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Design and synthesis of novel class of CK2 inhibitors: application of copper- and gold-catalysed cascade reactions for fused nitrogen heterocycles

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Abstract: Two classes of fused nitrogen heterocycles were designed as CK2 inhibitor candidates on the basis of previous structure–activity relationship (SAR) studies. Various dipyrrolo[3,2-*b*:2',3'-*e*]pyridine and benzo[*g*]indazole derivatives were prepared using transition-metal-catalysed cascade and/or multicomponent reactions. Biological evaluation of these candidates revealed that benzo[*g*]indazole is a promising scaffold for potent CK2 inhibitors. The inhibitory activities on cell proliferation of these potent CK2 inhibitors are also presented.

Introduction

The protein kinase CK2 (previously called casein kinase II) is a ubiquitous and highly pleiotropic serine/threonine kinase for more than 300 protein substrates. A number of reports have suggested that CK2 is a potential target for cancer treatment because CK2 is overexpressed in a wide variety of tumours,¹ and various small-molecule CK2 inhibitors have been developed.² CK2 predominantly forms a heterotetramer composed of two catalytic subunits (CK2 α and/or CK2 α') and two regulatory subunits (CK2 β).³ The majority of reported small-molecule CK2 inhibitors target the ATP-binding site of the catalytic subunits. A benzonaphthyridine derivative CX-4945 (**1**) has been reported as a potent ATP-competitive CK2 inhibitor, and is currently undergoing clinical trials for cancer treatment (Figure 1).⁴

We have also carried out structure–activity relationship (SAR) studies⁵ and crystallographic analyses⁶ of pyrazine-based CK2 inhibitors **2**^{5b} (Figure 1) to obtain structural information for the design of novel CK2 inhibitors. These inhibitors **2** bind to CK2 α with a planar horseshoe-shaped conformation to support favourable interactions via van der Waals contacts with hydrophobic residues (Leu45, Val53, Val66, Ile95, Phe113, His160, Met163, and Ile174) in CK2 α as well as hydrogen bonds with the surrounding residues. A nitrogen atom at the 4-position of a pyrazine ring interacts with the backbone NH group of Val116 in the hinge region. The carboxyl group, which is necessary for binding to CK2, interacts by a salt bridge and hydrogen bonds with the Lys68 ϵ -amino group and the backbone NH group of Asp175 in the activation loop, and a water molecule in the active site.

We recently identified 2-phenyl-1,3,4-thiadiazoles **3** and 3-phenyl-1,2-pyrazole derivatives **4** as CK2 inhibitor led by virtual screening of a compound library (Figure 1).⁷ Binding mode analysis of the thiadiazole-type inhibitor **3a** suggested that unfavourable repulsion exists between one of the thiadiazole nitrogen atoms and the backbone oxygen atom of CK2 α -Glu114 (Figure 2A). The pyrazole analogue **4** designed by replacement of the thiadiazole ring in **3b** [IC₅₀ = 3.4 μ M (CK2 α) and 1.2 μ M (CK2 α')] with a pyrazole ring would improve the CK2-binding affinity because the NH

group of the pyrazole could form an additional hydrogen bond with the Glu114 carbonyl oxygen (Figure 2B). The pyrazole-type inhibitor **4** showed more potent inhibitory activity toward CK2 [IC_{50} = 0.14 μ M (CK2 α) and 0.063 μ M (CK2 α')] than the thiadiazole **3b** did. Because the other tautomer, **4'**, may have unfavourable electrostatic repulsions with the binding region (Figure 2B), stabilization of tautomer **4** would enhance the inhibitory activity. In this study, we have designed and synthesized two classes of fused nitrogen heterocycles, using copper- or gold-catalysed cascade reactions. The *in vitro* inhibitory activities toward two subtypes of the CK2 catalytic subunit (CK2 α and CK2 α') and anti-proliferative activity of the potent inhibitors are also presented.

Results and discussion

Design

On the basis of our previous SAR studies, we designed two fused nitrogen heterocycles **5** and **6** as novel CK2 inhibitor candidates (Figure 3). The highly fused and rigid scaffold would fix the preferred planar conformation and decrease the entropic loss when incorporated into the binding pocket.⁶ In the dipyrrolo[3,2-*b*:2',3'-*e*]pyridine derivative **5**, a nitrogen atom of the pyridine core and a carboxyl group would form favourable electrostatic interactions with CK2, as the pyrazine-based CK2 inhibitors **2** do (Figure 3A).^{5a,6} The benzo[*g*]indazole derivatives **6** also have a highly fused and planar structure (Figure 3B). More importantly, in the benzo[*g*]indazole scaffold (a bridged analogue of the phenylpyrazole **4**) the desired tautomer of the pyrazole moiety would be preferred because of the fused benzene ring.⁸ The stabilization effect of this tautomer in **6** would enhance the chance of the NH group forming favourable interactions with the Glu114 carbonyl oxygen. Preliminary molecular modelling studies of **6a** (R^1 = Ph, R^2 = H) using the CK2 α -**3a** co-crystal structure suggested that the carboxyl group and two indazole nitrogens could form favourable interactions with CK2 α (Figure 3C).

Synthesis

We expected that the inhibitor candidates **5** and **6** could be synthesized using the transition-metal-catalysed cascade reactions which have been developed by us in recent years.⁹ The synthetic route to dipyrrolo[3,2-*b*:2',3'-*e*]pyridine derivatives **5a–e** is shown in Scheme 1. The dipyrrolo[3,2-*b*:2',3'-*e*]pyridine framework was constructed using copper-catalysed bis-cyclization of 2,6-diethynylpyridine-3,5-diamine **7**.^{9a} The reaction proceeded smoothly to give 1,7-bismesyldipyrrolo[3,2-*b*:2',3'-*e*]pyridine (**8**). One of the mesyl groups of **8** was selectively removed using Cs₂CO₃ and methanol to afford **9**. The copper-catalysed Ullmann-type coupling of **9**¹⁰ with several aryl or heteroaryl carboxylic acid esters, followed by successive deprotection of a mesyl group and an ester, yielded 1-aryldipyrrolo[3,2-*b*:2',3'-*e*]pyridines **5a–e**.

Benzo[*g*]indazole derivatives **6a–f** were prepared via a gold-catalysed three-component annulation and cyclization cascade^{9b} as a key step (Scheme 2). 4-Amino-3-bromobenzoic acid (**12**) was converted to the methyl ester **13** under acidic conditions. A Sandmeyer reaction using potassium iodide, followed by a Sonogashira coupling with trimethylsilylacetylene (TMS-acetylene) afforded the common intermediate **15**. A second Sonogashira coupling with several acetylenes, using tri-*tert*-butylphosphine as a ligand for palladium,¹¹ and removal of the TMS group gave methyl 3,4-diethynylbenzoates **17a–d**. A three-component annulation and cyclization cascade of **17** with a hydrazine derivative **18** and aldehydes **19**, using a catalytic amount of IPrAuCl [IPr = 1,3-bis(2,6-diisopropylphenyl)imidazol-2-ylidene] and AgOTf, afforded the desired 2,3-dihydrobenzo[*g*]indazole derivatives **20a–f**. The cleavage of a 4-methoxybenzyl (PMB) group and aromatization of a dihydropyrazole ring were achieved by treatment with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) or trifluoroacetic acid (TFA) to give the benzo[*g*]indazoles **21a–f**. Finally, full deprotection of the methyl carbamate and ester yielded the desired compounds **6a–f**.

The 3,4-unsubstituted congener **6g** (R¹ and R² = H) was prepared via a different synthetic route (Scheme 3), because of difficulties in using **17** bearing two terminal alkynes (R¹ = H) for the three-component annulation. The 5-aryl-1-benzylpyrazole derivative **26** was prepared according to

the traditional method for construction of pyrazoles from the acetophenone derivative **22**. A Sonogashira coupling of **26** with TMS-acetylene, followed by removal of a TMS group, afforded the cyclization precursor **27**. The alkyne **27** was heated with a catalytic amount of IPrAuCl and silver bis(trifluoromethanesulfonyl)imide (AgNTf₂) to give a benzo[g]indazole derivative **21g** via intramolecular hydroarylation of an alkyne, based on our three-component annulation and cyclization cascade. The entire deprotection of the benzyl group¹² and methyl ester yielded the desired compound **6g**.

Evaluation of CK2 inhibitory activity

The inhibitory activities of the synthesized analogues toward two subtypes of the catalytic subunit of CK2 (CK2 α and CK2 α') are summarised in Table 1. Although the dipyrrolo[3,2-*b*:2',3'-*e*]pyridine derivative bearing a benzoic acid, **5a**, had significantly less potent activity, its pyridine congener **5b** showed moderate activity toward both CK2 α and CK2 α' (IC₅₀ = 41 μ M and 26 μ M, respectively). The position of the carboxyl group also had a distinct effect on the activity: the analogue bearing a 4-carboxypyridine moiety, **5c**, exhibited higher activity toward CK2 α (IC₅₀ = 14 μ M) and CK2 α' (IC₅₀ = 12 μ M) than did the 3-, 5-, and 2-substituted analogues **5b**, **5d**, and **5e**.

In contrast, benzo[g]indazole derivatives gave more promising results. The 4-methoxybenzene-substituted analogue **6b** and the thiophene-substituted one **6d** were more potent than the parent pyrazole derivative **4**, despite the loss of the amino group on the pyrazole ring of **4**, which would form a favourable hydrogen bond with the Val116 carbonyl oxygen (Figure 2B). These results suggest that the benzo[g]indazole core would be a more suitable scaffold for potent CK2 inhibitors than the biaryl-type pyrazoles would be, presumably because of the rigidity of the molecule and/or the predominance of the preferable tautomer of the pyrazole ring. The introduction of an isopropyl group as R² reduced the binding affinity 100-fold relative to those of the unsubstituted analogues (compare **6b** with **6c**, and **6d** with **6e**). On the other hand, the absence of a

substituent at the 4-position ($R^1 = H$, **6g**) also lowered the activity relative to those of the other 4-substituted analogues. An aromatic group was preferable to an alkyl chain as the substituent R^1 , and the electrostatic and/or steric environment of the aromatic ring slightly affected the binding affinity. These observations correspond with the predicted binding affinities of -23.8 , -22.0 , and -20.7 kcal/mol for **6a**, **6f**, and **6g**, respectively.

Evaluation of cytotoxic activity

Compounds **4**, **6b**, and **6d**, which exerted potent CK2 inhibitory activities, were tested for anti-proliferative effects on colorectal cancer cells, HCT-116 (Figure 4). The cancer cells were treated with increasing concentrations of the compounds, and viabilities were measured by the 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS) assay. The pyrazole **4** had a lower than 50% inhibitory effect at 100 μM , whereas the benzo[g]indazoles **6b** and **6d** exhibited moderate inhibitory effects ($IC_{50} = 26.5$ μM and 21.0 μM , respectively), slightly lower than that of **1** ($IC_{50} = 6.7$ μM). These data are in linear correlation with the CK2 inhibitory effects.

Conclusion

In conclusion, we have designed dipyrrolo[3,2-*b*:2',3'-*e*]pyridine and benzo[g]indazole derivatives as novel CK2 inhibitor candidates, based on our previous SAR studies. A series of benzo[g]indazole analogues were efficiently prepared using our three-component cascade reaction. Evaluation of the resulting derivatives for CK2 inhibitory activities led to identification of novel potent inhibitors such as the thiophene-substituted benzo[g]indazole derivative **6d**. Benzo[g]indazole is a promising scaffold for highly potent CK2 inhibitors. These results will provide useful information for further studies on CK2 inhibitors with high selectivity toward the two isozymes CK2 α and CK2 α' .

Experimental section

General

Melting points were measured by a hot stage melting point apparatus (uncorrected). ^1H NMR spectra were recorded using a JEOL AL-400 or JEOL ECA-500 spectrometer, and chemical shifts are reported in δ (ppm) relative to TMS as internal standard. ^{13}C NMR spectra were recorded using a JEOL AL-400 or JEOL ECA-500 spectrometer and referenced to the residual solvent signal. ^1H NMR spectra are tabulated as follows: chemical shift, number of protons, multiplicity (b = broad, s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet), and coupling constant(s). Exact mass (HRMS) spectra were recorded on a JMS-HX/HX 110A mass spectrometer. Infrared (IR) spectra were obtained on a JASCO FT/IR-4100 FT-IR spectrometer with JASCO ATR PRO410-S. Compounds **7**,^{9a} **8**,^{9a} and **22**¹³ were prepared according to the literatures.

1-(Methylsulfonyl)-1,7-dihydrodipyrrolo[3,2-*b*:2',3'-*e*]pyridine (9). To the mixture of **8** (700 mg, 2.23 mmol) in MeOH/THF (1:1, 220 cm³) was added Cs₂CO₃ (2.18 g, 6.70 mmol). After stirring at rt for 12 h, NH₄Cl (835 mg, 15.6 mmol) was added and the mixture was stirred at rt for 1.5 h. It was concentrated in vacuo and the residue was diluted with CHCl₃. After filtration, the mixture was concentrated and the residue was chromatographed on silica gel (CHCl₃/MeOH = 10/1) to afford the title compound **9** (407 mg, 78%) as a white solid: mp 218–219 °C (decomp); δ_{H} (400 MHz; CDCl₃; Me₄Si) 3.05 (3H, s), 6.80–6.82 (1H, m), 6.97 (1H, dd, *J* 3.8, 0.6 Hz), 7.57–7.59 (1H, m), 7.65 (1H, d, *J* 3.8 Hz), 8.25 (1H, br s), and 8.46 (1H, s); δ_{C} (100 MHz; CDCl₃) 40.2, 103.1, 103.1, 110.7, 127.0, 128.8, 129.5, 131.3, 145.4, and 145.7. *Anal.* Found: C, 52.0; H, 3.9; N, 17.9. Calc. for C₁₀H₉N₃O₂S: C, 52.0; H, 3.8; N, 17.6%.

Representative procedure for coupling reactions of 9 with aryl halides 10a–e: synthesis of ethyl 4-{7-(Methylsulfonyl)dipyrrolo[3,2-*b*:2',3'-*e*]pyridin-1(*7H*)-yl}benzoate (11a). Under argon atmosphere, the mixture of **9** (50 mg, 0.21 mmol), ethyl 4-iodobenzoate (**10a**) (70 mg, 0.26 mmol),

CuI (4 mg, 0.021 mmol), (\pm)-*trans*-*N,N'*-dimethylcyclohexane-1,2-diamine **A** (0.007 cm³, 0.042 mmol), and K₃PO₄ (113 mg, 0.53 mmol) in 1,4-dioxane (1 cm³) was refluxed for 3 h. The mixture was diluted with EtOAc and filtered. The filtrate was concentrated and chromatographed on silica gel (hexane/EtOAc = 1/1) to afford the title compound **11a** (73 mg, 90%) as a white solid: mp 75–76 °C; IR (neat): $\nu_{\max}/\text{cm}^{-1}$ 1698 (C=O), 1278 (OCH₃); δ_{H} (400 MHz; CDCl₃; Me₄Si) 1.44 (3H, t, *J* 7.1 Hz), 3.06 (3H, s), 4.43 (2H, q, *J* 7.1 Hz), 6.96 (1H, d, *J* 3.7 Hz), 6.98 (1H, d, *J* 3.7 Hz), 7.58–7.61 (2H, m), 7.68 (1H, d, *J* 3.7 Hz), 7.72 (1H, d, *J* 3.7 Hz), 8.24–8.27 (2H, m), and 8.40 (1H, s); δ_{C} (100 MHz; CDCl₃) 14.3, 40.4, 61.2, 102.5, 105.0, 110.3, 123.1 (2C), 126.0, 127.0, 128.7, 129.4, 131.5 (2C), 132.1, 142.6, 145.8, 146.9, and 165.6; HRMS (FAB) *m/z* Calc. for C₁₉H₁₈N₃O₄S (MH⁺) 384.1013, found 384.1014.

Representative procedure for removal of protecting groups of 11a–e: Synthesis of 4-{dipyrrolo[3,2-*b*:2',3'-*e*]pyridin-1(7*H*)-yl}benzoic acid (5a). To the mixture of **11a** (60 mg, 0.16 mmol) in MeOH/THF (1:1, 16 cm³) was added Cs₂CO₃ (153 mg, 0.47 mmol). After stirring at rt for 12 h, the mixture was concentrated in vacuo. The residue was diluted with sat. aqueous NH₄Cl and the mixture was extracted with EtOAc twice. The combined extracts were washed with brine, dried over Na₂SO₄, and concentrated in vacuo. The crude product was dissolved with THF (3 cm³) and 3N NaOH (0.3 cm³) was added. The mixture was stirred at rt for 40 h and concentrated in vacuo. The residue was dissolved in water (*ca.* 1 cm³) and filtered. The filtrate was acidified with 3N HCl, then 28% aqueous NH₃ was added until the mixture was clear solution. The solution was freeze dried and the residue was purified by reverse phase HPLC [3–6% MeCN in H₂O (containing 0.1% NH₃); flow rate: 10 cm³ min⁻¹] to afford the title compound **5a** as an NH₃ salt (24.9 mg, 54%). Pale yellow solid: mp >300 °C; IR (neat): $\nu_{\max}/\text{cm}^{-1}$ 1602 (C=O); δ_{H} (500 MHz; DMSO-*d*₆; Me₄Si) 6.55–6.57 (1H, br m), 6.82 (1H, d, *J* 3.4 Hz), 7.65–7.67 (1H, m), 7.81 (2H, d, *J* 8.0 Hz), 7.98 (1H, d, *J* 3.4 Hz), 8.05 (1H, s), 8.16 (2H, d, *J* 8.0 Hz), and 11.03 (1H, s); δ_{C} (125 MHz; DMSO-*d*₆) 99.7, 100.4, 104.5, 122.3 (2C), 125.5, 126.6, 128.0, 129.6, 130.5, 131.1 (2C), 142.8, 144.3, 144.4, and 166.8; HRMS (FAB)

m/z Calc. for $C_{16}H_{10}N_3O_2$ $[M - H]^-$ 276.0779, found 276.0781.

Methyl 4-amino-3-bromobenzoate (13). To the mixture of 4-amino-3-bromobenzoic acid **12** (5.4 g, 25 mmol) in MeOH (30 cm³) was added sulphuric acid (0.83 cm³), then the mixture was refluxed for 20 h. The mixture was cooled to 0 °C and basified with sat. aqueous NaHCO₃ (ca. 100 cm³). The precipitates were collected and washed with water. Recrystallization from MeOH-H₂O afforded the title compound **13** (5.34 g, 93%) as a white solid. All spectral data were in good agreement with those reported by Bräse *et al.*¹⁴

Methyl 3-bromo-4-iodobenzoate (14). To the stirred suspension of **13** (5.0 g, 21.7 mmol) in 6N HCl (40 cm³) was added a solution of NaNO₂ (3.0 g, 43.4 mmol) in H₂O (20 cm³) dropwise over 15 min at 0 °C. After stirring at 0 °C for 1 h, a solution of KI (5.4 g, 32.6 mmol) in H₂O (16 cm³) was added dropwise to the reaction mixture over 15 min at 0 °C. The mixture was vigorously stirred at rt for 24 h in the dark. The precipitate was collected and washed with H₂O. The solid was dissolved in Et₂O, then the mixture was washed with aqueous Na₂S₂O₃, H₂O, and brine, dried over MgSO₄, and concentrated in vacuo. The residue was filtered through a silica gel pad with elution of hexane/EtOAc (50/1). The filtrate was concentrated and recrystallized from hot hexane to afford the title compound **14** (3.9 g, 53%) as colourless crystals. All spectral data were in good agreement with those reported.¹⁵

Methyl 3-bromo-4-[(trimethylsilyl)ethynyl]benzoate (15). Under argon atmosphere, Et₃N (7.3 cm³, 53 mmol) and TMS-acetylene (1.8 cm³, 12.7 mmol) were added to the mixture of **14** (3.6 g, 10.6 mmol), PdCl₂(PPh₃)₂ (190 mg, 0.27 mmol) and CuI (50 mg, 0.27 mmol) in THF (35 cm³), then the mixture was stirred at rt for 12 h. The mixture was concentrated in vacuo and the residue was diluted with Et₂O and filtered through a silica gel pad. The filtrate was concentrated in vacuo and the residue was chromatographed on silica gel (hexane/EtOAc = 20/1) to afford the title compound **15**

(3.19 g, 97%) as a yellow oil. All spectral data were in good agreement with those reported.¹⁶

Representative procedure for coupling reactions of 15 with 16a–d and removal of a TMS group: Synthesis of methyl 4-ethynyl-3-(thiophen-2-ylethynyl)benzoate (17c). Under argon atmosphere, **15** (938 mg, 3.01 mmol) was dissolved in toluene (3 cm³). To the solution were added PdCl₂(PhCN)₂ (35 mg, 0.09 mmol), CuI (11 mg, 0.06 mmol), *i*-Pr₂NH (1.3 cm³, 9.03 mmol), 2-ethynylthiophene (**16c**),¹⁷ and a solution of *t*-Bu₃P in toluene (1.0 M, 0.18 cm³) and the mixture was stirred at rt for 7 h. The resulting mixture was filtered through a silica gel pad with elution of hexane/EtOAc (9/1). The filtrate was concentrated in vacuo and chromatographed on silica gel (hexane/EtOAc = 9/1) to afford methyl 3-(thiophen-2-ylethynyl)-4-[(trimethylsilyl)ethynyl]benzoate (994 mg, 98%) as a yellow solid: mp 71–72 °C; IR (neat): $\nu_{\max}/\text{cm}^{-1}$ 2210, 2159 (C≡C), 1246 (OCH₃); δ_{H} (400 MHz; CDCl₃; Me₄Si) 0.29 (9H, s), 3.92 (3H, s), 7.03 (1H, dd, *J* 5.1, 3.7 Hz), 7.31–7.33 (2H, m), 7.54 (1H, d, *J* 8.2 Hz), 7.90 (1H, dd, *J* 8.2, 1.7 Hz), and 8.16 (1H, d, *J* 1.7 Hz); δ_{C} (100 MHz; CDCl₃) 52.5, 87.5, 91.2, 102.5, 102.6, 123.0, 126.3, 127.3, 128.1, 128.7, 129.5, 129.8, 132.3, 132.5, 132.7, and 166.0; HRMS (FAB) *m/z* Calc. for C₁₉H₁₉O₂SSi (MH⁺) 339.0870, found 339.0874.

The coupling product (475 mg, 1.40 mmol) was dissolved in MeOH (14 cm³). After addition of K₂CO₃ (388 mg, 2.80 mmol), the mixture was stirred at rt for 1 h. The resulting mixture was acidified with sat. aqueous citric acid and extracted with Et₂O. The extract was washed with H₂O and brine, dried over MgSO₄, and concentrated in vacuo. The residue was chromatographed on silica gel (hexane/EtOAc = 9/1) followed by recrystallization from CHCl₃-hexane to afford the title compound **17c** (259 mg, 70%) as pale yellow crystals: mp 75–76 °C; IR (neat): $\nu_{\max}/\text{cm}^{-1}$ 3246 (C≡CH), 2195 (C≡C), 1717 (C=O), 1244 (OCH₃); δ_{H} (500 MHz; CDCl₃; Me₄Si) 3.51 (1H, s), 3.93 (3H, s), 7.02–7.04 (1H, m), 7.33 (1H, s), 7.34–7.35 (1H, m), 7.58 (1H, d, *J* 8.0 Hz), 7.93 (1H, dd, *J* 8.0, 1.7 Hz), and 8.18 (1H, d, *J* 1.7 Hz); δ_{C} (125 MHz; CDCl₃) 52.4, 81.4, 84.1, 87.6, 90.7, 126.4, 127.2, 128.1, 128.4, 128.7, 130.1, 132.6, 132.6 (2C), and 165.8. *Anal.* Found: C, 72.4; H, 4.0. Calc. for

C₁₆H₁₀O₂S: C, 72.2; H, 3.8%.

Methyl 2-(4-methoxybenzyl)hydrazinecarboxylate (18). The mixture of methyl hydrazinecarboxylate (10.0 g, 111 mmol) and *p*-anisaldehyde (15 cm³, 122 mmol) in Et₂O (400 cm³) was stirred at 35 °C for 15 h. The reaction mixture was concentrated in vacuo until *ca.* 50 cm³ and the residue was diluted with toluene. The precipitate was collected and washed with hexane to afford methyl 2-(4-methoxybenzylidene)hydrazinecarboxylate (20.3 g, 88%) as a white solid: mp 123–124 °C; IR (neat): $\nu_{\max}/\text{cm}^{-1}$ 3252 (NH), 1712 (C=O), 1249 (OCH₃); δ_{H} (400 MHz; CDCl₃; Me₄Si) 3.84 (3H, s), 3.85 (3H, br s), 6.90 (2H, d, *J* 8.8 Hz), 7.63 (2H, d, *J* 8.8 Hz), 7.79 (1H, br s), and 7.90 (1H, s); δ_{C} (100 MHz; CDCl₃) 52.7, 55.2, 113.9 (2C), 126.4, 128.7 (2C), 144.8, 154.6, and 161.0. *Anal.* Found: C, 57.6; H, 5.6; N, 13.6. Calc. for C₁₀H₁₂N₂O₃: C, 57.7; H, 5.8; N, 13.5%.

The mixture of benzylidenehydrazine (5.0 g, 24.0 mmol) and 10% Pd/C (2.5 g) in EtOH (340 cm³) was stirred under hydrogen atmosphere at rt for 1.5 h. The reaction mixture was filtered through Celite and concentrated. The residue was chromatographed on silica gel (hexane/EtOAc = 2/3) followed by recrystallization from hexane to afford the title compound **18** (3.56 g, 71%) as colourless crystals: mp 63–64 °C; IR (neat): $\nu_{\max}/\text{cm}^{-1}$ 3301 (NH), 1684 (C=O), 1251 (OCH₃); δ_{H} (400 MHz; CDCl₃; Me₄Si) 3.73 (3H, br s), 3.80 (3H, s), 3.94 (2H, d, *J* 4.9 Hz), 4.15 (1H, br s), 6.15 (1H, br s), 6.86–6.88 (2H, m), and 7.25–7.27 (3H, m); δ_{C} (100 MHz; CDCl₃) 52.3, 55.1, 55.2, 113.8 (2C), 129.4, 130.1 (2C), 157.8, and 159.0. *Anal.* Found: C, 57.3; H, 6.5; N, 13.4. Calc. for C₁₀H₁₄N₂O₃: C, 57.1; H, 6.7; N, 13.3%.

Representative procedure for gold-catalysed three-component annulation and cyclisation cascade reactions of 17a–d with 18 and 19a,b: Synthesis of dimethyl 2-(4-methoxybenzyl)-4-(4-methoxyphenyl)-2,3-dihydro-1H-benzo[g]indazole-1,7-dicarboxylate (20b). Under argon atmosphere, the mixture of **17b** (20 mg, 0.069 mmol), a hydrazine **18** (5.8 mg, 0.028 mmol), paraformaldehyde **19a** (1.7 mg, 0.055 mmol as HCHO), IPrAuCl (2.1 mg, 3.5 μmol),

and AgOTf (0.9 mg, 3.5 μmol) in AcOH (0.35 cm^3) was stirred at 35 $^\circ\text{C}$ for 2 h, then the additional hydrazine **18** (5.8 mg, 0.028 mmol) and paraformaldehyde **19a** (1.7 mg, 0.055 mmol as HCHO) were added. After stirred at 35 $^\circ\text{C}$ for 2 h, further hydrazine **18** (5.8 mg, 0.028 mmol) and paraformaldehyde **19a** (1.7 mg, 0.055 mmol as HCHO) were added and stirred for 2 h. The reaction mixture was poured into sat. aqueous NaHCO_3 and the mixture was extracted with EtOAc twice. The combined extracts were washed with H_2O and brine, dried over Na_2SO_4 , and concentrated in vacuo. The residue was chromatographed on silica gel (hexane/EtOAc = 10/1 to 2/1) to afford the title compound **20b** [11.5 mg, 33% (41% rsm)] as a yellow oil: IR (neat): $\nu_{\text{max}}/\text{cm}^{-1}$ 1716, 1609 (C=O), 1251 (OCH₃); δ_{H} (500 MHz; CDCl_3 ; Me_4Si , 50 $^\circ\text{C}$) 3.75 (3H, s), 3.79 (2H, br s), 3.79 (3H, s), 3.86 (3H, s), 3.99 (3H, s), 4.34 (2H, br s), 6.76 (2H, d, J 8.6 Hz), 7.00 (2H, d, J 8.6 Hz), 7.19 (2H, d, J 8.6 Hz), 7.37 (2H, d, J 8.6 Hz), 7.81 (1H, s), 8.01–8.07 (2H, m), and 8.63 (1H, s); δ_{C} (100 MHz; CDCl_3) 52.3, 53.6, 55.2, 55.4, 57.7, 61.7, 113.5 (2C), 114.4 (2C), 124.8, 125.6, 125.8, 127.2, 127.5, 128.3, 129.2 (2C), 130.1, 131.1 (2C), 131.2, 131.5, 133.6, 136.2, 137.3, 157.0, 159.1, 159.5, and 167.1; HRMS (FAB) m/z Calc. for $\text{C}_{30}\text{H}_{29}\text{N}_2\text{O}_6$ (MH^+) 513.2020, found 513.2011.

Dimethyl 3-isopropyl-2-(4-methoxybenzyl)-4-(thiophen-2-yl)-2,3-dihydro-1H-benzo[g]-indazole-1,7-dicarboxylate (20e). Under argon atmosphere, the mixture of **17c** (40 mg, 0.15 mmol), a hydrazine **18** (35 mg, 0.17 mmol), isobutyraldehyde **19b** (0.027 cm^3 , 0.30 mmol), IPrAuCl (4.7 mg, 7.5 μmol), and AgOTf (1.9 mg, 7.5 μmol) in AcOH (1.5 cm^3) was stirred at 50 $^\circ\text{C}$ for 7 h. The reaction mixture was poured into sat. aqueous NaHCO_3 and the mixture was extracted with EtOAc twice. The combined extracts were washed with H_2O and brine, dried over Na_2SO_4 , and concentrated in vacuo. The residue was chromatographed on silica gel (hexane/EtOAc = 4/1) to afford the title compound **20e** (67.6 mg, 85%) as a yellow oil: IR (neat): $\nu_{\text{max}}/\text{cm}^{-1}$ 1717, 1612 (C=O); δ_{H} (400 MHz; CDCl_3 ; Me_4Si) 0.53 (3H, d, J 6.8 Hz), 0.61 (3H, d, J 6.8 Hz), 1.55–1.63 (1H, m), 3.77 (1H, d, J 12.2 Hz), 3.79 (3H, s), 3.86 (3H, s), 3.99 (3H, s), 4.12 (1H, d, J 12.2 Hz), 4.36 (1H, d, J 3.4 Hz), 6.84 (2H, d, J 8.5 Hz), 7.11 (1H, dd, J 4.8, 3.4 Hz), 7.21 (1H, d, J 3.7 Hz), 7.34–7.38 (3H, m), 7.90

(1H, s), 8.00–8.07 (2H, m), and 8.61 (1H, s); δ_C (100 MHz; CDCl₃) 16.0, 20.1, 30.9, 52.2, 53.3, 55.2, 62.6, 72.4, 113.4 (s, 2H), 124.9, 125.5, 125.7, 125.9, 126.0, 127.6, 127.7, 127.8, 128.5, 129.6, 131.1, 131.2, 131.6 (s, 2H), 133.6, 137.6, 141.3, 157.1, 159.2, and 167.0; HRMS (FAB) m/z Calc. for C₃₀H₃₁N₂O₅S (MH⁺) 531.1948, found 531.1946.

Deprotection and aromatisation of 20: 4-Phenyl-1H-benzo[g]indazole-7-carboxylic acid (6a). Compound **20a** (20 mg, 0.042 mmol) and anisole (0.022 cm³, 0.21 mmol) were dissolved in TFA (0.6 cm³). After stirring at 80 °C for 12 h, the mixture was poured into sat. aqueous NaHCO₃ and the mixture was extracted with EtOAc twice. The combined extracts were washed with H₂O and brine, dried over Na₂SO₄, and concentrated in vacuo. The crude product (**21a**) was dissolved in 1,4-dioxane (0.4 cm³), then 3N NaOH (0.083 cm³, 0.25 mmol) was added. After stirring at 40 °C for 12 h, NH₄Cl (22 mg, 0.42 mmol) was added and the mixture was stirred at rt for 1 h. The mixture was concentrated in vacuo and the residue was chromatographed on silica gel (CHCl₃/MeOH = 9/1) to afford the title compound **6a** (5.8 mg, 49%) as a white solid: mp >300 °C; IR (neat): $\nu_{\max}/\text{cm}^{-1}$ 1703 (C=O); δ_H (500 MHz; DMSO-*d*₆; Me₄Si) 7.48 (1H, dd, *J* 7.6, 7.6 Hz), 7.58 (2H, dd, *J* 7.6, 7.6 Hz), 7.78 (1H, s), 7.85 (2H, d, *J* 7.6 Hz), 8.17 (1H, d, *J* 8.6 Hz), 8.28 (1H, s), 8.53 (1H, d, *J* 8.6 Hz), and 8.70 (1H, s); δ_C (125 MHz; DMSO-*d*₆) 118.7, 121.0, 121.6 (2C), 123.3, 126.49, 126.53, 127.9, 128.2 (2C), 129.0 (2C), 130.4, 131.4, 131.7, 133.0, 139.1, and 167.8; HRMS (FAB) m/z Calc. for C₁₈H₁₁N₂O₂ [M – H][–] 287.0826, found 287.0820.

3-Isopropyl-4-(thiophen-2-yl)-1H-benzo[g]indazole-7-carboxylic acid (6e). The mixture of **20e** (65 mg, 0.12 mmol) and DDQ (83 mg, 0.37 mmol) in CH₂Cl₂/H₂O (5/1, 1.2 cm³) was stirred at rt for 18 h. The resulting mixture was diluted with CHCl₃, washed with H₂O, and dried over Na₂SO₄. The solvent was removed in vacuo and the residue was chromatographed on silica gel (CHCl₃) to afford the title compound **21e** (36.8 mg, 73%) as a white solid: mp 135–136 °C; IR (neat): $\nu_{\max}/\text{cm}^{-1}$ 1746, 1714 (C=O), 1262 (OCH₃); δ_H (500 MHz; CDCl₃; Me₄Si) 1.21 (6H, d, *J* 6.9 Hz), 2.99–3.04

(1H, m), 4.00 (3H, s), 4.20 (3H, s), 7.14–7.16 (2H, m), 7.45 (1H, d, *J* 4.6 Hz), 7.79 (1H, s), 8.20 (1H, dd, *J* 8.6, 1.7 Hz), 8.65 (1H, s), and 9.11 (1H, d, *J* 8.6 Hz); δ_{C} (125 MHz; CDCl₃) 22.0 (2C), 27.1, 52.3, 55.2, 123.1, 123.5, 125.9, 126.1, 126.3, 126.8, 126.9, 128.0, 128.5, 130.1, 131.1, 132.7, 138.8, 139.5, 152.5, 157.8, and 166.7; HRMS (FAB) *m/z* Calc. for C₂₂H₂₁N₂O₄S (MH⁺) 409.1217, found 409.1215.

The mixture of **21e** (30 mg, 0.073 mmol) and 3N NaOH (15 cm³, 0.44 mmol) in 1,4-dioxane was stirred at 40 °C for 12 h. After addition of NH₄Cl (39 mg, 0.73 mmol), the mixture was stirred at rt for 1 h, then concentrated in vacuo. The residue was chromatographed on silica gel (CHCl₃/MeOH = 9/1) to afford the title compound **6e** (19.9 mg, 81%) as a white solid: mp >300 °C; IR (neat): $\nu_{\text{max}}/\text{cm}^{-1}$ 1681 (C=O); δ_{H} (500 MHz; DMSO-*d*₆; Me₄Si) 1.15 (6H, d, *J* 6.9 Hz), 3.09–3.14 (1H, m), 7.23 (1H, dd, *J* 4.9, 3.7 Hz), 7.31 (1H, d, *J* 3.7 Hz), 7.62 (1H, s), 7.70 (1H, d, *J* 4.9 Hz), 8.17 (1H, d, *J* 8.6 Hz), 8.56 (1H, d, *J* 8.6 Hz), and 8.67 (1H, s); δ_{C} (125 MHz; DMSO-*d*₆) 22.7 (2C), 26.3, 116.0, 121.9, 122.9, 124.0, 126.3 (2C), 127.3, 127.45, 127.51, 128.7, 130.4, 130.6, 138.9, 140.3, 150.3, and 167.3; HRMS (FAB) *m/z* Calc. for C₁₉H₁₅N₂O₂S [M – H][–] 335.0860, found 335.0865.

Methyl 3-bromo-4-[3-(dimethylamino)acryloyl]benzoate (24). The mixture of **22** (5.53 g, 21.5 mmol) in dimethylformamide dimethylacetal (**23**) (17.3 cm³, 129 mmol) was stirred at 85 °C for 8 h in an open vessel. After cooled to rt, the reaction mixture was concentrated in vacuo and the residue was recrystallized from EtOAc-Et₂O to afford the title compound **24** (5.77 g, 86%) as yellow crystals: mp 102–103 °C; IR (neat): $\nu_{\text{max}}/\text{cm}^{-1}$ 1719 (C=O), 1640 (C=O); δ_{H} (500 MHz; CDCl₃; Me₄Si) 2.90 (4H, s), 3.11 (3H, br s), 3.93 (3H, s), 5.30 (1H, d, *J* 12.6 Hz), 6.74 (1H, br s), 7.40 (1H, br s), 7.97 (1H, dd, *J* 8.0, 1.7 Hz), and 8.24 (1H, d, *J* 1.7 Hz); δ_{C} (125 MHz; CDCl₃) 37.2, 45.1, 52.4, 95.0, 98.7, 128.2, 128.4, 129.3, 131.4, 134.1, 154.1, 157.2, and 165.4. *Anal.* Found: C, 50.3; H, 4.4; N, 4.5. Calc. for C₁₃H₁₄BrN₂O₃: C, 50.0; H, 4.5; N, 4.5%.

Methyl 4-(1-benzyl-1*H*-pyrazol-5-yl)-3-bromobenzoate (26). The mixture of **24** (312 mg,

1.0 mmol), BnNHNH₂ (**25**) (0.22 cm³, 2.0 mmol), and AcONa (205 mg, 2.5 mmol) in AcOH (1.0 cm³) was stirred at 60 °C for 5 h. The mixture was diluted with H₂O and cooled on ice. To the mixture was added NaOH until the pH was about 12, then the resulting mixture was extracted with Et₂O twice. The combined extracts were washed with 1N HCl, H₂O, and brine, dried over Na₂SO₄, and concentrated in vacuo. The residue was chromatographed on silica gel (hexane/EtOAc = 4/1) to afford the title compound **26** (248 mg, 67%) as a colourless oil: IR (neat): $\nu_{\text{max}}/\text{cm}^{-1}$ 1725 (C=O), 1283 (OCH₃); δ_{H} (400 MHz; CDCl₃; Me₄Si) 3.95 (3H, s), 5.19 (2H, s), 6.35 (1H, d, *J* 1.7 Hz), 6.92–6.94 (2H, m), 7.18–7.21 (4H, m), 7.63 (1H, d, *J* 1.7 Hz), 7.92 (1H, dd, *J* 7.9, 1.7 Hz), and 8.33 (1H, d, *J* 1.7 Hz); δ_{C} (125 MHz; CDCl₃) 52.5, 53.8, 107.7, 124.4, 127.1, 127.6 (2C), 128.0, 128.4 (2C), 132.1, 133.8, 133.8, 136.4, 136.5, 138.8, 140.8, and 165.1; HRMS (FAB) *m/z* Calc. for C₁₈H₁₆BrN₂O₂ (MH⁺) 371.0390, found 371.0383.

Methyl 4-(1-benzyl-1*H*-pyrazol-5-yl)-3-ethynylbenzoate (27). To the mixture of **26** (74 mg, 0.20 mmol), PdCl₂(PhCN)₂ (7.7 mg, 0.020 mmol), CuI (3.8 mg, 0.020 mmol) in toluene (0.5 cm³) were added *i*-Pr₂NH (0.084 cm³, 0.60 mmol) and TMS-acetylene (0.055 cm³, 0.40 mmol), and a solution of *t*-Bu₃P in toluene (1.0 M, 0.040 cm³) under argon atmosphere. After stirring at 20 °C for 4.5 h, the mixture was diluted with Et₂O. The resulting mixture was washed with H₂O and brine, dried over Na₂SO₄, and concentrated in vacuo. The residue was chromatographed on silica gel (hexane/EtOAc = 4/1) to afford methyl 4-(1-benzyl-1*H*-pyrazol-5-yl)-3-[(trimethylsilyl)ethynyl]benzoate (73 mg, containing inseparable impurities, <94%) as a yellow oil. This crude product was used for the next reaction without further purifications. δ_{H} (400 MHz; CDCl₃; Me₄Si) 0.16 (9H, s), 3.94 (3H, s), 5.27 (2H, s), 6.40 (1H, d, *J* 2.0 Hz), 6.91–6.93 (2H, m), 7.18–7.24 (4H, m), 7.60 (1H, d, *J* 1.7 Hz), 7.92 (1H, dd, *J* 8.0, 1.7 Hz), and 8.21 (1H, d, *J* 1.7 Hz).

The coupling product (73 mg) was dissolved in MeOH (2.0 cm³). After addition of K₂CO₃ (52 mg, 0.38 mmol), the mixture was stirred at rt for 1 h, then diluted with H₂O and extracted with

EtOAc. The extract was washed with brine, dried over Na₂SO₄, and concentrated in vacuo. The residue was chromatographed on silica gel (hexane/EtOAc = 4/1 to 2/3) to afford the title compound **27** (29.1 mg, 46%) as a yellow oil: IR (neat): $\nu_{\text{max}}/\text{cm}^{-1}$ 3289 (C≡CH), 1726 (C=O), 1295 (OCH₃); δ_{H} (400 MHz; CDCl₃; Me₄Si) 3.13 (1H, s), 3.94 (3H, s), 5.28 (2H, s), 6.42 (1H, d, *J* 2.0 Hz), 6.92–6.94 (2H, m), 7.18–7.26 (4H, m), 7.62 (1H, d, *J* 2.0 Hz), 7.96 (1H, dd, *J* 8.0, 2.0 Hz), and 8.26 (1H, d, *J* 1.7 Hz); δ_{C} (100 MHz; CDCl₃) 52.4, 53.9, 80.7, 82.1, 107.9, 122.9, 127.1 (2C), 127.5, 128.5 (2C), 129.5, 130.6, 130.7, 134.4, 137.0, 137.8, 139.0, 140.7, and 165.7; HRMS (FAB) *m/z* Calc. for C₂₀H₁₇N₂O₂ (MH⁺) 317.1285, found 317.1290.

Methyl 1-benzyl-1*H*-benzo[*g*]indazole-7-carboxylate (21g). Under argon atmosphere, the mixture of **27** (29 mg, 0.092 mmol), IPrAuCl (2.9 mg, 4.6 μmol), and AgNTf₂ (1.8 mg, 4.6 μmol) in xylene was stirred at 130 °C for 4 h. The resulting mixture was filtered and concentrated in vacuo. The residue was chromatographed on silica gel (hexane/EtOAc = 4/1) to afford the title compound **21g** (11.0 mg, 38%) as a white solid: mp 163–164 °C; IR (neat): $\nu_{\text{max}}/\text{cm}^{-1}$ 1715 (C=O), 1284 (OCH₃); δ_{H} (500 MHz; CDCl₃; Me₄Si) 3.96 (3H, s), 6.11 (2H, s), 7.09 (2H, d, *J* 7.4 Hz), 7.23–7.30 (3H, m), 7.60 (1H, d, *J* 9.2 Hz), 7.78 (1H, d, *J* 9.2 Hz), 8.05 (1H, dd, *J* 8.6, 1.7 Hz), 8.18 (1H, s), 8.20 (1H, s), and 8.65 (1H, d, *J* 1.7 Hz); δ_{C} (125 MHz; CDCl₃) 52.2, 56.3, 120.2, 122.1, 123.1, 123.6, 125.8, 126.1, 127.2, 128.8, 131.4, 131.5, 132.6, 133.9 (2C), 134.2 (2C), 135.3, 136.6, and 166.9; HRMS (FAB) *m/z* Calc. for C₂₀H₁₇N₂O₂ (MH⁺) 317.1285, found 317.1290.

1*H*-Benzo[*g*]indazole-7-carboxylic acid (6g). Under oxygen atmosphere, **21g** (11 mg, 0.035 mmol) and DMSO (0.025 cm³, 0.35 mmol) were dissolved in THF (0.35 cm³) and the mixture was cooled to 0 °C. To the mixture was added *t*-BuOK (27 mg, 0.24 mmol) and the resulting mixture was stirred for 15 min. After addition of H₂O (0.1 cm³), the mixture was stirred at 0 °C for 0.5 h and at rt for 0.5 h. The mixture was diluted with H₂O (*ca.* 0.5 cm³) and NH₄Cl (28 mg, 0.52 mmol) was added to it. After stirring at rt for 1 h, the mixture was freeze dried and the residue was purified by reverse

phase HPLC [3–6% MeCN in H₂O (containing 0.1% NH₃); flow rate: 10 cm³ min⁻¹] to afford the title compound **6g** as an NH₃ salt (3.3 mg, 41%). White solid: mp >300 °C; IR (neat): $\nu_{\max}/\text{cm}^{-1}$ 1689 (C=O); δ_{H} (400 MHz; DMSO-*d*₆; Me₄Si) 7.60 (1H, d, *J* 8.6 Hz), 7.76 (1H, d, *J* 8.6 Hz), 8.14 (1H, d, *J* 8.6 Hz), 8.17 (1H, s), 8.44 (1H, d, *J* 8.6 Hz), and 8.57 (1H, s); δ_{C} (125 MHz; DMSO-*d*₆) 119.5, 119.7, 121.4, 122.3, 123.0, 126.6, 129.9, 130.6, 131.1, 132.8, 133.9, and 168.0; HRMS (FAB) *m/z* Calc. for C₁₂H₇N₂O₂ [M – H]⁻ 211.0513, found 211.0523.

Molecular modeling study of CK2 α complexes with benzo[*g*]indazole **6a**, **6f**, and **6g**

Complex structures of CK2 α with **6a**, **6f**, and **6g** were modeled manually based on the crystal structure of CK2 α with **3a** using MOE. Each model was subjected to energy minimization applying the MMFF force field, and then the binding affinity was estimated by the MM/GBVI method.

CK2 kinase assay

CK2 α or CK2 α' kinase reaction was done in a reaction buffer [0.015 cm³; 20 mM MOPS pH 7.2, 25 mM β -glycerol phosphate, 5 mM EGTA, 1 mM sodium orthovanadate, 13.5 mM MgCl₂, 0.4 μ M PKA inhibitor cocktail, 0.2 mM CK2 substrate peptide (RRRDDDSDDD), 8.5 mU CK2 α enzyme, 90 μ M ATP, 2.2 nM [γ -³²P] ATP]. After incubation at 37 °C for 10 min, the reaction was terminated by the addition of 0.010 cm³ of 40% TCA. 0.005 cm³ of the solution was transferred onto a 96-well P81 UniFilter (Whatman), and each well was washed with 0.20 cm³ of 0.75% phosphoric acid solution 20 times. Residual radioactivity was measured using TOP count NXT (PerkinElmer) after 30-min incubation in 0.020 cm³ of Microscinti-0 (PerkinElmer).

Growth inhibition assay

HCT-116 cells were cultured in McCoy's 5A medium (GIBCO), supplemented with 10% (v/v) FBS at 37 °C in a 5% CO₂ incubator. Growth inhibition assays using HCT-116 cells were performed in 96-well plates (BD Falcon). HCT-116 cells were seeded at 5000 cells/well in 0.050 cm³ of culture

media and placed for 6 hr. Chemicals in DMSO were diluted 200-fold with the culture medium in advance. Following the addition of 0.040 cm³ of the fresh culture medium, 0.030 cm³ of the chemical diluents were also added to the cell cultures. The final volume of DMSO in the medium was equal to 0.125% (v/v). The cells under chemical treatment were incubated for a further 72 hr. The wells in the plates were washed twice with Phenol-red minus medium [McCoy's 5A medium (Thermo Scientific)]. After 1 hour incubation with 0.10 cm³ of the medium, the cell culture in each well was supplemented with 0.020 cm³ of the MTS reagent (Promega), followed by incubation for an additional 40 min. Absorbance at 490 nm of each well was measured using a Wallac 1420 ARVO SX multilabel counter (Perkin Elmer).

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References and notes

1. J. S. Duncan and D. W. Litchfield, *Biochim. Biophys. Acta*, 2008, **1784**, 33–47.
2. (a) J. W. Critchfield, J. E. Coligan, T. M. Folks and S. T. Butera, *Proc. Natl. Acad. Sci. U.S.A.*, 1997, **94**, 6110–6115; (b) H. L. Yim, Y. H. Lee, C. H. Lee and S. K. Lee, *Planta Med.*, 1999, **65**, 9–13; (c) E. Vangrevelinghe, K. Zimmermann, J. Schoepfer, R. Portmann, D. Fabbro and P. Furet, *J. Med. Chem.*, 2003, **46**, 2656–2662; (d) Z. Nie, C. Perretta, P. Erickson, S. Margosiak, R. Almassy, J. Lu, A. Averill, K. M. Yager and S. Chu, *Bioorg. Med. Chem. Lett.*, 2007, **17**, 4191–4195; (e) A. Chilin, R. Battistutta, A. Bortolato, G. Cozza, S. Zanatta, G. Poletto, M. Mazzorana, G. Zagotto, E. Uriarte, A. Guiotto, L. A. Pinna, F. Meggio and S. Moro, *J. Med.*

Chem., 2008, **51**, 752–759.

3. F. J. Lozeman, D. W. Litchfield, C. Pienning, K. Takio, K. A. Walsh and E. D. Krebs, *Biochemistry*, 1990, **29**, 8436–8447.
4. (a) F. Pierre, P. C. Chua, S. E. O'Brien, A. Siddiqui-Jain, P. Bourbon, M. Haddach, J. Michaux, J. Nagasawa, M. K. Schwaebe, E. Stefan, A. Vialettes, J. P. Whitten, K. T. Chen, L. Darjania, R. Stansfield, K. Anderes, J. Bliesath, D. Drygin, C. Ho, M. Omori, C. Proffitt, N. Streiner, K. Trent, W. G. Rice and D. M. Ryckman, *J. Med. Chem.*, 2011, **54**, 635–654; (b) R. Battistutta, G. Cozza, F. Pierre, E. Papinutto, G. Lolli, S. Sarno, S. E. O'Brien, A. Siddiqui-Jain, M. Haddach, K. Anderes, D. M. Ryckman, F. Meggio and L. A. Pinna, *Biochemistry*, 2011, **50**, 8478–8488.
5. (a) Y. Suzuki, J. Cluzeau, T. Hara, A. Hirasawa, G. Tsujimoto, S. Oishi, H. Ohno and N. Fujii, *Arch. Pharm.*, 2008, **341**, 554–561; for the initial report of pyrazine-based CK2 inhibitors, see: (b) N. Fuchi, Y. Iura, H. Kaneko, M. Yamada and Y. Sekitani, *Jpn. Pat.*, 145786, 2007.
6. (a) T. Nakaniwa, T. Kinoshita, Y. Sekiguchi, T. Tada, I. Nakanishi, K. Kitaura, Y. Suzuki, H. Ohno, A. Hirasawa and G. Tsujimoto, *Acta Crystallogr. Sect. F*, 2009, **65**, 75–79; (b) T. Kinoshita, Y. Sekiguchi, H. Fukada, T. Nakaniwa, T. Tada, S. Nakamura, K. Kitaura, H. Ohno, Y. Suzuki, A. Hirasawa, I. Nakanishi and G. Tsujimoto, *Mol. Cell. Biochem.*, 2011, **356**, 97–105.
7. Z. Hou, I. Nakanishi, T. Kinoshita, M. Yasue, R. Misu, Y. Suzuki, S. Nakamura, T. Kure, H. Ohno, K. Murata, K. Kitaura, A. Hirasawa, G. Tsujimoto, S. Oishi and N. Fujii, *J. Med. Chem.*, 2012, **55**, 2899–2903.
8. Lopez *et al.* have determined the tautomeric structure of various pyrazoles and indazoles by ¹³C NMR analysis, see: C. Lopez, R. M. Claramunt, A. Trofimenko and J. Elguero, *Can. J. Chem.*, 1993, **71**, 678–684.
9. (a) Y. Suzuki, Y. Ohta, S. Oishi, N. Fujii and H. Ohno, *J. Org. Chem.*, 2009, **74**, 4246–4251; (b) Y. Suzuki, S. Naoe, S. Oishi, N. Fujii and H. Ohno, *Org. Lett.*, 2012, **14**, 326–329.
10. J. C. Antilla, J. M. Baskin, T. E. Barder and S. L. Buchwald, *J. Org. Chem.*, 2004, **69**,

5578–5587.

11. T. Hundertmark, A. F. Littke, S. L. Buchwald and G. C. Fu, *Org. Lett.*, 2000, **2**, 1729–1731.
12. A. A. Haddach, A. Kelleman and M. V. Deaton-Rewolinski, *Tetrahedron Lett.*, 2002, **43**, 399–402.
13. R. Joyeau, L. D. S. Yadav and M. Wakselman, *J. Chem. Soc., Perkin Trans. 1*, 1987, 1899–1907.
14. K. Knepper, S. Vanderheiden and S. Bräse, *Eur. J. Org. Chem.*, 2006, 1886–1898.
15. J. García-Fortanet, S. L. Buchwald, *Angew. Chem., Int. Ed.*, 2008, **47**, 8108–8111.
16. T. Kawase, A. Konishi, Y. Hirao, K. Matsumoto, H. Kurata and T. Kubo, *Chem. Eur. J.*, 2009, **15**, 2653–2661.
17. R. Wu, J. S. Schumm, D. L. Pearson and J. M. Tour, *J. Org. Chem.*, 1996, **61**, 6906–6921.

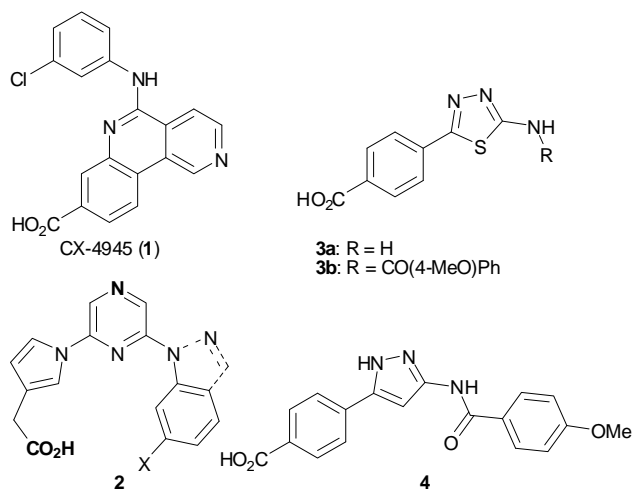


Figure 1. Structures of reported CK2 inhibitors

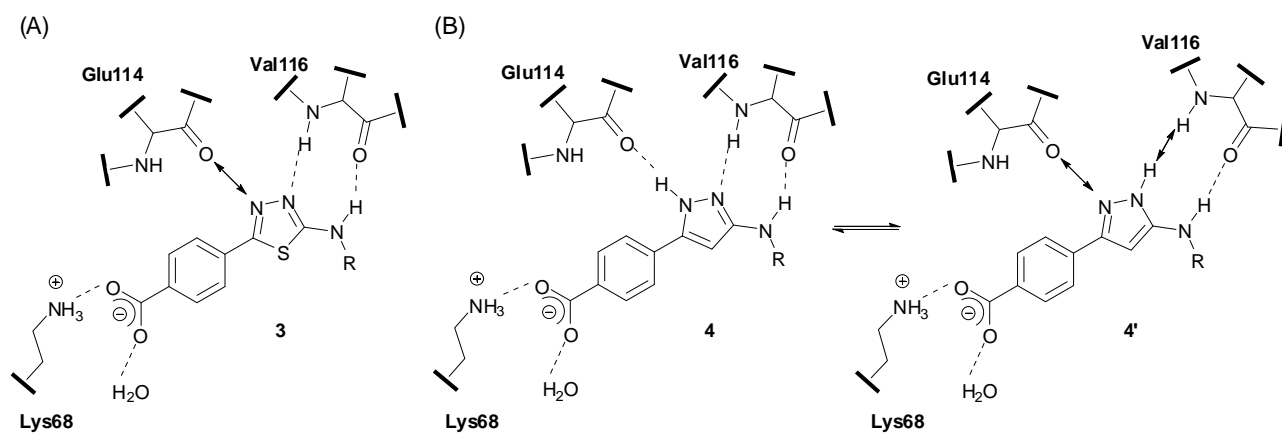


Figure 2. Plausible binding mode of thiaziazole **3** (A) and pyrazole **4** (B) with CK2 α (R = 4-methoxybenzoyl)

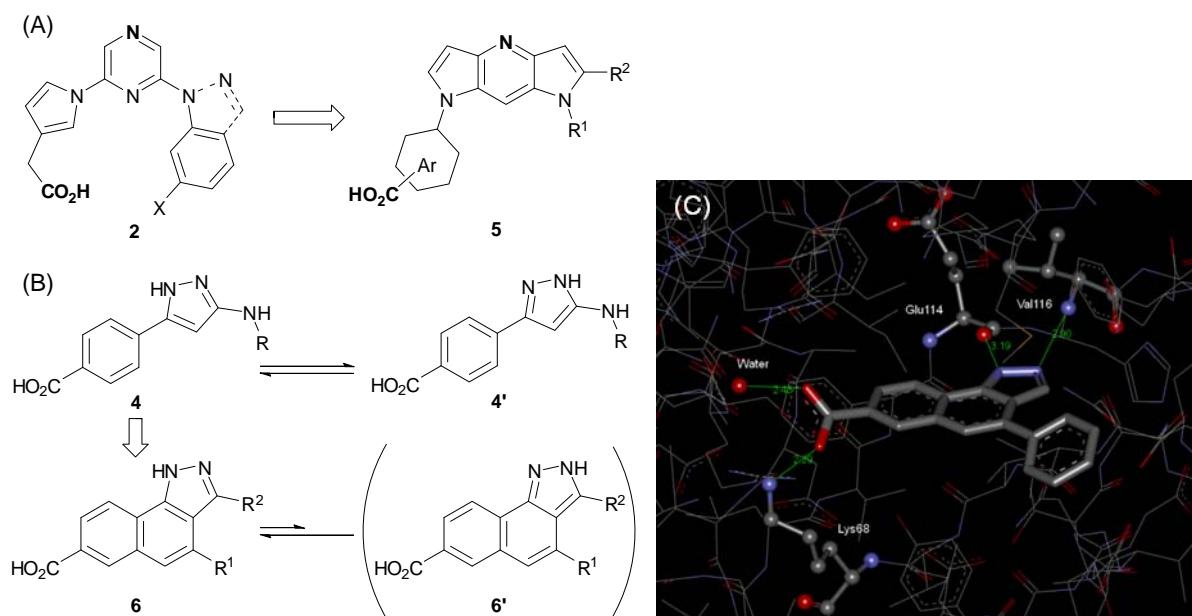


Figure 3. (A) Pyrazine-based CK2 inhibitors **2** and dipyrrolo[3,2-*b*:2',3'-*e*]pyridines **5**; (B) pyrazole-based CK2 inhibitor **4**, benzo[*g*]indazoles **6**, and their tautomers **4'** and **6'**; and (C) simulated binding mode of **6a** with CK2 α .

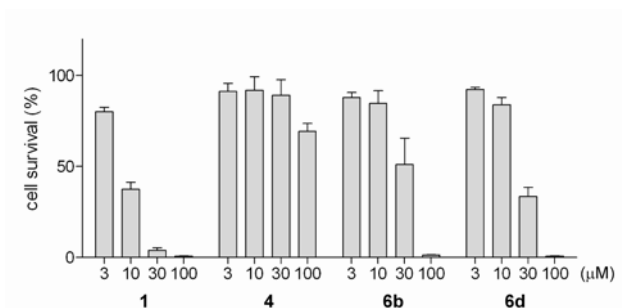
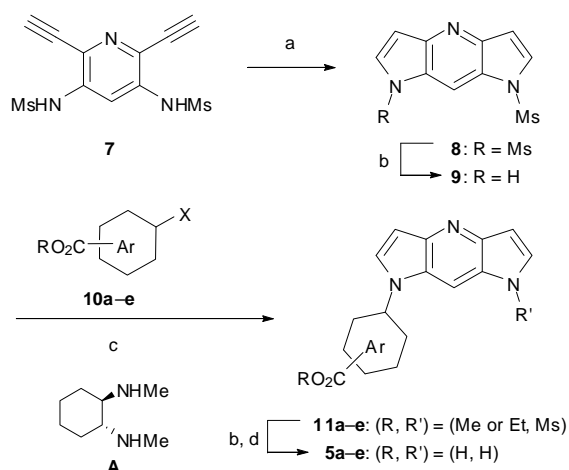
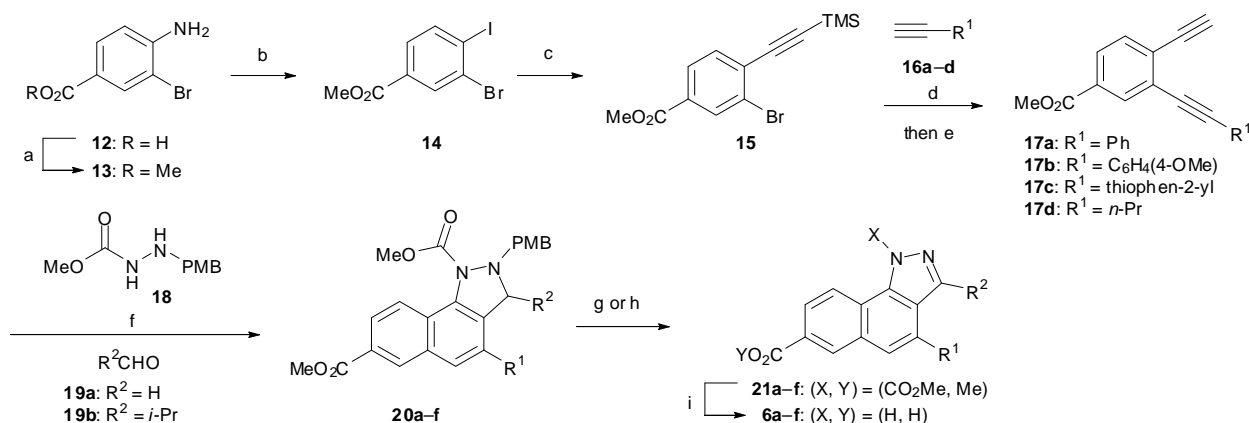


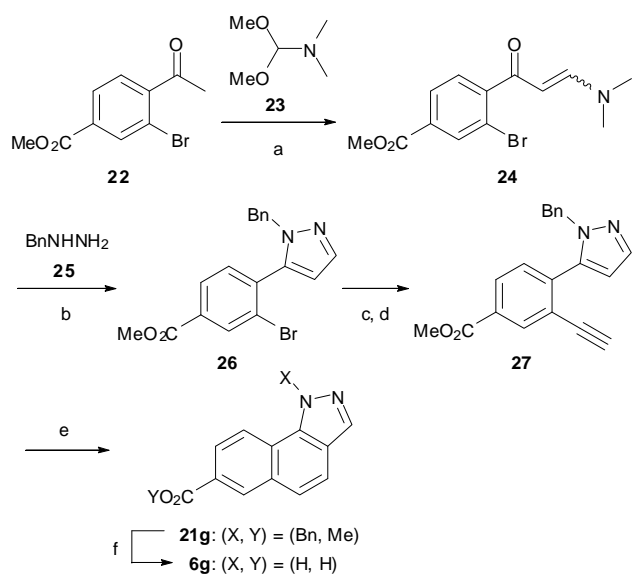
Figure 4. Inhibitory effects on cell proliferation of CK2 inhibitors toward HCT-116



Scheme 1. Synthesis of dipyrrolo[3,2-*b*:2',3'-*e*]pyridine derivatives **5a–e**. Reagents and conditions: (a) cat. CuI, Et₃N, 1,4-dioxane, 60 °C, 5 min; (b) Cs₂CO₃, MeOH/THF, rt, 12 h; (c) cat. CuI/(±)-**A**, K₃PO₄, 1,4-dioxane, reflux; (d) 3N NaOH, THF, rt, 40 h.

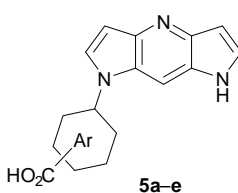
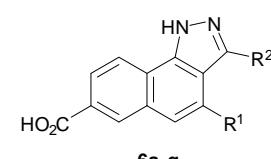
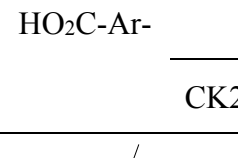
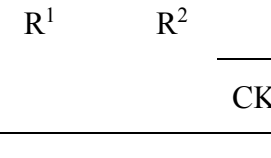
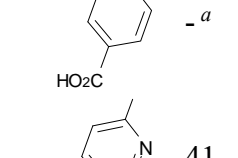
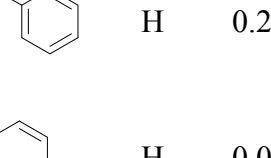
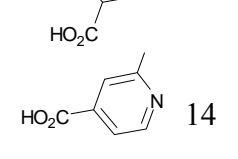
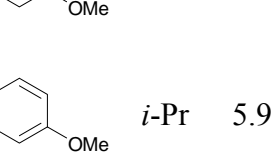
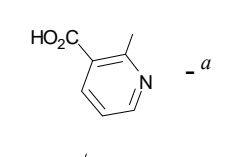
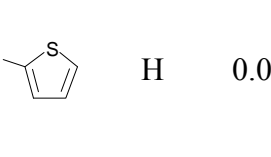


Scheme 2. Synthesis of benzo[*g*]indazole derivatives **6a–f** via three-component annulation and cyclization cascade. Reagents and conditions: (a) H₂SO₄, MeOH, reflux, 20 h; (b) NaNO₂, HCl, H₂O, 0 °C, 1 h, then KI, rt, 24 h; (c) TMS-acetylene, cat. PdCl₂(PPh₃)₂/CuI, Et₃N, THF, rt, 1.5 h; (d) cat. PdCl₂(PhCN)₂/*t*-Bu₃P/CuI, *i*-Pr₂NH, toluene, rt; (e) K₂CO₃, MeOH, rt, 1 h; (f) cat. IPrAuCl/AgOTf, AcOH, 35–80 °C; (g) anisole, TFA, 80 °C (for **21a**, **21b**, **21d** and **21f**); (h) DDQ, CH₂Cl₂/H₂O, rt (for **21c** and **21e**); (i) 3N NaOH, 1,4-dioxane, 40 °C.



Scheme 3. Synthesis of benzo[*g*]indazole derivative **6g**. Reagents and conditions: (a) 85 °C, 8 h; (b) AcONa, AcOH, 60 °C, 5 h; (c) TMS-acetylene, cat. PdCl₂(PPh₃)₂/CuI, Et₃N, THF, rt, 4.5 h; (d) K₂CO₃, MeOH, rt, 1 h; (e) cat. IPrAuCl/AgNTf₂, xylene, 130 °C; (f) *t*-BuOK, DMSO, THF/H₂O, O₂, rt, 0.5 h.

Table 1. Structures of the synthesized heterocyclic compounds and the CK2 inhibitory activities

compd	HO ₂ C-Ar-	IC ₅₀ (μM)		compd	R ¹	R ²	IC ₅₀ (μM)	
		CK2α	CK2α'				CK2α	CK2α'
5a		- ^a	- ^a	6a		H	0.20	0.12
5b		41	26	6b		H	0.089	0.067
5c		14	12	6c		<i>i</i> -Pr	5.97	7.56
5d		- ^a	- ^a	6d		H	0.040	0.042
5e		- ^a	- ^a	6e		<i>i</i> -Pr	3.36	2.52
1 (CX-4945)	-	0.014	0.014	6f	<i>n</i> -Pr	H	0.82	0.49
4	-	0.14	0.063	6g	H	H	2.2	1.0

^a Less than 20% inhibition was observed in the presence of 32 μM of compounds.