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2-(6-Phenyl-1*H*-indazol-3-yl)-1*H*-benzo[*d*]imidazoles: Design and synthesis of a potent and isoform selective PKC-ζ inhibitor

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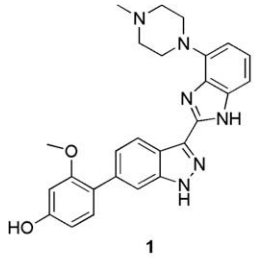
ABSTRACT

The inhibition of PKC-ζ has been proposed to be a potential drug target for immune and inflammatory diseases. A series of 2-(6-phenyl-1*H* indazol-3-yl)-1*H*-benzo[*d*]imidazoles with initial high crossover to CDK-2 has been optimized to afford potent and selective inhibitors of protein kinase c-zeta (PKC-ζ). The determination of the crystal structures of key inhibitor:CDK-2 complexes informed the design and analysis of the series. The most selective and potent analog was identified by variation of the aryl substituent at the 6-position of the indazole template to give a 4-NH_2 derivative. The analog displays good selectivity over other PKC isoforms (α, βII, γ, δ, ε, μ, θ, η and ι/λ) and CDK-2, however it displays marginal selectivity against a panel of other kinases (37 profiled).

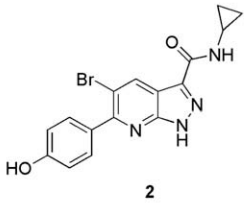
The PKC family of kinases is comprised of 11 members that are grouped into three categories based upon their molecular structure and mode of activation, conventional PKCs (α, βI, βII, γ), novel PKCs (δ, ε, μ, θ and η), and atypical PKCs (ζ and ι/λ).[1] Recent literature has implicated the atypical PKCs in important cellular signaling events, PKC-ζ in particular has been proposed as an intermediary in the activation of the NF-κb pathway and IL-4/Stat6 pathway.[2] The NF-κb pathway is important in immune and inflammatory diseases, therefore an inhibitor of PKC-ζ may serve to reduce the severity of these types of diseases.[3] Recently we identified compound **1**, a 6-phenyl-3-benzimidazole through a screening of our corporate compound collection. The compound was profiled against a variety of kinases and found to be especially active against PKC-ζ (IC_{50} = 22 nM) and CDK-2 (IC_{50} = 102 nM).

Thus, a key objective of our chemistry effort was to enhance the PKC-ζ selectivity of our lead. Recent publications by GlaxoSmithKline disclosed the identification of GSK-3 kinase inhibitors belonging to the 6-aryl-pyrazolo[3,4-*b*]-pyridine structural class, **2**, analogous to our 6-aryl indazoles.[4] In these publications the authors described their lead compound as possessing significant crossover onto CDK-2 despite possessing good GSK-3 activity. The strategy employed to enhance the selectivity of their lead was to explore variation of the 6-aryl ring by replacement with alternate heterocycles and substituted phenyl derivatives. Indeed, the Glaxo group was successful in identifying analogs which spared CDK-2, as well as other derivatives to provide a compound with excellent selectivity against a panel of 23 other kinases. Given



1

PKC-ζ, IC_{50} = 22 nM
CDK-2, IC_{50} = 102 nM



2

GSK-3, IC_{50} = 0.8 nM
CDK-2, IC_{50} = 5 nM

Figure 1. Structure and biological activity of PKC-ζ lead molecule and GSK compound.

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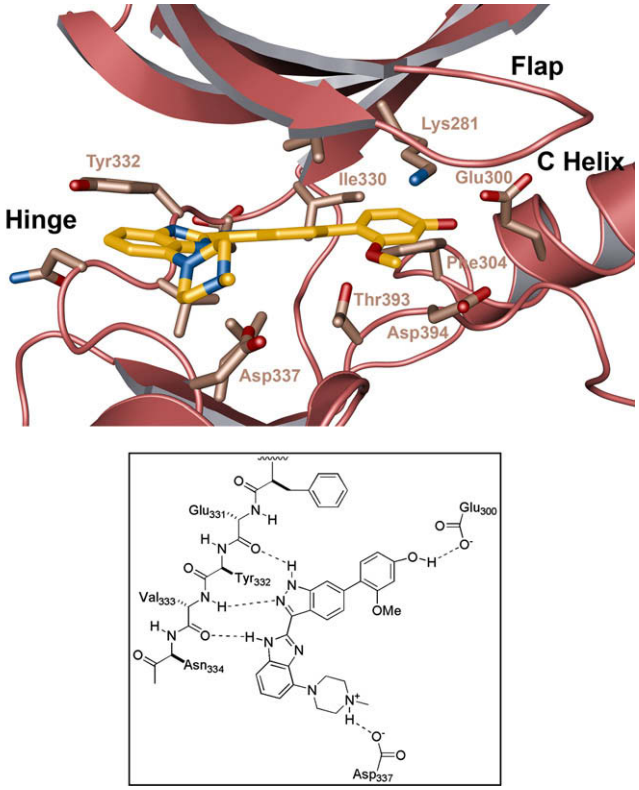
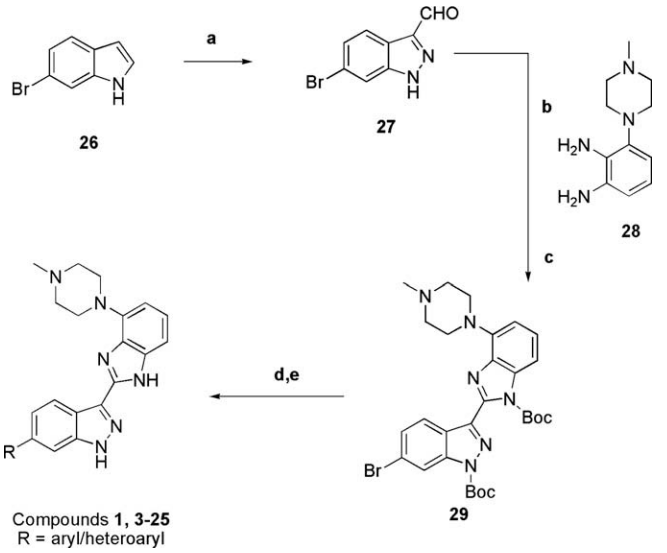


Figure 2. Schematic of binding interactions of compound **1** with PKC-ζ predicted by homology modeling and molecular docking.[8]



Scheme 1. Preparation of 6-aryl/heteroaryl indazole-benzimidazoles. Reagents and conditions: (a) $NaNO_2$, 2 N HCl, acetone:H_2O (1:1), 55%; (b) S, DMF, 80 °C, 71% or Na_2S_2O5, EtOH:H_2O (10:1), 85%; (c) Boc_2O, CH_3CN, DMAP, 95%; (d) $RB(OH)_2$, $PdCl_2$(dppf), CH_3CN:H_2O (10:1), Na_2CO_3, 50–70%; (e) 30% TFA, CH_2Cl_2, rt, 50–80% yield.

Glaxo's success in identifying a selective inhibitor of GSK-3, we sought to apply a similar strategy in the development of a selective PKC-ζ inhibitor.

Based upon the binding mode observed for similar compounds in other kinases, we surmised that compound **1** would form three hydrogen bonds to the hinge region of PKC-ζ and potentially an additional hydrogen bond between its phenol and Glu300 of helix C of the kinase (Fig. 2). A comparison of the protein sequences in the binding sites of CDK-2 with the PKC isoforms identified multiple residues that differ between CDK-2 and PKC-ζ as well as several amino acid differences between the PKC isoforms. Since helix C of kinases can be in at least two distinct conformations, depending on the state of the enzyme (active or inactive),[5] and since the structures of PKC family kinases were not available at the time of our work (since then, three different isoforms now have known structures),[6] we could not be certain of that hydrogen bond forming. In order to validate the assumptions of the binding mode and to test the hypothesis that variation of the C-6-substituent would lead to a CDK-2 sparing compound, a series of analogs were prepared (compounds **3**–**25**). Crystal structures of select analogs were then determined in complex with CDK-2.[7]

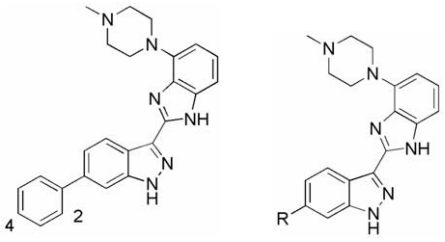
The 6-ary/heteroaryl indazole-benzimidazoles were prepared according to a modification of procedures described previously,[9] and as described in Scheme 1. Thus the 6-bromo-1*H*-indazole-3-carbaldehyde **27** was prepared by oxidation of 6-bromo-1*H*-indole **26** with $NaNO_2$/HCl in 55% yield.[9b] Formation of the benzimidazole ring system was accomplished via one of two routes: (i) elemental sulfur in DMF at 80 °C[9]; or (ii) sodium thiosulfate in EtOH:H_2O[10] using 3-(4-methylpiperazin-1-yl)benzene-1,2-diamine[5] as the diamine partner **28**. Following formation of the benzimidazole ring system the free NH of the indazole and benzimidazole were protected as their Boc-derivative in quantitative yield to give the key intermediate **29**. Suzuki cross-coupling of the bromide **29** with a range of aryl/heteroaryl boronic acids afforded the corresponding coupled products, which were then treated with 30% TFA in CH_2Cl_2, followed by purification via RP-HPLC to give the final compounds **1** and **3**–**25**.

Initially, we investigated the role of the 2-OMe and thus prepared compounds **3** and **4**; not surprisingly neither derivative displayed enhanced selectivity for PKC-ζ over CDK-2; rather, both compounds were more potent against both enzymes relative to compound **1** (Table 1). Subsequently, to further understand the role of the 4-OH substituent, an analog with the 4-OH converted to a 4-OMe **6**, and two analogs with the 4-OH removed entirely, were prepared (**7** and **8**). The analogs lost significant activity against both kinases, thus pointing to the need for a substituent capable of donating an H-bond. In order to probe the area further, analogs capable of making H-bonds but differing in size and donor/acceptor status were prepared **5, 9, 13, 15, 17, 20, 23**, and **25**, Table 1). Notably, compound **9** displayed very good PKC-ζ activity combined with a reduction in CDK-2 activity, thus resulting in a selectivity ratio of ~200-fold. Analogs of compound **9** were prepared to further investigate the area surrounding the 4-NH_2 (**11, 12, 15**, and **16**).

The data from these analogs suggests a high degree of sensitivity of the PKC-ζ kinase to steric and electronic variations. For instance, addition of the 2-OMe moiety to either the 4-OH (**1** and **4**) or the 4-NH_2 (**9** and **17**) both result in approximately a 4-fold loss of potency against PKC-ζ but also show a net 2-fold improvement in selectivity versus CDK-2. By contrast, substitution of 4-OH for 4-NH_2 (**1** and **17**, **4** and **9**) does not affect potency for PKC-ζ, but the change causes 70- to 100-fold weaker inhibition of CDK-2. To investigate the basis for the observed selectivity, we determined the crystal structures of key compounds either bound by CDK-2 alone (**9** and **17**) or the CDK-2:CyclinA complex (**9**; Fig. 3 and Supplementary Materials).

In both the active (CDK-2:CyclinA complex) and inactive (CDK-2 alone) forms of the crystal structures, the inhibitor binds in nearly the identical position, the largest difference being a ~10° rotation of the plane of the compound in the binding site when comparing **9** complexed with CDK-2:CyclinA and **17** bound by CDK-2 alone. The center of the rotation was approximately N1 of the indazole ring.

Table 1
Inhibition of hPKC-ζ and hCDK-2 by selected indazole analogs-phenyl derivatives[a]



Compound	R	PKC-ζ	CDK-2	Ratio
1	2-OMe, 4-OH	22	102	4.6
3	3-OMe, 4-OH	2.26	4.1	1.8
4	4-OH	5.6	13.3	2.4
5	4-CH_2OH	290	21,500	74
6	2-OMe, 4-OMe	12,400	10,000	0.8
7	2-OMe, 4-H	5520	10,000	1.8
8	2-OMe, 4-F	19,000	10,000	0.5
9	4-NH_2	5.18	1040	201
10	3-NH_2	1510	6100	4
11	3-F, 4-NH_2	55.5	1730	31
12	3-OMe, 4-NH_2,	51.3	247	4.8
13	4-CH_2NH_2	31,100	10,000	1.1
14	4-NHCOMe	8800	10,000	1.1
15	4-NHMe	585	109,000	186
16	4-NMe_2	1310	–	–
17	2-OMe, 4-NH_2	23.3	10,000	429
18	4-CO_2H	27,300	10,000	0.4
19	4-COMe	13,600	10,000	0.7
20	4-$CONH_2$	11,800	–	–
21	4-SO2NH_2	8930	–	–
22	5-Indolyl	4080	10,000	2.5
23	6-Quinolyl	6840	10,000	1.5
24	6-Indazole	1070	–	–
25	6-Benzofuran	3020	–	–

[a] Values are in nM.

While the compound positions were conserved, the conformation of the kinases in their DFG loop, flap, and C helix regions increasingly differed. In the structure with CDK-2 alone, helix C was observed in its inactive conformation with hydrophobic side chains Ile52 and Leu55 projecting inward towards the aniline substituent of the compound. Only the three predicted hinge hydrogen bonds are formed between **9** and the protein in its complex with CDK-2 alone. By contrast, in the CDK-2:CyclinA complex with **9**, six hydrogen bonds are formed in total, three between backbone atoms of the hinge region and the three adjacent nitrogens of the indazole and benzimidazole rings, one between the terminal piperazine nitrogen and Asp86, and the final bonds between the aniline nitrogen of **9** and the side chain of Glu51 (Glu300 in PKC-ζ) of helix C and the backbone nitrogen of Phe146 of the DFG loop. This binding mode agrees with both the SAR of the series as well as the model for the binding of 1 to PKC-ζ (Fig. 1).

As has been observed in previous reports of the transition from inactive to active CDK-2, helix C both pivots and rotates to present a different, hydrophilic face (Glu51) to the active site. While the structures of the CDK-2:CyclinA complex most closely matched the SAR for the series, those crystals typically diffracted X-rays to only around 3 Å resolution. By contrast, the crystals of CDK-2 alone routinely produce data to better than 2 Å resolution. For that reason, we primarily pursued structures of CDK-2 alone to probe the structural aspects of this series.

With the selectivity for CDK-2 (>200-fold) achieved, the next step was to profile the compounds against a panel of PKC isoforms, as well as against other relevant kinases. Indeed, when **9** was profiled against a panel of PKC isoforms good selectivity was achieved

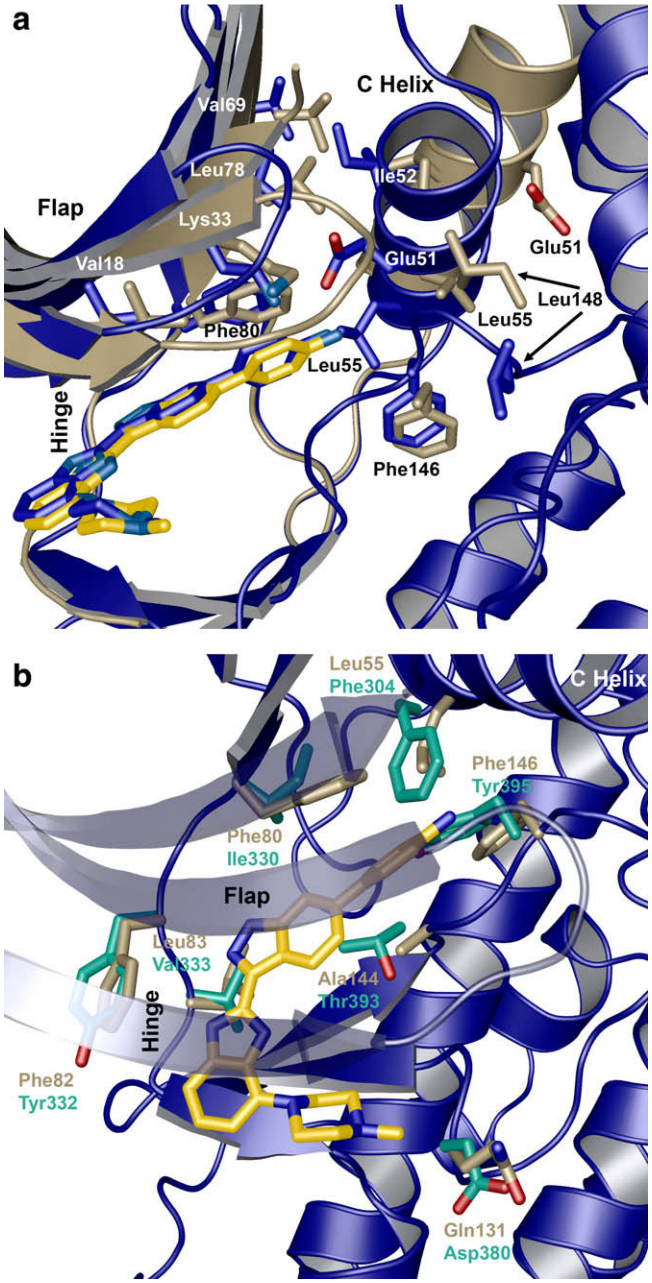


Figure 3. Crystal structure of compound **9** in complex with CDK-2:CyclinA. (a) Comparison of the structure of compound **9** bound to CDK-2 alone (tan) and in complex with CyclinA (blue). (b) CDK-2:CyclinA complex structure showing sites of amino acid variation between CDK-2 (tan) and PKC-ζ (green) are shown as elaborated side chains.

(Table 2), with only the PKC-ι isoform exhibiting less than 10-fold selectivity. Interestingly, PKC-ι is most closely related to PKC-ζ, only differing by a single amino acid in the binding site. However, the other PKC isoforms and CDK-2 differ from PKC-ζ at several other positions, many of which flank the 6-substituent of the inda-

Table 2
PKC isoform selectivity for compounds **1** and **9**[a]

Compound	ζ	α	βII	γ	δ	ε	η	ι	θ
1	1	109	126	1	6	88	1695	7	4
9	1	8000	20,671	711	918	1895	12,424	10	1218

[a] Values are fold selectivity (IC_{50}-PKC isoform/IC_{50}-PKC-ζ).

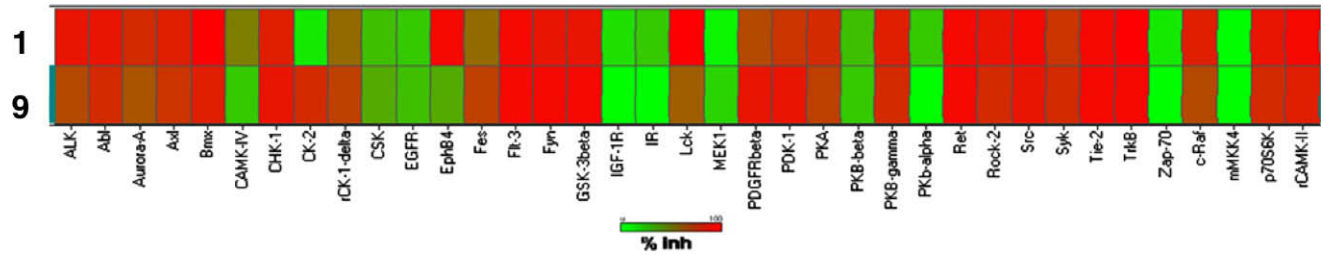


Figure 4. Selectivity profiling[12] of compounds **1** and **9** against 37 different kinases (upstate panel). Values are % inhibition at 10 μM using ATP = Km.

zole, poised to read out the changes discussed above (Fig. 3). In spite of this progress on selectivity against both closely and distantly related kinases, when **9** was profiled against a broader set of other kinases (Upstate panel), significant crossover was observed (Fig. 4).

While the molecular determinants of kinase selectivity can occasionally be linked to a single amino acid difference, in most cases multiple amino acid changes and subtle shifts of conformation of the protein govern the answer. Comparing the PKC-ζ docking model for **1** and the CDK-2:CyclinA complex with **9** reveals a tilt in the position of the compounds in the binding site (see Supplementary Materials). This shift allows the phenyl ring of **1** to rotate ~70° and its 4-OH group to come within 3.9 Å of the side chain of Asp394. Conversion of the hydroxyl moiety of **1** to an amine as in **9** and **17** would provide a second hydrogen bond donor that could, with slight shifts to the protein or ligand position, allow it to form an interaction with that Asp residue. Interestingly, CDK-2 and the PKC isoforms with decreased affinity for **9** and **17** compared to **1** all differ from PKC-ζ and PKC-ι at Phe304, Ile330, Thr393, and Tyr395: amino acid positions flanking the phenyl ring of the inhibitors (Fig. 1). However, comparison of amino acid identity across those positions in the broader panel of kinases assayed does not reveal a clear trend to predict selectivity in this series.

In conclusion, by varying the nature of the 6-substituent of the indazole-benzimidazole[11] **1**, a potent and highly PKC isoform selective compound **9** was identified.[13] The compound unfortunately does not possess the desired selectivity across a broader range of other kinases (<50% inhibition at 10 μM). Structure based design of selectivity against these other kinases is currently underway.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2008.11.105.

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7. The crystal structures of compounds **9** and **17** bound at the active sites of CDK-2 or CDK-2 in complex with CyclinA have been deposited in the Protein Databank (Accession codes: 3EZV, 3EZR, 3FX5). Data for those experiments were in part collected at IMCA-CAT (17-ID) beamline at the Advanced Photon Source and was supported by the companies of the Industrial Macromolecular Crystallography Association through a contract with the Center for Advanced Radiation Sources at the University of Chicago.
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