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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Screening of 1,2-furanonaphthoquinones 1,2,3-1*H*-triazoles for glycosidases inhibitory activity and free radical scavenging potential: an insight in anticancer activity

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Abstract

In many cancer cells, glycoproteins show abnormal glycosylation patterns which have been associated with tumor initiation, progression, and metastasis. Thus, the glycosidases involved in glycoprotein maturation represent good targets for the development of new anticancer agents. In a previous report, we synthetized and evaluated the cytotoxic effect of a novel series of nor-β-lapachone tethered to 1*H*-1,2,3-triazoles (1,2-FNQT, **9a**–**r**) against a panel of leukemia cell lines. Many 1,2-FNQT were active at low micromolar concentration and some were selective for cancer cells rather than normal ones. These results prompted us to investigate the mechanism of action that underlies their cytotoxic effect. Here, we tested if this effect could be attributed to the inhibition of cancer-related glycosidase activities, namely α-glucosidase and α-mannosidase. To evaluate enzyme selectivity, the same compounds were screened on other glycosidases of physiological relevance. In addition, we also studied the free radical scavenging activity of 1,2-FNQT, since redox metabolism plays a part in cancer development. Overall, the compounds were weak glycosidase inhibitors at 500 µM. The most active was **9i** (IC_{50} = 413.7 µM) for α-glucosidase activity. In contrast, many of the compounds decreased more than 40% the content of DPPH, a free radical reagent, at 500 µM. This reduction was positively correlated with 1,2-FNQT cytotoxic potency, but only in KG1 cells (acute myelogenous leukemia). In conclusion, the cytotoxic effect of 1,2-FNQT on leukemic cells does not seem to be related to glycosidases inhibition, but may be, at least in part, due to their free radical scavenging activity.

Keywords 1,2,3-triazoles · Nor-β-lapachone · Glycosidase inhibitors · Free radical scavenger · Leukemia · Cancer drug discovery

Introduction

In the last 20 years there has been a growing interest in understanding the relationship between glycosylation metabolism and cancer (Drake 2015). In part, this is due to different protein glycosylation patterns (e.g.,: neo, over, and underexpression of glycans) found in many types of cancer and their possible implication in neoplasic progression. Aberrant glycosylation has been associated with alterations in the expression of cell surface carbohydrates and protein folding, stability and degradation by cellular quality control systems. These effects may contribute to uncontrolled growth, invasion, immune evasion, and metastasis of cancer cells (Gerber-Lemaire and Juillerat-Jeanneret 2006; Stowell et al. 2015).

The modulation of key enzymes of glycosylation/deglycosylation metabolic pathways represent an interesting

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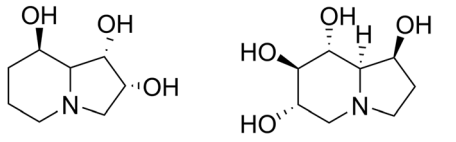

Swainsonine (**1**) Castanospermine (**2**)

Fig. 1 Chemical structures of glycosidase inhibitors swainsonine (**1**) and castanospermine (**2**)

approach for the development of new antitumoral drugs. Glycosidases, such as α-mannosidase and α-glucosidase, are suitable molecular targets since they participate in different steps of glycoprotein synthesis and maturation. In fact, many glycosidase inhibitors have shown antitumoral effect on in vitro and in vivo models and two of them (swainsonine (**1**) and castanospermine (**2**), Fig. 1) have already reached clinical trials (Brás et al. 2014; Vasconcelos-dos-Santos et al. 2015). Most of glycosidase inhibitors identified so far mimic the structure of natural substrates (i.e., mono and oligosaccharides), and thus have physicochemical properties that make them well-accepted by the organisms (Brás et al. 2014).

A major advance in medicinal chemistry occurred in 2002 with the development of Cu(I)-catalyzed azide-alkyne cycloaddition (CuAAC), a common type of "click" reaction (Rostovtsev et al. 2002). This reaction selectively produces 1*H*-1,2,3-triazoles at high yields with fewer by-products, according to the principles of "Click chemistry" (Kolb et al. 2001; de O Freitas et al. 2011). The triazole nucleus is a privileged scaffold where virtually any kind of molecule (natural or synthetic) can combine to form new compounds. There are many examples of biologically active triazoles with pharmacological potential to treat diabetes, cancer, viral and parasite infections, among other diseases (de O Freitas et al. 2011). 1*H*-1,2,3-triazoles, like the antibiotic tazobactam and the anticancer drug candidate carboxyamidotriazole, have already reached the late stages of clinical trials and probably will be become commercially available in the near future (Ferreira et al. 2013).

The "Click dawn" also had a significant impact in the development of mono and multivalent glycosidase inhibitors. Theoretically, the triazole nucleus itself could act as an inhibitor since its structure may mimic the charge and planarity of the transition states formed during glycosidase catalysis (Ferreira et al. 2010). Several 1*H*-1,2,3-triazole derivatives, especially those with carbohydrates moieties, showed moderate to high inhibition potencies against glycosidases activities. In previous reports, our group described the synthesis of new series of glycosidic and non-glycosidic 1*H*-1,2,3-triazoles and their effect on α-amylase and α-glucosidase activities in vitro (Ferreira et al. 2010; Senger et al. 2012; Gonzaga et al. 2014). Some compounds showed low IC_{50} values (<10 μM) that were in the same order of magnitude of acarbose, a commercial drug used in clinics as an antihyperglycemic agent. In addition, they also decreased post-prandial glycemia on an in vivo model suggesting that these triazoles, in the same way of acarbose, have a therapeutic potential to treat diabetes. Since then we have incorporated biologically active molecules in the triazole nucleus to evaluate other relevant pharmacological properties.

Lapachol (4-hydroxy-3-(3-methylbut-2-enyl)naphthalene-1,2-dione)) is a natural naphthoquinone found in large amounts in plant species of Bignoniaceae family (e.g., Tabebuia sp., popular known as "pau d'arco") of the Brazilian Cerrado. It also can be synthetized in good yield from lawsone (2-hydroxi-1,4-naphthoquinone) (Ferreira et al. 2011). Lapachol (**3**) is the precursor of α-lapachones and β-lapachones (**4** and **5**), both obtained from natural sources (Fig. 2). The semi-synthetic nor-β-lapachone (**6**) can be easily obtained from lawsone (Hussain et al. 2007). Many lapachol/lapachone derivatives show biological activities as antiparasitic (Salas et al. 2008), antiviral (Li et al. 1993), antimicrobial (Souza et al. 2013), anti-inflammatory (Lee et al. 2015), and anticancer (Epifano et al. 2014) agents. A recent review (Hussain and Green 2017) listed the lapachone derivatives patented in the period of 1997–2016. Among them several compounds showed antitumoral activity ($IC_{50} < 10$ μM) against different types of solid and hematological cancers models. According to clinicaltrials.gov, currently there is one β-lapachone analog (ARQ 761, ArQule, Woburn, MA, USA) under clinical trial for pancreatic cancer and other solid tumors.

In a previous paper (Cardoso et al. 2014), we described the synthesis of a new series of nor-β-lapachone tethered to 1*H*-1,2,3-triazoles (1,2-FNQT) (Fig. 3, **9a**–**r**) which showed high cytotoxic activity (IC_{50} values ranging from 0.05 to 83.44 μM) against acute leukemia cell lines from lymphoid (CCRF-CEM and MOLT-4) and myeloid (K562 and KG1) origins. Some compounds were even more potent than cisplatin and doxorubicin, commercial drugs used in clinics to treat different types of cancer. Although the mechanism of action of 1,2-FNQT is yet to be clarified, several studies suggest that naphthoquinones may exert their toxicity by acting as topoisomerase II inhibitors, arylation of the thiol groups of proteins, intercalation, induction of breaks in the DNA chain, and bioreductive alkylation via the formation of quinone methide or undergoing redox cycle to generate reactive oxygen species (Wellington 2015; de Paiva et al. 2015). In addition, the presence of 1,2,3-triazole nucleus could also confer another cytotoxic mechanism to 1,2-FNQT as glycosidase inhibitors. In order to test this hypothesis, we investigated the effect of the 1,2-FNQT compounds against α-mannosidase and α-glucosidase activities, both involved in glycoprotein biosynthesis.

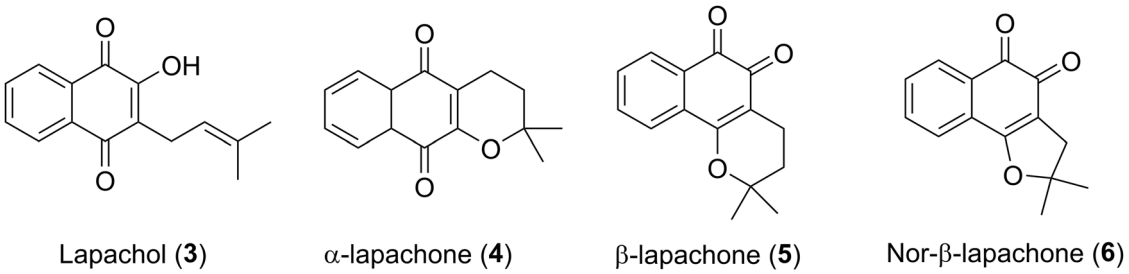

Fig. 2 Chemical structures of natural and semi-synthetic naphthoquinones

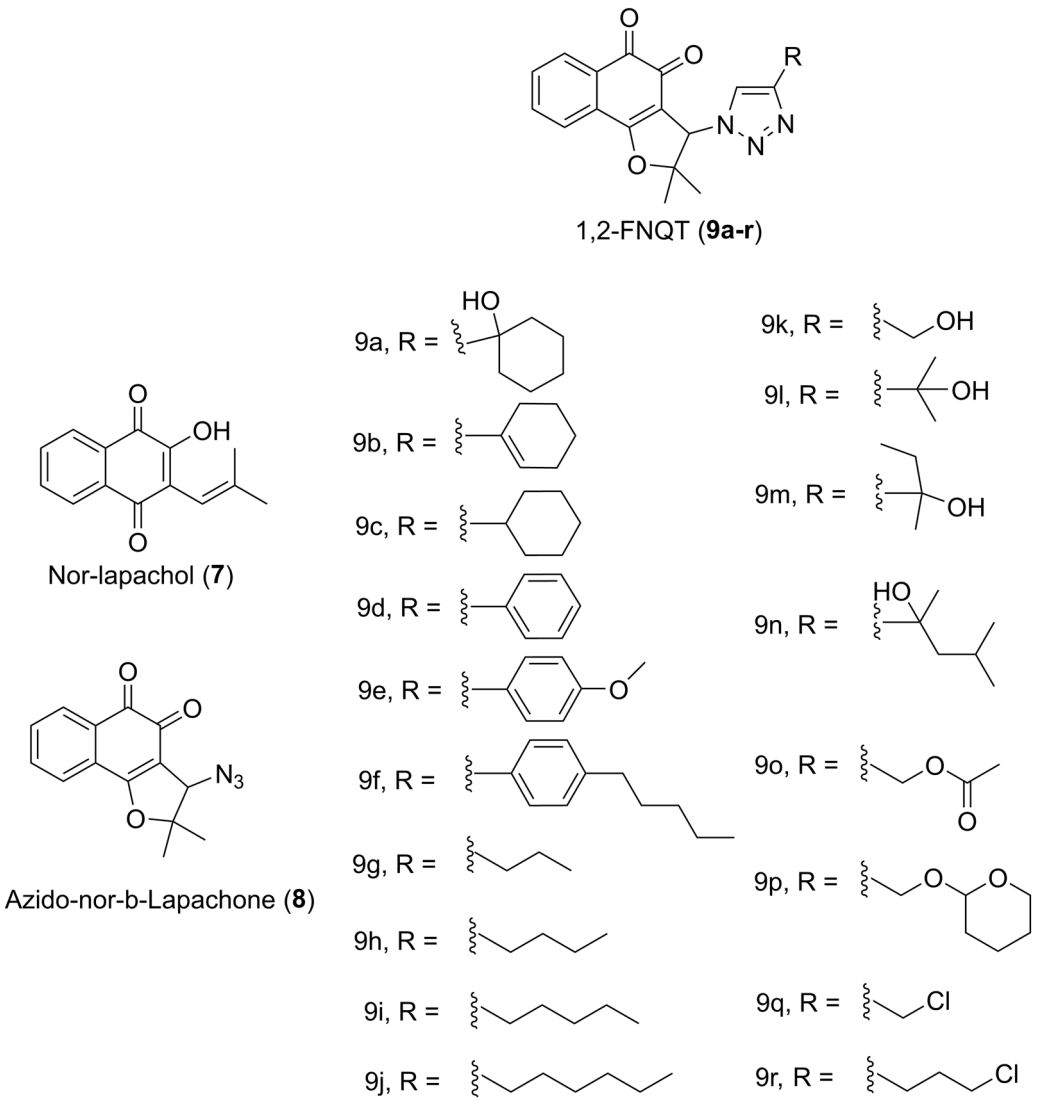

Fig. 3 Chemical structures of nor-lapachol (**7**), azido-nor-β-lapachone (**8**) and 1,2-FNQTs (**9a**–**r**)

Enzyme selectivity was evaluated by testing the compounds against physiological relevant glycosidase activities: α-amylase, key activity for diet carbohydrates digestion, and α-galactosidase, β-glucosidase and β-galactosidase, responsible for glycoprotein and glycolipid metabolism. Since quinone-based molecules may undergo redox cycling we also tested the free radical scavenging activity of 1,2-FNQT.

Materials and methods

Chemistry

Nor-β-lapachone (**6**), nor-lapachol (**7**), azido-nor-β-lapachone (**8**), and 1,2-furanonaphthoquinone triazoles (1,2-FNQTs, **9a**–**r**), were synthetized according to protocol described previously (Cardoso et al. 2014) (Fig. 3). All compounds were solubilized in 100% DMSO and diluted in milli Q water (Millipore Corporation) prior to the experiments.

In vitro enzymatic assays

All assays were performed in 96-well microplates using enzymes, substrates and inhibitors (acarbose, miglitol, 1-deoxynojirimycin, N-(n-nonyl)deoxynojirimycin, N-butyldeoxynojirimycin and castanospermine) from Sigma-Aldrich. The general protocol consisted in pre-incubating buffer, substrate and test compounds (1,2-FNQTs) or commercial inhibitors (positive control) at 500 µM for 5 min. Reaction was initiated after adding enzyme to reaction media (200 µL final volume). The same procedure was performed in the presence of DMSO instead of compound (negative control). A detailed description of reaction conditions of each enzymatic assay is shown in Table 1.

The rate of hydrolysis reaction of p-nitrophenol glycoderivatives (PNP-X and CNPG3) was determined by kinetic method at 405 nm on a FlexStation 3 Benchtop Multi-Mode Microplate Reader (Molecular Devices, Sunnyvale, CA). The results were expressed as mili absorbance units per minute ($mAU.min^{-1}$). Percental inhibition was calculated using the mean activity of negative control. All the experiments were repeated at least twice, each one in triplicate.

In order to calculate the IC_{50} value (compound concentration that inhibits 50% enzymatic activity), the assays were performed as described before, but in the presence of variable concentrations of 1,2-FNQT or commercial inhibitor. The IC_{50} value was determined by fitting residual activity data and inhibitor concentration to the four-parameter logistic equation: Residual Activity = min + (max-min)/(1 + ([I]/IC_{50})^(-Hillslope)).

Free radical scavenging activity (DPPH assay)

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenger assay was performed in 96-well microplates according to Rao and coworkers (Ranga Rao et al. 2009), with minor modifications. The reaction medium (200 µL) contained 500 µM of 1,2-FNQT, trolox (positive control, Sigma-Aldrich) or DMSO (negative control), and 100 µM ethanolic solution of DPPH (Sigma-Aldrich) in a 50 mM

Table 1 Reaction conditions for enzymatic assays

Enzymatic activity	Buffer	Substrate	Enzyme	Temperature
α-glucosidase	50 mM phosphate buffer, 100 mM NaCl, pH 7.0	p-nitrophenyl-α-D-glucopyronoside (PNP-α-Glu,1 mM)	α-glucosidase from *Saccharomyces cerevisiae* (0.065 U)	37 °C
α-mannosidase	50 mM HEPES buffer, pH 7.0	p-nitrophenyl-α-D-mannoside (PNP-Man, 5 mM)	α-mannosidase from *Canavalia ensiformis* (0.15 U)	25 °C
α-amylase	50 mM HEPES buffer, 5 mM $CaCl_2$,100 mM NaCl, pH 7.0	2-chloro-p-nitrophenyl-α-D-maltotrioside (CNPG3, 1 mM)	α-amylase from porcine pancreas (1.5–2.0 U)	37 °C
α-galactosidase	50 mM phosphate buffer, pH 6.5	p-nitrophenyl-α-D-galactopyranoside (PNP-α-Gal, 2 mM)	α-galactosidase from green coffee beans (0.01 U)	25 °C
β-glucosidase	50 mM acetate buffer, pH 5.0	p-nitrophenyl-β-D-glucopyranoside (PNP-β-Glu, 5 mM)	β-glucosidase from almonds (0.05 U)	37 °C
β-galactosidase	50 mM HEPES buffer pH 7.0	p-nitrophenyl-β-D-galactopyranoside (PNP-β-Gal, 4 mM)	β-galactosidase from *Aspergillus oryzae* (0.1 U)	30 °C

Note: Enzymatic unit (U) = 1 µmol of substrate converted into product per min

Tris-HCl buffer pH 7.4. The mixture was kept in the dark for 30 min. After incubation, DPPH was quantified by absorbance in a microplate reader at 517 nm. A second reading was performed in the absence of DPPH (blank) to subtract the intrinsic absorbance of the 1,2-FNQT compounds. The results were expressed as absorbance units. The percental of reduced DPPH was calculated using the mean absorbance of the negative control. All the experiments were repeated at least twice, each one in triplicate.

Statistical analysis

Graphs and statistical analysis were performed in Sigmaplot 12.0 software from Systat software Inc, USA and GraphPad Prism version 7.00 for Windows, GraphPad Software, La Jolla California USA, www.graphpad.com. Unpaired *t*-test and one-way ANOVA (followed by Dunnett´s multiple comparisons test) were used to compare mean enzymatic activities. $P < 0.05$ was considered statistically significant.

Results and discussion

Cancer related glycosidase activities

The 1,2-FNQT were tested at 500 µM against a panel of commercial glycosidases. Table 2 and 3 show their effects on α-glucosidase and α-mannosidase activities, respectively. These activities are responsible for sequential removal (trimming) of monosaccharides from newly synthesized glycoproteins in the endoplasmatic reticulum and Golgi apparatus during the second phase of glycoprotein maturation. In cancer cells, altered glycosylation may significantly change glycoprotein structure impairing its recognition and elimination by control mechanisms. Therefore, α-mannosidase and α-glucosidase activities have been used as potential targets for antitumoral agents (Gerber-Lemaire and Juillerat-Jeanneret 2006).

Most compounds inhibited less than 50% α-glucosidase activity (Table 2), except **9c** and **9i** which inhibition were statistically greater (**9i**, $P = 0.0067$) than or similar (**9c**, $P = 0.7932$) to acarbose, a well-known inhibitor of α-glucosidases. **9i** was the most active (66.2% inhibition) and therefore, it was selected for potency evaluation ($IC_{50} = 413.7 \pm 71.7$ µM). This IC_{50} value was in the same order of acarbose ($IC_{50} = 514.6 \pm 25.43$ µM) indicating equivalent potencies.

In order to perform a structure-activity relationship study we compared the inhibition profile of **9i** to structurally related compounds in 1,2-FNQT series. Compound **9i** has a n-pentyl chain attached to triazole core and differs from **9h** and **9j** solely by one methyl group. Despite their similarity, the inhibitory activity of **9i** was statistically higher than **9j** ($P = 0.0002$) and **9h** ($P < 0.0001$). This result may be an example of the so-called activity cliff, a medicinal chemistry term for a pair of compounds that share a high degree of structural similarity, but largely different activities (Senger et al. 2016). The elucidation of the binding mode of **9i** to α-glucosidase may help to understand this phenomenon and perhaps guide the development of more potent compounds.

Table 2 Screening of α-glucosidase activity inhibition by 1,2-FNQT at 500 µM

Compounds	$V_o \pm SD$ (mAU/min)	% inhibition
Negative control	375.0 ± 14.9	–
9i	126.6 ± 12.0	66.2
9c	174.4 ± 10.8	53.5
acarbose	177.0 ± 11.9	52.8
9b	212.0 ± 32.1	43.5
8	225.7 ± 8.5	39.8
9d	248.6 ± 25.7	33.7
9j	259.8 ± 13.0	30.7
9q	264.2 ± 12.3	29.6
6	285.8 ± 5.1	23.8
7	289.6 ± 7.3	22.8
9g	318.8 ± 17.6	15.0
9a	320.9 ± 15.6	14.4
9h	326.2 ± 14.5	13.0
9o	337.7 ± 10.9	10.0
9e	343.3 ± 19.6	8.5
9n	346.3 ± 15.4	7.7
9f	346.6 ± 8.6	7.6
9r	357.2 ± 16.2	4.8
9k	386.8 ± 2.2	−3.1
9p	389.4 ± 2.5	−3.8
9m	393.7 ± 7.7	−5.0
9l	402.0 ± 5.2	−7.2

All 1,2-FNQT compounds inhibited less than 50% α-mannosidase activity (Table 3). **9r** was the most potent among them (42.1% inhibition). This compound has a chlorine atom in its structure which may participate in electrostatic interactions with positive charged groups of the enzyme.

Low α-mannosidase inhibitory activity was also reported by Poláková and coworkers (Poláková et al. 2015). They synthetized a series of glycoconjugated 1*H*-1,2,3-triazoles and tested them against recombinant and commercial enzymes, homologs of human α-mannosidases. One of them was jack bean α-mannosidase, the same enzyme used in our work, which share structural and functional similarities with human α–mannosidase II, a well-studied target for cancer treatment (van den Elsen et al. 2001). The series of triazoles tested were weak inhibitors of this enzyme

Table 3 Screening of α-mannosidase activity inhibition by 1,2-FNQT at 500 μM

Compounds	$V_o \pm SD$ (mAU/min)	% inhibition
Negative control	4.9 ± 1.1	–
1-deoxynojirimycin	1.2 ± 0.4	66.0
N-(n-nonyl)deoxynojirimycin	1.9 ± 0.1	48.5
9r	2.2 ± 0.1	42.1
6	2.6 ± 0.8	30.7
9h	4.0 ± 0.2	30.1
acarbose	2.6 ± 0.8	27.9
9n	4.2 ± 0.8	27.9
N-butyldeoxynojirimycin	2.8 ± 0.9	23.2
9e	5.1 ± 0.0	11.5
9c	5.1 ± 0.4	11.5
8	3.4 ± 0.0	9.5
9a	5.2 ± 0.4	9.2
9f	5.3 ± 0.4	6.9
9l	5.4 ± 0.2	6.1
9k	5.4 ± 0.3	5.7
9i	5.5 ± 0.3	5.2
9q	3.5 ± 0.1	4.9
9p	5.4 ± 0.3	4.8
7	3.5 ± 0.1	3.6
miglitol	3.6 ± 0.4	1.6
9g	3.7 ± 0.3	−0.4
9b	5.8 ± 0.0	−1.4
9j	3.8 ± 0.2	−1.9
9d	5.9 ± 0.2	−3.1
castanospermine	3.9 ± 0.6	−7.5
9o	4.2 ± 0.2	−12.4
9m	6.6 ± 0.5	−16.2

($IC_{50} = 0.4$–1.1 mM), including a compound which had a phenyl group attached to N4 position of triazole nucleus, the same substituent found in **9d**.

Physiologically relevant glycosidase activities

Glycosidases are widely spread in the human organism and participate in a myriad of physiologically relevant reactions. Therefore, one of the major concerns in developing new glycosidase inhibitors is selectivity. In fact, low glycosidase activity is associated to digestive and lysosomal storage disorders (LSD). We addressed this issue by testing 1,2-FNQT against alpha and beta glycosidases activities which are related to carbohydrate digestion and glycoproteins/glycolipids metabolism. Screening results are shown in Tables 4–7 and will be discussed in more details in the following topics.

Table 4 Screening of α-amylase activity inhibition by 1,2-FNQT at 500 μM

Compounds	$V_o \pm SD$ (mAU/min)	% inhibition
Negative control	842.2 ± 50.2	–
acarbose	3.8 ± 0.4	99.6
8	623.6 ± 54.7	26.0
6	628.9 ± 85.5	25.3
9d	637.7 ± 31.6	24.3
9b	653.1 ± 0.32	22.5
9c	657.9 ± 49.5	21.9
9n	660.7 ± 43.6	21.5
9o	684.2 ± 71.9	18.8
9q	693.8 ± 68.1	17.6
9j	704.7 ± 19.0	16.3
9h	715.3 ± 37.1	15.1
9a	717.8 ± 33.3	14.8
9r	734.7 ± 64.6	12.8
9k	735.3 ± 90.3	12.7
7	742.3 ± 91.2	11.9
9m	765.5 ± 56.2	9.1
9l	771.3 ± 58.5	8.4
9p	787.7 ± 43.8	6.5
9e	801.5 ± 28.2	4.8
9g	804.9 ± 39.6	4.4
9f	820.8 ± 25.3	2.5
9i	821.3 ± 45.5	2.5

Alpha-amylase activity

Salivary and pancreatic α-amylase catalyze a key step in digestion of dietary polysaccharide, such as starch. They are responsible to initiate a cascade of events that culminates with the release of monosaccharides in the intestinal lumen and their absorption by mucosa. Both enzymes are pharmacological targets for oral anti-hyperglycemic drugs (e.g.: acarbose), since their inhibition avoid post-prandial hyperglycemic peak in diabetic patients. According to Table 4 no 1,2-FNQT compound inhibited more than 25% pancreatic α-amylase activity. These inhibitory responses are negligible when compared to acarbose ($IC_{50} = 4.18 \pm 0.4$ μM) suggesting that 1,2-FNQT series has very little effect on α-amylase.

β-glucosidase activity

Table 5 shows the effect of 1,2-FNQT and commercial inhibitors on β-glucosidase activity. Human β-glucosidase activity is responsible for the hydrolysis of β-D-glycosides from endogenous (e.g.: glycolipids) and exogenous (e.g.: flavonoids) sources. Low lysosomal activity is associated to Gaucher Disease, a LSD. Compound **9q** showed the highest

Table 5 Screening of β-glucosidase activity inhibition by 1,2-FNQT at 500 μM

Compounds	$V_o \pm SD$ (mAU/min)	% inhibition
Negative control	220.6 ± 26.8	–
miglitol	1.2 ± 0.1	99.4
castanospermine	9.4 ± 1.0	95.6
N-(n-nonyl)deoxynojirimycin	11.9 ± 1.3	94.4
1-deoxynojirimycin	20.5 ± 2.8	90.5
N-butyldeoxynojirimycin	106.9 ± 10.5	50.2
8	143.9 ± 47.1	38.2
9q	168.0 ± 14.5	27.9
9c	165.3 ± 5.7	21.3
9h	170.5 ± 3.2	18.9
9n	172.2 ± 15.3	18.0
9a	176.6 ± 11.7	16.0
9o	195.8 ± 18.8	15.9
9r	196.4 ± 5.4	15.7
9l	165.6 ± 11.8	15.5
7	181.9 ± 13.0	15.3
9i	180.0 ± 3.7	14.3
9k	169.2 ± 3.3	13.7
9m	173.5 ± 6.1	11.5
9e	186.6 ± 11.3	11.2
9d	175.6 ± 8.7	10.4
9p	176.9 ± 8.3	9.7
9g	211.9 ± 12.9	9.1
9j	215.3 ± 2.4	7.6
9b	181.3 ± 2.9	7.5
6	216.7 ± 7.3	7.0
9f	198.1 ± 2.1	−1.0
acarbose	239.6 ± 8.1	−11.6

Table 6 Screening of α-galactosidase activity inhibition by 1,2-FNQT at 500 μM

Compounds	$V_o \pm SD$ (mAU/min)	% inhibition
Negative control	15.8 ± 1.2	–
1-deoxynojirimycin	6.6 ± 0.5	56.3
9h	11.8 ± 0.8	24.4
8	12.8 ± 0.5	18.1
9q	13.5 ± 0.7	13.7
9r	13.6 ± 2.8	12.8
9k	14.6 ± 1.1	10.8
6	14.0 ± 0.3	10.3
9o	14.2 ± 1.3	9.2
N-butyldeoxynojirimycin	14.0 ± 1.9	8.3
9f	15.4 ± 1.6	6.1
9c	14.7 ± 1.7	6.1
castanospermine	14.3 ± 2.2	5.9
9i	14.9 ± 2.6	5.0
9e	14.9 ± 2.0	4.5
9a	15.1 ± 2.6	3.6
9g	15.3 ± 1.3	2.5
miglitol	14.9 ± 3.2	2.4
9l	16.2 ± 2.0	1.1
N-(n-nonyl)deoxynojirimycin	15.2 ± 2.4	0.4
9d	16.3 ± 1.5	0.1
9m	16.6 ± 1.5	−1.1
9p	16.6 ± 1.3	−1.4
7	15.5 ± 2.4	−1.8
9n	16 ± 1.7	−2.6
9b	16.8 ± 1.0	−2.9
9j	16.6 ± 1.4	−6.4
acarbose	17.7 ± 2.5	−16.0

inhibitory activity among the triazoles (27.9%). This compound has a chlorine atom on R1 moiety which may be involved in electrostatic interactions with positively charged groups of the enzyme. Other studies with glycoconjugated 1*H*-1,2,3-triazoles also reported low inhibitory activity against β-glucosidase (Rossi and Basu 2005; Périon et al. 2005; Dedola et al. 2010) suggesting that 1*H*-1,2,3-trizoles may not be good inhibitors of this enzyme. Interesting to note is the difference, though not statistically significant ($P = 0.0572$), in inhibitory activity between **8** (38.2%) and **6** (7.0%), which may indicate an important role of azido nitrogen to β-glucosidase inhibition.

α-galactosidase activity

In Table 6 are listed the activities of α-galactosidase in the presence of 1,2-FNQT series and commercial inhibitors. Human acid α-galactosidase A (α-Gal) catalyzes the hydrolysis of α-galactosyl moieties from glycolipids and glycoproteins in lysosomes. Low α-Gal activity leads to the deleterious symptoms observed in Fabry´s Disease (LSD) patients (Asano et al. 2000). All 1,2-FNQTs showed low inhibitory activity (<30%) against α-Gal activity.

β-galactosidase activity

In humans, most of β-galactosidase activity is associated to acid β-galactosidase (β-Gal), a lysosomal enzyme responsible for the hydrolysis of terminal β-galactose residues from a variety of substrates, such as ganglioside G_{M1} and keratin sulfate. B-Gal deficiency underlies the metabolic disorders found in GM1-gangliosidosis and Morquio B syndrome, both LSD diseases (Parenti et al. 2015). Table 7 shows the activities of β-galactosidase in the presence of 1,2-FNQT triazoles and commercial inhibitors. None of the compounds tested inhibited more than 20% the activity of

Table 7 Screening of β-galactosidase activity inhibition by 1,2-FNQT at 500 μM

Compounds	$V_o \pm SD$ (mAU/min)	% inhibition
Negative control	313.1 ± 11.9	–
9r	250.9 ± 15.5	18.9
9l	261.6 ± 7.7	15.3
9h	259.5 ± 15.0	15.3
miglitol	278.1 ± 13.8	14.1
9q	267.5 ± 15.9	13.6
9d	267.0 ± 10.5	13.5
9i	265.0 ± 6.3	13.5
9p	267.5 ± 18.0	13.4
9a	265.7 ± 10.3	13.2
1-deoxynojirimycin	283.3 ± 8.6	12.5
N-(n-nonyl)deoxynojirimycin	284.7 ± 9.5	12.1
9k	271.6 ± 9.6	12.0
9m	272.2 ± 16.4	11.8
6	272.9 ± 10.5	11.8
9b	274.4 ± 7.8	11.1
9o	275.1 ± 10.1	11.1
9f	275.5 ± 25.4	10.8
9c	274.4 ± 4.3	10.4
8	279.0 ± 9.9	9.9
castanospermine	292.8 ± 16.7	9.6
N-butyldeoxynojirimycin	292.6 ± 17.7	9.6
9e	280.0 ± 8.8	8.6
9n	286.2 ± 8.3	6.6
9j	290.5 ± 10.3	6.1
9g	295.1 ± 8.5	4.7
7	312.5 ± 8.9	3.5
acarbose	320.6 ± 9.5	1.0

β-galactosidase indicating that 1,2-FNQT are weak inhibitors of this enzyme.

Free radical scavenging activity

Reactive oxygen species (ROS) play a dual role in cancer. Increased levels of ROS have been detected in several stages of tumorigenesis, from transformation to metastasis, in different cancer models. ROS promote genetic instability, signaling disruption and other events that ensure cancer cell survival and proliferation (Galadari et al. 2017). In turn, toxic levels of ROS may overcome antioxidant defense and induce apoptosis due to oxidative stress. Thus, both antioxidant and pro-oxidant therapies have been explored in the treatment of neoplastic diseases (Tong et al. 2015).

Upregulation of ROS is a common feature in lymphocytic and myeloid leukemias. Some studies suggest that this increase is mainly due to the constitutive activation of NADPH oxidase system (NOXs), reduced levels of GSH and depletion of antioxidant proteins. This mild oxidative stress has been associated to leukemogenesis, resistance to chemotherapeutic drugs, relapse of leukemia and poor clinical outcome. In part, many anti-leukemic drugs, such as doxorubicin, vincristine and cytarabine, exert they cytotoxic activity by increasing the amount of ROS in cancer cells (Chen et al. 2017).

Table 8 Free radical scavenging activity of 1,2-FNQT at 500 μM

Compounds	Absorbance ± SD	% reduced DPPH
Negative control	0.599 ± 0.021	–
trolox	0.066 ± 0.002	89.0
9n	0.100 ± 0.000	83.3
9g	0.185 ± 0.006	69.2
9l	0.217 ± 0.015	63.9
9k	0.217 ± 0.006	63.8
9m	0.252 ± 0.025	58.0
9j	0.266 ± 0.002	55.7
9o	0.266 ± 0.012	55.7
9b	0.266 ± 0.011	55.6
9p	0.282 ± 0.026	52.9
9i	0.303 ± 0.008	49.4
9d	0.311 ± 0.024	48.0
9h	0.317 ± 0.001	47.1
9c	0.324 ± 0.001	45.9
9f	0.352 ± 0.028	41.3
9a	0.356 ± 0.073	40.7
8	0.378 ± 0.001	36.9
7	0.391 ± 0.047	34.8
9r	0.458 ± 0.010	23.5
9q	0.514 ± 0.007	14.3
6	0.550 ± 0.016	8.3
9e	0.578 ± 0.074	3.5

The presence of a quinone, a redox active group, in the core of 1,2-FNQT chemical structure instigated us to investigate whether this series of compounds could behave as antioxidant or pro-oxidant agents. To achieve this goal, we used a colorimetric assay based on DPPH radical reduction. This method allows a simple and fast detection of free radical scavengers or hydrogen donors in a wide variety of samples (Kedare and Singh 2011). In Table 8 are listed the absorbance values of DPPH radical pre-incubated with 500 μM of 1,2-FNQT and antioxidant trolox. Most triazoles were capable of reducing more than 40% of DPPH radical. These values were significantly higher than that observed with **6** alone ($P = 0.0001$) indicating that triazole nucleus and R ligands contribute significantly to the scavenger activity. **9n** showed the highest free radical scavenging activity among the triazole compounds (83.3%). This effect

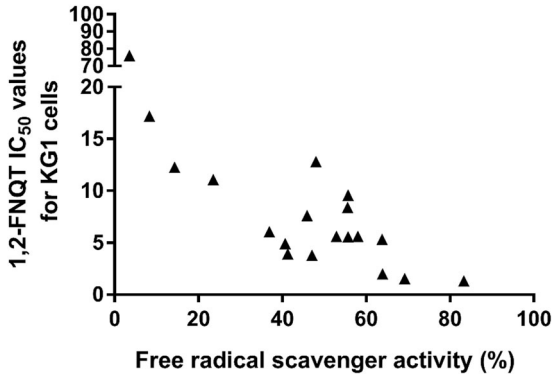

Fig. 4 Scatter plot of DPPH free radical scavenging activity × IC_{50} for KG1 leukemic cell line of 1,2-FNQT series

was statistically similar to that observed with trolox ($P = 0.49$), a hydrophilic analog of vitamin E, suggesting an antioxidant role for triazole compounds.

To investigate if the anti-leukemic activity of 1,2-FNQT could be due to their redox nature, we compared their IC_{50} values for each cancer cell line to their DPPH scavenging activity. Among the four different leukemic cell lines tested, only KG1 cells (acute myelogenous leukemia) showed a low negative correlation between IC_{50} values and free radical scavenging activity (Fig. 4). Correlation coefficient ($r = -0.61$) was calculated by Pearson *r* correlation method ($R^2 = 0.37$). This result suggests that the cytotoxic effect of 1,2-FNQ on KG1 cells could be, in part, related to their antioxidant properties. In order to test this hypothesis, we intend to perform a mechanistic study (e.g., by using ROS-sensitive probes and fluorescence microscopy) with KG1 cells in the presence of 1,2-FNQT.

Conclusions

In a previous report, we described the cytotoxic effect of a novel series of 1,2-furanonaphtoquinones 1*H*-1,2,3-triazoles (1,2-FNQ) on a panel of leukemic cell lines. These results led us to investigate how these compounds affect cancer cells. In this paper, we tested if the this could be due to inhibition of cancer-related glycosidases or/and to free radical scavenger activity. All 1,2-FNQ showed low inhibitory effect on commercial glycosidases, including those used as models for cancer drug discovery (α-glucosidase and α-mannosidase). Compound **9i**, which has a n-pentyl chain attached to triazole core, was the most active on α-glucosidase activity ($IC_{50} = 413.7\ \mu M$). Many of 1,2-FNQ showed DPPH free radical scavenging activity, which was positively correlated with their cytotoxic effect in KG1 leukemic cell lines. In summary, 1,2-FNQ seem to decrease leukemic cancer cells viability by mechanisms other than glycosidase inhibition. In part, this effect could be due to their redox properties, which is understandable, since reactive oxygen species play a pivotal role in cancer development.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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