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Structural Optimization of the Diarylurea PSNCBAM-1, an Allosteric Modulator of Cannabinoid Receptor 1

Rachel Dopart[1], Sri Sujana Immadi[2], Dai Lu[2], Debra A. Kendall[1,*]

[1] Department of Pharmaceutical Sciences, University of Connecticut, Storrs, Connecticut
[2] Rangel College of Pharmacy, Health Science Center, Texas A&M University, Kingsville, Texas

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ABSTRACT

Background: Structure–activity relationship studies improve the pharmacological and pharmacokinetic properties of a lead compound such as PSNCBAM-1, an allosteric modulator of the cannabinoid receptor 1.
Objectives: Here, several derivatives of PSNCBAM-1 were synthesized with the aim of reducing the number of rings within its structure and enhancing the solubility of the compounds. The derivatives studied contain substituents previously shown to enhance binding of agonists (ie, a cyano group and a pyrimidine ring), with a reduced number of rings compared with the parent compound, PSNCBAM-1.
Methods: The synthesized compounds were tested for the enhancement of the binding of orthosteric cannabinoid receptor 1 agonist CP55,940 in the presence of varying concentrations of each test compound. Select compounds were also tested for their effects on cannabinoid receptor 1 inverse agonist SR141716A binding. The compounds were also subjected to computational analysis of drug-like properties and solubility.
Results: Consistent with a positive allosteric modulator for orthosteric ligand binding, compounds LDK1317 (12a), LDK1320 (12b), LDK1321 (6a), LDK1323 (8a), and LDK1324 (6b) all enhanced the binding of agonist CP55,940 to some degree. Reduction in the number of rings did not abolish the activity. The new lead compounds LDK1317 (12a) and LDK1321 (6a) showed improved drug-like properties and enhanced solubility in silico.
Conclusions: In contrast to PSNCBAM-1, the synthesized compounds are analogs with fewer rings. The compounds LDK1317 (12a) and LDK1321 (6a) contained only 2 or 3 rings, respectively, and showed the binding parameters ($K_B = 110$ nM, $\alpha = 2.3$, and $K_B = 85$ nM, $\alpha = 5.9$). Further, the computationally predicted drug-like properties and solubility suggest these compounds are acceptable new lead compounds for further development of cannabinoid receptor 1 allosteric modulators. (*Curr Ther Res Clin Exp*. 2020; 81:XXX–XXX)

Introduction

The cannabinoid receptor 1 (CB_1) is a rhodopsin-like G protein coupled receptor,[1] and is the most abundant G protein coupled receptor in the brain.[2,3] Orthosteric ligands for CB_1 include the endogenous cannabinoids 2-arachidonyl glycerol and anandamide, as well as the phytocannabinoid Δ^9-tetrahydrocannabinol and synthetic compounds such as the agonist CP55,940 and the inverse agonist SR141716A.[4–10] CB_1 is implicated in pathways involving pain, hunger, emotional state, and neurodegenerative disease, making it an attractive therapeutic target for maladies influencing these pathways.[11–13]

In addition to orthosteric compounds, which bind the site where the endogenous ligands bind, several allosteric modulators for CB_1 have been identified such as ORG27569[14] and PSNCBAM-1.[15] An allosteric modulator binds to a site that is topographically distinct from the orthosteric site.[14,16] There are several advantages to targeting the allosteric site, such as subtype selectivity, spatiotemporal control, pathway selectivity, and a ceiling effect that may minimize overdose risk.[17–20]

PSNCBAM-1 is an allosteric modulator of CB_1 first characterized by Horswill et al[15] in 2007. It displayed properties characteristic of a compound that promotes an active conformation of CB_1 in

* Address correspondence to: Debra A. Kendall, PhD, Department of Pharmaceutical Sciences, 69 N Eagleville Rd, Storrs, CT 06269-3092.
E-mail address: debra.kendall@uconn.edu (D.A. Kendall).

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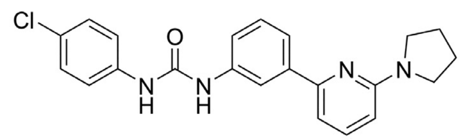
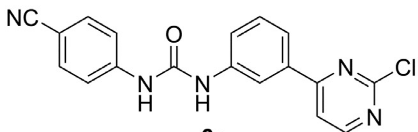


PSNCBAM-1



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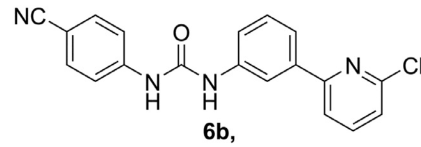

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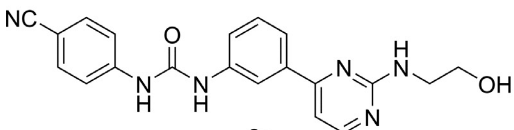

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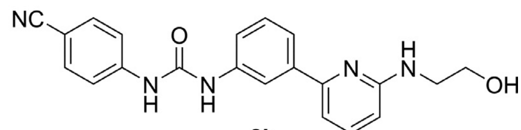

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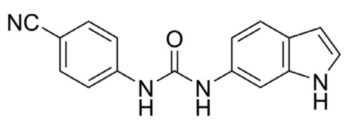

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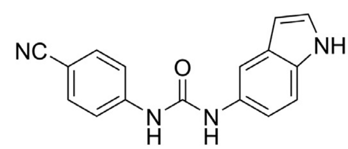

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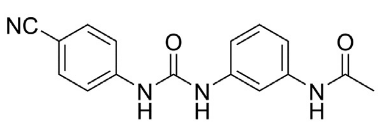
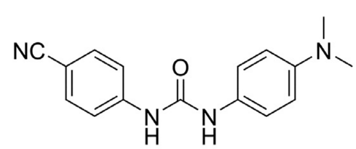


12a, LDK1317



12b, LDK1320

Figure 1. Structures of PSNCBAM-1 and the analogs tested for allosteric modulator binding.

that it enhanced binding of the CB_1 agonist CP55,940 while reducing the binding of the inverse agonist SR141716A.[15] However, PSNCBAM-1 had noncompetitive, inhibitory effects in GTPγS and cAMP assays, and caused reduced food intake and body weight in rats.[15]

Several structure–activity relationship studies have been performed on derivatives of PSNCBAM-1.[21–23] A finding from these studies is that a noncyclic substitution in the 2-pyrrolidinylpyridine position, such as a dimethylamino, is favored.[21] In addition, it was suggested that the electron-withdrawing cyano group may play additional roles such as replacing the water molecule in the receptor-ligand complex, which in turn improved the potency of the modulator compared with the original chloro group of PSNCBAM-1.[21,22] In binding experiments of CB_1 using agonist CP55,940 as the tracer, the half maximal effective concentration (EC_{50}) of the derivative of PSNCBAM-1 that was cyano-substituted was 55 nM, compared with the EC_{50} of PSNCBAM-1, which was 167 nM,[21] suggesting it is important for the cyano group to maintain its position. By shifting the substituent to the meta position, the affinity for CB_1 decreases.[22] The NH group of the urea also appears to be essential for the compound to influence CP55,940 binding.[23] Khurana et al[22] replaced the pyridine ring of PSNCBAM-1 with a pyrimidine ring. Two scaffolds were made where the nitrogens of the pyrimidine ring were in different positions. For both of these newly synthesized PSNCBAM-1 derivatives, they maintained the ability to positively modulate the binding of the orthosteric agonist CP55,940. Although the compounds had a lowered binding affinity, they showed a greater degree of positive cooperativity than the parent compound, indicated by a higher α value.[22] In the lead selection and optimization for central nervous system drug discovery, the preferred number of rings within a structure is up to 3.[24] The lead compound PSNCBAM-1 possesses 4 rings. In this study, several derivatives of PSNCBAM-1 with reduced numbers of rings (ie, 2 or 3) were synthesized and tested for their ability to potentiate orthosteric agonist CP55,940 binding. All compounds in this series have a cyano group that replaces the chloro group of the lead compound PSNCBAM-1, whereas some compounds in this series feature a pyrimidine ring as opposed to the pyridine ring of the lead compound (see Figure 1). Our work in reducing the rings of PSNCBAM-1 to optimize the scaffold met the end points of this study that aim at enhancing the drug-like properties[25] and improving aqueous solubility.

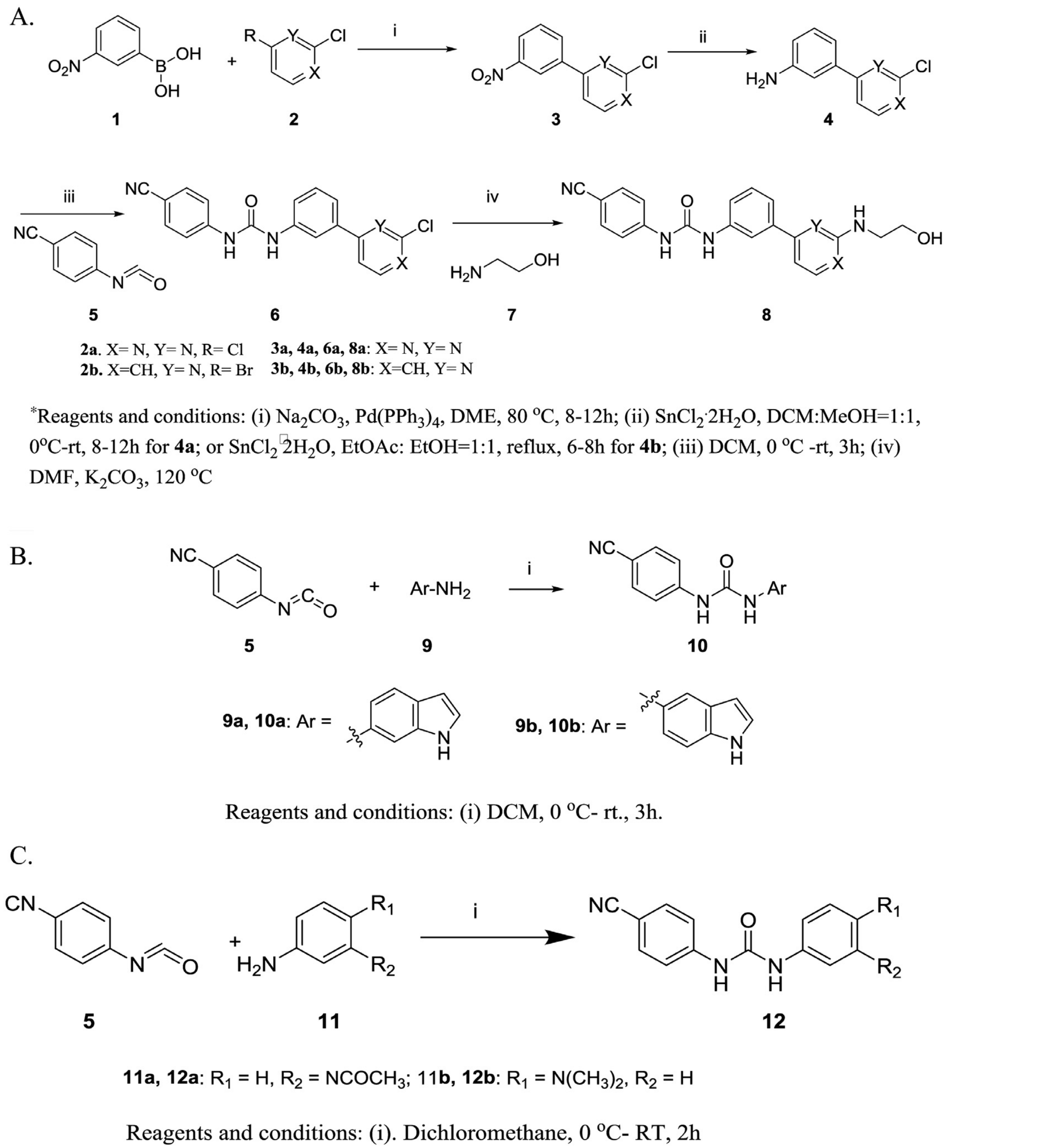


Figure 2. Methods by which target compounds were synthesized. (A) Scheme 1: Synthesis route for pyridinyl and pyrimidinyl biphenyl ureas 6 and 8.* (B) Scheme 2: Synthesis route for diaryl urea 10. (C) Scheme 3: Synthesis route for diaryl urea 12.

Materials and Methods

Compound synthesis

The target compounds were synthesized according to the methods illustrated in schemes A through C of Figure 2. Generally, the target compounds 6, 10, and 12 were prepared from coupling a commercially available (ie, 9) or synthesized arylamine (ie, 4) with 4-Cyanoisothiocyanate isocyanate (ie, 5). To synthesize the target compound 8, the diaryl urea 6 was further reacted with ethanolamine in heated anhydrous *N,N*-dimethyl formaldehyde in the presence of potassium carbonate.

General procedure A for the synthesis of diarylurea compounds (6a/6b, 10a/10b, and 12a/12b)

To the solution of 1.5 mmol of in anhydrous dichloromethane (5–8 mL) was added to the selected isocyanate 5 (1.8 mmol, 1.2 equivalent) at 0°C. The reaction mixture was stirred at 0°C for 10 minutes and then at room temperature for between 2 and 4 hours. The reaction was monitored by thin layer chromatography (30% acetone in hexane or 50%–70% ethyl acetate in hexane). After completion of the reaction, the suspension was filtered. The filtered solid was further washed with dichloromethane (2 mL) and diethyl ether (5 mL) successively and then dried in a vacuum oven to provide the desired compounds.

2-Chloro-4-(3-nitrophenyl)pyrimidine (3a). In a 3-neck round bottomed flask, argon gas was bubbled through a mixture of 3-nitrophenylboronic acid (1, 2.68 g, 16.10 mmol), 2,4-dichloropyrimidine (2a; 2 g, 13.42 mmol), sodium carbonate (4.26 g, 40.26 mmol), dimethoxyethane (80 mL), and water (7 mL) for 20 to 25 minutes. Then the palladium catalyst $Pd(PPh_3)_4$ (1.54 g, 1.34 mmol) was added, and the reaction mixture was refluxed for 12 hours and monitored by thin layer chromatography. Upon completion of the reaction, the mixture was allowed to cool to room temperature and was filtered through a small Celite (Sigma-Aldrich, St. Louis, Missouri) pad. The filtrate was washed with water (2 $\times$20 mL), and the organic compound was extracted with ethyl acetate (3 $\times$ 30 mL). The combined organic phase was washed with water, brine, and dried over sodium sulfate. Filtration and removal of solvent in vacuo provided the crude product, which was purified by silica gel CombiFlash (Teledyne Technologies, Thousand Oaks, California) chromatography (0%–70% dichloromethane in hexane) to afford the compound 3a (1.88 g [59.8%]) as a white solid; mp 147°C to 149°C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.95 (t, J = 1.8 Hz, 1H), 8.78 (d, J = 5.4 Hz, 1H), 8.52 (dt, J = 7.8, 0.9 Hz, 1H), 8.42 (ddd, J = 8.1, 2.1, 0.9 Hz, 1H), 7.74 to 7.79 (m, 2H). MS (ESI): m/z = 236.015 $[M+H]^+$.

2-Chloro-6-(3-nitrophenyl)pyridine (3b). The compound 3b was synthesized from 3-nitrophenylboronic acid (952 mg, 5.75 mmol), 2-bromo-6-chloropyridine 2b (850 mg, 4.42 mmol), sodium carbonate (1.39 g, 13.26 mmol), $Pd(PPh_3)_4$ (508 mg, 0.44 mmol), dimethoxyethane (25 mL), and water (2.3 mL) according to the procedure described for compound 3a. The crude compound was purified by CombiFlash chromatography (0%–30% ethyl acetate in hexane) to afford the compound 3b (540 mg [52.2%]) as white solid; mp 128°C to 129°C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.86 (t, J = 1.9 Hz, 1H, CH), 8.40 (dt, J = 7.8, 1.1 Hz, 1H, CH), 8.31 (ddd, J = 8.2, 2.1, 0.8 Hz, 1H, CH), 7.66 to 7.84 (m, 3H, CH), 7.38 (dd, J = 7.5, 1.0 Hz, 1H, CH). MS (ESI): m/z = 235.020 $[M+H]^+$.

3-(2-Chloropyrimidin-4-yl)aniline (4a). The 3-nitrophenyl) pyrimidine 3a (815 mg, 3.46 mmol) in the mixture of dichloromethane and methanol (1:1), was added stannous chloride dihydrate (4.68 g, 20.76 mmol) and stirred at 0°C for 1 hour and then at room temperature. The reaction was monitored by thin layer chromatography (30% ethyl acetate in hexane). Upon completion of the reaction, it was cooled to room temperature and condensed in vacuo. It was then treated with saturated sodium bicarbonate solution (60 mL) and the solid precipitated out. The solid was filtered under vacuum and the filtrate was extracted with ethyl acetate (2 $\times$ 40 mL). The organic layer was washed with water, brine, and dried over anhydrous sodium sulfate. Filtration and removal of solvent provided the crude solid, which was purified using silica gel CombiFlash chromatography (0%–30% ethyl acetate in hexane) to provide 4a (348 mg [48%]) as a light yellow solid; mp 101°C to 105°C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.63 (d, J = 6.0 Hz, 1H, CH), 7.63 (d, J = 5.3 Hz, 1H, CH), 7.50 (t, J = 3.0 Hz, 1H, CH), 7.40 (dt, J = 9.0, 3.0 Hz, 1H, CH), 7.31 (d, J = 9.0 Hz, 1H, CH), 6.86 (ddd, J = 7.8, 2.4, 0.9 Hz, 1H, CH), 3.9 (s, 2H, NH). MS (ESI): m/z = 206.041 $[M+H]^+$.

3-(6-Chloropyridin-2-yl)aniline (4b). The compound 3-nitrophenyl) pyridine 3b (100 mg, 0.43 mmol) in the mixture of ethyl acetate and ethanol (1:1) was added with stannous chloride dehydrate (298.3 mg, 2.98 mmol) at room temperature. The reaction mixture was then refluxed for 6 to 8 hours and monitored by thin layer chromatography (30% ethyl acetate in hexane). Upon completion of the reaction, the reaction mixture was cooled to room temperature and condensed in vacuo and treated with saturated sodium bicarbonate solution (60 mL) and filtered. The filtrate was then extracted with ethyl acetate (2 $\times$ 40 mL). The combined organic layer was washed with water, brine, and dried over anhydrous sodium sulfate. Filtration and removal of solvent provided the crude solid, which was purified using silica gel CombiFlash chromatography (0%–30% ethyl acetate in hexane) to provide 4b (68 mg [77%]) as a light yellow solid; mp 76°C to 80°C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.62 to 7.73 (m, 2H, CH), 7.42 (t, J = 1.8 Hz, 1H, CH), 7.23 to 7.34 (m, 3H, CH), 6.77 (dd, J = 7.8, 1.2 Hz, 1H, CH), 3.84 (brs, 2H, NH). MS (ESI): m/z = 205.045 $[M+H]^+$.

1-(3-(2-Chloropyrimidin-4-yl)phenyl)-3-(4-cyanophenyl)urea (6a, LDK1321). The compound 6a was synthesized from amine 4a (308.46 mg, 1.5 mmol), and the 4-cyanophenyl isocyanate 5 (259.43 mg, 1.8 mmol) in anhydrous dichloromethane according to general procedure A (see Figure 2). The crude compound was purified using CombiFlash chromatography (0%–50% ethyl acetate in hexane) to provide product 6a (302 mg [57%]) as a white solid; mp 241°C to 243°C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.79 (d, J= 5.4 Hz, 1H), 8.73 (s, 1H), 8.63 (s, 1H), 8.41 (t, J = 1.9 Hz, 1H), 8.01(d, J = 5.4 Hz, 1H), 7.68-7.88 (m, 6H), 7.52 (t, J = 7.9 Hz, 1H). MS (ESI): m/z = 350.073 $[M+H]^+$.

1-(3-(6-Chloropyridin-2-yl)phenyl)-3-(4-cyanophenyl)urea (6b, LDK13 24). The compound 6b was synthesized from amine 4b (145 mg, 0.71 mmol), and 4-cyanophenyl isocyanate 5 (122.5 mg, 0.85 mmol) in anhydrous dichloromethane according to general procedure A (see Figure 2). The crude compound was purified using CombiFlash chromatography (0%–50% ethyl acetate in hexane) to provide product 6b (180 mg [72%]) as a white solid; mp 180°C to 184°C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.20 (s, 1H, NH), 9.13 (s, 1H, NH), 8.19 (t, J = 1.8 Hz, 1H, CH), 7.96 (d, J = 3.3 Hz, 1H, CH), 7.95 (s, 1H, CH), 7.57 to 7.76 (m, 6H, CH), 7.45 (dd, J = 5.9, 2.4 Hz, 1H, CH), 7.42 to 7.47 (m, 1H, CH). MS (ESI): m/z = 349.078 $[M+H]^+$.

1-(4-Cyanophenyl)-3-(3-(2-((2-hydroxyethyl)amino)pyrimidin-4-yl) phenyl)urea (8a, LDK1323). The solution of 6a (50 mg, 0.14 mmol) in anhydrous dimethylformamide (2.5 mL) was added to ethanolamine 7 (17.71 mg, 0.29 mmol) and potassium carbonate (40 mg, 0.29 mmol). The reaction mixture was stirred and heated at 110°C for 8 hours. The reaction was monitored by thin layer chromatography (10% methanol in dichloromethane). Upon completion of the reaction, it was cooled to room temperature and quenched by adding 30 mL water and extracted with ethyl acetate (3 $\times$ 12 mL). The organic layer was washed with water, brined, and dried over anhydrous sodium sulfate. Filtration and removal of the solvent *in* vacuo provided the crude compound. The crude compound was purified using CombiFlash chromatography (0%–10% methanol in dichloromethane) to provide the product 8a (37 mg [70%]) as a light yellow solid; mp 189°C to 193°C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.27 (s, 1H), 9.04 (s, 1H), 8.35 (d, J = 5.1 Hz, 1H), 8.18 (s, 1H), 7.76 - 7.64 (m, 6H), 7.43 (t, J = 6.0 Hz, 1H), 7.07 (d, J = 4.5 Hz, 1H), 4.71 (t, J= 5.4 Hz, 1H), 3.59 (brs, 2H), 3.42 (brs, 2H). MS (ESI): m/z = 375.149 $[M+H]^+$.

1-(4-Cyanophenyl)-3-(3-(6-((2-hydroxyethyl)amino)pyridin-2-yl)phenyl)urea (8b, LDK1325). The compound 8b was synthesized from 6b (100 mg, 0.29 mmol) and ethanolamine 7 (35 mg, 0.58 mmol) in anhydrous dimethylformamide (5 mL) in the presence of potassium carbonate (80 mg, 0.58 mmol) according to the procedure for 8a. The crude compound was purified by CombiFlash chromatography (0%–10% methanol in dichloromethane) to provide product 8b (25 mg [23%]) as a light yellow solid; mp 225°C to 230°C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.46 (s, 1H, NH), 9.14 (s, 1H, NH), 8.04 (s, 1H, CH), 7.32-7.75 (m, 8H), 6.97 (d, J = 6.0 Hz, 1H), 6.56 (t, J = 5.1 Hz, 1H), 6.46 (d, J = 8.3 Hz, 1H), 4.76

(t, J = 5.2 Hz, 1H, OH), 3.59 (brs, 2H), 3.42 (brs, 2H). MS (ESI): m/z = 374.154 $[M+H]^+$.

1-(4-Cyanophenyl)-3-(1H-indol-6-yl)urea (10a, LDK1319). The compound 10a was synthesized from amine 9a (50 mg, 0.38 mmol), and 4-cyanophenyl isocyanate 5 (65 mg, 0.45 mmol) in anhydrous dichloromethane according to the general procedure A (see Figure 2). The pure product was obtained as a white solid (97 mg [92%]); mp 235°C to 238°C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.97 (s, 1H), 9.73 (s, 1H), 8.74 (s, 1H), 7.62 to 7.78 (m, 5H), 7.42 (d, J = 8.4 Hz, 1H), 7.24 (s, 1H), 6.86 (dd, J = 8.5, 1.7 Hz, 1H), 6.35 (s, 1H). MS (ESI): m/z = 277.101 $[M+H]^+$.

1-(4-Cyanophenyl)-3-(1H-indol-5-yl)urea (10b, LDK1318). The compound 10b was synthesized from amine 9b (50 mg, 0.38 mmol), and 4-cyanophenyl isocyanate 5 (66 mg, 0.46 mmol) in anhydrous dichloromethane according to the general procedure A (see Figure 2). The pure product was obtained as a white solid (96 mg [91.4%]); mp 238°C to 240°C. ^{1}H NMR (300 MHz, DMSO-d_6): δ10.99 (s, 1H, NH), 9.11 (s, 1H, NH), 8.60 (s, 1H, NH), 7.62 to 7.73 (m, 5H, CH), 7.30 to 7.33 (m, 2H, CH), 7.08 (dd, J = 8.7, 1.6 Hz, 1H, CH), 6.36 (s, 1H, CH). MS (ESI): m/z = 277.101 $[M+H]^+$.

N-(3-(3-(4-Cyanophenyl)ureido)phenyl)acetamide (12a, LDK1317). The biphenyl urea 12a was synthesized from *N*-(3-aminophenyl)acetamide 11a (0.050 g, 0.33 mmol) and 4-isocyanatobenzonitrile 5 (0.056 g, 0.39 mmol) in 8 mL anhydrous dichloromethane according to the general procedure A (see Figure 2). The crude product was purified by trituration with diethyl ether to provide 12a (85 mg [87.5%]) as a yellow solid; mp 240°C to 243°C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.94 (s, 1H), 9.10 (s, 1H), 8.90 (s, 1H), 7.77 (s, 1H), 7.72 (d, J = 8.55 Hz, 2H), 7.62 (d, J = 8.55 Hz, 2H), 7.20-7.16 (m, 3H), 2.02 (s, 3H). MS (APCI): m/z = 295.11 $[M+H]^+$.

1-(4-Cyanophenyl)-3-(4-(dimethylamino)phenyl)urea (12b, LDK1320). The biphenyl urea 12b was synthesized from dimethyl aniline 11b (0.1 g, 0.73 mmol) and 4-isocyanatobenzonitrile 5 (0.115 g, 0.80 mmol) in 10 mL dichloromethane according to the general procedure A (see Figure 2). The crude product was purified by multiple titrations with diethyl ether to provide 12b (85 mg [87.5%]) as a white solid; mp 219°C to 221°C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.06 (s, 1H), 8.49 (s, 1H), 7.59 to 7.77 (m, 4H), 7.25 (d, J = 8.94 Hz, 2H), 6.70 (d, J = 9.03 Hz, 2H), 2.83 (S, 6H). MS (APCI): m/z = 281.13 $[M+H]^+$.

Receptor expression and membrane preparation

Human embryonic kidney 293T cells were seeded at 1,000,000 cells/100-mm plate, and grown in Dulbecco's modified Eagle's medium containing 10% fetal bovine serum, and 3.5 mg/mL glucose. They were incubated at 37°C with 5% carbon dioxide. The next day, cells were transfected with 20 µg human CB_1 receptor cloned into pcDNA3.1 using the calcium phosphate method.[26] Transfected cells were harvested and the membranes were prepared as previously described[27] 21 hours after transfection.

Equilibrium binding assays

CB_1-expressing membrane preparations (5 µg) were incubated with 9 concentrations of the allosteric modulator (1 nM–10 µM). In each reaction, [^{3}H]CP55,940 (150.2 Ci/mmol; Perkin Elmer, Boston MA), a radiolabeled tracer that is an orthosteric agonist of CB_1, was also added at 0.5 nM. Or, for select allosteric compounds (LDK1321, LDK1323, or LDK 1324), [^{3}H]SR141716A (56 Ci/mmol; Perkin Elmer), a radiolabeled tracer that is an orthosteric inverse agonist of CB_1, was added at a concentration of 1 nM instead of the orthosteric agonist. Nonspecific binding was determined by treatment of the membranes with 10 µM of either untritiated CP55,940 (Bio-Techne, Minneapolis, Minnesota) or untritiated SR141716A (Bio-Techne, Minneapolis, Minnesota). Membranes were incubated for 60 minutes at 30°C. The reaction was terminated by the addition of 300 µL Tris-Mg2+-EDTA buffer with 5% bovine serum albumin. Harvesting of the mixture was performed with a Brandel cell (Brandel, Gaithersburg, MD) harvester with Whatman GF/C filter paper (Brandel, Gaithersburg, MD). Measurement of bound radioactivity was performed using liquid scintillation counting.

Table 1
Binding parameters of PSNCBAM-1 analogs.*

Compound code	K_B (nM)	α
PSNCBAM-1	55 (26–120)	3.6 (2.6–6.1)
6a, LDK1321	85 (41–180)	5.9 (2.8–12)
6b, LDK1324	300 (150–630)	5.6 (3.4–9.4)
8a, LDK1323	200 (19–1900)	2.4 (1.4–4.2)
8b, LDK1325	NB	NB
10a, LDK1319	NB	NB
10b, LDK1318	NB	NB
12a, LDK1317	110 (24–490)	2.3 (1.6–3.3)
12b, LDK1320	830 (240–3000)	5.4 (2.7–11)

α = cooperativity factor for the allosteric modulator tested; K_B = equilibrium dissociation; NB = no detectable binding of the orthosteric agonist [^{3}H]CP55,940 in the presence of the test compound up to 10 µM.

* The allosteric parameters, K_B and α, were determined using [^{3}H]CP55,940 as the orthosteric ligand. Values are presented with 95% CI in parentheses.

Data analysis

The binding data collected were subjected to nonlinear regression, fitted to a log (dose) versus response curve to determine the EC_{50} using Prism 7.02 (Graphpad Software, La Jolla, California). Binding analysis was performed in the graphs as the mean (SE) (error bars) and summarized in Table 1 as the corresponding 95% confidence limits. The physicochemical properties, including solubility were obtained from computational prediction using the ChemAxon program (Chemicalize, San Diego, California).

Results and Discussion

The compounds reported in this study (Figure 1) were generated to explore whether reducing the number of rings within the scaffold of PSNCBAM-1 was possible and to identify lead compounds with improved drug-like properties. Eight analogs of PSNCBAM-1 and the parent compound were tested using equilibrium binding for their ability to enhance binding of the CB_1 agonist CP55,940. For comparison, PSNCBAM-1 was tested and consistent with the literature value,[22] K_B = 55 nM. The cooperativity factor, α = 3.6, indicates positive cooperativity with agonist CP55,940 (Figure 3A and Table 1).

All 8 analogs also featured a cyano group, which was previously shown to enhance the allosteric properties of PSNCBAM-1–derived compounds.[22] Of these derivatives, 3 compounds displayed no binding: LDK1318 (10b), LDK1319 (10a), and LDK1325 (8b). The compounds LDK1318 (10b) and LDK1319 (10a) were structurally similar in that both featured an indole ring (Figure 1). LDK 1323 (8a) and LDK1325 (8b) featured an ethanolamine attached to the pyrimidine or pyridine ring, respectively (Figure 1). All of these compounds were optimized from PSNCBAM-1 with reduced number of rings (2 or 3 rings), which is preferred for therapeutic agents used in the central nervous system.[24]

Compounds LDK1321 (6a) and LDK1324 (6b) were designed to retain the 3 aromatic rings of PSNCBAM-1, whereas its pyrrolidinyl ring was removed. LDK1321 (6a) showed a K_B = 85 nM and α = 5.9 suggesting that this compound retained key features of receptor

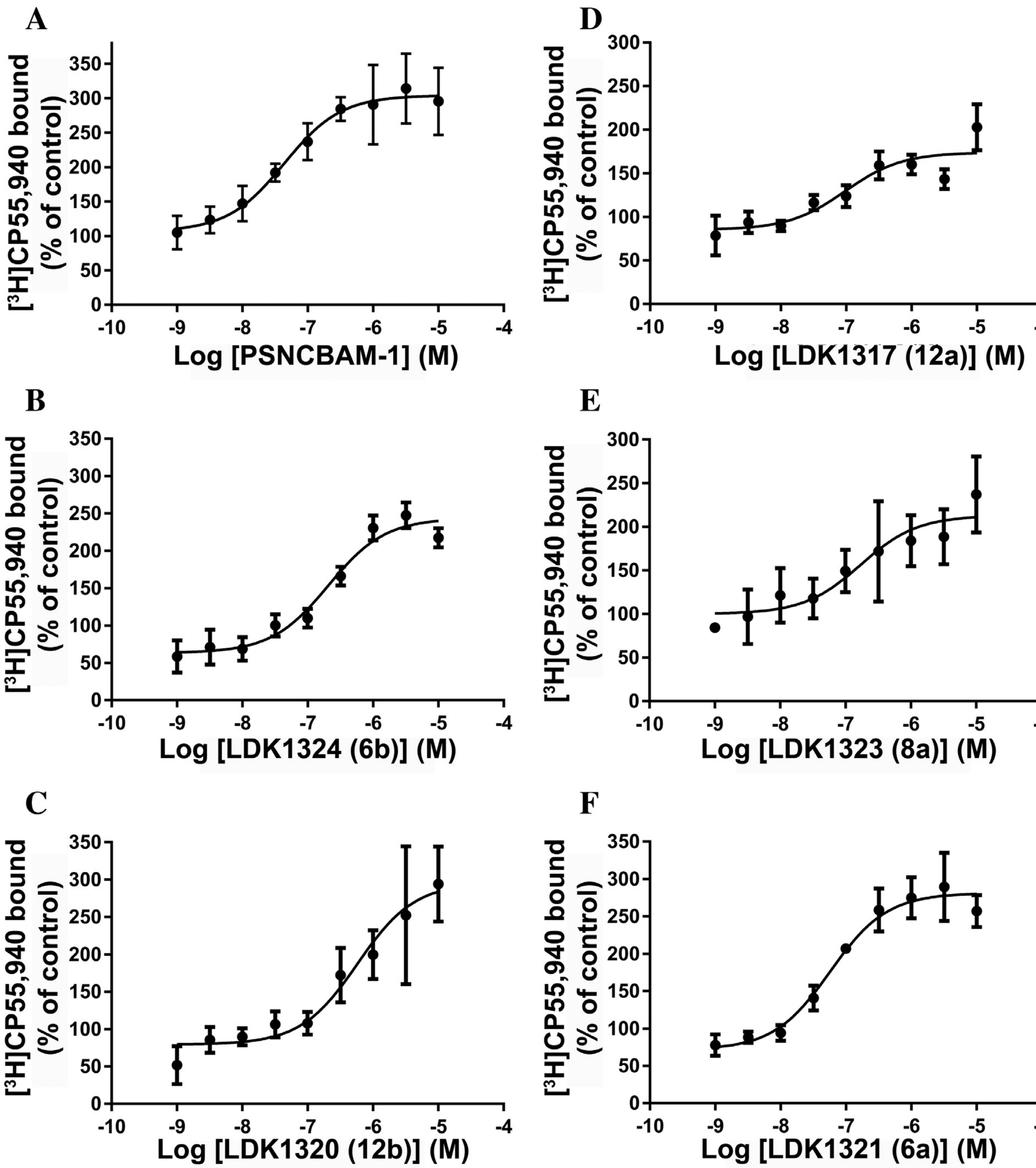


Figure 3. The influence of allosteric modulators on orthosteric agonist [^{3}H]CP55940-specific binding to cannabinoid receptor 1. Shown are binding assays with [^{3}H]CP55940 as a tracer with varying concentrations of (A) PSNCBAM-1, (B) LDK1324 (6b), (C) LDK1320 (12b), (D) LDK1317 (12a), (E) LDK1323 (8a), and (F) LDK1321 (6a). Results determined from at least 3 experiments performed in duplicate, and data are presented as the mean (SE) (error bars).

modulation of PSNCBAM-1 (Table 1). LDK1324 (6b) maintained the pyridine ring PSNCBAM-1 and also featured a chlorine substituent (Figure 1), and enhanced binding of CP55,940 (Figure 3B). In the CP55,940 binding experiments, the $K_B = 300$ nM and the cooperativity factor, $\alpha = 5.6$, indicative of positive cooperativity (Table 1). Although the K_B is higher than that of the parent compound, PSNCBAM-1 (55 nM), the cooperativity factor for LDK1324 (6b) is >1, indicating that this compound maintains the ability to positively enhance the binding of CP55,940 (Table 1).

LDK1320 (12b) featured a dimethylamino group that replaced the 2 rings (ie, the pyridine ring and the pyrrolidine ring) (Figure 1). LDK1320 (12b) enhanced binding of agonist CP55,940 (Figure 3C), and showed positive cooperativity, as indicated by its $\alpha = 5.4$ (Table 1). However, the $K_B = 830$ nM; thus, the binding affinity is weaker than PSNCBAM-1 (Table 1). Replacement of the pyridine ring (Figure 1) with an acetamide group maintains some enhancement of binding of CP55,940 as with LDK1317 (12a), which exhibited with a $K_B = 110$ nM and an $\alpha = 2.3$ (Figure 3D and Table 1). Results from testing these 2 compounds indicate that diarylureas that possess only 2 aromatic rings may still be capable of binding the allosteric site and cause positive binding cooperativity.

Compound LDK1323 (8a) featured a pyrimidine ring in place of the pyridine ring of the parent compound, and also had an ethanolamine group attached to the pyrimidine ring (Figure 1).

Table 2
Calculated physicochemical properties and solubility of the synthesized analogs of PSNCBAM-1.*

Compound code	Rings	Lipinski rule of 5 satisfaction	Log D (pH = 7.4)	Log D (pH = 1.7)	Log P (pH = 7.4)	Intrinsic solubility† (mg/mL) (pH = 7.4)
PSNCBAM-1	4	No	5.64	3.69	5.64	0.000203
6a, LDK1321	3	Yes	3.99	3.97	3.99	0.000207
6b, LDK1324	3	Yes	4.61	4.59	4.61	0.000219
8a, LDK1323	3	Yes	2.55	1.14	2.55	0.00381
8b, LDK1325	3	Yes	3.15	1.21	3.15	0.00402
10a, LDK1319	3	Yes	0.94	0.94	0.94	0.00758
10b, LDK1318	3	Yes	0.94	0.94	0.94	0.00758
12a, LDK1317	2	Yes	0.08	0.08	0.08	0.0493
12b, LDK1320	2	Yes	0.95	0.99	0.95	0.0873

* The parameters were obtained from computational prediction using ChemAxon (Chemicalize, San Diego, California).
† The intrinsic solubility is the equilibrium solubility of the compound at the pH where it is fully unionized.

This compound has a modest, but positive influence on the binding of agonist CP55,940 (Figure 3E). The compound in this series with both the lowest K_B (85 nM) and the highest cooperativity factor (5.9) was LDK1321 (6a) (Table 1). This compound, like LDK1323 (8a), has a pyrimidine ring instead of the pyridine ring of PSNCBAM-1, but with a chlorine replacing the pyrrolidine group (Figure 1), and was successful in enhancing the binding of agonist CP55,940 (Figure 3F).

Select compounds were also tested for their ability to decrease the binding of the inverse agonist SR141716A. LDK1323 (8a) displayed a modest decrease in SR141716A binding, whereas LDK1321 (6a) and LDK1324 (6b) demonstrated a robust, dose-dependent decrease of SR141716A binding (Figure 4A–C). All compounds with this orthosteric inverse agonist had K_B values in the micromolar range (1.4, 6.8, and 1.8 µM for LDK1321 (6a), LDK1323 (8a), and LDK1324 (6b), respectively), and cooperativity factors <1, which is indicative of negative binding cooperativity (Figure 4). This negative cooperativity of binding with an inverse agonist is a characteristic of other positive allosteric modulators of CB_1, including PSNCBAM-1.[14,15] Because the positive allosteric modulator stabilizes CB_1 in an activated form that enhances CP55,940 binding, one would expect the modulator to have a lower affinity for an inverse agonist (eg, SR141716A) in its presence relative to its absence and a negative cooperativity factor. As one would expect, SR141716A binding is decreased as one employs a higher concentration of allosteric modulator (Figure 4A–C).

For those where there is binding, it is established by the binding assay to CP55,940 with allosteric modulator that there is positive cooperativity. That is expected for an activated receptor. That the CP55,940 (agonist) binding has an $\alpha > 1.0$ argues that the allosteric modulator activates the receptor and therefore is positive allosteric modulator-like. That SR141716A (inverse agonist) binds an activated receptor less well and the $\alpha < 1.0$ agrees with that.[28]

To investigate whether the structural optimization improves the drug-like properties, the compounds shown in Table 1 were assessed with a computational program for their drug-like properties and solubility. The results are shown in Table 2. Compound LDK1317 (12a) ($K_B = 110$ nM, $\alpha = 2.3$) and LDK 1321 (6a) ($K_B = 85$ nM, $\alpha = 5.9$) can serve as lead compounds for further development of allosteric modulators from the diarylurea scaffold. Based on the computationally calculated drug-like properties of these molecules in Table 2, reducing the number of rings within the structures of the diarylurea analogs makes these satisfy the Lipinski rule of 5 and leads to improvement of the distribution constant (LogD) and intrinsic solubility of the compounds (Table 2). The calculated LogD values obtained in 2 different pH conditions (pH 7.4 and pH 1.7) indicates that the synthesized compounds could be ionized in acidic media (eg, the stomach) except compounds LDK1319 (10a) and LDK1318 (10b). The calculated LogD and LogP values obtained at pH = 7.4 are identical. This indicated that the compounds

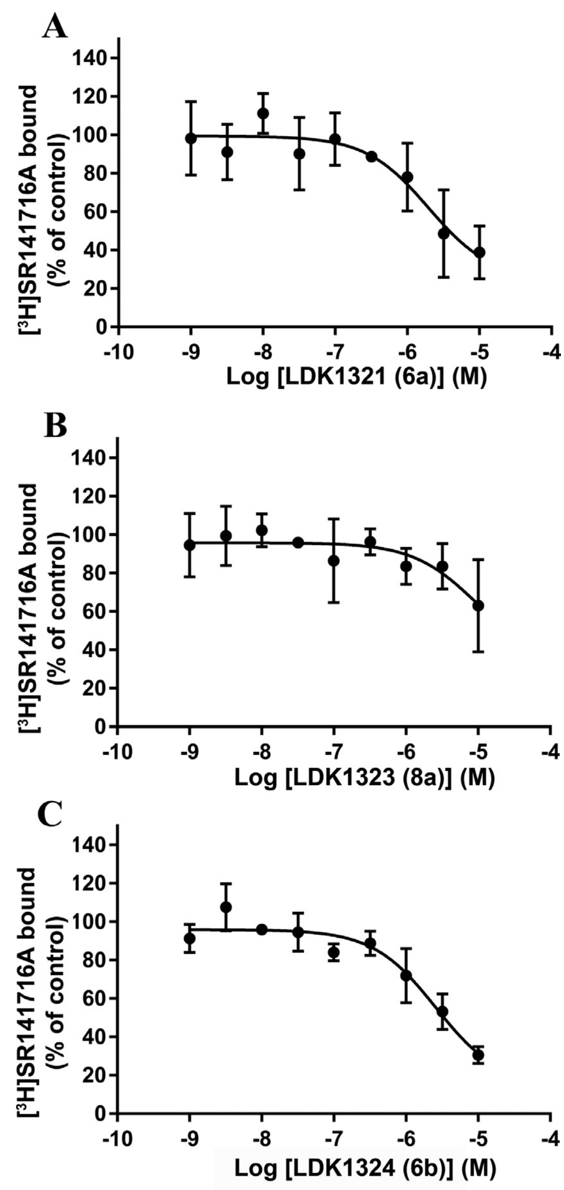


Figure 4. The influence of allosteric modulators on orthosteric inverse agonist [^{3}H]SR141716A-specific binding to cannabinoid receptor 1. Shown are equilibrium binding assays with [^{3}H]SR141716A as a tracer with varying concentrations of (A) LDK1321 (6a), (B) LDK1323 (8a), and (C) LDK1324 (6b). Results are determined from at least 3 experiments performed in duplicate, and data are presented as the mean (SE) (error bars).

are in neutral unionized forms in aqueous media at pH 7.4 (eg, the blood). Introducing ethanolamine into compounds LDK1321 (6a) and LDK1324 (6b) enhanced the calculated intrinsic solubility (ie, LDK1323 [8a] and LDK1325 [8b]) by approximately 20-fold with the cost that LDK1325 (8b) lost its binding affinity for the allosteric site. It is noteworthy that reducing the number of rings of diarylurea analogs to 2 significantly enhanced intrinsic solubility by about 400-fold (ie, PSNCBAM-1, 0.000203 mg/mL vs LDK1320 (12b), 0.0873 mg/mL) (Table 2).

Conclusions

In this preliminary study, it was found that that reducing the number of rings within the scaffold of PSNCBAM-1 is a viable approach to generate novel lead compounds for developing allosteric modulators of the CB_1 receptor. Fewer rings likely holds the key for improving the drug-like properties and solubility. By continuing structure–activity relationship studies based on the allosteric modulator scaffold of PSNCBAM-1, we can develop new lead compounds with improved drug-like properties that target the pharmacologically important CB_1 receptor.

Declaration of Competing Interest

The authors have indicated that they have no conflicts of interest regarding the content of this article.

Author Contributions

RD, SSI, DL and DAK participated in the research design. RD and SSI conducted experiments. RD, SSI, DL, and DAK analyzed the data. RD, SSI, DL and DAK contributed to the writing of the manuscript.

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