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SYNTHESIS OF NOVEL COUMARIN DERIVATIVES AND IN VITRO BIOLOGICAL EVALUATION AS POTENTIAL PTP 1B INHIBITORS

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Abstract – The aim of protein tyrosine phosphatase 1B (PTP 1B) inhibitors is to develop effective drug for diabetes and obesity. Coumarin becomes as a good skeleton, and is often applied in drug design and synthesis. In this paper, we have synthesized a series of novel coumarin derivatives to be as potential PTP 1B inhibitors. The inhibition rate of compound **9** was more than 80%, and the IC_{50} value was 49.2 μM, which would be considered for further study.

Insulin and leptin resistance is a major pathophysiological factor in the development of type 2 diabetes and obesity.[1,2] Currently diabetic patients are treated with various oral antihyperglycemic agents, however, over a period of time, type 2 diabetes mellitus subjects lose their response to these agents and thereby require insulin therapy.[3,4] Except incretin therapies, most of the available antihyperglycemic agents, including insulin promote weight gain, which further aggravates obesity associated cardiovascular risk and insulin resistance.[5,6] Thus, there is an urgent need to develop novel agents for glycemic control.[7,8]

PTP 1B is an active player in insulin and leptin resistance. This enzyme acts as a negative regulator of insulin and leptin signaling.[9-11] The role of PTP 1B in diabetes and obesity is well documented and has been reviewed extensively.[12,13] Recently inhibitors for PTP 1B mainly includes antisense nucleotides, thiophene-based inhibitors, heterocyclic carboxylic acid mimetics, oxidoreduction-type inhibitors, monocarboxylic acid inhibitors and allosteric PTP-1B inhibitors.[14]

Coumarins are a general structural class of aromatic heterocycle defined by the naturally occurring parent compound, which was first isolated in 1822 from Tonka beans (*Dipteryx odorata*).[15] They possess a wide range of biological activities and have long been considered attractive drug scaffolds being used in the treatment of viral infections, neurodegenerative diseases, oedema, inflammation, cancer, and as hepatoprotective agents and antioxidants.[16] Barrios has described an elegant coumarin-based amino acid that can be incorporated into peptides for substrate screening purposes.[17] In this paper, our substrate design uses the commercially available phenols to synthesize some coumarin-based derivatives referred to reported coumarin compound **1** (Km= 224 μM) (Figure 1), which is a potential non-peptide PTP 1B inhibitor.[18]

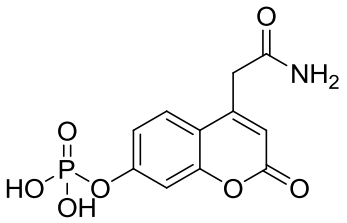
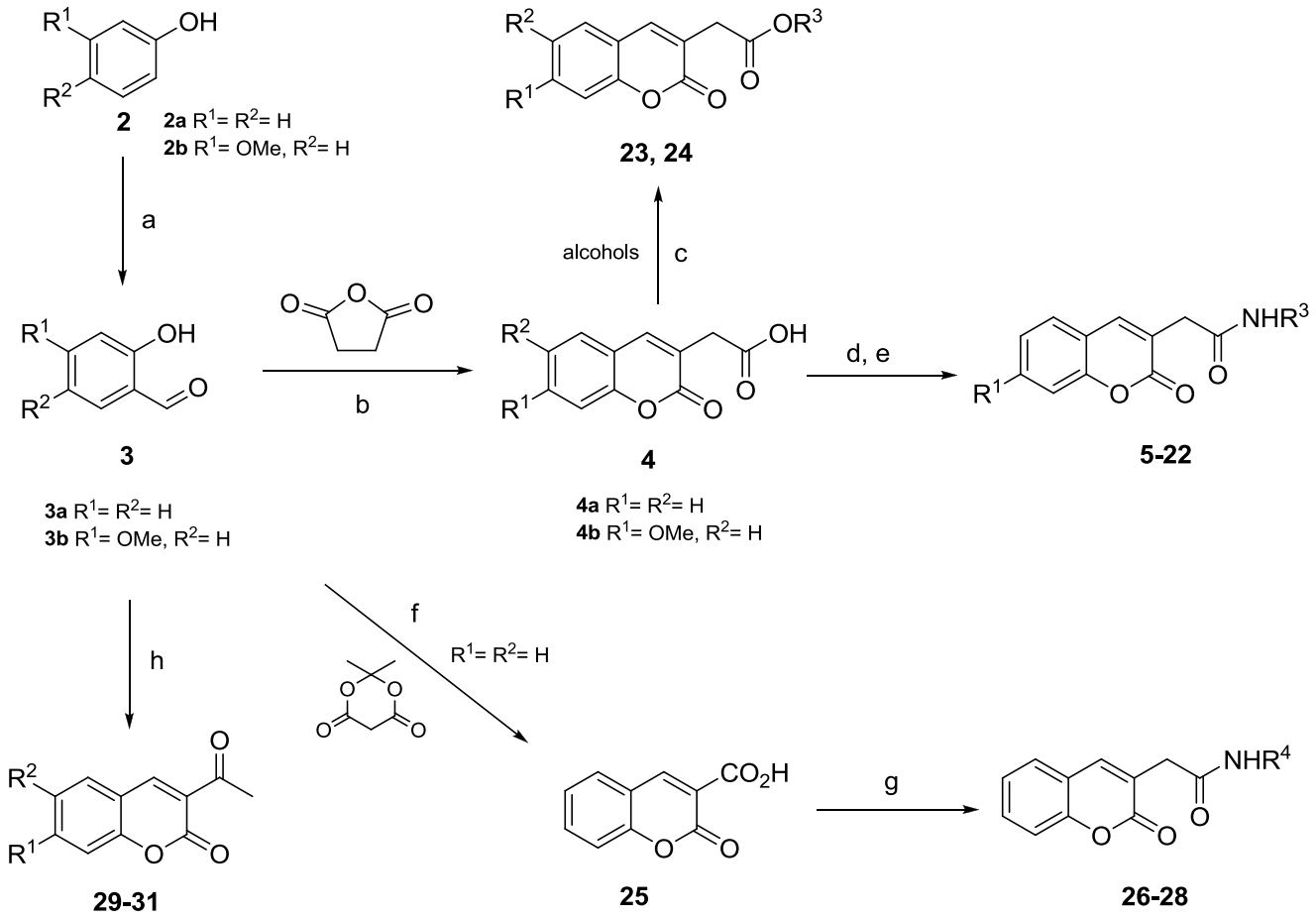


Figure 1. Coumarin compound **1**

We applied simple synthetic methods to get twenty-six coumarin derivatives **5-24** and **26-31**, which were purified by column chromatography or recrystallization. Their structures have been demonstrated by HRMS, IR and ^{1}HNMR analyses. Title Compounds were synthesized following the synthetic pathway described in Scheme 1.



Scheme 1. Reagents and conditions: (a) acetonitrile, $MgCl_2$, $(CH_2O)n$, triethylamine, reflux; (b) triethylamine, reflux; (c) DCC, DMAP, CH_2Cl_2, rt; (d) CDI, CH_2Cl_2, rt; (e) amine, CH_2Cl_2, rt; (f) H_2O, 75 °C; (g) CDI, CH_2Cl_2, amines, rt; (h) ethyl acetoacetate, piperidine, EtOH, reflux, 4 h.

Compounds **3** were synthesized by a published method.[19] In brief, phenols **2** as starting materials reacted with paraformaldehyde to yield salicylaldehydes **3**. Compounds **3** reacted with succinic anhydride to yield 2-(2-oxo-*2H*-chromen-3-yl)acetic acids **4** referring to literature.[20] By the method of acid-amine condensation, reacting acids **4** by CDI activated with aromatic amines yielded compounds **5-22**. Similarly, acids **4** reacted with alcohols yielded compounds **23**,**24** in the presence of both DMAP and DCC. The compound **25** was obtained using the literature procedures[21] with minor modifications. Briefly, salicylaldehyde **3a** reacted with meldrum's acid to yield 2-oxo-*2H*-chromene-3-carboxylic acid **25**. Reacting compound **25** by CDI activated with aromatic amines yielded compounds **26-28**. Compounds **29-31** were synthesized following the synthetic pathway described in Scheme 1.[22]

We postulated that coumarin could be used as a core structure allowing for the introduction of different substituents to obtain potential non-peptide PTP 1B inhibitors. Although the coumarin core is a part of many natural and synthetic compounds with wide pharmacological activity, there is only one paper

reporting about coumarin structures that inhibit PTP 1B.[18] All of these coumarin derivatives were evaluated for inhibition of recombinant human PTP 1B in vitro. The biological activity was detected by absorption spectrophotometry, and the substrate model was nitrobenzene phosphate (pNPP). Under the action of PTP 1B, the phosphate group of pNPP was removed, and generated *p*-nitrophenol. With alkaline condition, *p*-nitrophenol deprotonated to get yellow water soluble *p*-nitrophenolate, which had a strong absorption peak under 405 nm (Figure 2). If drug has inhibition to the PTP 1B, the dephosphorylation would be weaken or even disappeared. According to the changes of absorbance value, we could get the inhibition data of coumarin derivatives. The positive control was Na_3VO_4 (IC_{50}=0.7 μM),[23] results were shown in Tables 1-2.

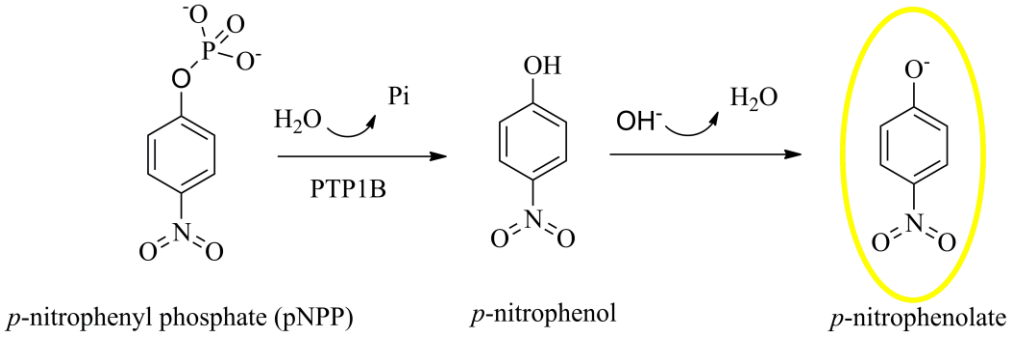
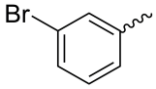
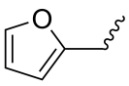
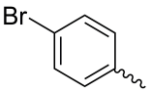
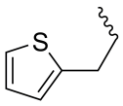
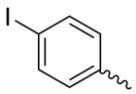
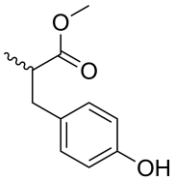
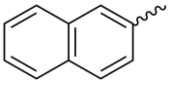
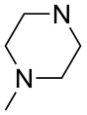
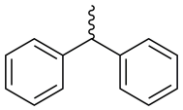
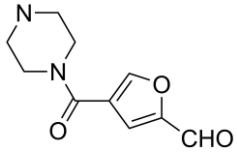
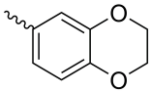
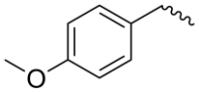
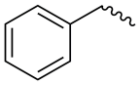
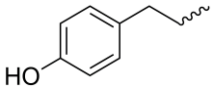
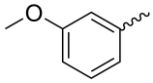
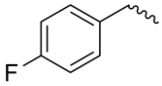
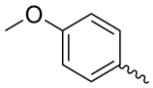
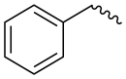
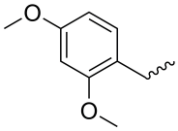
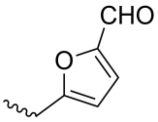


Figure 2. The screening model of PTP 1B in vitro

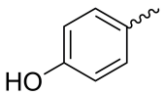
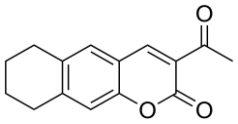
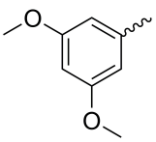
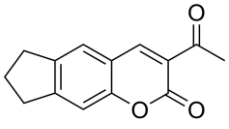
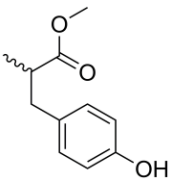
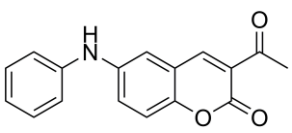
Table 1. Structure and inhibitory activity of derivatives **5-24** against PTP 1B

Compd.	R^1 (R^2)	R^3	inhibition ratio (%) in 0.5 mM	Compd.	R^1 (R^2)	R^3	inhibition ratio (%) in 0.5 mM
5	H		41.7%	**15**	H		66.1%
6	H		36.7%	**16**	H		ND
7	H		39.3%	**17**	H		74.6%
8	H		56.0%	**18**	H		2.6%

9	H		93.7% (IC_{50}=49.2 μM)[a]	**19**	H		0.2%
10	H		24.1%	**20**	MeO		78.1%
11	H		ND	**21**	MeO		ND
12	H		34.6%	**22**	MeO		28.7%
13	H		76.2%	**23**	H		ND
14	H		37.1%	**24**	H		75.6%

[a]The IC_{50} value of compounds was tested when its inhibition ratio exceeded 80%.
ND =Not done.

Table 2. Structure and inhibitory activity of derivatives **26-31** against PTP 1B

Compd.	R^4	inhibition ratio (%) in 0.5 mM	Compd.	Structure	inhibition ratio (%) in 0.5 mM
26		73.8%	**29**		31.9%
27		ND	**30**		29.7%
28		67.1%	**31**		70.9%

The inhibitory activity of coumarin derivatives was tested toward recombinant human PTP-1B by absorption spectrophotometry. As show in Table 1 to 2, firstly, we decided to modify the 3-position of the coumarin ring, and got two types of coumarin derivatives, **5-24** and **26-28**. There has no significant improvement for PTP1B in vitro, the inhibition rate of most of compounds were less than 80%. Whereas, compound **9** was the only one which had a modest inhibitory activity, which likely caused by the introduction of large hydrophobic substituent containing benzene ring. In contrast, introduction of hydrophilic group to 3-position, such as aldehyde, the inhibition rate for PTP1B dropped down to 0.19%. Additionally, substitution at 6-position of coumarin derivatives **20-22** and 7–position of coumarin derivatives **29-31** did not exhibit enhanced potency.

To get some insight to binding mode of coumarin derivatives, compound **9** (Figure 3) was docked into the active site of PTP 1B. The CDOCKER program was used to predict the binding modes of **9** to PTP 1B. As shown in Figure 4, the active site consists of S1 and S2 parts (left). Modeling of **9** into the active site of PTP 1B showed that the molecular mainly occupied S2 part. As shown in Figure 4 (right), coumarin ring could be interacting with Arg24, Arg254 amino acid residues in the S2 pocket and formed four hydrogen bonds. The amide group also could be interacting with Gln262 in the S2 pocket and developed one hydrogen bond. The benzene ring P2 developed an σ-π ligand with Arg24, however, other compounds bearing hydrophobic *N*-substituents (such as **8** and **11**) have not σ-π interaction with active pocket, which.led to very weak potency. Overall, compound **9** fitted well within S2 part, and formed five hydrogen bonds. Judging from what has been discussed above, we have come to recognize that the activity of coumarin derivatives might be improved to modify the other benzene ring P1 in order to extend it to the S1 part of PTP 1B activity pocket.

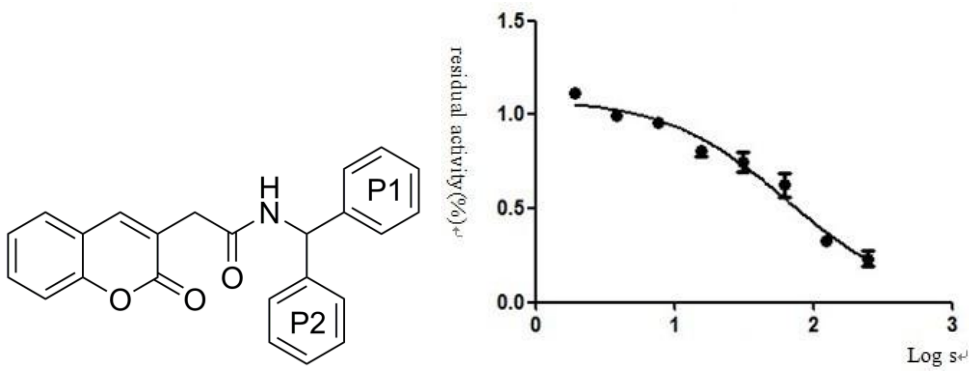


Figure 3. Structure of compound **9** for the docking study (left) and its detection curve of IC_{50} (right)

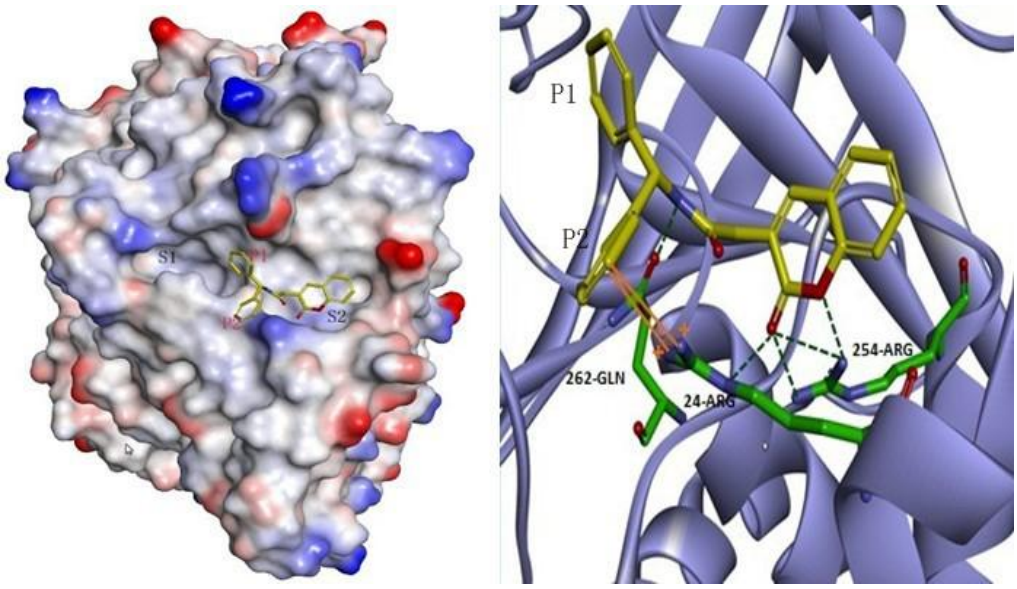


Figure 4. PTP 1B-binding electrostatic potential groove (left) and interactive map (right). Hydrogen bonds are shown as green dashed.

In conclusion, we have completed the synthesis and in vitro evaluation of a series of coumarin derivatives having a modest potency for inhibiting the recombinant human PTP 1B in vitro. The biological activity results told us that introduction of hydrophobic groups to 3-position of coumarin might improve the inhibition activity for PTP 1B. We have found that IC_{50} value of compound **9** is 49.2 μM. According to the docking results of computer simulation, modifying the benzene ring P1 of coumarin derivative **9** to extend it to the S1 part of PTP 1B activity pocket might improve the inhibitory activity of coumarin derivatives against PTP 1B in vitro. Further work is in progress to optimize the structure of compound **9** with the aim of increasing its potency as a potential PTP 1B inhibitor.

EXPERIMENTAL

Chemistry. General Procedures. All experiments involving water-sensitive compounds were conducted under scrupulously dry conditions. Melting points were determined using an SGW-X4B digital melting point apparatus and was uncorrected. ^{1}H NMR spectra was recorded on 400 MHz Bruker Avance DPX spectrometers. All NMR spectra were recorded in $CDCl_3$ or DMSO-d_6 at room temperature. Chemical shifts for ^{1}H spectra are quoted in ppm downfield from TMS. Coupling constants are referred to as *J* values. ESI mass spectra were acquired using a Bruker ESQUIRELCTM ESI ion trap spectrometer. HR-ESI mass spectra were obtained with an Agilent Technologies ESI-TOF spectrometer. FT-IR spectra were recorded in the region of 4000–400 cm^{-1} with a PerkinElmer spectrum 65 FT-IR using KBr pellets.

Preparation of Compound 3. The dry paraformaldehyde (116.7 mmol) was added in portions to a

mixture of phenol (33.3 mmol), Et_3N (93.3 mmol) and anhydrous $MgCl_2$ (103 mmol) in MeCN (300 mL). The mixture was refluxed for 8 h, cooled to room temperature, acidified with aqueous 3 N HCl solution, and extracted with Et_2O. The ether layer was washed with water, and brine, and dried ($MgSO_4$). Removal of solvent yielded a crude material which was purified by column chromatography to yield the products **3**.

Preparation of 2-(2-oxo-2*H*-chromen-3-yl)acetic acids 4.[20] A mixture of 0.3 mol succinic anhydride, 0.1 mol of salicylaldehyde **3**, and 0.13 mol of Et_3N was heated under stirring to the boiling point. After 1-1.5 h, abundant solid separated. The mixture was cooled and treated with concentrated hydrochloric acid, and the precipitate was filtered off and dried. The product was dissolved in a warm saturated aqueous solution of $NaHCO_3$, the solution was filtered, finely powdered activated charcoal was added to the filtrate, the mixture was stirred for 15 min and filtered, and the filtrate was acidified with concentrated hydrochloric acid. The precipitate **4** was filtered off, washed with water, and dried in air until constant weight.

Standard procedure A for Compound 5-22: refer to the method of acid amine condensation： 0.162 g (10 mmol) CDI and 10 mmol acid **4** in 10 mL CH_2Cl_2 stirred 30 min at room temperature, then the reaction mixture was dropwise added to amines (10 mmol) dissolved in 5 mL CH_2Cl_2 , stirred 2-3 h at room temperature. The product was separated by column chromatography over silica gel (eluted with MeOH/CH_2Cl_2= 1/20) to afford the product.

***N*-(3-Bromophenyl)-2-(2-oxo-*2H*-chromen-3-yl)acetamide (5).** Compound **5** was prepared according to standard procedure A by using compound **4** and 3-bromoaniline, obtained as a white solid in 67% yield. mp116.7-118.1 °C; IR (KBr, cm^{-1}): 3435, 1747, 1699, 1616, 1565, 1248, 998, 797, 758; ^{1}HNMR (400 MHz, $CDCl_3$): 8.52 (s, 1H), 7.86 (s, 1H), 7.80 (s, 1H), 7.58-7.51 (m, 2H), 7.42-7.31 (m, 3H), 7.21 (d, *J* = 8.0 Hz, 1H), 7.15 (t, *J* = 8.0 Hz, 1H), 3.64 (s, 2H); HRMS-ESI (*m/z*) calcd for $C_{17}H_{12}BrNO_3$ $[M+H]^+$ 358.0079, found 358.0081.

***N*-(4-Bromophenyl)-2-(2-oxo-*2H*-chromen-3-yl)acetamide (6).** Compound **6** was prepared according to standard procedure A by using compound **4** and 4-bromoaniline, obtained as a white solid in 64% yield. mp 246.5-248.2 °C; IR (KBr, cm^{-1}): 3334, 1714, 1698, 1607, 1544, 1489, 1395, 1248, 1085, 819, 749; ^{1}HNMR (400 MHz, DMSO-d_6): 10.31 (s, 1H), 8.01 (s, 1H), 7.72 (d, *J* = 7.6 Hz, 1H), 7.63-7.56 (m, 3H), 7.49 (d, *J* = 8.8 Hz, 2H), 7.43 (d, *J* = 8.0 Hz, 1H), 7.37 (t, *J* = 7.6 Hz, 1H), 3.63 (s, 2H); HRMS-ESI (*m/z*) calcd for $C_{17}H_{12}BrNO_3$ $[M+H]^+$ 358.0079, found 358.0083.

***N*-(4-Iodophenyl)-2-(2-oxo-*2H*-chromen-3-yl)acetamide (7).** Compound **7** was prepared according to standard procedure A by using compound **4** and 4-iodoaniline, obtained as a white solid in 68% yield. mp 240.2-241.8 °C; IR (KBr, cm^{-1}): 3334, 1714, 1698, 1605, 1538, 1246, 820, 749; ^{1}HNMR (400 MHz, DMSO-d_6): 10.28 (s, 1H), 8.01 (s, 1H), 7.73 (d, *J* = 7.6 Hz, 1H), 7.65-7.58 (m, 3H), 7.45-7.39 (m, 3H), 7.37 (t, *J* = 7.6 Hz, 1H), 3.63 (s, 2H); HRMS-ESI (*m/z*) calcd for $C_{17}H_{12}INO_3$ $[M+H]^+$ 405.9940, found

405.9949.

***N*-(Naphthalen-2-yl)-2-(2-oxo-*2H*-chromen-3-yl)acetamide (8).** Compound **8** was prepared according to standard procedure A by using compound **4** and naphthalen-2-amine, obtained as a white solid in 64% yield. mp 238.2-240.1 °C; IR (KBr, cm^{-1}): 3336, 1719, 1697, 1507, 1432, 1280, 859, 740; ^{1}HNMR (400 MHz, DMSO-d_6): 10.38 (s, 1H), 8.29 (s, 1H), 8.05 (s, 1H), 7.88-7.74 (m, 4H), 7.61 (d, J = 7.6 Hz, 2H), 7.48-7.36 (m, 4H), 3.70 (s, 2H); HRMS-ESI (*m/z*) calcd for $C_{21}H_{15}NO_3$ $[M+H]^+$ 330.1130, found 330.1137.

***N*-Benzhydryl-2-(2-oxo-*2H*-chromen-3-yl)acetamide (9).** Compound **9** was prepared according to standard procedure A by using compound **4** and diphenylmethanamine, obtained as a white solid in 58% yield. mp 240.8-242.6 °C; IR (KBr, cm^{-1}): 3292, 1728, 1644, 1242, 1074, 752, 697; ^{1}HNMR (400 MHz, DMSO-d_6): 9.01 (d, J = 8.4 Hz, 1H), 7.91 (s, 1H), 7.67 (dd, J = 7.6, 1.6 Hz, 1H), 7.58 (td, J = 7.6, 1.6 Hz, 1H), 7.31 (m, 12H), 6.13 (d, J = 8.4 Hz, 1H), 3.53 (s, 2H); HRMS-ESI (*m/z*) calcd for $C_{24}H_{19}NO_3$ $[M+H]^+$ 370.1443, found 370.1436.

***N*-(2,3-Dihydrobenzo[*b*][1,4]dioxin-6-yl)-2-(2-oxo-*2H*-chromen-3-yl)acetamide (10).** Compound **10** was prepared according to standard procedure A by using compound **4** and 2,3-dihydrobenzo[*b*][1,4]dioxin-6-amine, obtained as a white solid in 61% yield. mp 200.5-202.6 °C; IR (KBr, cm^{-1}): 3348, 1709, 1671, 1611, 1512, 1221, 1068, 755; ^{1}HNMR (400 MHz, $CDCl_3$): 8.21 (s, 1H), 7.85 (s, 1H), 7.53 (m, 2H), 7.37-7.28 (m, 2H), 7.17 (d, J = 2.4 Hz, 1H), 6.89 (dd, J = 8.8, 2.4 Hz, 1H), 6.77 (d, J = 8.4 Hz, 1H), 4.24 (s, 4H), 3.61 (s, 2H); HRMS-ESI (*m/z*) calcd for $C_{19}H_{15}NO_5$ $[M+H]^+$ 338.1028, found 338.1033.

***N*-Benzyl-2-(2-oxo-*2H*-chromen-3-yl)acetamide (11).** Compound **11** was prepared according to standard procedure A by using compound **4** and phenylmethanamine, obtained as a white solid in 71% yield. mp 184.6-186.4 °C; IR (KBr, cm^{-1}): 3283,1731, 1643, 1550, 1455, 1239, 1057, 765, 698; ^{1}HNMR (400 MHz, $CDCl_3$): 7.81 (s, 1H), 7.54-7.48 (m, 2H), 7.35-7.24 (m, 7H), 4.43 (d, J = 5.6 Hz, 2H), 3.51 (s, 2H); HRMS-ESI (*m/z*) calcd for $C_{18}H_{15}NO_3$ $[M+H]^+$ 294.1130, found 294.1137.

***N*-(3-Methoxyphenyl)-2-(2-oxo-*2H*-chromen-3-yl)acetamide (12).** Compound **12** was prepared according to standard procedure A by using compound **4** and 3-methoxyaniline, obtained as a white solid in 69% yield. mp 109.5-111.2 °C; IR (KBr, cm^{-1}): 3449, 3030, 2930, 1728, 1679, 1559, 1453, 1366, 1203, 972, 778; ^{1}HNMR (400 MHz, $CDCl_3$): 8.39 (s, 1H), 7.86 (s, 1H), 7.56-7.50 (m, 2H), 7.38-7.26 (m, 3H), 7.19 (t, J = 8.0 Hz, 1H), 7.00 (d, J = 7.6 Hz, 1H), 6.64 (dd, J = 8.0, 1.6 Hz, 1H), 3.79 (s, 3H), 3.64 (s, 2H); HRMS-ESI (*m/z*) calcd for $C_{18}H_{15}NO_4$ $[M+H]^+$ 310.1079, found 310.1073.

***N*-(4-Methoxybenzyl)-2-(2-oxo-*2H*-chromen-3-yl)acetamide (13).** Compound **13** was prepared according to standard procedure A by using compound **4** and (4-methoxyphenyl)methanamine, obtained as a white solid in 72% yield. mp 182.2-184.1 °C; IR (KBr, cm^{-1}): 3275, 1729, 1637, 1515, 1254, 1055,

750; ^{1}HNMR (400 MHz, $CDCl_3$): 7.81 (s, 1H), 7.50 (s, 2H), 7.35-7.21 (m, 4H), 6.86 (s, 2H), 6.56 (s, 1H), 4.37 (s, 2H), 3.79 (s, 3H), 3.49 (s, 2H); HRMS-ESI (*m/z*) calcd for $C_{19}H_{17}NO_4$ $[M+H]^+$ 324.1236, found 324.1240.

***N*-(2,4-Dimethoxybenzyl)-2-(2-oxo-*2H*-chromen-3-yl)acetamide (14).** Compound **14** was prepared according to standard procedure A by using compound **4** and (2,4-dimethoxyphenyl)methanamine, obtained as a yellow solid in 54% yield. mp 159.2-160.8 °C; IR (KBr, cm^{-1}): 3290, 1714, 1604, 1567, 1242, 1057, 762; ^{1}HNMR (400 MHz, DMSO-d_6): 8.27 (t, *J* = 5.2 Hz, 1H), 7.93 (s, 1H), 7.69 (d, *J* = 8.0 Hz, 1H), 7.59 (t, *J* = 7.6 Hz, 1H), 7.41 (d, *J* = 7.6 Hz, 2H), 7.36 (d, *J* = 8.4 Hz, 1H), 7.12 (d, *J* = 8.0 Hz, 1H), 6.53 (s, 1H), 6.48 (d, *J* = 8.0 Hz, 1H), 4.17 (d, *J* = 5.6 Hz, 2H), 3.78 (s, 3H), 3.74 (s, 3H), 3.43 (s, 2H); HRMS-ESI (*m/z*) calcd for $C_{20}H_{19}NO_5$ $[M+H]^+$ 354.1341, found 354.1348.

***N*-(Furan-2-ylmethyl)-2-(2-oxo-*2H*-chromen-3-yl)acetamide (15).** Compound **15** was prepared according to standard procedure A by using compound **4** and furan-2-ylmethanamine, obtained as a white solid in 62% yield. mp 150.2-152.0 °C; IR (KBr, cm^{-1}): 3290, 1729, 1713, 1646, 1614, 1458, 1242, 1060, 743; ^{1}HNMR (400 MHz, DMSO-d_6): 8.18 (t, *J* = 5.2 Hz, 1H), 7.91 (s, 1H), 7.69 (d, *J* = 7.6 Hz, 1H), 7.59 (t, *J* = 7.6 Hz, 1H), 7.42-7.32 (m, 3H), 6.95 (t, *J* = 3.6 Hz, 1H), 6.90 (s, 1H), 3.37 (s, 2H), 2.94 (t, *J* = 7.2 Hz, 2H); HRMS-ESI (*m/z*) calcd for $C_{16}H_{13}NO_4$ $[M+H]^+$ 284.0923, found 284.0921.

2-(2-Oxo-*2H*-chromen-3-yl)-*N*-(2-(thiophen-2-yl)ethyl)acetamide (16). Compound **16** was prepared according to standard procedure A by using compound **4** and 2-(thiophen-2-yl)ethanamine, obtained as a white solid in 56% yield. mp 149.2-151.1 °C, IR (KBr, cm^{-1}): 3290, 1730, 1716, 1647, 1555, 1244, 1187, 1060, 760, 700; ^{1}HNMR (400 MHz, DMSO-d_6): 8.18 (t, *J* = 5.2 Hz, 1H), 7.91 (s, 1H), 7.70 (d, *J* = 8.0 Hz, 1H), 7.59 (t, *J* = 7.6 Hz, 1H), 7.42-7.32 (m, 3H), 6.95 (t, *J* = 3.6 Hz, 1H), 6.90 (s, 1H), 3.37 (s, 2H), 3.31 (t, *J* = 7.2 Hz, 2H), 2.94 (t, *J* = 7.2 Hz, 2H); HRMS-ESI (*m/z*) calcd for $C_{17}H_{15}NO_3S$ $[M+H]^+$ 314.0851, found 314.0855.

Methyl 3-(4-hydroxyphenyl)-2-(2-(2-oxo-*2H*-chromen-3-yl)acetamido)propanoate (17). Compound **17** was prepared according to standard procedure A by using compound **4** and methyl 2-amino-3-(4-hydroxyphenyl)propanoate, obtained as a white solid in 46% yield. mp 162.4-164.3 °C, IR (KBr, cm^{-1}): 3317, 3242, 1737, 1722, 1716, 1610, 1243; ^{1}HNMR (400 MHz, $CDCl_3$): 7.68 (s, 1H), 7.53 (t, *J* = 7.6 Hz, 1H), 7.45 (d, *J* = 7.6 Hz, 1H), 7.36 (d, *J* = 8.0 Hz, 1H), 7.33 (d, *J* = 7.2 Hz, 1H), 6.94 (d, *J* = 8.4 Hz, 2H), 6.73 (d, *J* = 8.0 Hz, 1H), 6.59 (d, *J* = 8.0 Hz, 2H), 4.82 (dd, *J* = 13.2, 7.6 Hz, 1H), 3.89 (s, 1H), 3.74 (s, 1H), 3.73 (s, 3H), 3.52 (d, *J* = 14.0 Hz, 1H), 2.99 (d, *J* = 14.0 Hz, 1H); HRMS-ESI (*m/z*) calcd for $C_{21}H_{19}NO_6$ $[M+H]^+$ 382.1291, found 382.1296.

3-(2-(4-Methylpiperazin-1-yl)-2-oxoethyl)-*2H*-chromen-2-one (18). Compound **18** was prepared according to standard procedure A by using compound **4** and 1-methylpiperazine, obtained as a white solid in 60% yield. mp 123.3-125.2 °C; IR (KBr, cm^{-1}): 3009, 2935, 2858, 2800, 1726, 1659, 1226, 1063,

758; ^{1}HNMR (400 MHz, $CDCl_3$): 7.75 (s, 1H), 7.52-7.46 (m, 2H), 7.33 (d, *J* = 8.0 Hz, 2H), 7.28 (d, *J* = 8.0 Hz, 2H), 3.72-3.63 (m, 6H), 2.45 (d, *J* = 16.4 Hz, 4H), 2.33 (s, 3H); HRMS-ESI (*m/z*) calcd for $C_{16}H_{18}N_2O_3$ $[M+H]^+$ 287.1396, found 287.1341.

3-(2-(4-(Furan-2-carbonyl)piperazin-1-yl)-2-oxoethyl)-*2H*-chromen-2-one. (19). Compound **19** was prepared according to standard procedure A by using compound **4** and 4-(piperazine-1-carbonyl)furan-2-carbaldehyde, obtained as a white solid in 62% yield; mp 188.5-190.4 °C; IR (KBr, cm^{-1}): 3107, 2918, 1732, 1630, 1483, 1434, 1232, 1011, 757 cm^{-1}; ^{1}HNMR (400 MHz, DMSO-d_6): 7.88 (d, *J* = 8.0 Hz, 2H), 7.71 (d, *J* = 8.0 Hz, 1H), 7.60 (d, *J* = 7.2 Hz, 1H), 7.42 (d, *J* = 8.4 Hz, 1H), 7.36 (t, *J* = 7.6 Hz, 1H), 7.05 (d, *J* = 3.2 Hz, 1H), 6.65 (q, *J* = 1.6 Hz, 1H), 3.75-3.65 (m, 8H), 3.57 (m, 2H); HRMS-ESI (*m/z*) calcd for $C_{20}H_{18}N_2O_5$ $[M+H]^+$ 367.1294, found 367.1299.

2-(7-Methoxy-2-oxo-*2H*-chromen-3-yl)-*N*-(4-methoxybenzyl)acetamide (20). Compound **20** was prepared according to standard procedure A by using compound **4** and (4-methoxyphenyl)methanamine, obtained as a yellow solid in 58% yield. mp 189.6-191.4 °C; IR (KBr, cm^{-1}): 3282, 1725, 1640, 1611, 1513, 1240, 820; ^{1}HNMR (400 MHz, DMSO-d_6): 8.43 (t, *J* = 5.6 Hz, 1H), 7.86 (s, 1H), 7.60 (d, *J* = 8.4 Hz, 1H), 7.19 (d, *J* = 8.4 Hz, 2H), 7.00 (s, 1H), 6.96 (d, *J* = 8.8 Hz, 1H), 6.88 (d, *J* = 8.4 Hz, 2H), 4.22 (d, *J* = 5.6 Hz, 2H), 3.85 (s, 3H), 3.73 (s, 3H), 3.37 (s, 2H); HRMS-ESI (*m/z*) calcd for $C_{20}H_{19}NO_5$ $[M+H]^+$ 354.1341, found 354.1344.

***N*-(4-Hydroxyphenethyl)-2-(7-methoxy-2-oxo-*2H*-chromen-3-yl)acetamide (21).** Compound **21** was prepared according to standard procedure A by using compound **4** and 4-(2-aminoethyl)phenol, obtained as a white solid in 48% yield. mp 180.1-182.0 °C; IR (KBr, cm^{-1}): 3336, 1705, 1613, 1444, 1249, 1158, 1068, 829; ^{1}HNMR (400 MHz, DMSO-d_6): 9.16 (s, 1H), 8.03 (t, *J* = 5.6 Hz, 1H), 7.80 (s, 1H), 7.60 (d, *J* = 8.4 Hz, 1H), 7.00-6.94 (m, 4H), 6.66 (d, *J* = 8.4 Hz, 2H), 3.86 (s, 3H), 3.29 (s, 2H), 3.21 (dd, *J* = 6.4, 13.6 Hz, 2H), 2.59 (t, *J* = 7.2 Hz, 2H); HRMS-ESI (*m/z*) calcd for $C_{20}H_{19}NO_5$ $[M+H]^+$ 354.1341, found 354.1347.

***N*-(4-Fluorobenzyl)-2-(7-methoxy-2-oxo-*2H*-chromen-3-yl)acetamide (22).** Compound **22** was prepared according to standard procedure A by using compound **4** and (4-fluorophenyl)methanamine, obtained as a yellow solid in 48% yield. mp 236.4-238.3 °C; IR (KBr, cm^{-1}): 3280, 1723, 1643, 1612, 1509, 1211, 836; ^{1}HNMR (400 MHz, DMSO-d_6): 8.51 (s, 1H), 7.87 (s, 1H), 7.60 (d, *J* = 8.0 Hz, 1H), 7.31 (t, *J* = 8.0 Hz, 2H), 7.14 (t, *J* = 8.0 Hz, 2H), 7.01 (s, 1H), 6.96 (d, *J* = 8.0 Hz, 1H), 4.27 (s, 2H), 3.85 (s, 3H), 3.39 (s, 2H); HRMS-ESI (*m/z*) calcd for $C_{19}H_{16}FNO_4$ $[M+H]^+$ 342.1142, found 342.1139.

Standard procedure B for Compound 23, 24: To a stirred solution of the appropriate acid **4** (5 mmol) and alcohol (5.5 mmol) in CH_2Cl_2 (25 mL), DCC (5.5 mmol) and DMAP (0.5 mmol) were added. After 24 h stirring at room temperature, the reaction mixture was filtered and the solution washed with 10% citric acid (2×20 mL), water (15 mL), and brine (30 mL), dried over anhydrous Na_2SO_4, filtered and

concentrated under reduced pressure. The crude product was purified by column chromatography over silica gel (eluted with MeOH/CH_2Cl_2= 1/20) to afford the product.

Benzyl 2-(2-oxo-*2H*-chromen-3-yl)acetate (23). Compound **23** was prepared according to standard procedure B by using compound **4** and phenylmethanol, obtained as a white solid in 71% yield. mp 114.2-115.5 °C; IR (KBr, cm^{-1}): 3087, 2931, 1727, 1639, 1608, 1452, 1338, 1194, 756; ^{1}HNMR (400 MHz, $CDCl_3$): 7.65 (s, 1H), 7.51 (t, *J* = 8.0 Hz, 1H), 7.44 (d, *J* = 7.2 Hz, 1H), 7.37-7.32 (s, 6H), 7.27 (t, *J* = 7.2 Hz, 1H), 5.10 (s, 2H), 3.65 (s, 2H); HRMS-ESI (*m/z*) calcd for $C_{18}H_{14}O_4$ $[M+H]^+$ 295.0970, found 295.0974.

(5-Formylfuran-2-yl)methyl 2-(2-oxo-*2H*-chromen-3-yl)acetate (24). Compound **24** was prepared according to standard procedure B by using compound **4** and 5-(hydroxymethyl)furan-2-carbaldehyde, obtained as a white solid in 73% yield. mp 141.8-143.3 °C; IR (KBr, cm^{-1}): 3095, 2963, 1740, 1712, 1677, 1610, 1343, 1268, 1190, 759; ^{1}HNMR (400 MHz, DMSO-d_6): 9.60 (s, 1H), 8.04 (s, 1H), 7.71 (d, *J* = 7.6 Hz, 1H), 7.62 (t, *J* = 7.6 Hz, 1H), 7.53 (d, *J* = 3.2 Hz, 1H), 7.43 (d, *J* = 8.4 Hz, 2H), 7.38 (t, *J* = 7.6 Hz, 1H), 6.82 (d, *J* = 3.2 Hz, 1H), 5.23 (s, 2H), 3.69 (s, 2H); HRMS-ESI (*m/z*) calcd for $C_{17}H_{12}O_6$ $[M+H]^+$ 313.0712, found 313.0714.

Preparation of 2-oxo-*2H*-chromene-3-carboxylic acid 25. Salicylaldehyde **3a** with Meldrum's acid in water at 75 °C provided 3-carboxycoumarin derivatives. The reaction proceeds to completion in 2 h and the product precipitates during the course of the reaction and was separated by filtration. Yield 80%. ^{1}HNMR (400 MHz, $CDCl_3$): 12.32 (s, 1H), 9.00 (s, 1H), 7.82-7.76 (m, 2H), 7.51-7.47 (m, 2H). ESI-MS (*m/z*) $[M+H]^+$ 191.2.

***N*-(4-Hydroxyphenyl)-7-methoxy-2-oxo-*2H*-chromene-3-carboxamide (26).** Compound **26** was prepared according to standard procedure A by using compound **25** and 4-aminophenol, obtained as a white solid in 52% yield. ^{1}HNMR (400 MHz, DMSO-d_6): 9.83 (s, 1H), 8.52 (s, 1H), 7.63 (d, *J* = 8.0 Hz, 1H), 7.31 (d, *J* = 7.6 Hz, 1H), 7.22 (m, 4H), 6.73 (d, *J* = 8.0 Hz, 2H), 5.72 (s, 1H); HRMS-ESI (*m/z*) calcd for $C_{16}H_{11}NO_4$ $[M+H]^+$ 282.0766, found 282.0770.

***N*-(3,5-Dimethoxyphenyl)-7-methoxy-2-oxo-*2H*-chromene-3-carboxamide (27).** Compound **27** was prepared according to standard procedure A by using compound **25** and 3,5-dimethoxyaniline, obtained as a yellow solid in 54% yield. ^{1}HNMR (400 MHz, $CDCl_3$): 10.82 (s, 1H), 9.02 (s, 1H), 7.72 (dd, *J* = 7.2, 13.6 Hz, 2H), 7.52-7.40 (m, 2H), 7.00 (d, *J* = 2.0 Hz, 2H), 6.31 (s, 1H), 3.83 (m, 6H); HRMS-ESI (*m/z*) calcd for $C_{18}H_{15}NO_5$ $[M+H]^+$ 326.1028, found 326.1033.

Methyl 3-(4-hydroxyphenyl)-2-(2-oxo-*2H*-chromene-3-carboxamido)propanoate (28). Compound **28** was prepared according to standard procedure A by using compound **25** and methyl 2-amino-3-(4-hydroxyphenyl)propanoate, obtained as a white solid in 45% yield. ^{1}HNMR (400 MHz, $CDCl_3$): 9.22 (d, *J* = 7.2 Hz, 1H), 8.85 (s, 1H), 7.68-7.65 (m, 2H), 7.38 (dd, *J* = 8.4, 14.8 Hz, 2H), 7.09

(d, *J* = 8.4 Hz, 2H), 6.76 (d, *J* = 8.4 Hz, 2H), 5.04 (s, 1H), 4.95 (dd, *J* = 7.2, 12.8 Hz, 1H), 3.77 (s, 3H), 3.20 (dd, *J* = 5.2, 14.0 Hz, 1H), 3.09 (dd, *J* = 7.2, 14.0 Hz, 1H); HRMS-ESI (*m/z*) calcd for $C_{20}H_{17}NO_6$ $[M+H]^+$ 368.1134, found 368.1139.

Standard procedure C for Compound 29-31. The corresponding salicylaldehyde (10 mmol) and ethyl acetoacetate (10 mmol) were dissolved in EtOH. Piperidine (0.02 mmol) was added, and the mixture was refluxed for 4 h. Filtration of the cooled mixture and the subsequent washing of product with cold ethanol provided analytically pure crystals, or separated by column chromatography over silica gel (eluted with MeOH/CH_2Cl_2= 1/20) to afford the product.

3-Acetyl-6,7,8,9-tetrahydro-*2H*-benzo[*g*]chromen-2-one (29). Compound **29** was prepared according to standard procedure C by using ethyl acetoacetate and 3-hydroxy-5,6,7,8-tetrahydronaphthalene-2-carbaldehyde, obtained as a yellow solid in 56% yield. mp 94.8-95.6 °C; IR (KBr, cm^{-1}): 2939, 1729, 1683, 1622, 1551, 1365, 1219, 769; ^{1}HNMR (400 MHz, $CDCl_3$): 8.45 (s, 1H), 7.31 (s, 1H), 7.06 (s, 1H), 2.89-2.83 (m, 4H), 2.72 (s, 3H), 1.83 (m, 4H); HRMS-ESI (*m/z*) calcd for $C_{15}H_{14}O_3$ $[M+H]^+$ 243.1021, found 243.1022.

3-Acetyl-7,8-dihydrocyclopenta[*g*]chromen-2(*6H*)-one (30). Compound **30** was prepared according to standard procedure C by using ethyl acetoacetate and 6-hydroxy-2,3-dihydro-*1H*-indene-5-carbaldehyde, obtained as a yellow solid in 60% yield. mp 162.3-164.2 °C; IR (KBr, cm^{-1}): 2970, 1736, 1718, 1681, 1560, 1361, 1212, 954, 769; ^{1}HNMR (400 MHz, $CDCl_3$): 11.10 (s, 1H), 9.80 (s, 1H), 7.34 (s, 1H), 2.92 (m, 6H), 2.12 (s, 3H); HRMS-ESI (*m/z*) calcd for $C_{14}H_{12}O_3$ $[M+H]^+$ 229.0865, found 229.0869.

3-Acetyl-6-(phenylamino)-*2H*-chromen-2-one (31). Compound **31** was prepared according to standard procedure C by using ethyl acetoacetate and 2-hydroxy-5-(phenylamino) benzaldehyde, obtained as a red solid in 42% yield. mp 192.3-194.2 °C; IR (KBr, cm^{-1}): 3328, 1700, 1686, 1562, 1232, 1001; ^{1}HNMR (400 MHz, DMSO-d_6): 8.62 (s, 1H), 8.40 (s, 1H), 7.60 (d, *J* = 2.4 Hz, 1H), 7.42-7.36 (m, 2H), 7.26 (t, *J* = 7.6 Hz, 2H), 7.11 (d, *J* = 7.6 Hz, 2H), 6.87 (t, *J* = 7.2 Hz, 1H), 2.58 (s, 3H); HRMS-ESI (*m/z*) calcd for $C_{17}H_{13}NO_3$ $[M+H]^+$ 280.0974, found 280.0979.

Preparation of active protein tyrosine Phosphatase 1B. The catalytic domain of PTP1B, consisting of amino acids 1–321, was inserted into the EcoR I and Not I site of the pGEX-6P-1 vector R. Recombinant human PTP 1B was produced according to a procedure published previously with minor modification. Briefly, PTP 1B was expressed and processed in Transetta (DE3) cells, Bacterial cultures were grown in 800 mL LB medium at 37 °C for 4-5 h, which containing 100 μg/mL ampicillin until the OD600 reached 0.6-0.8. Then, the culture was transferred to 16 °C, and protein expression was induced for 12-16 h with 0.25 mol/L isopropyl-β-D-1-thiogalactopyranoside (IPTG). Harvested cells were resuspended in lysis buffer containing 50 mM Hepes-NaOH (pH=7.5), 10 mM NaCl, 10% glycerol, 1% β-Me with a JN-3000 PLUS low temperature ultra-high pressure cell disrupter (JNBIO, Guangzhou). The lysate was

centrifuged at 16000 rpm for 30 min at 4 °C to remove cell debris. The supernatant was then loaded twice onto a GST agarose column pre-equilibrated with lysis buffer. The FLAG peptide of PTP 1B was cut off by a PPase in the above buffer. Fractions containing PTP 1B were pooled and concentrated with a Millipore Ultrafree filtration device. The concentrated PTP 1B was loaded onto a Hi-Trap heparin (GE Healthcare) equilibrated with above buffer. Fractions containing PTP 1B were pooled and concentrated. The PTP 1B obtained with this method shows 38 kDa on 10% SDS-polyacrylamide gel electrophoresis, approximately 6-10 mg of PTP 1B were recovered from a 1000 mL culture and aliquots stored at -80 °C.

Biological activtity test[23]

Preliminary screening. PTP 1B purified are dissolved in enzyme activity buffer, made into 200 nM PTP 1B protein solution. And the substrate pNPP (bought from BIO BASIC INC) soluble are dissolved in enzyme activity buffer, made into 4 mM substrate reserve liquid. All synthesized compounds are dissolved in 95% DMSO respectively, made up to 50 mM for the test solution.

First of all, 50 μL PTP 1B protein solution is added to NUNC 96 orifice (Nunclon TM Surface) in turn, and 1 μL compound solution to be tested, shakes 1 min, incubates 30 min in 30 °C, then add the 50 μL pNPP, shakes 10 s. At the same time set positive and negative control, and join 50 μL PTP 1B protein solution, 1 μL 95% DMSO to the negative control, concussion 1 min, incubate 30 min in 30 °C, then add 50 μL pNPP, shake 10 s. Join 50 μL PTP 1B protein solution, 1 μL (5 mM) Na_3VO_4 to the positive control, shake 1 min, incubate 30 min in 30 °C, then add the 50 μL pNPP, shake 10 s. Use the VARIOSKAN FLASH (Thermo SCITIFIC) to measure the changes of absorbance with reaction time under 405 nm wavelength, make a measurement each 6 s, 60 times in all. Each compound takes parallel test for three times, and draw the response curve to compute the inhibitory activity for PTP 1B. Make a further IC_{50} value measurement of compound with the residual activity less than 20%.

Compound IC_{50} value measurement. First of all, add 50 μL PTP 1B protein, 2 μL different concentrations compound into the 96 orifice plate in turn, shake 1 min, incubate 30 min in 30 °C, then add the 50 μL pNPP, shake 10 s. The changes of absorbance with reaction time under 405 nm wavelength, make a measurement each 6 s, 60 points in all. Test it three times in parallel, and draw the response curve to calculate the enzyme inhibitory activity of different concentrations compound. Take use of software GraphPad Prism 5 to do the nonlinear fitting analysis, with the residual active value for ordinate, compound concentration on the numerical for x axis, then draw the curve plotting to calculate the compound's IC_{50} value at last.

Computational studies

In order to predict the interactions and binding modes of **9** synthesized inhibitors in the PTP 1B active site, the Discovery Studio 3.0 was used to perform the molecular docking studies. The crystal structure of PTP 1B with small molecule was acquired from the Protein Data Bank (PDB code 2NT7). As for the

preparation of the protein, the small molecule included in 2NT7 protein was extracted, hydrogen atoms were added and the deletion of crystal waters using Prepare Protein Structure module.

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