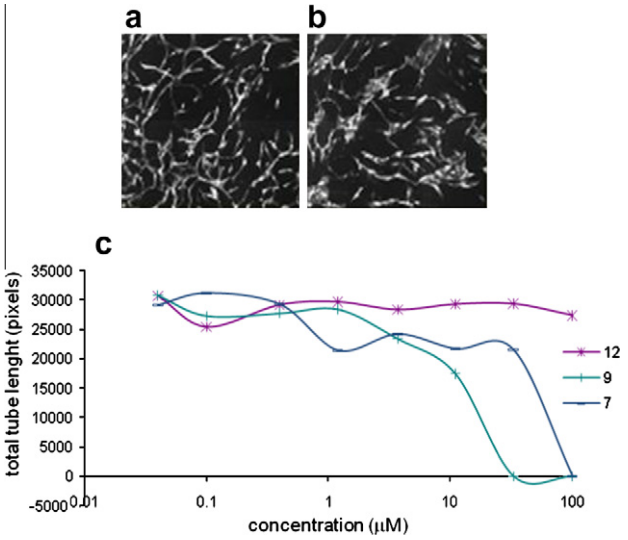


Figure 3. Capillary-like network formation. (a) The endothelial cells gradually interact to generate a capillary-like network. (b) Enone **9** inhibits tube formation (11 μM). (c) Quantitative analysis of the dose–response experiments for compounds **7**, **9** and **12**.

The SAR data reported in this study show that β-haloketone is potentially an effective replacement of the enone moiety, yielding compounds which show activities in enzymatic and cellular assays. Moreover, *E*-resorcylides **8** and **9** exhibit interesting biological activities, inhibiting with high selectivity PDGFRα kinase in the nanomolar range. This would suggest that potentially more potent kinase inhibitors could be obtained by introducing modifications at the C1′–C5′ chain in combination with other isosteric replacements for the *Z*-enone. Thus far we have not established a synthetic strategy to generate RAL analogues containing both the β-halo and desired diol functional groups. Synthetic approaches to these and other RAL analogues are underway.

Acknowledgments

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Supplementary data

Supplementary data (experimental details for the kinase assays) associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.12.100.

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22. *Selected analytical data for* **4**: white solid; ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.10–1.69 (m, 8H), 1.95 (dd, *J* 12.0 Hz, *J* 15.5 Hz, 1H), 2.08 (dd, *J* 11.0 Hz, *J* 15.5 Hz, 1H), 2.13–2.22 (m, 2H), 2.36 (td, *J* 4.5 Hz, *J* 12.0 Hz, 1H), 2.76 (t, *J* 12.0 Hz, 1H), 2.86–2.95 (m, 1H), 3.04 (td, *J* 3.0 Hz, *J* 12.0 Hz, 1H), 3.28 (dd, *J* 3.0 Hz, *J* 11.5 Hz, 1H), 3.80 (s, 3H), 4.35–4.44 (m, 1H), 5.57–5.65 (m, 1H), 6.26 (d, *J* 2.0 Hz, 1H), 6.36 (d, *J* 2.0 Hz, 1H), 12.01 (s, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 20.7, 22.3, 27.1, 31.5, 37.6, 38.0, 44.1, 44.4, 55.3, 55.4, 70.3, 99.1, 104.5, 110.8, 147.4, 164.1, 166.2, 171.1, 207.4. HRMS (ESI): found 413.0972 $[M+H]^+$, $C_{19}H_{26}O_5Br$ requires 413.0964.
23. *Photoisomerization procedure*: halide (0.01 mmol) dissolved in CH_3CN (3 mL) was irradiated with 350 nm light through Pyrex for 16 h. Volatiles were removed under diminished pressure. Chromatography of the residue (petroleum ether–acetone, 95:5) afforded the pure title compound. *Selected analytical data for* **5**: white solid; ^{1}H NMR (500 MHz, $CDCl_3$) 1.40 (d, *J* 6.5 Hz, 3H), 1.48–1.60 (m, 1H), 1.86 (dd, *J* 10.0 Hz, *J* 15.5 Hz, 1H), 2.03–2.14 (m, 1H), 2.15–2.41 (m, 5H), 2.63 (t, *J* 13.0 Hz, 1H), 2.95 (dd, *J* 2.5 Hz, *J* 13.0 Hz, 1H), 3.81 (s, 3H), 4.01–4.08 (m, 1H), 5.36–5.44 (m, 2H), 6.20 (d, *J* 2.5 Hz, 1H), 6.40 (d, *J* 2.5 Hz, 1H), 6.55–6.60 (m, 1H), 11.75 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) 19.9, 20.7, 28.9, 41.3, 44.3, 52.5, 53.7, 55.5, 69.7, 99.7, 104.0, 111.4, 129.6, 132.3, 141.3, 163.9, 165.7, 170.6, 205.6. HRMS (ESI): found 365.1155 $[M-H]^-$, $C_{19}H_{22}O_5Cl$ requires 365.1156. *Selected analytical data for* **6**: white solid; ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.40 (d, *J* 6.0 Hz, 3H), 1.50–1.57 (m, 1H), 1.96–2.12 (m, 2H), 2.13–2.40 (m, 5H), 2.75 (t, *J* 13.0 Hz, 1H), 3.06 (dd, *J* 2.0 Hz, *J* 13.0 Hz, 1H), 3.82 (s, 3H), 4.10 (t, *J* 11.0 Hz, 1H), 5.36–5.44 (m, 2H), 6.20 (d, *J* 2.0 Hz, 1H), 6.40 (d, *J* 2.0 Hz, 1H), 6.57 (d, *J* 11.5 Hz, 1H), 11.74 (s, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 19.8, 20.6, 28.8, 41.1, 44.3, 44.9, 53.2, 55.5, 70.3, 99.6, 103.9, 111.4, 129.5, 132.2, 141.2, 163.8, 165.6, 170.5, 205.6. HRMS (ESI): found 411.0816 $[M+H]^+$, $C_{19}H_{24}O_5Br$ requires 411.0807.
24. *Selected analytical data for* **10**: amorphous white solid; ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.50 (d, *J* 6.5 Hz, 3H), 1.85–2.20 (m, 4H), 2.21–2.29 (m, 1H), 2.34–2.42 (m, 1H), 2.72–2.80 (m, 1H), 2.83 (ddd, *J* 5.0 Hz, *J* 10.5 Hz, *J* 14.0 Hz, 1H), 3.80 (s, 3H), 5.39–5.54 (m, 2H), 5.98 (d, *J* 16.0 Hz, 1H), 6.18 (d, *J* 2.5 Hz, 1H), 6.38 (d, *J* 2.5 Hz, 1H), 6.64 (d, *J* 11.0 Hz, 1H), 6.89 (ddd, *J* 4.5 Hz, *J* 10.5 Hz, *J* 16.0 Hz, 1H), 11.80 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 19.0, 26.0, 27.4, 36.2, 37.3, 55.5, 70.6, 99.7,103.8, 111.1, 129.8, 132.5, 135.3, 141.4, 141.5, 163.7, 165.7, 170.4, 202.3. HRMS (ESI): found 331.1541 $[M+H]^+$, $C_{19}H_{23}O_5$ requires 331.1545.
25. Kinase assays were carried out by CEREP (www.cerep.fr) according to methods described previously. See: (a) Blume-Jensen, P.; Wernsted, C.; Heldin, C.-H.; Rönnstrand, L. *J. Biol. Chem.* **1995**, *270*, 14192; (b) Yung, Y.; Yao, Z.; Aebersold, D. M.; Hanoch, T.; Seger, R. *J. Biol. Chem.* **2001**, *276*, 35280; (c) Bardwell, A. J.; Abdollahi, M.; Bardwell, L. *Biochem. J.* **2003**, *370*, 1077; (d) Mody, N.; Campbell, D. G.; Morrice, N.; Peggie, M.; Cohen, P. *Biochem. J.* **2003**, *372*, 567; (e) Itokawa, T.; Nokihara, H.; Nishioka, Y.; Sone, S.; Iwamoto, Y.; Yamada, Y.; Cherrington, J.; McMahon, G.; Shibuya, M.; Kuwano, M.; Ono, M. *Mol. Cancer Ther.* **2002**, *1*, 295; (f) Dosil, M.; Wang, S.; Lemischka, I. R. *Mol. Cell. Biol.* **1993**, *13*, 6572; (g) Kirkin, V.; Mazitschek, R.; Krishnan, J.; Steffen, A.; Waltenberger, J.; Pepper, M. S.; Giannis, A.; Sleeman, J. P. *Eur. J. Biochem.* **2001**, *268*, 5530; (h) Meijer, L.; Skaltsounis, A. L.; Magiatis, P.; Polychronopoulos, P.; Knockaert, M.; Leost, M.; Ryan, X. P.; Vonica, C. A.; Brivanlou, A.; Dajani, R.; Crovace, C.; Tarricone, C.; Musacchio, A.; Roe, S. M.; Pearl, L.; Greengard, P. *Chem. Biol.* **2003**, *10*, 1255; (i) Robinson, M. J.; Cheng, M.; Khokhlatchev, A.; Ebert, D.; Ahn, N.; Guani, K.-L.; Stein, B.; Goldsmith, E.; Cobb, M. H. *J. Biol. Chem.* **1996**, *271*, 29734; (j) Songyang, Z.; Carraway, K. L.; Eck, M. J.; Harrison, S. C.; Feldman, R. A.; Mohammadi, M.; Schlessinger, J.; Hubbard, S. R.; Smith, D. P.; Eng, C.; Lorenzo, M. J.; Ponder, B. A. J.; Mayer, B. J.; Cantley, L. C. *Nature* **1995**, *373*, 536; (k) Baxter, R. M.; Secrist, J. P.; Vaillancourt, R. R.; Kazlauskas, A. *J. Biol. Chem.* **1998**, *273*, 17050.
26. The natural product was purchased from BioAustralis Fine Chemicals (http://www.tebu-bio.com).
27. In the present study the substrate phosphorylation was measured using TR-FRET assay systems, while a radiometric assay was used in Ref. 17. In addition, different phosphorylation substrates, preincubation times and temperatures, as well as a different order of addition of the reaction cocktail components were employed in the two studies. For details about the assay format used for the study described herein see the Supplementary data.
28. Two papers reporting on the crystal structures of complexes of RALs with a prototypical kinase of the targeted group have been published. (a) Rastelli, G.; Rosenfeld, R.; Reid, R.; Santi, D. V. *J. Struct. Biol.* **2008**, *164*, 18; (b) Ohori, M.; Kinoshita, T.; Yoshimura, S.; Warizaya, M.; Nakajima, H.; Miyake, H. *Biochem. Biophys. Res. Commun.* **2007**, *353*, 633.
29. Jurkat cells were cultured in RPMI 1640 medium supplemented with 10% Foetal Bovine Serum, penicillin (50 units/mL) and streptomycin (50 μg/mL, all from Sigma–Aldrich) in a humidified atmosphere of 5% CO_2 at 37 °C. To calculate the IC_{50} for each compound, Jurkat cells were seeded at 4×10^5 cells/mL in 96-well plates (100 μL/well) and treated in duplicate with eight different concentrations for each compounds. At 24 and 48 h post-treatment, cells were lysed and the ATP content in the well, used as a measure of viable cells, was determined using a thermostable firefly luciferase–based assay (CellTiter-Glo) from Promega according to the manufacture instructions. IC_{50} values were calculated from two independent experiments using percent of growth versus untreated control.
30. Evensen, L.; Micklem, D. R.; Link, W.; Lorens, J. B. *Cytometry A* **2010**, *77*, 41. and references cited therein.