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Inhibiting the cancer cell growth by maleopimarate amino imide bis-tetrazoles synthesized *via* the azido-Ugi reaction

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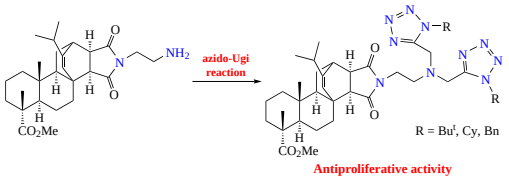
The azido-Ugi reaction of methyl maleopimarate *N*-(2-aminoethyl) imide with isocyanides, paraformaldehyde and trimethylsilyl azide in one step leads to 1,5-disubstituted diterpene bis-tetrazoles. The compounds demonstrate selective cytotoxicity against NCI-60 cancer cell panel.



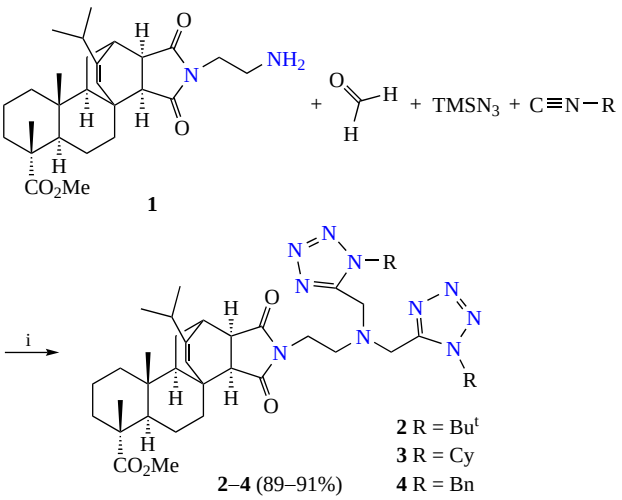
Keywords: azido-Ugi reaction, isocyanides, tetrazoles, abietane diterpenes, methyl maleopimarate, amino imides, anticancer activity.

Multicomponent reactions (MCRs) have found a wide application in modern organic chemistry, among them isocyanide-based MCRs occupy an important place due to the reactivity of the isocyano group, which allows the creation of C–C, C–N and other carbon–heteroatom bonds.[1–3] One of the most used MCRs is the four-component Ugi reaction between an amine, a carbonyl compound, an isocyanide and a carboxylic acid.[4–7] A modification of this classical reaction is the azido-Ugi condensation, in which hydrazic acid generated *in situ* from trimethylsilyl azide and alcohol is used instead of carboxylic acid.[8,9] The products of this reaction are the corresponding 1,5-disubstituted tetrazoles. Tetrazoles are considered to be structural analogs of *cis*-amide and *N*-alkyl-amide groups in peptides and have much higher metabolic stability and biological activity, which ensures their high demand in medicinal chemistry.[10–13] The pharmaceutically important *NH*-tetrazoles losartan and valsartan are angiotensin receptor blockers, 1,5-disubstituted tetrazoles cilostazol, nojiritetrazole and pentylenetetrazole are used in medical practice as anti-inflammatory and antidiabetic drugs, stimulants of blood circulation and respiration (see Online Supplementary Materials, Figure S1).[14–16]

The study of the behavior of terpenes in MCRs has been the focus of research in recent years. Using Ugi and Mannich MCRs based on abietic, dehydroabietic acids and diene adducts of levopimaric acid, new derivatives were synthesized with an activity against the influenza A virus (H1N1) and the SARS-CoV-2 pseudovirus, low toxicity and a high selectivity index.[17–19]

When we have started this research, no examples of the synthesis of bis-tetrazoles on a diterpene scaffold using the azido-Ugi reaction were known. When our compounds have already been synthesized and subjected to bioassay, the synthesis of bis-substituted tetrazoles demonstrating significant inhibition of acetyl- and butyrylcholinesterase enzymes based on dehydroabiethylamine, formaldehyde, $TMSN_3$ and isocyanides under ultrasonic irradiation has emerged.[20] The authors have noted that when using paraformaldehyde, benzyl isocyanide and $TMSN_3$, the reaction proceeded at room temperature with the formation of a monotetrazole derivative in low yield, but with other isocyanides under the same conditions or upon refluxing the reaction did not occur at all.

Herein, we describe an efficient one-step synthesis of 1,5-disubstituted bis-tetrazoles *via* the pseudo-seven-component azido-Ugi reaction based on methyl maleopimarate *N*-(2-aminoethyl) imide **1** and evaluation of an antiproliferative activity of the synthesized compounds **2**–**4** (Scheme 1). Our experiments showed that the azido-Ugi reaction of primary amine derivative **1** with *tert*-butyl-, cyclohexyl- or benzyl isocyanides, formaldehyde and $TMSN_3$ easily proceeded in methanol at room temperature for 48–72 h with the formation of products **2**–**4** in high yields (89–91%) (Scheme 1).†



Scheme 1 *Reagents and conditions*: i, MeOH, room temperature, 48–72 h.

† *General procedure for the synthesis of compounds* **2**–**4**. Methyl maleopimarate *N*-(2-aminoethyl) imide **1** (460 mg, 1 mmol) was dissolved in methanol (25 ml), the corresponding isocyanide (1.8 mmol), $TMSN_3$ (0.21 ml, 1.8 mmol) and paraformaldehyde (200 mg) were added, and the reaction was stirred at ambient temperature for 48–72 h (TLC monitoring). After completion of the reaction, the reaction mixture was poured into aqueous HCl (2 M), and the precipitate formed was filtered off, washed until neutral, and dried in air. The residue was purified by column chromatography (silica gel, hexane/ethyl acetate 10 → 30%).

Table 1 Anticancer screening data for compounds **2**–**4** in concentration 10 μM.[a]

Compound (NSC code)	Mean growth (%)	Most sensitive cell lines (growth %)
2 (D-850913/1)	–96.06	All 55 cell lines (–42.87 to –99.45)
3 (D-850908/1)	63.52	Leukemia HL-60(TB) (–12.29), MOLT-4 (12.91), RPMI-8226 (–69.48); Colon cancer HT29 (31.72); CNS cancer SNB-75 (1.92); Renal cancer RXF 393 (26.31); Prostate cancer PC-3 (30.79); Breast cancer MCF7 (26.18), MDA-MB-468 (8.59)
4 (D-850913/1)	74.81	Leukemia HL-60(TB) (–36.41), MOLT-4 (9.37), RPMI-8226 (–51.73); Breast cancer MDA-MB-468 (1.62)

[a] 55 Cell lines assay in 1 dose 10 μM concentration.

The structure of the synthesized compounds **2**–**4** was confirmed by mass spectrometry and NMR spectroscopy data. Mass spectra correspond to the molecular masses of bis-derivatives (quasi-molecule ion at m/z = 732.6, 785.6, 801.6 (100%, $[M]^+$). ^1H and ^{13}C NMR spectra demonstrate the expected structure skeleton of methyl maleopimarate amino imide, signals from the corresponding R residues, and ^{13}C NMR spectra contained a typical tetrazole resonances at δ_C = 150.6–152.7 ppm.

Compounds **2**–**4** were screened in the National Cancer Institute (NCI, Bethesda, USA) Developmental Therapeutic Program (DTP) at one dose assay (10^{-5} M) towards a panel NCI-60 representing different cancer types (leukemia, melanoma, lung, colon, CNS, ovarian, renal, prostate, and breast cancer) (Table 1 and Figures S4–S9). The anticancer activity results showed that compound **2** with *tert*-butyl fragment was effective against all the cell lines with growth percent in range from –42.87 (leukemia K-562) to –99.45 (melanoma UACC-257). Bis-tetrazole derivatives **3** and **4** with a cyclohexyl and benzyl residues, respectively, selectively acted mainly against leukemia cell lines HL-60(TB), MOLT-4, RPMI-8226 and breast cancer MDA-MB-468.

As one can see, the antiproliferative activity of the diterpene bis-tetrazoles is strongly influenced by the substituent in the tetrazole ring. It should be noted that the original maleopimaric acid, its methyl ester and amino imide analogs do not have antitumor activity;[21] at the same time, modifications of the maleopimaric acid carboxy group using the Ugi MCR led to the derivative active against leukemia, colon cancer and melanoma.[22] Next, it will be necessary to carry out more detailed studies of synthesized compounds for a deeper understanding of the mechanism of antitumor action.

In summary, the first example of the azido-Ugi reaction of methyl maleopimarate amino imide and aliphatic or aromatic isocyanides and $TMSN_3$ with the formation of bis-tetrazoles is presented. The screening of cytotoxicity on the NCI-60 panel revealed a selective action of tetrazoles with a cyclohexyl and benzyl residues against leukemia and breast cell lines. It is noteworthy that the diterpene bis-tetrazole with a *tert*-butyl residue was cytotoxic toward the total cancer cell panel. The future prospects of the current finding will include the regulation of maleopimarate amino imide chain and the type of isocyanide as well as the study of mechanism of anticancer activity of the lead compounds.

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Online Supplementary Materials

Supplementary data associated with this article can be found in the online version at doi: 10.1016/j.mencom.2024.06.012.

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