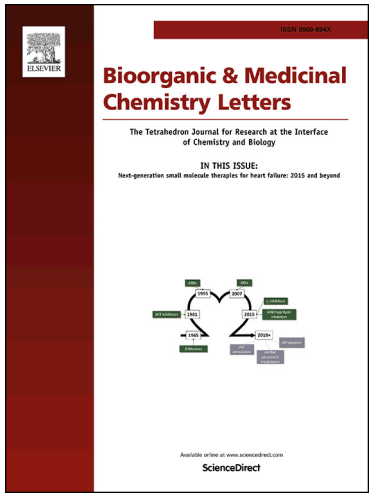

Design, synthesis and biological evaluation of novel HIV-1 protease inhibitors with pentacyclic triterpenoids as P2-ligands

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Design, synthesis and biological evaluation of novel HIV-1 protease inhibitors with pentacyclic triterpenoids as P2-ligands

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Abstract: The design, synthesis and SAR study of a new series of HIV-1 protease inhibitors with pentacyclic triterpenoids as P2 ligands and phenylsulfonamide as P2' ligands were discussed. These compounds exhibited micromolar inhibitory potency, among which compound **T1c** displayed HIV-1 protease inhibition with IC_{50} values of 0.12 μM, which was 67 times the inhibitory activity of its raw material Ursolic acid (8.0 μM).

Keywords: HIV-1 protease inhibitors; P2 ligand; pentacyclic triterpenoids

AIDS has been one of the most challenging problems in medicine since the first case was reported in 1981 in USA. [1] There were approximately 36.7 million people living with AIDS worldwide by the end of 2017. Miserably, the morbidity and mortality increased year by year. More than thirty kinds of drugs have been thus far approved for the treatment of HIV/AIDS targeting different steps of HIV viral life cycle. [2] However, there still exists severe problems, such as the emergence of extensively drug resistant and adverse effects. [3-5] Thus, there is an urgent need for new anti-HIV drug candidates with increased potency, improved pharmacokinetic properties, and reduced side effects.

Furthermore, as there exist lots of problems of traditional chemical drugs, compounds of plant origin are brought into focus. [6] Over 40% of antiviral drugs in clinic were natural products or designed using natural products as prototypes from 1981 to 2014. [7] Pentacyclic triterpenoids are a large family of natural compounds with varieties of biological activities such as antitumor, antiviral, antimicrobial, anti-inflammatory and so on. [8,9] Lots of pentacyclic triterpenoids and their saponin or modifying agents have been reported for their anti-HIV activity. [10-13]

HIV-1 protease is one of the most important enzymes in the life cycle of HIV-1 virus, which would influence maturation of HIV-1 if it is inhibited. In this work, the HIV-1 protease inhibitor Darunavir (DRV) was chosen as the lead compound and

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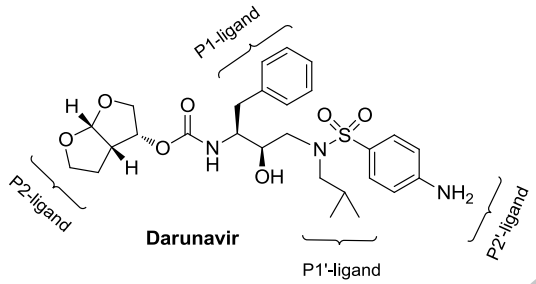
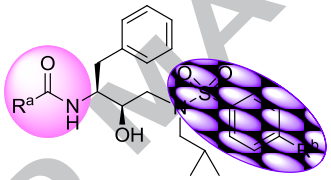
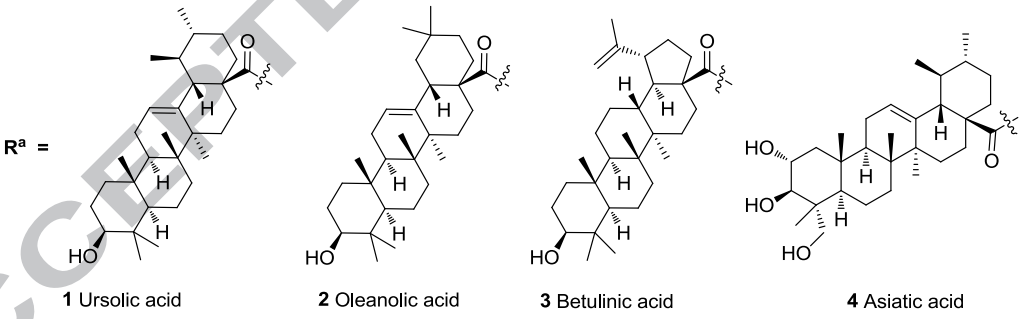
pentacyclic triterpenoids were introduced into P2 ligands by scaffold-hopping strategy. Meanwhile, phenylsulfonamide P2' ligands were investigated to maximize the ligand-binding site interactions in the protease active site (**Fig. 1**). A series of novel HIV-1 protease inhibitors were synthesized and biological evaluated.



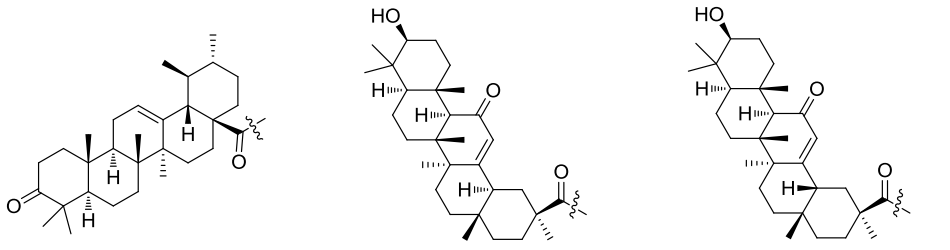
Lead compound



Target molecules



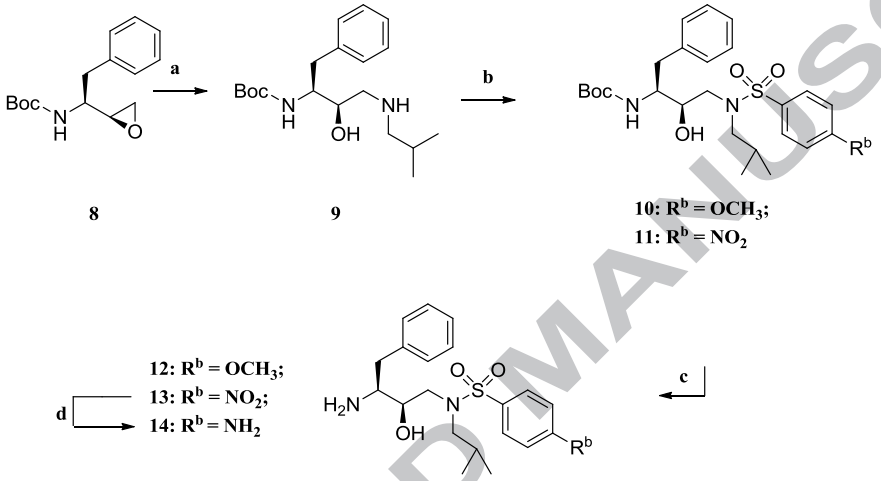
1 Ursolic acid **2** Oleanolic acid **3** Betulinic acid **4** Asiatic acid



5 Ursonic acid **6** 18alpha-Glycyrrhetinic acid **7** 18beta-Glycyrrhetinic acid

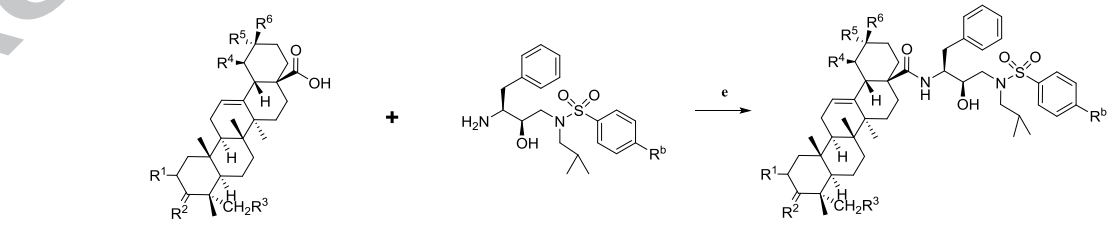
Fig. 1. Chemical structure of target molecules

Compounds **9-13** were prepared from commercial available material (2*S*, 3*S*)-1, 2-epoxy-3-(boc-amino)-4-phenylbutane (**8**) as reported in the literatures in **Scheme 1**. [14-16] Treatment of epoxide **8** with isobutylamine followed by coupling with sulfonyl chloride affording sulfonamides **10** and **11**. Removal of the Boc protection of **10** and **11** was treated by trifluoroacetic acid to afford intermediate compounds **12** and **13**. Catalytic hydrogenation of **13** over 10% Pd/C in methanol afforded the corresponding aminosulfonamide derivative **14**.



Scheme 1. Syntheses of amines **12-14**. Reagents and conditions: (a) *i*-$BuNH_2$, CH_3CN, 80 °C, 6 h; (b) Aryl sulfonyl chloride, DIEA, DMAP(Cat.), THF, 0 °C ~ r.t, 3- 5 h; (c) CH_2Cl_2-CF_3COOH (1:1), 0 °C ~ r.t, 3 h; (d) H_2 (gas), 50 psi, 10% Pd/C, CH_3OH, r.t, 2 h.

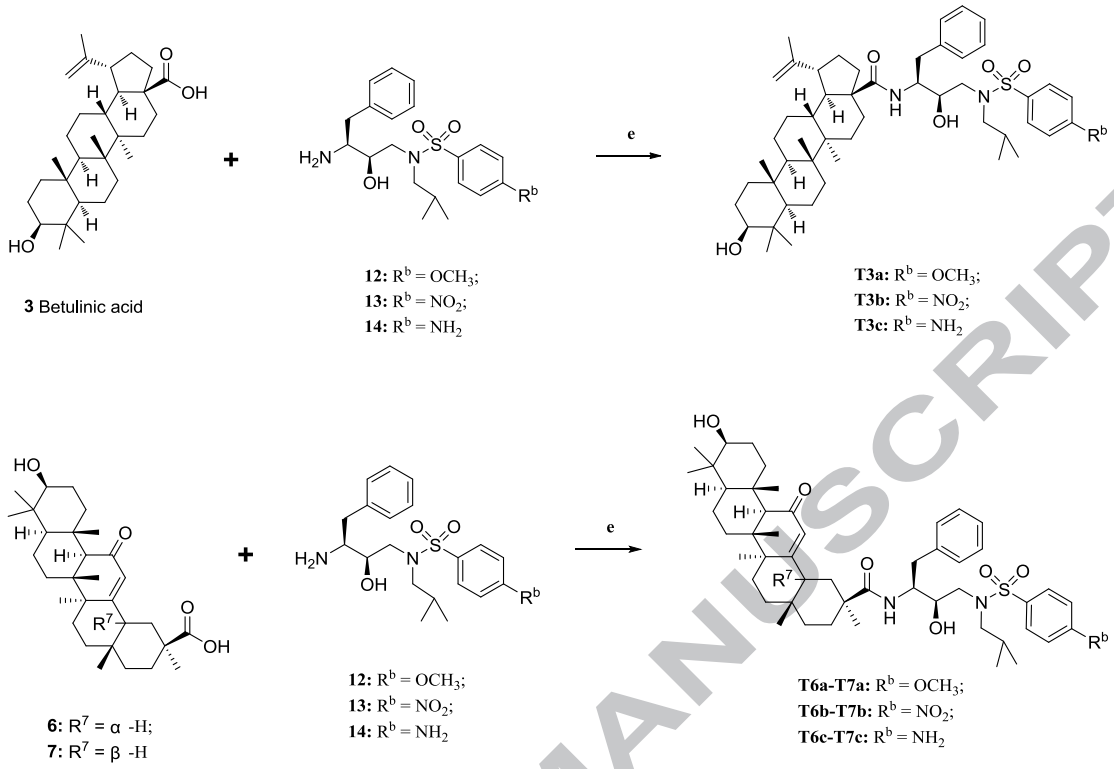
The syntheses of inhibitors **T1−T7** shown in **Scheme 2** were carried out by coupling acids **1−7** with amines **12−14** under an EDCI/HOBt/DMAP-mediated coupling method. [14-16] The structures of these compounds are shown in **Scheme 2**.



1: R^1 = H; R^2 = β -OH, α -H; R^3 = H; R^4 = CH_3; R^5 = CH_3; R^6 = H
2: R^1 = H; R^2 = β -OH, α -H; R^3 = H; R^4 =H; R^5 = CH_3; R^6 = CH_3
4: R^1 = α -OH; R^2 = β -OH, α -H; R^3 = OH; R^4 = CH_3; R^5 = CH_3; R^6 = H
5: R^1 = H; R^2 = O; R^3 = H; R^4 = CH_3; R^5 = CH_3; R^6 = H

12: R^b = OCH_3;
13: R^b = NO_2;
14: R^b = NH_2

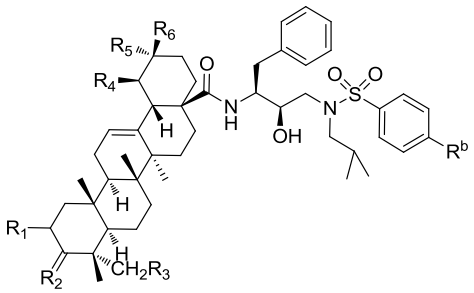
T1a-T5a: R^b = OCH_3;
T1b-T5b: R^b = NO_2;
T1c-T5c: R^b = NH_2



Scheme 2. Syntheses of inhibitors **T1**−**T7**. Reagents and conditions: (e) EDCI, HOBt, DMAP, anhydrous DMF, Ar, 0 °C ~ r.t, 12 h.

The inhibition potency of the synthetic compounds against HIV-1 protease was evaluated using a fluorescence resonance energy transfer (FRET) method. [17,18] Inhibitors were further evaluated in cytotoxicity assay using a cell counting kit-8 assay. [19] The results were presented in **Tables 1**, **2** and **3**, respectively.

Table 1 Enzymatic inhibitory activities and cytotoxicity of inhibitors with pentacyclic triterpenoids amine-acetamide P2 ligands (1)



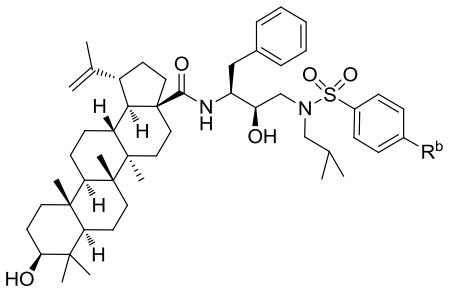
Compd.	R^1	R^2	R^3	R^4	R^5	R^6	R^b	IC_{50}[a] (μM)	CC_{50}[a] (μM)	SI[b]
T1a	H	*β*-OH,	H	CH_3	CH_3	H	OCH_3	0.46±0.12	＞100	＞217

T1b	H	α-H, β-OH, α-H	H	CH_3	CH_3	H	NO_2	0.40±0.15	＞100	＞250
T1c	H	α-H, β-OH, α-H	H	CH_3	CH_3	H	NH_2	0.12±0.04	＞100	＞833
T2a	H	α-H, β-OH, α-H	H	H	CH_3	CH_3	OCH_3	0.24±0.09	＞100	＞417
T2b	H	α-H, β-OH, α-H	H	H	CH_3	CH_3	NO_2	0.15±0.08	＞100	＞667
T2c	H	α-H, β-OH, α-H	H	H	CH_3	CH_3	NH_2	0.17±0.09	86.85	511
T4a	α-OH	α-H, β-OH, α-H	OH	CH_3	CH_3	H	OCH_3	0.15±0.09	＞100	＞667
T4b	α-OH	α-H, β-OH, α-H	OH	CH_3	CH_3	H	NO_2	0.17±0.11	＞100	＞588
T4c	α-OH	α-H, β-OH, α-H	OH	CH_3	CH_3	H	NH_2	0.30±0.19	＞100	＞333
T5a	H	O	H	CH_3	CH_3	H	OCH_3	0.37±0.29	＞100	＞270
T5b	H	O	H	CH_3	CH_3	H	NO_2	0.22±0.19	＞100	＞455
T5c	H	O	H	CH_3	CH_3	H	NH_2	2.37±0.79	＞100	＞42.2
DRV	-	-	-	-	-	-	-	0.57±0.17nM	＞100	＞175000

[a] Values were means of three independent experiments.

[b] SI = CC_{50}/IC_{50}

Table 2 Enzymatic inhibitory activities and cytotoxicity of inhibitors with pentacyclic triterpenoids amine-acetamide P2 ligands (2)

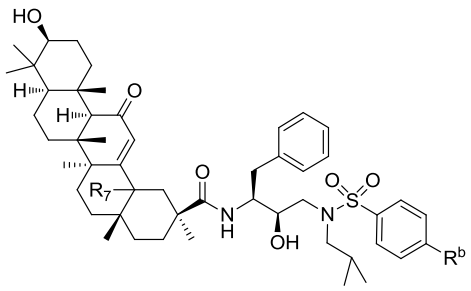


Compd.	R^b	IC_{50}[a] (μM)	CC_{50}[a](μM)	SI[b]
T3a	OCH_3	0.21±0.02	＞100	＞476
T3b	NO_2	1.05±0.45	＞100	＞95
T3c	NH_2	0.61±0.28	＞100	＞164
DRV	-	0.46±0.12 nM	＞100	＞217000

[a] Values were means of three independent experiments.

[b] SI = CC_{50}/IC_{50}

Table 3 Enzymatic inhibitory activities and cytotoxicity of inhibitors with pentacyclic triterpenoids amine-acetamide P2 ligands (3)



Compd.	R^7	R^b	IC_{50}[a] (μM)	CC_{50}[a] (μM)	SI[b]
T6a	*α*-H	OCH_3	1.85±1.76	＞100	＞54.1
T6b	*α*-H	NO_2	1.08±0.51	74.90	69.4
T6c	*α*-H	NH_2	0.49±0.51	＞100	＞204
T7a	*β*-H	OCH_3	0.48±0.32	＞100	＞208
T7b	*β*-H	NO_2	0.50±0.34	＞100	＞200
T7c	*β*-H	NH_2	0.58±0.42	＞100	＞172
DRV	-	-	0.46±0.12 nM	＞100	＞217000

[a] Values were means of three independent experiments.

[b] SI = CC_{50}/IC_{50}

As shown above, the series of pentacyclic triterpenoids derivatives exhibited unsatisfactory micromolar inhibitory potency. The reason might be that fragments of pentacyclic triterpenoids were massive which were not matched with the residues of the protease S2 subsite. [20] Furthermore, these fragments contained only a few hydroxyl groups which could not promote extensive H-bonding interactions involved directly or water-mediated with the backbone amino groups of residues Asp29 and Asp30 of PR in the corresponding S2 subsite. [21] However, there still existed some regularity. For example, inhibitors with asiatic acid amine-acetamide P2 ligands with more hydroxyl groups exhibited better inhibitory potency compared with ursonic acid series, which might make more additional hydrogen bond interactions with the backbone atoms and amino acid residues in the protease S2 subsite through oxygen atoms. In addition, inhibitors with 18 *β*- glycyrrhetinic acid amine-acetamide P2 ligands along with 4-methoxylphenylsulfonamide and 4- nitrophenylsulfonamide as P2' ligands exhibited better inhibitory potency compared with that of 18 *α*-glycyrrhetinic acid, which indicated that the steric configuration of these compounds might affect the combination between the inhibitors and the residues of the protease S2 subsite, i. e. **T7a** *vs* **T6a**, **T7b** *vs* **T6b**.

In summary, a new series of HIV-1 protease inhibitors with pentacyclic triterpenoids were designed and synthesized. Although compound **T1c** displayed 67 times the inhibitory activity of its raw material Ursolic acid (8.0 μM), these

compounds showed unsatisfactory micromolar inhibitory potency. But the results gave a conclusion that massive structures in P2 ligands might be not matched with the residues of the protease S2 subsite well, which was available for us to avoid introducing massive structures in the following designing of HIV-1 protease inhibitors. Furthermore, fragments of pentacyclic triterpenoids might be effective to other targets of HIV, rather than HIV protease, which should be studied further.

Acknowledgements

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Supplementary data

Supplementary data associated with this article can be found in the online version. The data include MOL files of the compounds described in this article.

References and notes

1. Friedman, K.A.; Laubenstein, L.; Marmor, M. *MMWR Morb Mortal Wkly Rep.* **1981**, *30*, 305.

2. Zhan, P.; Pannecouque, C.; Clercq, E. D.; Liu, X. *J. Med. Chem.* **2016**, *59*, 2849.

3. E. D. Clercq. Curr. Opin. *Pharmacol* **2010**, *10*, 507.

4. Clercq, E. D. *Adv. Pharmacol* **2013**, *67*, 317.

5. Clercq, E. D. *Pharmacol* **2013**, *86*, 711.

6. Xiao, S.; Tian, Z.; Wang, Y.; Si, L.; Zhang, L.; Zhou, D. *Med. Res. Rev.* **2018**, *38*, 951.

7. Newman, D. J.; Cragg, G. M. *J Nat Prod.* 2**016**, *79*, 629.

8. Kvasnica, M.; Urban, M.; Dickinson, N. J.; Sarek, J. *Nat. Prod. Rep.* **2015**, *32*, 1303.

9. Li, Z.; Min, Q.; Huang, H.; Liu, R.; Zhu, R.; Zhu, Q. *Bioorg Med Chem Lett.* **2018**, *28*, 1501.

10. Hirabayashi, K.; Iwata, S.; Matsumoto, H.; Mori, T.; Shibata, S.; Baba, M.; Ito, M.; Shigeta, S.; Nakashima, H.; Yamamoto N. *Chem Pharm Bull*. **1991**, *39*, 112.

11. Fields, A. P.; Bednarik, D. P.; Hess, A.; May, W. S. *Nature* **1988**, *333*, 278.

12. Sun, I.C.; Chen, C.H.; Kashiwada, Y.; Wu, J. H.; Wang, H. K.; Lee, K. H. *J Med Chem*. **2002**, *45*, 4271.

13. Bedoya, L. M.; Beltran, M.; Garcia-Perez, J.; Obregon-Calderon, P.; Callies, O.; Jimenez, I. A.; Bazzocchi, I. L.; Alcami, J. *Front in Pham.* **2018**, *9*, 358.

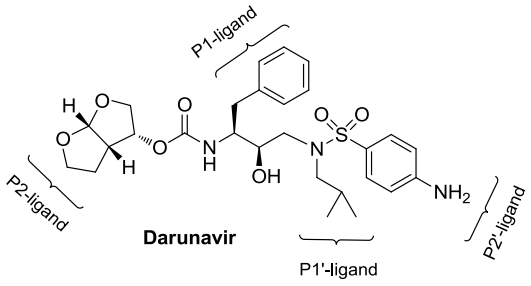
14. Yang, Z. H.; Bai, X.G. ; Zhou, L.; Wang, J. X.; Liu, H. T.; Wang Y. C. *Bioorg Med Chem Lett.* **2015**, *25*, 1880.

15. Bai, X.; Yang, Z.; Zhu, M.; Dong, B.; Zhou, L.; Zhang, G.; Wang, J.; Wang, Y. *Eur. J. Med. Chem.* **2017**, *137*, 30.

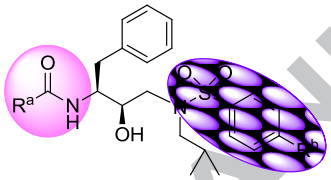
16. Ghosh, A. K.; Sridhar, P. R.; Leshchenko, S.; Hussain, A. K.; Li, J.; Kovalevsky, A. Y.; Walters,D. E.; Wedekind, J. E.; Tokars, V. G.; Das, D.; Koh, Y.; Maeda, K.; Gatanaga, H.; Weber, I. T.; Mitsuya, H. *J. Med. Chem.* **2006**, *49*, 5252.

17. Matayoshi, E. D.; Wang, G. T.; Krafft, G. A.; Erickson, J. *Science* **1990**, *247*, 954.

18. Gregson, S. J.; Howard, P. W.; Hartley, J. A.; Brooks, N. A.; Adams, L. J.; Jenkins, T. C.; Kelland, L. R.; Thurston, D. E. *J. Med. Chem.* **2001**, *44*, 737.
19. Tominaga, H.; Ishiyama, M.; Ohseto, F.; Sasamoto, K.; Hamamoto, T.; Suzuki, K.; Watanabe, M. *Anal. Commun.* **1999**, *36*, 47.
20. Parai, M. K.; Huggins, D. J.; Cao, H.; Nalam, M. N.; Ali, A.; Schiffer, C. A.; Tidor, B.; Rana, T. M. *J. Med. Chem.* **2012**, *55*, 6328.
21. Ghosh, A. K.; Parham, G. L.; Martyr, C. D.; Nyalapatla, P. R.; Osswald, H. L.; Agniswamy, J.; Wang, Y. F.; Amano, M.; Weber, I. T.; Mitsuya, H. *J. Med. Chem.* **2013**, *56*, 6792.

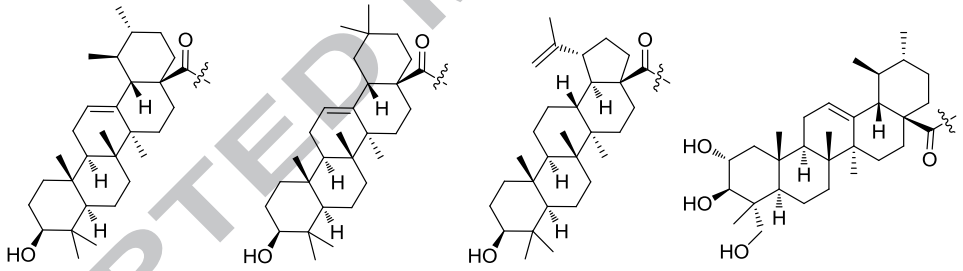


Lead compound

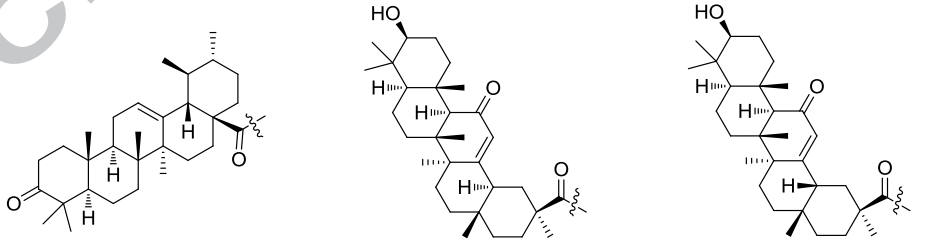


Target molecules

R^a =



1 Ursolic acid **2** Oleanolic acid **3** Betulinic acid **4** Asiatic acid



5 Ursonic acid **6** 18alpha-Glycyrrhetinic acid **7** 18beta-Glycyrrhetinic acid

R^b =