Chinese Chemical Letters 26 (2015) 334–338

Contents lists available at ScienceDirect

Chinese Chemical Letters

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

Original article

Efficient synthesis of panaxadiol derivatives using continuous-flow microreactor and evaluation of anti-tumor activity

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ARTICLE INFO

Article history:
Received 1 August 2014
Received in revised form 17 October 2014
Accepted 17 October 2014
Available online 13 November 2014

Keywords:
Flow chemistry
Microreactor
Panaxadiol
Anti-tumor activity

ABSTRACT

An efficient method has been developed for the synthesis of a series of (20*R*)-panaxadiol derivatives (**4a**–**w**) using a continuous-flow microreactor. The antitumor activities of the newly synthesized compounds were evaluated *in vitro* in two human prostate adenocarcinoma tumor cell lines (*i.e.*, PC-3 and LNCaP cells), and their cytotoxicities were evaluated using a standard MTT assay. Compounds **4c**, **4h**, **4p**, **4q** and **4s** exhibited higher antitumor activities toward PC-3 cell line than panaxadiol, which was used as a reference standard.

1. Introduction

The use of continuous-flow microreactor technology in organic chemistry has attracted considerable attention during the past few years as a valuable addition to traditional batch-processing methods [1–6]. Microreactor systems have excellent heat transfer characteristics because their surface area-to-volume ratio is much greater than that of a conventional reaction vessel. The good heat transfer characteristics of microreactor systems allow for the rapid dissipation of heat from the reaction mixture, which leads to an even heat distribution and avoids the formation of local hot-spots and overheating. Compared with traditional reaction systems, microreactor systems therefore provide enhanced levels of selectivity, with cleaner reaction profiles and shorter reaction times [7–15].

Ginsenosides can be found in a wide range of Panax ginseng plants, which are widely distributed throughout East Asia. Compounds belonging to this structural class have been reported to exhibit a range of interesting pharmacological properties [16–23], including antitumor [24–26], immunomodulatory [27], antioxidative [28], analgesic and anti-inflammatory activities [29]. (20*R*)-Panaxadiol (PD) (**1**, Fig. 1) is a protopanaxadiol-type compound bearing an aglycone, and this compound has been identified as an interesting lead for the synthesis of new derivatives with antitumor activity. A large number of structural modifications of **1** have been made with the aim of increasing its potency, and many efforts have culminated in the indenfication of panaxadiol analogs with higher antitumor activities [30].

In the course of our development of new panaxadiol derivatives (**4a**–**w**) to evaluate their antitumor activities, initially, long exposure time of Ginseng ketone **2** and benzaldehyde at 80 °C resulting in the formation of panaxadiol derivative **4i** in low yield along with several undesired products. Therefore, we postulated that this synthesis can also be achieved in a greener way by using continuous-flow microreactor. Herein, several new panaxadiol derivatives (**4a**–**w**) were synthesized under continuous flow conditions and evaluated their antitumor activities.

2. Experimental

Melting points were measured on a SGW X-1 microscopic melting-point apparatus. ^{1}H NMR and ^{13}C NMR spectra on a Bruker AV 400 MHz spectrometer were recorded in DMSO-d_6. Chemical shifts are reported in δ (ppm) units relative to the internal standard tetramethylsilane (TMS). Mass spectra were obtained on a Waters Quattro Micromass instrument using electrospray ionization (ESI) techniques. Infrared spectra (IR) were recorded with a nexus FT/IR-4200 spectro-meter. All the reactions were monitored by thin layer

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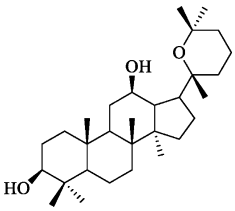
http://dx.doi.org/10.1016/j.cclet.2014.11.013



Panaxadiol **1**

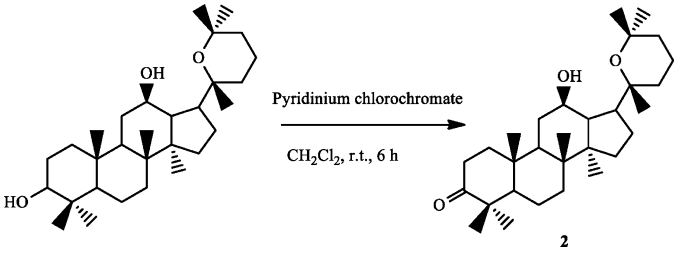
Fig. 1. Chemical structure of (20*R*)-panaxadiol.

chromatography (TLC) on pre-coated silica gel G plates at 254 nm under a UV lamp using ethyl acetate/petroleum ether as eluent. Flash chromatography separations were obtained on silica gel (300–400 mesh).

General synthetic procedure for compound **2**: a solution of (20*R*)-panaxadiol (1.0 g, 2.17 mmol) in CH_2Cl_2 (11.1 mL) and pyridinium chlorochromate (2812.1 mg, 13.0 mmol) was stirred for 6 h at room temperature. The solvent was removed under reduced pressure to give a white solid. The white solid was dissolved in ethyl ether and washed with $NaHCO_3$ (5%), dried ($MgSO_4$) and concentrated under reduced pressure to give the crude product. The crude products were chromatographied using silica gel and eluted with petroleum ether/ethyl acetate (8:1) to give the pure product **2** (0.9 g, 90%) (Scheme 1) [31].

General procedure for the preparation of panaxadiol derivatives **4a–w**: A solution of Ginseng ketone **2** (1 equiv.) in the appropriate ethanol was introduced into a 2 mL injection loop as stream 1, which was then mixed through a T-piece with a solution of aromatic aldehyde (**3a–w**) (1.20 equiv.) and 40% KOH in the appropriate ethanol as stream 2 (Scheme 2). The flow rate was set to 0.084 mL/min, and then mixture was passed through the flow reactor (internal volume 5 mL, 8 bar) at 80 °C around 60 min to collect the crude product at the outlet.

The reaction mixture was poured into ice water then neutralized with 2 mol/L aq. HCl, and washed with ice water, dried with anhyd. Na_2SO_4. Evaporation of solvent in vacuum gave the crude products, which were purified by silica gel column chromatography (ether/ethyl acetate 10:1) to afford corresponding products **4a–w** (40%–82%). The structure was confirmed by IR, ^{1}H NMR, ^{13}C NMR, and mass spectral.



Scheme 1. General synthetic procedure for compound **2**.

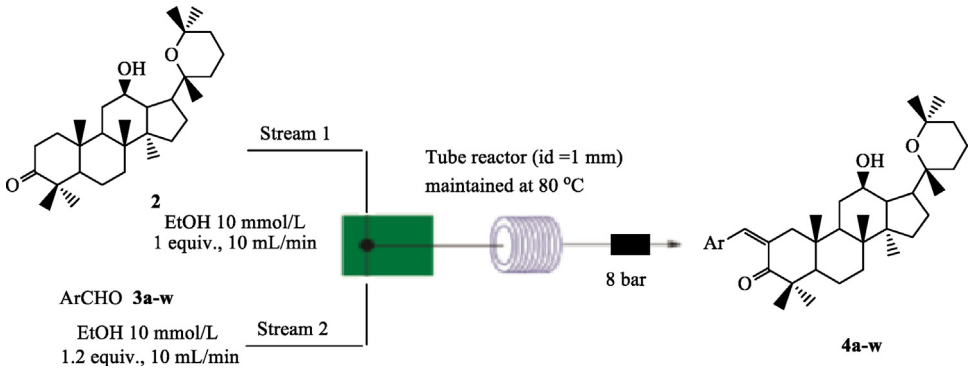
2.1. Biological studies

Cytotoxicity of these derivatives was evaluated on human prostate adenocarcinoma tumor cell lines PC-3 and LNCaP using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium (MTT) assay. Panaxadiol used as reference. In short, 1×10^6 cells/well were seeded into 96-well plates, 24 h later, and the cells were treated with serial dilutions of the compounds (0–100 μmol/L) for another 48 h. MTT solution (5 mg/mL, 10 μL) was added to each well, and the tumor cells were incubated at 37 °C in a humidified atmosphere of 5% CO_2 air for 4 h. At the end of incubation, the growth medium was removed and replaced with 100 μL of DMSO (at room temperature). After agitating on a vortex for 10 min, the absorbance was determined at 492 nm as reference on a Bio-Rad (model 550) microplate reader to calculate 50% inhibition concentration (IC_{50}). DMSO and MTT were purchased from Sigma Chemical Co., Ltd, USA.

3. Results and discussion

The synthesis of panaxadiol derivatives was carried out using the commercially available flow reactor, the Vapourtec R_2^+/R_4 combination (Fig. 2). The pressure within the system is maintained using an in-line 8 bar back-pressure regulator. Mixing of the reagent streams is achieved with a simple T-piece and the combined output is then directed through perfluoroalkoxy (PFA) tubing to the convection-flow coil (CFC) which can be precisely heated up to 150 °C with further use of back-pressure regulation should superheating of solvents be required. Following exit from the CFC the rapidly cooled flow line may then be directed to various scavenger (or reagent) cartridges which often consist of omnifit glass columns packed with appropriate immobilized species. The final flow stream can be collected and evaporated to afford the product.

Benzaldehyde (substrate **3**) was used as a model substrate for the optimization of aldol condensation reaction in the micro-reactor (Table 1). Several different reaction parameters were evaluated during the optimization experiments, including the temperature, flow rate and residence time, to determine their impact on the reaction yield. When the reaction was conducted at room temperature, the reaction proceeded with a short residence time to afford the desired product in a low yield (Table 1, entries 1 and 2). For the heated reactions (Table 1, entries 3–5), the results revealed that compound **4i** was formed in good yield at 80 °C, with



Scheme 2. Flow synthesis of derivatives **4a–w**.