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Original article

Natural sesquiterpene lactones as inhibitors of Myb-dependent gene expression: Structure–activity relationships

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ABSTRACT

c-*myb* is a proto-oncogene encoding a transcription factor which is highly expressed in hematopoietic progenitor cells. It regulates the expression of genes important for lineage determination, cell proliferation, and differentiation. Deregulation of c-*myb* expression is known to be involved in the development of human tumors, especially certain types of leukemia and breast and colon cancer. The c-Myb protein has thus been identified as an interesting therapeutic target. We recently discovered that some sesquiterpene lactones suppress Myb-dependent gene expression which is a new mechanism for these natural products' potential anti-cancer activity. We developed a test system to screen compounds for inhibitory activity on Myb-inducible reporter gene activation. Using this system we have now investigated 60 sesquiterpene lactones for their capacity to inhibit c-Myb-dependent gene activation. The IC_{50} values were in a range between 0.7 and >30 μM. The furanoheliangolide goyazensolide and the pseudoguaianolide helenalin acetate (IC_{50} = 0.6 and 0.7 μM, respectively) represent the most active inhibitors of c-Myb dependent gene expression found up to present. Control measurements for cell viability (MTS assay) proved that the observed activity on c-Myb dependent gene expression is not a function of cytotoxicity/unspecific cell damage.

Structure–activity relationships were investigated by a QSAR approach based on flexible alignment of the most active compounds and a common pharmacophore model. These investigations resulted in a QSAR model which indicates that the potency of inhibitory activity on c-Myb-dependent transcription does not only depend on the presence of reactive Michael-acceptor features but also on their optimal spatial arrangement in the molecule.

1. Introduction

Proto-oncogenes of the *myb*-family (A-*myb*, B-*myb*, and c-*myb*), encode transcription factors that regulate the expression of genes with important functions for lineage determination, cell proliferation, and differentiation [1–4]. The c-*myb* gene is expressed predominantly in the hematopoietic system and has been demonstrated in various studies to play a crucial role in the development of most lineages of this system [5–10]. Its expression is highest in the immature progenitors of all hematopoietic lineages. Down-regulation of *c-myb* is known to be essential for their terminal differentiation. Furthermore, *c-myb* is also expressed in some other tissues [11] where it plays a role, e.g., in the proliferation of colonic crypt progenitor cells [12].

The protein encoded by c-*myb* (c-Myb) is a transcription factor which regulates the expression of a large variety of genes, including genes involved in proliferation, cell survival and differentiation [13–16]. Furthermore, strong evidence has been presented that its deregulation plays a role in the development of certain leukemias and of breast and colon tumors. It has hence been suggested that suppression of c-Myb-induced gene activation might be a valuable therapeutic strategy [4].

In a recent communication, we demonstrated that some natural sesquiterpene lactones (STLs) isolated from plants of the Sunflower family (Asteraceae) are able to inhibit the activation of Myb-inducible genes [17]. We developed a fluorescence-based assay system that allows screening of compounds for their ability to suppress the activation of a Myb-inducible reporter gene. This system has now

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been used to screen a series of 60 natural STLs for their potency in inhibiting c-Myb activity and, on this basis, to study structure–activity relationships for this first group of soluble low-molecular weight Myb inhibitors using complementary *in silico* approaches.

2. Results and discussion

2.1. Differential inhibitory activity of sesquiterpene lactones on c-Myb dependent gene expression

We have recently developed a stable reporter cell line in which a doxycyclin-inducible expression system for c-Myb was combined with an eGFP reporter gene driven by the promoter and enhancer of the Myb-responsive chicken *mim-1* gene [17]. When Myb expression is induced by doxycyclin in these cells, the increase in fluorescence intensity can be used as a read-out for Myb activity. We have now used this reporter cell line to investigate the inhibitory activity of 60 natural STLs on the ability of c-Myb to stimulate the transcription of the reporter gene. The structures and activity data of the tested compounds are reported in Fig. 1 and Table 1.

Quite noteworthy, two compounds belonging to different subclasses of STLs, namely, helenalin acetate (**2**), a helenanolide-type pseudoguaianolide, and goyazensolide (**58**), a furanoheliangolide-type compound, were found to be the most active within the studied series. They displayed IC_{50} values of 0.7 and 0.6 μM, respectively, and thus interfered more potently with c-Myb activity than mexicanin I (**15**, 1.8 μM) and unesterified helenalin (**1**, 2.4 μM), both reported as potential c-Myb inhibitors in our previous study [17]. The IC_{50} values in the studied series of STLs ranged between 0.6 μM and >30 μM (which was the highest concentration tested, i.e. for compounds reported to show an IC_{50} above this cutoff value, no definite IC_{50} value could be determined). This latter category of compounds was thus considered inactive.

2.2. Cell viability measurements and relationships between biological activity data

Since STLs have long been known to cause cytotoxic cell damage, control measurements for cell viability in an MTS assay were carried out for all compounds with c-Myb IC_{50}s < 30 μM. The IC_{50} values for cell viability/cytotoxicity (Table 1) were generally significantly higher than those for c-Myb inhibition (e.g. 8.8 and 7.4 μM for goyazensolide **56** and helenalin acetate **2**), so that the observed inhibition of transcriptional activity was not solely due to unspecific cell damage.

However, since a linear correlation between the c-Myb inhibitory and the cytotoxicity data was found ($n = 60$, $R^2 = 0.57$; IC_{50} values >30 were arbitrarily set to 50; see Fig. 2A), there appears to be a direct relationship between these effects. Interestingly, a plot of the log selectivity index ($\log SI = pIC_{50}^{(c\text{-}Myb)} - pIC_{50}^{(MTS)}$) versus $pIC_{50}^{(c\text{-}Myb)}$ for compounds that allowed measurement of explicit IC_{50} values for both activities ($n = 16$) shows a strong positive correlation ($R^2_{linear} = 0.76$, see Fig. 2B) while there is no such correlation with $pIC_{50}^{(MTS)}$ ($R^2 = 0.03$; see Fig. 2C) indicating that selectivity increases as a function of c-Myb inhibitory potency rather than of basic cytotoxicity. This indicates that selective inhibition of the Myb-induced activation of the reporter gene can be differentiated from basic cytotoxicity so that it appears promising to investigate the chemical/structural reasons for selective c-Myb inhibition.

2.3. Structure–activity relationships

Potentially reactive structure elements such as α-methylene-γ-lactone (ML) and cyclopentenone (CP) or other conjugated enone (EN) groups are very common in natural sesquiterpene lactones and have been found essential for many biological activities of STLs [18–22] since they are known to inhibit the activity of many functional proteins [18,21]. It hence appears a very noteworthy initial observation that the presence of an ML group as such does not warrant c-Myb inhibitory activity. Several compounds possessing this structural feature were found essentially inactive in our assay. On the other hand, as has been found also in other SAR studies [19–22], the presence of at least one reactive structure element (CP, EN or ML) is required for activity since compounds **30** and **44** lacking any such feature are inactive. Thus, as already anticipated in our initial study [17], covalent protein modification is likely to represent the general mechanism of action.

On the other hand, we have already demonstrated in our previous study [17] that the reactive structure elements as found e.g. in helenalin (**1**) and its derivatives as well as in mexicanin I (**15**), namely, cyclopentene-2-one and α-methylene-γ-butyrolactone, are devoid of activity when tested as model chemicals on their own. Furthermore, we have also tested a 1:1 mixture of these two model chemicals and found it inactive so that a combined effect of the two reactive moieties independent of the sesquiterpene skeleton can definitely be ruled out. It thus becomes clear that the STL molecule bearing one or both partial structures in a chemical environment suitable to mediate interactions with the target by non-covalent binding is of great importance.

Comparing the activity of 11,13-dihydrohelenalin derivatives (**5**, **6**; only CP moiety) and a variety of 2,3-dihydropseudoguaianolides (**7**–**14**, **16**, **17**; only ML) with compounds of the helenalin and mexicanin I series (**1**–**4**, **15**; CP and ML), it can be concluded that the presence of both, an α,β-unsaturated ketone as well as a methylene lactone confers a much higher level of activity than the presence of either one alone.

The modulating influence of the STL skeleton on activity is nicely illustrated when comparing helenalin (**1**) and its acetate (**2**), isobutyrate (**3**) and methylbutyrates (mixture of 2-methylbutyrate and isovalerate, **4a** + **4b**). c-Myb induced reporter gene activity is maximally inhibited by the acetate **2** and activity decreases in the free alcohol **1** as well as the higher esters, being lowest in the methylbutyrates **4**. This effect related to the size of the ester group at C-6 is interesting to note since a similar decrease of activity is also observed for a compound with a larger ester group in the furanoheliangolide group (see below).

In the series of guaianolides and 4,5-secoguaianolides (xanthanolides) as well as the eudesmanolides, activity was moderate to low throughout. The highest activity among the guaianolides was observed with the simple guaianolide dehydrocostus lactone (**19**) which was more active than two compounds with an epoxide moiety and different mode of lactonization (**20**, **21**). Among the xanthanolides, 8-epixanthatin-1β,5β-epoxide (**23**) was the most (though only moderately) active derivative. In contrast to its monomeric xanthanolide congeners (**22**, **24**), compound **23** possesses an α,β-unsaturated keto group in addition to the methylene lactone moiety which apparently renders it somewhat more active. The two xanthanolide dimers **25** and **26** were inactive. Compound **26**, in addition to a further enone moiety, possesses the same reactive partial structures as **23**. It may hence be stated that its inactivity is probably related to its larger size. Among the eudesmanolides, isoalantolactone (**27**) was only slightly more active than its 2-hydroxy- and -acetoxy derivatives ivalin (**28**) and ivalin acetate (**30**) as well as its double bond isomer alantolactone (**31**) whereas some further compounds of this type (**32**,**33**) were inactive.

Within the relatively large and diverse group of germacranolides (**34**–**60**), considerable variability of activity (IC_{50} ranging from 0.63 to >30 μM) was observed. It is interesting to note that the most