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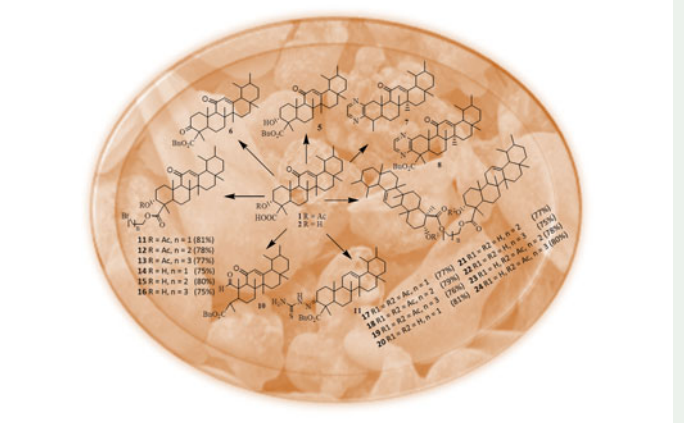
Synthesis of new boswellic acid derivatives as potential antiproliferative agents

Umair Shamraiz[a,b], Hidayat Hussain[a,c], Najeeb Ur Rehman[a], Sulaiman Al-Shidhani[a], Aasim Saeed[a,b], Husain Yar Khan[a], Ajmal Khan[a], Lucie Fischer[d], René Csuk[d], Amin Badshah[b], Ahmed Al-Rawahi[a], Javid Hussain[a] and Ahmed Al-Harrasi[a]

[a]UoN Chair of Oman's Medicinal Plants and Marine Natural Products, University of Nizwa, Nizwa, Sultanate of Oman; [b]Department of Chemistry, Quaid-i-Azam University, Islamabad, Pakistan; [c]Department of Bioorganic Chemistry, Leibniz Institute of Plant Biochemistry, Halle, Germany; [d]Organic Chemistry, Martin-Luther-University Halle-Wittenberg, Halle, Germany

ABSTRACT

In the current investigation, a series of heterocyclic derivatives of boswellic acids were prepared along with new monomers of 3-*O*-acetyl-11-keto-β-boswellic acid (AKBA, **1**) 11-keto-β-boswellic acid (KBA, **2**) and several new bis-AKBA and KBA homodimers and AKBA-KBA heterodimers. The effects of these compounds on the proliferation of different human cancer cell lines, viz., FaDu (pharynx carcinoma), A2780 (ovarian carcinoma), HT29 (colon adenocarcinoma), and A375 (malignant melanoma), have been evaluated. Thus, KBA homodimer **21** effectively inhibited the growth of FaDu, A2780, HT29, and A375 cells with EC_{50} values below 9 μM. In addition, compounds **7**, **8**, **11**, **12**, **15**, **16,** and **17** also exhibited cytotoxic effects for A2780, HT29, and A375 cancer cells. In particular, the pyrazine analog **8** was highly cytotoxic for A375 cancer cells with an EC_{50} value of 2.1 μM.



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CONTACT Hidayat Hussain hussainchem3@gmail.com; Ahmed Al-Harrasi aharrasi@unizwa.edu.om

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1. Introduction

Cancer is considered one of the most dreadful diseases in the world. This disease caused nearly 8.2 million deaths in 2012 and around 14.1 million new cases (Roy et al. 2016) were repoted. Unfortunately, current chemotherapeutic agents are often linked with various side effects, and especially the development of chemo-resistance towards these clinical drugs impedes an efficient and sustainable treatment of cancer (Roy et al. 2016). Frankincense has traditionally been used to treat cancer and several inflammatory diseases such as chronic pain syndrome, asthma, cerebral edema, arthritis, and chronic bowel disease (Takahashi et al. 2012; Roy et al. 2016). In addition, the gum resin of *Boswellia sacra* (frankincense) has also traditionally been used, for example, in Oman to treat severe muscle pain, colds, cough, dental infections, and stomach aches but also fever (Al-Ghassany 2008).

Frankincense consists – among many other compounds—of four major triterpenic acids known as boswellic acids, viz., 3-*O*-acetyl-11-keto-β-boswellic acid (AKBA, **1**, Figure 1), 11-keto-β-boswellic acid (KBA, **2**), β-boswellic acid (BA, **3**), and 3-*O*-acetyl-β-boswellic acid (ABA, **4**). These boswellic acids (BAs) showed interesting *in vitro* cytotoxic effects for various cancer cell lines, among them cancer of bladder (Frank et al. 2009), brain (Glaser et al. 1999), cervix (Qurishi et al. 2012), colon, liver (Liu et al. 2002), colorectum (Takahashi et al. 2012), leukemia (Shao et al. 1998), lung (Ravanan et al. 2011), melanoma (Zhao et al. 2003), meningioma (Park et al. 2002), myeloma (Kunnumakkara et al. 2009), neuroblastoma (Qurishi et al. 2012), pancreatic (Park et al. 2011), and prostate cancer (Syrovets et al. 2005). In addition, boswellic acids also demonstrated *in vivo* anticancer effects towards various cancers, for example colon cancer (Yuan et al. 2013), Ehrlich tumor (Agrawal et al. 2011), colorectal cancer (Takahashi et al. 2012), glioma (Ravanan et al. 2011), leukemia (Huang et al. 2000), pancreatic cancer (Park et al. 2011), and prostate cancer (Syrovets et al. 2005). Some clinical studies have also reported boswellic acids for treatment of brain tumor, lung cancer and breast cancer (Huang et al. 2000). An extensive metabolism and poor absorption, however, narrow the bioavailability of AKBA (Cheng et al. 2015), and therefore there is an urgent need of new derivatives of AKBA with effective biological actions and improved bioavailability. Dimers (Bednarczyk-Cwynar and Günther 2017) of oleanolic acid have been previously reported by Cheng et al. (2015) and Medina-O'Donnell et al. (2018).

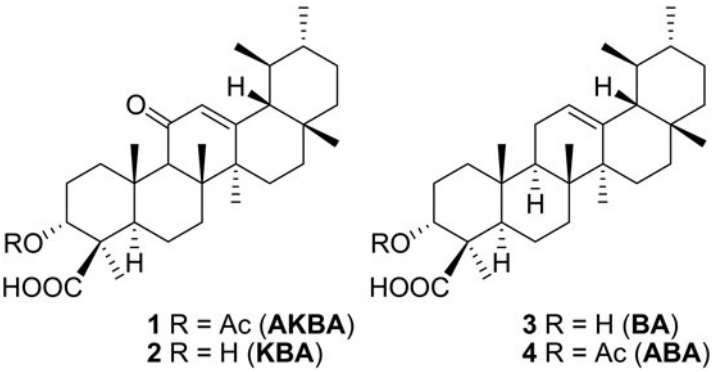
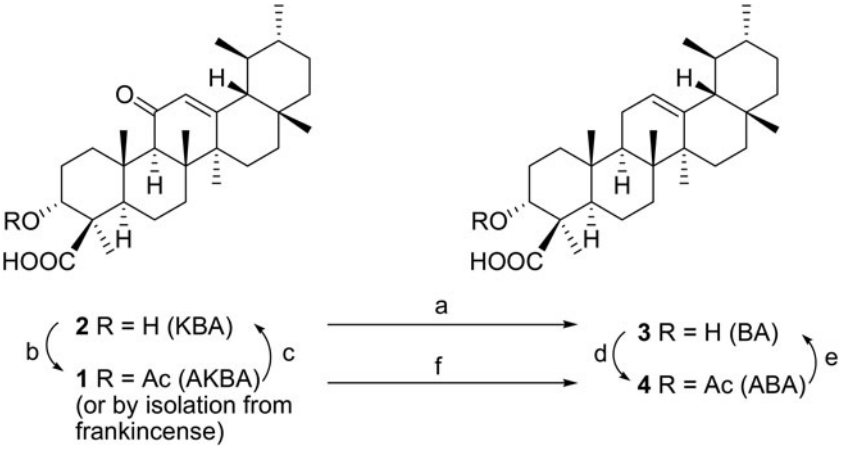


Figure 1. Structure of the most important β-boswellic acids.



Scheme 1. (Interconversion of the boswellic acids **1**–**4:** a) PtO_2, HOAc, H_2, 85 bar, 25 °C, 12 h, 87%; b) AcCl, pyridine, DMAP, 25 °C, 6 h, 91%; c) NaOH, EtOH, 25 °C, 12 h, 98%; d) AcCl, pyridine, DMAP, 25 °C, 6 h, 89%; e) NaOH (aq.), 25 °C, 12 h, 93%; f) PtO_2, HOAc, H_2, 85 bar, 25 °C, 12 h, 87% (Jauch and Bergmann2003; Wolfram et al.2017).

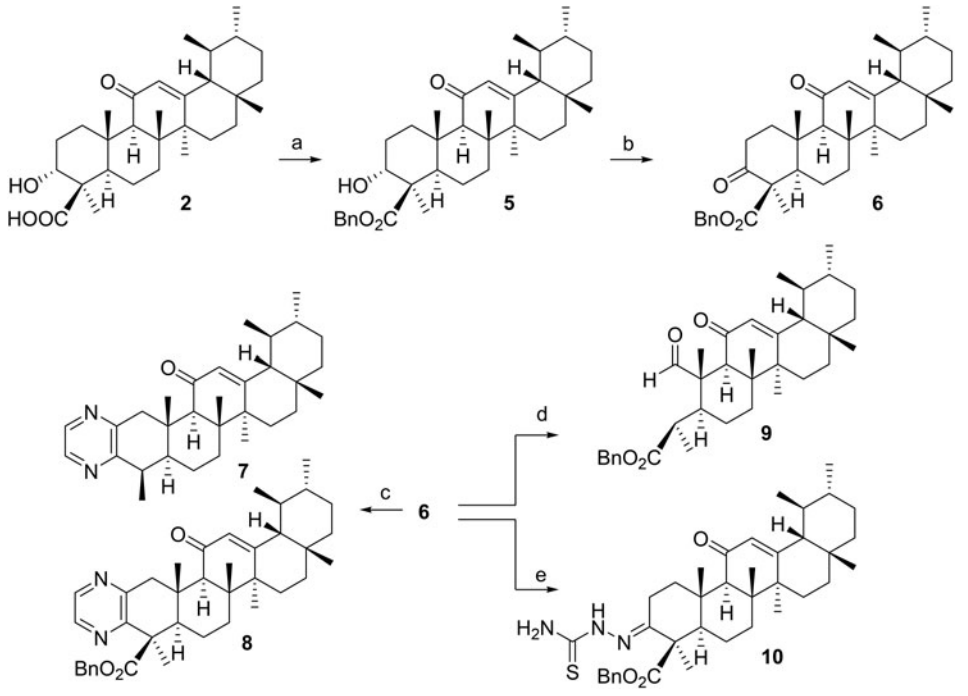
These dimers showed significant antiproliferative effects against various cancer cell lines (Cheng et al. 2015). Moreover, quite recently we have prepared several monomers and dimers of the triterpene acid myrrhanone B, many of which have shown potent anticancer effects against the different human cancer cell lines FaDu, A2780, HT29 and A375 (Saeed et al. 2018). These interesting results from oleanolic acid (dimers) and myrrhanone B (monomers and dimers) as well as the well-demonstrated anticancer activity of boswellic acids reported against different types of cancer cells has prompted us to synthesize various derivatives and dimers of some boswellic acids. Unfortunately, the concentration of the most active boswellic acid AKBA in extracts from *Boswellia* resins is in the range of 0.1–3%. Consequently, we used the reported procedure described by Jauch and Bergmann (2003) to obtain AKBA and KBA in larger amount (Scheme 1). This allowed the synthesis of several analogs of boswellic acids that were subjected to an *in vitro* cytotoxicity screening utilizing different human cancer cell lines [FaDu (pharynx carcinoma), A2780 (ovarian carcinoma), HT29 (colon adenocarcinoma) and A375 (malignant melanoma)] to identify new cytotoxic lead molecules.

2. Results and discussion

2.1. Synthesis

The concentration of AKBA (**1**) in *B. sacra* is very low while the concentration of KBA (**2**), BA, (**3**), or ABA, (**4**) is relatively higher. To prepare KBA in larger quantities we followed the procedure reported by Jauch and Bergmann (2003) to obtain AKBA. All boswellic acids **1–4** can easily be interconverted (Wolfram et al. 2017).

KBA (**2**) was esterified with $BnBr/K_2CO_3$ in DMF, and ester **5** was obtained in 91% yield (Scheme 2). Jones oxidation (CrO_3/aq. H_2SO_4) in acetone of the C-3 hydroxyl group gave 3-keto **6** in 87% yield. Compound **6** served as a valuable starting material



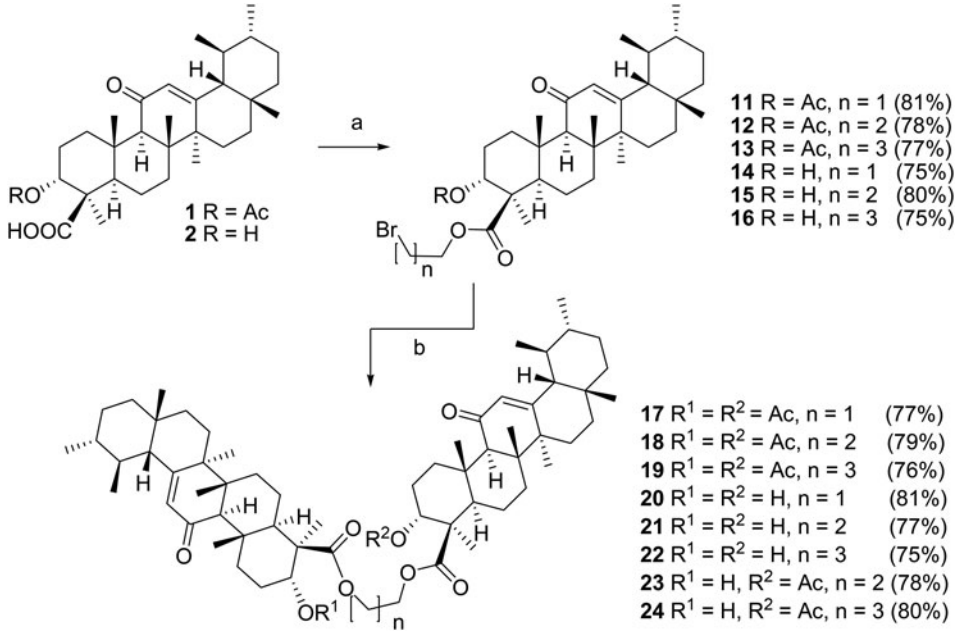
Scheme 2. Synthesis of compounds **5**–**10**: a) BnBr, K_2CO_3, DMF, rt, 12 h, 91%; b) Jones reagent, rt, 2 h, 87%; c) ethane-1,2-diamine, morpholine, S, reflux, 18 h, **7** (45%), **8** (42%); d) aniline, ^{t}BuOK, DMSO, rt, 48%; e) thiosemicarbazide, EtOH, reflux, 81%.

for the syntheses to follow. Thus, synthesis of the pyrazine derivatives (Csuk et al. 2011; Zorina et al. 2018) of AKBA (compounds **7** and **8**) was accomplished with 45% and 42% yield by reacting ketone **6** with ethylene diamine in morpholine as the solvent (Bhandari et al. 2014). Furthermore, ketone **6** gave upon reaction with potassium *tert*-butoxide in the presence of aniline not the corresponding indole but this reaction ended up with an opening of ring A and provided aldehyde **9** in 48% yield. The synthesis of semithiocarbazone **10** was accomplished by reacting **6** with semicarbazide. Monomers of boswellic acids **11–16** (Scheme 3) were prepared by esterifying boswellic acids **1** and **2** with three equivalents of the linkers 1,2-dibromoethane, 1,3-dibromopropane, and 1,4-dibromobutane in the presence of K_2CO_3 in DMF, respectively (Saeed et al. 2018). The bis and hetero dimers of boswellic acids **17–25** (ester-linked) were obtained in good yields by reacting the monomers **11–16** in the presence of K_2CO_3 in DMF with compounds **1** and **2**.

2.2.Cytotoxic activity and SAR studies

The compounds were subsequently screened for their cytotoxic activity for human cancer cell lines FaDu, A2780, HT29, and A375. The EC_{50} values of the boswellic acid analogs and dimers for these cell lines are summarized in Table 1. Compounds having EC_{50} values over 30 μM were considered as not active.

As evident from the data presented in Table 1, pyrazine analogs **7** and **8** demonstrated cytotoxic effects for A2780 (**7**: EC_{50} = 15.7 μM; **8**: EC_{50} = 13.7 μM), HT29 (**7**:



Scheme 3. Reagents and conditions: a) 1,2-dibromoethane (3 eq) or 1,3-dibromopropane (3 eq) or 1,4-dibromobutane (3 eq), K_2CO_3, DMF; b) compounds **1** or **2** (1 eq), 1,2-dibromoethane (1 eq) or 1,3-dibromopropane (1 eq) or 1,4-dibromobutane (1 eq), K_2CO_3, DMF.

Table 1. EC_{50} values (in μM, from sulforhodamine B assays performed at least thrice with three technical replicates each) of novel Boswellic acid derivatives and dimers against various cancer cell lines.

Compound	FaDu	A2780	HT29	A375
7	>30	15.7 ± 1.7	22.7 ± 2.2	12.8 ± 1.6
8	>30	13.7 ± 1.9	12.2 ± 1.8	2.1 ± 0.4
9	>30	17.9 ± 1.4	>30	19.4 ± 2.0
10	29.5 ± 2.1	9.3 ± 0.9	11.3 ± 0.8	10.8 ± 1.9
11	>30	16.3 ± 1.7	27.7 ± 3.1	12.1 ± 1.7
12, 13	>30	>30	>30	>30
14	17.2 ± 1.3	13.3 ± 1.3	12.5 ± 1.5	13.9 ± 1.6
15	16.3 ± 1.5	12.1 ± 1.5	12.8 ± 1.9	12.6 ± 1.4
16	21.7 ± 2.7	13.2 ± 1.6	14.7 ± 2.2	16.7 ± 2.0
17–19	>30	>30	>30	>30
20	8.6 ± 1.2	6.6 ± 1.2	6.2 ± 1.1	8.2 ± 1.7
21–24	>30	>30	>30	>30

Each value represents the mean ± SD; FaDu = pharynx carcinoma, A2780 = ovarian carcinoma), HT29 = colon adenocarcinoma, and A375 = melanoma human cancer cell lines; EC_{50} values >30 μM were considered inactive.

EC_{50} = 22.7 μM; **8**: EC_{50} = 12.2 μM) and A375 (**7**: EC_{50} = 12.8 μM; **8**: EC_{50} = 2.1 μM) tumor cells. It is interesting to note that compound **8** exhibited a much better activity than compound **7**. In particular, AKBA analog **8** showed six times stronger cytotoxic effect for A375 cancer cells (**8**: EC_{50} = 2.1 μM vs **7**: EC_{50} = 12.8 μM). These results suggest that an addition of a pyrazine group has a significant impact on the activity of boswellic acids. On the other hand, ring A modified *seco*-structure **9** was only active against cell lines A2780 (EC_{50} = 17.9 μM) and A375 (EC_{50} = 19.4 μM). This also indicated that a cleavage of ring A did not enhance activity. This parallels previous

findings for other triterpenoic acids (Csuk et al. 2011). Compound **7** (being decarboxylated at position C-23 compared to its parent compound), and compound **9**, which holds a modified ring A, have shown diminished activities as compared to compound **8**. This indicates that the presence of a carboxyl group and of an intact ring A is crucial for retaining the cytotoxic effects of the boswellic acid analogs. Further, the semithiocarbazone **10** demonstrated good cytotoxic effects for the three cancer cell lines A2780 ($EC_{50} = 9.3\,\mu M$), HT29 ($EC_{50} = 11.3\,\mu M$) and A375 ($EC_{50} = 10.8\,\mu M$). This result revealed that an incorporation of extra nitrogen into the AKBA skeleton enhances activity; this finding is in excellent agreement with previous findings (Wolfram et al. 2017).

Cytotoxic effects of the three monomers of AKBA **11–13** were not impressive at all. Monomers **12** and **13,** holding a bromopropyl or a bromobutyl group, were found to be inactive for all of the tested cancer cell lines ($EC_{50} > 30\,\mu M$). It was only monomer **11**, carrying a bromoethyl group, that was found to be active against at least three types of cancer cells: A2780 ($EC_{50} = 16.3\,\mu M$), HT29 ($EC_{50} = 27.7\,\mu M$), and A375 ($EC_{50} = 12.1\,\mu M$). On the other hand, the growth inhibitory activity of the three monomers of KBA viz., compounds **14–16** was very significant inasmuch as all these monomers were active against the tested tumor cell lines (FaDu, A2780, HT29 and A375) with EC_{50} values ranging from 12.1 to 21.7 μM.

Homodimers of AKBA **17**–**19** holding an ethylene, a trimethylene or a tetramethylene spacer were found to be inactive ($EC_{50} > 30\,\mu M$) against all the four cancer cell lines tested. Similarly, the two homodimers of KBA **21** and **22** having a trimethylene or a tetramethylene linker also turned out to be inactive ($EC_{50} > 30\,\mu M$). However, the KBA homodimer **20**, holding an ethylene group as linker, proved to be the most potent cytotoxic compound. This compound showed significant inhibitory effects on the proliferation of all the four types of cancer cells viz., FaDu (8.6 μM), A2780 ($EC_{50} = 6.6\,\mu M$), HT29 ($EC_{50} = 6.2\,\mu M$), and A375 ($EC_{50} = 8.2\,\mu M$). Finally, it may be noted that among all the tested compounds, only compounds **14–16** and **20** were effective in inhibiting the proliferation of pharynx carcinoma (FaDu) cells.

3. Conclusion

We have prepared ring A modified AKBA analogs (compounds **7**, **8**, **9**, and **10**), AKBA monomers (**11–13**) and KBA monomers (**14–16**) as well as homo- and heterodimers of AKBA and KBA (**17–24**). The most remarkable anti-proliferative activity was displayed by the pyrazine analog **8** against A375 (malignant melanoma) cells with an EC_{50} value as low as 2.1 μM. SAR studies revealed that the presence of an amine enhances the activity of boswellic acids. Moreover, it was also observed that decarboxylation or cleavage of ring A led to reduced cytotoxic activity. KBA homodimer **20** and KBA-AKBA heterodimer **23** exhibited cytotoxic effects while all the other dimers turned out to be inactive. In fact, **20** was the most potent compound showing significant cytotoxic effects against all the four cancer cell lines with $EC_{50} \leq 11.0\,\mu M$. It is interesting to note that compound **20** (holding an ethylene group as linker in its dimeric structure) was cytotoxic. This suggests that the length of the spacer plays a significant role in establishing the cytotoxic effects of KBA homodimers.

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Disclosure statement

The authors declared no conflict of interest.

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