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Research Article

# DESIGN, SYNTHESIS, AND CHARACTERIZATION OF THE SOME NOVEL 2‐AMINO-PYRIDINE‐3‐CARBONITRILE AND 2‐AMINO-4H-PYRAN‐3‐CARBONITRILE DERIVATIVES AGAINST ANTIMICROBIAL ACTIVITY AND ANTIOXIDANT ACTIVITY

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**ABSTRACT**

**Objective:** Investigation, the series of newer 2-amino-pyridine-3-carbonitrile and 2-amino-4H-pyran-3-carbonitrile derivative were synthesized and evaluated antimicrobial activities and antioxidant activity.

**Methods:** Novel synthesized chalcones were further condensation to give 2-amino-3-cyanopyridine and 2-amino-3-cyanopyrans in the presence of malononitrile, pyridine, and ammonia acetate. The product is characterized by conventional and instrumental methods. Pyridine and 4-H-Pyran and their analogs occupy prime position due to their diverse applications.

**Results:** The compounds A3C and B3C exhibited marked zone of inhibition with 30.02±0.02 mm and 29.06±0.01 mm, respectively. Docking studies suggested possible interactions with dihydrofolic reductase 4 with 9.15 and −9.67 kcal/mol, respectively. The $IC_{50}$ 30.28±0.01 exhibited A3C by 2,2-diphenylpicrylhydrazyl methods which is better among the series. The 2-amino-3-cyanopyridine derivatives were found good activity than 2-amino-3-cyanopyrane derivative. Among all synthesized compounds few having potent activity and some are near to the standard.

**Conclusion:** Antimicrobial activity and antioxidant of the newly synthesized pyrans and pyridines derivatives will definitely inspire future researchers for the preparation of new analogs.

**Keywords:** Dihydrofolic reductase, 2-amino-3- cyanopyridine compounds, 2-amino-3- cyanopyrans compounds, Zone of inhibition.

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## INTRODUCTION

Infections in humans influenced by poor hygienic, improper medications, and seasonal conditions. Illnesses is reinforcement of uncleanliness, accompanies water-related substances. Trends in developing countries lifestyle quite changes which burden on chronological disturbance in the body cannot tolerate during dosage remegin cause resistance. In world 170 billion tons meat is consumed by human, drug resistant bacteria and other microbes are passed through the food chain to the consumer, transfer the resistance to human pathogens. Due to change in human habitats may alteration defence immune system this emphasis on infectious diseases by WHO. These impacts, medicinal chemist's booster to developed newer drugs tragedy approaches. Architecture of pyridine and pyran nucleus was redesign of fluorinated chacones by Michael's acceptor principle [1]. Strategically approach of these nucleuses is co related with few of pathological functions in humans system as well as in living ecosystem. To enhance permeation of molecules rapidly across the barrier which influence the shape and size of molecules developed by fluorinated chalcones as precursors. The pyridine and pyran scaffolds skelton emerged near 600 drugs structures as integral part possessing more biological activities such as antiviral [2-4], sex pheromone [5], anti-proliferative [6], anticancer [7], antimicrobial [8,9], IKK-β inhibitors [10], A2A adenosine receptor antagonists [11], potent inhibitor of HIV-1 integrase [12], antitb [13], anti-inflammatory [14], as well as antihypertensive [15], and herbicides [16].

## METHODS

### Reagents and chemicals

All the chemicals purchased from various dealer which are analytical grade. Ketones were obtained from Alfa Aesar. Aldehydes procure from Avara chemicals, Silica gel-G for thin-layer chromatography from Merck. The solvent from desai chemicals. The spots were seen in UV lamp at 254 nm and iodine chamber. Further purification compounds done in column chromatography as mobile phase ethyl acetate:hexane melting points were measured using a capillary tube method with a digital melting point apparatus (EZmelt, Stanford Research Systems). Fourier-transform infrared (FTIR) spectra are detected with help of KBr pellets on OPUS FT-IR Spectrophotometer. Mass spectra were recorded on SHIMADZU Lab Solution (electrospray ionization mass spectrometry [ESI-MS]) spectrometer $^{1}H$ and $^{13}C$ NMR spectra were recorded on Bruker 400 MHz. The elemental analysis was performed with an Thermo Finnigan flash (EA 1112 CHNS Analyzer).

## RESULTS AND DISCUSSION

### Chemistry

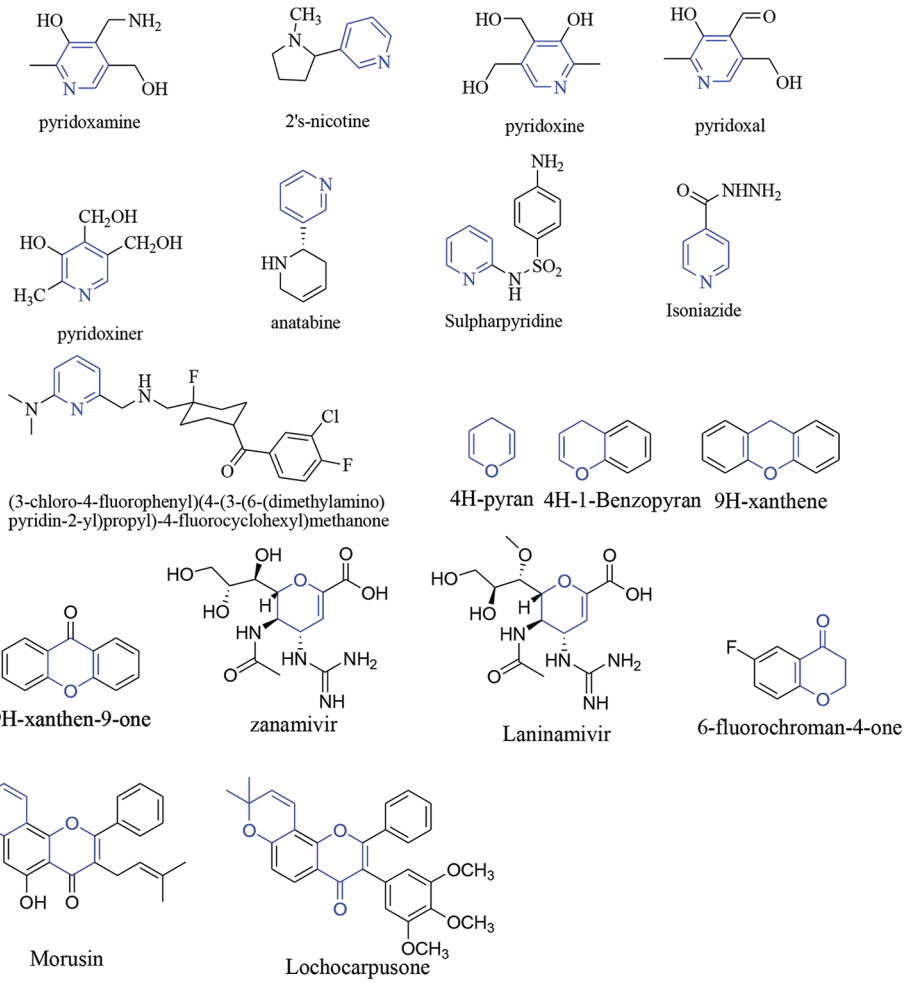
*2-amino-3-cyanopyridines (A1C-A10C; B1C-B10C)*

A synthesized chalcones (A1-A10/ B1-B10) and malononitrile were taken in equal (0.01 M) and ammonium acetate (0.03 M) in dimethylformamide (DMF) (30 ml) the contents should be condense for 8 h, after that crushed ice taken in clean beaker then mixture was decant slowly in it [17,18]. The solid obtained was filtered, washed with water and crystallized from DMF allowed to dry in desiccator.

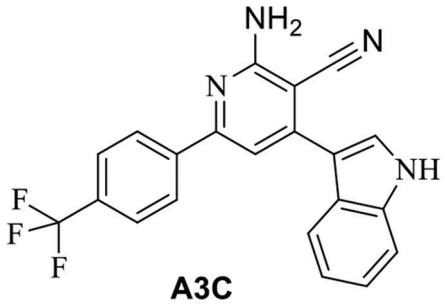
*The synthesis of 2-amino-3-cyanopyrans (A1D-A10D; B1D-B10D)*

A synthesized chalcones (A1-A10/ B1-B10) and malononitrile are taken in equal (0.01 M) and then dry pyridine (0.03 M) in DMF (30 ml) was condense for 15 h, after that crushed ice taken in clean beaker then mixture was decant slowly in it [19]. The solid obtained was filtered, washed with water and crystallized from DMF allowed to dry in desiccator.

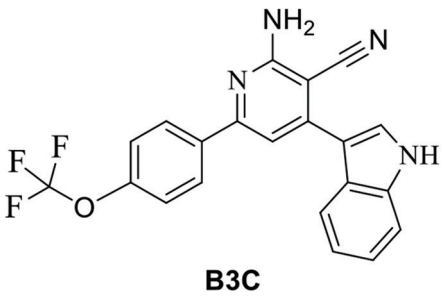
The 4'-trifluromethyl chalcones and 4'-trifluromethoxy chalcones treated with malononitrile cyclized to give 2-amino-3- cyanopyridine




in the present of salts reductive eliminations mechanisms, to cyclized 2-amino-3- cyanopyridine, and 2-amino-3- cyanopyrans in the presence of mild base addition. The yield of the compounds after recrystallization was in the range from 40 to 95%. The compounds were characterized spectrometer and elemental analysis within ±0.5% variation of calculated data. All the compounds showed absorption bands and peaks characteristic to chalcones in their IR, $^{1}$H NMR, $^{13}$C NMR for series of **C and D** compounds, and mass spectra.

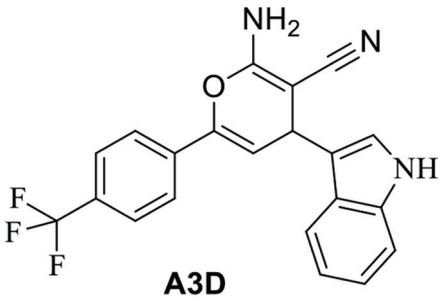



The IR spectrum of **A3C** illustrated sharpe nitrile band of 2-amino-3-cyanopyridine at 2221.88 and strong stretching band at 3635.05 accounting for (-$NH_2$), 1503.15 (-C=N Stretching), and 1173.15 (C-N stretching's). Moreover, 3730 (-NH in indole) and 1234.83 (-$CF_3$) bands absorpted bands, respectively. Two important peaks more intense at 1.94 singlet ppm and 7.90 ppm is amine and CH in pyridine ring the $^{1}$H NMR spectrum of **A3C**. In addition, three singlets in indole amine proton at $\delta$: 8.25 and two multiplets around 8.12–8.04 and 8.24–7.24 for 13 aromatic hydrogen. The $^{13}$C NMR spectra of compound **A3C** demonstrated three crucial peaks at 162, 158, 102, 97.00, and 86 which responsible to the five carbons cyclized to disappear of propenone moiety to 2-amino-3- cyano pyridine nucleus. The other $^{13}$C peaks include 158.21 (C-6), 111.07 (C-5), 152.54 (C-4), 88.30 (C-3), 117.00 (C-3$^{1}$), 162.17 (C-2), 140.11 (C-1''), 128.60 (C-2'', 6''), 125.59 (C- 3'',5''), 137.30 (C-4''), 123.77 (C-4''$^{-1}$,), 129.52 (C-6'), 125.30 (C-2'), 102.06 (C-3'), 130.30 (C-9'),114.06 (C-8'), 120.71 (C-7'), 122.02 (C-6'), 117.44 (C-5'), 125.56 (C-4') the mass spectrum ensured in positive mode of [M + H] peak at m/z 379.00. Based on the above spectral data, the compound **A3C** was confirmed as (2E)- 2-amino-4-(1H-indol-3-yl)-6-[4-(trifluoromethyl)phenyl]pyridine-3-carbonitrile.



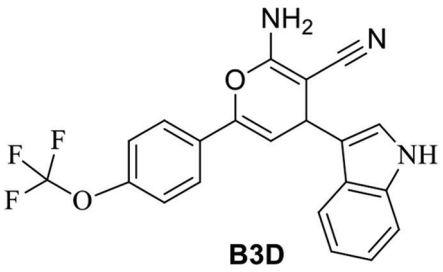

The IR spectrum of **B3C** illustrated sharpe nitrile band of 2-amino-3-cyano pyridine at 2186 and strong stretching band at 3340 accounting for (-$NH_2$), 1592 (-C=N Stretching), and 1355 (C-N stretching's). Moreover, absorption bands at 3731 (-NH in indole) and 1238 (-$CF_3$),

respectively. Two important peaks more intense at 1.94 singlet ppm and 7.84 ppm are amine and CH in pyridine ring the $^1$H NMR spectrum of **B3C**. Three singlets respond to the indole amine proton at *δ*: 8.40 and two multiplets around 8.39–8.38 and 8.12–6.98 for 13 aromatic protons 8.11 (1H, s, 5C), and 6.80 (2H, s, -$NH_2$). The $^{13}$C NMR spectra of compound **B3C** demonstrated three crucial peaks at 162, 158, 102, 97.00, and 86 responding to the five carbons form 2-amino-3- cyano pyridine nucleus and peaks of propenone moiety got vanished. The other $^{13}$C peaks include 158.58 (C-6), 111.88 (C-5), 152.1 (C-4), 88.74 (C-3), 117.00 (C-3$^1$), 160.18 (C-2), 130.23 (C-1''), 130.71 (C-2'', 6''), 121.88 (C-3'',5''), 139.61 (C-4''), 121.88 (C-4'''$^{-1}$), 100 (C-3'), 123.63 (C-2'), 137.35 (C-9'),114.41 (C-8'), 120.47 (C-7'), 122.69 (C-6'), 117.81 (C-4'), 123.63 (C-5') The mass spectrum of **B3C** recorded in positive mode has given an [M + H] peak at m/z 395.20 The spectral data consist with structure, the compound **B3C** was confirmed as (2E)- 2-amino-4-(1H-indol-3-yl)-6-[4-(trifluoromethoxyl)phenyl]pyridine-3-carbonitrile.




**A3D**

The IR spectrum of **A3D** illustrated sharpe nitrile band of 2-amino-3-cyano pyran at 2220 and strong stretching band at 3552 accounting for (-$NH_2$), 1135 (C-O-C pyran), and 1320 (C-N stretching's). Moreover, absorption bands at 3352 (-NH in indole) and 1235 (-$CF_3$), respectively. Two important peaks more intense at 6.86 singlet ppm and 5.34 ppm are amine and CH in pyran ring the $^1$H NMR spectrum of **A3D**. The three singlets peaks in indole amine proton at *δ*: 8.29 and two set of multiplets around 7.94 and 7.02 are aromatic protons of four proton and five protons, respectively, 4.99 (1H, d, *J* = 5.4 Hz 5C), 3.94 (1H, d, *J* = 5.4 Hz 4C), and 6.93 (2H, s, -$NH_2$). The $^{13}$C NMR spectra of compound **A3D** demonstrated three crucial peaks at 140, 88.99, 23.22, 45.59, and 117.44 respond to the five carbons of the propenone moiety got cyclized to 2-amino-3- cyano pyran nucleus. The other $^{13}$C peaks include 140.12 (C-6), 88.99 (C-5), 23.22 (C-6), 45.59 (C-3), 117.44 (C-3$^1$), 159.20 (C-2), 130.81 (C-1''), 128.88 (C-2'', 6''), 125.59 (C-3'',5''), 137.30 (C-4''), 123.77 (C-4'''$^{-1}$), 105.52 (C-3'), 125.30 (C-2'), 137.36 (C-9'),114.06 (C-8'), 120.71 (C-7'), 122.02 (C-6'), 117.00 (C-5'), 125.56 (C-4'). The mass spectrum of **A3D** ensured in positive mode [M +H] peak at m/z 382.45. Spectral data compile with structure, the compound **A3D** was confirmed as (2E)- 2-amino-4-(1H-indol-3-yl)-6-[4-(trifluoromethyl) phenyl]pyran-3-carbonitrile.




**B3D**

The IR spectrum of **B3D** illustrated sharpe nitrile band of 2-amino-3-cyano pyran at 2216 and strong stretching band at 3538 accounting for (-$NH_2$), 1139 (C-O-C pyran), 1350 (C-N stretching's), 3730 (-NH in indole), 1134 (-C-O-), and 1228 (-$CF_3$), respectively. Two important peaks more intense at 1.94 singlet ppm and 7.84 ppm are amine and CH in pyran ring the $^1$H NMR spectrum of **B3D** seen three singlets respond to the indole amine proton at *δ*: 8.29 and two multiplets around 7.68–6.83 for nine aromatic protons 4.99 (1H, d, *J* = 5.4 Hz 5C), 3.93 (1H, d, *J* = 5.4 Hz 4C), and 6.81 (2H, s, -$NH_2$). The $^{13}$C NMR spectra of compound **B3D** demonstrated three crucial peaks at include 140.81, 98.00, 33.30, 41.40, and 117.02 respond to the five carbons cyclized from propenone moiety to 2-amino-3- cyano pyrane nucleus. The other $^{13}$C peaks include 140.81 (C-6), 98.00 (C-5), 33.30 (C-4), 41.40 (C-3), 117.02 (C-3$^1$), 160.81 (C-2), 130.23 (C-1''), 130.34 (C-2'', 6''), 120.99 (C-3'',5''), 160.00 (C-4''), 121.88 (C-4'''$^{-1}$), 126.82 (C-2'), 105.8 (C-3'), 122.88 (C-2'),117.18 (C-5'), 120.47 (C-6'), 120.47 (C-7'), 114.42 (C-8'), 137.35 (C-9'). The mass spectrum of **B3D** recorded in positive mode ensured that [M + H] peak at m/z 398.30. The spectral data compiles with compound **B3D** were confirmed as (2E)- 2-amino-4-(1H-indol-3-yl)-6-[4-(trifluoromethoxyl)phenyl]pyran-3-carbonitrile.

*A1C: 2-amino-4-(2,3-dichlorophenyl)-6-[4-(trifluoromethyl)phenyl]pyridine-3-carbonitrile*

Yield: 60%; m.p. 120.34°C; $R_f$= 0.6 (20% Ethyl acetate in Hexane); IR (KBr, cm$^{-1}$): 3360 (-N-H), 2221 (-CN), 1595 (-C=N), 1321(-C-N-), 1600 (C=C Ar), 1255 (-$CF_3$), 3015 (Ar C-H stretching), 832 (C-Cl); $^1$H NMR (400MHz, CDCl3, ppm): *δ*: 7.67 (1H, s, 5C), 6.80 (2H, s, -$NH_2$), 8.10–8.04 (4H, m, C-2'', 3'', 5'', 6'', Ar-H), 7.66 –7.11 (3H, m, C- 4', 5', 6', Ar-H); ESI-MS [M+H]$^+$: 408.61and 409.61; Anal. Calcd for: $C_{19}H_{10}Cl_2F_3N_3$: C, 55.90; H, 2.47; N, 10.29; Cl, 17.37; F, 13.96; Found: C, 55.77; H, 2.59; N, 10.35; Cl, 17.42; F, 13.99.

*A2C: 2-amino-4-(2-chlorophenyl)-6-[4-(trifluoromethyl)phenyl]pyridine-3-carbonitrile*

Yield: 60%; m.p. 123.15°C; $R_f$= 0.6 (20% Ethyl acetate in Hexane); IR (KBr, cm$^{-1}$): 3350 (-N-H), 2220 (-CN), 1580 (-C=N), 1322 (-C-N-),1252 (-$CF_3$), 3010 (Ar C-H stretching), 1615 (C=C Ar), 846 (C-Cl); $^1$H NMR (400 MHz, $CDCl_3$, ppm): *δ*: 7.66 (1H, s, 5C), 6.80 (2H, s, -$NH_2$), 8.10–8.04 (4H, m, C-2'', 3'', 5'', 6'', Ar-H), 7.65–7.28 (4H, m, C-3', 4', 5', 6', Ar-H); ESI-MS [M+H]$^+$: 374.26 and 375.26; Anal. Calcd for: $C_{19}H_{11}ClF_3N_3$: C, 61.06; H, 2.97; N, 11.24; Cl, 19.49; F, 15.25; Found: C, 61.24; H, 3.06; N, 11.64; Cl, 9.51; F, 15.20.

*A3C: 2-amino-4-(1H-indol-3-yl)-6-[4-(trifluoromethyl)phenyl]pyridine-3-carbonitrile*

Yield: 50%; m.p. 130.42°C; $R_f$= 0.6 (20% Ethyl acetate in Hexane); IR (KBr, cm$^{-1}$): 3635 (-N-H), 2221 (-CN), 1503 (-C=N), 1322 (-C-N-),1234 (-$CF_3$), 3010 (Ar C-H stretching), 1438 (C=C Ar); $^1$H NMR (400MHz, $CDCl_3$, ppm): *δ*: 7.84 (1H, s, 5C), 6.80 (2H, s, -$NH_2$), 8.25 (1H, s, indole), 8.12–8.04 (4H, m, C-2'', 3'', 5'', 6'', Ar-H), 8.24 –7.24 (5H, m, C-2', 4', 5', 6', 7', Ar-H); ESI-MS [M+H]$^+$: 379.00 and 380.10; Anal. Calcd for: $C_{21}H_{13}F_3N_4$: C, 66.66; H, 3.46; N, 14.81; F, 15.06; Found: C, 66.60; H, 3.49; N, 14.96; F,15.09.

*A4C: 2-amino-6-(4-(trifluoromethyl)phenyl)-4-(2-nitrophenyl)pyridine-3-carbonitrile*

Yield: 56%; m.p. 141.21°C; $R_f$= 0.6 (20% Ethyl acetate in Hexane); IR (KBr, cm$^{-1}$): 3364 (-N-H), 2218 (-CN), 1586 (-C=N), 1324 (-C-N-),3010 (Ar C-H stretching), 3010 (Ar C-H stretching), 1615 (Ar C=C), 1249 (-$CF_3$), 1461 ($NO_2$); $^1$H NMR (400MHz, $CDCl_3$, ppm): *δ*: 7.78 (1H, s, 5C), 6.80 (2H, s, -$NH_2$), 8.10–8.04 (4H, m, C-2'', 3'', 5'', 6'', Ar-H), 7.66–7.42 (4H, m, C-3', 4', 5', 6', Ar-H); ESI-MS [M+H]$^+$: 385.40 and 386.40; Anal. Calcd for: $C_{19}H_{11}F_3N_4O_2$: C, 59.38; H, 2.88; N, 14.58; O, 8.33; F, 14.83; Found: C, 59.31; H, 3.01; N, 14.62; O, 8.47; F,14.96.

*A5C: 2-amino-6-(4-(trifluoromethyl)phenyl)-4-(4-nitrophenyl)pyridine-3-carbonitrile*

Yield: 80%; m.p. 141.37°C; $R_f$= 0.6 (20% Ethyl acetate in Hexane); IR (KBr, cm$^{-1}$): 3362 (-N-H), 2229 (-CN), 1587 (-C=N), 1321 (-C-N-), 3014 (Ar C-H stretching), 3028 (Ar C-H stretching), 1616 (Ar C=C), 1248 (-$CF_3$), 1463 ($NO_2$); $^1$H NMR (400 MHz, $CDCl_3$, ppm): *δ*: 8.04 (1H, s, 5C), 6.80 (2H, s, -$NH_2$), 7.66–7.53 (4H, m, C-2', 3', 5', 6', Ar-H), 8.16–8.07 (4H, m, C-2'', 3'', 5'', 6'', Ar-H); ESI-MS [M+H]$^+$: 385.17 and 386.17; Anal. Calcd

for: $C_{19}H_{11}F_3N_4O_2$: C, 59.38; H, 2.88; N, 14.58; O, 8.33; F, 14.83; Found: C, 59.26; H, 3.08; N, 14.50; O, 8.49; F, 14.80.

*A6C: 2-amino-4-(thiophen-2-yl)-6-[4-(trifluoromethyl)phenyl]pyridine-3-carbonitrile*

Yield: 70%; m.p. 121.89°C; $R_f$= 0.6 (20% Ethyl acetate in Hexane); IR (KBr, $cm^{-1}$): 3369 (-N-H), 2229 (-CN), 1575 (-C=N), 1366 (-C-N-), 1629 (Ar C=C), 3130 (Ar C-H stretching), 1258 (-$CF_3$), 633 (C-S); $^1$H NMR (400MHz, $CDCl_3$, ppm): *δ*: 7.66 (1H, s, 5C), 6.80 (2H, s, -$NH_2$), 7.63–7.09 (4H, m, C-3', 4', 5', Ar-H), 8.10 –7.65 (4H, m, C-2'', 3'', 5'', 6'', Ar-H); ESI-MS $[M+H]^+$: 346.06 and 347.06; Anal. Calcd for: $C_{17}H_{10}F_3N_3S$: C, 59.12; H, 2.92; N, 12.17; F, 16.50; Found: C, 59.19; H, 3.08; N, 12.12; F, 16.55.

*A7C: 2-amino-4-(furan-2-yl)-6-[4-(trifluoromethyl)phenyl]pyridine-3-carbonitrile*

Yield: 70%; m.p. 129.18°C; $R_f$= 0.6 (20% Ethyl acetate in Hexane); IR (KBr, $cm^{-1}$): 3352 (-N-H), 2236 (-CN), 1589 (-C=N), 1362 (-C-N-), 1616 (C=C Ar), 1236 (-$CF_3$), 1370 (-C-N), 3135 (Ar C-H stretching); $^1$H NMR (400 MHz, $CDCl_3$, ppm): *δ*: 7.66 (1H, s, 5C), 6.48 (2H, s, -$NH_2$), 8.10–7.72 (4H, m, C-2'', 3'', 5'', 6'', Ar-H), 7.65–6.49 (4H, m, C-3', 4', 5', Ar-H); ESI-MS $[M+H]^+$: 330.10 and 331.10; Anal. Calcd for: $_{C17}H_{10}F_2N_3O$: C, 65.81; H, 3.25; N, 13.54; O, 5.16; F, 12.25; Found: C, 65.16; H, 3.30; N, 13.50; O, 5.13; F, 12.29.

*A8C: 2-amino-4-(3-chlorophenyl)-6-[4-(trifluoromethyl)phenyl]pyridine-3-carbonitrile*

Yield: 75%; m.p. 120.69°C; $R_f$= 0.6 (20% Ethyl acetate in Hexane); IR (KBr, $cm^{-1}$): 3361 (-N-H), 2222 (-CN), 1583 (-C=N), 1326 (-C-N-),1248 (-$CF_3$), 3012 (Ar C-H stretching), 1617 (C=C Ar), 848 (C-Cl); $^1$H NMR (400MHz, $CDCl_3$, ppm): *δ*: 8.28 (1H, s, 5C), 6.80 (2H, s, -$NH_2$), 8.33–8.29 (4H, m, C-2'', 3'', 5'', 6'', Ar-H), 8.01–7.62 (4H, m, C-2', 4', 5', 6', Ar-H); ESI-MS $[M+H]^+$: 374.22 and 375.22; Anal. Calcd for: $C19H_{11}ClF_3N_3$: C, 61.06; H, 2.97; N, 11.24; Cl,9.49; F, 15.25; Found: C, 61.24; H, 3.07; N, 11.20; Cl, 9.53; F,15.29.

*A9C: 2-amino-6-(4-(trifluoromethyl)phenyl)-4-(3-nitrophenyl)pyridine-3-carbonitrile*

Yield: 58%; m.p. 147.31°C; $R_f$= 0.6 (20% Ethyl acetate in Hexane); IR (KBr, $cm^{-1}$): 3351 (-N-H), 2225 (-CN), 1590 (-C=N), 1326 (-C-N-), 3011 (Ar C-H stretching), 3015 (Ar C-H stretching), 1616 (Ar C=C), 1240 (-$CF_3$), 1465 ($NO_2$); $^1$H NMR (400MHz, $CDCl_3$, ppm): *δ* 7.66 (1H, s, 5C), 6.80 (2H, s, -$NH_2$), 7.65–7.34 (4H, m, C-2', 3', 5', 6', Ar-H), 8.10–8.04 (4H, m, C-2'', 3'', 5'', 6'', Ar-H); ESI-MS $[M+H]^+$: 385.33 and 386.33; Anal. Calcd for: $C_{19}H_{11}F_3N_4O_2$: C, 59.38; H, 2.88; N, 14.58; O, 8.33; F, 14.83; Found: C, 59.45; H, 3.03; N, 14.68; O, 8.43; F, 14.91.

*A10C: 2-amino-4-(1H-pyrrol-2-yl)-6-[4-(trifluoromethyl)phenyl]pyridine-3-carbonitrile*

Yield: 65%; m.p. 138.08°C; $R_f$= 0.6 (20% Ethyl acetate in Hexane); IR (KBr, $cm^{-1}$): 3342 (-N-H), 2186 (-CN), 1590 (-C=N), 1362 (-C-N-) 1612 (C=C Ar), 1226 (-$CF_3$), 1377 (C-N), 3129 (Ar C-H stretching); $^1$H NMR (400MHz, $CDCl_3$, ppm): *δ*: 7.72 (1H, s, 5C), 6.12 (2H, s, -$NH_2$), 8.10–8.07 (4H, m, C-2'', 3'', 5'', 6'', Ar-H), 7.65–6.13 (4H, m, C-3', 4', 5', Ar-H), 8.53 (1H, s, pyrrol); ESI-MS $[M+H]^+$: 329.34 and 330.34; Anal. Calcd for: $C_{18}H_{11}F_3N_4$: C, 63.53; H, 3.26; N, 16.46; F, 16.75; Found: C, 63.59; H, 3.25; N, 16.41; F, 16.77.

*B1C: 2-amino-4(2,3-dichlorophenyl)-6-[4-(trifluoromethoxy)phenyl] pyridine-3-carbonitrile*

Yield: 61%; m.p. 150.21°C; $R_f$= 0.6 (20% Ethyl acetate in Hexane); IR (KBr, $cm^{-1}$): 3321 (-N-H), 2142 (-CN), 1580 (-C=N), 1366 (-C-N-),1241 (-$CF_3$), 1612 (Ar C=C), 3025 (Ar C-H stretching), 1167 (-C-O-), 825 (C-Cl); $^1$H NMR (400MHz, $CDCl_3$, ppm): *δ*: 8.04 (1H, s, 5C), 6.80 (2H, s, -$NH_2$), 8.40 -8.38 (4H, m, C-2'', 3'', 5'', 6'', Ar-H), 7.66–7.00 (3H, m, C- 4', 5', 6', Ar-H); ESI-MS $[M+H]^+$: 425.13 and 426.13; Anal. Calcd for: $C_{19}H_{10}Cl_2F_3N_3O$: C, 53.80; H, 2.38; N, 9.91; O, 3.77; Cl, 16.72; F, 13.44; Found: C, 53.48; H, 2.16; N, 9.41; O, 3.34;Cl, 16.45; F, 13.18.

*B2C: 2-amino-4-(2-chlorophenyl)-6-[4-(trifluoromethoxy)phenyl]pyridine-3-carbonitrile*

Yield: 58%; m.p. 120.38°C; $R_f$= 0.6 (20% Ethyl acetate in Hexane); IR (KBr, $cm^{-1}$): 3332 (-N-H), 2146 (-CN), 1561 (-C=N), 1353 (-C-N-), 1600 (Ar C=C), 1234 (-$CF_3$), 3014 (Ar C-H stretching), 1158 (-C-O-), 844 (C-Cl); $^1$H NMR (400MHz, $CDCl_3$, ppm): *δ*: 8.04 (1H, s, 5C), 6.80 (2H, s, -$NH_2$), 8.40 -8.05 (4H, m, C-2'', 3'', 5'', 6'', Ar-H), 7.50–6.98 (4H, m, C-3', 4', 5', 6', Ar-H); ESI-MS $[M+H]^+$: 390.03 and 391.03; Anal. Calcd for: $C_{19}H_{11}ClF_3N_3O$: C, 58.55; H, 2.84; N, 10.78; O, 4.10; Cl, 9.10; F, 14.62; Found: C, 58.25; H, 2.41; N, 10.34; O, 4.12; F, 14.15; Cl, 9.08.

*B3C: 2-amino-4-(1H-indol-3-yl)-6-[4-(trifluoromethoxy)phenyl]pyridine-3-carbonitrile*

Yield: 57%; m.p. 126.36°C; $R_f$= 0.6 (20% Ethyl acetate in Hexane); IR (KBr, $cm^{-1}$): 3540 (-N-H), 2186 (-CN), 1592 (-C=N), 1355 (-C-N-),1620 (Ar C=C), 1238 (-$CF_3$), 3020 (Ar C-H stretching), 1149 (-C-O-); $^1$H NMR (400MHz, $CDCl_3$, ppm): *δ*: 8.11 (1H, s, 5C), 6.80 (2H, s, -$NH_2$), 8.40 (1H, s, indole), 8.39 -8.38 (4H, m, C-2'', 3'', 5'', 6'', Ar-H), 8.12–6.98 (5H, m, C-2', 4', 5', 6', 7', Ar-H); ESI-MS $[M+H]^+$: 397.29 and 398.29; Anal. Calcd for: $C_{21}H_{13}F_3N_4O$: C, 63.96; H, 3.32; N, 14.21; O, 4.06; F, 14.45; Found: C, 63.46; H, 3.19; N, 14.27; O, 4.09; F, 14.20.

*B4C: {2-{2-amino-3-cyano-6-[4-(trifluoromethoxy)phenyl]pyridin-4-yl} phenyl)azinic acid*

Yield: 58%; m.p. 131.25°C; $R_f$= 0.6 (20% Ethyl acetate in Hexane); IR (KBr, $cm^{-1}$): 3322 (-N-H), 2171 (-CN), 1582 (-C=N), 1356 (-C-N-), 1609 (Ar C=C), 1245 (-$CF_3$), 3012 (Ar C-H stretching), 1120 (-C-O-), 1458 ($NO_2$); $^1$H NMR (400 MHz, $CDCl_3$, ppm): *δ*: 8.04 (1H, s, 5C), 6.80 (2H, s, -$NH_2$), 8.40–8.37 (4H, m, C-2'', 3'', 5'', 6'', Ar-H), 7.78–6.98 (4H, m, C-3', 4', 5', 6', Ar-H); ESI-MS $[M+H]^+$: 401.32 and 402.32; Anal. Calcd for: $C_{19}H_{11}F_3N_4O_3$: C, 57.01; H, 2.77; N, 14.00; O, 11.99; F, 14.24; Found: C, 56.22; H, 2.54; N, 13.90; O, 12.19; F, 14.14.

*B5C: 2-amino-6-(4-(trifluoromethoxy)phenyl)-4-(4-nitrophenyl)pyridine-3-carbonitrile*

Yield: 76%; m.p. 198.16°C; $R_f$= 0.6 (20% Ethyl acetate in Hexane); IR (KBr, $cm^{-1}$): 3329 (-N-H), 2175 (-CN), 1575 (-C=N), 1362 (-C-N-), 1640 (Ar C=C), 1204 (-CF3), 3018 (Ar C-H stretching), 1120 (-C-O-), 1436 ($NO_2$); $^1$H NMR (400MHz, $CDCl_3$, ppm): *δ*: 8.04 (1H, s, 5C), 6.80 (2H, s, -$NH_2$), 8.16–6.99 (4H, m, C-2', 3', 5', 6', Ar-H), 8.39–8.38 (4H, m, C-2'', 3'', 5'', 6'', Ar-H); ESI-MS $[M+H]^+$: 401.17 and 402.17; Anal. Calcd for: $C_{19}H_{11}F_3N_4O_3$: C, 57.01; H, 2.77; N, 14.00; O, 11.99; F, 14.24; Found: C, 56.25; H, 2.53; N, 13.90; O, 12.02; F, 14.21.

*B6C: 2-amino-4-(thiophen-2-yl)-6-[4-(trifluoromethoxy)phenyl]pyridine-3-carbonitrile*

Yield: 68%; m.p. 145.61°C; $R_f$= 0.6 (20% Ethyl acetate in Hexane); IR (KBr, $cm^{-1}$): 3340 (-N-H), 2177 (-CN), 1564 (-C=N), 1352 (-C-N-), 1611 (Ar C=C), 3125 (Ar C-H stretching), 1266 (-$CF_3$), 1156 (-C-O-), 628 (C-S); $^1$H NMR (400MHz, $CDCl_3$, ppm): *δ*: 8.37 (1H, s, 5C), 6.80 (2H, s, -$NH_2$), 7.72–6.98 (4H, m, C-3', 4', 5', Ar-H), 8.40–8.37 (4H, m, C-2'', 3'', 5'', 6'', Ar-H); ESI-MS $[M+H]^+$: 362.06 and 363.06; Anal. Calcd for: $C_{17}H_{10}F_3N_3OS$: C, 56.51; H, 2.79; N, 11.63; O, 4.43; F, 15.77; Found: C, 56.01; H, 2.28; N, 11.23; O, 4.21; F, 15.77.

*B7C: 2-amino-4-(furan-2-yl)-6-[4-(trifluoromethoxy)phenyl]pyridine-3-carbonitrile*

Yield: 67%; m.p. 142.73°C; $R_f$= 0.6 (20% Ethyl acetate in Hexane); IR (KBr, $cm^{-1}$): 3338 (-N-H), 2221 (-CN), 1564 (-C=N), 1350 (-C-N-), 1632 (Ar C=C), 3126 (Ar C-H stretching), 1245 (-$CF_3$), 1160 (-C-O-), 1720 (C-O); $^1$H NMR (400MHz, $CDCl_3$, ppm): *δ*: 8.37 (1H, s, 5C), 6.48 (2H, s, -$NH_2$), 8.40 -8.38 (4H, m, C-2'', 3'', 5'', 6'', Ar-H), 7.72–6.49 (4H, m, C-3', 4', 5', Ar-H); ESI-MS $[M+H]^+$: 346.16 and 347.16; Anal. Calcd for: $C_{17}H_{10}F_3N_3O_2$: C, 59.14; H, 2.92; N, 12.17; O, 9.27; F, 16.51; Found: C, 59.40; H, 2.54; N, 12.05; O, 9.12; F, 16.21.

*B8C: 2-amino-4-(3-chlorophenyl)-6-[4-(trifluoromethoxy)phenyl]pyridine-3-carbonitrile*

Yield: 70%; m.p. 121.38°C; $R_f$= 0.6 (20% Ethyl acetate in Hexane); IR (KBr, $cm^{-1}$): 3320 (-N-H), 2136 (-CN), 1561 (-C=N), 1353 (-C-N-), 1611 (Ar C=C), 1234 (-$CF_3$), 3014 (Ar C-H stretching), 1158 (-C-O-), 843 (C-Cl); $^1$H NMR (400MHz, $CDCl_3$, ppm): $\delta$: 8.32 (1H, s, 5C), 6.80 (2H, s, -$NH_2$), 8.40–8.37 (4H, m, C-2'', 3'', 5'', 6'', Ar-H), 8.31–6.99 (4H, m, C-2', 4', 5', 6', Ar-H); ESI-MS $[M+H]^+$: 390.18 and 391.18; Anal. Calcd for: $C_{19}H_{11}ClF_3N_3O$: C, 58.55; H, 2.84; N, 10.78; O, 4.10; Cl, 9.10 F, 14.62; Found: C, 58.17; H, 2.54; N, 10.78; O, 4.03; Cl, 9.06; F, 14.22.

*B9C: 2-amino-6-(4-(trifluoromethoxy)phenyl)-4-(3-nitrophenyl)pyridine-3-carbonitrile*

Yield:54%; m.p. 130.28°C; $R_f$= 0.6 (20% Ethyl acetate in Hexane); IR (KBr, $cm^{-1}$): 3330 (-N-H), 2186 (-CN), 1580 (-C=N), 1370 (-C-N-), 1240 (-$CF_3$), 1604 (Ar C=C), 3019 (Ar C-H stretching), 1122 (-C-O-), 1448 (-$NO_2$); $^1$H NMR (400 MHz, $CDCl_3$, ppm): $\delta$: 8.04 (1H, s, 5C), 6.80 (2H, s, -$NH_2$), 7.62–6.98(4H, m, C-2', 3', 5', 6', Ar-H), 8.40–8.37 (4H, m, C-2'', 3'', 5'', 6'', Ar-H); ESI-MS $[M+H]^+$: 401.54 and 402.54; Anal. Calcd for: $C_{19}H_{11}F_3N_4O_3$: C, 57.01; H, 2.77; N, 14.00; O, 11.99; F, 14.24; Found: C, 56.01; H, 2.52; N, 13.89; O, 12.22; F, 14.16.

*B10C: 2-amino-(1H-pyrrol-2-yl)-6-[4-(trifluoromethoxy)phenyl]pyridine-3-carbonitrile*

Yield: 68%; m.p. 112.60°C; $R_f$= 0.6 (20% Ethyl acetate in Hexane); IR (KBr, $cm^{-1}$): 3345 (-N-H), 2167 (-CN), 1561 (-C=N), 1350 (-C-N-), 1625 (C=C Ar), 1231 (-$CF_3$), 1336 (C-N), 3121 (Ar C-H stretching) 1166 (-C-O-); $^1$H NMR (400MHz, $CDCl_3$, ppm): $\delta$: 7.72 (1H, s, 5C), 6.12 (2H, s, -$NH_2$), 8.53 (1H-pyrrol, s), 8.40–8.37 (4H, m, C-2'', 3'', 5'', 6'', Ar-H), 7.01–6.13 (4H, m, C-3', 4', 5', Ar-H); ESI-MS $[M+H]^+$: 345.41 and 346.41; Anal. Calcd for: $C_{17}H_{11}F_3N_4O$: C, 59.31; H, 3.22; N, 16.27; O, 4.65; F, 16.55; Found: C, 59.15; H, 3.61; N, 16.14; O, 4.24; F, 16.25.

*A1D:2-amino-4-(2,3-dichlorophenyl)-6-[4-(trifluoromethyl)phenyl]-4H-pyran-3-carbonitrile*

Yield: 60%; m.p. 179.1°C; $R_f$= 0.81 (20% Ethyl acetate in Hexane); IR (KBr, $cm^{-1}$): 3368 (-N-H), 2215 (-CN), 1149 (Pyran -C-O-C-), 1320 (-C-N-), 1610 (C=C Ar), 1215 (-$CF_3$), 3015 (Ar C-H stretching), 830 (C-Cl); $^1$H NMR (400 MHz, $CDCl_3$, ppm): $\delta$: 4.99 (1H, d, $J$ = 5.6 Hz 5C), 3.93 (1H, d, $J$ = 5.6 Hz 4C), 6.93 (2H, s, -$NH_2$), 7.94–7.91 (4H, m, C-2'', 3'', 5'', 6'', Ar-H), 7.30–6.93 (3H, m, C- 4', 5', 6', Ar-H); ESI-MS $[M+H]^+$: 411.21 and 412.21; Anal. Calcd for: $C_{19}H_{11}C_{l2}F_3N_2O$: C, 55.50; H, 2.70; N, 6.81; O, 3.89; Cl, 17.24; F, 17.24; Found: C, 55.21; H, 2.25; N, 6.12;O, 3.22; Cl, 17.25; F, 17.06.

*A2D: 2-amino-4-(2-chlorophenyl)-6-[4-(trifluoromethyl)phenyl]-4H-pyran-3-carbonitrile*

Yield: 60%; m.p. 139.66°C; $R_f$= 0.82 (20% Ethyl acetate in Hexane); IR (KBr, $cm^{-1}$): 3341 (-N-H), 2140 (-CN), 1130 (Pyran -C-O-C-), 1321 (-C-N-), 1599 (Ar C=C), 1230 (-$CF_3$), 3014 (Ar C-H stretching), 844 (C-Cl); $^1$H NMR (400 MHz, $CDCl_3$, ppm): $\delta$: 4.99 (1H, d, $J$ = 5.6 Hz 5C), 3.94–3.93 (1H, m, 4C), 6.93 (2H, s, -$NH_2$), 7.94–7.55 (4H, m, C-2'', 3'', 5'', 6'', Ar-H), 7.41–7.25 (4H, m, C-3', 4', 5', 6', Ar-H); ESI-MS $[M+H]^+$: 378.11 and 379.11; Anal. Calcd for: C19H12ClF3N2O: C, 60.57; H, 3.21; N, 7.44; O, 4.25; Cl, 9.41; F, 15.13; Found: C, 60.24; H, 3.12; N, 7.14;O, 4.08; Cl, 9.45; F, 16.11.

*A3D: 2-amino-4-(1H-indol-3-yl)-6-[4-(trifluoromethyl)phenyl]-4H-pyran-3-carbonitrile*

Yield: 60%; m.p. 145°C; $R_f$= 0.67 (20% Ethyl acetate in Hexane); IR (KBr, $cm^{-1}$): 3252 (-N-H), 2220 (-CN), 1135 (Pyran -C-O-C-), 1320 (-C-N-), 1235 (-$CF_3$), 3022 (Ar C-H stretching), 1629 (C=C Ar); $^1$H NMR (400MHz, $CDCl_3$, ppm): $\delta$: 4.99 (1H, d, $J$ = 5.4 Hz 5C), 3.94 (1H, d, $J$ = 5.4 Hz 4C), 6.93 (2H, s, -$NH_2$), 7.94–7.91 (4H, m, C-2'', 3'', 5'', 6'', Ar-H), 7.61–7.02 (5H, m, C-2', 4', 5', 6', 7', Ar-H), 8.29 (1H, s, indole); ESI-MS $[M+H]^+$: 382.45 and 383.45; Anal. Calcd for: $C_{21}H_{14}F_3N_3O$: C, 66.14; H, 3.70; N, 11.02 ;O, 4.20; F, 14.95; Found: C, 66.07; H, 3.32; N, 11.77;O, 4.05; F, 15.95.

*A4D: 2-amino-6-(4-(trifluoromethyl)phenyl)-4-(2-nitrophenyl)-4H-pyran-3-carbonitrile*

Yield: 62%; m.p. 142°C; $R_f$= 0.63 (20% Ethyl acetate in Hexane); IR (KBr, $cm^{-1}$): 3360 (-N-H), 2220 (-CN), 1135 (Pyran -C-O-C-), 1311 (-C-N-), 3020 (Ar C-H stretching), 1626 (Ar C=C), 1210 (-$CF_3$), 1455 (-$NO_2$); $^1$H NMR (400 MHz, $CDCl_3$, ppm): $\delta$: 4.99 (1H, d, $J$ = 5.2 Hz 5C), 3.94–3.93 (1H, m, 4C), 6.93 (2H, s, -$NH_2$), 7.94–7.66 (4H, m, C-2'', 3'', 5'', 6'', Ar-H), 7.57–7.27 (4H, m, C-3', 4', 5', 6', Ar-H); ESI-MS $[M+H]^+$: 389.07 and 390.06; Anal. Calcd for: $C_{19}H_{12}F_3N_3O_3$: C, 58.92; H, 3.12; N, 10.85; O, 12.39; F, 14.72; Found: C, 58.87; H, 3.05; N, 10.83; O, 12.13; F, 15.68.

*A5D: 2-amino-6-(4-(trifluoromethyl)phenyl)-4-(4-nitrophenyl)-4H-pyran-3-carbonitrile*

Yield: 60%; m.p. 122.21°C; $R_f$= 0.65 (20% Ethyl acetate in Hexane); IR (KBr, $cm^{-1}$): 3350 (-N-H), 2225 (-CN), 1154 (Pyran -C-O-C-), 1313 (-C-N-), 3021 (Ar C-H stretching), 1616 (Ar C=C), 1240 (-$CF_3$), 1460 (-$NO_2$); $^1$H NMR (400MHz, $CDCl_3$, ppm): $\delta$: 4.99 (1H, d, $J$ = 5.6 Hz 5C), 3.93 (1H, d, $J$ = 5.52 Hz 4C), 6.93 (2H, s, -$NH_2$), 7.93- 7.27 (4H, m, C-2', 3', 5', 6', Ar-H), 7.96–7.94 (4H, m, C-2'', 3'', 5'', 6'', Ar-H); ESI-MS $[M+H]^+$: 389.05 ad 386.05; Anal. Calcd for: $C_{19}H_{12}F_3N_3O_3$: C, 58.92; H, 3.12; N, 10.85; O, 12.39; F, 14.72; Found: C, 58.84; H, 3.06; N, 10.45;O, 12.21; F, 15.45.

*A6D: 2-amino-4-(thiophen-2-yl)-6-[4-(trifluoromethyl)phenyl]-4H-pyran-3-carbonitrile*

Yield: 72%; m.p. 157.89°C; $R_f$= 0.65 (20% Ethyl acetate in Hexane); IR (KBr, $cm^{-1}$): 3350 (-N-H), 2218 (-CN), 1144 (Pyran -C-O-C-), 1363 (-C-N-), 1625 (Ar C=C), 3125 (Ar C-H stretching), 1258 (-$CF_3$), 635 (C-S); $^1$H NMR (400MHz, $CDCl_3$, ppm): $\delta$: 4.99 (1H, d, $J$ = 4.96 Hz 5C), 3.93 (1H, d, $J$ = 4.8 Hz 4C), 6.86 (2H, s, -$NH_2$), 7.35–6.86 (4H, m, C-3', 4', 5', Ar-H), 7.94–7.92 (4H, m, C-2'', 3'', 5'', 6'', Ar-H); ESI-MS $[M+H]^+$: 349.05 and 350.05; Anal. Calcd for: $C_{17}H_{11}F_3N_2OS$: C, 58.62; H, 3.18; N, 8.04; O, 4.59; F, 16.36; Found: C, 58.24; H, 3.8; N, 8.15;O, 4.59; F, 17.38.

*A7D: 2-amino-4-(furan-2-yl)-6-[4-(trifluoromethyl)phenyl]-4H-pyran-3-carbonitrile*

Yield: 70%; m.p. 180.01°C; $R_f$= 0.64 (20% Ethyl acetate in Hexane); IR (KBr, $cm^{-1}$): 3350 (-N-H), 2253 (-CN), 1135 (Pyran -C-O-C-), 1360 (-C-N-), 1615 (C=C Ar), 1230 (-$CF_3$), 1365 (-C-N), 3115 (Ar C-H stretching); $^1$H NMR (400 MHz, $CDCl_3$, ppm): $\delta$: 4.99 (1H, d, $J$ = 5.84 Hz 5C), 4.17–4.16 (1H, m, 4C), 6.93 (2H, s, -$NH_2$), 7.94–7.91 (4H, m, C-2'', 3'', 5'', 6'', Ar-H), 7.47–6.23 (4H, m, C-3', 4', 5', Ar-H); ESI-MS $[M+H]^+$: 333.15 and 334.15; Anal. Calcd for: $C_{17}H_{11}F_3N_2O_2$: C, 61.45; H, 3.34; N, 8.43; O, 9.63; F, 17.15; Found: C, 61.11; H, 3.17; N, 8.35;O, 9.58; F, 18.18.

*A8D: 2-amino-4-(4-chlorophenyl)-6-[4-(trifluoromethyl)phenyl]-4H-pyran-3-carbonitrile*

Yield: 60%; m.p. 136.66°C; $R_f$= 0.65 (20% Ethyl acetate in Hexane); IR (KBr, $cm^{-1}$): 3327 (-N-H), 2220 (-CN), 1149 (Pyran -C-O-C-), 1320 (-C-N-), 1243 (-$CF_3$), 3028 (Ar C-H stretching), 1615 (C=C Ar), 844 (C-Cl); $^1$H NMR (400 MHz, $CDCl_3$, ppm): $\delta$: 4.99 (1H, d, $J$ = 5.6 Hz 5C), 3.93 (1H, d, $J$ = 5.6 Hz 4C), 6.93 (2H, s, -$NH_2$), 8.04–8.01 (4H, m, C-2'', 3'', 5'', 6'', Ar-H), 7.96–7.28 (4H, m, C-2', 4', 5', 6', Ar-H); ESI-MS $[M+H]^+$: 378.17 and 379.17; Anal. Calcd for: $C_{19}H_{12}ClF_3N_2O$: C, 60.57; H, 3.21; N, 7.44; O, 4.25; Cl, 9.41; F, 15.13; Found: C, 60.48; H, 3.09; N, 7.18;O, 4.51; Cl, 9.37; F, 15.15.

*A9D: 2-amino-6-(4-(trifluoromethyl)phenyl)-4-(3-nitrophenyl)pyrane-3-carbonitrile*

Yield: 60%; m.p. 184.33°C; $R_f$= 0.61 (20% Ethyl acetate in Hexane); IR (KBr, $cm^{-1}$): 3320 (-N-H), 2220 (-CN), 1131 (Pyran -C-O-C-), 1320 (-C-N-), 3020 (Ar C-H stretching), 3020 (Ar C-H stretching), 1625 (Ar C=C), 1236 (-$CF_3$), 1460 (-$NO_2$); $^1$H NMR (400MHz, $CDCl_3$, ppm): $\delta$: 4.99 (1H, d, $J$ = 5.6 Hz 5C), 3.93 (1H, d, $J$ = 5.6 Hz 4C), 3.93 (1H, d, $J$ = 8 Hz 4C), 6.93 (2H, s, -$NH_2$), 7.58–7.14 (4H, m, C-2', 3', 5', 6', Ar-H), 7.94–7.59 (4H, m, C-2'', 3'', 5'', 6'', Ar-H); ESI-MS $[M+H]^+$: 389.44 ad 386.44; Anal. Calcd for: $C_{19}H_{12}F_3N_3O_3$: C, 58.92; H, 3.12; N, 10.85;O, 12.39; F, 14.72; Found: C, 58.86; H, 3.07; N, 10.13;O, 12.19; F, 15.46.

*A10D: 2-amino-4-(1H-pyrrol-2-yl)-6-[4-(trifluoromethyl)phenyl] -4H-pyran -3-carbonitrile*

Yield: 60%; m.p. 151.3°C; $R_f$= 0.67 (20% Ethyl acetate in Hexane); IR (KBr, $cm^{-1}$): 3340 (-N-H), 2172 (-CN), 1129 (Pyran -C-O-C-), 1352 (-C-N-) 1615 (C=C Ar), 1218 (-$CF_3$), 1371 (C-N), 3120 (Ar C-H stretching); $^1$H NMR (400 MHz, $CDCl_3$, ppm):*δ*: 4.99 (1H, d, *J* = 5.8 Hz 5C), 3.93–3.94 (1H, m, 4C), 6.93 (2H, s, -$NH_2$), 7.94–7.91 (4H, m, C-2'', 3'', 5'', 6'', Ar-H), 7.30–6.04 (4H, m, C-3', 4', 5', Ar-H), 8.88 (1H, s, pyrrol); ESI-MS [M+H]$^+$: 332.15 and 333.15; Anal. Calcd for: $C_{17}H_{12}F_3N_3O$: C, 61.63; H, 3.65; N, 12.68; O, 4.83; F, 17.20; Found: C, 61.35; H, 3.31; N, 12.64;O, 4.81; F, 18.17.

*B1D:2-amino-4-(2,3-dichlorophenyl)-6-[4-(trifluoromethoxy) phenyl]-4H-pyran-3 carbonitrile*

Yield: 60%; m.p. 171.3°C; $R_f$= 0.7 (20% Ethyl acetate in Hexane); IR (KBr, $cm^{-1}$): 3320 (-N-H), 2115 (-CN), 1138 (Pyran -C-O-C-), 1362 (-C-N-), 1236 (-$CF_3$), 1620 (Ar C=C), 3032 (Ar C-H stretching), 1160 (-C-O-), 820 (C-Cl); $^1$H NMR (400MHz, $CDCl_3$, ppm): *δ*: 4.99 (1H, d, *J* = 5.6 Hz 5C), 3.93 (1H, d, *J* = 5.6 Hz 4C), 6.93 (2H, s, -$NH_2$), 7.68–7.65 (4H, m, C-2'', 3'', 5'', 6'', Ar-H), 7.26–7.0 (3H, m, C- 4', 5', 6', Ar-H); ESI-MS [M+H]$^+$: 427.10 and 428.10; Anal. Calcd for: $C_{19}H_{11}C_{l2}F_3N_2O_2$; C, 53.42; H, 2.60; N, 6.56; O, 7.49; Cl, 16.60; F, 13.34; Found: C, 53.21; H, 2.20; N, 6.16;O, 7.93; Cl, 15.11; F, 12.24.

*B2D: 2-amino-4-(2-chlorophenyl)-6-[4-(trifluoromethyl)phenoxy]-4H-pyran-3-carbonitrile*

Yield: 61%; m.p. 158.89°C; $R_f$= 0.71 (20% Ethyl acetate in Hexane); IR (KBr, $cm^{-1}$): 3327 (-N-H), 2216 (-CN), 1160 (Pyran -C-O-C-), 1350 (-C-N-), 1626 (Ar C=C), 1234 (-$CF_3$), 3027 (Ar C-H stretching), 1151 (-C-O-), 832 (C-Cl); $^1$H NMR (400MHz, $CDCl_3$, ppm):*δ*: 4.99 (1H, d, *J* = 5.6 Hz 5C), 3.94–3.93 (1H, m, Hz 4C), 6.93 (2H, s, -$NH_2$), 7.67–7.65 (4H, m, C-2'', 3'', 5'', 6'', Ar-H), 7.54–7.25 (4H, m, C-3', 4', 5', 6', Ar-H); ESI-MS [M+H]$^+$: 393.05 and 394.05; Anal. Calcd for: $C_{19}H11C_{l2}F_3N_2O_2$; C, 58.10; H, 3.08; N, 7.13; O, 8.15; Cl, 9.03; F, 14.51; Found: C, 58.99; H, 3.25; N, 7.05;O, 8.21; Cl, 9.08; F, 14.43.

*B3D: 2-amino-4-(1H-indol-3-yl)-6-[4-(trifluoromethoxy)phenyl]-4H-pyran-3-carbonitrile*

Yield: 60%; m.p. 154.07°C; $R_f$= 0.7 (20% Ethyl acetate in Hexane); IR (KBr, $cm^{-1}$): 3538 (-N-H), 2216 (-CN), 1139 (Pyran -C-O-C-), 1350 (-C-N-), 1615 (Ar C=C), 1228 (-$CF_3$), 3026 (Ar C-H stretching), 1134 (-C-O-); $^1$H NMR (400MHz, $CDCl_3$, ppm): *δ*: 4.99 (1H, d, *J* = 5.4 Hz 5C), 3.93 (1H, d, *J* = 5.4 Hz 4C), 6.81 (2H, s, -$NH_2$), 7.68–7.65 (4H, m, C-2'', 3'', 5'', 6'', Ar-H), 7.61–6.83 (5H, m, C-2', 4', 5', 6', 7', Ar-H), 8.29 (1H, s, indol); ESI-MS [M+H]$^+$: 398.33 and 399.33; Anal. Calcd for: $C_{21}H_{14}F_3N_3O_2$; C, 63.48; H, 3.55; N, 10.58; O, 8.05; F, 14.34; Found: C, 63.45; H, 3.53; N, 10.52;O, 3.22; F, 14.12.

*B4D: 2-amino-6-(4-(trifluoromethoxy)phenyl)-4-(2-nitrophenyl)pyran-3-carbonitrile*

Yield: 66%; m.p. 154.51°C; $R_f$= 0.68 (20% Ethyl acetate in Hexane); IR (KBr, $cm^{-1}$): 3317 (-N-H), 2201 (-CN), 1142 (Pyran -C-O-C-), 1350 (-C-N-), 1622 (Ar C=C), 1240 (-$CF_3$), 3031 (Ar C-H stretching), 1121 (-C-O-), 1458 ($NO_2$); $^1$H NMR (400MHz, $CDCl_3$, ppm): *δ*: 4.99 (1H, d, *J* = 5.2 Hz 5C), 3.94–3.93 (1H, m, Hz 4C), 6.93 (2H, s, -$NH_2$), 7.69–7.66 (4H, m, C-2'', 3'', 5'', 6'', Ar-H), 7.57- 6.82 (4H, m, C-3', 4', 5', 6', Ar-H); ESI-MS [M+H]$^+$ :404.65 and 405.65; Anal. Calcd for: $C_{19}H_{12}F_3N_3O_4$; C, 56.58; H, 3.00; N, 10.42;O, 15.87; F, 14.13; Found: C, 56.55; H, 3.08; N, 10.12;O, 15.07; F, 14.08.

*B5D: 2-amino-6-(4-(trifluoromethoxyl)phenyl)-4-(4-nitrophenyl)pyridine-3-carbonitrile*

Yield: 65%; m.p. 152.48°C; $R_f$= 0.67 (20% Ethyl acetate in Hexane); IR (KBr, $cm^{-1}$): 3341 (-N-H), 2225 (-CN), 1142 (Pyran -C-O-C-), 1358 (-C-N-), 1632 (Ar C=C), 1224 (-$CF_3$), 3022 (Ar C-H stretching), 1126 (-C-O-), 1439 ($NO_2$); $^1$H NMR (400MHz, $CDCl_3$, ppm): *δ*: 4.99 (1H, d, *J* = 5.6 Hz 5C), 3.93 (1H, d, *J* = 5.6 Hz 4C), 6.83 (2H, s, -$NH_2$), 7.67–7.60 (4H, m, C-2', 3', 5', 6', Ar-H), 7.95–7.94 (4H, m, C-2'', 3'', 5'', 6'', Ar-H); ESI-MS [M+H]$^+$: 404.23 and 405.23; Anal. Calcd for: $C_{19}H_{12}F_3N_3O_4$; C, 56.58; H, 3.00; N, 10.42; O, 15.87; F, 14.13; Found: C, 56.55; H, 3.06; N, 10.11;O, 15.77; F, 14.03.

*B6D: 2-amino-4-(thiophen-2-yl)-6-[4-(trifluoromethoxy)phenyl]-4H-pyran-3-carbonitrile*

Yield: 60%; m.p. 180.12°C; $R_f$= 0.67 (20% Ethyl acetate in Hexane); IR (KBr, $cm^{-1}$): 3342 (-N-H), 2225 (-CN), 1142 (Pyran -C-O-C-), 1348 (-C-N-), 1626 (Ar C=C), 3120 (Ar C-H stretching), 1250 (-$CF_3$), 1168 (-C-O-), 638 (C-S); $^1$H NMR (400MHz, $CDCl_3$, ppm): *δ*: 4.99 (1H, d, *J* = 4.8 Hz 5C), 3.93 (1H, d, *J* = 4.8 Hz 4C), 6.93 (2H, s, -$NH_2$), 8.03 -7.68 (4H, m, C-2'', 3'', 5'', 6'', Ar-H), 7.67–6.81 (4H, m, C-3', 4', 5', Ar-H); ESI-MS [M+H]$^+$: 365.16 and 366.16; Anal. Calcd for: $C_{17}H_{11}F_3N_2O_2S$; C, 56.04; H, 3.04; N, 7.69;O, 8.78; F, 15.64; Found: C, 56.02; H, 3.05; N, 7.90;O, 8.81; F, 15.60.

*B7D: 2-amino-4-(furan-2-yl-6-[4-(trifluoromethoxy)phenyl]-4H-pyran-3-carbonitrile*

Yield: 75%; m.p. 163.24°C; $R_f$= 0.68 (20% Ethyl acetate in Hexane); IR (KBr, $cm^{-1}$): 3320 (-N-H), 2220 (-CN), 1142 (Pyran -C-O-C-), 1351 (-C-N-), 1612 (Ar C=C), 1245 (-$CF_3$), 1160 (-C-O-C), 1715 (C-O); 3133 (Ar C-H stretching); $^1$H NMR (400MHz, $CDCl_3$, ppm): *δ*: 4.99 (1H, d, *J* = 4.8 Hz 5C), 3.93 (1H, d, *J* = 4.8 Hz 4C), 6.93 (2H, s, -$NH_2$), 7.68 -7.65 (4H, m, C-2'', 3'', 5'', 6'', Ar-H), 7.47–6.10 (4H, m, C-3', 4', 5', Ar-H); ESI-MS [M+H]$^+$: 349.04 and 350.04; Anal. Calcd for: $C1_8H_{12}F_3N_2O$; C, 59.84; H, 3.35; N, 7.75; O, 13.29; F, 15.78; Found: C, 59.82; H, 3.33; N, 7.77;O, 13.25; F, 15.75.

*B8D: 2-amino-4-(4-chlorophenyl)-6-[4-(trifluoromethoxy)phenyl]-4H-pyran-3-carbonitrile*

Yield: 62%; m.p. 158.89°C; $R_f$= 0.68 (20% Ethyl acetate in Hexane); IR (KBr, $cm^{-1}$): 3324 (-N-H), 2210 (-CN), 1158 (Pyran -C-O-C-), 1352 (-C-N-), 1620 (Ar C=C), 1230 (-$CF_3$), 3027 (Ar C-H stretching), 1150 (-C-O-), 830 (C-Cl), 1158 (-C-O-), 843 (C-Cl); $^1$H NMR (400 MHz, $CDCl_3$, ppm): *δ*: 4.99 (1H, d, *J* = 5.6 Hz 5C), 3.93 (1H, d, *J* = 5.6 Hz 4C), 6.93 (2H, s, -$NH_2$), 8.03–7.95 (4H, m, C-2'', 3'', 5'', 6'', Ar-H), 7.68–7.53 (4H, m, C-2', 4', 5', 6', Ar-H); ESI-MS [M+H]$^+$: 393.16 and 394.16; Anal. Calcd for: $C_{19}H_{12}ClF_3N_2O_2$; C, 58.10; H, 3.08; N, 7.13; O, 8.15; Cl, 9.03; F, 14.51; Found: C, 58.11; H, 3.07; N, 7.15;O, 8.14; Cl, 9.13; F, 14.52.

*B9D: 2-amino-6-(4-(trifluoromethoxy)phenyl)-4-(3-nitrophenyl)pyran-3-carbonitrile*

Yield: 77%; m.p. 159.47°C; $R_f$= 0.64 (20% Ethyl acetate in Hexane); IR (KBr, $cm^{-1}$): 3332 (-N-H), 2226 (-CN), 1134 (Pyran -C-O-C-), 1370 (-C-N-), 1240 (-$CF_3$), 1625 (Ar C=C), 3021 (Ar C-H stretching), 1120 (-C-O-), 1440 ($NO_2$); $^1$H NMR (400 MHz, $CDCl_3$, ppm): *δ*: 4.99 (1H, d, *J* = 5.6 Hz 5C), 3.93 (1H, d, *J* = 5.6 Hz 4C), 6.93 (2H, s, -$NH_2$), 7.59–7.14 (4H, m, C-2', 3', 5', 6', Ar-H), 7.68–7.65 (4H, m, C-2'', 3'', 5'', 6'', Ar-H); ESI-MS [M+H]$^+$: 404.12 and 405.12; Anal. Calcd for: $C_{19}H_{12}F_3N_3O_4$; C, 56.58; H, 3.00; N, 10.42; O, 15.87; F, 14.13; Found: C, 56.55; H, 3.02; N, 10.49;O, 15.80; F, 14.07.

*B10D: 2-amino-4-(1H-pyrrol-2-yl)-6-[4-(trifluoromethoxy)phenyl] -4H-pyran -3-carbonitrile*

Yield: 60%; m.p. 161.31°C; $R_f$= 0.65 (20% Ethyl acetate in Hexane); IR (KBr, $cm^{-1}$): 3340 (-N-H), 2227 (-CN), 1530 (Pyran -C-O-C-), 1350 (-C-N-), 1624 (Ar C=C), 1235 (-$CF_3$), 1328 (C-N), 3120 (Ar C-H stretching), 1165 (-C-O-); $^1$H NMR (400MHz, $CDCl_3$, ppm): *δ*: 4.99 (1H, d, *J* = 4.8 Hz 5C), 3.93 (1H, d, *J* = 4.8 Hz 4C), 6.93 (2H, s, -$NH_2$), 8.88 (1H, s, Pyrrol), 7.68–7.65 (4H,m, C-2'', 3'', 5'', 6'', Ar-H), 6.93–6.04 (4H, m, C-3', 4', 5', Ar-H); ESI-MS [M+H]$^+$: 348.16 and 349.16; Anal. Calcd for: $C_{17}H_{12}F_3N_3O_2$; C, 54.69; H, 3.31; N, 10.63; O, 12.14; F, 19.22; Found: C, 54.61; H, 3.12; N, 10.52;O, 12.12; F, 19.20.

**Antimicrobial screening**

The antimicrobial activity was performed against three varieties of couples strains. The organisms selected were give below:

| | |
|---|---|
| Gram-positive bacteria: | *Bacillus subtilis* (NCIM-2079), *Staphylococcus aureus* (NCIM-2079) |
| Gram-negative bacteria: | *Escherichia coli* (NCIM-2065), *Proteus vulgaris* (NCIM-2027) |
| Fungi: | *Candida albicans* (MDCC-227), *Aspergillus niger* (MTCC 5889) |

Before initiation of antimicrobial assay, fumigate the laboratory before 4 days then cleaned glassware were kept in hot air oven 160°C for 2 h, the media were sterilized and the antibiotic solution (standard) and synthesized compounds were kept ready. Meanwhile, composition of the nutrient agar medium was prepared. The weighed quantities of peptone, meat extract, and NaCl were dissolved in 1000 ml of distilled water adjust the pH 7.2 of the medium. The agar gets dissolved and the medium was distributed in conical flask each containing 25 ml. The media and sterile water required were sterilized by autoclaving at 121°C temperature and 15 lbs/sq. inch pressure for 20 min. Petri plates, test tubes, pipettes, and borer required for experiment were sterilized by dry heat sterilization using hot air oven. Eighteen hours old cultures of respective organisms were taken and sterile water was used for making the suspension of these organisms. 0.5 ml of this suspension was used as inoculum and pour plate technique was conducted. The inoculated agar medium was poured into sterile 10 cm diameter Petri dishes. Medium in the plates was allowed to solidify. The solutions of the test compounds in concentrations of 0.1 µg/ml were prepared in DMSO. The cups of 5 mm diameter were prepared using borer in the corresponding medium. In each plate, five wells were prepared. Three cups are of test compounds, one of standard compound and other one was used as control in each cup samples was poured and then plates were left for 45 min in a refrigerator for diffusion. After incubation for 18 h at 37°C, the plates were examined for inhibition zones [20]. The experiments were done thrice and the average diameter of the zones of inhibition was recorded.

**Biological activities**

*Antimicrobial activity*

Despite the availability of many antimicrobial agents available in the market still there is challenge to find newer molecules for their antimicrobial property due to existing molecules are not sufficient to defeat the infections because of resistance. Hence, the synthesized compounds (A1C-A10C, B1C-B10C, A1D-A10D, and B1D-B10D) were tested to study their antimicrobial behavior against couple of bacterial strains *Bacillus subtilis*, *Staphylococcus aureus, Escherichia coli, Proteus vulgaris,* and couple of fungal strains *Aspergillus niger and Candida albicans* at 0.1% concentration. Benzyl penicillin and fluconazole were used as standard drugs for antibacterial and antifungal activities. The results are summarized; the activity of some of the compounds is superior to the standard drugs. The study showed that the compounds exhibited varying degree of activities. In antimicrobial activity, the compounds A3C, A3D in A-series and in B3C and B3D in B-series shows more activity than benzyl penicillin against *S. aureus* with a zone of inhibition 26, 25 and 27, 24 mm, respectively. The compounds A3C, A3D in A-series and B3C in B-series were more activity than benzyl penicillin against *B. subtilis* with a zone of inhibition 30, 28 and 29 mm, respectively. The compounds A3C, A5D in A-series and B3C, B4C, B4D, and B5D in B-series show more activity than benzyl penicillin against *E. coli* with a zone of inhibition 24, 18, and 16, and 16, 17, and 17 mm, respectively.

The compounds A3C, A3D, A5D, and A6D in A-series and B1C, B2C, B3C, B6C, B1D, and B3D show more activity than benzyl penicillin against *P. vulgaris* with a ZI 25, 19, 19, 19 and 20, 20, 20, 20, 20, and 20 mm, respectively. In antimicrobial activity among all the compound A3C show more activity than benzyl penicillin against *B. subtilis, E. coli,* and *P. vulgaris* with a zone of inhibition 30, 24, and 25 mm, respectively. The compound B3C shows more activity against benzyl penicillin against *S. aureus* with a zone of inhibition 27 mm.

In antifungal activity, the compounds A3C, A3D in A-series and B4C, B5C, and B3D in B-series show more activity than fluconazole against *C. albicans* with zone of inhibition 26, 22 and 23, 23, 20 mm, respectively. The compounds A3D in A-series and B3C show more activity than fluconazole against *A. niger* with a zone of inhibition 26 and 26 mm, respectively. In antifungal activity, the compound A3C shows more activity than fluconazole against *C. albicans* with a zone of inhibition 26. The compounds B3C, A3D shows more activity than fluconazole against *A. niger* with a zone of inhibition 26 mm.


**Molecular docking studies**

To understand the mechanism of action of the antimicrobial activities of the newly synthesized compounds, we carried out molecular docking, which is used to predict the binding mode of ligands within the binding site of target proteins [21]. Taking into consideration the previously reported dihydrofolic reductase (DHFR) inhibitory activity of the structurally related pyridine and pyran antimicrobial agents methotrexate (MTX) [22], docked the synthesized compounds in MTX active site. To validate and specify the target protein for the antimicrobial activity of newly synthesized 2-amino-pyridine-3- carbonitrile derivative and 2-amino-4H-pyran-3-carbonitrile derivative, DHFR protein was retry in protein data bank [36]. Docking

**Table 1: Antioxidant activity of pyran and pyridine derivatives**

| Compound codes | $IC_{50}$ (µg/ml) | Compound codes | $IC_{50}$ (µg/ml) |
|---|---|---|---|
| A1C | 48.60±0.02 | A1D | 51.20±1.12 |
| A2C | 55.31±0.06 | A2D | 62.12±1.38 |
| A3C | 30.28±0.01 | A3D | 50.47±1.40 |
| A4C | 39.74±0.08 | A4D | 61.18±0.08 |
| A5C | 40.24±0.02 | A5D | 72.15±0.17 |
| A6C | 42.21±0.47 | A6D | 70.27±1.52 |
| A7C | 31.05±0.15 | A7D | 89.15±2.06 |
| A8C | 56.28±0.19 | A8D | 64.24±1.22 |
| A9C | 40.24±0.72 | A9D | 76.26±0.09 |
| A10C | 52.45±0.12 | A10D | 38.16±0.26 |
| B1C | 50.29±1.25 | B1D | 82.24±3.17 |
| B2C | 51.77±0.08 | B2D | 80.16±0.14 |
| B3C | 32.24±3.07 | B3D | 52.25±2.06 |
| B4C | 35.64±2.01 | B4D | 100.25±3.45 |
| B5C | 39.51±3.15 | B5D | 97.11±0.05 |
| B6C | 31.42±3.18 | B6D | 75.16±1.25 |
| B7C | 36.6±3.12 | B7D | 70±4.20 |
| B8C | 50.33±2.18 | B8D | 79.71±0.19 |
| B9C | 39.29±3.10 | B9D | 96.13±0.55 |
| B10C | 86.10±2.15 | B10D | 98.06±2.21 |
| Control | | - | |
| Standard ascorbic acid | | 0.0775±0.001825 | |

*n=3; Stdev. *One-way ANOVA comparison between chalcones, pyridine, and Pyrans; p≤0.0001

studies of A3C compound into the active site of 4DFR enzyme showed three hydrogen bonds with LEU24, ASN18, and ALA7 a good interaction with the active site (Fig. 1). The docking conformation of the active B3C compound shows good interactions with the active site residues of this protein. Compound B3C formed five hydrogen bond interactions between amino groups moiety, as it acts as a hydrogen bond donor with the chain A of ILE29, TYR100, GLU17, HIS45, and SER49 residues 50% strength. In addition, it showed Van der Waals interaction with THR46 (Fig. 2). Finally, there was a good correlation of data between *in silico* and *in vitro* profiles.

**Table 2: Antimicrobial activity of pyran and pyridine derivatives zone of inhibition (mm)**

| Compound code | *Staphylococcus aureus* | *Bacillus subtilis* | *Escherichia coli* | *Proteus vulgaris* | *Candida albicans* | *Aspergillus niger* |
|---|---|---|---|---|---|---|
| A1C | 22.04±0.02 | 26.03±0.01 | 15.03±0.01 | 20.13±0.02 | 20.13±0.02 | 23.04±0.02 |
| A2C | 20.04±0.001 | 25.03±0.01 | 14.04±0.05 | 18.12±0.11 | 19.06±0.01 | 24.05±0.01 |
| A3C | 26.05±0.02 | 30.02±0.02 | 24.23±0.15 | 25.01±0.11 | 20.06±0.01 | 19.06±0.01 |
| A4C | 23.02±0.01 | 26.05±0.05 | 20.06±0.01 | 20.02±0.02 | 20.08±0.01 | 23.03±0.01 |
| A5C | 25.06±0.08 | 26.3±0.26 | 17.05±0.05 | 22.05±0.01 | 20.07±0.01 | 23.03±0.02 |
| A6C | 23.03±0.01 | 28.4±0.11 | 14.02±0.07 | 20.04±0.01 | 18.08±0.01 | 15.05±0.01 |
| A7C | 22.05±0.01 | 25.02±0.05 | 21.06±0.02 | 21.05±0.01 | 17.05±0.04 | 20.02±0.01 |
| A8C | 10.04±0.02 | 10.3±0.28 | 11.07±0.03 | 12.06±0.01 | 09.05±0.19 | 10.04±0.76 |
| A9C | 9.03±0.01 | 8.5±0.16 | 10.07±0.02 | 12.05±0.04 | 11.18±0.56 | 12.06±0.01 |
| A10C | 12.05±1.77 | 12.03±0.02 | 11.3±0.17 | 10.05±0.04 | 12.04±0.31 | 12.04±0.08 |
| B1C | 23.05±0.01 | 26.04±0.01 | 15.3±0.17 | 20.04±0.05 | 21.04±0.01 | 23.05±0.05 |
| B2C | 24.08±0.005 | 26.3±0.17 | 15.36±0.15 | 20.12±0.01 | 19.04±0.04 | 24.04±0.01 |
| B3C | 27.02±0.015 | 29.06±0.01 | 16.07±0.02 | 20.15±0.01 | 12.05±0.04 | 16.06±0.05 |
| B4C | 25.05±0.01 | 26.03±0.03 | 16.07±0.01 | 19.03±0.03 | 23.03±0.02 | 22.06±0.01 |
| B5C | 25.04±0.05 | 26.05±0.01 | 15.07±0.05 | 19.05±0.04 | 23.04±0.03 | 20.07±0.02 |
| B6C | 23.04±0.02 | 27.08±0.01 | 14.04±0.02 | 20.04±0.03 | 18.04±0.06 | 16.08±0.01 |
| B7C | 22.02±0.01 | 26.05±0.05 | 15.05±0.01 | 19.05±0.04 | 18.03±0.03 | 20.06±0.01 |
| B8C | 11.04±0.03 | 10.05±0.01 | 10.03±0.01 | 8.04±0.03 | 10.07±0.15 | 12.06±0.02 |
| B9C | 11.04±0.01 | 13.06±0.01 | 8.03±0.02 | 12.04±0.03 | 11.07±0.07 | 12.11±0.69 |
| B10C | 10.12±0.13 | 14.06±0.17 | 11.06±0.01 | 10.17±0.15 | 10.08±0.05 | 10.17±0.27 |
| A1D | 21.07±0.54 | 23.37±1.10 | 13.03±0.11 | 14.04±0.02 | 19.02±0.03 | 21.1±0.01 |
| A2D | 11.68±0.54 | 15.07±1.06 | 12.03±0.15 | 16.33±0.57 | 17.03±0.03 | 23.01±0.01 |
| A3D | 25.06±0.01 | 28.39±0.58 | 14.06±0.30 | 18.97±0.06 | 18.05±0.04 | 24.04±0.04 |
| A4D | 18.06±0.01 | 19.01±0.02 | 16.9±0.34 | 18.08±0.05 | 19.07±0.05 | 22.3±0.01 |
| A5D | 21.05±0.05 | 25.86±0.30 | 18.7±0.43 | 19.05±0.01 | 18.03±0.02 | 21.1±0.05 |
| A6D | 19.03±0.07 | 17.97±0.06 | 15.23±0.05 | 19.03±0.01 | 16.07±0.23 | 20.23±0.25 |
| A7D | 20.05±0.56 | 18.46±0.55 | 14.13±0.11 | 18.03±0.03 | 18.07±0.05 | 22.16±0.15 |
| A8D | 11.03±0.01 | 12.36±0.32 | 8.07±0.11 | 9.1±0.26 | 14.01±0.56 | 14.5±0.11 |
| A9D | 12.04±0.01 | 13.08±0.10 | 12.04±0.02 | 11.04±0.02 | 14.07±0.01 | 15.05±0.08 |
| A10D | 15.06±0.01 | 12.99±0.07 | 11.24±0.31 | 14.06±0.01 | 12.07±0.03 | 12.04±0.21 |
| B1D | 20.06±0.01 | 23.08±0.02 | 16.06±0.01 | 17.06±0.01 | 19.05±0.4 | 20.04±0.03 |
| B2D | 14.05±0.02 | 16.05±0.04 | 12.83±0.29 | 15.05±0.01 | 14.13±0.15 | 20.05±0.04 |
| B3D | 24.04±0.02 | 26.00±0.07 | 15.02±0.02 | 20.07±0.01 | 20.26±0.11 | 24.04±0.03 |
| B4D | 19.06±0.02 | 17.06±0.01 | 17.04±0.03 | 16.2±0.75 | 17.23±0.05 | 20.3±0.36 |
| B5D | 19.07±0.07 | 16.05±0.04 | 17.04±0.04 | 19.03±0.03 | 15.6±0.10 | 18.75±0.48 |
| B6D | 19.05±0.17 | 18.04±0.03 | 15.04±0.03 | 19.07±0.10 | 18.2±0.17 | 21.96±0.22 |
| B7D | 20.06±0.01 | 19.05±0.04 | 16.05±0.04 | 20.03±0.03 | 19.26±0.05 | 20.01±0.01 |
| B8D | 11.05±0.50 | 5.07±0.01 | 07.05±0.04 | 8.03±0.55 | 12.6±0.02 | 15.07±0.02 |
| B9D | 14.05±0.79 | 15.08±0.70 | 14.08±0.12 | 11.4±0.17 | 04.05±0.33 | 10.04 ±0.12 |
| B10D | 10.05±0.01 | 10.08±0.01 | 11.05±0.04 | 10.02±0.18 | 12.03±0.02 | 16.04±0.19 |
| Control benzyl pencillin | 24.06±0.05 | 27.02±0.02 | 14.05±0.05 | 19.04±0.03 | | |
| Fluconazole | | | | | 19.05±0.04 | 24.41±0.52 |

*n=3; Stdev

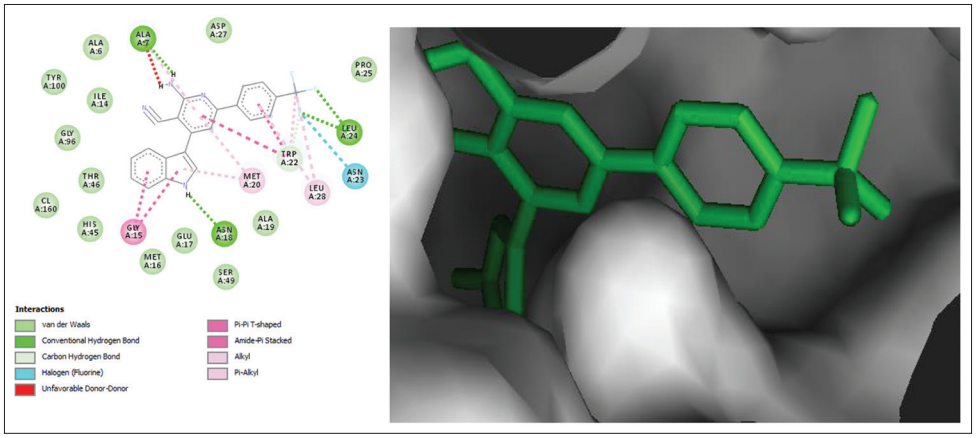



**Fig. 1: 2D and 3D interactions of A3C with ATP active site of methotrexate of 4 dihydrofolic reductase, which shown hydrogen bond and other interactions.**

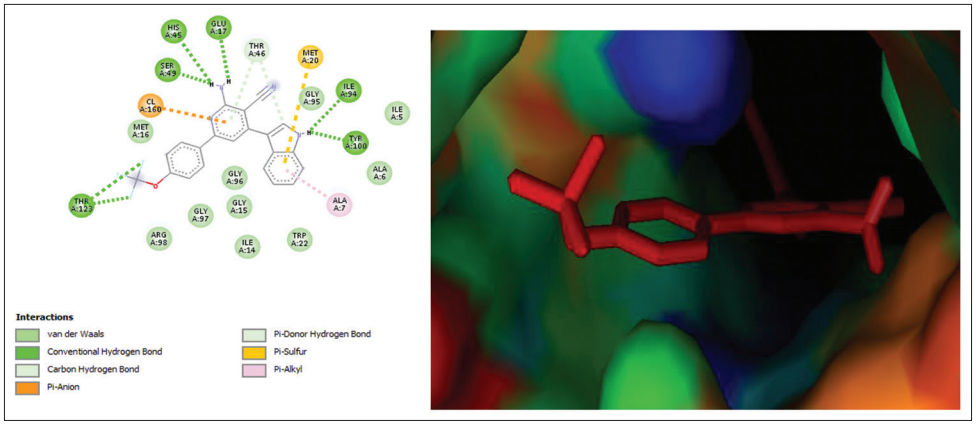



**Fig. 2: 2D and 3D interactions of B3C with Methotrexate active site of 4 dihydrofolic reductase, which shown hydrogen bond and other interactions**

### 2,2-diphenylpicrylhydrazyl (DPPH) of free radicals scavenging activity

The scavenging activity for DPPH free radicals was measured according to the procedure described [23]. An aliquot of 3 ml of 0.004% DPPH solution in methanol and 0.1 ml of synthetic compounds at various concentrations were mixed. The mixture was shaken vigorously and allowed to reach a steady state at room temperature for 30 min. Decolonization of DPPH was determined by measuring the absorbance at 517 nm. A control was prepared using 0.1 ml of respective vehicle in the place of synthesized compounds of three series along with ascorbic acid as standard. Three series of synthesized compounds were evaluated at different concentration such as 10 µg/ml, 30 µg/ml, 50 µg/ml, 70 µg/ml, and 100 µg/ml, respectively. There is a significant difference was observed where, $p<0.001$ One-way ANOVA competed with chalcones, pyridine, and pyrans derivatives. The mean $IC_{50}$ values for DPPH radical of the test compounds of three series were found to be in range 51±0.05–71±1.08 µg/ml, 55±0.06–31±0.15 µg/ml, and 100±3.45–51±1.12 µg/ml, respectively. The mean $IC_{50}$ value of ascorbic acid was found to be 0.0775±0.001825 µg/ml

## CONCLUSION

In investigation of novel series of Pyridine-3-carbonitrile and 4H-pyran-3-carbonitrile scaffolds was synthesized and evaluated as antimicrobial and antioxidant. The compounds A3C and B3C exhibited marked zone of inhibition with 30.02±0.02 mm and 29.06±0.01 mm relative potency, respectively, designed molecules possess good permeation into barrier of microbiota with zone of inhibition. Heterocyclic rings with less strain result in better activity. The docking studies revealed that these compounds might act through inhibition of DHFR, considered to be good candidates as novel antimicrobial agents, and further studies including preparation of novel analogues and toxicity testing are required for optimization of the activity which are being undertaken. Furthermore, the daily intake of this molecule will change ecology of the gut microbiota, decreasing the risk of infectious disease and bring to homeostasis in cell function.

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## AUTHORS' CONTRIBUTION

L. Surendra babu made a substantial contribution in conception, acquisition of data, interpretation of data, in drafting the article and revising it for ensuring critical academic content; gave final approval of the version to be published; and agreed to be held accountable for all aspects of the work. Prof .Y. Rajendra Prasad mentor who uplifting in difficult things and design of the work.

## CONFLICTS OF INTEREST

The author declared that there are no conflicts of interest related to this study by the authors.

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## REFERENCES

1. Available from: https://www.who.int/whr/1996/media_centre/press_release/en/index4.html.
2. Smith PW, Sollis SL, Howes PD, Cherry PC, Starkey ID, Cobley KN, *et al*. Dihydropyrancarboxamides related to zanamivir: A new series of inhibitors of influenza virus sialidases. 1. Discovery, synthesis, biological activity, and structure-activity relationships of 4-guanidino- and 4-amino-4H-pyran-6-carboxamides. J Med Chem 1998;41:787-97.
3. Henry GD. *De novo* synthesis of substituted pyridines. Tetrahedron 2004;60:6043-61.
4. Martínez-Grau A, Marco J. Friedländer reaction on 2-amino-3-cyano-4H-pyrans: Synthesis of derivatives of 4H-pyran [2, 3-b] quinoline, new tacrine analogues. Bioorg Med Chem Lett 1997;7:3165-70.
5. Bianchi G, Tava A. Synthesis of (2R)-(+)-2,3-dihydro-2,6-dimethyl-4H-pyran-4-one, a homologue of pheromones of a species in the *Hepialidae* family. Agric Biol Chem 1987;51:2001-2.
6. Abouzid KA, Al-Ansary GH, El-Naggar AM. Eco-friendly synthesis of novel cyanopyridine derivatives and their anticancer and PIM-1 kinase inhibitory activities. Eur J Med Chem 2017;134:357-65.
7. Dev S, Dhaneshwar S, Mathew B. Virtual combinatorial library design, synthesis and *in vitro* anticancer assessment of-2-amino-3-cyanopyridine derivatives. Comb Chem High Throughput Scree 2018;21:138-48.
8. Amala VE, Jeyaraj M. Determination of antibacterial, antifungal, bioactive constituents of Triphala by FT-IR and GC-MS analysis. Int J Pharm Pharm Sci 2014;6:123-6.
9. Gurunanjappa P, Nagamallu R, Kariyappa AK. Synthesis and antimicrobial activity of novel fused pyrazoles. Int J Pharm Pharm Sci 2015;7:379-81.
10. Movassaghi M, Hill MD, Ahmad OK. Direct synthesis of pyridine derivatives. JACS 2007;129:10096-7.
11. Li AH, Moro S, Forsyth N, Melman N, Ji X, Jacobson KA. Synthesis, CoMFA analysis, and receptor docking of 3,5-diacyl-2,4-dialkylpyridine derivatives as selective a3 adenosine receptor antagonists. J Med Chem 1999;42:706-21.
12. Vacher B, Bonnaud B, Funes P, Jubault N, Koek W, Assie MB, *et al*. Novel derivatives of 2-pyridinemethylamine as selective, potent, and orally active agonists at 5-HT1A receptors. J Med Chem 1999;42:1648-60.
13. Mahdavi SM, Habibi A, Dolati H, Shahcheragh SM, Sardari S, Azerang P, *et al*. Synthesis and antimicrobial evaluation of 4H-pyrans and schiff bases fused 4H-pyran derivatives as inhibitors of *Mycobacterium bovis* (BCG). Iran J Pharm Sci 2018;17:1229.
14. Murata T, Shimada M, Sakakibara S, Yoshino T, Masuda T, Sato H, *et al*. Synthesis and structure-activity relationships of novel IKK-beta

inhibitors. Part 3: Orally active anti-inflammatory agents. Bioorg Med Chem Lett **2004**;14:4019-22.
15. Baldwin JJ, Engelhardt EL, Hirschmann R, Ponticello GS, Atkinson JG, Wasson BK, *et al*. Heterocyclic analogs of the antihypertensive. Beta-adrenergic blocking agent (S)-2-[3-(tert-butylamino)-2-hydroxypropoxy]-3-cyanopyridine. J Med Chem **1980**;23:65-70.
16. Temple C Jr., Rener GA, Waud WR, Noker PE. Antimitotic agents: Structure-activity studies with some pyridine derivatives. J Med Chem 1992;35:3686-90.
17. Rateb NM, Zohdi HF. Atom-efficient, solvent-free, green synthesis of chalcones by grinding. Synth Commun 2009;39:2789-94.
18. Solankee A, Patel G, Solankee S. Synthesis and studies of chalcones and its cyanopyridine and acetyl pyrazoline derivatives. Rasayan J Chem 2008;1:591-5.
19. Gouda MA, Berghot MA, El Ghani GE, Khalil AE. Chemistry of 2-amino-3-cyanopyridines. Synth Commun 2014;44:297-330.
20. Babu LS, Shaik AB, Prasad YR. Synthesis, antibacterial, antifungal antitubercular activities and molecular docking studies of nitrophenyl derivatives. Int J Life Sci Pharma Res 2019;9:54-64.
21. Graziani Y, Erikson E, Erikson RL. Differential inhibitory effects of various flavonoids on the activities of reverse transcriptase and cellular DNA and RNA polymerases. Eur J Biochem 1983;135:583-9.
22. Protein Data Bank. Available from: http://www.rcsb.org/pdb.
23. Biswas NN, Acharzo AK, Anamika S, Khushi S, Bokshi B. Screening of natural bioactive metabolites and investigation of antioxidant, antimicrobial, antihyperglycemic, neuropharmacological, and cytotoxicity potentials of *Litsea polyantha* Juss. Ethanolic root extract. Evid Based Complement Altern Med 2017;2017:3701349.