Phytochemistry xxx (2017) 1–7

Contents lists available at ScienceDirect

Phytochemistry

journal homepage: www.elsevier.com/locate/phytochem

Viewpoint

Selected flavonoid compounds as promising inhibitors of protein kinase CK2α and CK2α′, the catalytic subunits of CK2

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ARTICLE INFO

Article history:
Received 10 July 2016
Received in revised form
21 December 2016
Accepted 22 December 2016
Available online xxx

Keywords:
Protein kinase CK2
Flavonoids
Inhibitors
Chrysoeriol

ABSTRACT

CK2 is a ubiquitous protein kinase involved in many cell functions. During the last years it became an interesting target in cancer research. A series of flavonoid compounds was tested as inhibitors of protein kinase CK2. Several substances were found to be highly active against both catalytic subunits with IC_{50} values below 1 μM in case of CK2α′. The most promising inhibitor we identified is chrysoeriol with IC_{50} values of 250 and 34 nM for CK2α and CK2α′, respectively.

1. Introduction

Reversible protein phosphorylation is an important post-translational modification of proteins regulating many processes in the cell. Approximately one third of the cellular proteome is phosphorylated, and several sites are often modified (Mann et al., 2002). Phosphorylation of proteins on hydroxyl side-chains of serine, threonine and tyrosine is recognized as a key mode of signal transduction and amplification in eukaryotic cells (Ahn and Resing, 2001; Hunter, 2007; Yang, 2005). The Human Genome Project revealed that approximately 20% of human genes code for signal transduction-related proteins including over 520 known protein kinases and 130 protein phosphatases (Yang, 2005). Aberrant expression and/or activation of many members of this group of proteins results in perturbation of signaling (Blume-Jensen and Hunter, 2001; Avendaño and Menèndez, 2015) and have been observed in various cancers and other proliferative diseases (Avendaño and Menèndez, 2015; Vogelstein et al., 2014).

Protein kinase CK2 is a ubiquitous, pleiotropic and constitutively active protein kinase, localized in both cytosolic and nuclear compartments of mammalian cell, where it phosphorylates hundreds of proteins at residues mainly located within negatively charged amino acid sequences (Meggio and Pinna, 2003; Salvi et al., 2009). The enzyme is traditionally classified as Ser/Thr protein kinase, however, examples of Tyr phosphorylation have also been documented (Marin et al., 1999; Vilk et al., 2008). CK2 has a tetrameric structure composed of two catalytic (α and/or α′) and two regulatory subunits (β). The catalytic subunits α/α′ are active even in the absence of the β subunits, whose major role is the stabilization of the holoenzyme and regulation of substrate selectivity (Bibby and Litchfield, 2005). It has many cellular targets and forms different signaling complexes which reflect the multifunctional nature of this enzyme. There are some peculiar properties associated with protein kinase CK2, which are not found in any other protein phosphotransferase: (1) the enzyme is constitutively active, (2) it can use ATP as well as GTP, and (3) it is found elevated in most investigated rapidly proliferating tissues and tumors (Ahmad et al., 2007; Duncan and Litchfield, 2008; Kim et al., 2007; Litchfield, 2003; Ruzzene and Pinna, 2010).

It is believed that CK2 promote tumorigenesis, because its protein content and/or activity are enhanced in many human cancers and rapidly proliferating tissues (Ahmad et al., 2007; Duncan and Litchfield, 2008; Kim et al., 2007; Ruzzene and Pinna, 2010; Trembley et al., 2009). Moreover, CK2 may play an important role in other human disorders due to Alzheimer's disease, ischemia, chronic alcohol exposure, or HIV infection (Blanquet, 2000; Caples

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http://dx.doi.org/10.1016/j.phytochem.2016.12.018
0031-9422/

et al., 2006). Many reports indicate that deregulated CK2 activity is associated with suppression of apoptosis, and inhibition of CK2 strongly enhances sensitivity of cancer cells to programmed cell death (Ahmad et al., 2007, 2008; Duncan and Litchfield, 2008; Götz et al., 2012; Hessenauer et al., 2011; Litchfield, 2003; Yde et al., 2007). Therefore, regulating CK2 activity may be a promising therapeutic intervention for cancer (Cozza et al., 2010; Duncan and Litchfield, 2008; Seeber et al., 2005).

In the last three decades numerous inhibitors addressed to the target CK2 were developed. Several potent and relatively specific inhibitors of protein kinase CK2 belong to the classes of tetrabromobenzimidazole/triazole derivatives, condensed polyphenolic compounds, and indoloquinazolines. The structural basis for their selectivity is provided by a hydrophobic pocket adjacent to the ATP/GTP binding site, which in CK2 is smaller than in the majority of other protein kinases (Cozza et al., 2010; Sarno et al., 2005). One of them, the benzonaphthyridine derivative CX-4945, has shown impressive activity in cell culture studies and has reached advanced clinical trials for the treatment of cancer (Pierre et al., 2011).

Flavonoids are most common and widely distributed group of natural phenolic compounds synthesized by plants that have varied effects on mammalian cell systems (Kumar and Pandey, 2013). Several studies demonstrate that flavonoids might be potent inhibitors of several protein kinases involved in various cellular responses including regulation of cell growth and proliferation (Batra and Sharma, 2013; Kumar and Pandey, 2013; Ravishankar et al., 2013). It has been shown that natural flavones and flavonols - including apigenin, luteolin, fisetin, kaempferol, quercetin, myricetin and coumestan derivative coumestrol – can inhibit the CK2 holoenzyme with K_i's in the sub-micromolar range (Liu et al., 2013; Lolli et al., 2012; McCarty, 2015).

In this report a series of twenty four flavonoids and four related compounds have been tested for their ability to modulate the activity of both α and α′ catalytic subunits of human protein kinase CK2.

2. Results and discussion

Twenty eight natural compounds were tested according their influence on human protein kinase CK2 (Figs. 1 and 2). Among them were twenty four aglycones and glycosides of flavonoids belonging to various classes - flavones, flavonols, flavanones and aurones. Apart from them we determined the activity of two pyromeconic acid, one caffeoylquinic acid and one phenylpropanoid derivatives.

In former studies we had described differences in the inhibition data using either catalytic subunit CK2α or CK2α′ (Janeczko et al., 2011, 2012). In present study we investigated 4 already described CK2 holoenzyme inhibitors (apigenin (**4**), luteolin (**5**), kaempferol (**7**), and quercetin (**8**)) and 24 previously undescribed compounds for their activity against human CK2α and CK2α′ subunits. Both proteins were overexpressed in *E. coli* and purified to homogeneity by affinity chromatography using glutathione-sepharose (GE Healthcare). The inhibitory effect was examined by increasing concentrations of the compound. As in the former study we used two different substrates. In Table 1 all results using the synthetic peptide RRRADDSDDDDD or yeast P2B as a substrate are summarized.

First of all we tested the flavonoid aglycones chrysoeriol (**1**), tricin (**3**), apigenin (**4**), luteolin (**5**), pedalitin (**2**), isokaempferide (**6**), kaempferol (**7**) and quercetin (**8**) for their influence on both CK2 catalytic subunits using yeast acidic ribosomal protein P2B (Fig. 3). The best inhibitory effect showed chrysoeriol (**1**), quercetin (**8**) and pedalitin (**2**) with IC_{50} values between 0.1 and 0.4 μM for CK2α′ as well as 0.5 and 7.0 μM for CK2α. The well known CK2 inhibitor apigenin possesses less effectiveness with IC_{50} values of 9.8 and 2.3 μM, for CK2α and CK2α′, respectively (Fig. 4). Luteolin (**5**) and kaempferol (**7**) decreased the phosphorylating activity weaker than chrysoeriol (**1**) but better than apigenin (IC_{50} = 1.0 μM). In all described cases the differences between the effect on both catalytic subunits are similar. CK2α′ is affected much stronger, up to 17-fold as in case of pedalitin (**2**). Within the tested compounds we also found examples that only CK2α′ was inhibited, like tricin (**3**, Fig. 4) and glycoside scutellarin (**9**). Otherwise aurone cernuoside (**10**) is the only inhibitor with a similar effect on both subunits. Fig. 3 represents the inhibitory activity of chrysoeriol (1), pedalitin (**2**), tricin (**3**), apigenin (**4**), scutellarin (**9**) and cernuoside (**10**).

Furthermore, some glycosides of luteolin, apigenin, scutellarein, isokaempferide, kaempferol and quercetin were also involved in this study. Both luteolin derivatives, 7-*O*-glucoside (**5a**) and 6-hydroxyluteolin 7-*O*-glucoside (**5b**), are better inhibitors of CK2α′ than luteolin (Fig. 4). Examining the CK2α activity in the presence of these compounds the inhibitory effect is lost in case of a glycosylation at position C-7 (**5a**), whereas the compound with a hydroxyl group at position C-6 (**5b**) possesses similar influence. The apigenin derivative with the *O*-linked glucose moiety at position C-7 (**4a**) affects the CK2α activities in a weaker extent than apigenin. Glucuronic acid attached at the same position (**4b**) revealed a further decrease of the phosphorylating activity of CK2α′. This effect was not seen when a methylated glucuronic acid (**4c**) is attached, anyway, this compound still exhibited inhibitory potential against CK2α′. In general, compounds with an *O*-linked glucose moiety revealed mixed results. In case of the scutellarein 7-*O*-glucoside (**9a**) the effect was similar like for the 7-*O*-glucuronide - scutellarin (**9**). On the other hand, the substitution with glucose lowered the inhibitory potency as in case of derivatives of kaempferol (**7a**), isokaempferide (**6a**) and quercetin (**8a**). Similar observation like the increased inhibitory activity of apigenin 7-*O*-glucuronide (**4b**) was made in case of isokaempferide 7-*O*-glucuronide (**6b**) having a 4–5 fold stronger effect on CK2α′.

Since protein kinase CK2 has over 300 known substrates we examined if the inhibitory effects described above could also be detected using another substrate. Therefore, we employed the synthetic peptide RRRADDSDDDDD which is often used in CK2 inhibitory studies. Quite contrary to the phosphorylation of P2B we obtained mixed results with the small peptide resembling the consensus sequence. In most cases CK2α′ was better inhibited than CK2α but this was not the rule as in case of substrate P2B. Chrysoeriol (**1**), apigenin (**4**), luteolin (**5**), pedalitin (**2**), kaempferol (**7**) possess higher inhibitory potency against both catalytic subunits compared to the IC_{50} obtained using P2B. One substance, scutellarin (**9**), has similar effect on the phosphorylating activities independently of which substrate was used. Tricin (**3**), quercetin (**8**) and cernuoside (**10**) were more active against CK2α′ subunit than CK2α. Glycosides of apigenin (**4a-c**) had similar or weaker effect towards CK2α′ than towards CK2α. Isokaempferide (**6**) inhibited the CK2α′ activity similarly independent from the used substrate. CK2α was moderately inhibited using the peptide substrate. The glucuronic derivative of isokaempferide (**6b**) similar like in case of P2B showed better inhibitory potential towards CK2α′ than the parent compound. Both derivatives of luteolin (**5a** and **5b**) possess the same inhibitory effect towards CK2α′ comparing phosphorylation of both substrates. CK2α was much better inhibited by the glucosylated derivative (**5b**) using P2B. Experiments with scutellarin (**9**) and scutellarein 7-*O*-glucoside (**9a**) gave opposite results than when tested with P2B. The derivative with a glucose moiety at position C-7 (**9a**) possess quite good inhibitory effect towards CK2α and slightly weaker effect on CK2α′.

Further tested compounds linarin (**11**), pectolinarin (**11a**), erigeroside (**13**), 6″-caffeoylerigeroside (**13a**) and chlorogenic acid (**14**) had very weak effect (IC_{50} values over 40 μM) on CK2 activity

Please cite this article in press as: Baier, A., et al., Selected flavonoid compounds as promising inhibitors of protein kinase CK2α and CK2α′, the catalytic subunits of CK2, Phytochemistry (2017), http://dx.doi.org/10.1016/j.phytochem.2016.12.018