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Pharmacokinetic, Physicochemical and Medicinal Properties of N-glycoside Anti-cancer Agent More Potent than 2-Deoxy-D-Glucose in Lung Cancer Cells

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Abstract: Acetylated N-xyloside of 1-naphthylamine (K8A) has been shown to be more potent than 2-deoxy-D-glucose in lung cancer cells and has therapeutic potential for further drug development. In this paper we evaluate and report cytotoxicity, pharmacokinetic, physicochemical and medicinal properties of this D-Xylose derivative (K8A) as a lead anticancer agent with greater therapeutic potential than 2-deoxy-D-glucose (2-DG). 2-DG has been in clinical trials for treatment of solid tumors and other types of cancer. We demonstrate using virtual tools that K8A has better "drug-likeness" than 2-DG and does not violate any Lipinski, Ghose, Veber, Egan or Muegge rules. On the other hand, 2-DG violates Ghose and Muegge rules. A "BOILEDegg evaluation", predicts that K8A has higher gastrointestinal absorption (HIA) than 2-DG and is not effluxed by P-glycoprotein (P-gp). Additionally, K8A does not penetrate the blood brain barrier (BBB) and is not a substrate of most Cytochrome P450 (CYP) enzymes. Importantly, K8A did not show false positive alert from PAINS screening enabling us to narrow down and rule out false targets. Importantly, K8A is more potent than 2-DG in H1299 and A549 lung cancer cells.

Key words: Anticancer agent, N-glycoside, 2-deoxy-D-glucose, pharmacokinetics, lung cancer.

1. Introduction

2-deoxy-D-glucose (2-DG) induces cellular stress [1-4] and cell death [5]. 2-DG inhibits glycolysis [6], disrupts N-glycosylation [7] and alters the pentose phosphate pathway (PPP) to cause oxidative [8] and endoplasmic reticulum (ER) stress [4]. Upon 2-DG phosphorylation, 2-DG-6-phosphate noncompetitively inhibits hexokinase, and competitively inhibits phosphoglucose isomerase (PGI) [6]. 2-DG has been in clinical trial for potential use as an anticancer agent [9, 10]. The use of 2-DG is however limited by high-dose systemic toxicity [11] because the drug discovery process ignored toxicity and *in silico* pharmacodynamic and pharmacokinetic indicators. For this reason, high concentrations are required to out-compete intracellular glucose concentrations in the human body. In most humans, glucose concentration varies from about 80 mg/dl to 110 mg/dl. Additionally, 2-DG promotes multiple prosurvival pathways [12] and can activate migration and invasion of glioblastomas [13], thus necessitating the need for drug discovery of alternative carbohydrate-based inducers of cellular stress [14]. One such molecule is acetylated N-xyloside of 1-naphthylamine (K8A) and has been shown to be more potent than 2-deoxy-D-glucose in lung cancer cells. K8A has therapeutic potential for

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further drug development.

Modern drug development involves evaluation of efficiency of potent molecules and their ability to reach targets in bioactive form. This involves cellular, animal and human clinical trials that are often costly and laden with safety risks. Currently, computer aided assessment of absorption, distribution, metabolism and excretion of drugs (ADME) has gained traction to mitigate these concerns [15]. Importantly, computer models provide predictive and reliable data fast and thus compliment experimental approaches. These computer models also predict pharmacokinetic, physicochemical and medicinal properties of small molecules and assist in optimization of lead compounds to patient drugs.

Free web tools are now available that give access to these predictive models and include molinspiration (http://www.molinspiration.com), Silicos-it can be traced online at (http://silicos-it.be.s3-website-eu-west-1.amazonaws.com/software/software.html), omicX found at (https://omictools.com/qed-tool), molsoft, (http://www.molsoft.com/mprop/) and swissADME (http://www.swissadme.ch) [16], among others. Molinspiration is a website that allows one to predict the bioactivity of a structure against several different common drug targets. Molinspiration offers broad range of cheminformatics software tools supporting molecule manipulation and processing. Silicos-it was developed to share information on open source cheminformatics with downloadable tools that aid in drug design and development. SwissADME is a more recent and comprehensive site run by the Swiss institute of bioinformatics (SIB) which provides bioinformatics services and resources for scientists worldwide. SIB has over 65 bioinformatics research groups and 800 scientists from the major Swiss schools of higher education and research institutes.

SwissADME enables assessment of ADME parameters of drug candidates and small molecules and provides information that allows early risk assessment in the drug development process. Notably, swissADME provides a platform to assess Lipinski's rule of five [17] for drug-likeness of oral bioavailability. Drug-likeness is a complex balance of molecular properties and structural features which determine whether an unknown molecule is like the known drugs. These molecular properties include hydrophobicity, electronic distribution, and hydrogen bonding characteristics, molecule size and flexibility. SwissADME includes "BOILEDegg evaluation" [18] that predict gastrointestinal absorption (HIA) and efflux/retention by P-glycoprotein (P-gp). "BOILEDegg" is a graphical evaluation of HIA as a function of the position of the small molecule in the WLOGP-versus-TPSA plot. Additionally, blood brain barrier (BBB) penetration and Cytochrome P450 (CYP) enzyme substrate-inhibition prediction can be made. Importantly, false positive results commonly seen in biochemical assays of small molecules are predicted with a fair degree of certainty [19].

2. Materials and Methods

SIB website http://www.swissadme.ch was accessed in a web browser that displays the submission page of SwissADME. Previously synthesized and characterized hit molecules K8 and K8A [20] with positive control (2-deoxyD-glucose) and other controls were input for estimation of ADME, physicochemistry, drug-likeness, pharmacokinetics and medicinal chemistry properties. The input zone comprises a molecular sketcher based on ChemAxon's Marvin JS (http://www.chemaxon.com) that allowed the user to draw and edit 2D chemical structures. The structures are transferred as a list to the right-hand side of the submission page, which is the actual input for computation. The list is made to contain one input molecule per line, defined by simplified molecular-input line-entry system (SMILES) and results are presented for each molecule in tables, graphically and as an excel spreadsheet.

We then synthesized and evaluated K8 and K8A

with D-xylose and naphthylamine as described previously [20]. Briefly, equimolar D-xylose and appropriate naphthylamine was refluxed in ethanol for 8 hours. This was followed by acetylation in pyridine with acetic anhydride in an ice bath for 3 hours and in the cold room (4 ^{0}C) for another 13 hours. The reactant mixture was then quenched by pouring into ice/water mixture, extracted, washed with aqueous sodium bicarbonate, water and brine, and purified by column chromatography (1:4 ethyl acetate/hexane solvent conditions). Bioactive K8A was characterized by ^{1}H NMR and ^{13}C NMR (supporting information).

We evaluated acetylated N-Naphthyl-β-D-Xylose (K8A) vs 2-DG in two lung cancer cell lines (Table 7). Cell viability was measured by using the 3-(4,5-dimethylthiazol-2-yl)-diphenyl tetrazolium bromide assay (MTT assay) after a 48-hour incubation period.

3. Results and Discussion

We evaluated our lead compound K8A, with controls that included 1-naphthylamine (K8A-aglycone), D-Xylose (K8A-glycone), acetylated D-Xylose and the positive control 2-deoxy-D-glucose. The structural features of these small molecules were entered in the SwissADME website (http://swissadme.ch) using the ChemAxon's Marvin JS structure drawing tool. Structural features of a pharmacophore influence the behavior of a molecule in humans, including bioavailability, transport properties, affinity to proteins, reactivity, toxicity and metabolic stability. Unique to swissADME is the bioavailability radar [16] that provides a graphical snapshot of the drug-likeness parameters of an orally available bioactive drug. The drug-likeness graph is presented as a hexagon (Fig. 1) with each of the vertices representing a parameter that define a bioavailable drug. The pink area within the hexagon represents the optimal range for each property (lipophilicity: XLOGP3 between -0.7 and +5.0, size: MW between 150 and 500 g/mol, polarity: TPSA between 20 and 130 Å^{2}, solubility: log S not higher than 6, saturation: fraction of carbons in the sp3 hybridization not less than 0.25, and flexibility: no more than 9 rotatable bonds).

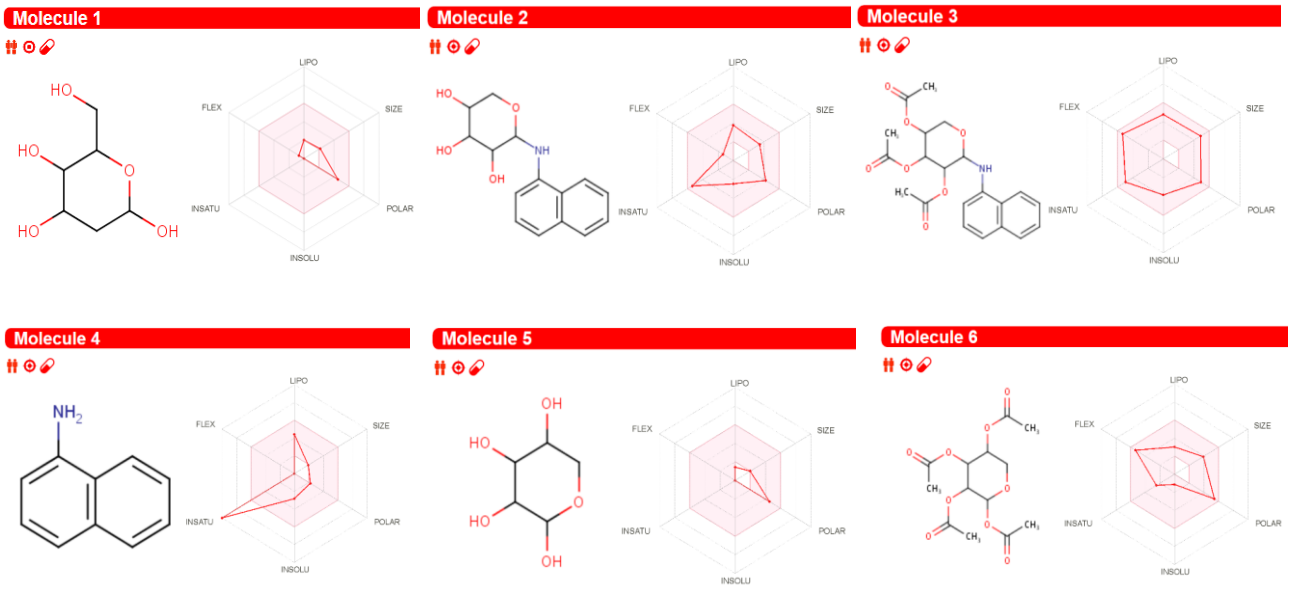


Fig. 1 The bioavailability radar of the small molecules evaluated using swissADME web tool. 2-deoxy-D-glucose (Molecule 1), K8 (Molecule 2), K8A (Molecule 3), 1-naphthylamine (Molecule 4), D-xylose (Molecule 5) and acetylated D-xylose (Molecule 6). (Lipophilicity (LIPO): XLOGP3 between -0.7 and +5.0, Molecular weight (SIZE): MW between 150 and 500 g/mol, Polarity (POLAR) TPSA between 20 and 130 Å^{2}, Solubility (INSOLU): log *S* not higher than 6, Saturation (INSATU): fraction of carbons in the sp3 hybridization not less than 0.25, and Flexibility (FLEX): no more than 9 rotatable bonds.

3.1 Drug-Likeness

2-deoxy-D-glucose (Molecule 1), K8 (Molecule 2) and K8A (Molecule 3) drug-likeness properties are represented by the red distorted hexagon within the pink shade (Fig. 1). Notably, 2-DG, K8 and K8A drug-likeness fall within parameters of a bioavailable drug. 1-naphthylamine (Molecule 4), D-xylose (Molecule 5) and acetylated D-xylose (Molecule 6) were negative controls with no bioactivity in cancer cells. Molecule 4 has high unsaturation indicated by an off-shoot of one of the saturation (INSATU) vertex (Fig. 1).

SwissADME also has computational filters that include Ghose [21], Egan [22], Veber [23], and Muegee [24] developed by leading pharmaceutical companies and cheminfomaticians to evaluate the drug-likeness of small molecules. The Ghose filter quantitatively characterizes small molecules based on computed physicochemical property profiles that include log P, molar refractivity (MR), molecular weight (MW), and number of atoms. Additionally, the Ghose [21, 25] filter include a qualitative characterization based on the presence of functional groups and important substructures. The qualifying range of calculated log P (ClogP) is between -0.4 and 5.6. For MW, the qualifying range is between 160 and 480. For MR, the qualifying range is between 40 and 130 and for the total number of atoms, the qualifying range is between 20 and 70 atoms in a small molecule. Our hit compound K8A met the Ghose qualifying criteria but not molecule 4 with a MW around 143, molecule 1 (2-DG) with a MR less than 40, and Molecule 5 with a MR of around 30 (Table 1).

Egan (pharmacia) filter [22] provides a prediction of drug absorption based on physical processes involved in membrane permeability of a small molecule. The descriptors in the Egan model are polar surface area (PSA) and AlogP98v with exclusion of redundant descriptors such as MW. PSA is a reference point for AlogP98 [25], since the latter descriptor is a ratio of lipophilicity to hydrophilicity which contains no information on the absolute measure of either factor. Importantly, the Egan computational model for human passive intestinal absorption (HIA) of small molecule accounts for active transport and efflux mechanisms and is therefore robust in predicting absorption of drugs. Exclusion of redundant descriptors by this model allowed K8A and all the small molecules to obey the Egan rules (Table 2).

Veber (GSK filter) [23] model characterizes molecules as drug-like if they have 10 or fewer rotatable bonds and a PSA equal to or less than 140 $Å^2$ with 12 or fewer H-bond donors and acceptors. K8A and all the small molecules evaluated met Veber criteria (Tables 1 and 2). Molecules with these properties have a high probability of good oral bioavailability in a rat model. Reduced PSA correlates better with increased permeation rate than lipophilicity does (ClogP). Conversely, increased rotatable bond count

Table 1 Physicochemical properties of the small molecules (MW; molecular weight, MR; molar refractivity, TPSA; total polar surface area).

Molecule	Small molecule	Canonical SMILES	Formula	MW	#Heavy atoms	#Aromatic heavy atoms	Fraction Csp3	#Rotatable bonds	#H-bond acceptors	#H-bond donors	MR	TPSA
Molecule 1	2-deoxy-D-glucose	OCC1OCC(C(C1O)O)O	C6H12O5	164.16	11	0	1	1	5	4	34.57	90.15
Molecule 2	K8	OC1COC(C(C1O)O)Nc1cccc2c1cccc2	C15H17NO4	275.3	20	10	0.33	2	4	4	74.94	81.95
Molecule 3	K8A	CC(=O)OC1C(OCC(C1OC(=O)C)OC(=O)C)Nc1cccc2c1cccc2	C21H23NO7	401.41	29	10	0.38	8	7	1	104.15	100.16
Molecule 4	1-Naphthylamine	Nc1cccc2c1cccc2	C10H9N	143.19	11	10	0	0	0	1	48.35	26.02
Molecule 5	D-Xylose	OC1COC(C(C1O)O)O	C5H10O5	150.13	10	0	1	0	5	4	29.77	90.15
Molecule 6	Acetylated D-Xylose	CC(=O)OC1COC(C(C1OC(=O)C)OC(=O)C)OC(=O)C	C13H18O9	318.28	22	0	0.69	8	9	0	68.72	114.43

Table 2 Drug-likeness evaluation of small molecules using swissADME shows the hit compounds K8A and K8 do not violate any of the drug-likeness criteria.

Molecule	Small Molecule	Lipinski violations	Ghose violations	Veber violations	Egan violations	Muegge violations
Molecule 1	2-deoxy-D-glucose	0	2	0	0	2
Molecule 2	K8	0	0	0	0	0
Molecule 3	K8A	0	0	0	0	0
Molecule 4	1-Naphthylamine	0	1	0	0	2
Molecule 5	D-Xylose	0	3	0	0	2
Molecule 6	Acetylated D-Xylose	0	0	0	0	0

has a negative effect on the permeation rate. Consequently, a threshold permeation rate is a prerequisite of oral bioavailability (F). Of note is the Abbot bioavailability score [26] that predicts the probability of a compound to have F > 10% based on the predominant charge at biological pH in a rat model. Abbot bioavailability model distinguishes compounds that are poorly permeable from those that are permeable in Caco-2 cells.

Muegge (Bayer filter) [24] model is a database-independent pharmacophore point filter that discriminates between drug-like and nondrug-like chemical matter. It is based on the observation that non-drugs are often less functionalized. Four functional motifs are defined to be important in drug-like molecules and include ketone, hydroxyl, sulfonyl and amine groups. Therefore, a minimum count of well-defined pharmacophore points is required to pass the filter. The occurrence of these functional motifs guarantees hydrogen-bonding capabilities that are essential for specific drug interactions with its targets. These functional groups can be combined to what Muegge model [24] refers to as pharmacophore points. The pharmacophore points include amine, amide, alcohol, ketone, sulfone, sulfonamide, carboxylic acid, carbamate, guanidine, amidine, urea, and ester functional groups. These pharmacophore points in small molecules potentially provide key interactions with the target protein. Bioactive K8 and K8A have an amine linker with hydroxyl groups on 2-DG. The hydroxyl groups in K8A are made available by esterases when the small molecule enters cells and likely provides these important hydrogen bonds.

3.2 PAINS, Brenk and Leadlikeness Screening

Complimentary to models that predict the drug-likeness of small molecules are models that exclude those that are likely to show false positives in biological assays. PAINS [27] is a screening computer model that identifies compounds that appear as hits (promiscuous compounds) in many biochemical high throughput screens. Notably, such compounds have been reported to be active in many different assays and are often reported in the literature as potential starting points for further exploration, whereas they may not be active. SwissADME evaluation did not post any PAINS alert of any of the molecules (Table 3).

In another selection model, Brenk [28] considered compounds that are smaller and less hydrophobic and not those defined by “Lipinski’s rule of 5” to widen opportunities for lead optimization. This was after exclusion of compounds with potentially mutagenic, reactive and unfavorable groups such as nitro groups, sulfates, phosphates, 2-halopyridines and thiols. Brenk model restricts the ClogP/ClogD between zero and four, the number of hydrogen-bond donors and acceptors to fewer than 4 and 7, respectively, and the number of heavy atoms to be between 10 and 27 [28]. Additionally, only compounds with limited complexity defined as fewer

Table 3 Medicinal chemistry evaluation of the small molecules.

Molecule	Small molecule	PAINS alerts	Brenk alerts	Leadlikeness violations	Synthetic Accessibility
Molecule 1	2-deoxy-D-glucose	0	0	1	3.58
Molecule 2	K8	0	0	0	3.81
Molecule 3	K8A	0	1	2	4.5
Molecule 4	1-Naphthylamine	0	1	1	1
Molecule 5	D-Xylose	0	0	1	3.8
Molecule 6	Acetylated D-Xylose	0	1	1	4.34

than eight rotatable bonds, fewer than five ring systems, and no ring systems with more than two fused rings are considered medicinal [28]. K8A with 3 ester groups flouted 1 Brenk rule, luckily however, these ester groups are cleaved off *in-vivo* to generate non-ester containing K8 (Table 3). Molecule 4 with an aniline moiety also flouted 1 Brenk rule (Table 3).

Conversely, Teague [29] and others propose that there is a great deal of precedent to suggest that libraries consisting of molecules with MW in the range 100 ± 350 and ClogP in the range 1 ± 3.0 are greatly superior to those considered drug-like compounds and are therefore lead-like [29]. With these stringent lead-like criteria K8A with a MW of 401 and 7 rotors failed two Leadlikeness criteria (Table 3). Of note, bioactive K8 passed all Leadlikeness criteria but all other controls failed (Table 3). Leadlikeness tests are intended to provide leads with high affinity in high-throughput screens that allow for the discovery and exploitation of additional interactions in the lead-optimization phase.

3.3 P-glycoprotein and CYP Enzyme Activity Prediction

SwissADME also enables the estimation for a chemical to be a substrate of p-glycoprotein (P-gp) or inhibitor of the cytochrome p450 isoenzymes (CYP isoenzymes). P-gp is extensively distributed and expressed in the intestinal epithelium where it pumps xenobiotics such as drugs back into the intestinal lumen and in the capillary endothelial cells composing the blood-brain barrier where it pumps them back into the capillaries. CYP isoenzymes are responsible for the biotransformation of drugs [30]. Drug metabolism via CYP isoenzymes is an important determinant of drug interactions that can lead to drug toxicities and reduced pharmacological effect. The models return "Yes" or "No" if the molecule under investigation has higher probability to be substrate or non-substrate of P-gp or inhibitor or non-inhibitor of a given CYP. All the small molecules returned "No" for P-gp substrate and "No" for most CYP isoenzymes. K8A returned "Yes" for CYP3A4 inhibition (Table 4). While many drugs are deactivated by CYP3A4, there are also some drugs which are activated by the enzyme. Now, a biological experiment will be required to determine if K8A is activated or deactivated by CYP3A4.

3.4 HIA and BBB Prediction

Pertinent to P-gp and CYP enzyme kinetics is human gastrointestinal absorption (HIA) and blood-brain barrier penetration (BBB). SwissADME "BOILEDegg" (Fig. 2) allows for evaluation of HIA as a function of the position of the small molecules in the WLOGP-versus-TPSA referential. The white region of the "BOILEDegg" is for high probability of passive absorption by the gastrointestinal tract, and the yellow region (yolk) is for high probability of brain penetration. Yolk and white areas are not mutually exclusive. In addition, the points are colored in blue if predicted as actively effluxed by P-gp (PGP+) and in red if predicted as non-substrate of P-gp (PGP−). K8 (Molecule 2) and K8A (Molecule 3) are predicted as absorbed by gastrointestines (white region) but are not brain penetrant (yolk) (Fig. 2). The anti-cancer agent 2-deoxyD-glucose (Molecule 1) and the control molecule

Table 4 Pharmacokinetic evaluation of the small molecules (GI: gastro-intestinal absorption, BBB: blood brain barrier, CYP: Cytochromes, P-gp: P-glycoprotein).

Molecule		GI absorption	BBB permeant	Pgp substrate	CYP1A2 inhibitor	CYP2C19 inhibitor	CYP2C9 inhibitor	CYP2D6 inhibitor	CYP3A4 inhibitor
Molecule 1	2-deoxy-D-glucose	Low	No	No	No	No	No	No	No
Molecule 2	K8	High	No	No	Yes	No	No	No	No
Molecule 3	K8A	High	No	No	No	No	No	No	Yes
Molecule 4	1-Naphthylamine	High	Yes	No	Yes	No	No	No	No
Molecule 5	D-Xylose	Low	No	No	No	No	No	No	No
Molecule 6	Acetylated D-Xylose	High	No	No	No	No	No	No	No

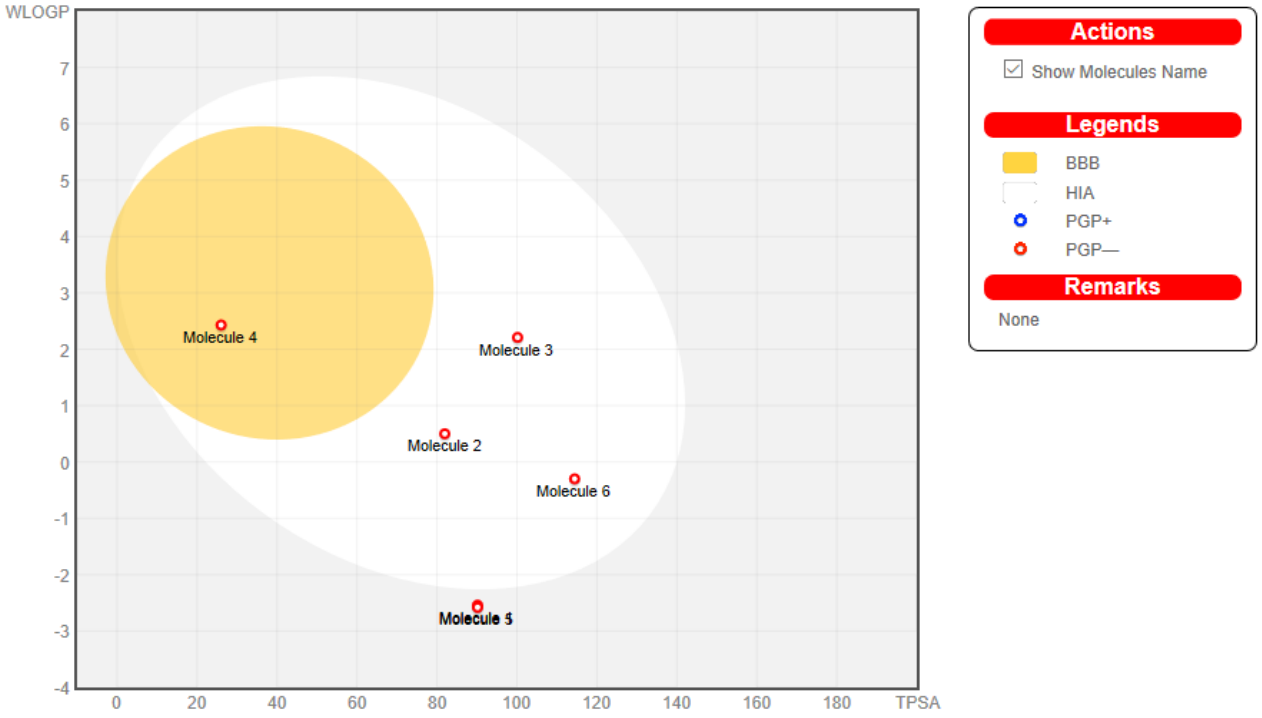


Fig. 2 The BOILED-Egg allows for evaluation of passive gastrointestinal absorption (HIA), brain penetration (BBB) and P-glycoprotein activity in presence of the molecule (P-gp).

D-xylose (Molecule 5) are predicted as not-absorbed by the intestines and the brain. 1-naphthylamine (Molecule 4) was predicted to be brain-penetrant. All the molecules evaluated are PGP negative and are not subject to active efflux (red dot).

HIA and BBB are dependent on water solubility and lipophilicity of the drug. Two topological methods to predict water solubility are included in SwissADME. The first one is an implementation of the ESOL [31] model and the second one is adapted from Ali et al. [32]. SwissADME third predictor for solubility was developed by SILICOS-IT. All predicted values are the decimal logarithm of the molar solubility in water (log *S*). Water solubility [31] of the small molecules ranged from highly soluble (Molecule 1) to very soluble (Molecule 6) using the ESOL [31] and other criteria [32] (Table 5). Conversely, lipophilicity evaluated as Consensus Log P indicated K8A (molecule 3) to be the most lipophilic (Consensus Log P = 2.38) whereas 2-DG was the least lipophilic

Table 5 Water solubility evaluation of the small molecules.

Molecule	Small molecule	ESOL Log S	ESOL Solubility (mg/ml)	ESOL Solubility (mol/l)	ESOL Class	Ali Log S	Ali Solubility (mg/ml)	Ali Solubility (mol/l)	Ali Class	Silicos-IT LogSw	Silicos-IT Solubility (mg/ml)	Silicos-IT Solubility (mol/l)	Silicos-IT class
Molecule 1	2-deoxy-D-glucose	0.83	1.10E+03	6.72E+00	Highly soluble	1.22	2.74E+03	1.67E+01	Highly soluble	1.82	1.08E+04	6.56E+01	Soluble
Molecule 2	K8	-2.47	9.29E-01	3.38E-03	Soluble	-2.4	1.09E+00	3.95E-03	Soluble	-2.6	6.84E-01	2.48E-03	Soluble
Molecule 3	K8A	-3.83	5.99E-02	1.49E-04	Soluble	-4.57	1.08E-02	2.69E-05	Moderately soluble	-4.48	1.33E-02	3.30E-05	Moderately soluble
Molecule 4	1-Naphthylamine	-2.82	2.18E-01	1.52E-03	Soluble	-2.43	5.29E-01	3.69E-03	Soluble	-3.69	2.93E-02	2.05E-04	Soluble
Molecule 5	D-Xylose	1.13	2.03E+03	1.35E+01	Highly soluble	1.69	7.34E+03	4.89E+01	Highly soluble	2.23	2.56E+04	1.71E+02	Soluble
Molecule 6	Acetylated D-Xylose	-1.14	2.30E+01	7.24E-02	Very soluble	-1.72	6.13E+00	1.93E-02	Very soluble	-0.37	1.35E+02	4.24E-01	Soluble

Table 6 Lipophilicity evaluation of the small molecules.

Molecule	Small molecule	iLOGP	XLOGP3	WLOGP	MLOGP	Silicos-IT Log P	Consensus Log P
Molecule 1	2-deoxy-D-glucose	0.56	-2.57	-2.54	-2.35	-1.44	-1.67
Molecule 2	K8	1.47	1.09	0.5	0.43	0.51	0.8
Molecule 3	K8A	3.34	2.81	2.21	1.64	1.89	2.38
Molecule 4	1-Naphthylamine	1.62	2.25	2.43	2.54	2.31	2.23
Molecule 5	D-Xylose	-0.39	-3.02	-2.58	-2.32	-1.7	-2
Molecule 6	Acetylated D-Xylose	2.94	-0.23	-0.3	-0.16	-0.04	0.44

Table 7 K8A Cytotoxicity in lung cancer cells.

Cell line	Cell type	D-xylose (K) (mM)	Acetylated D-xylose (KA) (mM)	K8 (mM)	K8A (mM)	2-deoxy-D-glucose (2-DG) (mM)
H1299	Lung	~5.54	~2.51	0.076±0.19	0.045±0.003	0.79±0.32
A549	Lung	~2.95	~7.96	0.122±0.26	0.029±0.005	3.87±0.58

(Consensus Log P = -1.67). Consensus Log P is the average value of all Log P evaluated with various lipophilicity criteria (Table 6).

3.5 Cytotoxicity of K8A

Cell viability studies revealed that K8A is more potent than 2-DG, in the two cancer cell lines tested. K8A cytotoxicity ranged between 0.03 mM and about 0.05 mM, whereas 2-DG cytotoxicity ranged between 1 mM and 4 mM in the same cell lines. K8 cytotoxicity (IC_{50}; 0.076 ± 0.19 mM) was about 10-fold higher than 2-DG (IC_{50}; 0.79 ± 0.32 mM) in H1299 cells. On acetylation (K8A), cytotoxicity improved about 20-fold higher (IC_{50}; 0.045 ± 0.003 mM) than 2-DG cytotoxicity in H1299 cell line, underscoring importance of increased lipophilicity with acetylation. Cytotoxicity of D-xylose and the acetylated form was very low ranging between 3 and 6 mM.

4. Conclusions

Small molecules that block the altered metabolism in cancer are emerging as potential anti-cancer agents. Carbohydrate derivatives such as 2-deoxy-D-glucose and K8A can be used for cellular energetics of which interruption can lead to cellular stress. Indeed K8A, a derivative of D-xylose was more potent than clinically-tested 2-deoxy-D-glucose. We used the swissADME virtual tools to further evaluate the hit compound K8A and demonstrated that K8A has better "drug-likeness" than 2-DG. A "BOILEDegg evaluation", predicts that K8A has higher gastrointestinal absorption (HIA) than 2-DG and is not effluxed by P-glycoprotein (P-gp). Additionally, K8A is lipophilic but does not penetrate the blood brain barrier

(BBB) and is not a substrate of most CYP enzymes. Of note is the moderate synthetic accessibility of K8A that provides medicinal chemists with opportunities for synthesis of numerous analogues. Importantly, K8A did not show false positive alert enabling us to rule out false targets with confidence of pursuing potential biologically relevant targets. Ultimately, biological evaluation is required to validate the pharmacokinetics and pharmacodynamics of any potential patient drug.

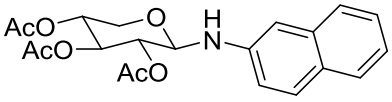
Acknowledgements

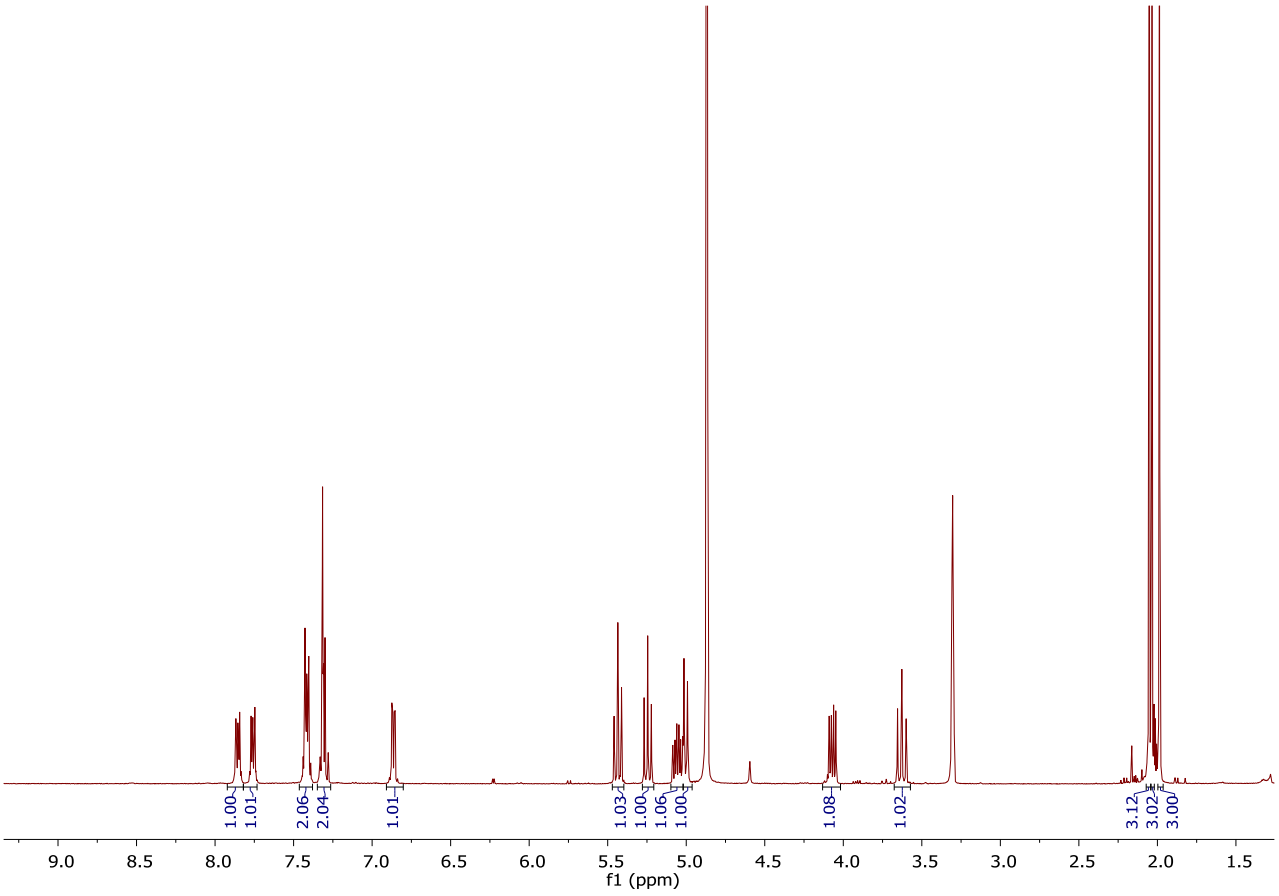
Synthesis, NMR characterization and cytotoxicity evaluation of K8A was done at the Department of Chemistry, Wayne State University with the help of Prof. Young-Hoon Ahn.

Supporting Information: NMR Data

^{1}H NMR of K8A

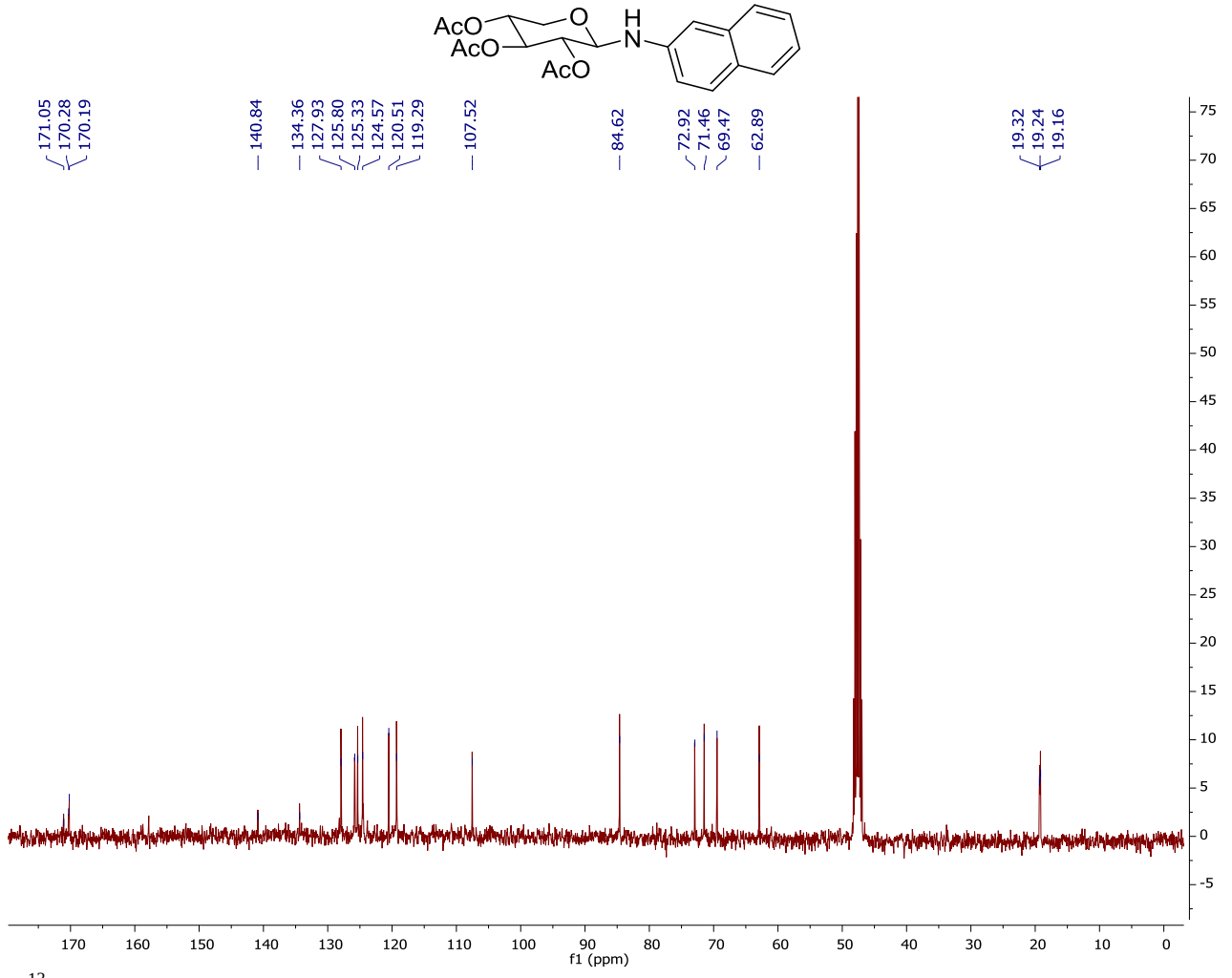
^{1}H NMR (400 MHz, CD_3OD) δ 7.90-7.82 (m, 1H), 7.80-7.70 (m, 1H), 7.46-7.38 (m, 2H), 7.35-7.26 (m, 2H), 6.89-6.83 (m, 1H), 5.44 (t, *J* = 9.5 Hz, 1H), 5.25 (t, *J* = 9.2 Hz, 1H), 5.05 (ddd, *J* = 10.6, 9.5, 5.6 Hz, 1H), 5.00 (d, *J* = 8.9 Hz, 1H), 4.07 (dd, *J* = 11.3, 5.6 Hz, 1H), 3.63 (t, *J* = 10.9 Hz, 1H), 2.05 (s, 3H), 2.03 (s, 3H), 1.99 (s, 3H).





^{13}C NMR of K8A (101 MHz, CD_3OD)

^{13}C NMR (101 MHz, CD_3OD) δ 171.05, 170.28, 170.19, 140.84, 134.36, 127.93, 125.80, 125.33, 124.57, 120.51, 119.29, 107.52, 84.62, 72.92, 71.46, 69.47, 62.89, 19.32, 19.24, 19.16. $[\alpha]D^{RT}$-45.9 (*c*, 0.5, methanol). HRMS calculated mass 424.1372, found mass 424.1372.



^{13}C NMR of K8A

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