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Green solvent-free synthesis of new *N*-heterocycle-L-ascorbic acid hybrids and their antiproliferative evaluation

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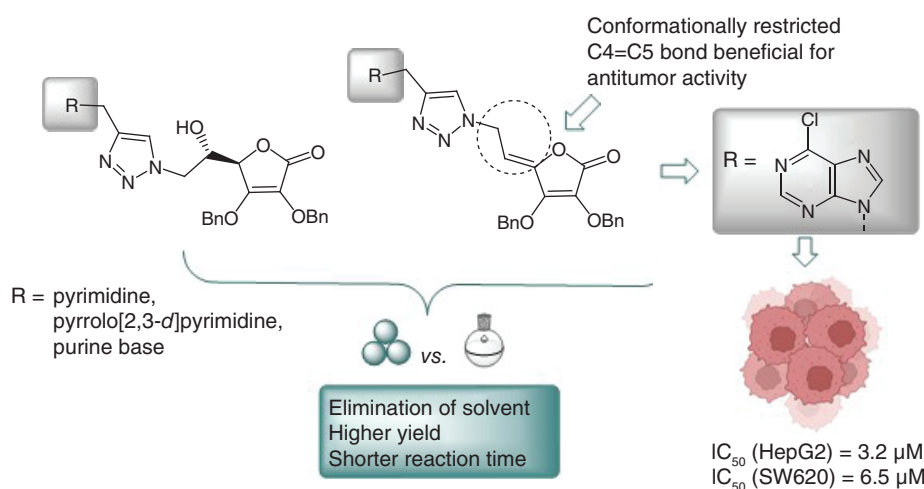
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Aim: The authors' aim was to improve the application of copper-catalyzed azide-alkyne cycloaddition in the synthesis of hybrids containing biologically significant nucleobases and L-ascorbic acid scaffolds by introducing an environmentally friendly and waste-free ball mill. **Results:** Two series of hybrids with a purine, pyrrolo[2,3-*d*]pyrimidine or 5-substituted pyrimidine attached to 2,3-dibenzyl-L-ascorbic acid via a hydroxyethyl- (**15a–23a**) or ethylidene-1,2,3-triazolyl (**15b–23b**) bridge were prepared by ball milling and conventional synthesis. The unsaturated 6-chloroadenine L-ascorbic acid derivative **16b** can be highlighted as a lead compound and showed strong antiproliferative activity against HepG2 (hepatocellular carcinoma) and SW620 (colorectal adenocarcinoma) cells. **Conclusion:** Mechanochemical synthesis was superior in terms of sustainability, reaction rate and yield, highlighting the advantageous applications of ball milling over classical reactions.

Graphical abstract:



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Keywords: 1,2,3-triazole • antitumor activity • CuAAC • green chemistry • L-ascorbic acid • mechanochemistry • nucleobase • purine • pyrimidine

To address the need for environmentally acceptable greener and sustainable development [1,2], mechanochemistry has emerged as a powerful technique with the potential to revolutionize the chemical and pharmaceutical

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industries [3–5]. Mechanochemical processes are performed by grinding, shearing, pulling or milling reagents [6], thereby avoiding potentially harmful and polluting organic solvents, minimizing waste production and improving safety [7,8]. Compared with conventional solution-based protocols, it offers several other advantages, such as shorter reaction time, improved yield, facilitation of reactions with poorly soluble reagents, changes in reactivity and selectivity and more efficient energy transfer to the reaction [9–13]. Recognizing the benefits of this method, in 2019 IUPAC included mechanochemistry in its list of ten chemical innovations that could change the world and have the potential to make our planet more sustainable [14,15]. Recent reports have demonstrated the application of mechanochemical ball milling in the synthesis of active pharmaceutical ingredients, such as silver sulfadiazine and dantrolene [16], hydantoin-based active pharmaceutical ingredients [17], tolbutamide, chlorpropamide, glibenclamide [18] and bismuth subsalicylate [19], opening new perspectives for the sustainable preparation of biologically significant scaffolds for the pharmaceutical industry [20,21].

The beneficial effects using solvent-free ball milling conditions have been described for a number of reactions involving the formation of new C–C, C–N, C–O and C–X bonds [22–28]. Copper-catalyzed azide-alkyne cycloaddition (CuAAC), which enables the formation of 1,4-disubstituted 1,2,3-triazoles [29–31], has proved to be irreplaceable in drug discovery, polymer chemistry, biochemistry, nanotechnology and material science [32,33]. Considering the importance of this reaction in organic synthesis, significant efforts have been made to increase its sustainability and to introduce mechanochemistry into CuAAC reactions using both standard catalytic systems and heterogeneous copper(0) catalysts, either as copper milling vessels or copper powder [34–39].

Moreover, the CuAAC reaction has been used for the synthesis of biologically important L-ascorbic acid derivatives, which showed antitumor and antiviral activity *in vitro* [40]. L-ascorbic acid (vitamin C) has attracted the interest of the scientific community for decades, which has led to a continuous increase in novel vitamin C derivatives with diverse pharmacological properties [41,42]. Vitamin C is a co-factor for a number of enzymes, including the Fe(II)- and α -ketoglutarate-dependent dioxygenases and is therefore involved in epigenetic regulation of gene activity [43,44]. At high concentrations, ascorbic acid has a selective toxic effect on carcinoma with *KRAS* or *BRAF* mutations by participating in cellular processes that lead to the inhibition of glycolysis and decreased adenosine triphosphate levels, causing cell death [45]. Ascorbic acid was also found to disrupt the redox balance in liver cancer cells (HepG2), leading to intracellular reductive stress and induction of apoptotic pathway [46].

In the authors' previous study [47–50], they found that nucleoside derivatives with pyrimidine or purine bases linked to 2,3-di-*O*-benzyl-L-ascorbic acid via hydroxyethyl- or ethylidene-linker showed marked antiproliferative activity against a number of tumor cells and an antiviral effect on varicella-zoster virus and cytomegalovirus (**Ia**, **Ib** and **IIa**, **IIb**, Figure 1). In addition, C-6-1,2,3-triazolyl-2,3-dibenzyl-L-ascorbic acid derivatives with aromatic and aliphatic fragments attached to the C-4 position of 1,2,3-triazole (**IIIa** and **IIIb**, Figure 1) showed antitumor activity against a variety of tumor cells [40]. Among them, a compound with a decyl substituent showed selective activity against breast cancer (MCF-7) cells in the nanomolar range without being toxic to normal cells. *p*-Bromophenyl- and *p*-pentylphenyl-substituted 1,2,3-triazolyl-L-ascorbic acid conjugates with free C-2 and C-3 hydroxyl groups had a selective cytotoxic effect on MCF-7 cells [51].

Although the application of mechanochemistry is an innovative and rapidly growing field in medicinal chemistry, to the authors' knowledge, ball milling has not yet been used for the synthesis of biologically relevant L-ascorbic acid derivatives. Considering the advantages of mechanochemistry over conventional synthesis and continuing their research on 1,2,3-triazolyl appended *N*-heterocycles, the authors' goal was to improve the applicability of CuAAC in the synthesis of *N*-heterocycle-L-ascorbic acid hybrids by introducing ball milling. Here the authors compare the sustainability, yield and reaction rate of the environmentally friendly solvent-free and CuAAC reaction in solution, which allows attachment of the nucleoside base to the vitamin C moiety via 1,2,3-triazole and yields two series of conformationally unrestricted hybrid molecules (**15a–23a**) and their unsaturated counterparts (**15b–23b**) (Figure 1).

Based on the authors' previous research on *N*-heterocycle-L-ascorbic acid conjugates (Figure 1) [40,47–50], they hypothesized that these new purine-, pyrrolo[2,3-*d*]pyrimidine- and 5-substituted pyrimidine-L-ascorbic acid hybrids (**15a–23a** and **15b–23b**) will exhibit antitumor activity.

Experimental section

General remarks

Solvents and reagents were purchased from commercial suppliers and used without additional purification. Progress of the reactions was monitored by thin-layer chromatography using precoated Merck (Darmstadt, Germany) silica

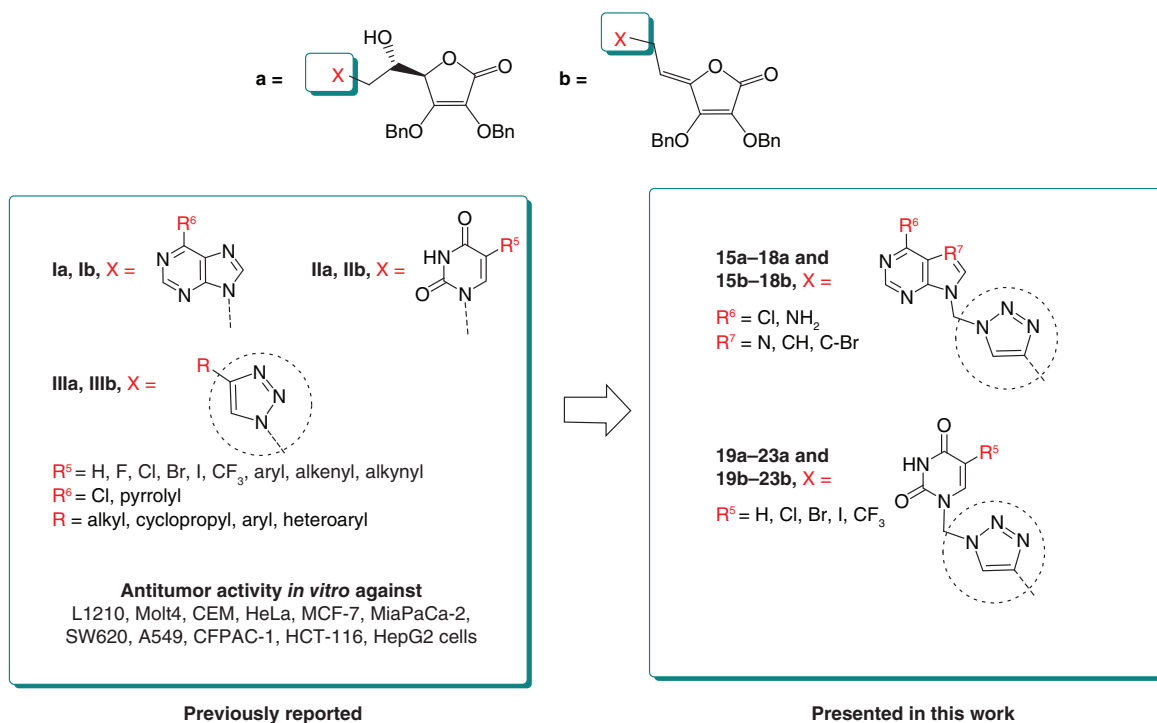


Figure 1. Design of *N*-heterocycle-L-ascorbic acid hybrids (15a–23a, 15b–23b) based on previously reported purine- (Ia, Ib), pyrimidine- (IIa, IIb) and 1,2,3-triazolyl (IIIa, IIb)-L-ascorbic acid derivatives with antitumor activity.

gel 60F-254 plates, and UV light (254 and 366 nm) was used for the detection of spots. Silica gel (0.063–0.2 mm) Fluka (Buchs, Switzerland) was used for column chromatography and glass columns were slurry-packed under gravity. ¹H and ¹³C NMR spectra were recorded on a Bruker 300 and 600 MHz NMR spectrometer (Bruker Biospin, Rheinstetten, Germany) at 298 K in DMSO-*d*₆. Chemical shifts were referenced to the DMSO solvent signal at δ 2.50 ppm for ¹H and δ 39.50 ppm for ¹³C NMR. Melting points were determined with Kofler hot-stage microscopy (Reichert, Wien, Austria). Mechanochemical reactions were performed in a polytetrafluoroethylene reaction vessel using stainless steel balls and mixer mill IST500 (InSolido Tehnologies, Zagreb, Croatia) operating at 30 Hz.

Experimental procedures for the synthesis of compounds

Precursors **1–3** [52], **4a** [53], **4b** [49], **5a** [40], **5b** [54], **6** [55], **7–9** [56], **10–13** [55] and **14** [57] for the synthesis of targeted derivatives were synthesized in accordance with the procedures in the literature, and the general procedure is given in the Supplementary Information.

General procedure for synthesis of hybrids 15a–23a & 15b–23b

Method A: the mechanochemical copper-catalyzed azide-alkyne cycloaddition reaction.

Azide (**5a** or **5b**, 1 equiv.), *N,N*-diisopropylethylamine (0.1 equiv.) and acetic acid (0.1 equiv.) were added in one-half of the reaction vessel and the other half was filled with the corresponding propargylated purine, pyrrolo[2,3-*d*]pyrimidine or pyrimidine base (**6–14**; 1 equiv.), copper(I) iodide (0.02 equiv.) and two 7 mm-diameter stainless steel balls. The vessel was sealed and positioned in the ball mill. The reaction mixture was ground for 3.5 h at 30 Hz and then purified by column chromatography using silica gel and CH₂Cl₂:methanol = 50:1 as the mobile phase. Compounds **15a–23a** and **15b–23b** were isolated as white solids.

Method B: the conventional copper-catalyzed azide-alkyne cycloaddition reaction in solution

The propargylated purine, pyrrolo[2,3-*d*]pyrimidine or pyrimidine base (**6–14**; 1.2 equiv.) and Cu(OAc)₂ (0.05 equiv.) were added to a solution of the corresponding azide (**5a** or **5b**; 1 equiv.) in methanol (5–10 ml). The reaction mixture was stirred at room temperature for 24 h. After the reaction was completed, the solvent was removed by vacuum evaporation. The remaining product was purified by column chromatography using silica gel

and CH₂Cl₂:methanol = 50:1 as the mobile phase. Compounds **15a–23a** and **15b–23b** were isolated as white solids.

*Preparation of 6-{4-[(6-amino-9H-purin-9-yl)methylene]-1,2,3-triazol-1-yl}-2,3-O,O-dibenzyl-L-ascorbic acid (**15a**)*

Compound **15a** (method A: 65.8 mg, 91%; method B: 203.8 mg, 71%; melting point [mp]: 113–115°C) was synthesized following the general procedure from compound **5a** (method A: 50.0 mg, 0.13 mmol; method B: 199.1 mg, 0.52 mmol) and compound **6** (method A: 22.5 mg, 0.16 mmol; method B: 100.00 mg, 0.58 mmol).

*Preparation of 2,3-O,O-dibenzyl-6-{4-[(6-chloro-9H-purin-9-yl)methylene]-1,2,3-triazol-1-yl}-L-ascorbic acid (**16a**)*

Compound **16a** (method A: 65.2 mg, 87%; method B: 365.3 mg, 68%; mp: 106–109°C) was synthesized following the general procedure from compound **5a** (method A: 50.0 mg, 0.13 mmol; method B: 357.0 mg, 0.94 mmol) and compound **7** (method A: 25.0 mg, 0.16 mmol; method B: 200.0 mg, 1.04 mmol).

*Preparation of 2,3-O,O-dibenzyl-6-{4-[(4-chloro-7H-pyrrolo[2,3-d]pyrimidine-7-yl)methylene]-1,2,3-triazol-1-yl}-L-ascorbic acid (**17a**)*

Compound **17a** (method A: 65.3 mg, 88%; method B: 273.3 mg, 68%; mp: 84–87°C) was synthesized following the general procedure from compound **5a** (method A: 50.0 mg, 0.13 mmol; method B: 357.0 mg, 0.94 mmol) and compound **8** (method A: 24.9 mg, 0.16 mmol; method B: 200.0 mg, 1.04 mmol).

*Preparation of 2,3-O,O-dibenzyl-6-{4-[(5-bromo-4-chloro-7H-pyrrolo[2,3-d]pyrimidine-7-yl)methylene]-1,2,3-triazol-1-yl}-L-ascorbic acid (**18a**)*

Compound **18a** (method A: 83.1 mg, 98%; method B: 432.0 mg, 79%; mp: 146–149°C) was synthesized following the general procedure from compound **5a** (method A: 50.0 mg, 0.13 mmol; method B: 319.2 mg, 0.84 mmol) and compound **9** (method A: 35.2 mg, 0.16 mmol; method B: 200.0 mg, 0.93 mmol).

*Preparation of 2,3-O,O-dibenzyl-6-{4-[(pyrimidine-2,4-dione-1-yl)methylene]-1,2,3-triazol-1-yl}-L-ascorbic acid (**19a**)*

Compound **19a** (method A: 64.7 mg, 94%; method B: 62.9 mg, 91%; mp: 119–122°C) was synthesized following the general procedure from compound **5a** (50.0 mg, 0.13 mmol) and compound **10** (21.8 mg, 0.16 mmol).

*Preparation of 2,3-O,O-dibenzyl-6-{4-[(5-chloropyrimidine-2,4-dione-1-yl)methylene]-1,2,3-triazol-1-yl}-L-ascorbic acid (**20a**)*

Compound **20a** (method A: 70.1 mg, 95%; method B: 49.2 mg, 66%; mp: 124–126°C) was synthesized following the general procedure from compound **5a** (50.0 mg, 0.13 mmol) and compound **11** (27.3 mg, 0.16 mmol).

*Preparation of 2,3-O,O-dibenzyl-6-{4-[(5-bromopyrimidine-2,4-dione-1-yl)methylene]-1,2,3-triazol-1-yl}-L-ascorbic acid (**21a**)*

Compound **21a** (method A: 68.4 mg, 86%; method B: 61.1 mg, 77%; mp: 116–120°C) was synthesized following the general procedure from compound **5a** (50.0 mg, 0.13 mmol) and compound **12** (34.4 mg, 0.16 mmol).

*Preparation of 2,3-O,O-dibenzyl-6-{4-[(5-iodopyrimidine-2,4-dione-1-yl)methylene]-1,2,3-triazol-1-yl}-L-ascorbic acid (**22a**)*

Compound **22a** (method A: 75.5 mg, 88%; method B: 74.0 mg, 86%; mp: 118–121°C) was synthesized following the general procedure from compound **5a** (50.0 mg, 0.13 mmol) and compound **13** (41.8 mg, 0.16 mmol).

*Preparation of 2,3-O,O-dibenzyl-6-{4-[(5-methylpyrimidine-2,4-dione-1-yl)methylene]-1,2,3-triazol-1-yl}-L-ascorbic acid (**23a**)*

Compound **23a** (method A: 68.7 mg, 97%; method B: 68.1 mg, 96%; mp: 94–97°C) was synthesized following the general procedure from compound **5a** (50.0 mg, 0.13 mmol) and compound **14** (25.5 mg, 0.7 mmol).

Preparation of (Z)-2,3-O,O-dibenzyl-6-{4-[(6-amino-9H-purin-9-yl)methylene]-1,2,3-triazol-1-yl}-4,5-didehidro-5,6-didehydroxy-L-ascorbic acid (15b)

Compound **15b** (method A: 62.4 mg, 85%; method B: 48.5 mg, 66%; mp: 90–93°C) was synthesized following the general procedure from compound **5b** (50.0 mg, 0.14 mmol) and compound **6** (29.09 mg, 0.17 mmol).

Preparation of (Z)-2,3-O,O-dibenzyl-6-{4-[(6-chloro-9H-purin-9-yl)methylene]-1,2,3-triazol-1-yl}-4,5-didehidro-5,6-didehydroxy-L-ascorbic acid (16b)

Compound **16b** (method A: 78.8 mg, 98%; method B: 58.8 mg, 77%; mp: 63–67°C) was synthesized following the general procedure from compound **5b** (50.0 mg, 0.14 mmol) and compound **7** (26.3 mg, 0.14 mmol).

Preparation of

(Z)-2,3-O,O-dibenzyl-6-{4-[(4-chloro-7H-pyrrolo[2,3-d]pyrimidine-7-yl)methylene]-1,2,3-triazol-1-yl}-4,5-didehidro-5,6-didehydroxy-L-ascorbic acid (17b)

Compound **17b** (method A: 67.3 mg, 89%; method B: 67.1 mg, 88%; mp: 56–60°C) was synthesized following the general procedure from compound **5b** (50.0 mg, 0.14 mmol) and compound **8** (26.1 mg, 0.17 mmol).

Preparation of

(Z)-2,3-O,O-dibenzyl-6-{4-[(5-bromo-4-chloro-7H-pyrrolo[2,3-d]pyrimidine-7-yl)methylene]-1,2,3-triazol-1-yl}-4,5-didehidro-5,6-didehydroxy-L-ascorbic acid (18b)

Compound **18b** (method A: 85.0 mg, 98%; method B: 83.2 mg, 95%; mp: 115–119°C) was synthesized following the general procedure from compound **5b** (50.0 mg, 0.14 mmol) and compound **9** (46.0 mg, 0.17 mmol).

Preparation of (Z)-2,3-O,O-dibenzyl-4,5-didehidro-5,6-didehydroxy-6-{4-[(pyrimidine-2,4-dione-1-yl)methylene]-1,2,3-triazol-1-yl}-L-ascorbic acid (19b)

Compound **19b** (method A: 64.3 mg, 91%; method B: 62.3 mg, 88%; mp: 143–145°C) was synthesized following the general procedure from compound **5b** (50.0 mg, 0.14 mmol) and compound **10** (23.1 mg, 0.17 mmol).

Preparation of (Z)-2,3-O,O-dibenzyl-6-{4-[(5-chloropyrimidine-2,4-dione-1-yl)methylene]-1,2,3-triazol-1-yl}-4,5-didehidro-5,6-didehydroxy-L-ascorbic acid (20b)

Compound **20b** (method A: 67.5 mg, 88%; method B: 64.2 mg, 85%; mp: 159–162°C) was synthesized following the general procedure from compound **5b** (50.0 mg, 0.14 mmol) and compound **11** (31.4 mg, 0.17 mmol).

Preparation of (Z)-2,3-O,O-dibenzyl-6-{4-[(5-bromopyrimidine-2,4-dione-1-yl)methylene]-1,2,3-triazol-1-yl}-4,5-didehidro-5,6-didehydroxy-L-ascorbic acid (21b)

Compound **21b** (method A: 73.5 mg, 90%; method B: 70.0 mg, 86%; mp: 162–164°C) was synthesized following the general procedure from compound **5b** (50.0 mg, 0.14 mmol) and compound **12** (36.6 mg, 0.17 mmol).

Preparation of

(Z)-2,3-O,O-dibenzyl-4,5-didehidro-5,6-didehydroxy-6-{4-[(5-iodopyrimidine-2,4-dione-1-yl)methylene]-1,2,3-triazol-1-yl}-L-ascorbic acid (22b)

Compound **22b** (method A: 70.0 mg, 80%; method B: 68.7 mg, 78%; mp: 90–93°C) was synthesized following the general procedure from compound **5b** (50.0 mg, 0.14 mmol) and compound **13** (43.9 mg, 0.17 mmol).

Preparation of (Z)-2,3-O,O-dibenzyl-4,5-didehidro-5,6-dideoxy-6-{4-[(5-methylpyrimidin-2,4-dione-1-yl)methyl]-1,2,3-triazol-1-yl}-L-ascorbic acid (23b)

Compound **23b** (method A: 63.0 mg, 87%; method B: 56.3 mg, 78%; mp: 77–80°C) was synthesized following the general procedure from compound **5b** (50.0 mg, 0.14 mmol) and compound **14** (25.5 mg, 0.17 mmol).

Cell culturing

The cell lines A549 (lung adenocarcinoma), CFPAC-1 (ductal pancreatic adenocarcinoma), HCT-116 (colorectal carcinoma), HeLa, HepG2, MCF-7 and SW620 were purchased from the American Type Culture Collection (VA, USA) and cultured as monolayers. They were allowed to proliferate in Dulbecco's modified Eagle medium in a humidified atmosphere at 37°C with 5% CO₂; 10% fetal bovine serum, 2 mM L-glutamine, 100 U/ml penicillin and 100 µg/ml streptomycin were added to Dulbecco's modified Eagle medium.

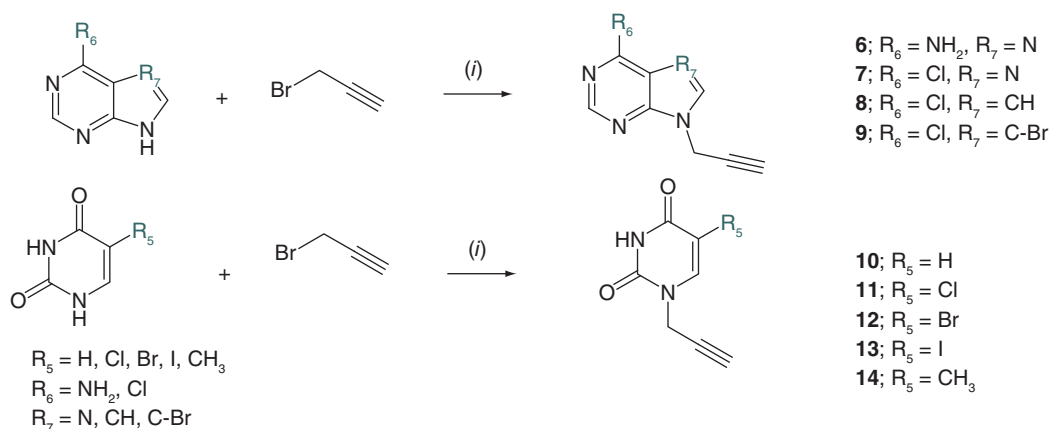


Figure 2. Synthesis of *N*-propargylated purines (**6** and **7**), pyrrolo[2,3-*d*]pyrimidines (**8** and **9**) and pyrimidine-2,4-diones (**10–14**) by propargylation of the corresponding base. Reagents and conditions: (i) sodium hydride, dimethylformamide, 24 h, room temperature.

Proliferation assay

The cells were seeded onto 96-well microtiter plates at a density of 3000 cells/well for carcinoma cell lines and 5000 cells/well for normal human fibroblasts. After 24 h, the cells were treated with five different concentrations (0.01–100 μm) of test compounds and further incubated for 72 h. The cytotoxic effect of DMSO (solvent) did not exceed 0.1%. After the incubation was completed, the MTT assay was performed and the percentage of cell growth was calculated from the measured absorbances [58]. Experiments were performed three-times for each compound and concentration. IC_{50} values were calculated using linear regression analysis.

Results & discussion

Chemistry

Novel purine, pyrrolo[2,3-*d*]pyrimidine and pyrimidine-6-(1,2,3-triazolyl)-6-deoxy- (**15a–23a**) and unsaturated 6-(1,2,3-triazolyl)-4,5-didehydro-5,6-dideoxy-L-ascorbic acid (**15b–23b**) derivatives were synthesized according to the procedures shown in Figures 2 & 3. The *N*-propargylated purines (**6** and **7**), pyrrolo[2,3-*d*]pyrimidines (**8** and **9**) and pyrimidines (**10–14**) were prepared by *N*-alkylation of the corresponding base with propargyl bromide and sodium hydride as deprotonating agent (Figure 2) [55,57,59].

First, the hydroxyl groups of L-ascorbic acid were selectively protected and deprotected to obtain the desired C-6 azido-L-ascorbic acid derivatives (**5a** and **5b**) [40,49,52–54]. The 5-OH and 6-OH groups of L-ascorbic acid were protected using AcCl and acetone, and the isopropylidene derivative **1** was isolated. Selective benzylation of the remaining 2-OH and 3-OH groups of **1** with BnCl and K_2CO_3 afforded compound **2**. After removal of isopropylidene protection under acidic conditions, compound **3** was isolated and the reaction of compound **3** with tosyl chloride gave mono- (**4a**) and ditosyl (**4b**) derivatives. The nucleophilic substitution reaction of NaN_3 with **4a** gave the desired 6-azido-6-deoxy-2,3-dibenzyl-L-ascorbic acid derivative (**5a**). Reaction of NaN_3 with ditosyl **4b** resulted in the formation of a C4=C5 double bond and the C-6-azido-4,5-didehydro-5,6-dideoxy-2,3-dibenzyl-L-ascorbic acid derivative (**5b**) was obtained. The (*Z*)-configuration of the C4=C5 bond was confirmed by the 2D rotating-frame Overhauser effect spectroscopy NMR spectrum (Supplementary Figure 19), in which a cross signal between the methylene proton of the OCH_2Ph benzyl group at C-3 of the lactone ring and the methane proton at C-5 was observed. These findings are consistent with the observations described for related purine and pyrimidine derivatives of L-ascorbic acid [47,54].

The target nucleoside mimetics **15a–23a** and **15b–23b** were prepared by copper-catalyzed azide-alkyne cycloaddition using azido-ascorbic acid analogs (**5a** or **5b**) and the corresponding *N*-propargylated bases (**6–14**) (Figure 2), employing both green chemistry mechanochemical reactions in a ball mill with copper(I) iodide (CuI)/acetic acid (HOAc)/*N,N*-diisopropylethylamine (DIPEA) (method A) and conventional solution-based click reactions with $\text{Cu}(\text{OAc})_2$ and methanol catalytic system (method B). A synthetic method using a mixture of water and organic solvents that was also applied in the synthesis of structurally related L-ascorbic acid derivatives [40,60] did

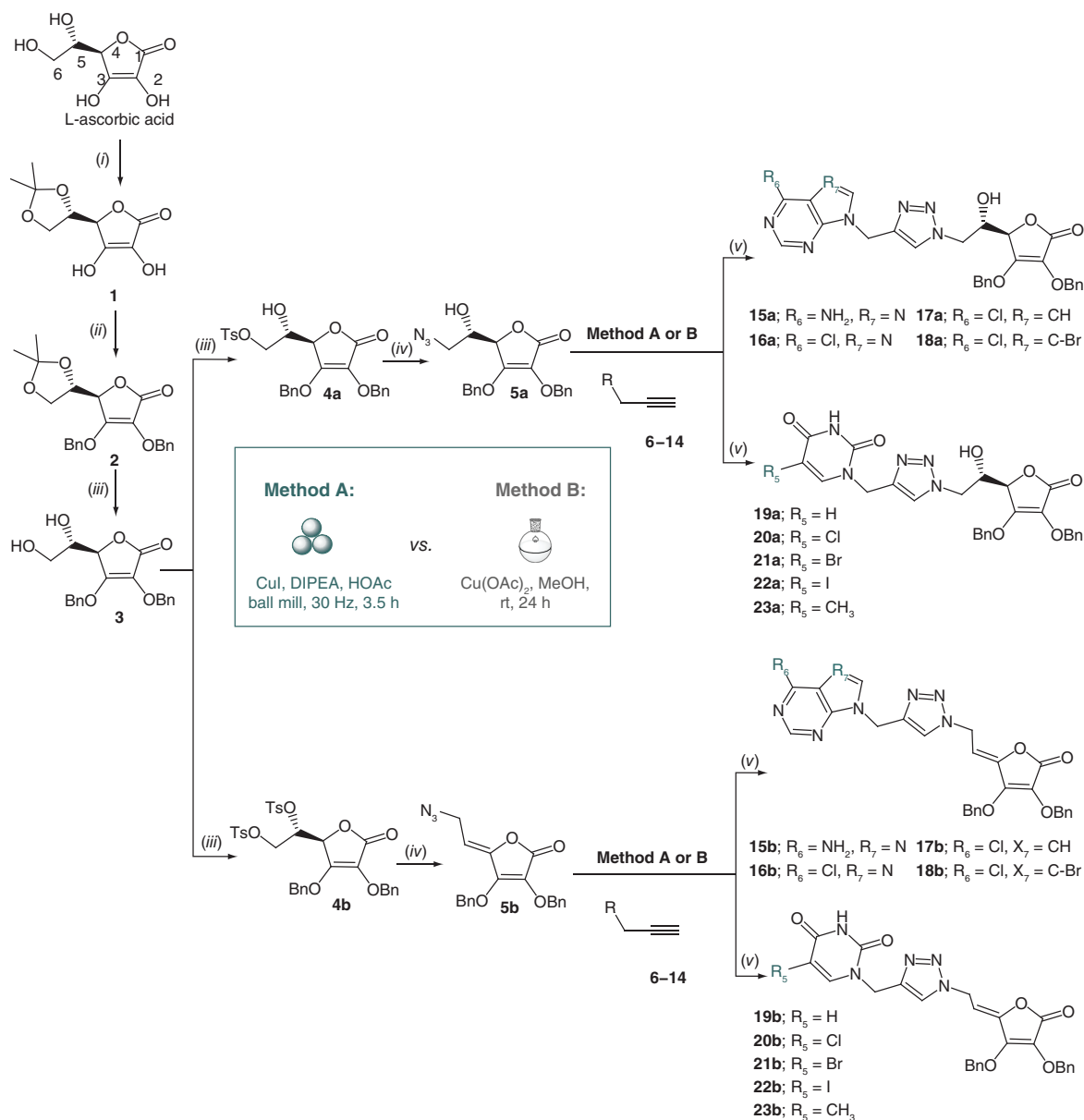


Figure 3. Synthesis of purine, pyrrolo[2,3-*d*]pyrimidine and pyrimidine-2,4-dione derivatives of 6-(1,2,3-triazolyl)-6-deoxy-L-ascorbic acid (**15a–23a**) and their unsaturated analogs (**15b–23b**). Reagents and conditions: (i) acetyl chloride, acetone, 4 h, room temperature; (ii) benzyl chloride, potassium carbonate, dimethylformamide, 60°C, overnight; (iii) acetic acid, methanol, 100°C, 24 h; (iv) *p*-toluenesulfonyl chloride, dichloromethane, pyridine, 0–25°C, 24 h; (v) method A: *N,N*-diisopropylethylamine, acetic acid, copper(I) iodide, mill, two stainless steel milling balls, polytetrafluoroethylene, 30 Hz, 3.5 h; method B: copper(II) acetate, methanol, room temperature, 24 h. rt: Room temperature.

not afford improved yields, so the Cu(OAc)₂/methanol system, in which methanol is both a solvent and a reducing agent, was chosen for the conventional click reactions.

Optimization of reaction parameters for the synthesis of compounds **22a** & **22b** by the mechanochemical click reaction

The model copper-catalyzed azide-alkyne cycloaddition reaction for the synthesis of compound **22a** and its C4=C5 unsaturated counterpart, **22b**, was conducted by grinding *N*-1 propargylated 5-iodouracil (**13**), the corresponding C-6-azido-L-ascorbic acid (**5a** or **5b**) and the copper catalyst in a ball mill with two stainless steel milling balls

Table 1. Comparison of yields in the preparation of compounds **22a** and **22b** by the solvent-free mechanochemical click reactions using two catalytic systems.

Catalytic system	Yield (%)	
	22a	22b
Cu(OAc) ₂ /Na ascorbate	54	39
Copper(I) iodide/ <i>N,N</i> -diisopropylethylamine/acetic acid	88	80

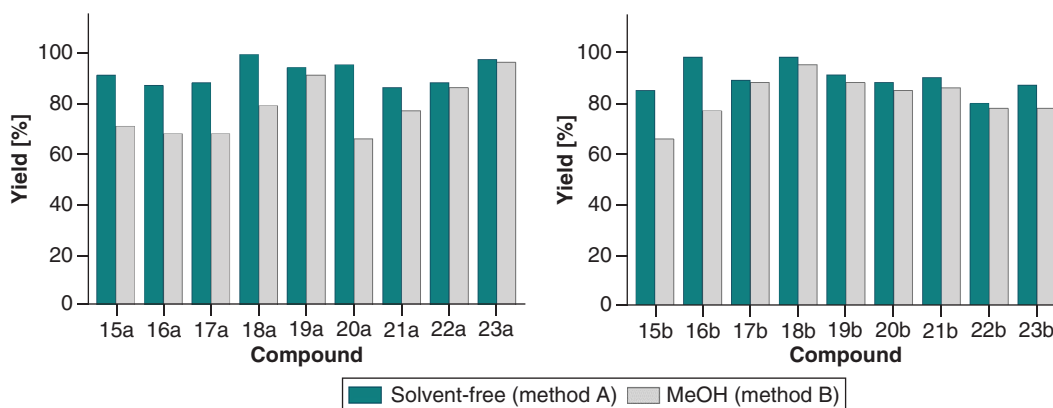


Figure 4. Comparison of yields obtained by solvent-free (method A) and the conventional click reaction in methanol (method B) in the preparation of compounds **15a–23a** (A) and their unsaturated counterparts **15b–23b** (B).

(7 mm), in a polytetrafluoroethylene vessel at 30 Hz. The influence of the two catalytic systems was investigated and the comparative results are summarized in Table 1.

The reactions catalyzed by the CuI/HOAc/DIPEA showed significantly better yields (88% and 80%) within 3.5 h of milling compared with reactions with Cu(OAc)₂/sodium ascorbate (54% and 39%) (Table 1), in which the required Cu(I) catalyst is generated *in situ* by the reduction of a Cu(II) salt with sodium ascorbate.

Therefore, the CuI/DIPEA/HOAc catalytic system was chosen for the synthesis of the remaining target compounds, **15a–23a** and **15b–23b** (method A). In this acid-base catalytic system, the amine ligand base (DIPEA) has the role of coordinating the active Cu(I) species and stabilizing it against oxidation and disproportionation and promotes the formation of copper(I) acetylide. Acetic acid protonates the C-Cu link of copper triazolide to form the 1,4-disubstituted 1,2,3-triazole product [61].

Conventional solution-based click reactions versus the mechanochemical click reaction

The compounds **15a–23a** and their unsaturated analogs **15b–23b** were prepared by both a solvent-free approach using ball milling (method A) and the conventional reaction in methanol (method B). The yields of the isolated products (**15a–23a** and **15b–23b**) were calculated and compared in Table 2 & Figure 4. In general, solvent-free reactions, performed by grinding the azide (**5a** or **5b**), *N*-propargylated base (**6–14**) and CuI/DIPEA/HOAc catalytic system in a ball mill using mechanochemical force showed better overall yields compared with conventional reactions in methanol using a magnetic stirrer and Cu(OAc)₂ as the copper(I) source. Moreover, the reaction time was significantly reduced from 24 h for conventional synthesis in solution (method B) to 3.5 h for the mechanochemical solvent-free reaction (method A).

Antiproliferative evaluation

Prepared *N*-heterocycle-L-ascorbic acid hybrids (**15a–23a** and **15b–23b**) were tested on seven human tumor cell lines: A549, CFPAC-1, HCT-116, HeLa, HepG2, MCF-7 and SW620. The results of antiproliferative activity are shown in Table 3. L-ascorbic acid and 5-fluorouracil were used as reference compounds (Table 3).

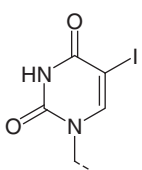
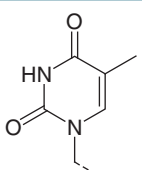
The influence of both the nucleobase attached to C-4 of the 1,2,3-triazole and the spacer between the lactone and the 1,2,3-triazole on antiproliferative activity was observed (Figure 5).

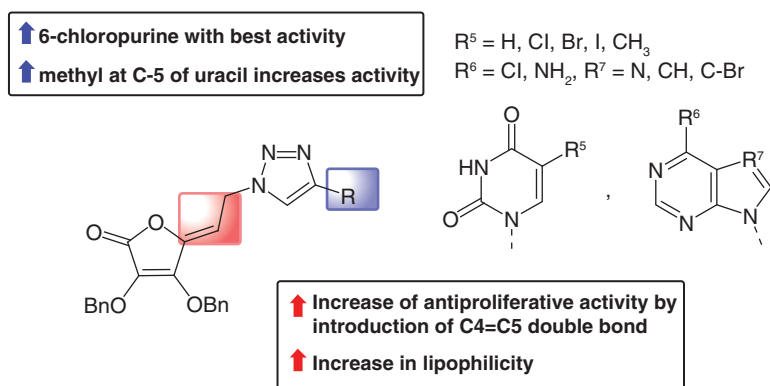
Thus, the uracil derivatives of L-ascorbic acid (**19a–23a**) with the hydroxyethylene spacer showed only slight or no antiproliferative activity, with the exception of 6-chloropurine (**16a**) and 7-deazapurines (**17a** and **18a**).

Table 2. Comparison of yields for the solvent-free ball milling (method A) and conventional (method B) click reaction in solution to afford compounds **15a–23a** and their unsaturated counterparts, **15b–23b**.

Base	Compound	Yield (%)		Compound	Yield (%)		
		Method A	Method B		Method A	Method B	
		15a–23a			15b–23b		
		15a	91	71	15b	85	66
		16a	87	68	16b	98	77
		17a	88	68	17b	89	88
		18a	99	79	18b	98	95
		19a	94	91	19b	91	88
		20a	95	66	20b	88	85
		21a	86	77	21b	90	86

Table 2. Comparison of yields for the solvent-free ball milling (method A) and conventional (method B) click reaction in solution to afford compounds **15a–23a** and their unsaturated counterparts, **15b–23b** (cont.).

Base	Compound	Yield (%)		Compound	Yield (%)	
		Method A	Method B		Method A	Method B
	22a	88	86	22b	80	78
	23a	97	96	23b	87	78

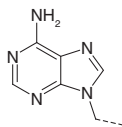
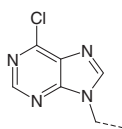
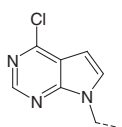
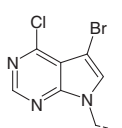
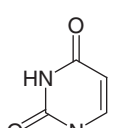
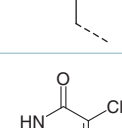
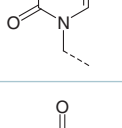
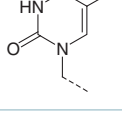
Figure 5. Insights into the structure–activity relationship of novel *N*-heterocycle-L-ascorbic acid hybrids.

Conversely, analogs **15b–23b**, containing a conformationally restricted ethylidene spacer, were more active. From the C4=C5 unsaturated derivatives, the 6-chloropurine analog **16b** showed the most pronounced cytostatic activity, particularly against HepG2 (IC₅₀: 3.2 μm) and SW620 (IC₅₀: 6.5 μm) cells. Interestingly, from the conformationally unconstrained purine and deazapurine derivatives (**15a–18a**), only the adenine analog **15a** was devoid of any antiproliferative activity.

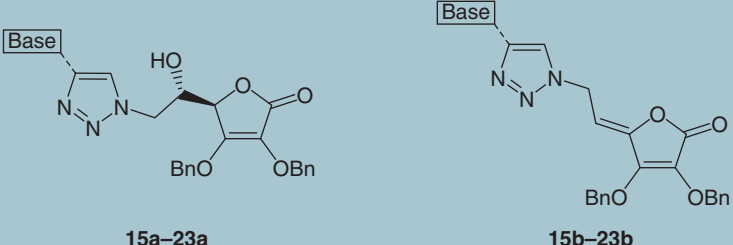
Considering the impact of nucleobase on the growth-inhibitory effect, it can be observed that purine and 7-deazapurines demonstrated higher inhibitory effect than uracil and 5-substituted uracil derivatives with activity in the following order: 6-chloropurine > 6-chloro-7-bromo-7-deazapurine > 6-chloro-7-deazapurine > adenine. Among the uracil and 5-substituted uracil derivatives, **23b** with an electron-donating methyl group displayed moderate inhibitory activity against all cell lines studied, with IC₅₀ in the range of 27.3–39.2 μm. Uracil and 5-substituted uracil derivatives (**19a–23a** and **19b–23b**) showed cytostatic activity in the following order: 5-methyluracil > uracil > 5-chlorouracil > 5-bromouracil > 5-iodouracil.

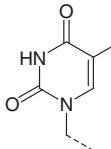
A relationship between the lipophilicity of the evaluated compounds and their antiproliferative activity was also observed. Purine and 7-deazapurine derivatives with better cytostatic effects were more lipophilic, with ClogP values

Table 3. Antiproliferative activity of *N*-heterocycle-L-ascorbic acid hybrids (15a–23a) and their unsaturated counterparts (15b–23b) against selected tumor cell lines and their index of molecular lipophilicity or hydrophilicity values.

Base	Compound	IC ₅₀ (μm) [†]								ClogP [‡]
		A549	CFPAC-1	HCT-116	HeLa	HepG2	MCF-7	SW620		
	15a	>100	>100	>100	>100	>100	>100	>100	>100	0.80
	15b	57.6 ± 4.3	49.2 ± 4.2	62.2 ± 5.1	52.5 ± 3.2	46.2 ± 2.6	47.1 ± 5.0	51.1 ± 5.6	51.1 ± 5.6	1.75
	16a	43.9 ± 3.1	37.3 ± 5.8	29.3 ± 2.2	25.4 ± 1.9	21.5 ± 1.6	30.0 ± 2.3	31.9 ± 3.3	1.45	
	16b	32.7 ± 2.3	27.5 ± 5.3	21.7 ± 5.0	20.4 ± 6.4	3.2 ± 0.3	19.5 ± 1.3	6.5 ± 0.4	6.5 ± 0.4	2.41
	17a	91.3 ± 8.8	>100	53.9 ± 4.2	95.6 ± 9.3	84.2 ± 4.9	78.4 ± 5.2	>100	>100	2.28
	17b	>100	>100	82.2 ± 7.8	93.4 ± 9.2	68.6 ± 8.1	87.1 ± 9.7	97.0 ± 8.6	97.0 ± 8.6	3.24
	18a	51.8 ± 3.7	51.8 ± 4.1	82.9 ± 6.4	51.4 ± 5.4	49.9 ± 4.5	61.6 ± 5.2	>100	>100	3.15
	18b	>100	70.3 ± 6.8	79.1 ± 9.7	50.4 ± 6.5	36.4 ± 5.2	70.4 ± 9.2	60.9 ± 5.7	60.9 ± 5.7	4.11
	19a	>100	>100	>100	>100	>100	>100	>100	>100	0.24
	19b	41.6 ± 8.2	41.5 ± 6.2	40.1 ± 5.3	30.3 ± 7.3	28.4 ± 4.4	47.3 ± 0.2	39.1 ± 1.8	39.1 ± 1.8	1.20
	20a	>100	>100	>100	>100	>100	>100	>100	>100	1.29
	20b	52.8 ± 5.5	54.8 ± 2.4	56.5 ± 7.2	43.0 ± 4.6	46.9 ± 2.5	39.8 ± 3.7	44.6 ± 5.4	44.6 ± 5.4	2.25
	21a	>100	>100	>100	>100	>100	>100	>100	>100	1.44
	21b	69.8 ± 3.4	61.1 ± 5.2	55.1 ± 1.8	50.4 ± 4.5	52.3 ± 3.0	65.3 ± 2.7	46.4 ± 0.3	46.4 ± 0.3	2.40
	22a	>100	98.3 ± 11.5	77.9 ± 9.2	76.6 ± 8.2	64.2 ± 9.1	85.2 ± 11.7	>100	>100	1.70
	22b	79.0 ± 11.7	71.8 ± 9.8	60.1 ± 8.2	54.4 ± 8.8	68.1 ± 2.1	35.1 ± 1.8	>100	>100	2.66

[†] 50% inhibitory concentration or compound concentration required to inhibit tumor cell proliferation by 50%.
[‡] Values of ClogP were calculated by ChemDraw Professional 15.0. Cell lines: A549 (lung adenocarcinoma), CFPAC-1 (ductal pancreatic adenocarcinoma), HCT-116 (colorectal carcinoma), HeLa (cervical carcinoma), HepG2 (hepatocellular carcinoma), MCF-7 (breast adenocarcinoma, metastatic), SW620 (colorectal adenocarcinoma, metastatic).

Table 3. Antiproliferative activity of *N*-heterocycle-L-ascorbic acid hybrids (**15a–23a**) and their unsaturated counterparts (**15b–23b**) against selected tumor cell lines and their index of molecular lipophilicity or hydrophilicity values (cont.).


Base	Compound	IC ₅₀ (μm) [†]							
		A549	CFPAC-1	HCT-116	HeLa	HepG2	MCF-7	SW620	ClogP [‡]
	23a	>100	>100	>100	>100	>100	>100	>100	0.74
	23b	39.2 ± 5.8	39.2 ± 6.2	45.6 ± 1.9	27.3 ± 2.2	31.5 ± 1.4	27.9 ± 0.9	37.5 ± 4.3	1.70
	L-ascorbic acid	>100	>100	>100	>100	>100	>100	>100	
	5-Fluorouracil	2.80	0.14	/	8.81	9.04	0.096	0.08	

[†] 50% inhibitory concentration or compound concentration required to inhibit tumor cell proliferation by 50%.
[‡] Values of ClogP were calculated by ChemDraw Professional 15.0. Cell lines: A549 (lung adenocarcinoma), CFPAC-1 (ductal pancreatic adenocarcinoma), HCT-116 (colorectal carcinoma), HeLa (cervical carcinoma), HepG2 (hepatocellular carcinoma), MCF-7 (breast adenocarcinoma, metastatic), SW620 (colorectal adenocarcinoma, metastatic).

ranging from 0.80 to 4.11 compared with 5-substituted pyrimidine analogs (ClogP: 0.24–2.66). Consistent with this finding, compounds **15b–23b** with a C4=C5 double bond, which were more lipophilic, exhibited enhanced inhibitory activity compared with their conformationally unrestricted analogs **15a–23a** with hydroxyethyl linker.

Conclusion

An eco-friendly, solvent-free copper-catalyzed azide-alkyne cycloaddition mechanochemical procedure for coupling purine and pyrimidine derivatives with L-ascorbic acid pharmacophores via 1,2,3-triazole was efficiently implemented. In this way, two series of new purine-, pyrrolo[2,3-*d*]pyrimidine- and 5-substituted pyrimidine-2,3-dibenzyl-L-ascorbic acid hybrids with hydroxyethylene (**15a–23a**) and ethylidene (**15b–23b**) linker were prepared by solvent-free synthesis along with a comparative synthesis in methanol using conventional magnetic stirring. Ball milling allowed us to efficiently perform the copper-catalyzed azide-alkyne cycloaddition reaction under sustainable reaction conditions and proved superior to conventional solution synthesis in terms of higher yields and shorter reaction time.

The new *N*-heterocycle-L-ascorbic acid hybrids showed predominantly moderate antiproliferative activity against the tumor cell lines tested, with the exception of the C4=C5 unsaturated 6-chloropurine derivative **16b**, which showed potent antiproliferative activity against the HepG2 and SW620 cell lines, with IC₅₀s of 3.2 and 6.5 μm, respectively. Further structural optimization of the purine/pyrrolo[2,3-*d*]pyrimidine and L-ascorbic acid hybrids is needed to achieve stronger inhibitory effects on HepG2 and SW620 cells and clarify their mode of action.

The combination of mechanochemistry with the copper-catalyzed azide-alkyne cycloaddition reaction for the synthesis of biologically significant *N*-heterocycle-L-ascorbic acid hybrids proved to be beneficial in promoting sustainable chemistry. In addition, insight into the relationship between the structure and activity of these molecules can serve as a guide for the design and synthesis of novel antitumor agents with selective and potent inhibitory activities on HepG2 and SW620 cells.

Future perspective

To address the need for environmentally sustainable development in line with green chemistry principles, mechanochemistry has the potential to revolutionize the drug discovery process. Recently, ball milling has been successfully applied to a number of chemical reactions, providing a cleaner, safer and more efficient alternative to solution-based protocols.

The structure of the most promising 6-chloroadenine derivative, **16b**, can be further optimized to provide candidates with stronger inhibitory activity on HepG2 and SW620 cells.

The application of mechanochemistry, as we demonstrated in the solvent-free ball milling copper-catalyzed azide-alkyne cycloaddition reaction for coupling of nucleobase and L-ascorbic acid pharmacophores, can open new perspectives for the sustainable preparation of biologically significant scaffolds for the pharmaceutical industry. Examples of the application of mechanochemistry, as an innovative and rapidly growing field in medicinal chemistry, may serve as a source of inspiration for generations of new biologically active chemical entities.

Summary points

Background

- The need for environmentally friendly, green and sustainable drug discovery processes justifies research in this area.
- Mechanochemistry as a cleaner, safer and more efficient alternative to solution protocols.
- Copper-catalyzed azide-alkyne cycloaddition is used for the coupling of two pharmacophores: L-ascorbic acid and nucleobase.
- L-ascorbic acid derivatives and nucleobase derivatives possess biological activity.

Synthesis of target compounds

- Two series of *N*-heterocycle-L-ascorbic acid hybrids with hydroxyethyl and the conformationally restricted ethylidene linker were prepared using copper-catalyzed azide-alkyne cycloaddition.
- A comparative study using solvent-free ball milling conditions along with conventional synthesis in methanol using magnetic stirring was carried out.
- The mechanochemical synthesis was found to be superior in terms of sustainability, reaction rate and yield.

Antitumor activity

- The conformationally restricted C4=C5 double bond has a beneficial effect on antitumor activity.
- Insight into structure–activity relationship revealed the influence of both nucleobase and spacer on antiproliferative activity.
- The unsaturated 6-chloroadenine derivative of L-ascorbic acid, **16b**, was the most active against hepatocellular carcinoma (HepG2) and colorectal adenocarcinoma (SW620) cells.

Supplementary data

To view the supplementary data that accompany this paper (paper (¹H and ¹³C NMR chemical shifts and spectra for new compounds as well as rotating-frame Overhauser effect spectroscopy NMR spectrum of compound **15b** indicative of Z-configuration) please visit the journal website at: www.future-science.com/doi/suppl/10.4155/fmc-2022-0047

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References

Papers of special note have been highlighted as: • of interest

1. Sheldon RA. Green solvents for sustainable organic synthesis: state of the art. *Green Chem.* 7(5), 267–278 (2005).
2. Anastas P, Eghbali N. Green chemistry: principles and practice. *Chem. Soc. Rev.* 39(1), 301–312 (2009).
3. Liu X, Li Y, Zeng L *et al.* A review on mechanochemistry: approaching advanced energy materials with greener force. *Adv. Mater.* (2022).
4. O'Neill RT, Boulatov R. The many flavours of mechanochemistry and its plausible conceptual underpinnings. *Nat. Rev. Chem.* 5(3), 148–167 (2021).
5. Han GF, Li F, Chen ZW *et al.* Mechanochemistry for ammonia synthesis under mild conditions. *Nat. Nanotechnol.* 16(3), 325–330 (2020).
6. Lukin S, Germann LS, Friščić T, Halasz I. Toward mechanistic understanding of mechanochemical reactions using real-time *in situ* monitoring. *Acc. Chem. Res.* (2022).
- **Article describing advantages of mechanochemistry over conventional synthesis.**
7. Varma RS. Greener and sustainable trends in synthesis of organics and nanomaterials. *ACS Sustain. Chem. Eng.* 4(11), 5866–5878 (2016).

- **Article describing advantages of mechanochemistry over conventional synthesis.**
- 8. Stolle A, Szuppa T, Leonhardt SES, Ondruschka B. Ball milling in organic synthesis: solutions and challenges. *Chem. Soc. Rev.* 40(5), 2317–2329 (2011).
- **Article describing advantages of mechanochemistry over conventional synthesis.**
- 9. Mateti S, Mathesh M, Liu Z *et al.* Mechanochemistry: a force in disguise and conditional effects towards chemical reactions. *Chem. Commun.* 57(9), 1080–1092 (2021).
- **Article describing advantages of mechanochemistry over conventional synthesis.**
- 10. Howard JL, Cao Q, Browne DL. Mechanochemistry as an emerging tool for molecular synthesis: what can it offer? *Chem. Sci.* 9(12), 3080–3094 (2018).
- **Article describing advantages of mechanochemistry over conventional synthesis.**
- 11. Andersen JM, Starbuck HF. Rate and yield enhancements in nucleophilic aromatic substitution reactions via mechanochemistry. *J. Org. Chem.* 86(20), 13983–13989 (2021).
- **Article describing advantages of mechanochemistry over conventional synthesis.**
- 12. Hernández JG, Bolm C. Altering product selectivity by mechanochemistry. *J. Org. Chem.* 82(8), 4007–4019 (2017).
- **Article describing advantages of mechanochemistry over conventional synthesis.**
- 13. Friščić T, Mottillo C, Titi HM. Mechanochemistry for synthesis. *Angew. Chemie Int. Ed.* 59(3), 1018–1029 (2020).
- **Article describing advantages of mechanochemistry over conventional synthesis.**
- 14. Gomollón-Bel F. Ten chemical innovations that will change our world: IUPAC identifies emerging technologies in chemistry with potential to make our planet more sustainable. *Chem. Int.* 41(2), 12–17 (2019).
- **Article describing advantages of mechanochemistry over conventional synthesis.**
- 15. Gomollón-Bel F, García-Martínez J. Emerging chemistry technologies for a better world. *Nat. Chem.* 14(2), 113–114 (2022).
- **Provides information about mechanochemistry in synthesis of active pharmaceutical ingredients.**
- 16. Sović I, Lukin S, Meštrović E *et al.* Mechanochemical preparation of active pharmaceutical ingredients monitored by *in situ* Raman spectroscopy. *ACS Omega* 5(44), 28663–28672 (2020).
- 17. Colacino E, Porcheddu A, Charnay C, Delogu F. From enabling technologies to medicinal mechanochemistry: an eco-friendly access to hydantoin-based active pharmaceutical ingredients. *React. Chem. Eng.* 4(7), 1179–1188 (2019).
- 18. Tan D, Štrukil V, Mottillo C, Friščić T. Mechanosynthesis of pharmaceutically relevant sulfonyl-(thio)ureas. *Chem. Commun.* 50(40), 5248–5250 (2014).
- 19. André V, Hardeman A, Halasz I *et al.* Mechanochemical synthesis of the metallodrug bismuth subsalicylate from Bi₂O₃ and structure of bismuth salicylate without auxiliary organic ligands. *Angew. Chem. Int. Ed. Engl.* 50(34), 7858–7861 (2011).
- **Provides information about mechanistic studies of milling reactions.**
- 20. Tan D, Loots L, Friščić T. Towards medicinal mechanochemistry: evolution of milling from pharmaceutical solid form screening to the synthesis of active pharmaceutical ingredients (APIs). *Chem. Commun.* 52(50), 7760–7781 (2016).
- 21. Pérez-Venegas M, Juaristi E. Mechanochemical and mechanoenzymatic synthesis of pharmacologically active compounds: A green perspective. *ACS Sustain. Chem. Eng.* 8(24), 8881–8893 (2020).
- **Provides information about beneficial effects using solvent-free ball milling conditions for reactions often used in organic synthesis.**
- 22. Achar TK, Bose A, Mal P. Mechanochemical synthesis of small organic molecules. *Beilstein J. Org. Chem.* 13(1), 1907–1931 (2017).
- 23. Kubota K, Seo T, Koide K, Hasegawa Y, Ito H. Olefin-accelerated solid-state C–N cross-coupling reactions using mechanochemistry. *Nat. Commun.* 10(1), 1–11 (2019).
- 24. Raston CL, Scott JL. Chemoselective, solvent-free aldol condensation reaction. *Green Chem.* 2(2), 49–52 (2000).
- 25. Tanaka K, Kishigami S, Toda F. Reformatsky and Luche reaction in the absence of solvent. *J. Org. Chem.* 56(13), 4333–4334 (2002).
- 26. Mack J, Shumba M. Rate enhancement of the Morita-Baylis-Hillman reaction through mechanochemistry. *Green Chem.* 9(4), 328–330 (2007).
- 27. Nielsen SF, Peters D, Axelsson O. The Suzuki reaction under solvent-free conditions. *Synth. Commun.* 30(19), 3501–3509 (2000).
- 28. Achar TK, Mal P. Radical-induced metal and solvent-free cross-coupling using TBAI-TBHP: oxidative amidation of aldehydes and alcohols with N-chloramines via C–H activation. *J. Org. Chem.* 80(1), 666–672 (2015).
- 29. Meghani NM, Amin HH, Lee BJ. Mechanistic applications of click chemistry for pharmaceutical drug discovery and drug delivery. *Drug Discov. Today* 22(11), 1604–1619 (2017).
- **The importance and applications of copper-catalyzed azide-alkyne cycloaddition.**
- 30. Thirumurugan P, Matosiuk D, Jozwiak K. Click chemistry for drug development and diverse chemical–biology applications. *Chem. Rev.* 113(7), 4905–4979 (2013).

31. Kolb HC, Sharpless KB. The growing impact of click chemistry on drug discovery. *Drug Discov. Today* 8(24), 1128–1137 (2003).
32. Neumann S, Biewend M, Rana S, Binder WH. The CuAAC: principles, homogeneous and heterogeneous catalysts, and novel developments and applications. *Macromol. Rapid Commun.* 41(1), e1900359 (2020).
33. Haldón E, Nicasio MC, Pérez PJ. Copper-catalysed azide-alkyne cycloadditions (CuAAC): an update. *Org. Biomol. Chem.* 13(37), 9528–9550 (2015).
- **Introducing mechanochemistry into copper-catalyzed azide-alkyne cycloaddition.**
34. Gonnet L, Baron M, Baltas M. Synthesis of biologically relevant 1,2,3- and 1,3,4-triazoles: from classical pathway to green chemistry. *Molecules* 26(18), 5667 (2021).
35. Thorwirth R, Stolle A, Ondruschka B, Wild A, Schubert US. Fast, ligand- and solvent-free copper-catalyzed click reactions in a ball mill. *Chem. Commun.* 47(15), 4370–4372 (2011).
36. Cummings AJ, Ravalico F, McColgan-Bannon KIS *et al.* Nucleoside azide-alkyne cycloaddition reactions under solvothermal conditions or using copper vials in a ball mill. *Nucleosides Nucleotides Nucleic Acids* 34(5), 361–370 (2015).
37. Rinaldi L, Martina K, Baricco F, Rotolo L, Cravotto G. Solvent-free copper-catalyzed azide-alkyne cycloaddition under mechanochemical activation. *Molecules* 20(2), 2837–2849 (2015).
38. Tireli M, Maračić S, Lukin S *et al.* Solvent-free copper-catalyzed click chemistry for the synthesis of *N*-heterocyclic hybrids based on quinoline and 1,2,3-triazole. *Beilstein J. Org. Chem.* 13(1), 2352–2363 (2017).
39. Sahu A, Agrawal RK, Pandey RK. Synthesis and systemic toxicity assessment of quinine-triazole scaffold with antiprotozoal potency. *Bioorg. Chem.* 88, 102939 (2019).
40. Mešćić Macan A, Harej A, Cazin I *et al.* Antitumor and antiviral activities of 4-substituted 1,2,3-triazolyl-2,3-dibenzyl-L-ascorbic acid derivatives. *Eur. J. Med. Chem.* 184, 111739 (2019).
- **Provides information about the biological importance of L-ascorbic acid and its derivatives.**
41. Mešćić Macan A, Gazivoda Kraljević T, Raić-Malić S. Therapeutic perspectives of vitamin C and its derivatives. *Antioxidants* 8(8), 247 (2019).
42. Doseděl M, Jirkovský E, Macáková K *et al.* Vitamin C – sources, physiological role, kinetics, deficiency, use, toxicity, and determination. *Nutrients* 13(2), 1–36 (2021).
43. Mikkelsen SU, Gillberg L, Lykkesfeldt J, Grønbaek K. The role of vitamin C in epigenetic cancer therapy. *Free Radic. Biol. Med.* 170, 179–193 (2021).
44. Yu X-X, Liu Y-H, Liu X-M *et al.* Ascorbic acid induces global epigenetic reprogramming to promote meiotic maturation and developmental competence of porcine oocytes. *Sci. Rep.* 8(1), 6132 (2018).
45. Yun J, Mullarky E, Lu C *et al.* Vitamin C selectively kills KRAS and BRAF mutant colorectal cancer cells by targeting GAPDH. *Science* (80-) 350(6266), 1391–1396 (2015).
46. Gao X, Wei K, Hu B, Xu K, Tang B. Ascorbic acid induced HepG2 cells' apoptosis via intracellular reductive stress. *Theranostics* 9(14), 4233 (2019).
- **Provides rationale for the design of pyrimidine and purine-L-ascorbic acid hybrids.**
47. Gazivoda T, Plevnik M, Plavec J *et al.* The novel pyrimidine and purine derivatives of L-ascorbic acid: synthesis, one- and two-dimensional ¹H and ¹³C NMR study, cytostatic and antiviral evaluation. *Bioorganic Med. Chem.* 13(1), 131–139 (2005).
48. Gazivoda T, Šokčević M, Kralj M *et al.* Synthesis and antiviral and cytostatic evaluations of the new C-5 substituted pyrimidine and furo[2,3-d]pyrimidine 4',5'-didehydro-L-ascorbic acid derivatives. *J. Med. Chem.* 50(17), 4105–4112 (2007).
49. Gazivoda T, Wittine K, Lovrić I *et al.* Synthesis, structural studies, and cytostatic evaluation of 5,6-di-O-modified L-ascorbic acid derivatives. *Carbohydr. Res.* 341(4), 433–442 (2006).
50. Gazivoda T, Raić-Malić S, Marjanović M *et al.* The novel C-5 aryl, alkenyl, and alkynyl substituted uracil derivatives of L-ascorbic acid: synthesis, cytostatic, and antiviral activity evaluations. *Bioorganic Med. Chem.* 15(2), 749–758 (2007).
51. Harej A, Macan AM, Štepanić V *et al.* The antioxidant and antiproliferative activities of 1,2,3-triazolyl-L-ascorbic acid derivatives. *Int. J. Mol. Sci.* 20(19), 4735 (2019).
52. Von Dallacker F, Sanders J. Derivate der L-ascorbinsäure 1, darstellung und eigenschaften der O2,O3-ethandiyl- und der O2,O3-dibenzyl-L-ascorbinsäuren. *Chem. Zeitung.* 109, 197–202 (1985).
53. Von Dallacker F, Sanders J. Derivate der L-ascorbinsäure 2, darstellung von deoxy-L-ascorbinsäuren. *Chem. Zeitung.* 109, 277–280 (1985).
54. Mešćić A, Šalić A, Gregorić T, Zelić B, Raić-Malić S. Continuous flow-ultrasonic synergy in click reactions for the synthesis of novel 1,2,3-triazolyl appended 4,5-unsaturated L-ascorbic acid derivatives. *RSC Adv.* 7(2), 791–800 (2017).
55. Lazrek HB, Taourirte M, Oulih T *et al.* Synthesis and anti-HIV activity of new modified 1,2,3-triazole acyclonucleosides. *Nucleosides Nucleotides Nucleic Acids* 20(12), 1949–1960 (2001).
56. Bistrovic A, Grbčić P, Harej A *et al.* Small molecule purine and pseudopurine derivatives: synthesis, cytostatic evaluations and investigation of growth inhibitory effect in non-small cell lung cancer A549. *J. Enzyme Inhib. Med. Chem.* 33(1), 271–285 (2018).

57. Krim J, Sillahi B, Taourirte M, Rakib EM, Engels JW. Microwave-assisted click chemistry: synthesis of mono and bis-1,2,3-triazole acyclonucleoside analogues of ACV via copper(I)-catalyzed cycloaddition. *ARKIVOC* 2009(13), 142–152 (2009).
58. Gazivoda T, Raić-Malić S, Krištafor V *et al.* Synthesis, cytostatic and anti-HIV evaluations of the new unsaturated acyclic C-5 pyrimidine nucleoside analogues. *Bioorg. Med. Chem.* 16(10), 5624–5634 (2008).
59. Bistrović A, Harej A, Grbčić P *et al.* Synthesis and anti-proliferative effects of mono- and bis-purinomimetics targeting kinases. *Int. J. Mol. Sci.* 18(11), 2292 (2017).
60. Gregorić T, Sedić M, Grbčić P *et al.* Novel pyrimidine-2,4-dione-1,2,3-triazole and furo[2,3-d]pyrimidine-2-one-1,2,3-triazole hybrids as potential anti-cancer agents: synthesis, computational and x-ray analysis and biological evaluation. *Eur. J. Med. Chem.* 125, 1247–1267 (2017).
61. Shao C, Wang X, Zhang Q, Luo S, Zhao J, Hu Y. Acid-base jointly promoted copper(I)-catalyzed azide-alkyne cycloaddition. *J. Org. Chem.* 76(16), 6832–6836 (2011).