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DESIGN AND SYNTHESIS OF AZA-β-CARBOLINE ANALOGS AND THEIR ANTIBACTERIAL EVALUATION

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Bacterial drug resistance has become a growing problem worldwide due to the excessive use of antibiotics in recent decades. Two small focused libraries of 5*H*-pyrimido[5,4-*b*]indole-4-carboxamides and 5*H*-pyrimido-[5,4-*b*]indole-4-ketones were designed as eudistomin Y_3 and 1-acetyl-β-carboline **(1-ABC)** analogs and prepared via application of Inverse Electron-Demand Diels-Alder (IEDDA) reaction of 1,3,5-triazines and 3-aminoindoles. Compounds **2a** and **2b** were discovered to have activity against *Mycobacterium bovis* BCG with Minimum Inhibitory Concentration (MICs) values of 25 and 50 μg/mL respectively while compound **2e** was against all three strains of *Candida albicans* tested with MIC values of 50 μg/mL. Moreover, compound **2e** demonstrated synergistic antibacterial activity with fluconazol, which suggested that future drug candidates from this class of compounds could be used in combination with existing drugs to treat *C. albicans* infections.

Keywords: drug design; aza-β-carboline analogs; biological screening; 5*H*-pyrimido[5,4-*b*]indoles.

1. INTRODUCTION

Bacterial drug resistance has become a growing problem worldwide because of the excessive use of antibiotics in recent decades [1 – 4]. Since the first discovery in 1961, methicillin-resistant *Staphylococcus aureus* (MRSA) has become the main pathogenic bacteria responsible for hospital and community acquired infections. MRSA showed resistance to most of the currently available antibiotics and became an enormous problem for health care providers [5 – 7]. Therefore, efforts to discover new antibacterial agents to tackle the clear unmet medical need of new treatment for antibiotic-resistant bacterial infections are much needed [2].

Natural products provide rich leads for most antibiotic discovery projects [8, 9], however their pharmacokinetic properties often require additional optimization before they could be developed as drug candidates. Unfortunately it is often difficult to prepare derivatives of complex natural products as required by medicinal chemistry for lead optimization efforts [2]. As part of our ongoing efforts to develop novel and efficient methodologies to access complex heterocycles as analogs of natural products, we became interested in β-carboline derivatives, which are tricyclic alkaloids with intriguing biological activities such as antibacterial and antifungal properties [11 – 17]. For example, β-carboline is the heterocyclic nucleus of all eudistomins that were isolated from the Korean *Ascidian Synoicum Sp.* Most eudistomins have antibacterial activities with several analogs showing MIC_{99} (the minimum concentration of the compound required to inhibit 99% of bacterial growth) value of as potent as 1.56 μg/mL against *S. aureus*; however few synthetic studies were reported with regard to their SAR of antibacterial activities. After examination of the limited eudistomin literature, we noticed that compounds with two or three bromine atoms on the distal aryl rings are more active, and compounds containing a carbonyl group at C-10 showed higher activities [18]. The reported SAR is limited but seems to indicate that the antibacterial activity of eudistomins could be further improved via preparation of new analogs.

The simplest member of the eudistomin family is 1-acetyl-β-carboline (**1-ABC**) which was first isolated in

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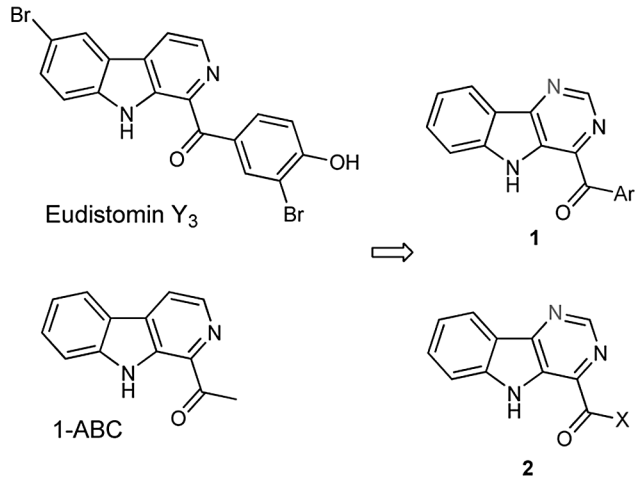


Fig. 1. Design of aza-eudistomin analogs.

1977 [19]. Even though **1-ABC** showed weak antibacterial activity against MRSA, it exhibited synergistic antibacterial effect when combined with ampicillin [20]. Therefore we rationalized that preparation of eudistomin analogs at C-10 position of amides and ketones could lead to compounds with improved antibacterial and/or synergistic antibacterial activities.

To validate initial screening hits, further optimization is often carried out in drug discovery programs to confirm that the hits could be advanced to leads with desired potency and physical properties [21 – 23]. One common strategy is to identify suitable places where a nitrogen atom could be introduced which may increase potency via new hydrogen bond formation and/or improve physical properties by increasing aqueous solubility, modulating LogP, and enhancing selectivity toward the desired target (Fig. 1). Consequently we developed an efficient method to prepare aza-β-carbolines in a single step via Electron-Demand Diels-Alder (IEDDA) reactions of aminoindoles and 1,3,5-triazines. We envisioned that the ready access of aza-β-carboline **5**, which was reported during development of the method of new aza-β-carboline synthesis, must enable us to rapidly study the SAR of C-10 region of eudistomins (Fig. 2), and results from such an exercise should be useful to guide future research in this area leading to new antibacterial and/or synergistic antibacterial agents.

2. EXPERIMENTAL PART

2.1. Materials and Methods

DMF was simply disposed with Na_2SO_4 and $MgSO_4$. Other commercial reagents were used as received without additional purification. All melting points were obtained on SGW X-4 apparatus (Shanghai Precision Instrument Co., Ltd, Shanghai, China). Mass spectra (MS) were taken in ESI mode on LCQ DECA XP Plus (Thermo Finnigan, California, USA). Analytical TLC was performed using 2.5 × 5 cm plated coated with a 0.25 mm thickness of silica gel GF_{254}. Column chromatography was performed using silica gel G (200 – 300 mesh). The ^{1}H NMR (300 MHz) and ^{13}C NMR (75 MHz) spectra were recorded on Varian spectrometer (Varian, Palo Alto, CA) with TMS as internal standard (δ scale). Multiplicities are indicated as follows: br = broad, s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet; integration and coupling constants (Hz) are determined with complete proton decoupling and expressed in ppm. Compounds **2a**, **2b**, **2c**, **2d, 2e**, **3**, **4** were synthesized according to our previously published procedures [24].

2.2. Synthesis

***Tert*-butyl-4-((5H-pyrimido[5,4-*b*]indole-4-carboxamido)methyl)piperidine-1-carboxylate (1a).** 5*H*-pyrimido-[5,4-*b*]indole-4-carboxylic acid (214 mg, 1.0 mmol) was dissolved in DMF (5 mL) and CDI (*N*,*N*′-Carbonyldiimidazole) (162 mg, 1.0 mmol) was added at room temperature (25°C). After 1h, N-(*tert*-butoxycarbonyl)-4-aminomethylpiperidine (214 mg, 1.0 mmol) was slowly added. When the reaction finished after about 1 h, it was quenched by addition of H_2O (20 mL). The aqueous phase was extracted with EtOAc (3 × 10 mL). The combined organic extracts were washed

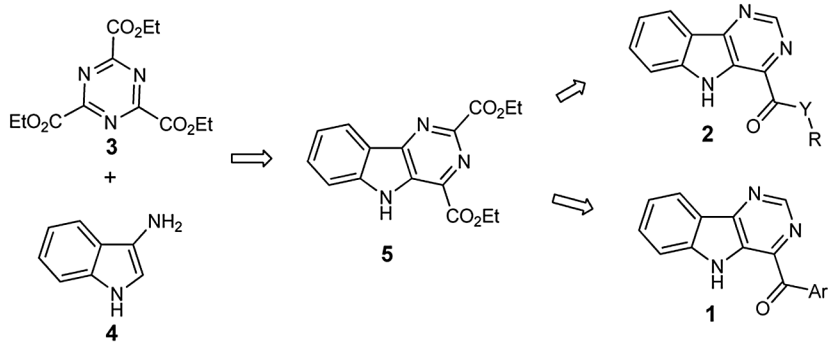


Fig. 2. Efficient synthetic strategies for aza-eudistomin analogs.

with brine, dried (Na_2SO_4) and evaporated to dryness. The residue was chromatographied on silica gel eluted with PE/CH_2Cl_2/EtOAc (5:5:3) to give the desired product as white solid in 89% yield (364 mg); m.p., 176 – 178°C. ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 9.96 (s, 1H), 9.11 (s, 1H), 8.41 (d, *J* = 8.1 Hz, 1H), 8.27 – 8.23 (m, 1H), 7.72 – 7.66 (m, 1H), 7.57 (d, *J* = 8.1 Hz, 1H), 7.42 – 7.37 (m, 1H), 4.17 – 4.14 (m, 2H), 3.46 (d, *J* = 6.6 Hz, 2H), 2.76 – 2.68 (m, 2H), 1.89 – 1.78 (m, 3H), 1.45 (s, 9H), 1.34 – 1.24 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm): 165.4, 154.6, 150.6, 148.3, 142.5, 136.1, 131.3, 128.9, 122.2, 121.2, 119.8, 112.1, 79.3, 44.5, 43.5, 36.6, 29.7, 28.3. ESI-MS: *m/z* 410 $[M+H]^+$.

***Tert*-butyl-4-(5H-pyrimido[5,4-*b*]indole-4-carboxamido) piperidine-1-carboxylate (1b).** 5*H*-pyrimido[5,4-*b*]-indole-4-carboxylic acid (214 mg, 1.0 mmol) was dissolved in DMF (5 ml) and CDI (*N*,*N*′-Carbonyldiimidazole) (162 mg, 1.0 mmol) was added at room temperature (25°C). After 1 h, *N*-Boc-4-piperidineamine (200 mg, 1.0 mmol) was slowly added. When the reaction finished after about 1 h, it was quenched by addition of H_2O (20 mL). The aqueous phase was extracted with EtOAc (3 × 10 mL). The combined organic extracts were washed with brine, dried (Na_2SO_4) and evaporated to dryness. The residue was chromatographied on silica gel eluting with PE/CH_2Cl_2/EtOAc (5:5:3) to give the desired product as light green solid in 76% yield (300 mg); m.p., 195 – 198°C. ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 9.94 (s, 1H), 9.11 (s, 1H), 8.41 (d, *J* = 8.1 Hz, 1H), 8.08 – 8.05 (m, 1H), 7.73 – 7.67 (m, 1H), 7.58 (d, *J* = 8.1 Hz, 1H), 7.42 – 7.37 (m, 1H), 4.21 – 4.10 (m, 3H), 3.03 – 2.95 (m, 2H), 2.09 – 2.03 (m, 2H), 1.61 – 1.57 (m, 2H), 1.48 (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm): 164.5, 154.6, 150.6, 148.2, 142.6, 136.0, 131.3, 128.9, 122.2, 121.1, 119.8, 112.1, 79.7, 46.6, 42.4, 31.8, 28.4. ESI-MS: *m/z* 396 $[M+H]^+$.

General procedure for the preparation of 5*H*-pyrimido[5,4-*b*]indole-4-carboxamides (1c – 1k). 5*H*-pyrimido-[5,4-*b*]indole-4-carboxylic acid was dissolved in DMF and CDI (*N*,*N*′-Carbonyldiimidazole) (1eq.) was added at room temperature (25°C). After 1h, amine (1.2eq.) was slowly added. The reaction was monitored by TLC or LC-MS. When the reaction finished, it was quenched by addition of H_2O. The aqueous phase was extracted with EtOAc. The combined organic extracts were washed with brine, dried (Na_2SO_4) and evaporated to dryness. The residue was chromatographied on silica gel eluting with PE/CH_2Cl_2/EtOAc to give the desired product.

***N*-Isobutyl-5*H*-pyrimido[5,4-*b*]indole-4-carboxamide (1c).** Purification by silica gel column chromatography (CH_2Cl_2: EtOAc =10:1) gave the desired product as white solid in 49% yield (63mg). m.p., 156 – 157°C. ^{1}H-NMR (300 MHz, DMSO-d_6): δ 12.00 (s, 1H), 9.18 (t, *J* = 6.3 Hz, 1H), 9.12 (s, 1H), 8.29 (d, *J* = 7.8 Hz, 1H), 7.82 (d, *J* = 8.4 Hz, 1H), 7.73 – 7.68 (m, 1H), 7.39 – 7.34 (m, 1H), 3.26 (t, *J* = 6.6 Hz, 2H), 2.03 – 1.94 (m, 1H), 0.94 (d, *J* = 6.9 Hz, 6H). ^{13}C-NMR (75 MHz, DMSO-d_6) δ 164.4, 149.6, 147.7 143.2, 137.2, 130.9, 128.0, 121.3, 120.6, 119.0, 113.5, 46.1, 28.3, 20.2. ESI-MS: *m/z* 269.0 $[M+H]^+$.

***N*-Cyclopropyl-5*H*-pyrimido[5,4-*b*]indole-4-carboxamide (1d).** Purification by silica gel column chromatography (CH_2Cl_2: EtOAc =10:1) gave the desired product as white solid in 70% yield (89mg). m.p., 203 – 204°C. ^{1}H-NMR (300 MHz, DMSO-d_6) δ: 12.00 (s, 1H), 9.17 (d, *J* = 5.1 Hz, 1H), 9.09 (s, 1H), 8.29 (d, *J* = 7.8 Hz, 1H), 7.85 – 7.81 (m, 1H), 7.74 – 7.68 (m, 1H), 7.39 – 7.34 (m, 1H), 3.09 – 3.03 (m, 1H), 0.80 – 0.76 (m, 4H); ^{13}C-NMR (75 MHz, DMSO-d_6) δ: 165.6, 149.5, 147.6, 143.2, 137.1, 130.8, 127.8, 121.3, 120.6, 119.0, 113.5, 22.8, 5.7. ESI-MS: *m/z* 253 $[M+H]^+$.

***N*-(*tert*-butyl)-5*H*-pyrimido[5,4-*b*]indole-4-carboxamide (1e).** Purification by silica gel column chromatography (CH_2Cl_2: EtOAc =10:1) gave the desired product as light yellow solid in 37% yield (46mg). m.p., 163 – 165°C. ^{1}H-NMR (300 MHz, DMSO-d_6) δ: 12.07 (s, 1H), 9.10 (s, 1H), 8.30 – 8.28 (m, 2H), 7.82 (d, *J* = 8.4 Hz, 1H), 7.73 – 7.68 (m, 1H), 7.39 – 7.34 (m, 1H), 1.52 (s, 9H); ^{13}C-NMR (75 MHz, DMSO-d_6) δ: 163.5, 149.7, 147.4, 143.2, 137.2, 130.8, 127.8, 121.3, 120.5, 118.9, 113.4, 50.7, 28.3. ESI-MS: *m/z* 269 $[M+H]^+$.

***N*-butyl-5*H*-pyrimido[5,4-*b*]indole-4-carboxamide (1f).** Purification by silica gel column chromatography (CH_2Cl_2: EtOAc =10:1) gave the desired product as white solid in 60% yield (77mg). m.p., 153 – 154°C. ^{1}H-NMR (300 MHz, DMSO-d_6) δ: 11.99 (s, 1H), 9.18 (t, *J* = 6.0 Hz, 1H), 9.10 (s, 1H), 8.28 (d, *J* = 7.8 Hz, 1H), 7.83 – 7.80 (m, 1H), 7.72 – 7.66 (m, 1H), 7.38 – 7.32 (m, 1H), 3.41 (q, *J* = 6.9 Hz, 2H), 1.65 – 1.55 (m, 2H), 1.42 – 1.30 (m, 2H), 0.92 (t, *J* = 7.2 Hz, 3H); ^{13}C-NMR (75 MHz, DMSO-d_6) δ: 164.2, 149.6, 147.6, 143.1, 137.2, 130.8, 128.0, 121.2, 120.5, 119.0, 113.4, 38.3, 31.2, 19.6, 13.6. ESI-MS: *m/z* 269 $[M+H]^+$.

***N*-(2-(Dimethylamino)-ethyl)-5*H*-pyrimido[5,4-*b*]indole-4-carboxamide (1g).** Purification by silica gel column chromatography (CH_2Cl_2: EtOAc=10:1) gave the desired product as light yellow solid in 58% yield (79mg). m.p., 168 – 169°C. ^{1}H-NMR (300 MHz, DMSO-d_6) δ: 12.00 (s, 1H), 9.12 (s, 1H), 9.00 (t, *J* = 8.7 Hz, 1H), 8.29 (d, *J* = 8.1 Hz, 1H), 7.82 (d, *J* = 8.1 Hz, 1H), 7.73 – 7.68 (m, 1H), 7.39 – 7.34 (m, 1H), 3.55 – 3.49 (m, 2H), 2.52 – 2.48 (m, 2H), 2.22 (s, 6H); ^{13}C-NMR (75 MHz, DMSO-d_6) δ: 164.1, 149.6, 147.7, 143.2, 136.9, 130.9, 127.9, 121.3, 120.6, 119.0, 113.5, 57.8, 45.1, 36.5. ESI-MS: *m/z* 284 $[M+H]^+$.

***N*-(2-Morpholinoethyl)-5*H*-pyrimido[5,4-*b*]indole-4-carboxamide (1h)**. Recrystallization (PE/EtOAc) gave the desired product as light yellow solid in 64% yield (98mg). m.p., 214 – 215°C. ^{1}H-NMR (300 MHz, DMSO-d_6) δ: 12.00 (s, 1H), 9.11 (s, 1H), 9.09 – 9.07 (m, 1H), 8.28 (d, *J* = 8.1 Hz, 1H), 7.82 (d, *J* = 8.4 Hz, 1H), 7.73 – 7.67 (m, 1H), 7.38 – 7.33 (m, 1H), 3.60 – 3.53 (m, 6H), 2.57 (d, *J* = 6.6 Hz, 2H), 2.49 – 2.44 (m, 4H); ^{13}C-NMR (75 MHz,

DMSO-d_6) δ: 164.3, 149.7, 147.7, 143.2, 137.0, 130.9, 128.0, 121.3, 120.6, 119.0, 113.5, 66.2, 57.1, 53.2, 35.8. ESI-MS: *m/z* 326 $[M+H]^+$.

Morpholino-(5*H*-pyrimido[5,4-*b*]indol-4-yl)methanone (1i). Recrystallization (PE/EtOAc) gave the desired product as light yellow solid in 62% yield (82mg). m.p., 230 – 232°C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 11.98 (s, 1H), 9.07 (s, 1H), 8.29 (d, *J* = 7.8 Hz, 1H), 7.71 – 7.70 (m, 2H), 7.39 – 7.34 (m, 1H), 3.81 – 3.76 (m, 4H), 3.74 – 3.71 (m, 2H), 3.62 – 3.59 (m, 2H). ^{13}C-NMR (75 MHz, DMSO-d_6) δ: 164.0, 148.2, 147.8, 142.4, 141.4, 130.7, 128.0, 121.3, 120.6, 119.4, 113.0, 66.5, 66.1, 47.0, 42.2. ESI-MS: *m/z* 283 $[M+H]^+$.

(4-Methylpiperazin-1-yl)(5*H*-pyrimido[5,4-*b*]indol-4-yl)methanone (1j). Purification by silica gel column chromatography (CH_2Cl_2: EtOAc=10:1) gave the desired product as light yellow solid in 56% yield (79mg). m.p., 177 – 179°C. ^{1}H-NMR (300 MHz, DMSO-d_6) δ: 11.97 (s, 1H), 9.07 (s, 1H), 8.28 (d, *J* = 7.8 Hz, 1H), 7.72 – 7.69 (m, 2H), 7.38 – 7.33 (m, 1H), 3.80 (t, *J* = 5.1 Hz, 2H), 3.65 (t, *J* = 5.1 Hz, 2H), 2.48 (t, *J* = 5.1 Hz, 2H), 2.32 (t, *J* = 5.1 Hz, 2H), 2.22 (s, 3H). ^{13}C-NMR (75 MHz, DMSO-d_6) δ: 163.9, 148.1, 147.9, 142.3, 141.8, 130.6, 127.9, 121.2, 120.5, 119.4, 112.9, 54.9, 54.2, 46.3, 45.5, 41.6. ESI-MS: *m/z* 296 $[M+H]^+$.

Ethyl-2-(5*H*-pyrimido[5,4-*b*]indole-4-carboxamido) acetate (1k). Recrystallization (PE/EtOAc) gave the desired product as light yellow solid in 49% yield (68mg). m.p., 221 – 223°C. ^{1}H NMR (300 MHz, DMSO-d$_6$) δ: 12.03 (s, 1H), 9.53 (t, *J* = 6.0 Hz, 1H), 9.15 (s, 1H), 8.31 (d, *J* = 7.8 Hz, 1H), 7.82 (d, *J* = 8.1 Hz, 1H), 7.74 – 7.69 (m, 1H), 7.40 – 7.35 (m, 1H), 4.20 – 4.13 (m, 4H), 1.23 (t, *J* = 6.9 Hz, 3H); ^{13}C NMR (75 MHz, DMSO-d$_6$) δ: 169.4, 164.9, 149.8, 147.7, 143.3, 136.3, 131.0, 127.9, 121.3, 120.7, 118.9, 113.4, 60.6, 41.0, 14.1. ESI-MS: *m/z* 299 $[M+H]^+$.

General Procedure for the preparation of 5*H*-pyrimido[5,4-*b*]indole-4-carboxamides (1l – 1o). 5*H*-pyrimido [5,4-*b*]indole-4-carboxylic acid was dissolved in CH_2Cl_2 and $(ClCO)_2$ (4eq.) and a drop of DMF were added on ice-cold bath, then warmed up to room temperature. After 6h, the appropriate aromatic amine (1.5eq.) was slowly added. The reaction was monitored by TLC or LC-MS. When the reaction finished, it was quenched by addition of H_2O. The precipitate was separated by filtration and recrystallized with PE/EtOAc to give the desired product.

***N*-(4-fluorophenyl)-5*H*-pyrimido[5,4-*b*]indole-4-carboxamide (1l).** Recrystallization (PE/EtOAc) gave the desired product as light yellow solid in 77% yield (110mg). m.p., 209 – 210°C. ^{1}H NMR (300 MHz, DMSO-d$_6$) δ: 12.15 (s, 1H), 11.13 (s, 1H), 9.21 (s, 1H), 8.32 (d, *J* = 7.8 Hz, 1H), 8.08 – 8.03 (m, 2H), 7.84 (d, *J* = 8.1 Hz, 1H), 7.76 – 7.710 (m, 1H), 7.41 – 7.36 (m, 1H), 7.30 – 7.24 (m, 2H); ^{13}C NMR (75 MHz, DMSO-d$_6$) δ: 163.0, 160.2(157.0), 149.9, 147.5, 143.3, 136.8, 134.4, 131.0, 128.2, 122.4, 122.3, 121.3, 120.7, 119.0, 115.4, 115.1, 113.5. ESI-MS: *m/z* 307 $[M+H]^+$.

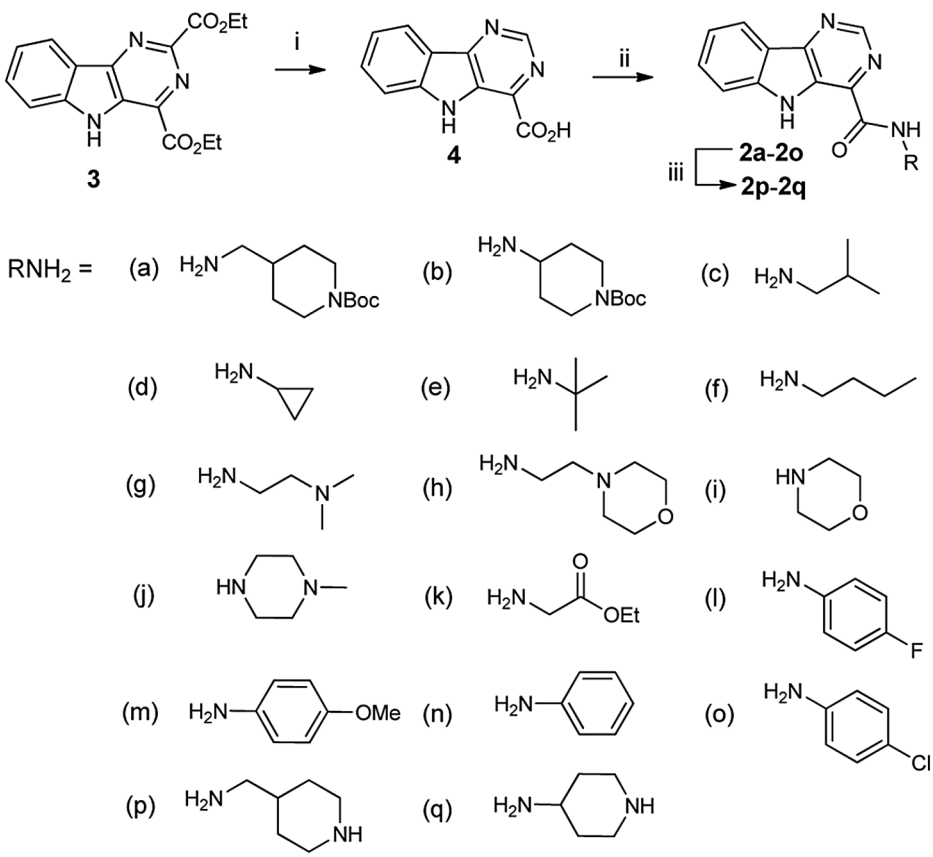
***N*-(4-methoxyphenyl)-5*H*-pyrimido[5,4-*b*]indole-4-carboxamide (1m).** Recrystallization (PE/EtOAc) gave the desired product as yellow solid in 71% yield (105mg). m.p., 213 – 215°C. ^{1}H-NMR (300 MHz, DMSO-d_6) δ: 12.14 (s, 1H), 10.93 (s, 1H), 9.20 (s, 1H), 8.32 (d, *J* = 7.8 Hz, 1H), 7.95 (d, *J* = 9.0 Hz, 2H), 7.84 (d, *J* = 8.4 Hz, 1H), 7.75 – 7.73 (m, 1H), 7.41 – 7.36 (m, 1H), 6.99 (d, *J* = 9.0 Hz, 2H), 3.78 (s, 3H); ^{13}C-NMR (75 MHz, DMSO-d_6) δ: 162.5, 155.9, 149.8, 147.5, 143.2, 137.2, 131.1, 131.0, 128.1, 121.9, 121.3, 120.7, 119.0, 113.8, 113.5, 55.2. ESI-MS: *m/z* 319 $[M+H]^+$.

***N*-Phenyl-5*H*-pyrimido[5,4-*b*]indole-4-carboxamide (1n).** Recrystallization (PE/EtOAc) gave the desired product as yellow solid in 68% yield (92mg). m.p., 193 – 198°C. ^{1}H-NMR (300 MHz, DMSO-d_6) δ: 12.16 (s, 1H), 11.00 (s, 1H), 9.21 (s, 1H), 8.32 (d, *J* = 7.8 Hz, 1H), 8.05 – 8.02 (m, 2H), 7.85 (d, *J* = 8.1 Hz, 1H), 7.76 – 7.71 (m, 1H), 7.45 – 7.36 (m, 3H), 7.21 – 7.16 (m, 1H); ^{13}C-NMR (75 MHz, DMSO-d_6) δ: 163.0, 150.0, 147.5, 143.3, 138.0, 136.9, 131.0, 128.7, 128.2, 124.3, 121.4, 120.8, 120.4, 119.0, 113.5. ESI-MS: *m/z* 289 $[M+H]^+$.

***N*-(4-Chlorophenyl)-5*H*-pyrimido[5,4-*b*]indole-4-carboxamide (1o).** Recrystallization (PE/EtOAc) gave the desired product as yellow solid in 75% yield (114mg). m.p., 261 – 263°C. ^{1}H-NMR (300 MHz, DMSO-d_6) δ: 12.15 (s, 1H), 11.18 (s, 1H), 9.21 (s, 1H), 8.32 (d, *J* = 8.1 Hz, 1H), 8.07 (d, *J* = 9.0 Hz, 2H), 7.84 (d, *J* = 8.4 Hz, 1H), 7.76 – 7.71 (m, 1H), 7.49 (d, *J* = 9.0 Hz, 2H), 7.42 – 7.36 (m, 1H); ^{13}C-NMR (75 MHz, DMSO-d_6) δ: 163.3, 150.0, 147.5, 143.3, 137.1, 136.8, 131.1, 128.6, 128.2, 128.0, 122.1, 121.4, 120.8, 119.0, 113.5. ESI-MS: *m/z* 323 $[M+H]^+$.

***N*-(Piperidin-4-ylmethyl)-5*H*-pyrimido[5,4-*b*]indole-4-carboxamide (1p).** *Tert*-butyl 4-((5H-pyrimido [5,4-*b*]indole-4-carboxamido)methyl)piperidine-1-carboxylate (182 mg, 0.44 mmol) was dissolved in CH_2Cl_2 (2 ml) and CF_3COOH (253mg, 2.22 mmol) was added in ice bath. After 2h, the reaction finished, it was quenched by addition of H_2O (10 ml). The organic phase was separated, and the aqueous phase was extracted with CH_2Cl_2 (2 × 5 ml). Then the aqueous phase was adjusted to pH 12 with 4N NaOH. The precipitate was separated by filtration and dried to give the desired product as light yellow solid (155 mg, 90%). Decomp. >160°C. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 9.32 (br.s, 1H), 9.08 (s, 1H), 8.28 (d, *J* = 8.1 Hz, 1H), 7.80 (d, *J* = 8.1 Hz, 1H), 7.70 – 7.65 (m, 1H), 7.35 – 7.30 (m, 1H), 3.32 – 3.27 (m, 2H), 2.94 – 2.90 (m, 2H), 2.45 – 2.37 (m, 2H), 1.74 – 1.71 (m, 1H), 1.64 – 1.60 (m, 2H), 1.11 – 1.08 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 164.5, 149.6, 147.4, 143.8, 137.3, 130.7, 128.6, 121.3, 120.3, 119.1, 113.7, 45.8, 44.7, 36.6, 30.9. ESI-MS: *m/z* 310 $[M+H]^+$.

N-(Piperidin-4-yl)-5*H*-pyrimido[5,4-*b*]indole-4-carbo xamide (1q). *Tert*-butyl-4-(5H- pyrimido[5,4-*b*]indole-4-carboxamido)piperidine-1-carboxylate (182 mg, 0.51 mmol) was dissolved in CH_2Cl_2 (2 ml) and CF_3COOH (288mg, 2.53 mmol) was added in ice bath. After 2h, the reaction fin-

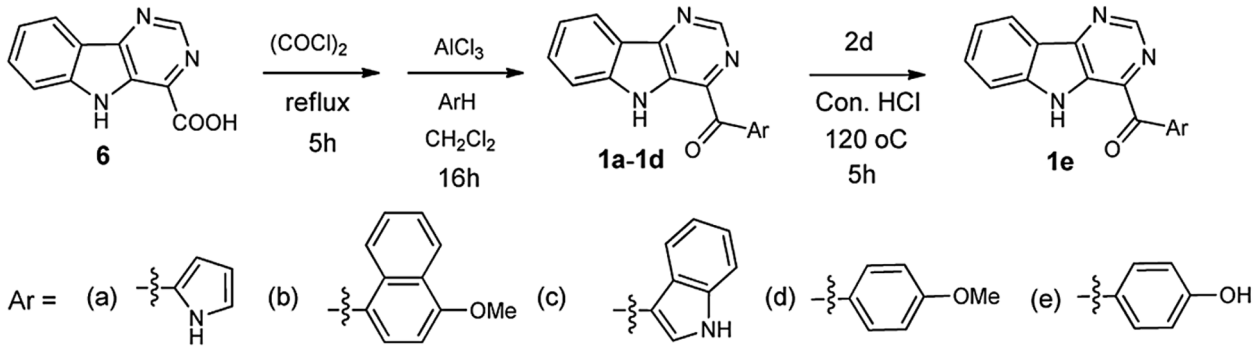


Scheme 1. Synthesis of 5*H*-pyrimido[5,4-*b*]indole-4- carboxamides **2**. Reagents and conditions: (i) Conc. HCl, reflux, 6h; (ii) CDI, DMF, aliphatic amines, 2h; or $(COCl)_2$, aromatic amines, 8h; (iii) **2a**-**2b**, Sat. HCl- EtOAc, 25°C, 2h.

ished, it was quenched by addition of H_2O (10 ml). The organic phase was separated, and the aqueous phase was extracted with CH_2Cl_2 (2 × 5 ml). Then the aqueous phase was adjusted to pH 12 with 4N NaOH. The precipitate was separated by filtration and dried to give the desired product as light yellow solid (123 mg, 82%). m.p., 195 – 197°C. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 11.97 (br.s, 1H), 9.11 (s, 1H), 8.94 – 8.91 (m, 1H), 8.29 (d, J = 8.1 Hz, 1H), 7.81 (d, J = 8.7 Hz, 1H), 7.73 – 7.68 (m, 1H), 7.39 – 7.34 (m, 1H), 3.99 – 3.96 (m, 1H), 3.01 – 2.97 (m, 2H), 2.60 – 2.50 (m, 2H), 1.82 – 1.77 (m, 2H), 1.66 – 1.53 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 163.4, 149.6, 147.6, 143.2, 137.3, 130.9, 128.1, 121.3, 120.6, 119.0, 113.5, 47.2, 45.2, 32.7. ESI-MS: *m/z* 296 $[M+H]^+$.

2.3 Biological Activity Testing

General biological activity assays. The Gram-positive bacterial strains used in this study included *S. aureus* (ATCC 6538), MRSAa (Chaoyang Hospital, Beijing, China), *Bacillus subtilis* (ATCC 6633), *Escherichia coli* (ATCC 25922), and *Micrococcus luteus* (ATCC 9341). All of the compounds were prepared as 4 mg/mL stock solution using sterile dimethyl sulfoxide (DMSO). The microbes were stored as glycerol stocks at –80°C and streaked onto Mueller-Hinton agar (MHA) for colony growth at 37°C. The antibacterial activities and minimum inhibitory concentrations (MICs) were determined in flat bottom, 96-well microtiter plates using a modified broth microdilution protocol according to the Clinical and Laboratory Standards Institute M7-A6, M-38A, and M-27A2 methods. Briefly, single colonies of bacteria were picked from MHA plate and adjusted to 10^5 CFU/mL with Mueller-Hinton Broth (MHB) as bacterial suspensions. Aliquots (2 μL) of two-fold serial dilutions of each compound (in DMSO) were added to each row on the 96-well plates containing 78 μL of bacterial suspension in each well. The 96-well plates were incubated aerobically at 37°C for 16 h before the results were recorded. MICs were defined as the lowest concentrations of the compounds that inhibited visible bacterial growth after 16 h of incubation. The MICs were tested twice in triplicate. Vancomycin was used as a positive control for *S. aureus*, MRSA, *M. luteus, B. subtilis*, and *E. coli* [25].The test strains used in the study of second-batch compounds included three *C. albican* (SC5314 from ATCC, G5 and 17# from clinical isolates), *Mycobacterium bovis* BCG (*M. bovis* BCG, Pasteur 1173P2), *Staphylococcus aureus* (*S. aureus*, ATCC 6538), *Enterococcus*



Scheme 2. Synthesis of 5*H*-pyrimido[5,4-*b*]indole-4-ketones **1**.

faecium (*E. faecium*, Chaoyang Hospital, Beijing, China), *Pseudomonas aeruginosa* (*P. aeruginosa*, PA14). *C. albicans* were grown on YPD medium and MIC assay performed in the RPMI 1640. The microbes were streaked onto Mueller-Hinton agar (MHA) for colony growth at 37°C and MIC determined in Mueller-Hinton Broth (MHB).

BCG inhibition activity in the THP-1 cell line. THP-1 cells were seeded into 96-well clear bottom plates and differentiated using 100 ng/ml phorbol 12-myristate 13-acetate (PMA). BCG (pMSP12: GFP and maintained with kanamycin) was grown to mid-log phase in Middlebrook 7H9 OADC and prepared for infection. The de-clumped bacteria were diluted with pre-warmed infection media (RPMI 1640, 1% glutamine, and 10% fetal calf serum) and were used to infect cells at an MOI of 10:1. After 3 h, the macrophages were gently washed to remove non internalized bacteria, and then RPMI incomplete medium was added. After 24 h, all of the media was aspirated and replaced with media containing testing compounds. Following a 2 days incubation period at 37°C and 5% CO_2, GFP fluorescence was quantified using an Envision Multilabel (Perkin Elmer) plate reader. All of the screening plates contained negative (DMSO) and positive (2.5 μg/mL rifampicin) controls.

TABLE 1. Antibacterial Activity of the First Batch of Compounds (MIC, μg/mL)

Comp.	M. bovis BCG	MRSA	SA	BS	E.f	PA	K.pneumoniae #249
2a	25	>100	>100	>100	>100	>100	>100
2b	50	>100	>100	>100	>100	>100	>100
2p	>100	>100	>100	>100	>100	>100	>100
2q	>100	>100	>100	>100	>100	>100	>100
1a	>100	>100	>100	>100	>100	>100	>100
1b	>100	>100	>100	>100	>100	>100	>100
1c	>100	>100	>100	>100	>100	>100	>100
1d	>100	>100	>100	>100	>100	>100	>100
1e	>100	>100	>100	>100	>100	>100	>100

3. RESULTS AND DISCUSSION

3.1. Chemistry

The synthesis of 5*H*-pyrimido[5,4-*b*]indole-4-carboxamides **2** is shown in Scheme 1. Compound **5** was prepared via IEDDA reaction using published method [24]. Selective mono-decarboxylation of compound **5** led to the key intermediate acid **6**, which could be used to access either **1-ABC** analogs or eudistomin Y_3 analogs. First, we prepared amide analogs of **1-ABC** as shown in Scheme 1. Coupling of acid **6** with aliphatic amines using CDI provided compounds **2a-2k**, while aromatic amines were coupled with acid **6** via its acyl chloride to give compounds **2l-2o**. Removal of Boc protecting groups of compounds **2a** and **2b** using hydrogen chloride in ethyl acetate gave compounds **2p** and **2q**.

The synthesis of eudistomin Y3 analogs **1** is shown in Scheme 2. Friedel-Crafts acylation of aromatic compounds was achieved by first converting acid **6** into its acyl chloride and then reacted with various aromatic compounds in the presence of aluminum trichloride to give compounds **1a – 1d**. Demethylation of compound **1d** using concentrated hydrogen chloride in sealed tube provided compound **1e.**

3.2. Biological Activity

The synthesized eudistomin Y_3 and **1-ABC** analogs were tested for antibacterial activity with the hope that any initial observed active compounds could help to guide us to design and prepare future analogs. To this end, compounds **2a**, **2b**, **2p**, **2q**, and **1a** – 1**e** were screened against *Mycobacterium bovis* BCG (M.B. BCG), MRSA, *Staphylococcus aureus* (S.A.), *Bacillus subtilis* (B.S.), *Enterococcus faecalis* (E.F.), *Pseudomonas aeruginosa* (P.A.), *Klebsiella peneumoniae* #249 (K.P. #249). As evident from the results presented in Table 1, compounds **2a** and **2b** showed antibacterial activity against *M. bovis* BCG with MICs values of 25 μg/mL and

TABLE 2. Antibacterial Activity of the Second Batch of Compounds (MIC, μg/mL)

Compd.	*C. albicans*				Antimicrobial			
	SC5314	G5	17#	G5 synergisticg[a]	*M. bovis* BCG	*S. aureus*	*E. faecium*	*P. aeruginosa*
2c	>100	>100	>100	>100	>100	>100	>100	>100
2d	50	>100	>100	>100	>100	>100	>100	>100
2e	50	50	50	6.25	>100	100	>100	>100
2f	100	>100	>100	>100	>100	>100	>100	>100
2g	100	>100	>100	>100	>100	>100	>100	>100
2h	>100	>100	>100	>100	>100	>100	>100	>100
2i	100	>100	>100	>100	>100	>100	>100	>100
2j	>100	>100	>100	>100	>100	>100	>100	>100
2k	100	>100	>100	>100	>100	>100	>100	>100
2l	>100	>100	>100	>100	>100	>100	>100	>100
2m	>100	>100	>100	>100	>100	>100	>100	>100
2n	>100	>100	>100	>100	>100	>100	>100	>100
2o	>100	>100	>100	>100	>100	>100	>100	>100
Control	0.39[b]	0.78[c], 100[b]	0.78[c], 100[b]	0.03[d]	0.0156[e]	0.625[f]	0.625[f]	0.039[g]

[a]Synergistic assay with the addition of 25 μg/mL ketoconazole; [b]MIC for fluconazole; [c]MIC for amphotericin B; [d] MIC for cyclosporine; [e] MIC for rifampicin; [f] MIC for vancomycin; and [g] MIC for ciprofloxacin.

50 μg/mL respectively. The results showed that ketones had no activity, while amides had certain activity. Therefore, in the next step, some more amides were synthesized to evaluate their activity.

To increase the chance of discovering new leads, additional new analogs were synthesized and evaluated for antibacterial activity against *Candida albicans* (*C. albicans*) from clinical isolates. As shown in Table 2, all aromatic amides were inactive. Among the aliphatic amides. Compound **2d** showed antibacterial activity against *C. albicans* with MICs values of 50ìg/mL, and compound **2e** was discovered with antibacterial activity against all three strains of *C. albicans* tested with MICs values of 50ìg/mL. In the same time, synergistic antibacterial activities were also evaluated. As a result, compound **2e** demonstrated synergistic antibacterial activity with fluconazole (6.25 and 25 μg/mL), which suggested that future drug candidates from this class of compounds could be used in combination with existing drugs to treat *C. albican* infections.

In conclusion, a strategy to access 5*H*-pyrimido[5,4-*b*]indole-4-carboxamides and 5*H*-pyrimido[5,4-*b*] indole-4-ketones as eudistomin Y_3 and **1-ABC** analogs was realized via application of IEDDA reaction of 1,3,5-triazines and 3-aminoindoles. Two small focused library of compounds were prepared and their biological evaluation was investigated. Compounds **2a** and **2b** were discovered to have antibacterial activity against *M. bovis* BCG with MICs values of 25 μg/mL and 50 μg/mL respectively; while compound **2e** was discovered with antibacterial activity against all three strains of *C. albicans* tested with MICs values of 50 μg/mL. Moreover, compound **2e** demonstrated synergistic antibacterial activity with fluconazol, which suggested that future drug candidates from this class of compounds could be used in combination with existing drugs to treat *C. albican* infections. Further optimization of these initial hits could lead to new candidate compounds as potential antibacterial agents.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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