Asian Journal of Chemistry; Vol. 33, No. 10 (2021), 2327-2332

ASIAN JOURNAL OF CHEMISTRY

https://doi.org/10.14233/ajchem.2021.23313

Synthesis and Anticancer Activity of Novel Amide Tagged Trifluoromethyl Indole and Pyrimido Indole Derivatives

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Received: 16 May 2021; *Accepted*: 12 July 2021; *Published online*: 20 September 2021; AJC-20502

A series of novel amide tagged trifluoromethyl indole and pyrimido indole derivatives **4a-e** & **5a-e** and **6a-d** & **7a-d** were synthesized from 4-methyl-2-(methylamino)-6-(trifluoromethyl)isophthalonitrile (**1**) on reaction with bromoethyl acetate to obtain **2a** and **2b** isomers. Compound **2a** treated with hydrazine hydrate followed by Schiff base reaction to get compounds **4a-e**. In another way, compound **2a** on reaction with aliphatic primary amine to get compounds **6a-d**. For cyclization, compounds **4a-e** & **6a-d** treated with trifluoroacetic acid to obtain compounds **5a-e** and **7a-d**, respectively. All the synthesized compounds **4a-e** & **5a-e** and **6a-d** & **7a-d** were tested for anticancer activity against four human cancer cell lines such as A549-lung cancer (CCL-185), MCF7-breast cancer (HTB-22), DU145-prostate cancer (HTB-81) and HeLa-cervical cancer (CCL-2). Compounds **9e** and **9f** were found to have promising anticancer activity at micromolar concentration.

Keywords: Trifluoromethyl indole, Pyrimido indole, Cyclization, Schiff base, Anticancer activity.

INTRODUCTION

Mostly the five/six membered hetero-ring fused benzene derivatives play a significant role in synthetic organic chemistry and in medicinal chemistry due to their extensive range of applications in pharmaceutical field [1-4]. Particularly pyrrole-fused benzene (indole) derivatives considered as an important intermediates in drugs, pesticides and also as potential pharmaceuticals [5]. The nitrogen containing heterocycles have been attained great attention due to their direct involvement in natural products and plays an important role in medicinal chemistry [6-16]. Among all these *N*-heterocycles indole received significant attention because indole derivatives exhibits wide range of biological activities like antifungal [17], antihistaminic [18], antimicrobial [19], antioxidant [20], plant growth regulator [21], anti-HIV [22], anticonvulsant [23], anti-inflammatory and analgesic [24], anticancer [25], *etc*. The biomedical importance of indoles has also been reviewed [26]. Indole derivatives are present in many proteins, amino acids, bioactive alkaloids and drugs, some of the indole moiety containing important drugs are shown in Fig. 1.

Trifluoromethyl, nitrile, amine groups present on indole moiety makes the changes about its selectively properties. In general, indoles substituted at 2nd or 3rd position [27-29], are known to exhibit certain bioactivity. Thus, in this work, incorporation of amide and Schiff base functional groups at 2nd position of indole is attempted. No work is reported on trifluoromethyl group containing indole scaffolds functionalized with amide and Schiff base derivatives. It was decided to synthesize some novel Schiff base, amide functionalized trifluoromethyl group containing indole and pyrimidoindole derivatives. All the synthesized compounds **4a-e** & **5a-e** and **6a-d** & **7a-d** were tested for anticancer activity against four human cancer cell lines *viz*. A549-lung cancer (CCL-185), MCF7-breast cancer (HTB-22), DU145-prostate cancer (HTB-81) and HeLa - cervical cancer (CCL-2).

EXPERIMENTAL

Melting points were recorded on Casia-Siamia (VMP-AM) melting point apparatus and are uncorrected. IR spectra were recorded on a Perkin-Elmer FT-IR 240-C spectrophotometer

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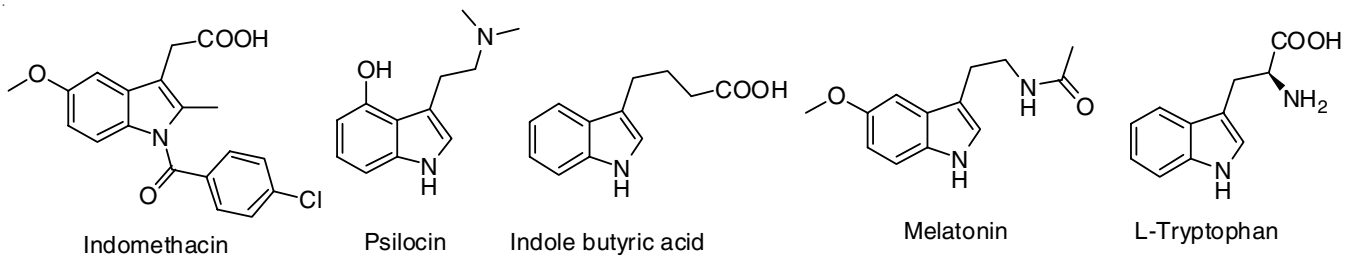
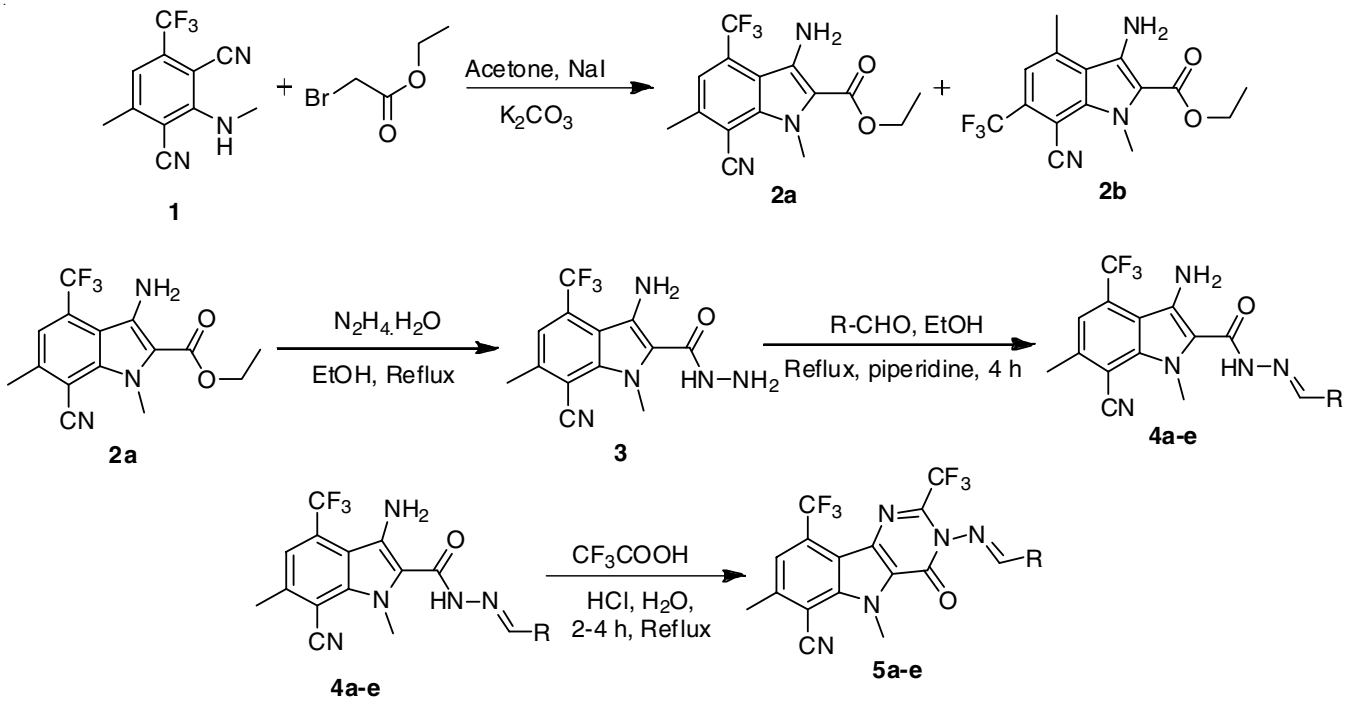


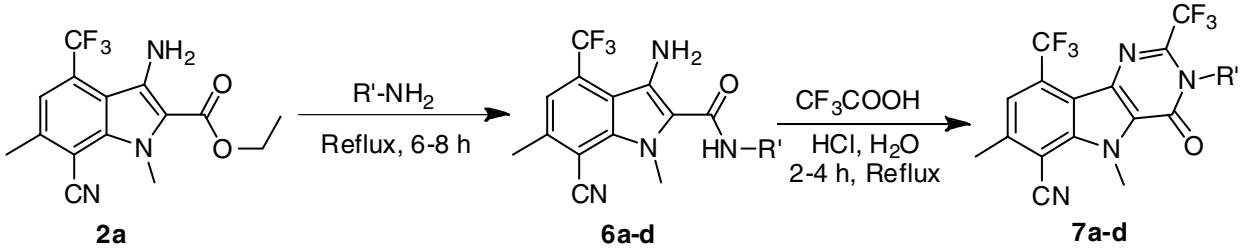
Fig. 1. Bioactive molecules containing indole framework

using KBr optics. ^{1}H NMR spectra were recorded on Bruker AV 300 MHz in $CDCl_3$ & DMSO-d_6 using TMS as internal standard. ESI spectra were recorded on Micro mass, Quattro LC using ESI+ software with capillary voltage 3.98 kV and ESI mode positive ion trap detector. All high-resolution spectra were recorded on QSTARXL hybrid MS/MS system (Applied Biosystems, USA) under electrospray ionization. All the reactions were monitored by TLC on precoated silica gel 60 F_{254}; spots were visualized with UV light. Merck silica gel (60-120 mesh) was used for column chromatography.

Synthesis: 4-Methyl-2-(methylamino)-6-(trifluoromethyl)-isophthalonitrile (**1**) on reaction with bromoethyl aceate in the presence of K_2CO_3 and acetone as a solvent media to obtain ethyl 3-amino-7-cyano-1,6-dimethyl-4-(trifluoromethyl)-1*H*-indole-2-carboxylate **2a** and **2b** as isomers [30]. Compound **2a** on reaction with hydrazine hydrate in ethanol to get 3-amino-7-cyano-1,6-dimethyl-4-(trifluoromethyl)-1*H*-indole-2-carbohydrazide (**3**), which further treated with substituted aromatic aldehydes to obtain Schiff base derivatives **4a-e** (**Scheme-I**). In another route, compound **2a** on reaction with different substituted aliphatic amines to get amide derivatives **6a-d**. Compounds **4a-e** and **6a-d** reacted with trifluoroacetic acid in the presence of HCl and water to get pyrimido indole derivatives (**Schemes-II**).



Scheme-I



Scheme-II

4-Methyl-2-(methylamino)-6-(trifluoromethyl)isophthalonitrile (1): Yield: 0.27 g, 45%; pale yellow solid; m.p.: 115 ºC; Anal. calcd. (found) % of $C_{11}H_8N_3F_3$: C, 55.23 (55.21); H, 3.37 (3.34); N, 17.57 (17.53). IR (KBr, ν_{max}, cm^{-1}): 3331 (NH), 2975 (C–H), 2350, 2232 (C≡N), 1570, 1121; ^{1}H NMR (200 MHz, $CDCl_3$, δ ppm): 2.55 (3H, s, $-CH_3$), 3.45 (3H, d, J_Z = 5.1 Hz, N-CH_3), 5.4 (1H, br s, NH), 6.9 (1H, s, H–C (4)); *m/z* (LSI-MS): 239 (MC, 100%).

Synthesis of 3-amino-7-cyano-1,6-dimethyl-4-(trifluoromethyl)-1*H*-indole-2-carbohydrazide (3): Ethyl 3-amino-7-cyano-1,6-dimethyl-4-(trifluoromethyl)-1*H*-indole-2-carboxylate (**2**) (3 mmol) was taken in 95% ethanol (30 mL) and hydrazine hydrate (5 mL) was added. The mixture was refluxed for 6 h and after cooling to room temperature, ethanol was removed under vacuum. The residue was washed with *n*-hexane and then water was added to give a yellow solid, which was filtered with water and dried. Colour: yellow solid; m.p.: 174-176 ºC; IR (KBr, ν_{max}, cm^{-1}): 3489, 3343 (NH_2), 3215, 1621 (CONH); ^{1}H NMR ($CDCl_3$, 300 MHz, δ ppm): 2.56 (3H, s, $-CH_3$), 3.43 (3H, d, *J* = 5.1 Hz, N-CH_3), 5.58 (br s, 2H, NH_2), 7.11 (s, 1H, Ar-H), 11.42 (br s, 1H, NHCO); MS (ESI): *m/z* [(M+H)$^+$]: 312. Anal. calcd. (found) % for $C_{13}H_{12}N_5OF_3$: C 50.16 (50.17), H 3.89 (3.90), N 22.50 (22.52).

Synthesis of (*E*)-3-amino-*N*'-benzylidene-7-cyano-1,6-dimethyl-4-(trifluoromethyl)-1*H*-indole-2-carbohydrazide derivatives (4a-e): 3-Amino-7-cyano-1,6-dimethyl-4-(trifluoromethyl)-1*H*-indole-2-carbohydrazide (**3**) (3 mmol) was taken in 95% ethanol (10 mL) and benzaldehyde (3 mmol) was added. To this piperidine (0.1 mL) was added as catalyst. The mixture was refluxed for 4 h and after cooling to room temperature the ethanol was removed under vacuum. The residue was washed with *n*-hexane and then water was added to give yellow solid which was filtered, washed with water and dried.

(*E*)-3-Amino-*N*′-benzylidene-7-cyano-1,6-dimethyl-4-(trifluoromethyl)-1*H*-indole-2-carbohydrazide (4a): Yellow solid; m.p.: 192-194 ºC; IR (KBr, ν_{max}, cm^{-1}): 3421, 3325 (NH_2), 1621 (CONH); ^{1}H NMR ($CDCl_3$, 300 MHz, δ ppm): 2.58 (3H, s, $-CH_3$), 3.41 (3H, s, N–CH_3), 5.65 (br s, 2H, NH_2), 7.15 (s, 1H, Ar-H), 7.28-7.31 (m, 3H, Ar-H), 7.43-7.45 (m, 2H, Ar-H), 8.35 (s, 1H, CH=N), 11.63 (br, 1H, NHCO); ^{13}C NMR (DMSO-d_6, 75 MHz, δ ppm): 21.5, 32.4, 121.2, 122.4, 124.6, 125.6, 126.8, 128.6, 130.5, 132.5, 134.1, 136.3, 140.4, 142.6, 144.8, 147.1, 148.5, 157.3;MS (ESI): *m/z* [(M+H)$^+$]: 400; HRMS *m/z* calcd. (found) for $C_{20}H_{16}N_5OF_3$ [(M+H)$^+$]: 400.0116 (400.0119).

(*E*)-3-Amino-7-cyano-1,6-dimethyl-*N*′-(4-methylbenzylidene)-4-(trifluoromethyl)-1*H*-indole-2-carbohydrazide (4b): Yellow solid; m.p.: 204-206 ºC; IR (KBr, ν_{max}, cm^{-1}): 3418, 3332 (NH_2), 1625 (CONH); ^{1}H NMR ($CDCl_3$, 300 MHz, δ ppm): 2.32 (3H, s, $-CH_3$), 2.57 (3H, s, $-CH_3$), 3.42 (3H, s, N-CH_3), 5.67 (br s, 2H, NH_2), 7.16 (s, 1H, Ar-H), 7.34 (dd, 2H, Ar-H), 7.53 (dd, 2H, Ar-H), 8.39 (s, 1H, CH=N), 11.64 (br, 1H, NHCO); ^{13}C NMR (DMSO-d_6, 75 MHz, δ ppm): 21.2, 23.4, 31.6, 121.7, 123.6, 124.3, 126.1, 127.8, 129.4, 130.5, 133.2, 134.6, 136.9, 140.3, 142.2, 145.4, 147.2, 149.2, 158.4; MS (ESI): *m/z* [(M+H)$^+$]: 414; HRMS *m/z* calcd. (found) for $C_{21}H_{18}N_5OF_3$ [(M+H)$^+$]: 414.0258 (414.0260).

(*E*)-3-Amino-*N*′-(4-chlorobenzylidene)-7-cyano-1,6-dimethyl-4-(trifluoromethyl)-1*H*-indole-2-carbohydrazide (4c): Yellow solid; m.p.: 211-213 ºC; IR (KBr, ν_{max}, cm^{-1}): 3412, 3314 (NH_2), 1629 (CONH); ^{1}H NMR ($CDCl_3$, 300 MHz, δ ppm): 2.58 (3H, s, $-CH_3$), 3.47 (3H, s, N-CH_3), 5.69 (br s, 2H, NH_2), 7.18 (s, 1H, Ar-H), 7.39 (dd, 2H, Ar-H), 7.58 (dd, 2H, Ar-H), 8.42 (s, 1H, CH=N), 11.68 (br, 1H, NHCO); ^{13}C NMR (DMSO-d_6, 75 MHz, δ ppm): 23.6, 31.4, 120.5, 122.4, 124.5, 126.8, 127.9, 129.5, 130.6, 133.4, 134.8, 137.5, 140.3, 142.6, 144.4, 146.6, 148.1, 158.8; MS (ESI): *m/z* [(M+H)$^+$]: 434; HRMS *m/z* calcd. (found) for $C_{20}H_{15}N_5OClF_3$ [(M+H)$^+$]: 434.0543 (434.0545).

(*E*)-3-Amino-*N*′-(4-bromobenzylidene)-7-cyano-1,6-dimethyl-4-(trifluoromethyl)-1*H*-indole-2-carbohydrazide (4d): Yellow solid; m.p.: 223-225 ºC; IR (KBr, ν_{max}, cm^{-1}): 3410, 3325 (NH_2), 1632 (CONH); ^{1}H NMR ($CDCl_3$, 300 MHz, δ ppm): 2.55 (3H, s, $-CH_3$), 3.46 (3H, s, N-CH_3), 5.65 (br s, 2H, NH_2), 7.14 (s, 1H, Ar-H), 7.38 (dd, 2H, Ar-H), 7.56 (dd, 2H, Ar-H), 8.41 (s, 1H, CH=N), 11.64 (br, 1H, NHCO); ^{13}C NMR (DMSO-d_6, 75 MHz, δ ppm): 23.2, 31.6, 121.3, 122.8, 124.5, 125.7, 127.6, 129.2, 131.4, 133.2, 134.7, 137.4, 140.2, 142.4, 144.3, 146.2, 148.8, 157.3; MS (ESI): *m/z* [(M+H)$^+$]: 479; HRMS *m/z* calcd. (found) for $C_{20}H_{15}N_5OBrF_3$ [(M+H)$^+$]: 479.0125 (479.0128).

(*E*)-3-Amino-7-cyano-*N*′-(3-methoxybenzylidene)-1,6-dimethyl-4-(trifluoromethyl)-1*H*-indole-2-carbohydrazide (4e): Yellow solid; m.p.: 201-203 ºC; IR (KBr, ν_{max}, cm^{-1}): 1657 (CONH); ^{1}H NMR ($CDCl_3$, 300 MHz, δ ppm): 2.52 (3H, s, $-CH_3$), 3.47 (3H, s, N-CH_3), 3.68 (3H, s, $-OCH_3$), 5.63 (br s, 2H, NH_2), 7.15 (s, 1H, Ar-H), 7.29 (s, 1H, Ar-H), 7.62-7.65 (m, 3H, Ar-H), 8.43 (s, 1H, CH=N), 11.66 (br, 1H, NHCO); ^{13}C NMR (DMSO-d_6, 75 MHz, δ ppm): 23.5, 31.8, 52.4, 120.6, 121.6, 123.7, 124.6, 125.8, 127.3, 128.4, 129.4, 132.5, 133.4, 134.6, 136.5, 141.3, 142.5, 144.6, 147.3, 148.2, 157.4; MS (ESI): *m/z* [(M+H)$^+$]: 430; HRMS *m/z* calcd. (found) for $C_{21}H_{18}N_5O_2F_3$ [(M+H)$^+$]: 430.01105 (430.01108).

Synthesis of (*E*)-3-(benzylideneamino)-5,7-dimethyl-4-oxo-2,9-*bis*(trifluoromethyl)-4,5-dihydro-3*H*-pyrimido[5,4-*b*]indole-6-carbonitrile derivatives (5a-e): (*E*)-3-Amino-*N*′-benzylidene-7-cyano-1,6-dimethyl-4-(trifluoromethyl)-1*H*-indole-2 carbohydrazide (**4**) (3 mmol) and trifluoroacetic acid (3 mmol) were taken. To this reaction mixture added catalytic amount of HCl and H_2O. The reaction mixture was refluxed at 100 ºC for 2-4 h and after cooling to room temperature, *n*-hexane was added and filtered washed with water to give a pale yellow colour solid and dried.

(*E*)-3-(Benzylideneamino)-5,7-dimethyl-4-oxo-2,9-*bis*(trifluoromethyl)-4,5-dihydro-3*H*-pyrimido[5,4-*b*]-indole-6-carbonitrile (5a): Light yellow solid; m.p.: 168-170 ºC; IR (KBr, ν_{max}, cm^{-1}): 1663 (CONH); ^{1}H NMR ($CDCl_3$, 300 MHz, δ ppm): 2.54 (3H, s, $-CH_3$), 3.49 (3H, s, N-CH_3), 5.61 (br s, 2H, NH_2), 7.16 (s, 1H, Ar-H), 7.56-7.58 (m, 3H, Ar-H), 7.78-7.80 (m, 2H, Ar-H), 8.82 (s, 1H, CH=N); ^{13}C NMR (DMSO-d_6, 75 MHz, δ ppm): 23.5, 31.8, 118.7, 120.8, 121.4, 122.7, 123.8, 125.6, 126.4, 129.7, 132.0, 133.5, 136.9, 138.4, 140.5, 142.7, 143.6, 147.6, 150.4, 161.2; MS (ESI): *m/z* [(M+H)$^+$]: 430; HRMS *m/z* calcd. (found) for $C_{22}H_{13}N_5O_2F_6$ [(M+H)$^+$]: 430.1105 (430.1108).

(*E*)-5,7-Dimethyl-3-((4-methylbenzylidene)amino)-4-oxo-2,9-*bis*(trifluoromethyl)-4,5-dihydro-3*H*-pyrimido[5,4-*b*]-indole-6-carbonitrile (5b): Pale yellow solid; m.p.: 198-200 ºC; IR (KBr, ν_{max}, cm^{-1}): 1632 (CONH); ^{1}H NMR ($CDCl_3$, 300 MHz, δ ppm): 2.34 (3H, s, -CH_3), 2.54 (3H, s, -CH_3), 3.48 (3H, s, N-CH_3), 5.62 (br s, 2H, NH_2), 7.15 (s, 1H, Ar-H), 7.26-7.28 (dd, 2H, Ar-H), 7.68-7.70 (dd, 2H, Ar-H), 8.86 (s, 1H, CH=N); ^{13}C NMR (DMSO-d_6, 75 MHz, δ ppm): 21.4, 23.5, 31.8, 118.7, 120.8, 121.4, 122.7, 123.8, 125.6, 126.4, 129.7, 132.0, 133.5, 136.9, 138.4, 140.5, 142.7, 143.6, 147.6, 150.4, 161.2; MS (ESI): *m/z* [(M+H)$^+$]: 492; HRMS *m/z* calcd. (found) for $C_{23}H_{15}N_5OF_6$ [(M+H)$^+$]: 492.0521 (492.0523).

(*E*)-3-((4-Chlorobenzylidene)amino)-5,7-dimethyl-4-oxo-2,9-*bis*(trifluoromethyl)-4,5-dihydro-3*H*-pyrimido[5,4-*b*]indole-6-carbonitrile (5c): Yellow solid; m.p.: 214-216 ºC; IR (KBr, ν_{max}, cm^{-1}): 1629 (CONH); ^{1}H NMR ($CDCl_3$, 300 MHz, δ ppm): 2.58 (3H, s, -CH_3), 3.42 (3H, s, N-CH_3), 5.61 (br s, 2H, NH_2), 7.11 (s, 1H, Ar-H), 7.32-7.34 (dd, 2H, Ar-H), 7.73-7.75 (dd, 2H, Ar-H), 8.89 (s, 1H, CH=N); ^{13}C NMR (DMSO-d_6, 75 MHz, δ ppm): 23.4, 31.3, 119.5, 120.5, 121.7, 122.8, 123.6, 125.2, 126.6, 129.1, 131.3, 132.8, 136.8, 138.8, 141.4, 142.6, 143.2, 148.8, 151.3, 160.4; MS (ESI): *m/z* [(M+H)$^+$]: 512; HRMS *m/z* calcd. (found) for $C_{22}H_{12}N_5OClF_6$ [(M+H)$^+$]: 512.0112 (512.0114).

(*E*)-3-((4-Bromobenzylidene)amino)-5,7-dimethyl-4-oxo-2,9-*bis*(trifluoromethyl)-4,5-dihydro-3*H*-pyrimido[5,4-*b*]-indole-6-carbonitrile (5d): Yellow solid; m.p.: 221-224 ºC; IR (KBr, ν_{max}, cm^{-1}): 1634 (CONH); ^{1}H NMR ($CDCl_3$, 300 MHz, δ ppm): 2.56 (3H, s, -CH_3), 3.41 (3H, s, N-CH_3), 5.64 (br s, 2H, NH_2), 7.14 (s, 1H, Ar-H), 7.36-7.38 (dd, 2H, Ar-H), 7.81-7.83 (dd, 2H, Ar-H), 8.91 (s, 1H, CH=N); ^{13}C NMR (DMSO-d_6, 75 MHz, δ ppm): 23.2, 31.2, 118.7, 120.2, 121.5, 122.4, 123.5, 125.3, 126.4, 128.2, 131.2, 133.2, 135.4, 138.7, 141.2, 142.8, 143.3, 148.4, 150.5, 161.2; MS (ESI): *m/z* [(M+H)$^+$]: 557; HRMS *m/z* calcd. (found) for $C_{22}H_{12}N_5OBrF_6$ [(M+H)$^+$]: 557.0245 (557.0248).

(*E*)-3-((3-Methoxybenzylidene)amino)-5,7-dimethyl-4-oxo-2,9-*bis*(trifluoromethyl)-4,5-dihydro-3*H*-pyrimido[5,4-*b*]indole-6-carbonitrile (5e): Light yellow solid; m.p.: 207-209 ºC; IR (KBr, ν_{max}, cm^{-1}): 1639 (CONH); ^{1}H NMR ($CDCl_3$, 300 MHz, δ ppm): 2.57 (3H, s, -CH_3), 3.42 (3H, s, N-CH_3), 3.69 (3H, s, -OCH_3), 5.62 (br. s., 2H, NH_2), 7.11 (s, 1H, Ar-H), 7.41-7.44 (m, 3H, Ar-H), 7.78 (s, 1H, Ar-H), 8.89 (s, 1H, CH=N); ^{13}C NMR (DMSO-d_6, 75 MHz, δ ppm): 23.2, 31.1, 52.6, 119.4, 120.4, 121.6, 122.2, 124.8, 125.2, 126.7, 128.1, 131.4, 133.6, 136.8, 138.3, 141.5, 142.6, 143.4, 147.3, 149.7, 159.2; MS (ESI): *m/z* [(M+H)$^+$]: 508; HRMS *m/z* calcd. (found) for $C_{23}H_{15}N_5OF_6$ [(M+H)$^+$]: 508.1102 (508.1104).

3-Amino-7-cyano-*N*-substituted-1,6-dimethyl-4-(trifluoromethyl)-1*H*-indole-2-carboxamide (6a-d): Ethyl 3-amino-7-cyano-1,6-dimethyl-4-(trifluoromethyl)-1*H*-indole-2-carboxylate (**2**) (3 mmol) and aliphatic primary amines (6 mmol) were refluxed for 6-8 h and after cooling to room temperature kept in crushed ice. The reaction mixturewas filtered, washed with *n*-hexane followed by water to give a white solid and dried.

3-Amino-7-cyano-*N*,1,6-trimethyl-4-(trifluoromethyl)-1*H*-indole-2-carboxamide (6a): White solid; m.p.: 156-158 ºC; IR (KBr, ν_{max}, cm^{-1}): 3496 (-NH_2), 3454 (-NH_2), 3341 (NHCO), 1636 (NHCO); ^{1}H NMR ($CDCl_3$, 300 MHz, δ ppm): 2.59 (3H, s, -CH_3), 3.05 (3H, s, -CH_3), 3.42 (3H, s, N-CH_3), 5.66 (br s, 2H, NH_2), 6.39 (br s, 1H, -CONH-), 7.16 (s, 1H, Ar-H); ^{13}C NMR (DMSO-d_6, 75 MHz, δ ppm): 21.6, 25.8, 32.3, 119.4, 121.2, 122.4, 123.7, 126.8, 128.6, 130.5, 134.1, 142.6, 144.8, 160.3; MS (ESI): *m/z* [(M+H)$^+$]: 311; HRMS *m/z* calcd. (found) for $C_{19}H_{15}N_4OF_3$ [(M+H)$^+$]: 311.0057 (311.0060).

3-Amino-7-cyano-*N*-ethyl-1,6-dimethyl-4-(trifluoromethyl)-1*H*-indole-2-carboxamide (6b): White solid; m.p.: 186-188 ºC; IR (KBr, ν_{max}, cm^{-1}): 3485 (-NH_2), 3435 (-NH_2), 3345 (-NHCO-), 1632 (-NHCO-); ^{1}H NMR ($CDCl_3$, 300 MHz, δ ppm): 1.14 (t, 3H, -CH_3), 2.52 (3H, s, -CH_3), 3.05 (3H, s, -CH_3), 3.42 (3H, s, N-CH_3), 3.51 (quintet, 2H, -CH_2-), 5.62 (br s, 2H, NH_2), 6.33 (br s, 1H, -CONH-),7.13 (s, 1H, Ar-H); ^{13}C NMR (DMSO-d_6, 75 MHz, δ ppm): 16.13, 26.2, 32.5, 36.4, 119.7, 121.4, 122.7, 124.5, 126.6, 127.7, 131.3, 133.4, 142.6, 144.8, 160.3; MS (ESI): *m/z* [(M+H)$^+$]: 325; HRMS *m/z* calcd. (found) for $C_{15}H_{15}N_4OF_3$ [(M+H)$^+$]: 325.0124 (325.0126).

3-Amino-7-cyano-1,6-dimethyl-*N*-propyl-4-(trifluoromethyl)-1*H*-indole-2-carboxamide (6c): White solid; m.p.: 192-194 ºC; IR (KBr, ν_{max}, cm^{-1}): 3478 (-NH_2), 3432 (-NH_2), 3324 (NHCO), 1629 (NHCO); ^{1}H NMR ($CDCl_3$, 300 MHz, δ ppm): 1.58-1.65 (m, 2H, -CH_2-), 2.52 (3H, s, -CH_3), 3.38-3.41 (m, 2H, -CH_2-), 3.43 (3H, s, N-CH_3), 5.61 (br s, 2H, NH_2), 6.40 (br s, 1H, -CONH-), 7.16 (s, 1H, Ar-H); ^{13}C NMR (DMSO-d_6, 75 MHz, δ ppm): 10.2, 22.4, 24.2, 32.5, 41.2, 118.6, 120.8, 122.3, 124.1, 126.3, 127.5, 130.4, 133.6, 142.7, 145.4, 161.3; MS (ESI): *m/z* [(M+H)$^+$]: 339; HRMS *m/z* calcd. (found) for $C_{15}H_{15}N_4OF_3$ [(M+H)$^+$]: 339.1042 (339.1045).

3-Amino-7-cyano-*N*-cyclopentyl-1,6-dimethyl-4-(trifluoromethyl)-1*H*-indole-2-carboxamide (6d): White solid; m.p.: 162-164 ºC; IR (KBr, ν_{max}, cm^{-1}): 3485 (-NH_2), 3446 (-NH_2), 3332 (NHCO), 1636 (NHCO); ^{1}H NMR ($CDCl_3$, 300 MHz, δ ppm): 1.53-1.58 (m, 2H, -CH_2-), 1.64-1.69 (m, 2H, -CH_2-), 1.74-1.81 (m, 2H, -CH_2-), 2.12-2.16 (m, 2H, -CH_2-), 2.54 (3H, s, -CH_3), 3.44 (3H, s, N-CH_3), 4.42-4.48 (m, 1H, -CH-), 5.66 (br s, 2H, NH_2), 6.42 (br s, 1H, -CONH-), 7.18 (s, 1H, Ar-H); ^{13}C NMR (DMSO-d_6, 75 MHz, δ ppm): 21.2, 24.2, 32.5, 35.1, 46.2, 120.8, 121.3, 123.4, 124.5, 126.7, 128.4, 130.2, 132.5, 142.3, 144.6, 160.4; MS (ESI): *m/z* [(M+H)$^+$]: 365; HRMS *m/z* calcd. (found) for $C_{18}H_{19}N_4OF_3$ [(M+H)$^+$]: 365.0521 (365.0524).

Synthesis of 5,7-dimethyl-4-oxo-3-substituted-2,9-*bis*-(trifluoromethyl)-4,5-dihydro-3*H*-pyrimido[5,4-*b*]indole-6-carbonitrile derivatives (7a-d): 3-Amino-7-cyano-*N*-1,6-trimethyl-4-(trifluoromethyl)-1*H*-indole-2-carboxamide (**6**) (4 mmol) and trifluoroacetic acid (4 mmol) were mixed with the catalytic amount of HCl and H_2O. The mixture was refluxed at 100 ºC for 2-4 h and after cooling to room temperature, *n*-hexane was added and filtered washed with water to give a pale white colour solid and dried.

3,5,7-Trimethyl-4-oxo-2,9-*bis*(trifluoromethyl)-4,5-dihydro-3*H*-pyrimido[5,4-*b*]indole-6-carbonitrile (7a): White solid; m.p.: 210-212 ºC; IR (KBr, ν_{max}, cm^{-1}): 3496 (-NH_2), 3454 (-NH_2), 3341 (NHCO), 1636 (NHCO); ^{1}H NMR ($CDCl_3$, 300 MHz, δ ppm): 2.52 (3H, s, -CH_3), 3.15 (3H, s, -CH_3),

3.61 (3H, s, N-CH_3), 7.32 (s, 1H, Ar-H); ^{13}C NMR (DMSO-d_6, 75 MHz, δ ppm): 23.1, 32.3, 35.2, 120.4, 121.6, 122.4, 123.7, 124.3, 126.8, 128.6, 130.5, 132.4, 134.3, 142.8, 145.9, 159.4; MS (ESI): *m/z* [(M+H)$^+$]: 389; HRMS *m/z* calcd. (found) for $C_{16}H_{10}N_4OF_6$ [(M+H)$^+$]: 389.0102 (389.0105).

3-Ethyl-5,7-dimethyl-4-oxo-2,9-*bis*(trifluoromethyl)-4,5-dihydro-3*H*-pyrimido[5,4-*b*]indole-6-carbonitrile (7b): White solid; m.p.: 198-200 ºC; IR (KBr, ν_{max}, cm^{-1}): 3475 (-NH_2), 3448 (-NH_2), 3329 (NHCO), 1631 (NHCO); ^{1}H NMR ($CDCl_3$, 300 MHz, δ ppm): 1.50 (t, 3H, -CH_3), 4.21 (q, 2H, -CH_2), 2.52 (3H, s, -CH_3), 3.15 (3H, s, -CH_3), 7.36 (s, 1H, Ar-H); ^{13}C NMR (DMSO-d_6, 75 MHz, δ ppm): 12.8, 24.5, 36.3, 38.5, 120.2, 121.4, 122.6, 123.1, 125.3, 126.8, 128.2, 130.4, 133.1, 134.6, 142.7, 145.6, 160.3; MS (ESI): *m/z* [(M+H)$^+$]: 403; HRMS *m/z* calcd. (found) for $C_{17}H_{12}N_4OF_6$ [(M+H)$^+$]: 403.0115 (403.0118).

5,7-Dimethyl-4-oxo-3-propyl-2,9-*bis*(trifluoromethyl)-4,5-dihydro-3*H*-pyrimido[5,4-*b*]indole-6-carbonitrile (7c): White solid; m.p.: 218-220 ºC; IR (KBr, ν_{max}, cm^{-1}): 3478 (-NH_2), 3435 (-NH_2), 3321 (NHCO), 1638 (NHCO); ^{1}H NMR ($CDCl_3$, 300 MHz, δ ppm): 1.06 (t, 3H, -CH_3), 1.62-1.65 (m, 2H, -CH_2), 2.51 (3H, s, –CH_3), 3.16 (3H, s, -CH_3), 4.42 (t, 2H, -CH_2), 7.39 (s, 1H, Ar-H); ^{13}C NMR (DMSO-d_6, 75 MHz, δ ppm): 11.8, 21.7, 24.5, 36.3, 46.3, 119.3, 121.2, 122.4, 123.5, 124.7, 126.5, 127.9, 131.3, 133.5, 135.2, 142.2, 145.6, 160.1; MS (ESI): *m/z* [(M+H)$^+$]: 417; HRMS *m/z* calcd. (found) for $C_{18}H_{14}N_4OF_6$ [(M+H)$^+$]: 417.0204 (417.0207).

3-Cyclopentyl-5,7-dimethyl-4-oxo-2,9-*bis*(trifluoromethyl)-4,5-dihydro-3*H*-pyrimido[5,4-*b*]indole-6-carbonitrile (7d): White solid; m.p.: 195-197 ºC; IR (KBr, ν_{max}, cm^{-1}): 3474 (-NH_2), 3436 (-NH_2), 3328 (-NHCO-), 1632 (-NHCO-); ^{1}H NMR ($CDCl_3$, 300 MHz, δ ppm): 1.61-1.65 (m, 4H, -CH_2-), 1.70-1.76 (m, 2H, -CH_2-), 1.94-1.98 (m, 2H, -CH_2-), 2.51 (3H, s, -CH_3), 3.16 (3H, s, -CH_3), 5.39-5.43 (m, 1H, -CH-), 7.35 (s, 1H, Ar-H); ^{13}C NMR (DMSO-d_6, 75 MHz, δ ppm): 23.4, 32.6, 36.3, 48.5, 120.3, 121.5, 122.6, 123.6, 124.8, 126.8, 127.6, 131.5, 132.4, 136.6, 142.5, 146.4, 148.2, 160.1; MS (ESI): *m/z* [(M+H)$^+$]: 443; HRMS *m/z* calcd. (found) for $C_{20}H_{16}N_4OF_6$ [(M+H)$^+$]: 443.0045 (443.0048).

Cytotoxicity assay: Cytotoxicity of the synthesized compounds **4a-e** & **5a-e** and **6a-d** & **7a-d** were screened on the basis of measurement of *in vitro* growth inhibition of tumor cell lines in 96-well plates by cell-mediated reduction of tetrazolium salt to water insoluble formazan crystals using 5-fluorouracil as a standard. The cytotoxicity was assessed using the MTT assay [31] against four human cancer cell lines *viz*. A549-lung cancer (CCL-185), MCF7-breast cancer (HTB-22), DU145-prostate cancer (HTB-81) and HeLa-cervical cancer (CCL-2). The IC_{50} (50% inhibitory concentration) values were calculated from the plotted absorbance data for the dose-response curves.

RESULTS AND DISCUSSION

4-Methyl-2-(methylamino)-6-(trifluoromethyl)isophthalonitrile (**1**) on reaction with bromoethyl aceate, thus mainly the mechanism is alkylation of amine with ethylbromoacetate and resultant product cyclized by abstraction of a proton from the active methylene by base and followed by attack on one of the nitrile functions in two ways to give two regioisomers in different proportions in a single pot. This type of cyclization is called Thorpe-Ziegler cyclization.

Each isomer has been identified based on ^{1}H NMR data with reference to the substituents present on benzene ring. A characteristic difference between two regioisomers **2a** and **2b** and is based on chemical shift of NH_2 protons and proton on C-5 carbon in ^{1}H NMR. In isomer **2b**, the NH_2 protons appeared as a broad peak at δ 4.75 ppm whereas in isomer **2b** at δ 5.4 ppm and proton on C-5 carbon at δ 7.21 ppm and δ 7.59 ppm. Compound **3** shows IR peaks at 3489, 3343 cm^{-1} (NH_2), 3215,

TABLE-1
SYNTHESIS OF SCHIFF'S BASE AND AMIDE FUNCTIONALIZED INDOLE AND PYRIMIDO INDOLE DERIVATIVES **4a-e**, **5a-e** & **6a-d**, **7a-d** AND THEIR ANTI CANCER ACTIVITY RESULTS

Compound	R	R'	A549	MCF7	DU145	HeLa
4a	-Ph	–	23.1	29.4	11.5	39.8
4b	-4CH_3-C_6H_5	–	52.3	62.2	42.8	56.1
4c	-4Cl-C_6H_5	–	28.4	46.3	19.8	25.3
4d	-4Br-C_6H_5	–	31.3	44.6	53.2	69.4
4e	-3OCH_3-C_6H_5	–	51.2	78.4	49.2	59.3
5a	-Ph	–	89.2	–	98.5	–
5b	-4CH_3-C_6H_5	–	–	–	–	113.8
5c	-4Cl-C_6H_5	–	–	110.4	95.4	–
5d	-4Br-C_6H_5	–	–	–	81.3	–
5e	-3OCH_3-C_6H_5	–	–	99.3	61.4	86.3
6a	–	-CH_3	8.5	9.4	7.5	11.6
6b	–	-CH_2CH_3	12.5	17.3	14.2	9.9
6c	–	-$CH_2CH_2CH_3$	15.2	21.5	31.7	23.8
6d	–	-Cyclopentyl	14.4	9.3	11.6	16.3
7a	–	-CH_3	114.5	–	–	120.4
7b	–	-CH_2CH_3	–	–	–	–
7c	–	-$CH_2CH_2CH_3$	118.5	–	–	–
7d	–	-Cyclopentyl	11.3	–	–	–
5-Fluorouracil (std control)	–	–	1.1	1.2	1.3	1.1

– indicates IC_{50} value > 120.4 µg/mL; Cell lines used: A549 = Lung cancer (CCL-185), MCF7 = Breast cancer (HTB-22), DU145 = Prostate cancer (HTB-81) and HeLa = Cervical cancer(CCL-2).

1621 cm^{-1} (CONH) respective to amide functional group. In ^1H NMR also disappearance of ester peaks, in compound **3**, at δ 11.42 ppm broad singlet was observed for –NHCO– enol type proton. Schiff base compound **4** shows imine proton at δ 8.35 ppm and in compound **5** disappearance of imine proton we may observe at the same in IR disappearance of two $–NH_2$ stretching frequency peaks also observed, this indicates the cyclization formation from Schiff base derivatives. Compound **6** shows IR peaks at 1636 cm^{-1} indicating amide formation and in proton NMR broad singlet at δ 6.39 ppm for amide proton. Cyclized compound **7** shows the disappearance of $–NH_2$ and –NHCO stretching frequency peaks and in proton NMR also disappearance of $–NH_2$ and –NHCO and thus, indicating the cyclization product of compound **7**.

Anticancer activity: Compounds **4a-e & 5a-e** and **6a-d & 7a-d** were screened for anticancer activity against four human cancer cell lines. Compounds **6a-d** shows better activity, compound **6a** considered as more potetnt as it shows good activity on all four cancer cell lines (7.5-11.6 µg/mL). Rest of the compounds **6b**, **6c**, **6d** exhibit in the range of 9.3 to 31.7 µg/mL. Schiff base compounds **4a-e** exhibits IC_{50} values in the range of 11.5-78.4 µg/mL. All compounds were screened up to the concentration of < 120.4 µg/mL. Compound **7b** doesn't shows activity against four cancer cell lines upto the concentration 120.4 µg/mL (Table-1).

The structure-activity relationship studies explains that the aliphatic amine tagged indole compounds showed better activity when compared with Schiff base tagged indole compounds. The presence of CF_3 group on indole increases the properties of lipid solubility and thereby enhances the transport mechanism and bioefficacy.

Conclusion

In conclusion, novel amide tagged trifluoromethyl indole and pyrimido-indole derivatives **4a-e & 5a-e** and **6a-d & 7a-d** were we designed and synthesized. Compounds **4a-e** showed moderate activity, while compounds **6a-d** showed promising activity results. Among all the synthesized compounds, compound **6a** showed better activity results at micromolar concentration and with respective to 5-fluorouracil as standard control.

ACKNOWLEDGEMENTS

The authors are thankful to the Department of Chemistry, GITAM University, Hyderabad, India for providing the laboratory facilities and chemicals.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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