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Antiangiogenic properties of substituted (*Z*)-(±)-2-(*N*-benzylindol-3-ylmethylene)quinuclidin-3-ol/one analogs and their derivatives

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ABSTRACT

In the past half century research efforts have defined a critical role for angiogenesis in tumor growth and metastasis. We previously reported that inhibition of a novel target, ENOX1, by a (*Z*)-2-benzylindol-3-ylmethylene) quinuclidin-3-ol, suppressed tumor angiogenesis. The present study was undertaken in order to establish structure–activity relationships for quinuclidine analogs. The angiogenesis inhibiting activity of a series of substituted (*Z*)-(±)-2-(*N*-benzylindol-3-ylmethylene)quinuclidin-3-ols (**1a–1k**), (*Z*)-2-benzylindol-3-ylmethylene)quinuclidin-3-ones (**2a–2h**), (*Z*)-(±)-2-(1*H*/*N*-methyl-indol-3-ylmethylene)quinuclidin-3-ols (**3a–3b**), and substituted (*Z*)-(±)-2-(*N*-benzenesulfonylindol-3-yl-methylene) quinuclidin-3-ols and their derivatives (**4a–4d**) that incorporate a variety of substituents in both the indole and *N*-benzyl moieties was evaluated using Human Umbilical Vein Endothelial Cells (HUVECs) subjected to in vitro cell migration scratch assays, tubule formation in Matrigel, cell viability and proliferation assays. In total, 25 different analogs were evaluated. Based on in vitro cell migration scratch assays, eight analogs were identified as potent angiogenesis inhibitors at 10 μM, a concentration that was determined to be nontoxic by colony formation assay. In addition, this approach identified a potent antiangiogenic ENOX1 inhibitor, analog **4b**.

Endothelial cells form the lining of all the blood vessels and play a critical role in tissue growth and wound healing. They achieve this by retaining the ability to divide and proliferate in an adult vascular system by the process of 'angiogenesis', where new blood vessels are formed from existing cells via development of small capillaries.[1] Development of these capillaries, also known as neovascularization, plays a critical role in cell division, proliferation and movement.[1,2]

In the past half century research efforts have established a critical role for angiogenesis in tumor growth and metastasis.[2] Without development of a capillary network by neovascularization, a tumor will not have sufficient nutrients to grow beyond a limited size.[3] Thus, angiogenesis constitutes an important point in the control of cancer progression,[3] and consequently, several major cancer therapy advances that target angiogenesis have been made in the recent years. These include development of antiangiogenesis drugs such as bevacizumab that binds to vascular endothelial growth factor (VEGF), a pro-angiogenic growth factor, and inhibits VEGF–VEGF receptor interactions. Sunitinib and Sorafenib, small molecule receptor tyrosine kinase inhibitors, represent two other examples.[3–5] Other drugs that are currently in clinical trials include, AZD2171, a potent, indole-ether quinazoline that also targets all VEGF receptors in renal cell cancer.[6] While these treatments have been shown to be effective in controlling initial cancer progression, their effects have been shown to be limited due to adaptation or previously existing resistance.[6] This ability of tumor cells to adapt to current antiangiogenesis drugs underscores the need to develop novel drugs that not only target tumor vasculature but also complement cytotoxic therapy.[6,7]

We previously described the synthesis of novel compounds consisting of combinations of indole, benzyl, and quinuclidine moieties.[8–10] In these studies, the effect of three specific compounds on cellular proliferation and tubule formation ability of endothelial cells was analyzed in detail.[8] Both phenotypic screening and analysis of biochemical pathways affected by non toxic doses of the active compounds identified the ECTO-NOX (ENOX) family of cell surface enzymes as potential targets for these indole analogs.[8] The ENOX family of enzymes exhibit cell surface protein disulfide-thiol interchange activity and oxidize NAD(P)H as an alternate substrate.[8] Enox enzymes play a critical role in cell proliferation

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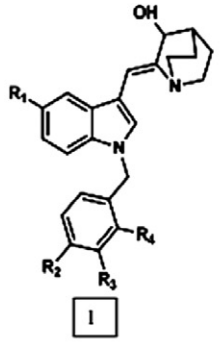
and cancer.[11] The family consists of constitutively expressed ENOX1 (previously called CNOX), which is ubiquitous in mammals and plants, a cancer specific ENOX2 (formally called tNOX) that is thought to be unregulated and responsive to inhibitors, and an age-related ENOX.[12,13]

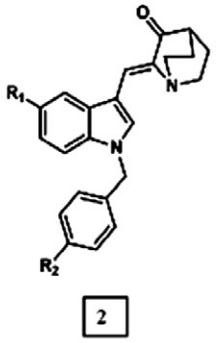
Our studies demonstrated that inhibition of ENOX activity by these indole compounds inhibited endothelial cell proliferation, inhibited the ability to form tubules, and synergistically increased radiation-mediated xenograft tumor growth delay. These observations led us to further investigate and establish the relationship between structure and antiangiogenesis activity of a series of (*Z*)-(±)-2-(*N*-benzylindol-3-ylmethylene)quinuclidin-3-ols (Fig. 1, **1a**–**1k**), (*Z*)-2-benzylindol-3-ylmethylene)quinuclidin-3-ones (**2a–2i**), (*Z*)-(±)-2-(1*H*/*N*-methyl-indol-3-ylmethylene)quinuclidin-3-ol (**3b**), and (*Z*)-(±)-2-(*N*-benzenesulfonylindol-3-yl-methylene)quinuclidin-3-ols and their derivatives (Fig. 1, **4a**–**4d**). In this study we describe the effect of 25 additional derivatives on cellular migration of HUVECs. The synthesis of these analogs has been previously described.[10]

Initially, 25 novel analogs (Fig. 1) were analyzed for their ability to inhibit the migration of HUVECs in an in vitro cell migration scratch assay at a fixed concentration (25 μM). The scratch assay was performed as described in Geng et al.[8] HUVECs were seeded in 24-well culture plates. When confluent, a scratch was created using a 10 μl sterile pipette tip. Immediately following scratching, cells were washed twice with PBS to remove cell debris. Analogs were added at a final concentration of 25 μM in 1 ml final volume of HUVEC medium. Cells were incubated at 37 °C for 20 h. Microscopic images were taken at 0 h (immediately after drug addition) and at 20 h and the samples analyzed for cellular migration in the presence or absence of the test compound. Among analogs **1a**–**1k**, introduction of either halogen, methyl or methoxy substituents



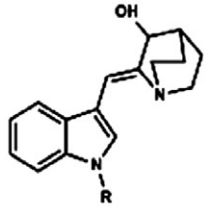
1

Compound	R_1	R_2	R_3	R_4
10	H	H	H	H
1a	NO_2	H	H	H
1b	Br	H	H	H
1c	CH_3	CH_3	H	H
1d	H	Cl	H	H
1e	H	F	H	H
1f	Br	F	H	H
1g	Br	H	H	Br
1h	Br	OCH_3	H	H
1i	OCH_3	H	H	H
1j	Br	H	CH_3	H
1k	H	CO_2CH_3	H	H



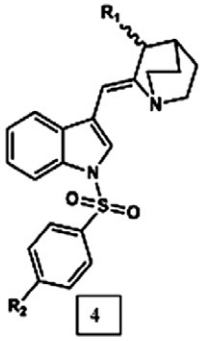
2

Compound	R_1	R_2
2a	H	H
2b	H	F
2c	Br	F
2d	Br	OCH_3
2e	Br	CH_3
2f	Br	Br
2g	H	CO_2CH_3
2h	H	CN



3

Compound	R
3a	H
3b	CH_3



4

Compound	R_1	R_2
4a	OH	CH_3
4b	=N-OH	H
4c	OCONHPh	H
4d	=N-NHCONH$_2$	H

Figure 1. Chemical structures of novel quinuclidine compounds: structures of potent antiangiogenic agents with various substituents incorporated into the indole and *N*-benzyl moieties.