Arch. Pharm. Res. Vol.20, No.5, pp. 476-479, 1997

Studies on the Synthesis and *in vitro* anti-Tumor Activity of Dihydroberberine Derivatives

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(Received June 10, 1997)

Three types of dihydroberberine derivatives such as spirobenzylisoquinoline, benzindenoazepine and cyclopropanated quinolizine species were synthesized from dihydroberberine for the investigation on their anti-tumor activity. Among them, cyclopropanated quinolizine species were more effective than spirobenzylisoquinoline and benzindenoazepine against P-388 and L-1210 leukemia cell.

Key words : Dihydroberberine, Dichlorocarbene addition, Antiproliperative activity

INTRODUCTION

Chemical transformations of berberine and dihydroberberine were well investigated by Hanaoka and his collegues (Hanaoka *et al.*, 1984 and 1986). In spite of broad investigation on their chemical reactivity, studies on the structure-activity relationship of their analogs were relatively unexplored.

There are strong possibilities that dihydroberberine related compound can be a good candidate for anti-tumor agent (Sufness *et al.*, 1979) when judging from the example of benzo[c]phenanthridine(Cho *et al.*, 1996 and Simeon *et al.*, 1989).

Our efforts (Chung *et al.*, 1990 and Woo *et al.*, 1996) to find a potential anti-tumor agent have made us investigate isoquinoline ring system such as dihydroberberine which has a chemical variety (Hanaoka *et al.*, 1984). Chemical-biological relationship of three types of dihydroberberine derivatives on their anti-tumor activity are described herein.

MATERIALS AND METHODS

Melting points were determined on a international melting point apparatus and are uncorrected. Column chromatography was carried out with alumina (Al_2O_3, neutral, 70~230 mesh, Merck) and silica gel(Kieselgel 60, 70~230 mesh, Merck). ^{1}H NMR spectra were recorded on Varian gemini-300 (300 MHz) and Brucker AC-100F (100 MHz) spectrophotometer using tetramethylsilane (TMS) as an internal standard unless otherwise stated. Infrared (IR) spectra were measured with a Brucker IFS28 spectrophotometer.

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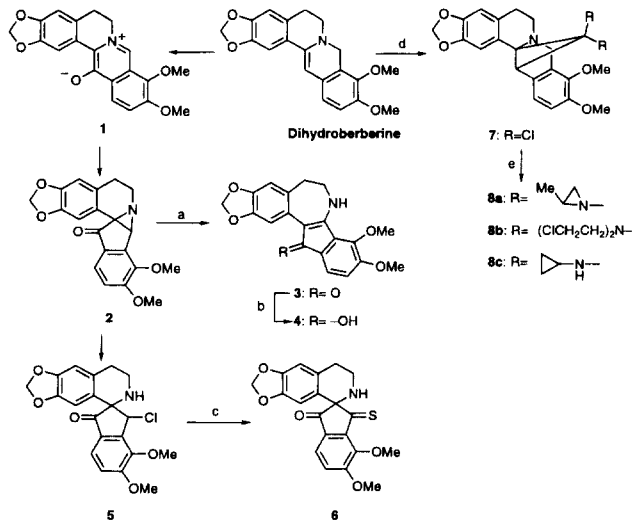
5,6,7,13-Tetrahydro-9,10-dimethoxy-2,3-methylenedioxy-benz[d]indeno[1,2-b]azepine-13-one[3]

A solution of **2** (80 mg, 0.23 mmol) and *p*-TsOH (100 mg, 0.57 mmol) in benzene (3 ml) was refluxed for 2hr. After the solvent being evaporated, the residue was extracted with EtOAc (3×5 ml), washed with sat. $NaHCO_3$ solution (2×5 ml) and brine (5 ml), dried over Na_2SO_4, filtered and concentrated in vacuo. The residue was purified by column chromatograpy (silica gel, CH_2Cl_2:EtOAc=19:1) to give 3 (56 mg, 70 %). mp 238~240°C, IR ν_{max} ($CHCl_3$) 3375, 1660 cm^{-1}; ^{1}H-NMR (in $CDCl_3$) δ 2.81-2.87 (2H, m, C5-H), 2.98-3.16 (2H, m, C6-H), 3.89 (3H, s, OMe), 3.97 (3H, s, OMe), 5.90 (2H, s, -OCH_2O-), 6.69 (1H, s, C4-H), 6.74 (1H, AB-q, J=8 Hz, C11-H), 7.21 (1H, AB-q, J=8 Hz, C12-H), 7.87 (1H, s, C1-H).

13-Hydroxy-10,11-dimethoxy-2,3-methylenedioxy-benz[d]- indeno[1,2-b]azepine [4]:

To a solution of **3** (100 mg, 0.28 mmol) in EtOH-CH_2Cl_2 (5 ml:2 ml) was added $NaBH_4$ (10.4 mg, 0.28 mmol). After the reaction being stirred for 7hr at room temperature, removal of solvent followed by extraction with CH_2Cl_2 (3×5 ml) and work-up gave the crude material. It was purified by column chromatography (silica gel, EtOAc) to give **4** (82 mg, 81%) as a white powder. mp 188-190°C; IR ν_{max} ($CHCl_3$) 3186 cm^{-1}; ^{1}H-NMR (in $CDCl_3$) δ 2.84 (2H, br-t, J=7 Hz, C5-H), 3.44 (2H, dt, J=3, 7 Hz, C6-H), 3.84 (6H, br-s, 2 x OMe), 4.23 (1H, br-s, NH), 5.97 (2H, s, -OCH_2O-), 6.63 (1H, s, C4-H), 6.79 (1H, AB-q, J=8 Hz, C11-H),



Schme 1. Reagents and conditions: (a) p-TsOH, benzene, 70%; (b) $NaBH_4$, EtOH-CH_2Cl_2, 81% (c) thiourea, EtOH, 71%; (d) Ph_3N, 30% NaOH, $CHCl_3$, 63% (e) R_2NH, 60% NaH in oil, 38~66%.

7.04 (1H, AB-q, J=8 Hz, C12-H), 7.45 (1H, s, C1-H).

2,3-Methylenedioxy-9,10-dimethoxy-8-thionorchotensane-13-one [6]

To a solution of **5** (80 mg, 0.21 mmol) in EtOH (10 ml) was dropwise added a solution of thiourea (32 mg, 0.42 mmol) in EtOH (2 ml).

The reaction was refluxed for 30 min, cooled to room temperature, diluted with H_2O (10 ml) and neutralized with 10% NaOH solution. After the solvent being removed, the residue was purified by column chromatography (silica gel, hexanes:EtOAc=1:1) to give 6 (56 mg, 71%). mp 186-188°C; IR ν_{max} ($CHCl_3$) 1768, 1343 cm^{-1}: ^{1}H-NMR (in $CDCl_3$) δ 2.81-2.88 (2H, m, C 5-H), 2.99-3.28 (2H, m, C6-H), 3.87 (3H, s, OMe), 3.97 (3H, s, OMe), 6.02 (2H, s, -OCH_2O-), 6.60 (1H, s, C4-H), 7.59 (1H, s, C1-H), 7.15 (1H, AB-q, J=8 Hz, C11-H), 7.67 (1H, AB-q, J=8 Hz, C12-H).

2,3-Methylenedioxy-9,10-dimethoxy-13,14-dichlorocarbeneldibenzo[a, g]quinoline [7]

To a solution of dihydroberberine (100 mg, 0.3 mmol) and Ph_3N (7.5 mg, 0.03 mmol) in $CHCl_3$ (3 ml) was added 30% NaOH solution (2 ml) at 0°C. The reaction was stirred for 1 hr at room temperature and stirred vigorously for 3 hr at 50°C. After the solvent being evaporated, the residue was extracted with CH_2Cl_2 (3× 5 ml), washed with H_2O (2×5 ml), and brine, dried over Na2SO4 and evaporated *in vacuo.*

The residue was purified by column chromatography (silica gel, benzene) to give **7** (76 mg, 63%). IR ν_{max} ($CHCl_3$) 740 cm^{-1}; ^{1}H-NMR (in $CDCl_3$) δ 2.72-2.79 (2H, m, C5-H), 3.30-3.40 (2H, m, C6-H), 3.72-3.76 (2H, m, C8-H), 3.87 (3H, s, OMe), 3.93 (3H, s, OMe), 5.66 (1H, s, C13-H), 5.95 (2H, s, -OCH_2O-), 6.61 (1H, s, C4-H), 6.85 (1H, AB-q, J=8 Hz, C11-H), 6.98 (1H, AB-q, J=8 Hz, C12-H), 7.35 (1H, s, C1-H).

General Procedure for 8a, 8b and 8c

To a solution of **7** (0.17-0.24 mmol) and each amine (2.5 eq.) in CH_2Cl_2 (2 ml) was added 60% NaH in oil (2.5 eq.). The reaction was stirred for 3 hr at room temperature and then quenched by addition of H_2O (1 drop). After the solvent being removed, the residue was extracted with CH_2Cl_2 (3×3 ml), washed with H_2O (2×3 ml) and brine, dried over Na_2SO_4 and evaporated in vacuo. The residue was purified by column chromatography (Al_2O_3:CH_2Cl_2=10: 1) to give **8a**, **8b** and μ8c, respectively.

2,3-Methylenedioxy-9,10-dimethoxy-13,14-di(2-methylaziridyl)carbeneldibenzo[a,g]quinoline [8a]

8a (42 mg, 38%) was obtained from **7** (100 mg, 0.24 mmol), 2-methylaziridine (42 ml, 0.60 mmol) and 60% NaH in oil (24 mg, 0.60 mmol) according to the procedure described above. mp 196-198°C; IR ν_{max} ($CHCl_3$) 2930 cm^{-1}; ^{1}H-NMR (in $CDCl_3$) δ 1.60 (6H, br-s, 2 x Me), 4.18 (6H, br-s, 2 x OMe), 5.31 (1H, s, C13-H), 6.09 (2H, s, -OCH_2O-), 6.82 (1H, AB-q, J=8 Hz, C11-H), 7.39 (1H, AB-q, J=8 Hz, C12-H), 7.80 (1H, s, C1-H).

2,3-Methylenedioxy-9,10-dimethoxy-13,14-di[bis(2-chloroethyl)amino]carbenedibenzo[a,g]quinoline [8b]

8b (45 mg, 66%) was obtained from **7** (100 mg, 0.24 mmol), bis (2-chloroethyl)-amine (107 mg, 0.60 mmol) and 60% NaH in oil (24 mg, 0.60 mmol) according to the procedure described above. mp 214-216°C; IR ν_{max} ($CHCl_3$) 2970 cm^{-1}; ^{1}H-NMR (in $CDCl_3$) δ 4.07 (8H, br-s, -$CH_2\underline{CH_2}Cl$), 4.34 (8H, br-s, 4 x -$\underline{CH_2}CH_2Cl$), 5.30 (1H, s, C13-H), 6.09 (2H, s, -OCH_2O-), 6.83 (1H, s, C 4-H), 7.33 (1H, AB-q, J=8 Hz, C11-H), 7.80 (1H, AB-q, J=8 Hz, C12-H), 8.21 (1H, s, C1-H).

2,3-Methylenedioxy-9,10-dimethoxy-13,14-di(cycloamino)-carbenedibenzo[a,g]quinoline [8c]

8c (43.7 mg, 56%) was obtained from **7** (70 mg, 0.17 mmol), cyclopropylamine (29 ml, 0.42 mmol) and 60 % NaH in oil (17 mg, 0.42 mmol) according to the procedure described above. mp 162-164°C IR ν_{max} ($CHCl_3$) 3350 cm^{-1}; ^{1}H-NMR (in $CDCl_3$) δ 1.28 (1H, br-s, 2 x cyclopropyl), 2.71 (2H, m, C5-H), 3.32 (2H, m, C6-H), 3.66 (2H, m, C8-H), 3.87 (3H, s, OMe), 3.93 (3H, s, OMe), 5.63 (1H, s, C13-H), 5.98 (2H, s, -OCH_2O-), 6.61 (1H, s, C4-H), 6.84 (1H, AB-q, J=8 Hz, C11-H), 6.98 (1H, AB-q, J=8 Hz, C12-H), 7.16 (1H, s, C1-H).

Antiproliferative Assays

Antiproliferative assays were performed essentially as reported previously (Denizot and Lang, 1986, Baek *et al.*, 1996 and Kil *et al.*, 1996).

Cells (P-388 and L-1210 leukemia cell) were seeded into microtiter plates and incubated overnight. Each compound dissolved in DMSO was added in serial dilution (the final DMSO concentrations in all assays did not exceed 1%). Then, 10 μl new media or containing each compound at the indicated concentrations were added and the plates were incubated for 48hrs. Cells were washed once before adding 50 μl FBS-free medium containing 5 mg/ml (MTT) concentration. After 4hrs incubation at 37°C, the medium was discarded and formazan blue formed into the cells was replaced by adding 100 μl of DMSO. Optical density was measured at 570 nm.

RESULTS AND DISCUSSION

Three types of dihydroberberine derivatives were synthesized (see **4**, **6** and **8**). The cycloberbine **2** made by conventional method was treated with HCl gas to give chloroketone **5**. Thioketone formation from **5** with thiourea was smoothly occurred to afford **6** in 71% yield. The cycloberbine **2** was faced to BC ring transformation. Treatment of **2** with *p*-toluenesulfonic acid (*p*-TsOH) in benzene provided the unsaturated benzindenoazepine **3** in 70% yield. Successive reduction of **3** gave aminoalcohol **4** in 81% yield.

On the other hand, electrophilic attack on dihydroberberine can be presumed because it has endocyclic double bond in C ring. Thus, addition of dichlorocarbene to dihydroberberine was attempted in phase transfer condition (Castro *et al.*, 1985, $CHCl_3$, 30% NaOH and Ph_3N) to give dichloro-cyclopropylquinolizine 7 in moderate yield. Although **7** can be used for various chemical trasformation such as C-ring homologation in reductive condition, biologically active amines were tentatively introduced to this system for aiming at the enhancement of activity. After all, 2-methylaziridine, cyclopropylamine and bis(2-chloroethyl) amine were introduced to cyclopropylquinolizine ring system to give **8a**, **8b** and **8c** in 38-66% yield.

The antiproliferative activities of each compound (dihydroberberine, **3**, **4**, **6**, **7**, **8a**, **8b** and **8c**) against P-388 and L-1210 leukemia cells were investigated by MTT test (Table I).

Any significant effect was not obtained from BC-ring transformed or spiro type compound **3**, **4** and **6** (ED_{50} >200 μg/ ml). This result is in accordance with the previous investigation on antimicrobial activity (Kim *et al.*, 1994).

A critical result was obtained from the addition product **7** and **8**. In special, non-substituted dichlorocyclopropylquinolizine 7 was most effective (ED_{50} 2.26 μg/ml to P-388 and 2.28 μg/ml to L-1210). These facts can be summarized as the followings: (i) chemical transformation of BC ring in dihydroberberine does not effect on anti-proliferative activity; (ii) The existance of cyclopropane on quinolizine ring plays an important role in antiproliferative activity against leukemia cell. Although this result demand a further investigation on the influence of cyclopropane ring, it shows a strong possibilty that addition product can be a good candidate for an anti cancer drug.

Table I. Inhibition of Tumor Cells Proliferation by Dihydroberberine and its Derivatives (**3, 4, 6, 7, 8a, 8b,** and **8c**) *in vitro*

Compound	ED_{50} (μg/ ml)	
	P-388	L-1210
Dihydroberberine	24.6	26.0
3	>200	>200
4	>200	>200
5	>200	>200
7	2.26	2.28
8a	8.1	20
8b	10.3	12.5
8c	17.5	7.37

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