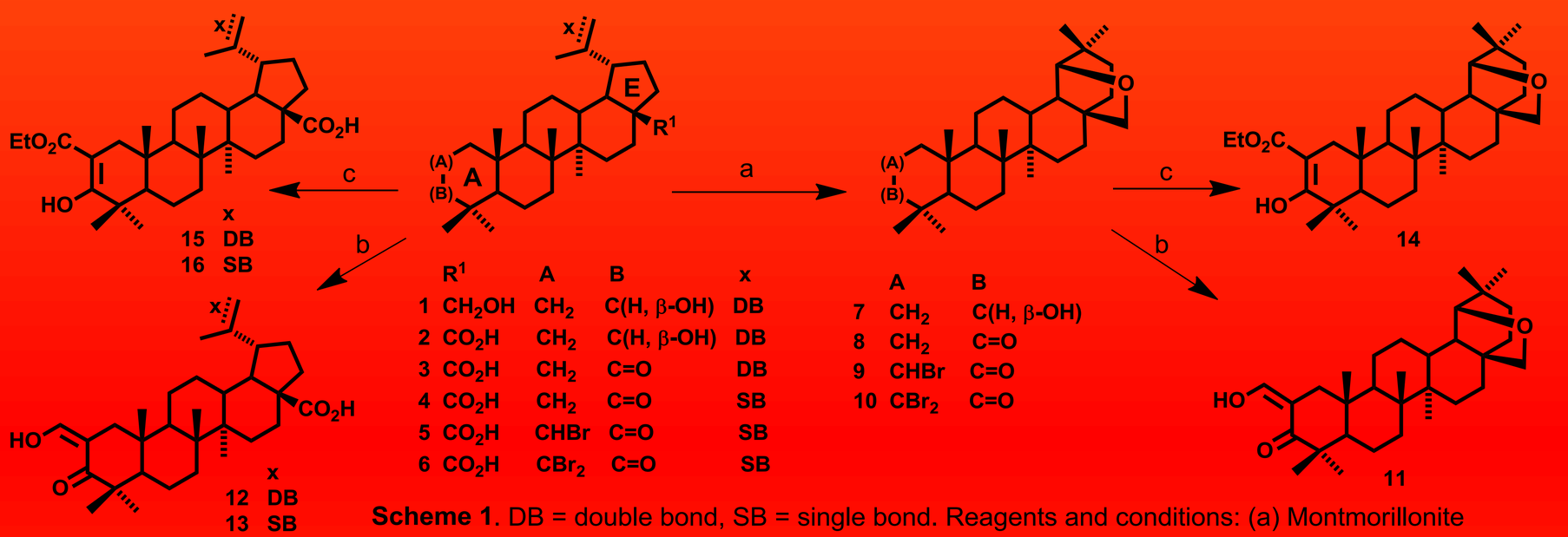
Triterpenoid Derivatives and Their Biological Activities

Milan Urban[a,b], Jan Sarek[a], Marian Hajduch[a], Jiri Rehulka[a], Petr Dzubak[a], Lucie Borkova[b]

[a] Institute of Molecular and Translational Medicine, Faculty of Medicine and Dentistry, Palacky University in Olomouc, Hnevotinska 5, Olomouc,77900, Czech Republic.

[b] Department of Organic Chemistry, Faculty of Science, Palacky University in Olomouc, 17. listopadu 1192/12, Olomouc, 771 46, Czech Republic.

e-mail: **urban@orgchem.upol.cz**



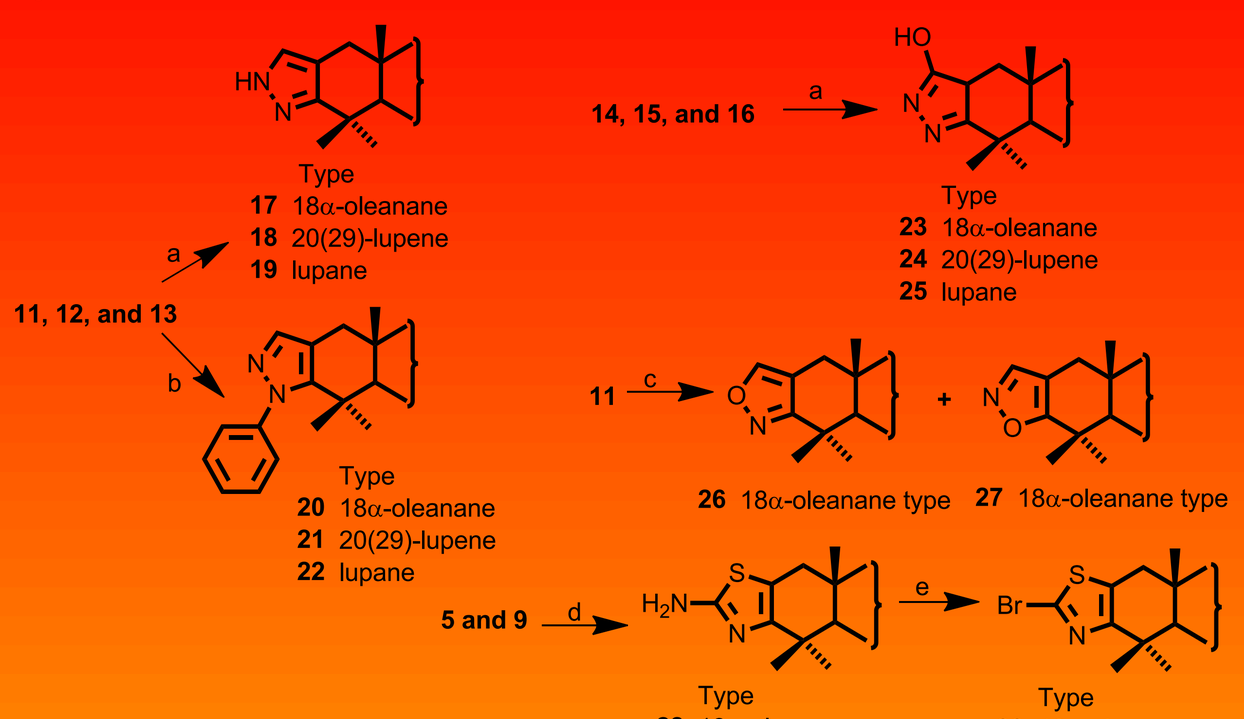
Scheme 1. DB = double bond, SB = single bond. Reagents and conditions: (a) Montmorillonite K10, $CHCl_3$, reflux; (b) HCOOEt, NaH, dioxane, reflux; (c) Et_2CO_3, NaH, dioxane, reflux.

Triterpenoids

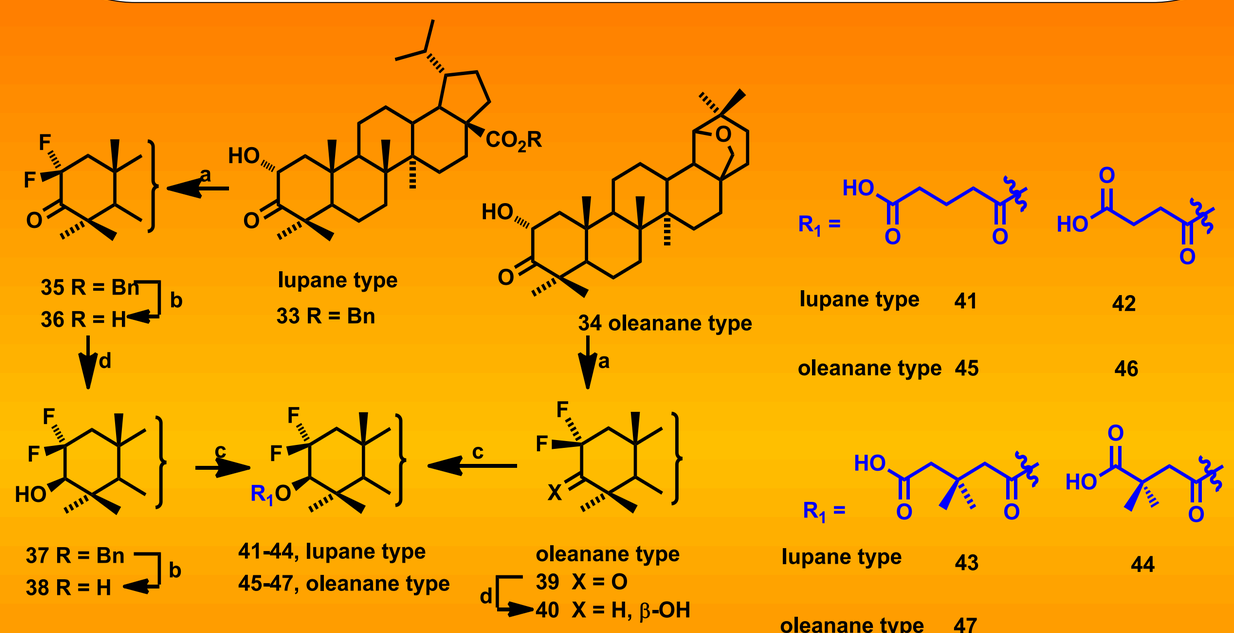
Triterpenoids are a large group of natural compounds that are found in numerous living organisms, and are particularly prevalent in plants. They often have a variety of biological activities.[1] Betulinic acid, for example, has strong anti-HIV and anti-cancer activities.[2]

Terpenoid Heterocycles & Difluoroderivatives

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] Hydroxyderivatives **33** and **34** were precursors for synthesis of cytotoxic difluorocompounds **35** – **47**.[5]



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) DAST, pyridine, $CHCl_3$, reflux, 11h; (b) Pd/C (10%), H_2 (0.5 MPa), THF, MeOH, r.t.; (c) anhydride of each diacid, DMAP, *sym*-collidine, reflux under Ar; (d) $NaBH_4$, MeOH, THF, 0 °C.

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
36	2.4	6.2	5.8	2.6	2.3	4.7	4.7	15.4	9.1
38	4.0	10.9	6.7	5.5	4.1	5.8	5.8	18.7	14.7
44	4.5	11.4	11.4	11.8	11.5	3.7	3.7	26.0	12.8

Table 1. Cytotoxic activity of selected compounds **4** - **6**, **12**, **18**, **19**, **29**, **33, 36, 38, and 44** against seven tumor and two normal fibroblast cell lines.

Biological Activity

The basic cytotoxicity screening was performed on CCRF-CEM cell line and the best compounds were 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**, heterocycles **18**, **19**, **29**, **33**, and fluoroderivatives **26**, **38**, and **44** had the highest activity and selectivity. This puts these derivatives among promising candidates for cancer treatment, therefore their *in vivo* activity is currently investigated. Cell cycle analysis in CCRF-CEM line showed, that compound **38** blocked or slowed down the cell cycle progression through G0/G1 or S-phase and decreased DNA synthesis.

Conclusions

We synthesized a set of triterpenoid derivatives 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, currently, the library serves as a database for a large QSAR study being performed.

Acknowledgement

Authors are grateful to the project CZ.1.07/2.2.00/28.0184, 2012-2015 - Biotrend coming from European Social Fund.

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