Available online at www.sciencedirect.com
ScienceDirect
Chinese Journal of Natural Medicines 2010, **8**(6): 0436–0440
Chinese Journal of Natural Medicines

Synthesis and Cytotoxicity of Oleanolic Acid/ *N*-aryl-*N'*-hydroxyguanidine Hybrids

CHEN Li[1*], ZHU Zi-Fei[1], MENG Fei[1], XU Chang-Sheng[1], ZHANG Yi-Hua[2]

[1]*Department of Natural Medicinal Chemistry, China Pharmaceutical University, Nanjing 210009, China;*
[2]*Center of Drug Discovery, China Pharmaceutical University, Nanjing 210009, China*

Available online 20 Nov. 2010

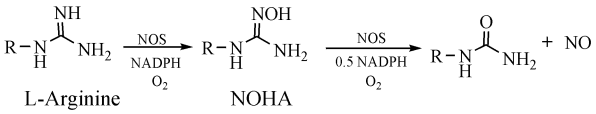
[ABSTRACT] AIM: To synthesize oleanolic acid/ *N*-aryl-*N'*-hydroxyguanidine hybrids and evaluate their antitumor activity. **METHODS:** Substituted *N*-aryl-*N'*-hydroxyguanidines were conjugated with C3-OH of OA via succinyl, giving eight target compounds, whose cytotoxicities against human cancer cells A549, HT-29, BEL-7402 and SMMC-7721 *in vitro* were evaluated by MTT assay. **RESULTS:** Eight novel compounds were synthesized and their structures were identified by IR, MS and ^{1}H NMR. The preliminary experimental results showed that **6b, 6e, 6g** and **6h** had strong cytotoxicity against SMMC-7721. **CONCLUSION:** These compounds are worthy of further studies.

[KEY WORDS] Oleanolic acid (OA); *N*-aryl-*N'*-hydroxyguanidine; Antitumor activity

[CLC Number] TQ460.31 **[Document code]** A **[Article ID]** 1672-3651(2010)06-0436-05

Oleanolic acid (OA) is widely found in plants and has been demonstrated to inhibit hepatitis in humans without apparent side-effects [1-2]. In recent years, it has been found that OA had marked antitumor effects and exhibited cytotoxic activity toward many cancer cell lines in culture [3-4].

Nitric oxide (NO), which is naturally generated from L-arginine under the catalysis of NO synthase (NOS) *in vivo*, exerts a wide range of biological effects [5-6]. N^{ω}-hydroxy-L-arginine (NOHA) is the natural NOS substrate.

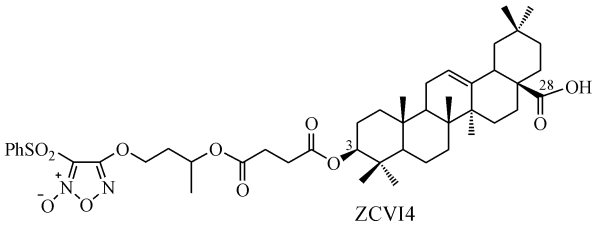


L-Arginine NOHA

R= HO_2C-CH(NH_2)-(CH_2)$_3$-

NO can also be generated from synthetic NO-donors, such as nitrate, furoxan, diazeniumdiolate, etc. [7-8]. Recent studies have shown that NO appears to be critical for the tumoricidal activity of the immune system. In general, it has been demonstrated that highly concentrated NO is cytotoxic and can induce the apoptosis of tumor cells, prevent tumors from metastasizing, and assist macrophage to kill tumor cells [9-10]. This fact provides a solid biological basis for the application of NO replacement therapy in clinic.

Recently, we reported a series of furoxan-based NO releasing derivatives of OA and evaluated their *in vitro* and *in vivo* activities. The results showed that several compounds exhibited strong selective cytotoxicity against HCC cells, which appeared to be associated with high levels of NO production in HCC cells *in vitro*. Within this series of active compounds, ZCVI4 was the most effective, greatly inhibiting the growth of HCC tumors inoculated without causing obvious morphological changes in mouse liver [11].

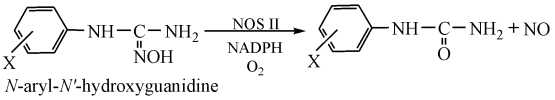


ZCVI4

Renodon-Corniere synthesized a series of *N*-aryl-*N'*-hydroxyguanidines used as the analogues of NOHA. Some of them could be oxidized by NOSs with the formation of NO,

[Received on] 08-Sep.-2010
[Research Funding] This project was supported by the National Natural Science Foundation of China (No. 2097210), the Natural Science Foundation of Jiangsu Province (No. BK2009302) and Tianjin Application Bases and Advanced Technology Research Program Key Projects (No. JCZDJC21400)
[*Corresponding author] CHEN Li: Associate Prof., Tel: 86-25-83271447, E-mail: chenliduo12@gmail.com
These authors have no any conflict of interest to declare.

and structure-activity relationship studies showed that NO formation rates were highly dependent on the size of the para-substituents of the phenyl ring. Several *N*-aryl *N'*-hydroxyguanidines bearing a relatively small, electron-donating para-substituents, such as H, F, Cl, CH_3, OH, OCH_3, and NH_2 that could favorably interact with a hydrophobic cavity close to the NOS catalytic site, led to NO formation rates between 8% and 41%, while others were close to zero with bigger substituents such as $C(CH_3)_3$, CH_2CO_2H, or OCH_2CO_2H. These results opened the way toward a new class of enzymatic NO donors [12-13].

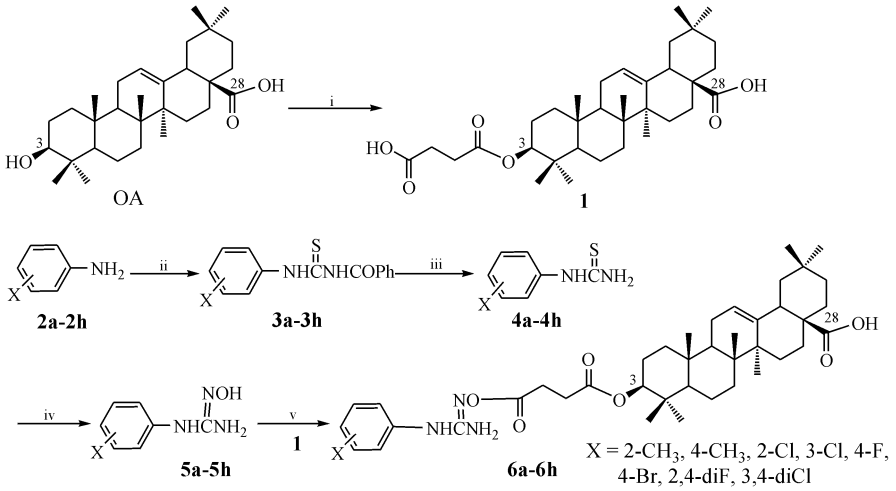


N-aryl-*N'*-hydroxyguanidine

In this study, another novel type of NO-OA hybrids was designed and synthesized by coupling a series of substituted *N*-aryl-*N'*-hydroxyguanidines to C3-OH of OA. Subsequently, their cytotoxicity against A549, HT-29, BEL-7402 and SMMC-7721 was evaluated *in vitro* by MTT assay, so as to investigate the influence on activities of the NO-OA hybrids caused by different types of NO donors.

1 Synthetic Route

Eight *N*-aryl *N'*-hydroxyguanidines (**5a-5h**) bearing various substituted phenyls were prepared starting from different substituted anilines according to the route in Scheme 1. The benzoyl thioureas (**3a-3h**) were obtained from the reaction of corresponding amines (**2a-2h**) with ammonium sulfocyanate and benzoyl chloride. Then the benzoyl was released with 10% NaOH, acidified with concentrated hydrochloric acid to give the thioureas (**4a-4h**) [14]. Finally, they were successfully converted to the desired substituted *N*-aryl-*N'*-hydroxyguanidines (**5a–5h**) by treatment with hydroxyamine catalyzed by mercuric oxide[15]. OA reacted with succinic anhydride in the presence of 4-*N*, *N*-dimethylaminopyridine (DMAP) to form succinyl oleanolic acid (**1**) in 57% yield [11]. Furthermore, **1** was esterified with **5a-5h** in the presence of dicyclohexylcarbodiimide (DCC)/DMAP to generate target compounds (**6a-6h**).



Scheme 1 Reagents and conditions: (i) Succine anhydride, DMAP, anhydrous CH_2Cl_2, reflux; (ii) NH_4SCN, PhCOCl; (iii) 10 % NaOH; (iv) $HONH_2$·HCl, HgO, $NaHCO_3$; (v) DCC, DMAP, anhydrous CH_2Cl_2, rt.

2 Experimental

2.1 *Materials and instrumentation*

Melting points were determined using a capillary apparatus (RDCSY-I) uncorrected. All of the compounds synthesized were purified by column chromatography (CC) on silica gel 60 (200-300 mesh) and thin-layer chromatography (TLC) on silica gel 60 F_{254} plates (250 μm; Qingdao Marine Chemical Company, China). Subsequently, they were routinely analyzed by IR (Shimadzu FTIR-8400S), ^{1}H NMR (Bruker ACF-300Q, 300 MHz), and MS (Hewlett-Packard 1100 LC/MSD spectrometer).

2.2 *General protocol for the synthesis of N-aryl-N'-hydroxyguanidines*

Benzoyl chloride (1.2 mol) was added to a freshly prepared solution of NH_4SCN (1.2-1.5 mol) in acetone and the mixture was heated under reflux for ~15 min. Heating was stopped and the appropriate aryl-amine (**2a-2h,** 1 mol), either neat or in acetone, was added as rapidly as possible while maintaining a vigorous reflux. Following the addition, the mixture was heated under reflux for 15 to 30 min, and then poured onto excess cracked ice with vigorous stirring. The resulting solid was collected and recrystallized from acetone. The products (**3a-3h)** are sufficiently pure to be used directly for hydrolysis to yield the thioureas (**4a-4h)**. A 0.2 mol solution of the thioureas (**4a-4h)** in anhydrous ethanol containing 1.1 equiv mercuric oxide and 1.5 equiv of hydroxylamine hydrochloride was heated under reflux for 1-15 h. The solvent was evaporated, and the residue was dissolved in water containing concentrated hydrochloric acid. The byproduct