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In vitro cytotoxicity and structure-activity relationship approaches of *ent*-kaurenoic acid derivatives against human breast carcinoma cell line

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ABSTRACT

In this study, *ent*-kaurenoic acid derivatives were obtained by microbial transformation methodologies and tested against breast cancer cell lines (MCF-7). A multivariate quantitative-structure activity relationship (QSAR) analysis was performed taking into account both microbial transformation derivatives and other analogues previously reported in literature to give some insight into the main features behind the cytotoxic activity displayed by kaurane-type diterpenes against MCF-7 cells. The partial least square regression (PLS) method was employed in the training set and the best PLS model was built with a factor describing 69.92% of variance and three descriptors (log*P*, ε_{HOMO} and ε_{HOMO-1}) selected by the Ordered Predictors Selection (OPS) algorithm. The QSAR model provided reasonable regression ($Q^2 = 0.64$, $R^2 = 0.72$, *SEC* = 0.29 and *SEV* = 0.33). The model was validated by leave-*N*-out cross-validation, y-randomization and external validation ($R^2_{pred} = 0.89$ and *SEP* = 0.27). The selected descriptors indicated that the activity was mainly related to electronic parameters (HOMO and HOMO-1 molecular orbital energies), as well as to log*P*. These findings suggest that higher activity values are directly related with both higher log*P* and frontier orbital energy values. The positive relationship between these orbitals and the activity suggests that the *ent*-kaurenoic acid analogues interaction with the target involves charge displacement, which is entirely consistent with the literature. Based on these findings, three compounds were proposed and one of them was synthesized and tested. The experimental result confirmed the activity predicted by the model.

1. Introduction

Mother nature has been considered the most promising inspiration source for preparation of novel pharmacologically active chemical entities (Newman and Cragg, 2016). Generally, natural compounds have been employed either in drug design processes or applied as starting scaffolds for structural modifications leading to the preparation of semi-synthetic derivatives, which have demonstrated to be even more potent and/or less toxic in comparison with their parent structures (Newman and Cragg, 2016; Simao et al., 2016).

Nowadays, *in silico* methods have become noticeable during the development of novel drugs, and quantitative-structure activity relationship (QSAR) studies have been employed to identify important chemical features and consequently propose descriptors related with the respective biological activity, thus assisting in the discovery of novel potential lead compounds (Danishuddin and Khan, 2016; Martinez et al., 2017). In fact, QSAR has been considered an important tool in the rational development and discovery of new drugs (Danishuddin and Khan, 2016; Martinez et al., 2017).

Among a plethora of bioactive natural compounds, kaurenoic acid (**1**; *ent*-kaur-16-en-19-oic acid; Fig. 1), a kaurane-type diterpene, is found in high amounts in some Brazilian medicinal plants genera such

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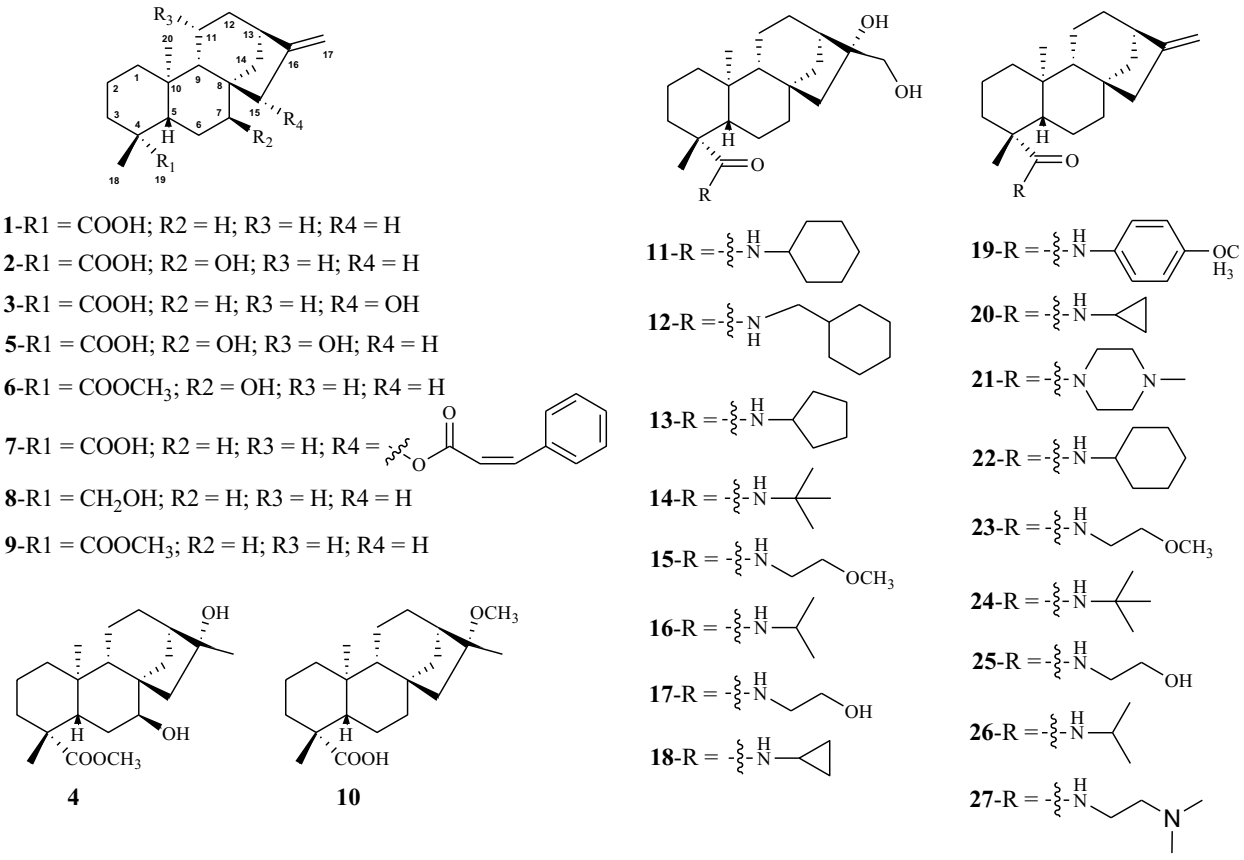

Fig. 1. Series of kaurenoic acid analogues employed in QSAR studies.

as *Copaifera* and *Mikania* (De Andrade et al., 2011; Simao et al., 2016). Compound **1**, in particular, has demonstrated to possess a wide spectrum of biological activities, displaying anti-inflamatory, analgesic, antispasmodic, antimicrobial, and antiparasitic activities (Batista et al., 2013; Mizokami et al., 2012; Moreira et al., 2016; Tirapelli et al., 2005). Moreover, this diterpene has also attracted considerable attention from the scientific community because this natural compound and its structural derivatives exhibit significant *in vitro* cytotoxic effects against several tumor cell lines (Batista et al., 2013; Hueso-Falcon et al., 2010; Lizarte Neto et al., 2013; Simao et al., 2016).

In 2010, Hueso-Falcon and coworkers (Hueso-Falcon et al., 2010) synthesized a series containing thirty-one *ent*-kaurane derivatives, from which four were considered as potent apoptosis inducers in human tumor cells through a caspase-dependent mode of action. In another study, by Batista and coworkers (Batista et al., 2013), the *in vitro* citotoxicity of oxidized/epoxidized kaurenoic acid analogues was assessed against ovary, breast, melanoma, lung, leukaemia, pancreas, colon and cervical cancer cells. From their results, two allylic alcohol derivatives showed to be the most potent ones, displaying very promising GI_{50} values, ranging from 0.71 to 8.90 μM (Batista et al., 2013). More recently, Simão and coworkers (Simao et al., 2016) investigated the ability of a series of amides and diols derivatives of compound **1** to inhibit the growth of a human breast carcinoma cell line. Among the obtained analogues, one diterpene exhibited significant activity, displaying an IC_{50} value of 7.84 μM and a selectivity index of about 50.7. It was also observed in this study that the presence of an amide containing an aromatic moiety in kaurenoic acid, instead of the carboxylic acid group, significantly increased the activity and the selectivity of the obtained derivative (Simao et al., 2016). Such findings brought an insight to investigate the structure–activity relationship displayed by kaurenoic acid structural analogues against tumor cell lines.

In the present study, kaurenoic acid derivatives were obtained through fungal transformation using *Aspergillus terreus* strains. Moreover, other kaurane-type diterpenes previously reported (De Andrade et al., 2011; Simao et al., 2016) were also employed to perform a multivariate data analysis (partial least squares regression - PLS), including QSAR studies against a human breast carcinoma cell line. Based upon the information obtained from QSAR analysis, three kaurenoic acid derivatives were proposed - one of them was synthesized and also evaluated against MCF-7 cell lines (compound **27**).

2. Results and discussion

2.1. Microbial transformation of kaurenoic acid (compound 1)

Fourteen-days incubation of compound **1**, isolated from *Copaifera* ssp, with *Aspergillus terreus* strains gave rise to four derivatives (**2, 3, 4** and **5**), which were isolated with a yield ranging from 2.2 to 24.3% based on their weights relative to the starting substrate. Due to the high rate converting of diterpene **2** from **1**, this metabolite was used as substrate to obtain its respective methyl ester (**6**), which was also evaluated against MCF-7 cells. The spectral data of all compounds are in agreement with that previously reported in the literature, **2** (Silva et al., 1999); **3** (Vichnewski et al., 1977); **4** (Rudolf et al., 2016); **5** (Marquina et al., 2009). The chemical structures of substrate (**1**) as well as of the derivatives obtained by microbial transformation are presented in Fig. 1.

As established in the scientific literature, microbial transformation is an area of great interest to the pharmaceutical and chemical industries, once it allows for the production of derivatives that are difficult to obtain by organic synthesis (dos Santos et al., 2018; Parshikov and Sutherland, 2014). Biotransformation studies of natural products using *Aspergillus* species are commonly found in the scientific literature and hydroxylation is the major reaction produced by these