European Journal of Medicinal Chemistry xxx (2017) 1–11

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Research paper

Synthesis and biological evaluation of new berberine derivatives as cancer immunotherapy agents through targeting IDO1

Yan–Xiang Wang [a, 1], Wei–Qiang Pang [a, 1], Qing–Xuan Zeng [a], Zhe–Song Deng [b], Tian–Yun Fan [a], Jian–Dong Jiang [a], Hong–Bin Deng [a, *], Dan–Qing Song [a, **]

[a] Institute of Medicinal Biotechnology, Chinese Academy of Medical Science & Peking Union Medical College, Beijing 100050, China
[b] Medical College, Nanchang University, Nanchang, Jiangxi 330006, China

ARTICLE INFO

Article history:
Received 6 October 2017
Received in revised form
28 October 2017
Accepted 30 October 2017
Available online xxx

Keywords:
Berberine
IDO1
Structure-activity relationship
Cancer immunotherapy
Small-molecule
AMPK

ABSTRACT

To discover small-molecule cancer immunotherapy candidates through targeting Indoleamine 2,3-dioxygenase 1 (IDO1), twenty–five new berberine (BBR) derivatives defined with substituents on position 3 or 9 were synthesized and examined for repression of IFN-γ-induced IDO1 promoter activities. Structure–activity relationship (SAR) indicated that large volume groups at the 9-position might be beneficial for potency. Among them, compounds **2f**, **2i**, **2n**, **2o** and **8b** exhibited increased activities, with inhibition rate of 71–90% compared with BBR. Their effects on IDO1 expression were further confirmed by protein level as well. Furthermore, compounds **2i** and **2n** exhibited anticancer activity by enhancing the specific lysis of NK cells to A549 through IDO1, but not cytotoxicity. Preliminary mechanism revealed that both of them inhibited IFN-γ-induced IDO1 expression through activating AMPK and subsequent inhibition of STAT1 phosphorylation. Therefore, compounds **2i** and **2n** have been selected as IDO1 modulators for small-molecule cancer immunotherapy for next investigation.

1. Introduction

Over the past few years, the cancer immunotherapy has made great progress, which showns significant efficacy in human solid tumors, with several drugs approved by the FDA, such as programmed cell death protein 1 (PD-1) antibody Keytruda® and Opdivo® [1,2]. Clinical benefit of these antibodies as single agent, however, has been limited to some factors such as a subset of patients, high cost, difficulty to generate a decent amount and efficacy not for all tumor types. These limitations call for the development of rational combination strategies or small-molecule therapeutics aiming to extend therapeutic benefit to a broader range of patients [3]. Meanwhile, as promising immunotherapy candidates, Indoleamine 2,3-dioxygenase 1 (IDO1) inhibitors including Epacadostat and Indoximod have already entered clinic trial. Especially, combination of small-molecule IDO inhibitors with PD-1 antibodies could significantly improve the objective response rate (ORR) in specific tumor treatment [4]. Phase I/II results showed that Epacadostat and Keytruda combination demonstrated an ORR of 53% (10 out of 19 patients) and a disease control rate of 74% (15 out of 19 patients) across multiple malignancies [5]. Since it is a single-chain catalytic enzyme with a well-defined biochemistry [6], IDO1 is considered to be an attractive target for small-molecule immunotherapeutics development. Therefore, it is a promising therapeutic strategy to develop small-molecule IDO1 inhibitors or modulators and provide new components for cancer immunotherapy combination.

A number of pathways are related to IDO1 expression [7], and IFN-γ is the major inducer of IDO1 expression. In an effort to discover and explore novel small-molecule immunotherapeutics through targeting IDO1, the high-throughput screening model on inhibition of IFN-γ-induced IDO1 promoter activity was then established in our laboratory. Given its inherent sensitivity, large signal dynamics, and simple set up, the reporter assay platform has been used as a high-throughput homogenous assay for screening IDO1 modulators in this study. Then, a library of natural products constructed in our lab was screened for their IDO1 regulating ability. Luckily, berberine (BBR, Fig. 1), as a Traditional Chinese Medicine used in China for decades against diarrhea, exhibited a moderate potency with inhibition rate of 17% at 10 μM [8–12]. The

* Corresponding author.
** Corresponding author.
E-mail addresses: hdeng@imb.pumc.edu.cn (H. Deng), songdanqingsdq@hotmail.com (D. Song).
[1] These authors made equal contribution to this work.

https://doi.org/10.1016/j.ejmech.2017.10.078

unique isoquinoline skeleton of BBR evokes our great interest to carry out structure-activity relationship (SAR) so as to discover small-molecule cancer-immunotherapy candidates targeting IDO1. Therefore, a series of novel BBR derivatives, including esters, amides and sulfonates on positions 3 and 9 as depicted in Fig. 1, were prepared and evaluated for their IDO1 effects, as well as primary mode of mechanism of the representative compounds.

2. Results and discussion

2.1. Synthesis

A total of twenty-five new BBR derivatives were designed and semi-synthesized as displayed in Schemes 1–3, taking commercial available BBR, palmatine (PMT) or jatrorrhizine (JTH) as the starting material respectively. As shown in Scheme 1, BBR esters **2a**–**q** and sulfonate **3a** were obtained by esterification and sulfonation of compound **1** using previously reported methods [13,14]. The key intermediate **4** was prepared with 2,4-dimethoxyaniline as the nucleophilic reagent as well as the solvent. Another key intermediate **5** with a free amine group was then acquired, and HCl/CH_3OH was selected to remove 2,4-dimethoxybenzyl in 80% yield. The desired products **6a**–**c** were obtained by amidation with corresponding acyl chloride using pyridine as the base with the yields of 33–37%.

As described in Scheme 2, compounds **8a** and **8b** were prepared through de-methylation and esterification from PMT using the procedures reported previously in overall yields of 25–33%. Similarly, as depicted in Scheme 3, the desired products **9a** and **9b** were obtained via esterification of JTH by 33–34% yields. All the final products were purified via flash column chromatography using CH_3OH/CH_2Cl_2 as the gradient eluent (see Table 1).

2.2. Pharmacological evaluation

2.2.1. SAR for down-regulated IFN-γ-induced IDO1 promoter activity

Thus, we screened all the newly-synthesized BBR analogues for their IDO1 regulating abilities by IDO1 promoter reporter assay. A549 cells were transfected with a pGL4-IDO1-luc vector and the inhibition rates of the BBR analogues on IFN-γ-induced IDO1 promoter activities are shown in Fig. 2.

First, SAR analysis was focused on the influence of substitutions on position 9 of ring D, and 17 new ester derivatives (**2a**–**q**) were prepared and tested. Compounds **2a**–**d** with different substituted benzene and heterocycle were examined for their abilities to inhibit IDO1 promoter activity, and their activities almost vanished compared with BBR. Then, three analogues **2e**–**g** with alkyl chain attached were synthesized and screened, and compound **2f** bearing 2-ethyl butyrate gave the most potent activity with the inhibitory rate of 90%. Compounds **2h**–**k** with cyclic groups attached were tested, and 2,2,3,3-tetramethylcyclopropane-1-carboxylate substituted analogue **2i** showed satisfactory inhibitory activity on IDO1 activity. It seems that tertiary carbon or quaternary carbon substituent with large volume might be beneficial for the inhibitory ability. Based on these results, the replacement of bridged-ring derivatives (**2l**–**o**) at position 9 were created and tested. Surprisingly, compounds **2n** and **2o** gave ideal activity with inhibitory rate of 71% and 89% respectively, which indicated that large-volume rigid structure might be favourable for the ability. The combination of aromatic ring and alkyl chain (compounds **2p** and **2q**) on position 9 resulted in an obvious drop of inhibitory activity. Moreover, the inhibitory activity of compounds **3a** and **6a**–**c** reduced significantly or lost completely, while the ester linker fragment was switched to sulfonate or amide.

Further SAR study was conducted for the substituents on position 3 of ring A according to the above SAR analysis. The methylenedioxy was opened while two analogues **8a**–**b** were created, and compound **8b** gave an satisfactory activity with inhibitory rate of 72% which indicated that ring A might not be essential for the activity maintaining. Moving the adamantate group from ring D to position 3 of ring A, compound **9b** gave acceptable potencies with inhibition rate of 55%.

Based on the screening results, compounds **2f**, **2i**, **2n**, **2o** and **8b** exhibited the potent activities for modulating IFN-γ-induced IDO1 promoter. In addition, as compounds **2c**, **2e**, **2q**, **6a** and **8a** possess different types of structure, all of them were selected as representative compounds to investigate their inhibitory effects on protein expression level of IDO1.

2.2.2. BBR analogues inhibited IFN-γ-induced IDO1 expression by protein level

In order to further confirm the regulating effect of BBR analogues, we examined whether they could block protein expression level of IDO1 or not. A549 cells were pre-treated with 10 μM of the indicated compounds for 2 h and then treated with IFN-γ for 24 h. IDO1 expression level in total cell lysate was detected by western blot. As shown in Fig. 3, treatment with compounds **2f**, **2i**, **2n**, **2o** and **8b** significantly reduced IFN-γ-induced IDO1 expression, which was consistent with the results of dual-luciferase reporter screening model.

2.2.3. Effects of BBR analogues on A549 cell viability

To exclude the BBR analogues-induced IDO1 down-regulation resulted from the potential cytotoxicity, the cytotoxic effects of the ten representative compounds on A549 cells were evaluated by MTT assay. As displayed in Fig. 4, the results revealed that compounds **2i**, **2n**, **6a** and **8a** showed no cytotoxic activities in A549 cells, much lower than that of other BBR analogues.

2.2.4. Effects of BBR analogues on killing activity of human NK cells toward A549 cells

To determine whether BBR analogues could enhances the specific lysis of NK cells to A549 cells, A549 cells were pre-

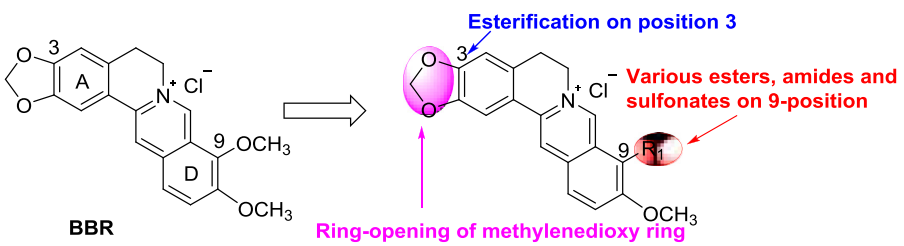
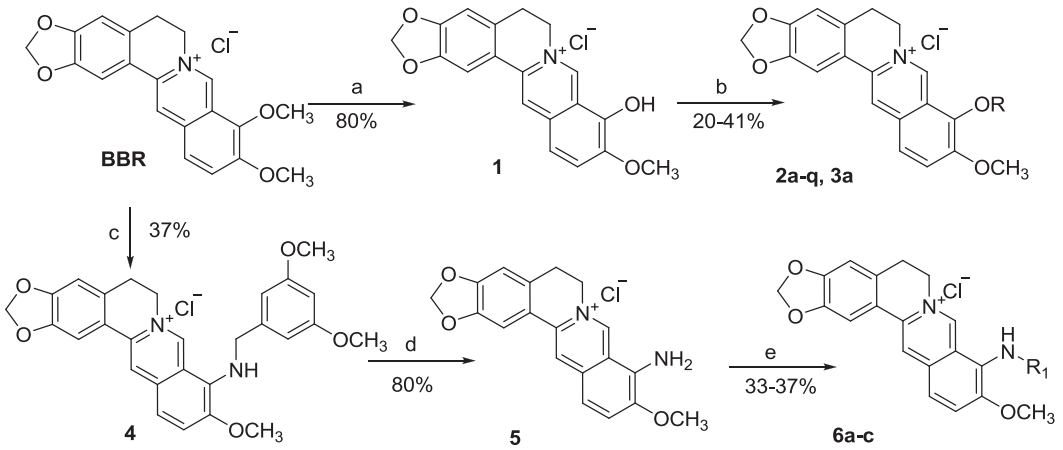
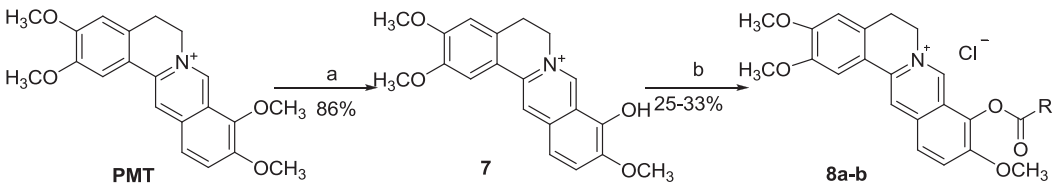


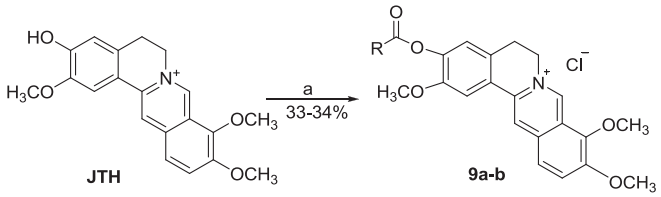
Fig. 1. Chemical structure of BBR, and structure modification strategy.



Scheme 1. Synthesis of compounds **2a**–**q**, **3a**, **6a**–**c**. Reagents and conditions: (a) 195 °C, 30–40 mmHg, 60 min; (b) RCOX/RSO_2Cl, triethylamine, CH_3CN, reflux. (c) 2,4-Dimethoxybenzylamine, 120 °C; (d) HCl, CH_3OH; (e) R_1COX, pyridine, CH_2Cl_2, reflux.



Scheme 2. Synthesis of compounds **8a**–**b**. Reagents and conditions: (a) 195 °C, 30–40 mmHg, 60 min; (b) RCOCl, triethylamine, CH_3CN, reflux.



Scheme 3. Synthesis of compounds **9a**–**b**. Reagents and conditions: (a) RCOX, triethylamine, CH_3CN, reflux.

treated with the indicated compounds with or without IFN-γ for 16 h. Cells were washed and then NK cells were co-cultured with A549 cells at 10:1, NK killing activity was assessed by LDH assay (Fig. 5A) and cell impedance assay (Fig. 5B), respectively. As shown in Fig. 5A, compounds **2i**, **2n** and **8b** significantly enhanced the specific lysis of NK cells to A549 cells. In consideration of the cytotoxic effects of the three compounds, we choose compounds **2i** and **2n** to assess its effects on killing activity of human NK cells toward A549 cells by cell impedance assay. 1-Methyl-D-tryptophan (1-MT) was used as a positive IDO1 inhibitor. As shown in Fig. 5B, significant reduction in the cell index of A549 cells was observed following compounds **2i** and **2n** treatment compared with BBR, and compound **2i** exhibited stronger activity than 1-MT.

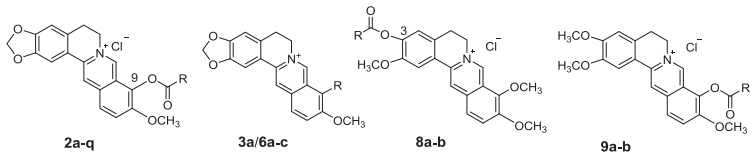
2.2.5. BBR analogues inhibited IFN-γ-induced STAT1 transcription activity by inhibiting STAT1 phosphorylation and activating AMPK

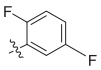
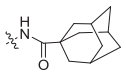
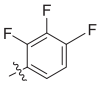
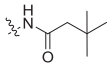
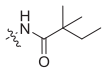
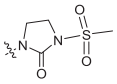
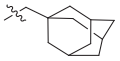
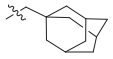
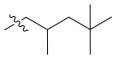
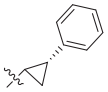
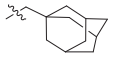
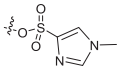
IFN-γ-induced IDO1 expression involves the activation of signal transducer and activator or transcription 1 (STAT1), which can be activated via tyrosine phosphorylation by Janus kinases 1 (JAK1). Activated STAT1 homodimerizes and translocates into the nucleus, where it binds to and activates IFN-γ-responsive specific promoters of IDO1 [15,16]. Considering that STAT1 plays a critical role in IFN-γ-induced IDO1 expression, preliminary mechanism study was carried out to verify if the ten selected compounds work through STAT1 pathway. STAT1 phosphorylation at residue Tyr701 is required for acting as an active transcription factor. Therefore, we asked whether BBR analogues interfered with STAT1 phosphorylation. The expression level of STAT1 and phosphorylation of STAT1 Tyr701 were detected by western blot. As shown in Fig. 6, stimulation of cells with IFN-γ alone resulted in a rapid increase in Tyr701 phosphorylation of STAT1, and this increase was remarkably inhibited by the treatment of compounds **2e**, **2f**, **2i**, **2n**, **2o** and **8b** respectively.

Recent studies have shown that adenosine 5′-monophosphate (AMP)–activated protein kinase (AMPK) activation suppresses STAT signaling [17,18]. AMPK kinase (AMPKK), an upstream kinase of AMPK, phosphorylates threonine 172 of the AMPK subunit α. Activated AMPK phosphorylates and inactivates acetyl-CoA carboxylase (ACC) [19,20]. To assess whether compounds **2e**, **2f**, **2i**, **2n**, **2o** and **8b**-induced STAT1 inactivation through AMPK pathway, AMPK activation was determined by assessing ACC phosphorylation on Ser79 and AMPK phosphorylation on Thr172 [21,22]. Western blot results (Fig. 7) demonstrated that compounds **2i**, **2n** and **8b** significantly increased IFN-γ-induced ACC and AMPK phosphorylation. Compounds **2i**, **2n** and **8b** induced AMPK activation and STAT1 dephosphorylation. Considering compounds **2i**, **2n** and **8b** markedly suppressed IFN-γ-induced IDO1 promoter activities, which demonstrated that they inhibited IFN-γ-induced IDO1 promoter activity by activating AMPK and subsequent inhibition of STAT1 phosphorylation, indicating these compounds had great potential as immunotherapy agents targeting inhibition of IDO1 at the transcriptional level.

Please cite this article in press as: Yan–Xiang-g. Wang, et al., Synthesis and biological evaluation of new berberine derivatives as cancer immunotherapy agents through targeting IDO1, European Journal of Medicinal Chemistry (2017), https://doi.org/10.1016/j.ejmech.2017.10.078

Table 1
Structures of all the newly synthesized BBR derivatives.



No	R	No	R	No.	R
2a		**2j**		**6a**	
2b		**2k**		**6b**	
2c		**2l**		**6c**	
2d		**2m**		**8a**	
2e		**2n**		**8b**	
2f		**2o**		**9a**	
2g		**2p**		**9b**	
2h		**2q**		**BBR**	-
2i		**3a**			

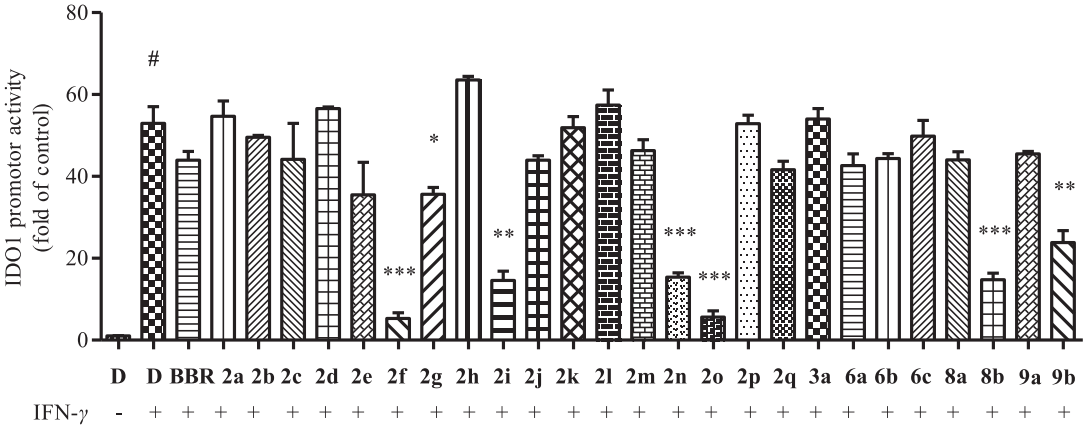


Fig. 2. Inhibition effect of BBR analogues on IFN-γ-induced IDO1 promoter activity. A549 cells were transfected with a pGL4-IDO1-luc vector and the pRL-CMV vector as an internal control, followed by pretreatment with 10 μmol/L of the indicated compounds for 2 h and stimulation with or without IFN-γ for 24 h. The level of luciferase activity was measured and normalized to Renilla luciferase activity. #$p < 0.001$ compared with untreated control group; *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ compared with the IFN-γ group.

Please cite this article in press as: Yan–Xiang-g. Wang, et al., Synthesis and biological evaluation of new berberine derivatives as cancer immunotherapy agents through targeting IDO1, European Journal of Medicinal Chemistry (2017), https://doi.org/10.1016/j.ejmech.2017.10.078

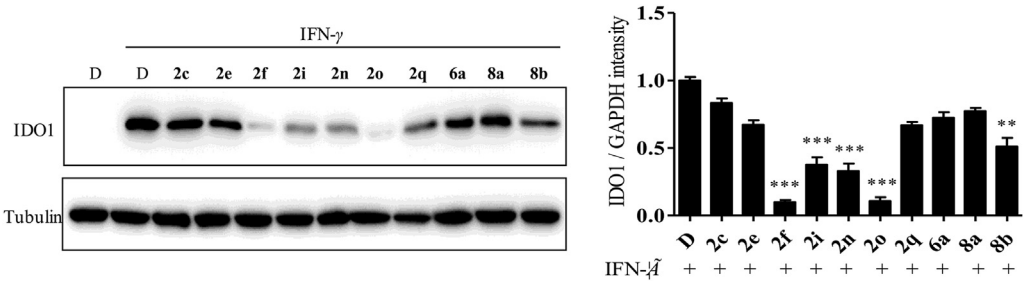


Fig. 3. BBR analogues inhibited IFN-γ-induced IDO1 expression. A549 cells were pre-treated with 10 μmol/L of the indicated compounds for 2 h, followed by IFN-γ (5 ng/mL) treatment for 24 h. The expression levels of IDO1 were measured by western blot. D: DMSO. GAPDH served as the loading control. $^{**}p < 0.01$, $^{***}p < 0.001$ compared with the IFN-γ group.

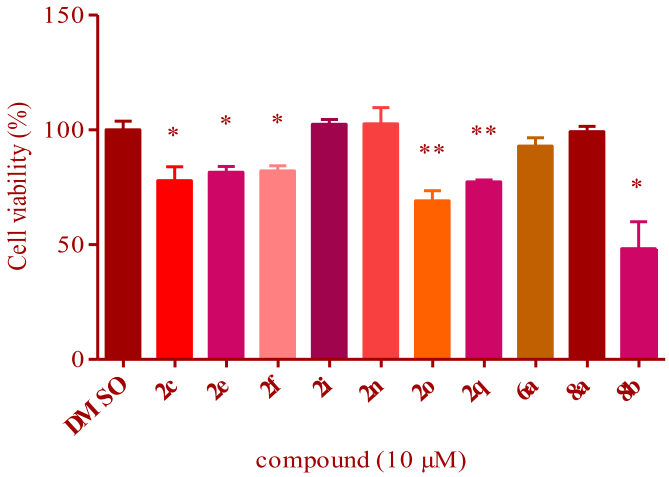


Fig. 4. Effects of BBR analogues on A549 cell viability. A549 cells were treated with 10 μmol/L of the indicated compounds for 24 h, cell toxicity was measured by MTT assay. Control cells were treated with 0.5% (v/v) DMSO. The results are presented as means ± standard error and represent three individual experiments. $^{*}p < 0.05$, $^{**}p < 0.01$ compared with the untreated control group.

3. Conclusions

Taking IDO1 as the target, a series of novel BBR derivatives including esters, amides and sulfonates on different positions were designed, prepared and examined for their activity for suppression of IFN-γ-induced IDO1 promoter expression. SAR analysis indicated that large volume substituent at the 9-position might be beneficial for enhancing the potency. Among them, compounds **2f**, **2i**, **2n**, **2o** and **8b** exhibited increased potency with inhibitory rate of 71–90% compared with BBR. Their activities were further confirmed by protein level. Furthermore, compounds **2i** and **2n** exhibited their anticancer activity by enhancing the specific lysis of NK cells to A549 cells, as through targeting IDO1, but not cytotoxicity. Preliminary mechanism revealed that compounds **2i** and **2n** inhibited IFN-γ-induced IDO1 expression through activating AMPK and subsequent inhibition of STAT1 phosphorylation. Thus, the results provided powerful information on further strategic optimization, and compounds **2i** and **2n** have been selected as promising IDO1 modulators for cancer immunotherapy for next investigation.

4. Experimental section

4.1. General

Melting point (mp) was obtained with CXM-300 melting point apparatus and uncorrected. The ^{1}H NMR spectra was performed on a Varian Inova 500 or 600 MHz spectrometer (Varian, San Francisco, CA) and ^{13}C NMR on a Bruker Avance III 500 or 600 spectrometer with Me_4Si as the internal standard, all the samples were dissolved in DMSO-d_6 before testing. ESI high-resolution mass spectra (HRMS) was recorded on an Autospec Ultima-TOF mass spectrometer (Micromass UK Ltd, Manchester, UK). Flash chromatography was performed on CombiflashRf 200 (Teledyne, Nebraska, USA), particle size 0.038 mm. Antibodies against IDO1, STAT1, phospho-STAT1 (Thr701), AMPKα, phospho-AMPK (Thr172) and phospho-ACC (Ser79) were purchased from Cell Signaling Technology (Danvers, MA, USA). MTT, α-Tubulin, GAPDH and β-actin antibody were obtained from Sigma (St. Louis, MO, USA). 1-MT (1-Methyl-D-tryptophan, IDO1 inhibitor) was purchased from Selleckchem (Shanghai, China).

4.2. Chemistry

4.2.1. General synthesis procedure for synthesis of compounds *2a–q* and *3a*

BBR (3.71 g, 10 mmol) was heated at 195–210 °C for 10–15 min under vacuum (30–40 mmHg) to afford the black oil, which was acidified with ethanol/concentrated HCl (95:5). The solvent was removed by evaporation, the residue was collected and then purified by flash chromatography over silica gel using CH_2Cl_2/CH_3OH as the gradient eluent, affording the title compound **1** (2.85 g, 80%) as a yellow solid.

To a stirred solution of **1** (100 mg, 0.28 mmol) in anhydrous CH_3CN, triethylamine (175 μL, 1.26 mmol) was added and heated to 70 °C. Then the RCOCl/RSO_2Cl (1.1–1.2 eq) was added and stirred for 5–6 h. The mixture was cooled to precipitate completely, filtrated and washed with CH_2Cl_2 to afford compounds **2a–q** and **3a**. Compounds **2a–g**, **2i**, **2n–q** and **3a** were gained following the same procedure using purchased acyl chloride or sulfuryl chloride as material. Compounds **2h**, **2j–m** were gained by the same procedure using purchased acid which was reflux in $SOCl_2$ to afford acyl chloride.

*4.2.1.1. 2,3-Methylenedioxy-9-((2,5-difluorobenzoyl)oxy)-10-methoxyprotoberberine chloride (**2a**).* Compound **1** (100 mg, 0.28 mmol) was treated with 2,5-difluorobenzoyl chloride (39 μL, 0.31 mmol) according to the general procedure to give the desired product **2a** as a yellow solid, yield: 43%; Mp: 184–186 °C (dec.); ^{1}H NMR (500 MHz) δ 10.07 (s, 1H), 9.14 (s, 1H), 8.37 (d, J = 9.3 Hz, 1H), 8.30 (d, J = 9.3 Hz, 1H), 8.10 (s, 1H), 7.85 (s, 1H), 7.84–7.77 (m, 1H), 7.67–7.61 (m, 1H), 7.11 (s, 1H), 6.19 (s, 2H), 4.92 (t, J = 6.4 Hz, 2H), 4.06 (s, 3H), 3.22 (t, J = 6.4 Hz, 2H); ^{13}C NMR (126 MHz) δ 160.1, 159.5, 157.6, 150.9, 150.7, 148.4, 145.2, 138.9, 133.6, 133.4, 131.5, 127.9, 126.5, 124.3, 121.6, 121.3, 121.0, 120.2, 119.4, 118.2, 109.1, 106.2, 102.8, 58.03, 56.0, 26.8; HRMS: calcd for $C_{26}H_{18}F_2NO_5Cl$ $[M - Cl]^+$

Please cite this article in press as: Yan–Xiang-g. Wang, et al., Synthesis and biological evaluation of new berberine derivatives as cancer immunotherapy agents through targeting IDO1, European Journal of Medicinal Chemistry (2017), https://doi.org/10.1016/j.ejmech.2017.10.078

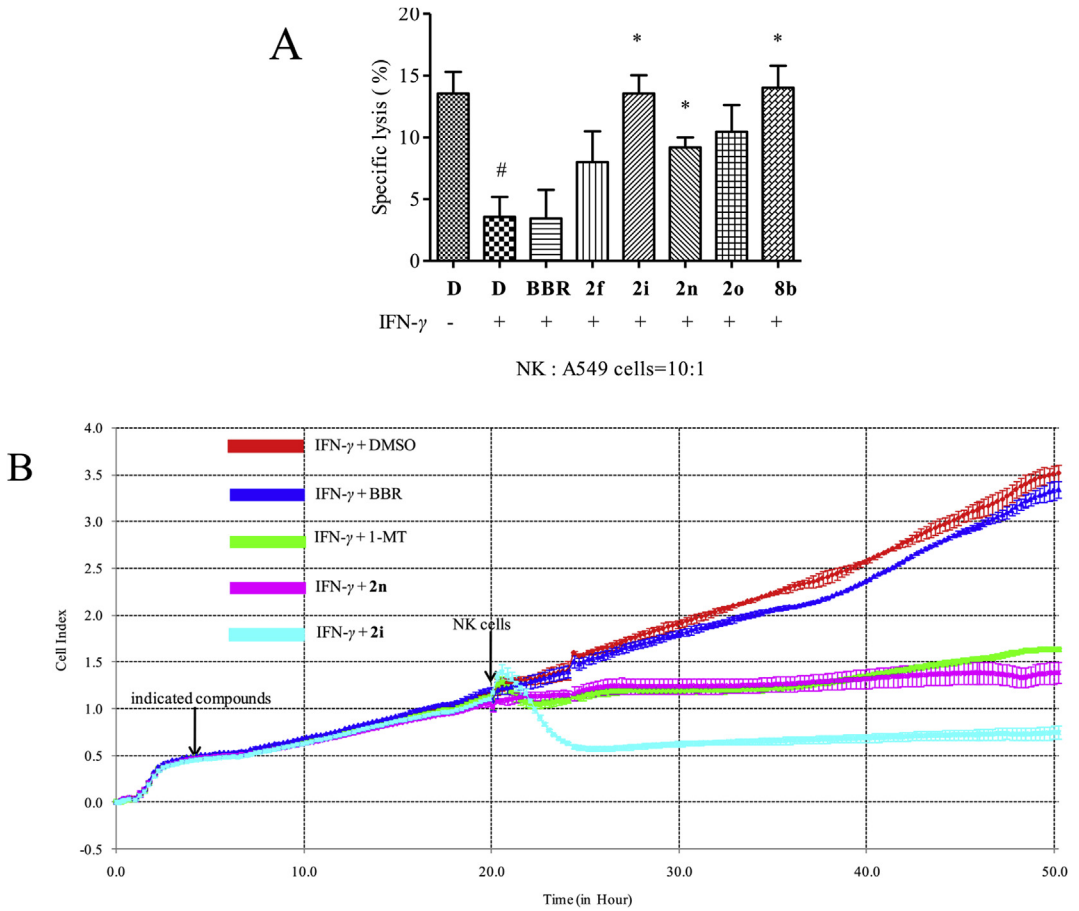


Fig. 5. BBR analogues induce killing activity of human NK cells toward A549 cells. A549 cells were pre-treated with the indicated compounds with or without IFN-γ (10 ng/mL) for 16 h. Cells were washed and then NK cells were co-cultured with A549 cells at 10:1 in RPMI 1640 plus 10% FBS. (A): After 4 h, the specific lysis of NK cells to A549 cells was determined by LDH releasing assay. All of the experiments were performed in triplicated and the results were calculated as means ± standard error. #$p < 0.05$ compared with untreated control group; *$p < 0.05$ compared with the IFN-γ group. (B): A549 cells were freshly plated in E16 plates and the killing activity of human NK cells toward A549 cells was measured by a cell impedance assay. Data was analyzed using the RTCA Software 1.2 program (Roche Diagnostics). All data is presented as the mean (n = 2) normalized cellular index ± SEM over time.

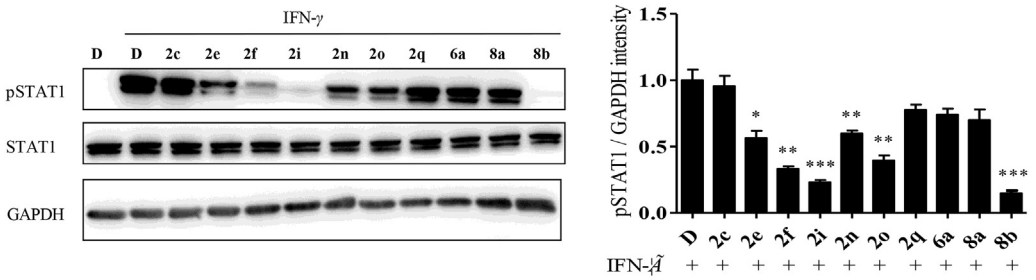


Fig. 6. BBR analogues suppressed IFN-γ-induced STAT1 phosphorylation. A549 cells were pre-treated with 10 μmol/L of the indicated compounds for 2 h, followed by IFN-γ (5 ng/mL) treatment for 24 h. The expression level of STAT1 and phosphorylation of STAT1 (Tyr701) were measured by western blot using corresponding antibodies. D: DMSO. GAPDH served as the loading control. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ compared with the IFN-γ group.

462.1148, found 462.1155.

*4.2.1.2. 2,3-Methylenedioxy-9-((2,3,4-trifluorobenzoyl)oxy)-10-methoxyprotoberberine chloride (**2b**).* Compound **1** (100 mg, 0.28 mmol) was treated with 2,3,4-trifluorobenzoyl chloride (40 μL, 0.31 mmol) according to the general procedure to give the desired product **2b** as a yellow solid, yield: 36%; Mp: 186–188 °C (dec.); ^{1}H NMR (500 MHz) δ 10.06 (s, 1H), 9.13 (s, 1H), 8.37 (d, $J = 9.2$ Hz, 1H), 8.30 (d, $J = 9.2$ Hz, 1H), 8.22–8.12 (m, 1H), 7.85 (s, 1H), 7.75–7.63 (m, 1H), 7.11 (s, 1H), 6.19 (s, 2H), 4.91 (t, $J = 6.3$ Hz, 2H), 4.06 (s, 3H), 3.22 (t, $J = 6.3$ Hz, 2H); ^{13}C NMR (126 MHz) δ 160.0, 154.7, 152.1, 150.9, 150.7, 148.4, 145.1, 140.5, 139.0, 133.6, 133.3, 131.5, 128.6, 128.0, 126.5, 121.6, 121.3, 121.0, 115.2, 114.2, 109.1, 106.2, 102.8, 58.0, 56.0, 26.8; HRMS: calcd for $C_{26}H_{17}F_3NO_5Cl$ $[M - Cl]^+$ 480.1053, found 480.1058.

*4.2.1.3. 2,3-Methylenedioxy-9-((6-fluoronicotinoyl)oxy)-10-methoxyprotoberberine chloride (**2c**).* Compound **1** (100 mg,

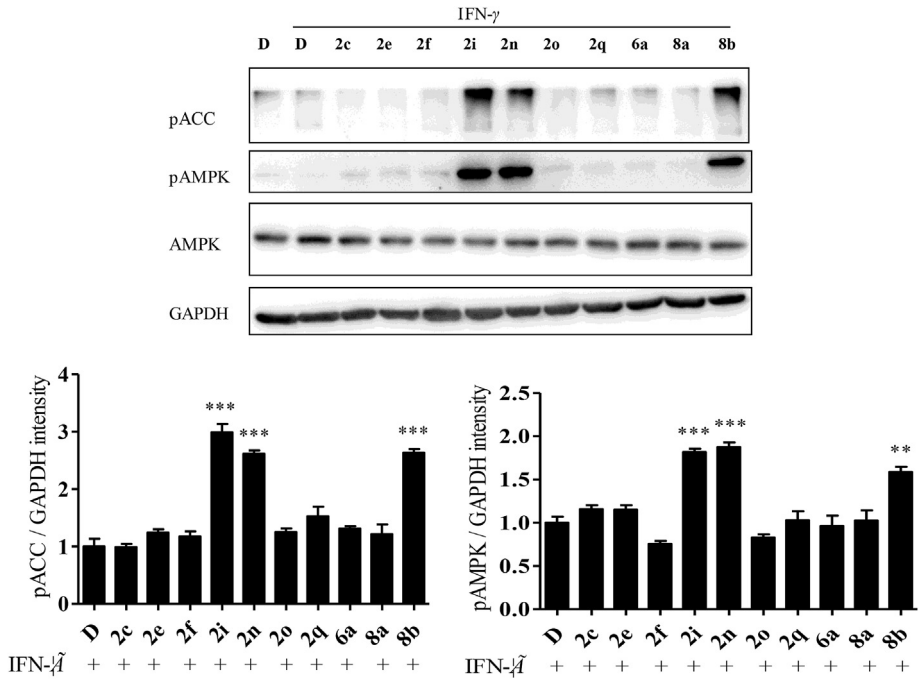


Fig. 7. BBR analogues induces AMPK activation. A549 cells were pre-treated with 10 μmol/L of the indicated compounds for 2 h, followed by IFN-γ (5 ng/mL) treatment for 24 h. The expression level of phosphor-ACC (Ser79), AMPK and phosphor-AMPK (Thr172) were measured by western blot using corresponding antibodies. D: DMSO. GAPDH served as the loading control. $^{**}p < 0.01$, $^{***}p < 0.001$ compared with the IFN-γ group.

0.28 mmol) was treated with 6-fluoropyridine-3-carbonyl chloride (54 mg, 0.34 mmol) according to the general procedure to give the desired product **2c** as a yellow solid, 39%; Mp: 187–189 °C (dec.); ^{1}H NMR (500 MHz) δ 10.07 (s, 1H), 9.17 (d, J = 2.4 Hz, 1H), 9.10 (s, 1H), 8.82–8.75 (m, 1H), 8.37 (d, J = 9.3 Hz, 1H), 8.29 (d, J = 9.3 Hz, 1H), 7.84 (s, 1H), 7.59–7.55 (m, 1H), 7.11 (s, 1H), 6.20 (s, 2H), 4.90 (t, J = 6.3 Hz, 2H), 4.05 (s, 3H), 3.21 (t, J = 6.4 Hz, 2H);^{13}C NMR (151 MHz) δ 166.2, 161.7, 151.3, 150.8, 150.5, 148.2, 144.9, 144.8, 138.8, 133.4, 133.2, 131.3, 127.7, 126.4, 123.5, 121.4, 121.1, 120.8, 111.0, 108.9, 106.0, 102.6, 57.8, 55.8, 26.6; HRMS: calcd for $C_{25}H_{18}FN_2O_5Cl$ $[M - Cl]^+$ 445.1194, found 445.1201.

*4.2.1.4. 2,3-Methylenedioxy-9-((3-methylsulfonyl-2-oxoimidazolidine-1-carbonyl)oxy) -10-methoxyprotoberberine chloride (**2d**).* Compound **1** (100 mg, 0.28 mmol) was treated with 1-chlorocarbonyl-3-methanesulfonyl-2-imidazolidinone (70 mg, 0.31 mmol) according to the general procedure to give the desired product **2d** as a yellow solid, yield: 34%; Mp: 147–149 °C (dec.); ^{1}H NMR (600 MHz) δ 9.96 (s, 1H), 9.03 (s, 1H), 8.30 (d, J = 9.4 Hz, 1H), 8.23 (d, J = 8.8 Hz, 1H), 7.79 (s, 1H), 7.09 (s, 1H), 6.16 (s, 2H), 4.91 (t, J = 6.3 Hz, 2H), 4.13 (s, 2H), 3.98–3.94 (m, 2H), 3.41 (s, 3H), 3.22 (t, J = 6.3 Hz, 3H); ^{13}C NMR (151 MHz) δ 151.1, 150.5, 150.1, 148.2, 147.9, 144.7, 138.8, 133.4, 132.6, 131.3, 127.8, 126.4, 121.5, 121.1, 120.8, 108.9, 106.0, 102.6, 57.8, 56.0, 41.6, 41.0, 29.3, 26.6; HRMS: calcd for $C_{24}H_{22}N_3O_8SCl$ $[M - Cl]^+$ 512.1122, found 512.1126.

*4.2.1.5. 2,3-Methylenedioxy-9-((3,3-dimethylbutanoyl)oxy)-10-methoxyprotoberberine chloride (**2e**).* Compound **1** (100 mg, 0.28 mmol) was treated with 2,2-dimethylbutyryl chloride (43 μL, 0.31 mmol) according to the general procedure to give the desired product **2e** as a yellow solid, yield: 37%; Mp: 205–207 °C (dec.); ^{1}H NMR (500 MHz) δ 9.47 (s, 1H), 9.10 (s, 1H), 8.30 (d, J = 9.2 Hz, 1H), 8.23 (d, J = 9.2 Hz, 1H), 7.83 (s, 1H), 7.12 (s, 1H), 6.19 (s, 2H), 4.98 (t, J = 6.3 Hz, 2H), 4.03 (s, 3H), 3.22 (t, J = 6.3 Hz, 2H), 1.83 (q, J = 7.4 Hz, 2H), 1.44 (s, 6H), 1.03 (t, J = 7.4 Hz, 3H); ^{13}C NMR (126 MHz) δ 175.3, 150.8, 150.6, 148.4, 144.4, 138.7, 134.4, 133.6, 131.6, 127.3, 126.5, 121.6, 121.4, 121.0, 109.1, 106.2, 102.8, 57.8, 56.4, 43.6, 33.5, 26.8, 24.9 (2), 9.6; HRMS: calcd for $C_{25}H_{26}NO_5Cl$ $[M - Cl]^+$ 420.1806, found 420.1813.

*4.2.1.6. 2,3-Methylenedioxy-9-((2-ethylbutanoyl)oxy)-10-methoxyprotoberberine chloride (**2f**).* Compound **1** (100 mg, 0.28 mmol) was treated with 2-ethylbutanoyl chloride (42 μL, 0.31 mmol) according to the general procedure to give the desired product **2f** as a yellow solid, yield: 27%; Mp: 200–201 °C (dec.); ^{1}H NMR (500 MHz) δ 9.72 (s, 1H), 9.09 (s, 1H), 8.30 (d, J = 9.2 Hz, 1H), 8.23 (d, J = 9.2 Hz, 1H), 7.83 (s, 1H), 7.11 (s, 1H), 6.19 (s, 2H), 4.96 (t, J = 6.3 Hz, 2H), 4.03 (s, 3H), 3.22 (t, J = 6.3 Hz, 2H), 2.85 (p, J = 6.6 Hz, 1H), 1.93–1.70 (m, 4H), 1.06 (t, J = 7.4 Hz, 6H); ^{13}C NMR (126 MHz) δ 173.2, 150.9, 150.6, 148.3, 144.7, 138.7, 134.1, 133.6, 131.5, 127.3, 126.5, 121.7, 121.3, 121.0, 109.1, 106.2, 102.8, 57.7, 56.2, 47.6, 26.8, 24.0 (2), 11.8 (2); HRMS: calcd for $C_{25}H_{26}NO_5Cl$ $[M - Cl]^+$ 420.1806, found 420.1816.

*4.2.1.7. 2,3-Methylenedioxy-9-((3,5,5-trimethylhexanoyl)oxy)-10-methoxyprotoberbe rine chloride (**2g**).* Compound **1** (100 mg, 0.28 mmol) was treated with 3,5,5-trimethylhexanoyl chloride (65 μL, 0.34 mmol) according to the general procedure to give the desired product **2g** as a yellow solid, 29%; Mp: 206–207 °C (dec.); ^{1}H NMR (500 MHz) δ 9.97 (s, 1H), 9.07 (s, 1H), 8.30 (d, J = 9.2 Hz, 1H), 8.22 (d, J = 9.2 Hz, 1H), 7.83 (s, 1H), 7.11 (s, 1H), 6.19 (s, 2H), 4.97 (t, J = 6.3 Hz, 2H), 4.03 (s, 3H), 3.23 (t, J = 6.3 Hz, 2H), 2.90 (dd, J = 15.6, 5.8 Hz, 1H), 2.69 (dd, J = 15.6, 8.1 Hz, 1H), 2.22–2.12 (m, J = 6.8 Hz, 1H), 1.45 (dd, J = 14.0, 4.1 Hz, 1H), 1.25 (dd, J = 14.0, 6.6 Hz, 1H), 1.14 (d, J = 6.6 Hz, 3H), 0.97 (s, 9H); ^{13}C NMR (126 MHz) δ 170.5, 151.0, 150.6, 148.3, 145.1, 138.7, 134.2133.6, 131.5, 127.3, 126.5, 121.8, 121.2, 121.0, 109.1, 106.2, 102.8, 57.8, 55.9, 50.6, 43.5, 31.45, 30.6 (3), 27.3, 26.8, 22.8; HRMS: calcd for $C_{28}H_{32}NO_5Cl$ $[M - Cl]^+$ 462.2275, found 462.2285.

Please cite this article in press as: Yan–Xiang-g. Wang, et al., Synthesis and biological evaluation of new berberine derivatives as cancer immunotherapy agents through targeting IDO1, European Journal of Medicinal Chemistry (2017), https://doi.org/10.1016/j.ejmech.2017.10.078

*4.2.1.8. 2,3-Methylenedioxy-9-((1-methylcyclopropane-1-carbonyl) oxy)-10-methoxy protoberberine chloride (**2h**).* Compound **1** (100 mg, 0.28 mmol) was treated with 1-methylcyclopro panecarboxylic acid (40 mg, 0.34 mmol) according to the general procedure to give the desired product **2h** as a brown solid, yield: 20%; Mp: 184–186 °C (dec.); ^{1}H NMR (500 MHz) δ 9.78 (s, 1H), 9.07 (s, 1H), 8.28 (d, J = 9.2 Hz, 1H), 8.20 (d, J = 9.2 Hz, 1H), 7.82 (s, 1H), 7.11 (s, 1H), 6.19 (s, 2H), 4.98 (t, J = 6.2 Hz, 2H), 4.04 (s, 3H), 3.22 (t, J = 6.2 Hz, 2H), 1.56 (s, 2H), 1.49 (s, 3H), 1.09 (d, J = 3.6 Hz, 2H); ^{13}C NMR (126 MHz) δ 173.6, 150.9, 150.6, 148.3, 144.9, 138.7, 134.5, 133.5, 131.5, 127.2, 126.5, 121.8, 121.3, 121.0, 109.1, 106.2, 102.8, 58.0, 56.0, 26.8, 19.6, 19.3, 18.1 (2); HRMS: calcd for $C_{24}H_{22}NO_5Cl$ $[M - Cl]^+$ 404.1493, found 404.1500.

*4.2.1.9. 2,3-Methylenedioxy-9-((2,2,3,3-tetramethylcyclopropane-1-carbonyl)oxy)-10- methoxyprotoberberine chloride (**2i**).* Compound **1** (100 mg, 0.28 mmol) was treated with 2,2,3,3-tetramethylcyclo propane-1-carbonyl chloride (48 μL, 0.31 mmol) according to the general procedure to give the desired product **2i** as a yellow solid, yield: 43%; Mp: 198–200 °C (dec.); ^{1}H NMR (500 MHz) δ 9.89 (s, 1H), 9.06 (s, 1H), 8.28 (d, J = 9.2 Hz, 1H), 8.19 (d, J = 9.2 Hz, 1H), 7.83 (s, 1H), 7.11 (s, 1H), 6.19 (s, 2H), 4.98 (t, J = 6.3 Hz, 2H), 4.02 (s, 3H), 3.23 (t, J = 6.3 Hz, 2H), 1.81 (s, 1H), 1.34 (s, 6H), 1.28 (s, 6H); ^{13}C NMR (126 MHz) δ 169.1, 150.9, 150.6, 148.3, 145.0, 138.6, 134.8, 133.5, 131.5, 126.9, 126.5, 121.9, 121.2, 121.1, 109.1, 106.2, 102.8, 57.7, 56.0, 35.2, 31.8 (2), 26.8, 23.8 (2), 17.2 (2); HRMS: calcd for $C_{27}H_{28}NO_5Cl$ $[M - Cl]^+$ 446.1962, found 446.1973.

*4.2.1.10. 2,3-Methylenedioxy-9-((cyclobutanecarbonyl)oxy)-10-methoxyprotoberbe rine chloride (**2j**).* Compound **1** (100 mg, 0.28 mmol) was treated with cyclobutanecarboxylic acid chloride (35 μL, 0.31 mmol) according to the general procedure to give the desired product **2j** as a brown solid, yield: 32%; Mp: 197–199 °C (dec.); ^{1}H NMR (500 MHz) δ 9.92 (s, 1H), 9.08 (s, 1H), 8.29 (d, J = 9.2 Hz, 1H), 8.21 (d, J = 9.2 Hz, 1H), 7.82 (s, 1H), 7.11 (s, 1H), 6.19 (s, 2H), 4.96 (t, J = 6.3 Hz, 2H), 4.05 (s, 3H), 3.82–3.73 (m, 1H), 3.22 (t, J = 6.3 Hz, 2H), 2.50–2.35 (m, 4H), 2.16–2.04 (m, 1H), 2.02–1.90 (m, 1H); ^{13}C NMR (126 MHz) δ 172.9, 151.0, 150.6, 148.3, 145.1, 138.7, 134.3, 133.5, 131.5, 127.2, 126.4, 121.8, 121.2, 121.0, 109.1, 106.2, 102.8, 57.9, 56.0, 37.7, 26.8, 25.4 (2), 18.6; HRMS: calcd for $C_{24}H_{22}NO_5Cl$ $[M - Cl]^+$ 404.1492, found 404.1496.

*4.2.1.11. 2,3-Methylenedioxy-9-((cyclohexanecarbonyl)oxy)-10-methoxyprotoberbe rine chloride (**2k**).* Compound **1** (100 mg, 0.28 mmol) was treated with cyclohexanecarboxylic acid chloride (41 μL, 0.31 mmol) according to the general procedure to give the desired product **2k** as a yellow solid, yield: 32%; Mp: 178–180 °C (dec.); ^{1}H NMR (500 MHz) δ 9.90 (s, 1H), 9.08 (s, 1H), 8.28 (d, J = 9.2 Hz, 1H), 8.21 (d, J = 9.2 Hz, 1H), 7.82 (s, 1H), 7.11 (s, 1H), 6.19 (s, 2H), 4.97 (t, J = 6.3 Hz, 2H), 4.03 (s, 3H), 3.23 (t, J = 6.3 Hz, 2H), 3.02–2.92 (m, 1H), 2.18–2.10 (m, 2H), 1.85–1.77 (m, 2H), 1.71–1.59 (m, 3H), 1.48–1.38 (m, 2H), 1.34 (td, J = 11.8, 3.8 Hz, 1H); ^{13}C NMR (126 MHz) δ 173.2, 150.9, 150.6, 148.3, 145.0, 138.7, 134.4, 133.5, 131.5, 127.2, 126.5, 121.8, 121.2, 121.0, 109.1, 106.2, 102.8, 57.9, 56.0, 42.5, 29.1 (2), 26.8, 26.0, 25.4 (2); HRMS: calcd for $C_{26}H_{26}NO_5Cl$ $[M - Cl]^+$ 432.1806, found 432.1809.

*4.2.1.12. 2,3-Methylenedioxy-9-((bicyclo[2.2.1]heptane-2-carbonyl) oxy)-10-methoxy protoberberine chloride (**2l**).* Compound **1** (100 mg, 0.28 mmol) was treated with bicyclo[2.2.1]heptane-2-carboxylic acid (48 mg, 0.34 mmol) according to the general procedure to give the desired product **2l** as a brown solid, yield: 31%; Mp: 202–204 °C (dec.); ^{1}H NMR (500 MHz) δ 9.90 (d, J = 5.3 Hz, 1H), 9.06 (s, 1H), 8.29 (d, J = 9.2 Hz, 1H), 8.21 (d, J = 9.2 Hz, 1H), 7.82 (s, 1H), 7.11 (s, 1H), 6.19 (s, 2H), 5.03–4.86 (m, 2H), 4.03 (s, 3H), 3.54–3.47 (m, 1H), 3.22 (t, J = 6.5 Hz, 2H), 2.83–2.77 (m, 1H), 2.36–2.32 (m, 1H), 1.90–1.81 (m, 1H), 1.73–1.53 (m, 5H), 1.48–1.41 (m, 1H), 1.33–1.19 (m, 1H); ^{13}C NMR (151 MHz) δ 172.3, 151.0, 150.9, 148.5, 143.8, 138.8, 134.4, 133.7, 130.6, 126.4, 125.5, 121.9, 120.6, 120.3, 107.9, 105.2, 102.3, 56.1, 55.9, 45.7, 40.7, 39.8, 37.0, 31.9, 28.8, 26.6, 24.2; HRMS: calcd for $C_{27}H_{26}NO_5Cl$ $[M - Cl]^+$ 444.1806, found 444.1809.

*4.2.1.13. 2,3-Methylenedioxy-9-((noradamantane-1-carbonyl)oxy)-10-methoxyproto berberine chloride (**2m**).* Compound **1** (100 mg, 0.28 mmol) was treated with 3-noradamantanecarboxylic acid (56 mg, 0.34 mmol) according to the general procedure to give the desired product **2m** as a yellow solid, yield: 36%; Mp: 201–203 °C (dec.); ^{1}H NMR (500 MHz) δ 9.62 (s, 1H), 9.08 (s, 1H), 8.29 (d, J = 9.2 Hz, 1H), 8.22 (d, J = 9.2 Hz, 1H), 7.84 (s, 1H), 7.12 (s, 1H), 6.19 (s, 2H), 4.98 (t, J = 6.3 Hz, 2H), 4.03 (s, 3H), 3.22 (t, J = 6.3 Hz, 2H), 3.08 (t, J = 6.9 Hz, 1H), 2.42–2.26 (m, 4H), 2.10–2.02 (m, 2H), 1.95–1.88 (m, 2H), 1.80–1.63 (m, 4H); ^{13}C NMR (126 MHz) δ 174.8, 150.8, 150.6, 148.4, 144.8, 138.6, 134.8, 133.6, 131.6, 127.2, 126.5, 121.7, 121.3, 121.0, 109.1, 106.2, 102.8, 58.0, 56.2, 54.4, 47.2 (2), 44.4, 43.8 (2), 37.6 (2), 34.7, 26.8; HRMS: calcd for $C_{29}H_{28}NO_5Cl$ $[M - Cl]^+$ 470.1962, found 470.1966.

*4.2.1.14. 2,3-Methylenedioxy-9-(2-(adamantan-1-yl)acetoxy)-10-methoxyberberine chloride (**2n**).* Compound **1** (100 mg, 0.28 mmol) was treated with 2-(1-adamantyl)acetyl chloride (66 mg, 0.31 mmol) according to the general procedure to give the desired product **2n** as a yellow solid, yield: 43%; Mp: 188–190 °C (dec.); ^{1}H NMR (500 MHz) δ 9.96 (s, 1H), 9.07 (s, 1H), 8.30 (d, J = 9.2 Hz, 1H), 8.21 (d, J = 9.2 Hz, 1H), 7.82 (s, 1H), 7.11 (s, 1H), 6.19 (s, 2H), 4.96 (t, J = 6.3 Hz, 2H), 4.05 (s, 3H), 3.23 (t, J = 6.3 Hz, 2H), 2.59 (s, 2H), 2.07–1.98 (m, 3H), 1.84–1.77 (m, 6H), 1.78–1.63 (m, 6H); ^{13}C NMR (126 MHz) δ 168.8, 150.9, 150.6, 148.3, 145.1, 138.7, 134.2, 133.6, 131.5, 127.3, 126.5, 121.8, 121.2, 121.0, 109.1, 106.2, 102.8, 57.7, 55.9, 48.7, 42.2 (3), 37.0 (3), 33.3, 28.7 (3), 26.8; HRMS: calcd for $C_{31}H_{32}NO_5Cl$ $[M - Cl]^+$ 498.2275, found 498.2278.

*4.2.1.15. 2,3-Methylenedioxy-9-((3-bromoadamantane-1-carbonyl) oxy)-10-methoxy protoberberine chloride (**2o**).* Compound **1** (100 mg, 0.28 mmol) was treated with 3-bromoadaman tane-1-carbonyl chloride (94 mg, 0.34 mmol) according to the general procedure to give the desired product **2o** as a yellow solid, yield: 39%; Mp: 191–193 °C (dec.); ^{1}H NMR (500 MHz) δ 9.63 (s, 1H), 9.08 (s, 1H), 8.29 (d, J = 9.2 Hz, 1H), 8.22 (d, J = 9.2 Hz, 1H), 7.83 (s, 1H), 7.12 (s, 1H), 6.19 (s, 2H), 4.99 (t, J = 6.3 Hz, 2H), 4.03 (s, 3H), 3.23 (t, J = 6.3 Hz, 2H), 2.78 (s, 2H), 2.45–2.35 (m, 4H), 2.34–2.26 (m, 2H), 2.26–2.13 (m, 4H), 1.84–1.74 (m, 2H); ^{13}C NMR (126 MHz) δ 173.0, 150.6 (2), 148.4, 144.6, 138.7, 134.3, 133.6, 131.6, 127.4, 126.5, 121.5, 121.4, 121.0, 109.1, 106.2, 102.8, 66.2, 58.0, 56.2, 49.4, 48.2 (2), 45.8, 37.1 (2), 34.3, 32.0 (2), 26.8; HRMS: calcd for $C_{30}H_{29}BrNO_5Cl$ $[M - Cl]^+$ 562.1224, found 562.1240.

*4.2.1.16. 2,3-Methylenedioxy-9-((1R,2R)-2-phenylcyclopropane-1-carbonyloxy)-10- methoxyprotoberberine chloride (**2p**).* Compound **1** (100 mg, 0.28 mmol) was treated with (1R,2R)-2-phenylcyclo propane-1-carbonyl chloride (45 μL, 0.31 mmol) according to the general procedure to give the desired product **2p** as a brown solid, yield: 34%; Mp: 172–174 °C (dec.); ^{1}H NMR (500 MHz) δ 9.95 (s, 1H), 9.09 (s, 1H), 8.30 (d, J = 9.2 Hz, 1H), 8.23 (d, J = 9.2 Hz, 1H), 7.83 (s, 1H), 7.41–7.32 (m, 4H), 7.32–7.25 (m, 1H), 7.11 (s, 1H), 6.19 (s, 2H), 4.96 (t, J = 6.3 Hz, 2H), 4.06 (s, 3H), 3.22 (t, J = 6.3 Hz, 2H), 2.85–2.79 (m, 1H), 2.45–2.39 (m, 1H), 1.84–1.77 (m, 1H), 1.77–1.71 (m, 1H); ^{13}C NMR (126 MHz) δ 170.8, 150.9, 150.6, 148.3, 145.1, 140.1, 138.7, 134.2, 133.5, 131.5, 129.2 (2), 127.4, 127.4, 126.9 (2), 126.5, 121.7, 121.3, 121.0, 109.1, 106.2, 102.8, 58.0, 56.0, 27.6, 26.8,

Please cite this article in press as: Yan–Xiang-g. Wang, et al., Synthesis and biological evaluation of new berberine derivatives as cancer immunotherapy agents through targeting IDO1, European Journal of Medicinal Chemistry (2017), https://doi.org/10.1016/j.ejmech.2017.10.078

24.6, 18.8; HRMS: calcd for $C_{29}H_{24}NO_5Cl$ [M − Cl]$^+$ 466.1649, found 466.1651.

*4.2.1.17. 2,3-Methylenedioxy-9-((2-methyl-2-phenylpropanoyl)oxy)-10-methoxyproto berberine chloride (**2q**).* Compound **1** (100 mg, 0.28 mmol) was treated with 2-methyl-2-phenyl propanoyl chloride (57 μL, 0.31 mmol) according to the general procedure to give the desired product **2q** as a yellow solid, yield: 35%; Mp: 198–200 °C (dec.); ^{1}H NMR (500 MHz) δ 9.23 (s, 1H), 9.08 (s, 1H), 8.25 (d, J = 9.2 Hz, 1H), 8.20 (d, J = 9.2 Hz, 1H), 7.82 (s, 1H), 7.66–7.61 (m, 2H), 7.52–7.47 (m, 2H), 7.41–7.35 (m, 1H), 7.12 (s, 1H), 6.19 (s, 2H), 4.90 (t, J = 6.3 Hz, 2H), 3.95 (s, 3H), 3.22 (t, J = 6.3 Hz, 2H), 1.84 (s, 6H); ^{13}C NMR (126 MHz) δ 174.1, 151.0, 150.7, 148.4, 144.2, 144.2, 138.7, 134.0, 133.6, 131.5, 129.2 (2), 127.8, 127.5, 126.8 (2), 126.6, 121.4, 121.4, 120.9, 109.09, 106.2, 102.8, 57.6, 56.5, 47.6 (2), 26.9, 26.8; HRMS: calcd for $C_{29}H_{26}NO_5Cl$ [M − Cl]$^+$ 468.1806, found 468.1809.

*4.2.1.18. 2,3-Methylenedioxy-9-((1-methyl-1H-imidazole-4-yl)sulfonyloxy)-10-methoxy protoberberine chloride (**3a**).* Compound **1** (100 mg, 0.28 mmol) was treated with 1-methyl-1H-imidazole-4-sulfonyl chloride (45 mg, 0.31 mmol) according to the general procedure to give the desired product **3a** as a yellow solid, yield: 28%; Mp: 181–183 °C (dec.); ^{1}H NMR (500 MHz) δ 9.54 (s, 1H), 9.07 (s, 1H), 8.30 (d, J = 9.2 Hz, 1H), 8.25 (d, J = 9.2 Hz, 1H), 8.22 (s, 1H), 8.05 (s, 1H), 7.82 (s, 1H), 7.13 (s, 1H), 6.19 (s, 2H), 4.88 (t, J = 6.3 Hz, 2H), 3.95 (s, 3H), 3.77 (s, 3H), 3.21 (t, J = 6.3 Hz, 2H); ^{13}C NMR (126 MHz) δ 152.7, 150.6, 148.2, 144.7, 141.6, 138.8, 133.9, 133.7, 132.1, 131. 3, 129.6, 128.6, 126.8, 122.0, 121.2, 120.7, 108.9, 106.0, 102.7, 57.7, 56.1, 34.5, 26.7; HRMS: calcd for $C_{23}H_{20}N_3O_6SCl$ [M − Cl]$^+$ 466.1067, found 466.1073.

*4.2.2. General synthesis procedure for synthesis of compounds **6a–c***

The solution of BBR (7.4 g, 20 mmol) in 2,4-dimethoxybenzylamine (15 mL, 78 mmol) was stirred at 120 °C for 6–8 h. The mixture was cooled to room temperature and washed with acetone (3 × 50 mL) to remove the remaining amine. The reside was purified by flash chromatography over silica gel using CH_2Cl_2/CH_3OH (96.5:3.5) as the gradient eluent to afford compound **4** (3.5 g, 37%). Mp: 239–240 °C (Dec.); ^{1}H NMR (500 MHz) δ 9.98 (s, 1H), 8.73 (s, 1H), 7.88 (d, J = 8.8, 1H), 7.77 (s, 1H), 7.52 (d, J = 8.8 Hz, 1H), 7.14 (d, J = 8.3 Hz, 1H), 7.09 (s, 1H), 6.52 (d, J = 2.2 Hz, 1H), 6.44 (t, J = 6.5 Hz, 1H), 6.42–6.39 (m, 1H), 6.17 (s, 2H), 4.80 (t, J = 6.2 Hz, 2H), 4.66 (d, J = 6.3 Hz, 2H), 3.87 (s, 3H), 3.77 (s, 3H), 3.71 (s, 3H), 3.21 (t, J = 6.3 Hz, 2H); ^{13}C NMR (126 MHz) δ 160.5, 158.5, 150.1, 148.2, 148.1, 147.1, 137.5, 136.3, 133.5, 130.9, 130.3, 124.8, 121.2, 120.5, 120.3, 118.0, 117.7, 109.1, 105.9, 104.8, 102.6, 98.9, 57.5, 56.0, 55.8, 55.6, 47.0, 27.3; HRMS: calcd for $C_{28}H_{27}N_2O_5Cl$ [M − Cl]$^+$ 471.1914, found 471.1919.

Compound **4** (3 g, 6.4 mmol) was dissolved in CH_3OH, and hydrochloric acid 3 mL was added. The mixture was stirred for 5–6 h, filtered and washed with 80% ethanol to afford compound **5** (1.8 g, 80%). Mp: 212–214 °C (Dec.); ^{1}H NMR (500 MHz) δ 10.19 (s, 1H), 8.64 (s, 1H), 7.84 (d, J = 8.6 Hz, 1H), 7.76 (s, 1H), 7.32 (d, J = 8.6 Hz, 1H), 7.08 (s, 1H), 6.89 (s, 2H), 6.16 (s, 2H), 4.70 (t, J = 6.3 Hz, 2H), 3.98 (s, 3H), 3.20 (t, J = 6.3 Hz, 2H); ^{13}C NMR (126 MHz) δ 149.8, 148.0, 147.0, 143.9, 138.1, 135.4, 132.1, 130.4, 123.0, 121.2, 119.8, 113.7, 113.3, 108.9, 105.6, 102.4, 56.9, 55.1, 27.2; HRMS: calcd for $C_{19}H_{17}N_2O_3Cl$ [M − Cl]$^+$ 321.12337, found 321.12352.

To a stirred solution of **5** (100 mg, 0.28 mmol) and pyridine (100 μL, 1.24 mmol) in anhydrous CH_2Cl_2 (5 mL), the RCOCl (2–3 eq) was added, and refluxed for 10–12 h. The solvent was removed by evaporation and purified by flash chromatography over silica gel using CH_2Cl_2/CH_3OH (95:5) as the gradient eluent to give **6a–c**.

*4.2.2.1. 2,3-Methylenedioxy-9-(adamantane-1-carboxamido)-10-methoxyprotoberbe rine chloride (**6a**).* Compound **5** (100 mg, 0.28 mmol) was treated with adamantane-1-carbonyl chloride (167 mg, 0.84 mmol) according to the general procedure to give the desired product **6a** as a yellow solid, yield: 35%; Mp: 242–243 °C (dec.); ^{1}H NMR (500 MHz) δ 9.40 (s, 1H), 9.29 (d, J = 4.6 Hz, 1H), 8.21 (s, 2H), 7.82 (s, 1H), 7.11 (s, 1H), 6.18 (s, 2H), 5.00 (t, J = 6.3 Hz, 2H), 4.07–3.96 (m, 3H), 3.21 (t, J = 6.3 Hz, 2H), 2.07 (s, 9H), 1.76 (s, 6H); ^{13}C NMR (126 MHz) δ 177.9, 155.2, 150.3, 148.1, 146.0, 137.8, 133.6, 131.2, 127.9, 125.7, 124.9, 122.9, 121.3, 120.9, 108.9, 106.0, 102.5, 57.5, 56.0, 41.3, 38.9, 36.6 (3), 28.2 (3), 26.8 (3); HRMS: calcd for $C_{30}H_{31}N_2O_4^+Cl$ [M − Cl]$^+$ 483.2278, found 483.2280.

*4.2.2.2. 2,3-Methylenedioxy-9-(3,3-dimethylbutanamido)-10-methoxyprotoberberine chloride (**6b**).* Compound **5** (100 mg, 0.28 mmol) was treated with 3,3-dimethylbutyryl chloride (116 μL, 0.84 mmol) according to the general procedure to give the desired product **6b** as a yellow solid, yield: 37%; Mp: 222–224 °C (dec.); ^{1}H NMR (500 MHz) δ 10.00 (s, 1H), 9.62 (s, 1H), 8.23 (d, J = 9.2 Hz, 1H), 8.20 (d, J = 9.2 Hz, 1H), 7.82 (s, 1H), 7.11 (s, 1H), 6.19 (s, 2H), 4.94 (t, J = 6.4 Hz, 2H), 4.03 (s, 3H), 3.22 (t, J = 6.4 Hz, 2H), 2.42 (s, 2H), 1.11 (s, 9H); ^{13}C NMR (126 MHz) δ 171.6, 154.9, 150.5, 148.3, 146.9, 138.0, 133.8, 131.3, 127.8, 125.9, 124.4, 122.7, 121.3, 121.1, 109.1, 106.1, 102.8, 57.5, 56.0, 49.6, 31.4 (3), 30.4, 26.97.; HRMS: calcd for $C_{25}H_{27}N_2O_4Cl$ [M − Cl]$^+$ 419.1965, found 419.1966.

*4.2.2.3. 2,3-Methylenedioxy-9-(2,2-dimethylbutanamido)-10-methoxyprotoberberine chloride (**6c**).* Compound **5** (100 mg, 0.28 mmol) was treated with 2,2-dimethylbutyryl chloride (77 μL, 0.56 mmol) according to the general procedure to give the desired product **6c** as a yellow solid, yield: 33%; Mp: 247–249 °C (dec.); ^{1}H NMR (600 MHz) δ 9.43 (s, 1H), 9.25 (s, 1H), 8.99 (s, 1H), 8.20 (s, 2H), 7.79 (s, 1H), 7.08 (s, 1H), 6.15 (s, 2H), 4.95 (s, 2H), 3.98 (s, 3H), 1.71 (d, J = 9.0 Hz, 2H), 1.30 (s, 6H), 0.93 (t, J = 7.6 Hz, 3H); ^{13}C NMR (151 MHz) δ 177.8, 155.2, 150.3, 148.1, 145.9, 137.8, 133.7, 131.1, 127.9, 125.7, 124.9, 122.9, 121.3, 120.9, 108.9, 106.0, 102.5, 57.3, 56.1, 43.2, 33.6, 26.8, 25.2 (2), 9.5; HRMS: calcd for $C_{25}H_{27}N_2O_4Cl$ [M − Cl]$^+$ 419.1965, found 419.1966.

*4.2.3. General synthesis procedure for synthesis of compounds **8a** and **8b***

PMT (3.87 g, 10 mmol) was heated at 195–210 °C for 10–15 min under vacuum (30–40 mmHg) to afford the black oil, which was acidified with ethanol/concentrated HCl (95:5). The solvent was removed by evaporation, the residue was collected and then purified by flash chromatography over silica gel using CH_2Cl_2/CH_3OH as the gradient eluent, affording the title compound **7** (3.2 g, 86%) as a yellow solid.

To a stirred solution of compound **7** (100 mg, 0.29 mmol) in anhydrous CH_3CN, triethylamine (175 μL, 1.26 mmol) was added and heated to 70 °C. Then the 2-(1-adamantyl)acetyl chloride (66 mg, 0.31 mmol) was added and stirred for 5–6 h. The mixture was cooled to precipitate completely, filtrated and washed by CH_2Cl_2 to afford compounds **8a–b**.

*4.2.3.1. 2,3,10-trimethoxy-9-((adamantane-1-carbonyl)oxy)protoberberine chloride (**8a**).* Compound **7** (100 mg, 0.29 mmol) was treated with adamantane-1-carbonyl chloride (61 mg, 0.31 mmol) according to the general procedure to give the desired product **8a** as a brown solid, yield: 25%; Mp: 158–160 °C (dec.); ^{1}H NMR (500 MHz) δ 9.51 (s, 1H), 9.20 (s, 1H), 8.29 (d, J = 9.2 Hz, 1H), 8.25 (d, J = 9.2 Hz, 1H), 7.76 (s, 1H), 7.12 (s, 1H), 5.00 (t, J = 6.3 Hz, 2H), 4.03 (s, 3H), 3.96 (s, 3H), 3.88 (s, 3H), 3.24 (t, J = 6.3 Hz, 2H), 2.18 (d, J = 3.0 Hz, 6H), 2.15–2.10 (m, 3H), 1.83–1.74 (m, 6H); ^{13}C NMR (126 MHz) δ 174.8, 152.2, 150.6, 149.4, 144.5, 138.9, 134.5, 133.7,

Please cite this article in press as: Yan–Xiang-g. Wang, et al., Synthesis and biological evaluation of new berberine derivatives as cancer immunotherapy agents through targeting IDO1, European Journal of Medicinal Chemistry (2017), https://doi.org/10.1016/j.ejmech.2017.10.078

129.4, 127.1, 126.5, 121.5, 121.0, 119.5, 111.9, 109.4, 57.9, 56.8, 56.5, 56.5, 41.6, 38.9 (2), 36.5 (2), 27.9 (2), 26.5; HRMS: calcd for $C_{31}H_{34}NO_5Cl$ $[M - Cl]^+$ 500.2432, found 500.2432.

*4.2.3.2. 2,3,10-trimethoxy-9-(2-(adamantan-1-yl)acetoxy)protoberberine chloride (**8b**).* Compound **7** (100 mg, 0.29 mmol) was treated with 2-(1-adamantyl)acetyl chloride (66 mg, 0.31 mmol) according to the general procedure to give the desired product **8b** as a brown solid, yield: 33%; Mp: 142–144 °C (dec.); ^{1}H NMR (600 MHz) δ 9.87 (s, 1H), 9.12 (s, 1H), 8.27 (d, J = 9.2 Hz, 1H), 8.21 (d, J = 9.2 Hz, 1H), 7.71 (s, 1H), 7.07 (s, 1H), 4.92 (t, J = 6.4 Hz, 2H), 4.02 (s, 3H), 3.92 (s, 3H), 3.85 (s, 3H), 3.21 (t, J = 6.4 Hz, 2H), 2.55 (s, 2H), 2.03–1.97 (m, 3H), 1.79–1.75 (m, 6H), 1.72–1.62 (m, 6H); ^{13}C NMR (151 MHz) δ 168.5, 152.1, 150.6, 149.2, 144.8, 138.8, 133.9, 133.5, 129.2, 127.0, 126.4, 121.5, 120.7, 119.2, 111.7, 109.3, 57.4, 56.6, 56.3, 56.0, 48.5, 42.0 (3), 36.7 (3), 33.0, 28.5 (3), 26.2; HRMS: calcd for $C_{32}H_{36}NO_5Cl$ $[M - Cl]^+$ 514.2588, found 514.2590.

*4.2.4. Synthesis of 3-((adamantane-1-carbonyl)oxy)-2,9,10-trimethoxyprotoberberine chloride (**9a**)*

To a stirred solution of JTH (100 mg, 0.29 mmol) in anhydrous CH_3CN, triethylamine (175 μL, 1.26 mmol) was added and heated to 70 °C. Then the adamantane-1-carbonyl chloride (61 mg, 0.31 mmol) was added and stirred for 5–6 h. The mixture was cooled to precipitate completely, filtrated and washed by CH_2Cl_2 to afford compound **9a** as a yellow solid, yield: 34%; Mp: 186–188 °C (dec.); ^{1}H NMR (500 MHz) δ 9.98 (s, 1H), 9.21 (s, 1H), 8.27 (d, J = 9.1 Hz, 1H), 8.09 (d, J = 9.1 Hz, 1H), 7.91 (s, 1H), 7.24 (s, 1H), 4.99 (t, J = 6.3 Hz, 2H), 4.12 (s, 3H), 4.10 (s, 3H), 3.95 (s, 3H), 3.24 (t, J = 6.3 Hz, 2H), 2.08–2.04 (m, 3H), 2.04–2.00 (m, 6H), 1.78–1.71 (m, 6H); ^{13}C NMR (126 MHz) δ 175.2, 151.5, 151.4, 146.6, 144.4, 142.5, 137.5, 133.3, 128.6, 127.4, 125.9, 124.4, 123.5, 122.4, 121.9, 110.7, 62.6, 57.7, 57.3, 56.0, 41.2, 39.0 (3), 36.5 (3), 27.9 (3), 26.06; HRMS: calcd for $C_{31}H_{34}NO_5Cl$ $[M - Cl]^+$ 500.2432, found 500.2434.

*4.2.5. Synthesis of 3-(2-(adamantan-1-yl)acetoxy)-2,9,10-trimethoxyprotoberberine chloride (**9b**)*

To a stirred solution of JTH (100 mg, 0.29 mmol) in anhydrous CH_3CN, triethylamine (175 μL, 1.26 mmol) was added and heated to 70 °C. Then the 2-(1-adamantyl)acetyl chloride (66 mg, 0.31 mmol) was added and stirred for 5–6 h. The mixture was cooled to precipitate completely, filtrated and washed by CH_2Cl_2 to afford compound **9b** as a yellow solid, yield: 33%; Mp: 149–151 °C (dec.); ^{1}H NMR (500 MHz) δ 9.99 (s, 1H), 9.26 (s, 1H), 8.26 (d, J = 9.1 Hz, 1H), 8.11 (d, J = 9.1 Hz, 1H), 7.94 (s, 1H), 7.25 (s, 1H), 5.00 (t, J = 6.3 Hz, 2H), 4.12 (s, 3H), 4.09 (s, 3H), 3.97 (s, 3H), 3.25 (t, J = 6.3 Hz, 2H), 2.34 (s, 2H), 2.04–1.96 (m, 3H), 1.77–1.64 (m, 12H); ^{13}C NMR (126 MHz) δ 169.3, 151.5, 151.4, 146.6, 144.4, 142.1, 137.4, 133.3, 128.6, 127.3, 126.1, 124.4, 123.6, 122.4, 122.0, 110.7, 62.6, 57.7, 57.2, 56.6, 48.4, 45.9, 42.2 (3), 36.9 (3), 33.3, 28.7 (3), 26.1; HRMS: calcd for $C_{32}H_{36}NO_5Cl$ $[M - Cl]^+$ 514.2588, found 514.2587.

4.3. Cell culture and western blot analysis

A549 human lung cancer cell lines were procured from ATCC. The cells were cultured in DME/F-12 (Hyclone, UT, USA) supplemented with 10% fetal bovine serum (Hyclone, UT, USA), 100 U/ml penicillin, and 100 μg/mL streptomycin sulfate, and incubated at 37 °C in a humidified atmosphere with 5% CO_2. Actived NK cells were purchased from Stemcell Technologies (Vancouver, BC, Canada).

Western blot was performed as described previously [23]. Briefly, A549 cells were washed with PBS and lysed in M2 lysis buffer (20 mM Tris-HCl, pH 7.5, 150 mM NaCl, 10 mM β-glycerophosphate, 5 mM EGTA, 1 mM sodium pyrophoshate, 5 mM NaF, 1 mM Na_3VO_4, 0.5% Triton X-100, and 1 mM DTT) supplemented with protease inhibitor cocktail (Sigma P8340). Proteins were separated by SDS-PAGE and were electrically transferred to a PVDF membrane. The membrane was probed with the appropriate primary antibody and with a HRP-conjugated secondary antibody. Blots were visualized by Tanon 5200 system (Tanon, Shanghai, China).

4.4. MTT assay

The effect of the indicated compounds on the cell viability of A549 cells was evaluated using the MTT assay [24]. Briefly, Cells were seeded (5×10^3/well) into 96-well plate and the investigated compounds were added at indicated concentrations for 48 h. Next, MTT solution at a concentration of 5 mg/mL was added to each well. After subsequent 4 h, the culture medium was removed and formazan crystals were dissolved with 150 μL DMSO. Finally, the absorbance was measured at 570 nm using a microplate reader (Multiskan FC, Thermo, USA).

4.5. IDO1 promoter activity assay

A549 cells were co-transfected with pGL4-IDO1-luc with the pRL-CMV plasmid using the Vigofect transfection reagent (Beijing, China) as instructed by the manufacturers. After 24 h of transfection, cells were pretreated with the indicated compounds (10 μmol/L) for 2 h and then stimulated with or without IFN-γ (10 ng/mL) for 24 h. Following IFN-γ treatment, the cells were lysed, and the luciferase activity was determined using the luciferase reporter assay system (Promega, Madison, CA, USA) according to manufacturer's protocols. The luciferase activity values were normalized to the expression of the *Renilla* luciferase, and presented as the percentages of luciferase activity.

4.6. LDH release assay

Cytotoxicity of human NK cells against A549 cells was assessed with LDH release assay, as previously described [25]. Briefly, A549 cells were freshly plated at 5×10^3/well in 96-well plates and pretreated with the indicated compounds (10 μmol/L) for 2 h and then stimulated with IFN-γ (10 ng/mL) for 16 h. A549 cells were washed and then were cocultured with NK cells at 1:10 in triplicate in RPMI 1640 plus 10% FBS. Four hours later, cytotoxicity assay was conducted using non-radioactive lactate dehydrogenase (LDH) release using a cytotoxicity detection kit (CytoTox 96, Promega, Madison, WI, USA) as the manufacturer's instructions. Spontaneous release and maximum release were determined by incubating target cells without effector cells in medium alone or in 0.5% NP40, respectively. The percent cytotoxicity was calculated as follows: (experimental release-spontaneous release)/(maximum release-spontaneous release) × 100%.

4.7. Cell impedance assay

A549 cells were freshly plated at 5×10^3/well in E16 plates (Roche Diagnostics, Basel, Switzerland). The plate was connected to an xCELLigence RTCA SP instrument (Roche Diagnostics) within a humidified cell culture incubator. After 4 h incubation and A549 cells were treated with the indicated compounds (10 μmol/L) with IFN-γ (10 ng/mL). After 16 h, A549 cells were washed and cocultured with NK cells at 1:10 in triplicate in RPMI 1640 plus 10% FBS. Data was analyzed using the RTCA Software 1.2 program (Roche Diagnostics). All data is presented as the mean normalized cellular index ± SEM over time.

Please cite this article in press as: Yan–Xiang-g. Wang, et al., Synthesis and biological evaluation of new berberine derivatives as cancer immunotherapy agents through targeting IDO1, European Journal of Medicinal Chemistry (2017), https://doi.org/10.1016/j.ejmech.2017.10.078

Acknowledgments

This work was supported by the CAMS initiative for innovative medicine (2016-12M-1-011) and the National Natural Science Foundation of China (81321004, 81473248 and 81773782).

Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.ejmech.2017.10.078.

References

[1] A. Hoos, Development of immuno-oncology drugs - from CTLA4 to PD1 to the next generations, Nat. Rev. Drug Discov. 15 (2016) 235–247.
[2] L. Chen, X. Han, Anti-PD-1/PD-L1 therapy of human cancer: past, present, and future, J. Clin. Invest. 125 (2015) 3384–3391.
[3] D.M. Pardoll, The blockade of immune checkpoints in cancer immunotherapy, Nat. Rev. Cancer 12 (2012) 252–264.
[4] C.G. Tara, H. Omid, C.S. David, M.B. Todd, S.W. Jeffrey, J.L. Jason, S.B. Ani, R.K. David, Z. Yufan, M. Janet, L. Lance, F.G. Thomas, Preliminary results from a Phase I/II study of epacadostat (incb024360) in combination with pembrolizumab in patients with selected advanced cancers, J. Immunother. Cancer 3 (2015). O7.
[5] L. Zhai, S. Spranger, D.C. Binder, G. Gritsina, K.L. Lauing, F.J. Giles, D.A. Wainwright, Molecular pathways: targeting IDO1 and other tryptophan dioxygenases for cancer immunotherapy, Clin. Cancer Res. 21 (2015) 5427–5433.
[6] A.E. Vilgelm, D.B. Johnson, A. Richmond, Combinatorial approach to cancer immunotherapy: strength in numbers, J. Leukoc. Biol. 100 (2016) 275–290.
[7] A.L. Mellor, D.H. Munn, IDO expression by dendritic cells: tolerance and tryptophan catabolism, Nat. Rev. Immunol. 4 (2004) 762–774.
[8] Y.H. Li, P. Yang, W.J. Kong, Y.X. Wang, C.Q. Hu, Z.Y. Zuo, Y.M. Wang, H. Gao, L.M. Gao, Y.C. Feng, N.N. Du, Y. Liu, D.Q. Song, J.D. Jiang, Berberine analogues as a novel class of the low-density-lipoprotein receptor up-regulators: synthesis, structure-activity relationships, and cholesterol-lowering efficacy, J. Med. Chem. 52 (2009) 492–501.
[9] Y.X. Wang, W.J. Kong, Y.H. Li, S. Tang, Z. Li, Y.B. Li, Y.Q. Shan, C.W. Bi, J.D. Jiang, D.Q. Song, Synthesis and structure-activity relationship of berberine analogues in LDLR up-regulation and AMPK activation, Bioorg Med. Chem. 20 (2012) 6552–6558.
[10] Y.X. Wang, H.G. Fu, Y.H. Li, J.D. Jiang, D.Q. Song, Synthesis and biological evaluation of 8-substituted berberine derivatives as novel anti-mycobacterial agents, Acta Pharm. Sin. B 2 (2012) 581–587.
[11] J. Yao, W. Kong, J. Jiang, Learning from berberine: treating chronic diseases through multiple targets, Sci. China Life Sci. 58 (2015) 854–859.
[12] E. Heidarian, M. Rafieian-Kopaei, A. Khoshdel, M. Bakhshesh, Metabolic effects of berberine on liver phosphatidate phosphohydrolase in rats fed on high lipogenic diet: an additional mechanism for the hypolipidemic effects of berberine, Asian Pac J. Trop. Biomed. 4 (2014) S429–S435.
[13] P. Yang, D.Q. Song, Y.H. Li, W.J. Kong, Y.X. Wang, L.M. Gao, S.Y. Liu, R.Q. Cao, J.D. Jiang, Bioorg. Med. Chem. Lett. 18 (2008) 4675–4677.
[14] Y.X. Wang, Y.P. Wang, H. Zhang, W.J. Kong, Y.H. Li, F. Liu, R.M. Gao, T. Liu, J.D. Jiang, D.Q. Song, Bioorg. Med. Chem. Lett. 19 (2009) 6004–6008.
[15] J.E. Darnell Jr., I.M. Kerr, G.R. Stark, Jak-STAT pathways and transcriptional activation in response to IFNs and other extracellular signaling proteins, Science 264 (1994) 1415–1421.
[16] J. Du, J. Zeng, X. Ou, X. Ren, S. Cai, Methylglyoxal downregulates Raf-1 protein through a ubiquitination-mediated mechanism, Int. J. Biochem. Cell Biol. 38 (2006) 1084–1091.
[17] A. Nerstedt, E. Cansby, M. Amrutkar, U. Smith, M. Mahlapuu, Pharmacological activation of AMPK suppresses inflammatory response evoked by IL-6 signalling in mouse liver and in human hepatocytes, Mol. Cell. Endocrinol. 375 (2013) 68–78.
[18] M. Dandapani, D.G. Hardie, AMPK: opposing the metabolic changes in both tumour cells and inflammatory cells? Biochem. Soc. Trans. 41 (2013) 687–693.
[19] G.R. Steinberg, B.E. Kemp, AMPK in health and disease, Physiol. Rev. 89 (2009) 1025–1078.
[20] S.P. Davies, N.R. Helps, P.T. Cohen, D.G. Hardie, 5'-AMP inhibits dephosphorylation, as well as promoting phosphorylation, of the AMP-activated protein kinase. Studies using bacterially expressed human protein phosphatase-2C alpha and native bovine protein phosphatase-2AC, FEBS Lett. 377 (1995) 421–425.
[21] J.S. Oakhill, R. Steel, Z.P. Chen, J.W. Scott, N. Ling, S. Tam, B.E. Kemp, AMPK is a direct adenylate charge-regulated protein kinase, Science 332 (2011) 1433–1435.
[22] K. Marcinko, G.R. Steinberg, The role of AMPK in controlling metabolism and mitochondrial biogenesis during exercise, Exp. Physiol. 99 (2014) 1581–1585.
[23] N. Zhang, L. Liu, Y. Dou, D. Song, H. Deng, Glycogen synthase kinase-3beta antagonizes ROS-induced hepatocellular carcinoma cell death through suppression of the apoptosis signal-regulating kinase 1, Med. Oncol. 33 (2016) 60.
[24] L. Liu, N. Zhang, Y. Dou, G. Mao, C. Bi, W. Pang, X. Liu, D. Song, H. Deng, Lysosomal dysfunction and autophagy blockade contribute to IMB-6G-induced apoptosis in pancreatic cancer cells, Sci. Rep. 7 (2017) 41862.
[25] T. Hayashi, T. Hideshima, M. Akiyama, N. Raje, P. Richardson, D. Chauhan, K.C. Anderson, Ex vivo induction of multiple myeloma-specific cytotoxic T lymphocytes, Blood 102 (2003) 1435–1442.

Please cite this article in press as: Yan–Xiang-g. Wang, et al., Synthesis and biological evaluation of new berberine derivatives as cancer immunotherapy agents through targeting IDO1, European Journal of Medicinal Chemistry (2017), https://doi.org/10.1016/j.ejmech.2017.10.078