Design and Synthesis of New 9-Substituted Norharmane Derivatives as Potential Sirt5 Inhibitors

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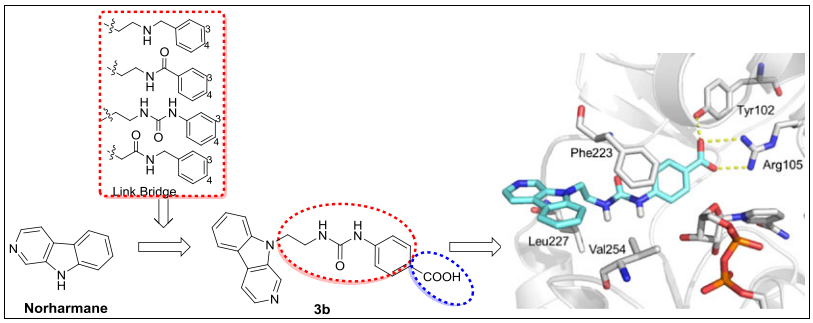


Sirt5 is a potential new drug target for the treatment of cancer, Alzheimer's disease, and Parkinson's disease. Given that norharmane is an important chemical synthon for some biologically important compounds and 9-substituted norharmane derivatives containing a negatively charged carboxyl group may accord with the characteristic of potential Sirt5 inhibitors, a series of novel 9-substituted norharmane derivatives were synthesized. The chemical structures and purities of all the target compounds were characterized by ^{1}H NMR, ^{13}C NMR, MS, and HPLC. By *in vitro* SIRT5 inhibitory assays, three compounds (**1a**, **3a**, and **3b**) show over 30% inhibition ratios at concentration of 100 μ*M*, and the most active compound **3b** has 35% and 52% inhibition ratios at 30 μ*M* and 100 μ*M*, respectively. Docking analysis showed that compound **3b** is likely to fit very well on the substrate binding site of Sirt5, and hence, we believe that compound **3b** can serve as a lead compound for further efforts to develop specific Sirt5 inhibitors.

INTRODUCTION

Sirtuins (Sirts) are known as a class of protein deacetylases, which carry out the deacetylation by using nicotinamide adenine dinucleotide as a cofactor. On the basis of sequence similarity, Sirts can be grouped into seven isoforms in mammals, Sirt1 to Sirt7 [1,2]. Among the seven Sirts, Sirt5 is a very interesting member, which only has weak deacetylation but has robust desuccinylation, demalonylation, and deglutarylation activities *in vitro* and *in vivo* [3–5]. Because these modifications by Sirt5 cover a broad range of pivotal protein substrates involved in cellular metabolism and metabolic energy homeostasis, aberrant activity of Sirt5 was considered to be a very critical factor for many human diseases, for example, cancer, Alzheimer's disease, and Parkinson's disease [6–8]. Therefore, Sirt5 may represent a fascinating drug target, and its inhibitors could be potential new treatments for associated diseases. However, up to now, only a few small-molecule Sirt5 inhibitors have been reported (Fig. 1), [9–11] and most of these compounds were initially investigated as inhibitors of other isoforms rather than Sirt5, except compound **I**. Thus, it is highly needed to develop novel Sirt5 inhibitors for characterizing the physiological function and therapeutical potentials.

By analyzing the crystal structure of Sirt5 in complex with its substrate, we found that there are three important features for substrate binding pocket, which would be useful in the design of new Sirt5 inhibitors (Fig. 2) [12]. First, three hydrophobic residues Leu227, Val254, and Phe223 form a triangle-shaped entrance for the pocket, which suggests that the compounds having triangle-shaped motif that can fit with this entrance could be potential inhibitors of Sirt5 [13]. Second, there are two specific non-hydrophobic residues, Arg105 and Tyr102, in the deep end of substrate binding pocket, which can interact with the carboxyl of the substrate by hydrogen-bonding and electrostatic interactions, indicating that some negatively charged groups, such as carboxyl and tetrazole, and some high polar groups, such as sulfonamide and isoxazol-3-ol, could be preferred chemical moieties to occupy this kind of pocket [14,15]. Third, the gate keeper residue Phe223 in loop S of Sirt5 may restrict large fragments to go into the deep end of the binding pocket, suggesting that it is better to use some rigid, linear chemical moieties or small aromatic rings as linkers to introduce the essential pharmacophore features into the deep end.

Norharmane and its derivatives are found to possess a variety of biological effects including anticancer and

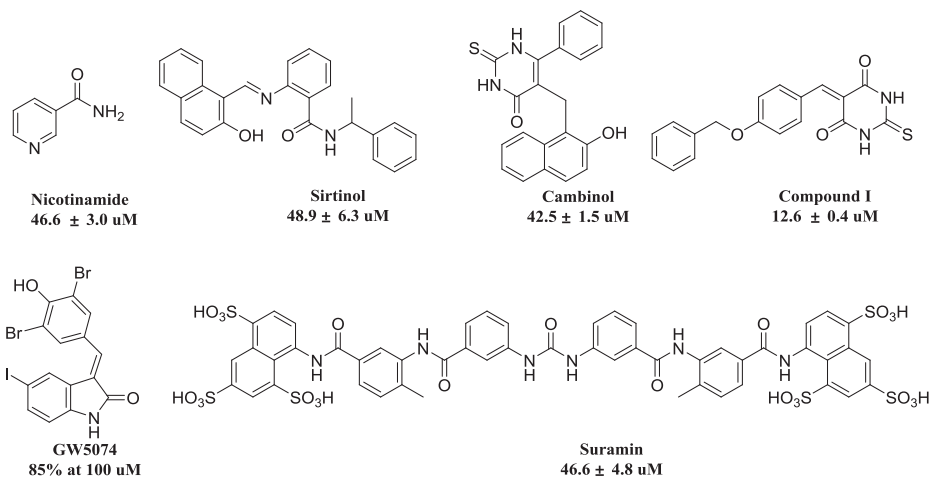


Figure 1. Known Sirt5 inhibitors and their activities.

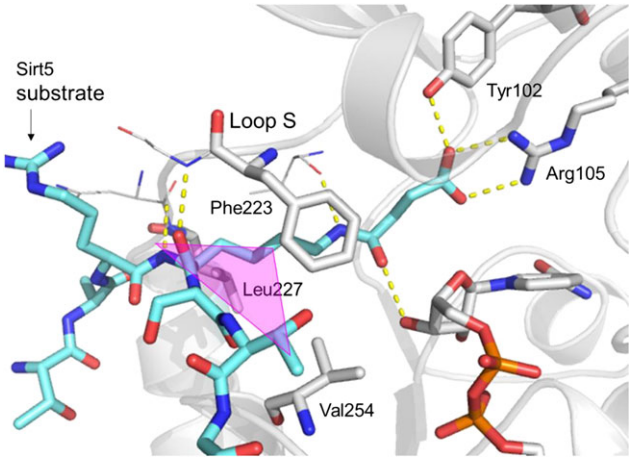


Figure 2. The features of substrate binding pocket of Sirt5 protein. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

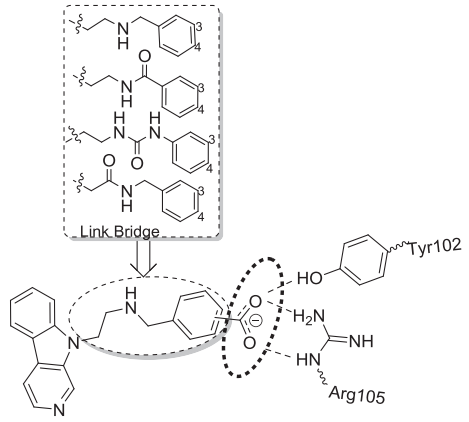


Figure 3. Schematic of structural modifications of 9-substituted norharmanes.

antimicrobial activities, and hence, norharmane is considered to be an important chemical synthon for some biologically important compounds [16–18]. Specifically, the 9-substituted norharmane derivatives seem to have a good match with the triangle-shaped entrance of the binding pocket of Sirt5. Thus, we thought that incorporation of a negatively charged carboxyl group to the terminal of substituents at the 9-position of norharmane by a rigid, linear-like linker with length of 6–8 atoms (Fig. 3) may make those derivatives to be potential Sirt5 inhibitors.

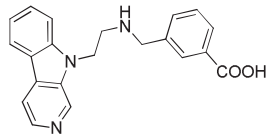
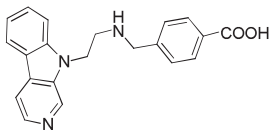
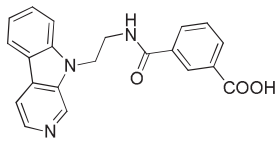
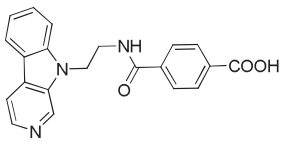
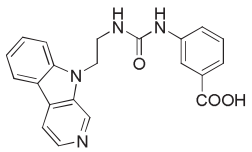
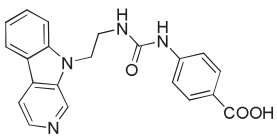
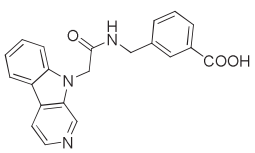
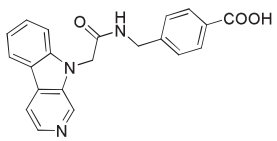
In order to examine our ideas, we first performed some chemical synthesis using the norharmane as raw material to obtain new 9-substituted norharmane derivatives. Then, the target compounds were tested by *in vitro* Sirt5 inhibitory assays. Molecular docking analysis was finally used to predict the possible binding modes of the most potent norharmane derivative.

RESULTS AND DISCUSSION

Chemistry. The novel 9-substituted norharmane derivatives shown in Tables 1 and 2 were synthesized by the synthetic routes outlined in Scheme 1 (**1a–c**, **2a–c**, and **3a–c**) and Scheme 2 (**4a–f**). Briefly, all of the target compounds were prepared from the commercially available norharmane (**7**), which reacted with 2-(*tert*-butoxycarbonylamino)ethyl-4-methylbenzenesulfonate (**6**) and ethyl 2-bromoacetate (**10**) to afford the compounds **8** and **11** in the presence of NaH. Then, the key intermediates **9** and **12** were obtained by the reaction of de-protection of amino and hydrolyzation of ester bond, respectively. Subsequently, target compounds **1a–c** were acquired through a two-step reductive amination reaction between intermediate **9** with various benzene formaldehydes. The compounds **2a** and **2b** were obtained by the hydrolysis reaction following the condensation of distinct benzoic acids with **9** in the presence of 1.0 equiv of 1-hydroxybenzotriazole (HOBT), 1.0 equiv of 1-(3-

Table 1

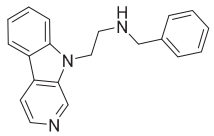
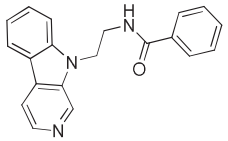
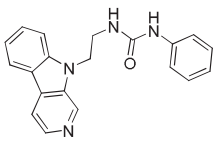
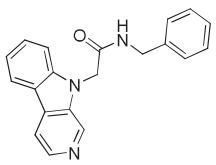
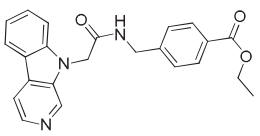
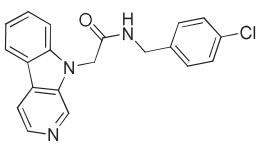
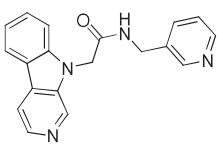
The Sirt 5 inhibitory activities of **1a**, **1b**, **2a**, **2b**, **3a**, **3b**, **4a**, and **4b**.

		% Inhibition (Sirtuin5)[a]		
Compound	Structure	10 μ*M*	30 μ*M*	100 μ*M*
1a		9	24	32
1b		10	19	22
2a		4	6	15
2b		11	19	25
3a		13	24	36
3b		19	35	52
4a		9	15	21
4b		6	17	25
Nicotinamide		—	—	76

[a]Each compound was tested twice; the data are presented as average values.

Table 2

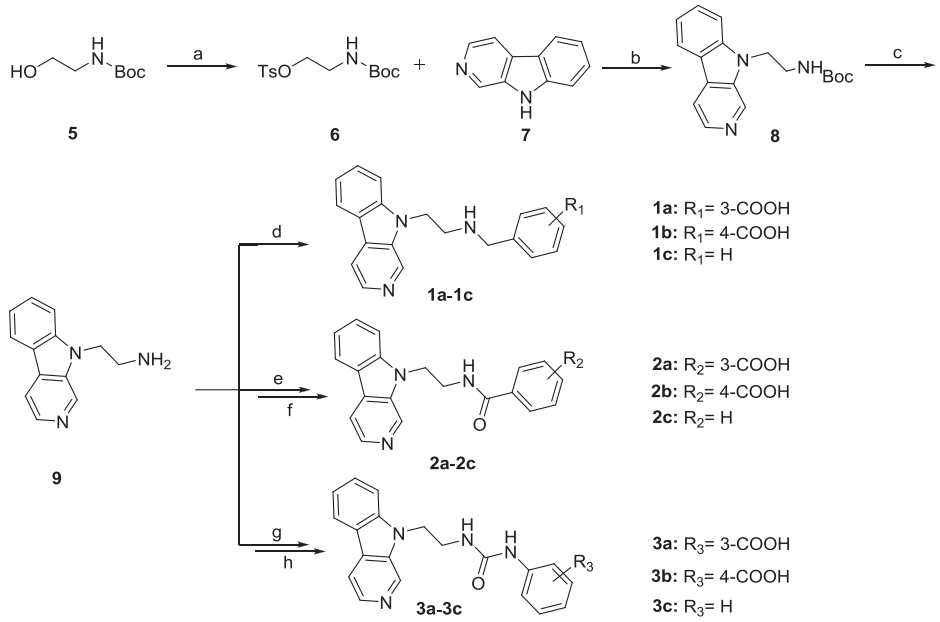
The Sirt5 inhibitory activities of **1c**, **2c**, **3c**, and **4c–f**.

		% Inhibition (Sirtuin5)[a]		
Compound	Structure	10 μ*M*	30 μ*M*	100 μ*M*
1c		4	8	10
2c		0	1	7
3c		0	7	10
4c		0	7	8
4d		6	10	12
4e		8	10	18
4f		10	15	24
Nicotinamide		—	—	76

[a]Each compound was tested twice; the data are presented as average values.

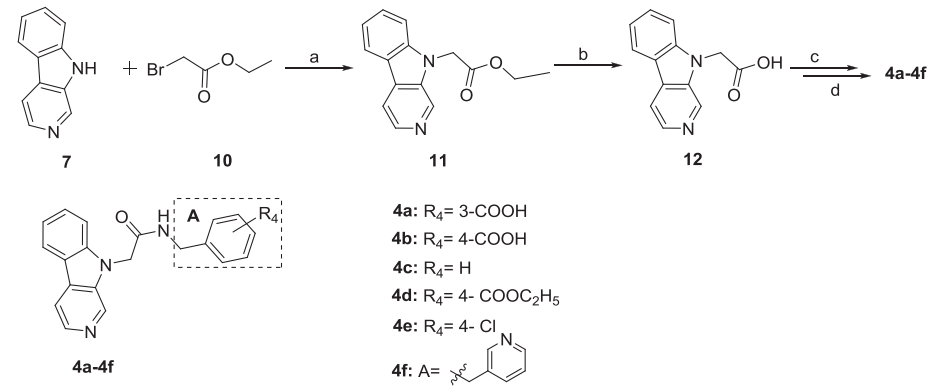
(dimethylamino)propyl)-3-ethylcarbodiimide hydrochloride (EDCI), and 1.5 equiv of *N*,*N*-diisopropylethylamine (DIEA), while compound **2c** was obtained firsthand through the condensation reaction. Similarly, **3a** and **3b** were acquired by the treatment of intermediate **9** with different isocyanates at refluxing CH_2Cl_2 and hydrolysis reaction of the esters in high yields [19]. The series of final compounds **4a–f** were synthesized using the same method as that for compounds **2a** or **2c** with yields ranging from 48% to quantitative.

Scheme 1. Synthetic routes for the target compounds **1a–c**, **2a–c**, and **3a–c**.



Reagents and conditions: (a) TsCl, Py, RT, 12h, 50%; (b) NaH, DMF, 45°C, 16h, 77%; (c) TFA, DCM, RT, 5h, 86%; (d) Hantzsch Ester, molecular sieve,TFA, DCM, 40°C,10h, 54%-63%; (e) HOBT, EDCI, DIEA, THF, Reflux,12h, 76%-84%; (f) For **2a** and **2b**: NaOH,H_2O/EtOH=1:1,85°C, 1.5h ,72%-81%; (g)BTC, Et_3N, DCM,RT-Reflux,2h,84%-98%; (h) For **3a** and **3b**: NaOH,H_2O/EtOH=1:1,100°C, 2h ,81%-88%

Scheme 2. Synthetic routes for the target compounds **4a–f**.



Reagents and conditions: (a) NaH, DMF, RT, 5h, 54%; (b) NaOH,H_2O/EtOH=1:1,100°C, 2h ,90%; (c) HOBT, EDCI, DIEA, DCM, RT, 12h, 58%-71%; (d) For **4a** and **4b**: NaOH,H_2O/EtOH=1:1,100°C, 2h ,80%-90%.

Biological activity. The inhibitory activities of target compounds **1a**, **1b**, **2a**, **2b**, **3a**, **3b**, **4a**, and **4b** against Sirt5 were evaluated using *in vitro* enzymatic inhibition assays through BPS Bioscience (San Diego, CA). All of these compounds were tested at concentrations of 10, 30, and 100 μ*M*, and nicotinamide was used as the positive control [20]. The Sirt5 inhibitory activities of these novel 9-substituted norharmane derivatives with different link bridges, including *N*-benzylpropan-1-amine, *N*-propylbenzamide, 1-phenyl-3-propylurea, and *N*-benzylpropionamide, are shown in Table 1. We can see from Table 1 that these compounds exhibited distinct inhibitory activities against Sirt5. Compounds **1a**, **3a**, and **3b** have over 30% inhibition ratios against Sirt5 at 100 μ*M*. The most potent compound **3b** shows 35% and 52% inhibition ratios at 30 and 100 μ*M*, respectively. These results indicated that compounds containing rigid link bridges like 1-phenyl-3-propylurea may possess high inhibitory activities against Sirt5. Subsequently, to further examine the importance of the

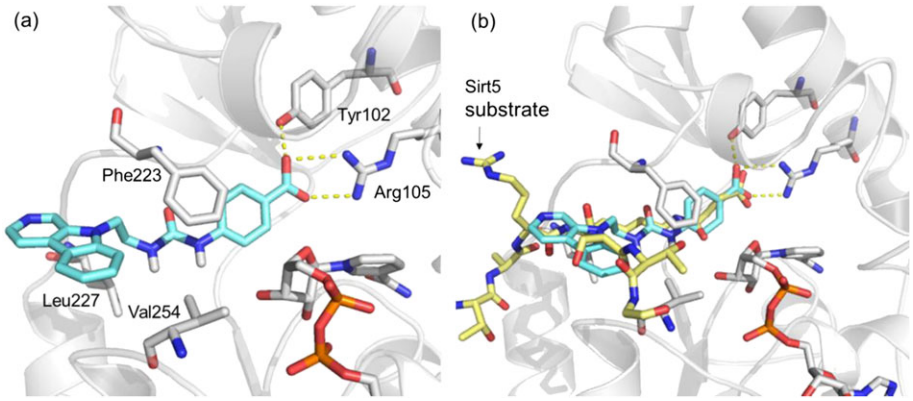


Figure 4. (a) The predicted binding mode of compound **3b** with Sirt5; (b) the binding mode of compound **3b** in comparison with the substrate of Sirt5. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

carboxyl group at the end of substituent, we synthesized compounds **1c**, **2c**, **3c**, and **4c–f** without the carboxyl group and evaluated their Sirt5 inhibitory activities. As shown in Table 2, the compounds without carboxyl groups only have low or no inhibitory activities against Sirt5.

Molecular docking. We further used molecular docking to analyze the possible binding mode of compound **3b**. The AutoDock Vina docking program was used here, and details about the docking method were given in the Experimental section. A total of six docking poses were generated for compound **3b**, and all of them have very similar binding modes, indicating those may be true binding modes. As shown in Figure 4a, the carboxyl group of compound **3b** forms ionic bonding interactions with Arg105 as well as tight hydrogen-bonding interactions with Arg105 and Tyr102, and the urea motif of compound **3b** seems to be parallel with Phe223 which may have polar–pi interactions between them. By overlapping the docking pose of compound **3b** with the co-crystal substrate, we can see that compound **3b** actually has a similar binding mode with that of Sirt5 substrate; for example, they both have interactions with Arg105 and Tyr102, and they also have good fit with the triangle-shaped entrance of the binding pocket formed by Phe223, Leu227, and Val254 (Fig. 4b).

CONCLUDING REMARKS

In this study, by analyzing the complex structure of Sirt5 with its substrate, we hypothesized that 9-substituted norharmane derivatives with a negatively charged carboxyl in the tail end of substituent may be potential Sirt5 inhibitors. A series of novel 9-substituted norharmane derivatives were then synthesized starting from the commercially available norharmane, followed by *in vitro* Sirt5 inhibitory assays to evaluate the inhibitory activities of these derivatives. The compound 4-(3-(2-(9*H*-pyrido [3,4-*b*]indol-9-yl)ethyl)ureido)benzoic acid (**3b**) bearing a rigid 1-phenyl-3-propylurea as link bridge exhibited most potent inhibitory activities, which has 35% and 52% inhibition ratios at 30 and 100 μ*M*, respectively. Finally, compound **3b** was observed by molecular docking to have good fit with the substrate binding site of Sirt5, especially have a very similar binding mode with Sirt5 substrate, and hence, we believe that compound **3b** can serve as a lead compound for further development of specific Sirt5 inhibitors.

EXPERIMENTAL

Chemistry methods. Unless otherwise noted, all starting materials, reagents, and solvents were purchased from commercial vendors and used as supplied without further purification. Analytical thin layer chromatography (TLC), performed on Qingdao Haiyang silica gel F-254, was used to monitor all of the reactions. Column chromatography, performed on Qingdao Haiyang silica gel 60 (300–400 mesh), was used to purify the intermediates and target compounds. All of the compound spots were visualized by UV light (254 nm). ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker AVANCE-III 400 MHz, respectively. Chemical shifts were expressed in parts per million (ppm, δ) relative to tetramethylsilane as an internal standard. Proton coupling patterns are described as singlet (s), doublet (d), triplet (t), quartet (q), multiplet (m), and broad (br). Low-resolution and high-resolution mass spectral (MS) data were acquired on an Agilent 1100 series LC-MS instrument with UV detection at 254 nm. HPLC analysis was conducted for all compounds listed in the tables on an UltiMate 3000 HPLC system (Dionex, USA). The ^{1}H NMR,^{13}C NMR, HRMS spectra and HPLC chromatograms of target compounds were given in Supporting Information.

2-(9H-Pyrido[3,4-b]indol-9-yl)ethan-1-amine (9). A mixture of *tert*-butyl (2-hydroxyethyl)carbamate (**5**, 5.00 g, 31.1 mmol) and TsCl (11.88 g, 62.2 mmol) in pyridine

(20 mL) was stirred at ambient temperature for 12 h. Upon completion of the reaction as determined by TLC, the solvent was removed *in vacuo*, and the crude mixture was extracted with ethyl acetate (3 × 200 mL). Then, the combined organic layers were washed with brine, dried, and concentrated. The residue was purified by column chromatography (eluent gradient PE/EA = 3/1) to give the intermediate 2-((*tert*-butoxycarbonyl)amino)ethyl 4-methylbenzenesulfonate (**6**,4.89 g, yield 50%) as a white solid.

Norharmane (**7**, 0.50 g, 2.98 mmol) and NaH (0.24 g, 5.96 mmol) in DMF (10 mL) was stirred at 45°C for 2 h, then 2-((*tert*-butoxycarbonyl)amino)ethyl 4-methylbenzenesulfonate (**6**,2.81 g,8.94 mmol) was slowly added to the mixture, and additional stirring at room temperature was continued for 16 h. When TLC indicated complete consumption of compound **7**, the reaction mixture was treated with ice water (200 mL) and extracted with EtOAc (3 × 150 mL). The combined extracts were washed with brine, dried, and concentrated. The residue obtained was purified by column chromatography (eluent gradient CH_2Cl_2/MeOH = 30/1) to give *tert*-butyl(2-(9*H*-pyrido[3,4-*b*]indol-9-yl)ethyl)carbamate (**8**, 0.74 g, yield 77%) as a light yellow solid. ^{1}H NMR (400 MHz, DMSO): δ 8.92 (s, 1H, ArH), 8.49 (d, 1H, J = 5.2 Hz,ArH), 8.17 (d, 1H, J = 8.0 Hz, ArH), 7.98 (d, 1H, J = 5.2 Hz, ArH), 7.62 (t, 1H, J = 7.6 Hz, ArH), 7.55 (d, 1H, J = 8.0 Hz, ArH), 7.33 (t, 1H, J = 7.6 Hz, ArH), 4.65 (s, br, 1H, NH), 4.59 (t, 2H, J = 5.6 Hz, N–CH_2), 3.62 (q, 2H, J = 6.0 Hz, NH–**CH_2**), 1.45 (s, 9H, $3CH_3$) ppm.

To a stirring solution of intermediate **8** (0.21 g,0.66 mmol) in CH_2Cl_2 (40 mL) was added trifluoroacetic acid (1.60 mL) at ambient temperature. The reaction mixture was stirred at room temperature for 5 h. After completion (monitored by TLC), the solvent was removed *in vacuo*, the crude residue was treated with 150 mL of ice water, and the pH was adjusted to >7 with saturated $NaHCO_3$. The water solution was treated with EtOAc (3 × 150 mL). The combined extracts were washed with brine, dried, and concentrated. The residue was not purified to give 2-(9*H*-pyrido[3,4-*b*]indol-9-yl)ethan-1-amine (**9**, 0.44 g, yield 86%) as a light yellow solid.

General procedure for the preparation of 1a–c. Catalytic amount of molecular sieve and trifluoroacetic acid was added to the solution of 2-(9*H*-pyrido[3,4-*b*]indol-9-yl) ethan-1-amine (**9**, 1.0 equiv), benzene formaldehydes (1.2 equiv), and hantzschester (1.2 equiv) in CH_2Cl_2 at room temperature, and the reaction was warmed to 40°C and reacted for 10 h. After completion (monitored by TLC), the reaction was filtered, and the crude residue was obtained by concentrating the filtrate *in vacuo*. Finally, the crude residue was purified by column chromatography (eluent gradient CH_2Cl_2/MeOH) to give the target compounds in 54–63% yield.

General procedure for the preparation of 2a–c. A mixture of substituted benzoic acids (1.0 equiv), 1-hydroxybenzotriazole (1.0 equiv), 1-(3-(dimethylamino) propyl)-3-ethylcarbodiimide hydrochloride (EDCI, 1.0 equiv), and *N*,*N*-diisopropylethylamine (1.5 equiv) in tetrahydrofuran was stirred at room temperature for 0.5 h. Next, the intermediate **9** (1.0 equiv) was added to the mixture, which was heated to reflux for 12 h. Then, the mixture was cooled to room temperature, and amount of water was added. The precipitate was filtered, washed with ice water and tetrahydrofuran, and purified by column chromatography (eluent gradient CH_2Cl_2/MeOH) to afford the products ethyl 3-((2-(9*H*-pyrido[3,4-*b*]indol-9-yl)ethyl)carbamoyl)benzoate, ethyl 4-((2-(9*H*-pyrido [3,4-*b*]indol-9-yl)ethyl)carbamoyl)benzoate, and compound **2c**. Then, the two compounds, which contain an ester, were undergone hydrolysis by NaOH in the solution of EtOH/H_2O (1:1) at 85°C for 1.5 h to give the target compounds **2a** and **2b** in high yields.

General procedure for the preparation of 3a–c. A solution of substituted anilines (1.0 equiv) dissolved in a spot of CH_2Cl_2 was slowly dripped into a stirred solution of triphosgene (0.33 equiv) in CH_2Cl_2 by using a constant-pressure dropping funnel. The catalytic quantity of Et_3N was then added slowly to the reaction mixture after the anilines were added, and the mixture was continued to stir for 0.5 h. After evaporation of the solvent, the residue was taken up in CH_2Cl_2, and intermediate **9** (1.0 equiv) was added directly to the residue. The reaction mixture was stirred at reflux for 2 h, and the solvent was subsequently removed *in vacuo*. The residue was extracted with ethyl acetate twice, and the combined organic layers were dried over $MgSO_4$ and concentrated. The crude product was purified by column chromatography (eluent gradient CH_2Cl_2/MeOH) to afford the ureasethyl 3-(3-(2-(9*H*-pyrido[3,4-*b*]indol-9-yl) ethyl)ureido)benzoate, ethyl 4-(3-(2-(9*H*-pyrido[3,4-*b*] indol-9-yl)ethyl)ureido)benzoate, and **3c** in high yields. Similarly, the two compounds, which contain an ester, were undergone hydrolysis by NaOH in the solution of EtOH/H_2O (1:1) at 100°C for 2 h to give the target compounds **3a** and **3b** in yields 81% and 88%, respectively.

3-(((2-(9H-Pyrido[3,4-b]indol-9-yl)ethyl)amino)methyl) benzoic acid (1a). Yield: 54%; HPLC purity: 98.58%. ^{1}H NMR (400 MHz, DMSO): δ 13.00 (s, 1H, COOH), 9.08 (s, 1H, ArH), 8.37 (d, 1H, J = 4.8 Hz, ArH), 8.28 (d, 1H, J = 4.8 Hz, ArH), 8.27 (d, 1H, J = 8.0 Hz, ArH), 8.13 (d, 1H, J = 5.2 Hz, ArH), 7.90 (s, 1H, ArH), 7.78 (d, 1H, J = 7.6 Hz, ArH), 7.74 (d, 1H, J = 8.4 Hz, ArH), 7.42 (d, 1H, J = 7.6 Hz, ArH), 7.35 (t, 1H, J = 7.6 Hz, ArH), 7.28 (t, 1H, J = 7.6 Hz, ArH), 4.60 (t, 2H, J = 6.4 Hz, N–CH_2), 3.77 (s, 2H, Ph–CH_2), 2.97 (t, 2H, J = 6.4 Hz, NH–**CH_2**) ppm. HRMS: *m*/*z* calcd for $C_{21}H_{20}N_3O_2$ $[M+H]^+$ 346.1550, found 346.1534.

4-(((2-(9H-Pyrido[3,4-b]indol-9-yl)ethyl)amino)methyl)benzoic acid (1b). Yield: 58%; HPLC purity: 96.63%. ^{1}H NMR (400 MHz, DMSO): δ 12.98 (s, 1H, COOH), 9.11 (s, 1H,

ArH), 8.45 (d, 1H, J = 5.2 Hz, ArH), 8.32 (d, 1H, J = 8.0 Hz, ArH), 8.21 (d, 1H, J = 5.2 Hz, ArH), 7.98 (d, 2H, J = 8.4 Hz, PhH), 7.77 (d, 1H, J = 8.4 Hz, ArH), 7.67 (t, 1H, J = 7.2 Hz, ArH), 7.57 (d, 2H, J = 8.0 Hz, PhH), 7.35 (t, 1H, J = 7.2 Hz, ArH), 4.80 (t, 2H, J = 6.8 Hz, N–CH_2), 4.26 (s, 2H, Ph–CH_2), 3.45 (t, 2H, J = 6.4 Hz, NH–**CH_2**) ppm. ^{13}C NMR (100 MHz, DMSO): δ 167.8, 167.6, 144.7, 142.1, 138.2, 137.2, 132.5, 131.7, 129.8, 129.2, 128.7, 127.7, 122.6, 121.0, 120.5, 115.3, 110.8, 46.3, 42.6 ppm. HRMS: m/z calcd for $C_{21}H_{20}N_3O_2$ $[M+H]^+$ 346.1549, found 346.1533.

***N*-Benzyl-2-(9H-pyrido[3,4-b]indol-9-yl)ethan-1-amine (1c).** Yield: 63%; HPLC purity: 98%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.08 (s, 1H, ArH), 8.41 (d, 1H, J = 5.2 Hz, ArH), 8.26 (d, 1H, J = 8.0 Hz, ArH), 8.08 (d, 1H, J = 5.2 Hz, ArH), 7.84–7.87 (m, 4H, ArH), 7.66 (t, 1H, J = 7.2 Hz, ArH), 7.49 (d, 2H, J = 8.0 Hz, ArH), 7.35 (t, 1H, J = 7.2 Hz, ArH), 4.78 (t, 2H, J = 6.8 Hz, ArH), 4.23 (s, 2H, N–CH_2), 3.42 (t, 2H, J = 6.4 Hz, Ph–CH_2), 2.61 (m, 1H, NH–**CH_2**) ppm. ^{13}C NMR (100 MHz, DMSO): δ 167.3, 141.1, 140.3, 137.3, 135.5, 131.7, 129.2, 128.7, 127.6, 127.3, 122.2, 121.1, 114.8, 109.4, 47.2, 43.3 ppm. LC-MS: m/z 302.2 $[M+H]^+$.

***3*-((2-(9H-Pyrido[3,4-b]indol-9-yl)ethyl)carbamoyl)benzoic acid (2a).** Yield: 53% for two steps; HPLC purity: 98.35%. ^{1}H NMR (400 MHz, DMSO): δ 13.02 (s, 1H, COOH), 9.03 (s, 1H, ArH), 8.84 (t, 1H, J = 6.0 Hz, NH), 8.35 (d, 1H, J = 5.2 Hz, ArH), 8.26 (d, 2H, J = 4.4 Hz, ArH), 8.12 (d, 1H, J = 5.2 Hz, ArH), 8.03 (d, 1H, J = 8.0 Hz, ArH), 7.85 (d, 1H, J = 8.0 Hz, ArH), 7.72 (d, 1H, J = 8.4 Hz, ArH), 7.58–7.50 (m, 2H, ArH), 7.26 (t, 1H, J = 7.6 Hz, ArH), 4.71 (t, 2H, J = 6.0 Hz, N–CH_2), 3.74 (q, 2H, J = 6.0 Hz, NH–**CH_2**) ppm. HRMS: m/z calcd for $C_{21}H_{18}N_3O_3$ $[M+H]^+$ 360.1343, found 360.1332.

***4*-((2-(9H-Pyrido[3,4-b]indol-9-yl)ethyl)carbamoyl)benzoic acid (2b).** Yield: 66% for two steps; HPLC purity: 100%. ^{1}H NMR (400 MHz, DMSO): δ 13.20 (s, br, 1H, COOH), 9.15 (s, 1H, ArH), 8.81 (t, 1H, J = 5.6 Hz, NH), 8.42 (d, 1H, J = 5.2 Hz, ArH), 8.29 (d, 1H, J = 5.2 Hz, ArH), 7.92 (d, 2H, J = 6.8 Hz, ArH), 7.79 (d, 1H, J = 4.4 Hz, ArH), 7.69–7.61 (m, 3H, ArH), 7.31 (t, 1H, J = 7.6 Hz, ArH), 4.75 (t, 2H, J = 6.4 Hz, N–CH_2), 3.76 (q, 2H, J = 6.0 Hz, NH–**CH_2**) ppm. ^{13}C NMR (100 MHz, DMSO): δ 167.2, 166.6, 142.2, 138.4, 136.8, 136.7, 133.4, 131.3, 129.6, 129.2, 127.7, 122.8, 120.7, 120.5, 115.7, 110.7, 42.6, 39.2 ppm. HRMS: m/z calcd for $C_{21}H_{18}N_3O_3$ $[M+H]^+$ 360.1344, found 360.1333.

***N*-(2-(9H-Pyrido[3,4-b]indol-9-yl)ethyl)benzamide (2c).** Yield: 84%; HPLC purity: 98.16%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.93 (s, 1H, ArH), 8.38 (t, 1H, J = 5.2 Hz, NH), 8.16 (d, 1H, J = 7.6 Hz, ArH), 7.96 (d, 1H, J = 5.2 Hz, ArH), 7.62–7.56 (m, 4H,Ar H),7.47 (t, 1H, J = 7.2 Hz, ArH), 7.38–7.30 (m, 3H, ArH), 6.69 (t, 1H, J = 5.2 Hz, ArH), 4.72 (t, 2H, J = 6.0 Hz, N–CH_2), 3.95 (q, 2H, J = 6.0 Hz, NH–**CH_2**) ppm. LC-MS: m/z 316.1 $[M+H]^+$.

***3*-(3-(2-(9H-Pyrido[3,4-b]indol-9-yl)ethyl)ureido)benzoic acid (3a).** Yield: 72% for two steps; HPLC purity: 98.28%. ^{1}H NMR (400 MHz, DMSO): δ 12.81 (s, br, 1H, COOH), 9.07 (s, 1H, ArH), 8.78 (s, 1H, Ph-NH), 8.38 (d, 1H, J = 5.2 Hz, ArH), 8.28 (d, 1H, J = 7.6 Hz, ArH),8.14 (d, 1H, J = 4.2 Hz, ArH), 8.03 (s, 1H, ArH), 7.75 (d, 1H, J = 8.4 Hz, ArH), 7.63–7.56 (m, 2H, ArH), 7.49 (d, 1H, J = 7.6 Hz, ArH), 7.35–7.27 (m, 2H, ArH), 6.31 (t, 1H, J = 5.2 Hz, CH_2–**NH**), 4.61 (t, 2H, J = 6.4 Hz, N–CH_2), 3.56 (q, 2H, J = 6.0 Hz, NH–**CH_2**) ppm. HRMS: m/z calcd for $C_{21}H_{19}N_4O_3$ $[M+H]^+$ 375.1452, found 375.1451.

***4*-(3-(2-(9H-Pyrido[3,4-b]indol-9-yl)ethyl)ureido)benzoic acid (3b).** Yield: 78% for two steps; HPLC purity: 99.32%. ^{1}H NMR (400 MHz, DMSO): δ 12.80 (s, br, 1H, COOH), 9.07 (s, 1H, ArH), 8.96 (s, 1H, Ph-NH), 8.38 (d, 1H, J = 4.8 Hz, ArH), 8.28 (d, 1H, J = 7.2 Hz, ArH), 8.15 (d, 1H, J = 4.2 Hz, ArH), 7.81 (d, 2H, J = 7.6 Hz, ArH), 7.75 (d, 1H, J = 8.0 Hz,), 7.61 (t, 1H, J = 6.8 Hz,), 7.46 (d, 2H, J = 7.2 Hz, ArH), 7.29 (t, 1H, J = 7.2 Hz,), 6.43 (t, 1H, J = 5.2 Hz, CH_2-**NH**), 4.61 (t, 2H, J = 6.4 Hz, N–CH_2), 3.56 (q, 2H, J = 6.0 Hz, NH–**CH_2**) ppm. HRMS: m/z calcd for $C_{21}H_{19}N_4O_3$ $[M+H]^+$ 375.1452, found 375.1450.

***1*-(2-(9H-Pyrido[3,4-b]indol-9-yl)ethyl)-3-phenylurea (3c).** Yield: 78%; HPLC purity: 98.88%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.81 (s, 1H, Ph-NH), 8.25 (d, 1H, J = 5.2 Hz, ArH), 8.12 (d, 1H, J = 7.6 Hz,), 7.86 (d, 1H, J = 5.2 Hz, ArH), 7.62–7.55 (m, 2H, ArH), 7.31 (t, 1H, J = 7.2 Hz, ArH), 7.20 (t, 2H, J = 7.6 Hz, ArH), 7.10–7.00 (m, 4H, ArH), 5.26 (t, 1H, J = 6.0 Hz, CH_2–**NH**), 4.53 (t, 2H, J = 6.0 Hz, N–CH_2), 3.66 (q, 2H, J = 6.0 Hz, NH–**CH_2**) ppm. ^{13}C NMR (100 MHz, DMSO): δ 156.0, 141.5, 140.8, 138.6, 136.8, 132.8, 129.1, 128.9, 127.9, 122.5, 121.7, 120.9, 120.1, 118.4, 115.1, 110.6, 43.1, 39.0 ppm. LC-MS: m/z 331.1 $[M+H]^+$.

***2*-(9H-Pyrido[3,4-b]indol-9-yl)acetic acid (12).** A mixture of norharmane (**7**, 0.50 g, 2.98 mmol) and NaH (0.24 g, 5.96 mmol) in DMF (10 mL) was stirred at ambient temperature for 2 h. Subsequently, ethyl 2-bromoacetate (**2**, 0.49 mL, 4.46 mmol) was slowly added to the mixture and additional stirring at room temperature was continued for another 5 h. Upon completion of the reaction as determined by TLC, The reaction mixture was then treated with ice water (250 mL) and extracted with EtOAc (3 × 150 mL). The combined extracts were washed with brine, dried, and concentrated. The residue obtained was purified by column chromatography (eluent gradient CH_2Cl_2/MeOH = 60/1) to provide ethyl 2-(9*H*-pyrido[3,4-*b*]indol-9-yl)acetate(**11**, yield 54%) as a light yellow solid. ^{1}H NMR (400 MHz, DMSO): δ 8.85 (s, 1H, ArH), 8.51 (d, 1H, J = 9.6 Hz, ArH), 8.17 (d, 1H, J = 7.6 Hz, ArH), 7.99 (d, 1H, J = 5.2 Hz, ArH), 7.63 (t, 1H, J = 7.6 Hz, ArH), 7.42 (d, 1H, J = 8.4 Hz, ArH), 7.35 (t, 1H, J = 7.6 Hz, ArH), 5.10 (s, 2H, N–CH_2), 4.24 (q, 2H, J = 7.2 Hz, OCH_2), 1.26 (t, 3H, J = 6.8 Hz, CH_3) ppm.

A mixture of ethyl 2-(9*H*-pyrido[3,4-*b*]indol-9-yl)acetate (**11**, 0.20 g, 0.79 mmol) and NaOH (0.10 g, 2.37 mmol) was reacted for 2 h in the solution of EtOH/H_2O (5 mL/5 mL) at 100°C. After evaporation of the organic solvent, the residue was treated with 150 mL of ice water, and the PH was adjusted to 6–7 with diluted HCl. At this time, a white solid was formed and collected by filtration. The crude product was dried in a vacuum oven to give the title compound **12** (0.16 g, yield 90%).

3-((2-(9H-Pyrido[3,4-b]indol-9-yl)acetamido)methyl)benzoic acid (4a). The title compound was prepared from intermediate **12** and ethyl 3-(aminomethyl)benzoate using the same method of compound **2a**, yield: 48%; HPLC purity: 98.68%. ^{1}H NMR (400 MHz, DMSO): δ 12.89 (s, br, 1H, COOH), 9.00 (s, 1H, ArH), 8.94 (t, 1H, J = 5.6 Hz, NH), 8.40 (d, 1H, J = 4.8 Hz, ArH), 8.28 (d, 1H, J = 7.6 Hz, ArH), 8.14 (d, 1H, J = 5.2 Hz, ArH), 7.91 (s, 1H, ArH), 7.84 (d, 1H, J = 7.6 Hz, ArH), 7.66–7.59 (m, 2H, ArH), 7.52 (d, 1H, J = 7.6 Hz, ArH), 7.45 (t, 1H, J = 7.6 Hz, ArH), 7.31 (t, 1H, J = 7.6 Hz, ArH), 5.26 (s, 2H, N–CH_2), 4.40 (d, 2H, J = 6.0 Hz, Ph–CH_2) ppm. ^{13}C NMR (100 MHz, DMSO): δ 167.8, 167.6, 141.8, 140.1, 139.2, 137.3, 133.3, 132.2, 131.5, 129.1, 128.8, 128.6, 128.4, 128.0, 122.3, 121.1, 120.2, 115.0, 110.6, 46.2, 42.6 ppm. HRMS: *m*/*z* calcd for $C_{21}H_{18}N_3O_3$ $[M+H]^+$ 360.1345, found 360.1334.

4-((2-(9H-Pyrido[3,4-b]indol-9-yl)acetamido)methyl)benzoic acid (4b). The title compound was prepared from intermediate **12** and ethyl 4-(aminomethyl)benzoate using the same method of compound **2a**, yield: 51%; HPLC purity: 96.34%. ^{1}H NMR (400 MHz, DMSO): δ 12.87 (s, br, 1H, COOH), 9.08 (s, 1H, ArH), 8.96 (t, 1H, J = 6.0 Hz, NH), 8.44 (d, 1H, J = 5.2 Hz, ArH), 8.32 (d, 1H, J = 8.0 Hz, ArH), 8.24 (d, 1H, J = 5.2 Hz, ArH), 7.89 (d, 2H, J = 8.0 Hz, ArH), 7.70–7.63 (m, 2H, ArH), 7.39 (d, 2H, J = 8.0 Hz, ArH), 7.34 (t, 1H, J = 6.8 Hz, ArH), 5.30 (s, 2H, N–CH_2), 4.40 (d, 2H, J = 5.6 Hz, Ph–CH_2) ppm. ^{13}C NMR (100 MHz, DMSO): δ 167.8, 167.6, 144.7, 142.1, 138.2, 137.2, 132.5, 131.7, 129.8, 129.2, 128.7, 127.7, 122.6, 121.0, 120.5, 115.3, 110.8, 46.3, 42.6 ppm. HRMS: *m*/*z* calcd for $C_{21}H_{18}N_3O_3$ $[M+H]^+$ 360.1343, found 360.1332.

N-Benzyl-2-(9H-pyrido[3,4-b]indol-9-yl)acetamide (4c). The title compound was prepared from intermediate **12** and phenylmethanamine using the same method of compound **2c**, yield: 66%; HPLC purity: 100%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.91 (s, 1H, NH), 8.55 (d, 1H, J = 8.4 Hz, ArH), 8.19 (d, 1H, J = 7.6 Hz, ArH), 7.99 (d, 1H, J = 5.2 Hz, ArH), 7.67 (t, 1H, J = 7.6 Hz, ArH), 7.49 (d, 1H, J = 8.4 Hz, ArH), 7.40 (t, 1H, J = 7.6 Hz, ArH), 7.70–7.08 (m, 5H, ArH), 6.04 (s, br, 1H, ArH), 5.11 (s, 2H, N–CH_2), 4.44 (d, 2H, J = 4.0 Hz, Ph–CH_2) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ 167.3, 141.1, 140.3, 137.3, 136.5, 131.7, 129.2, 128.7, 127.6, 127.3, 122.2, 121.7, 121.1, 114.8, 109.4, 47.2, 43.4 ppm. HRMS: *m*/*z* calcd for $C_{20}H_{18}N_3O$ $[M+H]^+$ 316.1444, found 316.1434.

N-(4-Chlorobenzyl)-2-(9H-pyrido[3,4-b]indol-9-yl)acetamide (4e). The title compound was prepared from intermediate **12** and (4-chlorophenyl)methanamine using the same method of compound **2c**, yield: 69%; HPLC purity: 97.52%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.70 (s, 1H, NH), 8.34–8.30 (m, 2H, ArH), 8.19 (s, 1H, ArH), 8.08 (d, 1H, J = 8.0 Hz, ArH), 7.83 (d, 1H, J = 5.2 Hz, ArH), 7.63 (t, 1H, J = 7.6 Hz, ArH), 7.44 (t, 2H, J = 8.0 Hz, ArH), 7.35 (t, 1H, J = 7.6 Hz, ArH), 7.13 (q, 1H, J = 4.8 Hz, J = 2.8 Hz, ArH), 6.84 (t, 1H, J = 6.0 Hz, ArH), 5.02 (s, 2H, N–CH_2), 4.37 (d, 2H, J = 6.0 Hz, Ph–CH_2) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ 167.3, 141.1, 140.3, 136.4, 135.6, 133.4, 131.6, 129.3, 129.2, 128.8, 128.7, 122.3, 121.7, 121.2, 114.8, 109.4, 47.2, 42.6 ppm. HRMS: *m*/*z* calcd for $C_{20}H_{17}N_3O$ $[M+H]^+$ 350.1055, found 350.1043.

N-(Pyridin-3-ylmethyl)-2-(9H-pyrido[3,4-b]indol-9-yl)acetamide (4f). The title compound was prepared from intermediate **12** and pyridin-3-ylmethanamine using the same method of compound **2c**, yield: 71%; HPLC purity: 99.84%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.80 (s, 1H, NH), 8.50 (d, 1H, J = 5.2 Hz, ArH), 8.39 (d, 1H, J = 4.8 Hz, ArH), 8.28 (s, 1H, ArH), 8.14 (d, 1H, J = 8.0 Hz, ArH), 7.92 (d, 1H, J = 5.2 Hz, ArH), 7.65 (t, 1H, J = 7.6 Hz, ArH), 7.46–7.43 (m, 2H, ArH), 7.38 (t, 1H, J = 8.0 Hz, ArH), 7.16 (q, 1H, J = 4.8 Hz, J = 2.8 Hz, ArH), 6.42 (s, br, 1H, ArH), 5.10 (s, 2H, N–CH_2), 4.41 (d, 2H, J = 6.0 Hz, Ph–CH_2) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ 167.6, 148.8, 148.7, 141.1, 140.1, 136.4, 135.3, 133.3, 131.5, 129.3, 123.5, 122.2, 121.6, 121.1, 114.7, 109.3, 47.1, 40.8 ppm. LC-MS: *m*/*z* 317.1 $[M+H]^+$.

Molecular docking methods. The AutoDock Vina program was used to predict the binding mode of compound **3b** to Sirt5. The crystal structure of Sirt5 in complex with succinyl-lysine substrate (PDB entry: 3RIY) was used as the docking template. Gasteiger–Marsili charges were added to the models of protein and compounds. The grid center were set as the center of substrate (x, y, z = −8.1, 4.5, −10.5), and the size was 25 Å × 25 Å × 25 Å, which encompassed the entire substrate binding site. The numbers of docking poses were set as 20. The other parameters for Vina were set as default.

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