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SYNTHESIS AND EVALUATION OF WATER-SOLUBLE DIMETHYLAMINOETHYL ETHERS OF METHOXSALEN FOR PROLIFERATIVE SKIN DISORDERS

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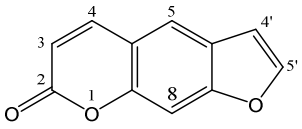
Abstract:
The natural product 8-methoxypsoralen (methoxsalen or 8-MOP) in combination with long wavelength ultraviolet light (UVA, 320-400 nm), also referred to as PUVA therapy, is used for the treatment of cutaneous proliferative disorders including psoriasis, vitiligo and mycosis fungoides.The use of 8-MOP (**3**)is limited by its poor water solubility and there remains a need to develop more water-soluble psoralens to enhance bioavailability following oral administration of the drug.In the present studies a water-soluble dimethylaminoethyl ether analog of 8-MOP was synthesizedand analyzed for biological activity. This analog, (8-[2-(N,N-dimethylamino)ethoxy]-psoralen hydrochloride (**1**) [or CAS name: 9-[2-(dimethylamino)ethoxy]-7*H*-furo[3,2-*g*][1]benzopyran-7-one, hydrochloride], was found to be significantly more active than **3**in keratinocyte growth inhibition assays (IC_{50} = 12 nM and 130 nM for **1** and **3**, respectively). The partially reduced dihydroderivative of **1**, 8-[2-(N,N-dimethylamino)ethoxy]-4',5'-dihydropsoralen hydrochloride (**2**) [or CAS name: 9-[2-(dimethylamino)ethoxy]-2,3-dihydro-7*H*-furo[3,2-*g*][1]benzopyran-7-one, hydrochloride]and the partially reduced 4',5'-dihydro-8-methoxypsoralen (**4**)lacking the water-solubilizing side-chain were significantly less active. As inhibitors of keratinocyte growth they ranked as IC_{50} = 13,000 nM and 70,000 nM for **2** and **4**, respectively, indicating that an unsaturated furan ring in the psoralen was required for maximal activity.Compound (**1**) was found to readily intercalate and damage DNA following UVA light treatment as determined by plasmid DNA nicking and unwinding experiments in neutral and alkaline agarose gels. Taken together, these data demonstrate that a water-soluble dimethylaminoethyl ether psoralen targets DNA, is highly active as a photosensitizer, and may be useful in the treatment of skin diseases involving abnormal keratinocyte proliferation.

Keywords: psoralens, methoxsalen, 8-MOP, photosensitizers, keratinocytes, plasmid-nicking

Introduction:
Linear furocoumarins, ubiquitous natural products also known as psoralens, have been used in combination with ultraviolet light for centuries in cosmetics and for the treatment of proliferative skin diseases such as, for example, psoriasis, vitiligo and mycosis fungoides.[i,ii]Terms such as photosensitization, photochemotherapy, photophoresis and PUVA (psoralens ultraviolet A) are commonly used to refer to such methods.[iii] The basic structure of psoralen, with the ring numbering structure used herein, is shown in Figure 1 below:

Figure 1:Position numbering in the psoralen (furocoumarin) ring system.

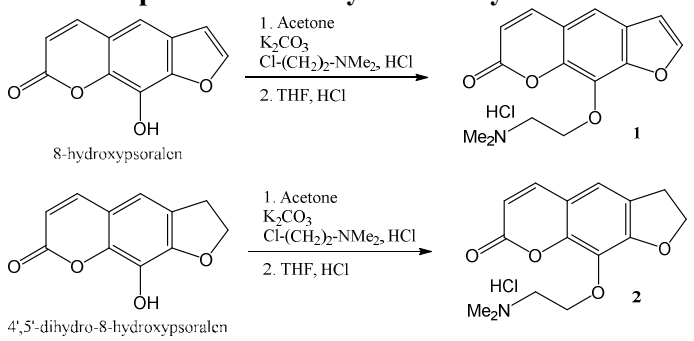


All psoralens contain two photo-activatable functional groups that absorb in the UVA range. These are an aryl-conjugated unsaturated pyrone (or the coumarin portion) and an aryl-conjugated vinyl ether (or the furan portion). Commercially available psoralens are typically highly lipophilic, non-nitrogenous, uncharged small molecules with minimal water solubility. Commercial psoralens are used in over-the-counter cosmetic creams, prescription pharmaceuticals, and as investigational candidates for many of the uses described above. Examples of commercial psoralens used cosmetically and medically include methoxsalen (also referred to as xanthotoxin, 8-methoxypsoralen or 8-MOP), trisoralen (also called 4,5',8-trimethylpsoralen, TMP, or trioxsalen), and bergaptan (alternatively referred to as 5-methoxypsoralen or 5-MOP). The phototherapeutic action of psoralens has been discussed elsewhere.[iv,v] It is generally thought that the highly lipophilic psoralens penetrate the target cell's membrane, intercalate into nuclear DNA, and photo crosslink the double helix through bis-cyclobutanes generated from the 3,4-double bond and the 4',5'-double bond [see above structure] to double bonds in DNA's pyrimidine bases. The consequences of psoralen-DNA adduct formation are target cell specific; in keratinocytes, DNA damage may render cells unable to proliferate or non-viable, while in melanocytes, this may trigger a proliferative response.[iii]
In many applications, the pharmacological utility of the parent psoralens is compromised by their insolubility in aqueous biological fluids. Various routes for the introduction of an aminomethyl [-CH_2-NH_2] group onto the furan ring have been described.[vi] However, while these basic loci can then be converted to water-soluble salts, the molecular architecture of the psoralen is altered by pendant attachments at C-5' and C-4'.Synthetic methods to obtain furan-substituted amino psoralens have been described previously.[vii,viii,ix] In the present work, the preparation and use of water-soluble dimethylaminoethyl ether psoralen compounds with a basic side chain attached on the phenyl ring are described.

Materials and Methods:
Sources of psoralens and preparation of aminoether derivatives of hydroxypsoralens.8-MOP(**3**)and 8-hydroxypsoralen (see Scheme 1) were obtained as research samples from the Elder Pharmaceutical Company, now Valeant Pharmaceuticals International (Laval, Quebec, Canada).4',5'-dihydro-8-methoxypsoralen, **4**,and 4',5'-dihydro-8-hydroxypsoralen (see Scheme 1) were prepared as described.[x] All reactants and solvents were of the highest purity commercial grade and were employed without further purification except for anhydrous acetone used in this study, which was dried over molecular sieves and distilled just prior to use.^{1}H-NMRs were obtained in DMSO-d_6 on a JEOL FX-90Q NMR spectrometer and were

calibrated against TMS (tetramethylsilane). IR spectra were recorded as solid samples on a ThermoFisher Nicolet iS10 FT-IR fitted with an ATR with a ZnSe plate. MS were recorded on a Bruker mocrOTOF-II mass spectrometer using direct infusion ESI in acetonitrile:water (9:1).

Scheme 1. Syntheses of the psoralen dimethylaminoethyl ethers



For the synthesis of the aminoether derivatives of the hydroxypsoralens (Scheme 1), a charge of either 8-hydroxypsoralen (0.303g, 1.5 mmol), or 4',5'-dihydro-8-hydroxypsoralen (0.306 g, 1.5 mmol) dissolved in 100 mL of anhydrous acetone containing a suspension of vacuum dried K_2CO_3 (3.5 g, 25.3 mmol), was placed in a 300 mL round-bottom flask equipped with an efficient magnetic stirrer. A slurry of 2-(N,N-dimethylamino)ethyl chloride hydrochloride salt (0.715 g, 5 mmol) in 40 mL of anhydrous acetone was added and the mixture stirred at reflux for three days. The acetone was removed *in vacuo* and the brown-tan solid treated with 50 mL of water at 70 ^oC and hot-filtered. The basic solution was exhaustively extracted with 3x50 mL portions of chloroform, dried ($MgSO_4$), and evaporated to a brown oil.The oil was taken-up in 40 mL of anhydrous tetrahydrofuran, filtered, chilled to 5^oC and treated with a slow-bubbling stream of HCl gas to precipitate the hydrochloride salt. The latter was recrystallized 2x from a 1:1 mixture of methanol and ethanol to give light beige solid products (yields 30-45% in multiple syntheses).

8-[2-(N,N-dimethylamino)ethoxy]-psoralen, hydrochloride salt (compound **1**): mp = 234-235°C [light beige solid]. Calculated for $C_{15}H_{16}ClNO_4$: C, 58.16%; H, 5.20%; N, 4.52%. Found: C, 58.05%; H, 5.18%; and N, 4.46%.HRMS (ESI) calculated for$C_{15}H_{16}NO_4$ 274.1074, found 274.1081 $(M+H)^+$. ^1H NMR (DMSO-d6), δ 2.95 [s, 6H, 2 Me]; 3.95 [t, *J* = 4.9Hz, 2H, N(CH2)]; 4.74 [t, *J* = 4.9Hz, 2H, O(CH2)]; 6.47 [d, *J* = 9.7Hz, 1H, H3]; 7.15 [d, *J* = 2.2 Hz, 1H, H4']; 7.77 [s, 1H, H5]; 8.17 [d, *J* = 2.2 Hz, 1H, H5']; 8.18 [d, *J* = 9.7Hz, 1H, H4]; 10.73 [s, 1H, $^+$NHMe2].IR (cm^{-1}): 1583, 1721, 3132.

8-[2-(N,N-dimethylamino)ethoxy]-4',5'-dihydropsoralen, hydrochloride salt (compound **2**): mp = 223-225°C [light beige solid]. Calculated for $C_{15}H_{18}ClNO_4$: C, 57.78%; H, 5.81%; N, 4.49%. Found: C, 57.69%; H, 5.92%; and N, 4.66%.HRMS (ESI) calculated for $C_{15}H_{18}NO_4$ 276.1230, found 276.1242 $(M+H)^+$. ^1H NMR (DMSO-d6), δ 2.90 [s, 6H, 2 Me]; 3.24-3.30 [m, 2H, H4']; 3.48 [t, *J* = 4.9Hz, 2H, N(CH2)]; 4.43 [t, *J* = 4.9Hz, 2H, O(CH2)]; 4.70-4.76 [m, 2H, H5']; 6.28 [d, J = 9.6Hz, 1H, H3]; 7.34 [s, 1H, H5]; 7.98 [d, *J* = 9.6Hz, 1H, H4]; 10.61 [s, 1H, $^+$NHMe2].

Keratinocyte growth inhibition studies.Photobiological activity was assayed using a keratinocyte cell line (PAM212 cells) grown in monolayer culture as previously described.[xi, xii] The PAM212 lineisa murinecutaneous squamous cell carcinoma with a long history of use as a surrogate for human skin hyperproliferative diseases.[xiii] Keratinocytes were grown in Dulbecco's Modified Eagle's medium supplemented with 10% newborn calf serum in a 5% carbon dioxide incubator at 37^oC.Cells were inoculated into 6-well Falcon plastic culture

dishes at 25,000 cells per well.After 24 hr, the medium was changed to fresh growth medium supplemented with increasing concentrations of the test compounds or control medium.After 30 min, culture plates were exposed to UVA light (320-400 nm) emitted from a bank of four BLB fluorescence light tubes (F40 BL, Sylvania) placed approximately 10 cm above the cell culture plates.The incident light on the culture plates was 2.4 mW/cm^2 as measured with an International Light UV radiometer, Model IL442A.The cells were exposed to 1.28 J/cm^2 of UVA.After irradiation, the cell culture medium was drained, the cells were refed with fresh growth medium and then re-incubated to allow for cell growth.After 4-5 days, cells on the plates were counted with a Coulter Counter. Data (see Figure 2) was presented as percent inhibition of cell growth when compared to controls. All data points represent means ± SEM (N = 3). In a typical experiment, control plates contained 5.5×10^4 cells.

Treatment of plasmid DNA with water-soluble dimethylaminoethyl ether psoralens and UVA light.

A purified 3451 bp pZeoSV plasmid was used to analyze the effects of the water-soluble dimethylaminoethyl ether psoralens on DNA double strand nicking and covalent-psoralen modifications as previously described in our laboratory.[xi, xiv] Plasmid DNA (75 ng) in 10-µL reaction mixtures containing TE buffer (10 mM Tris–HCl, 1 mM EDTA, pH 8) was treated with increasing concentrations of the psoralens in V-bottomed 96-well plates in the absence and presence of UVA light. Plasmid samples were then analyzed by electrophoresis using 1.2% agarose gels either in neutral gel buffer (40 mM Tris base, 20 mM acetic acid, 1 mM EDTA) or under denaturing conditions.

In the latter experiments, DNA was first mixed with 2 M NaOH supplement with 0.1 M EDTA, heated in a water bath at 90°C for 1 min, and cooled on ice prior to loading on 1.2% alkaline agarose gels prepared and electrophoresed in alkaline gel buffer (50 mM Tris base, 45 mM boric acid, 30 mM NaOH, 1 mM EDTA). Gels were washed, stained with ethidium bromide (0.5 µg/mL) and then photographed with an Eagle Eye II digital documentation system (Stratagene, San Diego, CA) following electrophoresis. See Figure 3.

Results and Discussion:

In initial studies, the effect of the dimethylaminoethyl ether psoralens on keratinocyte proliferation was assayed. Both compounds **1** and **2** caused a concentration-dependent inhibition of keratinocyte growth, but only when activated by UVA light (Figure2). Compound **1**, which contains anunsaturated furan ring, was significantly more active in inhibiting keratinocyte growth than **2** which contains a saturated furan ring (IC_{50} = 12 nM *vs.* 13,000 nM, respectively)(Table 1).In these experiments, compound **3** (a.k.a. 8-MOP) and compound **4** (a.k.a., dihydro-8-MOP) were used to compare activity with the water-soluble dimethylaminoethyl ether psoralens. Growth inhibition was dependent on UVA light, **3** (IC_{50} = 130 nM) was significantly more active than **4** (IC_{50} = 70,000 nM).

Figure 2. Inhibition of PAM212 cell growth with and without light

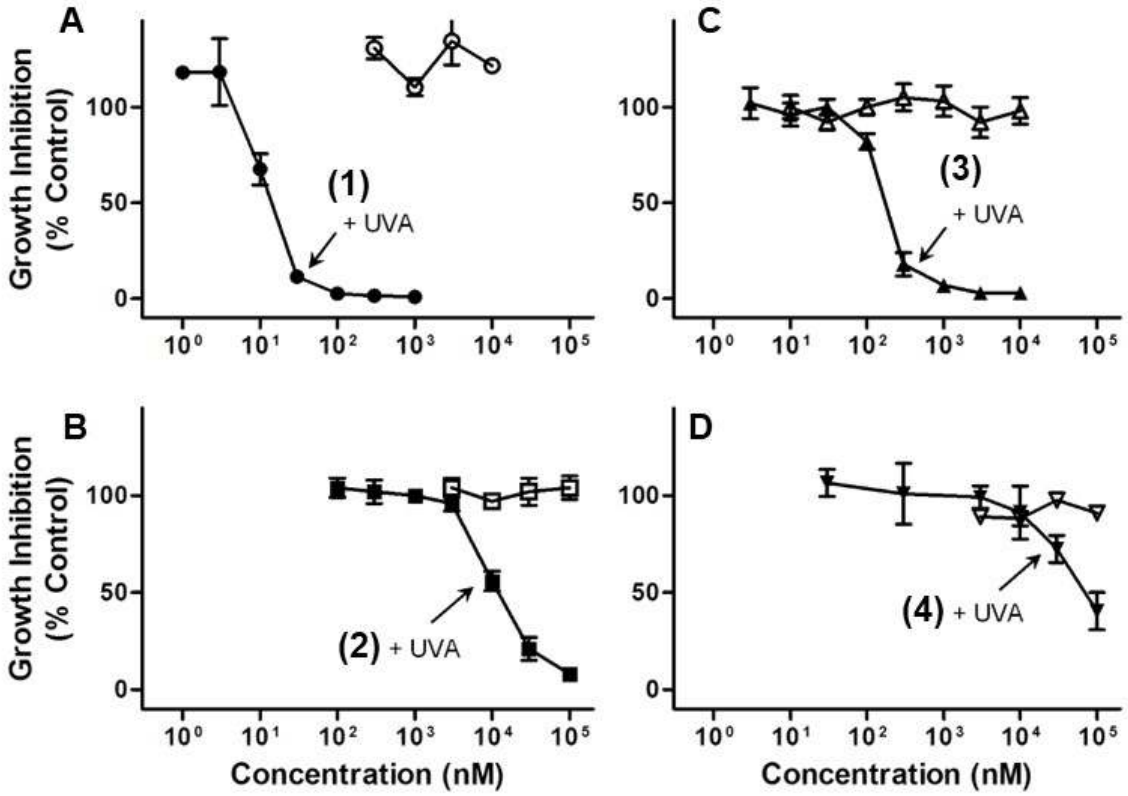
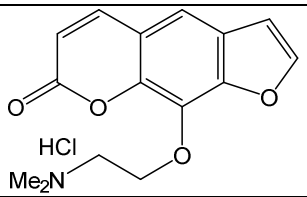
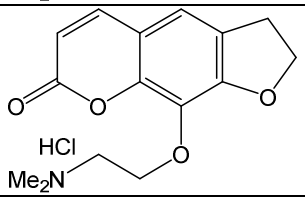
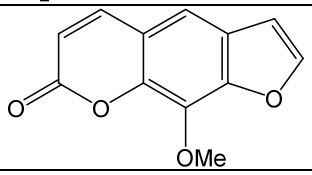
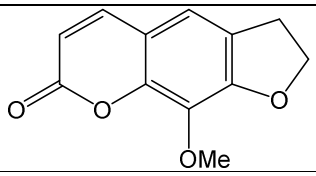


Table 1.Structures and growth inhibitory activities of 8-MOP derivatives

Compound	Structure	IC_{50} (nM) + UVA	- UVA
1		12	> 100,000
2		13,000	> 100,000
3		130	> 100,000

4		70,000	> 100,000

We next analyzed the activity ofthe dimethylaminoethyl ether psoralens in plasmid DNA unwinding assays to assess their ability to intercalate and modify DNA.In this assay, if the psoralens cause a strand nick in DNA, the plasmid will unwind and its migration in neutral agarose gels will be decreased.[xi,xiv] Alterations in double-strand migration in denaturing gels are indicative of covalent modifications in the plasmid DNA.Under neutral and alkaline conditions, none of the psoralens were found to alter migration of plasmids in the gels without UVA light (Figure3, panels A and C).In neutral agarose gels, both compounds **1** and **2** were found to alter migration of the plasmids.Compound **1** was significantly more active as evidenced by reduced migration in the gel at lower concentrations (1-10 μM for compound **1***vs.* 10-100 μM for compound **2**; Figure 3, panel B). Neither **3** nor **4**, when activated by UVA light, altered migration of the plasmids in the neutral gels.Compounds **1** and **2** also reduced migration of the plasmids in alkaline gels following UVA light treatment. The aminoethyl ether **1** was significantly more active than its furo-ring reduced analog, compound **2** (0.1-10 μM *vs.* 10-100 μM, respectively; Figure3, panel D).Under alkaline conditions, **3** but not **4** was active at concentrations from 1-10 μM in reducing migration of the plasmid after UVA light treatment.

Figure 3. Effects of psoralens and UVA light on plasmid DNA

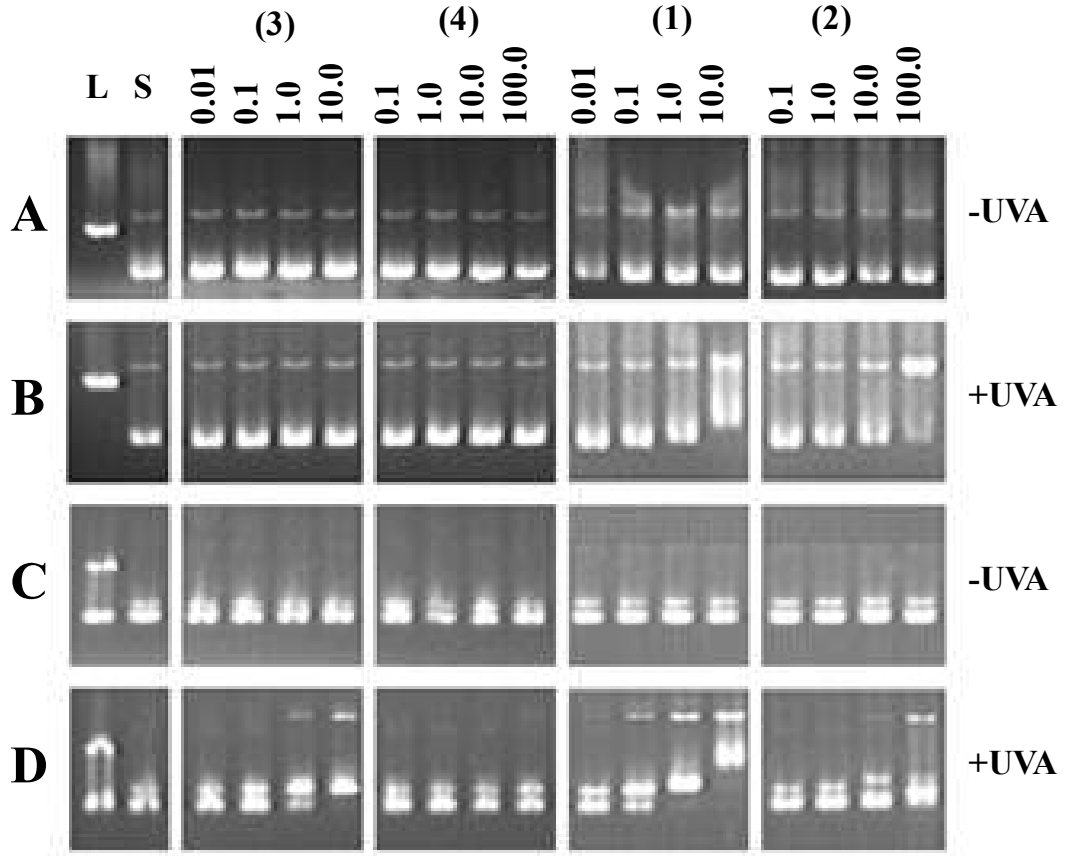


Figure 3 shows the results of plasmid DNA treated with increasing concentrations of compounds **1, 2, 3,** and **4**.Lane L shows products from the digestion with restriction endonuclease EcoRI to linearize the DNA (100 ng); in native gels, the linearized DNA migrates as one double-stranded band, whereas, in denaturing gels, it migrates as two well-separated single strands. Lane S shows untreated plasmid DNA (75 ng); in native gels, the intact plasmid DNA migrates mainly as one supercoiled double-stranded band, whereas, in denaturing gels, it migrates as two closely migrating single strands. Electrophoretic analysis was performed with neutral (panels A and B) or alkaline (panels C and D) agarose gels.Samples were treated without (panels A and C) or with (panels B and D) UVA light.Compounds **3** and **4** were used as controls for comparison with the water-soluble dimethylaminoethyl ether psoralens, **1** and **2.**

Our data demonstrate that the 8-MOP derivative,compound **1,**was significantly more active as an inhibitor of keratinocyte growth than the parent compound from which it was derived, compound **3**.This indicates that water solubility is, at least in part, an important determinant in the sensitivity of keratinocytes to the psoralens. A number of factors can contribute to increased activity of **1** when compared to **3** as an inhibitor of keratinocyte growth including increased uptake by the cells, nuclear localization, intercalation into DNA and DNA adduct formation following exposure to UVA light.Our demonstration that the dimethylaminoethyl ether psoralen modifies double stranded plasmid DNA to a much greater extent and at lower concentrations than **3** suggests that intercalation into DNA and the formation of mono- and bifunctional adducts is a key contributing factor for the increased activity of the dimethylaminoethyl ether**1**. As linear planar molecules containing conjugated ring structures, both **1** and **3**readily intercalate DNA and proximity of the unsaturated pyrane and/or furan rings to pyrimidine bases in DNA allows for the formation of DNA adducts following exposure to UVA light.[iv]

However, an additional factor controlling DNA interactions is the charge of the pendant modifications on the psoralen molecules. At physiological pH, the dimethylaminoethyl ether modification is positively charged and thus, more readily interacts with the negatively charged phosphates on the outer rim of DNA while positioning itself in the minor grove of DNA. An electrostatic binding contribution between the anionic DNA backbone and positively charged moieties pendant on the intercalator has been documented in bipyridine and phenazine intercalators.[xv,xvi] Thus, based on structure, **1** would be expected to be more efficient than **3** in interacting and modifying DNA and this is what we observed.

Compounds **2** and **4** contain saturated (reduced) furan rings. This results in non-planar structures with a more limited ability to fully intercalate into DNA. The saturated ring structures also disrupts π-π^* interactions with pyrimidine bases when compared to the unsaturated derivative and does not allow for the formation of bifunctional 2+2 cycloaddition adducts with the DNA. These compounds (**2** and **4**) are also expected to have a much more limited ability to absorb UVA light and participate in photosensitization reactions. These factors thus explain the limited ability of **2** and **4** to alter migration of plasmid DNA in agarose gels as well as inhibit keratinocyte growth.

In summary,a water-soluble dimethylaminoethyl ether psoralen, compound **1**,was identified that readily modifies and nicks DNA and is active as an inhibitor of keratinocyte cell growth in the nanomolar concentration range.In fact, this psoralen derivative was significantly more active than **3** the long-established pharmaceutical (a.k.a, methoxsalen, oxsoralen ultra, meladinine, and xanthotoxin) which is commonly used as a therapeutic to treat epidermal proliferative disorders.[v] Thus, **1** has potential as a phototherapeutic agent to treat various skin diseases.The fact that this psoralen derivative is water soluble may improve the

bioavailability of this class of compounds which could improve therapeutic efficacy when compared to the more lipophilic **3**.It is important to note that, in addition to DNA, additional cellular targets for photoactivated psoralens have been identified including proteins and lipids; modifications of these targets by **1** may also disrupt cellular functioning and contribute to the mechanism by which it inhibits keratinocyte growth.[xii,xvii,xviii,xix,xx,xxi] Future studies are needed to determine the precise mechanism of action of compound **1**and to assess its activity as a photosensitizer in clinical studies.

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References:

i. Dogra, S., Mahajan, R. *Indian J. Dermatol. Venereol. Leprol*.,**2015**, *81*, 124.
ii. Greb, J.E., Goldminz, A.M., Elder, J.T., Lebwohl, M.G., Gladman, D.D., Wu, J.J., Mehta, N.N., Finlay, A.Y., Gottlieb A.B. *Nat. Rev. Dis. Primers*.,**2016**, *2*, 16082.
iii. National Toxicology Program: Methoxsalen. *Rep. Carcinog*.,**2011**, *12*, 259.
iv Hearst, J.E. *Chemical Res. Toxicol.*, **1989**,*2,* 69.
v. Anderson, T.F., Voorhees, J.J.*Ann. Rev. Pharmacol. Toxicol.*,**1982**, *10*, 177.
vi. Bisagni, E. *J. Photochem Photobiol. B: Biol.*, **1992***, 14,* 23.
vii. Kaufman, K.D. **1981**,U.S. Patent #4,294,822.
viii. Hearst, J.E., Rapoport, H., Isaacs S., Shen, C.J.**1978**,U.S. Patent #4,124,598.
ix. Heindel, N.D.,Choudhuri, M. **1990**,U.S. Patent #4,950,770.
x. Heindel, N.D., Foster, N., Choudhuri, M. *J. Org. Chem*.,**1983**,*48*, 3817.
xi. Mariano, T.M., Vetrano, A.M., Gentile, S.L., Heck ,D.E., Whittemore, M.S., Guillon, C.D., Jabin, I., Rapp, R.D., Heindel, N.D., Laskin, J.D. *Biochem. Pharmacol.*,**2002**,*63*, 31.
xii. Yurkow, E.J., Laskin, J.D. *J. Biol. Chem.*,**1987**,*262*, 8439.
xiii. Ridd, K., Dhir, S., Smith, A.G., Gant, T.W.*Toxicol. in vitro*,**2010**, *24*, 910.
xiv. Martey, C.A., Vetrano, A.M., Whittemore, M.S., Mariano, T.M., Gentile, S.L., Heck, D.E., Laskin, D.L., Heindel, N.D., Laskin, J.D. *Biochem. Pharmacol.*, **2002**,*63*, 2001.
xv. Shade, C.M., Kennedy, R.D., Rouge, J.L. *Chemistry*, **2015**,*21,* 10983.
xvi. Liu, Y-J, Zeng, C-H, Huang, H-L.*Eur. J. Medicinal Chem.*, **2010**,*45,* 564.
xvii. Schmitt, I.M., Chimenti, S., Gasparro, F.P. *J. Photochem. Photobiol. B.*,**1995**,*27*, 101.
xviii. Anthony, F.A., Laboda, H.M., Costlow, M.E. *Photodermatol. Photoimmunol. Photomed*.,**1997**,*13*, 9.
xix. Meisenheimer, K.M., Koch, T.H. *Crit. Rev. Biochem. Mol. Biol*., **1997**,*32*, 101.
xx. Xia, W., Gooden, D., Liu, L., Zhao, S., Soderblom, E.J., Toone, E.J., Beyer, W.F. Jr., Walder, H., Spector, N.L.*PLoS One.*,**2014**,*9,* e88983.
xxi Van Aelst, B., Devloo, R., Zachée, P., t'Kindt, R., Sandra, K., Vandekerckhove, P., Compernolle, V. and Feys, H. B. *J. Biol. Chem.*, **2016**,*291*, 24364.

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