Derivatives of 2-Aminopyridines as Inhibitors of Multidrug Resistant *Staphylococcus Aureus* Strains

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Abstract

A new series of 2-aminopyridine derivatives were synthesised. N-acylation of 2-amino-3-chloro-5-(trifluoromethyl) pyridine and 2-amino-5-(trifluoromethyl) pyridine with series of acid chlorides afforded a total of fourteen (14) amide compounds. The structures of the new compounds have been established by their IR, NMR and mass spectra data. All the compounds were tested for their activity against four (4) multi-drug resistant (MDR) bacteria *Staphylococcus aureus* strains using microplate alamar blue assay. The MDR-*Staphylococcus aureus* strains employed for this study were Epidermic Methicilin Resistant *Staphylococcus aureus* (EMRSA-17), Methicilin Resistant *Staphylococcus aureus* (MRSA-252), Epidermic Methicilin Resistant *Staphylococcus aureus* (EMRSA-16) and Pakistani Drug resistant clinical isolate of *Staphylococcus aureus* (PRSA). Other bacteria strains also used include *Escherichia coli* (ATCC 2592), *Shigella flexenari* (ATCC 12022), *Staphylococcus aureus* (NCTC 6571) and *Pseudomonas aeruginosa* (NCTC 10662). The synthesised compounds exhibited very good activity against the four MDR-*Staphylococcus aureus* strains of which most of the compounds showed higher potencies for inhibiting the growth of the strains than vancomycin, the standard drug employed. The compounds reported here may serve as the starting point for the design and development of MDR-S.aureus inhibitors as antibacterial agents.

Keywords: N-acylation, Acid chlorides, 2, 4-difluoro-N-(5-(trifluoromethyl) pyridin-2-yl) benzamide, Staphylococcus aureus, Antibacterial

1. Introduction

Methicilin Resistant *Staphylococcus aureus* (MRSA) infections are a major public health problem producing a large number of deaths every year worldwide (NNIS, 2004). These important nosocomial and community-acquired pathogen has developed resistance to various antibiotics (Chen et al., 2010).

Pyridine nucleus plays central role in the development of different medical agents such as antimicrobial (Remuzon et al., 1992), antiviral (Tabarini et al., 2006), anticancer (Cai et al., 2003), antifungal (Patrick & Kinsman, 1996), antioxidant (Feng & SSNing, 2009), antidiabetic (Rajesh & Mukul, 2007), antitubercular and analgesic (Kasabe & Kasabe 2010). Aminopyridine and its derivatives are key intermediates for the synthesis of important pharmaceutical and agricultural products. Aminopyridine is used in the synthesis of antihistamines including methapyilene hydrochloride and pyrilamine maleate (Hettrer, 1946), synthesis of aldipem an anxiolytic (Dimsdale, 1988), preparation of the urinary tract analgesic phenazopyridine hydrochloride (Shreve, 1943), nevirapine a drug for treatment of AIDS (Merluzzi, 1990) and an antibacterial medicine, cephapirin sodium (Crast, et al., 1973).

MRSA being resistant to many antibiotics and the pyridine ring system reported as promising potential antibacterial agents, we became interested in the synthesis of 2-aminopyridine derivatives by *N*-acylation which is scarce in literature giving rise to amide analogues. 2-amino-3-chloro-5-(trifluoromethyl) pyridine and 2-amino-5-(trifluoromethyl) pyridine with selected acid chlorides in water reflux with acetonitrile and triethylamine (scheme 1 and scheme 2) gave the profile of the aminopyridine derivatives studied (Table 1).
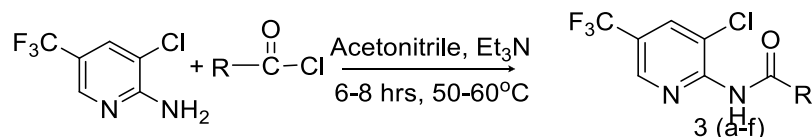
Scheme 1. Acylation of 2-amino-3-chloro-5-(trifluoromethyl) pyridine with acid chlorides
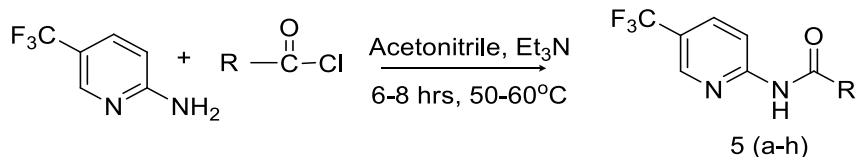
Scheme 2. Acylation of 2-amino-5-(trifluoromethyl) pyridine with acid chlorides

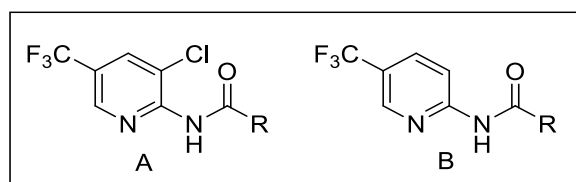## 2. Results and Discussion

The *N*-acylation of the selected aminopyridines with series of acid chlorides to give the afore mentioned amides analogues followed the SN_2 mechanism pathway (scheme 3) in which the nucleophile of the amino group attacks the carbonyl of the acid chloride to afford the expected amides using the polar aprotic solvent acetonitrile which prevents solvation and excess by-products, enhanced the yields of the amides.
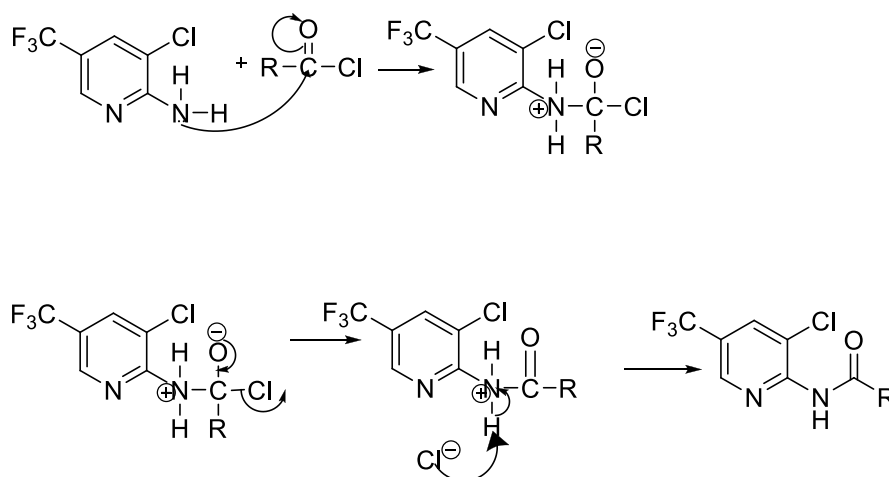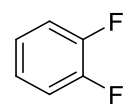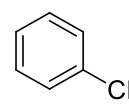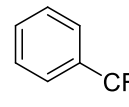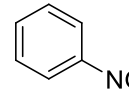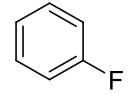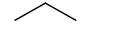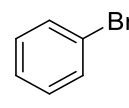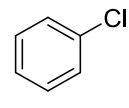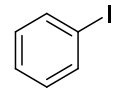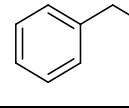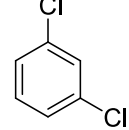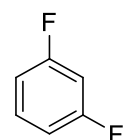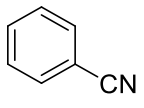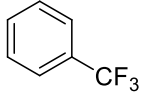
Scheme 3. General Mechanism of the synthetic reaction

Scheme 1 and scheme 2 reactions gave six (6) and eight (8) analogues which were coded as **3a-f** and **5a-h** respectively resulting in a total of fourteen (14) compounds. All the amides were prepared under mild conditions and the obtained products were easily purified by column chromatography on silica gel.

Table 1. Structural profile of the aminopyridine derivatives studied

COMPOUNDS	TYPE	R
3a	A	
3b	A	
3c	A	
3d	A	
3e	A	
3f	A	
5a	B	
5b	B	
5c	B	
5d	B	
5e	B	
5f	B	

| 5g | B | |
| 5h | B | |

Structure elucidation of representative member (compound 5a)

The identity of the final synthesised products was determined by ^{1}H-NMR and Infra Red (IR) spectroscopy and Mass Spectrometry (MS). Structure elucidation of a representative member (compound 5a) is presented below;
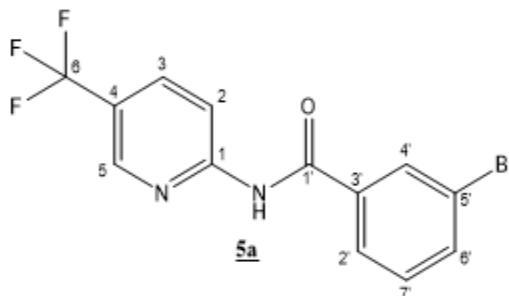
The ^{1}H-NMR was performed in a 400 MHz spectrometer and deuterated methanol MeOD was used as solvent. H5 was observed as a broad singlet and resonated at δ_H 8.45 (1H, d, $J_{2,5}$= 9.6 Hz). The signal at δ_H 8.15 was due to H4' being coupled to H6' (1H, d, $J_{4',6'}$ =2 Hz). H6' resonated as a doublet of doublet at δ_H 8.12 ($J_{6'4'}$ =2 Hz, $J_{6',7'}$=8 Hz). H2' resonated as a doublet at δ_H 7.97 being *ortho* coupled to H7' (1H, d, $J_{2'7'}$=7.6 Hz). H3 resonated at δ_H 7.79 as a doublet coupled to H5 (1H, d, $J_{3,5}$ =9.6 Hz) and H7' was found coupled with both 2' and 6' and appeared as a doublet of a doublet at δ_H 7.48($J_{7',2'}$= 7.6 Hz, $J_{7',6'}$= 8 Hz).; ^{13}C-NMR: δ 165.72 (C=O), 156.03 (C-1), 146.3(C-5), 137.07 (C-3'), 136.5 (C-6'), 135.97 (C-3), 131.7 (C-4'), 131.4 (C-7'), 127.7 (C-2', C-6) 122.9 (C-5'), 114.6 (C-2); EI-MS *m/z* (rel. abund. %): 346.8 [M$^+$+2] (2), 343.8 [M$^+$] (41), 314.8 (67), 235.9 (5), 184.7 (97), 182.8 (100), 154.8 (83), 103.9 (31), 82.8 (26), 75.9 (90), 57 (77); v_{max}: 3433.1, 3234.4, 31933.9, 3107.1, 2923.9, 2854.5, 1689.5, 1593.1, 1240.

The synthesised compounds were tested for their activity against four multi-Drug resistant (MDR) bacteria *S. aureus* strains and four other bacterial strains. The MDR- *S. aureus* strains employed for this study were Epidermic Methicilin Resistant *Staphylococcus aureus* (EMRSA)-17; methicilin resistant *Staphylococcus aureus* (MRSA)-252, Epidermic methicilin resistant *Staphylococcus aureus* (EMRSA)-16 and Pakistani drug resistant clinical isolate of *S. aureus* (PRSA) and the bacterial strains used were *E.coli* (ATCC 2592), *S. flexanari* (ATCC 12022), *S.aureus* (NCTC 6571) and *P. aeruginosa* (NCTC 10662) Table 2.

Table 2. Bioactivity of the compounds

BACTERIAL STRAINS	SYNTHESISED COMPOUNDS														CONTROL
	3a	3b	3c	3d	3e	3f	5a	5b	5c	5d	5e	5f	5g	5h	Vancomycin (20 µg /ml)
EMRSA-17	35.9	36.6	26.8	28.4	38.9	39.4	24.5	13.0	12.1	-	36.1	41.4	27.4	43.1	20.7
MRSA-252	16.7	9.6	8.9	-	20.2	33.6	-	-	-	-	24.7	18.3	18.0	15.0	18.6
EMRSA-16	28.4	29.0	21.4	11.6	31.7	-	-	-	-	-	32.5	31.1	26.5	27.5	23.7
PRSA	28.0	29.5	22.7	28.0	38.0	28.2	21.8	21.8	-	-	29.6	37.4	20.1	33.4	40.4
															Oflaxacin (50 µg /ml)
E.coli	-	5.7	5.2	-	-	3.8	1.3	1.9	-	4.3	1.8	3.6	6.5	4.5	87.7
S. flexenari	-	15.6	3.7	-	-	8.3	-	3.0	-	0.8	14.9	15.3	10.0	5.6	89.9
S. aureus	-	4.9	2.2	-	-	1.5	-	-	-	-	1.6	-	-	-	91.4
P. aureginosa	12.1	-	-	-	15.0	-	-	-	-	1.5	-	-	-	-	84.4

The antibacterial (EMRSA-17) inhibitory activity invitro Fig.1 afforded a two fold increase in the activity of the compounds against EMRSA-17 was observed with respect to vancomycin (20.7 μg /mL) the standard, when a fluorine compound was at the *meta* position C5' as in **5f** (41.4 μg /mL), and **3e** (38.9 μg /mL).
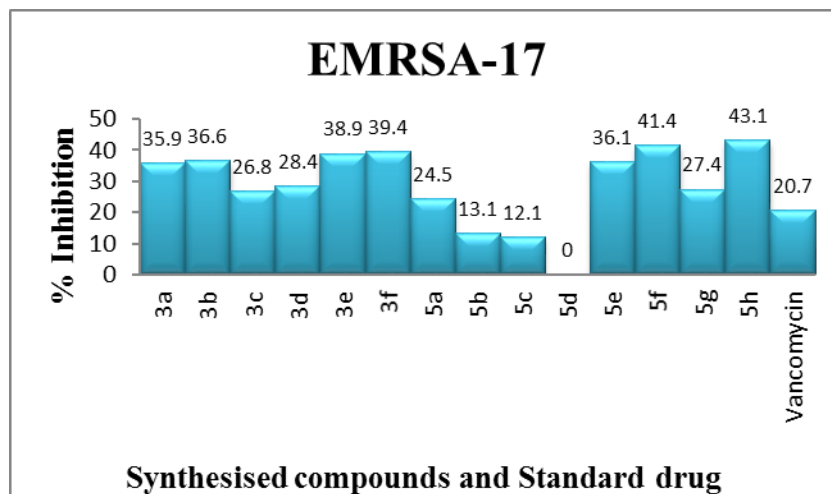

Figure 1. Inhibitory Activity against EMRSA-17

A slight decrease in activity was observed when in addition to the fluorine at the *meta* position another fluorine was at the *ortho* position C6' as observed in **3a** (35.9 μg /mL) An electron withdrawing substituent cyano (CN) at the *para* position C6' showed more increased activity when position C2 had chlorine as compared with **3b** (36.6 μg /mL) than when position C2 had no substituent as seen in **5g** (27.4 μg /mL). The absence of the benzylic ring system substituted with the presence of an electron donating alkyl chain was observed to increase the activity as seen in **3f** (39.4 μg /mL)., when the end of the alkyl chain was replaced with benzene; an electron withdrawing group as against the methyl that was an electron donating group, a total loss of activity was observed as in **5d.**

The synthesised compounds were tested for their antibacterial (EMRSA-16) activity Fig.2. Significant level of activity was observed for the compounds with respect to the standard vancomycin (23.7 μg/ mL) when the substituent at the *meta* position C5' was a halogen substituent fluorine as observed in **3a** (28.4 μg /mL), **3e** (31.7 μg /mL) and **5f** (31.1 μg /mL), though a total loss of activity was observed when the *meta* position C5' contained another halogen bromine and chlorine as in **5a** and **5b** respectively.
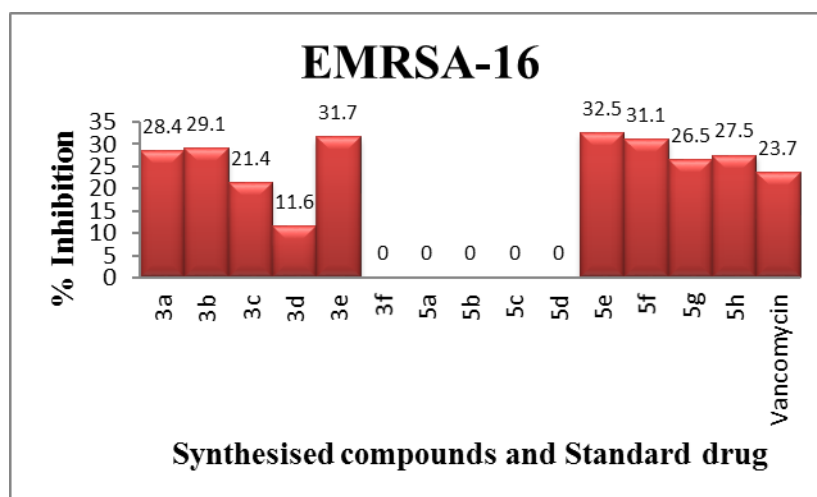
Figure 2. Inhibitory Activity against EMRSA-16

Moreso, with trifluoromethyl at position C6', significant activity was recorded similar to **3c** (21.4 μg/ mL) and **5h** (27.5 μg/ mL). This could have been due to the electron withdrawing ability of the halogen, fluorine present. When position C6' was substituted with yet another electron withdrawing group cyano (CN), same trend of significant activity was observed as in **3b** (29.1 μg /mL) and **5g** (26.5 μg /mL). The highest activity against EMRSA-16 was recorded for, when chlorine was the substituent at the *para* position C6' as reported in **5e** (32.5 μg /mL), the second chlorine atom at the *ortho* position C2' might have contributed to its activity because a halogen, iodine at the C6' position resulted to a total

loss in activity as in **5c**. When there was no substituent at both *meta* and *para* positions C5' and C6', a total loss of activity was observed compared to **3f** and **5d**.

Antibacterial (MRSA-252) inhibitory activity was performed Fig. 3. The highest activity against MRSA-252 was recorded when an alkyl chain was attached next to the carbonyl similar to **3f** (33.6 μg mL) with respect to the standard, vancomycin (18.6 μg /mL).

Figure 3. Inhibitory Activity against MRSA-252

The free rotation of the single bonds and the electron donating properties of the methyl and methine could have contributed. The next significant activity observed against MRSA-252 was when the *para* position C6' had chlorine a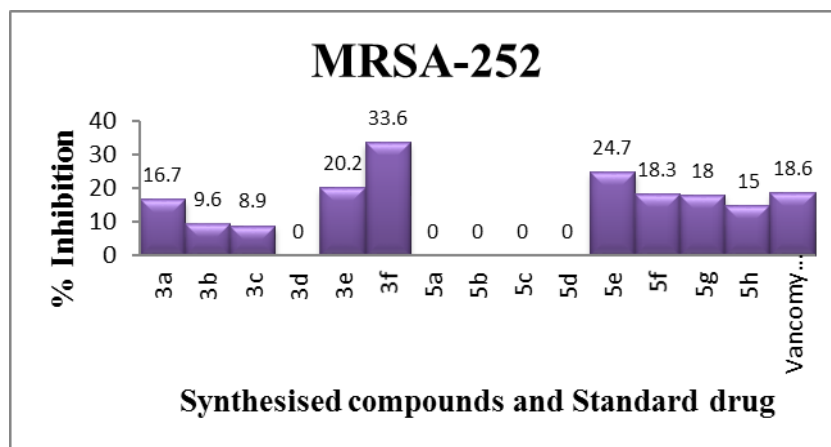nd *meta* position C5' had fluorine substituents as in **5e** (24.7 μg /mL), **3e** (20.2 μg /mL), **5f** (18.3 μg /mL) and **3a** (16.7 μg /mL) respectively. Again, a total loss of activity was also observed for MRSA-252 strain when there was no substituent at the *para* position C6' as seen in **5a**, **5b**, **5c** and **5d** with exception of **5f** which had fluorine at the *meta* position. A new trend of total loss of activity was observed for this strain of MDR *S.aureus* when position C6' substituent was a nitro group (NO_2) as in **3d**. This could be due to the electron donating effect of the lone pairs of electrons present in both nitrogen and oxygen; probably the mesomeric effect of both nitrogen and oxygen superseded its individual inductive effect for this particular strain. There was moderate activity observed when cyano and trifluoromethyl were at the *para* position C6' (18 μg /mL) and **5h** (15 μg /mL), a decrease in activity by half in addition to the *para* position still maintaining the cyano and trifluoromethyl was observed when chlorine was at position C2 similar to **3b** (9.6 μg /mL) and **3c** (8.9 μg /mL) respectively.

The antibacterial activity against PRSA Fig. 4 was observed to follow trend as the previous MDR-*S.aureus* strain afore mentioned.

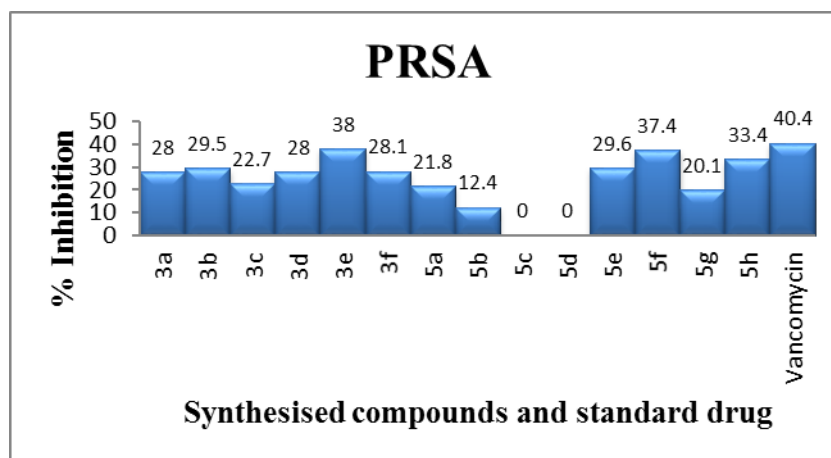Figure 4. Inhibitory Activity against PRSA

The most significant activity with respect to the standard, vancomycin (40.4 μg /mL) was noticed when the *meta* position C5' had fluorine similar to **3e** (38.0 μg /mL) and **5f** (37.4 μg /mL) followed by when the substituent at the *para* position C6' was trifluoromethyl and chlorine as in **5h** (33.4 μg/ mL) and **5e** (29.6 μg /mL) respectively.

3. Conclusion

It could be inferred that the electron withdrawing group at the *meta* position C5' with reference to fluorine especially and *para* position C6' with reference to the cyano and trifluoromethyl have a significant role and could be the key factor for the activities observed for the synthesised compounds against these MDR-*S.aureus* strains.

4. Experimental

4.1 General

Chemicals were supplied by Aldrich and Sigma. NMR spectra were recorded on Avance Bruker 300, 400, 500, 600 and cryprobes FT-NMR spectrometers. Chemical shifts are reported in parts per million and were recorded in deuterated methanol, Acetone and dichloromethane accordingly with tetramethylsilane as the internal standard. NMR multiplicities are indicated by the symbols: s (singlet), d (doublet), t (triplet), q (quartet), and m (multiplet). IR spectra were recorded on a shimadzu 8900 FT-IR spectrometer using KBr discs and pellets. Mass fragmentations were recorded using MAT 312 mass spectrometer.

4.2 N-Acylation

To Acetonitrile (10 ml), the respective aminopyridines (1mmol) and triethylamine (0.101 ml, 1mmol) were added. The mixture was placed on a hot plate magnetic stirrer and stirred for 20 min under water reflux before the corresponding acid chlorides (1mmol) were added. The mixture was inspected by TLC (Hexane-EtoAc). After being stirred for a further 4-6 hrs at 40-60 ^{o}C, it was concentrated to dryness. The residues were purified by silica gel gradient column chromatography (Hexane-EtoAc, 85:15) to afford the desired compounds (overall yield 65-85%). Their identity was confirmed by NMR and IR spectroscopy and MS spectrometry.

N-(3-chloro-5-(trifluoromethyl) pyridin-2-yl)-3, 4-difluorobenzamide (3a). Yield: (73%); m.p. 78-79 ^{0}C; IR: 3433 (N-H), 1670 (C=O); ^{1}H-NMR (MeOD): δ 8.72 (1H, brS, H5), 8.35 (1H, d, J=1.2Hz, H3), 7.95 (1H, d, J= 2Hz, H4'), 7.87 (1H, dd, J= 2, 8.8 Hz H2'), 7.45 (1H, d, J= 8.8Hz H7'); ^{13}C-NMR: δ 166.32 (C=O), 156.23 (C-1), 152.9 (C-6'), 152.7 (C-5'), 144.9 (C-5), 137.6 (C-3), 132.09 (C-3'), 126.6 (C-2'), 127.04 (C-2), 126.02 (C-4) 122.4 (C-6), 118.97 (C-4'), 118.74 (C-7'); MS: (m/z) 336 ([M+H]$^+$), $C_{13}H_6ClF_5N_2O$ (336.01)

N-(3-chloro-5-(trifluoromethyl) pyridin-2-yl)-4-cyanobenzamide (3b). Yield: (67%); m.p. 141-142 ^{0}C; IR: cm^{-1} 3440.8 (N-H), 1676 (C=O). ^{1}H-NMR (MeOD): δ 8.73 (1H, brS, H5), 8.37 (1H, brS, H3), 8.13 (2H, d, J= 8Hz, H5', H7') 7.93 (2H, d, J= 8Hz, H2', H4'); MS: (m/z) 325 ([M+H]$^+$), $C_{14}H_7ClF_3N_3O$ (325.02)

N-(3-chloro-5-(trifluoromethyl) pyridin-2-yl) 4(trifluoromethyl) benzamide (3c). Yield: (71%); m.p. 80-81 ^{0}C; IR: cm^{-1} 3433.1 (N-H), 1679.9 (C=O). ^{1}H-NMR (MeOD): δ 8.73 (1H, brS, H5), 8.37 (1H, d, J= 1.5Hz, H3), 8.17 (2H, d, J= 8.4 Hz, H2', H4'), 7.87 (2H, d, J= 8.4Hz, H5', H7'); ^{13}C-NMR: δ 165.26 (C=O), 153 (C-1), 144.66 (C-5), 138.17 (C-3'), 137 (C-3), 130.56 (C-6'), 129.84 (C-5', C-7'), 127.71 (C-2), 126.47 (C-2', C-4'); MS: (m/z) 368 ([M+H]$^+$), $C_{14}H_7ClF_6N_2O$ (368.02).

N-(3-chloro-5-(trifluoromethyl) pyridin-2-yl) 4-nitrobenzamide (3d). Yield: (67%); m.p. 108-109 ^{0}C; IR: cm^{-1} 3435 (N-H), 1685.7 (C=O). ^{1}H-NMR (MeOD): δ 8.75 (1H, brS, H5), 8.42 (2H, d, J= 8.4 Hz, H2', H4'), 8.39 (1H, d, J= 1.8Hz, H3), 8.21 (2H, d, J= 8.4 Hz, H5', H7'). MS: (m/z) 345 ([M+H] $^+$), $C_{13}H_7ClF_3N_3O_3$ (345.01).

N-(3-chloro-5-(trifluoromethyl) pyridin-2-yl) 3-fluorobenzamide (3e). Yield: (69%); m.p. 65-66 ^{0}C; IR: cm^{-1} 3438.8 (N-H), 1678 (C=O). ^{1}H-NMR (MeOD): δ 8.73 (1H, brS, H5), 8.35 (1H, brS, H3), 7.83 (1H, dd, J= 1.2Hz, 7.6Hz, H2'), 7.73 (1H, dd, J= 2 Hz, 9.6Hz, H6'), 7.59 (1H, dd, J= 7.6Hz, 9.6Hz, H7'), 7.41 (1H, dd, J=1.2Hz, 2Hz H4'); ^{13}C-NMR: δ 165.65 (C-F, C-5'), 162.5 (C=O), 153.0 (C-1), 144.9 (C-5), 137.58 (C-3), 137.0 (C-3'), 131.90 (C-7'), 126.5 (C-2), 126.03 (C-6), 125.08 (C-2'), 122.4 (C-4), 120.7 (C-4'), 115.9 (C-6'); MS: (m/z) 318 ([M+H]$^+$), $C_{13}H_7ClF_4N_2O$ (318.02).

N-(3-chloro-5-(trifluoromethyl) pyridin-2-yl) butyramide (3f). Yield: (74%); m.p. 128-129 ^{0}C; IR: cm^{-1} 3433.1 (N-H), 1689.5 (C=O); ^{1}H-NMR (MeOD): δ 8.64 (1H, brS, H5), 8.27 (1H, d, J=1.5, H3), 2.53 (2H, t, J= 7.4, H2'), 1.81 (2H, m, H3'), 1.05 (3H, t, J=7.4, H4'); ^{13}C-NMR: δ 172.01 (C=O), 152.77 (C-1), 144.38 (C-5), 136.55 (C-3), 124.99 (C-2'), 123.09 (C-6), 38.0 (C-2'), 19.18 (C-3'), 13.95 (C-4'); MS: (m/z) 266 ([M+H]$^+$), $C_{10}H_{10}ClF_3N_2O$ (266.04).

3-bromo-N-(5-(trifluoromethyl) pyridin-2-yl) benzamide (5a). Yield: (80%); m.p. 82-83 ^{0}C; IR: cm^{-1} 3433.1 (N-H), 1689.5 (C=O). ^{1}H-NMR (MeOD): δ8.66 (1H, brS, H5), 8.45 (1H, d, J= 8.8Hz, H2), 8.15 (1H, dd, J= 1.6, 2Hz, H4'), 8.12 (1H, dd, J=2, 8.8 Hz, H6'), 7.97 (1H, dd, J= 1.6, 7.6Hz, H2'), 7.79 (1H, d, J= 8.8 Hz, H3), 7.48 (1H, dd, J= 7.6, 8.8 Hz, H7'); ^{13}C-NMR: δ 165.72 (C=O), 156.03 (C-1), 146.3(C-5), 137.07 (C-3'), 136.5 (C-6'), 135.97 (C-3), 131.7 (C-4'), 131.4 (C-7'), 127.7 (C-2', C-6) 122.9 (C-5'), 114.6 (C-2); MS:(m/z) 344 ([M+H]$^+$), $C_{13}H_8BrF_3N_2O$ (343.98).

3-Chloro-N-(5-(trifluoromethyl) pyridin-2-yl) benzamide (5b). Yield: (65%); m.p. 132-133 ^{0}C; IR : cm^{-1} 3434.1 (N-H), 1691.59 (C=O); ^{1}H-NMR (MeOD): δ 8.00 (1H, dd, J=1.6, 2Hz, H4'), 7.93 (1H, dd, J= 1.2, 7.8Hz, H3), 7.64 (1H,

dd, J=1.6, 8.4 Hz, H6'),7.55 (1H, t, J=8.4, 8.8 Hz, H7'), 8.66 (1H, brS, H5), 8.45 (1H, d, J= 7.8, H2), 8.12 (1H, dd, J=2, 8.8Hz, H2'); ^{13}C-NMR: δ 165.82 (C=O), 156.0 (C-1), 146.3 (C-5), 136.8 (C-5'), 136.5 (C-3), 134.9 (C-3'), 133.02 (C-7'), 131.2 (C-6'), 128.8 (C-4'), 127.3 (C-2'), 114.5 (C-2); MS: (m/z) 299 ([M+H]$^+$), $C_{13}H_8ClF_3N_2O$ (300.03)

4-iodo-N-(5-(trifluoromethyl) pyridin-2-yl) benzamide (5c). Yield: (60%); m.p. 85-86 ^{0}C; IR:cm^{-1} 3400(N-H), 1656.7(C=O); ^{1}H-NMR (MeOD): δ 8.65 (1H, brS, H5), 8.45 (1H, d, J=8.8Hz, H2), 8.12 (1H, dd, J=2, 8.8Hz, H3), 7.93 (2H, dd, J= 1.6, 8.4Hz, H5', H7'), 7.74 (2H, dd, J=1.6, 8.4Hz, H2', H4'); MS: (m/z) 392 ([M+H]$^+$), $C_{13}H_8F_3IN_2O$ (391.96)

3-phenyl-N-(5-(trifluoromethyl) pyridin-2-yl)propanamide (5d). Yield: (62%); m.p. 130-131 ^{0}C; IR: cm^{-1} 3433 (N-H), 1664.5 (N-H); ^{1}H-NMR (MeOD): δ 8.55 (1H, brS, H5), 8.31 (1H, d, J=8.8Hz, H2), 8.03 (1H, dd, J=2, 8.8Hz, H3), 7.23 (4H, q, H5',H6',H8'H9'), 7.18 (1H,m, H7), 3.02 (2H, t, J=7.6 Hz, H2'), 2.78 (2H, t, J=7.6 Hz, H3'); MS:(m/z) : 294 ([M+H]$^+$), $C_{15}H_{13}F_3N_2O$ (294.10)

2, 4-dichloro-N-(5-(trifluoromethyl) pyridin-2-yl) benzamide (5e). Yield: (85%); m.p. 94-95 ^{0}C; IR: cm^{-1} 3429.2 (N-H), 1722.3(C=O); ^{1}H-NMR (MeOD): δ 8.72 (1H, brS, H5), 8.24 (1H, dd, J=2.4, 8.4Hz, H3), 7.82 (1H, dd, J=2, 8.4 Hz, H5') 7.64 (2H, d, J=8.4, H2, H4'), 7.49 (1H, d, J=2.4, H7'); ^{13}C-NMR: δ 168.67 (C=O), 155.49 (C-1), 147.07 (C-5), 137.79 (C-6'), 137.04 (C-4'), 134.42 (C-3), 133.3 (C-3'), 131.5 (C-2'), 130.75 (C-5'), 128.3 (C-7'), 124.4 (C-2); MS: (m/z) 334 ([M+H]$^+$), $C_{15}H_{13}F_3N_2O$ (333.99)

3, 5-difluoro-N-(5-(trifluoromethyl) pyridin-2-yl) benzamide (5f). Yield: (76%); m.p. 126-127 ^{0}C; IR: cm^{-1} 3344.3 (N-H), 1670 (C=O); ^{1}H-NMR (MeOD): δ 8.66 (1H, brs, H5), 8.44 (1H, d, J= 8.8Hz, H2), 8.13 (1H, dd, J=2, 8.8Hz, H3), 7.63 (2H, d, J= 2.4Hz, H2', H4'), 7.26 (1H, d, J= 2.4Hz H6'); ^{13}C-NMR: δ 164.62 (C-5', C-7'), 162.98 (C=O), 155.79 (C-1), 146.30 (C-5), 138.43 (C-5'), 136.61 (C-3), 122.72 (C-6), 112.24 (C-4', C-2'), 108.4 (C-6'); MS: (m/z) 302 ([M+H]$^+$), $C_{13}H_7F_5N_2O$ (302.05).

4-cyano-N-(5-(trifluoromethyl) pyridin-2-yl) benzamide (5g). Yield: (67%); m.p. 98-99 ^{0}C; IR: cm^{-1} 3367.5 (N-H), 1678.0 (C=O); ^{1}H-NMR (MeOD): δ 8.67 (1H, brs, H5), 8.47 (1H, d, J=8.8Hz, H2), 8.13 (3H, d, J=8.8Hz, H3, H5', H7'), 7.91 (2H, d, J= 8.8Hz, H2', H4'); ^{13}C-NMR: δ 165.91 (C=O), 155.8 (C-1), 146.3 (C-5), 138.8 (C-3'), 136.6 (C-3), 133.27 (C-5', C-7'), 129.69 (C-2', C-4'), 118.64 (C-8'), 116.32 (C-6'), 114.64 (C-2); MS: (m/z) 291 ([M+H]$^+$), $C_{14}H_3F_5N_3O$ (291.06).

4-(trifluoromethyl)-N-(5-(trifluoromethyl) pyridin-2-yl) benzamide (5h). Yield: (65%); m.p. 118-119 ^{0}C; IR: cm^{-1} 3365.3 (N-H), 1708.8 (C=O); ^{1}H-NMR (MeOD): δ 8.67 (1H, brs, H5), 8.48 (1H, d, J=8.8Hz, H2), 8.16 (2H, d, J= 8Hz, H5', H7'), 7.98 (1H, d, J=8.8Hz, H3), 7.86 (2H, d, J=8Hz, H2', H4'); MS: (m/z) 334 ([M+H]$^+$), $C_{14}H_8F_6N_2O$ (334.05).

4.3 Micro-Plate Alamar Blue Assay (MABA)

Antibacterial susceptibility testing was performed in a 96-well micro plate. Organisms were grown in Mueller-Hinton medium. Inoculums were adjusted to 0.5 McFarland turbidity index. The stock solutions of the compounds were prepared in DMSO in a 1:1 concentration, the stock solutions were serially diluted in Muller-Hinton broth to obtain the concentration of 20μg/ ml and the volume of the 96 well plates were made up to 200μl. 5 × 10^6 bacterial cells were added in each of the 96-well plate. The plate was then sealed and incubated at 37°C for 18-20 hrs, Alamar blue dye (10%) was dispensed in each well and shaken at 80 RPM in a shaking incubator at 37°C for 3-4 hrs. Oflaxacin was used as the positive control for the bacteria isolates and Vancomycin for the MDR- *S. aureus* (DMSO showed no cidal effect on the growth of the bacterial cells). All experiment was done in triplicate. Change in colour of Alamar dye from blue to pink indicated the growth of bacteria strains. Absorbance was recorded at 570 and 600 nm by the ELISA reader (SpectraMax M2, Molecular Devices, CA, USA).

Percentage difference in reduction of Alamar Blue between treated and control cells were calculated using the formular:

$$\% = \frac{(\varepsilon_{ox})\lambda_2 \, A\lambda_1 - (\varepsilon_{ox})\lambda_1 \, A\lambda_2}{(\varepsilon_{ox})\lambda_2 \, A'\lambda_1 - (\varepsilon_{ox})\lambda_1 \, A'\lambda_2} \times 100$$

Where $(\varepsilon_{ox})\lambda_2$ = molar extinction coefficient of Alamar blue oxidized form at 600 nm

$(\varepsilon_{ox})\lambda_1$= molar extinction coefficient of Alamar blue oxidized form at 570 nm

$A\lambda_1$ = Absorbance of test wells at 570 nm

$A\lambda_2$ = Absorbance of test wells at 600 nm

$A'\lambda_1$= Absorbance of control well at 570 nm

$A'\lambda_2$= Absorbance of control well at 600 nm

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