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Influence of Sugar Amine Regiochemistry on Digitoxigenin Neoglycoside Anticancer Activity

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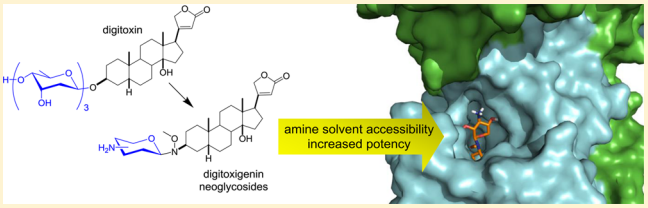
Supporting Information

ABSTRACT: The synthesis of a set of digitoxigenin neogluco/xylosides and corresponding study of their anticancer SAR revealed sugar amine regiochemistry has a dramatic effect upon activity. Specifically, this study noted sugar 3-amino followed by 4-amino-substitution to be most advantageous where the solvent accessibility of the appended amine within neoglycoside-Na^+,K^+-ATPase docked models correlated with increased anticancer potency. This study presents a preliminary model for potential further warhead optimization in the context of antibody-directed steroidal glycosides and extends the demonstrated compatibility of aminosugars in the context of neoglycosylation.



KEYWORDS: *Regiochemistry, sugar, digitoxigenin, neoglycosylation*

Steroidal glycosides (exemplified by digitoxin and digoxin, Figure 1) are a family of natural products that inhibit the Na^+,K^+-ATPase (NKA) catalytic alpha subunit, the corresponding positive inotropic effect of which serves as the basis for their use in treating congestive heart failure.[1−4] Consistent with retrospective studies that revealed a reduced recurrence rate of breast cancer among patients on digitalis,[5−9] up-regulation of NKA has been observed in many cancers including NSCLC, ovarian, pancreatic, colorectal, glioblastoma, and melanoma.[2−4] Recent research has revealed up to half of the NKA within cancer cells to reside within caveolae ("lipid rafts") and to mediate cell signaling processes via the oncogenic protein Src.[10−19] Inhibition of NKA within this context initiates kinase-dependent events involving EGFR, MAPK, PKC, or TGF-beta signaling pathways that induce cell apoptosis.[12−14,16,17]

Naturally occurring and semisynthetic steroidal glycosides display notable *in vitro* anticancer potency (including prostate,[20,21] lung,[22−26] breast,[27] colorectal,[28] leukemia, and myeloma[29]), efficacy in mouse models,[30] and importantly, more recently noted as triggering immunogenic cell death.[31] Yet, while specific analogues that display reduced ionotropic and increased cancer cell cytoxicity activity have been reported,[32] the narrow therapeutic index (by virtue of cardiotoxicity and neurotoxicity) has hampered steroidal glycoside clinical development for the treatment of cancer.[30,33−35] Steroidal aminoglycosides are attractive in this regard as the presence of an aminosugar reduces blood−brain barrier penetration and thereby offers potential for reduced neurotoxicity.[36−39] In addition, specific aminosugar-bearing analogues of digitoxigenin and proscillaridin have been employed in the context of cancer-targeting antibodies where the appended amino group also presents a convenient handle for antibody conjugation.[40,41] While some reported mono-[42−44] or oligosaccharide-based[45−48] structure−activity relationship (SAR) exists, there is a lack of fundamental anticancer SAR with respect to the sugar amine regiochemistry. Herein we describe a model

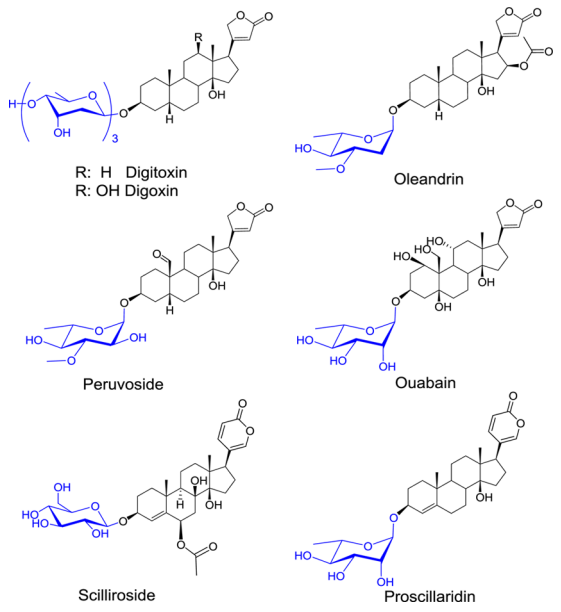


Figure 1. Representative naturally occurring cardenolides and bufadienolies with demonstrated anticancer activity (conjugated sugars highlighted in blue).

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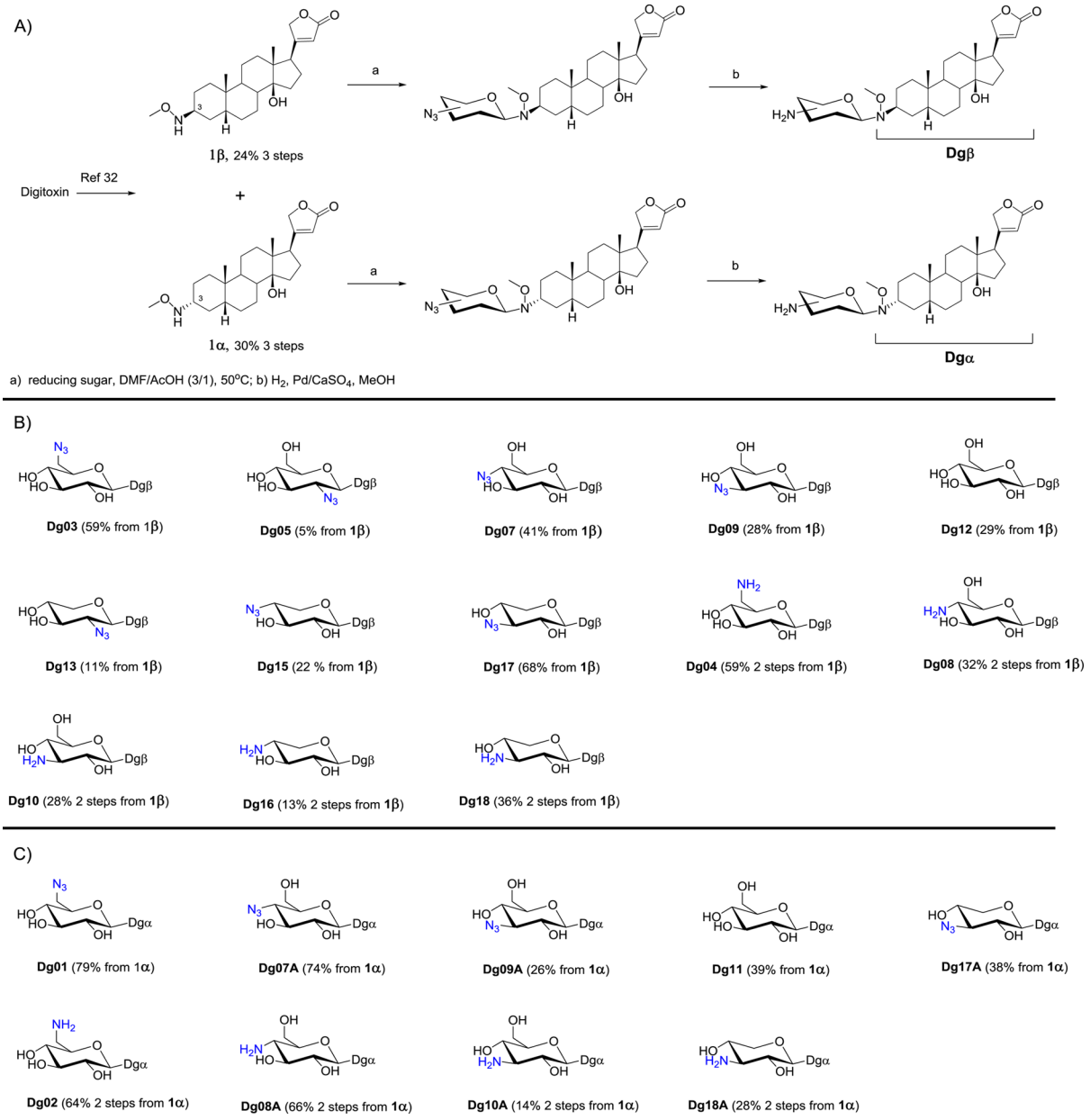

Figure 2. Overall synthetic strategy and corresponding steroidal neoglycoside products (sugar amino/azido functionalization highlighted in blue). (A) General synthetic scheme for neoaglycon (**1*α*** and **1*β***) synthesis and utilization beginning from digitoxin. (B) Digitoxigenin 3*S*-neoglycosides from aglycon **1*β***. (C) Digitoxigenin 3*R*-neoglycosides from aglycon **1*α***.

study on the impact of sugar amino substitution upon the anticancer activity of a series of digitoxigenin aminoxylosides and aminoglucosides. Consistent with computed NKA-ligand docked models, this study reveals digitoxigenin neoglycosylation with 3-aminosugars followed closely by 4-aminosugars as most advantageous where the determined potency parallels the predicted solvent accessibility of the sugar amine. Importantly, this study presents a preliminary model for potential further warhead optimization in the context of antibody-directed steroidal glycosides. In addition, this study also extends the demonstrated compatibility of aminosugars in the context of neoglycosylation.[49−52]

Preparation of digitoxigenin neoaglycon (Figure 2A) was accomplished as previously described from digitoxin to furnish 0.75 g of **1*β*** (24% yield) and 0.96 g of **1*α*** (30% yield).[32] The synthesis of aminosugar-containing neoglycosides was accomplished via a simple two step process beginning with neoglycosylation of both **1*α*** and **1*β*** with a set of azidosugars (2-azido-2-deoxy-D-glucose, 3-azido-3-deoxy-D-glucose, 4-azido-4-deoxy-D-glucose, 6-azido-6-deoxy-D-glucose, 2-azido-2-deoxy-D-xylose, 3-azido-3-deoxy-D-xylose, and 4-azido-4-deoxy-D-xylose) followed by reduction.[53] Comparator controls deriving from neoglycosylation of **1*α*** and **1*β*** with D-glucose were also generated to ultimately provide a cumulative set of 22 digitoxigenin neoglycosides for bioactivity assays (Figure 2B,C). The yield of neoglycosylation varied by the nature of the sugar employed with an average isolated yield of 40%. Neoglycoside product characterization revealed all the members to adopt 1,2-*trans*-anomer exclusively.

The set of 22 pure digitoxigenin neoglycosides and two parental aglycons were assessed *in vitro* against non-small cell lung cancer cell line A549 (Figure 3). Consistent with the prior single comparison of a 3*S*- and 3*R*-neoglucoside,[32] all 3*S*-digitoxigenin

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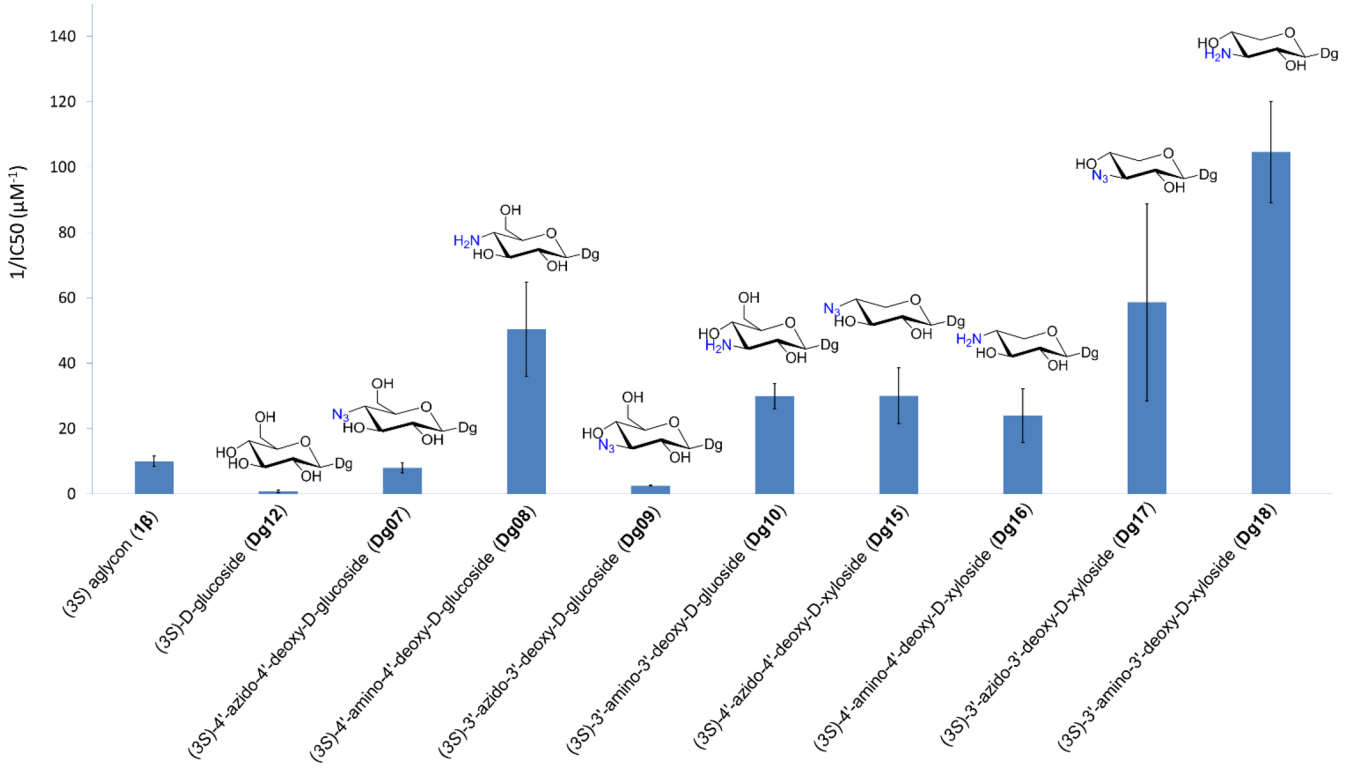

Figure 3. Anticancer activity of the most active digitoxigenin neoglycosides and comparator controls against the A549 (human lung adenocarcinoma) cancer cell line. Reciprocal IC_{50} values and the structures of the corresponding sugars conjugated for each **1α/1β** pairing are displayed. As a reference, the most active analogue in this series (**Dg18**) displayed an IC_{50} of 10 ± 1 nM, while the least active analogue represented (**Dg12**) displayed an IC_{50} of 1600 ± 400 nM. Three independent viability assays were conducted in triplicate of dose−response experiments over nine concentrations at 3-fold dilutions. IC_{50} values (and mean ± standard errors) can be found in the Supporting Information.

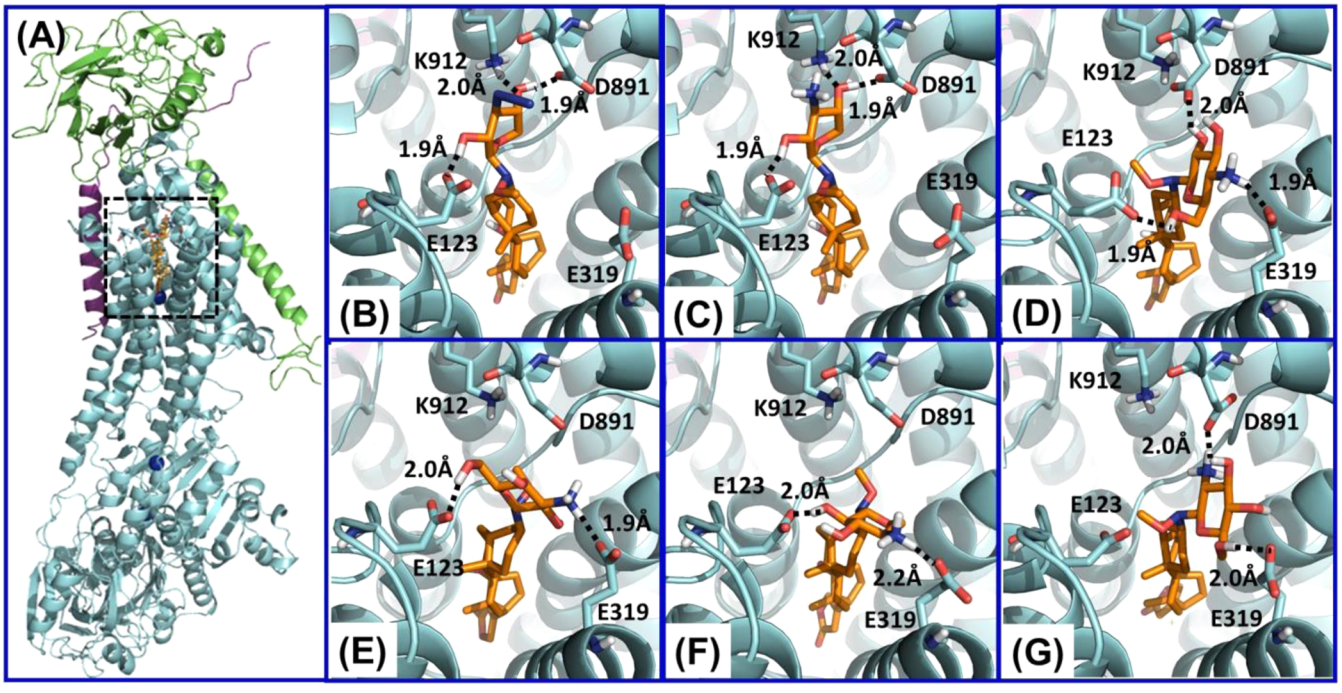

Figure 4. Human NKA-cardenolide neoglycoside docked models (cardenolide neoglycoside, orange ball and stick; NKA α1, β1, and γ subunits in cyan, green, and purple, respectively). Hydrogen bonds are illustrated as dotted lines with key distances in Å. (A) The overall NKA complex: (B) **Dg17**; (C) **Dg18**; (D) **Dg08**; (E) **Dg10**; (F) **Dg16**. (G) Predicted binding mode of a (3S)-2′-amino-2′-deoxy-D-glucoside (analogue not synthesized) predicts a potential rotation of sugar to afford the putative favorable electrostatic interaction between the glycosyl C2′-amine and NKA D891 side chain carboxylate.

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neoglycosides displayed notably greater potency than their 3*R* counterparts with differences of up to >1,000-fold between representative 3*S* and 3*R* pairings. Among the most active analogues, C3′-substitution presented a 3- to 5-fold enhancement in potency compared to C4′-functionalization. Among the pentose series amino and azido substitution was relatively interchangeable in the context of impact upon potency. In contrast, amino-substitution was found to be more advantageous in the context of the hexose series. The overall ranked order was **Dg18** (3′-amino-D-xyloside, IC_{50} 10 ± 1 nM) ≈ **Dg17** (3′-azido-D-xyloside, IC_{50} 17 ± 9 nM) ≥ **Dg08** (4′-amino-D-glucoside, IC_{50} 20 ± 6 nM) > **Dg10** (3′-amino-D-glucoside, IC_{50} 34 ± 4 nM) ≈ **Dg15** (4′-azido-D-xyloside, IC_{50} 34 ± 9 nM) ≈ **Dg16** (4′-amino-D-xyloside, IC_{50} 43 ± 8 nM) > **Dg07** (4′-azido-D-glucoside, IC_{50} 130 ± 25 nM) > **Dg09** (3′-azido-D-glucoside, IC_{50} 390 ± 18 nM). As is evident from the comparison of **Dg08** (4′-amino-D-glucoside, IC_{50} 20 ± 6 nM) and **Dg12** (D-glucoside, IC_{50} 1600 ± 400 nM), C4′-amino substitution presents the potential for dramatic improvement with a 80-fold difference.

In an effort to better understand the corresponding SAR, neoglycoside-NKA docked models were developed based upon previously determined human NKA ligand-bound structures.[54,55] Importantly, MD simulations of preferred binding modes track with experimentally determined inhibitor potency, suggesting this strategy to be potentially predictive for further inhibitor optimization. Notably, the side chains of four key NKA amino acids serve as the primary contributors to binding the sugar moiety in this model (E123, E319, D891, and K912) where a high degree of rotational freedom around the neoglycosidic bond leads to a divergence of predicted sugar orientations among the ligands compared. The conjugated pentose of the two most potent analogues **Dg17** and **Dg18** (Figure 4B,C, respectively) adopt an identical binding mode (with ligand sugar hydrogen bonding interactions contributed by the side chain carboxylate/amine of E123, D891, and K912) where the pentose C3′-azide/amine is notably fully solvent exposed. In contrast, a common feature of analogues with slightly lower potency (**Dg08**, **Dg10**, and **Dg16**; Figure 4D−F, respectively) is a key NKA E319 side chain carboxylate hydrogen bonding interaction with the corresponding sugar C3′ or C4′-amine, which effectively buries the carbohydrate amino group within the active site. Of these latter three analogues, a feature that further distinguishes the more potent **Dg08** (Figure 4D) from the less active counterparts **Dg10** and **Dg16** (Figure 4E,F, respectively) is the presence of an additional **Dg08** C2′-OH-NKA D891 side chain carboxylate hydrogen bonding interaction. Extrapolating from this computational model, a predicted buried sugar C2′-amine (by virtue of a hydrogen bond to the NKA D891-side chain carboxylate; Figure 4G) and additional sugar C6′-OH interaction with the NKA E319 side chain carboxylate may suggest C2′-aminosugar-derived analogues to display potencies similar to that of **Dg08**, **Dg10**, and **Dg16**.

Cumulatively, this focused SAR study further extends a streamlined strategy for employing aminosugars within the context of neoglycosylation and reveals the C3-glycosylation of digitoxigenin with C3′-amino/azido-xylose to offer the most dramatic improvements in anticancer activity. Molecular modeling highlights a correlation of determined anticancer activity with favored NKA ligand-binding site occupancy where the corresponding C3′-amine/azide in the most active analogues were fully solvent exposed. This latter point also supports the contention that the C3 glycosylation of cardenolides (and potentially bufadenolides) with simple C3′-amino/azidosugars present convenient chemoselective functionality for conjugation to cancer-targeting antibodies as a strategy to improve their therapeutic index and enable their use in the treatment of cancer.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsmedchemlett.5b00120.

Corresponding experimental details and compound characterization data (PDF)

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Notes

The authors declare the following competing financial interest(s): J.S.T. is a co-founder of Centrose (Madison, WI, USA).

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■ ABBREVIATIONS

CG, cardiac glycoside; ATPase, adenosine triphosphatase; NKA, Na^+,K^+-ATPase; NSCLC, non-small cell lung cancer; EGFR, epidermal growth factor receptor; MAPK, mitogen-activated protein kinase; PKC, protein kinase C; TGF-beta, transforming growth factor beta

■ REFERENCES

(1) Repke, K. R. H. Toward the discovery of digitalis derivatives with inotropic selectivity. *Drug Discovery Today* **1997**, *2*, 110−116.

(2) Mijatovic, T.; Van Quaquebeke, E.; Delest, B.; Debeir, O.; Darro, F.; Kiss, R. Cardiotonic steroids on the road to anti-cancer therapy. *Biochim. Biophys. Acta, Rev. Cancer* **2007**, *1776*, 32−57.

(3) Newman, R. A.; Yang, P.; Pawlus, A. D.; Block, K. I. Cardiac glycosides as novel cancer therapeutic agents. *Mol. Interventions* **2008**, *8*, 36−49.

(4) Prassas, I.; Diamandis, E. P. Novel therapeutic applications of cardiac glycosides. *Nat. Rev. Drug Discovery* **2008**, *7*, 926−935.

(5) Stenkvist, B.; Bengtsson, E.; Eriksson, O.; Holmquist, J.; Nordin, B.; Westman-Naeser, S. Cardiac glycosides and breast cancer. *Lancet* **1979**, *1*, 563.

(6) Stenkvist, B.; Bengtsson, E.; Dahlqvist, B.; Eriksson, O.; Jarkrans, T.; Nordin, B. Cardiac glycosides and breast cancer, revisited. *N. Engl. J. Med.* **1982**, *306*, 484.

(7) Goldin, A. G.; Safa, A. R. Digitalis and cancer. *Lancet* **1984**, *323*, 1134.

(8) Haux, J. Digitoxin is a potential anticancer agent for several types of cancer. *Med. Hypotheses* **1999**, *53*, 543−548.

(9) Stenkvist, B. Is digitalis a therapy for breast carcinoma? *Oncol. Rep.* **1999**, *6*, 493−496.

(10) Simons, K.; Toomre, D. Lipid rafts and signal transduction. *Nat. Rev. Mol. Cell Biol.* **2000**, *1*, 31−39.

(11) Ferrandi, M.; Molinari, I.; Barassi, P.; Minotti, E.; Bianchi, G.; Ferrari, P. Organ hypertrophic signaling within caveolae membrane subdomains triggered by ouabain and antagonized by PST 2238. *J. Biol. Chem.* **2004**, *279*, 33306−33314.

DOI: 10.1021/acsmedchemlett.5b00120
ACS Med. Chem. Lett. 2015, 6, 1053−1058

(12) Kometiani, P.; Liu, L.; Askari, A. Digitalis-induced signaling by Na^+/K^+-ATPase in human breast cancer cells. *Mol. Pharmacol.* **2005**, *67*, 929−936.
(13) Xie, Z. J.; Xie, J. The Na/K-ATPase-mediated signal transduction as a target for new drug development. *Front. Biosci., Landmark Ed.* **2005**, *10*, 3100−3109.
(14) Tian, J.; Cai, T.; Yuan, Z.; Wang, H.; Liu, L.; Haas, M.; Maksimova, E.; Huang, X. Y.; Xie, Z. J. Binding of Src to Na^+/K^+-ATPase forms a functional signaling complex. *Mol. Biol. Cell* **2006**, *17*, 317−326.
(15) Liang, M.; Tian, J.; Liu, L.; Pierre, S.; Liu, J.; Shapiro, J.; Xie, Z. J. Identification of a pool of non-pumping Na/K-ATPase. *J. Biol. Chem.* **2007**, *282*, 10585−93.
(16) Li, Z.; Xie, Z. The Na/K-ATPase/Src complex and cardiotonic steroid-activated protein kinase cascades. *Pfluegers Arch.* **2009**, *457*, 635−644.
(17) Prassas, I.; Karagiannis, G. S.; Batruch, I.; Dimitromanolakis, A.; Datti, A.; Diamandis, E. P. Digitoxin-induced cytotoxicity in cancer cells is mediated through distinct kinase and interferon signaling networks. *Mol. Cancer Ther.* **2011**, *10*, 2083−2093.
(18) Liu, C. C.; Fry, N. A.; Hamilton, E. J.; Chia, K. K.; Garcia, A.; Karimi Galougahi, K.; Figtree, G. A.; Clarke, R. J.; Bundgaard, H.; Rasmussen, H. H. Redox-dependent regulation of the Na^+-K^+ pump: new twists to an old target for treatment of heart failure. *J. Mol. Cell. Cardiol.* **2013**, *61*, 94−101.
(19) Reinhard, L.; Tidow, H.; Clausen, M. J.; Nissen, P. Na^+,K^+-ATPase as a docking station: protein-protein complexes of the Na^+,K^+-ATPase. *Cell. Mol. Life Sci.* **2013**, *70*, 205−222.
(20) Yeh, J. Y.; Huang, W. J.; Kan, S. F.; Wang, P. S. Inhibitory effects of digitalis on the proliferation of androgen dependent and independent prostate cancer cells. *J. Urol.* **2001**, *166*, 1937−1942.
(21) McConkey, D. J.; Lin, Y.; Nutt, L. K.; Ozel, H. Z.; Newman, R. A. Cardiac glycosides stimulate Ca^{2+} increases and apoptosis in androgen-independent, metastatic human prostate adenocarcinoma cells. *Cancer Res.* **2000**, *60*, 3807−3812.
(22) Wang, H. Y.; Xin, W.; Zhou, M.; Stueckle, T. A.; Rojanasakul, Y.; O'Doherty, G. A. Stereochemical survey of digitoxin monosaccharides: new anticancer analogues with enhanced apoptotic activity and growth inhibitory effect on human non-small cell lung cancer cell. *ACS Med. Chem. Lett.* **2011**, *2*, 73−78.
(23) Elbaz, H. A.; Stueckle, T. A.; Wang, H. Y.; O'Doherty, G. A.; Lowry, D. T.; Sargent, L. M.; Wang, L.; Dinu, C. Z.; Rojanasakul, Y. Digitoxin and a synthetic monosaccharide analog inhibit cell viability in lung cancer cells. *Toxicol. Appl. Pharmacol.* **2012**, *258*, 51−60.
(24) Frese, S.; Frese-Schaper, M.; Andres, A. C.; Miescher, D.; Zumkehr, B.; Schmid, R. A. Cardiac glycosides initiate Apo2L/TRAIL-induced apoptosis in non-small cell lung cancer cells by up-regulation of death receptors 4 and 5. *Cancer Res.* **2006**, *66*, 5867−5874.
(25) Mijatovic, T.; Mathieu, V.; Gaussin, J. F.; De Neve, N.; Ribaucour, F.; Van Quaquebeke, E.; Dumont, P.; Darro, F.; Kiss, R. Cardenolide-induced lysosomal membrane permeabilization demonstrates therapeutic benefits in experimental human non-small cell lung cancers. *Neoplasia* **2006**, *8*, 402−12.
(26) Pongrakhananon, V.; Stueckle, T. A.; Wang, H. Y.; O'Doherty, G. A.; Dinu, C. Z.; Chanvorachote, P.; Rojanasakul, Y. Monosaccharide digitoxin derivative sensitize human non-small cell lung cancer cells to anoikis through Mcl-1 proteasomal degradation. *Biochem. Pharmacol.* **2014**, *88*, 23−35.
(27) Winnicka, K.; Bielawski, K.; Bielawska, A.; Miltyk, W. Apoptosis-mediated cytotoxicity of ouabain, digoxin and proscillaridin A in the estrogen independent MDA-MB-231 breast cancer cells. *Arch. Pharmacal Res.* **2007**, *30*, 1216−1224.
(28) Felth, J.; Rickardson, L.; Rosen, J.; Wickstrom, M.; Fryknas, M.; Lindskog, M.; Bohlin, L.; Gullbo, J. Cytotoxic effects of cardiac glycosides in colon cancer cells, alone and in combination with standard chemotherapeutic drugs. *J. Nat. Prod.* **2009**, *72*, 1969−1974.
(29) Johansson, S.; Lindholm, P.; Gullbo, J.; Larsson, R.; Bohlin, L.; Claeson, P. Cytotoxicity of digitoxin and related cardiac glycosides in human tumor cells. *Anti-Cancer Drugs* **2001**, *12*, 475−483.
(30) Calderon-Montano, J. M.; Burgos-Moron, E.; Lopez-Lazaro, M. The in vivo antitumor activity of cardiac glycosides in mice xenografted with human cancer cells is probably an experimental artifact. *Oncogene* **2014**, *33*, 2947−1948.
(31) Menger, L.; Vacchelli, E.; Adjemian, S.; Martins, I.; Ma, Y.; Shen, S.; Yamazaki, T.; Sukkurwala, A. Q.; Michaud, M.; Mignot, G.; Schlemmer, F.; Sulpice, E.; Locher, C.; Gidrol, X.; Ghiringhelli, F.; Modjtahedi, N.; Galluzzi, L.; Andre, F.; Zitvogel, L.; Kepp, O.; Kroemer, G. Cardiac glycosides exert anticancer effects by inducing immunogenic cell death. *Sci. Transl. Med.* **2012**, *4*, 143ra99.
(32) Langenhan, J. M.; Peters, N. R.; Guzei, I. A.; Hoffmann, F. M.; Thorson, J. S. Enhancing the anticancer properties of cardiac glycosides by neoglycorandomization. *Proc. Natl. Acad. Sci. U. S. A.* **2005**, *102*, 12305−12310.
(33) Calderon-Montano, J. M.; Burgos-Moron, E.; Orta, M. L.; Maldonado-Navas, D.; Garcia-Dominguez, I.; Lopez-Lazaro, M. Evaluating the cancer therapeutic potential of cardiac glycosides. *BioMed Res. Int.* **2014**, *2014*, 794930.
(34) Slingerland, M.; Cerella, C.; Guchelaar, H. J.; Diederich, M.; Gelderblom, H. Cardiac glycosides in cancer therapy: from preclinical investigations towards clinical trials. *Invest. New Drugs* **2013**, *31*, 1087−1094.
(35) Menger, L.; Vacchelli, E.; Kepp, O.; Eggermont, A.; Tartour, E.; Zitvogel, L.; Kroemer, G.; Galluzzi, L. Trial watch: Cardiac glycosides and cancer therapy. *Oncoimmunology* **2013**, *2*, e23082.
(36) Caldwell, R. W.; Nash, C. B. Pharmacological studies of a new 4-aminosugar cardiac glycoside (ASI-222). *J. Pharmacol. Exp. Ther.* **1976**, *197*, 19−26.
(37) Mudge, G. H., Jr.; Lloyd, B. L.; Greenblatt, D. J.; Smith, T. W. Inotropic and toxic effects of a polar cardiac glycoside derivative in the dog. *Circ. Res.* **1978**, *43*, 847−854.
(38) Hutchinson, C. R.; Shekhani, M. S.; Prudent, J. R. Glycoside compounds and pharmaceutical compositions thereof. US Patent 8361973, Jan 29, 2013.
(39) Hutchinson, C. R.; Shekhani, M. S.; Prudent, J. R. Glycoside compounds and pharmaceutical compositions thereof. US Patent 8703723, April 22, 2014.
(40) Hutchinson, C. R.; Prudent, J. R.; Thorson, J. S. Extracellular targeted drug conjugates. US Patent 8470980, Jun 25, 2013.
(41) Sweeny, L.; Hartman, Y. E.; Zinn, K. R.; Prudent, J. R.; Marshall, D. J.; Shekhani, M. S.; Rosenthal, E. L. A novel extracellular drug conjugate significantly inhibits head and neck squamous cell carcinoma. *Oral Oncol.* **2013**, *49*, 991−997.
(42) Hinds, J. W.; McKenna, S. B.; Sharif, E. U.; Wang, H. Y.; Akhmedov, N. G.; O'Doherty, G. A. C3′/C4′-stereochemical effects of digitoxigenin alpha-L-/alpha-D-glycoside in cancer cytotoxicity. *ChemMedChem* **2013**, *8*, 63−69.
(43) Wang, H. Y. L.; Rojanasakul, Y.; O'Doherty, G. A. Synthesis and evaluation of the alpha-D-/alpha-L-rhamnosyl and amicetosyl digitoxigenin oligomers as antitumor agents. *ACS Med. Chem. Lett.* **2011**, *2*, 264−269.
(44) Wang, H. Y.; Qi, Z.; Wu, B.; Kang, S. W.; Rojanasakul, Y.; O'Doherty, G. A. C5′-alkyl substitution effects on digitoxigenin alpha-l-glycoside cancer cytotoxicity. *ACS Med. Chem. Lett.* **2011**, *2*, 259−263.
(45) Beale, T. M.; Taylor, M. S. Synthesis of cardiac glycoside analogs by catalyst-controlled, regioselective glycosylation of digitoxin. *Org. Lett.* **2013**, *15*, 1358−1561.
(46) Ueda, Y.; Mishiro, K.; Yoshida, K.; Furuta, T.; Kawabata, T. Regioselective diversification of a cardiac glycoside, lanatoside C, by organocatalysis. *J. Org. Chem.* **2012**, *77*, 7850−7857.
(47) Iyer, A. K.; Zhou, M.; Azad, N.; Elbaz, H.; Wang, L.; Rogalsky, D. K.; Rojanasakul, Y.; O'Doherty, G. A.; Langenhan, J. M. A direct comparison of the anticancer activities of digitoxin MeON-neoglycosides and *O*-glycosides: Oligosaccharide chain length-dependent induction of caspase-9-mediated apoptosis. *ACS Med. Chem. Lett.* **2010**, *1*, 326−330.
(48) Zhou, M.; O'Doherty, G. The de novo synthesis of oligosaccharides: application to the medicinal chemistry SAR-study of digitoxin. *Curr. Top. Med. Chem.* **2008**, *8*, 114−125.

DOI: 10.1021/acsmedchemlett.5b00120
ACS Med. Chem. Lett. 2015, 6, 1053−1058

(49) Nandurkar, N. S.; Zhang, J.; Ye, Q.; Ponomareva, L. V.; She, Q. B.; Thorson, J. S. The identification of perillyl alcohol glycosides with improved antiproliferative activity. *J. Med. Chem.* **2014**, *57*, 7478−7884.
(50) Goff, R. D.; Thorson, J. S. Neoglycosylation and neoglycorandomization: Enabling tools for the discovery of novel glycosylated bioactive probes and early stage leads. *MedChemComm* **2014**, *5*, 1036−1047.
(51) Zhang, J.; Ponomareva, L. V.; Marchillo, K.; Zhou, M.; Andes, D. R.; Thorson, J. S. Synthesis and antibacterial activity of doxycycline neoglycosides. *J. Nat. Prod.* **2013**, *76*, 1627−1636.
(52) Peltier-Pain, P.; Marchillo, K.; Zhou, M.; Andes, D. R.; Thorson, J. S. Natural product disaccharide engineering through tandem glycosyltransferase catalysis reversibility and neoglycosylation. *Org. Lett.* **2012**, *14*, 5086−5089.
(53) Zhang, J.; Singh, S.; Hughes, R. R.; Zhou, M.; Sunkara, M.; Morris, A. J.; Thorson, J. S. A simple strategy for glycosyltransferase-catalyzed aminosugar nucleotide synthesis. *ChemBioChem* **2014**, *15*, 647−651.
(54) Laursen, M.; Yatime, L.; Nissen, P.; Fedosova, N. U. Crystal structure of the high-affinity Na^+K^+-ATPase-ouabain complex with Mg^{2+} bound in the cation binding site. *Proc. Natl. Acad. Sci. U. S. A.* **2013**, *110*, 10958−10963.
(55) Laursen, M.; Gregersen, J. L.; Yatime, L.; Nissen, P.; Fedosova, N. U. Structures and characterization of digoxin- and bufalin-bound Na^+,K^+-ATPase compared with the ouabain-bound complex. *Proc. Natl. Acad. Sci. U. S. A.* **2015**, *112*, 1755−1760.

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