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PAPER

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

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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 (CK2α and/or CK2α′) and two regulatory subunits (CK2β).[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 (Fig. 1).[4]

We have also carried out structure–activity relationship (SAR) studies[5] and crystallographic analyses[6] of pyrazine-based CK2 inhibitors **2**[5b] (Fig. 1) to obtain structural information for the design of novel CK2 inhibitors. These inhibitors **2** bind to CK2α 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 CK2α, 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.

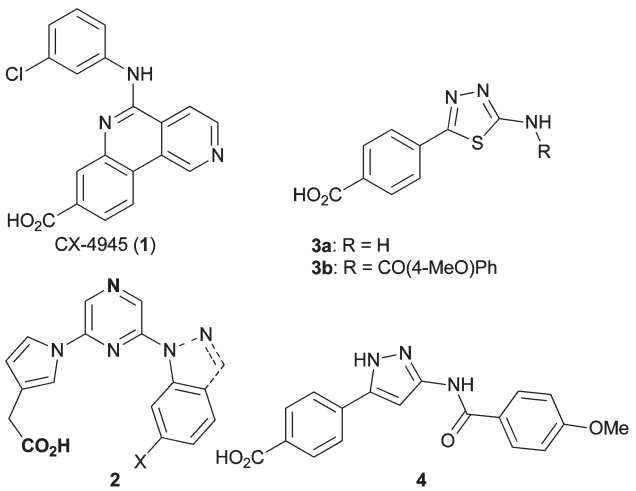

Fig. 1 Structures of reported CK2 inhibitors.

We recently identified 2-phenyl-1,3,4-thiadiazoles **3** and 3-phenyl-1,2-pyrazole derivatives **4** as CK2 inhibitors, led by virtual screening of a compound library (Fig. 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 CK2α-Glu114 (Fig. 2A). The pyrazole analogue **4**, designed by replacement of the thiadiazole ring in **3b** [IC_{50} = 3.4 μM (CK2α) and 1.2 μM (CK2α′)] 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

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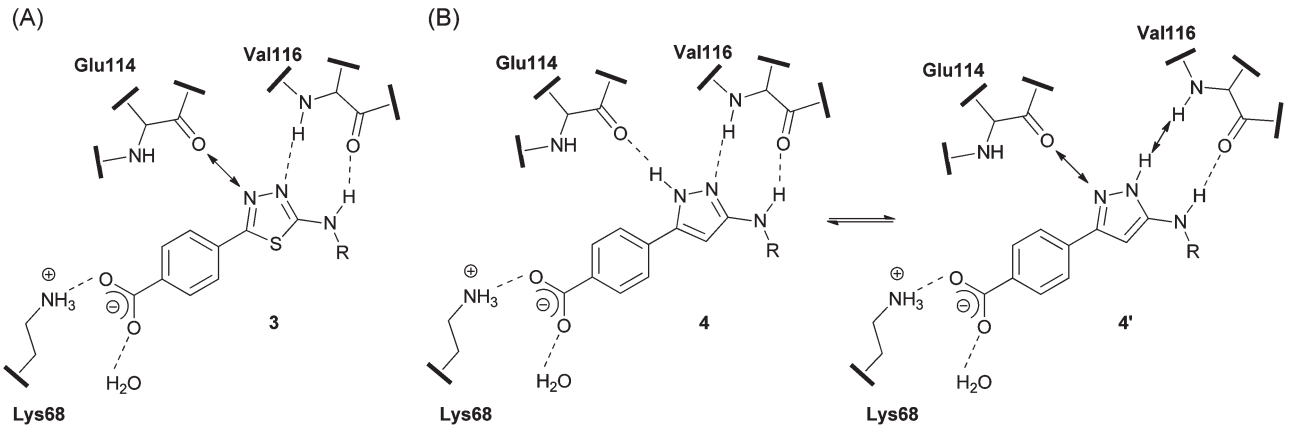


Fig. 2 Plausible binding mode of thiadiazole **3** (A) and pyrazole **4** (B) with CK2α (R = 4-methoxybenzoyl).

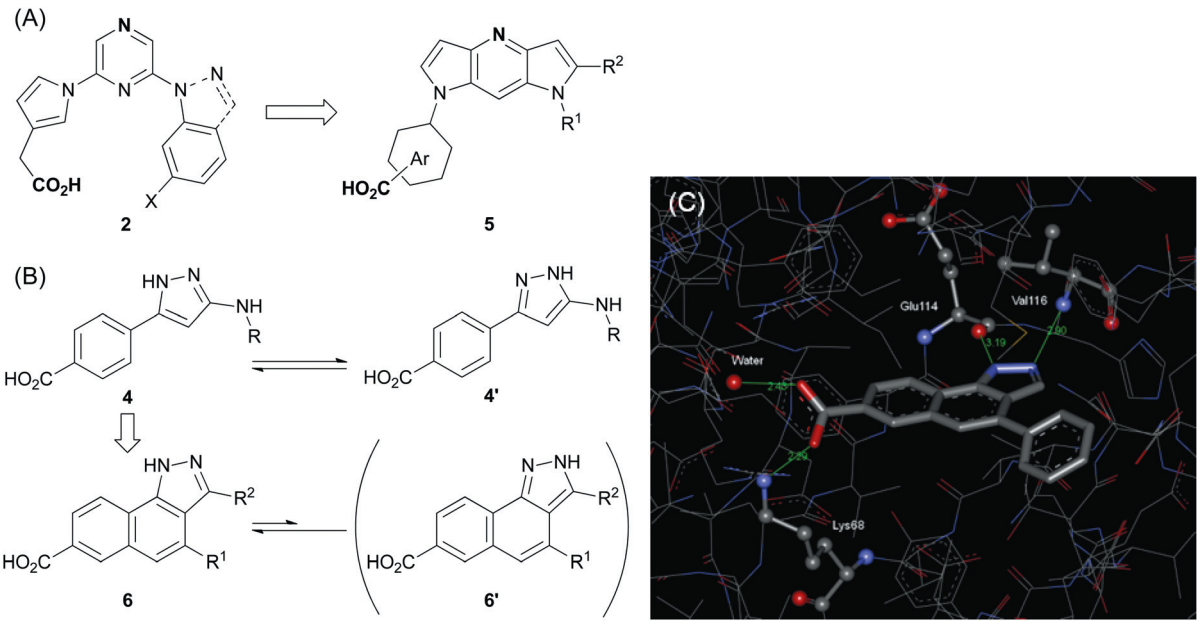


Fig. 3 (A) Pyrazine-based CK2 inhibitors **2** and dipyrrolo[3,2-*b*:2′,3′-*e*]pyridines **5**; (B) pyrazole-based CK2 inhibitor **4**, benzo[*g*]indazoles **6**, and their tautomers **4′** and **6′**; and (C) simulated binding mode of **6a** with CK2α.

(Fig. 2B). The pyrazole-type inhibitor **4** showed more potent inhibitory activity toward CK2 [IC_{50} = 0.14 μM (CK2α) and 0.063 μM (CK2α′)] than the thiadiazole **3b** did. Because the other tautomer, **4′**, may have unfavourable electrostatic repulsions with the binding region (Fig. 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 (CK2α and CK2α′) and anti-proliferative activity of the potent inhibitors are also presented.

Results and discussion

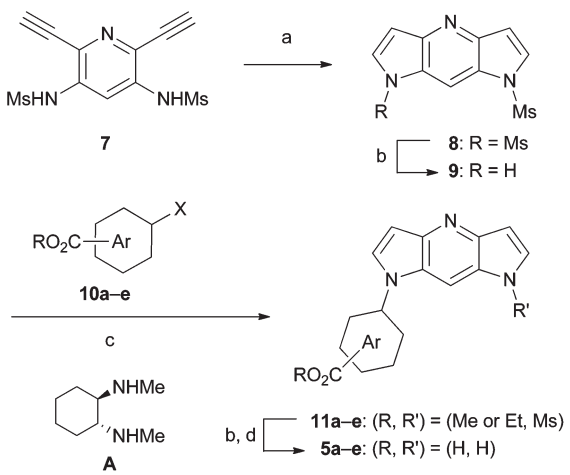
Design

On the basis of our previous SAR studies, we designed two fused nitrogen heterocycles **5** and **6** as novel CK2 inhibitor candidates (Fig. 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 (Fig. 3A).[5a,6] The benzo[*g*]indazole derivatives **6** also have a highly fused and planar structure (Fig. 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 CK2α–**3a** co-crystal structure suggested that the carboxyl group and two indazole nitrogens could form favourable interactions with CK2α (Fig. 3C).

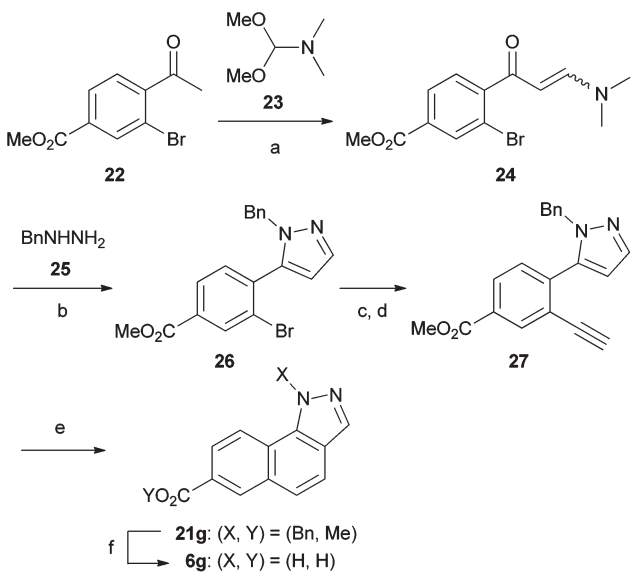
Synthesis

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 Cs_2CO_3 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**.

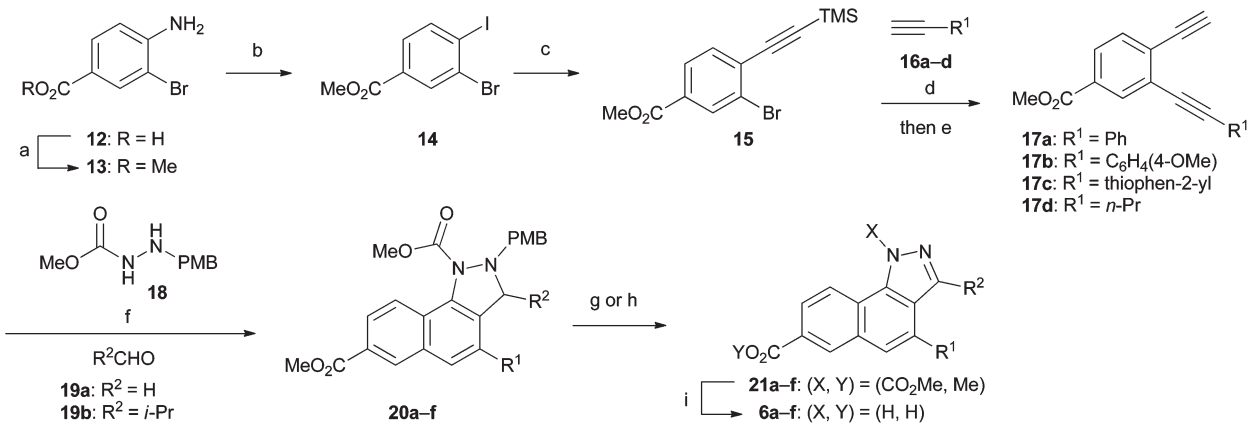
Benzo[*g*]indazole derivatives **6a–f** were prepared *via* a gold-catalysed three-component annulation and cyclization cascade[9b] as a key step (Scheme 2). 4-Amino-3-bromobenzoic acid (**12**) was converted to the methyl ester **13** under acidic conditions. A Sandmeyer reaction using potassium iodide, 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**.



Scheme 1 Synthesis of dipyrrolo[3,2-*b*:2′,3′-*e*]pyridine derivatives **5a–e**. Reagents and conditions: (a) cat. CuI, Et_3N, 1,4-dioxane, 60 °C, 5 min; (b) Cs_2CO_3, MeOH/THF, rt, 12 h; (c) cat. CuI/(±)-**A**, K_3PO_4, 1,4-dioxane, reflux; (d) 3 N NaOH, THF, rt, 40 h.



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. $PdCl_2(PPh_3)_2$/CuI, Et_3N, THF, rt, 4.5 h; (d) K_2CO_3, MeOH, rt, 1 h; (e) cat. IPrAuCl/$AgNTf_2$, xylene, 130 °C; (f) *t*-BuOK, DMSO, THF/H_2O, O_2, rt, 0.5 h.



Scheme 2 Synthesis of benzo[*g*]indazole derivatives **6a–f** *via* three-component annulation and cyclization cascade. Reagents and conditions: (a) H_2SO_4, MeOH, reflux, 20 h; (b) $NaNO_2$, HCl, H_2O, 0 °C, 1 h, then KI, rt, 24 h; (c) TMS-acetylene, cat. $PdCl_2(PPh_3)_2$/CuI, Et_3N, THF, rt, 1.5 h; (d) cat. $PdCl_2(PhCN)_2$/*t*-Bu_3P/CuI, *i*-Pr_2NH, toluene, rt; (e) K_2CO_3, 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, CH_2Cl_2/H_2O, rt (for **21c** and **21e**); (i) 3 N NaOH, 1,4-dioxane, 40 °C.

Finally, full deprotection of the methyl carbamate and ester yielded the desired compounds **6a–f**.

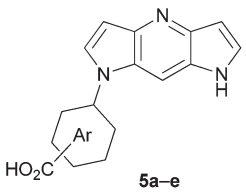
The 3,4-unsubstituted congener **6g** (R^1 and R^2 = H) was prepared *via* a different synthetic route (Scheme 3), because of difficulties in using **17** bearing two terminal alkynes (R^1 = H) for the three-component annulation. The 5-aryl-1-benzylpyrazole derivative **26** was prepared according to 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 ($AgNTf_2$) 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 group[12] and methyl ester yielded the desired compound **6g**.

Evaluation of CK2 inhibitory activity

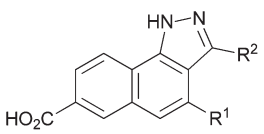
The inhibitory activities of the synthesized analogues toward two subtypes of the catalytic subunit of CK2 (CK2α and CK2α′) are summarised in Table 1. Although the dipyrrolo[3,2-*b*:2′,3′-*e*]-pyridine derivative bearing a benzoic acid, **5a**, had significantly less potent activity, its pyridine congener **5b** showed moderate activity toward both CK2α and CK2α′ (IC_{50} = 41 μM and 26 μM, 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 CK2α (IC_{50} = 14 μM) and CK2α′ (IC_{50} = 12 μM) 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 (Fig. 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 R^2 reduced the binding affinity 100-fold relative to those of the unsubstituted analogues (compare **6b** with **6c**, and **6d** with **6e**). On the other

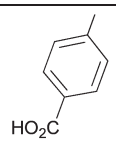
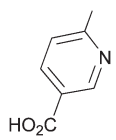
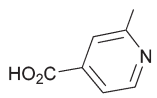
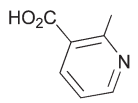
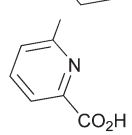
Table 1 Structures of the synthesized heterocyclic compounds and the CK2 inhibitory activities

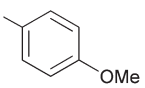
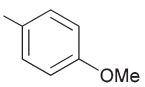


5a–e



6a–g

Compd	HO_2C-Ar-	IC_{50} (μM) CK2α	CK2α′
5a		—[a]	—[a]
5b		41	26
5c		14	12
5d		—[a]	—[a]
5e		—[a]	—[a]
1 (CX-4945)	—	0.014	0.014
4	—	0.14	0.063

Compd	R^1	R^2	IC_{50} (μM) CK2α	CK2α′
6a		H	0.20	0.12
6b		H	0.089	0.067
6c		*i*-Pr	5.97	7.56
6d		H	0.040	0.042
6e		*i*-Pr	3.36	2.52
6f	*n*-Pr	H	0.82	0.49
6g	H	H	2.2	1.0

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

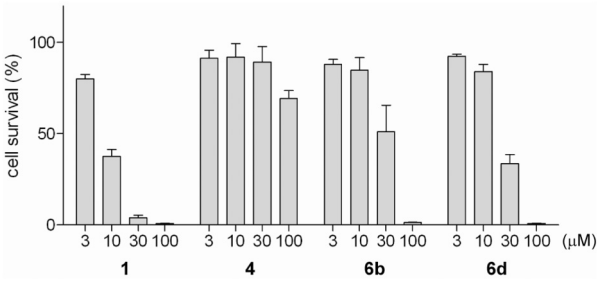


Fig. 4 Inhibitory effects on cell proliferation of CK2 inhibitors toward HCT-116.

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^{-1} 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 (Fig. 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)-2*H*-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 CK2α and CK2α′.

Experimental section

General

Melting points were measured by a hot stage melting point apparatus (uncorrected). ^{1}H 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 an 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. ^{1}H 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 cm^3) was added Cs_2CO_3 (2.18 g, 6.70 mmol). After stirring at rt for 12 h, NH_4Cl (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 $CHCl_3$. After filtration, the mixture was concentrated and the residue was chromatographed on silica gel ($CHCl_3$–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; $CDCl_3$; Me_4Si) 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; $CDCl_3$) 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. Calcd for $C_{10}H_9N_3O_2S$: 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(7*H*)-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), (±)-*trans*-*N,N*′-dimethylcyclohexane-1,2-diamine **A** (0.007 cm^3, 0.042 mmol), and K_3PO_4 (113 mg, 0.53 mmol) in 1,4-dioxane (1 cm^3) 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): ν_{max}/cm^{-1} 1698 (C═O), 1278 (OCH_3); δ_H (400 MHz; $CDCl_3$; Me_4Si) 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; $CDCl_3$) 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* Calcd for $C_{19}H_{18}N_3O_4S$ (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 cm^3) was added Cs_2CO_3 (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 NH_4Cl and the mixture was extracted with EtOAc twice. The combined extracts were washed with brine, dried over Na_2SO_4, and concentrated *in vacuo*. The crude product was dissolved with THF (3 cm^3) and 3 N NaOH (0.3 cm^3) was added. The mixture was stirred at rt for 40 h and concentrated *in vacuo*. The

residue was dissolved in water (*ca.* 1 cm^3) and filtered. The filtrate was acidified with 3 N HCl, then 28% aqueous NH_3 was added until the mixture was a clear solution. The solution was freeze dried and the residue was purified by reverse phase HPLC [3–6% MeCN in H_2O (containing 0.1% NH_3); flow rate: 10 cm^3 min^{-1}] to afford the title compound **5a** as an NH_3 salt (24.9 mg, 54%). Pale yellow solid: mp >300 °C; IR (neat): ν_{max}/cm^{-1} 1602 (C═O); δ_H (500 MHz; DMSO-d_6; Me_4Si) 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* Calcd 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 cm^3) was added sulphuric acid (0.83 cm^3), then the mixture was refluxed for 20 h. The mixture was cooled to 0 °C and basified with sat. aqueous $NaHCO_3$ (*ca.* 100 cm^3). The precipitates were collected and washed with water. Recrystallization from MeOH–H_2O 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 6 N HCl (40 cm^3) was added a solution of $NaNO_2$ (3.0 g, 43.4 mmol) in H_2O (20 cm^3) 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 H_2O (16 cm^3) 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 H_2O. The solid was dissolved in Et_2O, then the mixture was washed with aqueous $Na_2S_2O_3$, H_2O, and brine, dried over $MgSO_4$, 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, Et_3N (7.3 cm^3, 53 mmol) and TMS-acetylene (1.8 cm^3, 12.7 mmol) were added to the mixture of **14** (3.6 g, 10.6 mmol), $PdCl_2(PPh_3)_2$ (190 mg, 0.27 mmol) and CuI (50 mg, 0.27 mmol) in THF (35 cm^3), then the mixture was stirred at rt for 12 h. The mixture was concentrated *in vacuo* and the residue was diluted with Et_2O 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 cm^3). To the solution were added $PdCl_2(PhCN)_2$ (35 mg, 0.09 mmol), CuI (11 mg, 0.06 mmol), *i*-Pr_2NH (1.3 cm^3, 9.03 mmol), 2-ethynylthiophene (**16c**),[17] and a solution of *t*-Bu_3P in toluene (1.0 M, 0.18 cm^3) 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): ν_{max}/cm^{-1} 2210, 2159 (C≡C), 1246 (OCH_3); δ_H (400 MHz; $CDCl_3$; Me_4Si) 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; $CDCl_3$) 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* Calcd for $C_{19}H_{19}O_2SSi$ (MH^+) 339.0870, found 339.0874.

The coupling product (475 mg, 1.40 mmol) was dissolved in MeOH (14 cm^3). After addition of K_2CO_3 (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 Et_2O. The extract was washed with H_2O and brine, dried over $MgSO_4$, and concentrated *in vacuo*. The residue was chromatographed on silica gel (hexane–EtOAc = 9 : 1) followed by recrystallization from $CHCl_3$–hexane to afford the title compound **17c** (259 mg, 70%) as pale yellow crystals: mp 75–76 °C; IR (neat): ν_{max}/cm^{-1} 3246 (C≡CH), 2195 (C≡C), 1717 (C═O), 1244 (OCH_3); δ_H (500 MHz; $CDCl_3$; Me_4Si) 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; $CDCl_3$) 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. Calcd for $C_{16}H_{10}O_2S$: 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 cm^3, 122 mmol) in Et_2O (400 cm^3) was stirred at 35 °C for 15 h. The reaction mixture was concentrated *in vacuo* until *ca.* 50 cm^3 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): ν_{max}/cm^{-1} 3252 (NH), 1712 (C═O), 1249 (OCH_3); δ_H (400 MHz; $CDCl_3$; Me_4Si) 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; $CDCl_3$) 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. Calcd for $C_{10}H_{12}N_2O_3$: 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 cm^3) 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): ν_{max}/cm^{-1} 3301 (NH), 1684 (C═O), 1251 (OCH_3); δ_H (400 MHz; $CDCl_3$; Me_4Si) 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; $CDCl_3$) 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. Calcd for $C_{10}H_{14}N_2O_3$: 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-1*H*-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 °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 stirring at 35 °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): ν_{max}/cm^{-1} 1716, 1609 (C═O), 1251 (OCH_3); δ_H (500 MHz; $CDCl_3$; Me_4Si, 50 °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* Calcd for $C_{30}H_{29}N_2O_6$ (MH^+) 513.2020, found 513.2011.

Dimethyl 3-isopropyl-2-(4-methoxybenzyl)-4-(thiophen-2-yl)-2,3-dihydro-1*H*-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 μL, 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 °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): ν_{max}/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; $CDCl_3$) 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* Calcd for $C_{30}H_{31}N_2O_5S$ (MH^+) 531.1948, found 531.1946.

Deprotection and aromatisation of 20: 4-phenyl-1*H*-benzo[*g*]-indazole-7-carboxylic acid (6a). Compound **20a** (20 mg, 0.042 mmol) and anisole (0.022 cm^3, 0.21 mmol) were dissolved in TFA (0.6 cm^3). After stirring at 80 °C for 12 h, the 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 crude product (**21a**) was dissolved in 1,4-dioxane (0.4 cm^3), then 3 N NaOH (0.083 cm^3, 0.25 mmol) was added. After stirring at 40 °C for 12 h, NH_4Cl (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 ($CHCl_3$–MeOH = 9 : 1) to afford the title compound **6a** (5.8 mg, 49%) as a white solid: mp >300 °C; IR (neat): ν_{max}/cm^{-1} 1703 (C═O); δ_H (500 MHz; DMSO-d_6; Me_4Si) 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* Calcd for $C_{18}H_{11}N_2O_2$ $[M - H]^-$ 287.0826, found 287.0820.

3-Isopropyl-4-(thiophen-2-yl)-1*H*-benzo[*g*]indazole-7-carboxylic acid (6e). The mixture of **20e** (65 mg, 0.12 mmol) and DDQ (83 mg, 0.37 mmol) in CH_2Cl_2–H_2O (5 : 1, 1.2 cm^3) was stirred at rt for 18 h. The resulting mixture was diluted with $CHCl_3$, washed with H_2O, and dried over Na_2SO_4. The solvent was removed *in vacuo* and the residue was chromatographed on silica gel ($CHCl_3$) to afford the title compound **21e** (36.8 mg, 73%) as a white solid: mp 135–136 °C; IR (neat): ν_{max}/cm^{-1} 1746, 1714 (C═O), 1262 (OCH_3); δ_H (500 MHz; $CDCl_3$; Me_4Si) 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; $CDCl_3$) 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* Calcd for $C_{22}H_{21}N_2O_4S$ (MH^+) 409.1217, found 409.1215.

The mixture of **21e** (30 mg, 0.073 mmol) and 3 N NaOH (15 cm^3, 0.44 mmol) in 1,4-dioxane was stirred at 40 °C for 12 h. After addition of NH_4Cl (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 ($CHCl_3$–MeOH = 9 : 1) to afford the title compound **6e** (19.9 mg, 81%) as a white solid: mp >300 °C; IR (neat): ν_{max}/cm^{-1} 1681 (C═O); δ_H (500 MHz; DMSO-d_6; Me_4Si) 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* Calcd for $C_{19}H_{15}N_2O_2S$ $[M - H]^-$ 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 cm^3, 129 mmol) was stirred at 85 °C for 8 h in an open vessel. After cooling to rt, the reaction mixture was concentrated *in vacuo* and the residue was recrystallized from EtOAc–Et_2O to afford the title compound **24** (5.77 g,

86%) as yellow crystals: mp 102–103 °C; IR (neat): ν_{max}/cm^{-1} 1719 (C═O), 1640 (C═O); δ_H (500 MHz; $CDCl_3$; Me_4Si) 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; $CDCl_3$) 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. Calcd for $C_{13}H_{14}BrN_2O_3$: 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), $BnNHNH_2$ (**25**) (0.22 cm^3, 2.0 mmol), and AcONa (205 mg, 2.5 mmol) in AcOH (1.0 cm^3) was stirred at 60 °C for 5 h. The mixture was diluted with H_2O and cooled on ice. To the mixture was added NaOH until the pH was about 12, then the resulting mixture was extracted with Et_2O twice. The combined extracts were washed with 1 N HCl, 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 **26** (248 mg, 67%) as a colourless oil: IR (neat): ν_{max}/cm^{-1} 1725 (C═O), 1283 (OCH_3); δ_H (400 MHz; $CDCl_3$; Me_4Si) 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; $CDCl_3$) 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* Calcd for $C_{18}H_{16}BrN_2O_2$ (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), $PdCl_2(PhCN)_2$ (7.7 mg, 0.020 mmol), CuI (3.8 mg, 0.020 mmol) in toluene (0.5 cm^3) were added *i*-Pr_2NH (0.084 cm^3, 0.60 mmol) and TMS-acetylene (0.055 cm^3, 0.40 mmol), and a solution of *t*-Bu_3P in toluene (1.0 M, 0.040 cm^3) under argon atmosphere. After stirring at 20 °C for 4.5 h, the mixture was diluted with Et_2O. The resulting mixture was 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 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 purification. δ_H (400 MHz; $CDCl_3$; Me_4Si) 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 cm^3). After addition of K_2CO_3 (52 mg, 0.38 mmol), the mixture was stirred at rt for 1 h, then diluted with H_2O and extracted with EtOAc. The extract was washed with brine, dried over Na_2SO_4, 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): ν_{max}/cm^{-1} 3289 (C≡CH), 1726 (C═O), 1295 (OCH_3); δ_H (400 MHz; $CDCl_3$; Me_4Si) 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; $CDCl_3$) 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* Calcd for $C_{20}H_{17}N_2O_2$ (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 $AgNTf_2$ (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): ν_{max}/cm^{-1} 1715 (C═O), 1284 (OCH_3); δ_H (500 MHz; $CDCl_3$; Me_4Si) 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; $CDCl_3$) 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* Calcd for $C_{20}H_{17}N_2O_2$ (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 cm^3, 0.35 mmol) were dissolved in THF (0.35 cm^3) 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 H_2O (0.1 cm^3), the mixture was stirred at 0 °C for 0.5 h and at rt for 0.5 h. The mixture was diluted with H_2O (*ca.* 0.5 cm^3) and NH_4Cl (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 H_2O (containing 0.1% NH_3); flow rate: 10 cm^3 min^{-1}] to afford the title compound **6g** as an NH_3 salt (3.3 mg, 41%). White solid: mp >300 °C; IR (neat): ν_{max}/cm^{-1} 1689 (C═O); δ_H (400 MHz; DMSO-d_6; Me_4Si) 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* Calcd for $C_{12}H_7N_2O_2$ $[M - H]^-$ 211.0513, found 211.0523.

Molecular modeling study of CK2α complexes with benzo[*g*]-indazole 6a, 6f, and 6g. Complex structures of CK2α with **6a**, **6f**, and **6g** were modeled manually based on the crystal structure of CK2α 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

CK2α or CK2α′ kinase reaction was done in a reaction buffer [0.015 cm^3; 20 mM MOPS pH 7.2, 25 mM β-glycerol phosphate, 5 mM EGTA, 1 mM sodium orthovanadate, 13.5 mM $MgCl_2$, 0.4 μM PKA inhibitor cocktail, 0.2 mM CK2 substrate peptide (RRRDDDSDDD), 8.5 mU CK2α enzyme, 90 μM ATP, 2.2 nM [γ-^{32}P] ATP]. After incubation at 37 °C for 10 min, the reaction was terminated by the addition of 0.010 cm^3 of 40% TCA. 0.005 cm^3 of the solution was transferred onto a 96-well P81 UniFilter (Whatman), and each well was washed with 0.20 cm^3 of 0.75% phosphoric acid solution 20 times. Residual

radioactivity was measured using TOP count NXT (PerkinElmer) after 30 min incubation in 0.020 cm^3 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% CO_2 incubator. Growth inhibition assays using HCT-116 cells were performed in 96-well plates (BD Falcon). HCT-116 cells were seeded at 5000 cells per well in 0.050 cm^3 of culture media and placed for 6 h. Chemicals in DMSO were diluted 200-fold with the culture medium in advance. Following the addition of 40 μL of the fresh culture medium, 0.030 cm^3 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 h. The wells in the plates were washed twice with Phenol-red minus medium [McCoy's 5A medium (Thermo Scientific)]. After 1 h incubation with 0.10 cm^3 of the medium, the cell culture in each well was supplemented with 0.020 cm^3 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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Notes and references

1 J. S. Duncan and D. W. Litchfield, *Biochim. Biophys. Acta, Proteins Proteomics*, 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; (*b*) for the initial report of pyrazine-based CK2 inhibitors, see: 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: Struct. Biol. Cryst. Commun.*, 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 ^{13}C 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 and 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.