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LETTERS

Synthesis and biological evaluation of Trifluoromethoxyphenyl Indole Carboxamide analogs, ADME and toxicity prediction

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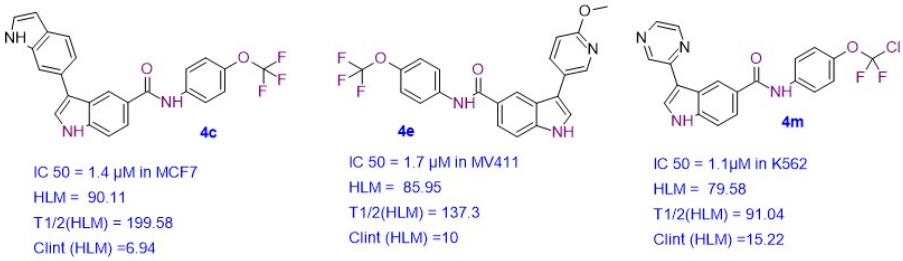
Article

ABSTRACT



We report synthesis, ADME profile, and biological evaluation of new analogues as effective Anticancer Agents. Trifluoromethoxyphenyl indole-5-carboxamide analogues (4a-4m) were developed as a class of strong inhibitors of BCR-ABL1 kinase. The compounds (4c, 4e, and 4m) showed good anticancer activity in cancer cell lines such as MCF7, MV411 and K562 with IC50 values of 1.4 µM, 1.7 µM, and 1.1 µM, respectively. In human liver microsomes, these substances likewise displayed a favorable ADME profile, good solubility, and minimal clearance. In an oncology program these analogues offer a promising beginning for the development of BCR-ABL1 kinase inhibitors.

Keywords: *Anticancer Agent, Docking Studies, CML, ADME, Indole Carboxamide*

INTRODUCTION

In the United States, blood cancer claims a life every nine minutes. According to estimates from the American Cancer Society, there will be over 2.1 million new cases of cancer detected in 2023, and six lakh cancer deaths are anticipated of which 186,000 will be due to leukemia.[1] Developing novel medications that target cancer cells specifically while also minimizing the side effects of chemotherapy is a significant challenge.[2] Targeted therapies have been developed for this purpose during the last ten years. "Targeted Cancer Therapies"[3,4] work by interfering with particular molecules primarily proteins involved in the development and spread of tumours, in order to prevent the growth and spread of cancer.[5,6] In other words, "molecular cancer targeted therapies" are cytotoxic chemicals that have a specific biological target.[7–11] In this sense, one could anticipate experiencing less severe side effects from the currently prevalent anticancer treatment. Thus, every process connected to cell division and growth may offer a therapeutic target for the treatment of cancer. Any attempt to rationalize the literature results in this area is hampered by the great variability of biological targets to find new anti-cancer drugs and the wide diversity of cell lines used to demonstrate an antitumor effect.

Tyrosine kinase inhibitors are a good class of molecules that are being explored extensively for potential application in different fields[12] with major emphasis as anti-cancer molecules.[13,14] These molecules are developed for specific inhibition of the tyrosine kinase enzymes that involved in the phosphorylation of tyrosine of proteins that participate in different metabolic pathways. The small size heterocyclic compounds are most explored molecular entities as tyrosine kinase inhibitors, and a variety of heterocycles have been reported in literature for use as tyrosine kinase inhibition.[15–18]

Considering the different periods of development of drugs, the four generations of TKI have been developed so far (figure 1). Novartis developed Imantinib,[19] a first-generation inhibitor. It prevents aberrant proteins from acting, even though complete alopecia was infrequently seen; hair loss was nevertheless occasionally reported. In 2006 the FDA approved Dasatinib, a second-generation inhibitor, for use in CML patients.[20] Imatinib is not as effective as nilotinib.[21] The multi-target inhibitor Dasatinib has 300 times the potency of Imantinib. Third-generation TKI multi-target inhibitor is Ponatinib.[20] The FDA authorized Asciminib as a medication for CML patients in 2021.[22] It binds to the BCR-ABL1 protein's myristyl site. These days, clinicians have the option of using Asciminib and Ponatinib to treat CML.[20]

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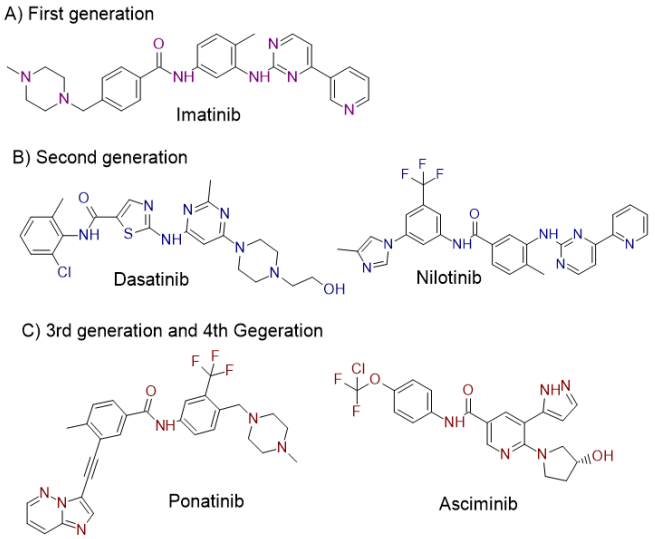


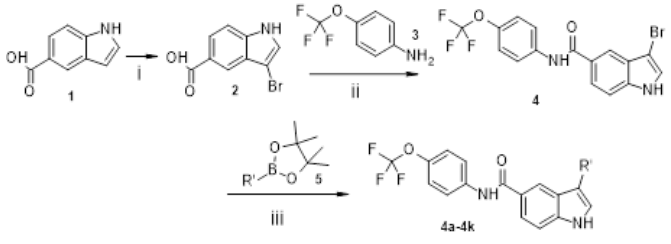
Figure 1. TKI anticancer drugs (A) Imantinib, (C) Asciminib, (B) Dasatinib, (C) Ponatinib, (B) Nilotinib.

It has been reported that a distinct structural "trifluromethyl substituent" is necessary for the inhibition of BCR-ABL1 kinase. Among the useful pharmacophore groups are aromatic amine, piperazine, and five and six membered heterocycles. It has been noted that 3-hydroxy pyrrolidine enhances the drug's water solubility and ADME. In this paper, we have suggested a novel BCR-ABL1 inhibitor that functions similarly to Asciminib. Numerous novel compounds are designed and synthesized. All of the compounds' anticancer properties were assessed using a leukemia cell line. LCMS and 1H-NMR techniques were used to characterize all synthesized compound.

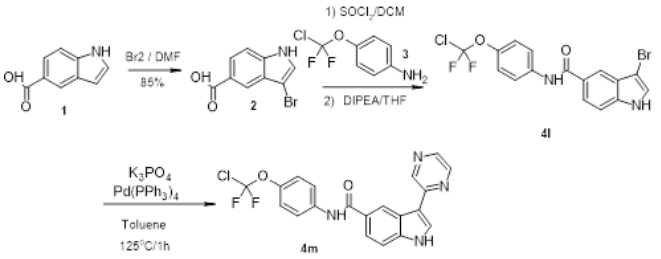
RESULTS AND DISCUSSION

CHEMISTRY

In this article novel series of Indole carboxamide analogs synthesized and their anticancer activity were tested. The designed target molecule (4a-4k) shown in Scheme.1 and (4l, 4m) in Scheme 2. were synthesized from 1H-Indole 5-Carboxylic acid in presence of Bromine and dry DMF.The compound 2 was treated with thionyl chloride to obtain acid chloride which then converted to 3 in presence of DIPEA and 4-trifluromethoxy aniline. The final target (4a-4n) synthesized through Suzuki Coupling using K_3PO_4, $Pd(PPh3)_4$ as catalyst. The Chemical Structure of all newly synthesized molecules were characterized by ^1H NMR, ^{13}C NMR and LCMS.



Scheme 1. Reagents and conditions: (I) Br_2, DMF, 2h, (II) a) $SOCl_2$, DCM. b) DIPEA, THF (III) K_3PO_4, Pd (PPh)$_4$, Toluene, 120℃



Scheme 2: Synthesis route *for 4l and 4m.*

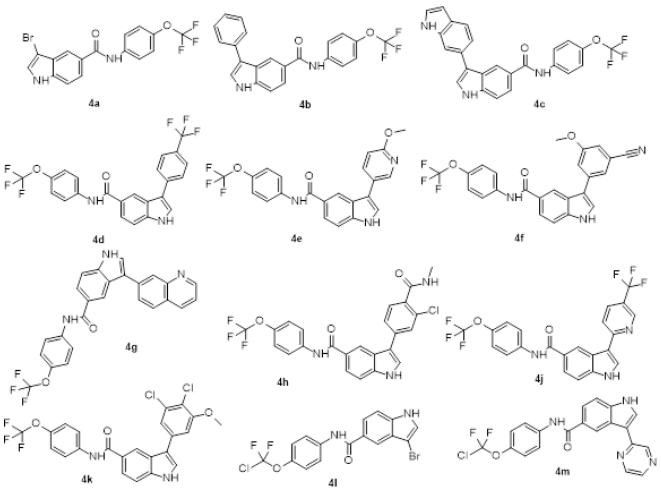


Figure 2. Planned approach of Indole carboxamide hybrids.

Table 1: ADME Parameters of Novel Compounds

Entry	Compound ID	MW	No of H-bond acceptors	No of H-bond donors	Log P	Log S
1	4a	399.16	5	2	2.70	-5.45
2	4b	396.36	5	2	2.81	-6.02
3	4c	435.40	5	3	2.74	-6.37
4	4d	464.36	8	2	3.24	-6.87
5	4e	427.28	7	2	3.31	-5.63
6	4f	464.36	8	2	3.24	-6.87
7	4g	447.41	6	2	3.00	-6.53
8	4h	487.56	6	3	3.29	-6.37
9	4j	465.35	9	2	2.77	-6.22
10	4k	495.28	6	2	3.77	-7.28
11	4l	415.60	4	2	2.99	-5.70
12	4m	414.79	6	2	2.56	-4.96
13	Asciminib	449.11	7	3	2.62	-4.49

Drug likeness and ADMETox prediction

Swiss ADME software was used to screen newly designed compounds for ADME parameters; the results are displayed in Table 1. Each of the novel compounds exhibited drug-like properties and had ADME values within a reasonable range. Asciminib was used as a reference compound to compare all of the predicted data.

***Insilco* molecular docking**

In silico molecular docking studies, as illustrated in Table 2, additional information about the binding interaction between the molecules and the target's active pocket is obtained.[23]

Table 2. Binding data of novel compounds

Entry	Compound	Docking Score	Entry	Compound	Docking Score
1	4a	-6.381	-	-	-
2	4b	-6.02	7	4g	-5.044
3	4c	-6.212	8	4h	-6.38
4	4d	-5.563	9	4k	-5.147
5	4e	-5.156	10	4l	-6.875
6	4f	-6.921	11	4m	-6.03

In Figure 3. A- Compound 4f Hydrogen bond interaction with the backbone oxygen of GLU481 is critical for binding at ABL1 Kinase. Water molecules present in the experimental crystal structure show H-bonding interaction with ligand MD dynamics simulations can reveal the role of water in mediating interactions with ARG351/TYR454 or ligand.

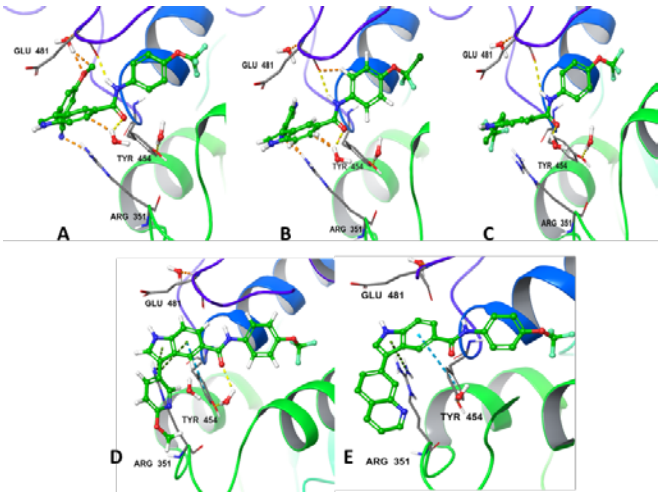


Figure 3. A-The binding mode of compound 4f with protein asciminib. B - The binding mode of compound 4m with protein asciminib, C- The binding mode of compound 4d with protein asciminib,D - The binding mode of compound 4e with protein asciminib.E- Shows 4g docking interaction with protein asciminib.

In Figure 3. B -Compound 4m Hydrogen bond interaction with the backbone oxygen of GLU481 is critical for binding at ABL1 Kinase. Crystal water show H-bonding interaction with ligand.MD dynamics simulations can reveal the role of water in mediating interactions with ARG351/TYR454 or ligand**.**

In Figure 3. C-Compound 4d Hydrogen bond interaction with the backbone oxygen of GLU481 is critical for binding at ABL1 Kinase. Crystal water show H-bonding interaction with ligand.MD dynamics simulations can reveal the role of water in mediating interactions with ARG351/TYR454 or ligand.

In Figure 3. D- Compound 4e Hydrogen bond interaction with GLU481 critical for binding at ABL1 Kinase is lost. Crystal water show H-bonding interaction with ligand. Indole ring of ligand is involved in pi-cation interactions with ARG351.Benzene ring of indole moiety shows pi-pi interaction with TYR454. MD dynamics simulations can reveal the role of water in mediating interactions with ARG351/TYR454 or ligand.

In Figure 3. E - Compound 4g Hydrogen bond interaction with GLU481 critical for binding at ABL1 Kinase is lost. Indole ring of ligand is involved in pi-cation interaction with ARG351.Benzene ring of indole moiety shows pi-pi interaction with TYR454. MD dynamics simulations can reveal the role of water in mediating interactions with ARG351/TYR454 or ligand.

Anticancer activity

The Indole carboxamide analogs were evaluated anticancer activity using the reference medications Asciminib and cisplatin. Synthesised novel molecules 4a–4m were assessed for anticancer activity in vitro using anti-proliferation assays in K562 (CML) and MV 411 (AML) cancer cell lines. Figure 4. Shows graph of target (4a-4m) on 8 point dose-response curve.

Cytotoxicity Analysis:

The effects of compounds on the activity of a BCR-ABL1 positive cell line are displayed in Table 4. For each compound's cell proliferation assay, the blood cancer cell lines K562 and MV411 were employed. (Table 4.) As a control substance, asciminib was used.

EXPERIMENTAL SECTION

The reactions were performed in flame-dried glassware under freshly distilled solvents. Experiment was carried out in an inert environment in a fume hood. TLC (Thin Layer Chromatography) was performed on Silica gel 60 $F_{524.}$ The Bruker AVANCE NEO 400 MHz instrument was used to record NMR spectra. The Acquity H-Class UPLC (Waters, Milford, USA) was utilised to acquire the mass spectra of every new compound. Compounds were purified using column chromatography (silica with a mesh size of 100–200). The melting point of each compound was recorded using a Mettler Toledo MP70-melting point instrument.

Table 4. Antiproliferative activity of new derivatives towards MV411 and K562 (4a-4m)

S.No.	Comp. Name	K562 IC_{50}(µM)	MV411 IC_{50}(µM)
1	**Cisplatin**	25.8	4.2
2	**4a**	4.01	4.0
3	**4b**	10.1	10.8
4	**4c**	10.1	4.6
5	**4d**	8.9	3.5
6	**4e**	2.05	1.7
7	**4f**	7.5	4.2
8	**4g**	11.9	5.5
9	**4h**	>30	>30
10	**4i**	15.8	6.5
11	**4j**	>30	7.0
12	**4k**	16.4	5.0
13	**4l**	>30	9.5
14	**4m**	1.1	>30
15	**Asciminib**	0.01	1.1

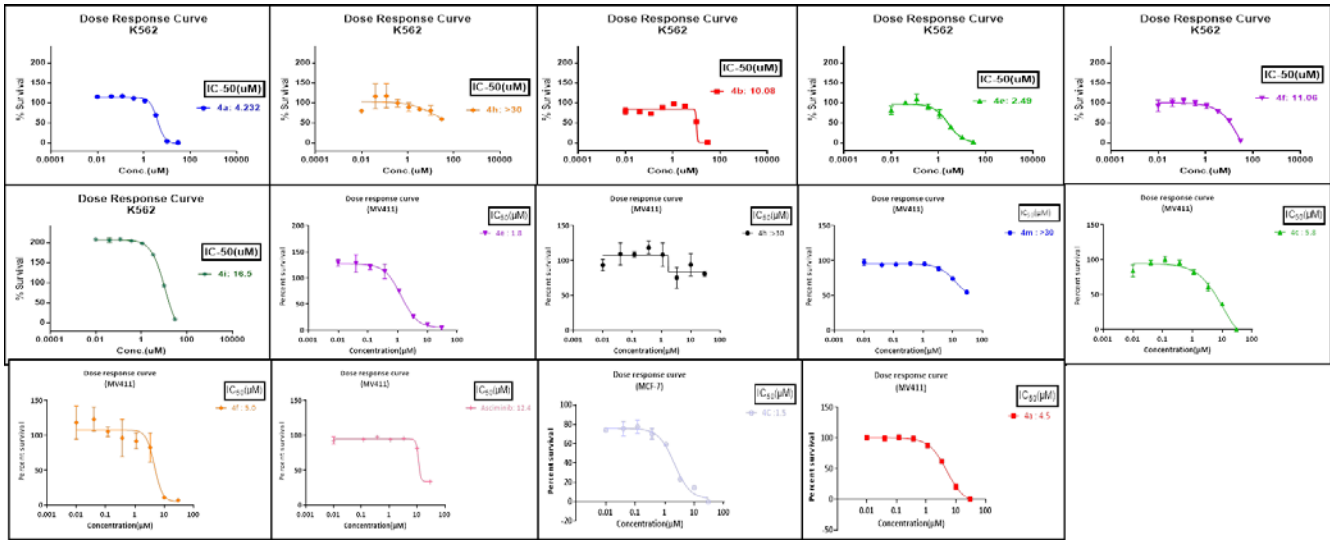


Figure 4. Shows graph of target (4a-4m) on 8 point dose-response curve

Synthesis of 3-bromo-1H-indole-5-carboxylic acid (2): Bromine (0.38 ml, 14.90 mmol, 1.2 eq) was added to a stirred solution of 1H-indole-5-carboxylic acid (2g, 12.42 mmol, 1.0 eq) in dry DMF (30 mL) at nitrogen atmosphere at 0°C. The reaction mixture were stirred for two hours at 0°C-RT. After 30 min sodium sulfite (Aq 5%) was used to quench the reaction, a solid residue was produced that was filtered and dried. The solid was used for next step without further purification. Yield: 2.4 g (crude), ^{1}H NMR (400 MHz, DMSO-d6) ppm: 12.57 (s, 1H,-COOH), 11.77 (s, 1H,-NH) 8.04 (s, 1H), 7.76 (d, 1H, J = 7.3, 7.65 (d, 1H,), 7.47 (d, 1H). EI-MS m/z 241 (M+H).

Synthesis of 3-bromo-N-(4-(trifluoromethoxy)phenyl)-1H-indole-5-carboxamide (3): To a stirred solution of 4-trifluoromethoxy aniline (1.0g, 3.96 mmol, 1.0 eq) in dry THF (20 ml) was added DIPEA (3.8 ml,19.8 mmol,5.0eq) under nitrogen atmosphere. Charged dropwise 5-bromo-6-chloronicotinoyl chloride (1.1 g, 3.96mmol, and 1.0 eq) in THF under nitrogen atmosphere dropwise at 0°C. The reaction mixture was stirred for 2 hr at RT. The reaction mixture was distilled and diluted with ice water 100 ml. The solid was precipitate out, filtered and dried under oven, which was used in next step without further purification. Yield: 1.2 g, off-white solid, 90%, ^{1}H NMR (400 MHz, DMSO-d_6) ppm 9.20 (s, 1 H,-NH) 8.65 - 8.77 (m, 2 H) 8.51 - 8.65 (m, 2 H) 8.08 (d, *J*=8.58 Hz, 1H) 7.64 (d, *J*=8.58 Hz 2 H). EI-MS m/z 400(M+H).

Synthesis of 3-phenyl-N-(4-(trifluoromethoxy)phenyl)-1H-indole-5-carboxamide (4) To a stirred solution of 3-bromo-N-(4-(trifluoromethoxy)phenyl)-1H-indole-5-carboxamide (200mg, 0.42 mmol, 1 eq.) in Toluene (8mL) was added phenyl bronic acid (0.50 mmol,1.2 eq.), K_3PO_4 (7.28 g, 1.26 mmol, 3.0 eq.) The reaction mixture was degassed with nitrogen for 20 min, then charged bis (triphenylphosphine) palladium chloride (15mg, 0.020 mmol, 0.05 eq.) The resulting reaction mixture was heated at 100°C for 16 h. The reaction mixture was filtered through celite and concentrated under reduced pressure. The crude product was purified by column chromatography so as to achieve the desired compounds.

3-bromo-N-(4-(trifluoromethoxy)phenyl)-1H-indole-5-carboxamide (4a): The yield was 40%, Brown solid, M.P.:168-170°C,LCMS 399(M+1), Chemical Formula: C16H10BrF3N2O2, Exact Mass: 397.99, Molecular Weight: 399.16.^{1}H NMR (400 MHz, DMSO-d_6) ppm 9.20 (s, 1 H,-NH) 8.65 - 8.77 (m, 1 H) 8.51 - 8.65 (m, 2 H) 8.08 (d, *J*=8.58 Hz, 2 H) 7.64 (d, *J*=8.58 Hz 2 H).

3-phenyl-N-(4-(trifluoromethoxy)phenyl)-1H-indole-5-carboxamide (4b): The yield was 38%, off-white solid, M.P.:146-148°C, LCMS 397 (M+1), Chemical Formula: C22H15F3N2O2, Exact Mass: 396.11, Molecular Weight: 396.367. ^{1}H NMR (400 MHz, DMSO-d_6) ppm 11.68 (br. s., 1 H,-NH) 10.40 (s, 1 H,-Amide NH) ,8.52 (s, 1 H) 7.91 (d, *J*=9.06 Hz, 2 H) 7.72 - 7.85 (m, 4 H) 7.44 - 7.64 (m, 3 H) 7.20 - 7.42 (m, 3 H).

N-(4-(trifluoromethoxy)phenyl)-1H,1'H-3,6'-biindole-5-carboxamide (4c): The yield was 10%, off-white solid, M.P.:105-107°C, LCMS 436 (M+1), Chemical Formula: C24H16F3N3O2,Exact Mass: 435.12,Molecular Weight: 435.40.^{1}H NMR (400 MHz, DMSO-d_6) ppm 11.57 (s, 1H,-NH) 11.09 (br. s., 1H,- NH) 10.39 (s, 1 H,Amide NH) 8.59 (s, 1 H) 7.92 (d, *J*=9.06 Hz, 2 H) 7.76 - 7.83 (m, 1 H) 7.67 - 7.76 (m, 2 H) 7.62 (d, *J*=8.11 Hz, 1 H) 7.55 (d, *J*=8.11 Hz, 1 H) 7.21 - 7.44 (m, 5 H) 6.44 (br. s., 1 H).

(R)-6-(3-hydroxypyrrolidin-1-yl)-N-(4-methoxycyclohexyl)-5-(1H-pyrazol-5-yl)nicotinamide (4d): The yield was 14%, off-white solid, M.P.: 224-226°C, LCMS 465 (M+1) Chemical Formula: C23H14F6N2O2, Exact Mass: 464.10, Molecular Weight: 464.36.^{1}H NMR (400 MHz, DMSO-d_6) ppm 11.89 (s, 1 H,-NH) 10.41 (s, 1 H,-NH),8.56 (s, 1 H) 7.96 - 8.03 (m, 3 H) 7.87 - 7.96 (m, 2 H) 7.78 - 7.87 (m, 3 H) 7.59 (d, *J*=8.58 Hz, 1 H) 7.37 (m, *J*=8.58 Hz, 2 H).

3-(6-methoxypyridin-3-yl)-N-(4-(trifluoromethoxy)phenyl)-1H-indole-5-carboxamide (4e): The yield was 36%, off-white solid, M.P.: 214-216°C, LCMS 428 (M+1), Chemical Formula: C22H16F3N3O3, Exact Mass: 427.11, Molecular Weight: 427.38.^{1}H NMR (400 MHz, DMSO-d_6) ppm 11.72 (br. s., 1 H,-

NH) 10.40 (br. s., 1 H,-NH) 8.61 (br. s., 1 H) 8.48 (br. s., 1 H) 8.08 (d, *J*=7.15 Hz, 1 H) 7.91 (d, *J*=7.63 Hz, 2 H) 7.82 (br. s., 2 H) 7.56 (d, *J*=7.63 Hz, 1 H) 7.22 - 7.46 (m, 2 H) 6.95 (d, *J*=8.11 Hz, 1 H) 3.91 (br. s., 3 H,-OCH3).

3-*(3-cyano-5-methoxyphenyl)-N-(4(trifluoromethoxy)phenyl)-1H-indole-5-carboxamide (4f)*:The yield was 76%, off- white solid, M.P.:232-234°C**,** LCMS 452.4 (M+1), Chemical Formula: C24H16F3N3O3, Exact Mass: 451.11,Molecular Weight: 451.40.^{1}H NMR (400 MHz, DMSO-d_6) ppm 11.88 (br. s., 1 H,-NH) 10.44 (s, 1 H,-NH) 8.52 (s, 1 H) 8.03 (d, *J*=2.86 Hz, 1 H) 7.93 (s, 2 H) 7.91 (s, 1 H) 7.74 - 7.87 (m, 2 H) 7.46 - 7.71 (m, 2 H) 7.18 - 7.46 (m, 3 H) 3.91 (s, 3 H, -OCH_3).

3-(quinolin-7-yl)-N-(4-(trifluoromethoxy)phenyl)-1H-indole-5-carboxamide (4g): The yield was 45%, Yellow solid, M.P.: 222-224°C**,** LCMS 448 (M+1), Chemical Formula: C25H16F3N3O2,Exact Mass: 447.12,Molecular Weight: 447.41.^{1}H NMR (400 MHz, DMSO-d_6) ppm 11.85 (br. s., 1H,-NH) 10.44 (s, 1 H,-NH) 8.89 (br. s., 1 H) 8.69 (s, 1 H) 8.52 (br. s., 1 H) 8.41 (br. s., 1 H) 8.23 (br. s., 1 H) 8.12 (br. s., 1 H) 8.05 (s, 1 H) 7.94 (m, *J*=9.06 Hz, 2 H) 7.84 (d, *J*=8.58 Hz, 1 H) 7.61 (d, *J*=8.58 Hz, 2 H) 7.37 (m, *J*=8.58 Hz, 2 H).

3(3chlor4(methylcarbamoyl)phenyl)N(4(trifluoromethoxy)phenyl)-1H-indole-5-carboxamide (4h): The yield was 36%, off-white solid, M.P.: 266-268°C, LCMS 488 (M+1), Chemical Formula: C24H17ClF3N3O3, Exact Mass: 487.09,Molecular Weight: 487.86,M.p.: 74 °C,^{1}H NMR (400 MHz, DMSO-d_6) ppm 11.88 (br. s., 1H,-NH) 10.44 (s, 1 H,-NH) 8.49 (s, 1 H) 8.34 (d, *J*=4.29 Hz, 1 H) 8.01 (s, 1 H) 7.92 (m, *J*=8.58 Hz, 2 H) 7.75 - 7.87 (m, 3 H) 7.48 - 7.71 (m, 2 H) 7.37 (m, *J*=8.58 Hz, 2 H) 2.78 (d, *J*=4.29 Hz, 3H,-NMe)

N-(4-(trifluoromethoxy)phenyl)-3-(5-(trifluoromethyl)pyridin-2-yl)-1H-indole-5-carboxamide (4j): The yield was 61%, off-white solid, M.P.:232-234°C, LCMS 466 (M+1), Chemical Formula: C22H13F6N3O2, Exact Mass: 465.09, Molecular Weight: 465.35,^{1}H NMR (400 MHz, DMSO-d_6) □ ppm 12.04 (br. s., 1 H,-NH) 10.42 (s, 1 H,-NH) 9.24 (s, 1 H) 8.59 (s, 1 H) 8.45 (d, *J*=7.63 Hz, 1 H) 8.17 (s, 1 H) 7.79 - 8.09 (m, 4 H) 7.62 (d, *J*=8.58 Hz, 1 H) 7.37 (d, *J*=8.58 Hz, 2H).

***3-(3,4-dichloro-5-methoxyphenyl)-N-(4-(trifluoromethoxy)phenyl)-1H-indole-5-carboxamide (4k)*:** The yield was 78%, off-white solid, M.P.:194-196°C, LCMS 495 (M+1), Chemical Formula: C23H15Cl2F3N2O3, Exact Mass: 494.04,Molecular Weight: 495.28,^{1}H NMR (400 MHz, DMSO-d_6) ppm 11.88 (br. s., 1 H) 10.44 (s, 1 H) 8.54 (s, 1 H) 8.03 (d, *J*=2.38 Hz, 1 H) 7.92 (d, *J*=9.06 Hz, 2 H) 7.82 (d, *J*=8.58 Hz, 1 H) 7.53 - 7.62 (m, 2 H) 7.46 (d, *J*=1.91 Hz, 1 H) 7.37 (d, *J*=8.11 Hz, 2 H) 4.02 (s, 3 H,-OMe).

***(R)-5-(3-chloro-4-(cyclopentylcarbamoyl)phenyl)-6-(3-hydroxypyrrolidin-1-yl)-N-(4-methoxycyclohexyl)nicotinamide (4l)*:** The yield was 44%, Brown solid, M.P.:108-110°C, LCMS 416 (M+1), Chemical Formula: C16H10BrClF2N2O2, Exact Mass: 413.96, Molecular Weight: 415.62,^{1}H NMR (400 MHz, DMSO-d_6) ppm 11.79 (br. s., 1 H,-NH) 10.44 (s, 1 H,-NH) 8.15 (s, 1 H) 7.93 (m, *J*=9.06 Hz, 2 H) 7.82 (dd, *J*=8.58, 1.43 Hz, 1 H) 7.70 (d, *J*=2.38 Hz, 1 H) 7.55 (s, 1 H) 7.36 (m, *J*=8.58 Hz, 2 H).

N-(4-(chlorodifluoromethoxy) phenyl)-3-(pyrazin-2-yl)-1H-indole-5-carboxamide (4m): The yield was 48%, off-white solid, M.P.: 258-260°C, LCMS: 415 (M+1), Chemical Formula: C20H13ClF2N4O2, Exact Mass: 414.07, Molecular Weight: 414.79. ^{1}H NMR (400 MHz, DMSO-d_6) ppm 12.01 (br. s., 1 H,-NH) 10.43 (s, 1 H,-NH) 9.28 (s, 2 H) 9.10 (s, 1 H) 8.57 (s, 1 H) 8.12 (d, *J*=2.38 Hz, 1 H) 7.81 - 8.01 (m, 4 H) 7.61 (d, *J*=8.58 Hz, 1 H) 7.37 (d, *J*=8.58 Hz, 3 H).

Anticancer activity

MV411 (ATCC® CRL-9591™) and K562 (ATCC® CCL-243™), Biphenotypic B Myelomonocytic and Chronic Myelogenous Leukemia CML cell lines respectively, were seeded in the medium (IMDM, Cat # 12440053 and RPMI, Cat # 11875093 with 10% FBS, Cat # 16000044; Gibco) at a cell count of 15000 cells per 100 µl per well in a 96 well edge plate (167425; Thermo Fisher).

Cells were allowed to grow at 37°C for 30 mins in 5% CO_2 (culture conditions) in a Nuaire incubator (humidified). Serially diluted compounds (100 µl) were added to the culture plate 30 mins and the cultures (MV411 & K562) were further incubated in culture conditions for 72 hr. Experiment was terminated at the completion of 72 hr exposure to the drug by adding 50 µl of Cell titer glow (G9243; Promega) and the plates were further incubated at room temperature for 10 min. Mixed the cells properly and transfer around 200µl to black bottom 96 Well Plate. Luminescence was then measured using a multimodal plate reader (Biotech Synergy Neo). Data analysis was done by subtracting the background luminescence (only medium blank) value from each reading and then normalizing with the vehicle control (DMSO treated cells) to obtain percent survival/proliferation. Percent survival at different doses was used to calculate IC_{50} by fitting the curve to the "four-parameter variable slope logistic model" using XL fit.

ADME Analysis of active compounds - HLM, Kinetic Solubility, MLM:

The most essential physicochemical characteristic of a medication is lipophilicity, which is crucial for its solubility, absorption, distribution, and binding to plasma proteins. The partition coefficient, or log-P, is used to quantify lipophilicity.

Table 5. Solubility data of selected compounds (4a-4m).

Molecule	Batch	Run 1(µM)	Run 2(µM)
4d	1	9.37	9.37
4e	1	18.75	18.75
4f	1	9.37	9.37
4k	1	2.34	2.34
4l	1	45.01	44.71
4m	1	47.24	47.71
4c	1	39.15	8.31

Due to more impact of kinetic solubility in drug discovery process for formulation, bioassays, absorption in our study newly synthesized compounds (4d,4e,4f,4k,4l,4m,4c) evaluated kinetic solubility data. HLM (Human liver microsomal) and MLM

(Mouse liver microsomal) data also evaluated (4d,4e,4f,4k,4m,4c) in table 6.

Table 6. ADME Data of (4c, 4d, 4e, 4f, 4k).

Molecule	Batch	HLM	MLM	HLM)	T 1/2(MLM)	Clint(HLM)	Clint(MLM)
4m	1	79.58	36.42	91.04	20.58	15.22	67.33
4c	1	90.11	94.46	199.58	364.49	6.94	3.80
4d	1	93.82	81.70	325.80	102.90	4.00	13.00
4e	1	85.95	70.36	137.30	59.10	10.00	23.00
4f	1	50.03	80.26	30.00	94.50	46.00	15.00
4k	1	85.92	86.69	137.00	145.50	10.00	10.00

CONCLUSION

In this study we have synthesized and biological evaluation of novel trifluoromethoxyphenyl indole carboxamide and chlorodifluoromethoxy phenyl)-3-(pyrazin-2-yl)-1H-indole-5-carboxamide analogues. The *invitro* biological evaluation of the compounds was done in human CML cell line K562 and the human AML cell line MV411. All of the novel compounds exhibited moderate to strong activities 4m exhibited an IC50 of 1.1µM in K562, 4e (1.7µM in MV411), and 4c (1.4µM in MCF-7) with Asciminib and Cisplatin as Standards. The toxicity and ADME profile of new trifluoromethoxyphenyl indole carboxamide analogs was assessed using the Swiss ADME. LCMS and ^{1}H NMR were used to characterize every synthesized compound. Compounds 4c, 4e, and 4m have shown appreciable anticancer activity. These analogues will envision the future oncology programs.

SUPPLEMENTARY MATERIAL

Supplementary material (NMR, LCMS spectra of compounds) of article can be found, in the online version, at journal site.

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CONFLICT OF INTEREST STATEMENT

The author declares that there is no conflict of interest, academic or financial, for publication of this article.

List of abbreviations:

LCMS- Liquid chromatography–mass spectrometry
HLM- Human liver microsomal
MLM- Mouse liver microsomal
CML- Chronic Myelogenous Leukemia
AML- Acute myeloid Leukemia
DMSO- Dimethyl sulfoxide
DMF- Dimethoxy ethane
IPA- Isopropyl alcohol
DIPEA- N, N-Isopropyl ethyl amine
TKI- Tyrosine Kinase Inhibitors
ADME- Absorption, Distribution, Metabolism, Excretion.
WBC- White Blood Cell
NMR- Nuclear Magnetic Resonance
UPLC- Ultra Performance Liquid Chromatography
DMF- N-N dimethylformamide
RT- Room Temperature

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