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In vitro and in vivo evaluation of fully substituted (5–(3-ethoxy-3-oxopropynyl)-4-(ethoxycarbonyl)-1,2,3-triazolyl-glycosides as original nucleoside analogs to circumvent resistance in myeloid malignancies

Hella Amdouni,[a‡] Guillaume Robert,[b‡] Mohsine Driowya,[a‡] Nathan Furstoss,[b] Camille Métier,[a] Alix Dubois,[b] Maeva Dufies,[b] Marwa Zerhouni,[b] François Orange,[c] Sandra Lacas-Gervais,[c] Khalid Bougrin,[d] Anthony R. Martin,[a] Patrick Auberger[b†] and Rachid Benhida[a†]**

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KEYWORDS: tri-substituted 1,2,3-triazoles, leukemia, anti-leukemic effects, myelodysplasic syndromes, autophagy.

ABSTRACT A series of nucleoside analogs bearing a 1,4,5-trisubstituted-1,2,3-triazole aglycone was synthesized using a straightforward click/electrophilic addition or click/oxidative coupling tandem procedures. SAR analysis, using cell culture assays, led to the discovery of a series of compounds belonging to the 5-alkynyl-1,2,3-triazole family that exhibits potent antileukemic effects on several hematologic malignancies including chronic myeloid leukemia (CML) and myelodysplastic syndromes (MDS) either sensitive or resistant to their respective therapy. Compound **4a** also proved efficient *in vivo* on mice xenografted with SKM1-R MDS cell line. Additionally, some insights in its mode of action revealed that this compound induced cell death by caspase and autophagy induction.

Introduction

Nucleoside analogs (NAs) constitute since decades a therapeutic armamentarium of choice in the treatment of malignant hemopathies. The anticancer nucleosides include several analogs of pyrimidine and purine derivatives.[1,2] The currently available analogues of purine are cladribine, clofarabine and fludarabine, and those of pyrimidine are cytarabine, gemcitabine and azacitidine. NAs mimic natural nucleosides by using their physiological nucleosides transporters to enter into the cells before being phosphorylated to their active triphosphate form inside the cell by specific kinases. These compounds are classified as anti-metabolites drugs that inhibit directly or indirectly the DNA and/or RNA synthesis thus inducing apoptotic cell death.[3,4]

Recently, we reported that acadesine (5-aminoimidazole-4-carboxamide-1-β-D-riboside, **Aca**) acts as a peculiar antineoplastic agent that inhibits CML progression of naïve and resistant cells.[5] Despite good efficiency in sensitive and resistant CML cells, the amount of acadesine needed to kill the cells was excessive (IC_{50} on K562 = 0.8 mM). However, its mode of action resulting in

an autophagic cell death remains of great interest.[5] Thus, the production of more potent analogs of acadesine that retain its original mode of action could be highly desirable. To ensure a rapid and straightforward access to a wide variety of acadesine analogs we replaced its original imidazole core by a more convenient 1,2,3-triazole structure (Figure 1).

Figure 1. From acadesine to potent analogs.

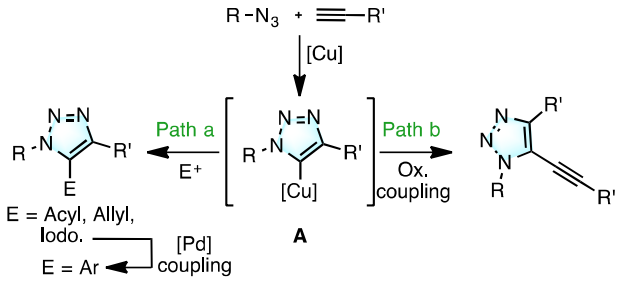
Herein, we report our efforts towards the discovery of potent analogs of acadesine and their biological evaluation against various hematological malignancies, including CML and myelodysplastic syndromes, either sensitive or resistant to their reference treatment. We also disclose some insights in the mode of action of our compounds and the *in vivo* evaluation of a representative structure on aggressive and resistant MDS mouse model is presented.

Results and discussion

Chemistry

We intended to develop potent antiproliferative compounds and at the same time envisioned to grant access to them through very short reaction sequences. For this purpose, we limited our investigations to single or two-step synthetic pathways. We based our syntheses on the copper catalyzed azide-alkyne cycloaddition reaction (CuAAC),[6-8] which is known for its robustness and versatility, and included it in modified procedures. Indeed, instead of using *regular* CuAAC which only yields 1,4-disubstituted 1,2,3-triazoles and narrows the accessible structural variety,

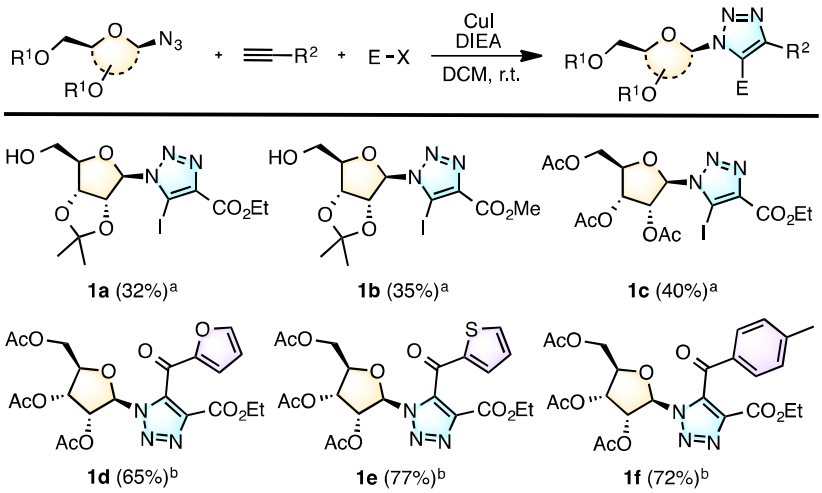
we employed *modified* CuAAC procedures that allow for the synthesis of 1,4,5-trisubstituted 1,2,3-triazoles, and thus expand the structural modularity. Moreover, the use of azido-sugars as 1,3-dipole would allow a direct access to fully functionalized triazolyl-nucleosides. It should be noted that in practice syntheses of such analogs have been limited to disubstituted 1,4-derivatives.[6-8] Fully decorated 1,2,3-triazoles can be obtained through several synthetic pathways;[9-11] herein, we report the use of two strategies, Scheme 1; both of them relying on the trapping of the 5-cuprated-1,2,3-triazole intermediate (**A**) generated during the CuAAC reaction. On the one hand, **A** can be trapped by various electrophiles.[12,13] (path a). When the electrophile is a halogen, a subsequent Pd-catalyzed coupling could be applied to increase the structural diversity. On the other hand, under oxidative conditions, the CuAAC reaction can yield, in a single step, 5-alkynyl-1,2,3-triazoles (path b).[14-16] Thus, we will resort to these two routes leading to fully decorated 1,2,3-triazoles to build up the library surveyed in the biology section.



Scheme 1. Synthetic access to the 1,2,3-trisubstituted triazolyl-nucleosides.

Path a, CuAAC / Electrophilic trapping. A first series of 1,4,5-trisubstituted 1,2,3-triazoles has been synthesized following a CuAAC/ electrophilic trapping sequence (Scheme 2). This methodology, previously reported by Wu[12] and Benhida,[13] takes advantage on the nucleophilicity of the 5-triazolyl copper intermediate of the CuAAC (**A**) to introduce, in a multicomponent fashion, an electrophile at the 5-position. In the present study, we used as electrophilic sources:

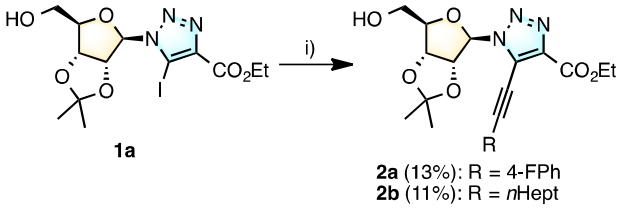
iodine (**1a-1c**) and acyl chlorides (**1d-1f**). Thus, starting from the conveniently protected 1-deoxy-1-azido-β-D-ribofuranose,[17,18] through this single-step method, we prepared 6 compounds (**1a-1f**) with non-optimized yields ranging from 32 to 77%.



Reaction conditions: [a] azide (1.0 mmol), alkyne (2.0 mmol), CuI (1.2 mmol), I_2 (2.0 mmol), CAN (1.0 mmol) and DIPEA (5.0 mmol) in THF. [b] azide (1.0 mmol), alkyne (2.0 mmol), CuI (1.2 mmol), acyl chloride (3.0 mmol) and DIEA (1.5 mmol), in THF.

Scheme 2. Synthesis of triazoles **1a-1f** through a CuAAC/Electrophilic trapping sequence

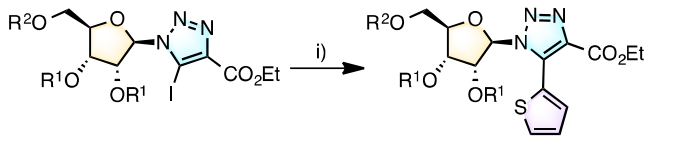
To broaden the structural variety of our acadesine analogs, **1a** was subjected to Sonogashira couplings using either *p*-fluorophenylacetylene or non-1yne as the alkyne (Scheme 3).



i) **1a** (1.0 mmol), alkyne (5.0 mmol), triethylamine (1.0 mmol), CuI (0.05 mmol) and $PdCl_2(PPh_3)_2$ (0.05 mmol), in anhydrous toluene at 60 °C.

Scheme 3. Functionalization of **1a** through Sonogashira coupling.

Finally, a Stille coupling (Scheme 4) was performed on **1a** and **1c** to afford the compounds **3a** and **3b** in 70% and 76% yield, respectively.

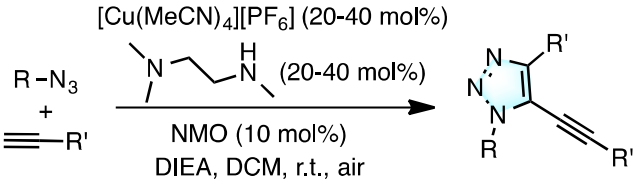


1a: R^1 = isopropylidene, R^2 = H **3a** (70%) : R^1 = isopropylidene, R^2 = H
1c: R^1 = R^2 = Ac **3b** (76%) : R^1 = R^2 = Ac

i) **1a** or **1c** (1.0 mmol), 2-(tributylstannyl)thiophene (2.0 mmol), CuI (0.05 mmol) and $PdCl_2(PPh_3)_2$ (0.1 mmol), in anhydrous toluene at 80 °C.

Scheme 4. Functionalization of **1a** and **1c** through a Stille coupling.

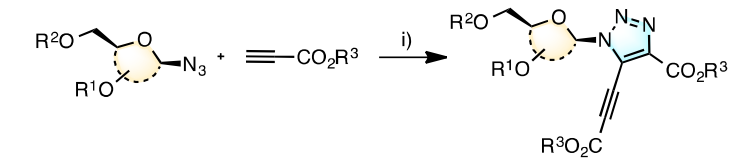
Path b, CuAAC / oxidative coupling. During his research on the cycloaddition of bromomagnesium acetylides, Krasiński evidenced 4-alkynyl-1,2,3-triazoles as trace byproducts.[19] In 2006, Porco Jr. developed a new methodology to gain access to this class of compound in decent yields (31-68 %).[14] The reported protocol relies on a formal CuAAC / oxidative coupling cascade (Scheme 5). This sequence was further explored by others and adapted to a wider range of substrates.[15,16]

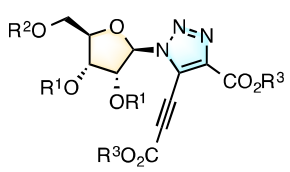


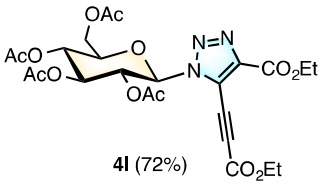
Scheme 5. CuAAC / Oxidative coupling developed by Porco Jr.

In the present study, we first tried to apply the reported conditions to the synthesis of compounds **4a**-**4o**. However, we eventually faced reactivity issues and very poor yields (<5%) were obtained when somewhat acidic alkynes, such as ethyl propiolate, were used. This prompted us to adapt the reaction conditions to our specific substrates. A rapid screening of the reaction parameters of this CuAAC / oxidative coupling revealed that copper cyanide was best suited as the Cu(I) source, *N,N*-di*iso*propylethylamine as the base/ligand, hydrogen peroxide as terminal oxidant[20] and tetrahydrofuran as the solvent (THF). Using our adapted protocol for the CuAAC / oxidative coupling, we next undertook the preparation of compounds **4a-4o**, dedicated

to biological evaluations. Thus a collection of 17 compounds was synthesized in poor to good yields (21-72%, Scheme 6).

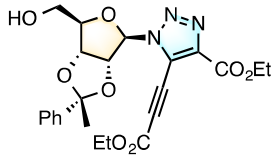




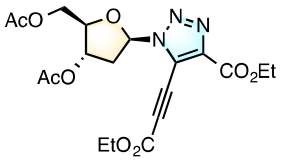


4l (72%)

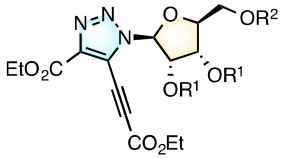
4a (64%): R^1 = isopropylidene, R^2 = H, R^3 = Et
4b (31%): R^1 = isopropylidene, R^2 = H, R^3 = Me
4c (60%): R^1 = isopropylidene, R^2 = H, R^3 = tBu
4d (49%): R^1 = isopropylidene, R^2 = Me, R^3 = Et
4e (63%): R^1 = isopropylidene, R^2 = Ac, R^3 = Et
4f (45%): R^1 = isopropylidene, R^2 = Ac, R^3 = Me
4g (42%): R^1 = cyclohexylidene, R^2 = H, R^3 = Et
4h (48%): R^1 = 4-heptylidene, R^2 = H, R^3 = Et
4i (63%): R^1 = R^2 = Ac, R^3 = Et
4j (21%) R^1 = R^2 = Ac, R^3 = tBu
4k (40%) R^1 = R^2 = Me, R^3 = Et



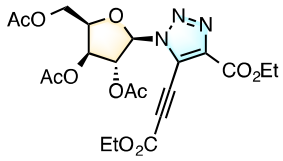
4m (47%)



4n (48%)



4p (45%): R^1 = isopropylidene, R^2 = H
4q (33%): R^1 = R^2 = Ac



4o (42%)

i): azide (1.0 mmol), alkyne (4.0 mmol), CuCN (1.2 mmol), H_2O_2 (5.0 mmol) and DIEA (2.0 mmol), in THF at 0 °C for 10 min. then r.t.

Scheme 6. Synthesis of triazoles **4a-4q** through a CuAAC/oxidative coupling reaction.

Biology

In vitro anti-leukemic effect of triazolyl nucleoside analogs in the chronic myelogenous leukemia K562 cell line. The first step in the investigation of the therapeutic potential of all the reported compounds was devoted to determine their activity on the viability of the K562 CML cell line. Cell viability assays were performed using the XTT assay. According to this first screen (Table 1) a robust structure-activity relationship was established. Indeed, the first 3 series of compounds (**1a-1f, 2a-2b** and **3a-3b**) did not exhibit inhibitory activities on K562 cell metabolism at 48 h (up to 10 μM). Remarkably, most of the compounds belonging to the fourth series (**4a-4q**) were highly efficient and displayed IC_{50} ranging from 0.15 to 2.50 μM. It is worth noting that these series of compounds are 320- to >5000-fold more active than the parent acadesine, used as a control (Table 1). Hence, concerning the aglycone ring, the structure-activity relationship revealed that substitution on the 5-position of the 1,2,3-triazole tremendously modulated the activity. The presence of halide, heteroaryl or acyl groups on this position resulted in no anti-proliferative efficiency (series **1**-**3**), while alkynyl substituents terminated by an ester function led to high activity (series **4**). Moreover, the nature of the ester (Me, Et, *t*Bu – **4a**-**4c**) did not influence significantly the IC_{50} values, albeit the *t*Bu substituent may be slightly less favorable (**4c** and **4j** *vs.* **4a**). Finally, it is worth noting that the 5-alkynyl substituent must be terminated by an ester function as its replacement by a non electron-withdrawing moiety induces a loss of activity (series **2**). Conversely to the aglycone moiety, the saccharide motif tolerates a broader variety of substituents without dramatically affecting the activity.[21] Indeed, various combinations of 2',3'-*O*-alkylidene, acetyl or methyl *O*-substitutions (**4a**-**4k**) did not significantly affect the IC_{50} although the best efficacies were observed with larger 2',3'-*O*-alkylidene (**4g**, **4h, 4m**). This may underpin the need for some hydrophobic bulk on this part of the scaffold. Regarding the nature of the saccharide, the replacement of the

ribofuranose structure (**4i**) by a glucopyranose (**4l**), a 2'-deoxyribose (**4n**), a xylose (**4o**) or even a L-ribose moiety (**4q** and **4p**) resulted in no notable variation of the IC_{50} value.

Table 1. Evaluation of the compounds efficiency against K562 cell lines.

Cpd	IC_{50} (μM)[a]	Cpd	IC_{50} (μM)[a]
1a	>10	**4e**	0.50 (0.01)
1b	>10	**4f**	0.15 (0.01)
1c	>10	**4g**	0.45 (0.01)
1d	>10	**4h**	0.15 (0.01)
1e	>10	**4i**	0.70 (0.04)
1f	>10	**4j**	2.50 (0.15)
2a	>10	**4k**	0.80 (0.06)
2b	>10	**4l**	0.70 (0.03)
3a	>10	**4m**	0.50 (0.02)
3b	>10	**4n**	0.20 (0.01)
4a	0.37 (0.01)	**4o**	0.20 (0.02)
4b	1.45 (0.01)	**4p**	0.20 (0.01)
4c	1.15 (0.04)	**4q**	0.20 (0.01)
4d	0.55 (0.02)	**Aca**	800

[a] IC_{50} determined by XTT assay (mitochondrial metabolism measurement) after 48 h incubation time. Standard deviation is given in parenthesis.

Following the first screen of all the compounds on the leukemic K562 cell line, we selected **4a** as a representative compound of the 4th series and get it screened on a panel of 60 cancer cell lines by the National Cancer Institute.[22] In line with the effect of **4a** on K562 cancer cells, it exhibited a strong activity, reported as the 50% growth inhibition, in most of the screened cancer cell lines and in particular in hematopoietic cell lines (see ESI for the full NCI_{60} results).

Table 2. GI_{50} of **4a** against selected cancer cell lines from NCI_{60} assay.

Cell lines	GI_{50}[a]	Cell lines	GI_{50}[a]
CCRF-CEM[b]	1.33	M14[c]	3.11
HL-60[b]	2.07	MDA-MB435[c]	2.03
K562[b]	1.28	SK-MEL-2[c]	12.20
MOLT-4[b]	3.07	SR[c]	1.22
RPMI-8226[b]	2.23	MCF7[d]	1.77
LOX IMVI[c]	1.71	BT-549[d]	3.96
MALME-3M[c]	2.39	MDA-MB468[d]	1.66

[a] Values in μM. [b] leukemia cell lines. [c] melanoma cell lines. [d] breast cancer cell lines.

Resistance to targeted therapies occurs in a significant percentage of CML patients. To complete these observations, we evaluated the capacity of our lead compound to eradicate imatinib sensitive *and* resistant CML cells. Thus, **4a** was assessed against the Ima-R cell line which derives from parental K562 after chronic exposition to imatinib for 6 months.[23,24] Importantly, compound **4a** was able to eliminate resistant cells as efficiently as sensitive cells (Figure 2) with an IC_{50} of 0.4 μM.

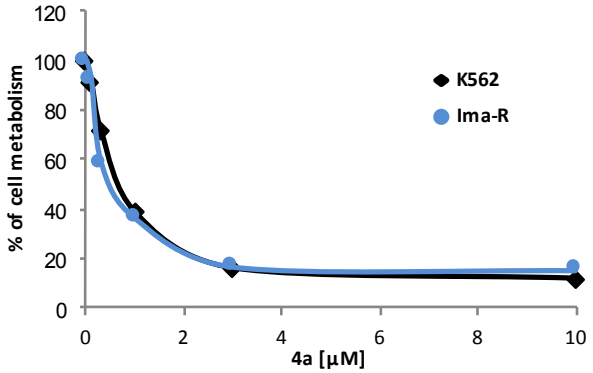


Figure 2. Comparison of the effect of **4a** on K562 and Ima-R cell lines.

We further extend our study to other hematopoietic cell lines. The SKM1–S and –R cells constitute a valuable model to study the resistance of myelodysplastic syndromes (MDS) cell lines to azacitidine (Aza), a gold standard drug used in the treatment for elderly patients, and classified as hypomethylating agent.[25,26] Hence, we assessed the effect of **4a** on the mitochondrial metabolism (IC_{50} – Table 2) and the cell viability (EC_{50} – Table 2) of both cell types, and the mitochondrial metabolism. We observed that the EC_{50} of **4a** were 0.24 and 0.40 µM for the SKM1-S and the SKM1-R, respectively. Its IC_{50} fall in the same range with values of 0.34 and 0.50 µM on the sensitive and resistant cell lines, respectively. We also confirm that azacitidine failed to kill resistant cells contrariwise to sensitive ones. Therefore, compound **4a** is highly efficient at least against two leukemic cell lines resistant to two different chemotherapies, imatinib and azacitidine.

Table 2. Evaluation of **4a** versus azacitidine in SKM1-S and SKM1-R cell lines.

	SKM1-S		SKM1-R	
	IC_{50} (µM)[a]	EC_{50} (µM)[b]	IC_{50} (µM)[a]	EC_{50} (µM)[b]
4a	0.34 (0.03)	0.24 (0.01)	0.50 (0.04)	0.40 (0.03)
Aza	0.50 (0.07)	0.51 (0.03)	>10	>10

[a] IC_{50} of the mitochondrial metabolism determined by XTT assay after 48 h incubation time. [b]DL_{50} determined by flow cytometry (DAPI incorporation) method after 48 h incubation time.

To gain insights into the mechanism of action of **4a**, we next analyzed the morphology of cells incubated for 24 h in the presence of **4a**. Electron microscopy images of SKM1 revealed the presence of autophagic vacuoles after 24 h treatment (Figure 3). Moreover, **4a** induced a striking disorganization of mitochondrial structure with an important swelling of mitochondrial crest, more particularly in sensitive cells. These observations suggest autophagy and mitochondrial impairment in the anti-leukemic effects of **4a**.

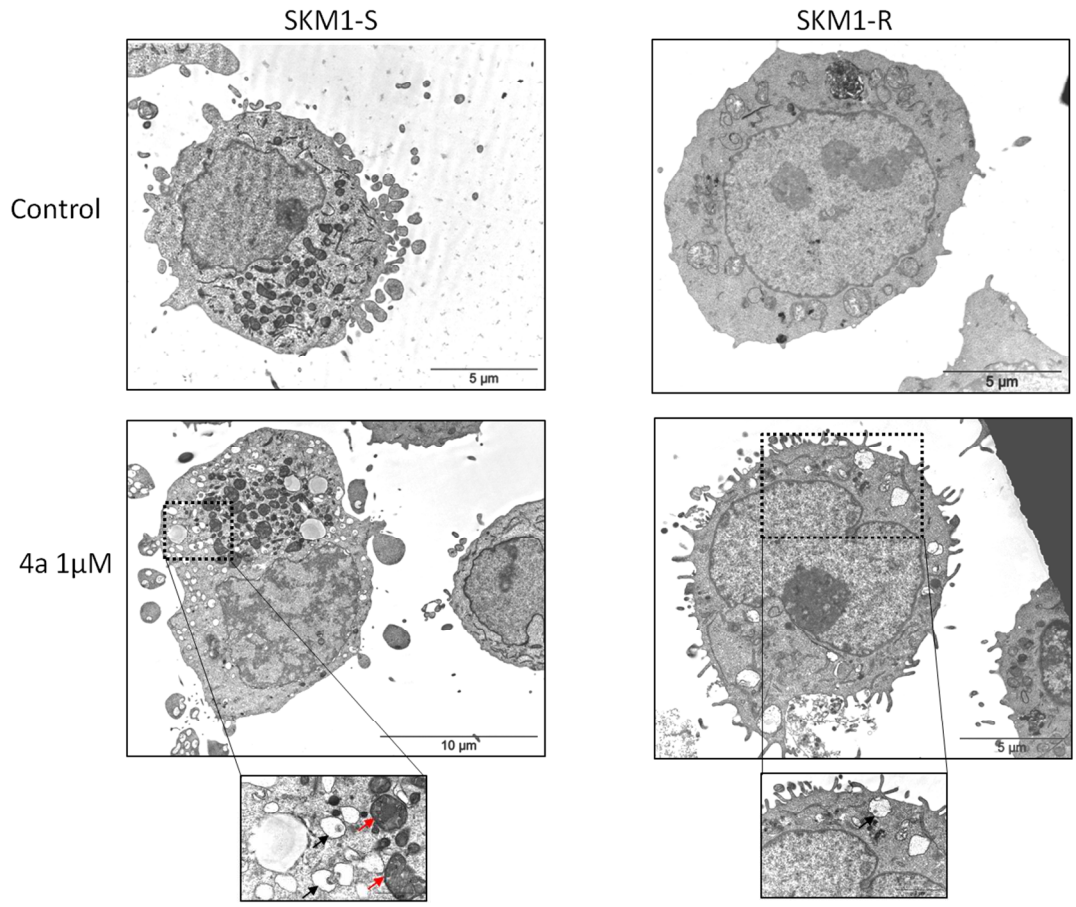


Figure 3. 4a induces the formation of autolysosomal vacuoles and disorganization of intramitochondrial structures.

We next compared the effect of azacitidine and **4a**, both at 1 µM, on the signaling pathways involved in cell death. We first confirmed that azacitidine is able to induce apoptotic cell death in sensitive SKM1-S cells with a robust cleavage of caspase 3. As expected, SKM1-R cells fail to respond to azacitidine treatment (Figure 4) even at 10 µM (Table 2). Meanwhile, compound **4a** induced both caspase activation and an increase in LC3 lipidation, a hallmark of autophagy induction (Figure 4). Resistant cells, SKM1-R, exhibit a low but detectable activation of caspase 3 under **4a** treatment.

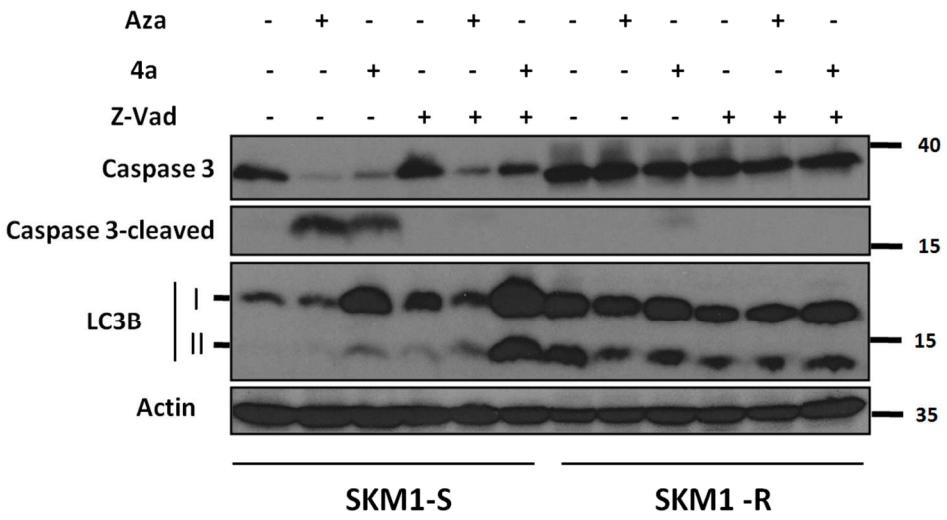


Figure 4. **4a** induces both apoptosis and autophagic cell deaths.

The results presented above show that resistant MDS cell lines harbor defect in apoptosis and are thus less sensitive to apoptosis-inducing agents as azacitidine. In this context, the original mode of cell death induced by **4a** could be used to circumvent resistance to apoptosis in cancer cells. To validate the effect of **4a** *in vivo*, we implanted highly aggressive human SKM1-R cells subcutaneously on the flank of athymic mice. These were separated in two groups, one receiving a vehicle and the other one receiving 5 mg/kg of **4a** five days a week. **4a** treatment was found to reduce the tumor volume by 50% as compared to untreated mice, after 35 days (Figure 5, left panel). Moreover, tumor free weight was comparable in both groups of mice indicating that compound **4a** did not induce acute toxicity in treated mice (Figure 5, right panel).

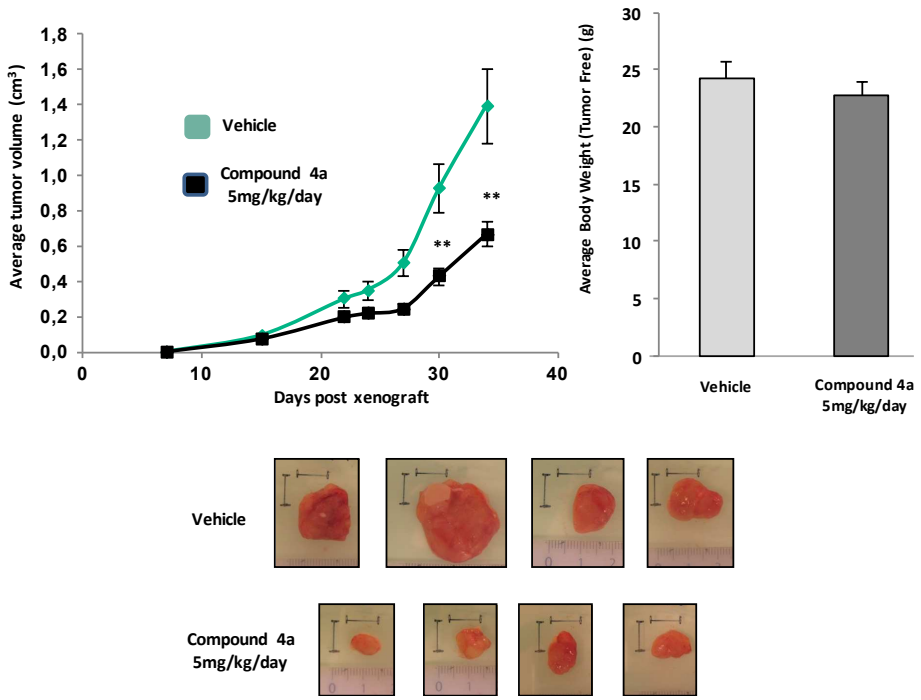


Figure 5. **4a** inhibits tumor growth in nude mice without any apparent acute toxicity.

Finally, the effect of compound **4a** was assessed in bone marrow samples from azacitidine resistant MDS and acute myeloid leukemia (AML) patients. Conversely to azacitidine which fails to induce cell death in these resistant cells as expected, compound **4a** killed these cells in a dose dependent manner (Figure 6A). Importantly, Zvad, a pan-caspase inhibitor was found to partially counteract the effect of **4a**, hence confirming that it induced cell death by both caspase-dependent and independent mechanisms.

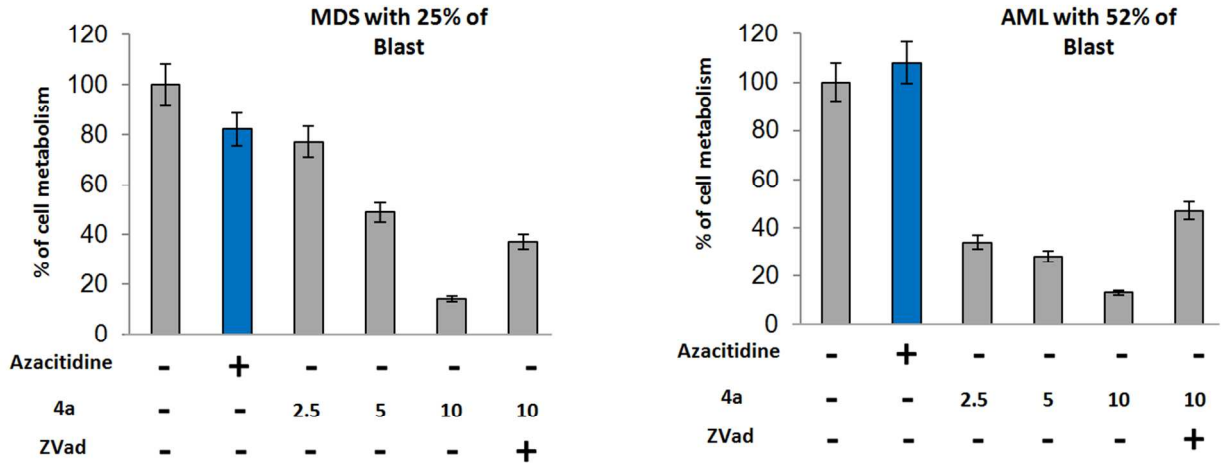


Figure 6. Effect of **4a** on primary cancer cells extracted from azacitidine-resistant patients.

CONCLUSION

In conclusion, we herein reported a series of nucleoside analogs bearing a 1,4,5-trisubstituted-1,2,3-triazole aglycone synthetized using a straightforward click/electrophilic addition or click/oxidative coupling tandem procedures. These original compounds acted as potent anti-leukemic agent in several hematopoietic cell lines. They exhibited strong activities compared to acadesine (>5000-fold) and were highly efficient on imatinib- and azacitidine-resistant myeloid cell lines. Moreover, we observed that one of our lead compound (**4a**), also induced tumor regression in nude mice xenotransplanted with azacitidine-resistant MDS cells, without significant toxicity. Finally, we established that the anti-leukemic effect of compound **4a** was related to its ability to induce both caspase activation and autophagy.

EXPERIMENTAL SECTION

Cell cultures. The human CML K562 cell line was provided by ATCC and was grown in RPMI 1630 medium (Lonza, Walkersville, MD, USA) in the presence of 5% FCS. The human MDS

SKM1, cell line was provided by DSMZ and was grown in RPMI 1630 medium (Lonza, Walkersville, MD, USA) in the presence of 10% FCS. All cell cultures were grown at 37°C under 5% CO_2, 50 units/ml penicillin and 50 mg/ml streptomycin to minimize contamination.

Isolation of bone marrow patient primary. Bone marrow samples were collected from patients suffering MDS or AML in the course of azacitidine (vidaza®) treatment as part of an institutionally approved cellular sample collection protocol. Informed consent has been obtained according to institutional guidelines. Mononuclear cells were isolated from bone marrow samples by density centrifugation (Ficoll-PaqueTM Plus), washed with PBS, 5% SVF, 2 mM EDTA and resuspended in cell culture medium (IMDM, 10% fetal bovine serum) and incubated overnight at 37°C in a 5% CO_2 incubator before CD34+ cells isolation. MDS or AML cells were labelled with CD34 microbeads isolated by magnetic positive selection (StemSepTM Human CD34 Selection Kit; StemCell, Vancouver, BC, Canada). Purity was estimated to at least 90% by FACS analysis. Experiments were performed using a StemSpanR SFEM medium (StemCell, Vancouver, BC, Canada) supplemented with 100 ng/ml human recombinant SCF, FLT3-L and 20 ng/ml human recombinant IL-3, IL-6 and G-CSF (Peprotech, Rocky Hill, NJ, USA).

Reagents and antibodies. Sodium fluoride and orthovanadate, phenylmethylsulfonyl fluoride, aprotinin and leupeptin were purchased from Sigma (Saint-Louis, MO, USA). Anti-anti-LC3-b, anti-caspase 3, and HRP conjugated anti-rabbit antibodies were from Cell Signaling Technology (Beverly, MA, USA). Anti-Hsp60 and anti-actin antibodies were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). HRP-conjugated anti-mouse and anti-goat antibodies were from Dakopatts (Glostrup, Denmark).

Measurement of cell metabolism (XTT). K562 cells (20.10^3 cells/100ml) were incubated in a 96-well plate with indicated concentration of STS for 6 h. About 50 µl of XTT reagent (sodium

3'-[1-(phenylaminocarbonyl)-3,4-tetrazolium]-bis (4–methoxy–6–nitro) benzene sulfonic acid hydrate) (MPP) was added to each well as described previously. The absorbance of the formazan product, reflecting cell viability, was measured at 490 nm. Each assay was performed in quadruplicate.

Measurement of cell death. After **4a** stimulation, cells were stained with propidium iodide. Then, stained cells were analyzed by flow cytometry.

Electronic microscopy. K562 cell pellets were collected, fixed with 1.6% glutaraldehyde, post-fixed in 1% OsO4, dehydrated in alcohol series, and embedded in epoxy resin. Thin sections were contrasted with uranyl acetate and lead citrate. Preparations were observed either with a Philips CM12 electron microscope operating at 80 kV (FEI, Eindhoven, The Netherlands) or with a Jeol 1400 (Tokyo, Japan) mounted with CCD cameras (Morada, Olympus SIS, Germany). Samples were analysed with Jeol 1200 XII Philipps electron microscope.

Western blot. After stimulation, cells were lysed at 4°C in lysis buffer. Lysates were centrifuged at 10 000g for 10 min at 4°C and supernatants were supplemented with concentrated SDS sample buffer. A total of 30 mg of protein were separated on 12% polyacrylamide gel and transferred onto polyvinylidene difluoride (PVDF) membrane (Immobilon-P, Millipore, Bedford, MA, USA). After blocking non-specific binding sites, the membranes were incubated with specific antibodies, washed three times and finally incubated with HRP-conjugated antibody for 1 h at room temperature. Immunoblots were revealed using the enhanced chemiluminescence detection kit (Amersham Biosciences, Uppsala, Sweden).

Tumor regression experiments in nude mice. Female Nude NMRI Mice (Janvier, Le Genest Saint Ile, France) were randomized into two experimental groups, each containing 7 animals.

Animals in both groups received a 200 µl injection of 1.10^6 SKM1-R leukemia cells on the left flank. When tumors reached 100 mm^3, animals were injected intraperitoneally with vehicle, or **4a** at a dose level of 5 mg/kg of body weight, 5 days a week. The growths of leukemic cells composing the tumor were visualized and quantified every 2 days in the animal with an electronic calliper.

General procedures. Reagents were obtained from commercial suppliers (Sigma-Aldrich or Alfa Aesar) and used as received. Anhydrous solvents were obtained according to standard procedures. The reactions were monitored by thin-layer chromatography (TLC, Merck silica gel 60 F254 plates) and revealed by visualization under UV light (254, 315 & 365 nm), then by spraying ethanolic solution of either vanillin/H_2SO_4 or *p*-anisaldehyde/H_2SO_4. Column chromatography purifications were performed on 40–63 µm silica gel. All NMR spectra (^1H, $^{13}C\{^1H\}$) were recorded on a 200 MHz Bruker Avance Spectrometer. Mass spectra (MS) were recorded on an Esquire 3000 Plus apparatus with ESI in both positive and negative mode. High resolution mass spectra (HRMS) were recorded on ESI-LTQOrbitrapU3000 RSLC spectrometer. HPLC analysis was performed on a Jasco LC-Net II /ADC apparatus using phenomenex columns (conditions: unless otherwise stated: 1.0 mL/min, gradient 75% A / 25% B (1 min.) then increasing to 0% A / 100% B in 6 min. and a plateau of 3 min. before returning to 75% A / 25% B in 1 min. A is water and B is CH_3CN, both containing 1‰ HCOOH). Purity of all compounds was found to be ≥95% as determined by HPLC using UV detection at 260 nm.

General procedure A: preparation of 5-iodo-1,2,3-triazoles (1a-1c). To a solution of azide (1.0 mmol) in THF or DCM (10.0 mL), were added successively the corresponding alkyne (2.0 mmol), CuI (1.2 mmol), I_2 (2.0 mmol), cerium ammonium nitrate (CAN) (1.0 mmol) and DIPEA (5.0 mmol). The reaction mixture was allowed to react at r.t. until complete consumption of the

starting material (0.5-1 h). Then, it was filtered on calcite and the solvent was removed under vacuum. The crude material was purified over a silica gel column (cyclohexane/ethyl acetate: 9/1 to 8/2) to yield the desired product.

General procedure B: preparation of 5-acylated triazoles (1d-1e). To a solution of azide (1.0 mmol) in THF or DCM (10.0 mL), were added successively the corresponding alkyne (2.0 mmol), CuI (1.2 mmol), acyl chloride (3.0 mmol) and DIPEA (1.5 mmol). The reaction mixture was allowed to react at r.t. until complete consumption of the starting material (3-5 h). Then, it was filtered on calcite and the solvent was removed under vacuum. The crude material was purified over a silica gel column eluted with cyclohexane/ethyl acetate: 9/1 to 8/2 to yield the desired product.

General procedure C: Sonogashira coupling (2a-2d). To a solution of 5-iodo-triazole (1.0 mmol) in anhydrous toluene at 60 °C were added the corresponding alkyne (5.0 mmol), triethylamine (1.0 mmol), CuI (0.05 mmol) and $PdCl_2(PPh_3)_2$ (0.05 mmol). The reaction mixture was allowed to react until complete consumption of the starting material (overnight). The reaction mixture was then filtered on calcite and the solvent was removed under vacuum. The crude material was purified over a silica gel column (cyclohexane/ethyl acetate: 9/1 to 7/3) to yield the desired product.

General procedure D: Stille coupling (3a, 3b)

To a solution of 5-iodo-triazole (1.0 mmol) in anhydrous toluene at 80 °C were added 2-(tributylstannyl)thiophene (2.0 mmol), CuI (0.05 mmol) and $PdCl_2(PPh_3)_2$ (0.1 mmol). The reaction mixture was allowed to react until complete consumption of the starting material (16 h). The reaction mixture was then filtered on calcite and washed with ethyl acetate. The filtrate was concentrated under reduced pressure and the crude residue purified on silica gel column

chromatography (cyclohexane/ethyl acetate: 8/2 to 3/7) followed by a recrystallization in diethylether to yield the desired product.

General procedure E: preparation of 5-alkynl-triazoles (4a-4q). To a solution of azide (1.0 mmol) in THF (10.0 mL) at 0 °C 10 min, were added successively the corresponding alkyne (4.0 mmol), CuCN (1.2 mmol), H_2O_2 (5.0 mmol) and DIPEA (2.0 mmol). The reaction mixture was allowed to react at r.t until complete consumption of the starting material (3-7h). The reaction mixture was then filtered on calcite and the solvent was removed under vacuum. The crude material was purified over a silica gel column eluted with cyclohexane/ethyl acetate: 9/1 to 7/3 to yield the desired product.

1-Deoxy-1-(5-iodo-4-(ethoxycarbonyl)-1*H*-1,2,3-triazol-1-yl)-2,3-*O*-isopropylidene-β-D-ribofuranose (1a). The title compound was obtained, according to the general procedure **A** and starting from the corresponding azide (6.0 g, 28.0 mmol), as a brown powder (4.0 g) in 32% yield. **^{1}H NMR** ($CDCl_3$, 200 MHz) δ 1.38-1.46 (m, 6H), 1.62 (s, 3H), 3.04 (br, 1H), 3.58-3.64 (m, 1H), 3.73-3.81 (m, 1H), 4.45 (q, *J* 7.1 Hz, 2H), 4.52-4.57 (m, 1H), 5.06 (dd, *J* 5.8 and 1.7 Hz, 1H), 5.38 (dd, *J* 5.8 and 1.9 Hz, 1H), 6.27 (d, *J* 1.9 Hz, 1H); **^{13}C{^{1}H} NMR** ($CDCl_3$, 50 MHz) δ 14.2, 25.1, 27.0, 61.8, 63.2, 82.0, 85.3, 89.6, 95.2, 113.9, 142.0, 156.7, 159.9; **HRMS** (ESI^+): calcd for $[M+H]^+$ $C_{13}H_{19}O_6N_3I$, 440.0313, found 440.0314; **HPLC** (λ_{260}) 97% purity.

1-Deoxy-1-(5-iodo-4-(ethoxycarbonyl)-1*H*-1,2,3-triazol-1-yl)-2,3-*O*-isopropylidene-β-D-ribofuranose (1b). The title compound was obtained, according to the general procedure **A** and starting from the corresponding azide (1.0 g, 4.6 mmol), as a pinkish powder (691 mg) in 35% yield. **^{1}H NMR** ($CDCl_3$, 200 MHz) δ 1.39 (s, 3H), 1.62 (s, 3H), 2.98 (m, 1H), 3.55-3.67 (m, 1H), 3.72-3.80 (m, 1H), 3.98 (s, 3H), 4.54 (t, *J* 3.2 Hz, 1H), 5.06 (dd, *J* 5.8 and 1.7 Hz, 1H), 5.39 (dd, *J* 5.8 and 1.9 Hz, 1H), 6.27 (d, *J* 1.8 Hz, 1H); **^{13}C{^{1}H} NMR** ($CDCl_3$, 50 MHz) δ 25.1, 27.0,

52.5, 63.1, 81.9, 85.3, 89.6, 95.2, 113.9, 141.9, 156.7, 160.3; **HRMS** (ESI^+): calcd for $[M+H]^+$ $C_{12}H_{17}O_6N_3I$, 426.0156, found 426.0157; **HPLC** (λ_{260}) 99% purity.

1-Deoxy-1-(5-iodo-4-(ethoxycarbonyl)-1*H*-1,2,3-triazol-1-yl)-2,3,5-tri-*O*-acetyl-β-D-ribofuranose (1c). The title compound was obtained, according to the general procedure **A** and starting from the corresponding azide (1.1 g, 3.7 mmol), as a brown powder (780 mg) in 40% yield. **^{1}H NMR** ($CDCl_3$, 200 MHz) δ 1.39 (t, *J* 7.1 Hz, 3H), 2.00 (s, 3H), 2.10 (s, 6H), 4.09 (dd, *J* 12.3 and 4.0 Hz, 1H), 4.33-4.46 (m, 4H), 5.74 (t, *J* 5.4 Hz, 1H), 6.07-6.15 (m, 2H); **^{13}C{^{1}H} NMR** ($CDCl_3$, 50 MHz) δ 14.1, 20.3, 20.4, 20.5, 61.6, 62.4, 70.8, 73.9, 81.4, 85.7, 90.2, 142.1, 159.8, 169.1, 169.3, 170.4; **HRMS** (ESI^+): calcd for $[M+H]^+$ $C_{16}H_{21}O_9N_3I$, 526.0317, found 526.0319; **HPLC** (λ_{260}) 97% purity.

1-Deoxy-1-(5-(furan-2-carbonyl)-4-(ethoxycarbonyl)-1*H*-1,2,3-triazol-1-yl)-2,3,5-tri-*O*-acetyl-β-D-ribofuranose (1d). The title compound was obtained, according to the general procedure **B** and starting from the corresponding azide (1.0 g, 3.3 mmol), as a white powder (1.13 g) in 65% yield. **^{1}H NMR** ($CDCl_3$, 200 MHz) δ 1.13 (t, *J* 7.1 Hz, 3H), 2.04 (s, 3H), 2.09 (s, 6H), 4.06 (dd, *J* 12.4 and 4.2 Hz, 1H), 4.09-4.28 (m, 3H), 4.38-4.40 (m, 1H), 5.68 (t, *J* 5.3 Hz, 1H), 6.12-6.20 (m, 2H), 6.64-6.65 (m, 1H), 7.28 (d, *J* 4.1 Hz, 1H), 7.66 (s, 1H); **^{13}C{^{1}H} NMR** ($CDCl_3$, 50 MHz) δ 13.8, 20.3, 20.4, 20.6, 61.7, 62.4, 70.7, 73.7, 81.6, 89.4, 113.5, 121.6, 135.9, 139.4, 148.7, 152.0, 159.5, 169.1, 169.4, 170.5, 171.8; **HRMS** (ESI^+): calcd for $[M+H]^+$ $C_{21}H_{24}O_{11}N_3$, 494.1405, found 494.1407; **HPLC** (λ_{260}) 99% purity.

1-Deoxy-1-(5-(thiophen-2-carbonyl)-4-(ethoxycarbonyl)-1*H*-1,2,3-triazol-1-yl)-2,3,5-tri-*O*-acetyl-β-D-ribofuranose (1e). The title compound was obtained, according to the general procedure **B** and starting from the corresponding azide (553 mg, 1.83 mmol), as a brown powder (717 mg) in 77% yield. **^{1}H NMR** ($CDCl_3$, 200 MHz) δ 1.03 (t, *J* 7.1 Hz, 3H), 2.01-2.06 (m, 9H),

4.00-4.27 (m, 4H), 4.32-4.36 (m, 1H), 5.62 (t, *J* 4.4 Hz, 1H), 6.07-6.10 (m, 2H), 7.10-7.15 (m, 1H), 7.42 (d, *J* 3.8 Hz, 1H), 7.85 (d, *J* 4.1 Hz, 1H); **^{13}C{^{1}H} NMR** ($CDCl_3$, 50 MHz) δ 13.5, 20.2, 20.3, 20.5, 61.6, 62.4, 70.7, 73.5, 81.7, 89.2, 128.7, 136.3, 136.9, 137.3, 138.6, 142.7, 156.6, 159.2, 169.0, 169.3, 170.3, 177.0; **HRMS** (ESI^+): calcd for $[M+H]^+$ $C_{21}H_{24}O_{10}N_3S$, 510.1177, found 510.1178; **HPLC** (λ_{260}) 96% purity.

1-Deoxy-1-(5-(*p*-methylbenzoyl)-4-(ethoxycarbonyl)-1*H*-1,2,3-triazol-1-yl)-2,3,5-tri-*O*-acetyl-β-D-ribofuranose (1f) The title compound was obtained, according to the general procedure **B** and starting from the corresponding azide (650 mg, 1.83 mmol), as a brown powder (552 mg) in 72% yield. **^{1}H NMR** ($CDCl_3$, 200 MHz) δ 0.98 (t, *J* 7.2 Hz, 3H), 2.01 (s, 3H), 2.07 (s, 6H), 2.42 (s, 3H), 3.99-4.26 (m, 4H), 4.31-4.35 (m, 1H), 5.64 (t, *J* 4.7 Hz, 1H), 6.11-6.15 (m, 2H), 7.27 (d, *J* 8.5 Hz, 2H), 7.64 (d, *J* 8.4 Hz, 2H); **^{13}C{^{1}H} NMR** ($CDCl_3$, 50 MHz) δ 13.1, 19.8, 19.9, 20.1, 21.4, 26.4, 61.1, 62.1, 70.3, 73.2, 81.2, 89.0, 129.2, 129,3, 133.3, 137.2, 138.3, 146.0, 168.7, 169.0, 169.8, 185.0; **HRMS** (ESI^+): calcd for $[M+H]^+$ $C_{24}H_{18}O_{10}N_3$, 518.1769, found 518.1772; **HPLC** (λ_{260}) >99% purity.

Ethyl 5-((4-fluorophenyl)ethynyl)-1-(2,3-*O*-isopropylidene-β-D-ribofuranosyl)-1*H*-1,2,3-triazole-4-carboxylate (2a). The title compound was obtained, according to the general procedure **C** starting from compound **1a** (200 mg, 0.46 mmol), as a brown oil (22 mg) in 11% yield. **^{1}H NMR** ($CDCl_3$, 200 MHz) δ 1.38 -1.46 (m, 6H), 1.61 (s, 3H), 3.25 (br, 1H), 3.64-3.72 (m, 1H), 3.81-3.87 (m, 1H), 4.45 (q, *J* 7.1 Hz, 2H), 4.52-4.61 (m, 1H), 5.06 (dd, *J* 5.8 and 1.36 Hz, 1H), 5.32 (dd, *J* 5.8 and 1.8 Hz, 1H), 6.35 (d, *J* 1.8 Hz, 1H), 7.06-7.15 (m, 2H), 7.57-7.10 (m, 2H); **^{13}C{^{1}H} NMR** ($CDCl_3$, 50 MHz) δ 14.3, 25.1, 27.0, 61.6, 63.3, 82.0, 85.5, 89.6, 93.6, 104.1, 113.8, 116.2 (d, *J* 22.4 Hz), 116.7 (d, *J* 3.6 Hz), 124.9, 134.2 (d, *J* 8.8 Hz), 134.3, 140.2,

159.7, 163.7 (d, *J* 253.3 Hz); **HRMS** (ESI^+): calcd for $[M+H]^+$ $C_{21}H_{23}O_6N_3F$, 432.1565, found 432.1566. **HPLC** (λ_{260}) 94% purity.

Ethyl 1-(2,3-*O*-isopropylidene-β-D-ribofuranosyl)-5-(non-1-yn-1-yl)-1*H*-1,2,3-triazole-4-carboxylate (2b). The title compound was obtained, according to the general procedure **C** starting from compound **1a** (200 mg, 0.46 mmol), as a brown oil (26 mg) in 13% yield. **^{1}H NMR** ($CDCl_3$, 200 MHz) δ 0.89 (t, *J* 6.5 Hz, 3H), 1.25-1.45 (m, 14H), 1.61-1.75 (m, 5H), 2.57 (t, *J* 7.0 Hz, 2H), 3.76-3.60 (m, 1H), 3.84 (dd, *J* 12.6 and 3.1 Hz, 1H), 4.44 (q, *J* 7.1 Hz, 2H), 4.51-4.59 (m, 1H), 5.06 (dd, *J* 5.8 and 1.5 Hz, 1H), 5.27 (dd, *J* 5.8 and 2.0 Hz, 1H), 6.28 (d, *J* 2.0 Hz, 1H); **^{13}C{^{1}H} NMR** ($CDCl_3$, 50 MHz) δ 14.1, 14.2, 19.9, 22.6, 25.1, 27.0, 27.9, 28.7, 28.8, 31.6, 61.5, 63.4, 64.4, 82.0, 85.6, 89.5, 93.4, 108.4, 113.7, 125.6, 139.8, 159.9; **HRMS** (ESI^+): calcd for $[M+H]^+$ $C_{22}H_{34}O_6N_3$, 436.2442, found 436.2444. **HPLC** (λ_{260}) 96% purity.

Ethyl 1-(2,3-*O*-isopropylidene-β-D-ribofuranosyl)-5-(thiophen-2-yl)-1*H*-1,2,3-triazole-4-carboxylate (3a). The title compound was obtained, according to the general procedure **D** and starting from **1a** (1.0 g, 2.2 mmol), as a white powder (630 mg) in 70% yield. **^{1}H NMR** ($CDCl_3$, 200 MHz) δ 1.31-1.38 (m, 6H), 1.52 (s, 3H), 3.36 (dd, *J* 8.4 and 4.8 Hz, 1H), 3.63-3.76 (m, 1H), 3.80-3.90 (m, 1H), 4.37 (q, *J* 7.1 Hz, 2H), 4.52 (br, 1H), 5.10 (dd, *J* 5.7 and 1.7 Hz, 1H), 5.42 (dd, *J* 5.8 and 2.2 Hz, 1H), 6.00 (d, *J* 2.2 Hz, 1H), 7.20 (dd, *J* 5.1 and 3.6 Hz, 1H), 7.43 (d, *J* 3.7 and 1.3 Hz, 1H), 7.66 (dd, *J* 5.1 and 1.2 Hz, 1H); **^{13}C{^{1}H} NMR** ($CDCl_3$, 50 MHz) δ 14.1, 25.1, 26.9, 61.5, 63.5, 82.2, 85.4, 89.2, 92.6, 113.7, 123.1, 127.4, 130.7, 132.5, 135.8, 137.1, 160.4; **HRMS** (ESI^+): calcd for $[M+H]^+$ $C_{17}H_{22}O_6N_3S$, 396.1224, found 396.1226; **HPLC** (λ_{260}) >99% purity.

Ethyl 1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)-5-(thiophen-2-yl)-1*H*-1,2,3-triazole-4-carboxylate (3b) The title compound was obtained, according to the general procedure **D** and

starting from **1c** (1.0 g, 1.9 mmol), as a white powder (701 mg) in 76% yield. **^{1}H NMR** ($CDCl_3$, 200 MHz) δ 1.31 (t, *J* 7.1 Hz, 3H), 2.06 (s, 6H), 2.09 (s, 3H), 4.14 (dd, *J* 13.0 and 5.0 Hz, 1H), 4.30-4.46 (m, 4H), 5.79-5.86 (m, 2H), 6.15 (dd, *J* 5.3 and 3.2 Hz, 1H), 7.22 (dd, *J* 5.1 and 3.7 Hz, 1H), 7.43 (d,d *J* 3.7 and 1.2 Hz, 1H), 7.65 (dd, *J* 5.1 and 1.2 Hz, 1H); **^{13}C{^{1}H} NMR** ($CDCl_3$, 50 MHz) δ 14.1, 20.4, 20.4, 20.6, 61.3, 62.5, 71.0, 74.0, 81.3, 87.9, 123.3, 127.4, 130.5, 132.2, 135.8, 137.3, 156.6, 160.4, 169.2, 169.3, 170.5; **HRMS** (ESI^+): calcd for $[M+H]^+$ $C_{20}H_{24}O_9N_3S$, 482.1228, found 482.1230; **HPLC** (λ_{260}) >99% purity.

1-Deoxy-1-(5-(3-ethoxy-3-oxoprop-1-yn-1-yl)-4-(ethoxycarbonyl)-1*H*-1,2,3-triazol-1-yl)-2,3-*O*-isopropylidene-β-D-ribofuranose (4a). The title compound was obtained, according to the general procedure **E** and starting from the corresponding azide (1.0 g, 4.6 mmol), as a brown powder (1.0 g) in 64% yield. **^{1}H NMR** ($CDCl_3$, 200 MHz) δ 1.32-1.46 (m, 9H), 1.61 (s, 3H), 2.86 (br, 1H), 3.66 (dd, *J* 12.4 and 4.7 Hz, 1H), 3.80 (dd, *J* 12.4 and 3.6 Hz, 1H), 4.34 (q, *J* 7.1 Hz, 2H), 4.45 (q, *J* 7.1 Hz, 2H), 4.52-4.57 (m, 1H), 5.04 (dd, *J* 5.9 and 1.8 Hz, 1H), 5.34 (dd, *J* 5.8 and 2.2 Hz, 1H), 6.29 (d, *J* 2.2 Hz, 1H); **^{13}C{^{1}H} NMR** ($CDCl_3$, 50 MHz) δ 13.9, 14.0, 25.1, 26.9, 62.1, 63.0, 67.5, 81.8, 85.1, 89.4, 93.8, 94.0, 114.1, 122.0, 142.7, 152.2, 156.7, 158.9; **HRMS** (ESI^+): calcd for $[M+H]^+$ $C_{18}H_{24}O_8N_3$, 410.1558, found 410.1558; **HPLC** (λ_{260}) >99% purity.

1-Deoxy-1-(5-(3-methoxy-3-oxoprop-1-yn-1-yl)-4-(methoxycarbonyl)-1*H*-1,2,3-triazol-1-yl)-2,3-*O*-isopropylidene-β-D-ribofuranose (4b). The title compound was obtained, according to the general procedure **E** and starting from the corresponding azide (231 mg, 1.1 mmol) as a brown powder (127 mg) in 31% yield. **^{1}H NMR** ($CDCl_3$, 200 MHz) δ 1.35 (s, 3H), 1.58 (s, 3H), 2.87 (br, 1H), 3.61 (dd, *J* 12.3 and 4.8 Hz, 1H), 3.74 (dd, *J* 12.3 and 3.9 Hz, 1H), 3.86 (s, 3H), 3.96 (s, 3H), 4.50 (br, 3H), 5.01 (d, *J* 5.9 Hz, 1H), 5.36 (d, *J* 5.9 Hz, 1H), 6.25 (br, 1H); **^{13}C{^{1}H}**

NMR ($CDCl_3$, 50 MHz) δ 25.0, 26.8, 52.7, 53.4, 62.7, 67.8, 81.7, 84.8, 89.4, 93.7, 114.0, 122.0, 142.4, 152.5, 159.2; **HRMS** (ESI^+): calcd for $[M+H]^+$ $C_{16}H_{20}O_8N_3$, 382.1245, found 382.1245; **HPLC** (λ_{260}) 96% purity.

1-Deoxy-1-(5-(3-*tert*-butoxy-3-oxoprop-1-yn-1-yl)-4-(*tert*-butoxycarbonyl)-1*H*-1,2,3-triazol-1-yl)-2,3-*O*-isopropylidene-β-D-ribofuranose (4c). The title compound was obtained, according to the general procedure **E** and starting from the corresponding azide (500 mg, 2.3 mmol) as a violet powder (600 mg) in 60% yield. **^{1}H NMR** ($CDCl_3$, 200 MHz) δ 1.39 (s, 3H), 1.54 (s, 9H), 1.62 (s, 12H), 2.87 (br, 1H), 3.67-3.85 (m, 2H), 4.52-4.57 (m, 1H), 5.04 (dd, *J* 5.8 and 1.8 Hz, 1H), 5.28-5.32 (m, 1H), 6.29 (d, *J* 2.2 Hz, 1H); **^{13}C{^{1}H} NMR** ($CDCl_3$, 50 MHz) δ 25.2, 27.0, 27.9, 28.0, 63.1, 65.9, 81.8, 83.6, 85.2, 85.3, 89.3, 93.7, 94.9, 114.0, 121.7, 143.6, 151.2, 157.9; **HRMS** (ESI^+): calcd for $[M+H]^+$ $C_{22}H_{32}O_8N_3$, 466.2184, found 466.2186; **HPLC** (λ_{260}) >99% purity.

1-Deoxy-1-(5-(3-ethoxy-3-oxoprop-1-yn-1-yl)-4-(ethoxycarbonyl)-1*H*-1,2,3-triazol-1-yl)-2,3-*O*-isopropylidene--5-*O*-methyl-β-D-ribofuranose (4d). The title compound was obtained, according to the general procedure **E** and starting from the corresponding azide (456 mg, 1.9 mmol) as a brown powder (396 mg) in 49% yield. **^{1}H NMR** ($CDCl_3$, 200 MHz) δ 1.33-1.46 (m, 9H), 1.60 (s, 3H), 3.22 (s, 3H), 3.39-3.33 (m, 2H), 4.34 (q, *J* 7.1 Hz, 2H), 4.40-4.53 (m, 3H), 4.98 (dd, *J* 6.0 and 2.4 Hz, 1H), 5.55 (dd, *J* 6.0 and 0.9 Hz, 1H), 6.27-6.34 (m, 1H); **^{13}C{^{1}H} NMR** ($CDCl_3$, 50 MHz) δ 13.9, 14.0, 25.1, 59.2, 63.0, 68.0, 72.2, 82.3, 84.2, 87.9, 92.9, 93.9, 114.1, 122.0, 143.0, 152.3, 159.1; **HRMS** (ESI^+): calcd for $[M+H]^+$ $C_{19}H_{26}O_8N_3$, 424.1714, found 424.1718; **HPLC** (λ_{260}) >99% purity.

1-Deoxy-1-(5-(3-ethoxy-3-oxoprop-1-yn-1-yl)-4-(ethoxycarbonyl)-1*H*-1,2,3-triazol-1-yl)-2,3-*O*-isopropylidene-5-*O*-acetyl-β-D-ribofuranose (4e). The title compound was obtained,

according to the general procedure **E** and starting from the corresponding azide (3.0 g, 11.7 mmol) as a yellow oil (3.3 g) in 63% yield. **^{1}H NMR** ($CDCl_3$, 200 MHz) δ 1.26-1.39 (m, 9H), 1.53 (s, 3H), 1.93 (s, 3H), 3.91-4.07 (m, 2H), 4.22-4.51 (m, 5H), 4.96 (dd, *J* 5.89 and 2.4 Hz, 1H), 5.57-5.45 (m, 1H), 6.25 (s, 1H); **^{13}C{^{1}H} NMR** ($CDCl_3$, 50 MHz) δ 13.8, 13.8, 20.4, 26.6, 61.8, 62.8, 63.2, 67.6, 81.7, 83.9, 86.7, 92.2, 93.7, 114.1, 122.0, 142.8, 152.0, 158.8, 170.1; **HRMS** (ESI^+): calcd for $[M+H]^+$ $C_{20}H_{26}O_9N_3$, 452.1664, found 452.1666; **HPLC** (λ_{260}) 99% purity.

1-Deoxy-1-(5-(3-methoxy-3-oxoprop-1-yn-1-yl)-4-(methoxycarbonyl)-1*H*-1,2,3-triazol-1-yl)-2,3-*O*-isopropylidene-5-*O*-acetyl-β-D-ribofuranose (4f). The title compound was obtained, according to the general procedure **E** and starting from the corresponding azide (3.0 g, 11.7 mmol) as an orange oil (2.2 g) in 45% yield. **^{1}H NMR** ($CDCl_3$, 200 MHz) δ 1.31 (s, 3H), 1.50 (s, 3H), 1.91 (s, 3H), 3.80 (s, 3H), 3.90 (s, 3H), 3.95-3.98 (m, 2H), 4.46 (dt, *J* 5.8, 5.7 and 2.4 Hz, 1H), 4.93 (dd, *J* 5.9 and 2.4 Hz, 1H), 5.50-5.53 (m, 1H), 6.22 (s, 1H); **^{13}C{^{1}H} NMR** ($CDCl_3$, 50 MHz) δ 20.4, 24.9, 26.8, 52.5, 63.1, 67.9, 81.6, 83.8, 86.7, 92.3, 93.4, 114.0, 122.0, 142.5, 152.4, 159.2, 170.0; **HRMS** (ESI^+): calcd for $[M+H]^+$ $C_{18}H_{22}O_9N_3$, 424.1351, found 424.1352; **HPLC** (λ_{260}) >99% purity.

1-Deoxy-1-(5-(3-ethoxy-3-oxoprop-1-yn-1-yl)-4-(ethoxycarbonyl)-1*H*-1,2,3-triazol-1-yl)-2,3-*O*-cyclohexylidene-β-D-ribofuranose (4g). The title compound was obtained, according to the general procedure **E** and starting from the corresponding azide (301 mg, 1.2 mmol) as a brown powder (610 mg) in 42% yield. **^{1}H NMR** ($CDCl_3$, 200 MHz) δ 1.33-1.47 (m, 6H), 1.91-1.53-1.91 (m, 10H), 3.68 (dd, *J* 12.6 and 4.7 Hz, 1H), 3.83 (dd, *J* 12.4 and 3.3 Hz, 1H), 4.35 (q, *J* 7.1 Hz, 2H), 4.47 (q, *J* 7.2 Hz, 2H), 4.57 (s, 1H), 5.03 (dd, *J* 5.8 and 1.6 Hz, 1H), 5.32 (dd, *J* 5.8 and 2.2 Hz, 1H), 6.31 (d, *J* 2.1 Hz, 1H); **^{13}C{^{1}H} NMR** ($CDCl_3$, 50 MHz) δ 13.9, 14.0, 23.6, 23.9,

24.8, 34.6, 36.8, 62.1, 63.1, 63.2, 67.6, 81.4, 84.9, 89.6, 94.1, 115.0, 122.0, 142.8, 152.2, 158.9; **HRMS** (ESI^+): calcd for $[M+H]^+$ $C_{21}H_{27}O_8N_3$, 450.1871, found 450.1872; **HPLC** (λ_{260}) >99% purity.

1-Deoxy-1-(5-(3-ethoxy-3-oxoprop-1-yn-1-yl)-4-(ethoxycarbonyl)-1*H*-1,2,3-triazol-1-yl)-2,3-*O*-(hept-4-ylidene)- β-D-ribofuranose (4h). The title compound was obtained, according to the general procedure **E** and starting from the corresponding azide (366 mg, 1.3 mmol) as a white powder (620 mg) in 48% yield. **^{1}H NMR** ($CDCl_3$, 200 MHz) δ 0.87-1.01 (m, 6H), 1.32-1.81 (m, 14H), 2.87 (m, 1H), 3.60-3.84 (m, 2H), 4.29-4.51 (m, 4H), 4.56 (br, 1H), 5.04 (d, *J 6.0* Hz, 1H), 5.31-5.33 (m, 1H), 6.31 (br, 1H); **^{13}C{^{1}H} NMR** ($CDCl_3$, 50 MHz) δ 14.0, 14.0, 14.3, 16.7, 17.5, 39.0, 39.1, 62.1, 63.1, 63.2, 67.5, 81.9, 85.5, 89.7, 94.0, 117.9, 122.1, 142.7, 152.2, 158.9; **HRMS** (ESI^+): calcd for $[M+H]^+$ $C_{22}H_{32}O_8N_3$, 466.2184, found 466.2185; **HPLC** (λ_{260}) >99% purity.

1-Deoxy-1-(5-(3-ethoxy-3-oxoprop-1-yn-1-yl)-4-(ethoxycarbonyl)-1*H*-1,2,3-triazol-1-yl)-2,3,5-tri-*O*-acetyl-β-D-ribofuranose (4i). The title compound was obtained, according to the general procedure **E** and starting from the corresponding azide (3.0 g, 9.8 mmol), as a white powder (3.1 g) in 63% yield. **^{1}H NMR** ($CDCl_3$, 200 MHz) δ 1.36 (t, *J* 7.1 Hz, 3H), 1.42 (t, *J* 7.1 Hz, 3H), 2.04 (s, 3H), 2.13 (s, 6H), 4.14 (dd, *J* 12.3 and 4.2 Hz, 1H), 4.29-4.50 (m, 6H), 5.72 (t, *J* 5.5 Hz, 1H), 6.05 (dd, *J* 5.5 and 3.5 Hz, 1H), 6.21 (d, *J* 3.45 Hz, 1H); **^{13}C{^{1}H} NMR** ($CDCl_3$, 50 MHz) δ 3.9, 14.0, 20.4, 20.5, 20.6, 62.0, 62.4, 63.1, 67.6, 70.7, 73.7, 81.6, 89.1, 94.0, 122.2, 143.0, 152.3, 158.9, 169.1, 169.4, 170.5; **HRMS** (ESI^+): calcd for $[M+H]^+$ $C_{21}H_{26}O_{11}N_3$, 496.1562, found 496.1564; **HPLC** (λ_{260}) >99% purity.

1-Deoxy-1-(5-(3-*tert*-butoxy-3-oxoprop-1-yn-1-yl)-4-(*tert*-butoxycarbonyl)-1*H*-1,2,3-triazol-1-yl)-2,3,5-*O*-tri-acetyl-β-D-ribofuranose (4j). The title compound was obtained, according to

the general procedure **E** and starting from the corresponding azide (679 mg, 13.3 mmol) as a pale violet powder (264 mg) in 21% yield. **^{1}H NMR** ($CDCl_3$, 200 MHz) δ 1.51 (s, 9H), 1.59 (s, 9H), 2.02 (s, 3H), 2.11 (s, 6H), 4.06-4.16 (m, 1H), 4.35-4.46 (m, 2H), 5.70 (t, *J* 5.5 Hz, 1H), 5.99-6.03 (m, 1H), 6.18 (d, *J* 3.0 Hz, 1H); **^{13}C{^{1}H} NMR** ($CDCl_3$, 50 MHz) δ 20.3, 20.4, 20.6, 27.8, 28.0, 62.4, 66.0, 70.6, 73.7, 81.4, 83.4, 85.2, 89.0, 94.7, 121.7, 143.7, 151.2, 157.8, 169.1, 169.3, 170.4; **HRMS** (ESI^+): calcd for $[M+H]^+$ $C_{25}H_{34}O_{11}N_3$, 552.2188, found 552.2189; **HPLC** (λ_{260}) 98% purity.

Ethyl 1-(2,3,5-tri-*O*-methyl-β-D-ribofuranosyl)-5-(3-ethoxy-3-oxoprop-1-yn-1-yl)-1*H*-1,2,3-triazole-4-carboxylate (4k). The title compound was obtained, according to the general procedure **E** and starting from the corresponding azide (217 mg, 1.0 mmol) as a brown powder (160 mg) in 40% yield. **^{1}H NMR** ($CDCl_3$, 200 MHz) δ 1.35 (t, *J* 7.1 Hz, 3H), 1.41 (t, *J* 7.1 Hz, 3H), 3.31 (s, 3H), 3.49 (s, 3H), 3.50 (s, 3H), 3.53 (d, *J* 2.2 Hz, 1H), 3.55 (d, *J* 1.2 Hz, 1H), 4.21 (t, *J* 5.2 Hz, 1H), 4.27-4.50 (m, 5H), 4.63 (dd, *J* 4.9 and 3.6 Hz, 1H), 6.17 (d, *J* 3.6 Hz, 1H); **^{13}C{^{1}H} NMR** ($CDCl_3$, 50 MHz) δ 13.9, 14.0, 58.4, 58.9, 59.4, 61.9, 63.0, 68.0, 72.5, 79.4, 82.1, 82.7, 89.6, 93.8, 122.2, 142.8, 152.2, 159.1; **HRMS** (ESI^+): calcd for $[M+H]^+$ $C_{18}H_{26}O_8N_3$, 412.1714, found 412.1716; **HPLC** (λ_{260}) 99% purity.

1-Deoxy-1-(5-(3-ethoxy-3-oxoprop-1-yn-1-yl)-4-(ethoxycarbonyl)-1*H*-1,2,3-triazol-1-yl)-2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranose (4l). The title compound was obtained, according to the general procedure **E** and starting from the corresponding azide (300 mg, 0.8 mmol) as a brown powder (326 mg) in 72% yield. **^{1}H NMR** ($CDCl_3$, 200 MHz) δ 1.33-1.44 (m, 6H), 1.85 (s, 3H), 2.01-2.04 (m, 9H), 4.01 (ddd, *J* 9.7, 4.7 and 2.4 Hz, 1H) 4.13-4.27 (m, 3H), 4.30-4.48 (m, 4H), 5.27 (t, *J* 9.7 Hz, 1H), 5.41 (t, *J* 9.2 Hz, 1H), 5.78-5.94 (m, 2H); **^{13}C{^{1}H} NMR** ($CDCl_3$, 50 MHz) δ 13.9, 13.9, 20.0, 20.4, 26.8, 30.0, 61.4, 62.0, 63.0, 67.3, 67.8, 69.2, 72.5, 75.2, 85.8,

94.3, 121.8, 143.2, 152.1, 158.8, 168.5, 169.0, 169.9, 170.4; **HRMS** (ESI^+): calcd for $[M+H]^+$ $C_{24}H_{30}O_{13}N_3$, 568.1773, found 568.1775; **HPLC** (λ_{260}) 99% purity.

Ethyl 5-(3-ethoxy-3-oxoprop-1-yn-1-yl)-1-((2R,3aR,4R,6R,6aR)-6-(hydroxymethyl)-2-methyl-2-phenyltetrahydrofuro[3,4-d][1,3]dioxol-4-yl)-1*H*-1,2,3-triazole-4-carboxylate (4m). The title compound was obtained, according to the general procedure **E** and starting from the corresponding azide (529 mg, 1.8 mmol) as a white powder (402 mg) in 47% yield. **^{1}H NMR** ($CDCl_3$, 200 MHz) δ 1.35-1.46 (m, 6H), 1.68 (s, 3H), 2.95-3.02 (m, 1H), 3.62-3.85 (m, 2H), 4.31-4.50 (m, 5H), 5.22 (dd, *J* 6.0 and 2.0 Hz, 1H), 5.44 (dd, *J* 6.0 and 2.4 Hz, 1H), 6.15 (d, *J* 2.4 Hz, 1H), 7.34-7.45 (m, 3H), 7.57-7.62 (m, 2H); **^{13}C{^{1}H} NMR** ($CDCl_3$, 50 MHz) δ 13.9, 14.0, 28.2, 62.1, 63.0, 63.2, 67.4, 82.3, 86.1, 88.8, 93.0, 94.0, 114.2, 121.9, 124.9, 128.4, 128.5, 142.5, 152.1, 158.9; **HRMS** (ESI^+): calcd for $[M+H]^+$ $C_{23}H_{26}O_8N_3$, 472.1714, found 472.1717; **HPLC** (λ_{260}) 98% purity.

1,2-Dideoxy-1-(5-(3-ethoxy-3-oxoprop-1-yn-1-yl)-4-(ethoxycarbonyl)-1*H*-1,2,3-triazol-1-yl)-3,5-di-*O*-acetyl-β-D-ribofuranose (4n). The title compound was obtained, according to the general procedure **E** and starting from the corresponding azide (630 mg, 1.8 mmol) as a brown oil (561 mg) in 48% yield. **^{1}H NMR** ($CDCl_3$, 200 MHz) δ 1.31-1.44 (m, 6H), 2.03 (s, 3H), 2.12 (s, 3H), 2.57-2.69 (m, 1H), 3.38-3.51 (m, 1H), 4.07-4.14 (m, 1H), 4.28-4.49 (m, 6H), 5.53 (s, 1H), 6.45 (t, *J* 6.2 Hz, 1H); **^{13}C{^{1}H} NMR** ($CDCl_3$, 50 MHz) δ 13.9, 14.0, 20.6, 20.8, 36.5, 61.9, 63.0, 63.1, 68.1, 74.2, 84.0, 87.6, 93.7, 122.0, 143.1, 152.3, 159.1, 170.2, 170.4; **HRMS** (ESI^+): calcd for $[M+H]^+$ $C_{19}H_{24}O_9N_3$, 438.1507, found 438.1508; **HPLC** (λ_{260}) >99% purity.

1-Deoxy-1-(5-(3-ethoxy-3-oxoprop-1-yn-1-yl)-4-(ethoxycarbonyl)-1*H*-1,2,3-triazol-1-yl)-2,3,5-tri-*O*-acetyl-β-D-xylofuranose (4o). The title compound was obtained, according to the general procedure **E** and starting from the corresponding azide (222 mg, 0.7 mmol) as a white

powder (145 mg) in 42% yield. **^{1}H NMR** ($CDCl_3$, 200 MHz) δ 1.37 (t, *J* 7.1 Hz, 3H), 1.43 (t, *J* 7.1 Hz, 3H), 2.08 (s, 3H), 2.14 (s, 3H), 2.16 (s, 3H), 4.26-4.51 (m, 6H), 4.70 (td, *J* 6.6 and 4.7 Hz, 1H), 5.42 (dd, *J* 4.7 and 1.9 Hz, 1H), 5.99 (t, *J* 2.1 Hz, 1H), 6.08 (d, *J* 2.3 Hz, 1H); **^{13}C{^{1}H} NMR** ($CDCl_3$, 50 MHz) δ 14.0, 14.1, 20.5, 20.6, 20.7, 61.3, 62.0, 63.0, 68.0, 74.5, 79.2, 79.9, 90.6, 94.0, 122.2, 143.0, 152.4, 159.0, 169.3, 170.0, 170.4; **HRMS** (ESI^+): calcd for $[M+H]^+$ $C_{21}H_{26}O_{11}N_3$, 496.1562, found 496.1563; **HPLC** (λ_{260}) 97% purity.

1-Deoxy-1-(5-(3-ethoxy-3-oxoprop-1-yn-1-yl)-4-(ethoxycarbonyl)-1*H*-1,2,3-triazol-1-yl)-2,3-*O*-isopropylidene-β-L-ribofuranose (4p). The title compound was obtained, according to the general procedure **E** and starting from the corresponding azide (306 mg, 0.7 mmol) as a brown powder (251 mg) in 45% yield. **^{1}H NMR** ($CDCl_3$, 200 MHz) δ 1.32-1.46 (m, 9H), 1.62 (s, 3H), 3.67 (dd, *J* 12.5 and 4.6 Hz, 1H), 3.82 (dd, *J* 12.5 and 3.6 Hz, 1H), 4.35 (q, *J* 7.1 Hz, 2H), 4.46 (q, *J* 7.1 Hz, 2H), 4.58-4.53 (m, 1H), 5.05 (dd, *J* 5.8 and 1.8 Hz, 1H), 5.34 (dd, *J* 5.8 and 2.2 Hz, 1H), 6.30 (d, *J* 2.2 Hz, 1H); **^{13}C{^{1}H} NMR** ($CDCl_3$, 50 MHz) δ 14.0, 14.3, 25.2, 27.0, 62.1, 63.1, 67.6, 81.8, 85.2, 89.4, 93.9, 94.1, 114.2, 122.0, 142.8, 152.2, 158.9; **HRMS** (ESI^+): calcd for $[M+H]^+$ $C_{18}H_{24}O_8N_3$, 410.1558, found 410.1559; **HPLC** (λ_{260}) >99% purity.

1-Deoxy-1-(5-(3-ethoxy-3-oxoprop-1-yn-1-yl)-4-(ethoxycarbonyl)-1*H*-1,2,3-triazol-1-yl)-2,3,5-tri-*O*-acetyl-β-L-ribofuranose (4q). The title compound was obtained, according to the general procedure **E** and starting from the corresponding azide (940 mg, 3.0 mmol) as a white powder (479 mg) in 33% yield. **^{1}H NMR** ($CDCl_3$, 200 MHz) δ 1.36 (t, *J* 7.1 Hz, 3H), 1.42 (t, *J* 7.1 Hz, 3H), 2.05 (s, 3H), 2.13 (s, 6H), 4.14 (dd, *J* 12.3 and 4.2 Hz, 1H), 4.29-4.53 (m, 6H), 5.72 (t, *J* 5.4 Hz, 1H), 6.05 (dd, *J* 5.4 and 3.6 Hz, 1H), 6.21 (d, *J* 3.6 Hz, 1H); **^{13}C{^{1}H} NMR** ($CDCl_3$, 50 MHz) δ 13.9, 14.2, 20.3, 20.4, 20.6, 62.0, 62.4, 63.1, 67.7, 70.7, 73.7, 81.7, 89.1, 94.0, 122.2,

143.0, 152.3, 158.9, 169.1, 169.4, 170.4; **HRMS** (ESI^+): calcd for $[M+H]^+$ $C_{21}H_{26}O_{11}N_3$, 496.1562, found 496.1566; **HPLC** (λ_{260}) 99% purity.

ASSOCIATED CONTENT

Supporting Information. The following files are available free of charge and contain: Preparation of the azido derivatives; for all the reported compounds: copies of ^1H and $^{13}C\{^1H\}$ NMR spectra, HPLC chromatogram, HRMS spectra; full NCI_{60} 5-doses screening of **1a**. (PDF file). Molecular formula strings (CSV file).

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ABBREVIATIONS

AML, acute myeloid leukemia; (P)-AMPK, (phosphorylated)-AMP activated protein kinase; Aza, azacitidine; BCR-ABL, breakpoint cluster region-abelson; CAN, cerium ammonium nitrate; CML, chronic myeloid leukemia; CuAAC, copper catalyzed azide-alkyne cycloaddition; DAPI, 4′,6-diamidino-2-phenylindole dihydrochloride; DIEA, di*iso*propylethylamine; EC_{50}, concentration yielding 50% of the expected effect; IC_{50}, concentration leading to 50% inhibition; LC3B, microtubule-associated proteins 1A/1B light chain 3B; MDS, myelodysplastic syndrome; NMO, N-methylmorpholine oxide; PBMC, peripheral blood mononuclear cells; SAR, structure-activity relationship; TKI, tyrosine kinase inhibitor; Zvad, *N*-Benzyloxycarbonyl-Val-Ala-Asp(O-Me) fluoromethyl ketone.

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(21) The structural variation on the 1-position of the azole showed some limitations as the replacement of the glycosyl unit of **4a** with a simple benzyl moiety lead to a complete loss of activity (76% of mitochondrial metabolism at 10 μM - XTT method).

(22) Compound **4a** was selected for in vivo assays for the following reasons : (a) in this model IC_{50} ranging from 0.1 to 0.3 are closely similar, (b) this compound exhibited better solubility compared to the other analogs.

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Table of contents graphic

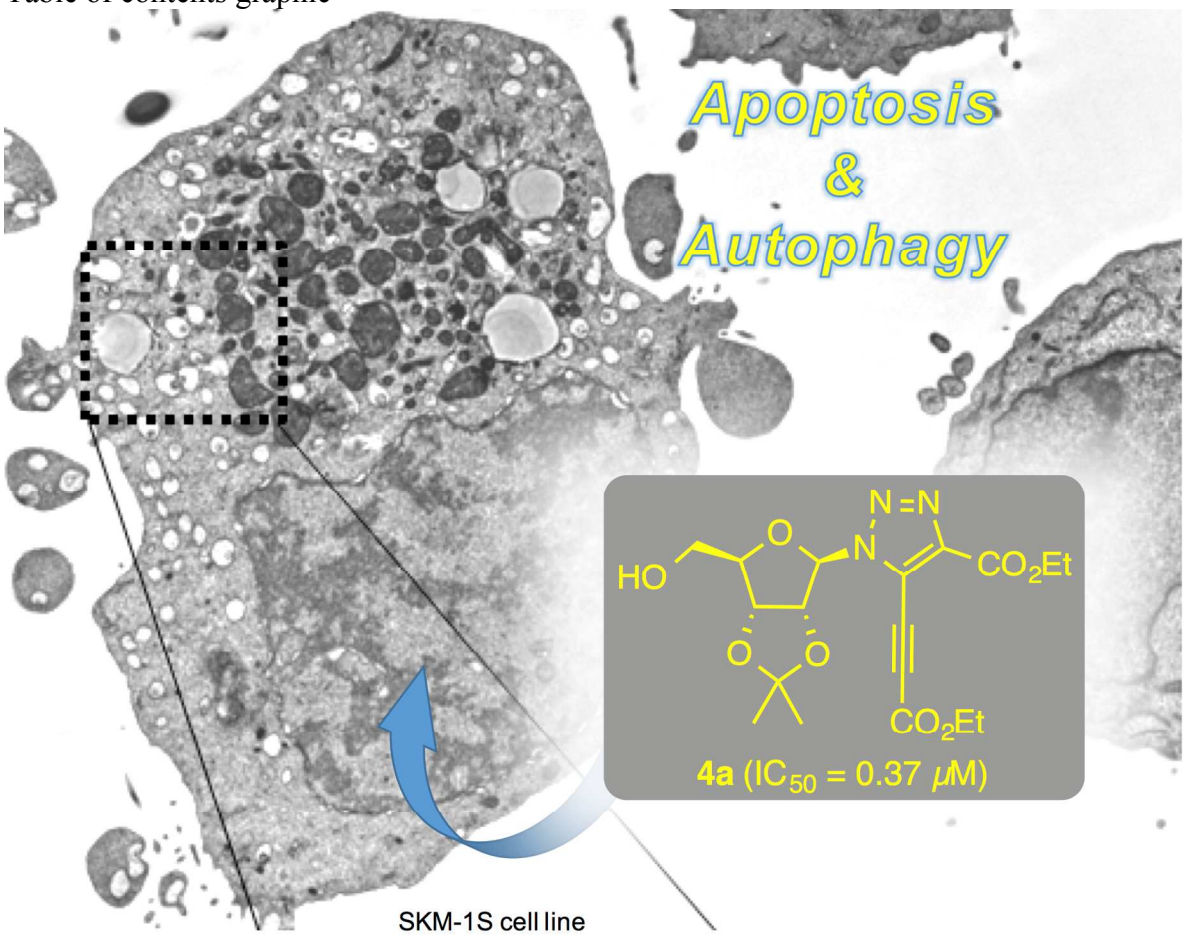

Figure 1. From acadesine to potent analogs.

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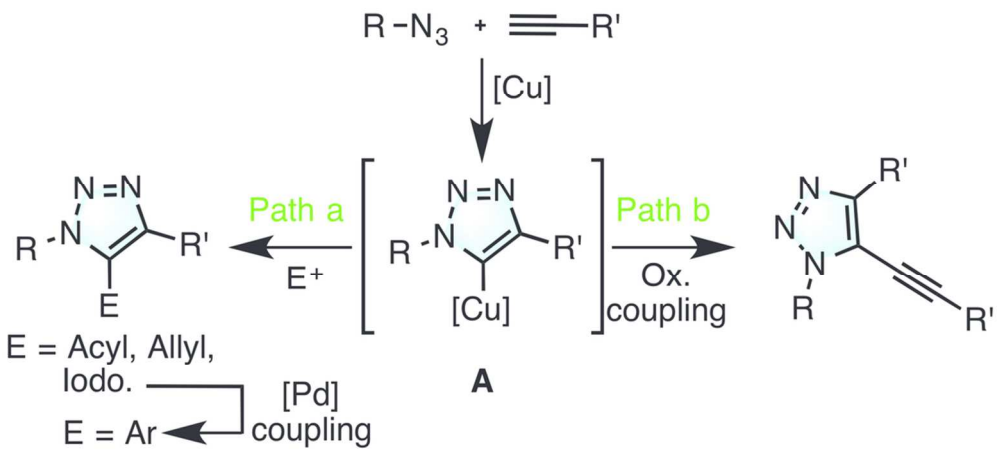

Scheme 1. Synthetic access to 1,2,3-trisubstituted triazolyl-nucleosides.

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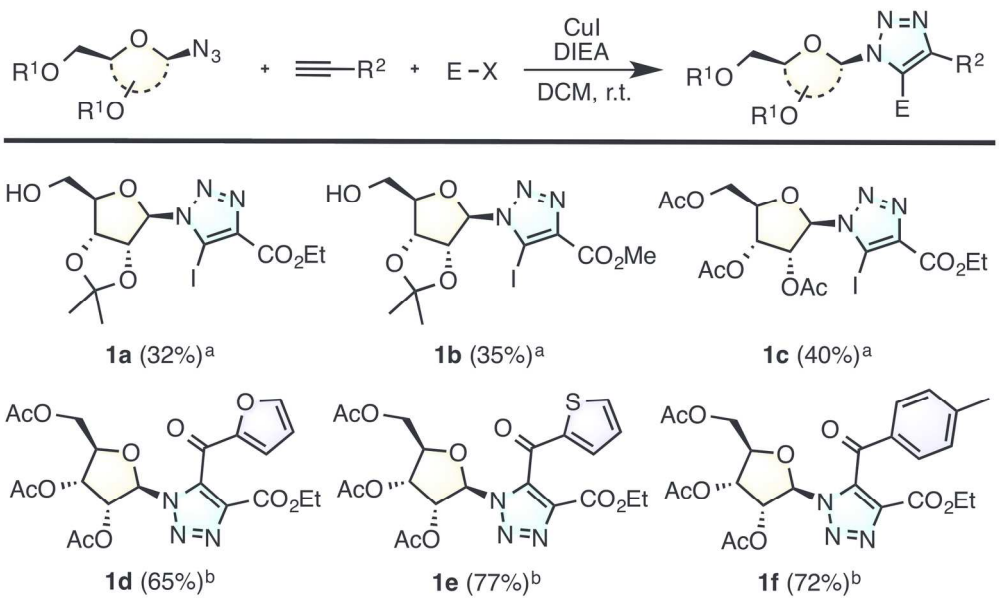
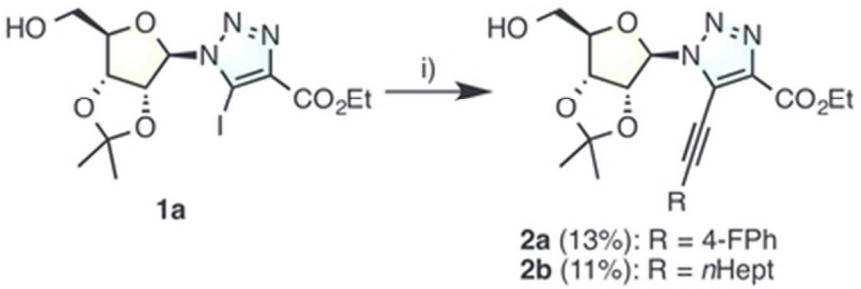


Scheme 2. Synthesis of triazoles 1a-1f through a CuAAC/Electrophilic trapping sequence

86x51mm (600 x 600 DPI)



Scheme 3. Functionalization of 1a through Sonogashira coupling.

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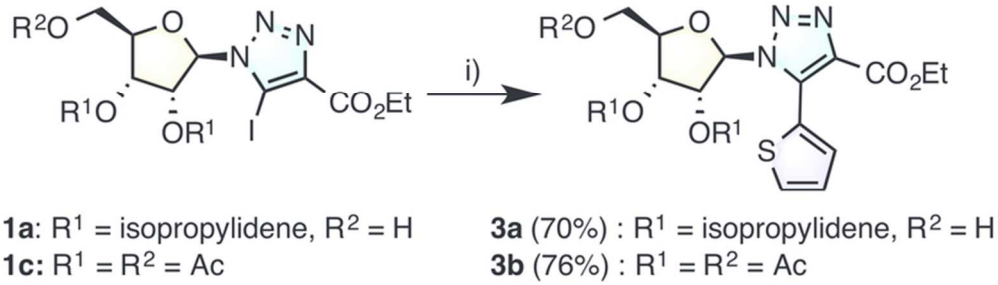

Scheme 4. Functionalization of 1a and 1c through a Stille coupling.

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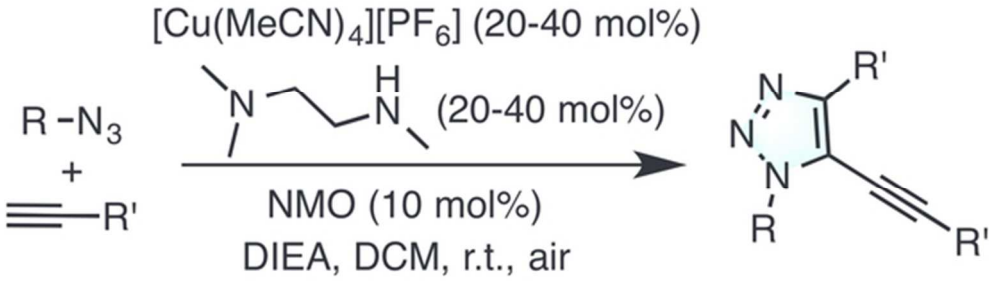
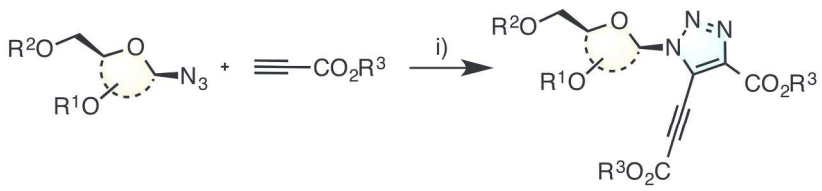
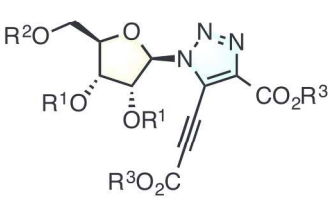


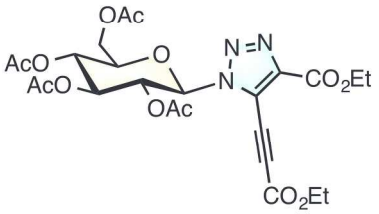
Scheme 5. CuAAC / Oxidative coupling developed by Porco Jr.

26x7mm (600 x 600 DPI)

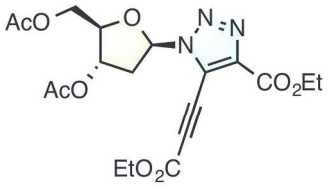




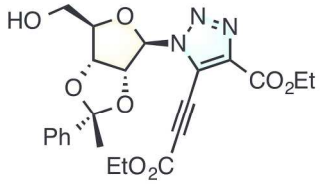
4a: R^1 = isopropylidene, R^2 = H, R^3 = Et
4b: R^1 = isopropylidene, R^2 = H, R^3 = Me
4c: R^1 = isopropylidene, R^2 = H, R^3 = tBu
4d: R^1 = isopropylidene, R^2 = Me, R^3 = Et
4e: R^1 = isopropylidene, R^2 = Ac, R^3 = Et
4f: R^1 = isopropylidene, R^2 = Ac, R^3 = Me
4g: R^1 = cyclohexylidene, R^2 = H, R^3 = Et
4h: R^1 = 4-heptylidene, R^2 = H, R^3 = Et
4i: R^1 = R^2 = Ac, R^3 = Et
4j: R^1 = R^2 = Ac, R^3 = tBu
4k: R^1 = R^2 = Me, R^3 = Et



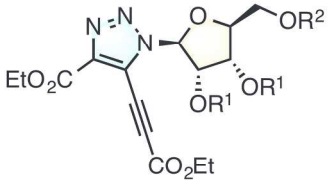
4l



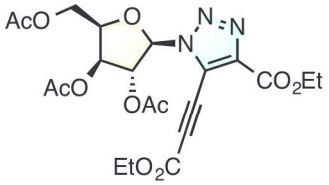
4n



4m



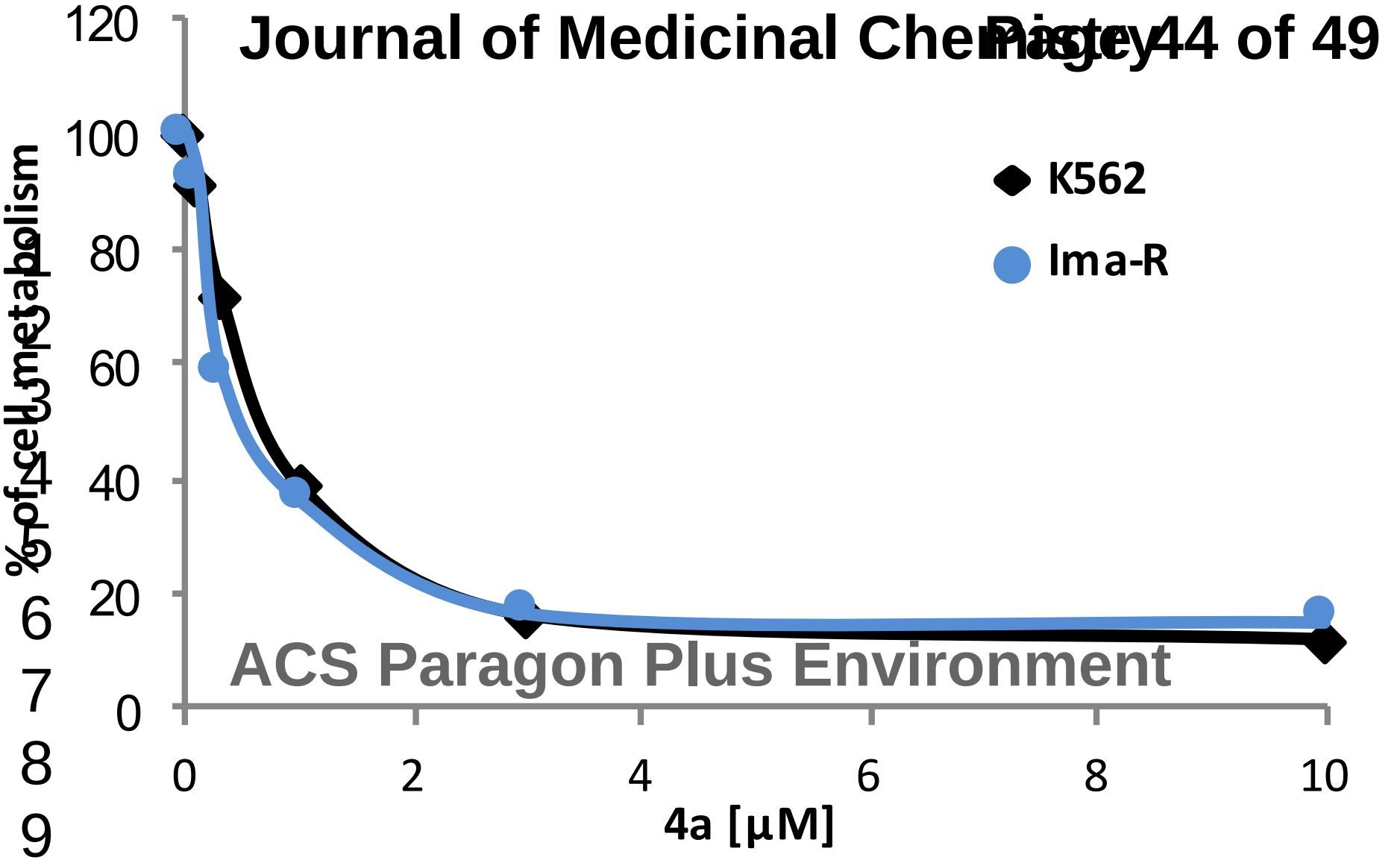
4p: R^1 = isopropylidene, R^2 = H
4q: R^1 = R^2 = Ac

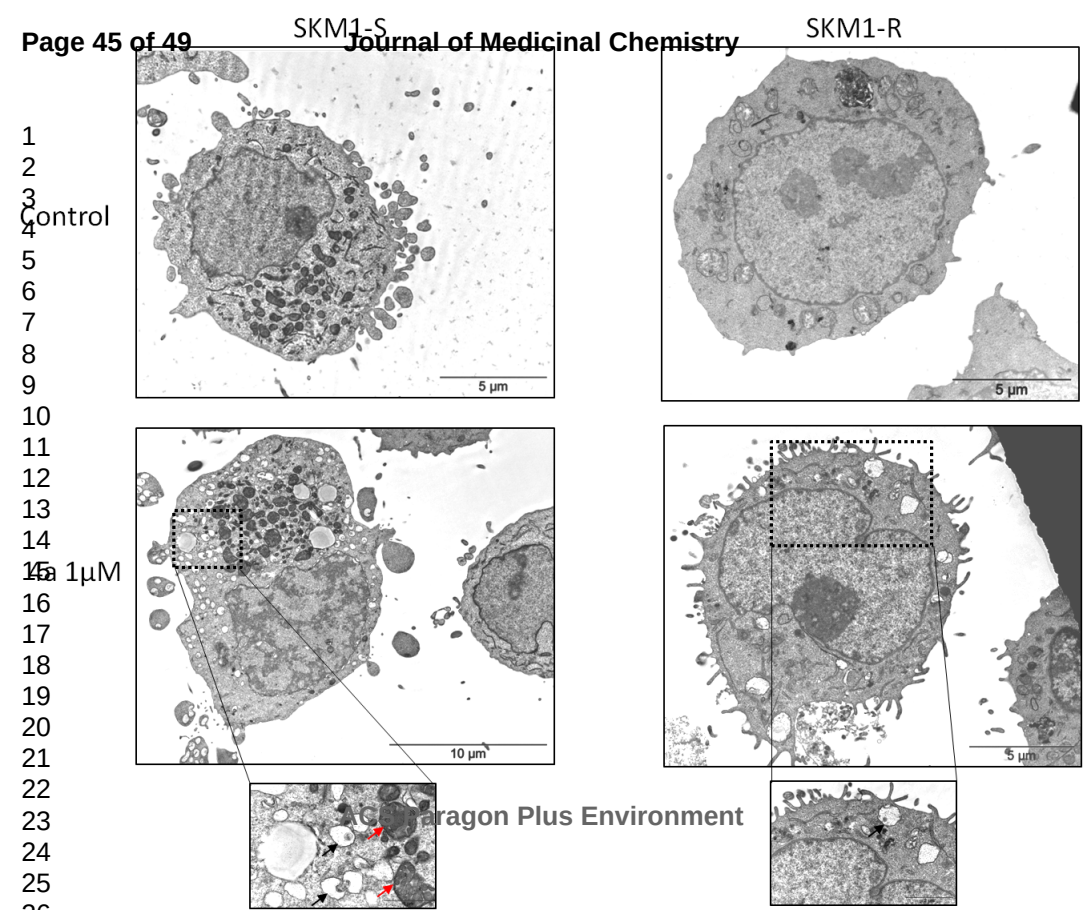


4o

Scheme 6. Synthesis of triazoles 4a-4q through a CuAAC/oxidative coupling reaction.

180x263mm (300 x 300 DPI)


% of cell metabolism
120
100
80
60
40
20
0
0
2
4
6
8
10
4a [μM]
K562
Ima-R
ACS Paragon Plus Environment



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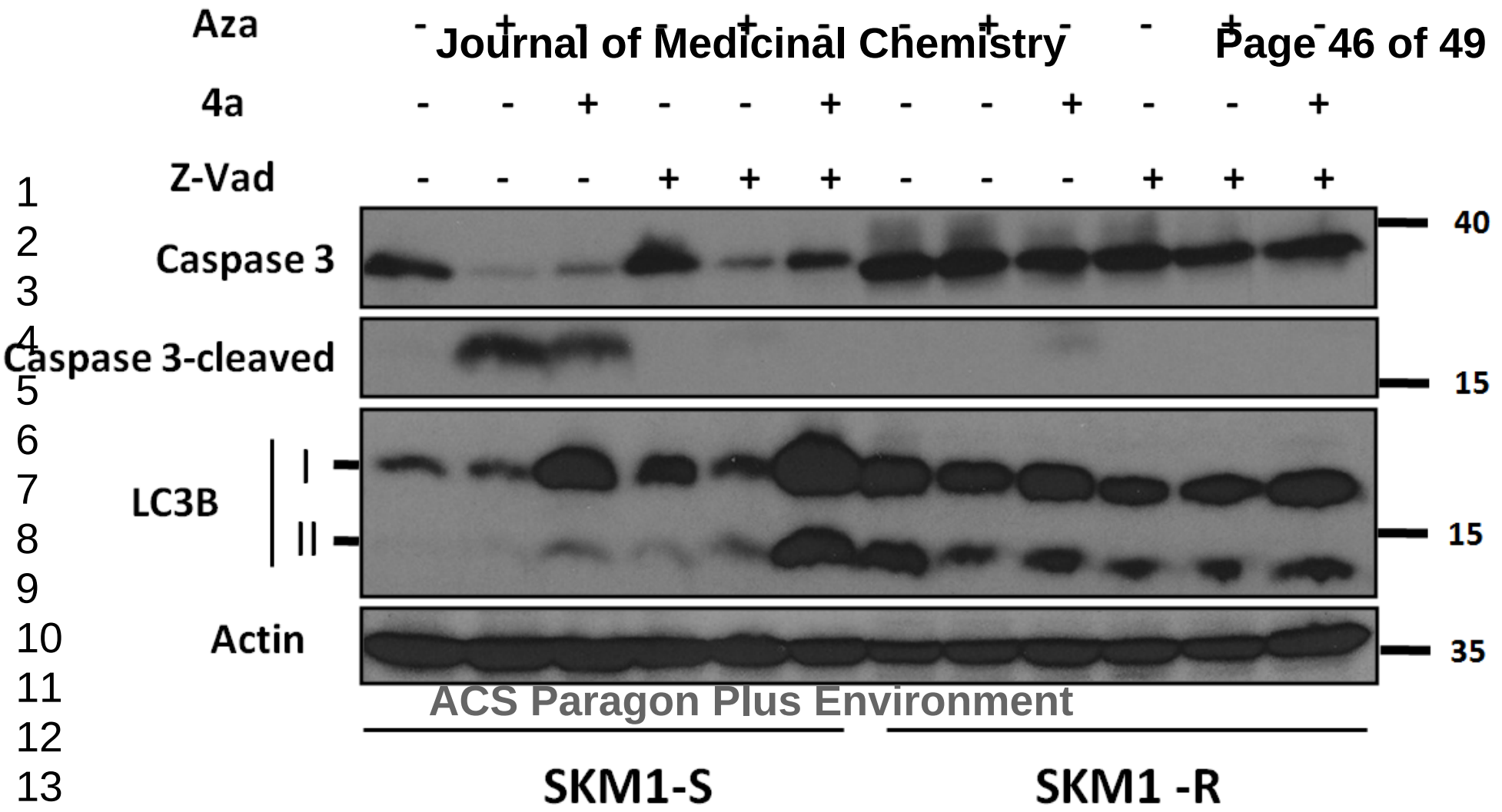

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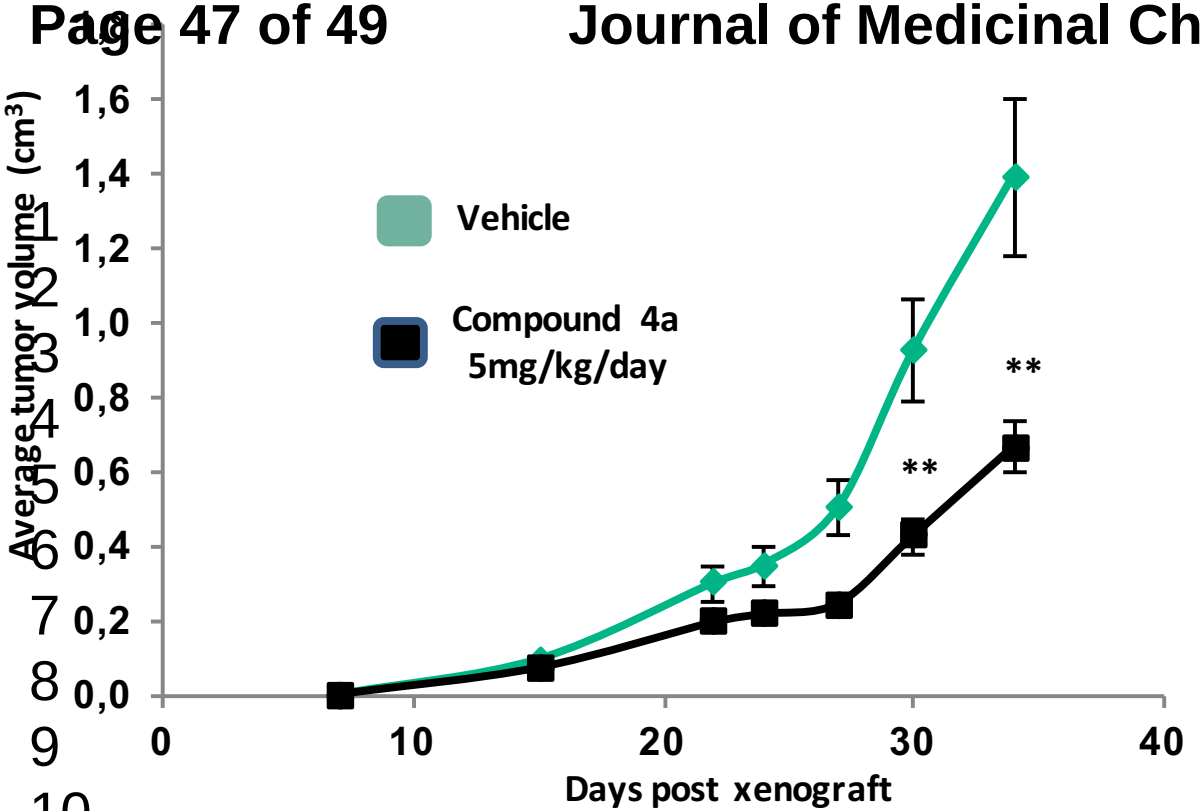

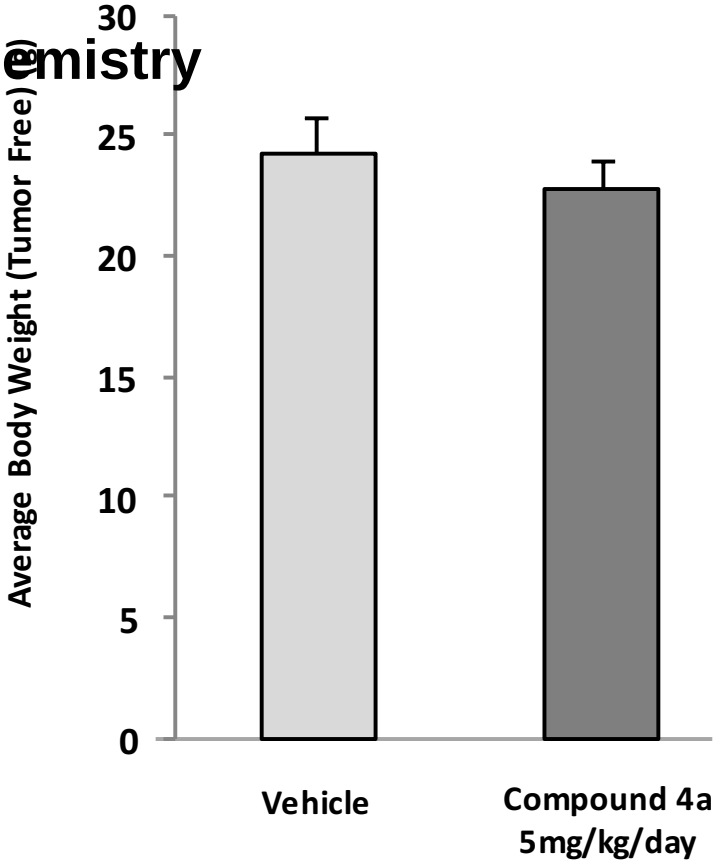

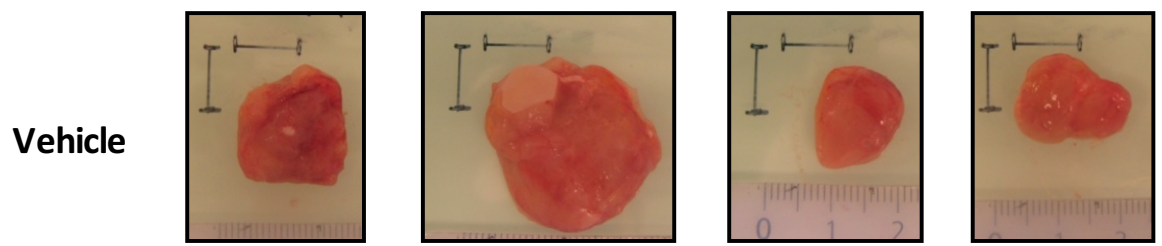

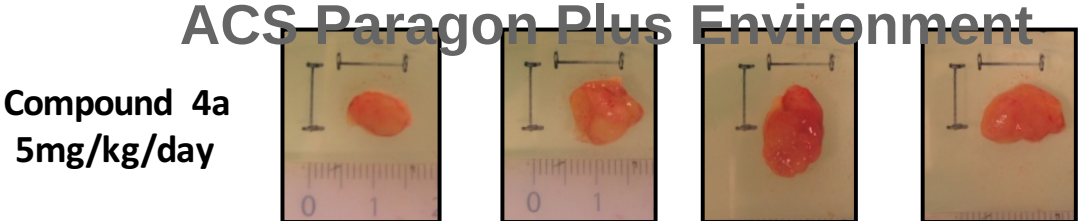

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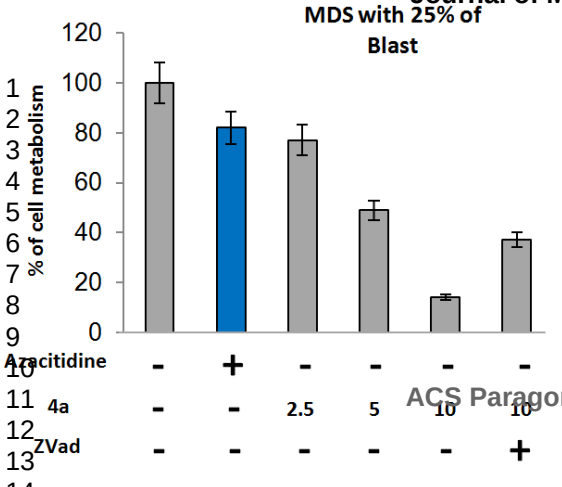

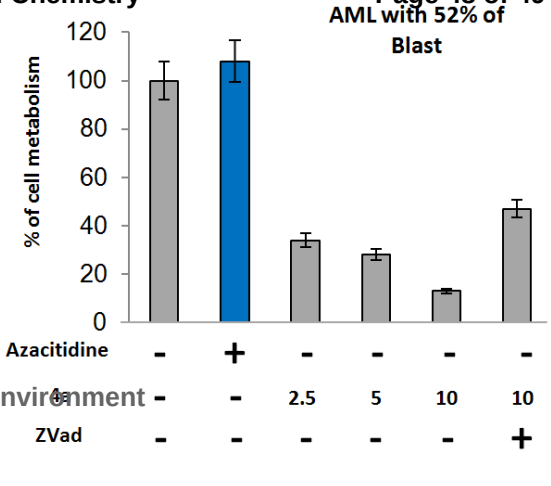

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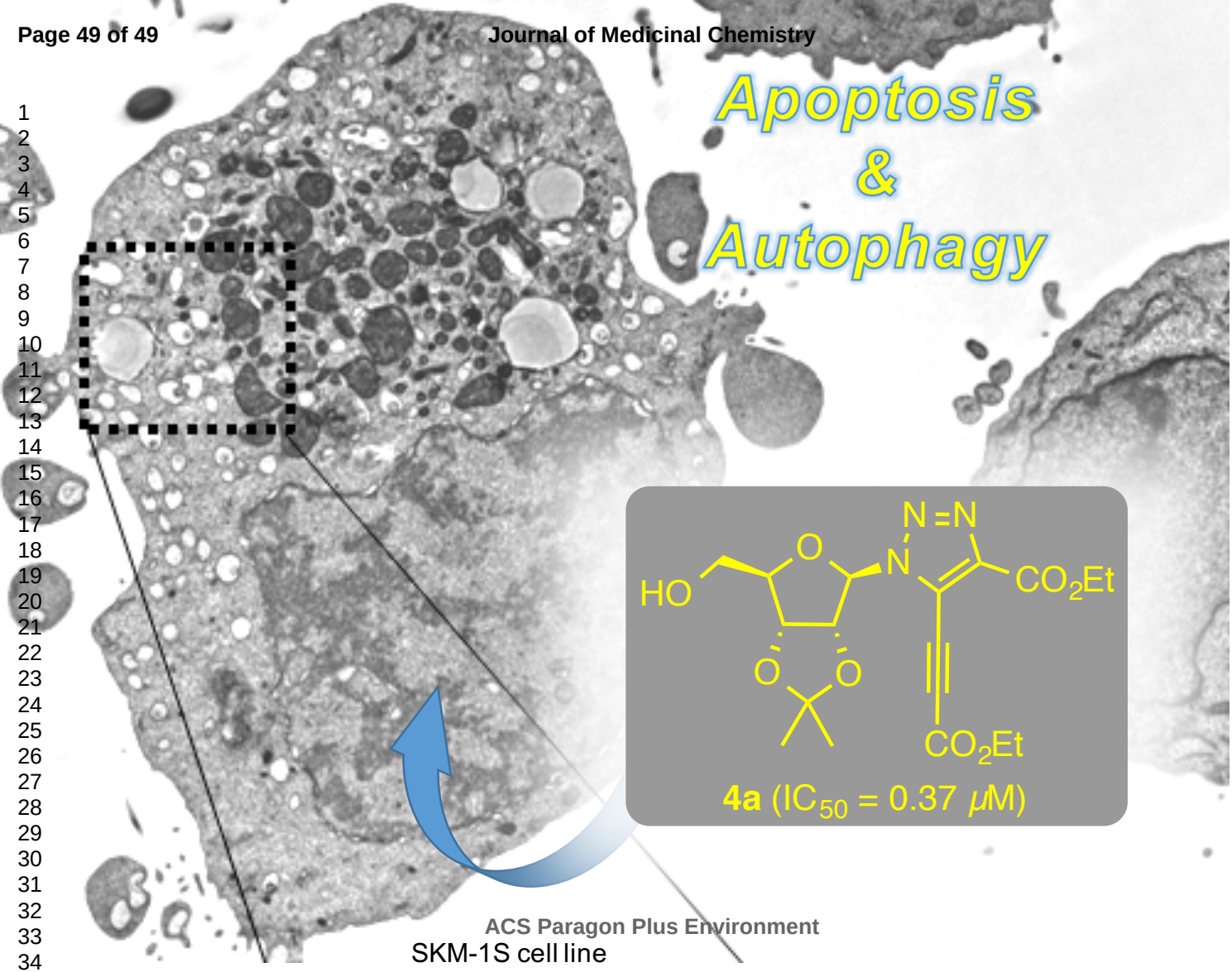
Apoptosis
&
Autophagy
HO
O
N
N=N
CO2Et
O
O
CO2Et
4a (IC50 = 0.37 μM)
SKM-1S cell line