DOI: 10.1002/cmdc.200((will be filled in by the editorial staff))

Rational Design of Novel Resorcylic Acid Lactone Analogues as Covalent MNK1/2 Kinase Inhibitors by Tuning the Reactivity of an Enamide Michael Acceptor

Jin Xu,[a,b] Anqi Chen,*[a] Joma Joy,[c] Vanessa Joanne Xavier,[a] Esther H. Q. Ong,[c] Jeffrey Hill,[c] and Christina L. L. Chai*[a,b]

Recent biological and computational advances in drug design have led to renewed interest in targeted covalent inhibition as an efficient and practical approach for the development of new drugs. As part of our continuing efforts in the exploration of the therapeutic potential of resorcylic acid lactones (RALs), we report herein the design, synthesis and biological evaluation of conveniently accessible RAL enamide analogues as novel covalent inhibitors of MNK kinases. In this study, we have successfully demonstrated that the covalent binding ability of RAL enamides can be tuned by attaching an electron-withdrawing motif, such as an acyl group, to enhance its reactivity towards the cysteine residues at the MNK1/2 binding sites. We have also shown that ^{1}H NMR spectroscopy is a convenient and effective tool for screening the covalent binding activities of enamides using cysteamine as a mimic of the key cysteine residue in the enzyme while mass spectrometry analysis serves to confirm covalent modification of the kinases. Preliminary optimisation of the initial hit has led to the discovery of enamides with low micromolar activity in MNK kinase assays. Cancer cell line assays have identified RAL enamides that inhibit the growth of cancer cells with similar potency as the natural product L-783,277.

Introduction

Historically, small molecules that are capable of covalently modifying proteins, DNA and other biological targets are disfavored candidates for drug development due to their potential off-target reactivities. Consequently, compounds containing a potentially reactive functionality would often be intentionally filtered off at the early stage of a drug discovery program as this could avoid indiscriminant reactivity that may lead to unfavorable toxicological events and unpredictable idiosyncratic adverse effects.[1] However, with recent advances in bio-informatics, structure-guided drug design, pharmacokinetics, preclinical metabolism and toxicology, targeted covalent inhibitors (TCIs) have emerged as an attractive approach for drug design and discovery with several covalent inhibitors being advanced into various stages of clinical trials for diverse indications.[2,3] The distinct advantages of covalent drugs over non-covalent ones are that they often have better potency, prolonged duration of action and are more effective against drug resistance caused by mutations.[2] Among the covalent drugs and drug candidates, those possessing electrophilic functionalities that are capable of modifying the non-catalytic cysteine residues within the active site of targets, especially of protein kinases, are most prominent.[4] To date, at least four cysteine-targeted kinase inhibitors have entered various stages of clinical trials for the treatment of advanced cancers.[5] All of these successful covalent inhibitors possess a substituted acrylamide motif which has been identified as a privileged functionality for covalent binding to cysteine residues.[2] More recently, Taunton's group has developed a cyanoacrylamide-based pyrrolopyrimidine as a covalent yet reversible inhibitor, which has opened opportunities for the rational design of reversible covalent inhibitors.[5,6]

Despite the few recent success stories involving TCI as drugs, the chemical scaffolds of covalent inhibitors are mostly based on *N*-containing heterocycles and the biological targets explored are limited. More diverse chemical structures and biological targets have yet to be investigated. Amongst many potential kinase targets, mitogen-activated protein (MAP) kinase interacting kinase 1 and 2 (MNK1/2) are emerging protein kinases for therapeutic exploration.[7, 8] These kinases selectively mediate the phosphorylation of eIF4E (a translation initiation factor) which is required for the proliferation of cancer cells but not normal cells, and hence these are attractive targets for the development of selective cancer therapeutics with minimal toxicity.[8] In addition, MNK1/2 are found to be involved in growth control, inflammation and viral translation *etc*, therefore providing numerous opportunities for other therapeutic indications.[9] A kinome wide bioinformatic analysis revealed that MNK1/2 fall within a group of

[a] *Ms Jin Xu, Dr. Anqi Chen, Ms Vanessa Joanne Xavier, Prof. Christina L. L. Chai*
*Institute of Chemical and Engineering Sciences (ICES), Agency for Science, Technology and Research (A*STAR), 8 Biomedical Grove, Neuros #07-01, Singapore 138665*
E-mail: phacllc@nus.edu.sg (C. L. L. Chai); chen_anqi@ices.a-star.edu.sg (A. Chen)
[b] *Ms Jin Xu, Prof. Christina L. L. Chai*
Department of Pharmacy, National University of Singapore, 18 Science Drive 4, Singapore 117543
[c] *Dr. Joma Joy, Ms Esther H. Q. Ong, Dr. Jeffrey Hill*
*Protein Biochemistry Enzymology, Experimental Therapeutic Centre (ETC), Agency for Science, Technology and Research (A*STAR), 31 Biopolis Way, Nanos Level 3, Singapore 13866*

Supporting information for this article is available on the WWW under http://www.chemmedchem.org or from the author.

46 kinases that are potential targets of *cis*-enone resorcylic acid lactone (RAL) natural products.[10] This class of compounds, exemplified by L-783,277 (**I**),[11] LL-Z1640-2 (**II**)[12] and hypothemycin (**III**)[13] (Figure 1), are known to form Michael adducts with a cysteine residue within the ATP binding site. Among them, LL-Z1640-2 has very recently been investigated in detail for its covalent inhibition of transforming growth factor-β activated kinase-1(TAK1).[14] Due to their high potency and diverse activities, the RALs have been extensively explored as a privileged scaffold for potential therapeutic applications.[15] Among them, E6201 (Figure 1, **IV**) derived from LL-Z1640-2 has entered clinical trials for anti-inflammatory[16] and anticancer[17] applications. Despite their high potency, natural RALs are limited by their low selectivity (multi-targeting) as well as limited resource from nature or chemical synthesis due to their long synthetic sequences.[18] As such, effort to retune the selectivity of resorcylic acid lactones while simplifying the structure has recently been reported.[19] As part of our continuing interest in exploring RALs as potential therapeutics,[20] we report herein the design, synthesis and biological evaluation of a group of conveniently accessible, novel enamide-based RAL analogues with the general structures (**V**) as covalent inhibitors of MNK1/2 kinases.

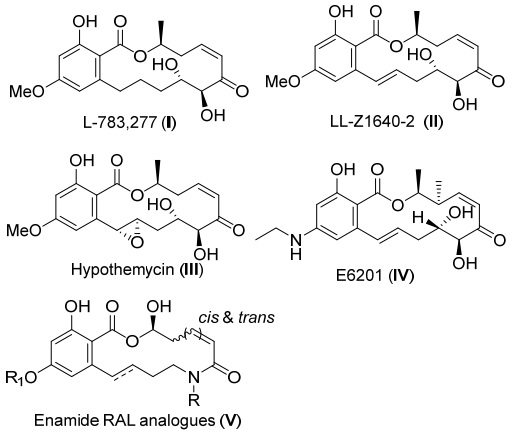


Figure 1. Structures of selected RAL compounds (I-IV) and designed enamide RAL analogues (V).

Results and Discussion

Design of enamide-based RAL inhibitors of MNK1/2

Our design of enamide-based covalent inhibitors of MNK kinases began with the analysis of X-ray crystal structures of MNK2-staurosphorine complex (pdb ID: 2hw7),[21] MNK1 (2hw6) and ERK2-hypothemycin complexes (pdb ID: 3c9w).[13] Superimposition of ERK2-hypothemycin with MNK2-staurophorine found good overlap between the ATP binding regions of the two kinases. Based on the structural comparison as well as sequence alignment, the respective covalent binding cysteine residues were identified as C225 in MNK2 and C190 in MNK1 within the ATP binding sites adjacent to the conserved and functionally important DFG/DFD motif. In particular, there is a good match between the C164 of ERK2 and C225 of MNK2 (Figure 2). It is known that in ERK2-hypothemycin structure, C164 covalently binds to the β-carbon of *cis*-enone motif of hypothemycin (**III**). It is therefore anticipated that *cis*-enone RAL compounds could behave similarly as covalent modifiers towards C225 of MNK2.[18a, 23] Based on this hypothesis and the fact that acrylamide motif is a moderate Michael acceptor towards cysteine,[2] it may be possible to design accessible RAL analogues (**V**) containing an enamide motif as novel covalent inhibitors of MNKs. The advantage of using the enamide as a Michael acceptor is its potential tunability of reactivity by chemical modification, e.g. *N*-acylation, thereby allowing adjustment of its potency.

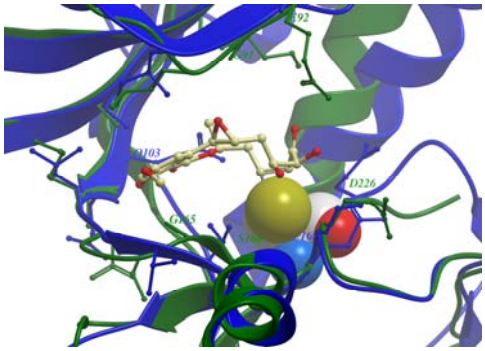
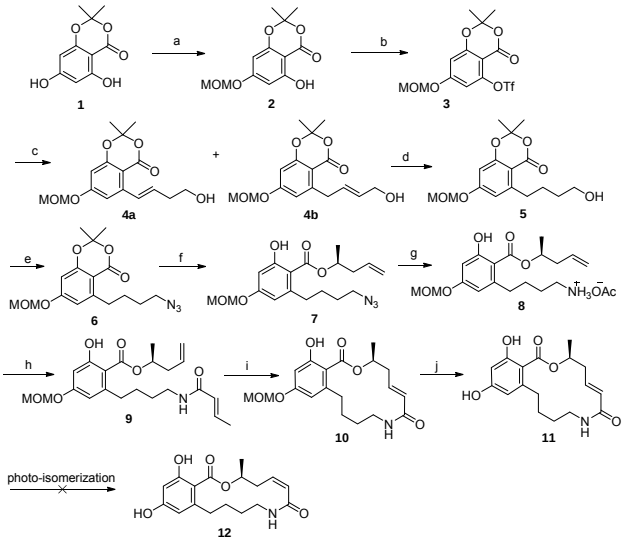


Figure 2. Superimposition of MNK2 (2hw7, green ribbon, C225 SH is shown as ball) with ERK2-hypothemycin (3c9w, sky-blue ribbon for ERK2, yellow-red stick for hypothemycin).

Chemistry – Synthesis of RAL Enamides

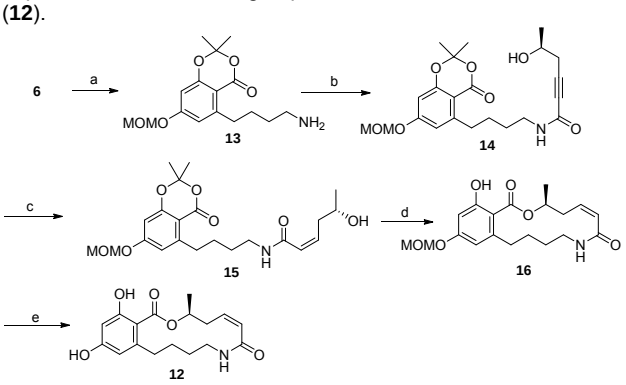
The synthesis of the N-H enamide RAL analogues (**11**, **12**) is described in Scheme 1 and 2. Selective MOM protection of the 4-phenol of the known resorcylic acid derivative **1** followed by triflate formation provided compound **3** in high yield. Subsequent Heck coupling of **3** with 4-buten-1-ol resulted in a mixture of alcohols **4a** and **4b** due to double bond migration under Pd catalysis.[23] However, the mixture was hydrogenated to give the same desired alcohol **5** which was converted to azide **6** under the Mitsunobu conditions. Transesterification of **6** with (*S*)-4-penten-2-ol using NaH as the base afforded azide **7.** This was converted to the amine salt **8** by reduction of the azide with PPh_3 followed by salt formation with acetic acid. Acylation of **8** using crotonyl chloride provided compound **9** which, after ring closing olefin metathesis (RCM), led to the thermodynamically more stable *trans*-enamide **10**. MOM deprotection of **10** afforded the *trans*-enamide **11**. Nevertheless, attempted photoisomerization of **11** to its *cis*-isomer (**12**) by UV light irradiation[25] under various conditions was unsuccessful.



Scheme 1. Synthesis of *trans*-enamide (**11**)**.** Reagents and conditions: (a) MOM-Cl (1.1 eq), Et_3N (2 eq), THF, 0 °C→rt, 6 h, 90%; (b) Tf_2O (1.2 eq), pyridine (3 eq), DCM, 0 °C →rt, 3 h, 78%; (c) 4-buten-1-ol **,** $Pd_2(dba)_3$ (4 mol%), iPr_2NEt, DMF, 90 °C, 2 h; (d) 10% Pd/C, H_2, EtOAc, rt, 16 h, 71% over 2 steps; (e) $(PhO)_2PON_3$ (1.7 eq), DIAD (1.7 eq), PPh_3 (2 eq), THF, rt, 18 h, 82%; (f) (*S*)-

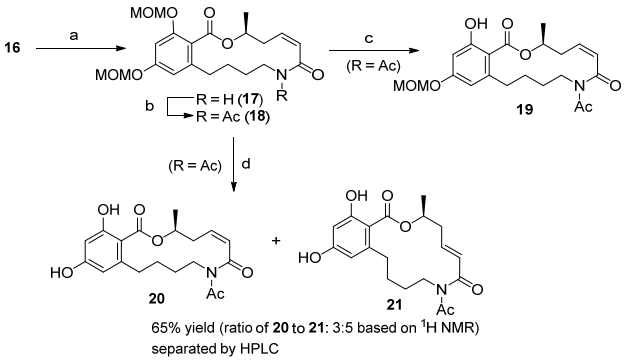
4-penten-2-ol, NaH (8 eq), THF, 0 °C, 77 %; (g) PPh_3 (2 eq), H_2O (4 eq), THF, rt, 16 h, then acetic acid, 76%; (h) Crotonyl chloride (1.2 eq), TEA (3 eq), DCM, -15 °C, 1 h, 78%; (i) Grubbs II (20 mol%), DCM, reflux, 8 h, 60%; (j) 2 N HCl/MeOH/DCM (1:4:1), 50 °C, 8 h, 64%.

In order to obtain the *cis*-enamide (**12**), an alternative route (Scheme 2) was sought. Thus, reduction of azide intermediate **6** followed by amide coupling with (*S*)-5-hydroxyhex-2-ynoic acid (see Supporting Information) provided alkyne (**14**). *cis*-Selective hydrogenation of the alkyne followed by base mediated macrolactonization of the open chain *cis*-enamide (**15**) and deprotection of the MOM group afforded the desired *cis*-enamide (**12**).



Scheme 2. Synthesis of *cis*-enamide (**12**). Reagents and conditions: (a) PPh_3 (2 eq), H_2O (4 eq), THF, rt, 24 h, 89%; (b) (*S*)-5-hydroxyhex-2-ynoic acid, EDC.HCl (1.3 eq), HOBt (1.3 eq), iPr_2NEt (3 eq), DMF, rt, 24 h, 75%; (c) H_2, 5% Pd/$CaCO_3$ (2 mol%, Pb posioned), quinoline (1.1 eq), EtOAc, rt, 30 min, 80%; (d) NaH (5 eq), THF/DMF, rt, 1.5 h, 75%; (e) 2 N HCl, MeOH, 50 °C, 4 h, quantitative yield.

Having achieved the synthesis of the *N*-H containing enamides (**11** and **12**), the synthesis of the *N*-acetyl compounds was investigated, making use of the advanced intermediate **16** obtained previously. MOM protection of the 2-phenol followed by base mediated acylation with acetic anhydride provided the desired *N*-acetyl enamide (**18**). Treatment **18** with trifluoroacetic acid led to the selective deprotection of MOM group at the C-2 position, providing the mono-MOM protected compound **19**. However, using BCl_3 as the deprotection reagent, a 3:5 mixture of *N*-acetyl *cis*- and *trans*-enamide (**20**) and (**21**) was obtained. This mixture was separated by preparative HPLC to afford pure **20** and **21** for biological studies (Scheme 3).



Scheme 3. Synthesis of *N*-acetyl enamides (**18 - 21**). Reagents and conditions: (a) MOM-Cl (1.5 eq), NaH (4 eq), THF, 0 °C, 1.5 h, 93%; (b) LDA (2.5 eq), Ac_2O (2 eq), -78 °C, 1 h, 59%; (c) 1M TFA, DCM, rt, 1 h, 70%; (d) BCl_3 (0.16 M), -10 to 0 °C, 2 h.

The synthesis of analogues **22**, **23** and **24** was achieved in analogous sequences as described in the Supporting Information. Overall nine compounds (Figure 3) have been prepared for biological evaluation.

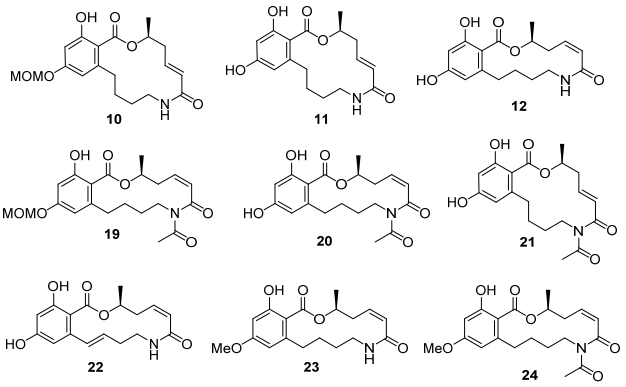


Figure 3. Synthetic enamide-based RAL analogues.

NMR studies of covalent interaction with cysteamine

The reactivity of the enamides as Michael acceptors was preliminarily assessed by ^{1}H-NMR spectroscopy using cysteamine as a model compound to mimic cysteine residues in enzymes.[25] This approach monitors the disappearance of the characteristic olefinic protons by ^{1}H-NMR spectroscopy and enables rapid assessment of potential compounds as covalent kinase inhibitors. When compounds **11** and **12** were treated with cysteamine respectively in DMSO-d_6, no discernible changes were observed in ^{1}H-NMR spectra even after 24 hours (Figure 4a for **12**). This suggests that these compounds may not be sufficiently reactive Michael acceptors. In sharp contrast, when acylated *cis*-enamide **19** was treated with cysteamine, rapid disappearance of the 7', 8' olefinic protons (within 5 min) was observed (Figure 4b), indicating that the introduction of an electron-withdrawing group onto the enamide nitrogen indeed effectively increased its reactivity. Similarly, both *cis* and *trans* *N*-acetyl enamides **20** and **21** also showed covalent adduct formation (Figure S1a and S1b in Supporting Information), suggesting their potential as cysteine covalent modifiers. The enhanced reactivity of *N*-acyl enamides indicated that the electronic properties and the reactivity of enamides can be tuned by *N*-acylation.

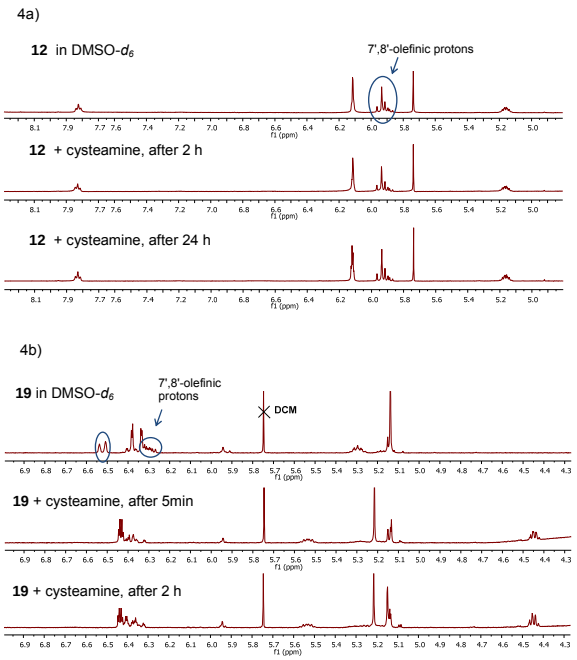


Figure 4. ^{1}H-NMR studies for the reactions of cysteamine with enamide **12** (Figure 4a) and *N*-acetyl enamide **19** (Figure 4b): No change was observed in ^{1}H NMR spectra of **12** after 24 hours**,** whilst the 7', 8' olefinic protons of **19**

disappeared after 5 min and not restored after 2 h, indicating the covalent modification is irreversible.

Inhibitor activities of enamide RAL analogues against MNK1/2 and eIF4E *p*-Ser209

The preliminary NMR studies suggested that *N*-acyl enamides could be promising covalent kinase inhibitors. The kinase inhibition activity (IC_{50} values) of the synthesized RAL analogues was determined against MNK1/2 (Table 1). In agreement with the NMR results, the N-H containing enamides **10**, **11** and **12** were essentially inactive (IC_{50} >40 μM) against both MNK1 and MNK2 although the 1',2'-olefin analogue **22** has slightly improved activity (27.6 μM) against MNK2. In contrast, the *N*-acetyl compound **19** showed greater potency against MNK2 with an IC_{50} value of 6.1 μM. The increase in potency is most likely due to a covalent modification of the protein as observed in the ^{1}H-NMR studies (vide supra) which was further confirmed by protein mass spectrometry analysis (*vide infra*).

Table 1. Inhibitor activity of synthetic enamide RAL analogues against kinases MNK1 and MNK2.[a],[b],[c]

Compd	IC_{50}[μM]	
	MNK2	MNK1
12	41.7±1.2	>50
19	6.1±1.4	15.9±3.5
20	1.3±0.1	8.9[d]
21	34.0±3.5	>50
22	27.6±11.7	>50
24	4.1±0.1	11.6±0.3

[a] Ccompounds **10**, **11**, and **23** have IC_{50} values > 50 μM against both MNK1 and 2. [b] Measurements were carried out in duplicate unless otherwise stated; values are the average ± standard error. [c] L-783,277: IC_{50} 0.061 ± 0.005 μM (MNK2) and 0.325 ± 0.109 μM (MNK1). [d Performed once only.

The fully deprotected compound (**20**) showed further improvement in potency with IC_{50} values of 1.3 μM and 8.9 μM for MNK 2 and MNK1 respectively (Table 1), suggesting a positive interaction of the 4-hydroxy of **20** with the binding site. The *trans*-analogue **21**, however displayed a much lower potency of *ca* 30 fold (for MNK2). Although both NMR (see supporting information) and protein mass analysis of *trans*-isomer **21** indicated it is a covalent modifier, the much weaker potency suggested its weak non-covalent interactions with the binding site residues of the kinase, which is likely due to its unfavorable conformation. The significant difference in biological activity between the *cis*- and *trans*-isomers has also been reported previously for other RAL enones.[11a,26] This indicates that the potency of a covalent inhibitor is governed by both its covalent binding ability and stereochemical interactions in the binding site.

Methylation of the 4-phenol (compound **24**) to mimic the resorcylic portion of the natural products (Figure 1, **I**-**III**) did not show further improvement in potency but instead the activity is marginally reduced as compared to **20** (IC_{50} 4.1 μM vs 1.3 μM against MNK2). In general, all the test compounds showed more potent inhibitory activities against MNK2 than MNK1. Although this preference in binding is not yet fully understood, it is likely caused by the structural difference within their catalytic domain (T-loop) and the C-termini domain between MNK1 and MNK2 which could determine their different binding behaviors.[21, 28] This trend of selectivity has been observed in RAL and other MNK inhibitors reported to date.[20, 23a]

The RAL enamides were further assessed in HeLa cells for the *in vitro* inhibition of phosphorylation of eIF4E, which reflect the level of phosphorylation on serine 209 of eIF4E by both MNK1 and MNK2. This assay, conducted at 10 μM of inhibitors, led to the identification of **20** and **24** as the most potent compounds with 90.4% and 79.4% inhibition respectively (Table 2), in good agreement with the results from the enzymatic assay on MNK2. The IC_{50} values of these two compounds were further determined on eIF4E phosphorylation and shown to be 1.6 μM and 4.7 μM respectively. These results confirmed that the target inhibition by **20** and **24** was effective in both biochemical and cellular assays.

Table 2. Inhibition of phosphorylation of Ser209 on eIF4E in HeLa cells.[a]

Compounds	**12**	**19**	**20**	**21**	**24**
% Inhibition[b]	3.4	44.6	90.4	28.8	79.4
IC_{50} (μM)	ND[b]	ND	1.6	ND	4.7

[a] Other synthetic compounds have no inhibition at 10 μM; ND: not determined. [b] Inhibition at a compound concentration of 10 μM.

Protein mass spectrometric analysis and time-dependent assay for the covalent modification of MNK1/2.

In order to confirm the covalent binding of the active enamides to the target kinases, protein mass spectrometry studies were carried out. This was investigated by incubating MNK1 or MNK2 respectively with the enamides followed by protein mass spectrometric analysis. The results confirmed that indeed the reference natural product L-783,277 (Figure 5a and 5b) and the *N*-acetyl enamides (**19**, **20** and **21**) all showed covalent modification of both MNK1 and MNK2 as is evidenced by the observation of a peak of the protein/ligand adduct (**19** shown in Figure 5c and 5d, and **20**, **21** shown in Supporting Information Figure S2). On the contrary, the free enamides **(11, 12**) did not give detectable adduct formation, in agreement with both NMR studies and bioassay results.

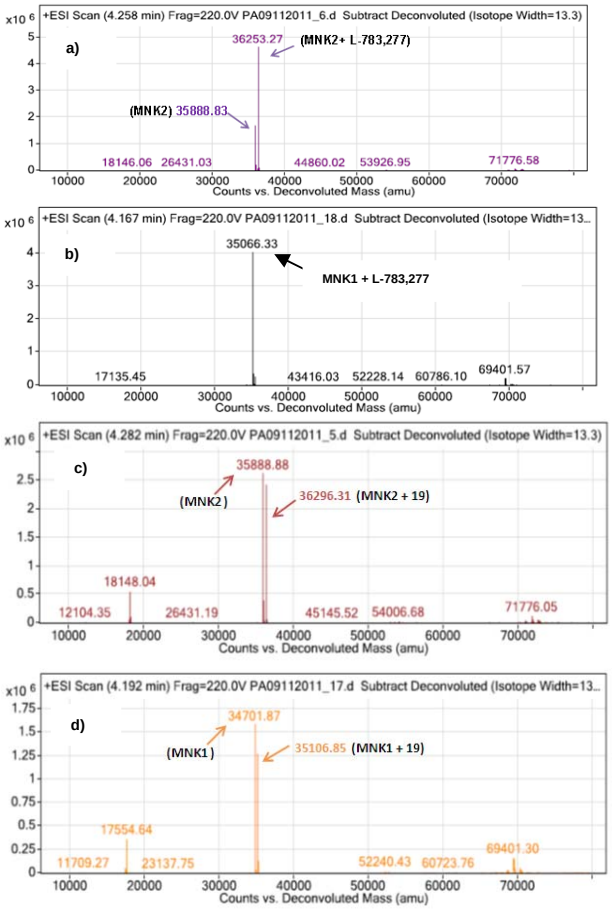


Figure 5. Mass spectra of MNK1/2 incubated with L-783,277 and **19** showing covalent adduct formation.

The irreversibility of inhibition of compounds **20** and **24** was revealed in a time-dependent assay. The results (Figure 6) showed that both **20** and **24** (each at two concentrations) reduced the MNK2 activity (expressed as the fluorescence polarization reading) as incubation time increased (5, 10, 20 and 40 min). This is known as a characteristic of irreversible inhibition.

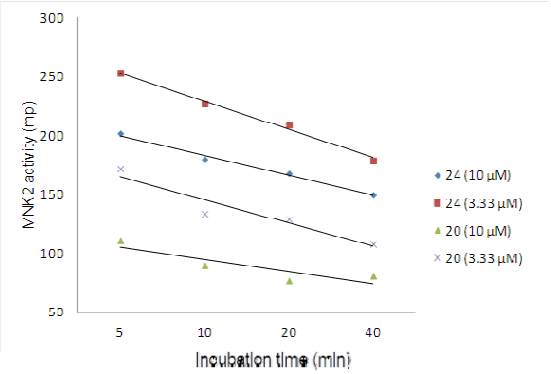


Figure 6 Time-dependent inhibition of MNK2 by **20** and **24** in kinase assay.

Cancer Cell anti-proliferative activities

Four most potent *N*-acetyl enamides were tested for their anti-proliferative activities in four cancer cell lines, benchmarked with the natural product L-783,277 (Table 3). The results showed these RAL enamide analogues are selective to cancer cell lines and **24** is most effective to MDAMB435s (human breast cancer) (GI_{50}: 8.5 μM), comparable to the natural product L-783,277 (GI_{50}: 7.5 μM). 4-*O*-Methylated analogue (**24**) is more active than the 4-phenol compound (**20**). Apart from MDAMB435s, some of the compounds also showed moderate activities against K562 and HeLa cell lines but all are largely inactive against lung cancer cell line A549 including the natural product L-783,277. It is encouraging that although our synthetic analogues are weaker in the kinase inhibition assay (e.g. against MNK2) when compared to L-783,277 (Table 1), they have comparable activities in the cell-based assay, especially for **24**. In addition, it is interesting to note that unlike the kinase inhibition activity (vide supra), both the *cis* and *trans* *N*-acetyl enamides (**20** and **21**) are active at the cellular level despite **21** being slightly weaker (as in MDAMB435s and K562). This is again in agreement with the reported cytotoxicity of natural *cis/trans*-enone RALs for which the *cis* and *trans* isomers show similar cellular activities.[29]

Table 3. Anti-proliferative activities (GI_{50}) of RAL enamides against selected cancer cell lines.[a]

Cell line	19	20	21	24	L783,277
MDAMB435s[d]	12.8±1.3	18.5±0.4	25.0±1.5	8.5±1.1	7.5[b]
HeLa[e]	>50	>50	35.1±0.1	38.8±1.2	40.7±0.7
K562[f]	31.4±0.3	19.5±0.1	29.5±0.2	10.1±0.01	Nt[c]
A549[g]	>50	>50	>50	>50	>50

[a] Measurements were carried out in duplicate unless otherwise stated. [b] Performed once only. [c] Not tested. [d] breast cancer. [e] cervical cancer. [f] leukemia. [g] lung cancer.

Conclusions

In summary, we have designed and synthesized a group of novel resorcylic acid enamide analogues as potential covalent inhibitors of protein kinases by incorporating the privileged RAL scaffold and an acrylamide motif as a Michael acceptor for cysteine residues in protein kinases. It is significant to find that unlike those well-known acyclic acrylamide-based covalent inhibitors, the macrocyclic enamides with free N-H, regardless of *cis* or *trans* stereochemistry, do not possess covalent binding ability. However, by tuning the reactivity of the enamide by *N*-acylation, the covalent binding capability was successfully restored. This has led to the discovery of RAL enamides **20** and **24** that display fairly potent covalent inhibition of MNK1/2 kinases. These conveniently accessible novel RAL analogues are as potent as the natural product L-783,277 in the growth inhibition of cancer cell lines. In the context of the resurging area of covalent drug discovery, our strategy of incorporating an enamide motif into a macrocyclic structure and tuning its activity electronically has the potential to be applied to the discovery of covalent kinase inhibitors for therapeutic applications.

Experimental Section

Chemistry

General experimental methods for chemistry

$^{1}H/^{13}C$-NMR spectra were recorded at 400/100 MHz on a Bruker 400 UltraShield spectrometer. All the proton and carbon spectra were referenced to the respective residual solvent peaks. High resolution mass spectra were obtained using the Agilent 6210 time-of-flight LC/MS. Low resolution mass spectra were obtained using Waters LC-MS system. Preparative HPLC was carried out using either a Waters preparative LC-MS system combining 2545 binary gradient module, with a 2545 SFO, 2767 sample manager and 2996 detector, or Agilent 1260 infinity series. Analytical HPLC traces were obtained from Agilent 1260 infinity analytical system with a binary pump. Flash silica gel column chromatography purification was carried out either manually or by using a Biotage SP1 purification system.

Synthesis of E-enamides:

5,7-Dihydroxy-2,2-dimethyl-4H-1,3-benzodioxin-4-one (1): To a solution of 2,4,6-trihydroxybenzoic acid monohydrate (1.0 g, 5.0 mmol) in acetone (10 mL) was added trifuoroacetic acid (4 mL) and trifuoroacetic anhydride (2.5 mL) slowly at 0 °C. The mixture was warmed to room temperature and continued stirring for 15 h and then refluxed for another 6 h before being cooled to ambient temperature. The mixture was concentrated under reduced pressure then diluted with EtOAc. Saturated sodium bicarbonate was added carefully to adjust the aqueous pH to 5~6. The organic phase was extracted with EtOAc (30 mL x 3), washed with brine, dried over $MgSO_4$ and concentrated to give a crude brown solid which after precipitation from DCM gave white solid. The solid was filtered and collected as pure product (**1**), and the filtrate was concentrated and purified by column chromatography (0-50% EtOAc/petroleum ether) to afford second batch of product. The combined yield was 657 mg (63 %) as a light brown solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.46 (1H, s, OH), 6.08 (1H, d, *J* = 2.1 Hz), 6.01 (1H, d, *J* = 2.1 Hz), 1.72 (6H, s, CH_3 x 2); ^{13}C NMR (100 MHz, $CDCl_3$) *δ* 167.2, 165.8, 164.1, 158.2, 107.6, 98.0, 96.2, 93.0, 25.6; HRMS (ESI-TOF) *m/z* calcd for $C_{10}H_{10}O_5Na$ $[M+Na]^+$:233.0420, found: 233.0419.

5-Hydroxy-7-(methoxymethoxy)-2,2-dimethyl-4H-benzo[d][1,3]dioxin-4-one (2): To a stirred solution of **1** (420 mg, 2.0 mmol) in anhydrous THF (20 mL) was added triethylamine (556 μL, 4.0 mmol) at 0 °C and the mixture was stirred for 20 min then MOM-Cl (228 uL, 2.4 mmol) was added portion-wise over 2 h. The mixture was stirred for another 3 hours upon slowly warmed up to room temperature, quenched by addition of sat. NH_4Cl, diluted with water and extracted 3 times with EtOAc, washed with brine, dried over $MgSO_4$ and concentrated to give the crude product which was purified by column chromatography (0-25 % EtOAc in hexane) to afford the title compound (**2**) (459 mg, 90 %) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.28 (d, *J* = 2.2 Hz, 1H), 6.13 (d, *J* = 2.2 Hz, 1H), 5.17 (s, 2H), 3.47 (s, 3H, OCH_3), 1.73 (s, 6H, CH_3 x 2). ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.3, 165.2, 162.9, 156.9, 107.0, 98.0, 95.9, 94.1, 93.8, 56.5, 25.7 (2C); ESI-MS *m/z* 253 $[M-H]^-$.

7-(Methoxymethoxy)-2,2-dimethyl-4-oxo-4*H*-benzo[*d*][1,3]dioxin-5-yl trifluoromethanesulfonate (3): To a stirred solution of **2** (1.85 g, 7.28 mmol) in anhydrous DCM (40 mL) was added anhydrous pyridine (1.77 mL, 21.8 mmol) after stirring at 0 °C for 15 min, trifluoromethane sulfonic

anhydride (1.48 mL, 8.7 mmol) was added dropwise at 0 °C and the mixture was slowly warmed up to room temperature and stirred for 3 h, then quenched by addition of sat. NH_4Cl. The DCM was removed under reduced pressure. The crude mixture was diluted with water and extracted 3 times with EtOAc, washed with brine, dried over $MgSO_4$ and concentrated to give a crude product which was purified by column chromatography (0-30 % EtOAc in petroleum ether) to afford the title compound (**3**) (2.1 g, 78%) as a white solid ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.67 (d, *J* = 2.3 Hz, 1H), 6.67 (d, *J* = 2.3 Hz, 1H), 6.66 (s, 1H), 5.22 (s, 2H), 3.50 (s, 3H), 1.74 (s, 6H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 163.2, 158.6, 157.0, 149.8, 120.4, 106.6, 106.5, 103.7, 94.7, 56.8, 25.6. ESI-MS *m/z* 387 $[M+H]^+$, 409 $[M+Na]^+$.

Compound 5: To the solution of aryl triflate **3** (1.5 g, 4.05 mmol) and 3-buten-1-ol (624 µL, 7.29 mmol) in DMF (20 mL) was added $Pd_2(dba)_3$ (147 mg, 4 mol%) and then diisopropylethylamine (1.42 mL). The mixture was degassed under reduced pressure and refilled with argon (repeat 3 times), stirred at 90 °C (equipped with a condenser) for 2 hours before being cooled to ambient temperature. The palladium precipitate was filtered off through Celite and the solvent was removed under reduced pressure. The crude intermediate was re-dissolved in EtOAc (20 mL) before 10% Pd/C (66 mg, 10 mol%) was added. The mixture was stirred under H_2 atomosphere (balloon) at room temperature for 16 hours. The Pd/C catalyst was removed by filtration and EtOAc evaporated under reduced pressure. The crude product was purified by column chromotography on silica gel (0-50 % EtOAc in petroleum ether) to afford the title compound (**5**) (colorless gum, 898 mg, 71%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.57 (d, *J* = 2.4 Hz, 1H), 6.46 (d, *J* = 2.4 Hz, 1H), 5.18 (s, 2H), 3.71 (t, *J* = 6.0 Hz, 2H), 3.47 (s, 3H, OCH_3), 3.03 (t, *J* = 7.8 Hz, 2H), 1.68 (s, 6H, CH_3x2), 1.70 - 1.64 (m, 4H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 162.6, 160.3, 159.0, 150.0, 113.5, 105.6, 105.0, 101.8, 94.1, 62.0, 56.4, 34.0, 32.2, 26.9, 25.6 (2C). ESI-MS *m/z* 311 $[M+H]^+$.

(*E*)-5-(4-Hydroxybut-1-en-1-yl)-7-(methoxymethoxy)-2,2-dimethyl-4*H*-benzo[*d*][1,3]dioxin-4-one (4a): **4a** was prepared from **3** (74 mg, 0.2 mmol) by Heck coupling following the procedure in the first step for the preparation of **5**. The mixture (**4a** + **4b**) was separated by repeated silica gel column chromotography to provide **4a** as sticky gum (34 mg, 55%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.43 (d, *J* = 15.8 Hz, 1H), 6.83 (d, *J* = 2.4 Hz, 1H), 6.52 (d, *J* = 2.4 Hz, 1H), 6.14 (dt, *J* = 15.8, 7.1 Hz, 1H), 5.21 (s, 2H), 3.79 (t, *J* = 6.1 Hz, 2H), 3.49 (s, 3H, OCH_3), 2.56 - 2.49 (m, 2H), 1.70 (s, 6H, CH_3 x 2). MS(ESI): *m/z* 309 $[M+H]^+$.

5-(4-Azidobutyl)-7-(methoxymethoxy)-2,2-dimethyl-4*H*-benzo[*d*][1,3]dioxin-4-one (6): To the solution of compound **5** (2.03 g, 6.55 mmol) and triphenylphosphine (3.44 g, 13.10 mmol) in THF (50 mL) was added diphenylphosphoryl azide (2.39 mL, 11.13 mmol) and then DIAD (2.19 mL, 11.13 mmol) under argon. The mixture was stirred at room temperature for 18 hours, THF was then removed under reduced pressure. The crude gum was triturated by vigorous stirring in 1:1 ether-petroleum ether (three times) to remove the PPh_3O precipitates. The combined organic solution was then concentrated and then subjected to silica gel column chromatography (0 - 30 % EtOAc in petroleum ether) to afford compound **6** (1.79 g, 82%) as a colorless gum. ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.57 (d, *J* = 2.4 Hz, 1H), 6.48 (d, *J* = 2.4 Hz, 1H), 5.19 (s, 2H), 3.49 (s, 3H, OCH_3), 3.31 (t, *J* = 6.4 Hz, 2H), 3.07 (t, *J* = 7.2 Hz, 2H), 1.69 (s, 6H, CH_3 x 2), 1.73 - 1.66 (m, 4H, overlapped). ^{13}C NMR (100 MHz, $CDCl_3$) δ 162.5, 160.0, 159.0, 149.3, 113.5, 105.7, 105.0, 101.9, 94.1, 56.5, 51.3, 34.1, 28.8, 28.0, 25.7. ESI-MS *m/z* 358 $[M+Na]^+$.

(*S*)-Pent-4-en-2-yl 2-(4-azidobutyl)-6-hydroxy-4-(methoxy-methoxy)benzoate (7): To a suspension of 60% NaH in mineral oil (112 mg, 4.66 mmol) in anhydrous THF (4 mL) was added the (*S*)-4-penten-2-ol (90 µL, 0.87 mmol) at 0 °C and stirred for 10 min, followed by the addition of a solution of compound **6** (195 mg, 0.58 mmol) in THF (1 mL). The mixture was stirred for 2 hours at 0 °C and then quenched by addition of saturated NH_4Cl. The organic phase was extracted with Et_2O (3×20 mL), washed with brine, dried over $MgSO_4$ and concentrated to give a crude product which was purified by column chromatography (0-30 % EtOAc in petroleum ether) to afford compound (**7**) (163 mg, 77%) as a colorless gum. ^{1}H NMR (400 MHz, $CDCl_3$) δ 11.70 (s, 1H, OH), 6.51 (d, *J* = 2.5 Hz, 1H), 6.37 (d, *J* = 2.5 Hz, 1H), 5.87 - 5.75 (m, 1H), 5.36 - 5.28 (m, 1H), 5.17 (s, 2H), 5.15 – 5.10 (m, 2H), 3.47 (s, 3H, OCH_3), 3.29 (br t, 2H), 3.02 – 2.79 (m, 2H), 2.56 - 2.39 (m, 2H), 1.70 - 1.62 (m, 4H), 1.39 (d, *J* = 6.3 Hz, 3H, CH_3). ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.7, 165.4, 161.4, 147.0, 133.2, 118.4, 111.4, 106.0, 101.9, 93.9, 71.8, 56.3, 51.5, 40.2, 36.3, 29.0, 19.5 (2C). ESI-MS *m/z* 362 $[M-H]^-$.

(*S*)-Pent-4-en-2-yl 2-(4-aminobutyl)-6-hydroxy-4-(methoxy-methoxy)benzoate acetic acid salt (8): To the solution of compound **7** (34 mg, 0.1 mmol) in THF (1 mL) and H_2O (10 µL) was added triphenylphosphine (52.4 mg, 0.2 mmol). The mixture was stirred at room temperature for 16 hours under argon. The solvent was then removed under reduced pressure. EtOAc (3 mL), H_2O (2 mL) and acetic acid was added until the solution reached ~pH 5. Extraction was repeated for three times between the organic phase and aqueous phase, the aqueous phase was then concentrated and dried under reduced pressure to afford amine salt **8** (30.3 mg, 76%) as a colorless gum. ^{1}H NMR (400 MHz, MeOD) δ 6.45 (d, *J* = 2.5 Hz, 1H), 6.43 (d, *J* = 2.5 Hz, 1H), 5.92 – 5.82 (m, 1H), 5.34 - 5.28 (m, 1H), 5.18 (s, 2H), 5.18 (s, 2H), 5.18 - 5.16 (m, 1H, overlapped), 5.14 – 5.08 (m, 1H), 3.44 (s, 1H, OCH3), 3.00 - 2.80 (m, 4H), 2.56 – 2.43 (m, 2H), 1.91 (s, 3H, CH_3), 1.73 – 1.63 (m, 4H), 1.39 (d, *J* = 6.3 Hz, 3H, CH_3). ESI-MS *m/z* 336 $[M-H]^-$.

(*S,E*)-Pent-4-en-2-yl 2-(4-(but-2-enamido)butyl)-6-hydroxy-4-(methoxymethoxy)benzoate (9): To the solution of compound **8** (30 mg, 0.075 mmol) in anhydrous DCM (2 mL) was added the triethylamine (31 µL, 0.225 mmol) and stirred for 10 min, the reaction mixture was cooled to -15 °C, followed by the addition of crotonyl chloride (8.6 µL, 0.09 mmol) dropwise. The mixture was stirred for 1 hour at -15 °C and then quenched by addition of saturated NH_4Cl. DCM was removed and EtOAc and water were added. The organic phase was extracted with EtOAc, washed with brine, dried over $MgSO_4$ and concentrated to give a crude product which was purified by column chromatography on silica gel (0-80 % EtOAc in petroleum ether) to afford compound (**9**) (24 mg, 78%) as white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 11.64 (s, 1H, OH), 11.64 (s, 1H), 6.82 (dq, *J* = 13.7, 6.8 Hz, 1H), 6.50 (d, *J* = 2.5 Hz, 1H), 6.36 (d, *J* = 2.5 Hz, 1H), 5.85 – 5.73 (m, 2H), 5.44 (*br* s, 1H, NH), 5.34 - 5.27 (m, 1H), 5.16 (s, 2H), 5.17 – 5.08 (m, 2H), 3.46 (s, 3H, OCH3), 3.36 - 3.30 (m, 2H), 3.01 - 2.78 (m, 2H), 2.54 – 2.37 (m, 2H), 1.84 (dd, *J* = 6.8, 1.5 Hz, 3H, CH3), 1.63 – 1.54 (m, 4H), 1.37 (d, *J* = 6.3 Hz, 3H, CH3). ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.6, 165.9, 165.3, 161.4, 147.3, 139.7, 133.2, 125.1, 118.4, 111.4, 106.0, 101.8, 93.9, 71.8, 56.3, 40.2, 39.4, 36.3, 29.7, 29.2, 19.5, 17.7. HRMS (ESI-TOF) *m/z* calcd for $C_{22}H_{30}NO_6$ $[M-H]^-$: 404.2079, found: 404.2067.

(*S,E*)-16-Hydroxy-14-(methoxymethoxy)-3-methyl-3,4,9,10,11,12 -hexahydro-1*H*-benzo[*l*][1,7]oxaazacyclotetradecine-1,7(8*H*)-dione (10): To the solution of **9** (50 mg, 0.123 mmol) in DCM (25 mL) was added Grubbs II catalyst (20.9 mg, 20 mol%). The mixture was heated to reflux under argon atmosphere for 8 hour before being cooled to ambient temperature. The catalyst was filtered off through a pad of Celite and the filtrate was concentrated to give the crude product which was purified by column chromatography on silica gel (0-100 % EtOAc in petroleum ether) to afford compound (**10**) (26.7 mg, 60%) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 11.75 (s, 1H, OH), 6.97 - 6.88 (m, 1H), 6.57 (t, *J* = 6.0 Hz, 1H, NH), 6.49 (d, *J* = 2.5 Hz, 1H), 6.34 (d, *J* = 2.5 Hz, 1H), 6.04 (d, *J* = 15.2 Hz, 1H), 5.46 - 5.37 (m, 1H), 5.16 (s, 2H), 3.46 (s, 3H, OCH_3), 3.48 - 3.40 (m, 1H), 3.30 – 3.21 (m, 1H), 3.11 – 3.02 (m, 1H), 2.75 – 2.66 (m, 2H), 2.58 - 2.49 (m, 1H), 1.72 – 1.57 (m, 4H), 1.40 (d, *J* = 6.3 Hz,

3H, CH_3). ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.0, 165.6, 165.5, 161.5, 147.7, 138.6, 127.4, 111.3, 105.9, 101.8, 93.9, 71.3, 56.3, 39.5, 38.2, 36.1, 29.8, 29.3, 19.8. HRMS (ESI-TOF) *m/z* calcd for $C_{19}H_{24}NO_6$ $[M-H]^-$: 362.1609, found: 362.1603.

(*S,E*)-14,16-Dihydroxy-3-methyl-3,4,9,10,11,12-hexahydro-1*H*-benzo[*l*][1,7]oxaazacyclotetradecine-1,7(8*H*)-dione (11): To a solution of **10** (9 mg, 0.0248 mmol) in methanol (1.2 mL) was added 2 N HCl (300 μL). The mixture was stirred at 50 °C for 8 hours before being cooled to ambient temperature. Methanol was removed under reduced pressure, and EtOAc (3 mL) and H_2O (2 mL) were added. The product was extracted with EtOAc (3 mL×3), washed with brine, dried with $MgSO_4$ and concentrated. The crude product was purified by column chromatography on silica gel (0-100% EtOAc in petroleum ether) to afford compound **11** (5.1 mg, 64%) as a white solid. ^1H NMR (400 MHz, acetone-d_6) δ 11.74 (s, 1H, OH), 9.21 (*br* s, 1H, OH), 7.69 (t, *J* = 5.6 Hz, 1H, NH), 6.90 - 6.80 (m, 1H), 6.28 (d, *J* = 2.4 Hz, 1H), 6.22 (d, *J* = 2.4 Hz, 1H), 6.18 (d, *J* = 15.3 Hz, 1H), 5.46 - 5.38 (m, 1H), 3.50 – 3.39 (m, 1H), 3.25 - 3.15 (m, 1H), 3.09 – 3.02 (m, 1H), 2.75 - 2.55 (m, 3H), 1.73 – 1.54 (m, 4H), 1.40 (d, *J* = 6.3 Hz, 3H, CH_3). ^{13}C NMR (100 MHz, acetone-d_6) δ 172.1, 166.6, 166.0, 163.3, 148.8, 138.7, 128.8, 111.8, 105.0, 101.9, 72.2, 39.8, 38.7, 36.9, 30.8, 30.3, 19.9. HRMS (ESI-TOF) *m/z* calcd for $C_{17}H_{20}NO_5$ $[M-H]^-$: 318.1347, found: 318.1338.

Synthesis of Z-enamides:

5-(4-Aminobutyl)-7-(methoxymethoxy)-2,2-dimethyl-4*H*-benzo[*d*][1,3]dioxin-4-one (13): To the solution of compound **6** (738 mg, 2.20 mmol) in THF (40 mL) and H_2O (0.4 mL) was added triphenylphosphine (1.15 g, 4.40 mmol). The mixture was stirred at room temperature for 24 hours under argon. The solvent was then removed under reduced pressure. The crude mixture was subjected to column chromatography on silica gel by 80-100% EtOAc firstly to elute PPh_3/PPh_3O, followed by 10-20% MeOH in DCM (with 0.1% Et_3N) to afford compound **13** (606 mg, 89%) as a colorless gum. ^1H NMR (400 MHz, $CDCl_3$) δ 6.57 (d, *J* = 2.4 Hz, 1H), 6.47 (d, *J* = 2.4 Hz, 1H), 5.18 (s, 2H), 3.48 (s, 3H, OCH_3), 3.08 – 3.02 (m, 2H), 2.74 (t, *J* = 6.7 Hz, 2H), 1.69 (s, 6H, CH_3x2), 1.67 – 1.51 (m, 4H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 162.4, 160.1, 158.9, 150.0, 113.5, 105.7, 104.9, 101.7, 94.1, 56.4, 41.7, 34.4, 33.5, 28.1, 25.7 (2C). ESI-MS *m/z* 310 $[M+H]^+$.

(*S*)-5-Hydroxy-*N*-(4-(7-(methoxymethoxy)-2,2-dimethyl-4-oxo-4*H*-benzo[*d*][1,3]dioxin-5-yl)butyl)hex-2-ynamide (14): A mixture of compound **13** (520 mg, 1.68 mmol), (*S*)-5-hydroxyhex-2-ynoic acid (258 mg, 2.02 mmol), EDCI (419 mg, 2.18 mmol) and HOBt (295 mg, 2.18 mmol) was dissolved in anhydrous DMF (25 mL). The reaction mixture was stirred at room temperature under argon for 24 hours. DMF was then removed under high vacuum and the crude product was purified by column chromatography on silica gel (0-60 % EtOAc in petroleum ether) to afford compound (**14**) (528 mg, 75%) as a colorless gum. ^1H NMR (400 MHz, $CDCl_3$) δ 6.64 (*br* s, 1H, NH), 6.56 (d, *J* = 2.4 Hz, 1H), 6.47 (d, *J* = 2.4 Hz, 1H), 5.18 (s, 2H), 4.06 - 3.98 (m, 1H), 3.48 (s, 3H, OCH_3), 3.40 - 3.34 (m, 2H), 3.03 - 2.95 (m, 2H), 2.52 - 2.37 (m, 2H), 1.69 (s, 6H, CH_3 x 2), 1.66 - 1.60 (m, 4H), 1.28 (d, *J* = 6.2 Hz, 3H, CH_3). ^{13}C NMR (100 MHz, $CDCl_3$) δ 162.7, 160.5, 159.1, 153.5, 149.6, 113.7, 105.4, 105.1, 102.0, 94.1, 83.7, 77.6, 65.8, 56.5, 38.7, 33.9, 29.2, 28.6, 28.0, 25.6, 22.5 (2C). ESI-MS *m/z* 420 $[M+H]^+$.

(*S,Z*)-5-Hydroxy-*N*-(4-(7-(methoxymethoxy)-2,2-dimethyl-4-oxo-4*H*-benzo[*d*][1,3]dioxin-5-yl)butyl)hex-2-enamide (15): To a solution of compound **14** (388 mg, 0.926 mmol) in EtOAc (20 mL) was added quinoline (131 mg, 1.02 mmol). The mixture was degassed under nitrogen gas, then added 5% Pd/$CaCO_3$ (lead poisoned, 46 mg, 2 mol% of Pd). The resulting mixture was degassed again under H_2 gas, then stirred under H_2 balloon at room temperature for 30 min. The catalyst was filtered off through Celite and the solvent evaporated under reduced pressure. The crude product was purified by silica gel column chromatography to give *cis*-olefin product **15** (313 mg, 80%) as a colorless gum. ^1H NMR (400 MHz, $CDCl_3$) δ 6.80 (*br* s, 1H, NH), 6.57 (d, *J* = 2.3 Hz, 1H), 6.48 (d, *J* = 2.3 Hz, 1H), 6.16 – 6.07 (m, 1H), 6.01 (d, *J* = 11.6 Hz, 1H), 5.19 (s, 2H), 4.02 – 3.93 (m, 1H), 3.49 (s, 3H, OCH_3), 3.45 – 3.38 (m, 2H), 3.04 – 2.97 (m, 2H), 2.75 – 2.57 (m, 2H), 1.70 (s, 6H, CH_3x2), 1.68 – 1.61 (m, 4H), 1.25 (d, *J* = 6.2 Hz, 3H, CH_3). ^{13}C NMR (100 MHz, $CDCl_3$) δ 167.0, 162.7, 160.5, 159.0, 149.7, 139.6, 126.1, 113.7, 105.4, 105.1, 102.0, 94.1, 67.1, 56.5, 38.2, 37.8, 34.0, 28.7, 28.1, 25.65, 25.64, 23.7. ESI-MS *m/z* 422 $[M+H]^+$.

(*S,Z*)-16-Hydroxy-14-(methoxymethoxy)-3-methyl-3,4,9,10,11,12-hexahydro-1*H*-benzo[*l*][1,7]oxaazacyclotetradecine-1,7(8*H*)-dione (16): To the suspension of 60% NaH in mineral oil (5 mg, 83 μmol) in anhydrous THF (1 mL) was added the solution of **15** (7 mg, 16.6 μmol) in THF (0.5 mL) at 0 °C and the mixture was stirred for 90 min upon warmed up to room temperature before being quenched by addition of saturated NH_4Cl. The organic phase was extracted with EtOAc (2 mL x 3), washed with brine, dried over $MgSO_4$ and concentrated to give a crude product which was purified by column chromatography on silica gel (0-100 % EtOAc in petroleum ether) to afford compound (**16**) (4.5 mg, 75%) as a white solid. ^1H NMR (400 MHz, $CDCl_3$) δ 11.70 (s, 1H, OH), 6.50 (d, *J* = 2.5 Hz, 1H), 6.40 (d, *J* = 2.5 Hz, 1H), 5.99 (dd, *J* = 11.5, 1.7 Hz, 1H), 5.90 (dt, *J* = 11.4, 5.7 Hz, 1H), 5.54 (*br* s, 1H, NH), 5.41 – 5.33 (m, 1H), 5.17 (s, 2H), 3.84 - 3.75 (m, 1H), 3.46 (s, 3H, OCH_3), 3.50 - 3.39 (m, 1H), 3.30 - 3.22 (m, 1H), 2.98 – 2.86 (m, 1H), 2.78 - 2.70 (m, 1H), 2.44 – 2.37 (m, 1H), 1.94 - 1.51 (m, 4H), 1.43 (d, *J* = 6.3 Hz, 3H, CH_3). ^{13}C NMR (100 MHz, Acetone-d_6) δ 172.2, 167.9, 166.0, 162.4, 148.4, 136.3, 128.4, 128.4, 110.1, 101.9, 94.7, 73.2, 56.3, 40.7, 36.0 (2C), 28.1, 27.7, 21.1. ESI-MS *m/z* 362 $[M-H]^-$.

(*S,Z*)-14,16-Dihydroxy-3-methyl-3,4,9,10,11,12-hexahydro-1*H*-benzo[*l*][1,7]oxaazacyclotetradecine-1,7(8*H*)-dione (12): Compound **12** was prepared by MOM deprotection of **16** following the procedures for the synthesis of **11**. 30 mg of **16** afforded 28.1 mg (quantitative yield) of **12**. ^1H NMR (400 MHz, MeOD) δ 6.23 (d, *J* = 2.4 Hz, 1H), 6.14 (d, *J* = 2.4 Hz, 1H), 6.03 (dd, *J* = 11.7, 1.5 Hz, 1H), 5.97 (ddd, *J* = 11.7, 10.3, 4.8 Hz, 1H), 5.37 - 5.28 (m, 1H), 3.58 - 3.52 (m, 1H), 3.34 - 3.25 (m, 1H, overlapped), 3.18 – 3.07 (m, 1H), 3.02 – 2.95 (m, 1H), 2.72 - 2.63 (m, 1H), 2.52 - 2.45 (m, 1H), 1.81 - 1.55 (m, 4H), 1.40 (d, *J* = 6.3 Hz, 3H, CH_3); ^{13}C NMR (100 MHz, MeOD) δ 172.6, 170.4, 165.9, 163.6, 148.3, 137.3, 127.7, 110.3, 106.3, 101.7, 73.0, 40.9, 36.7, 36.3, 28.5 (2C), 21.0. HRMS (ESI-TOF) *m/z* calcd for $C_{17}H_{20}NO_5$ $[M-H]^-$: 318.1347, found: 318.1339.

(*S,Z*)-14,16-Bis(methoxymethoxy)-3-methyl-3,4,9,10,11,12-hexahydro-1*H*-benzo[*l*][1,7]oxaazacyclotetradecine-1,7(8*H*)-dione (17): To the suspension of 60% NaH in mineral oil (146 mg, 3.66 mmol) in anhydrous THF (2 mL) was added the solution of **16** (332 mg, 0.915 mmol) in THF (0.5 mL) at 0 °C and the mixture was stirred for 10 min followed by addition of MOM-Cl (104 μL, 1.37 mmol). The mixture was stirred for another 90 min at 0 °C, quenched by addition of sat. NH_4Cl, diluted with water and extracted with EtOAc (3mL x 3). The combined organic phases were washed with brine, dried over $MgSO_4$ and concentrated to give a crude product which was purified by column chromatography on silica gel (0-100 % EtOAc in petroleum ether) to afford compound (**17**) (347 mg, 93 %) as a white solid. ^1H NMR (400 MHz, $CDCl_3$) δ 6.67 (d, *J* = 2.2 Hz, 1H), 6.51 (d, *J* = 2.2 Hz, 1H), 6.04 (br s, 1H, NH), 6.00 (d, *J* = 11.8 Hz, 1H), 5.96 – 5.88 (m, 1H), 5.44 – 5.37 (m, 1H), 5.15 (s, 2H), 5.15 (d, *J* = 6.7 Hz, 2H), 5.09 (d, *J* = 6.7 Hz, 1H), 3.46 (s, 3H), 3.45 (s, 3H), 3.40 – 3.15 (m, 3H), 2.79 - 2.72 (m, 1H), 2.63 - 2.55 (m, 1H), 2.36 – 2.28 (m, 1H), 1.68 – 1.47 (m, 4H), 1.44 (d, *J* = 6.5 Hz, 3H, CH_3). ^{13}C NMR (100 MHz, $CDCl_3$) δ 167.9, 167.1, 159.0, 156.1, 142.7, 131.1, 128.8, 117.1, 111.3, 101.4, 94.7, 94.3, 70.2, 56.3, 56.2, 38.1, 35.7, 32.9, 27.1, 26.6, 18.9. HRMS (ESI-TOF) *m/z* calcd for $C_{21}H_{29}NO_7Na$ $[M+Na]^+$: 430.1836, found: 430.1841.

(*S,Z*)-8-Acetyl-14,16-bis(methoxymethoxy)-3-methyl-3,4,9,10,11,12-hexahydro-1*H*-benzo[*l*][1,7]oxaazacyclotetradecine-1,7(8*H*)-dione (18): To the solution of **17** (30 mg, 0.074 mol) in THF (2 mL) was added freshly prepared LDA (1 M in THF, 184 μL) dropwise over 30 min at -78 °C. At the same time, acetic anhydride (1 M in THF, 147 μL) was also added dropwise over 30 – 40 min. The mixture was stirred for another 20 min at -78 °C then quenched by the addition of saturated aq. NH_4Cl at *ca* 0 °C, then warmly up to room temperature followed by addition of EtOAc and water. The mixture was extracted with EtOAc, and the extract was washed with brine, dried over $MgSO_4$ and concentrated under reduced pressure. The crude product was purified by column chromatography on silica gel (0-80 % EtOAc in petroleum ether) to afford compound (**18**) (19.5 mg, 59 %) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.68 (d, *J* = 2.2 Hz, 1H), 6.53 (d, *J* = 2.2 Hz, 1H), 6.45 - 6.38 (m, 1H), 6.35 (dd, *J* = 11.7, 1.3 Hz, 1H), 5.53 – 5.45 (m, 1H), 5.19 – 5.12 (m, 4H), 4.00 - 3.93 (m, 1H), 3.54 - 3.49 (m, 1H), 3.49 (s, 3H, OCH_3), 3.48 (s, 3H, OCH_3), 3.16 (dt, *J* = 16.5, 9.1 Hz, 1H), 2.72 - 2.62 (m, 2H), 2.52 – 2.45 (m, 1H), 2.45 (s, 3H, CH_3), 1.72 - 1.57 (m, 4H), 1.45 (d, *J* = 6.4 Hz, 3H, CH_3). ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.2, 169.6, 168.0, 158.8, 155.1, 143.4, 141.5, 125.2, 118.9, 109.8, 101.1, 94.7, 94.4, 70.9, 56.2, 56.1, 43.9, 36.3, 33.1, 28.8, 27.2, 26.6, 20.5. HRMS (ESI-TOF) *m/z* calcd for $C_{23}H_{31}NO_8Na$ $[M+Na]^+$: 472.1942, found: 472.1956.

(*S,Z*)-8-Acetyl-16-hydroxy-14-(methoxymethoxy)-3-methyl-3,4,9,10,11,12-hexahydro-1*H*-benzo[*l*][1,7]-oxaazacyclotetradecine-1,7(8*H*)-dione (19): To the solution of **18** (2 mg, 4.5 μmol) in DCM (1 mL) was added 1 M TFA in DCM (200 μL). The mixture was stirred at room temperature for 30 min, a few drops of sat. $NaHCO_3$ was added to adjust the pH ~7. After removal of DCM, EtOAc and water were added. The organic phase was extracted with EtOAc and the extracts were washed with brine, dried over $MgSO_4$ and concentrated under reduced pressure. The crude product was purified by column chromatography on silica gel (0-50 % EtOAc in petroleum ether) to afford compound (**19**) (1.3 mg, 70 %) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 11.85 (s, 1H, OH), 6.50 (d, *J* = 2.5 Hz, 1H), 6.33 (d, *J* = 2.5 Hz, 1H), 6.32 (dd, *J* =11.6, 1.9 Hz, 1H, overlapped), 6.12 (ddd, *J* = 11.6, 10.2, 3.7 Hz, 1H), 5.55 - 5.47 (m, 1H), 5.16 (s, 2H), 4.08 – 4.02 (m, 1H), 3.46 (s, 3H, OCH_3), 3.37 - 3.28 (m, 1H), 3.12 – 2.93 (m, 2H), 2.74 - 2.66 (m, 1H), 2.55 - 2.45 (m, 1H), 2.43 (s, 3H, $COCH_3$), 1.98 – 1.87 (m, 1H), 1.62 - 1.45 (m, 3H, overlapped), 1.43 (d, *J* = 6.2 Hz, 3H, CH_3). ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.0, 171.3, 170.7, 165.8, 161.6, 147.1, 140.4, 127.0, 111.0, 105.7, 101.8, 93.9, 72.3, 56.3, 44.4, 36.4, 36.0, 29.8, 28.7, 26.2, 20.6. HRMS (ESI-TOF) *m/z* calcd for $C_{21}H_{26}NO_7$ $[M-H]^-$: 404.1716, found: 404.1715.

(*S,Z*)-8-Acetyl-14,16-dihydroxy-3-methyl-3,4,9,10,11,12-hexahydro-1*H*-benzo[*l*][1,7]oxaazacyclotetradecine-1,7(8*H*)-dione (20) and (*S,E*)-8-Acetyl-14,16-dihydroxy-3-methyl-3,4,9,10,11,12-hexahydro-1*H*-benzo[*l*][1,7]-oxaazacyclotetradecine-1,7(8*H*)-dione (21): To compound **17** (4.2 mg, 9.3 μmol) was added boron trichloride solution (0.16 M in DCM, 0.5 mL) at -10 °C. The mixture as stirred for 2 hours then quenched by addition of sat. $NaHCO_3$ to pH ~7. After removal of DCM, EtOAc and water were added. The organic phase was extracted with EtOAc, washed with brine, dried over $MgSO_4$ and concentrated under reduced pressure. The crude product was purified by column chromatography on silica gel (0-50 % EtOAc in petroleum ether) to afford a mixture of *cis* (**20)** and *trans* products (**21)** (2.2 mg, 65%) as a white solid, which after HPLC purification gave two pure products: t_R 14.4 min (**21**), t_R 15.2 min (**20**). HPLC condition: Gemini C18, 10x100 mm, acetonitrile in H_2O, flow rate: 6 mL/min, gradient: 10% (2 min) - 10% to 70% (20 min) – 70% (5 min).

Compound 20: ^{1}H NMR (400 MHz, $CDCl_3$) δ 11.90 (s, 1H, OH), 6.32 (dd, *J* = 11.6, 2.0 Hz, 1H), 6.28 (d, *J* = 2.6 Hz, 1H), 6.18 (d, *J* = 2.6 Hz, 1H), 6.12 (ddd, *J* = 11.6, 10.2, 3.7 Hz, 1H), 5.57 - 5.48 (m, 1H), 4.09 – 4.01 (m, 1H), 3.38 – 3.28 (m, 1H), 3.11 – 2.93 (m, 2H), 2.73 – 2.66 (m, 1H), 2.52 – 2.45 (m, 1H), 2.43 (s, 3H, $COCH_3$), 1.99 – 1.87 (m, 1H), 1.60 - 1.45 (m, 3H, hidden),1.43 (d, *J* = 6.2 Hz, 3H, CH_3). ^{13}C NMR (100 MHz, $CDCl_3$) δ 140.5, 127.0, 110.3, 101.7, 72.3, 44.4, 36.4, 35.9, 29.8, 28.6, 26.2, 20.6 (quaternary carbons are not shown). HRMS (ESI-TOF) *m/z* calcd for $C_{19}H_{22}NO_6$ $[M-H]^-$: 360.1453, found: 360.1451.

Compound 21: ^{1}H NMR (400 MHz, MeOD) δ 6.94 (dt, *J* = 15.3, 7.4 Hz, 1H), 6.66 (d, *J* = 15.3 Hz, 1H), 6.24 (d, *J* = 2.4 Hz, 1H), 6.18 (d, *J* = 2.4 Hz, 1H), 5.56 - 5.49 (m, 1H), 3.93 – 3.85 (m, 1H), 3.74 – 3.65 (m, 1H), 3.07 – 2.98 (m, 1H), 2.82 – 2.62 (m, 3H), 2.37 (s, 3H, $COCH_3$), 1.87 – 1.79 (m, 1H), 1.64 – 1.54 (m, 2H), 1.54 - 1.45 (m, 1H), 1.47 (d, *J* = 6.4 Hz, 3H, CH_3). ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.5, 147.3, 141.9, 128.5, 109.1, 101.1, 70.8, 43.3, 38.2, 34.5, 29.5, 26.3, 26.2, 18.8 (some quaternary carbons are not shown). HRMS (ESI-TOF) *m/z* calcd for $C_{19}H_{22}NO_6$ $[M-H]^-$: 360.1453, found: 360.1469.

(*S*,5*Z*,11*E*)-14,16-Dihydroxy-3-methyl-3,4,9,10-tetrahydro-1*H*-benzo[l][1,7]oxaazacyclotetradecine-1,7(8*H*)-dione (22): Compound **22** was prepared from **4a** following the procedures for the synthesis of **12**. ^{1}H NMR (400 MHz, Acetone-d_6) δ 12.05 (s, 1H, OH), 7.12 (dd, *J* = 15.3, 1.4 Hz, 1H), 6.87 (br s, 1H, NH), 6.49 (d, *J* = 2.5 Hz, 1H), 6.30 (d, *J* = 2.5 Hz, 1H), 5.95 (dd, *J* = 11.5, 2.0 Hz, 1H), 5.86 – 5.77 (m, 2H), 5.33 - 5.24 (m, 1H), 3.73 - 3.62 (m, 2H), 3.05 – 2.94 (m, 1H), 2.87 – 2.75 (m, 1H), 2.35 - 2.23 (m, 2H), 1.43 (d, *J* = 6.1 Hz, 3H, CH_3). ^{13}C NMR (100 MHz, MeOD) δ 171.5, 168.4, 165.2, 162.6, 143.4, 136.1, 134.1, 130.1, 126.1, 107.9, 102.3, 101.6, 72.2, 38.2, 35.3, 31.9, 20.0. HRMS (ESI-TOF) *m/z* calcd for $C_{17}H_{18}NO_5$ $[M-H]^-$: 316.1190, found: 316.1200.

(*S,Z*)-16-Hydroxy-14-methoxy-3-methyl-3,4,9,10,11,12-hexahydro-1*H*-benzo[*l*][1,7]oxaazacyclotetradecine-1,7(8*H*)-dione (23): Compound **23** was prepared from **29** (Scheme S2) following the same procedures for the synthesis of **12**. ^{1}H NMR (400 MHz, $CDCl_3$) δ 11.86 (s, 1H), 6.33 (d, *J* = 2.6 Hz, 1H), 6.30 (d, *J* = 2.6 Hz, 1H), 5.99 (dd, *J* = 11.6, 1.8 Hz, 1H), 5.89 (td, *J* = 11.6, 4.4 Hz, 1H), 5.53 (br s, 1H, NH), 5.40 - 5.32 (m, 1H), 3.80 (s, 3H, CH_3), 3.84 - 3.75 (m, 1H), 3.50 – 3.39 (m, 1H), 3.30 - 3.21 (m, 1H), 2.97 - 2.88 (m, 1H), 2.77 - 2.68 (m, 1H), 2.43 – 2.37 (m, 1H), 1.94 – 1.61 (m, 4H), 1.43 (d, *J* = 6.3 Hz, 3H, CH_3). ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.3, 167.6, 166.0, 164.0, 146.8, 136.3, 127.0, 109.4, 105.1, 98.7, 71.9, 55.3, 40.3, 35.9, 35.0, 27.1, 26.7, 21.1. HRMS (ESI-TOF) *m/z* calcd for $C_{18}H_{22}NO_5$ $[M-H]^-$: 332.1503, found: 332.1513.

(*S,Z*)-8-Acetyl-16-hydroxy-14-methoxy-3-methyl-3,4,9,10,11,12-hexahydro-1*H*-benzo[*l*][1,7]oxaazacyclotetradecine-1,7(8*H*)-dione (24): Compound **24** was prepared from **23** following the same procedures for the synthesis of **19**. ^{1}H NMR (400 MHz, $CDCl_3$) δ 11.98 (s, 1H, OH), 6.34 (d, *J* = 2.6 Hz, 2H), 6.32 (dd, *J* = 11.6, 2.3 Hz, 2H), 6.24 (d, *J* = 2.6 Hz, 1H), 6.12 (ddd, *J* = 11.6, 10.2, 3.7 Hz, 1H), 5.55 - 5.46 (m, 1H), 4.05 (dt, *J* = 14.0, 6.4 Hz, 1H), 3.79 (s, 3H, OCH_3), 3.36 – 3.27 (m, 1H), 3.12 – 2.93 (m, 2H), 2.73 - 2.66 (m, 1H), 2.53 – 2.46 (m, 1H), 2.43 (s, 3H, $COCH_3$)1.98 – 1.87 (m, 1H), 1.60 - 1.43 (m, 3H, hidden), 1.43 (d, *J* = 6.2 Hz, 3H, CH_3). ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.0, 171.4, 170.7, 166.3, 164.1, 147.0, 140.4, 127.0, 110.3, 104.7, 99.0, 72.3, 55.3, 44.4, 36.4, 36.0, 29.8, 28.7, 26.2, 20.6. HRMS (ESI-TOF) *m/z* calcd for $C_{20}H_{24}NO_6$ $[M-H]^-$: 374.1609, found: 374.1596.

Biological evaluations

Proton NMR studies of selected enamides with cysteamine: Test compounds (typically a few milligrams) were dissolved in DMSO-d_6 (0.5 mL) in a standard NMR tube and the ^{1}H NMR spectra were recorded. Cysteamine (~10 equivalents) was added to the solution and the proton spectra of the mixture were recorded at a few time-points from 5 minutes to 1 day, depending on the reactivity of the compound as judged by the spectra obtained.

Protein mass spectroscopy analysis: 10 µM of the test compounds (or DMSO as a negative control) were incubated with 5 µM of unactivated MNK1/2 in Tris buffer for 3 hours at room temperature on a shaker before being subjected to MS analysis. The treated protein samples were analyzed on an Agilent LC 1200 series coupled to an Agilent LC/TOF 6224. 5 µL of the protein sample were eluted from a Zorbax column (SB-C18 5 µM, 150x0.5 mmID) at a flow rate of 20 µL/min, column temperature of 45 °C, with a linear gradient from 10% to 90%B over 7 min, mobile phase A being 0.2% formic acid in water and mobile phase B being 0.2% formic acid in acetonitrile. The spectra were acquired with a dual ESI source having a capillary voltage set at 3500V and using the reference mass of 922.009798 and 121.050873. The molecular weight of the proteins was determined by deconvolution using Agilent MassHunter Qualitative Analysis B.04 Software.

MNK kinase activity assay: MNK1 and MNK2 genes were cloned into PGEX-6P-1 (cloning sites: BamHI-SalI) by GenScript USA Inc. The plasmids were transformed into BL21 (DE3) cells. MNK 1 and MNK 2 were induced with 1.0 mM IPTG and grown at 25 °c for 6 hours. The proteins were purified using affinity chromatography on a Bio-Scale Mini Profinity GST cartridge. The GST tag was cleaved using PreScission protease. The proteins were concentrated to 10-15 mg/mL (equivalent to 285-428 µM) and flash-frozen in liquid nitrogen and stored in buffer at -80 ^{o}C. The IMAP 10000-tp FP assay kit was purchased from Molecular Devices and JH3 peptide (5-FAM-TATKSG**S**TTKNRFVV-NH_2) was synthesized by GenScript USA Inc. Buffer B contains: 20 mM HEPES/KOH pH 7.4, 10 mM $MgCl_2$, 0.5 mM DTT. IMAP progressive binding solution was prepared freshly on the assay day according to the description in the assay kit.

2.0 µL of 5.0 µM activated MNK 2 (or 4.0 µL of 5.0 µM activated MNK 1), 2.0 µl of compound at 10X concentrations, and buffer B to a total volume of 20 µL were added to a 384-well plate and incubated at 30 ^{o}C for 1 hour. 2.0 µL of 1.0 µM JH3 and 2.0 µL of 200 µM ATP were then added to each well and the plate was incubated at 30 ^{o}C for another 2 hours for MNK2 (or 6 hours for MNK1). The reaction was terminated with 60 µL of 1xIMAP binding solution. After 30 min incubation at room temperature, fluorescence polarization was measured by the Safire^2 microplate reader. The raw data were analyzed using GraphPad Prism 5.0.
Alphascreen® SureFire® eIF4E p-Ser209 assay: Hela cells were cultured in DMEM (Invitrogen) supplemented with 10% **(***v/v*) Fetal Bovine Serum (FBS)-US grade (Invitrogen), 100 UmL-1 penicillin (Invitrogen), and 100 gmL-1 streptomycin (Invitrogen). Cells were cultured in a humidified 5% CO_2 incubator at 37 °C, and then seeded at 30,000 cells per well in a 96-well plate and incubated for 24 hours before treatment with compounds. After 24 hours, the culture media was removed and the cells were treated with various concentration of compound (61nM – 100 µM) at a final concentration of 1% DMSO for 2 hours in a humidified 5% CO_2 incubator at 37 °C prior to cell lysis. The compounds were maintained in the DMEM media (without FBS and antibiotics) for the duration of the experiment. At the end of the 2 hours incubation, the medium was removed and the cells were lysed with freshly prepared 1X lysis buffer (supplied in the Alphascreen® SureFire® Kit) and agitated on a plate shaker at 350 rpm for 20 mins at room temperature.

The Alphascreen® SureFire® assay (Perkin Elmer, cat#TGREIF4S500) was performed in 384 well white Proxiplates according to the manufacturer instructions. 4 µL of lysate were incubated with 5 µL of Acceptor Mixture for 2 hours at room temperature before 2 µL of Donor Mixture was added under subdued lighting. Plates were further incubated for 2hours at room temperature and read using a PerkinElmer EnVision plate reader using AlphaScreen® settings. Raw data were presented as 'Envision Units'. Percentage inhibition were calculated based on the % reduction in 'Envision Units' after treatment with 10 µM of compound as compared to 1% DMSO control. IC_{50} measurements were performed using GraphPad Prism Version 5.0.

Cell anti-proliferative assay (WST-8): HeLa, A549, K562 and MCF-7 cells were grown in RPMI media (Invitrogen) with 5% fetal bovine serum added with 1% penicillin- streptomycin (Invitrogen). The cells were grown till 70% confluency, cultured in a humidified 5% CO_2 incubator at 37 °C, then harvested and seeded at 7500 cells per well in a 96 well plate. The plate was incubated for the cells to attach for 24 hours. The drug was dosed into the plate with 8 dilution points starting from 100 µM with 3 fold serial dilution. Drug controls and blank controls with media alone were prepared alongside the sample. The plate was then left to incubate for another 48 hours. After that, the reagent from the WST-8 cell proliferation analysis kit (Bio Vision) was added to all wells and was left to incubate according to manufacturer's protocol for 4 hours. At the end of the incubation time, the absorbance of the added reagent was measured with a spectrophotometer (Tecan Infinte M1000) at 440 nm. Viable cells will cleave the tetrazolium salt in the reagent to formazan by cellular mitochondrial dehydrogenase. The eventual formazan dye is measured and is proportional to the amount of viable cells. The raw data were analyzed again using GraphPad Prism 5.0.

Acknowledgements

We thank Agency for Science, Technology and Research (A*STAR), Singapore for supporting this work. We are grateful to Prof. Mei Lin Go at National University of Singapore for valuable discussions. We also thank Ms Boping Liu from Experimental Therapeutic Centre (ETC) for supplying MNK1/2 proteins and technical assistance.

Keywords: resorcylic acid lactone · covalent kinase inhibitor · enamide · MNK kinase· Michael acceptor· anti-cancer

[1] M. H. Potashman, M. E. Duggan, *J. Med. Chem.* **2009**, *52*, 1231-1246.
[2] J. Singh, R. C. Petter, T. A. Baillie, A. Whitty, *Nat. Rev. Drug Discovery*, **2011**, *10*, 307-317.
[3] D. S. Johnson, E. Weerapana, B. F. Cravatt, *Future Med. Chem.* **2010**, *2*, 949–964.
[4] a) J. Singh, E. M. Dobrusin, D. W. Fry, T. Haske, A. Whitty, D. J. McNamara, *J. Med. Chem.* **1997**, *40*, 1130-1135; b) W. Zhou, W. Hur, U. McDermott, A. Dutt, W. Xian, S. B. Ficarro, J. Zhang, S. V. Sharma, J. Brugge, M. Meyerson, J. Settleman, N. S Gray, *Chemistry & Biology* **2010**, *17*, 285-295; c) A. Wissner, E. Overbeek, M. F. Reich, M. B Floyd, B. D. Johnson, N. Mamuya, E. C. Rosfjord, C. Discafani, R. Davis, X. Shi, S. K. Rabindran, B. C. Gruber, F. Ye, W. A. Hallett, R. Nilakantan, R. Shen, Y.-F. Wang, L. M. Greenberger, H.-R. Tsou. *J. Med. Chem.* **2003**, *46*, 49–63; d) M. Hagel, *Nature Chem. Biol.* **2010**, *7*, 22–24; e) M. S. Cohen, C. Zhang, K. M. Shokat, J. Taunton, *Science* **2005**, *308*, 1318-1321.
[5] I. M. Serafimova, M. A. Pufall, S. Krishnan, K. Duda, M. S. Cohen, R. L. Maglathlin, J. M. McFarland, R. M. Miller, M. Frodin, J. Taunton *Nat. Chem. Biol.* **2012**,*8*, 471-476.
[6] C.-U. Lee, T. N. Grossmann, *Angew. Chem. Int. Ed.* **2012**, *51*, 2-4.
[7] a) T. Ueda, M. Sasaki, A. J. Elia, I. I. C. Chio, K. Hamada, R. Fukunaga, T. W. Mak, *PNAS* **2010**, *107*, 13984-13990; b) I. Reymundo, O. Rabal, A. Salgado, A. Corrionero, J. Fominaya, J. Pastor, J. R Bischoff, *J. Med. Chem.* **2010**, *53*, 6618–6628.
[8] a) N. Hay, *PNAS* **2010**, *107*, 13975–13976; b) S. Lim, T. Y. Saw, M. Zhang, M. R. Jane, K. Nacro, J. Hill, A. Q. Lim, C.-T. Chang, D. A. Fruman, D. A. Rizzieri, S. Y. Tan, H. Fan, C. T. H. Chuah, S. T. Ong. *PNAS*, **2013**, E2298-2307.
[9] A. J. Waskiewicz, A. Flynn, C. G. Pround, J. A. Cooper, *EMBO J.* **1997**, *16*, 1909 – 1920.
[10] A. Schirmer, J. Kennedy, S. Murli, R. Reid, D. V. Santi, *PNAS* **2006**, *103*, 4234–4239.
[11] a) A. Zhao, S. H. Lee, M. Mojena, R. G. Jenkins, D. R. Patrick, H. E. Huber, M. A. Goetz, O. D. Hensens, D. L. Zink, D. Vilella, A.W. Dombrowski, R. B. Lingham, L Huang, *J. Antibiot.* **1999**, *52*, 1086–1094; b) P. Y. Dakas, R. Jogireddy, G. Valot, S. Barluenga, N. Winssinger, *Chem. Eur. J.* **2009**, *15*, 11490-11497.
[12] J. Ninomiya-Tsuji, T. Kajino, K. Ono, T. Ohtomo, M. Matsumoto, M. Shiina, M. Mihara, M. Tsuchiya, K. Matsumoto, *J. Biol. Chem.* **2003**, *278*, 18485–18490.
[13] G. Rastelli, R. Rosenfeld, R. Reid, D. V Santi, *J. Strut. Bio.* **2008**, *164*, 18-23.
[14] a) J. Wu, F. Powell, N. A. Larsen, Z. Lai, K. F. Byth, J. Read, R.-F. Gu, M. Roth, D. Toader, J. C. Saeh, H. Chen, *ACS Chem. Bio.* **2012***, 8*, 643-650;

b) R. Scholz, C. L. Sidler, R. F. Thali, N. Winssinger, P. C. F. Cheung, D. Neumann, *J. Bio. Chem* **2010**, *285*, 25753-25766; c) A. Hamidi, V. von Bulow, R. Hamidi, N. Winssinger, S. Barluenga, C.-H. Heldin, M. Landstrom, *J. Bio. Chem*. **2012**, *287*, 123-133.

[15] a) N. Winssinger, S. Barbluenga, *Chem. Commun.* **2007**, *1*, 22-36; b) S. Barbluenga, P. Y. Dakas, M. Boulifa, E. Moulin, N. Winssinger, *C. R. Chimie* **2008***, 11*, 1306-1317; c) N. Winssinger, S. Barluenga, PCT WO **2011**/036299 A1.

[16] a) Y. Shen, R. Boivin, N. Yoneda, H. Du, S. Schiller, T. Matsushima, M. Goto, H. Shirota, F. Gusovsky, C. Lemelin, Y. Jiang, Z. Zhang, R. Pelletier, M. Ikemori-Kawada, Y. Kawakami, A. Inoue, M. Schnaderbeck, Y. Wang *Bioorg. Med. Chem. Lett.* **2010**, *20*, 3155–3157; b) M. Goto, J. Chow, K. Muramoto, K. Chiba, S. Yamamoto, M. Fujita, H. Obaishi, K. Tai, Y. Mizui, I. Tanaka, D. Young, H. Yang, Y. J. Wang, H. Shirota, F. Gusovsky, *J. Pharmacol. Exp. Ther.* **2009**, *331*, 485–495.

[17] Eisai Inc. Clinicaltrials.gov, Identifier: NCT00794781, 19 Nov. **2008**.

[18] a) S. Barluenga, R. Jogireddy, G. K. Koripelly, N. Winssinger, *ChemBioChem*, **2010**,*11*, 1692-1699; b) E. Leproult, S. Barluenga, D. Moras, J.-M. Wurtz, N. Winssinger, *J. Med. Chem.* **2011**, *54*, 1347-1355.

[19] C. Zambaldo, K. K. Sadhu, G. Karthikeyan, S. Barluenga, J.-P. Daguer, N. Winssinger, *Chem. Sci.* **2013**, *4*, 2088-2092.

[20] J. Xu, A. Chen, M.-L. Go, K. Nacro, B. Liu, C. L. Chai, *ACS Med. Chem. Lett.* **2011**, *2*, 662-666.

[21] R. Jauch, S. Jäkel, C. Netter, K. Schreiter, B. Aicher, H. Jäckle, M. C. Wahl, *Structure* **2005**, *13*, 1559–1568.

[22] a) R. Jogireddy, P. Y. Dakas, G. Valot, S. Barluenga, N. Winssinger, *Chem. Eur. J.* **2009**, *15*, 11498-11506; b) R. Jogireddy, S. Barluenga, N. Winssinger, *ChemMedChem.* **2010**, *5*, 670-673.

[23] J. Tan, Z. Zhang, Z. Wang, *Org. Biomol. Chem.* **2008***, 6*, 1344-1348.

[24] C. Napolitano, P. McArdle, P. V. Murphy, *J. Org. Chem.* **2010**, *75*, 7404–7407.

[25] C. Avonto, O. Taglialatela-Scafati, F. Pollastro, A. Minassi, V. Di Marzo, L. De Petrocellis, G. Appendino, *Angew. Chem. Int. Ed.* **2011**, *50*, 467 –471.

[26] M. Liniger, C. Neuhaus, T. Hofmann, L. Fransioli-Ignazio, M. Jordi, P. Drueckes, J. Trappe, D. Fabbro, K.-H. Atlmann, *ACS Med. Chem. Lett.* **2010**, *2*, 22 - 27.

[27] J. L. Parra, M. Buxade, C. G. Proud, *J. Biol. Chem.* **2005**, *280*, 37623-37833.

[28] S. Ayers, T. N. Graf, A. F. Adcock, D. J. Kroll, S. Matthew, E. J. C. de Blanco, Q. Shen, S. M. Swanson, M. C. Wani, C. J. Pearce, N. H. Oberlies, *J. Nat. Prod.* **2011**, *74*, 1126-1131.

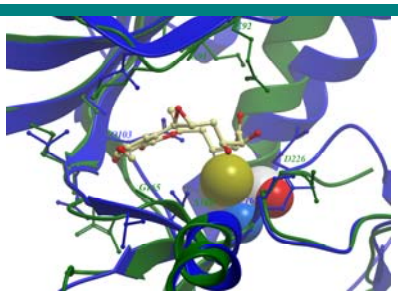
Received: ((will be filled in by the editorial staff))
Published online: ((will be filled in by the editorial staff))

Entry for the Table of Contents (Please choose one layout)

Layout 1:

FULL PAPERS

Reactivity tunable Michael acceptor: Enamide analogues of resorcylic acid lactone (RAL) as novel covalent inhibitors of MNK1/2 kinases were developed in this study. The covalent binding ability of such enamides can be tuned by attaching an electron-withdrawing motif to enhance its reactivity towards the cysteine residues at the MNK1/2 binding sites.



Jin Xu, Anqi Chen, Joma Joy,Vanessa Joanne Xavier, Esther H. Q. Ong, Jeffrey Hill, and Christina L. L. Chai**

Page No. – Page No.

Rational Design of Novel Resorcylic Acid Lactone Analogues as Covalent MNK1/2 Kinase Inhibitors by Tuning the Reactivity of an Enamide Michael Acceptor