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Total Synthesis and Evaluation of New B-homo Palmatine and Berberine Derivatives as p300 Histone Acetyltransferase Inhibitors

Zhongzhen Yang,[a] Yong Zhang,[a] Xin Chen,[b] Weijian Li,[a] Guo-Bo Li,*[a] Yong Wu*[a]

Abstract: Palmatine and berberine are structurally similar isoquinoline alkaloids exhibiting a broad range of biological activities, which were found recently to have inhibitory activity against the p300 histone acetyltransferase (HAT), a potential therapeutic target for treating transcriptional activator-driven malignancies and diseases. Here, we report the first total synthesis of the B-homo palmatine (**11a**) and berberine (**11b**) derivatives, which were synthesized from 3,4-dimethoxybenzaldehyde (**1a**) and benzo[*d*][1,3]dioxole-5-carbaldehyde (**1b**) in nine steps in 13.8% and 16.9% overall yields, respectively. A number of other new B-homo palmatine and berberine derivatives were also prepared. These derivatives displayed good inhibitory activity against p300 HAT; **12a** manifests the most potent inhibition with an IC_{50} value of 0.42 μM. Cell-based assays revealed that **12a** exhibited certain inhibitory activity to the HCG27, HT1080, and Z-138 cell lines, and no visible activity to other cancer cell lines tested, reflecting that **12a** has low cytotoxicity, and acts against some types of cancer cells.

Introduction

Natural products have been a rich source of useful starting materials for drug discovery.[1] During the last three decades, a third of new medicines approved by the US Food and Drug Administration (FDA) were natural products or direct derivatives of natural products.[1-2] Natural products that were developed as clinically useful drugs have been considered to have the 'metabolite-likeness' property, *i.e.* which are biologically active but also likely to be substrates of transporter systems that can deliver them to the intracellular site of action.[1a] Notably, natural products possess special chemical scaffolds, diverse pharmacophores and/or advanced stereochemistry, which may interrogate some difficult molecular targets, such as protein–protein interactions, featureless binding sites, and transcription factors.[3]

Palmatine and berberine are tetracyclic isoquinoline alkaloids (Figure 1a) found in such plants as *Coptis chinensis* (Chinese name: Huang-Lian), *Phellodendron amurense*, and *Corydalis yanhusuo*.[4] Many studies revealed that palmatine and berberine exhibit a wide range of biological activity and have therapeutic potential against bacterial infection, diabetes, cancer, arrhythimia, hypertension, inflammation, flavivirus, and liver-related diseases.[5] We recently identified that palmatine and berberine have p300 histone acetyltransferase (HAT) inhibition with IC_{50} values of 1.05 μM and 9.2 μM, respectively (Figure 1a);[6] p300 HAT is a crucial transcription factor for gene regulation, and its dysfunctions contribute to the development of multiple diseases, including cancer.[7] Since p300 HAT is a relatively difficult target (probably due to its tight-binding substrate Ac-CoA or CoA),[8] there is a lack of potent p300 HAT inhibitors at present, the development of new palmatine and berberine derivatives would be an impetus to discover more potent p300 HAT inhibitors and to develop potential new agents for the treatment of associated diseases.

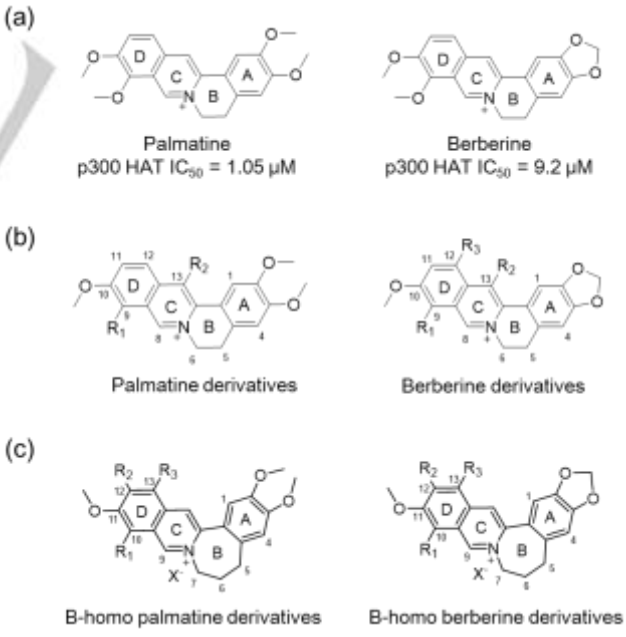

Figure 1. (a) Chemical structure of palmatine and berberine and their potency to p300 HAT. (b) The representative reported palmatine and berberine derivatives. (c) The B-homo palmatine and berberine derivatives presented in this work.

The previous studies mainly focused on modifications of the peripheral functional groups of palmatine and berberine, which led to various substituted derivatives (Figure 1b).[9] We are more interested in modifying the core tetracycle of palmatine and berberine to obtain

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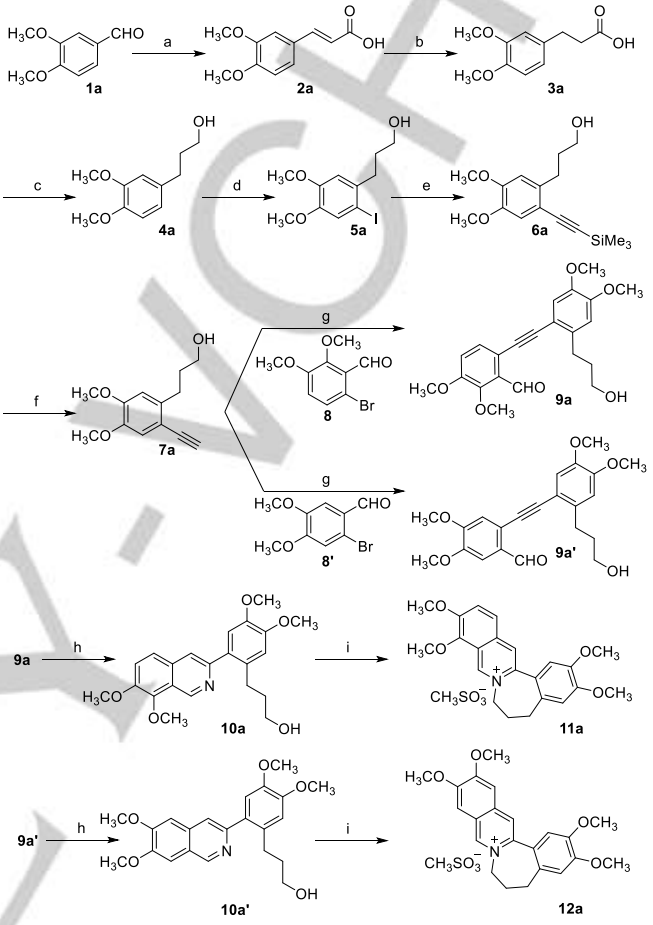
novel derivatives, such as the B-homo palmatine and berberine derivatives (Figure 1c), with aim to expand the diversity of naturally derived chemical scaffolds; to the best of our knowledge, there are no reports of such tetracycle-modified derivatives whose synthesis would be a challenge for us. Molecular docking analyses (for details see Experimental section) indicated that the B-homo palmatine and berberine derivatives are likely to have hydrogen-bonding, electrostatic and hydrophobic interactions with the catalytically important residues, including Arg1410, Thr1411, Trp1466, Tyr1467, Asp1399, and Pro1458 (Figure S1 in Supplementary Information), which is similar as that observed for palmatine.[6] Therefore, the establishment of a feasible synthetic route for the B-homo palmatine and berberine derivatives could not only expand the diversity of palmatine and berberine derivatives but also help identify new potent p300 HAT inhibitors. We here report first total synthesis of new B-homo palmatine and berberine derivatives, and *in vitro* evaluation of their activity against the p300 HAT.

Results and Discussion

Total Synthesis of B-homo Palmatine Derivatives

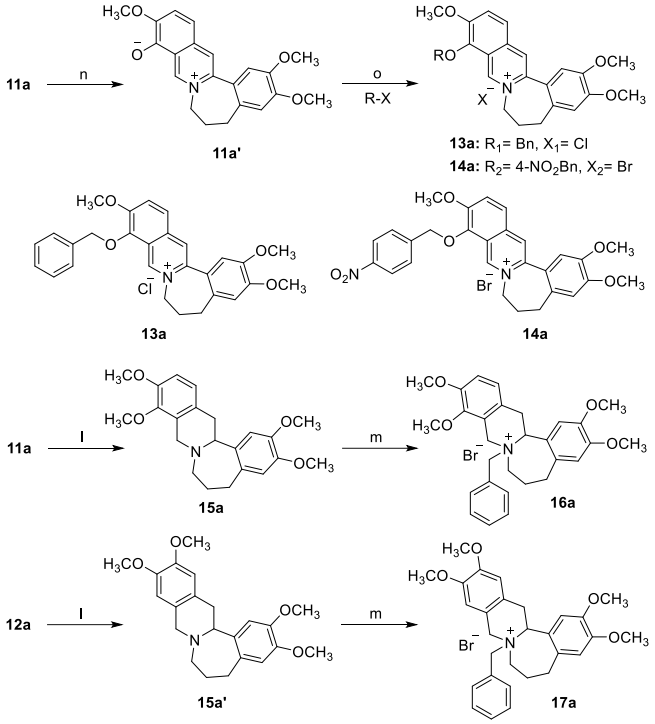
The synthetic routes for new B-homo palmatine derivatives are described in Schemes 1-2. Briefly, 3,4-dimethoxybenzaldehyde (**1a**) was used as the starting material to prepare the intermediates **2a** and **3a** according to the reported procedures [10] (Scheme 1). Then, reduction of **3a** using $NaBH_4$ gave the alcohol **4a** in 88.6% yield. The alcohol **4a** was converted to the intermediate **5a** into the presence of I_2 and CF_3COOAg according to the literature procedure.[11] Sonogashira reaction of **5a** with trimethylsilyacetylene (TMSA) in presence of triethylamine with $Pd(PPh_3)_2Cl_2$ and CuI afforded the intermediate **6a** (Scheme 1). Then, **6a** was treated with tetrabutylammonium fluoride trihydrate (TBAF) to remove the TMS group, leading to the C-C coupling product **7a** (Scheme 1). Further Sonogashira reaction of **7a** with **8** in presence of triethylamine with $Pd(PPh_3)_2Cl_2$ and CuI afforded the intermediate **9a** (Scheme 1). The cycloaddition of alkyene **9a** and ammonium acetete with $AgNO_3$ provided the 3-phenylisoquinoline derivative **10a** (Scheme 1).[12]

It is worthy of note that the cyclization reaction of **10a** was one of the most important step in this synthetic routes. Initially, we have tried out the cyclization reaction of **10a** in the presence of triphenylphosphine in DCM/CCl_4 (V/V = 2/1) [12] and attempted to synthesize **11a** with **10a** in the presence of dichlorosulfoxide in acetonitrile [9a], but unfortunately, we failed to get intermediate **11a**. Finally, the cyclization reaction of **10a** with methanesulphonyl chloride gave the B-homo palmatine **11a**. Together, this synthetic route for **11a** was achieved in nine steps in 13.8% over yield (Scheme 1, Table S1 in Supporting Information). The B-homo palmatine derivative **12a** was synthesized from compound **7a** and **8'** using the similar conditions (g-i) as that for the preparation of **11a** (Scheme 1). The total yield of **12a** was 13.6% in nine steps (Table S1 in Supporting Information).



Scheme 1. Synthetic routes for B-homo palmatine derivatives **11a** and **12a**. (a) $CH_2(COOH)_2$, pyridine, piperidine, 115 ℃, 2h, 75.5%; (b) H_2, 10% Pd/C, rt, 16 h, 78.7%; (c) $NaBH_4$, I_2, THF, 0-60 ℃, 10h 88.6%; (d) I_2, CF_3COOAg, $CHCl_3$, rt, 0.5 h, 90.0%; (e) TMSA, $PdCl_2(PPh_3)_2$, CuI, Et_3N, 50 ℃, 5 h; (f) TBAF, THF, rt, 0.5 h, 88.7% (total yield of steps e and f); (g) $PdCl_2(PPh_3)_2$, CuI, Et_3N, 70 ℃, 3 h, 62.9% for **9a** (65.3% for **9a'**); (h) NH_4OAc, $AgNO_3$, *t*-BuOH, rt, 15 h, 61.1% for **10a** (59.5% for **10a'**); (i) CH_3SO_2Cl, Et_3N, CH_2Cl_2, 0 - 50 ℃, 5 h, 85.2% for **11a** (83.4% for **12a**).

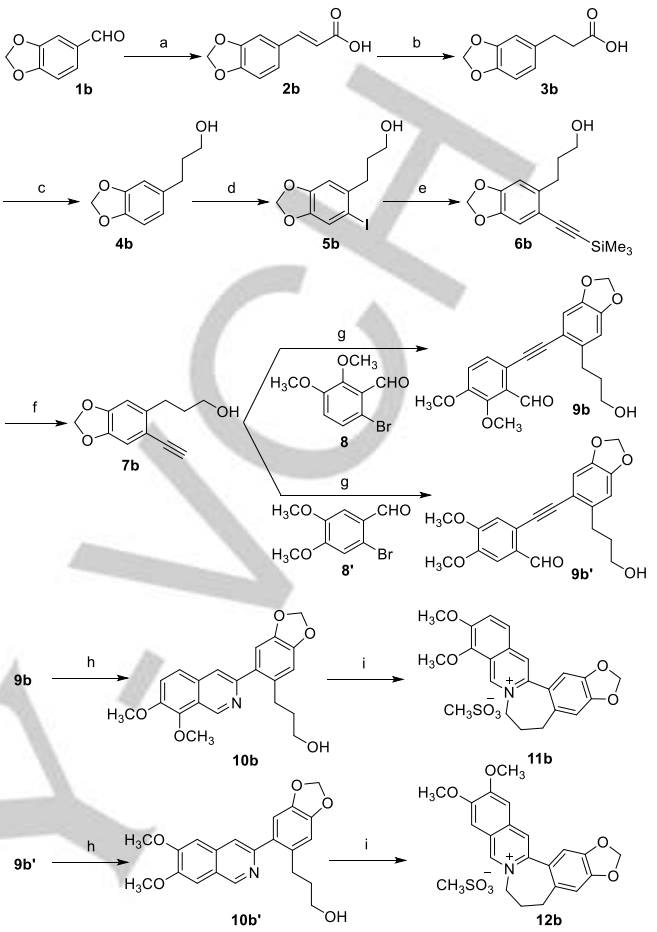
Four other B-homo palmatine derivatives (**13a**, **14a**, **16a**, and **17a**) were further synthesized as shown in Scheme 2. Compounds **13a** and **14a** were synthesized by the vacuum reaction of **11a**, followed by the etherification of **11a'** by treatment with alkyl halides. Compound **16a** was synthesized by $NaBH_4$ reduction of **11a**, followed by the reaction of **15a** with (bromomethyl)benzene. Compound **17a** was prepared from **12a** using the identical conditions (l, m) as that for **16a**. The overall yields for these prepared palmatine derivatives are summarized in Table S1 (see Supplementary Information).



Scheme 2. Synthetic routes for B-homo palmatine derivatives **13a**, **14a**, **16a**, and **17a**. (n) 180 ℃, in vacuum, 2 h, 90.6%; (o) alkyl halides (R-X), CH_3CN, 85 ℃, 3 h, 20.7% for **13a** (18.2% for **14a**); (l) $NaBH_4$, K_2CO_3, CH_3OH, 0 - 65 ℃, 3 h; (m) BnBr, CH_3CN, 50 ℃, 3 h, total yield of steps l and m : 82.1% for **16a** (84.6% for **17a**).

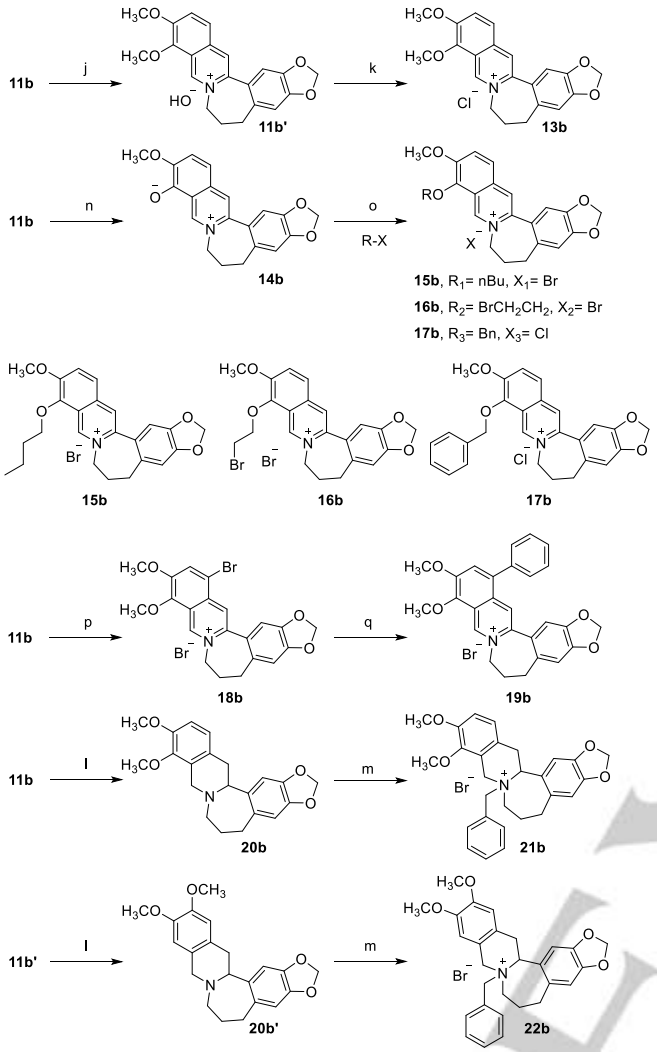
Total Synthesis of B-homo Berberine Derivatives

The synthesis of B-homo berberine derivatives is outlined in Schemes 3-4. The B-homo berberine **11b** and its derivative **12b** were prepared from the starting compound benzo[*d*][1,3]dioxole-5-carbaldehyde (**1b**) using the conditions (a-i) that for the synthesis of **11a** and **12a** (Scheme 3). **11b** and **12b** were obtained by nine steps in 16.9% and 15.2% over yield, respectively (Scheme 3, Table S1).



Scheme 3. Synthetic routes for B-homo berberine derivatives **11b** and **12b**. (a) $CH_2(COOH)_2$, pyridine, piperidine, 115 ℃, 2h, 77.2%; (b) H_2, 10% Pd/C, rt, 16 h, 74.3%; (c) $NaBH_4$, I_2, THF, 0-60 ℃, 10h; (d) I_2, CF_3COOAg, $CHCl_3$, rt, 0.5 h, 84.2% (total yield of steps c and d); (e) TMSA, $PdCl_2(PPh_3)_2$, CuI, Et_3N, 50 ℃, 5 h, 74.2%; (f) TBAF, THF, rt, 0.5 h, 98.3%; (g) $PdCl_2(PPh_3)_2$, CuI, Et_3N, 70 ℃, 3 h, 70.3% for **9b** (68.6% for **9b'**); (h) NH_4OAc, $AgNO_3$, *t*-BuOH, rt, 15 h, 78.5% for **10b** (74.1% for **10b'**); (i) CH_3SO_2Cl, Et_3N, CH_2Cl_2, 0 - 50 ℃, 5 h, 87.3% for **11b** (85.3% for **12b**).

Scheme 4 shows the synthetic routes for preparing berberine derivatives **13b**, **15b, 16b, 17b**, **19b**, **21b**, and **22b**. Compound **13b** was prepared by the reactions of **11b** with CaO, followed by the acidification of **11b'** by treatment with HCl in ethyl acetate.[13] Compounds **15b**, **16b**, and **17b** were synthesized by the vacuum reaction of **11b** at 180 ℃, followed by the etherification of **14b** by treatment with alkyl halides (Scheme 4). Compound **18b** was prepared by the halogenation of **11b** with Br_2, which was then converted to **19b** under Suzuki reaction conditions (Scheme 4). Compound **21b** was synthesized by $NaBH_4$ reduction of **11b**, followed by the reaction of **20b** with (bromomethyl)benzene. Similarly, compound **22b** was prepared from compound **12b** using the identical conditions (l, m) as for **21b**. The overall yields for these berberine derivatives are summerized in Table S1 (see Supplementary information).

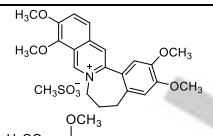
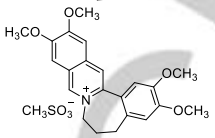
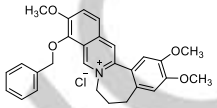
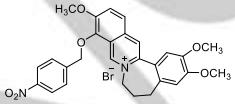
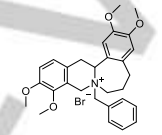
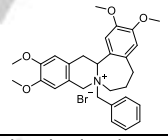


Scheme 4. Synthetic routes for berberine derivatives **13b**, **15b, 16b, 17b**, **19b**, **21b**, and **22b**. (j) CaO, CH_3OH/H_2O (pH = 9-10), rt - 60 ℃, 3 h; (k) HCl / ethyl acetate, DCM, rt, 1 h, total yield of steps j and k: 35.7% for **13b**; (n) 180 ℃, in vacuum, 2 h, 95.1%; (o) alkyl halides (R-X), CH_3CN, 85 ℃, 3 h for **15b** (23.2%), **16b** (21.5%), and **17b** (18.7%); (p) Br_2, CH_3COOH, 100 ℃, 8 h, 58.6%; (q) Na_2CO_3, $Pd(PPh_3)_4$, toluene, EtOH, H_2O, 100 ℃, 14 h, 20.2% for **19b**; (l) $NaBH_4$, K_2CO_3, CH_3OH, 0 - 65 ℃, 3 h; (m) BnBr, CH_3CN, 50 ℃, 3 h, total yield of steps l and m: 84.0% for **21b** (79.8% for **22b**).

Biological Evaluation of the B-homo Palmatine and Berberine Derivatives

The inhibitory activities (IC_{50}) of the B-homo palmatine and berberine derivatives to p300 HAT were tested using an isotope labelling method as described in Experimental section. The IC_{50} values, the calculated logP (clogP, predicted using the ALOGPS 2.1 program[14]) and ligand efficiency (LE) [15] values of the palmatine and berberine derivatives are in Table 1-2. We observed that of these derivatives, the molecular weights are less than 500, number of hydrogen-bond donors less than 5, number of hydrogen-bond acceptors less than 10, and the lipid-water partition coefficients (clogP) less than 5 (Table 1), partly reflecting that they are drug-like compounds.

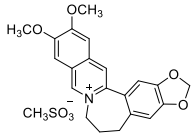
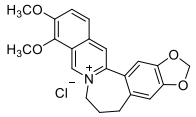
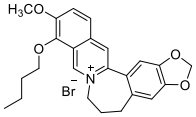
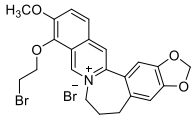
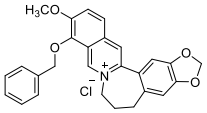
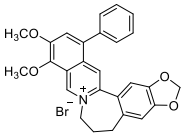
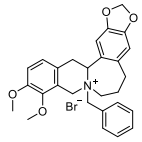
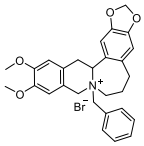
Table 1. The calculated logP values, the inhibitory activities (IC_{50}) and LE values to p300 HAT of the new B-homo palmatine derivatives.

Cpd	Chemical Structure	clogP[a]	p300 HAT IC_{50} (μM)	LE[b]
11a		0.58	4.5	0.28
12a		0.47	0.42	0.33
13a		1.91	4.7	0.23
14a		1.89	9.4	0.20
16a		4.33	4.7	0.22
17a		4.32	7.4	0.21

[a] logP was calculated using the ALOGPS 2.1 program [14] (http://www.vcclab.org/lab/alogps/; available on October 10, 2017).
[b] The ligand efficiency (LE) is calculated using the formula: LE = 1.4*(-logIC_{50})/N, where N is the number of non-hydrogen atoms.[15]

The inhibition assays revealed that **11a**, which has a heptatomic B-ring, displayed a slight lower inhibitory activity (IC_{50} =4.6 μM, Table 1) against p300 HAT than palmatine (IC_{50} = 1.05 μM), which has a hexatomic B-ring (Figure 1a); under the same assay conditions, C646, a known p300 HAT inhibitor,[16] showed IC_{50} of 2.6 μM. Small differences between the minimum energy conformations of **11a** and palmatine (particularly in their A- and B-ring) were observed (Figure S2 in Supplementary Information). Notably, shifting the methoxyl group from the R_1 position to the R_2 position led to compound **12a**, which has markedly increased inhibitory activity (IC_{50} = 0.42 μM, Table 1) against p300 HAT, comparing with **11a** (IC_{50} = 4.5 μM) and C646 (IC_{50} = 2.6 μM); the ligand efficicency (LE) of **12a** is 0.33. Compounds **13a** and **14a**, which bears aromatic substituents at the R_1 position, displayed comparable inhibitory activity to **11a** (IC_{50} = 4.5 μM, Table 1). Interestingly, **16a** and **17a**, which both have a benzyl moiety on the quaternary amine, displayed IC_{50} values of 4.7 μM and 7.4 μM, respectively, comparable to that of **11a** (IC_{50} = 4.5 μM, Table 1). The LE values of **13a**, **14a**, **16a**, and **17a** are less than that of **12a** (Table 1).

Table 2. The calculated logP values, the inhibitory activities (IC_{50}) and LE values to p300 HAT of the new B-homo berberine derivatives.

Cpd	Chemical Structure	clogP	p300 HAT IC_{50} (μM)	LE
12b		-0.01	1.8	0.31
13b		0.1	13.3	0.26
15b		1.15	6.7	0.25
16b		0.95	3.5	0.27
17b		1.44	2.5	0.25
19b		1.51	11	0.22
21b		4.03	34	0.19
22b		4.07	44	0.18

Similarly, comparing with berberine (IC_{50} = 9.2 μM), **13b** exhibited slight lower inhibitory activity against p300 HAT with an IC_{50} value of 13.3 μM (Table 2), which may due to the small difference in their minimum energy conformations (Figure S3 in Supplementary Information). Compound **12b**, with the methoxyl group at R_2 position, manifested IC_{50} of 1.8 μM (Table 2), similar with that observed for the palmatine derivative **12a** (IC_{50} = 0.42μM, Table 1). Compounds **15b**, **16b**, and **17b**, which have different substituents at R_1 position, showed better inhibitory activities than **13b** (Table 2), consistent with that observed for the palmatine derivatives (Table 1). Compounds **19b**, which has the benzene moiety at R_3 position, displayed IC_{50} value of 11 μM. Notably, **21b** and **22b** (Table 2), which have a benzyl moiety on the quaternary amine, showed lower inhibitory activity against p300 HAT than that of the corresponding palmatine derivatives **13a** and **12a** (Table 1). Taken together, these SAR studies revealed that the B-homo palmatine and berberine derivatives have micromolar inhibition against p300 HAT, among which **12a** manifests the most potent inhibitory activity (IC_{50} = 0.42 μM).

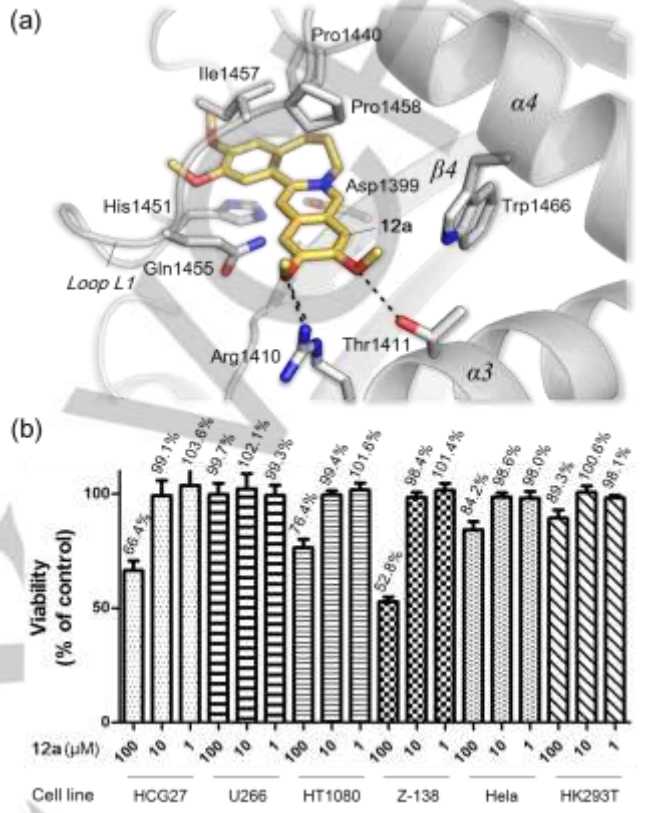


Figure 2. The possible binding mode of **12a** with p300 HAT and its cellular inhibitory activity against cancer cells. (a) The predicted binding mode of **12a** with p300 HAT. (b) Cellular inhibitory activity of **12a** against various cancer cell lines at different concentrations (100 μM, 10 μM, and 1 μM). The results revealed that **12a** has certain inhibitory activity to HCG27, HT1080, and Z138 cell lines at 100 μM, and no visible activity to other cancer cell lines.

We then performed molecular docking studies for **12a** to investigate its possible binding mode with p300 HAT. The docking results showed that **12a** is likely to form hydrogen-bonding interactions with Arg1410 (α3) and Thr1411 (α3), electrostatic interactions with Asp1399 (β4), π-π stacking interactions with His1451 (loop L1), and hydrophobic interactions with Gln1455 (loop L1), Ile1457 (loop L1), Pro1458 (loop L1), and Pro1440 (loop L1) (Figure 2a). Comparison of **12a** with its highly similar derivative **11a** reveals that they may bind in a similar manner to the p300 HAT domain, involving the catalytically important residues on loop L1, α3 and β4 (Figure S4 in Supplementary Information). Indeed, it is a big challenge to obtain p300:inhibitor complex crystal structures;[17] in future research, we will attempt crystallization for the complex of p300:**12a** using the strategy reported by Lasko *et al* [7] to confirm the

inhibition mode and provide structural basis for further inhibitor optimization.

We next tested the *in vitro* inhibitory activity of **12a** against several cancer cell lines, including HCG27 (gastric carcinoma), U266 (myeloma), HT1080 (epithelial, fibrosarcoma), Z-138 (mantle cell lymphoma), and Hela (adenocarcinoma), as well as the HEK293T (human embryonic kidney cells) for control. The results indicated that **12a** exhibited 33.6%, 23.6%, and 47.2% inhibition to the HCG27, HT1080, and Z-138 cell lines at 100 μM, respectively (Figure 2b); when treated with low concentrations of **12a**, no visible activity was observed for these cell lines (Figure 2b). We also observed that **12a** has no or low activity to other tested cancer cell lines as well as the normal HEK293T cells (Figure 2b). These results indicated that **12a** has low cytotoxicity, and it only acts against some types of cancer cells. For comparison, we tested the inhibitory activity of barberine and palmatine against Z-138 cell lines, which is the most sensitive tested cell lines to **12a**. We observed that barberine and palmatine displayed 32.5% and 21.2% inhibition to Z-138 at 100 μM, respectively, slightly less potent than **12a**. In general, the tested cancer cells are not so sensitive to **12a**, reflecting that p300 HAT may not be an addiction target to these cancer cells, for which one possible reason may be the CBP protein, the paralog of p300, compensating the acetyltransferase activity of p300 when inhibited by **12a**. Hence, p300 HAT inhibitors are possibly effective against CBP-deficient cancer cells as reported by Ogiwara *et al*.[18] Besides, one may speculate that the low inhibitory activity of **12a** in cells may be due to its chemical structure, since the presence of a quaternary amine may possibly decrease the cell membrane permeability (except being carrier-mediated).[19]

Conclusions

In conclusion, this work reported the first total synthesis of new B-homo palmatine and berberine derivatives, which involves nine to eleven steps with good overall yield (Table S1 in Supplementary Information). These synthetic routes will be useful in exploring structurally unique compounds, in particular the tetracycle-modified palmatine and berberine derivatives. The SAR studies revealed that these derivatives exhibit potent inhibition to p300 HAT, some of which (*e.g.* **12a**) are more potent than the known inhibitor C646, providing natural product-derived compounds for further structural optimization. Overall, this study will aid further efforts to explore structurally unique palmatine and berberine derivatives and to develop drug-like p300 HAT specific inhibitors.

Experimental Section

General Experimental Procedures

Unless otherwise noted, all reactions were carried out in oven dried glass flask under an air atmosphere. Solvents were purified and dried according to standard methods prior to use. All the reactions were monitored by thin-layer chromatography (TLC) and were visualized using UV light. The product purification was done using silica gel column chromatography. Thin layer chromatography (TLC) characterization was performed with precoated silica gel GF254 (0.2mm), while column chromatography characterization was performed with silica gel (100-200mesh). All new compounds were characterized by NMR spectroscopy, and melting point (if solids). ^{1}H NMR spectra were recorded at 400 or 600 MHz (Varian) and ^{13}C NMR spectra were recorded at 150 MHz (Varian). Chemical shifts are reported in ppm downfield from $CHCl_3$ (δ = 7.26 ppm) for ^{1}H NMR and relative to the central $CDCl_3$ resonance (δ = 77.0 ppm) for ^{13}C NMR spectroscopy. Then the signal due to residual DMSO appearing at δH 2.50 ppm and the central resonance of the DMSO - d_6 appearing at δC 39.5(2) ppm were used to reference ^{1}H and ^{13}C NMR spectra, respectively. Coupling constants are given in Hz. Chemical shifts (δ) were reported as parts per million (ppm) downfield from tetramethylsilane and the following abbreviations were used to identify the multiplicities: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad and all combinations thereof can be explained by their integral parts. LC-MS spectra were recorded on an Agilent 6400 series Triple Quadrupole LC-MS. Melting points (m.p.) were recorded on an INESA SGW X-4 melting point apparatus. Commercial reagent were purchased from Adamas Reagent Co., Ltd., Best reagent and Astatech Chemical Technology Co., Ltd. etc. All reagents were directly used without further purification. High-resolution mass spectra were recorded using the EI method with a double focusing magnetic mass analyzer. Unless otherwise noted, all products are isolated yields. The ^{1}H and ^{13}C NMR spectra of the prepared berberine derivatives are given in the Supplementary information.

Synthesis of palmatine derivatives

*3-(3,4-Dimethoxyphenyl)acrylic acid (**2a**)*

The compound **2a** was prepared according to a procedure of Manral *et al* [10a]. 18.8 g (180.6 mmol) of malonic acid was added to stirred solution of the 3,4-dimethoxybenzaldehyde **1a** (90.3 mmol) in pyridine (150 mL) and piperidine (9.0 mL). The mixture was heated to temperature of 115 ℃ for 2 h under reflux. After cooling, the mixture was poured into cold water and acidified with 2 mol*L^{-1} hydrochloric acid in an ice bath. The precipitated pale pink crystals **2a** were filtered, washed with cooled water and dried at 45 ℃, which was used in the next step without further purification. **2a**: yield 75.5%, pale pink solid, m.p. = 181 - 183 ℃.

*3-(3,4-Dimethoxyphenyl)propanoic acid (**3a**)*

The compound **3a** was prepared according to a modified procedure of Haadsma-Svensson *et al* [10b]. In a heat gun dried 250 mL Schlenk flask, equipped with a balloon with hydrogen, was placed 10% Pd/C (1.5 g, 10 mol%) under hydrogen atmosphere. A warm ethanol solution of dimethoxyphenylpropenoic acid **2a** (14.0 g, 67.2 mmol) was added and the mixture was stirred at rt for 16 h and then filtered over Celite. The solvent was removed under reduced pressure to afford compound **3a** (11.1 g) as a white solid, which was used without further purification in the next step. **3a**: yield 78.7%, white solid, m.p. = 95 - 97 ℃.

*3-(3,4-Dimethoxyphenyl)propan-1-ol (**4a**)*

$NaBH_4$ (5.9 g, 157.0 mmol) was added to compound **3a** (11.0 g, 52.3 mmol) in THF (80 mL) portionwise at room temperature. Then a solution of I_2 (13.3 g, 52.3 mmol) dissolved in THF (40 mL) was added dropwise over 30 min. After the addition of I_2 was completed and gas evolution had ceased, the reaction was heated to 60 ℃ and stirred for another 10 h. After the reaction was completed as monitored by TLC, methanol (30 mL) was added cautiously at 0 ℃.

Then the mixture was pour to H_2O (200 mL), extracted with DCM (80 mL×3). The organic layers were combined, washed with brine, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (petroleum ether/ethyl acetate = 12:1) to give compound **4a** as a pale yellow oil. **4a**: yield 88.6 %, pale yellow oil; ^{1}H NMR (400 MHz, DMSO-d_6) δ 6.82 (d, *J* = 8.0 Hz, 1H), 6.77 (d, *J* = 2.0 Hz, 1H), 6.67 (dd, *J* = 8.0, 2.0 Hz, 1H), 4.44 (t, *J* = 5.2 Hz, 1H), 3.72 (s, 3H), 3.69 (s, 3H), 3.43 – 3.36 (m, 2H), 2.56 – 2.49 (m, 2H), 1.72 – 1.63 (m, 2H).

*3-(2-Iodo-4,5-dimethoxyphenyl)propan-1-ol (**5a**)*

Compound **5a** was prepared according to the general procedure of He *et al* [11]. Compound **4a** (17.0 g, 86.6 mmol), I_2 (29.2 g, 113.9 mmol), CF_3COOAg (27.7 g, 123.5 mmol) were added in $CHCl_3$ (200 mL) stirred at room temperature for 30 min. Then the solvent was removed under reduced pressure, residue was purified by silica gel column chromatography (petroleum ether/acetone = 12:1) afforded compound **5a** (25.1 g) as a pale yellow solid. **5a**: yield 90.0%, Pale yellow solid, m.p. = 106 - 108 ℃; ^{1}H NMR (600 MHz, DMSO-d_6) δ 7.23 (s, 1H), 6.90 (s, 1H), 4.51 (t, *J* = 5.4 Hz, 1H), 3.74 (s, 3H), 3.72 (s, 3H), 3.46 – 3.41 (m, 2H), 2.63 – 2.59 (m, 2H), 1.69 – 1.61 (m, 2H).

*3-(4,5-Dimethoxy-2-((trimethylsilyl)ethynyl)phenyl)propan-1-ol (**6a**)*

Compound **5a** (6.6 g, 20.5 mmol), trimethylsilylacetylene (4.0 mL, 30.7 mmol), $PdCl_2(PPh_3)_2$ (230.2 mg, 0.33 mmol), CuI (42.9 mg, 0.226 mmol) was added in 100 mL Et_3N at 50 ℃ for 5 h. Then the solvent was removed under reduced pressure and the residue was diluted with EtOAc (30 mL) and water (15 mL). The organic layer was separated and the aqueous layer was washed two times with EtOAc (20 mL). The combined organic layer was dried over Na_2SO_4 and concentrated under reduced pressure to afford compound **6a**. **6a** was used without further purification in the next step. **6a**: Dark brown oil.

*3-(2-Ethynyl-4,5-dimethoxyphenyl)propan-1-ol (**7a**)*

Compound **6a** (7 g, 21.7 mmol) and TBAF (567 mg, 2.2 mmol) was added in THF (150 mL) at room temperature for 30 min. Then the solvent was removed under reduced pressure, the residue was purified by silica gel flash column chromatography (petroleum ether/acetone = 8:1) to afforded compound **7a** (4.3 g) as a yellowish white solid. **7a**: yield 88.7%, yellowish white solid, m.p. = 80 – 82 ℃; ^{1}H NMR (400 MHz, DMSO-d_6) δ 6.94 (s, 1H), 6.83 (s, 1H), 4.49 (br s, 1H), 4.16 (s, 1H), 3.76 (s, 3H), 3.72 (s, 3H), 3.42 (t, *J* = 6.4 Hz, 2H), 2.68 (t, *J* = 7.6 Hz, 2H), 1.70 (p, *J* = 7.6 Hz, 2H).^{13}C NMR (100 MHz, DMSO-d_6) δ 149.86, 146.99, 138.46, 115.54, 112.88, 112.78, 82.94, 82.64, 60.81, 56.05, 55.93, 34.20, 30.57.

*6-((2-(3-Hydroxypropyl)-4,5-dimethoxyphenyl)ethynyl)-2,3-dimethoxybenzaldehyde (**9a**)*

The compound **9a** was prepared according to a procedure of Reddy *et al* [12].Triethylamine (25 mL) was added to a mixture of $PdCl_2(PPh_3)_2$ (80.0 mg, 0.12 mmol), CuI (10.4 mg, 0.054 mmol), **7a** (1.0 g, 4.5 mmol) and 6-bromo-2,3-dimethoxybenzaldehyde **8** (1.2 g, 4.9 mmol) under an inert atmosphere. The reaction mixture was heated to 70 ℃ for 3 h. After the completion of the reaction (monitored by TLC), Et_3N was removed under reduced pressure. The residue was then diluted with EtOAc (50 mL) and water (25 mL). The organic layer was separated and the aqueous layer was washed two times with EtOAc (50 mL). The combined organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (petroleum ether/ethyl acetate = 3:1) to obtain pure **9a**. **9a**: Yield 62.9%, yellow oil; ^{1}H NMR (400 MHz, Chloroform-*d*) δ 10.56 (s, 1H), 7.37 (d, *J* = 8.4 Hz, 1H), 7.10 (d, *J* = 8.4 Hz, 1H), 7.03 (s, 1H), 6.75 (s, 1H), 3.95 (s, 3H), 3.93 (s, 3H), 3.91 - 3.89 (m, 6H), 3.73 (t, *J* = 6.0 Hz, 2H), 3.06 – 2.96 (m, 2H), 2.28 (br s, 1H), 2.02 – 1.92 (m, 2H).^{13}C NMR (100 MHz, Chloroform-*d*) δ 190.68, 152.90, 152.00, 149.80, 146.95, 138.30, 130.17, 129.76, 117.16, 116.21, 114.83, 114.24, 111.88, 92.41, 89.32, 62.35, 56.22, 56.12, 55.98, 34.34, 30.79.

*2-((2-(3-Hydroxypropyl)-4,5-dimethoxyphenyl)ethynyl)-4,5-dimethoxybenzaldehyde (**9a'**)*

The compound **9a'** was prepared according to the procedure of compound **9a** with **7a** and 2-bromo-4,5-dimethoxybenzaldehyde **8'**. **9a'**: Yield 65.3%, Pale yellow solid, m.p. = 148 – 149 ℃; ^{1}H NMR

(400 MHz, Chloroform-*d*) δ 10.50 (s, 1H), 7.40 (s, 1H), 7.07 (s, 1H), 7.01 (s, 1H), 6.77 (s, 1H), 3.99 (s, 3H), 3.95 (s, 3H), 3.91 (s, 3H), 3.90 (s, 3H), 3.72 (t, *J* = 6.0 Hz, 2H), 2.96 – 2.88 (m, 2H), 1.97 – 1.92 (m, 2H).^{13}C NMR (100 MHz, Chloroform-*d*) δ 190.73, 153.83, 150.01, 149.57, 147.07, 138.07, 129.80, 122.18, 114.54, 114.29, 113.44, 112.03, 108.36, 94.25, 87.23, 62.18, 56.42, 56.21, 56.14, 56.01, 34.11, 31.00.

*3-(2-(7,8-Dimethoxyisoquinolin-3-yl)-4,5-dimethoxyphenyl)propan-1-ol (**10a**)*

The compound **10a** was prepared according to a procedure of Reddy *et al* [12]. ^{t}BuOH (100 mL) was added to a mixture of $AgNO_3$ (60.3 mg, 0.36 mmol), ammonium acetate (410.0 mg, 5.3 mmol) and **9a** (1.4g, 3.6 mmol) under an inert atmosphere. The resulting mixture was stirred at room temperature for 10h and the reaction was monitored by TLC. After completion, the reaction was quenched by the addition of $NaHCO_3$ (1.17 g, 13.9 mmol) at room temperature and stirring was continued for additional 5 h. The mixture was then filtered through a cotton plug, washed with EtOAc (50 mL) and dried over anhydrous Na_2SO_4. The filtrate was evaporated under reduced pressure and the residue was purified through silica gel column chromatography (petroleum ether/ethyl acetate = 1:1) to obtain the pure isoquinoline **10a** (796 mg). **10a**: Yield: 61.1%, pale yellow solid, m.p. = 109 – 110 ℃; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.43 (s, 1H), 7.83 (s, 1H), 7.77 (d, *J* = 9.2 Hz, 1H), 7.74 (d, *J* = 9.2 Hz, 1H), 7.01 (s, 1H), 6.91 (s, 1H), 4.50 (t, *J* = 5.2 Hz, 1H), 4.00 (s, 3H), 3.98 (s, 3H), 3.82 (s, 3H), 3.77 (s, 3H), 3.33 – 3.26 (m, 2H), 2.75 – 2.66 (m, 2H), 1.68 – 1.58 (m, 2H).^{13}C NMR (101 MHz, DMSO-d_6) δ 151.12, 148.54, 148.42, 146.57, 145.56, 142.66, 132.70, 132.19, 131.57, 123.09, 121.87, 120.80, 119.26, 113.94, 113.22, 61.31, 60.37, 56.87, 55.60, 48.51, 34.41, 30.14.

*3-(2-(6,7-Dimethoxyisoquinolin-3-yl)-4,5-dimethoxyphenyl)propan-1-ol (**10a'**)*

The compound **10a'** was prepared according to the procedure of compound **10a**. **10a'**: yield 59.5%, Yellow oil, ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.09 (s, 1H), 7.71 (s, 1H), 7.50 (s, 1H), 7.37 (s, 1H), 6.97 (s, 1H), 6.89 (s, 1H), 4.55 (t, *J* = 4.8 Hz, 1H), 3.93 (s, 6H), 3.81 (s, 3H), 3.76 (s, 3H), 3.31 – 3.24 (m, 2H), 2.77 – 2.64 (m, 2H), 1.65 – 1.55 (m, 2H).^{13}C NMR (100 MHz, DMSO-d_6) δ 152.93, 151.70, 150.04, 148.30, 146.53, 132.66, 132.64, 132.60, 122.83, 118.72, 118.69, 113.87, 113.22, 105.40, 105.01, 60.34, 55.77, 55.68, 55.57, 48.51, 34.36, 30.15.

*2,3,10,11-Tetramethoxy-6,7-dihydro-5H-benzo[3,4]azepino[1,2-b]isoquinolin-8-ium methanesulfonate (**11a**)*

Methanesulphonyl chloride (322 µl, 4.2 mmol) was added into the solution of **10a** (796 mg, 2.1 mmol) and triethylamine (865 µl, 6.3 mmol) in 100 mL CH_2Cl_2 at 0 °C. The resulting mixture was allowed to warm to 50 °C for 5 h. After the reaction was completed as monitored by TLC, the mixture was diluted with 20 mL CH_2Cl_2. The CH_2Cl_2 layer was washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (CH_2Cl_2/MeOH = 30:1) to give compound **11a** (826 mg) as a yellow solid. **11a**: yield 85.2%, yellow solid, m.p. = 168 - 170 ℃ (decomposed); ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.08 (s, 1H), 8.63 (s, 1H), 8.26 (d, *J* = 8.8 Hz, 1H), 8.10 (d, *J* = 8.8 Hz, 1H), 7.31 (s, 1H), 7.11 (s, 1H), 5.05 (br s, 1H), 4,25 – 4.17 (br s, 1H), 4.12 (d, *J* = 1.7 Hz, 3H), 4.09 (s, 3H), 3.87 (s, 6H), 2.76 (br s, 1H), 2.66 (br s, 1H), 2.50 (br s, 1H), 2.32 (br s, 1H).^{13}C NMR (100 MHz, DMSO-d_6) δ 150.95, 150.59, 147.85, 145.82, 143.77, 143.67, 132.48, 131.38, 126.67, 125.44, 123.60, 123.21, 122.29, 113.48, 112.62, 61.98, 57.39, 57.15, 56.02, 55.84, 31.37, 27.76. LC-MS (ESI): *m/z* calculated for $C_{22}H_{24}NO_4^+$ [M-$CH_3SO_3^-$]$^+$: 366.2, found: 366.2.

*2,3,11,12-Tetramethoxy-6,7-dihydro-5H-benzo[3,4]azepino[1,2-b]isoquinolin-8-ium methanesulfonate (**12a**)*

The compound **12a** was prepared according to the procedure for preparing compound **11a**. **12a**: yield 83.4%, pale yellow solid, m.p. = 122 – 123 ℃; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.76 (s, 1H), 8.41 (s, 1H), 7.75 (s, 1H), 7.74 (s, 1H), 7.29 (s, 1H), 7.11 (s, 1H), 4.73 (br s, 1H), 4.22 (br s, 1H), 4.07 (s, 3H), 4.02 (s, 3H), 3.87 (s, 6H), 2.77 (br s, 1H), 2.60 (br s, 1H), 2.50 (br s, 1H), 2.33 (br s, 1H).^{13}C NMR (100 MHz, Methanol-*d4*) δ 160.02, 154.71, 153.19, 150.04, 146.57, 146.26, 138.48, 132.98, 125.01, 124.92, 124.68, 114.40, 113.63, 107.46, 106.45, 58.34, 57.53, 57.11, 56.94, 56.66, 33.09, 29.40. LC-MS (ESI): *m/z* calculated for $C_{22}H_{24}NO_4^+$ [M-$CH_3SO_3^-$]$^+$: 366.2, found: 366.2.

*2,3,11-Trimethoxy-6,7-dihydro-5H-benzo[3,4]azepino[1,2-b]isoquinolin-8-ium-10-olate (**11a'**)*

Compound **11a'** (268 mg, 0.58 mmol) was placed in the round bottom flask and heated at 180 °C in vacuum for 2 h to give the crude product, which was purified by silica gel column chromatography (CH_2Cl_2/MeOH = 10:1) to give the compound **11a'** as a dark red solid (184 mg). **11a'**: yield 90.6%, Dark red solid, m.p. > 200 ℃; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.60 (s, 1H), 7.97 (s, 1H), 7.64 (d, *J* = 8.1 Hz, 1H), 7.22 (s, 1H), 7.11 – 6.91 (m, 2H), 4.74 (br s, 1H), 4.15 – 4.00 (m, 1H), 3.95 – 3.80 (m, 9H), 2.73 (br s, 1H), 2.60 (br s, 1H), 2.39 (br s, 1H), 2.17 (br s, 1H).

*10-(Benzyloxy)-2,3,11-trimethoxy-6,7-dihydro-5H-benzo[3,4] azepino[1,2-b]isoquinolin-8-ium chloride (**13a**)*

(Chloromethyl)benzene (360.8 mg, 2.85 mmol) was added to compound **11a'** (200 mg, 0.57 mmol) in 10 mL CH_3CN at room temperature. The resulting mixture was refluxed at 85 °C for 3 h. After the reaction was completed as monitored by TLC, then the mixture was concentrated under reduced pressure. The residue was

purified by neutral Al_2O_3 column chromatography (dichloromethane /CH_3OH = 100:1) to give compound **13a** (56.4 mg) as a yellow solid. **13a**: yield 20.7%, yellow solid, m.p. = 78 – 79 ℃; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.93 (s, 1H), 8.63 (s, 1H), 8.27 (d, *J* = 8.8 Hz, 1H), 8.12 (d, *J* = 8.8 Hz, 1H), 7.58 (d, *J* = 7.2 Hz, 2H), 7.50 – 7.23 (m, 4H), 7.10 (s, 1H), 5.39 (s, 2H), 5.06 (br s, 1H), 4.35 – 4.20 (m, 1H), 4.12 (s, 3H), 3.87 (s, 6H), 2.77 (br s, 1H), 2.62 (br s, 1H), 2.46 – 2.20 (m, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 151.03, 150.91, 147.83, 145.61, 143.44, 141.85, 136.28, 132.29, 131.22, 129.15, 128.47, 128.30, 126.36, 125.47, 123.94, 123.11, 122.88, 113.44, 112.59, 75.28, 57.39, 57.10, 56.01, 55.82, 31.42, 27.71. LC-MS (ESI): *m/z* calculated for $C_{28}H_{28}NO_4^+$ [M-Cl$^-$]$^+$: 442.2, found: 442.1.

*2,3,11-Trimethoxy-10-((4-nitrobenzyl)oxy)-6,7-dihydro-5H-benzo[3,4]azepino[1,2-b]isoquinolin-8-ium bromide (**14a**)*

1-(Bromomethyl)-4-nitrobenzene (616 mg, 2.85 mmol) was added to compound **11a'** (200 mg, 0.57 mmol) in 10 mL CH_3CN at room temperature. The resulting mixture was refluxed at 85 °C for 3 h. After the reaction was completed as monitored by TLC, then the mixture was concentrated under reduced pressure. The residue was purified by neutral Al_2O_3 column chromatography (dichloromethane /CH_3OH = 100:1) to give compound **14a** (54.3 mg) as a yellow solid. **14a**: yield 18.2%, yellow solid, m.p. = 185 – 186 ℃, ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.04 (s, 1H), 8.65 (s, 1H), 8.28 (d, *J* = 8.4 Hz, 3H), 8.15 (d, *J* = 8.4 Hz, 1H), 7.91 (d, *J* = 8.4 Hz, 2H), 7.32 (s, 1H), 7.11 (s, 1H), 5.53 (s, 2H), 5.06 (br s, 1H), 4.20 (br s, 1H), 4.09 (s, 3H), 3.92 – 3.82 (m, 6H), 2.77 (s, 1H), 2.67 (s, 1H), 2.46 (s, 1H), 2.33 (s, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 150.97, 150.75, 147.86, 147.26, 145.63, 144.40, 143.72, 141.74, 132.42, 131.36, 129.39, 126.40, 125.54, 124.22, 123.52, 123.14, 122.54, 113.46, 112.62, 74.05, 57.53, 57.17, 56.03, 55.86, 31.44, 27.75. LC-MS (ESI): *m/z* calculated for $C_{28}H_{27}N_2O_6^+$ [M-Br$^-$]$^+$: 487.2, found: 487.2.

*2,3,10,11-Tetramethoxy-5,6,7,9,14,14a-hexahydrobenzo[3,4] azepino[1,2-b]isoquinoline (**15a**)*

$NaBH_4$ (74.1 mg, 1.96 mmol) was added to compound **11a** (226 mg, 0.49 mmol) and K_2CO_3 (62 mg, 0.49 mmol) in CH_3OH (10 mL) portionwise at 0 °C. The resulting mixture was refluxed at 65 °C for 3 h. After the reaction was completed as monitored by TLC, then the mixture was concentrated and pour to H_2O (10 mL), extracted with DCM (15 mL×3). The organic layers were combined, washed with brine, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure to give compound **15a** (221 mg) as pale yellow oil which was used in the next step without further purification.

*2,3,11,12-Tetramethoxy-5,6,7,9,14,14a-hexahydrobenzo[3,4] azepino[1,2-b]isoquinoline (**15a'**)*

$NaBH_4$ (74.1 mg, 1.96 mmol) was added to compound **12a** (226 mg, 0.49 mmol) and K_2CO_3 (62 mg, 0.49 mmol) in CH_3OH (10 mL) portionwise at 0 °C. The resulting mixture was refluxed at 65 °C for 3 h. After the reaction was completed as monitored by TLC, then the mixture was concentrated and pour to H_2O (10 mL), extracted with DCM (15 mL×3). The organic layers were combined, washed with brine, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure to give compound **15a'** (208 mg) as pale yellow oil which was used in the next step without further purification.

*8-Benzyl-2,3,10,11-tetramethoxy-6,7,8,9,14,14a-hexahydro-5H-benzo[3,4]azepino[1,2-b]isoquinolin-8-ium bromide (**16a**)*

Benzyl bromide (484 mg, 2.83 mmol) was added to compound **15a** (221 mg) in 10 mL CH_3CN at room temperature. The resulting mixture was stirred at 50 °C for 3 h. After the reaction was completed as monitored by TLC, then the mixture was concentrated under reduced pressure. The residue was purified by silica gel column chromatography (dichloromethane /CH_3OH = 50:1) to give and to give compound **16a** (218.2 mg) as a cream white solid. **16a**: yield 82.1%, cream white solid, m.p. = 152 – 154 ℃; ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.66 (d, *J* = 6.8 Hz, 2H), 7.58 – 7.45 (m, 3H), 7.25 (s, 1H), 7.11 (d, *J* = 8.8 Hz, 1H), 7.07 – 6.94 (m, 2H), 5.46 (dd, *J* = 13.2, 6.8 Hz, 1H), 5.23 (d, *J* = 15.2 Hz, 1H), 4.88 (d, *J* = 13.2 Hz, 1H), 4.56 (d, *J* = 13.2 Hz, 1H), 3.94 (d, *J* = 15.2 Hz, 2H), 3.86 – 3.73 (m, 9H), 3.71 – 3.64 (m, 1H), 3.61 (s, 3H), 3.49 – 3.41 (m, 1H), 3.30 – 3.16 (m, 2H), 2.94 – 2.82 (m, 1H), 2.67 – 2.52 (m, 1H), 2.06 (d, *J* = 13.2 Hz, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 150.66, 148.98, 146.88, 144.87, 133.49, 132.86, 130.38, 128.96, 127.69, 124.07, 123.71, 123.07, 120.42, 115.99, 115.22, 113.41, 73.58, 61.57, 60.00, 59.42, 55.98, 55.72, 55.66, 54.94, 32.06, 28.94, 22.82. LC-MS (ESI): *m/z* calculated for $C_{29}H_{34}NO_4^+$ [M-Br$^-$]$^+$: 460.2, found: 460.1.

*8-Benzyl-2,3,11,12-tetramethoxy-6,7,8,9,14,14a-hexahydro-5H-benzo[3,4]azepino[1,2-b]isoquinolin-8-ium bromide (**17a**)*

The compound **17a** was prepared according to the procedure of compound **16a**. **17a**: yield 84.6%, cream white solid, m.p. = 219 – 221 ℃; ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.72 – 7.59 (m, 2H), 7.56 – 7.48 (m, 3H), 7.30 (s, 1H), 7.00 (s, 1H), 6.87 (s, 1H), 6.78 (s, 1H), 5.50 (dd, *J* = 13.2, 4.8 Hz, 1H), 5.21 (d, *J* = 14.8 Hz, 1H), 4.84 (d, *J* = 13.2 Hz, 1H), 4.50 (d, *J* = 13.2 Hz, 1H), 3.87 (d, *J* = 14.8 Hz, 2H), 3.81 (s, 3H), 3.79 (s, 3H), 3.74 (s, 3H), 3.73 – 3.71 (m, 1H), 3.69 (s, 3H), 3.46 – 3.39 (m, 1H), 3.26 – 3.14 (m, 2H), 2.96 – 2.84 (m, 1H), 2.66 – 2.52 (m, 1H), 2.09 (d, *J* = 14.8 Hz, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 148.91, 148.61, 147.98, 146.87, 133.43, 132.88, 130.21, 128.98, 127.68, 123.94, 122.34, 118.25, 116.04, 115.16, 111.21, 110.32, 73.99, 63.24, 61.23, 55.74, 55.63, 55.54, 54.39, 32.19, 29.62, 22.85. LC-MS (ESI): *m/z* calculated for $C_{29}H_{34}NO_4^+$ [M-Br$^-$]$^+$: 460.2, found: 460.1.

Synthesis of berberine derivatives

*3-(Benzo[d][1,3]dioxol-5-yl)acrylic acid (**2b**)*

The compound **2b** was prepared according to the literature procedure of Yang *et al* [20]. 16.6 g (159.9 mmol) of malonic acid was added to stirred solution of the benzo[d][1,3]dioxole-5-carbaldehyde **1b** (79.9 mmol) in pyridine (140 mL) and piperidine (7.2 mL). The mixture was heated to temperature of 115 ℃ for 2 h under reflux. After cooling, the mixture was poured into cold water and acidified with 2 mol*L^{-1} hydrochloric acid in an ice bath. The precipitated white crystals **2b** were filtered, washed with cooled water and dried at 45 ℃, which was used in the next step without further purification. **2b**: yield 77.2%, pale white solid, m.p. > 200 ℃.

*3-(Benzo[d][1,3]dioxol-5-yl)propanoic acid (**3b**)*

In a heat gun dried 250 mL Schlenk flask, equipped with a balloon with hydrogen, was placed 10% Pd/C (1.2 g, 10 mol%) under hydrogen atmosphere. A warm ethanolic solution of dimethoxyphenylpropenoic acid **2b** (12.0 g, 62.4 mmol) was added and the mixture was stirred at rt for 16 h and then filtered over Celite. The solvent was removed under reduced pressure to afford compound **3b** (9.0 g) as a white solid, which was used without further purification in the next step. **3b**: yield 74.3%, white solid, m.p. = 82 - 84 ℃.

*3-(Benzo[d][1,3]dioxol-5-yl)propan-1-ol (**4b**)*

$NaBH_4$ (7.58 g, 202 mmol) was added to compound **3b** (13.1 g, 67.6 mmol) in 80 mL THF portionwise at 0 ℃. Then a solution of I_2 (17.0 g, 67.6 mmol) dissolved in THF (180 mL) was added dropwise over 30 min. After the addition of I_2 was completed and gas evolution had ceased, the reaction was heated to 60 ℃ and stirred for another 4 h. After the reaction was completed as monitored by TLC, 26 mL methanol was added cautiously at 0 ℃. Then the mixture was pour to 200 mL H_2O, extracted with DCM (80 mL × 3). The organic layers were combined, washed with brine, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure to give compound (16.7 g) as yellow oil which was used in the next step without further purification. **4b**: yellow oil; ^{1}H NMR (400 MHz, Chloroform-*d*) δ 6.72 (d, *J* = 7.6 Hz, 1H), 6.69 (d, *J* = 2.4 Hz, 1H), 6.63 (dd, *J* = 7.6, 2.4 Hz, 1H), 5.90 (s, 2H), 3.63 (t, *J* = 6.4 Hz, 2H), 2.61 (t, *J* = 7.6 Hz, 2H), 2.51 (s, 1H), 1.88 – 1.78 (m, 2H).

*3-(6-Iodobenzo[d][1,3]dioxol-5-yl)propan-1-ol (**5b**)*

Compound **5b** was prepared according to the general procedure of He *et al* [11].Compound **4b** (12.3 g, 68.6 mmol), I_2 (21.1 g, 82.3 mmol), CF_3COOAg (20 g, 89.2 mmol) was added in $CHCl_3$ (200 mL) stirred at -5 °C to room temperature for 30 min. Then the solvent was removed under reduced pressure, the residue was purified by silica gel column chromatography (petroleum ether/acetone = 12:1) afforded compound (17.7 g) as a light yellow solid. **5b**: yield 84.2%, light yellow solid, m.p. = 28 - 30 ℃; ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.22 (s, 1H), 6.76 (s, 1H), 5.94 (s, 2H), 3.70 (t, *J* = 6.4 Hz, 2H), 2.77 – 2.70 (m, 2H), 1.93 (s, 1H), 1.86 – 1.77 (m, 2H).

*3-(6-((Trimethylsilyl)ethynyl)benzo[d][1,3]dioxol-5-yl)propan-1-ol (**6b**)*

Compound **5b** (4.24 g, 13.9 mmol), trimethylsilylacetylene (2.95 mL, 20.9 mmol), $PdCl_2(PPh_3)_2$ (155.5 mg, 0.22 mmol), CuI (29 mg, 0.15 mmol) was added in 35 mL Et_3N at 50 °C for 5 h. Then the solvent was removed under reduced pressure and the residue was diluted with EtOAc (30 mL) and water (15 mL). The organic layer was separated and the aqueous layer was washed two times with EtOAc (20 mL). The combined organic layer was dried over Na_2SO_4 and concentrated under reduced pressure. The crude product was purified by silica gel column chromatography (petroleum ether/EtOAc = 5:1) to afford compound **6b** as yellow oil (2.7 g). **6b**: yield 74.2%, yellow oil; ^{1}H NMR (400 MHz, Chloroform-*d*) δ 6.87 (s, 1H), 6.66 (s, 1H), 5.93 (s, 2H), 3.60 (t, *J* = 6.4 Hz, 2H), 2.81 (t, *J* = 7.2 Hz, 2H), 1.98 (s, 1H), 1.89 – 1.80 (m, 2H), 0.24 (s, 9H).

*3-(6-Ethynylbenzo[d][1,3]dioxol-5-yl)propan-1-ol (**7b**)*

Compound **6b** (2.23 g, 8.46 mmol) and TBAF (222 mg, 0.85 mmol) in THF (20 mL) at room temperature for 30 min. Then the solvent was removed under reduced pressure and the residue was diluted with EtOAc (30 mL) and 1M HCl (50 mL). The organic layer was separated and the aqueous layer was washed two times with EtOAc (20 mL). The combined organic layer was dried over Na_2SO_4 and concentrated under reduced pressure to afford compound **7b** (1.7g). **7b** was used without further purification in the next step. **7b**: yield 98.3%, yellow oil.

*6-((6-(3-Hydroxypropyl)benzo[d][1,3]dioxol-5-yl)ethynyl)-2,3-dimethoxybenzaldehyde (**9b**)*

The compound **9b** was prepared according to the procedure of compound **9a**. Triethylamine (25 mL) was added to a mixture of $PdCl_2(PPh_3)_2$ (98.3 mg, 0.14 mmol), CuI (13.3 mg, 0.07 mmol), **7b** (1.36 g, 5.6 mmol) and 6-bromo-2,3-dimethoxybenzaldehyde **8** (1.47 g, 7.2 mmol) under an inert atmosphere. The reaction mixture was heated to 70 ℃ for 3 h. After the completion of the reaction (monitored by TLC), Et_3N was removed under reduced pressure. The residue was then diluted with EtOAc (50 mL) and water (25 mL). The organic layer was separated and the aqueous layer was washed two times with EtOAc (50 mL). The combined organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (petroleum ether/ethyl acetate = 3:1) to obtain pure **9b**(1.43g). **9b**: Yield 70.3%, yellow oil; ^{1}H NMR (400 MHz, Chloroform-*d*) δ 10.53 (s, 1H), 7.32 (d, *J* = 8.4 Hz, 1H), 7.07 (d, *J* = 8.4 Hz, 1H), 6.96 (s, 1H), 6.71 (s, 1H), 5.94 (s, 2H), 3.94 (s, 3H), 3.90 (s, 3H), 3.71 (t, *J* = 6.0 Hz, 2H), 2.98 (t, *J* = 7.6 Hz, 2H), 2.63 (br s, 1H), 1.97 – 1.88 (m, 2H).

*2-((6-(3-Hydroxypropyl)benzo[d][1,3]dioxol-5-yl)ethynyl)-4,5-dimethoxybenzaldehyde (**9b'**)*

The compound **9b'** was prepared according to the procedure of compound **9b** with **7b** and 2-bromo-4,5-dimethoxybenzaldehyde **8'**. **9b'**: Yield 68.6%, light yellow solid, m.p. = 164 – 166 ℃; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.36 (s, 1H), 7.31 (s, 1H), 7.24 (s, 1H), 7.14 (s, 1H), 6.91 (s, 1H), 6.05 (s, 2H), 4.58 (t, *J* = 5.6 Hz, 1H), 3.90 (s, 3H), 3.85 (s, 3H), 3.46 (q, *J* = 5.6 Hz, 2H), 2.84 – 2.76 (m, 2H), 1.78 – 1.68 (m, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 189.71, 153.65, 149.36, 148.34, 145.41, 140.05, 128.99, 120.87, 114.73, 113.65, 111.17, 109.46, 108.12, 101.52, 93.65, 87.30, 60.27, 56.15, 55.67, 34.12, 30.86.

*3-(6-(7,8-Dimethoxyisoquinolin-3-yl)benzo[d][1,3]dioxol-5-yl)propan-1-ol (**10b**)*

The compound **10b** was prepared according to the procedure of compound **10a**. ^{t}BuOH (36 mL) was added to a mixture of $AgNO_3$ (63.0 mg, 0.37 mmol), ammonium acetate (429.0 mg, 5.6 mmol) and **9b** (1.4g, 3.7 mmol) under an inert atmosphere. The resulting mixture was stirred at room temperature for 10h and the reaction was monitored by TLC. After completion, the reaction was quenched by the addition of $NaHCO_3$ (1.17 g, 13.9 mmol) at room temperature and stirring was continued for additional 5 h. The mixture was then filtered through a cotton plug, washed with EtOAc (50 mL) and dried over anhydrous Na_2SO_4. The filtrate was evaporated under reduced pressure and the residue was purified through silica gel column chromatography (petroleum ether/ethyl acetate = 1:1) to obtain the pure isoquinoline **10b** (1.07 g). **10b**: Yield: 78.5%, yellow foamed solid, m.p. = 45 – 46 ℃; ^{1}H NMR (400 MHz, Chloroform-*d*) δ 9.61 (s, 1H), 7.70 (s, 1H), 7.63 (d, *J* = 8.8 Hz, 1H), 7.60 (d, *J* = 8.8 Hz, 1H), 6.86 (s, 1H), 6.83 (s, 1H), 6.01 (s, 2H), 4.11 (s, 3H), 4.04 (s, 3H), 3.65 – 3.57 (m, 2H), 2.75 (t, *J* = 6.8 Hz, 2H), 2.00 – 1.92 (m, 2H).^{13}C NMR (100 MHz, DMSO-d_6) δ 150.98, 148.67, 146.98, 145.63, 145.26, 142.72, 134.31, 133.47, 131.57, 123.14, 121.97, 120.89, 119.49, 110.14, 109.55, 101.01, 61.34, 60.28, 56.92, 34.27, 28.96.

*3-(6-(6,7-Dimethoxyisoquinolin-3-yl)benzo[d][1,3]dioxol-5-yl)propan-1-ol (**10b'**)*

The compound **10b'** was prepared according to the procedure of compound **10b**. **10b'**: yield 74.1%, yellow oil; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.08 (s, 1H), 7.66 (s, 1H), 7.49 (s, 1H), 7.34 (s, 1H), 6.92 (s, 1H), 6.89 (s, 1H), 6.03 (s, 2H), 3.92 (s, 6H), 3.26 (t, *J* = 6.4 Hz, 2H), 2.65 (t, *J* = 7.6 Hz, 2H), 1.91 (s, 1H), 1.62 – 1.52 (m, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 152.96, 151.48, 150.11, 148.82, 146.81, 145.17, 134.13, 133.91, 132.59, 122.90, 118.82, 110.04, 109.48, 105.42, 105.00, 100.94, 60.21, 55.78, 55.70, 34.20, 28.88.

*10,11-Dimethoxy-6,7-dihydro-5H-[1,3]dioxolo[4'',5'':4',5']benzo [1',2':3,4]azepino[1,2-b]isoquinolin-8-ium methanesulfonate (**11b**)*

The compound **11b** was prepared according to the procedure of compound **11a**. **11b**: yield 87.3%, yellow solid, m.p. = 106 – 108 ℃; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.08 (s, 1H), 8.55 (s, 1H), 8.25 (d, *J* = 8.8 Hz, 1H), 8.10 (d, *J* = 8.8 Hz, 1H), 7.29 (s, 1H), 7.08 (s, 1H), 6.16 (s, 2H), 5.05 (br s, 1H), 4.22 (br s, 1H), 4.12 (s, 3H), 4.08 (s, 3H), 2.74 (br s, 1H), 2.64 (br s, 1H), 2.42 (br s, 1H), 2.30 (br s, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 150.71, 149.49, 146.71, 145.92, 143.79, 143.25, 133.17, 132.39, 126.64, 125.60, 124.58, 123.70, 122.37, 109.99, 109.49, 101.96, 61.99, 57.16, 31.27, 27.91. LC-MS (ESI): *m/z* calculated for $C_{21}H_{20}NO_4^+$ [M-$CH_3SO_3^-$]$^+$: 350.1, found: 350.1.

*11,12-Dimethoxy-6,7-dihydro-5H-[1,3]dioxolo[4'',5'':4',5']benzo [1',2':3,4]azepino[1,2-b]isoquinolin-8-ium methanesulfonate (**12b**)*

The compound **12b** was prepared according to the procedure of compound **11b**. **12b**: yield 85.3%, yellow solid, m.p. = 136 – 138 ℃; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.89 (s, 1H), 8.37 (s, 1H), 7.80 (s, 1H), 7.76 (s, 1H), 7.25 (s, 1H), 7.07 (s, 1H), 6.16 (s, 2H), 4.79 (br s, 1H), 4.18 (br s, 1H), 4.06 (s, 3H), 4.01 (s, 3H), 2.74 (br s, 1H), 2.56 (br s, 1H), 2.28 (s, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 162.63, 157.49, 154.62, 151.88, 151.01, 149.12, 141.29, 138.19, 129.88, 128.65, 128.03, 114.85, 114.68, 111.85, 110.92, 107.11, 62.03, 61.61, 61.57, 36.50, 33.10. LC-MS (ESI): *m/z* calculated for $C_{21}H_{20}NO_4^+$ [M-$CH_3SO_3^-$]$^+$: 350.1, found: 350.1.

*10,11-Dimethoxy-6,7-dihydro-5H-[1,3]dioxolo[4'',5'':4',5']benzo [1',2':3,4]azepino[1,2-b]isoquinolin-8-ium hydroxide (**11b'**)*

The compound **11b'** was prepared according to the literature procedure of Wang *et al* [13]. CaO was added to the compound **11b** (80 mg, 0.18 mmol) dissolved in methanol (5 mL) and water (1 mL) portionwise to adjust pH to 9-10, the suspension was then stirred at 60 °C for 3 h. The resulting mixture was pour to H_2O (10 mL), extracted with DCM (10 mL × 4). The organic layers were combined, washed with brine, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. Crude product was used in the next step without further purification.

*10,11-Dimethoxy-6,7-dihydro-5H-[1,3]dioxolo[4'',5'':4',5']benzo [1',2':3,4]azepino[1,2-b]isoquinolin-8-ium chloride (**13b**)*

The crude **11b'** was then dissolved in 5 mL dichloromethane, and the solution of HCl in ethyl acetate was added. The resulting mixture was stirred at room temperature for 1 h, and filtered to afford compound **13b** as a yellow solid. **13b**: yield 35.7%, yellow solid, m.p. = 116 – 118 ℃; ^{1}H NMR (400 MHz, Methanol-d_4) δ 9.95 (s, 1H), 8.38 (s, 1H), 8.17 (d, *J* = 8.8 Hz, 1H), 8.04 (d, *J* = 8.8 Hz, 1H), 7.22 (s, 1H), 6.96 (s, 1H), 6.10 (s, 2H), 4.98 – 4.93 (m, 1H), 4.41 (br s, 1H), 4.23 (s, 3H), 4.13 (s, 3H), 2.80 (br s, 2H), 2.43 (br s, 2H). ^{13}C NMR (100 MHz, Methanol-d_4) δ 150.94, 150.45, 147.61, 145.32, 144.52, 144.09, 133.12, 126.59, 125.41, 124.56, 123.10, 122.84, 109.50,

109.10, 102.11, 61.20, 57.58, 56.27, 31.59, 28.15. LC-MS (ESI): *m/z* calculated for $C_{21}H_{20}NO_4^+$ [M-Cl$^-$]$^+$: 350.1, found: 350.1.

*11-Methoxy-6,7-dihydro-5H-[1,3]dioxolo[4",5":4',5']benzo[1',2':3,4] azepino[1,2-b]isoquinolin-8-ium-10-olate (**14b**)*

The compound **14b** was prepared according to the procedure of compound **11a'**. **14b**: yield 95.1%, dark red solid, m.p. > 220 ℃; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.29 (s, 1H), 7.57 (s, 1H), 7.31 (d, *J* = 8.0 Hz, 1H), 7.16 (s, 1H), 6.97 (s, 1H), 6.49 (d, *J* = 8.0 Hz, 1H), 6.10 (s, 2H), 4.57 (br s, 1H), 3.88 (br s, 1H), 3.75 (s, 3H), 2.69 (br s, 1H), 2.40 (br s, 2H), 2.04 (br s, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 149.42, 148.58, 146.46, 145.94, 139.65, 132.24, 131.57, 126.11, 122.47, 122.44, 121.60, 121.55, 120.63, 109.27, 102.42, 101.59, 55.89, 53.96, 30.20, 28.40.

*10-Butoxy-11-methoxy-6,7-dihydro-5H-[1,3]dioxolo[4",5":4',5']benzo [1',2':3,4]azepino[1,2-b]isoquinolin-8-ium bromide (**15b**)*

1-Bromobutane (390.5 mg, 2.85 mmol) was added to compound **14b** (191.2 mg, 0.57 mmol) in 10 mL CH_3CN at room temperature. The resulting mixture was refluxed at 85 ℃ for 3 h. After the reaction was completed as monitored by TLC, then the mixture was concentrated under reduced pressure. The residue was purified by neutral Al_2O_3 column chromatography (dichloromethane /CH_3OH = 100:1) to give compound **15b** (62.4 mg) as a yellow solid. **15b**: yield 23.2%, yellow solid, m.p. = 182 – 183 ℃; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.96 (s, 1H), 8.56 (s, 1H), 8.25 (d, *J* = 9.2 Hz, 1H), 8.09 (d, *J* = 9.2 Hz, 1H), 7.30 (s, 1H), 7.08 (s, 1H), 6.16 (s, 2H), 5.10 (br s, 1H), 4.31 (br s, 2H), 4.22 (br s, 1H), 4.07 (s, 3H), 2.74 (br s, 1H), 2.63 (br s, 1H), 2.42 (br s, 1H), 2.27 (br s, 1H), 1.97 – 1.83 (m, 2H), 1.56 – 1.46 (m, 2H), 0.98 (t, *J* = 7.6 Hz, 3H). ^{13}C NMR (100 MHz, Methanol-d_4) δ 152.36, 151.79, 148.96, 146.50, 145.39, 145.16, 134.53, 134.50, 127.80, 126.87, 125.95, 124.50, 124.34, 110.92, 110.46, 103.49, 75.60, 59.05, 57.60, 33.29, 33.01, 29.55, 20.17, 14.26. LC-MS (ESI): *m/z* calculated for $C_{24}H_{26}NO_4^+$ [M-Br$^-$]$^+$: 392.2, found: 392.3.

*10-(2-Bromoethoxy)-11-methoxy-6,7-dihydro-5H-[1,3]dioxolo [4",5":4',5']benzo[1',2':3,4]azepino[1,2-b]isoquinolin-8-ium bromide (**16b**)*

1,2-Dibromoethane (535.4 mg, 2.85 mmol) was added to compound **14b** (191.2 mg, 0.57 mmol) in 10 mL CH_3CN at room temperature. The resulting mixture was refluxed at 85 °C for 3 h. After the reaction was completed as monitored by TLC, then the mixture was concentrated under reduced pressure. The residue was purified by neutral Al_2O_3 column chromatography (dichloromethane /CH_3OH = 100:1) to give compound **16b** (64.1 mg) as a yellow solid. **16b**: yield 21.5%, yellow solid, m.p. = 130 - 131 ℃; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.02 (s, 1H), 8.55 (s, 1H), 8.26 (d, *J* = 9.2 Hz, 1H), 8.11 (d, *J* = 9.2 Hz, 1H), 7.31 (s, 1H), 7.09 (s, 1H), 6.16 (s, 2H), 5.01 (br s, 1H), 4.67 – 4.50 (m, 2H), 4.26 (br s, 1H), 4.13 (t, *J* = 5.2 Hz, 2H), 4.08 (s, 3H), 2.75 (br s, 1H), 2.62 (br s, 1H), 2.42 (br s, 1H), 2.29 (br s, 1H). ^{13}C NMR (100 MHz, Methanol-d_4) δ 152.16, 151.91, 149.04, 146.60, 145.57, 144.02, 134.55, 134.49, 127.68, 126.89, 125.89, 124.96, 124.33, 110.90, 110.51, 103.53, 75.24, 59.24, 57.64, 44.53, 33.04, 29.51. LC-MS (ESI): *m/z* calculated for $C_{22}H_{21}BrNO_4^+$ [M-Br$^-$]$^+$: 442.1, found: 442.0.

*10-(Benzyloxy)-11-methoxy-6,7-dihydro-5H-[1,3]dioxolo[4",5":4',5'] benzo[1',2':3,4]azepino[1,2-b]isoquinolin-8-ium chloride (**17b**)*

(Chloromethyl)benzene (360.8 mg, 2.85 mmol) was added to compound **14b** (191.2 mg, 0.57 mmol) in 10 mL CH_3CN at room temperature. The resulting mixture was refluxed at 85 °C for 3 h. After the reaction was completed as monitored by TLC, then the mixture was concentrated under reduced pressure. The residue was purified by neutral Al_2O_3 column chromatography (dichloromethane /CH_3OH = 100:1) to give compound **17b** (49.2 mg) as a yellow solid. **17b**: yield 18.7%, yellow solid, m.p. = 60 – 72 ℃, ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.87 (s, 1H), 8.51 (s, 1H), 8.27 (d, *J* = 9.2 Hz, 1H), 8.08 (d, *J* = 9.2 Hz, 1H), 7.56 (d, *J* = 7.2 Hz, 2H), 7.41 – 7.32 (m, 3H), 7.30 (s, 1H), 7.08 (s, 1H), 6.16 (s, 2H), 5.38 (d, *J* = 4.8 Hz, 2H), 5.00 (br s, 1H), 4.21 (br s, 1H), 4.11 (s, 3H), 2.75 (br s, 1H), 2.57 (br s, 1H), 2.28 (br s, 2H). ^{13}C NMR (100 MHz, Methanol-d_4) δ 153.00, 151.88, 149.03, 146.44, 145.27, 143.82, 137.67, 134.35, 134.28, 130.50, 129.89, 129.62, 127.59, 126.77, 125.80, 125.12, 124.94, 110.79, 110.48, 103.52, 76.95, 58.92, 57.59, 33.01, 29.43. LC-MS (ESI): *m/z* calculated for $C_{27}H_{24}NO_4^+$ [M-Cl$^-$]$^+$: 426.2, found: 426.2.

*13-Bromo-10,11-dimethoxy-6,7-dihydro-5H-[1,3]dioxolo[4",5":4',5'] benzo[1',2':3,4]azepino[1,2-b]isoquinolin-8-ium bromide (**18b**)*

Br_2 (538.6 mg, 3.37 mmol) was added to compound **11b** (298.5 mg, 0.67 mmol) in 10mL CH_3COOH at room temperature. The resulting mixture was then refluxed at 100 °C for 8 h. After the reaction was completed as monitored by TLC and then cooled to room temperature, the precipitates were filtered and washed with 10% $Na_2S_2O_3$ solution, then with H_2O to afford the crude product **18b** (200 mg) as a yellow solid which was used in the next step without further purification. **18b**: yield 58.6%, yellow soild; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.13 (s, 1H), 8.58 (s, 1H), 8.23 (s, 1H), 7.43 (s, 1H), 7.10 (s, 1H), 6.17 (s, 2H), 5.08 (br s, 1H), 4.24 (br s, 1H), 4.15 – 4.11 (m, 6H), 2.76 (br s, 1H), 2.62 (br s, 1H), 2.46 – 2.35 (m, 1H), 2.30 (br s, 1H).

*10,11-Dimethoxy-13-phenyl-6,7-dihydro-5H-[1,3]dioxolo[4",5":4',5'] benzo[1',2':3,4]azepino[1,2-b]isoquinolin-8-ium bromide (**19b**)*

15mL toluene, 5mL C_2H_5OH and 5mL H_2O were added to a mixture of Na_2CO_3 (84 mg, 0.79 mmol), $Pd(PPh_3)_4$ (23 mg, 0.02 mmol), phenylboronic acid (96.4 mg, 0.79 mmol) and compound **18b** (200 mg, 0.39 mmol) under an inert atmosphere. The resulting mixture was refluxed at 100 °C for 14 h. After the reaction was completed as monitored by TLC, the mixture was then filtered, washed with EtOAc

(10 mL × 4). The filtrate was washed with brine, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (dichloromethane /CH_3OH = 20:1) to afford compound **19b** (40.4 mg) as a yellow solid. **19b**: yield 20.2%, yellow solid, m.p. = 77 – 78 ℃; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.18 (s, 1H), 8.13 (s, 1H), 7.97 (s, 1H), 7.69 (d, *J* = 7.2 Hz, 2H), 7.61 (t, *J* = 7.2 Hz, 2H), 7.54 (t, *J* = 7.2 Hz, 1H), 7.28 (s, 1H), 7.05 (s, 1H), 6.12 (d, *J* = 15.6 Hz, 1H), 5.12 (br s, 1H), 4.23 (br s, 1H), 4.17 (s, 3H), 4.15 (s, 3H), 2.72 (br s, 1H), 2.64 (br s, 1H), 2.43 (br s, 1H), 2.27 (br s, 1H).^{13}C NMR (100 MHz, DMSO-d_6) δ 150.31, 149.56, 146.79, 143.37, 136.55, 135.68, 133.29, 130.21, 130.16, 129.73, 129.08, 128.63, 126.70, 124.57, 122.76, 109.77, 109.47, 101.93, 62.10, 57.25, 57.10, 31.24, 27.81. LC-MS (ESI): *m/z* calculated for $C_{27}H_{24}NO_4^+$ [M-Br$^-$]$^+$: 426.2, found: 426.2.

*10,11-Dimethoxy-5,6,7,9,14,14a-hexahydro-[1,3]dioxolo[4'',5'':4',5'] benzo[1',2':3,4]azepino[1,2-b]isoquinoline (**20b**)*

$NaBH_4$ (74.1 mg, 1.96 mmol) was added to compound **11b** (218.2 mg, 0.49 mmol) and K_2CO_3 (62 mg, 0.49 mmol) in CH_3OH (10 mL) portionwise at 0 ℃. The resulting mixture was refluxed at 65 ℃ for 3 h. After the reaction was completed as monitored by TLC, then the mixture was concentrated and pour to H_2O (10 mL), extracted with DCM (15 mL×3). The organic layers were combined, washed with brine, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure to give compound **20b** (200 mg) as pale yellow oil which was used in the next step without further purification.

*11,12-Dimethoxy-5,6,7,9,14,14a-hexahydro-[1,3]dioxolo[4'',5'':4',5'] benzo[1',2':3,4]azepino[1,2-b]isoquinoline (**20b'**)*

The compound **20b'** was prepared according to the procedure of compound **20b**. Crude product **20b'** (218 mg, yellow oil) was used in the next step without further purification.

*8-Benzyl-10,11-dimethoxy-6,7,8,9,14,14a-hexahydro-5H-[1,3]dioxolo [4'',5'':4',5']benzo[1',2':3,4]azepino[1,2-b]isoquinolin-8-ium bromide (**21b**)*

Benzyl bromide (484 mg, 2.83 mmol) was added to compound **20b** (200 mg) in 10 mL CH_3CN at room temperature. The resulting mixture was stirred at 50 ℃ for 3 h. After the reaction was completed as monitored by TLC, then the mixture was concentrated under reduced pressure. The residue was purified by silica gel column chromatography (dichloromethane /CH_3OH = 50:1) to give and to give compound **21b** (216.7 mg) as a light yellow solid. **21b**: yield 84%, light yellow solid, m.p. = 180 – 182 ℃; ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.68 (dd, *J* = 7.2, 2.0 Hz, 2H), 7.58 – 7.51 (m, 3H), 7.20 (s, 1H), 7.11 (d, *J* = 8.8 Hz, 1H), 7.06 – 6.99 (m, 2H), 6.08 (s, 2H), 5.52 (dd, *J* = 13.2, 4.8Hz, 1H), 5.37 (d, *J* = 15.2 Hz, 1H), 4.91 (d, *J* = 13.2 Hz, 1H), 4.61 (d, *J* = 13.2 Hz, 1H), 3.95 – 3.84 (m, 2H), 3.79 (s, 3H), 3.67 (d, *J* = 13.2 Hz, 1H), 3.60 (s, 3H), 3.44 (d, *J* = 15.2 Hz, 1H), 3.28 – 3.17 (m, 2H), 2.86 (dd, *J* = 15.2, 4.8 Hz, 1H), 2.67 – 2.53 (m, 1H), 2.04 (d, *J* = 15.2 Hz, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 150.66, 148.01, 145.87, 144.85, 135.35, 132.83, 130.37, 128.95, 127.73, 125.06, 124.10, 123.02, 120.43, 113.39, 111.94, 111.83, 101.67, 73.32, 61.34, 59.99, 59.38, 55.98, 54.81, 32.30, 28.70, 22.61. LC-MS (ESI): *m/z* calculated for $C_{28}H_{30}NO_4^+$ [M-Br$^-$]$^+$: 444.2, found: 444.1.

*8-Benzyl-11,12-dimethoxy-6,7,8,9,14,14a-hexahydro-5H-[1,3]dioxolo [4'',5'':4',5']benzo[1',2':3,4]azepino[1,2-b]isoquinolin-8-ium bromide (**22b**)*

The compound **22b** was prepared according to the procedure of compound **21b**. 2**2b**: yield 79.8%, light yellow solid, m.p. = 168 – 169 ℃; ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.68 – 7.59 (m, 2H), 7.58 – 7.49 (m, 3H), 7.19 (s, 1H), 7.00 (s, 1H), 6.86 (s, 1H), 6.77 (s, 1H), 6.08 (s, 2H), 5.46 (dd, *J* = 13.2, 4.8 Hz, 1H), 5.23 (d, *J* = 15.2 Hz, 1H), 4.76 (d, *J* = 13.2 Hz, 1H), 4.56 (d, *J* = 13.2 Hz, 1H), 3.84 (d, *J* = 15.2 Hz, 2H), 3.79 (d, *J* = 4.8 Hz, 1H), 3.74 (s, 3H), 3.68 (s, 3H), 3.41 (d, *J* = 15.2 Hz, 1H), 3.26 – 3.12 (m, 2H), 2.86 (dd, *J* = 15.2, 4.8 Hz, 1H), 2.65 – 2.53 (m, 1H), 2.06 (d, *J* = 15.2 Hz, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 153.75, 153.14, 151.04, 140.47, 138.04, 135.41, 134.18, 132.80, 130.41, 127.44, 123.33, 117.15, 116.98, 116.34, 115.44, 106.83, 68.46, 66.47, 60.76, 60.71, 59.51, 37.60, 34.56, 27.78. LC-MS (ESI): *m/z* calculated for $C_{28}H_{30}NO_4^+$ [M-Br$^-$]$^+$: 444.2, found: 444.1.

Biological Evaluation

Enzymatic Inhibition Assays

The p300 HAT inhibitory assays were performed using isotope-labelled ^{3}H-Ac-CoA and substrate peptide by Shanghai ChemPartner Co., Ltd (http://www.chempartner.com/). The materials include p300 (BPS, Cat. No. 50071), Ac-CoA (Sigma, Cat. No. A2056), ^{3}H-Ac-CoA (PerkinElmer, Cat. No. NET290), and C646 (Calbiochem, Cat. No. 382113) for positive control. The substrate solution and stop solution were prepared by Shanghai ChemPartner. The reaction is started by the addition of 10 μL substrate solution, followed by 60 mins incubation at room temperature, then adding 10 μL stop solution to stop the reactions. Then, 25 μL reaction solutions are transferred to the Flashplate, incubated for 1 hour, and washed three times using *d*H_2O and 0.1% Tween-20 prior to reading on Microbeta. The inhibition rate is calculated using the formula: Inh%= (Max-Signal)/(Max-Min)*100. By using Graphpad Prism 5, the IC_{50} values were calculated from dose-response curves obtained at 10 different concentrations for each compound.

Molecular Docking Simulations

The molecular docking simulations in this study were carried out using AutoDock Vina.[21] The chemical structures of B-homo palmatine and berberine derivatives were prepared as described

previously.[22] The X-ray crystal structure of human p300 HAT complexed with a bisubstrate inhibitor, Lys-CoA (PDB ID: 3BIY) [23] was used as the docking template. The Lys-CoA, water molecules, and other solvent molecules were removed. The binding site was set as a square grid. The grid center was set to coordinates of [x, y, z = -17.63, 15.95, 1.48] and the grid size was set to 25Å × 25Å × 23Å encompassing the entire p300 HAT domain. The number of docking poses was set as 10, and the other parameters for Vina were set as default. The docking results were analysed using the PyMol program.

Cell-based Assays.

All cell lines were cultured in the RPMI1640 or DMEM medium supplemented with 10% (v/v) fetal bovine serum (FBS) and 100 U/ml of penicillin and streptomycin (purchased from Sigma-Aldrich) at 37°C in 5% CO_2. All compounds were prepared to 100 mM stock solution in 100% dimethyl sulfoxide (DMSO), stored at -20 ℃ and diluted in culture medium before use. Cell viability was carried out using MTT assay as previously reported.[24] Briefly, the cultured cancer cell lines were seeded in 96-well plants at 2500-4000 cells/well (depending on cell type) in the presence or absence of tested compounds. After a 72 h incubation, the MTT reagent was added for a 3-4 h incubation, and DMSO was used to dissolve the oxidative products. Finally, the absorption (OD) values of the dissolved cells were measured at 570 nm using a SpectraMAX M5 microplate spectrophotometer. All experiments were performed in triplicate.

Acknowledgments

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Supplementary Material

Supplementary data associated with this article can be found, in the online version, at.

Keywords: total synthesis • palmatine • berberine • p300 hat • natural product.

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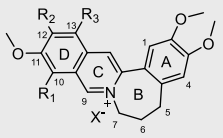

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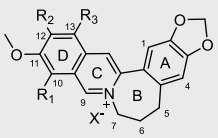

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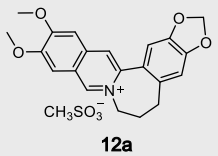

12a
p300 HAT IC_{50} = 0.42 μM

New B-homo palmatine and berberine derivatives have been prepared, which exhibited potent inhibition to p300 histone acetyltransferase.

Zhongzhen Yang, Yong Zhang, Xin Chen, Weijian Li, Guo-Bo Li, Yong Wu**

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Total Synthesis and Evaluation of New B-homo Palmatine and Berberine Derivatives as p300 Histone Acetyltransferase Inhibitors