Bioorganic & Medicinal Chemistry 16 (2008) 7399–7409

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

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

Illudalic acid as a potential LAR inhibitor: Synthesis, SAR, and preliminary studies on the mechanism of action

Qing Ling [a,†], Yue Huang [a,†], Yueyang Zhou [b], Zhengliang Cai [a], Bing Xiong [a], Yahui Zhang [b], Lanping Ma [a], Xin Wang [a], Xin Li [a], Jia Li [b,*], Jingkang Shen [a,*]

[a] State Key Laboratory of Drug Research, Shanghai Institute of Materia Medica, Chinese Academy of Sciences, 555 Zuchongzhi Road, Shanghai 201203, PR China
[b] National Center for Drug Screening, 189 Guoshoujing Road, Shanghai 201203, PR China

ARTICLE INFO

Article history:
Received 24 April 2008
Revised 8 June 2008
Accepted 10 June 2008
Available online 13 June 2008

Keywords:
Human leukocyte common antigen-related phosphatase (LAR)
Illudalic acid
Inhibitor
Mechanism
Structure–activity relationship (SAR)
Synthesis

ABSTRACT

A novel synthesis of the human leukocyte common antigen-related (LAR) phosphatase inhibitor, illudalic acid, has been achieved by a route more amenable to structure modifications. A series of simpler analogues of illudalic acid was synthesized and evaluated for potency in inhibiting LAR. The structure–activity relationship (SAR) study has shown that the 5-formyl group and the hemi-acetal lactone are crucial for effective inhibition of LAR activity, and are the key pharmacophores of illudalic acid. The fused dimethylcyclopentene ring moiety evidently helps to enhance the potency of illudalic acid against LAR. A preliminary study of the mechanism of action of illudalic acid against LAR was conducted using electrospray ionization mass spectrometry (ESI-MS) and molecular docking techniques. The results are in full agreement with the described mechanism.

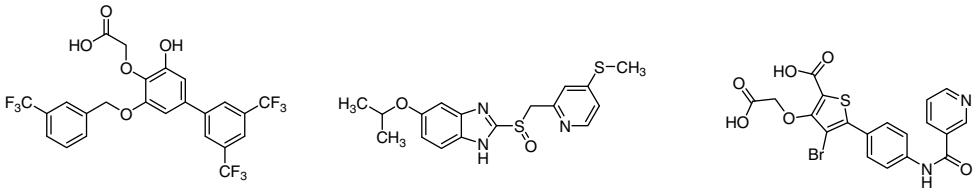
1. Introduction

Protein tyrosine phosphatases (PTPs) remove phosphate from tyrosine-phosphorylated proteins and protein tyrosine kinases (PTKs) phosphorylate cellular substrates at tyrosine residues.[1–5] PTPs, PTKs, and their corresponding substrates are integrated within elaborate signal transducing networks that play significant roles in regulating cellular growth, differentiation, metabolism, cell cycles, cell–cell communication, cell migration, gene transcription, ion-channel activity, the immune response, and survival.[6–11] PTPs, which contain a highly conserved active site (i.e., the pTyr-binding site) with the signature motif (H/V)**C**(X)$_5$**R**(S/T),[12,13] are considered to be a superfamily that consists of specific phosphatases, dual specificity phosphatases, and the low-molecular-weight phosphatases. Tyrosine-specific phosphatases can be further divided into two groups: receptor-like and intracellar PTPs. Following the progress in defining biological function, many PTPs have been proposed as targets for therapeutic intervention, such as anticancer, regulation of regenerative neurite outgrowth, immune response, and treatment of type II diabetes, obesity, etc.[14–24] However, compared with PTK inhibitors, which have been developed as therapeutic agents, the task of identifying substrates for PTPs still presents a challenge.[25] Therefore, potent and selective PTP inhibitors would be valuable as a means of enhancing our understanding of the interaction between PTPs and substrates.

Among the known PTPs, human leukocyte common antigen-related phosphatase (LAR)[26–30] is a receptor-like transmembrane phosphatase whose extracellular structure includes three immunoglobulin-like domains and eight fibronectin type III-like domains. The intracellular structure consists of two tandem phosphatase catalytic domains, a membrane proximal domain (D1), and a membrane distal domain (D2). Recently, LAR raised a lot of interest as it was shown to regulate neurite growth and nerve regeneration in a transgenic animal model.[31–34] In addition, LAR is potentially involved in the development of diabetes and cancer, and may be an attractive target in the treatment of those diseases.[35–38] Although LAR is likely to become significant as a drug target in various human pathologies, very little is known about the mechanisms of action of LAR at present.[29] Hence, the development of potent enzyme-specific inhibitors is particularly important because they may serve both as tools to study the role of LAR and as therapeutic agents. However, no novel compounds that selectively inhibit LAR have been reported[39–44] despite the identification of myriad inhibitors of other PTPs such as PTP1B[44–57] (Fig. 1). By high throughput screening of a library of 44,145 synthetic compounds and 19,960 natural product extracts, we have

* Corresponding authors. Tel.: +86 21 50801313; fax: +86 21 50801522 (J.L.); tel.: +86 21 50806600; fax: +86 21 50807088 (J.S.).
E-mail addresses: jli@mail.shcnc.ac.cn (J. Li), jkshen@mail.shcnc.ac.cn, kxiaosimm123@163.com (J. Shen).
† These two authors contributed equally to this work.

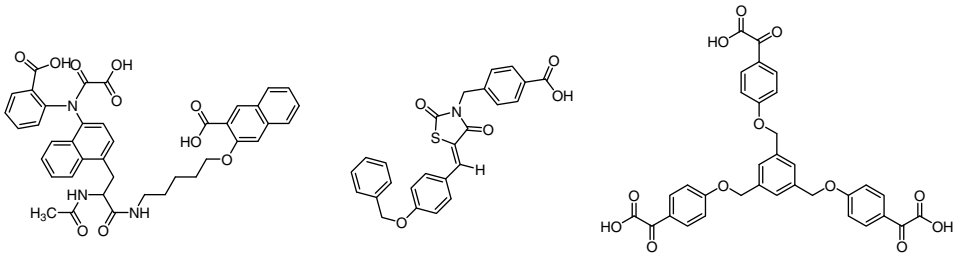
0968-0896/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmc.2008.06.014



IC_{50}[43] PTP1B 2.0 μM; LAR 116 μM; CD45 0.28 μM

IC_{50}[39] PTP1B 55.4 μM; LAR 18.2 μM; TCPTP 0.68 μM

IC_{50}[41] PTP1B 0.82 μM; LAR 320 μM



Ki[44] PTP1B 0.022 μM; LAR 1.3 μM; TCPTP 0.049 μM

IC_{50}[42] PTP1B 1.6 μM; LAR 27 μM

IC_{50}[40] PTP1B 13 μM; LAR 41 μM; Yersinia PTPase 1.7 μM

Figure 1. Structures of some reported none-selective LAR inhibitors.

previously identified illudalic acid **1** isolated from the Basidiomycete, *Clitocybe illudens*, as the first potent selective small molecular inhibitor of LAR (IC_{50} = 1.1 μM).[58] However, the quantity of illudalic acid isolated from natural extracts was very limited and resupplies of the compound for further biological evaluations had to be accomplished via a total synthesis. Total synthesis of **1** was achieved by Woodward starting from commercially available indan,[59] but we decided to take advantage of readily available starting material **2**[60] for SAR study. Herein, we give a full account of our study of the design, synthesis, and structure–activity relationship (SAR), as well as preliminary studies on the mechanism of action of illudalic acid against LAR.

2. Results and discussion

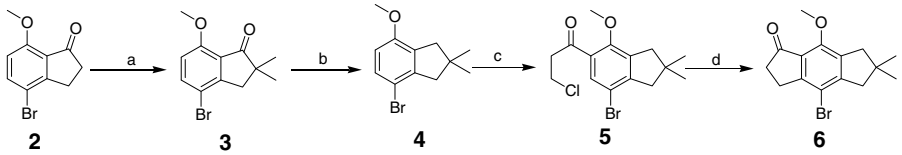
2.1. Chemistry

In our synthetic approach, we took good advantage of the regioselective Friedel–Crafts acylation reaction of suitably substituted bromo-dihydroindene with 3-chloropropanoyl chloride, followed by Friedel–Crafts alkylation for the construction of the key carbon skeleton **6** (Scheme 1).

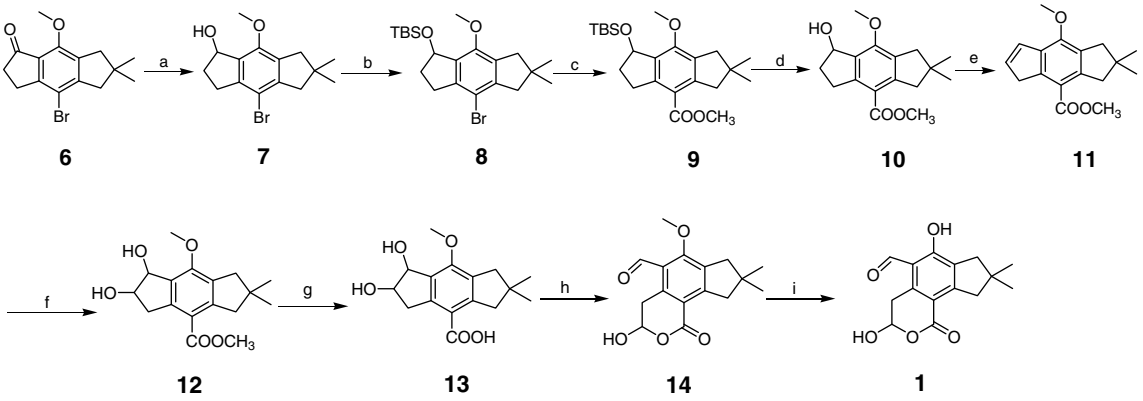
Dihydroindan-1-one **2** equipped with methoxyl and bromo substituents was obtained according to the method of Michael et al.,[60,61] starting from readily available 3-methoxycinnamic acid. The alkylation reaction of **2** with excess methyl iodide in the presence of NaH produced its dimethyl derivative **3**, which was transformed into bromo-dihydroindene **4** via Clemmensen reduction by refluxing in an ethanolic solution of concentrated hydrochloric acid in the presence of excess amalgamated zinc. Friedel–Crafts acylation of compound **4** with 3-chloropropionyl chloride gave the chloro-containing ketone **5**. Cyclization of **5** in concentrated sulfuric acid at 90 °C for 8 h led to the key carbon skeleton **6**.

The conversion of hydrindacen-1-one **6** to the target product **1** is shown in Scheme 2. The cyclic ketone **6** was reduced with sodium borohydride in methanol at 0 °C to give the acid-labile alcohol **7**, the hydroxy group of which was protected by treatment with *tert*-butyldimethylsilyl chloride (TBSCl), 4-dimethylaminopyridine (DMAP), and imidazole in dry CH_2Cl_2 to give TBS ether **8**. The halogen–metal exchange in **8** was accomplished using metalation by treatment with *n*-BuLi at −78 °C, followed by addition of an excess of methyl chloroformate to produce **9** in excellent yield.[62,63] The TBS protecting group was removed by treatment with HIO_4 solution in THF–H_2O giving **10**.

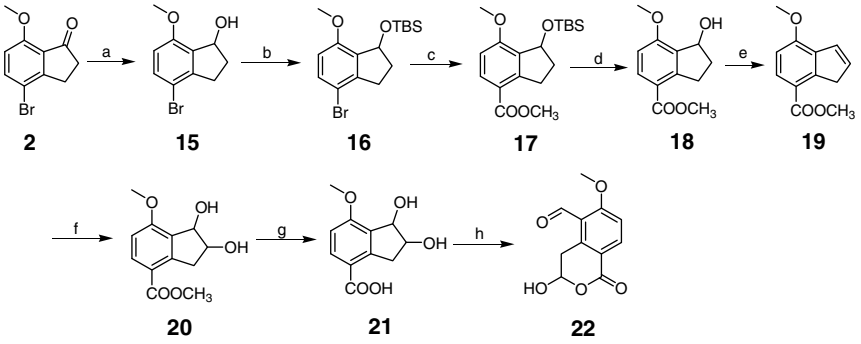
The monohydroxyl product **10** dehydrated to afford olefin **11** as white crystal in the presence of *p*-toluenesulfonic acid (*p*-TsOH). Dihydroxylation of the olefin occurred efficiently by the treatment of **11** with AD-mix-α in the presence of methanesulfonamide in *t*-BuOH–H_2O,[64] followed by saponification with 2 N KOH in ethanol to generate the requisite acid **13**. Subjecting the above acid **13** to



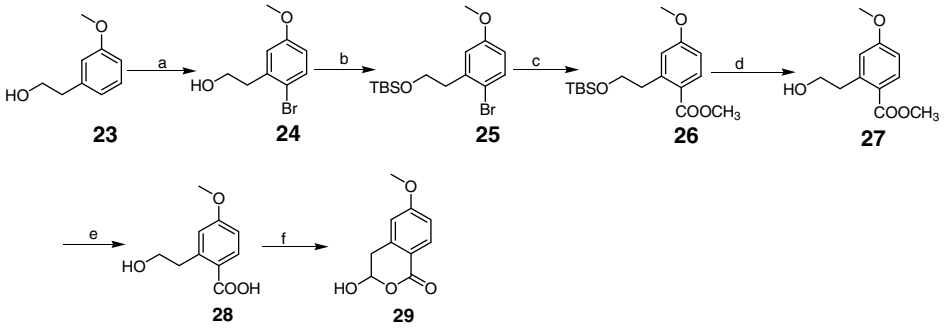
Scheme 1. Reagents and conditions: (a) CH_3I, NaH, THF, 0 °C to rt; (b) Zn/Hg, concd HCl, C_2H_5OH, reflux; (c) $SnCl_4$, $ClCH_2CH_2COCl$, CH_3NO_2, 0 °C to rt; (d) concd H_2SO_4, 90 °C.



Scheme 2. Reagents and conditions: (a) $NaBH_4$, CH_3OH, 0 °C; (b) TBSCl, imidazole, DMAP, CH_2Cl_2, rt; (c) *n*-BuLi, $ClCO_2CH_3$, THF, −78 °C to rt; (d) HIO_4, THF–H_2O, rt; (e) *p*-TsOH, toluene, 80 °C; (f) AD-mix-α, $CH_3SO_2NH_2$, *t*-BuOH–H_2O, 0 °C to rt; (g) 2 N KOH, C_2H_5OH, reflux; (h) $NaIO_4$, 1,4-dioxane–H_2O, rt; (i) BBr_3, CH_2Cl_2, −78 °C to rt.



Scheme 3. Reagents and conditions: (a) $NaBH_4$, CH_3OH, 0 °C; (b) TBSCl, imidazole, DMAP, CH_2Cl_2, rt; (c) *n*-BuLi, $ClCO_2CH_3$, THF, −78 °C to rt; (d) HIO_4, THF–H_2O, rt; (e) *p*-TsOH, toluene, 80 °C; (f) AD-mix-α, $CH_3SO_2NH_2$, *t*-BuOH–H_2O, 0 °C to rt; (g) 2 N KOH, C_2H_5OH, 80 °C; (h) $NaIO_4$, 1,4-dioxane–H_2O, rt.



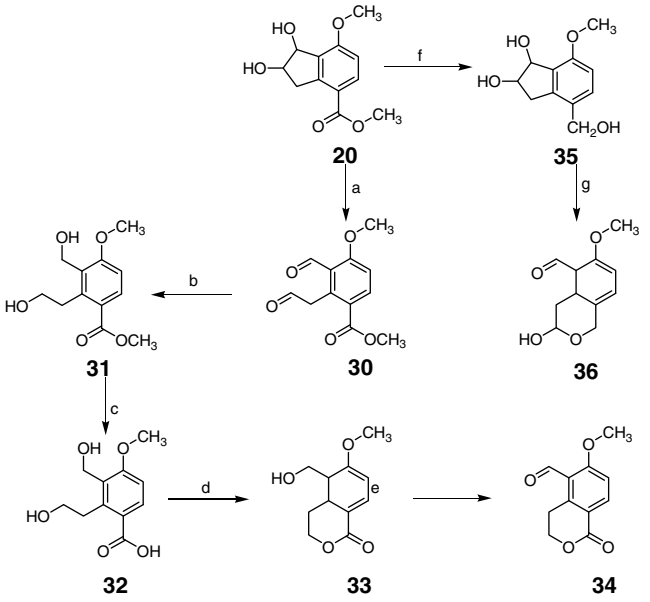
Scheme 4. Reagents and conditions: (a) Br_2, $CHCl_3$, 0 °C; (b) TBSCl, imidazole, DMAP, CH_2Cl_2, rt; (c) *n*-BuLi, $ClCO_2CH_3$, THF, −78 °C to rt; (d) HIO_4, THF–H_2O, rt; (e) 2 N KOH, C_2H_5OH, 80 °C; (f) PCC, CH_2Cl_2, rt.

sodium metaperiodate furnished the dialdehyde, which existed as the cyclized lactol **14** both in the solid state and in solution. Treatment of illudalic acid methyl ether **14** with boron tribromide in methylene chloride gave the major product, illudalic acid **1**. The route lends itself to the preparation of various analogues for a possible biological evaluation.

The simpler analogue **22** was prepared directly from **2** through sequential manipulations similar to those performed in converting **6** to **14** (Scheme 3). The synthetic pathway affording the final compound **29** is depicted in Scheme 4. Treatment of the starting material **23** with bromine gave the product **24**, which was then protected as **25** following the procedure for **1**. Likewise, the conversion of **25** to **26** was performed by bromo-metal exchange reaction. After deprotection and saponification, the corresponding product **28** was subjected to oxidation over pyridinium chlorochromate (PCC) to furnish **29**. Scheme 5 illustrates the synthesis of analogues **30**–**34** and **36** from their precursor **20**. Firstly, **20** was converted to alcohol **35** by reduction with lithium aluminium hydride (LAH), followed by oxidation cleavage to form the target compound **36**. The same oxidation cleavage conditions applied to **20** gave the desired **30**, which was reduced by $NaBH_4$ to generate the diol **31**. The saponification of **31** converted **31** to **32**, which, in turn, was transformed into the lactone **33** in high yield by treatment with *p*-TsOH. The aldehyde product **34** was derived from **33**.

2.2. Biological results

All analogues of illudalic acid were measured for their potency in inhibiting LAR with *p*-nitrophenyl phosphate (*p*NPP) as a substrate at room temperature and pH 6.0 according to procedures



Scheme 5. Reagents and conditions: (a) $NaIO_4$, 1,4-dioxane–H_2O, rt; (b) $NaBH_4$, CH_3OH, 0 °C; (c) 2 N KOH, C_2H_5OH, 80 °C; (d) *p*-TsOH, CH_2Cl_2, rt; (e) PCC, CH_2Cl_2, rt; (f) LAH, Et_2O, reflux; (g) $NaIO_4$, 1,4-dioxane–H_2O, rt.

described previously.[58] The synthetic illudalic acid (IC_{50} = 1.3 μM) displayed identical activity and selectivity as the natural product. The results are shown in Table 1.

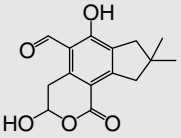
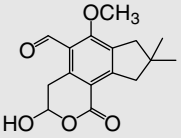
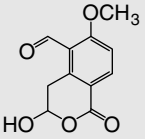
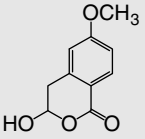
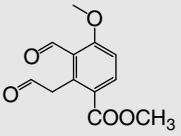
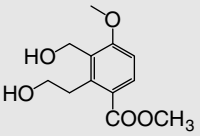
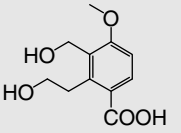
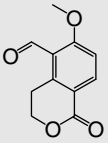
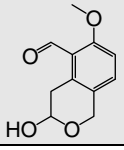
The methyl ether derivative **14** with an IC_{50} of 1.5 μM exhibited almost the same potency against LAR as its parent **1**. To understand the SAR of illudalic acid, we initially prepared its simpler analogue **22**, in which the fused dimethylcyclopentene ring of **14** was removed, resulting in a 14-fold decrease in potency against LAR (IC_{50} = 21.85 μM).

On the basis of the structure of compound **22**, the 5-formyl and 3-hydroxyl groups were removed to afford compounds **29** and **34**, respectively, which led to a significant loss of activity. On the other hand, compound **36** in which the 1-carbonyl group was removed showed poor activity. To further understand the key structure scaffold, compounds **30**–**32** were synthesized. In fact, structure **37** is the open style of hemi-acetal lactone structure moiety found in compound **22** (Scheme 6). With respect to the structure of **37**, we prepared compounds **30**–**32**, in which two aldehyde groups were reduced to two hydroxymethyl groups, the carboxylic acid group was converted to a methyl ester or two aldehyde groups, and the carboxylic acid groups were converted to two hydroxymethyl and methyl ester groups simultaneously. To our surprise, none of these compounds showed any activities against LAR up to 100 μM. The above results strongly implied that the 5-formyl group and the hemi-acetal lactone are crucial for effective inhibition of LAR activity, and are an absolute requirement for the activity of illudalic acid. The fused dimethylcyclopentene ring moiety evidently helps to enhance the potency of illudalic acid against LAR. The SAR of illudalic acid provides quite useful information for designing analogues with more potent activity against LAR.

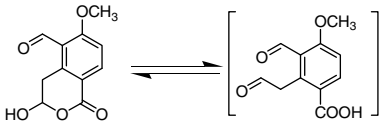
2.3. Mechanism of action

In testing bioactivity, we found an interesting phenomenon: compound **1** and its analogues showed inhibitory activity against LAR only after these compounds were pre-incubated with LAR enzyme at pH 8 and the enzyme activity was then analyzed at pH 6. Otherwise, none of these compounds show any LAR inhibitory activity if they are incubated and tested at pH 6.

Table 1
LAR-inhibiting activity of inhibitor illudalic acid analogues **14**, **22**, **29**–**32**, **34**, and **36**

Compound	Structure	MW	IC_{50}[b] (μM)
1		276	1.30 ± 0.06
14		290	1.53 ± 0.29
22		222	21.85 ± 1.02
29		194	<5% at 20 μg/mL[a]
30		236	<5% at 20 μg/mL[a]
31		240	<5% at 20 μg/mL[a]
32		226	<5% at 20 μg/mL[a]
34		206	<5% at 20 μg/m[a]
36		208	20% at 20 μg/mL[a]

[a] % Inhibition at the given concentration.
[b] IC_{50} values were determined by regression analyses and expressed as means ± SD of three replicates.



Scheme 6.

We performed two experiments to prove **1** as an irreversible inhibitor of LAR. First, we plotted enzyme activity versus reaction time. As shown in Figure 2A, without the inhibitor, the plot is a lin-

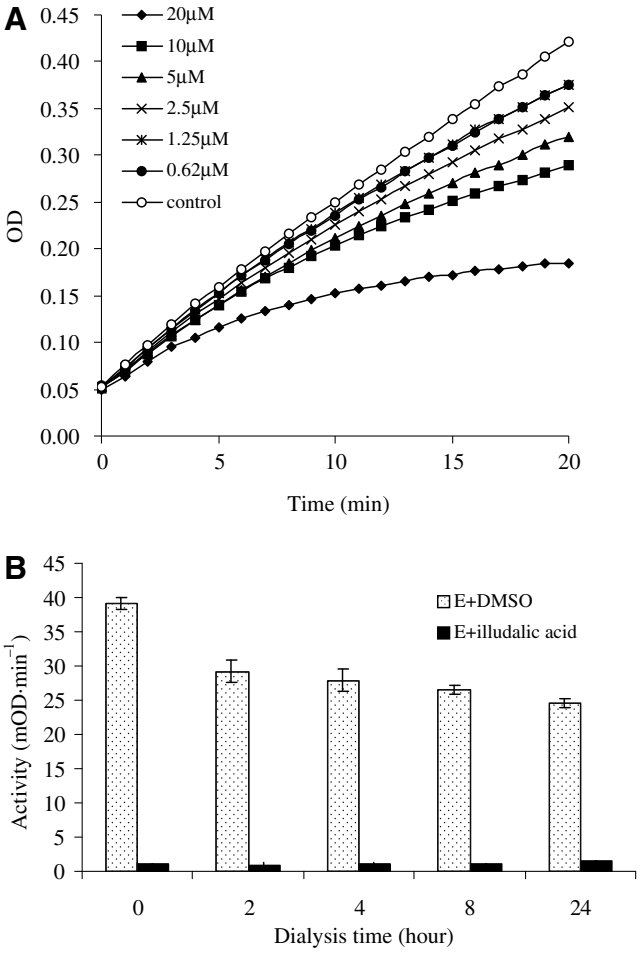
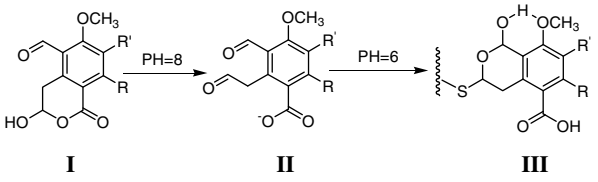


Figure 2. Characterization of illudalic acid. (A) Time-dependent inhibition of LAR by illudalic acid. Progress curves for LAR reaction with *p*NPP in the presence of increasing concentrations of illudalic acid. The assay system containing 2% DMSO was defined as control. (B) Irreversible inhibition of LAR by illudalic acid. The activities of PTP-LAR pre-incubation with illudalic acid or DMSO were detected after dialysis for the indicated time.

ear line. However, in the presence of various concentrations of **1**, the plot shows a curve for a while and a straight line later, and the slope of the straight line is smaller than the plot without inhibitor and will decrease dependent on the increased concentrations. This result suggested **1** as an irreversible or a slow tight-binding inhibitor. To exclude the possibility that this compound is a slow tight-binding inhibitor, we dialyzed the enzyme–inhibitor complex and examined the recovery of the enzyme activity. As shown in Figure 2B, the activity of LAR could not recover after 24 h of dialysis. With these results, we can suggest that the inhibitor inhibits LAR irreversibly.

This implies that the interaction of inhibitor and LAR involves covalent binding. Enzymological and structural biological studies have led to the explanation that phosphatases in the PTP superfamily share a common mechanism of catalysis that involves the nucleophilic Cys residue in the PTP signature motif.[65,66] At the same time, the SAR of **1** revealed that the 5-formyl group and the hemi-acetal lactone group are crucial for inhibitory activity against LAR. On the basis of the above information we propose a mechanism of action of illudalic acid and its analogues against LAR (Scheme 7).

The hemi-acetal lactone moiety of structure **I** firstly opens up via hydrolysis when incubated with LAR enzyme at pH 8. The newly produced free acetaldehyde group of structure **II** undergoes



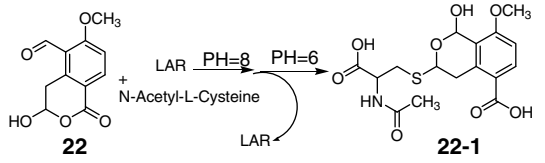
Scheme 7.

nucleophilic attack by the thiol group of the Cys residue[65,66] at the base of the active site leading to the formation of a hemi-acetal. The hydroxyl group of the hemi-acetal further attacks the 5-formyl group to give a new hemi-acetal and results in a fused six-membered ring structure. The H-bond binding between the hydroxyl group of the new hemi-acetal and the adjacent oxygen of the methoxyl group on the aryl moiety produces a new fused six-membered ring, thereby stabilizing the whole complex structure **III**. The interaction between the Cys residue and the inhibitor plays a critical role in irreversibly forming a covalent complex.

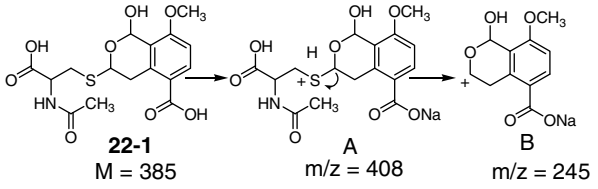
The employment of mass spectrometric techniques to illuminate the mechanistic aspects of biological events is attracting widespread attention.[67–69] These techniques are useful since they can simultaneously measure the masses of numerous products accurately, rapidly, and with great sensitivity, thus enabling the possibility of understanding the nature of intermediates and transformations in biological events.[70]

To shed light on the above action mechanism, we investigated the process by ESI-MS/MS studies of enzymatically and synthetically produced covalent complexes. The inhibitor **22** was incubated with LAR and excess LAR simulacrum *N*-acetyl-L-cysteine at pH 8 for 10 min (Scheme 8), and the pH was then adjusted to 6 (the same condition as that of the bioactivity assay). Then the product **22**-**1** of inhibitor **22** and simulacrum *N*-acetyl-L-cysteine were analyzed by ESI-MS/MS under low-resolution conditions (positive ion mode) after the denatured LAR enzyme was filtered away.

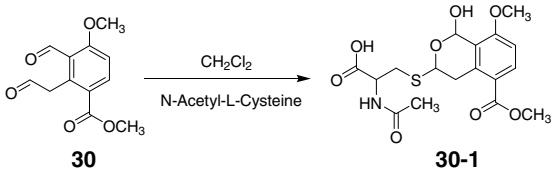
The resulting spectra correlate extremely well, indicating that the generated component is identical to the product **22**-**1**. The proposed fragmentation of the covalent complex **22**-**1** is illustrated in Scheme 9. The prominent features are observed in the fragmentation of the molecule **22**-**1**. Likewise, a synthetic version of the covalent complex **30**-**1** was prepared by the treatment of compound **30** with *N*-acetyl-L-cysteine in CH_2Cl_2 (Scheme 10) and analyzed by ESI-MS/MS under low-resolution conditions. The proposed fragmentation processes illustrated in Scheme 11 are consistent with the molecular structure of the covalent complex **30**-**1**. ESI-MS/MS



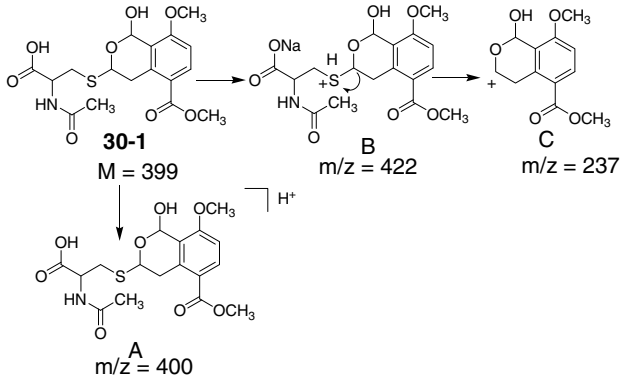
Scheme 8.



Scheme 9.



Scheme 10.

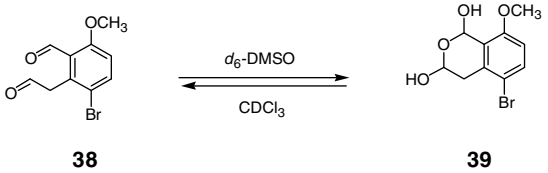


Scheme 11.

studies of the enzymatically and synthetically produced covalent complexes support this proposed mechanism of action of illudalic acid and its analogues with LAR. Another important piece of evidence also strongly sustained our proposal. The proton nuclear magnetic resonance spectrum of compound **38** was measured in $CDCl_3$; it showed two aldehydic protons at δ 10.48 ppm and δ *9.77 ppm,* respectively, which fully supported the dialdehyde structure **38**. However, when it was measured in the aprotic polar solvent, DMSO-d_6, the chemical shift signals in the spectrum were completely changed corresponding to a cyclic hemi-acetal structure **39** (Scheme 12), in which the two aldehydic protons disappeared and the two proton signals at δ 5.92 ppm and δ *5.40–5.44* ppm were consistent with a hemi-acetal structure. It is suggested that the formation of a cyclic thiohemiacetal intermediate could be the driving force for the interaction between **1** and LAR.

To further reinforce our hypothesis and gain more insight into the structural and mechanistic aspects of illudalic acid and its analogues and their interaction with LAR, a complex model of the inhibitor illudalic acid and LAR was constructed by molecular docking based on the LAR crystal structure (Brookhaven Protein Data Bank, http://www.rcsb.org./pdb).[71] The active molecule bound well into the LAR active site and formed favorable H-bonding and steric interactions with various active site amino acid residues inside the active site of LAR. The complex model and bound conformation of **1** are shown in Figure 3.

According to the proposed mechanism of action, the structure of the hemi-acetal lactone has changed to a new fused hemi-acetal ring system upon binding to LAR. Therefore we used the active structure version of **1** for docking purposes. The residue Cys1522 in the center of the LAR active site covalently binds with 3-C of **1**. The carboxyl group interacts with the amino acid residues,



Scheme 12.

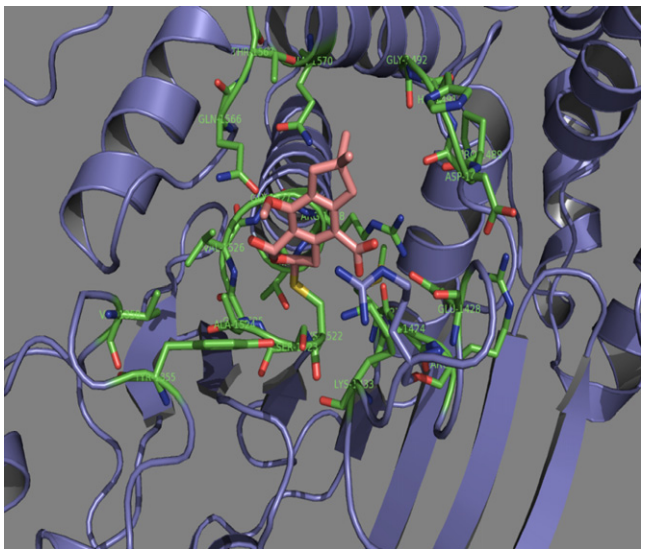


Figure 3. Three-dimensional structural model of the inhibitor, illudalic acid 1, bound to LAR derived from the molecular docking simulation.

Arg1528 and Glu1428, while the two oxygen atoms from the fused hemi-acetal ring system are surrounded by the near amino acid residues Ala1524, Gly1525, Val526, and Gly1527 and form several H-bonding interactions. At the same time, the phenolic hydroxyl group interacts with residue Gln1566 via a strong H-bond. A number of H-bonds efficiently enhance the enzyme–inhibitor interaction and stabilize the LAR–illudalic acid complex within the active site to a great extent. As expected, the molecular docking analysis elucidates the inhibition mechanism of illudalic acid and its analogues, and further validates our proposed mechanism of action. Although the results of the above studies support our assumption, the exact binding mode of the **1** with LAR should be obtained from X-ray crystallography experiments.

3. Conclusions

In summary, we have successfully synthesized illudalic acid and related simpler analogues to evaluate their SAR with respect to LAR. It has been found that the 5-formyl group and the hemi-acetal lactone play an extremely important role in effective inhibition of LAR activity, and are the key pharmacophores of illudalic acid. The phenolic hydroxy group seems to tolerate further modification, while the fused dimethylcyclopentene ring moiety evidently helps to enhance the potency of illudalic acid against LAR. The mechanism of action of illudalic acid and its analogues against LAR was elucidated by electrospray ionization mass spectrometry (ESI-MS) studies and the molecular docking method. Based on SAR, further efforts to modify illudalic acid with the aim of obtaining higher potency as well as specificity are now in progress and will be reported in due course.

4. Experimental

4.1. Chemistry

The ^{1}H NMR (300 MHz or 400 MHz) spectra were recorded on a Varian Mercury-300 or -400 High Performance Digital FT-NMR with TMS as internal standard. The chemical shifts were reported in δ (ppm) using the δ 7.26 signal of $CDCl_3$ (^{1}H NMR) as internal standard and spectra are given as shift (multiplicity, proton counts, and coupling constants). The ^{13}C NMR (100 MHz) spectra were determined with a Varian Mercury-400 High Performance Digital

FT-NMR. The LC–MS was carried out on a Thermo Finnigan LCQDECAXP and low-resolution EI-MS was measured on a MAT-95 spectrometer and HREI-MS on a MAT-77 spectrometer. TLC was carried out with glass pre-coated silica gel GF254 plates. Spots were visualized under UV light. All the solvents and reagents were used directly as obtained commercially unless otherwise noted.

4.1.1. 4-Bromo-7-methoxy-2,2-dimethyl-2,3-dihydroinden-1-one (3)

To a suspension of NaH 60% (1000 mg, 25 mmol) in dry 1,2-dimethoxyethane (5 mL) was added a solution of 4-bromo-7-methoxy-2,3-dihydroinden-1-one **2** (2000 mg, 8.3 mmol) in dry 1,2-dimethoxyethane (5 mL). After stirring for 10 min, the reaction mixture was cooled to 0 °C and CH_3I (1.55 mL, 25 mmol) was added dropwise. The mixture was allowed to warm to room temperature and stirred overnight. The reaction solution was quenched with 50 mL of water and extracted with Et_2O (3× 20 mL). The combined organic layer was washed with brine (20 mL), dried over anhydrous Na_2SO_4, and evaporated to dryness in vacuum. The residue was purified by chromatography on silica gel using petroleum ether/EtOAc to afford **3** (2000 mg, 90%) as needle crystal. Mp: 172–175 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.67 (d, 1H, J = 8.9 Hz), 6.75 (d, 1H, J = 8.9 Hz), 3.90 (s, 3H), 2.87 (s, 2H), 1.20 (s, 6H). EI-MS (m/z): 268 (M^+).

4.1.2. 4-Bromo-7-methoxy-2,2-dimethyl-2,3-dihydro-1H-indene (4)

To newly prepared amalgamated zinc (40.4 g) were added distilled water (13.4 mL), EtOH (3.8 mL), and concd HCl (40.6 mL). The mixture was stirred at a refluxing temperature. A solution of **3** (6200 mg, 23 mmol) in EtOH (48.7 mL) was added dropwise slowly to the above refluxing mixture. The resulting reaction mixture was refluxed overnight. The mixture was allowed to cool to room temperature and filtered. The solid was washed with water (3× 20 mL) and Et_2O (3× 20 mL). The organic phase was separated and the aqueous phase was extracted with Et_2O (3× 20 mL). The combined organic layers were washed with water (2× 20 mL), saturated sodium bicarbonate (20 mL), and brine (20 mL), dried over anhydrous Na_2SO_4, and evaporated to dryness under reduced pressure. The product was purified by chromatography on silica gel using petroleum ether/EtOAc to provide **4** (4900 mg, 84%) as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.20 (d, 1H, J = 8.5 Hz), 6.60 (d, 1H, J = 8.5 Hz), 3.80 (s, 3H), 2.76 (s, 2H), 2.75 (s, 2H), 1.18 (s, 6H). EI-MS (m/z): 254 (M^+).

4.1.3. 1-(7-Bromo-4-methoxy-2,2-dimethyl-2,3-dihydro-1H-inden-5-yl)-3-chloropropan-1-one (5)

Compound **4** (2000 mg, 7.8 mmol) was dissolved in the dry CH_3NO_2 (20 mL) and cooled to 0 °C. A solution of 3-chloropropionyl chloride (0.75 mL, 7.8 mmol) in dry CH_3NO_2 (5 mL) was added dropwise under argon atmosphere. The reaction mixture was allowed to warm to room temperature and stirring was continued for an additional 8 h. The mixture was quenched with 2 mL of concd HCl and 2 mL of ice and water and extracted with CH_2Cl_2 (3× 15 mL). The combined organic layer was washed with 1 N HCl (15 mL) and brine (10 mL), dried over anhydrous Na_2SO_4, and concentrated in vacuo. The product was purified by column chromatography over silica gel using petroleum ether/EtOAc as eluent to afford **5** (2050 mg, 76%) as a yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.64 (s, 1H), 3.85 (t, 2H, J = 6.7 Hz), 3.80 (s, 3H), 3.41 (t, 2H, J = 6.7 Hz), 2.90 (s, 2H), 2.70 (s, 2H), 1.20 (s, 6H). EI-MS (m/z): 344 (M^+).

4.1.4. 4-Bromo-8-methoxy-6,6-dimethyl-2,3,6,7-tetrahydro-s-indacen-1(5H)-one (6)

A solution of **5** (100 mg, 0.29 mmol) in 2 mL of CH_2Cl_2 was added dropwise to 0.237 mL of conc. H_2SO_4 at room temperature. The resulting mixture was stirred at 85 °C for 2.5 h. The mixture was allowed to warm to room temperature and 10 mL of ice and water were added, and the mixture was extracted with Et_2O (3× 10 mL). The combined organic layer was washed with saturated sodium bicarbonate (10 mL) and brine (10 mL), dried over anhydrous Na_2SO_4, and concentrated in vacuo. The product was purified by column chromatography over silica gel using petroleum ether/EtOAc as eluent to afford **6** (18 mg, 25%) as an orange crystal. Mp: 89–90 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.9 (s, 3H), 2.96 (m, 2H), 2.85 (s, 2H), 2.78 (s, 2H), 2.70 (m, 2H), 1.18 (s, 6H); ^{13}C NMR (400 MHz, $CDCl_3$): δ 203.3, 155.4, 153.8, 153.7, 135.4, 128.8, 112.3, 61.1, 49.1, 44.9, 39.7, 37.1, 28.7, 28.7, 26.5. EI-MS (m/z): 308 (M^+).

4.1.5. 4-Bromo-8-methoxy-6,6-dimethyl-1,2,3,5,6,7-hexahydro-s-indacen-1-ol (7)

A solution of **6** (250 mg, 0.81 mmol) in absolute MeOH (15 mL) was cooled to 0 °C and $NaBH_4$ (63 mg, 1.65 mmol) was added in one portion. After stirring at 0 °C for 2 h, the reaction solution was quenched with 20 mL of water and extracted with CH_2Cl_2 (3× 15 mL). The combined organic layers were washed with brine (10 mL), dried over anhydrous Na_2SO_4, and evaporated in vacuo to give **7** (249 mg, 98%) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.48 (m, 1H), 3.88 (s, 3H), 3.03 (m, 1H), 2.87 (s, 2H), 2.78 (m, 1H), 2.70 (s, 2H), 2.44 (m, 1H), 2.04 (m, 1H), 1.18 (s, 3H), 1.17 (s, 3H). EI-MS (m/z): 310 (M^+).

4.1.6. (4-Bromo-8-methoxy-6,6-dimethyl-1,2,3,5,6,7-hexahydro-s-indacen-1-yloxy)-*tert*-butyldimethylsilane (8)

A solution of **7** (100 mg, 0.32 mmol), TBSCl (97 mg, 0.64 mmol), imidazole (88 mg, 1.28 mmol) and 1.7 mg of DMAP in 15 mL of dry CH_2Cl_2 was stirred at room temperature for 36 h under argon atmosphere. The reaction was quenched with 20 mL of water and extracted with CH_2Cl_2 (3× 15 mL). The combined organic phases were washed with brine (10 mL), dried over anhydrous Na_2SO_4, and concentrated to dryness. The residue was purified by chromatography on silica gel using petroleum ether/EtOAc to obtain **8** (133 mg, 97%) as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ 5.48 (m, 1H), 3.88 (s, 3H), 3.03 (m, 1H), 2.87 (s, 2H), 2.78 (m, 1H), 2.70 (s, 2H), 2.44 (m, 1H), 2.04 (m, 1H), 1.18 (s, 3H), 1.17 (s, 3H), 0.90 (s, 9H), 0.14 (s, 3H), 0.12 (s, 3H). EI-MS (m/z): 424 (M^+).

4.1.7. Methyl 1-(*tert*-butyldimethylsilyloxy)-8-methoxy-6,6-dimethyl-1,2,3,5,6,7-hexahydro-s-indacene-4-carboxylate (9)

Compound **8** (100 mg, 0.24 mmol) was dissolved in dry THF (10 mL) and cooled to −78 °C. A solution of *n*-BuLi in hexane (1.6 M, 0.222 mL, 0.36 mmol) was added dropwise by syringe under argon atmosphere. Stirring was continued for an additional 20 min, and then the methyl chloroformate (0.055 mL, 0.72 mmol) was added dropwise. After 30 min, the reaction mixture was allowed to warm to room temperature, quenched with 10 mL of H_2O, and extracted with EtOAc (3× 15 mL). The combined organic layer was washed with saturated sodium bicarbonate (10 mL), dried over anhydrous Na_2SO_4, and concentrated in vacuo. The product was purified by column chromatography over silica gel using petroleum ether/EtOAc as eluent to afford **9** (85 mg, 90%) as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ 5.37 (m, 1H), 3.88 (s, 3H), 3.83 (s, 3H), 3.29 (m, 1H), 3.10 (m, 1H), 3.00 (s, 2H), 2.77 (s, 2H), 2.21 (m, 1H), 2.04 (m, 1H), 1.16 (s, 3H), 1.11 (s, 3H), 0.90 (s, 9H), 0.14 (s, 3H), 0.12 (s, 3H). EI-MS (m/z): 404 (M^+).

4.1.8. Methyl 1-hydroxy-8-methoxy-6,6-dimethyl-1,2,3,5,6,7-hexahydro-s-indacene-4-carboxylate (10)

A solution of **9** (100 mg, 0.25 mmol) in 10 mL of THF was treated with 1 N HIO_4 (1 mL) at room temperature. The reaction mixture was diluted with 10 mL of H_2O and extracted with EtOAc

(3× 15 mL). The combined organic extracts were washed with saturated sodium bicarbonate (10 mL), dried over anhydrous Na_2SO_4, and evaporated in vacuo. The crude product was purified by silica chromatography using petroleum ether/EtOAc as eluent to give **10** (69 mg, 96%) as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ 5.42 (m, 1H), 3.97 (s, 3H), 3.85 (s, 3H), 3.30 (m, 1H), 3.08 (m, 1H), 3.00 (s, 2H), 2.80 (s, 2H), 2.40 (m, 1H), 2.03 (m, 1H), 1.16 (s, 3H), 1.13 (s, 3H). EI-MS (*m*/*z*): 290 (M^+).

4.1.9. Methyl 8-methoxy-2,2-dimethyl-1,2,3,5-tetrahydro-s-indacene-4-carboxylate (11)

p-Toluenesulfonic acid (0.50 mg) was added to a solution of **10** (100 mg, 0.34 mmol) in dry toluene (8 mL) under nitrogen and the mixture was stirred at 80 °C for 10 min. After cooling to room temperature, the reaction mixture was concentrated to a total volume of 2 mL and applied to a short column of silica gel (petroleum ether/EtOAc elution) to give **11** (84 mg, 95%) as a colorless crystal. Mp: 78–79 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.03 (dt, 1H, J = 6 Hz and 2 Hz), 6.52 (dt, 1H, J = 6 Hz and 2 Hz), 3.97 (s, 3H), 3.90 (s, 3H), 3.70 (s, 2H), 3.10 (s, 2H), 2.78 (s, 2H), 1.17 (s, 6H). EI-MS (*m*/*z*): 272 (M^+).

4.1.10. Methyl 1,2-dihydroxy-8-methoxy-6,6-dimethyl-1,2,3,5,6,7-hexahydro-s-indacene-4-carboxylate (12)

To a solution of AD-mix-α (515 mg) in 2 mL of H_2O and 2 mL of *t*-BuOH was added methane sulfonamide (35 mg). The mixture was cooled to 0 °C, **11** was added (100 mg, 0.36 mmol), and then stirred overnight at room temperature. To the reaction mixture was added solid sodium sulfite (551 mg) at 0 °C and the mixture was allowed to warm to room temperature, stirred for another 30 min, and extracted with CH_2Cl_2 (3× 15 mL). The combined organic layers were washed with 2 N KOH (2× 10 mL) and brine (10 mL), dried over Na_2SO_4, and evaporated to dryness under reduced pressure. The product was purified by chromatography on silica gel using petroleum ether/EtOAc to obtain **12** (101 mg, 90%) as a white solid. Mp: 158–160 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 5.19 (m, 1H), 4.45 (m, 1H), 3.98 (s, 3H), 3.86 (s, 3H), 3.33 (m, 2H), 3.01 (s, 2H), 2.80 (s, 2H), 1.16 (s, 3H), 1.14 (s, 3H). EI-MS (*m*/*z*): 306 (M^+).

4.1.11. 1,2-Dihydroxy-8-methoxy-6,6-dimethyl-1,2,3,5,6,7-hexahydro-s-indacene-4-carboxylic acid (13)

Compound **12** (100 mg, 0.326 mmol) was dissolved in a methanolic solution of 2 N KOH (5 mL). The solution was heated at 80 °C with stirring for 2.5 h, most of the solvent was removed, and the solution was diluted with saturated KH_2PO_4 solution (10 mL) and extracted with EtOAc (5× 10 mL). The combined organic extracts were dried over anhydrous Na_2SO_4, concentrated to provide **13** (91 mg, 95%) as a pale yellow solid. Mp: 170–172 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 5.19 (m, 1H), 4.45 (m, 1H), 4.02 (s, 3H), 3.38 (m, 2H), 3.07 (s, 2H), 2.80 (s, 2H), 1.16 (s, 3H), 1.14 (s, 3H). EI-MS (*m*/*z*): 292 (M^+).

4.1.12. 3-Hydroxy-6-methoxy-8,8-dimethyl-1-oxo-1,3,4,7,8,9-hexahydro-cyclopenta[*h*]isochromene-5-carbaldehyde (14)

Compound **13** (100 mg, 0.342 mmol) was dissolved in 30% aqueous dioxane (6 mL) and treated with $NaIO_4$ (88 mg, 0.411 mmol). This mixture was stirred for a few minutes at room temperature and a white precipitate appeared. Stirring was continued for a total period of 2 h. The reaction mixture was diluted with 10 mL of H_2O and extracted with CH_2Cl_2 (4× 10 mL). The combined organic phases were washed with brine, dried over anhydrous Na_2SO_4, and evaporated to give **14** (99 mg, 98%) as a pale yellow solid. Mp: 145–147 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 10.54 (s,1H), 5.84 (t, 1H, J = 3.9 Hz), 4.00 (s, 3H), 3.61 (m, 2H), 3.24 (q, 2H), 2.87 (s, 2H), 1.24 (s, 6H); ^{13}C NMR (400 MHz, $CDCl_3$): δ 191.8, 164.2, 164.1, 157.7, 139.1, 134.2, 124.3, 117.4, 94.8, 61.1, 49.5, 44.9, 39.9, 31.6, 28.7. EI-MS (*m*/*z*): 290 (M^+).

4.1.13. 3,6-Dihydroxy-8,8-dimethyl-1-oxo-1,3,4,7,8,9-hexahydro-cyclopenta[*h*]isochromene-5-carbaldehyde (1)

Compound **14** (100 mg, 0.342 mmol) was dissolved in dry CH_2Cl_2 (10 mL), cooled to −60 °C, and then BBr_3 (100 mg, 0.342 mmol) was added under argon atmosphere. After stirring at room temperature for 3 h, the reaction was quenched with 10 mL of ice and water and extracted with CH_2Cl_2 (3× 15 mL). The combined organic extracts were washed with brine (10 mL), dried over anhydrous Na_2SO_4, and evaporated in vacuo. The crude product was purified by silica chromatography using petroleum ether/EtOAc as eluent to give **1** (41.8 mg, 44%) as a white solid. Mp: 200–210 °C dec (lit. mp > 200 °C dec). ^{1}H NMR (300 MHz, $CDCl_3$): δ 12.41 (s, 1H), 10.25 (s, 1H), 5.95 (t, 1H, J = 3.9 Hz), 3.51 (m, 2H), 3.22 (s, 2H), 2.72 (s, 2H), 1.19 (s, 6H); ^{13}C NMR (400 MHz, DMSO-d_6): δ 196.2, 162.8.1, 161.3, 156.0, 142.3, 130.0, 116.6, 114.1, 94.4, 49.9, 42.5, 40.1, 30.4, 28.8. EI-MS (*m*/*z*): 276 (M^+), 258, 243 (100%); HRMS-EI: *m*/*z* $C_{15}H_{16}O_5$; Calcd: 276.0998, found: 276.0999.

4.1.14. 4-Bromo-7-methoxy-2,3-dihydro-1H-inden-1-ol (15)

Compound **15** was prepared according to the procedure as described for **7** in 98% yield as a pale yellow solid. Mp: 79–81 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.34 (d, 1H, J = 8.7 Hz), 6.61 (d, 1H, J = 8.7 Hz), 5.52 (m, 1H), 3.85 (s, 3H), 3.05 (m, 1H), 2.80 (m, 1H), 2.47 (m, 1H), 2.04 (m, 1H). EI-MS (*m*/*z*): 241 (M^+).

4.1.15. (4-Bromo-7-methoxy-2,3-dihydro-1H-inden-1-yloxy)(*tert*-butyl)dimethylsilane (16)

Compound **16** was prepared according to the procedure as described for **8** in 98% yield as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.32 (d, 1H, J = 8.7 Hz), 6.56 (d, 1H, J = 8.7 Hz), 5.43 (m, 1H), 3.78 (s, 3H), 3.07 (m, 1H), 2.78 (m, 1H), 2.30 (m, 1H), 2.00 (m, 1H), 0.90 (s, 9H), 0.14 (s, 3H), 0.08 (s, 3H). EI-MS (*m*/*z*): 356 (M^+).

4.1.16. Methyl 1-(*tert*-butyldimethylsilyloxy)-7-methoxy-2,3-dihydro-1H-indene-4-carboxylate (17)

Compound **17** was prepared according to the procedure as described for **9** in 88% yield as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.95 (d, 1H, J = 8.7 Hz), 6.71 (d, 1H, J = 8.7 Hz), 5.37 (m, 1H), 3.85 (s, 3H), 3.84 (s, 3H), 3.40 (m, 1H), 3.16 (m, 1H), 2.24 (m, 1H), 1.98 (m, 1H), 0.90 (s, 9H), 0.14 (s, 3H), 0.08 (s, 3H). EI-MS (*m*/*z*): 336 (M^+).

4.1.17. Methyl 1-hydroxy-7-methoxy-2,3-dihydro-1H-indene-4-carboxylate (18)

Compound **18** was prepared according to the procedure as described for **10** in 96% yield as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.98 (d, 1H, J = 8.7 Hz), 6.76 (d, 1H, J = 8.7 Hz), 5.45 (m, 1H), 3.95 (s, 3H), 3.91 (s, 3H), 3.43 (m, 1H), 3.16 (m, 1H), 2.24 (m, 1H), 2.02 (m, 1H). EI-MS (*m*/*z*): 222 (M^+).

4.1.18. Methyl 7-methoxy-3H-indene-4-carboxylate (19)

Compound **19** was prepared according to the procedure as described for **11** in 95% yield as a colorless crystal. Mp: 49–51 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.91 (d, 1H, J = 8.8 Hz), 7.02 (m, 1H), 6.83 (d, 1H, J = 8.8 Hz), 6.56 (m, 1H), 3.94 (s, 3H), 3.91 (s, 3H), 3.76 (m, 2H). EI-MS (*m*/*z*): 204 (M^+).

4.1.19. Methyl 1,2-dihydroxy-7-methoxy-2,3-dihydro-1H-indene-4-carboxylate (20)

Compound **20** was prepared according to the procedure as described for **12** in 90% yield as a white solid. Mp: 130–132 °C. ^{1}H

NMR (300 MHz, $CDCl_3$): δ 8.01 (d, 1H, J = 8.8 Hz), 6.79 (d, 1H, J = 8.8 Hz), 5.20 (m, 1H), 4.52 (m, 1H), 3.93 (s, 3H), 3.86 (s, 3H), 3.40 (m, 2H). EI-MS (m/z): 238 (M^+).

4.1.20. 1,2-Dihydroxy-7-methoxy-2,3-dihydro-1H-indene-4-carboxylic acid (21)

Compound **21** was prepared according to the procedure as described for **13** in 95% yield as a pale yellow solid. Mp: 143–145 °C. ^{1}H NMR (300 MHz, DMSO):δ 7.86 (d, 1H, J = 8.8 Hz), 6.91 (d, 1H, J = 8.8 Hz), 4.75 (m, 1H), 4.09 (m, 1H), 3.84 (s, 3H), 2.91 (m, 2H). EI-MS (m/z): 224 (M^+).

4.1.21. 3-Hydroxy-6-methoxy-1-oxoisochroman-5-carbaldehyde (22)

Compound **22** was prepared according to the procedure as described for **14** in 99% yield as a pale yellow solid. Mp: 186–188 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 10.60 (s, 1H), 8.35 (d, 1H, J = 8.9 Hz), 7.05 (d, 1H, J = 8.9 Hz), 5.90 (t, 1H, J = 3.9 Hz), 4.00 (s, 3H), 3.62 (m, 2H). EI-MS (m/z): 222 (M^+).

4.1.22. 2-(2-Bromo-5-methoxyphenyl)ethanol (24)

2-(3-Methoxyphenyl)ethanol **23** (200 mg, 1.3 mmol) was dissolved in 4 mL of $CHCl_3$ and cooled to 0 °C. To the solution was added dropwise bromine (0.074 mL, 1.4 mmol). After stirring for 1 h at 0 °C, the reaction was quenched with 10% sodium metabisulfite, extracted with $CHCl_3$ (3× 5 mL). The combined organic extracts were washed with brine (10 mL) and dried over anhydrous Na_2SO_4, and evaporated in vacuo to give **24** (279 mg, 92%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.44 (m, 1H), 6.82 (m, 1H), 6.66 (m, 1H), 3.88 (t, 2H, J = 6.7 Hz), 3.78 (s, 3H), 2.98 (t, 2H, J = 6.7 Hz). EI-MS (m/z): 229 (M^+).

4.1.23. (2-Bromo-5-methoxyphenethoxy)(*tert*-butyl)dimethylsilane (25)

Compound **25** was prepared according to the procedure as described for **8** in 96% yield as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.41 (m, 1H), 6.81 (m, 1H), 6.63 (m, 1H), 3.84 (t, 2H, J = 6.8 Hz), 3.77 (s, 3H), 2.92 (t, 2H, J = 6.8 Hz), 0.86 (s, 9H), 0.10 (s, 6H). EI-MS (m/z): 344 (M^+).

4.1.24. Methyl 2-(2-(*tert*-butyldimethylsilyloxy)ethyl)-4-methoxybenzoate (26)

Compound **26** was prepared according to the procedure as described for **9** in 75% yield as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.93 (m, 1H), 6.78 (m, 2H), 3.87–3.83 (m, 8H), 3.20 (t, 2H, J = 6.4 Hz), 0.85 (s, 9H), 0.10 (s, 6H). EI-MS (m/z): 324 (M^+).

4.1.25. Methyl 2-(2-hydroxyethyl)-4-methoxybenzoate (27)

Compound **27** was prepared according to the procedure as described for **10** in 94% yield as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.93 (m, 1H), 6.79 (m, 2H), 3.91 (t, 2H, J = 6.3 Hz), 3.86 (s, 3H), 3.84 (s, 3H), 3.22 (t, 2H, J = 6.3 Hz). EI-MS (m/z): 210 (M^+).

4.1.26. 2-(2-Hydroxyethyl)-4-methoxybenzoic acid (28)

Compound **28** was prepared according to the procedure as described for **13** in 96% yield as a white solid. Mp: 131–132 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.03 (m, 1H), 6.82 (m, 2H), 3.94 (t, 2H, J = 6.3 Hz), 3.87 (s, 3H), 3.25 (t, 2H, J = 6.3 Hz). EI-MS (m/z): 196 (M^+).

4.1.27. 3-Hydroxy-6-methoxyisochroman-1-one (29)

To a solution of PCC (27 mg, 0.124 mmol) in CH_2Cl_2 (5 mL) was added dropwise a solution of **28** (12.1 mg, 0.062 mmol) in CH_2Cl_2 (3 mL). After stirring for 2 h, the reaction mixture was diluted with anhydrous Et_2O (10 mL) and filtered. The solid was washed with 5 mL of CH_2Cl_2 and the filtrate was evaporated to dryness under reduced pressure. The product was purified by chromatography on silica gel using petroleum ether/EtOAc to provide **34** (3.6 mg, 30%). Mp: 80–81 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.07 (m, 1H), 6.90 (m, 1H), 6.76 (m, 1H), 5.90 (m, 1H), 3.87 (s, 3H), 3.16 (m, 2H). EI-MS (m/z): 194 (M^+).

4.1.28. Methyl 3-formyl-4-methoxy-2-(2-oxo-ethyl)benzoate (30)

Compound **30** was prepared according to the procedure as described for **14** in 94% yield as a white solid. Mp: 163–164 °C.^{1}H NMR (300 MHz, $CDCl_3$): δ 10.56 (s, 1H), 9.80 (s, 1H), 8.20 (d, 1H, J = 9 Hz), 7.00 (d, 1H, J = 9 Hz), 4.61 (s, 2H), 3.98 (s, 3H), 3.85 (s, 3H). EI-MS (m/z): 236 (M^+).

4.1.29. Methyl 2-(2-hydroxyethyl)-3-(hydroxymethyl)-4-methoxybenzoate (31)

Compound **31** was prepared according to the procedure as described for **7** in 60% yield as a white solid. Mp: 120–122 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.12 (d, 1H, J = 8.8 Hz), 6.92 (d, 1H, J = 8.8 Hz), 4.73 (s, 2H), 4.51 (t, 2H, J = 6 Hz), 3.94 (s, 3H), 3.82 (s, 3H), 3.15 (t, 2H, J = 6 Hz). EI-MS (m/z): 240 (M^+).

4.1.30. 2-(2-Hydroxyethyl)-3-(hydroxymethyl)-4-methoxybenzoic acid (32)

Compound **32** was prepared according to the procedure as described for **13** in 90% yield as a pale yellow solid. Mp: 150–152 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.13 (d, 1H, J = 8.8 Hz), 6.93 (d, 1H, J = 8.8 Hz), 4.73 (s, 2H), 4.51 (t, 2H, J = 6 Hz), 3.94 (s, 3H), 3.14 (t, 2H, J = 6 Hz). EI-MS (m/z): 226 (M^+).

4.1.31. 5-(Hydroxymethyl)-6-methoxy-1-oxoisochroman (33)

To a solution of **32** (18 mg, 0.079 mmol) in dry CH_2Cl_2 (4 mL) was added *p*-toluenesulfonic acid (1 mg). After stirring at room temperature for 1 h, the reaction was quenched with 10 mL of water and extracted with CH_2Cl_2 (3× 15 mL). The combined organic extracts were washed with brine (10 mL), dried over anhydrous Na_2SO_4, and evaporated in vacuo. The crude product was purified by silica chromatography using petroleum ether/EtOAc as eluent to give **33** (16 mg, 96%) as a white solid. Mp: 96–98 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.12 (d, 1H, J = 8.8 Hz), 6.93 (d, 1H, J = 8.8 Hz), 4.73 (s, 2H), 4.51 (t, 2H, J = 6.1 Hz), 3.95 (s, 3H), 3.14 (t, 2H, J = 6.1 Hz). EI-MS (m/z): 210 (M^+).

4.1.32. 6-Methoxy-1-oxoisochroman-5-carbaldehyde (34)

Compound **34** was prepared according to the procedure as described for **29** in 78% yield. Mp: 137–138 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 10.60 (s, 1H), 8.36 (d, 1H, J = 9 Hz), 7.05 (d, 1H, J = 9 Hz), 4.46 (t, 2H, J = 6 Hz), 4.02 (s, 3H), 3.49 (t, 2H, J = 6 Hz). EI-MS (m/z): 206 (M^+).

4.1.33. 3-Hydroxy-6-methoxy-1H-isochroman-5-carbaldehyde (36)

To the suspension of LAH (12.7 mg, 0.336 mmol) in dry THF (5 mL) was added dropwise a solution of **20** (20 mg, 0.084 mmol) in dry THF (3 mL). The mixture was refluxed for 2 h and then was allowed to warm to room temperature. Two milliliters of water, 2 mL of 1 N NaOH, and 3 mL of EtOAc were added dropwise with ice bath cooling. The resulting precipitate was filtered and washed with EtOAc (3× 3 mL). The filtrate was extracted with EtOAc (3× 6 mL), washed with brine (10 mL), dried over Na_2SO_4, and evaporated in vacuo. The residue was applied to silica gel chromatography using petroleum ether/EtOAc as eluent to provide 4-(hydroxymethyl)-7-methoxy-2,3-dihydro-1H-indene-1,2-diol **35** (20 mg, 0.095 mmol). Compound **35** was dissolved in 30% aqueous dioxane (5 mL) and treated with $NaIO_4$ (25 mg, 0.116 mmol). This mixture was stirred for a few minutes at room temperature and a white precipitate appeared. Stirring was continued for a total

period of 2 h. The reaction mixture was diluted with 10 mL of H_2O and extracted with CH_2Cl_2 (4× 8 mL). The combined organic phases were washed with brine, dried over anhydrous Na_2SO_4, and evaporated to give **36** (19.4 mg) as a white solid. Mp: 180–181 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 10.26 (s, 1H), 7.20 (d, 1H, J = 8.6 Hz), 6.86 (d, 1H, J = 8.6 Hz), 5.37 (t, 1H, J = 4.2 Hz), 4.73 (m, 2H), 3.89 (s, 3H), 3.23 (m, 2H); ^{13}C NMR (400 MHz, $CDCl_3$): δ 192.0, 162.3, 134.2, 130.9, 126.4, 122.4, 109.5, 91.7, 62.4, 55.8, 33.2. EI-MS (m/z): 210 (M^+).

4.1.34. 3-Bromo-6-methoxy-2-(2-oxo-ethyl)-benzaldehyde (38)

Compound **38** was prepared from **2** according to the procedure as described for **30**. ^{1}H NMR (300 MHz, $CDCl_3$): δ 10.48 (s, 1H), 9.77 (s, 1H), 7.75 (d, 1H, J = 9 Hz), 6.90 (d, 1H, J = 9 Hz), 4.37 (s, 2H), 3.93 (s, 3H). EI-MS (m/z): 255 (M^+). **39** ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.72 (d, 1H, J = 8.7 Hz), 6.84 (d, 1H, J = 8.7 Hz), 5.92 (s, 1H), 5.44–5.40 (m, 1H), 3.76 (s, 3H), 2.95–2.72 (m, 2H).

4.2. Enzymatic assay

4.2.1. Inhibition assay

Recombinant LAR catalytic domain was expressed and purified according to the previous report.[58] The enzymatic activities of the LAR catalytic domain were determined at 30 °C by monitoring the hydrolysis of pNPP. Dephosphorylation of pNPP generates product pNP, which was monitored at an absorbance of 405 nm by the EnVision multilabel plate reader (PerkinElmer Life Sciences, Boston, MA, USA). In a typical inhibition assay, 2 μL DMSO solution with or without inhibitor pre-incubated with 20 μL enzyme solution containing 50 mM Tris, pH 8.0, 50 mM NaCl, 2 mM EDTA, 2 mM dithiothreitol, and 300 nM recombinant LAR for 10 min, then the enzymatic reaction was initiated through adding 78 μL substrate buffer containing 50 mM MES, pH 6.0, 2 mM EDTA, 2 mM dithiothreitol, and 2 mM pNPP. Absorbance at 405 nm was continuously monitored and the initial rate of the hydrolysis was determined using the early linear region of the enzymatic reaction kinetic curve. The IC_{50} was calculated with Prism 4 software (Graphpad, San Diego, CA, USA) from the non-linear curve fitting of the percentage of inhibition (% inhibition) versus the inhibitor concentration [I] by using the following equation: % Inhibition = $100/(1 + [IC_{50}/[I]]^k)$, where k is the Hill coefficient.

4.2.2. Dialysis

The dialysis method was used to determine the reversibility on the inhibition of illudalic acid on LAR. LAR (1500 nM) was pre-incubated with illudalic acid (20 μM) or DMSO (2%) for 10 min according to the method mentioned above. Then 1.5 mL samples were dialyzed against enzyme solution for indicated time, 60 μL samples were taken out, and their phosphatase activities were determined.

4.3. ESI-MS/MS studies

5 μL inhibitor **22** (17 mM) was incubated with 90 μL LAR (32 μM) and 5 μL LAR simulacrum *N*-acetyl-L-cysteine (30 mM) at pH 8.0 for 10 min (Scheme 8), and the pH was then adjusted to 6 by adding 400 μL substrate solution (the same condition as that of the inhibition assay). LAR enzyme was removed from the reaction mixture by using Amicon Ultra 5K centrifugal filter devices (Millipore Corporation) spinning at 7500*g* for 10 min. The obtained sample was analyzed by ESI-MS/MS under low-resolution conditions (positive ion mode).

4.4. Computational docking study

The three-dimensional crystal structure of LAR was retrieved from the PDB database (the access PDB code: 1LAR). The software AutoDock4[72] was adopted to dock the ligand **1** into the binding site of LAR which located around Cys1522. The Lamarckian genetic algorithm (LGA) was applied to deal with the protein–ligand interactions. A Solis and Wets local search was performed for the energy minimization on a user-specified proportion of the population. Due to the covalent interaction hypothesis we proposed, we first removed the side chain of residue Cys1522 and performed the docking, then a list of conformations having 3-**C** of ligand **1** within 1.5 Å of sulfur atom of Cys1522 were collected. Finally the conformation with lowest predicted free energy was picked for later interaction analysis. All the protein and ligand structures were prepared in graphics software AutoDock tool according with default parameters. To explore the conformational space of ligands, some parameters in AutoDock4 were set up as follows.

The overall translation step was set to 0.2 Å, and the overall rotation and torsion rotation step were set to 5° in the docking study. The number of GA generations, the number of energy evaluations, and the number of docking runs were set to 370,000, 1,500,000, and 50, respectively.

Acknowledgements

This work was supported by the Hi-tech Research and Development Program of China through Grant Nos. 2006AA02Z315 (L.-P.M.) and 2007AA09Z402 (J.L.).

References and notes

1. Fischer, E. H.; Charbonneau, H.; Tonks, N. K. *Science* **1991**, *13*, 1497.
2. Burke, T. R., Jr.; Zhang, Z. Y. *Biopolymers* **1998**, *47*, 225.
3. Zhang, Z. Y. *Acc. Chem. Res.* **2003**, *36*, 385.
4. Alonso, A.; Sasin, J.; Bottini, N.; Friedberg, I.; Friedberg, I.; Osterman, A.; Godzik, A.; Hunter, T.; Dixon, J.; Mustelin, T. *Cell* **2004**, *117*, 699.
5. Zhang, Z. Y. *Biochim. Biophys. Acta* **2005**, *1754*, 100.
6. Hunter, T. *Cell* **2000**, *100*, 113.
7. Tonks, N. K.; Neel, B. G. *Curr. Opin. Cell Biol.* **2001**, *13*, 182.
8. Kappert, K.; Peters, K. G.; Bohmer, F. D.; Ostman, A. *Cardiovasc. Res.* **2005**, *65*, 587.
9. Stoker, A. W. *J. Endocrinol.* **2005**, *185*, 19.
10. Tonks, N. K. *Nat. Rev. Mol. Cell Biol.* **2006**, *7*, 833.
11. Wang, W. J.; Kuo, J. Ch.; Ku, W.; Lee, Y. R.; Lin, F. Ch.; Chang, Y. L.; Lin, Y. M.; Chen, Ch. H.; Huang, Y. P.; Chiang, M. J.; Yeh, Sh. W.; Wu, P. R.; Shen, Ch. H.; Wu, Ch. T.; Chen, R. W. *Mol. Cell* **2007**, *27*, 701.
12. Zhang, Z. Y.; Wang, Y.; Wu, L.; Stuckey, J. A.; Schubert, H. L.; Saper, M. A.; Dixon, J. E. *Biochemistry* **1994**, *33*, 15266.
13. Tabernero, L.; Aricescu, A. R.; Jones, E. Y.; Szedlacsek, S. E. *FEBS* **2008**, *275*, 867.
14. Li, L.; Dixon, J. E. *Semin. Immunol.* **2000**, *12*, 75.
15. Zhang, Z. Y. *Curr. Opin. Chem. Biol.* **2001**, *5*, 416.
16. Zhang, Z. Y.; Lee, S. Y. *Exp. Opin. Invest. Drug* **2003**, *12*, 223.
17. Tonks, N. K. *FEBS Lett.* **2003**, *546*, 140.
18. Lund, I. K.; Andersen, H. S.; Iversen, L. F.; Olsen, O. H.; Moller, K. B.; Pedersen, A. K.; Ge, Y.; Holsworth, D. D.; Newman, M. J.; Axe, F. U.; Moller, N. P. *J. Biol. Chem.* **2004**, *279*, 24226.
19. Bourdeau, A.; Dube, N.; Tremblay, M. L. *Curr. Opin. Cell Biol.* **2005**, *17*, 203.
20. Bialy, L.; Waldmann, H. *Angew. Chem. Int. Ed.* **2005**, *44*, 3814.
21. Dewang, P. M.; Hsu, N. M.; Peng, S. Z.; Li, W. R. *Curr. Med. Chem.* **2005**, *12*, 1.
22. Hansen, S. K.; Cancilla, M. T.; Shiau, T. P.; Kung, J.; Chen, T.; Erlanson, D. A. *Biochemistry* **2005**, *44*, 7704.
23. Tautz, L.; Pellecchia, M.; Mustelin, T. *Expert Opin. Ther. Targets* **2006**, *10*, 157.
24. Ventura, J. J.; Nebreda, A. R. *Clin. Trans. Oncol.* **2006**, *8*, 153.
25. Tiganis, T.; Bennett, A. M. *Biochem. J.* **2007**, *402*, 1.
26. Streuli, M.; Krueger, N. X.; Hall, L. R.; Schlossman, S. F.; Saito, H. *J. Exp. Med.* **1988**, *168*, 1523.
27. Cheng, A.; Dubé, N.; Gu, F.; Tremblay, M. L. *Eur. J. Biochem.* **2002**, *269*, 1050.
28. Mooney, R. A.; Levea, C. M. *Curr. Top. Med. Chem.* **2003**, *3*, 809.
29. Chagnon, M. J.; Uetani, N.; Tremblay, M. L. *Biochem. Cell Biol.* **2004**, *82*, 664.
30. Heneberg, P.; Dráber, P. *Curr. Med. Chem.* **2005**, *12*, 1859.
31. Yang, T.; Bernabeu, R.; Xie, Y.; Zhang, J. L.; Massa, S. M.; Rempel, H. C.; Longo, F. M. *J. Neurosci.* **2003**, *23*, 3353.
32. Dunah, A. W.; Hueske, E.; Wyszynski, M.; Hoogenraad, C. C.; Jaworski, J.; Pak, D. T.; Simonetta, A.; Liu, G. S.; Sheng, M. *Nat. Neurosci.* **2005**, *8*, 458.
33. Yang, T.; Massa, S. M.; Longo, F. M. *J. Neurobiol.* **2006**, *66*, 1420.
34. Haapasalo, A.; Kim, D. Y.; Carey, B. W.; Turunen, M. K.; Pettingell, W. H. *J. Biol. Chem.* **2007**, *282*, 9063.
35. Tsai, A. Y. M.; Itoh, M.; Streuli, M.; Thai, T.; Saito, H. *J. Biol. Chem.* **1991**, *266*, 10534.
36. Mooney, R. A.; Kulas, D. T.; Bleyle, L. A.; Novak, J. S. *Biochem. Biophys. Res. Commun.* **1997**, *235*, 709.

37. Ahmad, F.; Goldstein, B. J. *J. Biol. Chem.* **1997**, *272*, 448.
38. Zabolotny, J. M.; Kim, Y.-B.; Perone, J. K.; Pani, M. A.; Boss, O.; Klamar, L. D.; Kamatkar, S.; Shulman, G. I.; Kahn, B. B.; Neel, B. G. *Proc. Natl. Acad. Sci. U.S.A.* **2001**, *98*, 5187.
39. Hamaguchi, T.; Takahashi, A.; Kagamizono, T.; Manaka, A.; Sato, M.; Osada, H. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 2657.
40. Chen, Y. T.; Seto, C. T. *J. Med. Chem.* **2002**, *45*, 3946.
41. Wan, Z. K.; Lee, J. B.; Xu, W. X.; Erbe, D. V.; Joseph-McCarthy, D.; Followsa, B. C.; Zhang, Y. L. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 4941.
42. Maccari, R.; Paoli, P.; Ottana, R.; Jacomelli, M.; Ciurleo, R.; Manao, G.; Steindl, T.; Langer, T.; Vigoritaa, M. G.; Camicib, G. *Bioorg. Med. Chem.* **2007**, *15*, 5137.
43. Bhattarai, B. R.; Shrestha, S.; Ham, S. W.; Kim, K. R.; Cheon, H. G.; Leea, K. H.; Cho, H. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 5357.
44. Szczepankiewicz, B. G.; Liu, G.; Hajduk, P. J.; Abad-Zapatero, C.; Pei, Z.; Xin, Z.; Lubben, T. H.; Trevillyan, J. M.; Stashko, M. A.; Ballaron, S. J.; Liang, H.; Huang, F.; Hutchins, C. W.; Fesik, S. W.; Jirousek, M. R. *J. Am. Chem. Soc.* **2003**, *125*, 4087.
45. Pei, Z.; Liu, G.; Lubben, T. H.; Szczepankiewicz, B. Z. *Curr. Pharm. Des.* **2004**, *10*, 3481.
46. Hoffman, H. E.; Blair, E. R.; Johndrow, J. E.; Bishop, A. C. *J. Am. Chem. Soc.* **2005**, *127*, 2824.
47. Combs, A. P.; Yue, E. W.; Bower, M.; Ala, P. J.; Wayland, B.; Douty, B.; Takvorian, A.; Polam, P.; Wasserman, Z.; Zhu, W.; Crawley, M. L.; Pruitt, J.; Sparks, R.; Glass, B.; Modi, D.; McLaughlin, E.; Bostrom, L.; Li, M.; Galya, L.; Blom, K.; Hillman, M.; Gonneville, L.; Reid, B. G.; Wei, M.; Becker-Pasha, M.; Klabe, R.; Huber, R.; Li, Y.; Hollis, G.; Burn, T. C.; Wynn, R.; Liu, P.; Metcalf, B. *J. Med. Chem.* **2005**, *48*, 6544.
48. Na, M.; Jang, J.; Njamen, D.; Mbafor, J. T.; Fomum, Z. T.; Kim, B. Y.; Oh, W. K.; Ahn, J. S. *J. Nat. Prod.* **2006**, *69*, 1572.
49. Lee, H.; Xie, L.; Luo, Y.; Lee, S.-Y.; Lawrence, D. S.; Wang, X. B.; Sotgia, F.; Lisanti, M. P.; Zhang, Z. Y. *Biochemistry* **2006**, *45*, 234.
50. Srinivasan, R.; Uttamchandani, M.; Yao, S. Q. *Org. Lett.* **2006**, *8*, 713.
51. Adams, D. R.; Abraham, A.; Asano, J.; Breslin, C.; Dick, C. A. J.; Ixkes, U.; Johnston, B. F.; Johnston, D.; Kewnay, J.; Mackay, S. P.; MacKenzie, S. J.; McFarlane, M.; Mitchell, L.; Spinks, D.; Takano, Y. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 6579.
52. Vidal, D.; Blobel, J.; Pérez, Y.; Thormann, M.; Pons, M. *Eur. J. Med. Chem.* **2007**, *42*, 1102.
53. Feng, Y.; Carroll, A. R.; Addepalli, R.; Fechner, G. A.; Avery, V. M.; Quinn, R. J. *J. Nat. Prod.* **2007**, *70*, 1790.
54. Boutselis, I. G.; Yu, X.; Zhang, Z. Y.; Borch, R. F. *J. Med. Chem.* **2007**, *50*, 856.
55. Zhang, X. Y.; Bishop, A. C. *J. Am. Chem. Soc.* **2007**, *129*, 3812.
56. Vazquez, J.; Tautz, L.; Ryan, J. J.; Vuori, K.; Mustelin, T.; Pellecchia, M. *J. Med. Chem.* **2007**, *50*, 2137.
57. Seo, Ch.; Choi, Y. H.; Sohn, J. H.; Ahn, J. S.; Yim, J. H.; Lee, H. K.; Oh, H. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 772.
58. Yang, X. N.; Li, J. Y.; Zhou, Y. Y.; Shen, Q.; Chen, J. W.; Li, J. *Biochim. Biophys. Acta* **2005**, *1726*, 34.
59. Woodward, R. B.; Hoye, T. R. *J. Am. Chem. Soc.* **1977**, *99*, 8007.
60. Michael, D. M.; John, F. D.; Arthur, A. H. *J. Med. Chem.* **1994**, *37*, 105.
61. Tatsuo, H. *J. Org. Chem.* **1975**, *40*, 3037.
62. Yasuharu, Y.; Hajime, M.; Minoru, H.; Kazuhiko, M. *Tetrahedron* **2004**, *60*, 9425.
63. Kim, D. W.; Ko, Y. K.; Sung Hoon Kim, S. H. *Synthesis* **1992**, 1203.
64. Sharpless, K. B.; Amberg, W.; Bennani, Y. L.; Crispino, G. A.; Hartung, J.; Jeong, K. S.; Kwong, H. L.; Morikawa, K.; Wang, Z. M.; Xu, D. Q.; Zhang, X. L. *J. Org. Chem.* **1992**, *57*, 2768.
65. Zhang, Z. Y. *Curr. Top. Cell. Regul.* **1997**, *35*, 21.
66. Zhang, Z. Y. *Crit. Rev. Biochem. Mol. Biol.* **1998**, *33*, 1.
67. Wang, H.; Wu, Y.; Zhao, Z. *J. Mass Spectrom.* **2001**, *36*, 58.
68. Ramtohul, Y. K.; Martin, N. I.; Silkin, L.; James, M. N. G.; Vederas, J. C. *J. Chem. Soc., Perkin Trans.* **2002**, *11*, 1351.
69. Dizdaroglu, M.; Jaruga, P.; Rodriguez, H. *Crit. Rev. Oxidative Stress Aging* **2003**, *1*, 165.
70. Tabei, K.; Feng, X. D.; Venkatesan, A. M.; Abe, T.; Hideki, U.; Mansour, T. S.; Siegel, M. M. *J. Med. Chem.* **2004**, *47*, 3674.
71. Nam, H. J.; Poy, F.; Krueger, N. X.; Saito, H.; Frederick, C. A. *Cell* **1999**, *97*, 449.
72. Morris, G. M.; Goodsell, D. S.; Halliday, R. S.; Huey, R.; Hart, W. E.; Belew, R. K.; Olson, A. J. *J. Comput. Chem.* **1998**, *19*, 1639.