Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2014, 6(2):442-445

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

Synthesis and cytotoxic activity of tanshinone I derivatives having azacyclo moiety

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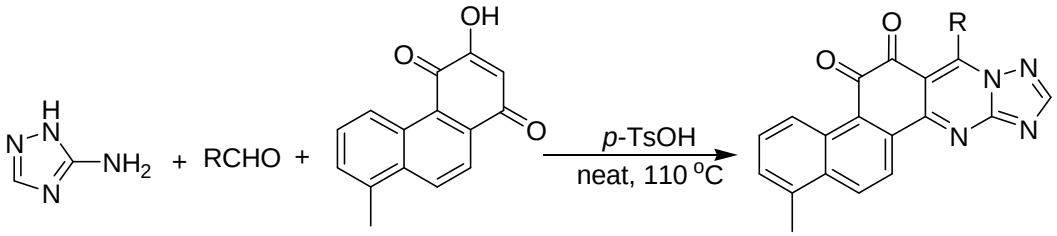
ABSTRACT

Three-component coupling of 3-amino-1,2,4-triazole, aldehydes and 3-hydroxy-8- methyl-1,4-phenanthrenequinone has been achieved using a catalytic amount of p-TsOH under solvent-free conditions to produce a novel series of tanshinone I derivatives having azacyclo in good yields and with high regioselectivity. These compounds are found to exhibit potent antitumoral properties.

INTRODUCTION

Tanshinone I is a abietane-type norditerpenoid quinones isolated from the roots of *Salvia miltiorrhiza*, a well-known traditional Chinese medicine with varied pharmacological activities such as antibacterial and anti-dermatophytic [1,2], anti-inflammatory [3,4], anticancer [5] and hepatic-protection [6,7] activities. In recent years, Tanshinone I have attracted of the attention of chemists and clinicians due to excellent anticancer activity. Tanshinones showed a broad spectrum of cytotoxic effects on cell lines derived from human carcinomas of the colon, ovary, lung, mouth, and breast [8]. However，its clinical use is limited because of the high lipid solubility, short half-life and low bioavailability. Therefore, the researchers have continuously attempted to make structural modification with tanshinone I, so as to achieve novel Tanshinone I analogues which have good physicochemical properties and pharmacokinetic parameters.

Multicomponent reactions (MCRs) typically involve more than two reactants to combine in a sequential manner giving highly selective products while retaining majority of the atoms of the starting material. MCRs have received considerable attention because of its wide range of applications in pharmaceutical chemistry for the creation of structural diversity and combinatorial libraries for drug discovery [9-12]. As a consequence of our continued interest in the synthesis of novel heterocycles employing domino protocols, herein we report for the first time a facile synthesis of a library of novel 6-substituted-12-methyl-naphtho[1,2-*h*]1,2,4]- triazolo[5,1-*b*] quinazoline-7,8- diones *via* the one-pot three-component domino reactions of 3-amino-1,2,4-triazole, aldehydes and 3-hydroxy-8-methyl-1,4-phenanthrenequinone in the presence of *p*-TsOH (Scheme 1). Most importantly, these previously unreported novel series of tanshinone I derivatives having azacyclo exhibited different range of significant cytotoxic activities.



1 **2** **3** **4**

Scheme 1

EXPERIMENTAL SECTION

General

IR spectra were determined on FTS-40 infrared spectrometer. NMR spectra were determined on Bruker AV-400 spectrometer at room temperature using TMS as internal standard. Chemical shifts (d) are given in ppm and coupling constants (*J*) in Hz. Mass spectra were recorded on a Finnigan LCQ Advantage mass spectrometer. Elemental analysis were performed by a Vario-III elemental analyzer. Melting points were determined on a XT-4 binocular microscope and were uncorrected. Commercially available reagents were used throughout without further purification unless otherwise stated

General procedure for the synthesis of compounds 4

To a mixture of 3-amino-1,2,4-triazole (1 mmol), aldehydes (1 mmol) and 3-hydroxy-8- methyl-1,4-phenanthrenequinone (1 mmol), *p*-TsOH (0.1 mmol) was added. The mixture was stirred at 110 °C for an appropriate time (Table 2). After completion of the reaction (TLC), the reaction mixture was treated with water (10 mL) and extracted with CH_2Cl_2 (2 × 10 mL), filtered and the solvent evaporated in vacuo. Solvent was evaporated and the crude product puried by silica gel column chromatography using chloroform: ethyl acetate (*v:v* = 10:1) as eluent to afford the pure product **4**.

6-Phenyl-12-methyl-naphtho[1,2-h]1,2,4]- triazolo[5,1-b] quinazoline-7,8-diones: brown red power, m.p. >300 °C; IR (KBr): *v* 3059, 2951, 2866, 1682, 1579, 1518, 1466, 1372, 13339, 1310, 1269, 1230, 1008, 720, 619 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) *δ*: 9.25 (d, 1H, *J* = 8.4 Hz), 8.90 (d, 1H, *J* = 8.0 Hz), 8.55 (s, 1H), 8.14 (d, 1H, *J* = 8.0 Hz), 7.64-7.33 (m, 7H), 2.70 (s, 3H); MS (ESI): m/z 391 $[M+H]^+$; Anal. Calc. for $C_{24}H_{14}N_4O_2$: C 73.84, H 3.61, N 14.35; found: C 73.90, H 3.54, N 14.43.

6-(2,4-Dichlorophenyl)-12-methyl-naphtho[1,2-h]1,2,4]-triazolo[5,1-b] quinazoline- 7,8-diones: brown red power, m.p. >300 °C; IR (KBr): *v* 3068, 2933, 2871, 1693, 1582, 1520, 1471, 1350, 1311, 1268, 1008, 806, 727, 621 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) *δ*: 9.24 (d, 1H, *J* = 8.8 Hz), 8.95 (d, 1H, *J* = 8.0 Hz), 8.56 (s, 1H), 8.15 (d, 1H, *J* = 8.0 Hz), 7.59-7.29 (m, 3H), 7.36 (d, 1H, *J* = 8.0 Hz), 7.28 (d, 1H, *J* = 8.4 Hz), 2.73 (s, 3H); MS (ESI): m/z 459 $[M+H]^+$; Anal. Calc. for $C_{24}H_{12}Cl_2N_4O_2$: C 62.76, H 2.63, N 12.20; found: C 62.86, H 2.59, N 12.17.

*6-(4-Methylphenyl)-12-methyl-naphtho[1,2-h]1,2,4]-triazolo[5,1-b]quinazoline-7,8-diones:*brown red power, m.p. >300 °C; IR (KBr): *v* 3081, 2923, 2870, 1695, 1582, 1522, 1490, 1376, 1301, 1269, 742, 641 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) *δ*: 9.25 (d, 1H, *J* = 8.8 Hz), 8.80-8.76 (m, 2H), 8.17 (d, 1H, *J* = 7.6 Hz), 7.64-7.40 (m, 5H), 7.34 (d, 1H, *J* = 8.0 Hz), 2.73 (s, 3H), 2.45 (s, 3H); MS (ESI): m/z 405 $[M+H]^+$; Anal. Calc. for $C_{25}H_{16}N_4O_2$: C 74.25, H 3.99, N 13.85; found: C 74.18, H 4.04, N 13.79.

6-(Thiophen-2-yl)-12-methyl-naphtho[1,2-h]1,2,4]-triazolo[5,1-b]quinazoline-7,8-diones: brown red power, m.p. >300 °C; IR (KBr): *v* 3099, 2956, 2876, 1699, 1586, 1519, 1480, 1400, 1302, 1279, 1183, 1076, 726, 486 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) *δ*: 9.22 (d, 1H, *J* = 8.8 Hz), 8.82 (s, 1H), 8.77 (d, 1H, *J* = 7.6 Hz), 8.17-7.31 (m, 6H), 2.70 (s, 3H); MS (ESI): m/z 397 $[M+H]^+$; Anal. Calc. for $C_{22}H_{12}N_4O_2S$: C 66.66, H 3.05, N 14.13; found: C 66.69, H 2.98, N 14.19.

12-Methyl-naphtho[1,2-h]1,2,4]- triazolo[5,1-b]quinazoline-7,8-diones: brown red power, m.p. >300 °C; IR (KBr): *v* 3072, 2930, 2869, 1690, 1593, 1585, 1518, 1474, 1342, 1302, 1274, 1001 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) *δ*: 9.23 (d, 1H, *J* = 8.8 Hz), 8.99 (s, 1H), 8.81-8.77 (m, 2H), 8.26 (d, 1H, *J* = 8.0 Hz), 7.58 (dd, 1H, *J* = 2.0, 8.0 Hz), 7.33 (dd, 1H, *J* = 2.0, 8.0 Hz)), 2.70 (s, 3H); MS (ESI): m/z 315 $[M+H]^+$; Anal. Calc. for $C_{18}H_{10}N_4O_2$: C 68.79, H 3.21, N 17.83; found: C 68.83, H 3.12, N 17.92.

Cytotoxicity assay

Cell viability for all cell lines was determined using the 3-[4,5- demethylthiazol-2,5-diphenyl-2*H*- tetrazolium bromide (MTT) colorimetric assay. Compounds **4a**-**4e** were subjected to cytotoxic evaluation against SGC7901 and HepG2 cell lines employing the colorimetric method. Tanshinone Iwas used as the reference substance.

MTT was dissolved in saline to make the concentration of 5 mg/μL as a stock solution. Cancer cells (3×10^3 cells) suspended in 100 mg/well of MEM medium containing 10% fetal calf serum were seeded onto a 96-well culture plate. After 24 h pre-incubation at 37 ^oC in a humidified atmosphere of 5% CO_2/95% air to allow cells attachment, various concentrations of test solution (10 μL/well) as listed in Table 3 were added and then incubated for 48 h under the above condition. At the end of the incubation, 10 μL of tetrazolium reagent was added into each well and then incubated at 37 ^oC for 4 h. The supernatant was decanted, and DMSO (100 μL/well) was added to allow formosan solubilization. The optical density (OD) of each well was detected by a microplate reader at 550 nm and for correction at 595 nm. Each determination represents the average means of six replicates. The 50% inhibition concentration (IC_{50}) was determined by curve fitting.

RESULTS AND DISCUSSION

In a typical reaction procedure, *p*-TsOH (10 mol%) was added to a mixture of 3-amino-1,2,4- triazole (1 mmol), benzaldehyde (1 mmol), 3-hydroxy-8-methyl-1,4- phenanthrenequinone (1 mmol) at 110 °C under solvent-free condition, and the reaction was monitored using TLC (chloroform: ethyl acetate, 10:1, *v/v*). After 3 h, TLC showed the starting reactant was completely consumed. The reaction mixture was then diluted with dichloromethane, filtered and the solvent evaporated in vacuo. The solid residue was further purified by flash chromatography using chloroform: ethyl acetate 10:1 as the eluent to give the final product **4a** (84% yield).

In order to extend the scope of this work, the described methodology was examined on different arylaldehydes (Table 1). The reactions were simply performed by stirring 3-amino-1,2,4-triazol, aldehydes, 2-hydroxy-1,4-naphthoquinone (1 mmol) under solvent-free condition in the presence of 10 mol% of *p*-TsOH at 110 °C. As shown in Table 1, a range of aromatic aldehydes, heterocyclic aldehyde and paraformaldehyde gave the expected products in high yields. The structures of the isolated products **4** were deduced on the basis of IR, ^1H NMR, mass spectrometry and elemental analysis It should be outlined the simplicity of the proposed procedure, cheap commercially available reagents being used without resorting to particulal experimental precautions.

Table 1. Preparation of 6-substituted-12-methyl-naphtho[1,2-*h*]1,2,4]- triazolo[5,1-*b*] quinazoline- 7,8-diones

Entry	R	Time/ h	Product	Yield/ %
1	C_6H_5	3	**4a**	84
2	2,4-Cl_2-C_6H_3	4	**4b**	80
3	4-Me-C_6H_4	3	**4c**	79
4	Thiophen-2-yl	3	**4d**	76
5	H	2	**4e**	85

All synthesized compounds were evaluated for their antiproliferative activities against the human gastric carcinoma cell line SCG7901, hepatoma cell line HepG2, and their IC_{50} values in micromolar concentration are represented in Table 2. It is clearly observed that all the tested compounds showed moderate to good antiproliferative activities against the tested cancer cell lines.

Table 2. Cytotoxic activities of 6-substituted-12-methyl-naphtho[1,2-*h*]1,2,4]- triazolo[5,1-*b*] quinazoline-7,8-diones

Compd	IC_{50} (μM)	
	SGC7901	HepG2
4a	5.56 ± 0.26	7.20 ± 1.62
4b	5.76 ± 0.42	6.82 ± 0.36
4c	3.54 ± 0.14	2.46 ± 0.24
4d	3.84 ± 0.32	4.64 ± 0.64
4e	1.88 ± 0.16	2.32 ± 0.14
Tanshinone I	9.96 ± 0.68	13.72 + 0.73

CONCLUSION

In summary, we have developed a novel method for the synthesis of 6-substituted- 12-methyl-naphtho[1,2-*h*]1,2,4]-triazolo[5,1-*b*] quinazoline-7,8-diones by means of a three- component reaction between 3-amino-1,2,4-triazole, aldehydes and 3-hydroxy-8-methyl-1,4- phenanthrenequinone. using a catalytic amount of *p*-TsOH under neat conditions. This method is simple and convenient to prepare a wide range of tanshinone I derivatives having

azacyclo in a single-step operation which are found to possess interesting antitumor properties.

Acknowledgement
The author wish to thank Xinxiang Medical University for providing financial support.

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