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Design, Synthesis and Activity Evaluation of Some Novel Indole Derivatives

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A series of novel indole derivatives as CDK4 inhibitors were designed and synthesized though the condensation reaction between the indolic acid and the corresponding substituted amine. The key step of our synthetic process is the efficient condensation reaction conducted by two different methods. The MTT and kinase assays were conducted used to assess the antitumor activity and cyclin-dependent kinases (CDKs) inhibitory activity. The most active compound **8b** has an IC_{50} of 10-25 μM for the inhibition of four different tumor cells and CDK4. The higher activities of **8b** were influenced by more conformational freedom resulted form the non-planar structure and by the stronger hydrogen bonding capability. Thus, the strategy we adapt to design potent, non-toxic CDK4 inhibitors is successful.

Keywords: Indole derivatives, Condensation reaction, Activity evaluation.

INTRODUCTION

Searching for new small molecule inhibition of cyclin-dependent kinases (CDKs) is an area of major current interest in the antitumor field[1]. CDKs perform a vital role in the different checkpoints of the eukaryotic cells division cycle. In particular, CDK4/cyclin D1 controls the restriction point, a keeper governing the G1-to-S transition. At this checkpoint, CDK4-cyclin D1phosphorylatesthe retinoblastoma protein (pRB). Phosphorylation leads to the release of E2F, which stimulates the G1-to-S transition across the restriction point where the cell is committed to division[2-5]. Recent researches have found that deregulation of CDK4-cyclin D1 kinase may play a particularly important role in tumorigenesis[6]. Cyclin D1 and Cdk4 genes are often amplified or overexpressed in many types of cancers[7,8], intimating that CDK4-cyclin D1 kinase would be a good breakthrough point in the cancer treatment.

The natural pigment fascaplysin 1 (Fig. 1) isolated from the Fijian sponge *Fascaplysinopsis* Bergquist sp. in 1988 is a famous CDK4 specific inhibitor[9,10]. Importantly, it also actives on cancer cell lines[11]. However, fascaplysin is highly toxic due to intercalating with DNA resulting from its planar structure[12]. Thus, our study is aimming at devising a potent, non-toxic (non-planar) fascaplysin-based CDK4 inhibitor. Our design strategy is to open rings of fascaplysin to seek analogues that have more conformational freedom. Intermediate is a good pointcut. Compound **2** (Fig. 1) is one intermediate in the pathway of fascaplysin synthesis by Radchenko in 1997[13]. It has been reported the weaker CDK4-cyclin D1 inhibitory activity[14]. Taking into account that fascaplysin actions on CDK4 through competing the hydrogen binding sites with ATP, reduced activity of the intermediate may be due to the lack of hydrogen bonding capability caused by changes in the spatial structure. We consider that whether the following measures can be taken to prevent the reduction, such as flipping the amide bond, extending carbon chain, changing the hydrogen type from donor to acceptor. Hence, a series of indole derivatives based on fascaplysin were synthesized and the synthesis and biological activity were reported in this paper. These compounds are not planar and then the intercalation in DNA is very unlikely.

We chose the inexpensive and commercially available indolic acid as the raw material. The target compounds were prepared by the treatment ofthe corresponding substituted amine with acylated indolic acid, or with indolic acid in the presence of CDI through the condensation reaction. The synthetic route ofnovel CDK4-cyclin D1 inhibitors (**4a-12b**) is shown in **Scheme-I**.

EXPERIMENTAL

Melting points were determined on an YRT-3 digital melting point apparatus and without correction. ^{1}H NMR and ^{13}C NMR spectra were recorded in DMSO-d_6 or $CDCl_3$ solution on a Brucker DRX-300M spectrometer operating at 400 MHz. Chemical shifts were reported in δ (ppm) units relative to the internal standard tetramethylsilane (TMS) *via* the solvent signal (2.49 for ^{1}H or 39.78 for ^{13}C). Mass spectra was measured on

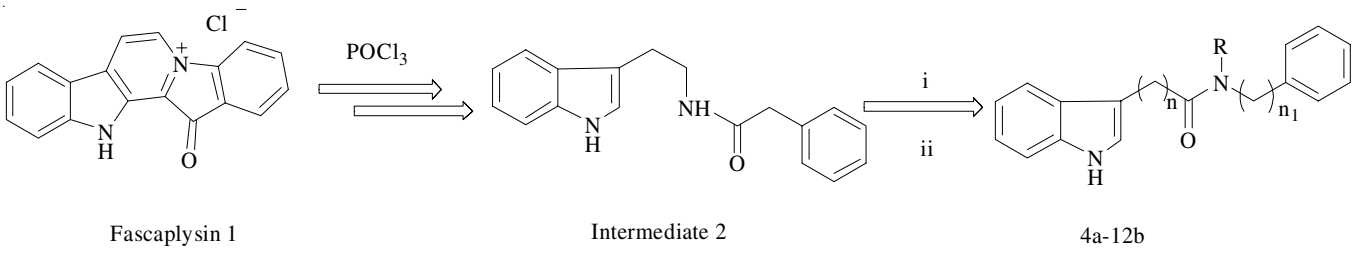
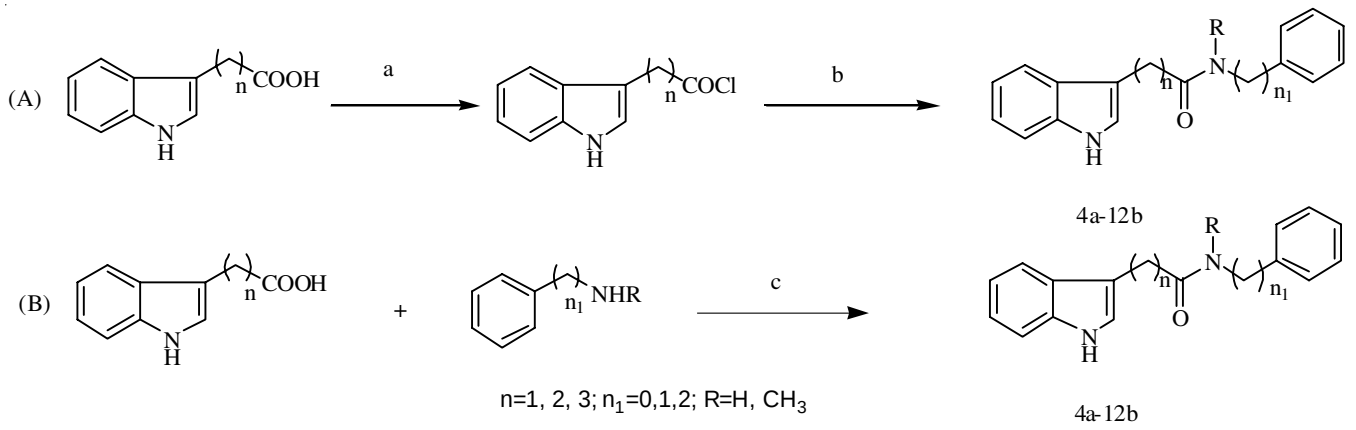


Fig. 1. Strategy adopted to convert intermediate **2** into the **4a-12b**. i:flip the of the intermediate **2**; ii:extend carbon chain of the products flipped



Scheme-1: Synthetic route to target compounds **4a-12b**; Reagents and conditions: Method A, a) Dry THF, $(COCl)_2$, 50 °C, 1h; b) Amine, Dry THF, rt, 4 h; Method B, c) CDI, rt

a HP-5988 mass spectrometer and optical rotation was detected by WXG-4 polarimeters. All chemicals and solvents used were of reagent grade purified and dried by standard methods before use. TLC monitoring all the reaction was performed on precoated silica gel G plates at 254/365 nm under a UV lamp, with petrol ether/ethyl acetate/triethylamine as the mobile phase.

General procedure for the synthesis of the compounds of series N-substituted amines: To a solution of corresponding amine (5 mmol) and triethylamine (4 mL) in CH_2Cl_2 (30 mL) cooled to 0 °C, ethyl chloroformate (0.75 mL, 6.25 mmol) was added dropwise over10 min with stirring. The reaction mixture was stirred for an additional 0.5 h at 0 °C, then allowed to warm to room temperature and stirred for an additional 1.5 h. The solvent was removed *in vacuo*. Afterwards, a solution of the product above (3.2 mmol) in THF (8 mL) was slowly added to a suspension of $LiAlH_4$ (35.1 mmol) in THF (30 mL) cooled to 0°C under argon. After the addition was completed, the reaction mixture was allowed to warm to room temperature, then refluxed for 3 h. After allowing to cool to room temperature, the reaction was quenched by the addition of water (5 mL) and 15 % aqueous KOH (w/v, 1 mL). The precipitate was removed by filtration and washed with ether (90 mL), then with MeOH (16 mL). The combined filtrates were dried over Na_2SO_4, then the solvent removed in vacuo and the residues purified by flash chromatography ($EtOAc/CH_2Cl_2$, 1:1) to give N-substituted amines.

General Procedure for the synthesis of compounds 4a-12b:

Method A: The corresponding acid (2 mmol) was heated at 50 °C in oxalyl chloride (1 mL) for 1 h in a 25 mL round-bottomed flask. The oxalyl chloride was then evaporated and the resulting solid was dissolved in anhydrous THF (5 mL). The reaction was cooled in an ice bath and aniline (1 mmol) in the presenceof TEA (3 mL) was added dropwise. After addition, the mixture was warmed to room temperature and stirred for 4 h. After the completion of reaction, the product was extracted with ethyl acetate. The organic layer was washed with 5 % $NaHCO_3$ and 10 % HCl followed by distilled water. Finally the organic layer was dried over anhydrous $MgSO_4$. The product was purified by chromatography on silica gel to give compound **4a-12b** .

Method B: Acid (1 mmol) and carbonyl diimidazole (CDI) (1 mmol) were dissolved in 3 mL of THF and stirred at room temperature. After 0.5 h, amine was added and the reaction mixture stirred overnight (monitored by TLC). Then the mixture was poured into water (20 mL), extracted by ethyl acetate and the combined organic solvent was dried over $MgSO_4$, filtered and concentrated in vacumn. The crude product was purified by recrystallization in petrol ether and ethyl acetate to give compound **4a-12b**.

2-(1*H*-Indol-3-yl)-N-phenyl acetamide(4a): White solid; 81 % yield; m.p. 156-157 °C; ^{1}H NMR (400 MHz $CDCl_3$): δ: 8.48 (1H, s, NH), 7.61 (1H, d, J = 8.0 Hz), 7.47 (1H, s, CONH), 7.42 (1H, d, J = 8.0 Hz), 7.34 (2H, d, J = 8.0 Hz), 7.27 (1H, d, J = 7.6 Hz), 7.23 (2H, t, J_1 = 7.6 Hz, J_2 = 8.0 Hz), 7.17 (2H, t, J_1 = 6.4 Hz, J_2 = 7.2 Hz), 7.05 (1H, t, J_1 = 7.2 Hz, J_2 = 7.2 Hz), 3.90 (2H, s, CH_2); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 165.4, 141.8, 136.0, 131.6, 128.7, 125.9, 122.8, 121.7, 120.9, 120.8, 118.4, 117.4, 111.8, 111.6, 104.1, 55.3; H RMS (ESI): (M + H) 251.1179 (calculated 251.3031), error = 1.2 ppm.

2-(1*H*-Indol-3-yl)-N-methyl-N-phenyl acetamide (4b): White solid; 73 % yield; m.p. 172-173 °C; ^{1}H NMR (400 MHz $CDCl_3$): δ:8.61 (1H, s, NH), 7.66 (1H, d, *J* = 7.6 Hz), 7.42 (1H, d, *J* = 7.2 Hz), 7.31 (2H, d, *J* = 7.6 Hz), 7.27 (1H, d, *J* = 7.6 Hz), 7.19 (2H, t, J_1 = 7.2 Hz, J_2 = 7.4 Hz), 7.11 (2H, t, J_1 = 7.6 Hz, J_2 = 7.2 Hz), 7.05 (1H, t, J_1 = 7.2 Hz, J_2 = 7.2 Hz), 3.89 (2H, s, CH_2), 2.81 (3H, s, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ:163.7, 142.1, 136.2, 131.5, 128.7, 125.9, 122.8, 121.1, 120.8, 120.8, 118.4, 118.4, 111.8, 111.6, 104.2, 47.5, 34.3 H RMS (ESI): (M + H) 265.2736 (calculated 265.3296), error = 0.8 ppm.

2-(1*H*-Indol-3-yl)-N-benzyl acetamide (5a): Yellow solid; 65 % yield; m.p. 154-155 °C; ^{1}H NMR (400 MHz $CDCl_3$): δ:10.9 (1H, s, NH), 8.4 (1H, s, CONH), 7.54 (1H, d, *J* = 7.6 Hz), 7.33 (1H, d, *J* = 8.0 Hz), 7.26 (2H, d, *J* = 6.0 Hz), 7.21 (4H, t, J_1 = 7.6 Hz, J_2 = 6.4 Hz), 7.06 (1H, t, J_1 = 7.6 Hz, J_2 = 7.2 Hz), 6.95 (1H, d, *J* = 7.2 Hz), 4.39 (2H, d, *J* = 4.8 Hz, CH_2), 3.81 (2H, s, CH_2); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 176.3, 140.1, 138.5, 132.1, 129.8, 129.8, 127.7, 127.7, 126.5, 123.8, 121.5, 120.7, 119.6, 117.4, 112.8, 52.3, 30.9 , H RMS(ESI): (M + H) 265.1335 (calculated 265.3296), error = 0.8 ppm.

2-(1*H*-Indol-3-yl)-N-methyl-N-benzyl acetamide (5b): Yellow solid; 51 % yield; m.p.160-162 °C; ^{1}H NMR (400 MHz $CDCl_3$): δ:10.9 (1H, s, NH), 7.56 (1H, d, *J* = 7.2 Hz), 7.34 (1H, d, *J* = 7.6 Hz), 7.25 (2H, d, *J* = 6.0 Hz), 7.22 (4H, t, J_1 = 7.6 Hz, J_2 = 7.2 Hz), 7.11 (1H, t, J_1 = 7.2 Hz, J_2 = 7.2 Hz), 6.90 (1H, d, *J* = 7.2 Hz), 4.40 (2H, d, *J* = 6.4 Hz, CH_2), 3.82 (2H, s, CH_2), 2.97 (3H, s, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 175.1, 139.3, 138.5, 131.1, 129.8, 129.8, 128.1, 127.7, 126.5, 123.8, 122.5, 120.6, 119.1, 117.4, 112.8, 55.3, 32.6, 28.5 , H RMS(ESI): (M + H) 279.1600 (calculated 279.3562), error = 0.8 ppm.

2-(1*H*-Indol-3-yl)-N-phenethyl acetamide (6a): White solid; 89 % yield; m.p. 101-102 °C; ^{1}H NMR (400 MHz $CDCl_3$): δ:10.8 (1H, s, NH), 8.7 (1H, t, J_1= 4.8 Hz, J_2 = 5.6 Hz, CONH), 7.52 (2H, d, *J* = 7.6 Hz), 7.34 (2H, d, *J* = 8.0 Hz), 7.17 (1H, d, *J* = 7.6 Hz), 7.06 (3H, t, J_1 = 6.8 Hz, J_2 = 6.0 Hz), 6.96 (2H, t, J_1 = 7.6 Hz, J_2 = 7.2 Hz), 3.33 (2H, q, J_1 = 7.2 Hz, J_2 = 6.8 Hz, J_3 = 6.8 Hz, CH_2), 2.81 (2H, t, J_1 = 7.6 Hz, J_2 = 7.2 Hz, CH_2); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 170.6, 136.2, 136.1, 131.6, 128.5, 128.5, 127.9, 127.9, 126.7, 122.8, 122.7, 120.5, 118.6, 111.4, 108.9, 50.3, 47.2, 33.9. H RMS(ESI): (M + H) 279.3532 (calculated 279.3562), error = 0.6 ppm.

2-(1*H*-Indol-3-yl)-N-methyl-N-phenethyl acetamide (6b): White solid; 76 % yield; m.p. 145-146 °C; ^{1}H NMR (400 MHz $CDCl_3$): δ: 10.8 (1H, s, NH), 7.61 (2H, d, *J* = 7.2 Hz), 7.43 (2H, d, *J* = 7.6 Hz), 7.20 (1H, d, *J* = 7.6 Hz), 7.05 (3H, t, J_1 = 6.8 Hz, J_2 = 7.2 Hz), 6.92 (2H, t, J_1 = 7.2 Hz, J_2 = 7.2 Hz), 3.31 (2H, q, J_1 = 7.6 Hz, J_2 = 7.2 Hz, J_3 = 6.8 Hz, CH_2), 3.01 (3H, s, CH_3), 2.81 (2H, t, J_1 = 7.2 Hz, J_2 = 7.2 Hz, CH_2); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 169.6, 136.2, 136.1, 131.6, 128.3, 128.3, 127.7, 127.6, 126.1, 122.8, 122.8, 120.5, 118.6, 111.4, 108.7, 52.1, 49.3, 33.3, 32.1. H RMS(ESI): (M + H) 293.3797 (calculated 293.3828), error = 0.6 ppm.

3-(1*H*-Indol-3-yl)-N-phenyl propionamide (7a): Light yellow powder; 87 % yield; m.p. 135-136°C; ^{1}H NMR (400 MHz $CDCl_3$): δ: 8.04 (1H, s, NH), 7.60 (1H, d, *J* = 8.0 Hz), 7.36 (3H, t, J_1 = 8.0 Hz, J_2 = 7.6 Hz), 7.35 (1H, *d*, *J* = 7.6 Hz), 7.30 (1H, d, *J* = 8.0 Hz), 7.19 (2H, d, *J* = 6.8 Hz), 7.12 (1H, t, J_1 = 7.6 Hz, J_2 = 7.2 Hz), 7.06 (1H, t, J_1 = 7.2 Hz, J_2 = 7.2 Hz), 6.94 (1H, s, *J* = CONH), 3.17 (2H, t, J_1 = 7.2 Hz, J_2 = 7.2 Hz, CH_2), 2.70 (2H, t, J_1 =7.2 Hz, J_2 = 7.2 Hz, CH_2); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 171.0, 139.4, 136.1, 131.6, 127.7, 127.7, 125.1, 122.3, 121.7,120.9, 120.9, 120.5 119.4, 113.2, 111.0, 34.1, 26.1; H RMS(ESI): (M + H) 265.1335 (calculated 265.3296), error = 1.1 ppm.

3-(1*H*-Indol-3-yl)-N-methyl-N-phenyl propionamide (7b): Light yellow powder; 83 % yield; m.p. 143-144 °C; ^{1}H NMR (400MHz $CDCl_3$): δ: 8.04 (1H, s, NH), 7.63 (2H, d, *J* = 7.2 Hz), 7.44 (1H, d, *J* = 8.0 Hz), 7.36 (3H, t, J_1 = 7.2 Hz, J_2 = 7.6 Hz), 7.31 (1H, d, *J* = 7.2 Hz), 7.25 (1H, d, *J* = 8.0 Hz), 7.11 (1H, t, J_1 = 7.6 Hz, J_2 = 7.6 Hz), 7.04 (1H, t, J_1 = 7.6 Hz, J_2 = 7.2 Hz), 3.18 (2H, t, J_1 = 7.6 Hz, J_2 = 7.2 Hz, CH_2), 2.82 (3H, s, CH_3); 2.64 (2H, t, J_1 = 7.2 Hz, J_2 = 7.2 Hz, CH_2); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 170.1, 139.4, 136.0, 131.6, 127.8, 127.7, 125.2, 122.3, 121.6, 120.9, 120.9, 120.4, 119.6, 113.1, 111.2, 35.2, 32.1, 26.6; H RMS(ESI): (M + H) 279.3538 (calculated 279.3562), error = 1.1 ppm.

3-(1*H*-Indol-3-yl)-N-benzyl propionamide (8a): White solid; 66 % yield; m.p. 119-120 °C; ^{1}H NMR (400 MHz $CDCl_3$): δ: 8.04 (1H, s, NH), 7.60 (1H, d, *J* = 8.0 Hz), 7.36 (1H, d, *J* = 8.4 Hz), 7.20 (2H, t, J_1 = 7.2 Hz, J_2 = 7.6 Hz), 7.12 (2H, d, *J* = 7.6 Hz), 7.08 (3H, t, J_1 = 7.6 Hz, J_2 = 5.6 Hz), 6.96 (1H, d, *J* = 7.6 Hz), 5.90 (1H, s, CONH), 4.36 (2H, d, *J* = 8.0 Hz, CH_2), 3.14 (2H, t, J_1 = 6.4 Hz, J_2 = 6.8 Hz, CH_2), 2.61 (2H, t, J_1 = 6.8 Hz, J_2 = 7.2 Hz, CH_2); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 171.9, 139.6, 136.3, 131.6, 128.2, 128.2, 127.1, 127.1, 126.6,122.2, 120.9, 118.1, 113.7, 112.1, 111.3, 41.2, 36.3, 21.1; H RMS (ESI): (M + H) 279.1492 (calculated 279.3562), error = 0.7 ppm.

3-(1*H*-Indol-3-yl)-N-methyl-N-benzyl propionamide (8b): White solid; 59 % yield; m.p. 123-125 °C; ^{1}H NMR (400 MHz $CDCl_3$): δ: 8.11 (1H, s, NH), 7.57 (1H, d, *J* = 7.6 Hz), 7.26 (1H, d, *J* = 8.0 Hz), 7.19 (2H, t, J_1 = 7.6 Hz, J_2 = 7.6 Hz), 7.11 (2H, d, *J* = 7.2 Hz), 7.04 (3H, t, J_1 = 7.2 Hz, J_2 = 7.2 Hz), 6.96 (1H, d, *J* = 7.6 Hz), 4.44 (2H, d, *J* = 7.2 Hz, CH_2), 3.12 (2H, t, J_1 = 7.2 Hz, J_2 = 6.8 Hz, CH_2), 3.02 (3H, s, CH_3); 2.65 (2H, t, J_1 = 7.2 Hz, J_2 = 7.2 Hz, CH_2); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 171.2, 137.6, 136.3, 131.6, 129.2, 129.2, 127.5, 127.5, 126.6, 122.2, 120.8, 118.0, 113.7, 112.3, 111.1, 47.2, 35.3, 32.2, 21.5; H RMS(ESI): (M + H) 293.3757 (calculated 293.3828), error = 0.7 ppm.

3-(1*H*-Indol-3-yl)-N-phenethyl propionamide (9a): White solid; 91 % yield; m.p. 156-158 °C; ^{1}H NMR (400 MHz $CDCl_3$): δ: 10.8 (1H, s, NH),7.99 (1H, t, J_1 = 7.6 Hz, J_2 = 7.2 Hz, CONH), 7.53 (2H, d, *J* = 7.6 Hz), 7.33 (2H, d, *J* = 8.0 Hz), 7.11 (1H, d, *J* = 8.8 Hz), 7.06 (3H, t, J_1 = 6.8 Hz, J_2 = 6.0 Hz), 6.97 (1H, t, J_1 = 7.2 Hz, J_2 = 7.6 Hz), 3.33 (2H, q, J_1 = 7.2 Hz, J_2 = 6.0 Hz, J_3 = 6.8 Hz, CH_2), 2.92 (2H, t, J_1 = 7.6 Hz, J_2 = 7.2 Hz, CH_2), 2.80 (2H, t, J_1 = 7.2 Hz, J_2 = 6.8 Hz, CH_2), 2.44 (2H, t, J_1 = 7.6 Hz, J_2 = 7.6 Hz, CH_2); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 171.7, 140.6, 136.2, 130.1, 128.7, 128.7, 127.2, 127.1, 122.5,122.0, 118.2, 118.1, 113.9, 111.9, 111.3, 41.2, 36.4, 25.3, 21.0; H RMS (ESI): (M + H) 293.3525 (calculated 293.3828), error = 0.9 ppm.

3-(1*H*-Indol-3-yl)-N-methyl-N-phenethyl propionamide (9b): White solid; 88 % yield; m.p. 175-176 °C; ^{1}H NMR (400 MHz $CDCl_3$): δ: 10.8 (1H, s, NH), 7.67 (2H, d, *J* =

7.2 Hz), 7.31 (2H, d, J = 7.6 Hz), 7.13 (1 H, d, J = 8.0 Hz), 7.01 (3H, t, J_1 = 7.2 Hz, J_2 = 7.2 Hz), 6.89 (1H, t, J_1 = 7.6 Hz, J_2 = 7.6 Hz), 3.54 (2H, q, J_1 = 7.2 Hz, J_2 = 7.2 Hz, J_3 = 6.8 Hz, CH_2), 3.01 (2H, t, J_1 = 7.2 Hz, J_2 = 7.2 Hz, CH_2), 2.96 (3H, s, CH_3), 2.77 (2H, t, J_1 = 7.6 Hz, J_2 = 7.2 Hz, CH_2), 2.41 (2H, t, J_1 = 7.2 Hz, J_2 = 7.6 Hz, CH_2); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 170.3, 140.6, 136.2, 130.1, 127.9, 127., 127.3, 127.2, 122.1, 122.1, 118.3, 118.1, 112.9, 111.3, 111.0, 51.4, 35.3, 33.1, 25.3, 21.7; H RMS (ESI): (M + H) 307.3790 (calculated 307.4093), error = 0.9 ppm.

4-(1*H*-Indol-3-yl)-N-phenyl butyramide (10a): Yellow powder; 93 % yield; m.p.102-103 °C; ^{1}H NMR (400 MHz $CDCl_3$): δ: 8.05 (1H, s, NH), 7.59 (1H, d, J = 7.6 Hz), 7.45 (2H, d, J = 7.2 Hz), 7.33 (2H, d, J = 8.4 Hz), 7.29 (2H, t, J_1 = 7.6 Hz, J_2 = 7.2 Hz), 7.19 (1H, t, J_1 = 6.8 Hz, J_2 = 7.6 Hz), 7.10 (2H, t, J_1 = 7.2 Hz, J_2 = 7.2 Hz), 6.93 (1H, s, CONH), 2.84 (2H, t, J_1 = 7.2 Hz, J_2 = 7.2 Hz, CH_2), 2.37 (2H, q, J_1 = 7.2 Hz, J_2 = 7.6 Hz, J_3 = 6.8 Hz, CH_2), 2.13 (2H, t, J_1 = 7.2 Hz, J_2 = 7.6 Hz, CH_2); ^{13}C NMR (100MHz, $CDCl_3$) δ: 171.2, 139.4, 136.3, 128.6, 128.6, 127.2, 127.1, 122.8,122.3, 119.1, 118.3, 118.1, 114.0, 111.3, 111.3, 40.1, 25.9, 24.3; H RMS (ESI): (M + K) 317.1051 (calculated 317.4826), error = 4.1 ppm.

4-(1*H*-Indol-3-yl)-N-methyl-N-phenyl butyramide (10b): Yellow powder; 86 % yield; m.p. 135-136 °C; ^{1}H NMR (400 MHz $CDCl_3$): δ: 8.15 (1H, s, NH), 7.60 (1H, d, J = 7.6 Hz), 7.35 (2H, d, J = 7.2 Hz), 7.31 (2H, d, J = 8.4 Hz), 7.29 (2H, t, J_1 = 7.6 Hz, J_2 = 7.2 Hz), 7.19 (1H, t, J_1 = 6.8 Hz, J_2 = 7.6 Hz), 7.13 (2H, t, J_1 = 7.2 Hz, J_2 = 7.2 Hz), 2.93 (3H, s, CH_3), 2.84 (2H, t, J_1 = 7.2 Hz, J_2 = 7.2 Hz, CH_2), 2.37 (2H, q, J_1 = 7.2 Hz, J_2 = 7.6 Hz, J_3 = 6.8 Hz, CH_2), 2.13 (2H, t, J_1 = 7.2 Hz, J_2 = 7.6 Hz, CH_2); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 171.5, 139.6, 136.3, 128.6, 128.6, 127.2, 127.1, 122.8,122.3, 119.1, 118.3, 118.1, 114.0, 111.3, 111.3, 36.1, 34.0, 25.9, 24.3; H RMS(ESI): (M + K) 331.1316 (calculated 331.4732), error = 4.0 ppm.

4-(1*H*-Indol-3-yl)-N-benzyl butyramide (11a): White solid; 67 % yield; m.p.122-123°C; ^{1}H NMR (400 MHz $CDCl_3$): δ: 8.14 (1H, s, NH), 7.55 (1H, d, J = 7.6 Hz), 7.31 (2H, d, J = 7.6 Hz), 7.28 (1H, d, J = 7.6 Hz), 7.24 (2H, t, J_1 = 5.6 Hz, J_2 = 6.8 Hz), 7.16 (1H, t, J_1 = 7.6 Hz, J_2 = 7.6 Hz), 7.08 (1H, t, J_1 = 7.6 Hz, J_2 = 7.2 Hz), 6.90 (1H, s, CONH), 5.75 (1H, *d*, J = 7.2 Hz), 4.46 (2H, d, J = 5.6 Hz, CH_2), 2.79 (2H, t, J_1 = 7.2 Hz, J_2 = 7.2 Hz, CH_2), 2.24 (2H, t, J_1 = 7.6 Hz, J_2 = 7.6 Hz, CH_2), 2.07 (2H, q, J_1 = 7.2 Hz, J_2 = 7.2 Hz, J_3 = 7.2 Hz, CH_2); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 172.1, 139.8, 136.3, 131.7, 128.3, 128.3, 127.2, 126.7, 122.4, 120.8, 120.8, 118.3, 118.1, 114.1, 111.3, 42.0, 35.2, 26.2, 24.3; H RMS (ESI): (M + H) 293.1648 (calculated 293.3828), error = 0.3 ppm.

4-(1*H*-Indol-3-yl)-N-methyl-N-benzyl butyramide (11b): White solid; 55 % yield; m.p.129-130 °C; ^{1}H NMR (400 MHz $CDCl_3$): *d*: 8.27 (1H, s, NH), 7.56 (1H, d, J = 7.2 Hz), 7.52 (2H, d, J = 7.6 Hz), 7.32 (1H, d, J = 7.6 Hz), 7.24 (2H, t, J_1 = 7.2 Hz, J_2 = 6.8 Hz), 7.16 (1H, t, J_1 = 7.6 Hz, J_2 = 7.6 Hz), 7.08 (1H, t, J_1 = 7.6 Hz, J_2 = 7.2 Hz), 5.75 (1H, d, J = 7.2 Hz), 4.46 (2H, d, J = 5.6 Hz, CH_2), 3.01 (3H, s, CH_3), 2.69 (2H, t, J_1 = 7.6 Hz, J_2 = 7.6 Hz, CH_2), 2.31 (2H, t, J_1 = 7.6 Hz, J_2 = 7.6 Hz, CH_2), 2.11 (2H, q, J_1 = 7.2 Hz, J_2 = 7.6 Hz, J_3 = 7.2 Hz, CH_2); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 171.4, 139.6, 137.3, 131.7, 128.2, 128.2, 127.2, 126.7, 121.3,120.1, 120.1, 118.7, 118.3, 115.6, 111.3, 41.9, 35.3, 32.4, 27.2, 24.3; H RMS (ESI):(M + H) 307.4313 (calculated 307.4094), error = 0.3 ppm.

4-(1*H*-Indol-3-yl)-N-phenethyl butyramide (12a): White solid; 95 % yield; m.p.124-125°C; ^{1}H NMR (400 MHz $CDCl_3$): δ: 10.8 (1H, s, NH), 7.92 (1H, t, J_1 = 5.2 Hz, J_2 = 4.8 Hz, CONH), 7.53 (1H, d, J = 8.0 Hz), 7.49 (1H, d, J = 7.6 Hz), 7.32 (2H, d, J = 8.0 Hz), 7.14 (1H, d, J = 7.2 Hz), 7.06 (3H, t, J_1 = 7.6 Hz, J_2 = 7.6 Hz), 6.97 (2H, t, J_1 = 7.2 Hz, J_2 = 7.2 Hz), 3.33 (2H, dd, J_1 = 6.8 Hz, J_2 = 6.4 Hz, CH_2), 2.82 (2H, t, J_1 = 7.6 Hz, J_2 = 7.2 Hz, CH_2), 2.66 (2H, t, J_1 = 7.2 Hz, J_2 = 7.6 Hz, CH_2), 2.15 (2H, q, J_1 = 6.8 Hz, J_2 = 7.2 Hz, J_3 = 7.6 Hz, CH_2), 1.91 (2H, t, J_1 = 7.6 Hz, J_2 = 7.6 Hz, CH_2); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 171.2, 136.3, 128.7, 128.3, 127.2, 122.5, 122.2, 120.9, 120.8, 118.2, 118.2, 118.1, 114.2, 111.9, 111.3, 40.6, 35.4, 26.1, 25.3, 24.3; H RMS (ESI): (M + H) 307.3983 (calculated 307.4094), error = 0.6 ppm.

4-(1*H*-Indol-3-yl)-N-methyl-N-phenethyl butyramide (12b): White solid; 83 % yield; m.p.141-142 °C; ^{1}H NMR (400 MHz $CDCl_3$): δ: 10.5 (1H, s, NH), 7.61 (1H, d, J = 7.6 Hz), 7.49 (1H, d, J = 7.6 Hz), 7.39 (2H, d, J = 8.0 Hz), 7.16 (1H, d, J = 7.6 Hz), 7.12 (3H, t, J_1 = 7.2 Hz, J_2 = 7.6 Hz), 6.97 (2H, t, J_1 = 7.2 Hz, J_2 = 7.2 Hz), 3.21 (2H, dd, J_1 = 6.4 Hz, J_2 = 6.4 Hz, CH_2), 2.90 (3H, s, CH_3), 2.77 (2H, t, J_1 = 7.6 Hz, J_2 = 7.2 Hz, CH_2), 2.67 (2H, t, J_1 = 7.2 Hz, J_2 = 7.6 Hz, CH_2), 2.13 (2H, q, J_1 = 7.2 Hz, J_2 = 7.2 Hz, J_3 = 7.6 Hz, CH_2), 2.01 (2H, t, J_1 = 7.2 Hz, J_2 = 7.6 Hz, CH_2); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 170.2, 136.3, 129.1, 128.5, 127.2, 122.4, 122.3, 120.9, 120.9, 118.1, 118.1, 118.0, 115.2, 111.9, 111.6, 45.1, 37.4, 32.7, 26.1, 25.5, 24.3; H RMS (ESI): (M + H) 321.4248 (calculated 321.4359), error = 0.6 ppm.

Biological activity cell culture and MTT assay: LS174T, A549, Hela, BeL-7402 were maintained in DMEM, supplemented with 10 % FBS, at 37 °C under 5 % CO_2 in the air. Cell proliferation was determined by standard MTT assay in LS174T, A549, Hela, BeL-7402. Cells were inoculated in 96-well plates at the density of 2×10^5 cells per well. Attached cells were incubated for 24 h prior to compounds addition at different concentrations and then the incubation period was extended for another 48 h. The cell survival fraction was determined with 10 ul MTT (5 mg mL^{-1} in PBS) per well. Cells were incubated further for 4 h at 37 °C. Finally the crystals formed were dissolved by the addition of DMSO and measured by the absorbance at 490 nm.

Kinase assays and IC_{50} determination: The kinase assays measure the IC_{50}s of the compounds through the depletion in ATP concentrations occurring as a result of phosphorylation of retinoblastoma and histone H1 by CDK4 and CDK2, respectively[10,15]. Protein complexes of CDK4-cyclin D1 and CDK2-cyclin A were extracted and purified according to description of the CDK4-cyclin D1 or CDK2-cyclin A kinase activity spectrum quantitative detection kit (GENMED SCIENTIFICS INC. U.S.A). Protein content was measured in Bradford assay. The compounds were dissolved in DMSO as 10 mM stock solutions. Compounds were further diluted in kinase buffer in order to obtain the desired concentrations. Purified enzyme complexes were pretreated first according to the requirements of the kit. The assay was run in a 96 well

format and was performed in 25 uL of kinase buffer with the enzymatic solution, substrate solution (in case of CDK4-cyclin D1 or CDK2-cyclin A) added sequentially. The plate was incubated for 3 min at 30 °C in a humidified incubator. Then the 20 μL pretreated sample was added and determined by microplate reader at 30 °C for 0.5 h. In the case of the CDK4-cyclin D1 assay, the compounds fascaplysin (Merck) with known IC_{50} values were used to validate the assay. For the CDK2-cyclin A assay, flavopiridol (Sigma) were used as standards for the assay.

RESULTS AND DISCUSSION

The strategy we adopt is to maintain most of the key interactions thought to occur between fascaplysin and CDK4 to enhance the success. We designed a series of such compounds proceeding from the intermediate before closing the loop in the fascaplysin synthesis. All compounds in this series could be synthesized by two different methods. Most yields are more than 70 %. Some compounds have been previously synthesized, while the application as CDK4 inhibitiors has not been studied. Spectroscopy data and melting point agreed with those of the literature[16-19].

All compounds were tested for *in vitro* cytotoxicity against four human tumor cell lines such as LS174T, A549, Hela and BeL-7402. The inhibitory effects of compounds were quantified using the MTT assay and IC_{50}s for cell growth inhibition were determined. The results were showed in Table-1. These results showed that all the compounds (**4a-12b**) have different degrees of cytotoxicity. In particular, **8b** is most potent and shows no differential cytotoxicity against four different tumor cells and CDK4 with the IC_{50} of 10-25 μM. The results is consistent with that seen in kinase assays. The kinase assays measure the IC_{50} of all compounds through the depletion in ATP concentrations occurring as a result of phosphorylation by CDKs of GST-pRB152 (a substrate for both CDK4-cyclin D1 and CDK2-cyclin A). The results for the inhibition are also shown in Table-1. Again we see that all compounds are CDK4 active compared to CDK2. The IC_{50}s were less than 60 μM for three of compounds . **8b** also shows the most powerful inhibition to CDK4 with an IC_{50} of 17 μM. Therefore, it is likely that **8b** inhibits cell proliferation by inhibiting the activity of CDK4.

In addition, the product of flipping the amide bond **8a** falls a little short of the activities. But the activity would increase significantly when we methylated the amide group (**8b**). In contrast, when we changed the carbon chains length at both ends of amide bond, the opposite result was obtained. With the extension of the carbon chains, the activity decreased badly, such like **10a-12b**. A possibility we predict is that methylation of the amino group may lead compound itself to combine with CDK4 more strongly. However, the extension of the carbon chains resists its inserting into the CDK4 binding pocket.

Conclusion

In summary, our studies of structure-activity relationship suggests that breaking the planar structure and changing the type of hydrogen bonds from donor to acceptor of the lead compound fascaplysin may greatly improve the activity, which will contribute to the discovery of a series of prospective non-toxic (non-planar) fascaplysin-based CDK4 inhibitor. In addition, the higher activity of **8b** compared other indole derivatives also proves the success of the efforts to flip the amide bond and extending carbon chain. The application of these results and further mechanism studies are underway in our laboratory.

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TABLE-1
ACTIVITY OF FASCAPLYSIN ANALOGUES IN DIFFERENT *in vitro* ASSAYS

Compound	n	n_1	R	CDK4-cyclin D1	CDK2- cyclin A	IC_{50} (μmol L^{-1})			
						LS174T	A549	Hela	BeL-7402
Fascaplysin	-	-	-	0.54 ± 0.1	513 ± 11	0.92 ± 0.1	0.73 ± 0.1	1.51 ± 1	0.61±0.1
4a	1	0	H	126 ± 9	1267 ± 37	153 ± 5	95 ± 3	134 ± 5	112 ± 4
4b	1	0	CH_3	117 ± 6	1020 ± 34	110 ± 4	103 ± 4	146 ± 5	108 ± 6
5a	1	1	H	64 ± 7	625 ± 20	67 ± 4	51 ± 7	87 ± 8	76 ± 6
5b	1	1	CH_3	59 ± 5	584 ± 24	55 ± 2	91 ± 6	121 ± 7	94 ± 3
6a	1	2	H	88 ± 7	763 ± 26	87 ± 4	105 ± 5	136 ± 4	97 ± 5
6b	1	2	CH_3	73 ± 8	731 ± 21	58 ± 5	81 ± 3.2	96 ± 7	88 ± 3
7a	2	0	H	103 ± 7	987 ± 31	105 ± 6	115 ± 8	141 ± 9	93 ± 3
7b	2	0	CH_3	36 ± 3	784 ± 18	28 ± 4	12 ± 1	52 ± 3	35 ± 2
8a	2	1	H	85 ± 6	1125 ± 27	78 ± 4	95 ± 3	126 ± 6	87 ± 4
8b	2	1	CH_3	17 ± 2	832 ± 17	11 ± 1	16 ± 2	25 ± 3	14 ± 1
9a	2	2	H	95 ± 7	584 ± 24	77 ± 3	94 ± 6	122 ± 4	59 ± 3
9b	2	2	CH_3	91 ± 6	913 ± 23	101 ±6	123 ± 8	151 ± 9	87 ± 3
10a	3	0	H	136 ± 8	950 ± 34	130 ± 5	103 ± 4	156 ± 5	118 ± 7
10b	3	0	CH_3	141 ± 7	1320 ± 35	87 ± 6	115 ± 8	147 ± 9	76 ± 3
11a	3	1	H	171 ± 5	937 ± 27	159 ± 5	95 ± 4	133 ± 6	113 ± 4
11b	3	1	CH_3	156 ± 3	821 ± 23	175 ± 6	121 ± 8	134 ± 8	92 ± 4
12a	3	2	H	257 ± 9	1410 ± 31	187 ± 6	202 ± 9	275 ± 9	117 ± 4
12b	3	2	CH_3	233 ± 6	1190 ± 26	265 ± 4	207 ± 8	131 ± 5	211 ± 6

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