Cytotoxic Heterocyclic Triterpenoids Derived From Betulin and Betulinic Acid.

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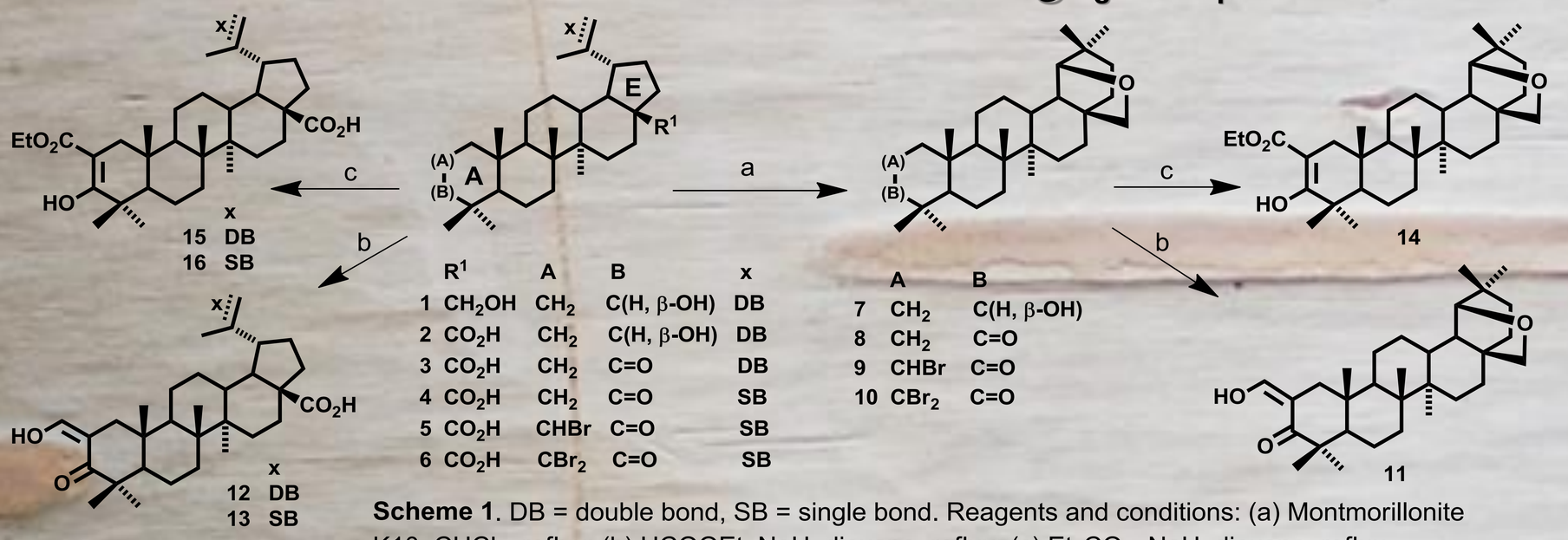


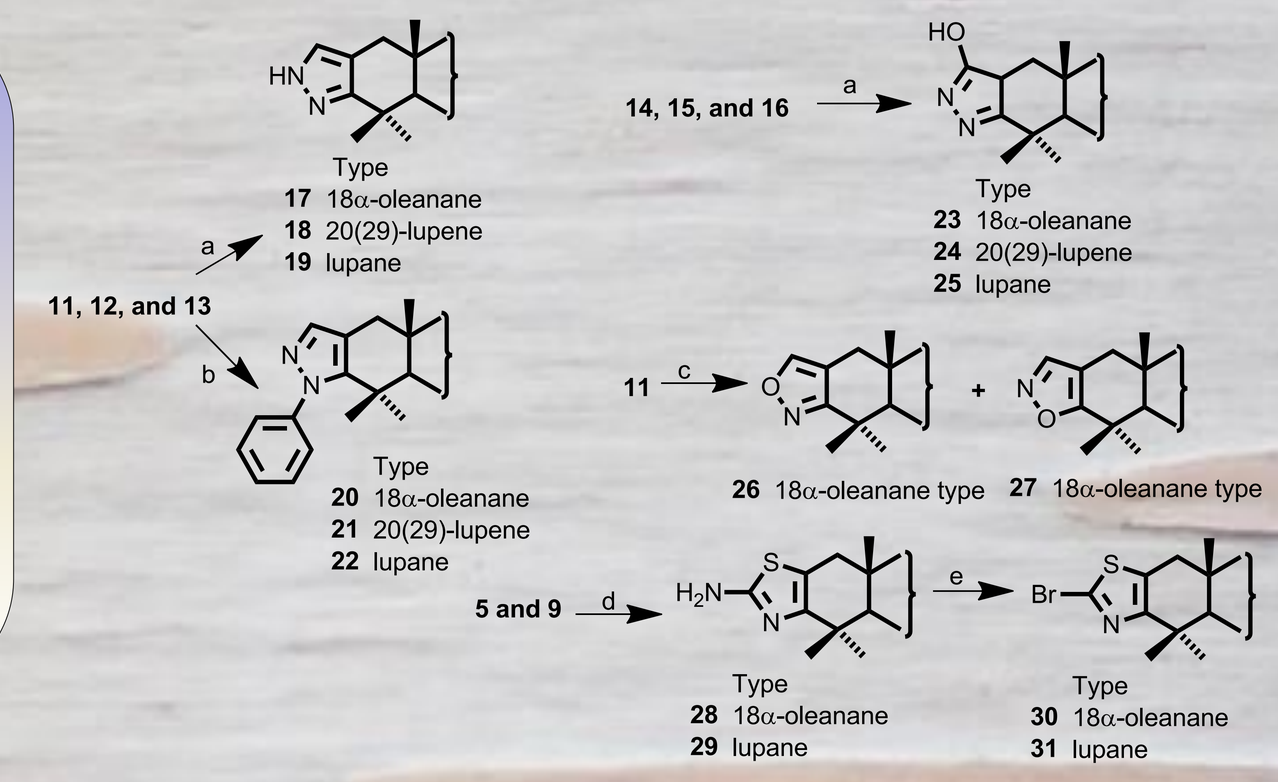
Scheme 1. DB = double bond, SB = single bond. Reagents and conditions: (a) Montmorillonite K10, $CHCl_3$, reflux; (b) HCOOEt, NaH, dioxane, reflux; (c) Et_2CO_3, NaH, dioxane, reflux.

Triterpenoids.

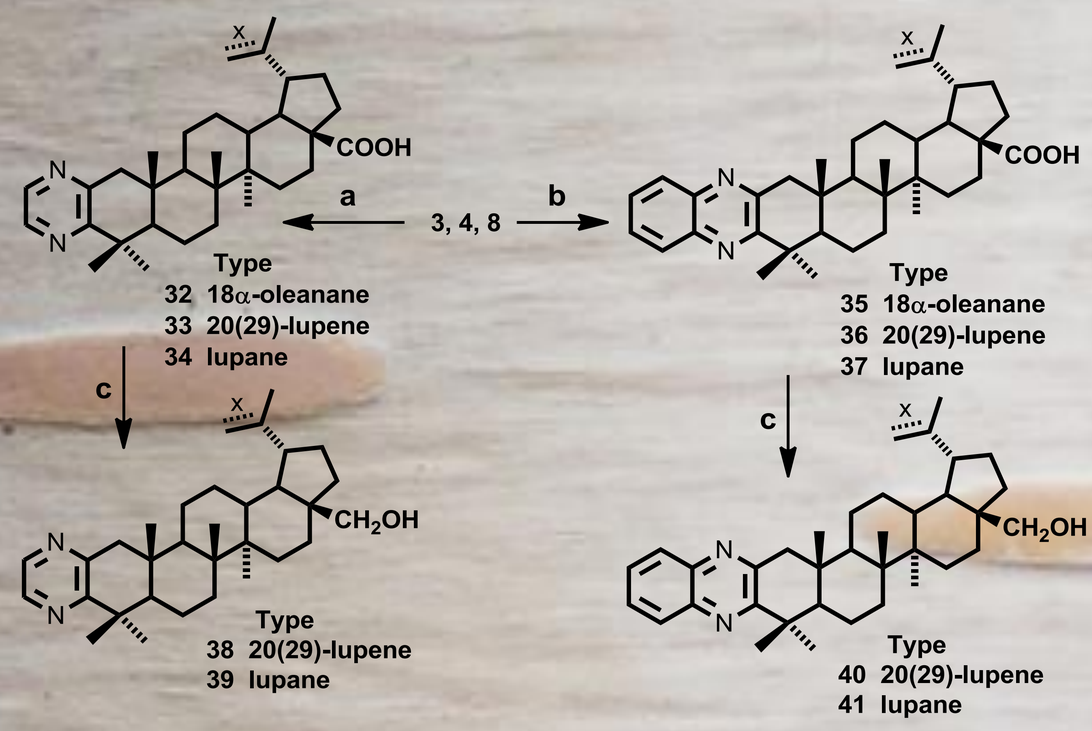
Triterpenoids are a large group of natural compounds that are found in numerous living organisms, and are particularly prevalent in plants. They often have a variety of biological activities.[1] Betulinic acid, for example, has strong anti-HIV and anti-cancer activities.[2]

Triterpenoid heterocycles.

The aim of this work was to synthesize a set of heterocyclic derivatives of lupane, lup-20(29)-ene, and 18α-oleanane, and to investigate their cytotoxicity. Starting from betulin (**1**) and betulinic acid (**2**), we prepared various precursors such as ketones, α–diketones, α-bromoketones, β-oxoesters, and 2-hydroxymethylene-3-oxo compounds **3** - **16**. Condensation of these intermediates with ethylene diamine, phenylene diamine, hydrazine, phenylhydrazine, hydroxyl-amine, or thiourea yielded the heterocycles **17** - **31**. Several structures were previously known, however, this study was the first to describe their biological activities.[3,4]



Scheme 2. Reagents and conditions: (a) $N_2H_4.H_2O$, dioxane, refl.; (b) Phenylhydrazine, AcOH, refl.; (c) $NH_2OH.HCl$, EtOH, pyridine, refl.; (d) Thiourea, morpholine, reflux; (e) isoamylnitrite, TBAB, $CHCl_3$, refl.



Scheme 3. Reagents and conditions: (a) ethylenediamine, sulfur, morpholine, refl.; (b) 1,2-phenylenediamine, sulfur, morpholine, refl.; (c) i: CH_2N_2, Et_2O, $CHCl_3$, then ii: LAH, dioxane, refl.

Compound	IC$_{50}$ (μmol/L)								
	CEM	CEM-DNR	A549	K562	K562-TAX	HCT116	HCT116p53$^{-/-}$	BJ	MRC-5
4	3.7	9.4	5.9	2.5	4.0	4.1	6.1	22.6	11.7
5	1.4	11.4	11.9	1.6	1.7	4.3	4.1	17.8	14.6
6	1.0	7.0	8.4	0.9	1.1	2.4	2.6	13.3	8.0
12	3.5	29.6	17.6	8.4	12.7	12.1	13.7	55.5	29.9
18	2.8	10.4	5.2	2.6	3.0	4.5	3.3	35.8	14.1
19	2.6	8.2	3.6	3.5	2.7	3.9	2.8	22.4	13.4
29	3.5	11.2	7.0	4.8	6.9	5.1	4.3	24.9	15.7
33	5.2	25.1	0.25	0.77	8.0	13.7	11.2	19.6	21.8

Table 1. Cytotoxic activity of selected compounds **4** - **6**, **12**, **18**, **19**, **29**, and **33** against seven tumor and two normal fibroblast cell lines.

Cytotoxic activity.

The basic cytotoxicity screening was performed on CCRF-CEM cell line and the most promising compounds were further tested on seven cancer cell lines with/without MDR phenotype and non tumor MRC-5 and BJ fibroblasts. The preferential cytotoxicity to cancer cell lines, particularly to hematological tumors was observed, acids **4** - **6** and heterocycles **18**, **19**, **29**, **33** showed the highest activity and selectivity. This puts these derivatives among promising candidates for cancer treatment, therefore their *in vivo* activity is currently investigated.

Conclusions.

We synthesized a set of heterocyclic triterpenoids in order to expand our library of active compounds. The activity was measured on multiple cell lines. Among the new compounds, several had cytotoxicity in low micromolar range and their *in vivo* activity is investigated. Currently, the library serves as a database for a large QSAR study being performed.

Acknowledgement

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