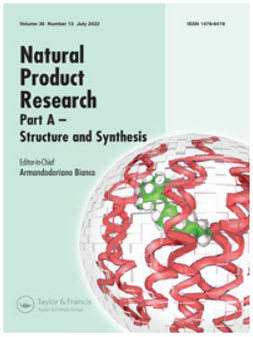

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Synthesis of ursolic acid arylidene-hydrazide hybrid compounds and investigation of their cytotoxic and antimicrobial effects

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Synthesis of ursolic acid arylidene-hydrazide hybrid compounds and investigation of their cytotoxic and antimicrobial effects

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ABSTRACT

In this study, 13 new hybrid compounds **(7a-m)** were synthesised starting from ursolic acid, and their cytotoxic activities were investigated on the BEAS-2B and A549 cell lines. In addition, the synthesised compounds were tested against *Staphylococcus aureus*, *Escherichia coli*, and *Candida albicans* to determine their antimicrobial properties. The hybrid compounds that exhibited the lowest cytotoxicity against the BEAS-2B were **7k, 7b,** and **7g**. The cytotoxicity of the compounds against A549 was evaluated, the IC_{50} value of **7k**, **7b,** and **7g** are found as 0.15 μM, 0.31 μM, and 0.26 μM, respectively. The results showed that the selectivity of **7k** was 7 times higher than doxorubicin against the A549 cells. According to the antimicrobial activity studies **7c** is found as the most effective compound against *S. aureus*. Almost all compounds showed a similar inhibition potential against *E. coli and C. albicans*.

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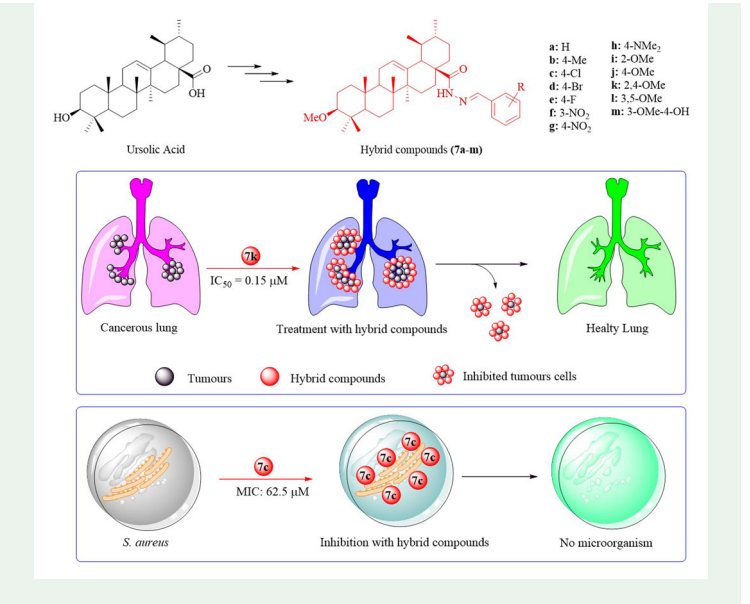

1. Introduction

Terpenes are secondary metabolites that play a crucial role in the defense mechanisms of plants. They can be classified as mono-, sesqui-, di-, sester-, or triterpenes. Ursolic acid, oleanolic acid, and betulinic acid (Figure 1) are all well-known triterpenes, and they are all associated with a number of biological activities (Ahmad et al. 2010; Khajeh et al. 2015; Birgani et al. 2018; Fan et al. 2021; Kazakova et al. 2021; Nguyen et al. 2021; Popov et al. 2021; Zhang et al. 2021; Zhao et al. 2021; Zhong et al. 2021; Zhou et al. 2021; Pereira et al. 2022).

Prior studies have reported that ursolic acid and its synthetic derivatives exert anti-cancer (Lin and Ye 2020), anti-diabetic (Hussain et al. 2017), anti-arrhythmic (Hussain et al. 2021), anti-hyperlipidemic (Khajeh et al. 2015), antimicrobial (Pereira et al. 2022), anti-hypercholesterolemic (Hao et al. 2020), and anti-cardiovascular effects (Mlala et al. 2019). Morever, ursolic acid and its synthetic derivatives also exhibit anti-microbial properties in relation to a broad spectrum of bacteria, human immunodeficiency virus (HIV), hepatitis C virus (HCV), and *Plasmodium protozoa* strains known to cause malaria (Wozniak et al. 2015).

Cancer is recognised as a major health problem worldwide. Indeed, it is one of the leading causes of death, being responsible for around 10 million deaths in 2019 alone. Globally, approximately one in six deaths are caused by cancer (Anonymous 2021; Ferlay et al. 2021). Based on the global cancer rates, the 10 most common types of cancers are lung, breast, colorectal, prostate, stomach, liver, esophagus, cervix uteri, thyroid, and bladder cancer (Ferlay et al. 2019).

Lung cancer has been determined to be the most common type of cancer in men worldwide, accounting for 15.5% of the total number of new cancer cases diagnosed in 2018. By contrast, breast cancer is the most common type of cancer in women worldwide, and it was responsible for 25.4% of all new cancer cases diagnosed in

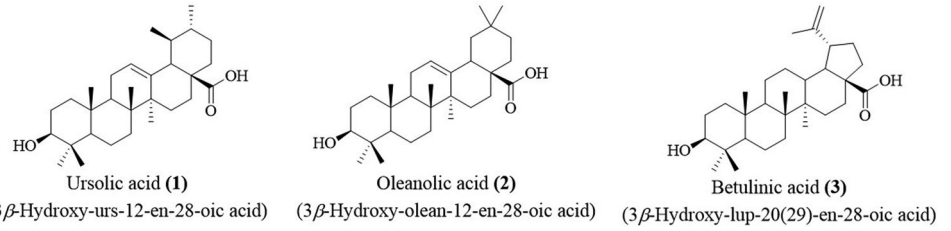

Figure 1. Ursolic, oleanolic, and betulinic acids.

2018. Yet, when all cancer-related deaths are taken into account, lung cancer is the most common cause of death among both genders worldwide, resulting in 18% of all cancer deaths (Ferlay et al. 2021; Merkouri et al. 2021).

Hybrid molecules are defined as chemical compounds that fulfill different biological functions and, further, that contain two or more structural domains that exhibit dual activity (Meunier 2008). Natural products and their semi-synthetic derivatives constitute a large percentage of the drugs used for the treatment of various diseases (Newman and Cragg 2007). However, due to the toxicity and poor bioavailability of anti-cancer drugs, it is considered necessary to improve the pharmacokinetic properties of new anti-cancer drugs. Although an increasing number of anticancer agents are being developed, their low selectivity and multi-drug resistance often prohibit successful cancer treatment (Kaya et al. 2017). Thus, medical professionals require new, potent, and selective anti-cancer agents capable of destroying tumor cells or, at the very least, stopping their proliferation (Pattnaik et al. 2017; Jin et al. 2019; Piccoli et al. 2020). The secondary metabolites of plants have traditionally been used in the treatment of cancer. Nowadays, the semi-synthetic derivatives of natural products comprise the majority of anti-cancer agents (Fu et al. 2014; Tuncay et al. 2018; Şenol et al. 2020).

2. Results and discussion

2.1. Syntheses

Prior studies have found that both hydrazide and hydrazone compounds exhibit a wide variety of biological activities (Reddy and Kathale 2017; El-Sayed et al. 2018; Jin et al. 2019; Mohamed 2019; Settypalli et al. 2019). When these compounds are combined with natural products, potentially biologically active hybrid molecules with two or more effects are formed (Pawełczyk et al. 2016; Pertino et al. 2018; Chen et al. 2019; Sheng et al. 2019; Sun et al. 2019). In light of this, in the present study, 13 new hybrid compounds were synthesised from the natural product ursolic acid (Figure 2). Moreover, the *in vitro* cytotoxic activities of these compounds were investigated in relation to BEAS-2B human non-tumorigenic lung epithelial cell lines and A549 adenocarcinomic human alveolar basal epithelial cell lines. In addition, the synthesised compounds were tested against *Staphylococcus aureus, Escherichia coli, and Candida albicans* to determine their anti-microbial properties.

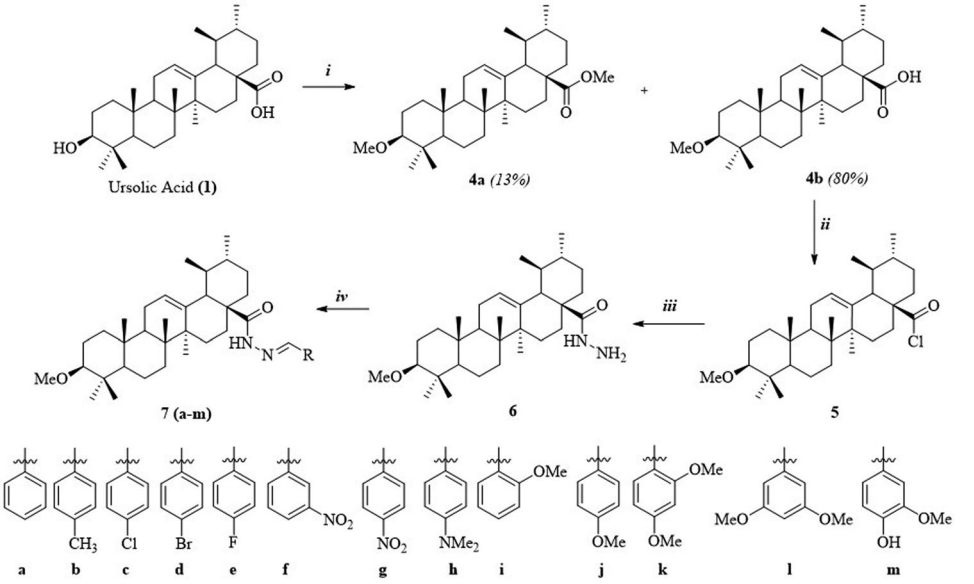

Figure 2. Syntheses of triterpenoid-hybrid compounds.

Initially, ursolic acid, a commercially available natural product, was converted into 3-methyl ursolic acid **(4 b)** using iodomethane in the presence of sodium hydride. During this reaction compound **4a** was obtained as a minor product. Next, compound **4b** was converted into acyl halide **(5),** which was then used to synthesise the hydrazide compound **(6)**. Finally, the triterpene-arylidene-hydrazide hybrid compounds **7(a-m)** were synthesised starting from hydrazide **(6)** and using 13 different aromatic aldehydes.

2.2. Cytotoxicity assay

The cytotoxic effects of compounds **6** and **7(a-m)** were investigated with regard to the BEAS-2B and A549 cell lines. The cytotoxic effects and IC_{50} values of the compounds in terms of the relative viability of the BEAS-2B and A549 cells at 0.1 μM, 0.5 μM, 1 μM, 5 μM, and 10 μM concentrations are given in Table S1 and S2, respectively. Furthermore, a relative cell viability graph showing the cytotoxic effects of the compounds on the BEAS-2B and A549 cells are presented in Figures S1 and S2, respectively.

The biological activity results of the synthesised compounds were compared with doxorubicin. The IC_{50} value of the cytotoxic effect of doxorubicin on the BEAS-2B cells was 0.16 μM (Emerce et al. 2019), while the IC_{50} values of the cytotoxic effects of the synthesised compounds on the same cell line ranged from 0.16 μM to 2.35 μM. More specifically, the hybrid compounds that exhibited the lowest cytotoxicity against the BEAS-2B cells are **7k** (IC_{50}= 2.35 μM), **7 b** (IC_{50}= 2.29 μM), **7i** (IC_{50}= 1.84 μM), **7g** (IC_{50}=

1.67 μM) and **7d** (IC_{50} = 1.46 μM). When the cytotoxic effects of compound **7k** and doxorubicin were compared, it was determined that **7k** had a toxic effect that was almost 15 times lower than the toxic effect of doxorubicin in the same healthy cells. In terms of compounds **7b**, **7i**, **7g**, and **7d** compounds, the cytotoxic effect was found to be reduced 14-, 11-, 10-, and 9- fold, respectively. The IC_{50} value of the cytotoxic effect of doxorubicin against the A549 lung cancer cells was determined to be 0.07 μM (Kashkin et al. 2010), while the IC_{50} values of the cytotoxic effects of the synthesised compounds against the same cell line ranged from 0.12 μM to 0.60 μM. When compared with the BEAS-2B cell line, the IC_{50} values of, the cytotoxicity of compounds **7k, 7b, 7i, 7g,** and **7d** in relation to the A549 cells, were were 0.15 μM, 0.31 μM, 0.45 μM, 0.26 μM, and 0.60 μM, respectively. When the cytotoxic effects of compound **7k** and doxorubicin were compared, **7k** was found to exert half the inhibitory effect of doxorubicin on the same cancer cells. Moreover, the toxicity of compound **7k** on healthy cells was 15 times lower than that of doxorubicin. Therefore, the selectivity (TD_{50}/ED_{50}) of compound **7k** was approximately seven times higher than the standard. In addition, the selectivity of compounds **7b, 7g, 7i,** and **7d** was 3, 3, 2, and 1.5 times higher, respectively, then that of doxorubicin.

2.3. Antimicrobial activity assay

Anti-microbial characteristics of synthesised compounds were tested against *S. Aureus, E. Coli, and C. Albicans* and the results were compared to standards such as amphotericin B, ciprofloxacin, and vancomycin. The tested compounds showed antibacterial activity against *S. aureus and E. coli*, at 62.5–250 μM and 125–250 μM concentrations, respectively. In addition, the tested compounds showed anti-fungal activity against *C. Albicans* at 125–250 μM concentration. The MIC values belonging to anti-microbial activity results were given in Table S3.

2.4. Structure activity relationship

According to the *in vitro* tests results, the structure-activity relationships were observed as follows. The selectivity for anticancer activity of compound **7a**, which include an unsubstituted benzene ring, is 5.5. Compounds **7k, 7b,** and **7g** have higher selectivity than **7a**, and these values are 15, 7 and 6 for **7k**, **7b,** and **7g,** respectively. In compounds **7k, 7b,** and **7g**, the substituents are 2,4-dimethoxy, 4-methyl and 4-nitro, respectively. Compounds **7e, 7c, 7f**, and **7d** have much lower selectivity than **7a** and the substituents in these compounds are 4-fluoro, 4-chloro-, 3-nitro- and, 4-bromo, respectively. It was observed that halogenated derivatives are unsuitable for the targeted anti-cancer activity, but electron-donating methoxy groups at the 2,4-position of the benzene ring are the optimum substituents. The lower selectivity of compound **7l** and **7f** explains why conjugation with the hydrazide group is important for activity. When the antimicrobial effects were evaluated, it was observed that the halogenated derivatives showed better activity than the others. The chloro- substituted **7c** compound having the highest antimicrobial activity.

3. Experimental

The experimental procedure for syntheses and biological activities, as well as all corresponding spectra, are described in the Supplementary Information. The structures of all synthesised compounds were well characterised by ^{1}H NMR, ^{13}C NMR, HRMS, and HPLC analyses.

4. Conclusion

In this study, according to the *in vitro* test results, the hybrid molecule **7k** may be a more selective potential anticancer drug when compared to doxorubicin. On the BEAS-2B and A549 cells, the hybrid compound **7k** is seven times more selective and effective than doxorubicin. Furthermore, anti-microbial activity investigations revealed that compound **7c** is the most efficient compound against *S. aureus* at 62.5 μM concentration when compared to standard antimicrobial agents. Almost all compounds showed a similar inhibition potential against *E. coli* and *C. Albicans* at 125 μM concentration. Finally, the hybrid compound **7k** appears to be a viable option for further research as an anti-cancer drug.

Disclosure statement

No potential conflict of interest was reported by the authors.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

Ahmad FB, Moghaddam MG, Basri M, Abdul Rahman MB. 2010. Enzymatic synthesis of betulinic acid ester as an anticancer agent: Optimization study. Biocatal Biotransform. 28(3):192–200.

Anonymous 2021. Worldwide cancer data and global cancer statistics for the most common cancers. World Cancer Research Fund, American Institute for Cancer Research. [accessed 2021 Oct 24]. https://www.wcrf.org/dietandcancer/worldwide-cancer-data/.

Birgani GA, Ahangarpour A, Khorsandi L, Moghaddam HF. 2018. Anti-diabetic effect of betulinic acid on streptozotocin-nicotinamide induced diabetic male mouse model. Braz J Pharm Sci.

54(2), 17171. https://www.scielo.br/j/bjps/a/Nbjvt5hGCD756YFLhgdDVVn/?format=pdf&lang=en.
Chen Z, Huang KY, Ling Y, Goto M, Duan HQ, Tong XH, Liu YL, Cheng YY, Morris-Natschke SL, Yang PC, et al. 2019. Discovery of an oleanolic acid/hederagenin-nitric oxide donor hybrid as an EGFR tyrosine kinase inhibitor for non-small-cell lung cancer. J Nat Prod. 82(11):3065–3073.
El-Sayed HA, Moustafa AH, Abd El-Moneim M, Awad HM, Esmat A. 2018. A new series of hydrazide-hydrazones based 2-oxonicotinonitriles as antimicrobial agents: Design, synthesis and antimicrobial evaluation. Afinidad. 75(584):305–310.
Emerce E, Gürbüz P, Doğan ŞD, Kadioglu E, Süntar I. 2019. Cytotoxic activity-guided isolation studies on Fumana procumbens (Dunal) Gren. & Godr. Rec Nat Prod. 13(3):189–198.
Fan JP, Lai XH, Zhang XH, Yang L, Yuan TT, Chen HP, Liang XR. 2021. Synthesis and evaluation of the cancer cell growth inhibitory activity of the ionic derivatives of oleanolic acid and ursolic acid with improved solubility. J Mol Liq. 332:115837.
Ferlay J, Colombet M, Soerjomataram I, Mathers C, Parkin DM, Pineros M, Znaor A, Bray F. 2019. Estimating the global cancer incidence and mortality in 2018: GLOBOCAN sources and methods. Int J Cancer. 144(8):1941–1953.
Ferlay J, Colombet M, Soerjomataram I, Parkin DM, Pineros M, Znaor A, Bray F. 2021. Cancer statistics for the year 2020: an overview. Int J Cancer. 149(4):778–789.
Fu LF, Lin QX, Liby KT, Sporn MB, Gribble GW. 2014. An efficient synthesis of methyl 2-cyano-3,12-dioxoursol-1,9-dien-28-oate (CDDU-methyl ester): analogues, biological activities, and comparison with oleanolic acid derivatives [Article. Org Biomol Chem. 12(28):5192–5200.
Hao WJ, Kwek E, He ZY, Zhu HY, Liu JH, Zhao YM, Ma KY, He WS, Chen ZY. 2020. Ursolic acid alleviates hypercholesterolemia and modulates the gut microbiota in hamsters. Food Funct. 11(7):6091–6103.
Hussain H, Green IR, Ali I, Khan IA, Ali Z, Al-Sadi AM, Ahmed I. 2017. Ursolic acid derivatives for pharmaceutical use: a patent review (2012-2016). Expert Opin Ther Pat. 27(9):1061–1072.
Hussain S, Taha M, Rahim F, Hayat S, Zaman K, Iqbal N, Selvaraj M, Sajid M, Bangesh MA, Khan F. 2021. Synthesis of benzimidazole derivatives as potent inhibitors for alpha-amylase and their molecular docking study in management of type-II diabetes. J Mol Struct. 1232: 130029.
Jin XY, Chen H, Li DD, Li AL, Wang WY, Gu W. 2019. Design, synthesis, and anticancer evaluation of novel quinoline derivatives of ursolic acid with hydrazide, oxadiazole, and thiadiazole moieties as potent MEK inhibitors. J Enzyme Inhib Med Chem. 34(1):955–972.
Kashkin KN, Musatkina EA, Komelkov AV, Favorskaya IA, Trushkin EV, Shleptsova VA, Sakharov DA, Vinogradova TV, Kopantzev EP, Zinovyeva MV, et al. 2010. Expression profiling and putative mechanisms of resistance to doxorubicin of human lung cancer cells. Dokl Biochem Biophys. 430(1):20–23.
Kaya B, Kaplancıklı ZA, Yurttaş L, Çiftçi GA. 2017. Synthesis and biological evaluation of some new pyrimidine bearing 2,5-disubstituted 1,3,4-oxadiazole derivatives as cytotoxic agents. Turk J Biochem. 42(2): 131–137. https://doi.org/10.1515/tjb-2016-0240.
Kazakova O, Rubanik L, Smirnova I, Poleschuk N, Petrova A, Kapustsina Y, Baikova I, Tret'yakova E, Khusnutdinova E. 2021. Synthesis and in vitro activity of oleanolic acid derivatives against Chlamydia trachomatis and Staphylococcus aureus. Med Chem Res. 30(7):1408–1418.
Khajeh M, Moghaddam MG, Danesh AZ, Khajeh B. 2015. Response surface modeling of betulinic acid pre-concentration from medicinal plant samples by miniaturized homogenous liquid-liquid extraction and its determination by high performance liquid chromatography. Arab J Chem. 8(3):400–406.
Lin WM, Ye HY. 2020. Anticancer activity of ursolic acid on human ovarian cancer cells via ROS and MMP mediated apoptosis, cell cycle arrest and downregulation of PI3K/AKT pathway. J Buon. 25(2):750–756.
Merkouri C, Grapsa D, Tourkantonis I, Gkiozos I, Charpidou A, Tournas G, Stratakos G, Politi E, Syrigos K. 2021. Cytology-histology concordance for diagnosis, histological subtyping and molecular profiling of lung cancer. J Thorac Oncol. 16(3):S463–S463.
Meunier B. 2008. Hybrid molecules with a dual mode of action: dream or reality? Acc Chem Res. 41(1):69–77.

Mlala S, Oyedeji AO, Gondwe M, Oyedeji OO. 2019. Ursolic acid and its derivatives as bioactive agents. Molecules. 24(15):2751.
Mohamed NA. 2019. Antimicrobial itaconimido aromatic hydrazide derivatives for inhibition of the thermal degradation of rigid PVC. Polym Bull. 76(5):2341–2365.
Newman DJ, Cragg GM. 2007. Natural products as sources of new drugs over the last 25 years. J Nat Prod. 70(3):461–477.
Nguyen NH, Pham DD, Le TTV, Nguyen TAT, Huynh DL, Duong TH, Sichaem J. 2021. Synthesis and alpha-glucosidase inhibitory activity of ursolic acid, lupeol, and betulinic acid derivatives. Chem Nat Compd. 57(6):1038–1041.
Pattnaik B, Lakshmi JK, Kavitha R, Jagadeesh B, Bhattacharjee D, Jain N, Mallavadhani UV. 2017. Synthesis, structural studies, and cytotoxic evaluation of novel ursolic acid hybrids with capabilities to arrest breast cancer cells in mitosis. J Asian Nat Prod Res. 19(3):260–271.
Pawełczyk A, Olender D, Sowa-Kasprzak K, Zaprutko L. 2016. Hybrid compounds strategy in the synthesis of oleanolic acid skeleton-NSAID derivatives. Molecules. 21(4):420.
Pereira VV, Pereira NR, Pereira RCG, Duarte LP, Takahashi JA, Silva RR. 2022. Synthesis and antimicrobial activity of ursolic acid ester derivatives. Chem Biodivers. 19(1). e202100566.
Pertino MW, Petrera E, Alche LE, Schmeda-Hirschmann G. 2018. Synthesis, antiviral and cytotoxic activity of novel terpenyl hybrid molecules prepared by click chemistry. Molecules. 23(6):1343.
Piccoli M, Ghiroldi A, Monasky MM, Cirillo F, Ciconte G, Pappone C, Anastasia L. 2020. Reversine: a synthetic purine with a dual activity as a cell dedifferentiating agent and a selective anticancer drug. Curr Med Chem. 27(21):3448–3462.
Popov SA, Wang C, Qi Z, Shul'ts EE, Turks M. 2021. Synthesis and antioxidant activity of new N-containing hybrid derivatives of gallic and ursolic acids. Chem Nat Compd. 57(6):1042–1046.
Reddy A, Kathale NE. 2017. Synthesis and anti-inflammatory activity of hydrazide-hydrazones bearing anacardic acid and 1,2,3-triazole ring based hybrids. Orient J Chem. 33(6):2930–2936.
Şenol H, Çokuludağ K, Sena Aktaş A, Atasoy S, Dağ A, Topçu G. 2020. Synthesis of new fatty acid derivatives of oleanane and ursane triterpenoids and investigation of their in vitro cytotoxic effects on 3T3 fibroblast and PC3 prostate cancer cell lines. Org Commun. 13(3): 114–126.
Settypalli T, Chunduri VR, Maddineni AK, Begari N, Allagadda R, Kotha P, Chippada AR. 2019. Design, synthesis, in silico docking studies and biological evaluation of novel quinoxaline-hydrazide hydrazone-1,2,3-triazole hybrids as alpha-glucosidase inhibitors and antioxidants. New J Chem. 43(38):15435–15452.
Sheng LX, Huang JY, Liu CM, Zhang JZ, Cheng KG. 2019. Synthesis of oleanolic acid/ursolic acid/glycyrrhetinic acid-hydrogen sulfide donor hybrids and their antitumor activity. Med Chem Res. 28(8):1212–1222.
Sun L, Chu XW, Liu CM, Sheng LX, Chen ZX, Cheng KG. 2019. Antiproliferative activity of ursolic acid/glycyrrhetinic acid-uracil/thymine hybrids. Med Chem Res. 28(6):892–899.
Tuncay S, Senol H, Guler EM, Ocal N, Secen H, Kocyigit A, Topcu G. 2018. Synthesis of oleanolic acid analogues and their cytotoxic effects on 3T3 cell line. Med Chem. 14(6):617–625.
Wozniak L, Skapska S, Marszalek K. 2015. Ursolic acid-A pentacyclic triterpenoid with a wide spectrum of pharmacological activities. Molecules. 20(11):20614–20641.
Zhang XF, Wang HY, Xu YR, Luan MZ, Zhao FL, Meng QG. 2021. Advances on the anti-inflammatory activity of oleanolic acid and derivatives. Mini Rev Med Chem. 21(15):2020–2038.
Zhao LJ, Duan X, Cao WW, Ren X, Ren GY, Liu PP, Chen JL. 2021. Effects of different drying methods on the characterization, dissolution rate and antioxidant activity of ursolic acid-loaded chitosan nanoparticles. Foods. 10(10):2470.
Zhong Y, Li HN, Zhou L, Su HS, Cheng MS, Liu Y. 2021. Synthesis and antitumor activity evaluation of oleanolic acid saponins bearing an acetylated L-arabinose moiety. Carbohydr Res. 503:108311.
Zhou D, Bao Q, Fu SB. 2021. Anticancer activity of ursolic acid on retinoblastoma cells determined by bioinformatics analysis and validation. Ann Transl Med. 9(20):1548–1548.