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Novel and efficient synthesis of 22-alkynyl-13,24(23)-cyclo-18,21-dinorchol-22-en-20(23)-one analogues

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ABSTRACT

The efficient synthesis of some 22-alkynyl-13,24(23)-cyclo-18,21-dinorchol-22-en-20(23)-ones was investigated. 22-Iodocyclo-18,21-dinorcholenones were prepared from cyclo-18,21-dinorcholenones using I_2/DMAP/pyridine system firstly. The cross coupling reaction of 22-iodocyclo-18,21-dinorcholenones and 1-alkynes was carried out efficiently catalyzed by tetrakis(triphenylphosphine) palladium/cuprous iodide in the presence of base diisopropylethylamine. This strategy offered a very straightforward and efficient method for access to conjugated alkynyl cyclo-18,21-dinorcholenones from the cyclo-18,21-dinorcholenones and 1-alkynes in excellent overall yields. Evaluation of the synthesized compounds for cytotoxicity against KB, HeLa, MKN-28 and MCF-7 cell lines showed that the 22-alkynylcyclodinorchoenones possessing hydroxylethyl and hydroxylmethyl mono-substituted side chain at the end of alkynyl group have significantly inhibition activity.

1. Introduction

Pentacyclic steroids and their derivatives are a very important class of steroids. They can be categorized as follows: the fusion of carbocyclic ring such as benzene or cyclohexane or cyclopentane to the steroid nucleus, the fusion of a carbocyclic ring to a heterosteroid skeleton, the fusion of heterocycle ring containing nitrogen, oxygen or sulfur to the steroid nucleus, the spiro-pentacyclic steroids and the bridged-pentacyclic steroids.

More and more studies showed pentacyclic steroids and their derivatives display very important pharmacological and biological properties. Many of them were tested and/or evaluated in potential drug discovery such as α-substituted-α,β-unsaturated pentacyclic steroid ketone [1–5]. The structure–activity relationship of the pentacyclic steroids indicates the carbocyclic or heterocyclic ring fused plays an important role of biological activity. Therefore, the preparation of pentacyclic steroids has attracted considerable attention from medicinal and synthetic organic chemists. A number of preparations for pentacyclic steroids and their derivatives were reported recently. Especially, when pentacyclic steroids were further modified, the unexpectedly special biologically properties were observed [1,2,6,7]. It is known that natural products which contain conjugated eneynes or enediynes exhibit an exceptional biological profile due to their unique molecular structure, striking mode of action and high potency [8,9], and play important ecological roles [10,11]. Our group has been involved in several projects aimed at the development of highly convergent syntheses of functionalized steroids, privileged structures in drug discovery. Our contribution in this field has been attained mainly by coupling reactions, as Sonogashira reaction that typically lead to conjugated enyne skeletons, with a secondary transformation in steroid nucleus. This methodology enables generation of various heterocyclic systems or fused rings of steroids. In our previous work, we reported the preparation of D-ring unsaturated 17-alkynyl steroids by $Pd(PPh_3)_4$/AgOAc-catalyzed coupling of steroidal 17-triflates and alkynes [12]. Since D-ring unsaturated 17-alkynyl steroids with conjugated double and triplet bond can be subsequently converted into pentacyclic steroids and 17-oxosteroid derivatives at the side chain of D-ring, this general method provides a highly efficient route to these biologically important compounds. In continuation of our studies on developing new steroids with conjugated enynes, our attention was focused on functionalized pentacyclic steroid scaffolds, which produce a variety of pentacyclic steroid derivatives with cyclo-18,21-dinorcholenone core structure (Fig. 1). For this reason, simple methods that can install this unsaturated hydrocarbon moiety are highly desirable. Thus, some conjugated alkynyl cyclo-18,21-dinorcholenones were obtained from the cyclo-18,21-dinorcholenones and 1-alkynes as a useful precursor for the preparation of many interesting steroids here (Scheme 1 and Tables 1 and 2). Evaluation of the synthesized compounds for cytotoxicity against KB, HeLa, MKN-28 and MCF-7 cell lines showed that the 22-alkynylcyclodinorchoenones possessing

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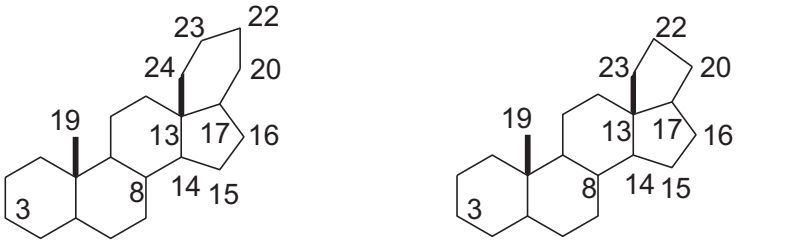
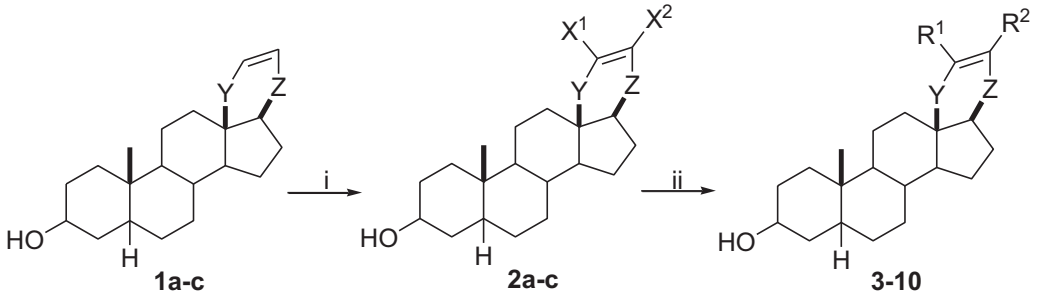


Fig. 1. Structures of 13,24-cyclo- and 13,23-cyclo-18,21-dinorcholane.



Reaction conditions: i. I_2, DMAP, pyridine, CCl_4, rt, 15 h; ii. $Pd[(C_6H_5)_3P]_4$, CuI, 1-Alkyne, DIPEA, THF, N_2, reflux, 4 h.

Scheme 1. Synthesis of 22-alkynyl-13,24(23)-cyclo-18,21-dinorcholenones **3**–**10**. Reaction conditions: (i) I_2, DMAP, pyridine, CCl_4, rt, 15 h; (ii) $Pd[(C_6H_5)_3P]_4$, CuI, 1-alkyne, DIPEA, THF, N_2, reflux, 4 h.

Table 1
Preparation of 22-iodocyclo-18,21-dinorcholenones **2a**–**c**.

Entry	3-OH	5-H	Y	Z	X^1	X^2	**2** (yield, %)	
1	α	β	CH_2	C=O	H	I	**2a**	98
2	β	α	CH_2	C=O	H	I	**2b**	93
3	α	α	C=O	N/A	I	H	**2c**	98

hydroxylethyl and hydroxylmethyl mono-substituted side chain at the end of alkynyl group have significantly inhibition activity.

2. Experimental

2.1. General

All reactions under nitrogen were run. All melting points were determined in a Yanaco melting point apparatus and are uncorrected. IR spectra were recorded in a Nicolet FT-IR 5DX spectrometer. The ^{1}H NMR (300 MHz) and ^{13}C NMR (75 MHz) spectra were recorded in a Bruker ACF-300 spectrometer with TMS as internal reference in $CDCl_3$ solutions. The *J* values are given in hertz. Only discrete or characteristic signals for the ^{1}H NMR are reported. The elemental analyses were performed in a Perkin-Elmer 240C instrument. Optical rotations were determined in a Perkin-Elmer 343 polarimeter. Flash chromatography was performed on silica gel (230–400 mesh) eluting with ethyl acetate–hexanes mixture.

2.2. Organic synthesis

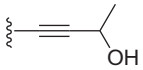
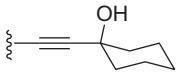
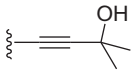
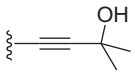
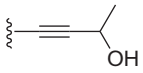
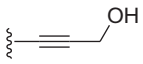
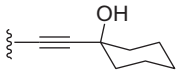
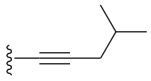
2.2.1. Preparation of the starting material 3-hydroxy-13,24-cyclo-18,21-dinorchol-22-en-20-one and 3-hydroxy-13,23-cyclo-18,21-dinorchol-20-en-23-one

(3α,5α)-3-Hydroxy-13,23-cyclo-18,21-dinorchol-20-en-23-one, (3α,5β) and (3β,5α)-3-hydroxy-13,24-cyclo-18,21-dinorchol-22-en-20-one were prepared using Dr. Covey's method (*Washington University School of Medicine* in *St. Louis*) [2].

2.2.2. Preparation of 3-hydroxy-22-iodo-13,24-cyclo-18,21-dinorchol-22-en-20-one and 3-hydroxy-22-iodo-13,23-cyclo-18,21-dinorchol-20-en-23-one

*2.2.2.1. (3α,5β)-3-Hydroxy-22-iodo-13,24-cyclo-18,21-dinorchol-22-en-20-one (**2a**).* The mixture of (3α,5β)-3-hydroxy-13,24-cyclo-18,21-dinorchol-22-en-20-one (328 mg, 1.0 mmol), 4-dimethylaminopyridine (9.8 mg, 0.08 mmol) and iodine (508 mg, 2.0 mmol) in pyridine (3.0 mL) and CCl_4 (10 mL) was stirred for 12 h at room temperature. After the mixture was diluted with EtOAc (15 ml), the resulting mixture was washed with sat. $Na_2S_2O_3$, water, 10% HCl and brine, dried over Na_2SO_4. Removal of solvent, the residue was purified by flash chromatography (silica gel, EtOAc/hexanes, 1/6) to give product (3α,5β)-3-hydroxy-22-iodo-13,24-cyclo-18,21-dinorchol-22-en-20-one (**2a**) (445 mg, 98%) as a white solid, mp 210–212 °C (EtOAc–hexanes); $[\alpha]_D^{20} = +47.47^\circ$ (c = 0.990, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) 7.59 (m, 1H), 3.63 (m, 1H), 2.50 (t, J = 10.2 Hz, 1H), 0.92 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): 194.38, 157.02, 101.62, 71.29, 56.84, 55.72, 47.96, 41.74, 39.81, 36.06, 35.64, 35.15, 34.41, 33.89, 30.28, 29.95, 27.23, 26.68, 26.26, 26.01, 23.18, 20.08; IR (NaCl, film, cm^{-1}): 3392, 2246, 1674, 1597, 1447, 1330, 910, 731;

Table 2
Synthesis of 22-alkynyl-13,24(23)-cyclo-18,21-dinorcholenones **3**–**10**.

Entry	3-OH	5-H	Y	Z	R^1	R^2	**Product**	(yield, %)
1	α	β	CH_2	C=O	H		**3**	92
2	α	β	CH_2	C=O	H		**4**	92
3	β	α	CH_2	C=O	H		**5**	93
4	α	α	C=O	N/A		H	**6**	86
5	α	α	C=O	N/A		H	**7**	90
6	α	α	C=O	N/A		H	**8**	80
7	α	α	C=O	N/A		H	**9**	92
8	α	α	C=O	N/A		H	**10**	94

Anal. Calcd. for $C_{22}H_{31}IO_2$: C, 58.15; H, 6.88; Found C, 58.10; H, 7.02.

*2.2.2.2. (3β,5α)-3-Hydroxy-22-iodo-13,24-cyclo-18,21-dinorchol-22-en-20-one (**2b**).* Following the above procedure using (3β,5α)-3-hydroxy-13,24-cyclo-18,21-dinorchol-22-en-20-one as a starting material, the title compound **2b** (93%) was obtained as a white solid, mp 188–190 °C (EtOAc–hexanes); $[\alpha]_D^{20} = +13.95°$ (c = 0.43, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) 7.59 (q, J = 3.0 Hz, 1H), 3.60 (m, 1H), 2.49 (t, J = 10.2 Hz, 1H), 0.80 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): 194.39, 156.90, 101.80, 71.05, 56.98, 55.84, 53.69, 48.05, 44.75, 38.05, 36.93, 35.50(2C), 33.89, 31.98, 31.39, 30.11, 28.37, 27.29, 26.12, 20.66, 12.34; IR (NaCl, film, cm^{-1}): 3400, 2245, 1672, 1596, 1444, 1331, 907, 731; Anal. Calcd. for $C_{22}H_{31}IO_2$: C, 58.15; H, 6.88; Found C, 58.03; H, 7.10.

*2.2.2.3. (3α,5α)-3-Hydroxy-22-iodo-13,23-cyclo-18,21-dinorchol-20-en-23-one (**2c**).* Following the above procedure using (3α,5α)-3-hydroxy-13,23-cyclo-18,21-dinorchol-20-en-23-one as a starting material, the title compound **2c** (98%) was obtained as a white solid, mp 215–217 °C (EtOAc–hexanes); $[\alpha]_D^{20} = +65.73°$ (c = 0.25, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) 7.93 (d, J = 3.6 Hz, 1H), 4.05 (s, 1H), 2.86 (dd, J = 3.3, 9.9 Hz, 1H), 2.30 (m, 1H), 0.92 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): 206.75, 170.23, 101.86, 66.10, 56.80, 54.88, 53.82, 53.37, 38.93, 36.15, 35.64, 32.91, 32.77, 32.09, 32.01, 29.72, 28.75, 28.14, 25.29, 19.41, 10.94; IR (NaCl, film, cm^{-1}): 3392, 2248, 1702, 1578, 1447, 1271, 909, 731; Anal. Calcd. for $C_{21}H_{29}IO_2$: C, 57.28; H, 6.64; Found C, 57.10; H, 6.52.

2.2.3. General procedure for the coupling reaction catalyzed by $Pd[(C_6H_5)_3P]_4$/CuI from 22-iodocyclo-18,21-dinorcholenones and 1-alkynes

To the mixture of compound **2a**–**c** (1.00 mmol), CuI (19 mg, 0.10 mmol), $Pd[(C_6H_5)_3P]_4$ (60 mg, 0.05 mmol) and DIPEA (390 mg, 3.00 mmol) in dried THF (10 mL) was added 1-alkyne (1.50 mmol) by syringe at room temperature under nitrogen. The resultant mixture was stirred under refluxing and nitrogen for 4 h. After the mixture was diluted with DCM (15 ml) and the solid was removed by filtered, the residue was purified by flash chromatography (silica gel, EtOAc/hexanes, 1/10–1/4) to give product **3**–**10**.

*2.2.3.1. (3α,5β)-3-Hydroxy-22-(3-(3-hydroxybut-1-ynyl))-13,24-cyclo-18,21-dinorchol-22-en-20-one (**3**).* Following the general procedure, the title compound **3** was obtained as needles solid, 92% in yield, mp 220–222 °C (EtOAc–hexanes); $[\alpha]_D^{20} = +13.79°$ (c = 0.435, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) 7.11 (m, 1H), 4.67 (m, 1H), 3.63 (bs, 1H), 3.28 (dd, J = 4.8, 15.3 Hz, 1H), 1.48 (d, J = 6.3 Hz, 6H), 0.92 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): 198.35, 152.39, 122.34, 94.59, 78.10, 71.60, 58.41, 58.35, 57.60, 56.40, 47.74, 41.95, 40.04, 36.02, 35.59, 35.40, 34.52, 34.27, 30.39, 27.20, 26.77, 26.40, 26.15, 24.12, 24.01, 23.22, 20.25; IR (NaCl, film, cm^{-1}): 3400, 2244, 1670, 1448, 1365, 1165, 1081, 911, 731; Anal. Calcd. for $C_{27}H_{38}O_3$: C, 78.98; H, 9.33; Found C, 78.88; H, 9.06.

*2.2.3.2. (3α,5β)-3-Hydroxy-22-(3-(2-(1-hydroxycyclohexyl)ethynyl))-13,24-cyclo-18,21-dinorchol-22-en-20-one (**4**).* Following the general procedure, the title compound **4** was obtained as needles solid, 90% in yield, mp 222–224 °C (MeOH); $[\alpha]_D^{20} = +12.01°$ (c = 0.267, $CHCl_3$); ^{1}H NMR (300 MHz, CD_3OD) 7.24 (m, 1H), 3.59 (m, 1H), 1.01 (s, 3H); ^{13}C NMR (75 MHz, DMSO-d_6): 196.96, 152.91, 121.38, 96.63, 77.82, 69.72, 66.85, 56.94, 55.37, 46.89, 41.42, 39.63, 36.14, 35.11, 34.97, 34.18(2C), 33.58, 30.27, 26.56(2C), 26.09, 25.79, 25.47, 24.83, 23.13(2C), 22.57(2C), 19.69; IR (NaCl, film, cm^{-1}): 3402, 2244, 1680, 1448, 1365, 1165, 1081, 911, 731; Anal. Calcd. for $C_{30}H_{42}O_3$: C, 79.96; H, 9.39; Found C, 79.78; H, 9.20.

*2.2.3.3. (3β,5α)-3-Hydroxy-22-(3-(3-hydroxy-3-methylbut-1-ynyl))-13,24-cyclo-18,21-dinorchol-22-en-20-one (**5**).* Following the general procedure, the title compound **5** was obtained as needles solid, 89% in yield, mp 216–218 °C (EtOAc–hexanes); $[\alpha]_D^{20} = -4.75°$ (c = 0.36, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) 7.09 (m, 1H), 3.62 (m, 1H), 3.34 (bs, 1H), 1.55 (s, 6H), 0.81 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): 198.11, 151.95, 122.51, 97.16, 76.41, 71.04,

65.12, 57.47, 56.10, 53.75, 47.55, 44.79, 37.98, 36.97, 35.53, 35.33, 34.00, 32.01, 31.30(3C), 28.41, 27.08, 26.36, 26.11, 20.63, 12.35; IR (NaCl, film, cm^{-1}): 3414, 2243, 1670, 1445, 731; Anal. Calcd. for $C_{27}H_{38}O_3$: C, 78.98; H, 9.33; Found C, 79.22; H, 9.18.

*2.2.3.4. (3α,5α)-3-Hydroxy-22-(3-(3-hydroxy-3-methylbut-1-ynyl))-13,23-cyclo-18,21-dinorchol-22-en-23-one (**6**).* Following the general procedure, the title compound **6** was obtained as needles solid, 86% in yield, mp 120–122 °C (EtOAc–hexanes); $[\alpha]_D^{20} = +60.29°$ ($c = 0.59$, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$) 7.60 (d, $J = 3.3$ Hz, 1H), 4.09 (s, 1H), 3.82 (s, 1H), 2.74 (m, 1H), 1.56 (s, 6H), 0.912 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): 209.81, 165.48, 128.24, 100.50, 73.04, 66.42, 64.98, 57.28, 57.04, 54.10, 49.86, 39.16, 36.35, 35.81, 33.09, 32.95, 32.31, 32.25, 31.13, 30.98, 30.16, 28.84, 28.34, 25.50, 19.66, 11.22; IR (NaCl, film, cm^{-1}): 3401, 2245, 1687, 1448, 1360, 1165, 908, 732; Anal. Calcd. for $C_{26}H_{36}O_3$: C, 78.75; H, 9.15; Found C, 78.48; H, 8.96.

*2.2.3.5. (3α,5α)-3-Hydroxy-22-(3-(3-hydroxybut-1-ynyl))-13,23-cyclo-18,21-dinorchol-22-en-23-one (**7**).* Following the general procedure, the title compound **7** was obtained as needles solid, 90% in yield, mp 115–117 °C (EtOAc–hexanes); $[\alpha]_D^{20} = +82.21°$ ($c = 0.62$, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$) 7.61 (d, $J = 3.9$ Hz, 1H), 4.69 (m, 1H), 4.08 (s, 1H), 3.63 (d, $J = 4.2$ Hz, 1H), 2.75 (m, 1H), 2.30 (m, 1H), 2.20 (s, 1H), 1.49 (d, $J = 6.6$ Hz, 3H), 0.91 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): 209.98, 165.74, 128.08, 97.80, 74.78, 66.44, 58.24, 57.30, 56.90, 53.94, 49.86, 39.13, 36.34, 35.74, 33.01, 32.92, 32.27, 32.24, 30.10, 28.79, 28.32, 25.53, 23.91, 19.58, 11.11; IR (NaCl, film, cm^{-1}): 3418, 2247, 1694, 1613, 1447, 1115, 908, 732; Anal. Calcd. for $C_{25}H_{34}O_3$: C, 78.49; H, 8.96; Found C, 78.40; H, 8.76.

*2.2.3.6. (3α,5α)-3-Hydroxy-22-(3-(3-hydroxyprop-1-ynyl))-13,23-cyclo-18,21-dinorchol-22-en-23-one (**8**).* Following the general procedure, the title compound **8** was obtained as needles solid, 80% in yield, mp 110–113 °C (EtOAc–hexanes); $[\alpha]_D^{20} = +55.71°$ ($c = 0.70$, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$) 7.64 (d, $J = 3.9$ Hz, 1H), 4.44 (s, 2H), 4.07 (s, 1H), 2.76 (m, 1H), 2.49 (bs, 1H), 2.30 (m, 1H), 0.91 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): 209.75, 166.13, 128.11, 93.79, 76.73, 66.60, 57.40, 56.93, 54.03, 51.38, 49.97, 39.28, 36.44, 35.85, 33.09, 33.00, 32.33(2C), 30.16, 28.97, 28.40, 25.64, 19.62, 11.90; IR (NaCl, film, cm^{-1}): 3400, 2248, 1694, 1446, 908, 731; Anal. Calcd. for $C_{24}H_{32}O_3$: C, 78.22; H, 8.75; Found C, 78.20; H, 8.66.

*2.2.3.7. (3α,5α)-3-Hydroxy-22-(3-(2-(1-hydroxycyclohexyl)ethynyl))-13,23-cyclo-18,21-dinorchol-22-en-23-one (**9**).* Following the general procedure, the title compound **9** was obtained as needles solid, 92% in yield, mp 150–152 °C (EtOAc–hexanes); $[\alpha]_D^{20} = +74.43°$ ($c = 0.46$, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$) 7.60 (d, $J = 3.6$ Hz, 1H), 4.08 (s, 1H), 3.75 (m, 1H), 2.73 (m, 1H), 2.33–2.29 (m, 2H), 0.91 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): 209.85, 165.42, 128.32, 99.69, 74.78, 68.42, 66.38, 57.24, 57.03, 54.05, 49.82, 39.58(2C), 39.10, 36.31, 35.76, 33.06, 32.89, 32.30, 32.24, 30.16, 28.81, 28.32, 25.50, 25.16, 23.15(2C), 19.66, 11.23; IR (NaCl, film, cm^{-1}): 3419, 2243, 1689, 1614, 1447, 907, 732; Anal. Calcd. for $C_{29}H_{40}O_3$: C, 79.77; H, 9.23; Found C, 79.42; H, 9.18.

*2.2.3.8. (3α,5α)-3-Hydroxy-22-(3-(4-methylpent-1-ynyl))-13,23-cyclo-18,21-dinorchol-22-en-23-one (**10**).* Following the general procedure, the title compound **10** was obtained as needles solid, 94% in yield, mp 110–112 °C (EtOAc–hexanes); $[\alpha]_D^{20} = +87.70°$ ($c = 0.37$, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$) 7.55 (d, $J = 3.6$ Hz, 1H), 4.05 (s, 1H), 2.73 (m, 1H), 2.35 (m, 1H), 2.27 (d, $J = 6.6$ Hz, 2H), 1.00 (d, $J = 6.6$ Hz, 6H), 0.92 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): 209.72, 164.49, 129.09, 95.55, 72.36, 66.45, 57.00, 56.95, 54.08, 49.57, 39.17, 36.35, 35.81, 33.10, 32.83, 32.25, 30.22, 28.93, 28.63, 28.34, 27.97, 25.47, 22.01(3C), 19.61, 11.09; IR (NaCl, film, cm^{-1}): 3393, 2247, 1701, 1612, 1448, 909, 732; Anal. Calcd. for $C_{27}H_{38}O_2$: C, 82.18; H, 9.71; Found C, 82.12; H, 9.58.

2.3. Cytotoxic activity against human epidermoid carcinoma cell line KB, human cervical carcinoma cell line HeLa, human gastric carcinoma cell line MKN-28 and human breast carcinoma cell line MCF-7

All tumor cell lines tested were purchased from Shanghai Institute of Cell Biology, Chinese Academy of Science. The cell lines were cultured in RPMI 1640 medium with 10% newborn calf serum. It was maintained in a humidified incubator with an atmosphere of 95% air and 5% CO_2 at 37 °C. The cells were continuously passaged once every 3–4 days. Growing cells were collected on experiments.

DMSO was used as latent solvent with the highest concentration less than 0.1% in solution of the drug. The control groups of doxorubicin, blank (1640) and DMSO solvent were set up at the same time. Proliferative activity was evaluated by colorimetric sulforhodamine B (SRB) assay. Briefly, cells were plated in 96-well plates. After cell adhering, they were treated with different compounds in a dose-dependent way for 44 h. Then the cells were fixed by 10% TDA for 1 h and stained by SRB for 10 min. After washed with acetic acid to remove the excess dye, protein bounding dye was dissolved in 10 mM Tris and detected by a Model Elx 800 Autoplate reader (Bio-Tek Instruments, USA).

3. Results and discussion

3.1. Chemistry

To initiate our studies, we first prepared three 22-iodo-18,21-dinorcholenones in 93–98% yields through iodination of the corresponding 18,21-dinorcholenones using iodine in the presence of DMAP and pyridine in CCl_4 at room temperature for 15 h (Scheme 1 and Table 1). Recently, the construction of conjugated enynes via palladium-catalyzed coupling reaction of 1-alkynes with 1-haloalkenes or alkenyl triflates has attracted considerable interest [13,14]. Among of them, it is convenient of palladium-catalyzed cross-coupling reactions of terminal alkynes with α-halo-α,β-unsaturated ketones. Typically these coupling reactions are carried out using a palladium catalyst such as $Pd(PPh_3)_2Cl_2$, $Pd(PPh_3)_4$, $Pd(OAc)_2$ and a copper salt as co-catalyst in the presence of an amine base. To make a detailed investigation for the cross-coupling reaction of 22-iodo cyclo-18,21-dinorcholenone **2a** and 3-butyn-2-ol, firstly, (3α,5β)-3-hydroxy-22-iodo-13,24-cyclo-18,21-dinorchol-22-en-20-one 2a was treated with 1.5 equiv. of 3-butyn-2-ol in THF in the presence of 10% $Pd(PPh_3)_2Cl_2$ (0.05 equiv.), CuI (0.10 equiv.), and DIPEA (3.00 mmol) under a nitrogen atmosphere to give (3α,5β)-3-hydroxy-22-(3-(3-hydroxybut-1-ynyl))-13,24-cyclo-18,21-dinorchol-22-en-20-one **3** in 85% yield (Table 3, entry 1). The use of $Pd(PPh_3)_4$ (0.05 equiv.) or Pd/C (0.05 equiv.)-PPh_3 (0.12 equiv.) in the place of $Pd(PPh_3)_2Cl_2$ gave the yield of **3** to 92%, 72% respectively (Table 3, entries 2 and 3). Therefore, it was evident that $Pd(PPh_3)_4$ (5 mol%) was the most effective catalyst for this transformation. But Pd/C–CuI–PPh_3 is a less expensive catalytic system than $Pd(PPh_3)_4$–CuI or $Pd(PPh_3)_2Cl_2$–CuI catalytic system. When copper iodide was replaced with silver halides for the coupling reaction of sensitive vinyl triflates and 1-alkyne [15], the desired conjugated enyne product was efficiently obtained in good yield. Thus, we tested this reaction using the above said 22-iodo-18,21-dinorcholenone **2a** and 3-butyn-2-ol in THF under nitrogen in the presence of $Pd(PPh_3)_4$ using silver iodide as a co-catalyst. It

Table 3
Pd/Ag or Cu salt-catalyzed cross-coupling reaction of 22-iodo cyclo-18,21-dinorcholenone **2a** with 3-butyn-2-ol to give compound **3**[a].

Entry	[Pd] catalyst	Co-catalyst	Base	Solvent	Yield (%)[b]
1	$PdCl_2(PPh_3)_2$	CuI (10 mol%)	$^iPr_2NEt$	THF	85
2	$Pd(PPh_3)_4$	CuI (10 mol%)	$^iPr_2NEt$	THF	92
3	Pd/C-PPh_3	CuI (10 mol%)	$^iPr_2NEt$	THF	72
4	$Pd(PPh_3)_4$	AgI (20 mol%)	$^iPr_2NEt$	THF	76
5	$Pd(PPh_3)_4$	$AgBF_4$ (20 mol%)	$^iPr_2NEt$	THF	82
6	$Pd(PPh_3)_4$	AgOAc (20 mol%)	$^iPr_2NEt$	THF	86
7	$Pd(PPh_3)_4$	CuI (10 mol%)	$^iPr_2NEt$	DMF	86
8	$Pd(PPh_3)_4$	CuI (10 mol%)	$^iPr_2NEt$	MeCN	80
9	$Pd(PPh_3)_4$	CuI (10 mol%)	Et_3N	THF	88
10	$Pd(PPh_3)_4$	CuI (10 mol%)	Pyridine	THF	62
11	$Pd(PPh_3)_4$	CuI (10 mol%)	K_2CO_3	THF	70
12	$Pd(PPh_3)_4$	CuI (10 mol%)	Cs_2CO_3	THF	87
13	$Pd(PPh_3)_4$	CuI (20 mol%)	$^iPr_2NEt$	THF	92
14	$Pd(PPh_3)_4$	CuI (5 mol%)	$^iPr_2NEt$	THF	80

[a] Reaction conditions: 22-iodo cyclo-18,21-dinorcholenone **2a**/3-butyn-2-ol/base = 1/1.5/3.0. Refluxing, nitrogen; 4 h
[b] Isolated yield based on 22-iodo cyclo-18,21-dinorcholenone **2a**.

was found that $Pd(PPh_3)_4$/AgI catalysts in THF gave product **3** in 76% yield (Table 3, entry 4). Besides silver iodide, fluoroborate or acetate [12,16] proved to be efficient in this reaction too, although lower yields were obtained (Table 3, entries 5 and 6). Afterwards, we examined various bases ($^iPr_2NEt$, Et_3N, pyridine, K_2CO_3 and Cs_2CO_3) and solvents (DMF, MeCN and THF) (Table 3, entries 2 and 7–12). Among these tested conditions, $^iPr_2NEt$, Et_3N and caesium carbonate could promote efficiently this reaction in THF as solvent in high yield. However, the weak base pyridine and potassium carbonate promoted limitedly this coupling reaction in lower yields (Table 3, entries 10 and 11). Furthermore, the co-catalyst copper iodide concentration was between 10 and 20 mol% to yield product **3** in 92% (Table 3, entry 13). But the result showed that yields of product **3** were obviously different when cuprous iodide concentration was reduced from 10% to 5 mol%. Under general and optimized conditions: 22-iodo cyclo-18,21-dinorcholenones **2a**–**c**, 1-alkynes, $Pd(PPh_3)_4$, CuI and $^iPr_2NEt$ in the mole ratios of 1:1.5:0.05:0.10:3.0 in THF under reflux), a series of 22-iodo cyclo-18,21-dinorcholenones **2a**–**c** and 1-alkynes were tested toward this coupling reaction. As indicated in Table 3, various 22-iodo cyclo-18,21-dinorcholenones and 1-alkynes were effective for this coupling reaction, and the desired products **3**–**10** could be obtained in excellent yields (Table 3, entries 1–8).

3.2. Biological activity

Covey and coworkers [2] reported the starting material 3-hydroxy-13,24-cyclo-18,21-dinorchol-22-en-20-one (**1a** and **1b**) shows modest cytotoxicity on the [^{35}S]TBPS as IC_{50} (373 ± 20–1570 ± 140 nM) at the GABA receptors. However, some studies showed those natural and synthetic steroids with αβ unsaturated ketone or enyne structural core give the potency against human cancer cell lines [17]. Thus, all compounds synthesized as described above were subjected to in vitro cytotoxic evaluation against KB (human epidermoid carcinoma), HeLa (human cervical carcinoma), MKN-28 (human gastric cancer) and MCF-7 (human breast carcinoma) cell lines. Doxorubicin which exhibits cytotoxicity against KB, HeLa, MKN-28 and MCF-7 cell lines, was used as reference. The results are summarized in Table 4.

From the data shown in Table 4, most of the new compounds showed a measurable anti-cancer activity against KB, HeLa, MKN-28 and MCF-7 cell lines tested. Although this is a preliminary screening, the results showed compound **10** containing only isobutyl side chain without hydroxyl was found inactive to all the tumor cells tested (IC_{50} value > 80 μg/ml). Similar results were found for the compounds **4**–**6** and **9** containing hydroxyl multi-substituted side chain at the end of alkynyl group for all cell lines. However, the cytotoxicity data of 22-alkynylcyclodinorchoenone possessing hydroxylethyl side chain at the end of alkynyl group showed significant cytotoxicity activity (IC_{50} value 23.2 ± 0.8, 32.0 ± 2.6, 21.6 ± 2.4 and 46.8 ± 3.5 μg/ml for KB, HeLa, MKN-28 and MCF-7, respectively). Similar results were also found for the compounds **3** and **7** containing hydroxyl mono-substituted side chain at the end of alkynyl group for all cell lines. The above results indicated that the end of 22-alkynyl group with hydroxyl mono-substituted side chain must be present to retain cytotoxic activity. This may indicate that the anticancer properties depend not only on hydroxyl group of the end of 22-alkynyl side chain but also on the moieties attached to the side chain.

Table 4
The in vitro cytotoxic activity (IC_{50}, in μM) of compounds against human carcinoma cell lines (KB, HeLa, MKN-28, and MCF-7)[a].

Compound	Cancer cell lines		IC_{50} (μg/ml)	
	KB	HeLa	MKN-28	MCF-7
3	32.0 ± 4.6	34.0 ± 0.5	22.2 ± 3.2	26.5 ± 0.5
4	76.0 ± 1.3	57.8 ± 3.2	52.2 ± 0.6	48.2 ± 1.8
5	>80	72.4 ± 3.2	70.3 ± 2.5	59.5 ± 3.0
6	>80	>80	>80	73.2 ± 2.6
7	45.2 ± 4.5	56.2 ± 0.8	60.4 ± 1.3	52.4 ± 0.7
8	23.2 ± 0.8	32.0 ± 2.6	21.6 ± 2.4	46.8 ± 3.5
9	>80	76.2 ± 0.8	>80	>80
10	>80	>80	>80	>80
Doxorubicin[b]	0.024	0.22	0.20	0.23

KB: human epidermoid carcinoma; HeLa: human cervical carcinoma; MKN-28: human gastric cancer; MCF-7: human breast cancer.
[a] The results are the average mean of eight replicate determinations ± SD.
[b] Used as reference.

4. Conclusion

In summary, we have successfully developed a novel and operationally simple coupling reaction for highly efficient synthesis of 22-alkynyl-13,24(23)-cyclo-18,21-dinorchol-22-en-20(23)-ones. Firstly, 22-Iodocyclo-18,21-dinorcholenones were prepared from cyclo-18,21-dinorcholenones using I_2/DMAP/pyridine system. The cross coupling reaction of 22-iodocyclo-18,21-dinorcholenones and 1-alkynes was carried out efficiently catalyzed by tetrakis(triphenylphosphine) palladium/cuprous iodide in the presence of base diisopropylethylamine. This strategy offered a very straightforward and efficient method for access to conjugated alkynyl cyclo-18,21-dinorcholenones from the cyclo-18,21-dinorcholenones and 1-alkynes in excellent overall yields, which are key intermediates for the preparation of some biologically important modified 22-side chain pentacyclic steroids and structurally related fused pentacyclic steroids.

The preliminary results showed that those 22-alkynylcyclodinorchoenones possessing hydroxylethyl and hydroxylmethyl mono-substituted side chain at the end of alkynyl group have significant impact on inhibiting human epidermoid carcinoma cell line KB, human cervical carcinoma cell line HeLa, human gastric cancer cell line MKN-28 and human breast carcinoma cell line MCF-7. Due to the structural features of our novel compounds, this mechanism of action cannot be discarded. Further research on the structure–activity relationship, their possible mechanism of inhibiting proliferation of cancer cell lines and the development of 22-alkynylcyclodinorchoenones as promising anticancer agents is ongoing.

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