Tetrahedron 67 (2011) 2251–2259

Contents lists available at ScienceDirect

Tetrahedron

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

Syntheses and characterizations of novel pyrrolocoumarin probes for SNAP-tag labeling technology

De-Sheng Mei [a], Yi Qu [b], Jin-Xiang He [b], Lei Chen [a], Zhu-Jun Yao [a,*]

[a] State Key Laboratory of Bioorganic and Natural Products Chemistry, Shanghai Institute of Organic Chemistry, Chinese Academy of Sciences, 345 Lingling Road, Shanghai 200032, China
[b] Key Laboratory for Advanced Materials and Institute of Fine Chemicals, East China University of Science and Technology, 130 Meilong Road, Shanghai 200237, China

ARTICLE INFO

Article history:
Received 4 December 2010
Received in revised form 20 January 2011
Accepted 26 January 2011
Available online 2 February 2011

Keywords:
Pyrrolocoumarin
Fluorescent probe
SNAP-tag
Protein labeling
Synthesis

ABSTRACT

SNAP-tag technology is a revolutionary protein labeling technology employing in various biological studies. Since low signal/noise ratio and severe overlap between the FRET donors/acceptors often occurred in applying present fluorescent probes and thus limited the further applications, development of new fluorescent probes with excellent fluorescent properties is still of request by today's SNAP-tag technology. In this paper, a number of SNAP-tag protein probes have been developed by incorporating a novel pyrrolocoumarin fluorophore recently developed by our group. Examination of these novel synthetic compounds shows all these materials possess satisfactory fluorescent properties. Among these, probe **7** exhibits the most excellent characters, and its quantum yield, maximum emission wavelength and Stocks shift reach to 0.44, 534 nm and 112 nm, respectively. Further analysis of structure-property relationship indicates that the probes with a longer C3-substituted alkyl (such as pentyl) give stronger fluorescence.

1. Introduction

Many real-time investigations of a target protein including its position and evolution in the complicated situations require the use of specific labeling techniques.[1] In the last decade, the green fluorescent protein (GFP) and its variants have emerged as the most powerful tool in the optical research of protein functions due to their bright clear fluorescence, which is detectable even in single cells.[2] However, the monotone fluorescent properties of GFPs hinders them from further application in some cases.[3]

SNAP-tag technology is a different protein labeling technology emerged for overcoming the above mentioned disadvantages.[4] SNAP-tag can react with its ligand (O^6-benzylguanine derivatives, BG) specifically and rapidly in vivo and vitro. Because BG can carry a variety of fluorescent groups, a wavelength variable protein labeling tag (SNAP-tag) with different fluorescent groups (as requested) can be prepared. As a revolutionary self-labeled protein technology, SNAP-tag has been successfully applied in the researches of protein location, protein quantitative analysis and protein–protein interactions.[5] However, current SNAP-tag technology is still far from perfection. Two major shortcomings existed: (i) Low ratio of signal/noise, which results from the intrinsic fluorescence of cells and (ii) severe overlap between the FRET (fluorescence resonance energy transfer) donors and acceptors in many protein–protein interaction experiments.[6] These problems are generated by the poor fluorescent properties of many present SNAP-tag probes. The low signal/noise ratio is due to the poor fluorescence intensity of probes, and the spectral overlap between FRET donors and acceptors is caused by small Stocks shifts of the probes. The Stocks shifts of most commercially available probes are usually below 30 nm. Therefore, development of small-molecule fluorophores with intensive fluorescent and larger Stocks shift is of urgency for ideal SNAP-tag probes.

2. Design of novel probes for SNAP-tag technology

Recently, we developed a novel series of pyrrolocoumarin fluorescent probes with satisfactory fluorescent properties and notable large Stocks shifts.[7] As a representative, compound **A** showed a large Stock shift (113 nm) and intense fluorescence ($\Phi = 0.55$, $\lambda_{em} = 523$ nm). These properties indicate that **A** might be able to apply into the fluorescent probes for those complicated biological studies. In this study, as one application, fluorophore **A** was successfully incorporated into a number of small-molecule probes, which are designed for future protein function studies with SNAP-tag technology (Fig. 1).

To achieve an ideal SNAP-tag probe, we examined the combinations of couple easily available substituted sites and several

* Corresponding author. Tel.: +86 21 5492 5133; fax: +86 21 5492 5124; e-mail address: yaoz@sioc.ac.cn (Z.-J. Yao).

0040-4020/$ – see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tet.2011.01.075

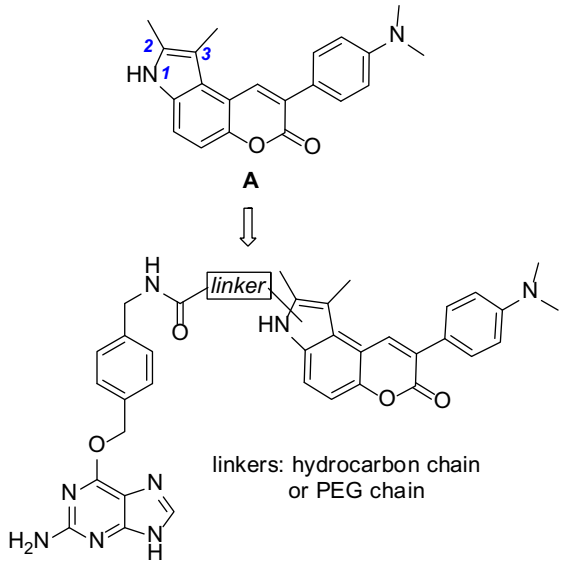


Fig. 1. Design of novel protein probes based on SNAP-tag technology.

commonly used linker chains. We chose to alter pyrrole nitrogen and C3 site of **A** as the anchors, and CH_2, $(CH_2)_3$, $(CH_2)_5$ as the linkers between the fluorescent core and BG moiety. Since many reports used poly(glycol) chain as the linker, we also applied similar linkages in this work. According to such design, we obtained two groups of new probes, N^1-substituted probes **1**–**4** (group I, Fig. 2) and C3-substituted probes **5**–**8** (group II, Fig. 2).

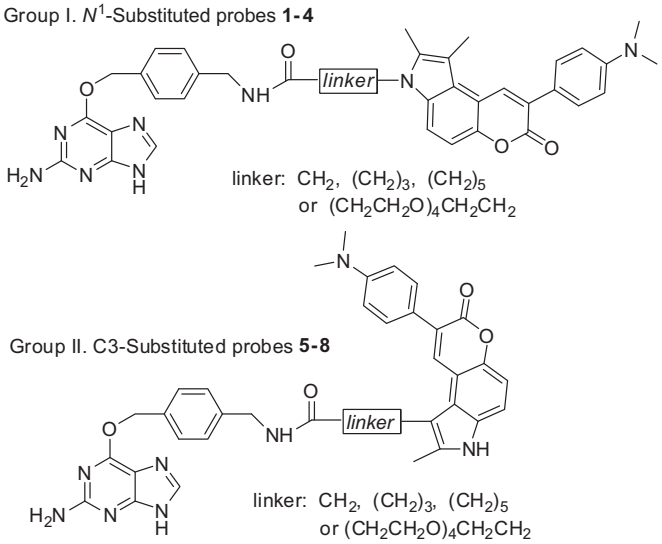
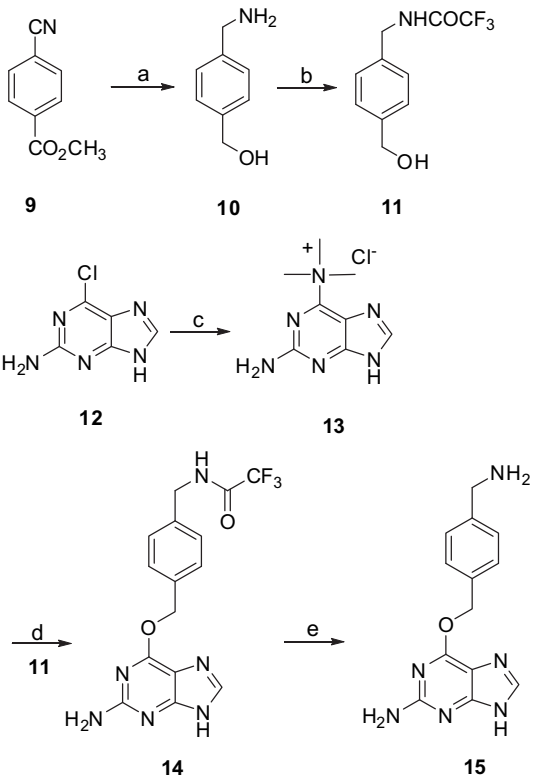


Fig. 2. Novel protein probes based on SNAP-tag technology.

3. Chemical synthesis of fluorescent probes

3.1. Synthesis of O^6-[4-(aminomethyl)benzyl]guanine

The common BG moiety used in this study, O^6-[4-(aminomethyl)benzyl]guanine (**15**), was synthesized with slight modifications of the known report (Scheme 1).[8] The starting material, methyl 4-cyanobenzoate (**9**) was reduced with lithium aluminum



Scheme 1. Synthesis of O^6-[4-(aminomethyl)benzyl]guanine **15**. Reagents and conditions: (a) $LiAlH_4$, THF, 65%; (b) $(CF_3CO)_2O$, Py, DCM, 81%; (c) $N(CH_3)_3$, DMF, 81%; (d) *t*-BuOK, DMSO, 34%; (e) K_2CO_3, MeOH, 76%.

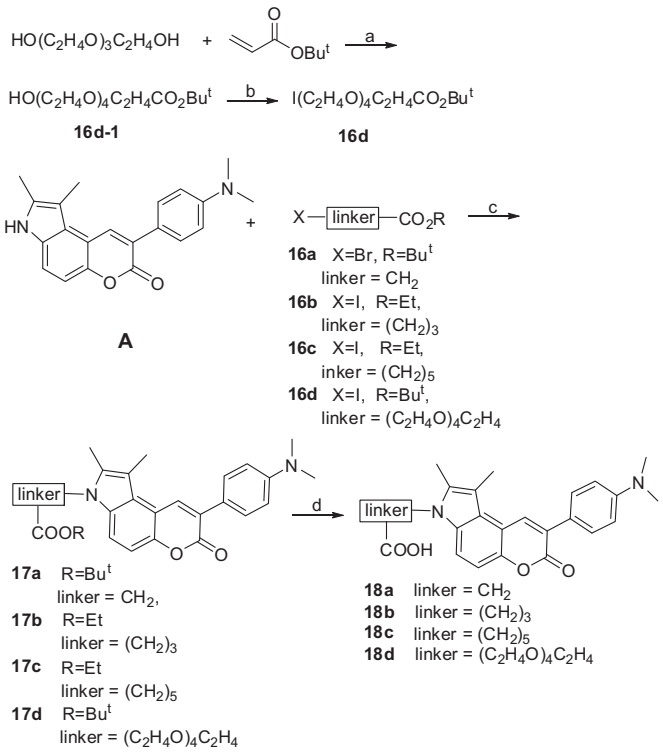
hydride to give amino alcohol **10**, whose amino group was protected with trifluoroacetic anhydride (TFAA), affording the benzyl alcohol **11**. In parallel, ammonium salt **13** was prepared from the reaction of 2-chloro-6-aminopurine (**12**) with trimethylamine. Then, ammonium salt **13** reacted with **11** in the presence of *t*-BuOK to yield the BG intermediate **14**. Final deprotection of **14** with potassium carbonate in methanol afforded the requested BG derivative **15** in 76% yield.

3.2. Synthesis of N^1-substituted pyrrolocoumarin carboxylic acids

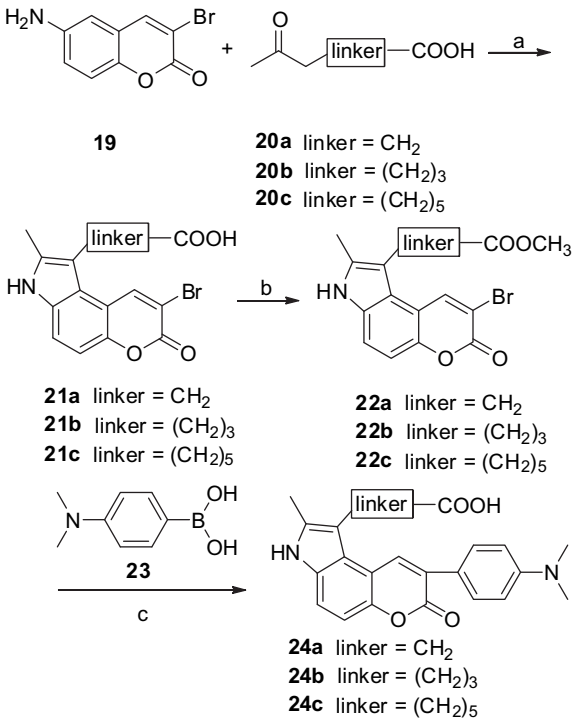
Synthesis of N^1-substituted pyrrolocoumarin carboxylic acids **18a**–**d** is outlined in Scheme 2. Because the linker ω-halo carboxylic ester **16d** is not commercially available, we prepared it prior to the first step of N-alkylation. Reaction of tetraglycol reacted with *tert*-butyl acrylate in NaH/DMF followed by iodination of the remaining hydroxyl group afforded the iodide **16d** in high yield. With all the linkers in hand, the fluorescent core **A** reacted in parallel with ω-halo carboxylic esters **16a**–**d** in the presence of NaH, affording the corresponding N^1-substituted pyrrolocoumarin carboxylic esters **17a**–**d**. Hydrolysis of esters **17a**–**d** in acid or base solution yielded the acids **18a**–**d** in 53–93% yields.

3.3. Synthesis of C3-substituted pyrrolocoumarin carboxylic acids

Parallel syntheses of the C3-substituted pyrrolocoumarin carboxylic acids **24a**–**c** using linear hydrocarbon linkers **20a**–**c** are shown in Scheme 3. Fischer indolization of aniline **19** with methyl acetones **20a**–**c** via a two-step procedure afforded the corresponding intermediates **21a**–**c** in acceptable yields. Suzuki couplings of bromides **22a**–**c** (prepared from the corresponding acids



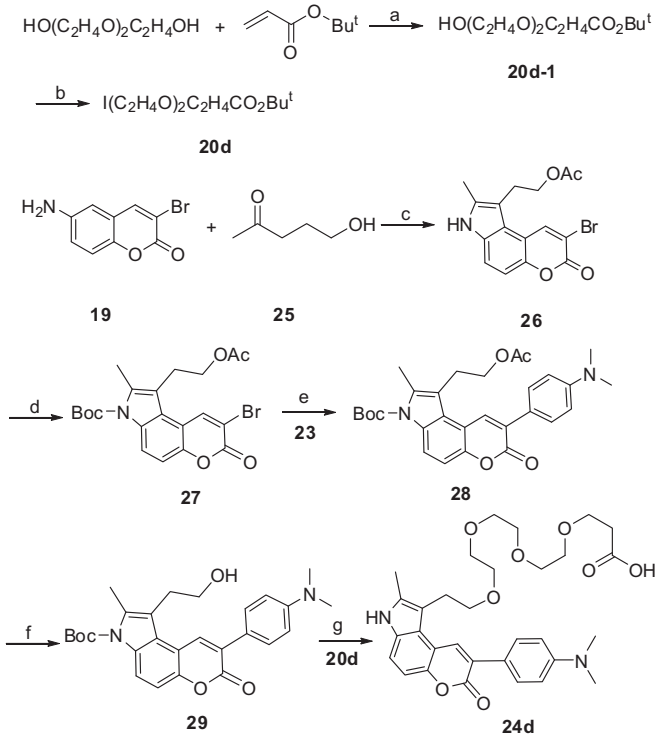
Scheme 2. Synthesis of N^1-substituted pyrrolocoumarin carboxylic acids **18a**–**d**. (a) NaH, THF, 55%; (b) I_2, PPh_3, imidazole, 80%; (c) NaH, DMF, 34–67%; (d) deprotection, 53–93%. (See Experimental section for the details).



Scheme 3. Synthesis of C3-substituted pyrrolocoumarin carboxylic acids **24a**–**c**. (a) (i) $NaNO_2$, HCl; (ii) $SnCl_2$, HCl; (iii) HOAc, heating, 35–42%; (b) $SOCl_2$, MeOH, 37–96%; (c) $Pd(PPh_3)_4$, K_2CO_3, EtOH/toluene/H_2O (1:1:2), 50–61%.

21a–**c**) with boric acid **23** afforded the carboxylic acids **24a**–**c** in the presence of catalytic amount of $Pd(PPh_3)_4$ under N_2 atmosphere.

For the case using the polyether chain, strongly acidic conditions are unsuitable for our synthesis. Instead of the above approach, an alternative indirect strategy was adopted in the synthesis of the C3-substituted pyrrolocoumarin carboxylic acid **24d** (Scheme 4). Oxa Michael addition of triglycol with *tert*-butyl acrylate using NaH as a base followed by iodination afforded the linker iodide **20d** in a high yield. In a parallel experiment, Fischer indolization was employed to construct the core structure of **26**, whose indole NH was then protected with Boc_2O, yielding the required building block **27** in 78% yield. Suzuki reaction of bromide **27** with boric acid **23** afforded fluorophore **28**. Deprotection of the *N*-Boc functionality of **28** with potassium carbonate gave alcohol **29**. Finally, O-alkylation of compound **29** with iodide **20d** under basic conditions followed by deprotection of *N*-Boc with TFA afforded **24d** in 41% yield.



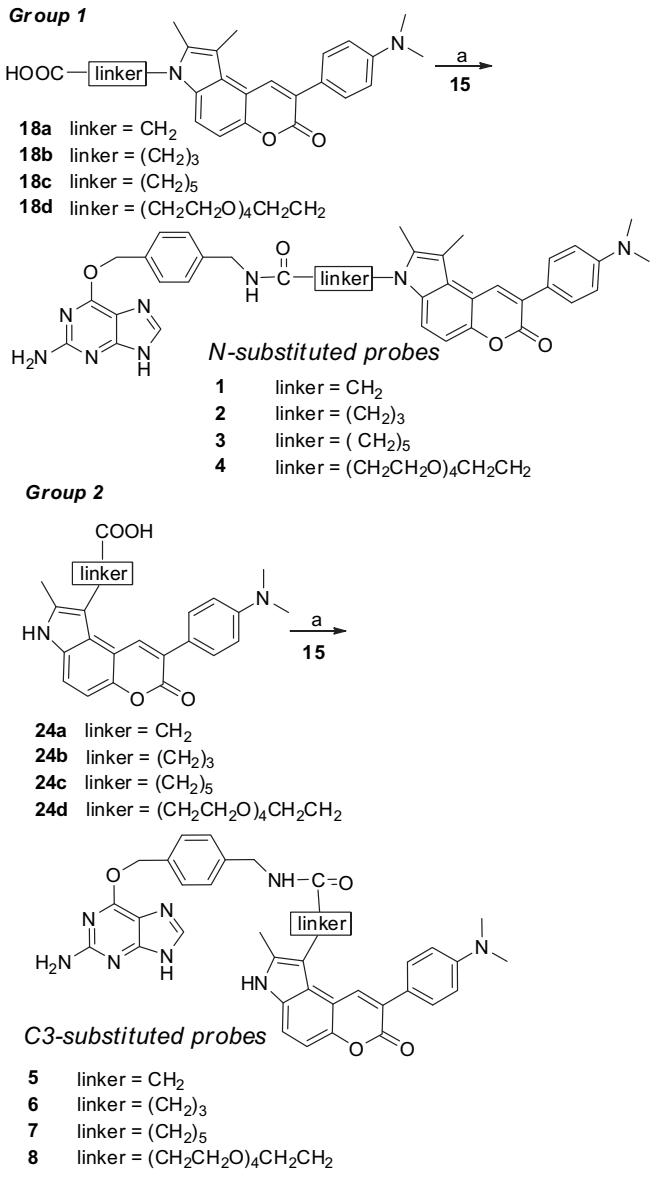
Scheme 4. Synthesis of C3-substituted pyrrolocoumarin carboxylic acid **24d**. (a) NaH, THF, 62%; (b) I_2, PPh_3, imidazole, 80%; (c) (i) $NaNO_2$, HCl; (ii) $SnCl_2$, HCl; (iii) HOAc, 40%; (d) BOC_2O, DMAP, 78%; (e) $Pd(PPh_3)_4$, K_2CO_3, 84%; (f) K_2CO_3, CH_3OH, 86%; (g) (i) *t*-BuOK, DMF; (ii) TFA, DCM, 41%.

3.4. Synthesis of small-molecule SNAP-tag probes

Parallel reactions of the pyrrolocoumarin carboxylic acids **18a**–**d** and **24a**–**d** with common BG derivative **15** were accomplished in the presence of EDCI/HOBt, providing the target SNAP-tag probes **1**–**8** in 31–96% yields, respectively (Scheme 5).

4. Optical characterizations and discussion

Optical properties of all above synthesized probes **1**–**8** were examined by standard characterizations with comparison of their parent dye **A** (Table 1). To our delight, all probes exhibit satisfactory quantum yields (0.26–0.44), and retain large Stocks shifts (112–120 nm), as well as similar emission wavelengths (533–538 nm). These data mention that linkage of the BG moiety with the parent dye **A** in the final probes **1**–**8** does not severely affect the original optical properties through either pyrrole N^1- or C3-substitution. Among these probes, probe **7** possesses the most attractive characters. Its quantum yield, Stocks shift and maximum emission wavelength reach to 0.44, 112 nm and 534 nm, respectively. Probe **7** is thus an ideal tool potentially useful for



Scheme 5. Synthesis of **1**–**8**. (a) EDCI, HOBt, DIPEA, DMF, 31–95%. (See Experimental section for details).

Table 1
Optical properties of parent compound **A** and novel probes **1**–**8**[a]

Compound	λ_{ex} (nm)	λ_{em} (nm)	Φ	Stocks shift (nm)
A	421	537	0.53	116
1	421	536	0.26	112
2	421	533	0.29	120
3	422	534	0.34	112
4	421	535	0.30	114
5	419	538	0.38	119
6	422	537	0.30	115
7	422	534	0.44	112
8	422	538	0.38	116

[a] All measurements were performed in DMF; quantum yields were measured and calculated relative to 9,10-diphenylanthracene in cyclohexane as the standard (excited at 372 nm).

labeling the functional proteins in future biological studies (Fig. 3). Such results also indicate that our initial design of incorporating pyrrolocoumarin dye **A** into the SNAP-tag probes is successful in the optical property stage.

Fig. 3. Fluorescence photograph of probe **7** in DMF (0.1 μM).

Based on the fluorescent properties of these compounds with substitution at different positions with different linkers, the structure–property relationship is also analyzed and concluded. It is found that the probes with a longer hydrocarbon or polyglycol linker gave higher quantum yields (**3** vs **1** or **2**, **7** vs **5** or **6**). This mentions that the longer linker may help to decrease the unfavorable optical interactions between the newly introduced BG group and the parent fluorescent core. By comparison to the corresponding probes having a hydrocarbon chain (**4** vs **3**, **8** vs **7**), introduction of the polyglycol chain seems to decrease quantum yield. As compared to those probes branching a linker at their pyrrole nitrogen, the corresponding C3-substituted probes usually offer higher quantum yields (**5** vs **1**, **6** vs **2**, **7** vs **3**, and **8** vs **4**).

5. Conclusion

Functional research of proteins has been keeping a hot field for centuries, but it still confronts some most basic problems and keeps challenging to us today. SNAP-tag technology enables the specific, covalent attachment of reporters, including fluorescent dyes, to a certain protein of interest in live cells, offering a powerful tool for visualization of biological events inside live cells. Many present problems in SNAP-tag technology are resulted from the poor fluorescent properties of probes. In this study, we have synthesized and characterized a series of novel pyrrolocoumarin derivatives as potentially useful fluorescent probes for the protein investigations with SNAP-tag technology. Examination of fluorescent properties shows that all these probes possess good to excellent fluorescent properties. Among these, probe **7** exhibits the most promising optical characters. Structure–property relationship of these compounds is also discussed in this work. To achieve ideal fluorescent properties, it is suggested that the pyrrole C3-position is the better anchor position for locating a relatively longer hydrocarbon-linked BG moiety. These foundings will be very helpful for future design of similar small-molecule probes with excellent fluorescent properties.

6. Experimental section

6.1. General methods

For thin-layer chromatography (TLC), silica gel plates GF_{254} were used and compounds were visualized by irradiation with UV light or iodine. FT-IR spectra were recorded on an AVATAR-360 spectrophotometer. ^1H and ^{13}C NMR spectra were recorded on Bruker DRX-400, or Bruker DRX-300 spectrometers with TMS as the internal standard. HRMS were measured by using either FTMS-7 or IonSpec 4.7 spectrometers. UV–vis spectra were recorded with a Varian Cary 500 spectrophotometer and fluorescence emission spectra were measured on a Varian Cary Eclipse fluorescence spectrophotometer. Reference compound **A** was synthesized according to our previous work,[7] and the known intermediates **10**,[9] **11**,[10] **13**,[11] **15**,[8] **16b**,[12] **16c**,[13] **20c**,[15] and **23**[14] were synthesized according to the corresponding reference procedures, respectively.

*6.1.1. [8-(4-Dimethylamino-phenyl)-1,2-dimethyl-7-oxo-7H-pyrano [3,2-e]indol-3-yl]-acetic acid tert-butyl ester (**17a**).* A solution of **A** (200 mg, 0.6 mmol) in DMF (7.0 mL) at 0 °C was treated with NaH (60% dispersion in mineral oil, 33 mg, 0.8 mmol). After being stirred at this temperature for additional 30 min, *tert*-butyl bromoacetate **16a** (0.23 mL, 1.2 mmol) was added. The resulting solution was warmed to room temperature and stirred for 12 h. The reaction mixture was diluted with EtOAc and quenched with 1 M HCl. The organic phase was separated, washed with brine, dried over anhydrous $MgSO_4$, and concentrated under reduced pressure. The residue was purified by flash column chromatography (petroleum ether/EtOAc=10/1) to afford **17a** as a yellow solid (160 mg, 60%). Mp 195–197 °C. IR (KBr) ν_{max} 3314, 1715, 1165 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6) δ (ppm) 8.41 (s, 1H), 7.67 (d, J=8.8 Hz, 2H), 7.58 (d, J=9.2 Hz, 1H), 7.08 (d, J=9.2 Hz, 1H), 6.79 (d, J=8.8 Hz, 2H), 4.99 (s, 2H), 2.95 (s, 6H), 2.47 (s, 3H), 2.28 (s, 3H), 1.42 (s, 9H). ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm) 168.0, 160.2, 150.2, 148.4, 136.1, 134.5, 132.7, 128.9 (2C), 124.3, 122.8, 122.3, 112.4, 111.8 (2C), 111.7, 108.4, 107.3, 81.6, 45.2, 40.1 (2C), 27.6 (3C), 11.5, 9.6. MS (ESI) m/z 447.2 $[M+H]^+$. HRMS (ESI) m/z calcd for $C_{27}H_{31}N_2O_4$ $[M+H]^+$: 447.2289; found, 447.2278.

*6.1.2. [8-(4-Dimethylamino-phenyl)-1,2-dimethyl-7-oxo-7H-pyrano [3,2-e]indol-3-yl]-acetic acid (**18a**).* A solution of **17a** (130 mg, 0.29 mmol) in CH_2Cl_2 (20 mL) was cooled to 0 °C and treated with TFA (0.85 mL). The reaction was warmed to room temperature and stirred overnight. The volatiles were removed under reduced pressure. The residue was purified by flash column chromatography (dichloromethane/methanol=25/1) to afford **18a** as a yellow solid (100 mg, 96%). Mp: 145–147 °C. IR (KBr) ν_{max} 3430, 2918, 1704, 1612, 1186 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6) δ (ppm) 8.36 (s, 1H), 7.66 (d, J=8.8 Hz, 2H), 7.56 (d, J=8.8 Hz, 1H), 7.05 (d, J=8.8 Hz, 1H), 6.78 (d, J=8.8 Hz, 2H), 4.98 (s, 2H), 2.94 (s, 6H), 2.44 (s, 3H), 2.29 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm) 170.4, 160.2, 150.1, 148.5, 136.2, 134.5, 132.7, 128.9 (2C), 124.1, 123.0, 122.3, 112.4, 111.9 (2C), 111.6, 108.3, 107.2, 44.3, 40.1 (2C), 11.6, 9.6. MS (ESI) m/z 391.0 $[M+H]^+$. HRMS (ESI) m/z calcd for $C_{23}H_{21}N_2O_4$ $[M-H]^-$: 389.1492; found, 389.1506.

*6.1.3. 4-[8-(4-Dimethylamino-phenyl)-1,2-dimethyl-7-oxo-7H-pyrano[3,2-e]indol-3-yl]-butyric acid ethyl ester (**17b**).* Compound **17b** was prepared using the above procedure for **17a**. Column chromatography mobile phase: petroleum ether/EtOAc=3/1. Yellow solid (67%). Mp: 150–152 °C. IR (KBr) ν_{max} 2916, 1736, 1697, 1607 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6) δ (ppm) 8.42 (s, 1H), 7.68 (d, J=8.8 Hz, 2H), 7.64 (d, J=8.8 Hz, 1H), 7.10 (d, J=8.8 Hz, 1H), 6.80 (d, J=8.8 Hz, 2H), 4.18 (t, J=7.2 Hz, 2H), 4.04 (q, J=7.2 Hz, 2H), 2.96 (s, 6H), 2.48 (s, 3H), 2.39 (s, 3H), 2.34 (t, J=7.2 Hz, 2H), 1.90–1.86 (m, 2H), 1.16 (t, J=7.2 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm) 172.3, 160.2, 150.2, 148.3, 135.5, 134.4, 131.9, 128.9 (2C), 124.0, 122.8, 122.0, 112.4, 111.8 (2C), 111.6, 108.1, 106.9. 59.9, 41.8, 40.1 (2C), 30.3, 25.0, 14.0, 11.5, 9.6. MS (ESI) m/z 447.1 $[M+H]^+$. HRMS (ESI) m/z calcd for $C_{27}H_{31}N_2O_4$ $[M+H]^+$: 447.2290; found, 447.2278.

*6.1.4. 4-[8-(4-Dimethylamino-phenyl)-1,2-dimethyl-7-oxo-7H-pyrano[3,2-e]indol-3-yl]-butyric acid (**18b**).* To a solution of **17b** (100 mg, 0.22 mmol) in THF (4.0 mL) and water (4.0 mL) was added LiOH hydrate (24.0 mg, 0.28 mmol) at 0 °C. The resulting solution was stirred overnight. The mixture was adjusted to *p*H 7 with 2 M HCl. The solvent were removed under reduced pressure. The residue was purified by flash column chromatography (dichloromethane/methanol=50/1) to afford **18b** as a yellow solid (86 mg, 92%). Mp 228–230 °C. IR (KBr) ν_{max} 2916, 1701, 1607 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6) δ (ppm) 12.20 (br, 1H), 8.42 (s, 1H), 7.68 (d, J=8.8 Hz, 2H), 7.64 (s, J=8.8 Hz, 1H), 7.09 (s, J=8.8 Hz, 1H), 6.80 (d, J=8.8 Hz, 2H), 4.18 (t, J=6.8 Hz, 2H), 2.96 (s, 6H), 2.48 (s, 3H), 2.39 (s, 3H), 2.27 (t, J=6.8 Hz, 2H), 1.86–1.83 (m, 2H). ^{13}C NMR (75 MHz, DMSO-d_6) δ (ppm) 173.9, 160.2, 150.2, 148.3, 135.6, 134.6, 131.9, 128.9 (2C), 124.1, 122.9, 122.1, 112.6, 111.9 (2C), 111.6, 108.2, 106.9, 41.9, 40.3 (2C), 30.4, 25.2, 11.6, 9.7. MS (ESI) m/z 419.0 $[M+H]^+$. HRMS (MALDI) m/z calcd for $C_{25}H_{26}N_2O_4Na$ $[M+Na]^+$: 441.1790; found, 441.1785.

*6.1.5. 6-[8-(4-Dimethylamino-phenyl)-1,2-dimethyl-7-oxo-7H-pyrano[3,2-e]indol-3-yl]-hexanoic acid ethyl ester (**17c**).* Compound **17c** was prepared using the above procedure for **17a**. Column chromatography mobile phase: petroleum ether/EtOAc=3/1. Yellow solid (67%). Mp 150–152 °C. IR (KBr) ν_{max} 3436, 2923, 1729, 1698, 1609 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6) δ (ppm) 8.35 (s, 1H), 7.65 (d, J=8.8 Hz, 2H), 7.55 (d, J=8.8 Hz, 1H), 7.03 (d, J=8.8 Hz, 1H), 6.77 (d, J=8.8 Hz, 2H), 4.08 (t, J=7.2 Hz, 2H), 4.00 (q, J=7.2 Hz, 2H), 2.94 (s, 6H), 2.42 (s, 3H), 2.34 (s, 3H), 2.23 (t, J=7.2 Hz, 2H), 1.61–1.48 (m, 4H), 1.29–1.24 (m, 2H), 1.13 (t, J=7.2 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm) 172.7, 160.2, 150.2, 148.3, 135.5, 134.5, 131.9, 128.9 (2C), 123.9, 122.8, 121.9, 112.5, 111.7 (2C), 111.6, 108.0, 106.7, 59.6, 42.5, 40.3 (2C), 33.3, 29.6, 25.7, 24.1, 14.0, 11.5, 9.7. MS (ESI) m/z 475.1 $[M+H]^+$. HRMS (ESI) m/z calcd for $C_{29}H_{35}N_2O_4$ $[M+H]^+$: 475.2609; found, 475.2591.

*6.1.6. 6-[8-(4-Dimethylamino-phenyl)-1,2-dimethyl-7-oxo-7H-pyrano[3,2-e]indol-3-yl]-hexanoic acid (**18c**).* Compound **18c** was prepared as the above procedure for **18b**. Column chromatography mobile phase: dichloromethane/methanol=50/1. Yellow solid (53%). Mp 142–144 °C. IR (KBr) ν_{max} 3434, 2919, 1704, 1609 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6) δ (ppm) 11.99 (br, 1H), 8.39 (s, 1H), 7.67 (d, J=8.8 Hz, 2H), 7.59 (d, J=8.8 Hz, 1H), 7.06 (d, J=8.8 Hz, 1H), 6.79 (d, J=8.8 Hz, 2H), 4.12 (t, J=7.2 Hz, 2H), 2.95 (s, 6H), 2.46 (s, 3H), 2.37 (s, 3H), 2.19 (t, J=7.2 Hz, 2H), 1.65–1.48 (m, 4H), 1.33–1.23 (m, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm) 174.3, 160.2, 150.2, 148.3, 135.6, 134.5, 131.9, 128.9 (2C), 124.0, 122.9, 122.0, 112.6, 111.8 (2C), 111.6, 108.1, 106.7, 42.6, 40.3 (2C), 33.5, 29.7, 25.8, 24.2, 11.6, 9.7. MS (ESI) m/z 445.1 $[M+H]^+$. HRMS (MALDI) m/z calcd for $C_{27}H_{30}N_2O_4$ Na $[M+Na]^+$: 469.2101; found, 469.2098.

*6.1.7. tert-Butyl 1-hydroxy-3,6,9,12-tetraoxapentadecan-15-oate (**16d–1**).* To a solution of tetraethylene glycol (20.0 g, 103 mol) in anhydrous THF (54.0 mL) were added NaH (60% dispersion in mineral oil, 42 mg, 1.05 mmol) and *tert*-butyl acrylate (5.2 mL, 36.0 mol). The resulting solution was stirred for 20 h at room temperature. The solvent was removed under reduced pressure. The residue was purified by flash column chromatography (petroleum ether/EtOAc=1/1) to afford **16d–1** as a colorless oil (9.3 g, 80%). IR (KBr) ν_{max} 3445, 2873, 1729 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ (ppm) 3.65–3.53 (m, 18H), 2.43 (t, J=8.4 Hz, 2H), 1.37

(s, 9H). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 170.7, 80.3, 72.6, 70.4 (2C), 70.3, 70.2, 70.1, 70.0, 66.7, 61.3, 36.0, 27.9 (3C). MS (ESI) *m/z* 340.2 $[M+NH_4]^+$. HRMS (ESI) *m/z* calcd for $C_{15}H_{30}O_7Na$ $[M+Na]^+$: 345.1883; found, 345.1884.

*6.1.8. tert-Butyl 1-iodo-3,6,9,12-tetraoxapentadecan-15-oate (**16d**).* To a solution of **16d**–**1** (1.2 g, 3.6 mmol) in THF (30 mL) were added PPh_3 (2.8 g, 10.8 mmol) and imidazole (780 mg, 11.5 mmol) slowly at 0 °C. After the mixture was stirred at same temperature for 2 min, I_2 (2.9 g, 11.5 mmol) was added. The resulting brown-black slurry was stirred overnight at 0 °C. Saturated aqueous $Na_2S_2O_3$ was added to quench the reaction. The resultant mixture was filtered, and the filtrate was concentrated under reduced pressure. The residue was extracted with ethyl acetate. The extract was successively washed with 1 M HCl and brine, and concentrated. The residue was purified by flash column chromatography (petroleum ether/EtOAc=10/1) to afford **16d** as a colorless oil (1.2 g, 82%). IR (KBr) ν_{max} 2870, 1731 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 3.70–3.55 (m, 16H), 3.19 (t, *J*=7.2 Hz, 2H), 2.42 (t, *J*=6.4 Hz, 2H), 1.38 (s, 9H). ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm) 170.5, 80.0, 71.7, 70.4 (2C), 70.3 (2C), 70.2 (2C), 70.0, 66.7, 36.1, 27.9 (3C). MS (ESI) *m/z* 450.0 $[M+NH_4]^+$. HRMS (ESI) *m/z* calcd for $C_{15}H_{29}IO_6Na$ $[M+Na]^+$: 455.0897; found,455.0901.

*6.1.9. tert-Butyl-1-(8-(4-(dimethylamino)phenyl)-1,2-dimethyl-7-oxopyrano[3,2-e]indol-3(7H)-yl)-3,6,9,12-tetraoxapentadecan-15-oate (**17d**).* Compound **17d** was prepared as the above procedure for **17a**. Column chromatography mobile phase: petroleum ether/EtOAc=2/1. Yellow oil (34%). IR (KBr) ν_{max} 2867, 1709, 1610 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 8.47 (s, 1H), 7.71 (d, *J*=8.8 Hz, 2H), 7.38 (d, *J*=8.8 Hz, 1H), 7.10 (d, *J*=8.8 Hz, 1H), 6.80 (d, *J*=8.8 Hz, 2H), 4.29 (t, *J*=6.0 Hz, 2H), 3.74–3.49 (m, 16H), 3.00 (s, 6H), 2.50–2.40 (m, 5H), 2.40 (s, 3H), 1.44 (s, 9H). ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm) 171.0, 161.8, 150.7, 149.4, 135.8, 135.3, 132.6, 129.4 (2C), 125.6, 123.9, 122.7, 112.5, 112.2 (2C), 111.9, 109.1, 107.5, 80.4, 70.8, 70.5 (2C), 70.4 (2C), 70.3 (2C), 70.1, 66.8, 43.5, 40.4, 36.2, 28.0 (2C), 12.1, 10.2. MS (MALDI) *m/z* 637.3 $[M+H]^+$. HRMS (MALDI) *m/z* calcd for $C_{36}H_{48}N_2O_6Na$ $[M+Na]^+$: 659.3318; found, 659.3302.

*6.1.10. 1-(8-(4-(Dimethylamino)phenyl)-1,2-dimethyl-7-oxopyrano[3,2-e]indol-3(7H)-yl)-3,6,9,12-tetraoxapentadecan-15-oic acid (**18d**).* Compound **18d** was prepared as the above procedure for **18a**. Column chromatography mobile phase: dichloromethane/methanol=50/1. Yellow oil (80%). IR (KBr) ν_{max} 2919, 1707, 1609 cm^{-1}. ^{1}H NMR(300 MHz, $CDCl_3$) δ (ppm) 8.47 (s, 1H), 7.71 (d, *J*=8.7 Hz, 2H), 7.40 (d, *J*=8.7 Hz, 1H), 7.10 (d, *J*=8.7 Hz, 1H), 6.82 (d, *J*=8.7 Hz, 2H), 4.29 (t, *J*=4.8 Hz, 2H), 3.75–3.56 (m, 16H), 3.01 (s, 6H), 2.63 (t, *J*=7.6 Hz, 2H), 2.50 (s, 3H), 2.40 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm) 175.2, 161.9, 150.6, 149.4, 135.8, 135.5, 132.7, 129.4 (2C), 125.5, 124.0, 122.9, 112.8, 112.5 (2C), 112.3, 109.4, 107.7, 71.1, 70.8 (2C), 70.7 (2C), 70.6, 70.4, 70.3, 66.6, 43.7, 40.7 (2C), 35.1, 29.9, 12.4, 10.4. MS (ESI) *m/z* 579.2 $[M-H]^-$. HRMS (MALDI) *m/z* calcd for $C_{32}H_{40}N_2O_8Na$ $[M+Na]^+$: 603.2688; found, 603.2677.

*6.1.11. 2-(8-Bromo-2-methyl-7-oxo-3,7-dihydropyrano[3,2-e]indol-1-yl)acetic acid (**21a**).* To a solution of the 6-amino-3-bromocoumarin (**19**) (239 mg, 1.0 mmol) in HCl (37%, 1.0 mL) was added $NaNO_2$ (83 mg, 1.20 mmol) in H_2O (0.5 mL) at −5 °C. After being stirred at this temperature for 30 min, the resulting mixture was treated with a pre-cooled (−10 to −5 °C) solution of stannous chloride dihydrate (452 mg, 2.0 mmol) in HCl (37%, 0.5 mL). The mixture was stirred at −5 °C for an additional 5 h. The reaction mixture was filtered. The solid was added into a solution of **20a** (278 mg, 2.4 mmol) in HOAc (5 mL). The mixture was then heated to 80 °C for 4 h. The solvent was evaporated in vacuo, and the residue was diluted with H_2O (10 mL) and EtOAc (50 mL). The mixture was adjusted to *p*H 8 by the addition of saturated $NaHCO_3$ solution. The organic phase was separated and washed with brine, dried over anhydrous Na_2SO_4, and concentrated. The residue was purified by flash column chromatography (dichloromethane/methanol=25/1) to afford **21a** as a yellow solid (117 mg, 35%). Mp 238–240 °C. IR (KBr) ν_{max} 3292, 1708 cm^{-1}. ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm) 12.40 (s, 1H), 11.54 (s, 1H), 8.84 (s, 1H), 7.59 (d, *J*=8.8 Hz, 1H), 7.09 (d, *J*=8.8 Hz, 1H), 3.83 (s, 2H), 2.41 (s, 3H). ^{13}C NMR (75 MHz, DMSO-d_6) δ (ppm) 173.2, 156.8, 149.1, 143.0, 137.5, 131.6, 122.0, 115.7, 110.2, 108.6, 107.5, 105.4, 31.1, 11.3. MS (ESI) *m/z* 335.9 $[M+H]^+$. HRMS (ESI) *m/z* calcd for $C_{14}H_{10}BrNO_4$ Na $[M+Na]^+$: 357.9687; found, 357.9685.

*6.1.12. Methyl 2-(8-bromo-2-methyl-7-oxo-3,7-dihydropyrano[3,2-e]indol-1-yl)acetate (**22a**).* To a solution of **21a** (100 mg, 0.3 mmol) in dry MeOH (2.1 mL) was added $SOCl_2$ (34 006DL, 0.47 mmol). The resulting mixture was refluxed for 1.5 h, and then cooled down to room temperature. The solvent was removed under reduced pressure. The residue was purified by flash column chromatography (petroleum ether/EtOAc=3/1) to afford **22a** as a yellow solid (100 mg, 96%). Mp 269–270 °C. IR (KBr) ν_{max} 3438, 1695 cm^{-1}. ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm) 11.58 (s, 1H), 8.79 (s, 1H), 7.59 (d, *J*=8.8 Hz, 1H), 7.08 (d, *J*=8.8 Hz, 1H), 3.94 (s, 2H), 3.61 (s, 3H), 2.41 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm) 172.1, 156.8, 149.1, 142.9, 137.7, 131.7, 121.9, 115.8, 110.2, 108.8, 107.6, 104.6, 51.8, 30.7, 11.3. MS (MALDI) *m/z* 350.0 $[M+H]^+$. HRMS (MALDI) *m/z* calcd for $C_{15}H_{12}BrNO_4$ $[M+H]^+$ 350.0034; found, 350.0025.

*6.1.13. 2-(8-(4-(Dimethylamino)phenyl)-2-methyl-7-oxo-3,7-dihydropyrano[3,2-e]indol-1-yl)acetic acid (**24a**).* To a mixture of **22a** (240 mg, 0.69 mmol), **23** (230 mg, 1.35 mmol), K_2CO_3 (470 mg, 2.06 mmol), and $Pd(PPh_3)_4$ (40 mg, 0.034 mmol) was added the mixture solvent EtOH/toluene/H_2O (1.7 mL, 1.7 mL, 3.4 mL) under nitrogen. The reaction was heated at 90 °C for 6 h. The solvents were evaporated under reduced pressure, the residue was diluted with H_2O (10 mL) and EtOAc (50 mL). The organic solution was washed with saturated brine, dried over anhydrous Na_2SO_4, and concentrated. The residue was purified by flash column chromatography (dichloromethane/methanol=10/1) to afford **24a** as a yellow solid (130 mg, 50%). Mp 180–181 °C. IR (KBr) ν_{max} 3399, 1678 cm^{-1}. ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm) 12.36 (s, 1H), 11.41 (s, 1H), 8.49 (s, 1H), 7.72 (d, *J*=8.8 Hz, 2H), 7.49 (d, *J*=8.8 Hz, 1H), 7.06 (d, *J*=8.8 Hz, 1H), 6.78 (d, *J*=8.8 Hz, 2H), 3.87 (s, 2H), 2.97 (s, 6H), 2.41 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm) 173.4, 160.2, 150.2, 148.2, 136.5, 134.9, 131.4, 128.9 (2C), 123.7, 122.7, 122.5, 114.1, 111.7 (2C), 111.0, 108.4, 105.4, 31.5, 11.2. MS (ESI) *m/z* 377.1 $[M+H]^+$. HRMS (ESI) *m/z* calcd for $C_{22}H_{21}N_2O_4$ $[M+H]^+$: 377.1491; found, 377.1496.

*6.1.14. 4-(8-Bromo-2-methyl-7-oxo-3,7-dihydropyrano[3,2-e]indol-1-yl)butanoic acid (**21b**).* Compound **21b** was prepared as the above procedure for **21a**. Column chromatography mobile phase: dichloromethane/methanol=25/1. Yellow solid (35%). Mp 169–171 °C. IR (KBr) ν_{max} 3418, 1729 cm^{-1}. ^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm) 12.19 (s, 1H), 11.39 (s, 1H), 8.75 (s, 1H), 7.53 (d, *J*=8.4 Hz, 1H), 7.00 (d, *J*=8.4 Hz, 1H), 2.79 (t, *J*=6.9 Hz, 2H), 2.35–2.29 (m, 5H), 1.73–1.69 (m, 2H). ^{13}C NMR (75 MHz, DMSO-d_6) δ (ppm) 174.9, 157.3, 149.4, 142.9, 136.7, 132.2, 121.8, 116.1, 111.7, 110.7, 108.8, 108.5, 33.3, 25.6, 24.3, 11.7. MS (ESI) *m/z* 362.0 $[M-H]^-$. HRMS (MALDI) *m/z* calcd for $C_{16}H_{14}BrNO_4$ Na $[M+Na]^+$: 385.9999; found, 385.9998.

*6.1.15. Methyl 4-(8-bromo-2-methyl-7-oxo-3,7-dihydropyrano[3,2-e]indol-1-yl)butanoate (**22b**).* Compound **22b** was prepared as the above procedure for **22a**. Column chromatography mobile phase:

petroleum ether/EtOAc=3/1. Yellow solid (85%). Mp 202–204 °C. IR (KBr) ν_{max} 3272, 1732, 1689 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6) δ (ppm) 11.41 (s, 1H), 8.74 (s, 1H), 7.56 (d, J=8.8 Hz, 1H), 7.05 (d, J=8.8 Hz, 1H), 3.59 (s, 3H), 2.84 (t, J=7.2Hz, 2H), 2.42–2.36 (m, 5H), 1.80–1.76 (m, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm) 173.2, 156.8, 148.9, 142.3, 136.3, 131.7, 121.2, 115.6, 111.0, 110.1, 108.3, 107.9, 51.2, 32.5, 25.1, 23.7, 11.2. MS (ESI) m/z 399.8 $[M+Na]^+$. HRMS (ESI) m/z calcd for $C_{17}H_{17}BrNO_4$ $[M+H]^+$: 378.0346; found, 378.0336.

*6.1.16. 4-(8-(4-(Dimethylamino)phenyl)-2-methyl-7-oxo-3,7-dihydropyrano[3,2-e]indol-1-yl)butanoic acid (**24b**).* Compound **24b** was prepared as the above procedure for **24a**. Column chromatography mobile phase: dichloromethane/methanol=50/1. Yellow solid (55%). Mp 204–205 °C. IR (KBr) ν_{max} 3314, 1685, 1608 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6) δ (ppm) 12.06 (s, 1H), 11.29 (s, 1H), 8.33 (s, 1H), 7.67 (d, J=8.8 Hz, 2H), 7.47 (d, J=8.8 Hz, 1H), 7.04 (d, J=8.8 Hz, 1H), 6.80 (d, J=8.8 Hz, 2H). 2.97 (s, 6H), 2.92 (t, J=7.2 Hz, 2H), 2.38 (s, 3H), 2.30 (t, J=7.2 Hz, 2H), 1.86 (t, J=7.2 Hz, 2H). ^{13}C NMR (75 MHz, DMSO-d_6) δ (ppm) 174.8, 160.7, 150.7, 148.9, 135.9, 134.9, 132.1, 129.4 (2C), 124.7, 123.2, 122.4, 114.7, 112.3 (2C), 111.6, 111.4, 108.7, 40.9 (2C), 33.6, 26.0, 24.7, 11.7. MS (ESI) m/z 403.1$[M-H]^-$. HRMS (ESI) m/z calcd for $C_{24}H_{23}N_2O_4$ $[M-H]^-$: 403.1671; found, 403.1663.

*6.1.17. 6-(8-Bromo-2-methyl-7-oxo-3,7-dihydro-pyrano[3,2-e]indol-1-yl)-hexanoic acid (**21c**).* Compound **21c** was prepared as the above procedure for **21a**. Column chromatography mobile phase: dichloromethane/methanol=25/1. Yellow solid (35%). Mp 169–171 °C. IR (KBr) ν_{max} 3312, 1717 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6) δ (ppm) 11.96 (s, 1H), 11.38 (s, 1H), 8.58 (s, 1H), 7.56 (d, J=8.8 Hz, 1H), 7.05 (d, J=8.8 Hz, 1H), 2.80 (s, 2H), 2.37 (s, 3H), 2.21 (t, J=7.2 Hz, 2H), 1.59–1.36 (m, 6H). ^{13}C NMR (75 MHz, DMSO-d_6) δ (ppm) 174.3, 156.7, 148.9, 142.3, 135.9, 131.6, 121.2, 115.5, 111.8, 110.0, 108.1, 107.6, 40.3, 33.7, 29.5, 28.4, 24.3, 11.3. MS (ESI) m/z 392.0 $[M+H]^+$. HRMS (MALDI) m/z calcd for $C_{18}H_{19}NO_4Br$ $[M+H]^+$: 392.0491; found, 392.0492.

*6.1.18. Methy 6-(8-bromo-2-methyl-7-oxo-3,7-dihydropyrano[3,2-e]indol-1-yl)hexanoate (**22c**).* Compound **22c** was prepared as the above procedure for **22a**. Column chromatography mobile phase: petroleum ether/EtOAc=6/1. Yellow solid (55%). Mp 141–142 °C. IR (KBr) ν_{max} 3330, 1694 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6) δ (ppm) 11.39 (s, 1H), 8.58 (s, 1H), 7.56 (d, J=8.8 Hz, 1H), 7.05 (d, J=8.8 Hz, 1H), 3.56 (s, 3H), 2.80 (t, J=7.2 Hz, 2H), 2.37 (s, 3H), 2.30 (t, J=7.2 Hz, 2H), 1.60–1.36 (m, 6H). ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm) 173.2, 156.8, 149.0, 142.4, 136.0, 131.7, 121.3, 115.6, 111.7, 110.1, 108.2, 107.7, 51.1, 40.1, 33.3, 29.5, 28.2, 24.3, 11.3. MS (ESI) m/z 406.0 $[M+H]^+$. HRMS (ESI) m/z calcd for $C_{19}H_{20}BrNO_4Na$ $[M+Na]^+$: 428.0484; found, 428.0468.

*6.1.19. 6-(8-(4-(Dimethylamino)phenyl)-2-methyl-7-oxo-3,7-dihydropyrano[3,2-e]indol-1-yl)hexanoic acid (**24c**).* Compound **24c** was prepared as the above procedure for **24a**. Column chromatography mobile phase: dichloromethane/methanol=50/1. Yellow solid (61%). Mp 194–195 °C. IR (KBr) ν_{max} 3258, 2924, 1685 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6) δ (ppm) 11.96 (br, 1H), 11.25 (s, 1H), 8.28 (s, 1H), 7.64 (d, J=8.4 Hz, 2H), 7.46 (d, J=8.4 Hz, 1H), 7.03 (d, J=8.4 Hz, 1H), 6.81 (d, J=8.4 Hz, 2H), 2.97 (s, 6H), 2.86 (t, J=6.8 Hz, 2H), 2.38 (s, 3H), 2.20 (t, J=6.8 Hz, 2H), 1.64–1.40 (m, 6H). ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm) 174.3, 160.2, 150.2, 148.4, 135.0, 134.6, 131.5, 128.8 (2C), 124.1, 122.8, 121.9, 114.1, 111.8 (3C), 110.9, 108.0, 40.3 (2C), 33.8, 30.1, 28.7, 24.9, 24.5, 11.2. MS (ESI) m/z 433.0 $[M+H]^+$. HRMS (ESI) m/z calcd for $C_{26}H_{29}N_2O_4$ $[M+H]^+$: 433.2126; found, 433.2122.

*6.1.20. tert-Butyl 3-(2-(2-(2-hydroxyethoxy)ethoxy)ethoxy)propanoate (**20d**–**1**).* Compound **20d**–**1** was prepared as the above procedure for **16d**–**1**. Column chromatography mobile phase: petroleum ether/EtOAc=1/2. Colorless oil (62%). IR (KBr) ν_{max} 3444, 2873, 1728 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ (ppm) 3.66–3.56 (m, 14H), 2.62 (br, 1H), 2.44 (t, J=5.2 Hz, 2H), 1.38 (s, 9H). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 170.8, 80.3, 72.5, 70.5, 70.4, 70.2 (2C), 66.8, 61.5, 36.1, 28.0 (3C). MS (ESI) m/z 301.0 $[M+Na]^+$. HRMS (ESI) m/z calcd for $C_{13}H_{26}O_6Na$ $[M+Na]^+$: 301.1619; found, 301.1622.

*6.1.21. tert-Butyl 3-(2-(2-(2-iodoethoxy)ethoxy)ethoxy)propanoate (**20d**).* Compound **20d** was prepared as the above procedure for **16d**. Column chromatography mobile phase: petroleum ether/EtOAc=20/1. Colorless oil (79%). IR (KBr) ν_{max} 2870, 1729 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ (ppm) 3.77–3.60 (m, 12H), 3.26 (t, J=6.8 Hz, 2H), 2.51 (t, J=6.4 Hz, 2H), 1.45 (s, 9H). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 170.8, 80.4, 71.9, 70.6 (2C), 70.5, 70.4, 70.2, 66.9, 36.3, 28.1 (3C). MS (ESI) m/z 410.9 $[M+Na]^+$. HRMS (ESI) m/z calcd for $C_{13}H_{25}IO_5Na$ $[M+Na]^+$: 411.0635; found, 411.0639.

*6.1.22. 2-(8-Bromo-2-methyl-7-oxo-3,7-dihydropyrano[3,2-e]indol-1-yl)ethyl acetate (**26**).* Compound **26** was prepared as the above procedure for **21a**. Column chromatography mobile phase: petroleum ether/EtOAc=4/1. Yellow solid (20%). Mp 211–212 °C. IR (KBr) ν_{max} 3350, 1736, 1700 cm^{-1} ^1H NMR (400 MHz, DMSO-d_6) δ (ppm) 11.53 (s, 1H), 8.93 (s, 1H), 7.57 (d, J=8.8 Hz, 1H), 7.07 (d, J=8.8 Hz, 1H), 4.17 (t, J=7.2 Hz, 2H), 3.16 (t, J=7.2 Hz, 2H), 2.40 (s, 3H), 1.98 (s, 3H). ^{13}C NMR (75 MHz, DMSO-d_6) δ (ppm) 190.5, 176.8, 169.0, 162.5, 157.3, 151.6, 141.7, 135.7, 130.2, 128.5, 127.9, 127.1, 83.8, 44.2, 40.6, 31.2. MS (ESI) m/z 385.9 $[M+Na]^+$. HRMS (MALDI) m/z calcd for $C_{16}H_{14}NO_4$ BrNa $[M+Na]^+$: 386.0004; found, 385.9998.

*6.1.23. tert-Butyl 1-(2-acetoxyethyl)-8-bromo-2-methyl-7-oxopyrano[3,2-e]indole-3(7H)-carboxylate (**27**).* To a solution of **26** (66 mg, 0.18 mmol) in THF (3.0 mL) was added di-*tert*-butyl dicarbonate (0.09 mL, 0.42 mmol) and 4-*N*,*N*-dimethylaminopyridine (33 mg, 0.27 mmol) at 0 °C. The resulting mixture was stirred at 0 °C for further 5 min. Then, 1 M HCl (0.22 mL) was added to quench the reaction. The solvent was removed under reduced pressure. Water was added, and the mixture was extracted with ethyl acetate. The extract was dried over Na_2SO_4, filtered, and concentrated to dryness under reduced pressure. The residue was purified by flash column chromatography (petroleum ether/EtOAc=10/1) to afford **27** as a white solid (69 mg, 82%). Mp 178–179 °C. IR (KBr) ν_{max} 2978, 1728 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ (ppm) 8.92 (s, 1H), 8.38 (d, J=8.8 Hz, 1H), 7.20 (d, J=8.8 Hz, 1H), 4.26 (t, J=7.2 Hz, 2H), 3.22 (t, J=7.2 Hz, 2H), 2.61 (s, 3H), 2.06 (s, 3H), 1.70 (s, 9H). ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm) 170.9, 157.2, 150.9, 149.8, 141.6, 138.1, 132.4, 124.1, 119.3, 113.5, 112.0, 111.1, 110.6, 85.1, 63.4, 28.2 (3C), 25.1, 20.9, 13.8. MS (MALDI) m/z 464.1$[M+H]^+$.

*6.1.24. tert-Butyl 1-(2-acetoxyethyl)-8-(4-(dimethylamino)phenyl)-2-methyl-7-oxopyrano[3,2-e]indole-3(7H)-carboxylate (**28**).* Compound **28** was prepared as the above procedure for **24a**. Column chromatography mobile phase: petroleum ether/EtOAc=8/1. Yellow solid (88%). Mp 173–174 °C. IR (KBr) ν_{max} 3446, 1717, 1609 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6) δ (ppm) 8.40 (s, 1H), 8.25 (d, J=9.2 Hz, 1H), 7.68 (d, J=8.8 Hz, 2H), 7.28 (d, J=9.2 Hz, 1H), 6.80 (d, J=8.8 Hz, 2H), 4.26 (t, J=6.4 Hz, 2H), 3.30 (t, J=6.8 Hz, 2H), 2.97 (s, 6H), 2.56 (s, 3H), 1.91 (s, 3H), 1.66 (s, 9H). ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm) 170.8, 160.9, 150.5 (2C), 150.1, 137.3, 133.6, 132.3, 129.4 (2C), 126.8, 124.5, 117.8, 114.0, 112.3, 112.2, 112.0, 111.9 (2C), 84.7, 63.5, 40.5, 28.3 (3C), 25.4, 20.9, 13.8. MS (MALDI) m/z 505.2 $[M+H]^+$. HRMS (MALDI) m/z calcd for $C_{29}H_{33}N_2O_6$ $[M+H]^+$: 505.2336; found, 505.2333.

*6.1.25. tert-Butyl 8-(4-(dimethylamino)phenyl)-1-(2-hydroxyethyl)-2-methyl-7-oxopyrano[3,2-e] indole-3(7H)-carboxylate (**29**).* A mixture of **28** (145 mg, 0.29 mmol) and K_2CO_3 (80 mg, 0.58 mmol) in MeOH (14.0 mL) and CH_2Cl_2 (14.0 mL) was stirred at room

temperature for 2 h. Water (60 mL) was added. The resulting solution was extracted with CH_2Cl_2 (20 mL×3). The organic phase was dried over Na_2SO_4 and concentrated. The residue was purified by flash column chromatography (petroleum ether/EtOAc=4/1) to afford **29** as a yellow solid (114 mg, 86%). Mp 155–157 °C. IR (KBr) ν_{max} 3291, 1678 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6) δ (ppm) 8.47 (s, 1H), 8.24 (d, J=9.2 Hz, 1H), 7.69 (d, J=8.8 Hz, 2H), 7.26 (d, J=9.2 Hz, 1H), 6.80 (d, J=8.8 Hz, 2H), 4.87 (br, 1H), 3.65 (s, 2H), 3.10 (t, J=6.8 Hz, 2H), 2.97 (s, 6H), 2.54 (s, 3H), 1.65 (s, 9H). ^{13}C NMR (75 MHz, DMSO-d_6) δ (ppm) 159.6, 150.4, 149.6, 149.5, 136.4, 133.4, 131.5, 129.2 (2C), 129.1, 125.3, 124.4, 122.2, 117.2, 115.4, 111.7 (2C), 111.4, 110.9, 84.4, 60.5, 40.1 (2C), 28.7, 27.6 (3C), 13.5. MS (MALDI) m/z 463.2 $[M+H]^+$. HRMS (MALDI) m/z calcd for $C_{27}H_{31}N_2O_5$ $[M+H]^+$: 463.2235; found, 463.2228.

*6.1.26. 3-{2-[2-(2-{2-[8-(4-Dimethylamino-phenyl)-2-methyl-7-oxo-3,7-dihydro-pyrano[3,2-e]indol-1-yl]-ethoxy}-ethoxy)-ethoxy]-ethoxy}-propionic acid (**24d**).* To a solution of **29** (60 mg, 0.13 mmol) in anhydrous DMF (10 mL) was added *t*-BuOK (19 mg, 0.20mmol) at room temperature. After being stirred for 30 min, compound **20d** (85 mg, 0.26 mmol) was added. The reaction mixture was stirred for 4 h. The solvent was evaporated under reduced pressure. The residue was diluted with H_2O (5 mL) and EtOAc (10 mL), and the organic phase was separated and concentrated. TFA (0.2 mL) and THF (2.0 mL) was added to the residue at 0 °C, and the resulting solution was warmed to room temperature and stirred overnight. The reaction mixture was concentrated. The residue was diluted with H_2O (5 mL) and EtOAc (10 mL), the mixture was adjusted to *p*H 7 with saturated aqueous $NaHCO_3$. The organic phase was separated and concentrated. The residue was purified by flash column chromatography (dichloromethane/methanol=20/1) to afford **24d** as a yellow oil (30 mg, 41%). IR (KBr) ν_{max} 3361, 2920, 1706 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ (ppm) 8.36 (s, 1H, 8.36), 7.67 (d, J=8.4 Hz, 2H), 7.34 (d, J=8.8 Hz, 1H), 7.04 (d, J=8.4 Hz, 1H), 6.81 (d, J=8.4 Hz, 2H), 4.26–3.45 (m, 16H), 3.18 (t, J=7.6 Hz, 2H), 2.98 (s, 6H), 2.55 (t, J=6.0 Hz, 2H), 2.43 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 174.9, 161.5, 149.3 (2C), 137.7, 134.9, 132.6, 129.3 (2C), 125.3, 122.2 (2C), 112.6, 112.3 (2C), 112.1, 109.3, 108.3, 70.8, 70.6 (2C), 70.4, 70.3, 70.0, 66.4, 62.5, 43.6, 40.6, 34.8, 29.3, 10.6. MS (ESI) m/z 565.2$[M-H]^-$. HRMS (MALDI) m/z calcd for $C_{26}H_{29}N_2O_4$ $[M+H]^+$: 566.2612; found, 566.2628.

*6.1.27. N-[4-(2-Amino-9H-purin-6-yloxymethyl)-benzyl]-2-[8-(4-dimethylamino-phenyl)-1,2-dimethyl-7-oxo-7H-pyrano[3,2-e]indol-3-yl]-acetamide (**1**).* To a solution of compound **15** (52 mg, 0.19 mmol), compound **18a** (75 mg, 0.19 mmol), EDC·HCl (55 mg, 0.29 mmol), and HOBt (38 mg, 029 mmol) in anhydrous DMF (3 mL) was added DIPEA (67 μL, 0.40 mmol) at room temperature. The reaction was stirred overnight. The solvent was removed in vacuo. The residue was purified by flash column chromatography (dichloromethane/methanol=25/1) to afford **1** as a yellow solid (85 mg, 69%). Mp 287–290 °C. IR (KBr) ν_{max} 3392, 1610 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6) δ (ppm) 12.41 (s, 1H), 8.69 (br, 1H), 8.67 (s, 1H), 7.81 (s, 1H), 7.69 (d, J=8.8 Hz, 2H), 7.60 (d, J=8.8 Hz, 1H), 7.46 (d, J=7.6 Hz, 2H), 7.29 (d, J=7.6 Hz, 2H), 7.11 (d, J=8.8 Hz, 1H), 6.81 (d, J=8.8 Hz, 2H), 6.29 (s, 2H), 5.46 (s, 2H), 4.93 (s, 2H), 4.31 (d, J=5.2 Hz, 1H), 2.96 (s, 6H),2.35 (s, 3H). ^{13}C NMR (75 MHz, DMSO-d_6) δ (ppm) 167.9, 160.7, 160.3, 160.1, 155.7, 150.8, 149.0, 139.3, 138.3, 137.1, 135.9, 135.1, 133.3, 129.5 (2C), 129.0 (2C), 127.9 (2C), 124.7, 123.2, 122.8, 114.0, 113.0, 112.3 (2C), 112.2, 108.8, 107.6. 66.9, 46.4, 41.1, 40.8 (2C), 12.2, 10.4. MS (ESI) m/z 640.8 [M—H]$^-$. HRMS (MALDI) m/z calcd for $C_{36}H_{35}N_8O_4$ $[M+H]^+$: 643.2783; found, 643.2775.

*6.1.28. N-[4-(2-Amino-9H-purin-6-yloxymethyl)-benzyl]-4-[8-(4-dimethylamino-phenyl)-1,2-dimethyl-7-oxo-7H-pyrano[3,2-e]indol-3-yl]-butyramide (**2**).* Probe **2** was prepared as the above procedure for **1**. Column chromatography mobile phase: DCM/MeOH=50/1. Yellow solid (85%). Mp 262–263 °C. IR (KBr) ν_{max} 3274, 1610 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6) δ (ppm) 12.41 (s, 1H), 8.45 (s, 1H), 8.36 (br, 1H), 7.80 (s, 1H), 7.69 (d, J=8.8 Hz, 2H), 7.65 (d, J=8.8 Hz, 2H), 7.45 (d, J=7.6 Hz, 2H), 7.27 (d, J=7.6 Hz, 2H), 7.09 (d, J=8.8 Hz, 1H), 6.81 (d, J=8.8 Hz, 2H), 6.28 (s, 2H), 5.44 (s, 2H), 4.27 (d, J=5.2 Hz, 2H), 4.18 (t, J=6.8 Hz, 2H), 2.96 (s, 6H), 2.38 (s, 3H), 2.20 (t, J=6.4 Hz, 2H), 1.90–1.87 (m, 2H). ^{13}C NMR (75 MHz, DMSO-d_6) δ (ppm) 171.3, 160.2, 159.5 (2C), 150.3 (2C), 148.4, 139.5, 138.2, 135.7, 135.1, 134.6, 131.9, 128.9 (2C), 128.5 (2C), 127.3 (2C), 124.1, 122.8, 122.1, 112.7, 111.8 (3C), 111.6, 108.2, 106.8, 66.58, 42.27, 41.90, 40.1 (2C), 31.8, 25.8, 11.6, 9.7. MS (MALDI) m/z 671.7 $[M+H]^+$. HRMS (ESI) m/z calcd for $C_{38}H_{38}N_8O_4Na$ $[M+Na]^+$: 693.2889; found, 693.2908.

*6.1.29. 6-[8-(4-Dimethylamino-phenyl)-1,2-dimethyl-7-oxo-7H-pyrano[3,2-e]indol-3-yl]-hexanoic acid 4-(2-amino-9H-purin-6-yloxymethyl)-benzylamide (**3**).* Probe **3** was prepared as the above procedure for **1**. Column chromatography mobile phase: DCM/MeOH=50/1. Yellow solid (46%). Mp 195–198 °C. IR (KBr) ν_{max} 3391, 2922, 1609 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6) δ (ppm) 8.44 (s, 1H), 8.29 (br, 1H), 7.93 (s, 1H), 7.68 (d, J=8.4 Hz, 2H), 7.61 (d, J=8.8 Hz, 1H), 7.45 (d, J=8.0 Hz, 2H), 7.25 (d, J=8.0 Hz, 2H), 7.08 (d, J=8.8 Hz, 1H), 6.79 (d, J=8.4 Hz, 2H), 6.44 (s, 2H), 5.45 (s, 2H), 4.24 (d, J=5.2 Hz, 2H), 4.14 (t, J=6.8 Hz, 2H), 2.96 (s, 6H),2.46 (s, 3H), 2.38 (s, 3H), 2.19 (t, J=7.2 Hz, 2H), 1.65–1.53 (m, 4H), 1.29–1.27 (m, 2H). ^{13}C NMR (75 MHz, DMSO-d_6) δ (ppm) 171.9, 160.2, 159.3 (2C), 155.6, 150.2, 148.3, 139.6, 138.7, 135.7, 135.0, 134.6, 131.9, 128.9 (2C), 128.5 (2C), 127.3 (2C), 124.1, 122.8, 122.0, 112.7, 111.8 (3C), 111.6, 108.1, 106.7, 66.7, 42.6, 41.7, 40.3 (2C), 35.1, 29.7, 25.9, 24.9, 11.6, 9.8. MS (MALDI) m/z 721.6 $[M+Na]^+$. HRMS (MALDI) m/z calcd for $C_{40}H_{42}N_8O_4Na$ $[M+Na]^+$: 721.3215; found, 721.3221.

*6.1.30. N-[4-(2-Amino-9H-purin-6-yloxymethyl)-benzyl]-3-{2-[2-(2-{2-[8-(4-dimethylamino-phenyl)-1,2-dimethyl-7-oxo-7H-pyrano[3,2-e]indol-3-yl]-ethoxy}-ethoxy)-ethoxy]-ethoxy}-propionamide (**4**).* Probe **4** was prepared as the above procedure for **1**. Column chromatography mobile phase: DCM/MeOH=50/1. Yellow solid (54%). Mp 142–143 °C. IR (KBr) ν_{max} 3411, 2864, 1609 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6) δ (ppm) 8.45 (s, 1H), 8.32 (t, J=6.0 Hz, 1H), 7.83 (s, 1H), 7.68 (d, J=8.8 Hz, 2H), 7.64 (d, J=8.8 Hz, 1H), 7.43 (d, J=8.0 Hz, 2H), 7.25 (d, J=8.0 Hz, 2H), 7.08 (d, J=8.8 Hz, 1H), 6.80 (d, J=8.8 Hz, 2H), 6.27 (s, 2H), 5.45 (s, 2H), 4.35 (t, J=5.2 Hz, 2H), 4.25 (d, J=5.6 Hz, 2H), 3.66–3.32 (m, 18H), 2.95 (s, 6H), 2.40 (s, 3H), 2.36 (t, J=6.4 Hz, 2H). ^{13}C NMR (75 MHz, DMSO-d_6) δ (ppm) 170.1, 160.3, 159.6 (2C), 150.3 (2C), 148.5, 139.4 (2C), 136.5, 135.2, 134.7, 132.3, 128.9 (2C), 128.5 (2C), 127.3 (2C), 124.2, 122.9, 122.3, 113.0, 111.9 (3C), 111.6, 108.1, 106.8, 70.1, 69.8 (2C), 69.7 (2C), 69.5 (2C), 66.9, 66.2, 43.1, 41.9, 40.4 (2C), 36.2, 11.7, 9.99. MS (MALDI) m/z 855.4 $[M+Na]^+$. HRMS (MALDI) m/z calcd for $C_{45}H_{53}N_8O_8$ $[M+H]^+$: 833.3977; found, 833.3981.

*6.1.31. N-[4-(2-Amino-9H-purin-6-yloxymethyl)-benzyl]-2-[8-(4-dimethylamino-phenyl)-2-methyl-7-oxo-3,7-dihydro-pyrano[3,2-e]indol-1-yl]-acetamide (**5**).* Probe **5** was prepared as the above procedure for **1**. Column chromatography mobile phase: DCM/MeOH=20/1. Yellow solid (31%). Mp 172–173 °C. IR (KBr) ν_{max} 3409, 1642 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6) δ (ppm) 12.41 (s, 1H), 11.39 (s, 1H), 8.52 (s, 1H), 8.26 (t, J=5.6 Hz, 1H), 7.80 (s, 1H), 7.66 (d, J=8.8 Hz, 2H), 7.48 (d, J=8.8 Hz, 1H), 7.29 (d, J=8.0 Hz, 2H), 7.16 (d, J=8.0 Hz, 2H), 7.05 (d, J=8.8 Hz, 1H), 6.73 (d, J=8.8 Hz, 2H), 6.72 (s, 2H), 5.39 (s, 2H), 4.25 (d, J=5.6 Hz, 1H), 3.83 (s, 2H), 2.93 (s, 6H), 2.43 (s, 3H). ^{13}C NMR (75 MHz, DMSO-d_6) δ (ppm) 170.9, 160.3, 159.6 (2C), 150.1, 148.4, 139.3, 136.8, 135.2, 135.1, 131.6, 129.0 (2C), 128.2 (2C), 127.1 (2C), 124.4, 123.7, 122.9, 122.8, 119.0, 114.0, 111.7 (2C), 111.3, 109.6, 108.3, 105.9, 66.5, 42.1, 40.3 (2C), 32.8, 11.4. MS

(MALDI) *m*/*z* 629.3 $[M+H]^+$. HRMS (MALDI) *m*/*z* calcd for $C_{35}H_{33}N_8O_4$ $[M+H]^+$: 629.2617; found, 629.2619.

*6.1.32. N-[4-(2-Amino-9H-purin-6-yloxymethyl)-benzyl]-4-[2-methyl-8-(4-dimethylamino-phenyl)-7-oxo-3,7-dihydro-pyrano[3,2-e]indol-1-yl]-butyramide (**6**).* Probe **6** was prepared as the above procedure for **1**. Column chromatography mobile phase: DCM/MeOH=20/1. Yellow solid (96%). Mp 210–211 °C. IR (KBr) ν_{max} 3450, 1610 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6) δ (ppm) 11.24 (s, 1H), 8.35–8.31 (m, 2H), 7.92 (s, 1H), 7.69 (d, *J*=8.0 Hz, 2H), 7.48–7.42 (m, 3H), 7.25 (d, *J*=7.6 Hz, 2H), 7.04 (d, *J*=8.4 Hz, 1H), 6.77 (d, *J*=8.0 Hz, 2H), 6.39 (s, 2H), 5.48 (s, 2H), 4.25 (d, *J*=5.6 Hz, 1H), 2.92–2.90 (m, 8H), 2.36 (s, 3H), 2.23 (t, *J*=3.6 Hz, 2H), 1.88–1.86 (m, 2H). ^{13}C NMR (75 MHz, DMSO-d_6) δ (ppm) 172.3, 160.8, 159.8 (2C), 156.0, 150.7, 148.9, 140.2 (2C), 135.9, 135.4, 135.0, 132.1, 129.5 (2C), 129.0 (2C), 127.8 (2C), 124.7, 123.3, 122.4, 114.7, 112.3 (3C), 111.9, 111.5, 108.6, 67.3, 42.3, 41.3 (2C), 39.2, 26.7, 25.0, 11.8. MS (MALDI) *m*/*z* 657.3 $[M+H]^+$.

*6.1.33. 6-[8-(4-Dimethylamino-phenyl)-2-methyl-7-oxo-3,7-dihydro-pyrano[3,2-e]indol-1-yl]-hexanoic acid 4-(2-amino-9H-purin-6-yloxymethyl)-benzylamide (**7**).* Probe **7** was prepared as the above procedure for **1**. Column chromatography mobile phase: DCM/MeOH=20/1. Yellow solid (45%). Mp 170–171 °C. IR (KBr) ν_{max} 3407, 1610 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6) δ (ppm) 12.42 (s, br, 1H), 11.26 (s, 1H), 8.29 (s, 1H), 8.25 (t, *J*=5.6 Hz, 1H), 7.82 (s, 1H), 7.64 (d, *J*=8.8 Hz, 2H), 7.47–7.41 (m, 3H), 7.22 (d, *J*=8.0 Hz, 2H), 7.03 (d, *J*=8.8 Hz, 1H), 6.82 (d, *J*=8.8 Hz, 2H), 6.27 (s, 2H), 5.44 (s, 2H), 4.23 (d, *J*=6.0 Hz, 1H), 2.94 (s, 6H), 2.86 (t, *J*=7.2 Hz, 3H), 2.37 (s, 3H), 2.14 (t, *J*=7.2 Hz, 3H), 1.66–1.43 (m, 6H). ^{13}C NMR (75 MHz, DMSO-d_6) δ (ppm) 172.0, 160.2, 159.6 (2C), 150.2, 148.4, 139.5, 135.1, 135.0, 134.6, 131.5, 128.8 (2C), 128.5 (2C), 127.2 (2C), 124.4, 124.1, 122.7, 121.9, 119.1, 114.1, 111.9 (3C), 110.9, 109.6, 108.1, 66.5, 41.7, 40.1 (2C), 35.4, 30.1, 28.7, 25.3, 24.9, 11.3. MS (MALDI) *m*/*z* 685.3 $[M+H]^+$. HRMS (MALDI) *m*/*z* calcd for $C_{39}H_{41}N_8O_4$ $[M+H]^+$: 685.3247; found, 685.3245.

*6.1.34. N-[4-(2-Amino-9H-purin-6-yloxymethyl)-benzyl]-3-{2-[2-(2-{2-[8-(4-dimethylamino-phenyl)-2-methyl-7-oxo-3,7-dihydro-pyrano[3,2-e]indol-1-yl]-ethoxy}-ethoxy)-ethoxy]-ethoxy}-propionamide (**8**).* Probe **8** was prepared as the above procedure for **1**. Column chromatography mobile phase: DCM/MeOH=20/1. Yellow solid (76%). Mp 155–156 °C. IR (KBr) ν_{max} 2922, 1609 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6) δ (ppm) 8.49 (s, 1H), 8.31 (t, *J*=5.6 Hz, 1H), 7.94 (s, 1H), 7.68 (d, *J*=8.8 Hz, 2H), 7.64 (d, *J*=8.8 Hz, 1H), 7.44 (d, *J*=8.0 Hz, 2H), 7.25 (d, *J*=8.0 Hz, 2H), 7.08 (d, *J*=8.8 Hz, 1H), 6.80 (d, *J*=8.8 Hz, 2H), 6.42 (s, 2H), 5.46 (s, 2H), 4.34 (t, *J*=5.2 Hz, 2H), 4.25 (d, *J*=6.0 Hz, 1H), 3.65–3.40 (m, 14H), 3.08 (t, *J*=4.8 Hz, 2H), 2.96 (s, 6H), 2.40 (s, 3H), 2.35 (t, *J*=6.0 Hz, 2H). ^{13}C NMR (75 MHz, DMSO-d_6) δ (ppm) 170.5, 160.7, 160.1 (2C), 150.7, 149.1, 139.8 (2C), 137.9, 135.6, 135.0, 132.7, 129.5 (2C), 128.9 (2C), 127.7 (2C), 124.7 (2C), 123.2, 122.3, 113.6, 112.3 (3C), 111.6, 109.2, 108.6, 70.5, 70.2 (2C), 70.1, 69.9, 67.3, 67.0, 61.9, 43.5, 42.3, 41.2 (2C), 36.6, 29.8, 10.5. MS (MALDI) *m*/*z* 841.4 $[M+Na]^+$. HRMS (MALDI) *m*/*z* calcd for $C_{44}H_{51}N_8O_8$ $[M+H]^+$: 819.3807; found, 819.3824.

Acknowledgements

Financially support from Ministry of Science and Technology of China (2010CB833200, 2009ZX09103-101), and the National Natural Sciences Foundation of China (21032002, 90713044, 20921091) is greatly appreciated.

Supplementary data

^1H and ^{13}C NMR copies of new compounds (PDF). This material is available free of charge via the internet at http://www.elsevier.com or from the authors. Supplementary data associated with this article can be found in online version at doi:10.1016/j.tet.2011.01.075. These data include MOL files and InChIKeys of the most important compounds described in this article.

References and notes

1. Nicolle, O.; Rouillon, A.; Guyodo, H.; Tamanai-Shacoori, Z.; Chandad, F.; Meuric, V.; Bonnaure-Mallet, M. *FEMS Immunol. Med. Microbiol.* **2010**, *59*, 357.
2. (a) Chalfie, M.; Tu, Y.; Euskirchen, G.; Ward, W. W.; Prasher, D. C. *Science* **1994**, *263*, 802; (b) Errampalli, D.; Leung, K.; Cassidy, M. B.; Kostrzynska, M.; Blears, M.; Lee, H.; Trevors, J. T. *J. Microbiol. Methods* **1999**, *35*, 187.
3. Krusemark, C. J.; Belshaw, P. J. *Org. Biomol. Chem.* **2007**, *5*, 2201.
4. Damoiseaux, R.; Keppler, A.; Johnsson, K. *Chembiochem* **2001**, *2*, 285.
5. (a) Pick, H.; Jankevics, H.; Vogel, H. *J. Mol. Biol.* **2007**, *374*, 1213; (b) Jansen, L. E.; Black, B. E.; Foltz, D. R.; Cleveland, D. W. *J. Cell Biol.* **2007**, *176*, 795; (c) Kikuchi, K. *Chem. Soc. Rev.* **2010**, *39*, 2048.
6. Maurel, D.; Comps-Agrar, L.; Brock, C.; Rives, M. L.; Bourrier, E.; Ayoub, M. A.; Bazin, H.; Tinel, N.; Durroux, T.; Prézeau, L.; Trinquet, E.; Pin, J. P. *Nat. Methods.* **2008**, *5*, 561.
7. Chen, L.; Hu, T. S.; Yao, Z. J. *Eur. J. Org. Chem.* **2008**, *36*, 6175.
8. Keppler, A.; Gendreizig, S.; Gronemeyer, T.; Pick, H.; Vogel, H.; Johnsson, K. *Nat. Biotechnol.* **2003**, *21*, 86.
9. Dogan, Z.; Paulini, R.; Rojas Stütz, J. A.; Narayanan, S.; Richert, C. *J. Am. Chem. Soc.* **2004**, *126*, 4762.
10. Naito, R.; Takeuchi, M.; Morihira, K.; Hayakawa, M.; Ikeda, K.; Shibanuma, T.; Isomura, Y. *Chem. Pharm. Bull.* **1998**, *46*, 1274.
11. Schirrmacher, R.; Wängler, B.; Schirrmacher, E.; August, T.; Rösch, F. *Synthesis* **2002**, *4*, 538.
12. Sparks, S. M.; Chow, C. P.; Zhu, L.; Shea, K. J. *J. Org. Chem.* **2004**, *69*, 3025.
13. Ellwood, A. R.; Porter, M. J. *J. Org. Chem.* **2009**, *74*, 7982.
14. Reiff, A. L.; Garcia-Frutos, E. M.; Gil, J. M.; Anderson, O. P.; Hegedus, L. S. *Inorg. Chem.* **2005**, *44*, 9162.
15. Burgstahler, A. W.; Weigel, L. O.; Bell, W. J.; Rust, M. K. *J. Org. Chem.* **1975**, *40*, 3456.