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Gram-Scale Laboratory Synthesis of UM171, a Potent Agonist of Human Hematopoietic Stem Cell Self-Renewal

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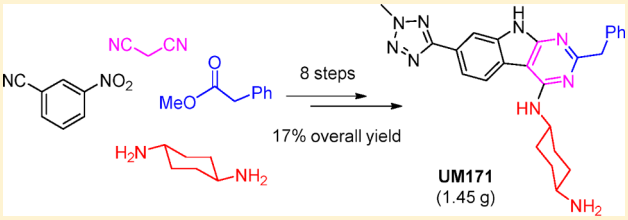
Supporting Information

ABSTRACT: A short economic synthesis of pyrimidoindole derivative UM171, a potent agonist for the ex vivo expansion of hematopoietic stem cells, has been developed in this work. A unique [3,3]-sigmatropic rearrangement upon a hydroxamic precursor followed by base-promoted cyclization was successfully applied as the key step, furnishing the fully functionalized indole nucleus. The current synthesis is not only capable of affording UM171 in gram quantities but also offers great flexibility in late-stage diversification. Three new analogues of UM171 were synthesized accordingly in excellent yields using the common indole-carboxamide intermediate.



INTRODUCTION

Allogeneic hematopoietic stem cell (HSC) transplant is an effective therapeutic protocol for numerous hematologic malignancies.[1] However, a certain ratio of patients are burdened by the absence of an identical human leukocyte antigen (HLA) donor and thus will be ruled out from the therapy, and it is almost impossible to find a healthy source of donor tissue that is immunologically compatible.[2] Fortunately, cord blood (CB) transplants have solved this problem by offering several advantages, such as the reduced need for HLA matching, thereby extending transplantation availability to nearly all patients, and the decreased risk of chronic graft-versus-host disease, the most important determinant of long-term quality of life in transplant patients.[3] On the other hand, CB transplants suffer from limited progenitor cell doses, leading to delayed neutrophil engraftment and increased mortality; thus, ex vivo growth of CB HSCs is important for clinical demand.[4] These challenges can be addressed by finding the provision of an unlimited and renewable source of functional hematopoietic stem cells from a variety of backgrounds to be used in as replacements of primary tissues.

Pyrimidoindoles continue to serve as a privileged type of heterocycle in drug discovery due to the frequent presence as part of biological interactions. Because they exhibit a broad spectrum of biological properties, this fascinating family has received significant attention in biomedical research. For example, a variety of medicinal materials using pyrimidoindole building blocks[5,6] were successfully applied in neuroprotective,[7] anti-inflammatory,[8] antihypertensive,[9] and tyrosine kinase inhibition.[10] More importantly, some pyrimidoindole derivatives were recently found to show exciting agonistic effects on self-renewal of hematopoietic stem cells. After the discovery of purine derivative StemReginine 1 (SR1), a breakthrough in HSC research was made by Fares and co-workers in 2014, revealing that pyrimidoindole derivative UM171 (**1**, Figure 1) enables

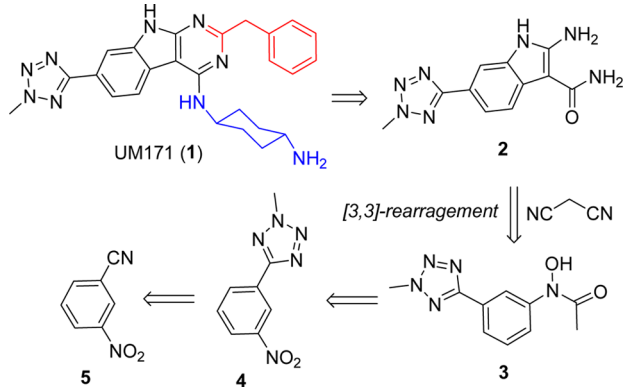


Figure 1. New design for the synthesis of UM171 (**1**).

significant ex vivo expansion of cord cells and thus enhances the ability of HSC self renewal.[11] The reported synthesis of UM171 started from relatively expensive materials and employed a number of strong acidic conditions, which led to low-yield generation of key carboxamide intermediate **2** (see Figure 1) and eventually resulted in poor accessibility of the synthesis and high cost of the final product.[12] UM 171 is currently available through several commercial suppliers at a very high price (~1 mg for 1000 USD).[13] To acquire more economic material of UM171 for various stem cell investigations, in this work we successfully developed a new short economic gram-scale synthesis of UM171 in a much higher overall yield in which pivotal carboxamide intermediate **2** was prepared through a unique [3,3]-sigmatropic rearrangement with much higher efficiency.

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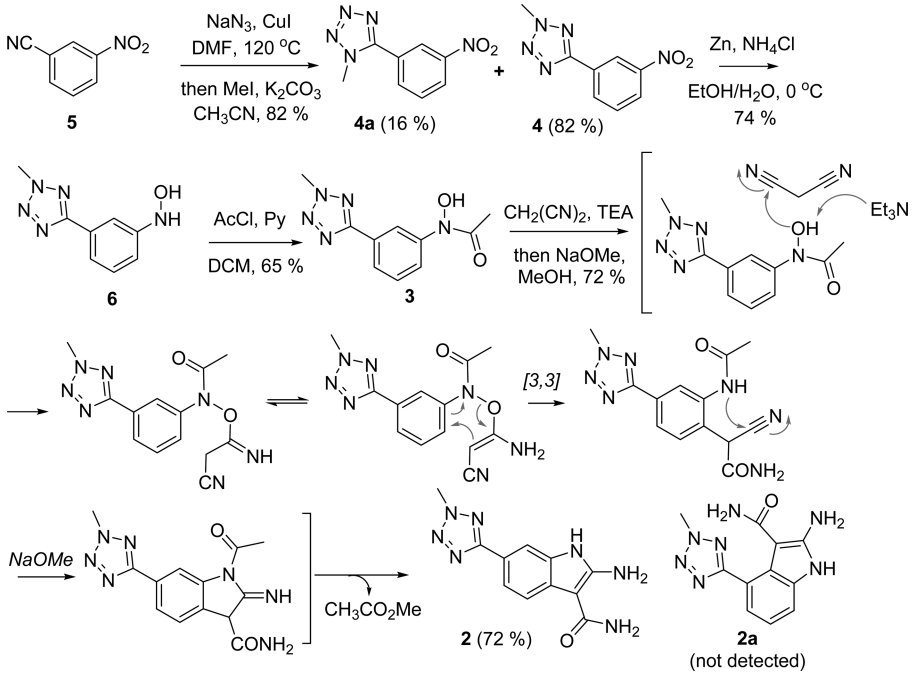
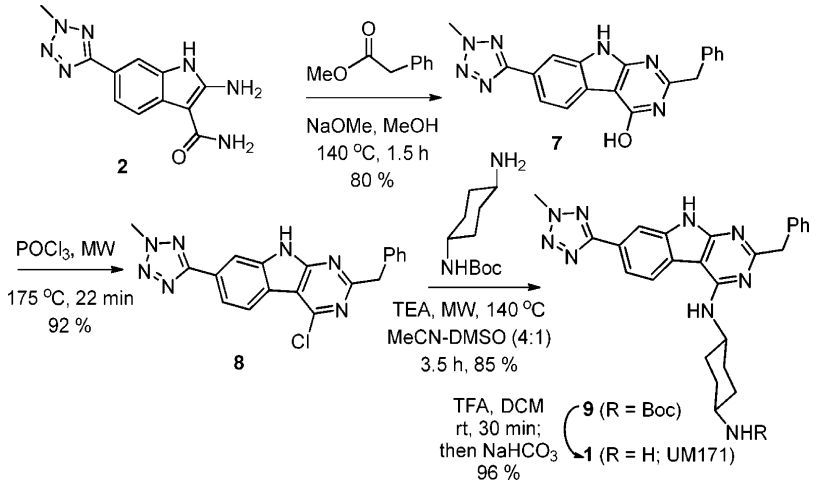
Scheme 1. Synthesis of the Fully Functionalized Indole Nucleus 2



Scheme 2. Completion of the Synthesis of UM 171 (1)



RESULTS AND DISCUSSION

Our retrosynthetic analysis is shown in Figure 1. It was envisioned that the pivotal carboxamide **2** could be achieved from the corresponding hydroxamic acid **3** via a unique [3,3]-sigmatropic rearrangement in the presence of malonitrile (see text below for more details).[14] Compound **3** could be prepared from nitrobenzene derivative **4** by a partial reduction.[15] The essential tetrazole moiety was designed to be generated by a CuI-catalyzed cycloaddition of the commercially available inexpensive materials 3-nitrobenzoonitrile (**5**) and sodium azide.[16]

The new synthesis of UM171 (**1**) commenced with the preparation of carboxamide **2** (Scheme 1). Treatment of 3-nitrobenzonitrile (**5**) with sodium azide and a catalytic amount of CuI[16] followed by *N*-alkylation with iodomethane and potassium carbonate afforded N^2-methyltetrazole **4** (82%) and the other minor N^1-methyl-tetrazole isomer **4a** (16%), which were elucidated by corresponding ^{1}H NMR and NOESY experiments. Partial reduction of the nitro group in **4** into the corresponding hydroxylamine functionality was achieved by careful treatment of **4** with zinc and aqueous ammonium chloride at 0 °C, affording hydroxylamine **6** in 74% yield. It is noteworthy here that the control of the reaction temperature at 0 °C was very important for this transformation, as higher reaction temperature (above 0 °C) led to an over-reduction aniline product. For the *O*,*N*-diacetylated byproduct in selective *N*-acetylation of **6** with acetyl chloride to be minimized, the reaction conditions were carefully optimized, including adjustment of the reaction temperature and additional time lengths of acetyl chloride (through a mechanical pump). Finally, mono-*N*-acetylation of **6** was accomplished, giving expected hydroxamic acid **3** in 65% yield. The crucial transformation of *N*-acetylated hydroxylamine **3** into fully functionalized carboxamide **2** was carried out by the reaction with malonitrile in the presence of triethylamine (via a unique [3,3]-sigmatropic rearrangement)[14] followed by treatment with sodium methoxide under reflux (intramolecular cyclization), affording **2** (72%) as the sole product. The absence of the other

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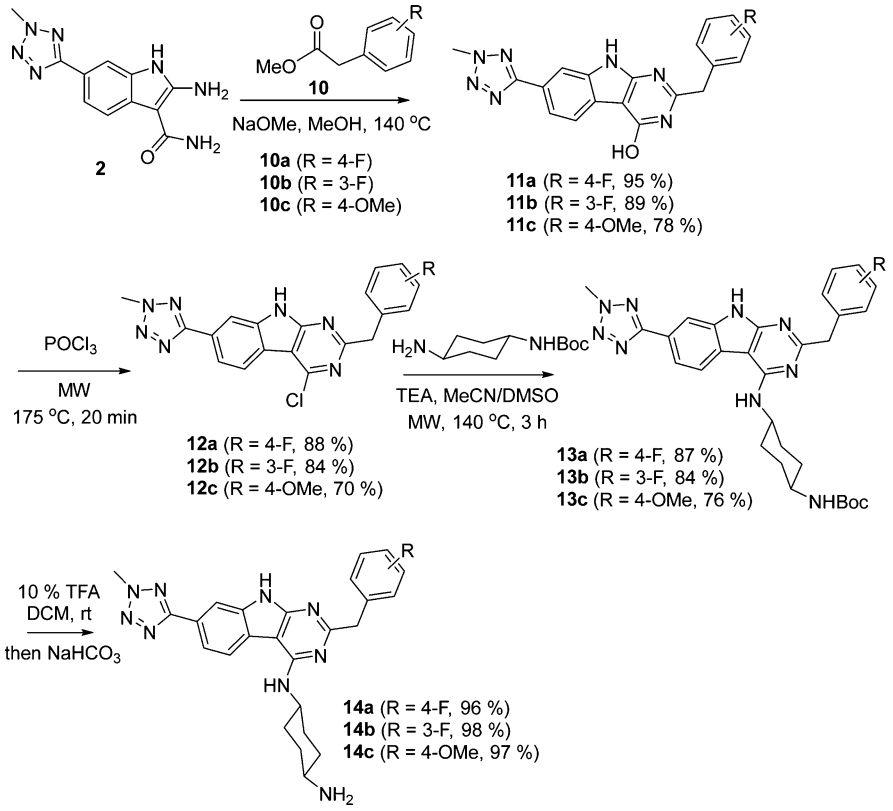
Scheme 3. Representative Synthesis of New Analogues 14a−14c



possible regioisomer **2a** in this cyclization reaction might be due to the unfavorable hindrance caused by the *ortho*-tetrazole moiety.

The conversion of **2** into UM171 (**1**) was completed using a modified procedure[12] in which microwave irradiation conditions were applied to shorten reaction times as well as reduce the byproducts in several steps (Scheme 2). Condensation of indole-carboxamide **2** with methylphenylacetate was carried out in methanol by the aid of sodium methoxide, giving hydroxypyrimidoindole **7** in 80% yield. Chlorination of **7** was carried out by treatment with phosphorus oxychloride[17] under microwave irradiation at 175 °C for 25 min, providing chlorinated pyrimidoindole **8** in 92% yield. The subsequent substitution of **8** with mono *N*-Boc-protected cyclohexane-1,4-diamine was also carried out in the presence of trimethylamine under microwave irradiation at 140 °C for 3.5 h in a mixture of MeCN-DMSO (4:1).[18] Our initial attempts in methanol or DMF at various temperatures suffered from poor conversion, low yield of the product, and the production of side products.[19] Finally, application of a mixture of acetonitrile and DMSO (4:1) solved the problem of substrate solubility and thus significantly improved the reaction, affording pyrimidoindole **9** in 85% yield. Final removal of the *N*-Boc group in **9** was carried out by treatment with 10% TFA in DCM and then workup with aqueous $NaHCO_3$, giving UM171 (**1**, as a free base) in 96% yield (overall yield of 17% in 8 steps).

As the newly established synthesis is capable of providing a relatively large quantity of indole-carboxamide intermediate **2**, a further late-stage modification of UM171 became much easier and more practical. To take advantage of the flexibility of this synthesis, we synthesized three new representative analogues **14a−14c** with variable substituent(s) on the phenyl group of UM171 (Scheme 3). These final products were provided in good to excellent yields from the common carboxamide **2**. Undoubtedly, this new synthesis of UM171 is qualified to serve as a discovery tool for future generation of new analogues of UM171.

■ CONCLUSIONS

In summary, we have developed a short, economic, and flexible synthesis of UM171, an extremely potent agonist of human hematopoietic stem cell self-renewal. A unique [3,3]-sigmatropic rearrangement followed by a basic cyclization has been successfully applied as the key step to furnish the fully functionalized indole nucleus. This synthesis is advantageous to afford UM171 in gram quantities and flexible for late-stage diversification upon the easily available common indole intermediate. Three representative new analogues of UM171 were synthesized accordingly in excellent yields. The reported new route, as well as its methodology, is believed to be useful in future discovery of novel agonists of cord blood stem cell growth as well as for the corresponding animal studies that demand a large amount of material.

■ EXPERIMENTAL SECTION

General. Melting points were determined using a micro melting point apparatus and are uncorrected; IR spectra were obtained as KBr pellets. NMR spectra were recorded on 400 and 500 MHz (δ in ppm) NMR spectrometers. Mass spectrometry was performed in ESI mode. HRMS was recorded in ESI mode on an LTQ FT analyzer. All of the solvents were distilled and freshly dried. All of the glassware was dried at 220 °C. Inert medium was provided wherever needed.

*2-Methyl-5-(3-nitrophenyl)-2H-tetrazole (**4**).* A mixture of 3-nitrobenzonitrile (**5**, 18.5 g, 125 mmol), NaN_3 (8.94 g, 137.5 mmol), and CuI (4.76 g, 25 mmol) in DMF (250 mL) was heated for 8 h under nitrogen. After completion of the reaction, the mixture was cooled to room temperature, diluted with EtOAc, and treated with aq HCl (1 M,

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100 mL). The volatiles were evaporated. The residue was treated with 1 M aq NaOH to pH 10 and stirred for 30 min. The resulting basic aqueous solution was washed with ethyl acetate to remove the organic phase. The aqueous phase was acidified again to pH 2 and extracted with EtOAc (5 × 200 mL). The combined organic layers were dried over anhydrous Na_2SO_4, filtered, and concentrated to give a crude yellow solid.

The above crude product was treated with MeI (10.90 mL, 175 mmol) in the presence of K_2CO_3 (19.35 g, 140 mmol) in MeCN under reflux for 2 h. The mixture was concentrated, and the residue was partitioned between EtOAc (1500 mL) and water (500 mL). The aqueous layer was again extracted with EtOAc (3 × 750 mL). The combined organic layers were dried over anhydrous Na_2SO_4, filtered, and concentrated. The residue was purified by flash column chromatography on silica gel (petroleum ether/EtOAc from 4:1 to 2:1) to afford **4** (21.01 g, 82%) and **4a** (4.10 g, 16%). **4**: light yellow solid; mp 100−101 °C; IR (KBr) ν_{max} 1738, 1540, 1512, 1449, 1366, 1346, 1229, 1217, 1077, 1052, 729 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ 8.77−8.69 (m, 1H), 8.46 (d, J = 7.8 Hz, 1H), 8.41−8.34 (m, 1H), 7.87 (t, J = 8.0 Hz, 1H), 4.47 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 162.3, 148.2, 132.3, 131.1, 128.3, 124.9, 120.5, 39.9; MS (m/z) 206.1; HRMS calcd for $C_8H_8O_2N_5$ $[M + H]^+$ 206.0673, found 206.0671. 1-Methyl-5-(3-nitrophenyl)-1*H*-tetrazole (**4a**): yellowish solid; mp 148−150 °C; IR (KBr) ν_{max} 3096, 2863, 1524, 1549, 1350, 1295, 1123, 914, 728 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ 8.69−8.62 (m, 1H), 8.51−8.44 (m, 1H), 8.33−8.26 (m, 1H), 7.93 (t, J = 8.0 Hz, 1H), 4.22 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 152.6, 148.0, 135.1, 130.9, 125.7, 125.4, 123.62, 35.14; MS (m/z) 206.1; HRMS calcd for $C_8H_8O_2N_5$ $[M + H]^+$ 206.0673, found 206.0671.

*N-(3-(2-Methyl-2H-tetrazol-5-yl)phenyl)hydroxylamine (**6**).* A mixture of **4** (17.42 g, 85 mmol), ammonium chloride (45.475 g, 850 mmol) in water (200 mL), and EtOH (1200 mL) was stirred at 0 °C. Zinc (42.5 g, 650 mmol) was added in portions over a period of 5 h. After completion of the reaction (6 h), the solution was filtered over a pad of Celite. Ethanol was removed under reduced pressure prior to extraction with dichloromethane (5 × 200 mL). The combined organic layers were dried over anhydrous Na_2SO_4, filtered, and concentrated. Crystallization of the crude material at 10−20 °C from DCM afforded **6** (12 g, 74%) as a white powder. Mp 88−89 °C; IR (KBr) ν_{max} 3286, 1614, 1592, 1478, 1464, 1354, 801, 756, 690 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ 8.54 (s, 1H), 8.49 (d, J = 2.1 Hz, 1H), 7.57 (s, 1H), 7.43 (d, J = 7.6 Hz, 1H), 7.33 (t, J = 7.8 Hz, 1H), 6.95 (d, J = 8.1 Hz, 1H), 4.41 (s, 3H); ^{13}C NMR (100 MHz, DMSO), δ 164.5, 152.8, 129.4, 127.2, 117.1, 114.8, 110.4, 39.6; MS (m/z) 190.0; HRMS calcd for $C_8H_8ON_5$ $[M - H]^-$ 190.0734, found 190.0733.

*N-Hydroxy-N-(3-(2-methyl-2H-tetrazol-5-yl)phenyl)acetamide (**3**).* To a solution of compound **6** (11.46 g, 60 mmol) and pyridine (12.07 mL, 150 mmol) in dry dichloromethane (220 mL) was added dropwise acetyl chloride (4.70 mL, 66 mmol) in dry dichloromethane (50 mL) at 0 °C. The mixture was stirred at 0 °C until completion of the reaction (4 h). The reaction was quenched with saturated aq $NaHCO_3$ (85 mL) and extracted with dichloromethane (4 × 100 mL). The combined organic phases were dried over anhydrous Na_2SO_4, filtered, and concentrated. The residue was purified by silica gel flash chromatography (1:1 petroleum ether/ethyl acetate) to give **3** (9.00 g, 65%) as a yellow oil; IR (KBr) ν_{max} 3162, 2919, 2850, 1739, 1644, 1465, 1374, 1228, 757 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ 10.82 (s, 1H), 8.40 (s, 1H), 7.85−7.78 (m, 2H), 7.55 (t, J = 8.0 Hz, 1H), 4.43 (s, 3H), 2.26 (s, 3H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 170.4, 164.0, 142.4, 129.5, 127.1, 122.1, 121.4, 117.2, 39.6, 22.7; MS (m/z) 256.1 $[M + Na]^+$; HRMS calcd for $C_{10}H_{11}O_2N_5Na$ $[M + Na]^+$ 256.0805, found 256.0804.

*2-Amino-6-(2-methyl-2H-tetrazol-5-yl)-1H-indole-3-carboxamide (**2**).*[12] To a solution of **3** (8.85 g, 38 mmol) and malonitrile (2.51 g, 38 mmol) in dichloromethane (190 mL) was added triethylamine (5.286 mL, 38 mmol) in dichloromethane (38 mL) at 0 °C. Then, the reaction mixture was allowed to warm to room temperature and stirred for 1 h. The volatiles were evaporated. The resulting residue was redissolved in methanol (300 mL) and treated with NaOMe (38 mmol). The reaction mixture was heated at reflux for 3 h. After completion of the reaction, the volatiles were removed under reduced pressure. The crude mixture was purified by flash column chromatography on silica gel (25:1 DCM/MeOH). Desired compound **2** (7.00 g, 72%) was afforded as a white solid. ^1H NMR (400 MHz, DMSO-d_6) δ 10.79 (s, 1H), 7.81 (s, 1H), 7.71−7.60 (m, 2H), 7.02 (s, 2H), 6.59 (s, 2H), 4.38 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 168.3, 165.5, 154.0, 132.5, 121.6, 118.4, 116.8, 116.6, 107.6, 86.8, 39.44; MS (m/z) 256.2 $[M - H]^-$; HRMS calcd for $C_{11}H_{12}ON_7$ $[M + H]^+$ 258.1098, found 258.1097.

*2-Benzyl-7-(2-methyl-2H-tetrazol-5-yl)-9H-pyrimido[4,5-b]indol-4-ol (**7**).*[12] A microwave tube was charged with **2** (1.49 g, 5.8 mmol), methyl 2-phenylacetate (1.24 mL, 8.7 mmol), methanol (14 mL), and 12.5% sodium methoxide (3.75 mL 20.38 mmol). The reaction solution was heated at 140 °C under microwave irradiation (CEMloader v1.11, Discover v2.2 $\mu\lambda$ = 150 W) for 1 h. Then, the second batch of methyl 2-phenylacetate (0.62 mL, 4.35 mmol) and sodium methoxide (1.88 mL, 10.19 mmol) were loaded and heated for an additional 30 min. The mixture was allowed to cool to room temperature, and water (6 mL) and AcOH (24 mL) were added. The slurried mixture was stirred for 30 min. The solid was filtered, washed with cold MeOH (3 × 10 mL), and dried in a vacuum. Title compound **7** (1.66 g, 80%) was obtained as a brown solid. ^1H NMR (400 MHz, DMSO-d_6) δ 12.48 (s, 1H), 12.39 (s, 1H), 8.07 (d, J = 8.3 Hz, 2H), 7.92 (dd, J = 8.1, 1.4 Hz, 1H), 7.45−7.37 (m, 2H), 7.34 (t, J = 7.5 Hz, 2H), 7.25 (t, J = 7.2 Hz, 1H), 4.43 (s, 3H), 4.03 (s, 2H); MS (m/z) 356.1 $[M - H]^-$; HRMS calcd for $C_{19}H_{16}ON_7$ $[M + H]^+$ 358.1411, found 358.1410.

*2-Benzyl-4-chloro-7-(2-methyl-2H-tetrazol-5-yl)-9H-pyrimido-[4,5-b]indole (**8**).*[12] A mixture of **7** (1.61 g, 4.50 mmol) and $POCl_3$ (32 mL) was heated at 175 °C under microwave irradiation ($\mu\lambda$ = 150 W) for 22 min. The mixture was allowed to cool and then poured into ice water (500 mL). Subsequently, 50% aq NaOH was added to neutralize the acid until reaching pH 8. The mixture was extracted with EtOAc (3 × 150 mL). The combined organic phases were dried over anhydrous Na_2SO_4, filtered, concentrated, and dried in vacuum. The resulting brown solid **8** (1.55 g, 92%) was pure enough for analysis. ^1H NMR (400 MHz, DMSO-d_6) δ 12.93 (s, 1H), 8.37 (d, J = 8.3 Hz, 1H), 8.22 (s, 1H), 8.08 (dd, J = 8.3, 1.2 Hz, 1H), 7.33 (dt, J = 12.9, 7.3 Hz, 4H), 7.23 (t, J = 7.1 Hz, 1H), 4.46 (s, 3H), 4.30 (s, 2H); MS (m/z) 376.2 $[M + H]^+$; HRMS calcd for $C_{19}H_{15}N_7Cl$ $[M + H]^+$ 376.1072, found 376.1071; found isotopic mass for ^{37}Cl 378.1014.

*tert-Butyl (4-((2-Benzyl-7-(2-methyl-2H-tetrazol-5-yl)-9H-pyrimido[4,5-b]indol-4-yl)amino)cyclohexyl)carbamate (**9**).*[12] A mixture of chloropyrimidindole **8** (1.52 g, 4.0 mmol), mono-*N*-Boc cyclohexane-1,4-diamine (1.71 g, 8 mmol), and triethylamine (1.11 mL, 8 mmol) in MeCN/DMSO (4:1, 60 mL) was heated under microwave irradiation ($\mu\lambda$ = 150 W) at 140 °C for 3.5 h. The reaction mixture was cooled to room temperature. The volatiles were evaporated, and the residue was poured into water (150 mL). The mixture was extracted with EtOAc (4 × 100 mL). The combined organic phases were dried over anhydrous Na_2SO_4, filtered, and concentrated. Purification from silica gel flash column chromatography (50:1 DCM/methanol) afforded **9** (1.90 g, 85%) as a yellow solid. Mp 358−360 °C (dec); IR (KBr) ν_{max} 3003, 2970, 2932, 1738, 1679, 1521, 1453, 1365, 1228, 1217; ^1H NMR (400 MHz, DMSO-d_6) δ 12.02 (s, 1H), 8.44 (d, J = 8.3 Hz, 1H), 8.07 (d, J = 1.0 Hz, 1H), 7.89 (dd, J = 8.2, 1.3 Hz, 1H), 7.39 (d, J = 7.1 Hz, 2H), 7.29 (t, J = 7.5 Hz, 2H), 7.20 (t, J = 7.3 Hz, 1H), 6.86−6.73 (m, 2H), 4.44 (s, 3H), 4.23 (m, 1H), 4.05 (s, 2H), 3.30−3.23 (m, 1H), 1.94 (d, J = 10.1 Hz, 2H), 1.85 (d, J = 10.2 Hz, 2H), 1.59 (q, J = 12 Hz, 2H), 1.40 (s, 9H), 1.33 (d, J = 12.3 Hz, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 166.0, 164.9, 157.2, 156.2, 154.9, 139.3, 136.3, 129.3 (2C), 128.0 (2C), 126.0, 122.4, 121.7, 121.3, 117.9, 108.55, 93.5, 77.4, 48.8, 48.7, 45.7, 39.6, 31.8 (2C), 30.85 (2C), 28.29 (3C); MS (m/z) 554.5 $[M + H]^+$; HRMS calcd for $C_{30}H_{36}O_2N_9$ $[M + H]^+$ 554.2986, found 554.2983.

*N^1-(2-Benzyl-7-(2-methyl-2H-tetrazol-5-yl)-9H-pyrimido[4,5-b]-indol-4-yl)cyclohexane-1,4-diamine (UM 171, **1**).*[12] Treatment of **9** (1.84 g, 3.33 mmol) with 10% TFA in DCM (180 mL) at room temperature for 45 min. The reaction mixture was concentrated under reduced pressure to dryness. The residue was redissolved in DCM (50 mL) and treated with saturated aq $NaHCO_3$ to pH 8. The aqueous phase was extracted with ethyl acetate (5 × 75 mL). The combined organic phases were dried over anhydrous Na_2SO_4, filtered, and

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concentrated. The crude material was recrystallized from MeOH to afford **UM171** (**1**, 1.45 g, 96%) as a yellowish solid; IR (KBr) ν_{max} 2920, 2850, 1738, 1688, 1604, 1454, 1204, 689 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ 12.06 (s, 1H), 8.43 (d, J = 8.1 Hz, 1H), 8.08 (s, 1H), 7.89 (d, J = 7.9 Hz, 1H), 7.39 (d, J = 6.7 Hz, 2H), 7.29 (s, 2H), 7.21 (d, J = 6.9 Hz, 1H), 6.83 (d, J = 7.3 Hz, 1H), 4.43 (s, 3H), 4.24 (m, 1H), 4.06 (s, 2H), 3.33−3.26 (m, 2H), 2.96 (m, 1H), 1.94 (m, 4H), 1.60 (q, J = 12.0 Hz, 2H), 1.43 (q, 12 Hz, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 165.9, 164.9, 157.2, 156.1, 139.3, 136.3, 129.3 (2C), 128.0 (2C), 126.0, 122.4, 121.8, 121.3, 117.9, 108.6, 93.6, 49.1, 48.4, 45.7, 39.6, 30.5 (2C), 29.9 (2C); MS (m/z) 454.5 $[M + H]^+$; HRMS calcd for $C_{25}H_{28}N_9$ $[M + H]^+$ 454.2462, found 454.2459.

*2-(4-Fluorobenzyl)-7-(2-methyl-2H-tetrazol-5-yl)-9H-pyrimido-[4,5-b]indol-4-ol (**11a**).* The synthesis of **11a** was carried out by following the same procedure for compound **7**. Brown solid (356 mg, 95%); mp 342 °C (dec); IR (KBr) ν_{max} 3127, 2816, 1671, 1590, 1380, 1224, 923, 904 cm^{-1};^1H NMR (500 MHz, DMSO-d_6) δ 12.56 (s, 2H), 8.10 (s, 1H), 8.07 (d, J = 8.2 Hz, 1H), 7.91 (dd, J = 8.2, 1.3 Hz, 1H), 7.43 (dd, J = 8.5, 5.6 Hz, 2H), 7.16 (t, J = 8.9 Hz, 2H), 4.42 (s, 3H), 4.03 (s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 164.9, 161.2 (d, J = 242.6 Hz), 159.6, 159.0, 155.3, 135.7, 132.7 (d, J = 3.0 Hz), 131.02 (d, J = 8.1 Hz, 2C), 123.8, 122.2, 120.9, 119.2, 115.2 (d, J = 21.3 Hz, 2C), 109.4, 98.1, 44.2, 39.5; MS (m/z) 376.2 $[M + H]^+$; HRMS calcd for $C_{19}H_{15}FN_7O$ $[M + H]^+$ 376.1317, found 376.1319.

*2-(3-Fluorobenzyl)-7-(2-methyl-2H-tetrazol-5-yl)-9H-pyrimido-[4,5-b]indol-4-ol (**11b**).* The synthesis of **11b** was carried out by following the same procedure for compound **7**. Brown solid (334 mg, 89%); mp 326−327 °C (dec); IR (KBr) ν_{max} 3052, 2818, 1674, 1587, 1548, 1448, 1378, 1227, 907 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6) δ 12.59 (s, 2H), 8.09 (s, 1H), 8.07 (d, J = 8.2 Hz, 1H), 7.91 (dd, J = 8.2, 1.3 Hz, 1H), 7.38 (dt, J = 7.8, 7.2 Hz, 1H), 7.28−7.19 (m, 2H), 7.12−7.05 (m, 1H), 4.42 (s, 3H), 4.07 (s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 164.9, 162.1 (d, J = 243.4 Hz), 159.2, 159.0, 155.2, 139.1 (d, J = 7.9 Hz), 135.7, 130.4 (d, J = 8.4 Hz), 125.3, 123.8, 122.2, 120.9, 119.3, 116.0 (d, J = 21.6 Hz), 113.7 (d, J = 20.6 Hz), 109.4, 98.2, 39.6 (detected by HMQC), 39.1; MS (m/z) 376.2 $[M + H]^+$; HRMS calcd for $C_{19}H_{15}FN_7O$ $[M + H]^+$ 376.1317, found 376.1318.

*2-(4-Methoxybenzyl)-7-(2-methyl-2H-tetrazol-5-yl)-9H-pyrimido-[4,5-b]indol-4-ol (**11c**).* The synthesis of **11c** was carried out by following the same procedure for compound **7**. Light brown solid (300 mg, 78%); mp 310−311 °C (dec); IR (KBr) ν_{max} 2916, 1671, 1588, 1511, 1443, 1376, 1247, 1024, 904 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6) δ 12.43 (s, 2H), 8.12−8.03 (m, 2H), 7.91 (dd, J = 8.2, 1.3 Hz, 1H), 7.31 (d, J = 8.6 Hz, 2H), 6.90 (d, J = 8.7 Hz, 2H), 4.42 (s, 3H), 3.95 (s, 2H), 3.72 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 164.9, 160.2, 159.0, 158.2, 155.3, 135.6, 130.1 (2C), 128.4, 123.8, 122.2, 120.9, 119.3, 113.9 (2C), 109.3, 98.0, 55.1, 39.6 (detected by HMQC), 39.4; MS (m/z) 388.2 $[M + H]^+$; HRMS calcd for $C_{20}H_{18}N_7O_2$ $[M + H]^+$ 388.1516, found 388.1517.

*4-Chloro-2-(4-fluorobenzyl)-7-(2-methyl-2H-tetrazol-5-yl)-9H-pyrimido[4,5-b]indole (**12a**).* The synthesis of **12a** was carried out by following the same procedure for compound **8**. Brown solid (232 mg, 88%); mp 275−277 °C; IR (KBr) ν_{max} 3339, 3188, 2929, 1779, 1682, 1605, 1535, 1383, 1233 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6) δ 12.56 (s, 2H), 8.10 (s, 1H), 8.07 (d, J = 8.2 Hz, 1H), 7.91 (dd, J = 8.2, 1.3 Hz, 1H), 7.43 (dd, J = 8.5, 5.6 Hz, 2H), 7.16 (t, J = 8.9 Hz, 2H), 4.42 (s, 3H), 4.03 (s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 165.9, 164.2, 161.0 (d, J = 242.3 Hz), 157.4, 151.8, 138.7, 134.3, 131.0 (d, J = 8.0 Hz), 126.1, 123.0, 119.6, 119.4, 115.1 (d, J = 21.2 Hz, 2C), 109.7, 108.8, 43.9, 39.6; MS (m/z) 394.2 $[M + H]^+$; HRMS calcd for $C_{19}H_{14}ClFN_7$ $[M + H]^+$ 394.0978, found 394.0979; found isotopic mass for ^{37}Cl 396.0952.

*4-Chloro-2-(3-fluorobenzyl)-7-(2-methyl-2H-tetrazol-5-yl)-9H-pyrimido[4,5-b]indole (**12b**).* The synthesis of **12b** was carried out by following the same procedure for compound **8**. Brown solid (251 mg, 84%); mp 297−298 °C; IR (KBr) ν_{max} 3107, 2917, 1613, 1559, 1531, 1448, 1400, 1228, 1037, 903 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6) δ 12.93 (s, 1H), 8.34 (dd, J = 8.3, 0.6 Hz, 1H), 8.19 (d, J = 0.6 Hz, 1H), 8.06 (dd, J = 8.3, 1.4 Hz, 1H), 7.39−7.33 (m, 1H), 7.24−7.15 (m, 2H), 7.06 (td, J = 8.5, 1.9 Hz, 1H), 4.45 (s, 3H), 4.33 (s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 165.4, 164.2, 162.1 (d, J = 243.5 Hz), 157.3, 151.8, 140.9 (d, J = 7.5 Hz), 138.7, 130.2 (d, J = 8.2 Hz), 126.2, 125.4, 122.9, 119.6, 119.3, 116.0 (d, J = 21.4 Hz), 113.2 (d, J = 20.9 Hz), 109.7, 108.8, 44.3, 39.6; MS (m/z) 394.2 $[M + H]^+$; HRMS calcd for $C_{19}H_{14}ClFN_7$ $[M + H]^+$ 394.0978, found 394.0979; found isotopic mass for ^{37}Cl 396.0954.

*4-Chloro-2-(4-methoxybenzyl)-7-(2-methyl-2H-tetrazol-5-yl)-9H-pyrimido[4,5-b]indole (**12c**).* The synthesis of **12c** was carried out by following the same procedure for compound **8**. Brown solid (198 mg, 70%); mp 285−286 °C; IR (KBr) ν_{max} 3136, 2962, 1734, 1604, 1560, 1537, 1511, 1404, 1381, 1330, 1234, 1035, 902 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6) δ 12.88 (s, 1H), 8.31 (d, J = 8.2 Hz, 1H), 8.18 (s, 1H), 8.03 (dd, J = 8.3, 1.2 Hz, 1H), 7.26 (d, J = 8.6 Hz, 2H), 6.86 (d, J = 8.7 Hz, 2H), 4.44 (s, 3H), 4.20 (s, 2H), 3.70 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 166.4, 164.3, 157.9, 157.4, 151.8, 138.7, 130.2 (2C), 126.0, 122.9, 119.5, 119.3, 113.8 (2C), 109.7, 108.6, 55.0, 44.0, 39.6; MS (m/z) 406.2 $[M + H]^+$; HRMS calcd for $C_{20}H_{17}ClN_7O$ $[M + H]^+$ 406.1178, found 406.1179; found isotopic mass for ^{37}Cl 408.1153.

*tert-Butyl (4-((2-(4-Fluorobenzyl)-7-(2-methyl-2H-tetrazol-5-yl)-9H-pyrimido[4,5-b]indol-4-yl)amino)cyclohexyl)carbamate (**13a**).* The synthesis of **13a** was carried out by following the same procedure for compound **9**. Yellowish solid (248 mg, 87%); mp 308−310 °C (dec); IR (KBr) ν_{max} 3345, 2932, 1678, 1608, 1585, 1511, 1453, 1382, 1255, 1160, 905 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6) δ 12.00 (s, 1H), 8.44 (d, J = 8.3 Hz, 1H), 8.07 (d, J = 1.0 Hz, 1H), 7.89 (dd, J = 8.2, 1.4 Hz, 1H), 7.41 (dd, J = 8.6, 5.7 Hz, 2H), 7.11 (dd, J = 12.4, 5.4 Hz, 2H), 6.84−6.73 (m, 2H), 4.43 (s, 3H), 4.24−4.17 (m, 1H), 4.05 (s, 2H), 3.31−3.22 (m, 1H), 1.94 (d, J = 11.1 Hz, 2H), 1.86 (d, J = 11.2 Hz, 2H), 1.59 (q, J = 12 Hz, 2H), 1.40 (s, 9H), 1.37−1.30 (m, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 165.7, 164.9, 160.8 (d, J = 241.3 Hz), 157.1, 156.1, 154.9, 136.3, 135.4 (d, J = 2.9 Hz), 131.0 (d, J = 7.9 Hz, 2C), 122.4, 121.7, 121.3, 117.9, 114.6 (d, J = 21.0 Hz, 2C), 108.6, 93.5, 77.4, 48.8 (d, J = 2.6 Hz, 2C), 44.6, 39.6, 31.8 (2C), 30.8 (2C), 28.3 (3C); MS (m/z) 572.5 $[M + H]^+$; HRMS calcd for $C_{30}H_{35}FN_9O_2$ $[M + H]^+$ 572.2892, found 572.2897.

*tert-Butyl (4-((2-(3-Fluorobenzyl)-7-(2-methyl-2H-tetrazol-5-yl)-9H-pyrimido[4,5-b]indol-4-yl)amino)cyclohexyl)carbamate (**13b**).* The synthesis of **13b** was carried out by following the same procedure for compound **9**. Yellowish solid (232 mg, 84%); mp 287−289 °C; IR (KBr) ν_{max} 3340, 2931, 1679, 1608, 1585, 1526, 1451, 1381, 1260 1168, 905 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ 12.04 (s, 1H), 8.45 (d, J = 8.3 Hz, 1H), 8.07 (d, J = 1.0 Hz, 1H), 7.89 (dd, J = 8.2, 1.4 Hz, 1H), 7.36−7.30 (m, 1H), 7.25−7.17 (m, 2H), 7.03 (td, J = 8.2, 2.1 Hz, 1H), 6.81 (d, J = 7.9 Hz, 2H), 4.43 (s, 3H), 4.26−4.18 (m, 1H), 4.08 (s, 2H), 3.30−3.22 (m, 1H), 1.94 (d, J = 16.0 Hz, 2H), 1.85 (d, 16.0 Hz, 2H), 1.60 (q, J = 12.0 Hz, 2H), 1.40 (s, 9H), 1.36−1.28 (m, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 165.3, 164.8, 162.0 (d, J = 242.8 Hz), 157.0, 156.1, 154.9, 142.0 (d, J = 7.9 Hz), 136.3, 129.8 (d, J = 8.4 Hz), 125.4, 122.4, 121.8, 121.3, 117.9, 115.9 (d, J = 21.2 Hz), 112.8 (d, J = 20.8 Hz), 108.6, 93.5, 77.4, 48.8, 48.7, 45.0, 39.6, 31.7 (2C), 30.8 (2C), 28.3 (3C); MS (m/z) 572.4 $[M + H]^+$; HRMS calcd for $C_{30}H_{35}FN_9O_2$ $[M + H]^+$ 572.2892, found 572.2890.

*tert-Butyl (4-((2-(4-Methoxybenzyl)-7-(2-methyl-2H-tetrazol-5-yl)-9H-pyrimido[4,5-b]indol-4-yl)amino)cyclohexyl)carbamate (**13c**).* The synthesis of **13c** was carried out by following the same procedure for compound **9**. Yellowish solid (194 mg, 76%); mp 274−275 °C; IR (KBr) ν_{max} 3338, 2934, 1676, 1610, 1585, 1528, 1510, 1440, 1390, 1244, 1173, 1040, 904 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6) δ 11.98 (s, 1H), 8.43 (d, J = 8.3 Hz, 1H), 8.07 (d, J = 1.0 Hz, 1H), 7.89 (dd, J = 8.2, 1.4 Hz, 1H), 7.30 (d, J = 8.7 Hz, 2H), 6.89−6.83 (m, 2H), 6.78 (t, J = 9.0 Hz, 2H), 4.43 (s, 3H), 4.27−4.20 (m, 1H), 3.97 (s, 2H), 3.71 (s, 3H), 3.31−3.24 (m, 1H), 1.95 (d, J = 10.7 Hz, 2H), 1.86 (d, J = 10.8 Hz, 2H), 1.60 (q, J = 8.0 Hz, 2H), 1.40 (s, 9H), 1.37−1.29 (m, 2H); ^{13}C NMR (100 MHz, DMSO) (100 MHz, DMSO-d_6) δ 166.3, 164.9, 157.7, 157.2, 156.2, 154.9, 136.3, 131.3, 130.2 (2C), 122.3, 121.7, 121.4, 117.9, 113.4 (2C), 108.5, 93.44, 77.4, 55.0, 48.8, 48.7, 44.8, 39.6, 31.8 (2C), 30.8 (2C), 28.3 (3C); MS (m/z) 584.5 $[M + H]^+$; HRMS calcd for $C_{31}H_{37}N_9O_3$ $[M + H]^+$ 584.3092, found 584.3085.

*N^1-(2-(4-Fluorobenzyl)-7-(2-methyl-2H-tetrazol-5-yl)-9H-pyrimido[4,5-b]indol-4-yl)cyclohexane-1,4-diamine (**14a**).* The synthesis of **14a** was carried out by following the same procedure for

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compound **1**. Light yellow solid (93 mg, 96%); mp 269−269 °C; IR (KBr) ν_{max} 2925, 1685, 1608, 1585, 1508, 1432, 1383, 1260, 1132, 994, 909 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6) δ 8.43 (d, J = 8.0 Hz, 1H), 8.07 (s, 1H), 7.89 (d, J = 7.8 Hz, 1H), 7.41 (s, 2H), 7.12 (d, J = 8.4 Hz, 2H), 6.78 (d, J = 7.2 Hz, 1H), 4.43 (s, 3H), 4.26−4.16 (m, 1H), 4.05 (s, 2H), 2.74 (dd, J = 14.6, 12.4 Hz, 1H), 1.91 (t, J = 13.9 Hz, 4H), 1.58 (q, J = 12 Hz, 2H), 1.27 (q, J = 12 Hz, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 166.0, 165.0, 161.0 (d, J = 242.4), 157.3, 156.4, 136.5, 135.6 (d, J = 2.8 Hz), 131.2 (d, J = 7.9 Hz, 2C), 122.6, 121.9, 121.5, 118.1, 114.8 (d, J = 21.0, 2C), 108.8, 93.7, 49.8, 49.0, 44.8, 39.6, 33.5 (2C), 30.6 (2C); MS (m/z) 472.4 $[M + H]^+$; HRMS calcd for $C_{25}H_{27}FN_9$ $[M + H]^+$ 472.2368, found 472.2360.

*N^1-(2-(3-Fluorobenzyl)-7-(2-methyl-2H-tetrazol-5-yl)-9H-pyrimido[4,5-b]indol-4-yl)cyclohexane-1,4-diamine (**14b**).* The synthesis of **14b** was carried out by following the same procedure for compound **1**. Light yellow solid (108 mg, 98%); mp 250−252 °C; IR (KBr) ν_{max} 3447, 2927, 1679, 1607, 1585, 1540, 1434, 1381, 1261, 1205, 1135, 1047, 994, 908 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6) δ 8.43 (d, J = 8.2 Hz, 1H), 8.07 (s, 1H), 7.88 (d, J = 8.2 Hz, 1H), 7.32 (dd, J = 14.4, 7.6 Hz, 1H), 7.27−7.14 (m, 2H), 7.06−6.98 (m, 1H), 6.80 (d, J = 7.9 Hz, 1H), 4.42 (s, 3H), 4.26−4.17 (m, 1H), 4.08 (s, 2H), 2.87−2.75 (m, 1H), 2.06−1.81 (m, 4H), 1.59 (q, J = 8 Hz, 2H), 1.32 (q, J = 8 Hz, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 165.3, 164.8, 162.0 (d, J = 242.7 Hz). 157.1, 156.1, 142.1 (d, J = 7.6 Hz), 136.3, 129.8 (d, J = 8.3 Hz), 125.4 (d, J = 2.2 Hz), 122.4, 121.8, 121.3, 117.9, 116.0 (d, J = 21.0 Hz), 112.8 (d, J = 20.8 Hz)., 108.6, 93.6, 49.4, 48.7, 45.1, 39.6, 32.1 (2C), 30.2 (2C); MS (m/z) 472.4 $[M + H]^+$; HRMS calcd for $C_{25}H_{27}FN_9$ $[M + H]^+$ 472.2368, found 472.2367.

*N^1-(2-(4-Methoxybenzyl)-7-(2-methyl-2H-tetrazol-5-yl)-9H-pyrimido[4,5-b]indol-4-yl)cyclohexane-1,4-diamine (**14c**).* The synthesis of **14c** was carried out by following the same procedure for compound **1**. Yellow solid (104 mg, 97%); mp 277−279 °C; IR (KBr) ν_{max} 3332, 2953, 1682, 1613, 1514, 1459, 1435, 1301, 1251, 1204, 1132, 1035, 905, 836 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6) δ 12.27 (s, 1H), 8.47 (d, J = 8.3 Hz, 1H), 8.12 (s, 1H), 7.94 (dd, J = 15.1, 5.9 Hz, 4H), 7.31 (d, J = 8.5 Hz, 2H), 7.14 (s, 1H), 6.87 (d, J = 8.5 Hz, 2H), 4.44 (s, 3H), 4.25 (m, 1H), 4.04 (s, 2H), 3.72 (s, 3H), 3.05 (m, 1H), 2.02 (m, 4H), 1.64 (q, J = 8.0 Hz, 2H), 1.50 (q, J = 8.0 Hz, 2H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 164.7, 158.3, 158.0, 157.8, 155.8, 136.4, 130.7, 130.2 (2C), 122.7, 121.8, 121.1, 118.2, 113.5 (2C), 108.8, 93.6, 55.0, 49.0, 48.6, 44.0, 39.6, 29.6 (2C), 29.4 (2C); MS (m/z) 484.3 $[M + H]^+$; HRMS calcd for $C_{26}H_{30}N_9O$ $[M + H]^+$ 484.2568, found 484.2563.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.joc.6b01094.

^1H and ^{13}C NMR spectral copies of the synthetic compounds (PDF)

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Notes

The authors declare no competing financial interest.

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