Natural Product Research

Formerly Natural Product Letters

ISSN: 1478-6419 (Print) 1478-6427 (Online) Journal homepage: http://www.tandfonline.com/loi/gnpl20

Betulinic acid derivatives: a new class of α-glucosidase inhibitors and LPS-stimulated nitric oxide production inhibition on mouse macrophage RAW 264.7 cells

Narayanarao Gundoju, Ramesh Bokam, Nageswara Rao Yalavarthi, Rajaram Azad & Mangala Gowri Ponnapalli

To cite this article: Narayanarao Gundoju, Ramesh Bokam, Nageswara Rao Yalavarthi, Rajaram Azad & Mangala Gowri Ponnapalli (2018): Betulinic acid derivatives: a new class of α-glucosidase inhibitors and LPS-stimulated nitric oxide production inhibition on mouse macrophage RAW 264.7 cells, Natural Product Research, DOI: 10.1080/14786419.2018.1462182

To link to this article: https://doi.org/10.1080/14786419.2018.1462182

NATURAL PRODUCT RESEARCH, 2018
https://doi.org/10.1080/14786419.2018.1462182

Taylor & Francis
Taylor & Francis Group

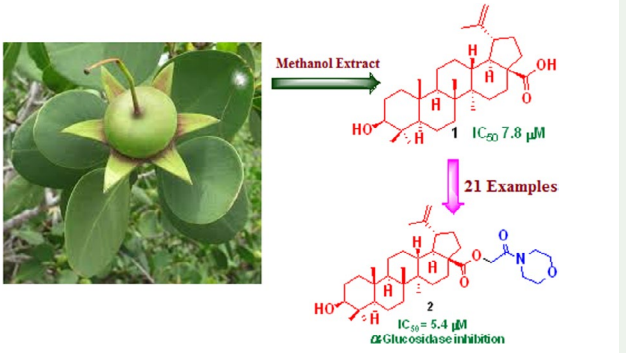
Betulinic acid derivatives: a new class of α-glucosidase inhibitors and LPS-stimulated nitric oxide production inhibition on mouse macrophage RAW 264.7 cells

Narayanarao Gundoju[a], Ramesh Bokam[a], Nageswara Rao Yalavarthi[a], Rajaram Azad[b] and Mangala Gowri Ponnapalli[a]

[a]Natural Products Chemistry Division, Indian Institute of Chemical Technology, Hyderabad, India; [b]Department of Animal Biology, University of Hyderabad, Hyderabad, India

ABSTRACT

Chemical manipulation studies were conducted on betulinic acid (**1**), twenty-one new rationally designed analogues of 1 with modifications at C-28 were synthesized for their evaluation of inhibitory effects on α-glucosidase and LPS-stimulated nitric oxide production in mouse macrophage RAW 264.7 cells. Compound **2** (IC_{50} = 5.4 μM) exhibited an almost 1.4-fold increase in α-glucosidase inhibitory activity on yeast α-glucosidase while analogues **5** (IC_{50} 16.4 μM) and **11** (IC_{50} 16.6 μM) exhibited a 2-fold enhanced inhibitory activity on NO-production than betulinic acid.



ARTICLE HISTORY
Received 10 February 2018
Accepted 3 April 2018

KEYWORDS
Sonneratia caseolaris; betulinic acid; α-glucosidase inhibition; LPS-stimulated nitric oxide production inhibition

1. Introduction

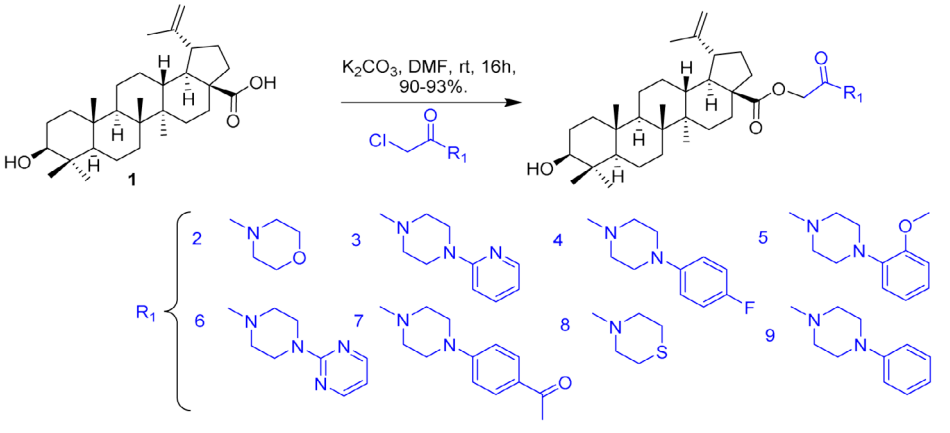
Betulinic, oleanolic, ursolic, and boswellic acids are abundant pentacyclic triterpenoids that occur in many edible fruits and vegetables. Among various pentacyclic triterpenoids, betulinic acid derivatives are widely studied group of compounds of natural origin due to their

CONTACT Mangala Gowri Ponnapalli pmgowri@yahoo.com, mangala@iict.res.in
Supplemental data for this article can be accessed at https://doi.org/10.1080/14786419.2018.1462182.

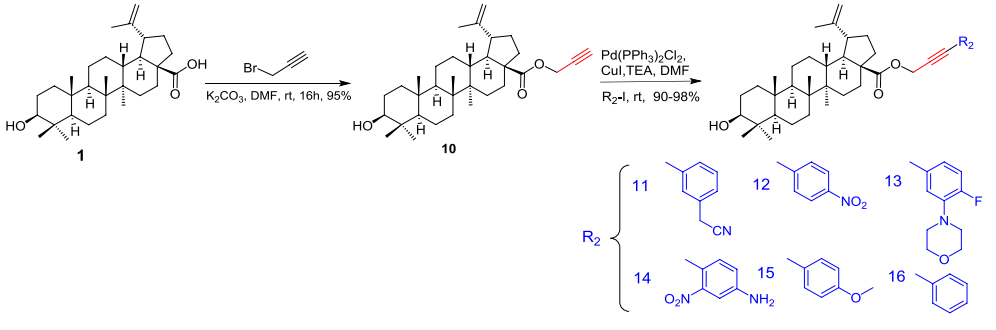
broad spectrum of biological profiles (Koehn and Carter 2005). Several triterpenoids are known to possess significant α-glucosidase (AGH) inhibitors and anti-inflammatory agents (Yu et al. 2014). As part of our ongoing program on Indian mangrove flora, we reported the α-glucosidase inhibitory and antihyperglycemic activity of the crude methanol extract and their metabolites from the fresh fruits of *Sonneratia caseolaris* (Ashok Kumar et al. 2010). A careful reexamination of the same extract led to the isolation of betulinic acid (Chao-Min et al. 2016) in substantial quantities. Herein, we report the isolation of betulinic acid from the underutilized fruits of *S. caseolaris* and semi synthesis of 21 new rationally designed N, O, S containing heterocyclic derivatives for their inhibitory effects on α-glucosidase and LPS induced NO inhibition on mouse macrophage RAW 264.7 cell lines.

2. Results and discussion

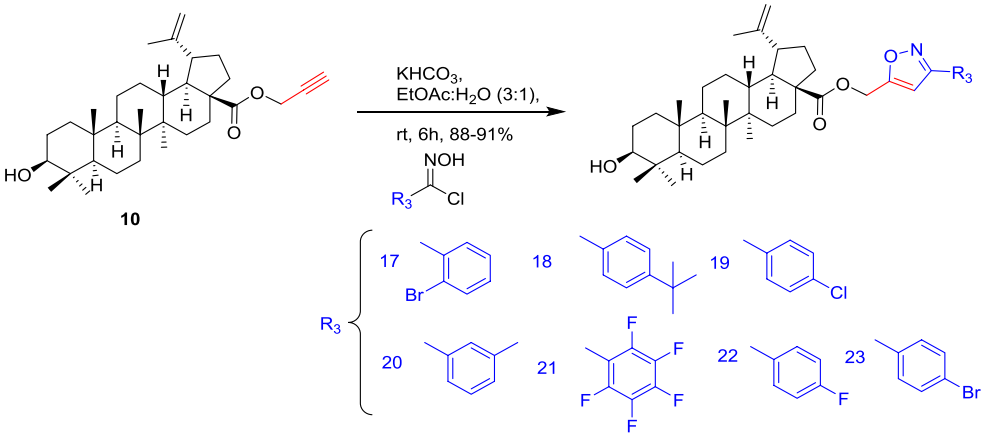
The structural modifications described in this paper were focused on the introduction of various nitrogen, oxygen and sulfur containing heterocyclic substituted methyl esters as new scaffolds (Schemes 1–3) at the C-28 region of the betulinic acid, which was isolated as major metabolite from the fruits of *Sonneratia caseolaris*. Prior pharmacological studies on



Scheme 1. Synthesis of heterocyclic amide derivatives on betulinic acid.



Scheme 2. Synthesis of phenyl acetylene derivatives on betulinic acid.



Scheme 3. Synthesis of isoxazole derivatives on betulinic acid.

betulinic acid demonstrated that the introduction of nitrogen-containing heterocyclic moieties will be beneficial for the development of new therapeutic agents (Qiu et al. 2009; Urban et al. 2012; Xu et al. 2012; Dang Thi et al. 2014; Gao et al. 2014). On the basis of promising *in vitro* cytotoxicity of piperazine, isoxazole (Jun et al. 2012), and acetylenic derivatives in a related series of pentacyclic lupane triterpenoids (Boryczka et al. 2013), we incorporated heterocyclic scaffolds into the betulinic acid template. Methyl ester analogues (**2**–**9**) were synthesized in excellent yields (90–93%) by the reaction between BA and different chloroacetyl amides in the presence of K_2CO_3 in DMF as solvent at room temperature. Chloroacetyl morpholine, chloroacetyl thiomorpholine, and Chloroacetyl N-substituted piperazines were synthesized by the reaction of chloroacetyl chloride with morpholine, thiomorpholine, and N-substituted piperazines in the presence of DCM using TEA as a base. The products were confirmed by the application of spectroscopic methods. BA was treated with propargyl bromide in the presence of K_2CO_3 in DMF solvent at room temperature to afford propargyl ester (**10**, 95% yield) as the key intermediate step for new semi synthetic derivatives (Schemes 2–3). The new series of analogues (**11**–**16**) were synthesized by a Sonogashira coupling (Sonogashira et al. 1975) using terminal alkyne C–H with substituted aryl iodides. The coupling reaction was catalyzed by Pd $(PPh_3)_2Cl_2$ and CuI in DMF solvent at room temperature to afford the product in excellent yields (90–98%) (Scheme 2). These products were easily identified by the absence of terminal methine at δ ~2.43 ppm in the ^{1}H NMR.

Furthermore, new analogues (Scheme 3) of betulinic acid with 3, 5-disubstituted isoxazole moieties were synthesized since isoxazoles are often encountered medicinally important pharmacophores (Cicchi et al. 2003; Jager and Colinas 2003; Grunanger and Vita-Finzi 1991). In general, uncatalyzed 1, 3-dipolar cycloaddition of nitrile oxides with acetylenes have been known for a long period, but its application to the synthesis of 3, 5-disustituted isoxazoles are scarce owing to low yields of isoxazole products (Huisgen 1984), side reactions and the possibility of both regioisomers. We have developed a methodology to improve yields, and wide scope of functional group on betulinic acid for a regioselective, experimentally convenient one-pot, three steps uncatalyzed 1, 3-dipolar cycloaddition of nitrile oxides with terminal acetylene. Moreover, an aldehyde is first converted into the corresponding aldoxime by the reaction with hydroxylamine. Without isolation, the aldoxime is transformed to the

chloroaldoxime. We have standardized the protocol with $KHCO_3$ and EtOAc: H_2O (3:1) at room temperature for 6 h to accomplish compounds (**17**–**23**) in good to excellent yields (88–91%), whereupon nitrileoxides are generated *in situ* and further reacted without isolation. The formation of 3, 5-disubstituted isoxazole moiety was confirmed by the presence of characteristic isoxazole proton singlet at δ ~6.66 ppm in the ^{1}H NMR and at δ ~102.0 ppm in the ^{13}C NMR.

The AGH inhibitory effects of BA analogues (**2**–**23**) were evaluated according to the modified method by Tomoyuki et al. (1999) and results are summarized in Table S1. Compound **2** (IC_{50} = 5.4 μM) exhibited an almost 1.4-fold increase in AGH inhibitory activity compared to BA (IC_{50} = 7.8 μM) whereas replacement of morpholine by thiomorpholine in the analogue **8** (IC_{50} = 42.2 μM) showed eight fold decrease in the AGH inhibitory activity, which strongly suggested that morpholine is essential for inhibiting α-glucosidase action compared to thiomorpholine substituent. Compounds **3** (IC_{50} = 8.9 μM), **15** (IC_{50} = 9.4 μM), and **23** (IC_{50} = 10.8 μM) displayed potent AGH inhibition compared to other derivatives. 2-Pyridyl (**3**), *p*-methoxy (**15**), and *p*-bromo (**23**) substituents played a prime role to increase AGH inhibition compared to other substituents in piperazine, acetylenic, and isoxazole analogues respectively. Compounds **4**–**14** and **16**–**22** were found to be less or inactive against AGH inhibition compared to BA.

The cell viability of BA analogues (**1**–**23**) on macrophage RAW 264.7 cell lines were determined by MTT assay (Figure S94). After 24 h of incubation, these compounds displayed cell viability > 90%. The anti-inflammatory activity of BA and its derivatives (**1**–**23**) were evaluated against LPS-stimulated NO production in RAW 264.7 cells. As shown Table S2, analogue **5** (IC_{50} = 16.4 μM) and **11** (IC_{50} = 16.6 μM) significantly suppress the NO production compared to that of betulinic acid (IC_{50} = 31.1 μM). Our results suggested that *O*-anisyl (**5**) substituent in the piperazine amide and *m*-cyanomethylphenyl acetylene (**11**) are essential functionalities for the mediation of anti-inflammatory activity while other derivatives showed the less potent or inactive NO-production inhibitory activity compared to BA (**1**).

3. Conclusions

Our findings demonstrated that BA was encountered as a major metabolite from the underutilized edible fruits of *S. caseolaris*. 21 New BA analogues were designed and synthesized for SAR studies. Our study revealed that certain ester functionalities enhanced AGH inhibition of BA significantly compared to NO production inhibition. Further studies are required to exploit AGH inhibitory potential of these analogues.

Acknowledgments

We are thankful to UGC for financial support and awarding SRF to NRG and director, Dr S. Chandrasekhar for constant encouragement and support. This work was supported by the Science and Engineering Research Board, New Delhi, India through a grant awarded to MGP (Grant number: EMR/2015/002319).

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

This work was supported by the UGC; SERB, Government of India [grant number EMR/2015/002319].

References

Ashok Kumar T, Viswanadh V, Mangala Gowri P, Ali AZ, Radhakrishnan SVS, Sachin Bharat A, Madhusudana K, Madhusudana Rao J. 2010. Oleanolic acid – an α-glucosidase inhibitory and ant hyperglycemic active compound from the fruits of *Sonneratia caseolaris*. Open Access J Med Aromatic Plants. 1:19–23.

Boryczka S, Bebenek E, Wietrzyk J, Kepinska K, Jastrzebska M, Kusz J, Nowak M. 2013. Synthesis, structure and cytotoxic activity of new acetylenic derivatives of betulin. Molecules. 18:4526–4543.

Chao-Min W, Hsiao-Ting C, Zong-Yen W, Yun-Lian J, Ching-Lin S, Chang-Hung Ch. 2016. Antibacterial and synergistic activity of pentacyclic triterpenoids isolated from *Alstonia scholaris*. Molecules. 21:139.

Cicchi S, Cordero FM, Giomi D. 2003. Progress in heterocyclic chemistry. 15:261.

Dang Thi TA, Kim Tuyet NT, Pham C, Thanh Nguyen H, Ba Thi C, Doan Duy T, Dhooghe M, Van Nguyen T. 2014. Synthesis and cytotoxic evaluation of novel ester-triazole-linked triterpenoid-AZT conjugates. Bioorg Med Chem Lett. 24:5190–5194.

Gao C, Dai FJ, Cui HW, Peng SH, He Y, Wang X, Yi ZF, Qiu WW. 2014. Synthesis of novel heterocyclic ring-fused 18b-glycyrrhetinic acid derivatives with antitumor and antimetastatic activity. Chem Biol Drug Des. 84:223–233.

Grunanger P, Vita-Finzi P. 1991. In: Taylor EC, Weissberger A, editors. Vol. 49, The chemistry of heterocyclic compounds: isoxazoles. New York: Wiley-Interscience; p. 1–416.

Huisgen R. 1984. In: Padwa A, editor. Vol. 1, 1, 3-dipolar cycloaddition chemistry. New York: Wiley; p. 1–176.

Jager V, Colinas PA. 2003. In: Padwa A, Pearson WH, editors. Vol. 59, The chemistry of heterocyclic compounds: synthetic applications of 1, 3-dipolar cycloaddition chemistry toward heterocycles and natural products. New York: Wiley; p. 361–472.

Jun X, Zhenxi L, Jian L, Fan Y, Ting L, Mingyao L, Wen WQ, Jie T. 2012. Synthesis and biological evaluation of heterocyclic ring-fused betulinic acid derivatives as novel inhibitors of osteoclast differentiation and bone resorption. J Med Chem. 55:3122–3134.

Koehn FE, Carter GT. 2005. The evolving role of natural products in drug discovery. Nat Rev Drug Discov. 4:206–220.

Qiu WW, Shen Q, Yang F, Wang B, Zou H, Li JY, Li J, Tang J. 2009. Synthesis and biological evaluation of heterocyclic ring-substituted maslinic acid derivatives as novel inhibitors of protein tyrosine phosphatase 1B. J Bioorg Med Chem Lett. 19:6618–6622.

Sonogashira K, Tohda Y, Hagihara NA. 1975. Convenient synthesis of acetylenes: catalytic substitutions of acetylenic hydrogen with bromoalkenes, iodoarenes and bromopyridines. Tetrahedron Lett. 16:4467.

Tomoyuki O, Toshiro M, Yutaka O. 1999. Inhibitory effect of α-glucosidase inhibitors varies according to its origin. J Agric Food Chem. 47:550–553.

Urban M, Vlk M, Dzubak P, Hajduch M, Sarek J. 2012. Cytotoxic heterocyclic triterpenoids derived from betulin and betulinic acid. Bioorg Med Chem. 20:3666–3674.

Xu J, Li Z, Luo J, Yang F, Liu T, Liu M, Qiu WW, Tang J. 2012. Synthesis and biological evaluation of heterocyclic ring-fused betulinic acid derivatives as novel inhibitors of osteoclast differentiation and bone resorption. J Med Chem. 55:3122–3134.

Yu MH, Shi ZF, Yu BW, Pi EH, Wang HY, Hou AJ, Lei C. 2014. Triterpenoids and α-glucosidase inhibitory constituents from *Salacia hainanensis*. Fitoterapea. 98:143–148.