Available online at www.sciencedirect.com
ScienceDirect
Bioorganic & Medicinal Chemistry 14 (2006) 6733–6736
Bioorganic & Medicinal Chemistry

Synthesis and in vitro cytotoxicity of haloderivatives of noscapine

Akhilesh Kumar Verma,[a,*] Sandhya Bansal,[b] Jaspal Singh,[a] Rakesh Kumar Tiwari,[a] V. Kasi Sankar,[a] Vibha Tandon[b] and Ramesh Chandra[a,b,*]

[a]*Synthetic Organic Chemistry Research Laboratory, Dr. B. R. Ambedkar Center for Biomedical Research, University of Delhi, Delhi 110007, India*
[b]*Medicinal Chemistry Research Laboratory, Dr. B. R. Ambedkar Center for Biomedical Research, University of Delhi, Delhi 110007, India*

Received 5 May 2006; revised 26 May 2006; accepted 26 May 2006
Available online 19 June 2006

Abstract—Three haloderivatives of noscapine **2**–**4** were synthesized chemoselectively and their in vitro cytotoxicity was assessed by MTT assay on U-87 human glioblastoma cell lines. At 50 μM concentration after 72 h, 9-chloronoscapine **2**, 9-bromonoscapine **3** (EM011), and 9-iodonoscapine **4** killed 87.8%, 51.2%, and 56.8% cells, respectively, however noscapine kills only 40% of the cells; revealing 9-chloronoscapine as a potential cytotoxic agent than noscapine and 9-bromonoscapine (EM011). At low concentration (1 μM) 9-bromonoscapine (46.7%) and 9-chloronoscapine (45.7%) did not show any significant difference.

1. Introduction

Microtubule-targeting agents such as the vinca alkaloids (vinblastine, vincristine, vindesine, etc.) and taxanes (paclitaxel and docetaxel) are important chemotherapeutic drugs for the treatment of cancer.[1,2] Antitumor agents that affect microtubule dynamics are of great medical interest and are now commonly used in current chemotherapy regimens.[3,4] The clinical use of these drugs has been hampered, however, by the side effects and limited effectiveness, increased drug resistance in tumors,[5] poor bioavailability, and poor solubility.[6] Thus, there is still a need for effective microtubule-directed drugs with improved solubility and therapeutic index.

Noscapine **1** is a naturally occurring phthalideisoquinoline alkaloid obtained from opium. It has been used orally in humans as an antitussive agent and displays a favorable toxicity profile.[7] Additionally, it has been known for some time that noscapine can act as a weak anticancer agent in certain in vivo models.[8] Recently, Joshi et al. have performed several studies to evaluate the mechanism of action of this anticancer effect and found that noscapine can disrupt tubulin dynamics.[9] Noscapine inhibits the progression of murine lymphoma, melanoma, and human breast tumors implanted in nude mice with little or no toxicity to the kidney, heart, liver, bone-marrow, spleen, or small intestine and does not inhibit primary humoral immune responses in mice. The lead compound noscapine is currently undergoing phase I/II clinical trials for cancer treatment at University of Southern California, USA. EM011, a brominated derivative of noscapine, possesses 5- to 10-fold higher anticancer activity in comparison to noscapine in preclinical models and it is potently effective against vinblastine-sensitive line CEM.[10–12] Although noscapine appears to be a weak inhibitor of microtubule polymerization, its low cost and ready availability allow for further exploration of this natural product.

In the present work, we are reporting the chemoselective synthesis of 9-halonoscapines **2**–**4** without effecting lactone ring and their in vitro cytotoxicity on U-87 human glioblastoma cell lines.

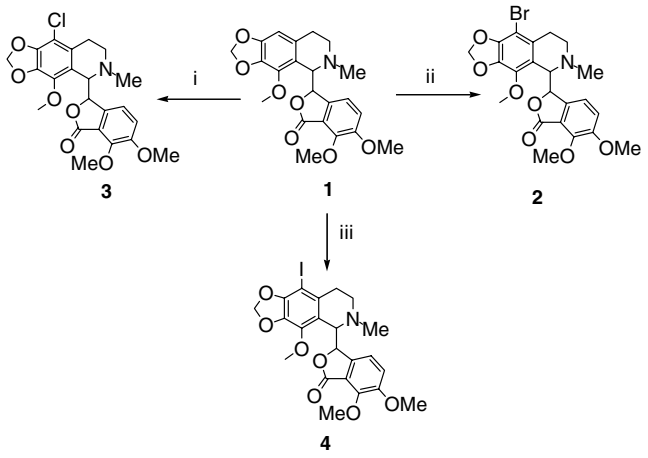
2. Results and discussion

2.1. Synthesis

3-(9-Chloro-4-methoxy-6-methyl-5,6,7,8,-tetrahydro-[1,3]-dioxolo [4,5-*g*] isoquinoline-5-yl)-6,7-dimethoxy-3*H*-isobenzofurane-1-one **2** was prepared by the chlorination

Keywords: Synthesis; Noscapine; Halo-noscapine; U-87 glioblastoma cell line; Cell proliferation.
* Corresponding authors. Tel.: +91 11 55782976/27666272; fax: +91 11 27666248; e-mail: akhilesh682000@yahoo.com

0968-0896/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmc.2006.05.069



Scheme 1. Reagents and conditions: (i) SO_2Cl_2, $CHCl_3$, 25 °C, 10 h; (ii) Br_2/H_2O, 25 °C, 2 h; (iii) ICl, $CHCl_3$, 25 °C, 10 h.

of noscapine (RS) **1** by dropwise addition of sulfuryl chloride at 0–5 °C with vigorous stirring followed by stirring at 25 °C for 8–10 h in 92% yield (Scheme 1).[13–15] The chlorination takes place regioselectively at 9 position. Iododerivative of noscapine 3-(9-iodo-4-methoxy-6-methyl-5,6,7,8,-tetrahydro-[1,3]dioxolo[4,5-*g*]isoquinoline-5-yl)-6,7-dimethoxy-3*H*-isobenzofurane-1-one **4** was prepared by the iodination of noscapine **1** using iodinemonochloride in chloroform and HCl at 0–5 °C in 74% yield (Scheme 1). Completion of the reaction was monitored by thin-layer chromatography. 9-Bromonoscapine 3-(9-bromo-4-methoxy-6-methyl-5,6,7,8,-tetrahydro-[1,3]dioxolo [4,5-*g*] isoquinoline-5-yl)-6,7-dimethoxy-3*H*-isobenzofurane-1-one **3** was prepared as described previously.[16] Disappearance of one aromatic singlet proton of C-9 at ~6.30 ppm in the ^{1}H NMR of the starting compound confirms the chlorination and iodination at C-9 position. Structures of compounds **2**–**4** were fully characterized by the ^{1}H NMR, ^{13}C NMR, and mass spectroscopic data.

2.2. Cell cytotoxicity

The cytotoxicity of synthesized derivatives of noscapine was investigated using human glioma cell line U-87 by using MTT assay[17,18] studying their effect on cell survival and cell proliferation. For survival studies cells were incubated with noscapine and its analogues **2**–**4** continuously and then washed to remove the drug and cell survival was determined following the addition of 1, 10, and 50 μM drug. At concentrations of 1, 10, and 50 μM noscapine killed 18%, 27%, and 40% of the cells, respectively. On the other hand, chloronoscapine **2**, bromonoscapine **3**, and iodonoscapine **4** at 1, 10, and 50 μM concentrations killed 45.7%, 61.5%, 87.8% (chloroderivative **2**); 46.7%, 47.1%, 51.2% (bromoderivative **3**); and 34.4%, 46.0%, 56.8% (iododerivative **4**) cells, respectively (Figs. 1 and 2). At low concentration (1 μM) bromonoscapine (46.7%) and chloronoscapine (45.7%) showed more toxicity than iodonoscapine (34.4%).

log *P* of all the haloderivatives were calculated using Chem 3D software and was found to be 3.18, 3.45, and 3.98 for compound **2** (9-chloronoscapine), **3** (9-bromonoscapine), and **4** (9-iodonoscapine), respectively.

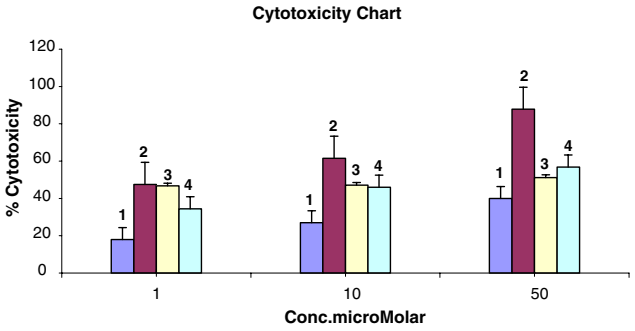


Figure 2. Showing the cytotoxicity of haloderivatives 1–4 at different concentrations.

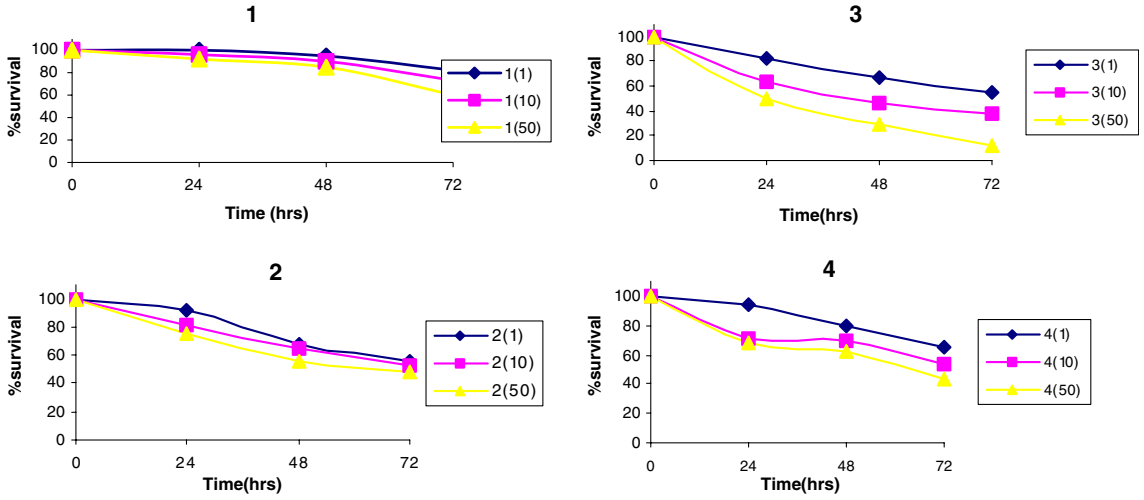


Figure 1. Cytotoxicity of noscapine and its analogues **2**–**4** using the MTT assay on human malignant glioma cells U87. Cells were grown in the presence of drugs at various concentrations up to 72 h and metabolic activity of these cells was measured using MTT. Results are shown as the % survival.

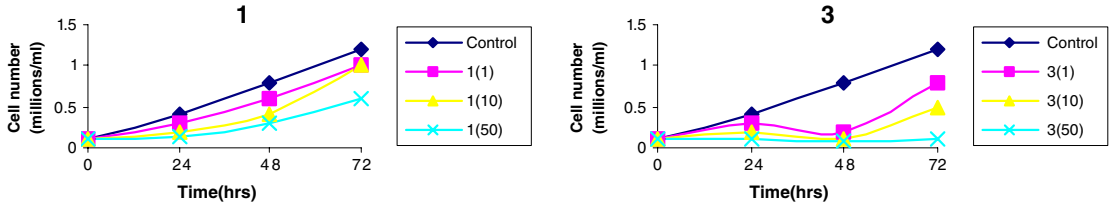


Figure 3. Effects of 1, 10, and 50 μM of noscapine (**1**) and chloronoscapine (**3**) on growth kinetics of U87 cells. Cells were grown in presence of these drugs and number of proliferating cells was counted using hemocytometer at different time intervals. Results are shown as number of cells (in millions per milliliter).

log *P* values of compounds **2**–**4** suggested the more solubility of compound **2** in comparison to compounds **3** and **4**.

2.3. Cell proliferation

Effects on proliferation of exponentially growing cells were also studied. In these experiments, cells were incubated with noscapine **1** and 9-chloronoscapine **2** for 24, 48, and 72 h to observe growth. Kinetics of cell growth treated with noscapine **1** and chloronoscapine **2** differ significantly from the kinetics of nontreated cells and doubling time was not comparable to that of nontreated cells (Fig. 3).

3. Conclusion

In conclusion, we have synthesized haloderivatives of isoquinoline alkaloid noscapine chemoselectively in good yields, and their in vitro cytotoxicity was assessed by MTT assay on U-87 human glioblastoma cell line. 9-Chloronoscapine **2** was found to be the most cytotoxic agent killing 87.8% cells at 50 μM concentration after 72 h. The enhanced cytotoxicity of 9-chloronoscapine **2** in comparison to 9-bromonoscapine (EM011) and its ready synthesis lend hope that it can be taken up for the development of novel anticancer agent. Further studies are ongoing and the results will be reported in due course.

4. Experimental

4.1. General

All reagents used were of AR grade. Melting points were determined using a Thomas Hoover melting point apparatus and are uncorrected. ^{1}H (300 MHz) and ^{13}C NMR (75 MHz) spectra were recorded on a Bruker 300 NMR spectrometer in $CDCl_3$ (with TMS for ^{1}H and chloroform-*d* for ^{13}C as internal references) unless otherwise stated. Mass spectrum was recorded on Hybrid Quadrupole-TOF LC\MS\MS mass spectrometer (Q. Star XL). Infrared spectra (υ_{max}) were recorded on Perkin-Elmer FTIR spectrophotometer as thin films on KBr plates (for oils) or KBr discs (for solids). Column chromatography was performed on silica gel (230–400 mesh). The reactions were monitored by thin-layer chromatography (TLC) using aluminum sheets with silica gel 60 F_{254} (Merck). All of the reactions were carried out under nitrogen atmosphere.

4.2. Synthesis of 3-(9-chloro-4-methoxy-6-methyl-5,6,7,8,-tetrahydro-[1,3]dioxolo[4,5-*g*]isoquinoline-5-yl)-6,7-dimethoxy-3*H*-isobenzofurane-1-one (2)

To a stirred solution of noscapine (1 g, 2.4187 mm) in chloroform (50 ml), sulfuryl chloride (3 equiv) in 30 ml chloroform was added dropwise at 0–5 °C. Reaction mixture was allowed to come at room temperature and stirring was continued for 8–10 h. Completion of the reaction was monitored by thin-layer chromatography. The reaction mixture was poured into 200 ml of water and extracted twice with chloroform. The organic extract was washed with brine and dried over anhydrous sodium sulfate. After removal of the solvent in vacuo, the residue was purified with column chromatography using chloroform/methanol (5%) as an eluent to give the desired product **3** as white needles, Yield 92%, mp 168.2–168.1 °C. ^{1}H NMR (300 MHz; $CDCl_3$; Me_4Si): 7.03 (d, J = 8.0 Hz, 1H) 6.31 (d, J = 8.1 Hz, 1H), 6.02 (s, 2H), 5.51 (d, J = 4.32 Hz, 1H), 4.34 (d, J = 4.3 Hz, 1H), 4.09 (s, 3H), 3.98 (s, 3H), 3.88 (s, 3H), 2.75–2.61 (m, 2H), 2.51–2.44 (m, 4H), 2.00–1.94 (m, 1H); ^{13}C (75 MHz; $CDCl_3$; Me_4Si): 167.5, 152.3, 147.5, 139.3, 134.6, 126.1, 120.3, 118.4, 108.6, 102.4, 93.5, 81.7, 64.3, 61.8, 59.4, 57.8, 54.9, 46.1, 45.2, 39.8, 20.6, 18.3. LC–MS *m*/*z*: 448.4 (M+1).

4.3. Synthesis of 3-(9-iodo-4-methoxy-6-methyl-5,6,7,8,-tetrahydro-[1,3]dioxolo[4,5-*g*]isoquinoline-5-yl)-6,7-dimethoxy-3*H*-isobenzofurane-1-one (4)

To a stirred solution of noscapine (1 g, 2.4187 mm) in chloroform (50 ml), iodinemonochloride (3 equiv) in 30 ml chloroform was added dropwise at 0 °C. Reaction mixture was allowed to come at room temperature and stirring was continued for 10 h. TLC monitored completion of the reaction. The reaction mixture was poured in 200 ml of water and extracted twice with chloroform. The organic extract was washed with brine and dried over anhydrous sodium sulfate. After removal of the solvent in vacuo, the residue was purified with column chromatography using $CHCl_3$/MeOH (5%) as an eluent to give the desired

product (**4**): white powder, Yield 74%, mp 186–190 °C. δ_H (300 MHz; $CDCl_3$; Me_4Si): 7.15 (d, $J = 8.1$ Hz, 1H), 6.93 (d, $J = 8.1$ Hz, 1H), 6.11 (s, 2H), 5.42 (d, $J = 4.8$ Hz, 1H), 4.26 (d, $J = 4.8$ Hz, 1H), 3.85 (s, 3H), 3.76 (s, 3H), 3.72 (s, 3H), 2.78–2.72 (m, 2H), 2.55–2.50 (m, 2H), 2.32 (s, 3H), ^{13}C (75 MHz; $CDCl_3$; Me_4Si): 168.2, 155.3, 151.8, 148.4, 146.6, 143.1, 140.3, 120.4, 119.6, 113.3, 101.5, 85.9, 82.3, 61.9, 56.7, 55.7, 54.5, 54.2, 51.2, 39.8, 30.1, 18.7. LC–MS *m*/*z*: 539.9 (M+1).

4.4. Cell culture

The human glioma cell line U 87 was maintained as monolayers at 37 °C in 25 cm^2 tissue culture flasks (Tarsons, India) using Dulbecco's modified Eagle's medium (Sigma) supplemented with 5% fetal calf serum (Biologicals, Israel). Cells were passaged routinely in exponential growth phase twice a week using 0.05% trypsin–EDTA solution in phosphate-buffered saline (PBS) for trypsinization. All experiments were performed with asynchronously growing cells in the exponential growth phase (24 h after plating).

4.5. Cytotoxicity assay

Cytotoxicity was determined using the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide] assay[17,18] using a 96-well microtiter plate. Three thousand cells per well were plated in 200 µl of the complete medium and treatment with these ligands was performed 24 h after plating. For % survival determination, cells were exposed continuously with varying concentrations of drug and MTT assays were performed at the end of the fourth day. At the end of the treatment, control and treated cells were incubated with MTT at a final concentration of 0.5 mg/ml for 2 h at 37 °C and then the medium was removed. The cells were lysed and the formazan crystals were dissolved using 150 µl DMSO. The absorbance was read at 570 nm using 630 nm as reference wavelength using ELISA reader.

4.6. Proliferation kinetics

U 87 cells were seeded at 7000–8000 cells/cm^2, and their proliferation kinetics was studied at 24 h intervals following trypsinization and counting total cells per flask using a hemocytometer.

Acknowledgments

A.K.V. gratefully acknowledges financial support from Department of Science and Technology, New Delhi; J.S. thanks CSIR for JRF; R.K.T. thanks Jean & Ashit Ganguly Trust for SRF.

References and notes

1. Checchi, P. M.; Nettles, J. H.; Zhou, J.; Snyder, J. P.; Joshi, H. C. *Trends Pharmacol. Sci.* **2003**, *24*, 361.
2. Jordan, M. A.; Wilson, L. *Nat. Rev. Cancer* **2004**, *4*, 253.
3. Wilson, L.; Jordan, M. A. *Chem. Biol.* **1995**, *2*, 569.
4. Li, Q.; Sham, H. L. *Expert Opin. Ther. Patent* **2002**, *12*, 1663.
5. Simon, S. M.; Schindler, M. *Proc. Natl. Acad. Sci. U.S.A.* **1994**, *91*, 3497.
6. Van Zuylen, L.; Verweij, J.; Sparreboom, A. *Invest. New Drugs* **2001**, *19*, 125.
7. Dahlstrom, B.; Mellstrand, T.; Lofdahl, C.-G.; Johansson, M. *Eur. J. Clin. Pharmacol.* **1982**, *22*, 535.
8. (a) Lettre, H. *Ann. N.Y. Acad. Sci.* **1954**, *58*, 1264; (b) Lettre, H.; Albrecht, M. *Naturwissenschaften* **1942**, *30*, 184.
9. (a) Ye, K.; Ke, Y.; Keshava, N.; Shanks, J.; Kapp, J. A.; Tekmal, R. R.; Petros, J.; Joshi, H. C. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 1601; (b) Joshi, H. C.; Zhou, J. *Drug News Perspect.* **2000**, *13*, 543; (c) Ke, Y.; Ye, K.; Grossniklaus, H. E.; Archer, D. R.; Joshi, H. C.; Kapp, J. A. *Cancer Immunol. Immunother.* **2000**, *49*, 217; (d) Landen, J. W.; Lang, R.; McMahon, S. J.; Rusan, N. M.; Yvon, A.-M.; Adams, A. W.; Sorcinelli, M. D.; Campbell, R.; Bonaccorsi, P.; Ansel, J. C.; Archer, D. R.; Wadsworth, P.; Armstrong, C. A.; Joshi, H. C. *Cancer Res.* **2002**, *62*, 4109.
10. Zhou, J.; Gupta, K.; Aggarwal, S.; Aneja, R.; Chandra, R.; Panda, D.; Joshi, H. C. *Mol. Pharmacol.* **2003**, *63*, 799.
11. Zhou, J.; Liu, M.; Luthra, R.; Jones, J.; Aneja, R.; Chandra, R., et al. *Cancer Chemother. Pharmacol.* **2005**, *55*, 461.
12. Aneja, R.; Zhou, J.; Vangapandu, S. N.; Zhou, B.; Chandra, R.; Joshi, H. C. *Blood* **2006**, *107*, 2486.
13. Green, R. H. *Tetrahedron Lett.* **1997**, *38*, 4697.
14. Marchent, J. R.; Shirali, S. S. *Curr. Sci.* **1977**, *46*, 12–13.
15. Stokker, G. E.; Deana, A. A.; deSolms, S. J.; Schultz, E. M.; Smith, R. L.; Cragoe, E. J., Jr.; Baer, J. E.; Ludden, C. T.; Russo, H. F.; Scriabine, A.; Sweet, C. S.; Watson, L. S. *J. Med. Chem.* **1980**, *23*, 1414.
16. Aggarwal, S.; Ghosh, N. N.; Aneja, R.; Joshi, H.; Chandra, R. *Helv. Chim. Acta* **2002**, *85*, 2458.
17. Zhang, X.; Kiechle, F. *J. Clin. Ligand Assay* **1998**, *21*, 62.
18. Turner, P. R.; Denny, W. A. *Mutat. Res.* **1996**, *355*, 141.