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Brief Article

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Structure-Based Design of Novel Potent Protein Kinase CK2 (CK2) Inhibitors with Phenyl-azole Scaffolds

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Supporting Information

ABSTRACT: Protein kinase CK2 (CK2) is a ubiquitous serine/threonine protein kinase for hundreds of endogenous substrates. CK2 has been considered to be involved in many diseases, including cancers. Herein we report the discovery of a novel ATP-competitive CK2 inhibitor. Virtual screening of a compound library led to the identification of a hit 2-phenyl-1,3,4-thiadiazole compound. Subsequent structural optimization resulted in the identification of a promising 4-(thiazol-5-yl)benzoic acid derivative.

INTRODUCTION

Protein kinase CK2 (CK2) (previously called casein kinase II) is a ubiquitous, essential, and highly pleiotropic serine/threonine-selective kinase for >300 protein substrates. Many of the substrates are implicated in various important cellular functions, including signal transduction and gene expression.[1,2] CK2 typically forms tetrameric complexes consisting of two catalytic α subunits (α or α') and two regulatory β subunits in various combinations.[3] CK2α' subtype is exclusively expressed in the brain and testis, while ubiquitous expression of CK2α subtype has been reported.[4] Being different from most protein kinases whose functions are activated only in response to specific stimuli, CK2 is constitutively active. It has been well demonstrated that CK2 is usually overexpressed in several cancer cells compared with corresponding normal tissues.[5] CK2 regulates many antiapoptotic signaling cascades to ensure that cancer cells can survive apoptosis.[6] Another mechanism by which CK2 can promote tumor phenotypes is reported to impair the function of tumor suppressor proteins such as promyelocytic leukemia (PML).[7] It has also been reported that a CK2 inhibitor may enhance the efficacy of antitumor agents such as melphalan and imatinib.[8,9] Thus, CK2 has a multifunctional role that creates a favorable cellular environment for tumor progression, making it a potential target for cancer treatment.

The relatively small ATP binding site of CK2 compared with those of other protein kinases has facilitated the design of selective small-molecule ATP-competitive inhibitors.[10] Several classes of ATP-competitive CK2 inhibitors have been reported (Figure 1).[11−18] Representative examples include coumarin derivatives (including quercetin and apigenin),[11,12] emodin,[13] 4,5,6,7-tetrabromo-1*H*-benzotriazole (TBB),[14] (5-oxo-5,6-dihydroindolo[1,2-*a*]quinazolin-7-yl)acetic acid (IQA),[15] and pyrazolo[1,5-*a*][1,3,5]triazine derivatives.[16] More recently, the benzonaphthyridine derivative **31** (CX-4945) has been reported to be a first-in-class, orally bioavailable ATP-competitive CK2 inhibitor and is currently in clinical trials for the treatment of cancer.[17,18] ATP-noncompetitive CK2 inhibitors targeting the CK2β subunit or CK2α−CK2β interaction have also been reported, providing opportunities for the development of allosteric inhibitors.[19]

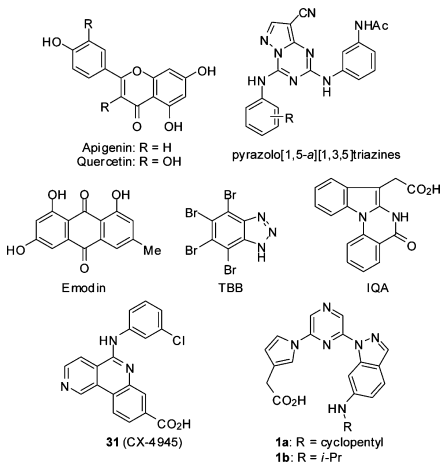

Figure 1. Structures of representative CK2 inhibitors.

Alternatively, two potent CK2 inhibitors (**1a**,**b**) containing a 2,6-disubstituted pyrazine framework have been identified.[20] Their derivatives were synthesized for structure−activity relationship studies.[21] The binding mode of pyrazine-based inhibitors **1a** and **1b** to CK2α or CK2α' was determined by X-ray crystallography: the inhibitors were bound to the ATP binding site through three hydrogen bonds.[22,23] In the present work, we report the structure-based design of CK2 inhibitors with a 5-phenylthiazole scaffold on the basis of the structure of the CK2α−**1b**[23] and CK2α−adenylyl-imidodiphosphate (AMPPNP)[24] complexes.

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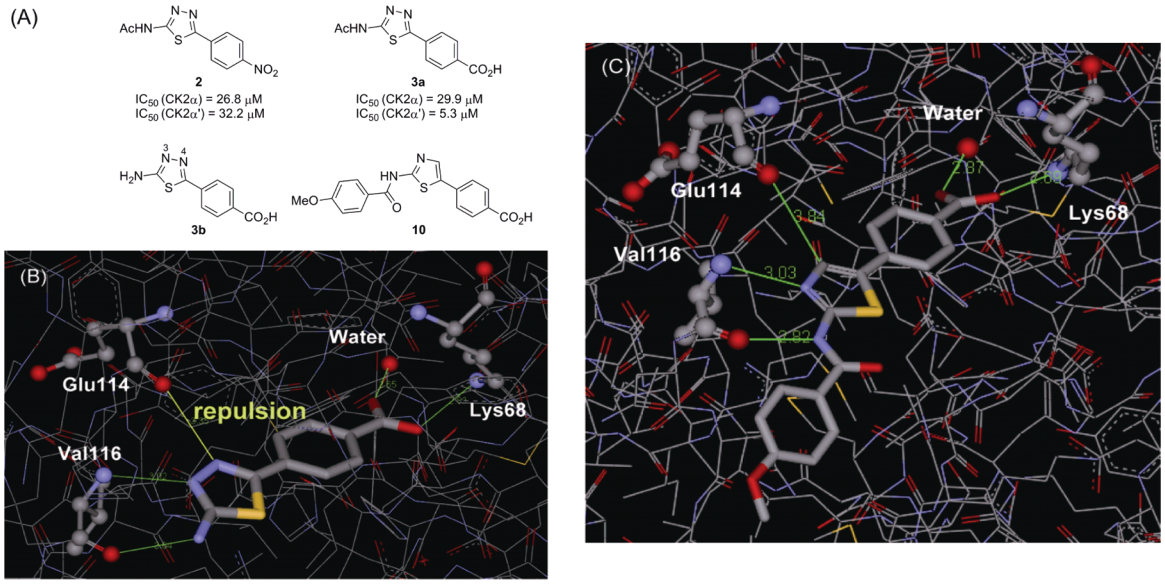

Figure 2. Structures and binding mode of thiadiazole- and thiazole-based CK2 inhibitors. (A) Structures of the 2-amino-1,3,4-thiadiazole-based inhibitors. (B) Crystal structure of the CK2α−thiadiazole **3b** complex. (C) Simulated binding mode of thiazole **10** with CK2α.

RESULTS AND DISCUSSION

For the development of new classes of CK2 inhibitors, a structure-based virtual screening of an approximately three million compound database was undertaken based on the structure of the CK2α−**1b** (PDB 3AT4) and CK2α−AMPPNP (PDB 1JWH) complexes. Among several candidate compounds selected, thiadiazole **2** exhibited moderate CK2 inhibitory activities with an IC_{50} of 26.8 μM toward CK2α and 32.2 μM toward CK2α' (Figure 2A). During our initial structure-based design, we focused on the similar binding mode of **1b** and **2** with CK2 under molecular modeling in which the nitro group of **2** was accommodated at the identical site with the carboxyl group of **1b**. Both polar groups are likely to be essential for the interaction with Lys68 of CK2. As expected, the analogue **3a**, in which the nitro group of **2** was replaced with a carboxyl group, exhibited equipotent inhibitory activities against CK2α and CK2α', respectively [IC_{50}(CK2α) = 29.9 μM; IC_{50}(CK2α') = 5.3 μM].

To confirm the binding mode of 2-phenyl-1,3,4-thiadiazole-type compounds with CK2α, we determined the crystal structure of CK2α complexed with **3b**, which is an *N*-acyl group-deficient analogue of **3a** (Figure 2B). Compound **3b** was bound to the ATP binding pocket, which was surrounded by hydrophobic amino acids. Several hydrogen bonds were observed between CK2α and the inhibitor. The carboxyl group formed a salt bridge with Lys68 and an additional water-mediated hydrogen bond. The amino group was bound with the carbonyl oxygen of Val116, while the thiadiazole nitrogen at the 3-position interacted with the backbone NH group of Val116.

On the basis of the docking simulation experiment using the CK2α−**3b** complex, a series of aminothiadiazoles **3** bearing various acyl groups with higher scores for favorable interactions were designed and synthesized (Scheme 1). Briefly, condensation of commercially available methyl 4-formylbenzoate **4** with thiosemicarbazide afforded the corresponding thiosemicarbazone, which was converted to the aminothiadiazole **5** by $FeCl_3$-mediated oxidative cyclization followed by treatment with pyridine.[25] One-pot transformation of **5** (including treatment with various acyl chloride followed by ester hydrolysis) afforded the desired compounds **3** and **S1**−**S37** (Supporting Information (SI)).

Among 55 compounds evaluated for in vitro CK2 inhibitory activities (**3a**, **3c**−**3s**, **S1**−**S37**, SI), 18 compounds exhibited moderate inhibitory activities (>90% inhibition at 32 μM against CK2α or CK2α'; see Table 1 and SI). The inhibitory activities of amide derivatives varied significantly depending on the acyl group structure. The inhibitory activities of the benzoyl derivatives relied on the substitution pattern of the benzene ring. Replacement of the benzene ring by a thiophene (**3f**) or a furan ring (**3g**) retained the potency, whereas replacement by a pyridine ring (**S9**, SI) resulted in the loss of activities. Acyl derivatives bearing a relatively small alkyl group (**3a** and **3h**−**k**) exhibited moderate potency. Most carbamate derivatives (**3l**−**s**) displayed moderate potency. Among 18 compounds exhibiting moderate inhibitory activities, compound **3e** with a 4-methoxybenzoyl group displayed the lowest IC_{50} value and was employed for the optimization process.

We next focused on the five-membered heterocyclic moiety of **3e**. The crystal structure of the CK2α−**3b** complex revealed a potential electrostatic repulsion between the thiadiazole nitrogen at the 4-position and the backbone carbonyl oxygen of Glu114 (Figures 2B and 3A). When simulations of the phenyl-azole derivatives **29** and **30** with a thiazole or pyrazole core substructure were carried out,[26] higher binding affinity toward CK2α was expected for thiazole **29** and pyrazole **30** compared to that of thiadiazole **3a** (see SI). Replacement with a thiazole ring could remove unfavorable repulsion and append a weak but favorable interaction between CH and the backbone oxygen atom of Glu114 to improve binding affinity and potency (Figures 2C and 3B). Alternatively, an NH group of a pyrazole ring in **21** could also form an additional hydrogen bond with the Glu114 carbonyl oxygen (Figure 3C). The pyrazole **21** could be rearranged to the tautomer **21'**, which may form disfavored electrostatic repulsions with CK2α. 2-Amino-oxadiazole **14** and 5-aminothiazole derivative **28** (Scheme 1) were also designed as reference scaffolds. A potential electrostatic repulsion between the thiazole nitrogen in **28** and the Glu114 carbonyl group was expected.

Using the standard synthetic methods for five-membered heterocycles, the phenyl-azole compounds **10**, **14**, **21**, and **28**

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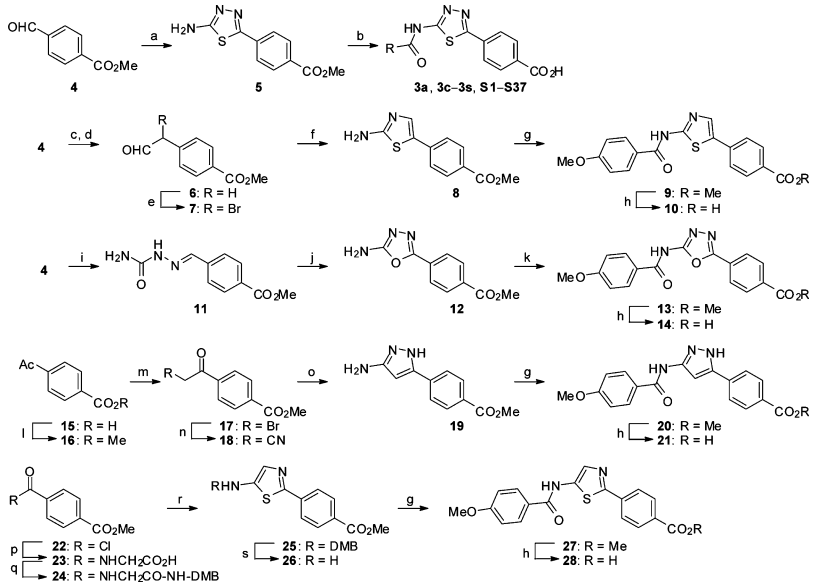
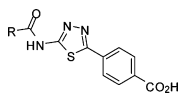
Scheme 1. Synthesis of Phenyl-azole CK2 Inhibitors[a]



[a]Reagents and conditions: (a) thiosemicarbazide, EtOH, H_2O, 70 °C, then $FeCl_3 \cdot 6H_2O$, 75 °C; (b) RCOCl, Et_3N, THF, 65 °C (or sonication), then LiOH·H_2O, H_2O, rt; (c) KO*t*-Bu, $MeOCH_2PPh_3Cl$, THF, 0 °C to rt; (d) conc. HCl aq, THF, rt; (e) Br_2, CH_2Cl_2, rt; (f) thiourea, EtOH, reflux; (g) 4-methoxybenzoyl chloride, Et_3N, THF, rt; (h) LiOH·H_2O, H_2O, THF, rt; (i) semicarbazide hydrochloride, NaOAc, MeOH, H_2O, rt; (j) Br_2, AcOH, 60 °C; (k) 4-methoxybenzoyl chloride, NaH, THF, rt; (l) H_2SO_4, MeOH, 60 °C; (m) *p*-TsOH·H_2O, NBS, MeCN, 85 °C; (n) KCN, EtOH, H_2O, 0 °C to rt; (o) hydrazine monohydrate, MsOH, EtOH, reflux; (p) glycine, $NaHCO_3$, H_2O, rt; (q) 2,4-dimethoxybenzylamine, $(i\text{-Pr})_2NEt$, HBTU, DMF, CH_2Cl_2, rt; (r) Lawesson's reagent, toluene, 110 °C; (s) TFA/CH_2Cl_2 (1:4), rt.

Table 1. CK2 Inhibitory Activities of Aminothiadiazole Derivatives



compd	R	IC50 [μM][a] CK2α	CK2α′	compd	R	IC50 [μM][a] CK2α	CK2α′
3a	methyl	29.9	5.3	**3k**	(*E*)-but-2-en-2-yl	3.6	2.9
3c	phenyl	4.4	3.3	**3l**	2-methoxyethoxy	6.8	4.3
3d	4-fluorophenyl	6.6	1.7	**3m**	allyloxy	8.1	2.7
3e	4-methoxy-phenyl	3.4	1.2	**3n**	*n*-propoxy	5.7	3.0
3f	2-thienyl	6.4	5.8	**3o**	isopropoxy	14.1	8.2
3g	2-furyl	5.1	1.8	**3p**	*n*-butoxy	7.7	3.1
3h	hydroxymethyl	5.1	2.8	**3q**	isobutoxy	5.1	5.7
3i	methoxymethyl	6.0	4.3	**3r**	*n*-pentyloxy	4.5	2.4
3j	cyclopropyl	3.9	1.6	**3s**	*n*-hexyloxy	6.1	2.1

[a]Inhibition values were determined by the CK2 kinase assay.

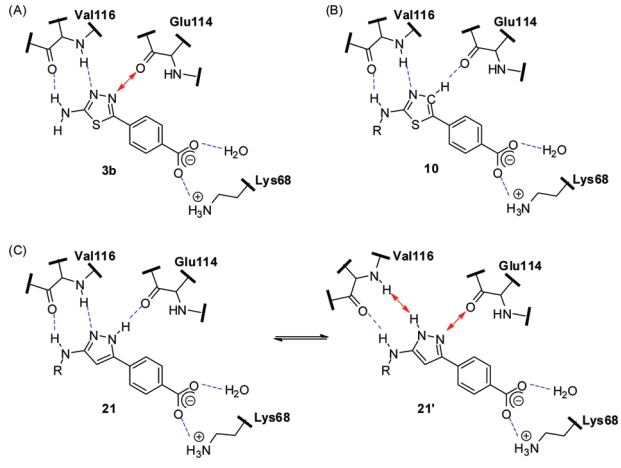


Figure 3. Two-dimensional view of a plausible binding mode of thiadiazole **3b** (A), thiazole **10** (B), and pyrazole **21** (C) with CK2α. R = 4-methoxybenzoyl.

were prepared (Scheme 1). The synthesis of 2-aminothiazole **10** started from methyl 4-formylbenzoate **4**. Wittig reaction of **4** with methoxymethyltriphenylphosphonium ylide followed by acid treatment yielded phenylacetaldehyde **6**. Bromination of **6** followed by condensation with the thiourea led to the 2-aminothiazole **8**. Acylation of **8** with 4-methoxybenzoyl chloride followed by ester hydrolysis provided the desired compound **10**. For the synthesis of the 2-amino-1,3,4-oxadiazole analogue **14**, the reaction of **4** with semicarbazide hydrochloride afforded semicarbazone **11**, which was cyclized to oxadiazole **12** by Br_2-mediated oxidative cyclization.[27] Acylation of **12** with 4-methoxybenzoyl chloride under standard conditions using Et_3N resulted in low yields, which was overcome by use of NaH as the base to yield **13**. Ester hydrolysis of **13** afforded the desired compound **14**. The 3-aminopyrazole **21** was derived from commercially available 4-acetylbenzoic acid **15**. Methyl ester formation followed by α-bromination with *N*-bromosuccinimide (NBS) in the presence of *p*-TsOH gave α-bromoketone **17**.[28] After cyanation of **17** with KCN, MsOH-catalyzed condensation of β-keto nitrile **18** with hydrazine monohydrate yielded aminopyrazole **19**.[29] The acylation and ester hydrolysis of **19** under standard conditions yielded the desired compound **21**. For the synthesis of 5-aminothiazole **28**, condensation of 4-(chlorocarbonyl)-benzoate **22** with glycine followed by 2,4-dimethoxybenzylamine coupling afforded *N*-dimethoxybenzyl-protected amide **24**. Cyclization of **24** in the presence of Lawesson's reagent,[30] and the subsequent manipulation afforded the compound **28**.

The in vitro inhibitory activities of the compounds toward CK2α and CK2α′ are shown in Table 2. As expected from the

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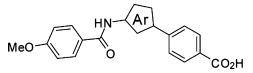
Table 2. CK2 Inhibitory Activities of 4-Arylbenzoate Derivatives



compd	NH–Ar	IC_{50} [μM][a] CK2α	CK2α'
1a	–	0.11	0.19
3e		3.4	1.2
10		0.032	0.046
14		74	32
21		0.14	0.063
28		50	25

[a]Inhibition values were determined by the CK2 kinase assay.

simulation experiment, 2-aminothiazole **10** exhibited significantly potent inhibitory activities toward CK2α and CK2α', which was approximately 100-fold more active compared with the parent thiadiazole **3e**. 3-Aminopyrazole **21** also showed more potent inhibitory activities than **3e**, but it was less potent than thiazole **10**.[31] Oxadiazole **14** showed less inhibitory activities, implying a significant contribution from the sulfur atom. 5-Aminothiazole **28**, in which the CH and N positions on the thiazole ring of **10** were interchanged, decreased the potency, providing additional evidence to support the proposed binding mode of **10** with CK2α.

The potent 2-aminothiazole **10** was screened against a panel of 70 kinases to assess its selectivity profile. At 0.30 μM, compound **10** exhibited a >50% inhibitory effect against 11 kinases, including CK2α and CK2α' (see SI). Highly potent inhibition was observed only against four kinases [84% inhibition for CK2α; >100% inhibition for CK2α'; 82% inhibition for dual-specificity tyrosinephosphorylation-regulated kinase 1B (DYRK1B); 76% inhibition for FMS-like receptor tyrosine kinase 3 (FLT3) in the presence of 30 nM compound **10**]. Although 2-amino-5-phenyl-thiazole and -thiadiazole scaffolds have been reported as cyclin-dependent kinase 6 (CDK6) and AKT1 inhibitors, respectively,[32,33] the thiazole **10** exerted significantly less inhibition at 3 μM against both kinases (32% inhibition for CDK6; 0.8% inhibition for AKT1), indicating that this is relatively selective for CK2.

Compounds **1a**, **3e**, **10**, and **21** were tested for their inhibitory effect on the proliferation of cancer cell lines: lung cancer cells A549, colorectal cancer cells HCT-116, and breast cancer cells MCF-7 (Table 3). Compound **31** (CX-4945, Figure 1) has been shown to be effective against these cell lines.[17] 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. Thiazole **10** was found to be slightly more potent compared with **1a** and **31** toward all the cell lines tested, whereas no inhibitory effects were observed at 30 μM of thiadiazole **3e** and pyrazole **21**. The reason for the apparently incompatible bioactivities of **3e** and **21** needs to be clarified. It is also possible that compound **10** exerts its cell growth inhibition by a multikinase inhibition mechanism for CK2 and other kinases such as (phosphoprotein 70 ribosomal protein S6 kinase) p70S6K.[34]

Table 3. Inhibitory Effects on Cell Proliferation of CK2 Inhibitors toward A549, HCT116, and MCF-7

compd	IC_{50} [μM][a] A549	HCT116	MCF-7
31	8.2	5.2	6.5
1a	36	24	75
3e	>30	>30	>30
10	2.6	1.6	2.4
21	>30	>30	>30

[a]IC_{50} values were derived from the dose–response curves generated from triplicate data points. Cytotoxic activity against cell lines after 72 h exposure to the compound.

CONCLUSION

In conclusion, we undertook a structure-based virtual screening for novel CK2 inhibitors using X-ray crystal structures of pyrazine-based CK2 inhibitor–CK2α and AMPPNP–CK2α complexes which led to a 2-phenyl-1,3,4-thiadiazole-type hit compound **2**. The subsequent structure-based scaffold hopping of five-membered nitrogen heterocycles identified a highly potent 4-(2-aminothiazol-5-yl)benzoate-type CK2 inhibitor **10**. The improved potency of **10** was rationalized by potential multiple favorable interactions with the CK2α ATP binding pocket. The thiazole-based inhibitor **10** with a relatively high selectivity toward CK2 exhibited potent cytotoxicity, indicating that it could be a promising candidate for cancer treatment. Further studies to improve CK2 inhibitory activities and kinase selectivity are now in progress.

ASSOCIATED CONTENT

Supporting Information

Experimental procedures, spectral characterization data, a simulation procedure, and a crystallographic information file. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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ABBREVIATIONS USED

CK2, protein kinase CK2; PML, promyelocytic leukemia; TBB, 4,5,6,7-tetrabromo-1*H*-benzotriazole; IQA, (5-oxo-5,6-dihydroindolo[1,2-*a*]quinazolin-7-yl)acetic acid; AMPPNP, adenylyl-imidodiphosphate; NBS, *N*-bromosuccinimide; DYRK1B, dual-specificity tyrosinephosphorylation-regulated kinase 1B; FLT3, FMS-like receptor tyrosine kinase 3; CDK6, cyclin-dependent kinase 6; MTS, 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2*H*-tetrazolium; p70S6K, phosphoprotein 70 ribosomal protein S6 kinase

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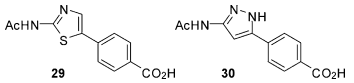

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