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Novel third-generation water-soluble noscapine analogs as superior microtubule-interfering agents with enhanced antiproliferative activity

Maged Henary [a,*], Lakshminarayana Narayana [a,b], Shazia Ahad [c], Sushma R. Gundala [b], Rao Mukkavilli [d], Vibhuti Sharma [b], Eric A. Owens [a], Yogesh Yadav [a,b], Mulpuri Nagaraju [a], Donald Hamelberg [a], Vibha Tandon [e,f], Dulal Panda [c], Ritu Aneja [b,**]

[a] Department of Chemistry and Biochemistry, Georgia State University, Petit Science Center, 100 Piedmont Ave., Atlanta, GA 30303, USA
[b] Department of Biology, Georgia State University, Petit Science Center, 100 Piedmont Ave., Atlanta, GA 30303, USA
[c] Department of Biosciences and Bioengineering, Indian Institute of Technology Bombay, Mumbai 400076, India
[d] Advinus Therapeutics Limited, Bangalore, Karnataka, India
[e] Department of Chemistry, University of Delhi, India
[f] Special Centre of Molecular Medicine, Jawaharlal Nehru University, Delhi, India

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ABSTRACT

Noscapine, an opium-derived 'kinder-gentler' microtubule-modulating drug is in Phase I/II clinical trials for cancer chemotherapy. However, its limited water solubility encumbers its development into an oral anticancer drug with clinical promise. Here we report the synthesis of 9 third-generation, water-soluble noscapine analogs with negatively charged sulfonato and positively charged quaternary ammonium groups using noscapine, 9-bromonoscapine and 9-aminonoscapine as scaffolds. The predictive free energy of solvation was found to be lower for sulfonates (**6a–c**; **8a–c**) compared to the quaternary ammonium-substituted counterparts, explaining their higher water solubility. In addition, sulfonates showed higher charge dispersability, which may effectively shield the hydrophobicity of isoquinoline nucleus as indicated by hydrophobicity mapping methods. These *in silico* data underscore efficient net charge balancing, which may explain higher water solubility and thus enhanced antiproliferative efficacy and improved bioavailability. We observed that **6b**, **8b** and **8c** strongly inhibited tubulin polymerization and demonstrated significant antiproliferative activity against four cancer cell lines compared to noscapine. Molecular simulation and docking studies of tubulin-drug complexes revealed that the brominated compound with a four-carbon chain (**4b**, **6b**, and **8b**) showed optimal binding with tubulin heterodimers. Interestingly, **6b**, **8b** and **8c** treated PC-3 cells resulted in preponderance of mitotic cells with multipolar spindle morphology, suggesting that they stall the cell cycle. Furthermore, *in vivo* pharmacokinetic evaluation of **6b**, **8b** and **8c** revealed at least 1–2-fold improvement in their bioavailability compared to noscapine. To our knowledge, this is the first report to demonstrate novel water-soluble noscapine analogs that may pave the way for future pre-clinical drug development.

1. Introduction

Over the past few decades, microtubule-active drugs have met with abundant success in the oncology clinic for a wide-spectrum of malignancies [1,2]. Beyond the two major classes of tubulin-binding drugs, namely, vincas (that depolymerize microtubules) and taxanes (that overpolymerize microtubules), the "middle-path" drugs such as noscapine, 2-ME, griseofulvin, are currently a topic of intense investigation both for their clinical utility as well as from a mechanistic standpoint [2–4]. Essentially, these "middle-path" drugs do not overpolymerize or depolymerize microtubules over a broad concentration range, rather subtly attenuate microtubule dynamics. Unlike microtubule polymerizing (taxanes) or microtubule depolymerizing (vincas) drugs, these middle-path agents are also referred to as microtubule modulating drugs (noscapines). They withstand the harsh effects on the microtubules over a wide span of concentration by increasing the pause phase of

* Corresponding author.
** Corresponding author. Tel. +1 404 413 5417.
E-mail address: raneja@gsu.edu (R. Aneja).

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microtubules, which in turn, helps them offer a wider therapeutic window with lower toxicity than classical tubulin binding drugs. No wonder microtubule-drugs currently occupy a major segment of the ever-expanding armamentarium of clinical chemotherapeutic regimens. Nonetheless, several impediments associated with their clinical use, such as non-specific toxicity, drug resistance, and water insolubility, have resulted in a sub-optimal realization of their clinical potential [5,6]. Thus, in the wake of these pharmacological challenges, new anticancer drug discovery, synthesis and development constitute an active area of intense research.

Noscapine, an innocuous cough-suppressant, was identified for its previously unrecognized tubulin-binding activity and chemotherapeutic benefits in the late 1990s [7,8]. The promising anticancer activity of noscapine coupled with its non-toxic attributes facilitated its quick inclusion into Phase 1/2 clinical trials [9]. Ever since, several groups including ours, have been actively engaged in the synthesis of *in silico* guided, more potent noscapine analogs with potentially better pharmacological profiles [10,11]. Recently, we reported the synthesis of second-generation 7-position benzofuranone noscapine analogs that offered better antiproliferative activity than the founding molecule [12–14]. Although first-pass *in vitro* experiments remain feasible with several more potent synthetic noscapine analogs, water insolubility has emerged to be a major issue for *in vivo* experimentation. Essentially, diminution of aqueous solubility can be ascribed to the presence of substituted isoquinoline and isobenzofuranone ring systems, which confer highly hydrophobic structural characteristics. This lack of adequate solubility thus poses a challenge for further drug development as low water solubility directly impacts absorption and distribution profiles of the test agents, thus compromising bioavailability. Thus the solubility characteristics of a drug are profoundly crucial at early drug development stage, in particular for animal studies. Given that the partition coefficient and TPSA (Topological Polar Surface Area) are the main descriptors of aqueous solubility of a drug, integrating knowledge of these parameters is often sought for fine-tuning the physicochemical profiles of drugs.

Here we describe rational design, and chemical modification of noscapine and its known congeners to successfully yield novel water-soluble analogs by incorporation of certain charged functional groups namely alkyl quaternary ammonium salt and alkyl sulfonates. Our data demonstrate that introduction of a charged species on the noscapine core greatly improved aqueous solubility, which reflected as enhanced bioavailability compared to noscapine and *in vitro* efficacy in reducing the proliferation of cancer cells. These data offer compelling grounds to further investigate the preclinical activity and pharmacokinetics of these novel water-soluble noscapine analogs.

2. Materials and methods

2.1. General

NMR spectroscopy was performed on a Bruker Avance (400 MHz) spectrometer located in the Department of Chemistry NMR facility and the solvents for the NMR experiments (99.8% CD_3OD-d_4, DMSO-d_6 and $CDCl_3$) were obtained from Cambridge Isotope Laboratories (Andover, MA) including TMS as the internal calibration standard. The reactions were followed using silica gel 60 F_{254} thin layer chromatography plates (Merck EMD Millipore, Darmstadt, Germany). Open column chromatography was utilized for the purification of all final compounds using 60-200u, 60A classic column silica gel (Dynamic Adsorbents, Norcross, GA). The melting points were determined with a Mel-temp melting point apparatus and are given as uncorrected values. High-resolution accurate mass spectra (HRMS) were obtained either at the Georgia State University Mass Spectrometry Facility using a Waters Q-TOF micro (ESI-Q-TOF) mass spectrometer or utilizing a Waters Micromass LCT TOF ES + Premier Mass Spectrometer. HPLC analyses were carried out on a Waters 1525 Binary HPLC pump/waters 2487 dual absorbance detector system using a Waters Delta-Pak 5 μm 100A 3.9 × 150 mm reversed phase C_{18} column. All reported yields refer to pure isolated compounds. Chemical and solvents were of reagent grade and used as obtained from Alfa Aesar (Ward Hill, MS) and Sigma Aldrich (St. Louis, MO) without further purification. The determined purity of all the final synthesized compounds were >95% as estimated by HPLC or determined by elemental analysis.

2.2. Chemical synthesis

2.2.1. (*S*)-3-((R)-9-bromo-4-methoxy-6-methyl-5,6,7,8-tetrahydro-[1,3]dioxolo[4,5-g]isoquinolin-5-yl)-6,7-dimethoxyisobenzofuran-1(3H)-one (2)

(*S*)-3-((*R*)-9-bromo-4-methoxy-6-methyl-5,6,7,8-tetrahydro-[1,3]dioxolo[4,5-g]isoquinolin-5-yl)-6,7-dimethoxyisobenzofuran-1(3H)-one (2) was synthesized from 1 and 1.4 g was obtained in 82% yield following the reported procedure [12].

2.2.2. (*S*)-3-((R)-9-amino-4-methoxy-6-methyl-5,6,7,8-tetrahydro-[1,3]dioxolo[4,5-g]isoquinolin-5-yl)-6,7-dimethoxyisobenzofuran-1(3H)-one (3)

Sodium azide (0.99 g, 15.231 mmoles, 2 equiv) was added to a solution of 9-bromo noscapine (3.0 g, 6.092 mmoles) in anhydrous DMSO (20 mL) followed by the addition of Cu_2O (872 mg, 6.092 mmoles, 1.0 equiv) and L-proline (912 mg, 7.917 mmoles, 1.3 equiv). The reaction mixture was stirred at 100 °C for 24 h while monitoring by TLC. The mixture was then quenched by the addition of aq. NH_4Cl solution and was extracted with DCM (2 × 60 mL). The organic layer was then washed with water (2 × 30 mL), dried over anhydrous Na_2SO_4, concentrated and purified by flash chromatography to obtain 2.1 g of the desired product. Yield: 81%; mp: 124 °C; ^{1}H NMR (400 MHz, CD_3OD) δ: 7.21 (d, J = 8.0 Hz, 1H), 6.24 (d, J = 8.4 Hz, 1H), 5.95 (s, 2H), 5.67 (s, 1H), 4.41 (s, 1H), 3.98 (s, 3H), 3.88 (s, 3H), 3.86 (s, 3H), 2.62 (t, J = 4.0 Hz, 1H), 2.48 (s, 3H), 2.45 (m, 2H), 1.83 (m, 1H); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 6.96 (d, J = 8.0 Hz, 1H), 6.16 (d, J = 8.4 Hz, 1H), 5.93 (d, J = 2.0 Hz, 2H), 5.61 (d, J = 3.6 Hz, 1H), 4.37 (d, J = 4.0 Hz, 1H), 4.07 (s, 3H), 3.87 (s, 3H), 3.86 (s, 3H), 3.79 (br s, 2H), 2.60 (m, 1H), 2.51 (s, 3H), 2.45 (m, 1H), 2.35 (m, 1H), 1.70 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 168.7, 152.4, 147.1, 141.0, 134.5, 133.1, 132.2, 121.2, 119.8, 118.1, 117.3, 116.2, 101.8, 82.3, 63.6, 61.1, 60.0, 57.4, 49.2, 46.3, 20.0; HRMS $(M+H)^+$: m/z Calcd. for $C_{22}H_{25}N_2O_7$, 429.1662; found 429.1646.

2.2.3. 3-(((R)-5-((S)-4,5-dimethoxy-3-oxo-1,3-dihydroisobenzofuran-1-yl)-4-methoxy-6-methyl-5,6,7,8-tetrahydro-[1,3]dioxolo[4,5-g]isoquinolin-9-yl)amino)-N,N,N-trimethylpropan-1-aminium bromide (4a)

Triethyl amine (0.7 mL, 4.67 mmoles) was added to a solution of 9-amino noscapine (1.0 g, 2.335 mmoles) in anhydrous DMF (10 mL) followed by the addition of 3-bromopropyltrimethylammoniumbromide (609.2 mg, 2.335 mmoles) and the reaction mixture was stirred at 90 °C for 17 h while monitoring by TLC. The reaction mixture was then concentrated at 60 °C under reduced pressure and the crude product was purified by flash chromatography to obtain 820 mg of the desired product. Yield: 67%; mp: 48 °C; ^{1}H NMR (400 MHz, CD_3OD) δ: 7.29 (d, J = 8.4 Hz, 1H), 6.58 (d, J = 8.0 Hz, 1H), 5.89 (d, J = 2.8 Hz, 2H), 5.69 (d, J = 2.8 Hz, 1H), 4.44 (d, J = 3.2 Hz, 1H), 3.95 (s, 3H), 3.90 (s, 3H), 3.70 (s, 3H), 3.48 (dt, J = 2.0 Hz and 8.0 Hz, 2H), 3.35 (t, J = 8.0 Hz, 2H), 3.15 (s, 9H), 2.91