Pergamon

Bioorganic & Medicinal Chemistry Letters 10 (2000) 1021–1023

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

The First Synthesis of Clausenamine-A and Cytotoxic Activities of Three Biscarbazole Analogues Against Cancer Cells

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Received 5 January 2000; accepted 28 February 2000

Abstract—Clausemine-A (**3**), isolated from the stem and root bark of *Clausena excavata*, was synthesized using Suzuki cross-coupling and Oxidative coupling as the key step. Compound **3**, and the other two structurally related biscarbazoles **1** and **2**, showed potent cytotoxic activities against a variety of human cancer cell lines in vitro.

Introduction

Several biscarbazoles with diverse biological activities have been isolated from different natural sources over the past decades.[1] For example, dimeric *O*-demethylmurrayafoline A (**1**) was found to exhibit antiplasmodial activity against *P. falciparum* in vitro.[2] Recently, clausenamine-A (**3**)[3] was isolated from the stem and root bark of *Clausena excavata*, which is used as folk medicine for detoxication caused by the poisonous snakebite in China. We[4] have synthesized two optically pure biscarbazoles through oxidative coupling of phenolic monomer. As a continuation of our work, we report herein on the first synthesis of clausenamine-A (**3**) and its cytotoxic activity. The activities of structurally related analogues **1** and **2** were also described.

Synthesis

To achieve a general and regiospecific synthesis of clausenamine-A (**3**), previously we chose the similar manner as in the preparation of **1** and **2**, which involved the oxidative coupling of phenolic monomer. However, owing to the electron-donating effect of the two methoxy groups in the benzene ring, we failed to get the desired coupling product through $Pd(OAc)_2$ catalyzed cyclization of *N*-(2,4-dimethoxy)-phenyl-2-benzyloxy-4-methylaniline and cyclization of 4-methyl-cyclohexane-1,2-dione-1-phenylhydrazone.[4] Therefore, another synthetic route starting from the commercially available 6-amino-*m*-cresol (**5**) was employed, as depicted in Scheme 1. There are three major stages involved in this approach: (i) Suzuki cross-coupling of boronic acid moiety and bromination moiety (**7**+**8**); (ii) regioselective bromination of the coupling product (**9**→**10**); (iii) palladium(0) mediated cyclization of amino bromide moiety (**10**→**11**).

After trying to use several protected groups, tosylate (**6**)[5] was employed as the electron-withdrawing group (Scheme 2). Compound **7** was gained through electrophilic aromatic bromination[6] of **6** using bromodimethylsulfonium bromide generated in situ. The brominate position substituted in the *ortho* position of the amino group was confirmed by NOE effects (94% yield). The required biphenyl (**9**) is prepared quantitatively by Suzuki cross coupling of 3,5-dimethoxyphenyl boronic acid (**8**)[7] and **7** with 5% $Pd(PPh_3)_4$, 2 N aqueous Na_2CO_3 in benzene.[8] Analysis of the electron distributing of **9** through the semiempirical MO calculation using the hyperchem programs disclosed that the phenyl owned two methoxy groups is electron abundant, which fits for the electrophilic bromination procedure. Bromination of **9** using 48% HBr in DMSO afforded compound **10** in 79.5% yield.[6] The five-membered ring closure was successfully performed through a palladium(0) mediated cyclization[9] of amino bromide **10** which provided 9-H-carbazole **11** in 97% yield. Deprotection of tosylate group gave the monomer **4**. Oxidative coupling of the phenolic monomer **4** was completed by aerial treatment of **4** with $(t\text{-BuO})_2$ in chlorobenzene to provide the racemic clausenamine-A (**3**) in 90% yield.[10] The structure of **3** was fully characterized by spectroscopic analysis such as ^{1}HNMR, IR, MS, and so on.[11]

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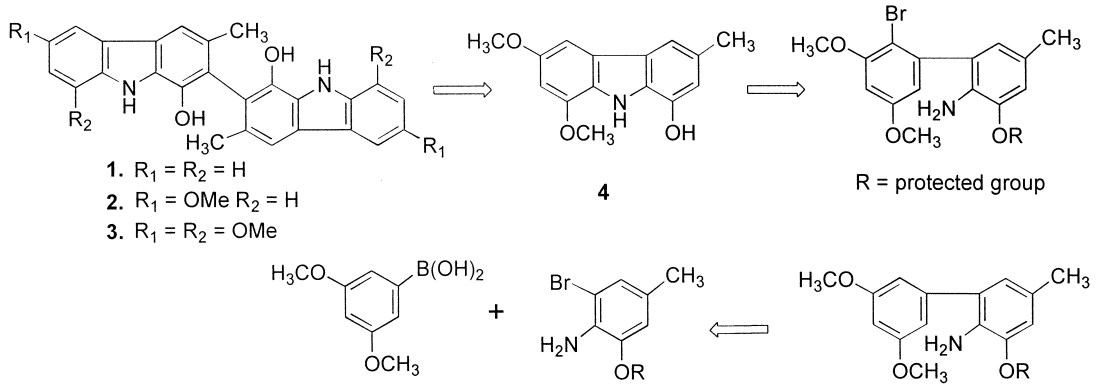
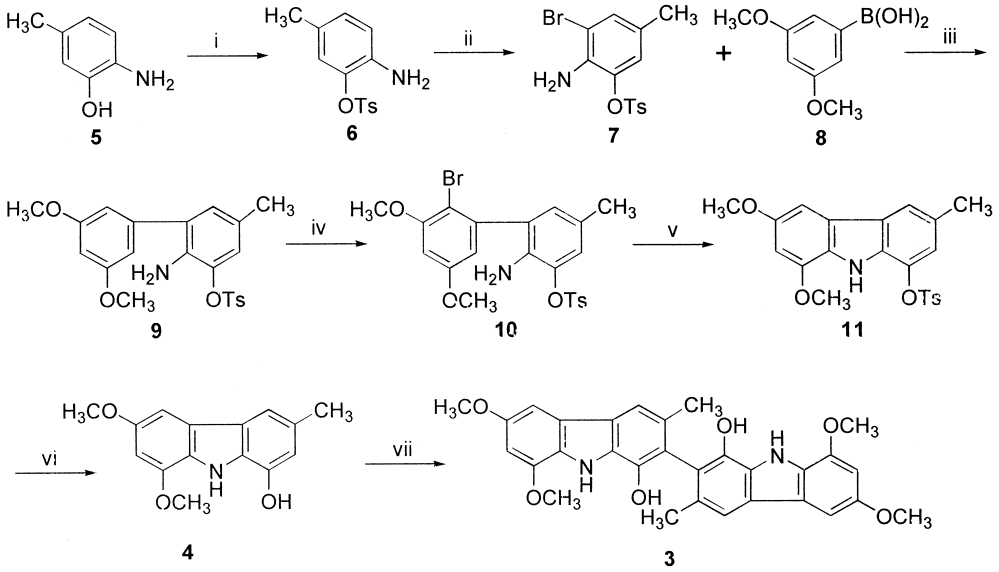
0960-894X/00/$ - see front matter
PII: S0960-894X(00)00158-X



Scheme 1.



Scheme 2. Reagents and conditions: (i) TsCl, Et_3N, CH_2Cl_2, rt, 84%; (ii) 48% HBr, DMSO, rt, 94%; (iii) 5% $Pd(PPh_3)_4$, 2 N Na_2CO_3, benzene, 3,5-dimethoxyphenyl boronic acid, ethanol, reflux, 99%; (iv) 48% HBr, DMSO, rt, 79.5%; (v) 1.2 equiv $Pd(PPh_3)_4$, Na_2CO_3, toluene, reflux, 97%; (vi) KOH, H_2O, EtOH, reflux, 88%; (vii) $(t\text{-BuO})_2$, chlorobenzene, reflux, 90%.

Cytotoxicity assays

With clausenamine-A (**3**) and its analogues **1** and **2**[4] at hand, their cytotoxic activities against cancer cells were evaluated in the National Cancer Institute (USA) for the disease-oriented antitumor screen in vitro,[12] which determines a test agent's effect growth parameters against a panel of approximately 60 human tumor cell lines. The cytotoxic effects of **3** and the related compounds **1** and **2** are obtained as TGI or GI_{50} values, which represent the molar drug concentration required to cause total growth inhibition and half growth inhibition, respectively. The results are presented in Table 1.

As shown in Table 1, these biscarbazoles exhibited from strong to moderate cytotoxic activities against a variety of human cancer cell lines. Compound **2** showed strong cytotoxic effects against leukemia cell lines, including HL-60 (0.032 μM), while *O*-demethylmurrayafoline A (**1**) has less activity compared with compounds **2** and **3**. In the MOLT-4, RPMI-8266 and SR assay, the GI_{50} of compound **1** is better than the other two. Clausenamine-A (**3**), which has two methoxy groups at the 6,6′,8,8′ position, proved to be the moderate active compound in

Table 1. The selected cancer cell growth inhibitory activity of compounds **1**–**3** in vitro (GI_{50} values in μM)

Cell lines[a]	**1**[b]	**2**[b]	**3**
HL-60	32.9	0.0321	2.82
K-562	12.3	4.04	9.70
MOLT-4	3.62	3.23	8.59
RPMI-8266	3.85	3.61	10.5
SR	3.16	3.97	9.01
NIC-H23	12.4	4.48	8.20
HCT-15	10.1	4.20	8.28
HT29	6.04	3.71	8.03
SF-295	17.4	5.11	10.2
SK-MEL-5	16.5	4.34	8.38
OVCAR-5	15.0	3.82	9.03
A-498	15.9	4.78	7.82
CAKI-1	14.7	4.27	9.89
UO-31	13.0	3.78	6.84
MCF-7	11.2	4.49	8.68
MDA-MB-435	15.0	4.34	12.0
MDA-N	14.7	4.50	8.83

[a]Hl-60, K-562, MOLT-4, RPMI-8266, SR, leukemia cell lines; NIC-H23, nonsmall cell lung cancer; HCT-15, HT29, colon cancer cell lines; SF-295, CNS; SK-MEL-5, melanoma; OVCAR-5, A-498, CAKI-1 and UO-31, ovarian and renal cancer cell lines; MCF-7, MDA-MB-435 and MDA-N, breast cancer cell lines.
[b]The spectroscopic data of compound **1** and **2** see ref 4.

this study. All these biscarbazoles showed fairly good activities against leukemia, nonsmall cell lung, colon, CNS, melanoma, ovarian, renal, prostate, and breast cancer.

In summary, for the first time we have accomplished the preparation of clausenamine-A (**3**). Clausemanine-A and its analogues exhibited important cytotoxic activities against a variety of human cancer cell lines in vitro.

Acknowledgements

We are grateful to the National Science Foundation of China (29772052) and the Chinese Academy Science (KT951-A1-506-05) for financial support of this work. We also thank V. L. Narayanan and E. D. Sauaville for the anticancer evaluation of compounds **1**–**3** at NIH.

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11. The spectroscopic data of compound **3**: ^{1}H NMR (300 MHz, $CDCl_3$) 8.69 (s, 2H,-NH), 7.60 (s, 2H, 4-H), 7.04 (d, $J=1.68$ Hz, 2H, 5-H), 6.45 (d, $J=1.64$ Hz, 2H, 7-H), 5.73 (s, 2H, -OH), 3.91 (s, 6H, $-OCH_3$), 3.63 (s, 6H, $-OCH_3$), 2.15 (s, 6H, $-CH_3$). FT–IR (KBr): 3398, 2932, 1595 cm^{-1}. MS *m*/*z* (EI, 70 EV): 512(100), 497, 482, 256. HRMS calcd for $C_{30}H_{28}N_2O_6$ (M+): 512.1947, found 512.1970.
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