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Design, synthesis and anticancer activity of matrine–1*H*-1,2,3-triazole–chalcone conjugates

Lihui Zhao, Lina Mao, Ge Hong, Xiaojiao Yang, Tianjun Liu *

Institute of Biomedical Engineering, Chinese Academy of Medical Sciences & Peking Union Medical College, Tianjin Key Laboratory of Biomedical Materials, Tianjin 300192, China

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ABSTRACT

A series of novel matrine–1*H*-1,2,3-triazole–chalcone conjugates was synthesized and their anticancer activity against A549, Bel-7402, Hela, and MCF-7 cancer cells was evaluated. Most of the conjugates displayed higher potency than their components. Compounds **6h** and **6i** exhibited more potent anticancer activity than 5-fluorouracil against the four tested human cancer cell lines and lower cytotoxicity to NIH3T3 normal cells. Flow cytometry tests demonstrated that compound **6h** could induce apoptosis of A549 cells in a concentration-dependent manner. Moreover, **6h** could efficiently suppress human tumor growth in mouse xenograft model without causing obvious toxicities.

Chemotherapy is one of the most effective approaches used for treating cancers in clinic. However, the lack of selectivity and development of drug-resistance diminish the efficacy of cancer chemotherapy.[1] This dilemma makes it urgent to develop new anticancer agents with high potency and less toxicity.

Matrine, a quinolizidine alkaloid, is an important active compound in the root of *Sophora flavescens* Ait (also known as Kushen) which was used as traditional Chinese herb for thousands of years in the treatment of liver diseases,[2] cardiovascular diseases,[3] asthma[4] as well as tumors.[5] While chalcones, with a scaffold of 1,3-diphenyl-2-propenone, possessing a variety of biological activities,[6] are widely spread in most natural fruits and vegetables as biological precursor of flavonoids. Notably, anticancer activity of both matrine and chalcones has been confirmed, but efficacy of their conjugates in anticancer activity has not been reported. The 1*H*-1,2,3-triazole is an important structure contained in a huge amount of active compounds mainly because of its metabolic stability, bioactivity and convenient synthesis.[7] Clinically used drugs containing a 1,2,3-triazole moiety include β-lactam antibiotics Tazobactam and Cefatrizine, as well as calcium channel blocker Carboxyamidotriazole (CAI) (Fig. 1).

In our pursuit of natural product derivatives with high anticancer potency and inspired by the idea of molecular hybridization,[8] we combined matrine, chalcones with 1*H*-1,2,3-triazole to generate a series of novel matrine–1*H*-1,2,3-triazole–chalcone conjugates (Fig. 2). In the present study, we reported their synthesis and preliminary anticancer activity studies in vitro and in vivo, and some of them showed potent anticancer activity.

The matrine–1*H*-1,2,3-triazole–chalcone conjugates were synthesized efficiently by Cu(I)-catalyzed azide/alkyne 'click' reaction between 13-azido matrine and corresponding 4′-propargyloxy chalcones.[9] The 13-azido matrine was prepared in excellent yield by Michael addition reaction between sophocarpine and excessive trimethylsilyl azide in the presence of acetic acid at ambient temperature.[10] 4′-Propargyloxychalcones were prepared by Claisen–Schmidt condensation reaction between substituted acetophenones and various benzaldehydes.[11] Substituted acetophenones (**1a** and **1b**) was prepared via Williamson ether synthesis. The synthetic route was depicted in Scheme 1. For activity comparison, compounds **7a** and **7b** were also prepared starting from **1a** and **1b**, respectively. All target compounds were characterized by IR, HRMS, ^{1}H NMR, and ^{13}C NMR (experimental details and spectra were in Supplementary data).[12]

Anti-proliferative activity against four cancer cell lines (human lung cancer cells A549, human cervical epithelial carcinoma cells Hela, human hepatocellular carcinoma cells Bel-7402 and human breast cancer cells MCF-7) and one normal cell line (mouse embryo fibroblasts NIH3T3) was evaluated based on a CCK-8 assay methodology (see Supplementary data). The IC_{50} values of matrine,

* Corresponding author. Tel./fax: +86 22 87893236.
E-mail address: liutianjun@hotmail.com (T. Liu).

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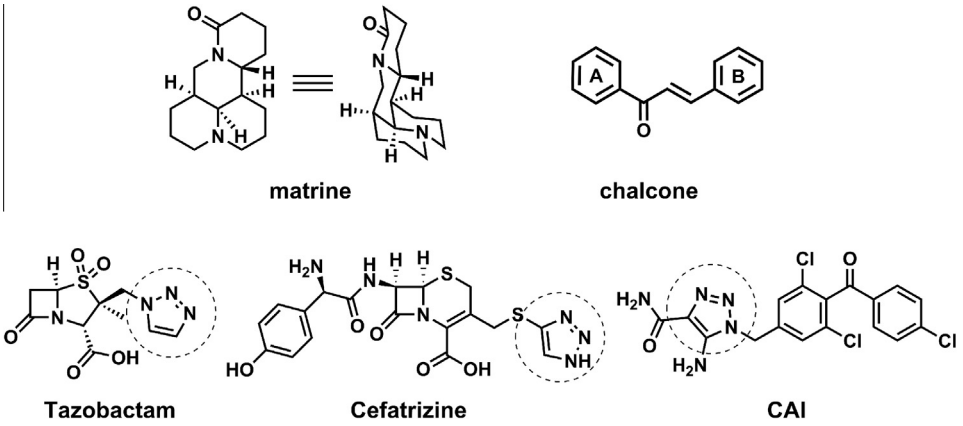


Figure 1. Chemical structures of matrine, chalcone and clinically used drugs contained 1,2,3-triazole moiety.

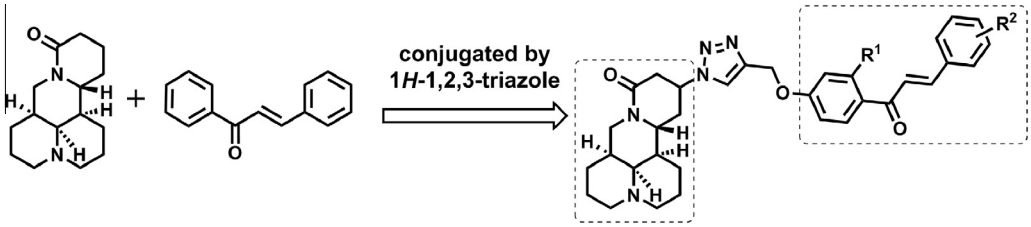
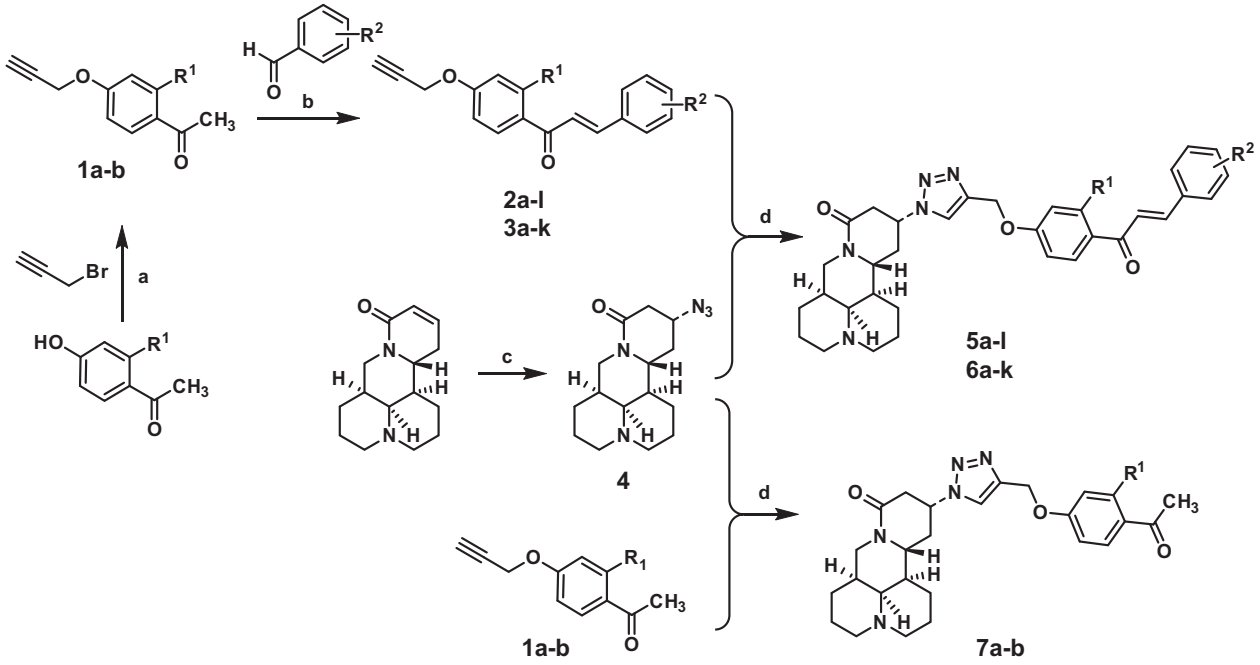


Figure 2. Design of matrine–1*H*-1,2,3-triazole–chalcones conjugates.



Scheme 1. Synthetic route of the conjugates **5**, **6** and **7**. Reagent and reaction conditions: (a) propargyl bromide, K_2CO_3, Me_2CO, rt −60 °C, 0.5–2.0 h; (b) KOH, EtOH, N_2, 25–60 °C, 6–24 h; (c) Me_3SiN_3, AcOH, DBU, toluene, N_2, rt, 36 h; (d) $CuSO_4{\cdot}5H_2O$, sodium ascorbate, THF/H_2O (1:1), rt, 4–48 h.

some representative chalcones and their conjugates were listed in Table 1.

Table 1 shows that matrine, chalcones or a simple mixture of them displayed little cytotoxicity against normal cell NIH3T3, or cancer cell like A549, Hela and MCF-7 (IC_{50} >50 μM), while matrine–1*H*-1,2,3-triazole–chalcone conjugates showed moderate to potent anticancer activity to tested cell lines and relatively weak toxicity toward NIH3T3 cells. The synergetic anticancer effect toward cells could be interpreted from the following points: in one case, the conjugate has a larger molecular skeleton with more binding sites than the components, which cause a strong affinity to the target sites; in another case, the conjugate contains both matrine and chalcone species, so the multi-target binding sites not only for matrine, chalcone alone, but also novel binding site

Table 1
Results of cell growth inhibition caused by conjugates and selected intermediates

Compd	R^1	R^2	IC_{50}[a] (μM)				
			NIH3T3	A549	Bel-7402	Hela	MCF-7
5a	H	H	19.38 ± 1.17	32.18 ± 3.89	20.73 ± 3.02	25.39 ± 2.62	17.24 ± 1.34
5b	H	4-Cl	31.30 ± 2.31	18.89 ± 1.30	21.25 ± 2.32	19.30 ± 1.73	14.32 ± 2.07
5c	H	4-NO_2	35.72 ± 2.94	14.46 ± 1.16	16.22 ± 1.52	6.06 ± 0.63	11.52 ± 0.98
5d	H	4-$OCHF_2$	27.33 ± 4.01	>50	ND[b]	>50	ND
5e	H	4-Me	14.85 ± 2.53	32.42 ± 3.27	35.55 ± 3.29	19.91 ± 1.81	26.75 ± 2.90
5f	H	4-Br	30.91 ± 3.83	24.51 ± 2.75	22.67 ± 1.73	17.14 ± 3.21	22.73 ± 2.92
5g	H	4-F	39.80 ± 4.02	18.74 ± 1.94	22.18 ± 3.12	18.74 ± 1.43	26.85 ± 3.49
5h	H	4-MeO	23.12 ± 3.10	>50	ND	>50	ND
5i	H	2-Cl	88.02 ± 7.68	14.25 ± 1.21	21.79 ± 2.56	13.52 ± 1.54	23.75 ± 2.84
5j	H	3-Cl	38.15 ± 4.78	11.87 ± 1.07	13.72 ± 1.03	15.23 ± 1.29	10.25 ± 1.05
5k	H	3,4-Di-Cl	19.38 ± 1.33	8.72 ± 0.62	11.93 ± 1.04	11.42 ± 1.01	13.30 ± 0.91
5l	H	3,4-Di-MeO	41.06 ± 5.05	>50	ND	>50	ND
6a	OH	H	>100	16.82 ± 1.19	17.33 ± 1.48	17.27 ± 1.29	11.97 ± 1.40
6b	OH	4-Cl	75.24 ± 9.52	15.64 ± 1.28	14.71 ± 0.99	10.72 ± 0.67	12.67 ± 1.16
6c	OH	4-F	36.12 ± 4.74	18.30 ± 1.95	9.02 ± 0.82	19.79 ± 1.84	5.71 ± 0.72
6d	OH	4-MeO	29.58 ± 1.24	>50	>50	>50	>50
6e	OH	4-Me	20.95 ± 2.30	>50	ND	>50	ND
6f	OH	4-*t*-Butyl	20.43 ± 2.03	36.46 ± 3.21	ND	36.27 ± 3.12	ND
6g	OH	3-CF_3	34.84 ± 4.11	8.18 ± 0.93	12.14 ± 1.01	12.72 ± 1.08	8.87 ± 0.63
6h	OH	3-Cl	39.21 ± 4.31	5.01 ± 0.59	5.60 ± 0.37	7.31 ± 0.64	6.25 ± 0.53
6i	OH	3,4-Di-Cl	48.28 ± 5.82	8.08 ± 0.94	12.44 ± 1.28	6.63 ± 0.17	7.03 ± 0.63
6j	OH	3,4-Di-MeO	13.34 ± 1.54	>50	ND	>50	ND
6k	OH	3,4,5-Tri-MeO	15.21 ± 2.21	>50	ND	>50	ND
7a	H	—	42.88 ± 5.34	>50	>50	>50	>50
7b	OH	—	61.80 ± 7.20	>50	>50	>50	>50
2a			72.88 ± 8.34	>50	ND	>50	>50
2k			61.80 ± 7.20	>50	ND	>50	>50
3a			66.56 ± 7.30	>50	ND	>50	>50
3h			79.51 ± 8.19	>50	ND	>50	>50
3i			94.67 ± 9.03	>50	ND	>50	>50
Matrine			ND	>50	>50	>50	>50
Combination of **3a** and matrine[c]			ND	>50	>50	>50	>50
Paclitaxel			20.01 ± 2.38	2.82 ± 0.31	ND	0.38 ± 0.03	ND
5-Fluorouracil			22.36 ± 2.09	40.38 ± 4.61	19.91 ± 2.68	8.93 ± 0.79	9.56 ± 1.01

[a] Data were shown as mean ± SD.
[b] Not tested.
[c] Molar ratio of **3a** and matrine is 1:1.

with new action mode would occur, while each component has no this advantage; thirdly, the conjugate is not physical addition in physical properties like hydrophilicity, so the transmembrane behavior for the conjugates will be different from that of each component. The ensemble effect above would account for the difference in cytotoxicity for the conjugate and its corresponding components. In the series of conjugates, 13-substituted matrine with poor anticancer activity, would provide a geometry to help the location of chalcone moiety, and the cytotoxicity difference is from the contribution of the chalcone moiety. Both the position and types of substituent on chalcone moiety have dominant influence on potency, as well as tumor-selectivity. Some structure–activity relationships (SARs) could be extracted. First of all, that structural integrity of chalcone moiety was essential for the anticancer activity of the conjugate (**7a**, **7b**), reveals the double bond of α,β-unsaturated moiety is of great importance to the anticancer activity, which was consistent with the reported literature.[11b] Secondly, conjugates with a 2′-OH in A ring of chalcone moiety showed more potent anticancer activity than those without –OH substitution (**5a** vs **6a**, **5b** vs **6b**, **5g** vs **6c**, **5j** vs **6h**), probably because 2′-OH in A ring could induce intramolecular hydrogen bond with chalcone carbonyl group which may change electron density distribution of α,β-unsaturated moiety or change conformer of the conjugate. Thirdly, conjugates with electron-withdrawing groups (EWGs) in B ring of chalcone moiety showed better anticancer activity than those substituted with electron-donating groups (EDGs) (**5c** vs **5h**, **5k** vs **5l**, **6c** vs **6d**, **6i** vs **6j**), mainly because EWGs could reduce electron density of B ring as well as α,β-unsaturated moiety, thus decreasing reactivity of α,β-unsaturated double bond as a Michael reaction acceptors in organisms. On the contrary, conjugates (**5d**, **5h**, **5l**, **6d**, **6e**, **6j**, **6k**) with EDGs (like 4-Me, 4-MeO, 4-$OCHF_2$, 3,4-di-MeO and 3,4,5-tri-MeO) in B ring, have poor activity with IC_{50} more than 50 μM toward tested cancer cells. Finally, conjugates substituted with 4-NO_2 or 4-F in B ring showed selectivity among cancer cells, for compound **5c** and **6c** were the most potent than other conjugates against Hela and MCF-7, respectively. Whether matrine or triazole is essential to activity needs further study.

Table 1 shows that compound **6h** is 8.0-fold more potent (IC_{50} 5.01 ± 0.59 μM) than 5-fluorouracil (IC_{50} 40.38 ± 4.61 μM) and is comparable to paclitaxel (IC_{50} 2.82 ± 0.31 μM) against A549 cells. Besides, compound **6h**, with a relatively broad anticancer spectrum, has less cytotoxicity (IC_{50} 39.21 ± 4.31 μM) than 5-fluorouracil (IC_{50} 22.36 ± 2.09 μM) and paclitaxel (IC_{50} 20.01 ± 2.38 μM) against NIH3T3 cells. Thus compound **6h** was chosen for further studies.

Flow cytometry assay with Annexin V-FITC/propidium iodide double staining in A549 cells (results in Fig. 3) suggested that **6h** was capable to induce apoptosis in a concentration-dependent manner, and percentage of both early and late apoptosis reached to more than 30% in the high concentration treatment group (two-fold IC_{50} of compound **6h**, incubation for 24 h). This was similar to that of the building blocks, matrine and chalcone. Matrine could significantly increase rate of apoptosis in A549 cells from 28.4% to 43.3% at 15–50 μM,[13] while chalcone and their synthetic derivatives, could also induce apoptosis in various cancer cell

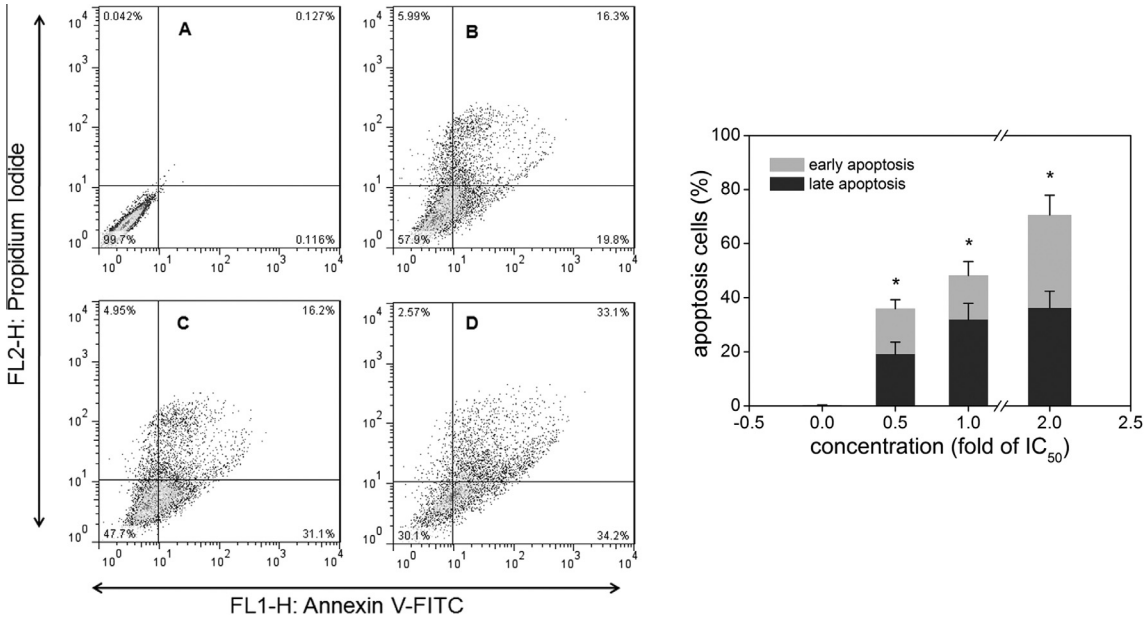


Figure 3. Apoptosis of A549 cells induced by compound **6h** after 24 h incubation. Experiments were done in triplicate. Left: representative graph of flow cytometry. (A) Control (0.1% DMSO); (B) half IC_{50} of **6h**; (C) IC_{50} of **6h**; (D) two-fold IC_{50} of **6h**. For each scatter diagram, lower right corner represents early apoptosis; upper right corner represents late apoptosis. Right: statistical data of apoptotic cells at different concentrations. $^{*}P$ <0.01, compared to control.

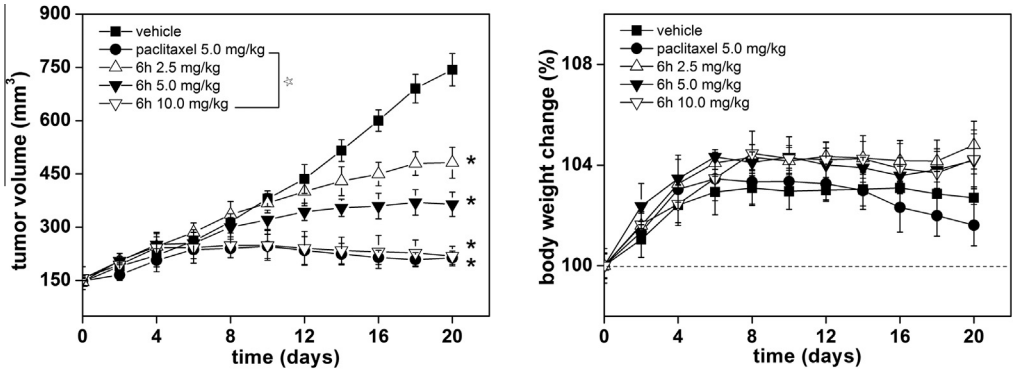


Figure 4. Tumor volume and mouse body weight change in xenograft model of A549 cells after treatment with compound **6h**. Values were expressed by mean ± SE. Left: tumor volume of different groups. Right: body weight change in each group. Tail vein injection every 3 days; n = 8 in treatment groups (**6h** and paclitaxel group), n = 6 in the vehicle group (saline plus 0.2% Tween 80). $^{*}P$ <0.01, compared to the vehicle; $^{\star}P$ >0.05, compared to paclitaxel group.

lines.[14] The conjugates can strongly induce apoptosis of A549 cells, which could be a reason for their anti-proliferative activity.

The antitumor efficacy of **6h** in vivo was studied in the xenograft nude mouse model of A549 cells (details in Supplementary data). The results in Figure 4 indicated that treatment with 5.0 mg/kg and 10.0 mg/kg of compound **6h** markedly suppressed tumor growth, while increase ratio of the body weight of tumor baring mice was relatively higher than that of the paclitaxel group or the vehicle group. This demonstrated that **6h** probably have a favorable safety profile in vivo. No significant difference was observed in tumor volume between the 10.0 mg/kg group and paclitaxel group after 8 days of treatment (P >0.05). Here percentage of tumor growth inhibition (% TGI, calculated by [1 − change of tumor volume in treated group/change of tumor volume in vehicle group] × 100) after 20 days treatment was dose-dependent (see Supplementary data). The values of TGI were 43.9%, 64.8% and 89.6% for the dosage 2.5 mg/kg, 5 mg/kg and 10 mg/kg, respectively. Particularly, after 16 days of treatment TGI in the group of 10.0 mg/kg **6h** increased to as high as 85.4%. These results suggested that compound **6h** could inhibit tumor growth in vivo without causing obvious toxicity.

In summary, conjugation of matrine, 1*H*-1,2,3-triazol and chalcones could form novel anticancer agents with synergistic effect, in which the double bond of α,β-unsaturated moiety plays a dominant role; adding 2′-OH in A ring or substituting B ring of chalcone with EWGs could increase anticancer activity of matrine–triazole–chalcone conjugates. Among the conjugates compound **6h** could inhibit proliferation and induce apoptosis of human cancer cells, and exhibited promising anticancer efficacy in vitro and in vivo, which would be a potential scaffold for further development of new anticancer agent.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2015.04.051.

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12. *General procedure for synthesis compound **5**, **6** and **7***: to a stirred solution of compound **2**, **3** or **1** (0.30 mmol) and compound **4** (0.3 mmol in 1.5 mL THF) in 1.5 mL water, freshly prepared 5% $CuSO_4$ (1 mol %) and sodium ascorbate (5 mol %) were added and this mixture was stirred for 4–48 h at room temperature. After completion of the reaction monitored by TLC, THF was removed under reduced pressure. The residue was purified by silica gel column chromatography (300–400 mesh) using ethyl acetate–ethanol–ammonia (95:4:1) to afford the pure product. A representative compound **5a** and its spectrum data are as follows:
(4^1S,7aS,13aR,13bR)-12-(4-((4-cinnamoylphenoxy)methyl)-1H-1,2,3-triazol-1-yl)dodecahydro-1H-dipyrido[2,1-f:3′,2′,1′-ij][1,6]naphthyridin-10(4^1H)-one (**5a**): Light-yellow solid, 143 mg, yield 86.6%; mp: 95.0–96.0 °C; IR (KBr, cm^{-1}): 2933.6, 2766.0, 1634.9, 1601.8, 1170.0; ^1H NMR (300 MHz, DMSO-d_6) δ 8.34 (s, 1H), 8.16 (d, 2H, J = 8.6 Hz), 7.97 (d, 1H, J = 15.4 Hz), 7.88 (d, 2H, J = 8.6 Hz), 7.69 (d, 1H, J = 15.4 Hz), 7.43 (m, 2H), 7.19 (m, 3H), 5.29 (s, 2H), 5.05 (m, 1H), 4.16 (dd, 1H, J_1 = 3.8 Hz, J_2 = 12.5 Hz), 3.63 (m, 1H), 2.85 (m, 3H), 2.70 (m, 2H), 2.45 (m, 1H), 2.11 (m, 1H), 2.04 (br, 1H), 1.84 (br, 3H), 1.59 (br, 4H), 1.44 (m, 1H), 1.31 (br, 4H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 187.3, 165.2, 162.2, 143.6, 142.7, 135.2, 131.4, 131.2, 130.9, 129.4, 129.3, 124.2, 122.7, 115.4, 64.1, 63.9, 62.6, 57.7, 53.3, 52.1, 50.9, 42.5, 38.1, 36.3, 31.9, 28.7, 27.4, 22.0, 21.5; HRMS (ESI-TOF) m/z Calcd for $C_{33}H_{38}N_5O_3^+$ 552.2969, found 552.2974.
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