ChemComm

ROYAL SOCIETY OF CHEMISTRY

COMMUNICATION

View Article Online
View Journal | View Issue

Cite this: *Chem. Commun.*, 2022, 58, 6693

Received 28th March 2022,
Accepted 17th May 2022

DOI: 10.1039/d2cc01759h

rsc.li/chemcomm

AI and computational chemistry-accelerated development of an alotaketal analogue with conventional PKC selectivity†

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The protein kinase C (PKC) family consists of ten isozymes and is a potential target for treating cancer, Alzheimer's disease, and HIV infection. Since known natural PKC agonists have little selectivity among the PKC isozymes, a new scaffold is needed to develop PKC ligands with remarkable isozyme selectivity. Taking advantage of machine-learning and computational chemistry approaches, we screened the PubChem database to select sesterterpenoids alotaketals as potential PKC ligands, then designed and synthesized alotaketal analogues with a different ring system and stereochemistry from the natural products. The analogue exhibited a one-order higher affinity for PKCα-C1A than for the PKCδ-C1B domain. Thus, this compound is expected to serve as the basis for developing PKC ligands with isozyme selectivity.

Isozymes of protein kinase C (PKC), a family of serine/threonine kinases, are key enzymes in intracellular signal transduction that are mainly activated by the second messenger 1,2-diacylglycerol (DAG).[1] Inter-organism chemical communication *via* PKCs has also been proposed.[2] DAG-responsive PKC isozymes are classified into calcium-dependent conventional PKCs (α, βI, βII, and γ) and calcium-independent novel PKCs (δ, ε, η, and θ).[1] Compounds that selectively activate these isozymes are expected to be sources of therapeutic agents against cancer,[3] Alzheimer's disease,[4] and HIV infection.[5] However, such compounds are quite limited; Wender's simplified analogue[6] of bryostatin 1 (bryolog)[7] and (3*R*)-1-hexyl-indolinelactam-V,[8] Irie's simplified analogue of aplysiatoxin[9] (10-Me-aplog-1[10]), and Kozikowski's benzolactam analogue[11] are known as rare examples with moderate isozyme selectivity (Fig. 1). While selective PKC ligands with novel skeletons are sought for new sources, efficient screening of PKC ligands from compound libraries is difficult because a high-throughput screening method for PKC ligands without [^{3}H]phorbol 12,13-dibutyrate[12] has not yet been developed.[13]

Molecular docking and dynamics simulations are often employed for virtual screening[14] and also predicting interactions between proteins and natural products.[15] Focusing on the physicochemical properties of natural products, computational tools have also been used to develop bioactive molecules based on natural products.[16] Sakakibara and colleagues have developed a comprehensively applicable machine learning approach to predict protein–compound interactions, utilizing general biological data including amino acid sequences and mass spectrometry (MS) data.[17] We envisioned that such machine learning approaches could be used to select candidate compounds from a library of 97 million compounds in the PubChem database[18] to search for PKC ligands with novel skeletons. In this study, we report the synthesis and evaluation of PKC surrogate binding of the simplified analogues of alotaketals[19,20] based on an *in silico* screening method and computational chemistry simulations to create new PKC skeletons.

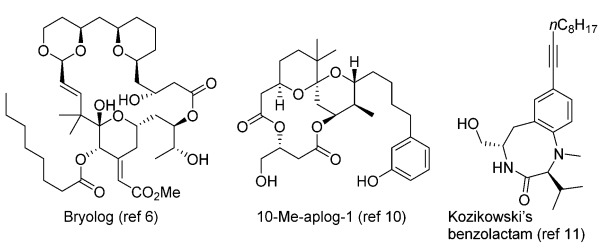

Fig. 1 Isozyme-selective PKC ligands.

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† Electronic supplementary information (ESI) available: Experimental procedures, characterization data, and NMR spectra. See DOI: https://doi.org/10.1039/d2cc01759h

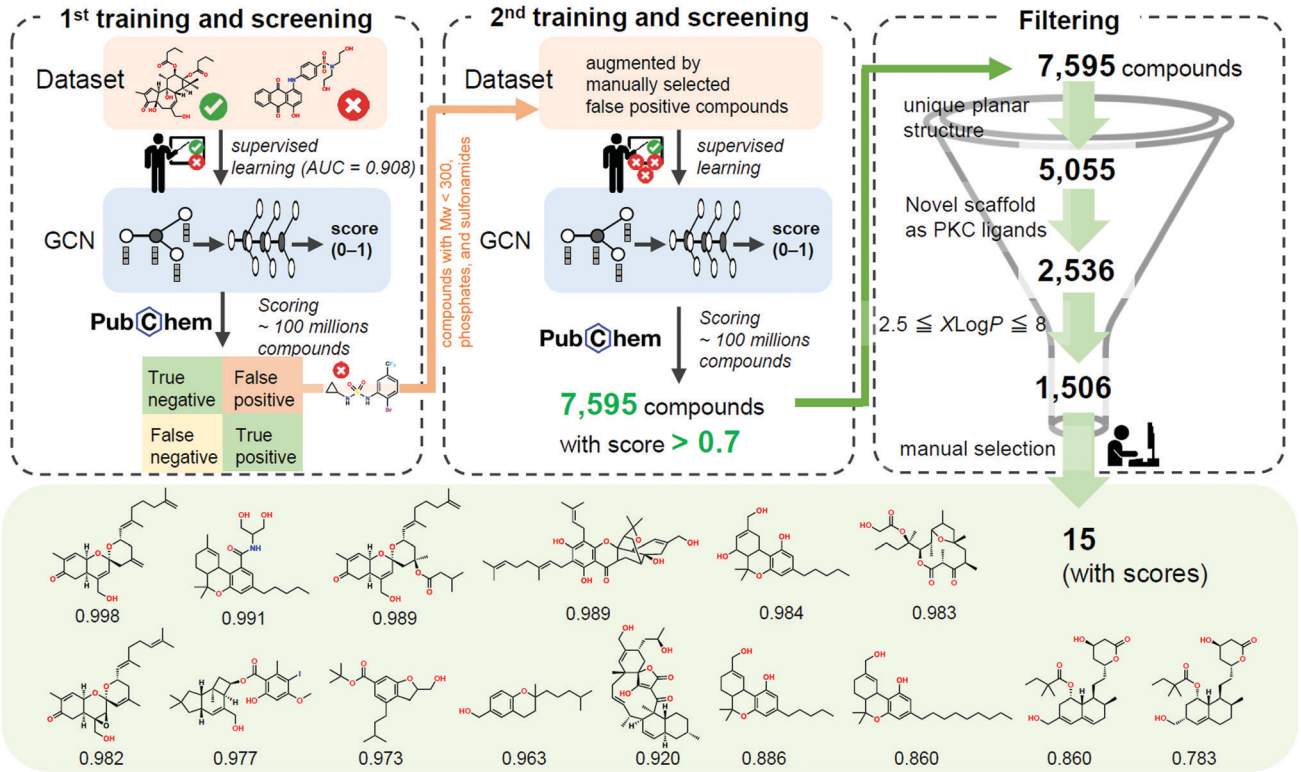


Fig. 2 Machine learning-aided screening of PKC ligands.

Initially, an *in silico* screening system for PKC ligands was constructed by using a machine-learning model based on graph-convolutional neural networks (GCN),[21] which is widely used for *in silico* screening of compounds due to its ability to extract effective features from chemical structures and further exhibited superior performance in our benchmark experiment compared with conventional machine-learning models (see the ESI†). The model was trained with 339 positive examples and 936 negative examples classified with a threshold of 10 nM of K_i, the binding inhibition constant, for the PKCδ-C1B domain peptide (Fig. 2). We evaluated the classification performance of the model by splitting the data set into training and test data sets, and confirmed that the area under the curve (AUC) was 0.908. Then, the whole data set was used to train the model for screening.

The first screening was then performed using the PubChem database, which consisted of 97 million compounds,[18] and then among predicted results with high scores, the compounds with MW $<$ 300, containing sulfonamides or phosphates, were fed back into the dataset as negative examples to reduce false positives. The second screening, based on the first screening, gave 7595 compounds (score $>$ 0.7). Removing compounds with known PKC ligand scaffolds and duplicated compounds gave 2536 compounds, followed by filtering with a criterion of hydrophobicity ($2.5 \leq X\mathrm{Log}P \leq 8.0$) and manual selection with domain-specific knowledge to reduce the final number of compounds to **15**. Three of the **15** hit compounds share a sesterterpene scaffold; these were alotaketals A and B isolated from marine sponge *Hamigera* sp.[19] and 7,8-epoxyphorbaketal A.[20] The former two compounds were first described as cAMP-signalling agonists in 2009,[19] while the latter was described as a Nurr1 activator/LXR antagonist in 2012.[20] In 2016, Anderson *et al.* reported that natural alotaketal congeners, including alotaketal C, induced HIV provirus expression, presumably through PKC activation, though the report did not confirm direct binding of alotaketals to PKC C1 domains.[22]

Therefore, we performed docking and molecular dynamics simulations of alotaketal A with the PKCδ-C1B domain[23] (Fig. 3a). The predicted binding mode of alotaketal A with the

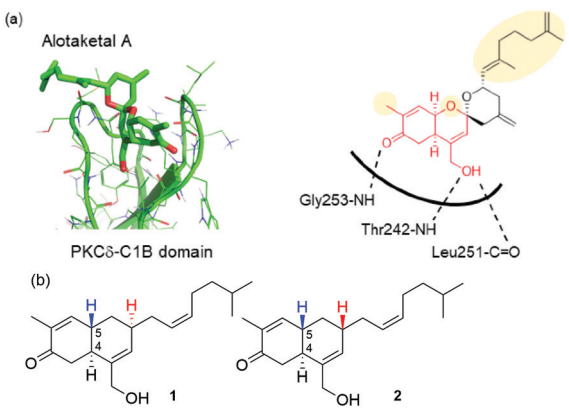
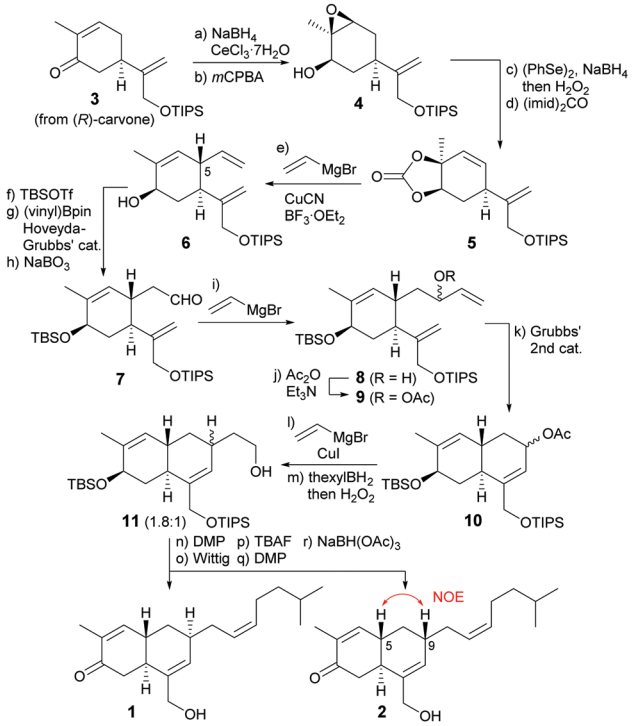


Fig. 3 (a) Docking and molecular dynamics simulation of alotaketal A with PKCδ-C1B domain; (b) simplified compounds **1** and **2**.

PKCδ-C1B domain had a hydrogen-bonding network similar to other PKC ligands such as DAG and phorbol esters, implying alotaketal A to be a direct binder of PKC. This simulation also suggested that (i) the fused bicyclic skeleton (shown as red) would be essential for interaction with the PKCδ-C1B domain (Thr242-NH, Leu251-C═O, Gly253-NH), and (ii) the side chain and a methyl group would be necessary for appropriate hydrophobicity.

Taking these suggestions and synthetic accessibility into account, we designed simplified bicyclic analogues of alotaketals, in which an oxygen atom in the dihydropyran ring was replaced with a carbon atom, and the side chain was a (*Z*)-6-methylhept-2-enyl group. We first performed docking and molecular dynamics simulations of the eight possible stereoisomers (compounds **S1–S6**, **1**, and **2**, see ESI,† Fig. S3 and Table S1) to predict their free energy of binding, ΔG^0, for the PKCδ-C1B domain in the presence of phospholipid bilayer. The simulations suggested that (4*S*,5*S*)-*trans*-fused molecules (**1** and **2**) have a slightly higher affinity for the PKCδ-C1B domain than the natural product-like (4*S*,5*R*)-*cis*-fused molecules (**S1** and **S2**). Furthermore, the simulations suggested that the other four stereoisomers with 6*R*-configuration (**S3–S6**) are practically inactive. Based on this result, we selected **1** and **2** (Fig. 3b) as target compounds because we expected that they would have the necessary structure for binding to PKC and represent different isozyme preferences from natural products because of their unnatural configuration.

The synthesis of the designed analogues started from Luche reduction[24] of a known compound **3** which was prepared from (*R*)-carvone in three steps (Scheme 1).[25] The resultant allyl alcohol was converted to epoxide **4** through stereoselective epoxidation. Regioselective ring-opening of **4** by treatment with *in situ*-generated phenyl selenide was followed by oxidative *syn* elimination to give a diol,[26] which was converted to carbonate **5**. A vinyl group was stereoselectively installed by Cu-mediated S_N2' alkylation[27] to give **6**. The 5*R* configuration was robustly established by this transformation. After silylation of **6**, the mono-substituted olefin among three double bonds was selectively converted to aldehyde **7** by cross-metathesis with vinylboronic acid pinacol ester and oxidative treatment.[28] A vinyl group was introduced to give allyl alcohol **8**, which was an inseparable diastereo-mixture. After acetylation, a six-membered ring was constructed by ring-closing metathesis to give *trans*-fused bicyclic compound **10**,[29] followed by Cu-catalyzed allylic α-substitution[30] for elongation of two-carbon units and hydroboration. The obtained alcohol **11** was converted to analogues **1** and **2** by a five-step sequence including Dess–Martin oxidation,[31] Wittig reaction, removal of silyl groups, oxidation, and chemoselective reduction.[25] Although two diastereomers could not be separated through these transformations, HPLC (ODS) purification provided pure **1** and **2**. The configurations at positions 5 and 9 was confirmed by the NOE experiment as shown in Scheme 1. The green chemistry metrics of this synthetic route were evaluated by following Roschangar's report (see ESI,† Table S3).[32] These metrics would serve as a guide in improving the synthesis of **1**.



Scheme 1 Synthesis of **1** and **2**. Reagents and conditions: (a) $NaBH_4$, $CeCl_3{\cdot}7H_2O$, MeOH, −78 °C, quant., dr >19 : 1; (b) *m*CPBA, CH_2Cl_2, −20 °C, 90%, dr 21 : 1; (c) $(PhSe)_2$, $NaBH_4$, EtOH then H_2O_2, THF, reflux; (d) 1,1′-carbonyldiimidazole, CH_2Cl_2, 46% (2 steps); (e) (vinyl)MgBr, CuCN, $BF_3{\cdot}OEt_2$, THF, −78 °C, 98%, dr >19 : 1; (f) TBSOTf, 2,6-lutidine, CH_2Cl_2, 94%; (g) (vinyl)Bpin, Hoveyda–Grubbs 2nd gen. cat., CH_2Cl_2, reflux; (h) $NaBO_3{\cdot}4H_2O$, THF, H_2O; (i) (vinyl)MgBr, THF, 0 °C, 42% (3 steps), dr 1.3 : 1, 27% of starting material was recovered (BRSM 57%); (j) Ac_2O, Et_3N, DMAP, CH_2Cl_2, 98%; (k) Grubbs' 2nd gen. cat., CH_2Cl_2, reflux, 98%; (l) (vinyl)MgBr, CuI, THF-Me_2S (10 : 1), −30 to 0 °C; (m) thexylborane, THF, −20 °C, 30% (2 steps); (n) Dess–Martin periodinane, pyridine, CH_2Cl_2 0 °C; (o) (4-methylpentyl)(triphenyl)phosphonium bromide, NaHMDS, THF, −78 to 15 °C; (p) TBAF, THF, 50 °C; (q) Dess–Martin periodinane, $NaHCO_3$, CH_2Cl_2, 40% (4 steps), *E*/*Z* >19 : 1; (r) $NaBH(OAc)_3$, benzene-AcOH (10 : 1), 0 °C to r.t., 71%.

With designed analogues **1** and **2** in hand, the binding affinity of **1** and **2** to PKC isozymes was evaluated by a competitive binding assay using [^{3}H]phorbol 12,13-dibutyrate ([^{3}H]PDBu)[12] and synthetic PKC C1 peptides.[33] The C1A peptides and the C1B peptides were used as conventional and novel PKC surrogates, respectively, since these domains are the main binding sites for natural PKC ligands such as phorbol esters.[33,34] Compound **1** exhibited the highest binding affinity for PKCα-C1A with a K_i value of 62 nM (Table 1). On the other hand, the binding affinity of **2** was approximately 30 times weaker than **1**. A similar tendency was observed for the other PKC isozymes. These results indicate that the direction of a side chain is important for binding to PKC C1 domains to control the steric effect between the side chain and β12 loop of the PKC C1 domain. The binding affinity for conventional PKC isozymes of **1** was *ca.* 10–30 and 50–100 times lower than 10-Me-aplog-1 and PDBu, respectively (Table 1). Further structural optimization including the side chain of **1** is necessary. Interestingly,

Table 1 K_i values of **1** and **2** for inhibition of the specific [^{3}H]PDBu binding to PKC C1 peptides

PKC C1 peptides	K_i values (nM)[a]			
	1	**2**	[^{3}H]PDBu (K_d)[b]	10-Me-aplog-1[c]
α-C1A[d]	62 (11)	1800 (290)	1.1	4.7
β-C1A[d]	77 (5.6)	1800 (90)	1.3	12
γ-C1A[d]	150 (3)	4100 (110)	1.5	5.5
δ-C1B[e]	460 (23)	>4000	0.53	0.46
ε-C1B[e]	590 (24)	>7000	0.81	2.0
η-C1B[e]	280 (21)	>4000	0.45	0.45
θ-C1B[e]	520 (45)	>6000	0.72	0.54
Ratio[f]	0.13	—	2.1	10.2

[a] Values in parenthesis are standard deviations. [b] Ref. 33 [c] Ref. 10 [d] Conventional PKC. [e] Novel PKC. [f] K_i for α-C1A/K_i for δ-C1B.

PKC isozyme selectivity was observed, in which the binding affinity of **1** to conventional PKCs was 10 times higher compared to novel PKCs. Most PKC activators are not selective among seven PKC isozymes as exemplified in PDBu, (−)-indolactam-V, ingenol 3-benzoate, and bryostatin 1,[35] while 10-Me-aplog-1,[10] some indolines,[8] and ten-membered analogues[36] of indolactam-V have selectivity toward novel PKC isozymes. In contrast, **1** has a rare selectivity toward conventional PKC isozymes (Table S2 in ESI†). Such rare isozyme selectivity of **1** could not be explained at present since the tertiary structures of PKC C1 domains in complex with a PKC ligand (phorbol 13-acetate) other than PKCδ-C1B were not determined.

In summary, we developed a new PKC ligand **1** with a novel skeleton through *in silico* screening, design, synthesis, and evaluation. Based on molecular modelling of alotaketals with PKCδ-C1B domains, which were selected by *in silico* screening, we designed *trans*-decalin-type analogues and synthesized and evaluated their binding affinity to the C1 domains of all PKC isozymes. The developed analogue **1** showed isozyme selectivity for the C1A domains of conventional PKC isozymes. We are now synthesizing several analogues based on these results to develop more potent ligands with superior selectivity for conventional PKC isozymes.

This work was supported by JSPS KAKENHI Grant No. 17H06405 (to K. I., C. T., and R. C. Y.)

Conflicts of interest

There are no conflicts to declare.

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