Synthesis and SAR of Tetracyclic Inhibitors of Protein Kinase CK2 Derived from Furocarbazole W16

Lukas Kröger,[a] Constantin G. Daniliuc,[b] Deeba Ensan,[a] Sebastian Borgert,[a] Christian Nienberg,[a] Miriam Lauwers,[c] Michaela Steinkräger,[c] Joachim Jose,[a] Markus Pietsch,[c] and Bernhard Wünsch[a, d]

The serine/threonine kinase CK2 modulates the activity of more than 300 proteins and thus plays a crucial role in various physiological and pathophysiological processes including neurodegenerative disorders of the central nervous system and cancer. The enzymatic activity of CK2 is controlled by the equilibrium between the heterotetrameric holoenzyme CK2αβ and its monomeric subunits CK2α and CK2β. A series of analogues of W16 ((3αR,4,10S,10aS)-4-((S)-(4-benzyl-2-oxo-1,3-oxazolidin-3-yl)carbonyl)-10-(3,4,5-trimethoxy-phenyl)-4,5,10,10a-tetrahydrofuro[3,4-b]carbazole-1,3(3aH)-dione ((+)-3a) was prepared in an one-pot, three-component Levy reaction. The stereochemistry of the tetracyclic compounds was analyzed. Additionally, the chemically labile anhydride structure of the furocarbazoles 3 was replaced by a more stable imide (9) and N-methylimide (10) substructure. The enantiomer (−)-3a (Ki = 4.9 μM) of the lead compound (+)-3a (Ki = 31 μM) showed a more than sixfold increased inhibition of the CK2α/CK2β interaction (protein-protein interaction inhibition, PPII) in a microscale thermophoresis (MST) assay. However, (−)-3a did not show an increased enzyme inhibition of the CK2α/CK2β holoenzyme, the CK2α subunit or the mutated CK2αC336S subunit in the capillary electrophoresis assay. In the pyrrolocarbazole series, the imide (−)-9a (Ki = 3.6 μM) and the N-methylimide (−)-10a (Ki = 2.8 μM) represent the most promising inhibitors of the CK2α/CK2β interaction. However, neither compound could inhibit enzymatic activity. Unexpectedly, the racemic tetracyclic pyrrolocarbazole (±)-12, with a carboxy moiety in the 4-position, displays the highest CK2α/CK2β interaction inhibition (Ki = 1.8 μM) of this series of compounds.

Introduction

A multitude of physiological processes is affected by the phosphorylation state of proteins, which is controlled by various protein kinases and phosphatases.(1) Introduction of the very polar phosphate moiety induces a conformational change of the protein and thus a modification of its biological and pharmacological properties. In particular, phosphorylation of substrate proteins displays a very important mechanism of inter- and intracellular signal transduction. Moreover, kinases control cellular processes such as metabolism, transcription, and cell cycle.[12–14] Due to their key role in several physiological processes, various protein kinases emerged as promising targets for the development of novel drugs.[15,16] The kinase inhibitor imatinib, which was introduced into the market in 2001, paved the way for the development of protein kinase inhibitors. Today, such inhibitors are approved for the treatment of cancer, inflammation and rheumatoid arthritis.[17–19]

The protein kinase CK2 (previously known as casein kinase 2, CK2) was detected in several eukaryotic organisms.[9,10] The human CK2 transfers a phosphate moiety from ATP or GTP to serine or threonine residues in various proteins.[11] It forms a heterotetramer consisting of two CK2α and two CK2β subunits. The monomeric subunits and the tetrameric holoenzyme are postulated to be in an equilibrium, which controls the enzymatic properties of CK2 in the cell.[12] Each CK2α subunit is bound to a CK2β dimer, forming contacts with both CK2β subunits.[13] The CK2α subunit contains the ATP binding site and is able to transfer a phosphate group to a substrate, even as a monomer.[14] However, stability, catalytic activity and selectivity of CK2α are regulated by the CK2β subunit,[15] with enzymatic activity and substrate spectrum being different from those of the tetramer holoenzyme.[16] According to Pinna,[17] three classes of CK2 substrates are distinguished on the basis of the quaternary structure of the active enzyme. Class I substrates (e.g., inhibitor-2 of protein phosphatase-1) are phosphorylated by both the CK2 holoenzyme and the CK2α/CK2β′ subunits, whereas class II substrates, such as calmodulin, are exclusively

---

[a] Dr. L. Kröger, D. Ensan, S. Borgert, Dr. C. Nienberg, Prof. J. Jose, Prof. B. Wünsch
Institut für Pharmazeutische und Medizinische Chemie
Westfälische Wilhelms-Universität Münster
Corrensstraße 48, 8149 Münster (Germany)
E-mail: wuensch@uni-muenster.de

[b] Dr. C. G. Daniliuc
Organisch-Chemisches Institut
Westfälische Wilhelms-Universität Münster
Corrensstraße 40, 48149 Münster (Germany)

[c] M. Lauwers, Dr. M. Steinkräger, Dr. M. Pietsch
Medizinische Fakultät, Universität Köln
Gieseler Straße 24, 50931 Köln (Germany)

[d] Prof. B. Wünsch
Cells-in-Motion Cluster of Excellence (EXC 1003–CM1)
Westfälische Wilhelms-Universität Münster
Waldeyerstraße 15, 48149 Münster (Germany)

Supporting information for this article is available on the WWW under https://doi.org/10.1002/cmdc.202000040

© 2020 The Authors. Published by Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim.
This is an open access article under the terms of the Creative Commons Attribution License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited.
accepted by the CK2α/CK2α′ subunits. Addition of CK2β subunit inhibits the phosphorylation of the latter substrates, which is mitigated by polycationic compounds, such as polylysine. Both effects were shown to be mediated by the N-terminal acidic loop (D(30)EPEELED) of CK2β. In contrast, phosphorylation of class III substrates, among them eukaryotic initiation factor 2β (eIF2β) and pancreatic transcription factor (PDX-1), requires the presence of the CK2β subunit with an intact N-terminal acidic loop. The acidic loop is thought to interact with a cluster of basic residues in the class III substrates located up to 33 residues away from the phosphoacceptor site, with addition of polycations preventing holoenzyme-dependent substrate phosphorylation most probably by competing with the basic cluster of the substrate for binding to the acidic loop of CK2β.

The presence of CK2 is essential for the survival of eukaryotic cells, since it modulates the activity of more than 300 proteins by phosphorylation. Due to the large number of substrates accepted by CK2, it plays a crucial role in various physiological and pathophysiological processes including regulation of the cell cycle, proliferation, angiogenesis, repairing of DNA damage, embryogenesis, suppression of apoptosis and influence on CNS activity.

It has been shown that CK2 is involved in several CNS disorders. An increased expression of CK2 was observed in neurons containing pathological neurofibrillary tangles (Alzheimer’s disease) and it contributes to the formation of Levy bodies (Parkinson’s disease). In particular, its role in the development of cancer is well investigated. CK2 is upregulated in almost all kinds of cancer, promoting cell proliferation and preventing apoptosis. Because of the high number of substrates and its constitutive activity, CK2 was considered to be "non-druggable" for many years. Recently, the development of the CK2 inhibitor silmitasertib (1, CX-4945, Figure 1) refuted this assumption. The ATP-competitive CK2 inhibitor 1 is in late stage clinical trials for the treatment of cholangiocarcinoma (bile duct cancer) and in early clinical trials for the treatment of various other cancers including hematological and lymphoid malignancies.

In general, kinase inhibitors competing with ATP for binding to the ATP binding site (e.g., silmitasertib) suffer from low selectivity, since the ATP binding site is highly conserved in different kinases. Inhibition of the interaction between the CK2α and CK2β subunits (protein-protein interaction, PPI) represents a further strategy to inhibit the kinase CK2. Very recently, the non-ATP-competitive ligand CAM187 (2) was reported to bind to the CK2α subunit and inhibit the interaction with the CK2β subunit (IC50 = 44 μM). Although 2 was able to affect this PPI, it did not significantly inhibit CK2 activity. The same behavior was observed for the cyclic 13 amino acid peptide Pc and its derivatives, which were designed as CK2β mimetics to inhibit CK2 subunit association.

In this project, the furocarbazole W16 ((+)−3a, Figure 1) served as lead compound for the optimization of the inhibition of CK2α and CK2β subunit interaction. With an IC50 value of 30–40 μM, W16 ((+)−3a) shows weak inhibition of CK2α/CK2β interaction. On the other hand, inhibition (IC50 = 20 μM) of the catalytic activity of monomeric CK2α was observed. Compound ((+)−3a with the rigid tetracyclic furocarbazole framework represents a rather large molecule, which violates Lipinski’s rule of 5. In particular its molecular weight of 610 Da exceeds the defined upper limit of 500 Da. However, for inhibition of PPIs larger molecules are required (e.g., cyclic peptide Pc has a molecular weight of 1,409 Da), since the compounds are supposed to interact with the large surface of a protein rather than with a deep binding pocket. The furocarbazole ((+)−3a is a
very good starting point, since it allows diverse structural modifications. In this report, we will focus on the furan moiety and the stereochemistry, since the relationships between the configuration and the PPI inhibition (PPII) within this compound class have not been investigated yet.

Results and Discussion

Synthesis of tetracyclic furo- and pyrrolocarbazoles

The furocarbazole derivative (+)-3a had been synthesized as shown in Scheme 1 by Levy reaction of (indol-2-yl)acetamide (S)-4, 3,4,5-trimethoxybenzaldehyde (5) and maleic anhydride (6). To compare both the stereochemistry and the biological activity of new analogues with those of the lead compound (+)-3a, the Levy reaction should be performed as reported in literature. For this purpose, (indol-2-yl)acetamide (S)-4 was prepared by reaction of (2-nitrophenyl)acetyl chloride with Meldrum’s acid, followed by aminolysis of the reactive triacyl intermediate with (S)-4-benzyloxazolidin-2-one and final reduction with Zn/NH₄Cl (details see the Supporting Information). The three-component Levy reaction of (indol-2-yl)acetamide (5)-4, benzaldehyde 5 and maleic anhydride (6) in the presence of CuSO₄·5H₂O at reflux temperature for 24 h led to a mixture of diastereomeric products 3. Column chromatographic separation of the mixture resulted in pure (+)-3c and a mixture of (+)-3a and (+)-3d, which were separated by recrystallization. Unexpectedly, the fourth diastereomer 3b could not be detected or isolated.

The structure including the stereochemistry of (+)-3a was confirmed by comparison of its spectroscopic data with those given in the literature for the lead compound (+)-3a. The absolute configuration of (+)-3d was determined unequivocally by X-ray crystal structure analysis. (Figure 2) Moreover, the cis,cis,trans-configuration of the substituents at ring B was confirmed by the down-field shift of the 4-H signal (δ = 6.02 ppm) and the small coupling constant between 4-H and 3a-H (J = 1.8 Hz; Table S1 in the Supporting Information). Similar NMR data obtained for (+)-3c (δ(4-H) = 6.07 ppm, J(4-H/3a-H) = 2.7 Hz, Table S1) clearly show the same relative cis,cis,trans-configuration of the substituents in ring B. As the S configuration of C-4 in the oxazolidine ring is pre-defined by...
the reactant (S)-4, the absolute S-3aR,4R,10S,10aS configuration for (+)-3c was unequivocally assigned.

According to the mechanism of the Levy reaction including a Diels-Alder reaction as key step, the cis,cis,cis-configured stereoisomers (+)-3a and 3b are expected to be the primary products. However, it has been shown that high temperature, prolonged reaction times, polar solvents and bases lead to epimerization of the kinetically formed cis,cis,cis-configured diastereomers into the thermodynamically favored cis,cis,trans-configured diastereomers. This epimerization nicely explains the formation of the cis,cis,trans-configured products (+)-3c and (+)-3d. A complete epimerization of 3b may be responsible for the exclusive isolation of (+)-3d.

The enantiomer (−)-3a was prepared in the same manner (36% yield) starting the Levy reaction with the enantiomer (R)-4. Here, the corresponding diastereomers (−)-3c and (−)-3d could not be isolated. Altogether, the high reactivity (instability) and fast epimerization which already occurred during recrystallization experiments of 3 stimulated the bio-isosteric replacement of the cyclic anhydride substructure by cyclic imides.

Thus, reaction of (indol-2-yl)acetamide (S)-4 with 3,4,5-trimethoxybenzylaldehyde (S) and maleimide (7) or N-methylmaleimide (8) in boiling toluene provided the diastereomeric cis,cis,cis-configured pyrrolocarbazoles (+)-9a/(−)-9b and (+)-10a/(−)-10b, respectively. (Scheme 2) The corresponding enantiomers (−)-9a, (−)-9b, (−)-10a, and (−)-10b were prepared by the same Levy-reaction using enantiomeric indol-2-yl)acetamide (R)-4 as starting material.

As described above, the relative cis,cis,cis configuration of the tetracyclic pyrrolocarbazoles 9a,b and 10a,b was confirmed by the high-field shift of the 4-H signal (δ = 5.35–5.51 ppm) and the large coupling constant between 4-H and 3a-H (J = 11.5–11.6 Hz). However, an X-ray crystal structure analysis was needed to correctly assign the absolute configuration. In Figure 3, the crystal structure of (−)-10b is displayed proving the absolute R:3aS,4S,10S,10aS configuration. Based on this absolute configuration of (−)-10b, the absolute configuration of the other stereoisomers (+)-10a, (−)-10a and (−)-10b as well as that of the corresponding maleimide derivatives (+)-9a, (+)-9b, (−)-9b and (−)-9b could be assigned unambiguously by comparing spectroscopic data and specific optical rotation of the compounds (Table S2).

During purification and recrystallization, the pyrrolocarbazoles 9a,b and 10a,b turned out to be much more stable than the corresponding furrocabazoles 3. Formation of C-4 epimers was not observed. Even heating to reflux of an acetonitrile
solution of (+)-10a with and without DIPEA or TFA led only to small amounts of C-4-epimer (analyzed by $^1$H NMR spectroscopy). Since this epimerization was accompanied by the formation of several side products, another strategy was pursued for the synthesis of the corresponding cis,cis,trans-configured stereoisomers 9c and 9d.

For the synthesis of cis,cis,trans-configured stereoisomers 9c and 9d (Scheme 3), racemic tetracyclic ester (±)-11 \cite{55,56} was hydrolyzed with NaOH to afford carboxylic acid (±)-12 in 96% yield. Activation of acid (±)-12 with oxalyl chloride and subsequent coupling with S-configured phenylalaninol (S)-13 led to enantiomerically pure amides (+)-14c and (−)-14d. The oxazolidinone moiety of (+)-9c and (−)-9d was established by cyclization of the amidoalcohols (+)-14c and (−)-14d with CDI.

The relative and absolute configuration of (+)-14d was determined by X-ray crystal structure analysis. The structure of (+)-14d displayed in Figure 4 clearly shows cis,cis,trans-configuration of the substituents at ring B. Moreover, the R configuration of the N-substituent coming from (R)-phenylalaninol (R)-13 and 3aS,4R,10S,10aS configuration of the four centers of chirality in the tetracyclic ring system of (+)-14d are shown. Careful comparison of NMR spectra including ROESY 2D spectra allowed the unequivocal assignment of the absolute configuration for the remaining isomers (−)-14d, (+)-14c, and

---

Scheme 3. Synthesis of cis,cis,trans-configured pyrrolocarbazole derivatives (+)-9c and (−)-9d. Reagents and reaction conditions: a) NaOH, H$_2$O, THF, RT, 30 min, 96%; b) 1, (COCl)$_2$, DMF, CH$_2$Cl$_2$, RT, 3 h; c) DIPEA, RT, 1 h; (+)-14c (28%), (−)-14d (26%); c) CDI, DMF, 60°C, 16 h; (+)-9c (20%), (−)-9d (42%). Absolute configuration of the products: (+)-9c 5-3aS,4R,10S,10aS configuration; (−)-9d 5-3aR,4S,10R,10aR configuration. The enantiomers (−)-9c and (+)-9d were prepared in the same manner.

---

Figure 3. X-ray crystal structure of (+)-10b. Compound (+)-10b crystallized in the hexagonal space-group P6$_3$. Thermal ellipsoids are shown with 20% probability. The R configuration of C21 in the oxazolidine ring and cis,cis,cis-configuration of the substituents in ring B are shown (R-3aS,4S,10S,10aS configuration). The Flack parameter was refined to 0.04(9).
interaction site, the lead compound W16 ((+)-3a) was prepared and pharmacologically evaluated. In the MST assay, a $K_i$ value of 31 µM was found which is very close to the reported $IC_{50}$ value of 30–40 µM.31 The diastereomer (+)-3d displayed a similar affinity as (+)-3a. Unfortunately, the inhibition of the CK2zα/CK2β interaction by the stereoisomer ((+)-3c could not be recorded due to solubility problems. The solubility of these relatively large molecules (molecular weight, MW, of 610 Da and, thus, exceeding Lipinski’s upper limit of MW = 500 Da) is a general problem. Unexpectedly, the enantiomer (−)-3a revealed an approximately sixfold increased inhibition of the CK2zα/CK2β interaction compared to the lead compound W16 ((+)-3a). This result clearly indicates the major role of the stereochemistry of these complex molecules on their biological activity (Table 1).

Due to the high reactivity (low stability) of ligands 3, the reactive anhydride substructure of 3 was replaced by a chemically more stable imide substructure. In the class of secondary imides 9, strong inhibition of the CK2zα/CK2β interaction was observed, in particular for those compounds with the same stereochemistry as (+)-3a and (−)-3a. (It should be noted, that the stereodescriptor for the center of chirality in 3a-position is changed upon exchange of the O-atom by a N-atom.) The affinities of (−)-9a ($K_i = 3.6$ µM) and (+)-9b ($K_i = 4.9$ µM) are in the same low-micromolar range as the dissociation constant of (−)-3a ($K_i = 4.9$ µM). Again, some of the test compounds ((+)-9c and (−)-9c precipitated during the MST assay due to low solubility (Table 1).

Analysis of the inhibition of the CK2zα/CK2β interaction by the tertiary imides 10 containing an additional methyl moiety at the N-atom also resulted in dissociation constants in the low micromolar range, with (−)-10a ($K_i = 2.8$ µM) and (−)-10b ($K_i = 3.7$ µM) being the most potent tertiary imides (Table 1).

It can be concluded that chemical stabilization of the anhydrides 3 by imides 9 and 10 is well tolerated without loss of inhibition of the CK2 subunit interaction. Moreover, the stereochemistry of the tetracyclic system appears to be crucial for the biological activity.

In addition to the final products 3, 9, and 10, the biological activity of the racemic ester (±)-11, the racemic carboxylic acid (±)-12 and the 2-hydroxyethylamines 14 was assessed in the MST assay. With a $K_i$ value of 1.8 µM the racemic acid (±)-12 exhibited the most potent inhibition of the CK2zα/CK2β interaction among the analyzed compounds, whereas the intermediates 14 showed no activity (Table 1).

**Inhibition of the enzymatic activity**

In addition to analyzing the influence of the test compounds on the CK2zα/CK2β interaction, the inhibition of the enzymatic activity of the holoenzyme (CK2zαβ2), the CK2zα subunit, and the mutated CK2zα C336S subunit was investigated in a capillary electrophoresis (CE) assay. In this assay, the decapeptide RRRDDDDDDDD, a known class I substrate suitable for assaying the kinase activity of both the CK2 holoenzyme and the catalytically active CK2 subunits,49,53,56 was reacted with ATP in

---

**Pharmacological evaluation**

**Inhibition of the CK2zα/CK2β interaction**

The inhibition of the interaction between the CK2zα and the CK2β subunit was determined in a microscale thermophoresis (MST) assay. At first the $K_D$ value of the CK2zα/CK2β interaction was determined by addition of a constant amount of fluorescently labeled CK2β subunit to increasing concentrations of the CK2zα subunit and by analysis of the thermophoretic shift. This experiment led to a $K_D$ value of 12 ± 1 nM (n = 4) for the CK2zα/CK2β interaction.37 A significantly increased dissociation constant $K_D'$ of the two CK2 subunits in the presence of test compound (50 or 100 µM) indicated an inhibition of the CK2zα/CK2β interaction: (Table S4) Because of a low solubility of some derivatives, the test compounds were also investigated at lower concentrations (10 or 20 µM), which, however, resulted in non-significantly changed dissociation constant $K_D'$. (Table S4) A re-analysis of all those MST experiments with significantly increased values of $K_D'$ by fitting the $K_D'$ value of the CK2zα/CK2β-interaction in the absence of test compound to a global value ($K_D = 11$ nM) yielded $K_i$ values for the PPII by the test compounds which are summarized in Table 1.

In order to ensure the validity of the MST method for determination of $K_i$ values of test compounds at the CK2zα/CK2β interaction, the lead compound W16 ((+)-3a) was prepared and pharmacologically evaluated. In the MST assay, a $K_i$ value of 31 µM was found which is very close to the reported $IC_{50}$ value of 30–40 µM.31 The diastereomer (+)-3d displayed a similar affinity as (+)-3a. Unfortunately, the inhibition of the CK2zα/CK2β interaction by the stereoisomer ((+)-3c could not be recorded due to solubility problems. The solubility of these relatively large molecules (molecular weight, MW, of 610 Da and, thus, exceeding Lipinski’s upper limit of MW = 500 Da) is a general problem. Unexpectedly, the enantiomer (−)-3a revealed an approximately sixfold increased inhibition of the CK2zα/CK2β interaction compared to the lead compound W16 ((+)-3a). This result clearly indicates the major role of the stereochemistry of these complex molecules on their biological activity (Table 1).

Due to the high reactivity (low stability) of ligands 3, the reactive anhydride substructure of 3 was replaced by a chemically more stable imide substructure. In the class of secondary imides 9, strong inhibition of the CK2zα/CK2β interaction was observed, in particular for those compounds with the same stereochemistry as (+)-3a and (−)-3a. (It should be noted, that the stereodescriptor for the center of chirality in 3a-position is changed upon exchange of the O-atom by a N-atom.) The affinities of (−)-9a ($K_i = 3.6$ µM) and (+)-9b ($K_i = 4.9$ µM) are in the same low-micromolar range as the dissociation constant of (−)-3a ($K_i = 4.9$ µM). Again, some of the test compounds ((+)-9c and (−)-9c precipitated during the MST assay due to low solubility (Table 1).

Analysis of the inhibition of the CK2zα/CK2β interaction by the tertiary imides 10 containing an additional methyl moiety at the N-atom also resulted in dissociation constants in the low micromolar range, with (−)-10a ($K_i = 2.8$ µM) and (−)-10b ($K_i = 3.7$ µM) being the most potent tertiary imides (Table 1).

It can be concluded that chemical stabilization of the anhydrides 3 by imides 9 and 10 is well tolerated without loss of inhibition of the CK2 subunit interaction. Moreover, the stereochemistry of the tetracyclic system appears to be crucial for the biological activity.

In addition to the final products 3, 9, and 10, the biological activity of the racemic ester (±)-11, the racemic carboxylic acid (±)-12 and the 2-hydroxyethylamines 14 was assessed in the MST assay. With a $K_i$ value of 1.8 µM the racemic acid (±)-12 exhibited the most potent inhibition of the CK2zα/CK2β interaction among the analyzed compounds, whereas the intermediates 14 showed no activity (Table 1).
the presence of enzyme, which transfers a phosphate group to Ser7 of the decapetide. Due to the additional negative charge of the phosphate group, the decapetide and the phosphorylated decapetide can be separated by CE. A reduced amount of phosphorylated decapetide, that is, product, indicates inhibition of the kinase.\(^{39}\) The mutated CK2α\(^{c}\) subunit was included into this study, since this subunit has also kinase activity and can also form an active holoenzyme, but is more stable than the wild-type CK2α subunit. The data on the inhibition of the kinase activities of CK2αβ, CK2α subunit and mutated CK2α\(^{c}\) subunit are summarized in Table 1.

In the first screening with a concentration of test compound of 10 μM only some of the stereoisomeric furcarbazoles 3 showed more than 50% inhibition of the CK2 holoenzyme, the CK2α subunit, and the mutated CK2α\(^{c}\) subunit. Therefore, IC\(_{50}\) values for the inhibition of CK2αβ were determined only for the furcarbazoles 3. Interestingly, the lead compound (±)-3a (W-16) exhibited the strongest inhibition of CK2αβ (IC\(_{50}\) = 1.9 μM). The enantiomer (−)-3a (IC\(_{50}\) = 2.7 μM) was only slightly less potent and the inhibitory activities of the diastereomers (±)-3c and (±)-3d were also in the same range.

The analogues imides 9 and 10, the synthesis educts (±)-11, (±)-12 and the 2-hydroxyethylamides 14 did not inhibit the kinases at a relevant extent.

**Conclusion**

In order to investigate SAR, four stereoisomeric furcarbazoles 3 resulting from partial epimerization at C-4, eight stereoisomeric pyrrolocarbazoles 9 and four stereoisomeric N-methyl-pyrrolo-carbazoles 10 were prepared in an one-pot, three-component Levy reaction. The relative and absolute configuration of the different products was assigned unequivocally by X-ray crystal structure analysis and NMR spectroscopy.

The stereochemistry has a high impact on the CK2α/CK2β interaction inhibition (protein protein interaction inhibition, PPI) as the enantiomer (−)-3a (K\(_i\) = 4.9 μM) is more than 6-fold more active than the lead compound (±)-3a (K\(_i\) = 31 μM) in the

---

**Table 1. CK2α/CK2β interaction inhibition and inhibition of the activities of holoenzyme CK2αβ, the CK2α subunit and the mutated CK2α\(^{c}\) subunit.**

| Compd. | X | Config. | Inhibition of CK2α/CK2β interaction (K, μM)\(^{[a]}\) | Inhibition (%) of CK2αβ \([c = 10 \mu M]\)\(^{[b]}\) | Inhibition (%) of CK2α \([c = 10 \mu M]\)\(^{[b]}\) | Inhibition (%) of CK2α\(^{c}\) \([c = 10 \mu M]\)\(^{[b]}\) |
|--------|---|---------|---------------------------------|----------------|----------------|----------------|
| (+)-3a  | O | S       | R-S-S-S                          | 31 ± 14        | 84 ± 5          | 1.9            | 76 ± 4         | 85 ± 13        |
| (−)-3a  | O | R       | S-R-R-R                          | 4.9 ± 1.8      | 89 ± 3          | 2.7            | 73 ± 0         | 93 ± 2         |
| (+)-3c  | O | S       | R-S-S-S                          | prec.          | 66 ± 16         | 6.5            | 65 ± 4         | 52 ± 38        |
| (−)-3c  | O | S       | R-S-S-S                          | 42 ± 6         | 92 ± 7          | 3.8            | 43 ± 13        | n.s.           |
| (+)-9a  | NH| S       | S-S-S-S                          | n.s.           | 20 ± 4          | n.d.           | n.s.           | n.s.           |
| (−)-9a  | NH| R       | R-R-R-R                          | 3.6 ± 0.6      | n.d.            | n.d.           | n.s.           | n.s.           |
| (+)-9b  | NH| R       | S-S-S-S                          | 4.9 ± 0.8      | 12 ± 7          | n.d.           | n.d.           | n.s.           |
| (−)-9b  | NH| S       | R-R-R-R                          | 4.4 ± 0.3      | n.d.            | n.d.           | n.d.           | n.s.           |
| (+)-9c  | NH| S       | S-R-S-S                          | prec.          | < 50            | n.d.           | n.d.           | n.d.           |
| (−)-9c  | NH| R       | R-S-R-R                          | prec.          | < 50            | n.d.           | n.d.           | n.d.           |
| (+)-9d  | NH| S       | R-R-R-R                          | n.s.           | < 50            | n.d.           | n.d.           | n.d.           |
| (−)-9d  | NH| S       | R-R-R-R                          | n.s.           | < 50            | n.d.           | n.d.           | n.d.           |
| (+)-10a| NCH\(_{3}\)| S     | S-S-S-S                          | 2.8 ± 0.9      | 26 ± 7          | n.d.           | 41 ± 11        | 53 ± 15        |
| (−)-10a| NCH\(_{3}\)| R     | R-R-R-R                          | 8.5 ± 2.9      | 29 ± 14         | n.d.           | n.d.           | 30 ± 23        |
| (+)-10b| NCH\(_{3}\)| R     | R-S-S-S                          | 7.2 ± 1.5      | 31 ± 9          | n.d.           | n.d.           | n.s.           |
| (−)-10b| NCH\(_{3}\)| S     | R-R-R-R                          | 3.7 ± 0.7      | 15 ± 11         | n.d.           | n.d.           | n.s.           |
| (±)-11 | NH| CO\(_{2}\)Et | RS-RR-RS-RS                     | 32 ± 17        | n.d.            | n.d.           | n.d.           | n.s.           |
| (±)-12 | NH| CO\(_{2}\)H | RS-RR-RS-RS                     | 1.8 ± 0.8      | n.d.            | n.d.           | n.d.           | n.s.           |
| (+)-14c| NH| S       | S-R-S-S                          | n.s.           | < 50            | n.d.           | n.d.           | n.d.           |
| (−)-14c| NH| R       | R-R-R-R                          | n.s.           | < 50            | n.d.           | n.d.           | n.d.           |
| (+)-14d| NH| R       | S-R-R-R                          | n.s.           | < 50            | n.d.           | n.d.           | n.d.           |
| (−)-14d| NH| S       | R-R-R-R                          | n.s.           | < 50            | n.d.           | n.d.           | n.d.           |

\(\text{[a]}\) Mean ± SEM values of 2–4 separate experiments resulting from a global fit of all included data sets. A global K\(_i\) value of 11 nM was calculated for the CK2α/CK2β interaction in this global analysis. [\(\text{[b]}\) Mean value ± standard deviation (SD) of three independent experiments. *prec. = precipitation; n.s. = not significant; n.d. = not determined]
Oxygen and moisture sensitive reactions were carried out under nitrogen dried with silica gel with moisture indicator (orange gel, www.chemmedchem.org). Isolera® (Biotage, USA); chemical shifts (δ) are reported in parts per million (ppm) (CDCl₃, 1H), 3.61 (s, 3H, 4-OCH₃), 4.02 (t, 2H, J = 9.0, 1H, 5-H), 4.27 (dd, J = 8.7, 1H, 1CH, 1CH₂), 4.46 (t, J = 8.6 Hz, 1H, 2OCH₃), 4.64 (s, 2H, J = 9.3/2.6 Hz, 1H, 3-H), 4.72–4.80 (m, 1H, PhCH₂CH₂), 4.89 (d, J = 8.5 Hz, 1H, 10-H), 6.07 (d, J = 2.7 Hz, 1H, 4-H), 6.33 (s, 2H, 2-H, 6-H), 6.90 (d, J = 8.0/6.9/1.0 Hz, 1H, 8-H), 7.04–7.09 (m, 3H, 9-H, 2-H, 6-H), 7.10 (d, J = 8.1/6.9 Hz, 1H, 7-H), 7.12–7.18 (m, 1H, 3-H, 5-H), 7.17–7.21 (m, 1H, 4-H), 7.47 (dt, J = 8.2/1.0 Hz, 1H, 6-H), 10.82 (s, 1H, 5-H).

For recrystallization, the previously obtained mixture of (+)-3a and (+)-3d was dissolved in a mixture of ethyl acetate and tert-butyl methyl ether under reflux. The solution was allowed to cool down to room temperature. The formed precipitate was filtered off and washed with cold tert-butyl methyl ether (2 x 10 mL) to give (+)-3a. The filtrate was concentrated in vacuo to give (+)-3d.
The second obtained fraction was also purified by flash column chromatography (ethyl acetate/cyclohexane 1:1) and was added to the solution and the mixture was heated to reflux for 22 h (oil bath temperature 130 °C). After cooling to room temperature, the mixture was filtered and the filter was washed with CH₂Cl₂ (3 x 10 ml). The filtrate was concentrated in vacuum and the residue was purified by flash column chromatography (ethyl acetate/cyclohexane = 3:1, 5 ml, h = 11 cm, V = 20 ml) using a silica gel column. The purified product was obtained as a colorless solid, mp 228-230 °C.

Under N₂, indole (R)-4 (168 mg, 0.50 mmol), maleimide (7, 146 mg, 1.50 mmol) and 3,4,5-trimethoxybenzaldehyde (5, 147 mg, 0.75 mmol) were dissolved in dry toluene (10 ml) in a pressure resistant Schlenk tube. Crushed CuSO₄·5 H₂O (12.6 mg, 0.05 mmol) was added to the solution and the mixture was heated to reflux for 16 h (oil bath temperature 130 °C). After cooling to room temperature, the mixture was filtered and the filter was washed with CH₂Cl₂ (3 x 10 ml). The filtrate was concentrated in vacuum and the residue was purified by flash column chromatography (ethyl acetate/cyclohexane = 3:1, 5 ml, h = 11 cm, V = 20 ml) using a silica gel column. The purified product was obtained as a colorless solid, mp 228-230 °C.

Under N₂, indole (R)-4 (167 mg, 0.50 mmol), N-vinylmaleimide (8, 166 mg, 1.49 mmol) and 3,4,5-trimethoxybenzaldehyde (5, 146 mg, 0.75 mmol) were dissolved in dry toluene (15 ml) in a pressure resistant Schlenk tube. Crushed CuSO₄·5 H₂O (12.9 mg, 0.05 mmol) was added to the solution and the mixture was heated to reflux for 22 h (oil bath temperature 130 °C). After cooling to room temperature, the mixture was filtered and the filter was washed with CH₂Cl₂ (3 x 10 ml). The filtrate was concentrated in vacuum and the residue was purified by flash column chromatography (ethyl acetate/cyclohexane = 3:1, 5 ml, h = 11 cm, V = 20 ml) using a silica gel column. The purified product was obtained as a colorless solid, mp 228-230 °C.
Microscale thermophoresis

The proteins CK2α<sup>1–335</sup> and CK2β<sup>1–195</sup> were recombinantly expressed and purified as described in literature, with the exception of the first purification step on phosphocellulose, where the protein was eluted using high salt buffer (1 M NaCl, 25 mM Tris/HCl, pH 8.5) without applying a gradient. CK2β<sup>1–195</sup> was fluorescently labelled using the Nanotemper Monolith<sup>TM</sup> NT.115 Protein Labeling Kit RED-NHS according to the manufacturer's manual. To quantify the protein-protein interaction in the presence of potential inhibitors, the investigated compounds were first dissolved in DMSO (2-10 mM) and then 50-fold diluted in ITC buffer (25 mM Tris-HCl, 500 mM NaCl, pH 8.5) containing 0.1% (v/v) Tween 20 to concentrations of 40, 100 or 200 μM in 2% (v/v) DMSO, i.e. twice the final concentrations: 20, 50 and 100 μM, respectively, in 1% (v/v) DMSO, 0.05% (v/v) Tween 20. Fluorescently labeled CK2β<sup>1–195</sup> in ITC buffer was added to the mixture to a concentration of 40 nM, followed by a centrifugation step to remove aggregates. A volume of 10 μl of this mixture was then added to the same volume of CK2α<sup>1–335</sup> in ITC buffer (16 serial dilutions between 0.305 and 10000 μM) to obtain final concentrations of fluorescently labelled CK2β<sup>1–195</sup> and CK2α<sup>1–335</sup> of 20 nM and 0.1526 - 5000 nM, respectively. MST traces were recorded at room temperature using a Nanotemper Monolith<sup>TM</sup> NT.115 Series Instrument with Monolith<sup>TM</sup> NT.115 standard treated capillaries and normalized to initial fluorescence (M.O. Affinity Analysis, Nanotemper). The change in normalized fluorescence (ΔF<sub>norm</sub>) was plotted against the CK2α<sup>1–335</sup> concentration and analyzed<sup>50</sup> in a double reciprocal plot (1/v) versus (1/K<sub>v</sub> + 1/v<sub>v</sub>). The calculated value resulted in dissociation constants (K<sub>sec</sub>) of 11 nM that is, the same value as previously found<sup>57</sup> by applying the same methodology, and the K<sub>v</sub> values shown in the fifth column of Table 1. Data was analyzed and statistically evaluated with the program GraphPad Prism v.5.04 for Windows (GraphPad Software, San Diego, CA, USA).

Capillary electrophoresis assay to determine the enzyme inhibition

Enzymatic activities with or without inhibitors were determined for the holoenzyme (CK2α<sub>α</sub>β<sub>β</sub>), the CK2α<sub>α</sub> subunit, and the mutated CK2α<sup>1–335/C335S</sup> subunit by a capillary electrophoresis assay described before.<sup>59</sup> For this purpose, CK2α<sub>α</sub> as well as (CK2α<sub>α</sub>β<sub>β</sub>) holoenzyme were purified after recombinant expression in E. coli BL21(DE3) and purified according the protocol of Grankowski et al.<sup>60</sup> The mutated CK2α<sup>1–335/C335S</sup> subunit was purified by Ni-NTA affinity chromatography using an N-terminally attached His<sub>6</sub> tag. Successful purification was controlled by SDS-PAGE. Enzymatic activity was determined in the presence of 60 μM ATP and 114 μM of the substrate peptide RRRLDSSDDDD. For both CK2α<sub>α</sub> subunits an assay buffer containing 100 mM NaCl instead of 60 mM NaCl as for the holoenzyme, and 20 mM MgCl<sub>2</sub> instead of 10 mM MgCl<sub>2</sub> was applied. For the holoenzyme, 1 μg was added, whereas for CK2α<sub>α</sub> and the mutated CK2α<sup>1–335/C335S</sup> subunit 0.25 μg was added each. For each compound inhibition was determined three times independently at an initial concentration of 10 μM and the mean value and the standard deviation (SD) were calculated. For compounds showing more than 60% inhibition at a concentration of 10 μM with respect to the enzyme without inhibitor, but the same amount of DMSO used for solving, an IC<sub>50</sub> value was determined again in three independent experiments.

Supporting Information

Characteristic NMR data, a summary of the specific optical rotation, determination of enantiomeric purity by chiral HPLC, synthesis of (S)-4 and (R)-4 and the X-ray crystal structure analysis of (±)-3d, (±)-10b and (±)-14d. CCDC-1951235, CCDC-1951236 and CCDC-1951237 contain the supplementary crystallographic data for these compounds. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/structures. In addition, dissociation constants obtained by MST for the CK2α<sup>1–335</sup>/CK2β<sup>1–195</sup> interaction in the absence and presence of test compounds are provided. Finally, all H and 13C NMR spectra of the compounds are displayed.

Acknowledgements

Financial support by the Deutsche Forschungsgemeinschaft (DFG) is gratefully acknowledged. Open access funding enabled and organized by Projekt DEAL.

Conflict of Interest

The authors declare no conflict of interest.

Keywords: CK2 inhibitors · enzyme inhibition · epimerization · kinases · stereochemistry · tetracyclic systems

[1] J. A. Endicott, M. E. M. Noble, L. N. Johnson, Annu. Rev. Biochem. 2012, 81, 587–613.
[2] J. Pines, Semin. Cell Biol. 1994, 5, 399–408.
[3] M. Karin, J. Biol. Chem. 1995, 270, 16483–16486.
[4] G. Schimmack, R. A. Defrazono, N. Musi, Diabetes Obes. Metab. 2006, 8(6), 591–602.
[5] K. S. Bhullar, N. O. Lagaron, E. M. McGowan, I. Parmar, A. Jha, B. P. Hubbard, H. P. V. Rupasinghe, Mol. Cancer 2018, 17, 48.
[6] A. Aroa, E. M. Scholar, J. Pharmacol. Exp. Ther. 2005, 315, 971–979.
[7] M. Deininger, E. Buchdunger, B. J. Druker, Blood 2005, 105, 2640–2653.
[8] N. Iqbal, N. Iqbal, Chemother. Res. Pract. 2014, 2014, 357027.
[9] D. W. Litchfield, Biochem. Bio. 2003, 369, 1–15.
[10] L. M. Alvarez, J. Revuelta-Cervantes, I. Dominguez, John Wiley & Sons, Inc, Oxford, 2013, 131–168.
[11] O. Marin, F. Meggio, S. Sarno, L. Cesaro, M. A. Pagano, L. A. Pinna, J. Biol. Chem. 1999, 274, 29260–29265.
[12] O. Filhol, J.-L. Martiel, C. Cochet, EMBO Rep. 2004, 5, 351–355.
[13] K. Niefnd, B. Guerra, I. Ermakova, O. G. Issinger, EMBO J. 2001, 20, 3320–3331.
[14] G. M. Mathaway, M. J. Zoller, J. A. Traugh, J. Biol. Chem. 1981, 256, 11442–11446.
[15] V. Martel, O. Filhol, A. Nueda, C. Cochet, Ann. N. Y. Acad. Sci. 2002, 973, 272–277.
[16] A. P. Bidwai, J. C. Reed, C. V. Glover, Arch. Biochem. Biophys. 1993, 300, 265–270.
[17] L. A. Pinna, J. Cell Sci. 2002, 115, 3873–3878.
[18] S. Sarno, P. Ghisellini, L. Cesaro, R. Battistutta, L. A. Pinna, Mol. Cell. Biochem. 2001, 227, 13–19.
