European Journal of Medicinal Chemistry 64 (2013) 432–441

Contents lists available at SciVerse ScienceDirect

European Journal of Medicinal Chemistry

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

Original article

Synthesis and activity evaluation of tilorone analogs as potential anticancer agents

Dingshan Zhou [a], Wei Tuo [a], Hao Hu [a], Jianrong Xu [b], Hongzhuan Chen [b], Zhigang Rao [a], Yuling Xiao [a], Xianming Hu [a], Peng Liu [a,*]

[a] State Key Laboratory of Virology, Ministry of Education Key Laboratory of Combinatorial Biosynthesis and Drug Discovery, Wuhan University School of Pharmaceutical Sciences, Wuhan 430071, China
[b] Department of Pharmacology, Institute of Medical Sciences, Shanghai Jiao Tong University School of Medicine, Shanghai 200025, China

ARTICLE INFO

Article history:
Received 23 January 2013
Received in revised form
8 March 2013
Accepted 24 March 2013
Available online 16 April 2013

Keywords:
Anticancer agent
Tilorone
Fluorenone
Isoflavone
Pharmacophore modeling

ABSTRACT

Tilorone is an interferon inducer with anticancer activity. Twenty-two novel tilorone analogs were synthesized by improvements of fluorenone skeleton, side chains and amino groups to screen new anticancer agents. *In vitro* evaluation showed that ten new compounds had better anticancer activities than tilorone. Among them, **2c** ($IC_{50} < 7$ μM against cancer cell lines and $IC_{50} > 35$ μM against non-cancer cell lines) and **5d** ($IC_{50} < 10$ μM against cancer cell lines and $IC_{50} > 53$ μM against non-cancer cell lines) exhibited the best anticancer activities and selectivities. Pharmacophore modeling of highly active compounds was carried out by Molecular Operating Environment (MOE) to generate a visualized model for compound design in future study.

1. Introduction

Tilorone (or 2,7-bis[2-diethylaminoethoxy]fluoren-9-one, Fig. 1) is the first synthetic, small molecular weight interferon inducer in possession of a broad range of therapeutical functions, such as resistance to systemic candidiasis, antiviral effect and anticancer activity [1–6]. These activities are mainly achieved by DNA strand intercalation that alters DNA chemobiological properties and further activates interferon expression [7–9].

Many previous studies showed that the modification of tilorone's fluorenone skeleton and side chain (Fig. 1) enhanced anticancer activity [10–16]. In general, the fluorenone skeleton was optimized by substitution with differently rigid planar rings [9,13,17–20], while the modification of the side chain was mostly derived from length extension, addition of carbonyl group and shift of linkage site [10,11,14,16] as well as change of *N*-alkyl groups [10–20]. It is thus of considerable interest to investigate the necessities of rigid skeleton and tertiary amino groups as well as the influence of extra hydroxyl groups.

On the basis of these considerations, twenty-two novel tilorone analogs containing rotational isoflavone skeleton with anticancer activity [21–24], hydroxyl side chains and non-tertiary amino groups have been designed, synthesized and assayed for cytotoxicity and anticancer activity. After *in vitro* activity evaluation, the highly active compounds were implemented pharmacophore modeling by Molecular Operating Environment (MOE) [25].

2. Chemistry

The compounds **1d** (Scheme 1) and **6c**–**m** (Scheme 2) were synthesized via a three-step procedure starting from daidzein (D) and 2,7-dihydroxy-9-fluorenone (F), respectively. This procedure included hydroxyethylation of phenolic hydroxyls by ethylene chlorohydrin, further chlorination of alcoholic hydroxyls by thionyl chloride and final nucleophilic substitution of chlorides by corresponding amines [26]. Note that in the first step, ethylene chlorohydrin in excess needed to be used due to its hydrolyzation during the reaction. The compounds **4c** and **4d** (Scheme 2) were produced via a similar procedure to **6c**–**m**, where the only difference was ethylation on one of the two phenolic hydroxyls by diethylsulfate prior to the three-step procedure. The synthesis of the compounds

* Corresponding author. Tel.: +86 27 68759887; fax: +86 27 68759850.
E-mail addresses: zhoudingshan@whu.edu.cn (D. Zhou), liupenghk@gmail.com (P. Liu).

0223-5234/$ – see front matter © 2013 Elsevier Masson SAS. All rights reserved.
http://dx.doi.org/10.1016/j.ejmech.2013.03.050

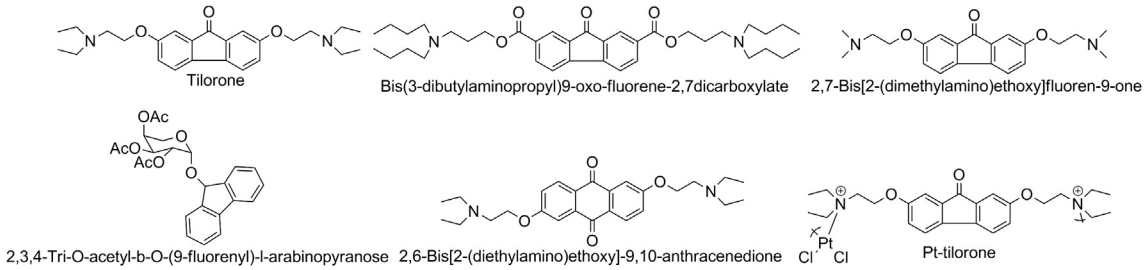
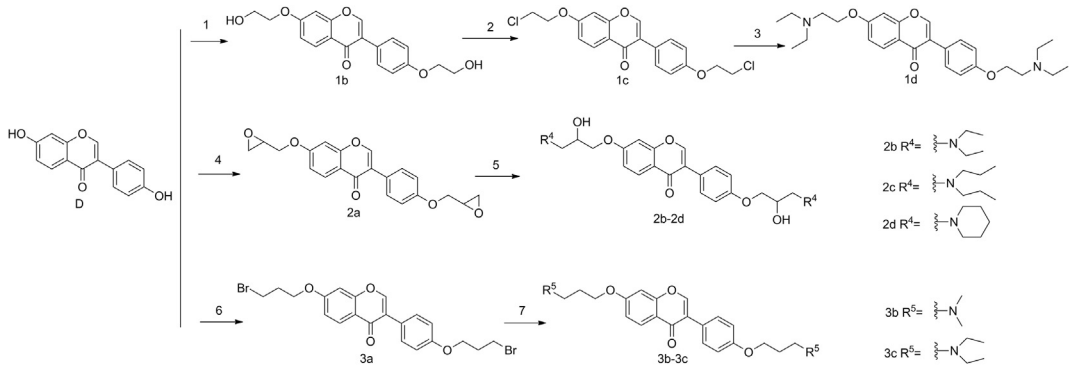
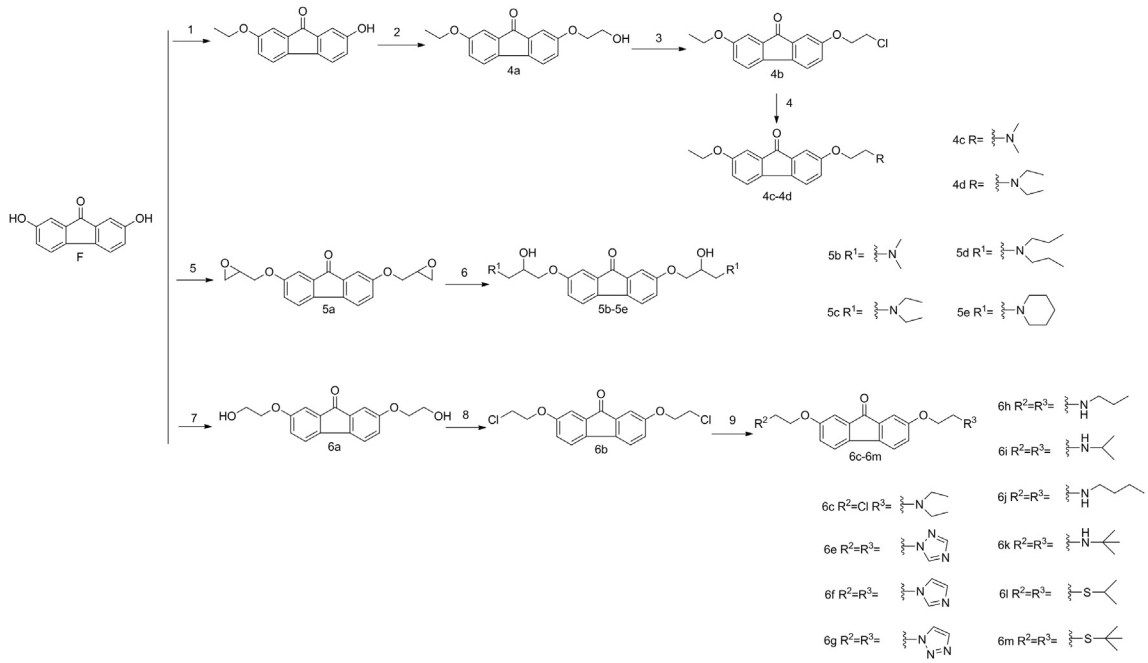


Fig. 1. Structures of tilorone and its active derivatives.



Scheme 1. Synthesis of compounds **1d**, **2b**–**d**, **3b** and **3c**. Reagents and conditions: (1) NaOH/ethylene chlorohydrin/DMSO, 70 °C; (2) thionyl chloride, 60 °C; (3) potassium carbonate/diethylamine/$CHCl_3$, reflux; (4) epichlorohydrin/NaOH/DMSO, 70 °C; (5) potassium carbonate/amines/$CHCl_3$, reflux; (6) NaOH/1,3-dibromopropane/DMSO, 70 °C; (7) potassium carbonate/amines/DMF, 70 °C.

2b–**d** (Scheme 1) and **5b**–**e** (Scheme 2) involved epoxypropylation of starting compounds (D and F) by epichlorohydrin followed by ring-opening reaction of epoxide using appropriate amines. The compounds **3b** and **3c** (Scheme 1) were obtained by bromopropylation and nucleophilic substitution. Bromopropylation was performed with an excess of 1,3-dibromopropane (five equivalents) in order to reduce by-products. Our attempts indicated that direct bromoethylation by 1,2-dibromoethane could not be used to prepare **1d** and **6c**–**m**, resulted in a large amount of polymolecular by-products.



Scheme 2. Synthesis of compounds **4c**, **4d**, **5b**–**e** and **6c**–**m**. Reagents and conditions: (1) NaOH/diethylsulfate/water, R.T.; (2) NaOH/ethylene chlorohydrin/DMSO, 70 °C; (3) thionyl chloride, 60 °C; (4) potassium carbonate/amines/DMF, 70 °C; (5) epichlorohydrin/NaOH/DMSO, 70 °C; (6) potassium carbonate/amines/DMF, 70 °C; (7) NaOH/ethylene chlorohydrin/DMSO, 70 °C; (8) thionyl chloride, 60 °C; (9) potassium carbonate/amines/DMF, 70 °C.