Indian Journal of Chemistry
Vol. 62, February 2023, pp. 99-106
DOI: 10.56042/ijc.v62i2.71260

Microwave-assisted solution phase synthesis of novel pyridine carboxamides in neat water and ADMET and protein-compounds interaction analysis and antibacterial activity

Visagamoorthy Babu[a,]*, Prabu Dharman[a] & K Anver Basha[b,]*

[a]Research & Development Centre, Bharathiar University, Coimbatore-641 046, Tamil Nadu, India

[b]P G and Research Department of Chemistry, C. Abdul Hakeem College, Melvisharam 632 509, Tamil Nadu, India

*E-mail: vishag0717@gmail.com (VB), kanverbasha@rediffmail.com (KAB)

Received 30 December 2021; accepted (revised) 10 January 2023

A range of novel 5-substituted-pyridine-2-carboxamides have been designed and synthesized by microwave-assisted solution phase synthesis in neat water. Their structures have been confirmed by ^{1}H NMR, ^{13}C NMR and LCMS analysis. The present work describes a simple, rapid, and efficient synthesis of compound **2a**-**2h** in good yield under mild reaction condition. Shorter reaction time and convenient workup make this methodology more practical. This protocol proceeds smoothly and avoids the use of toxic organic solvents. ADMET analysis have been performed for all the synthesized compounds and observed that all the eight compounds taken for the investigation have been shown to satisfy Lipinski's rule of 5. The molecular interaction study is performed using the AutoDock software. All the 8 test compounds are found to have higher binding affinity than the control compound chloramphenicol to the *E. Coli* DraE adhesion. In addition antibacterial activities of all eight compounds have been determined.

Keyword: Pyridine, Carboxamides, Antibacterial activity, ADMET

Practical applications of pyridine derivatives in medicinal and pesticidal chemistry have been achieved during past two decades. Heterocyclic compounds (containing Nitrogen, sulphur and oxygen atom) occupied enormous significance in the drug discovery process field (Valverde and Torroba, 2005)[1]. The pyridine ring is very important in the structure of many biologically active compounds, also pyridines containing heterocyclic compounds are predominant in many bioactive molecules[2,3]. Imidazopyridine derivatives are useful as therapeutic or preventive agent for acid related diseases[4], anticoccidial[5,6] and antiprotozoal[7] agents etc., Further, both solid and solution phase amide syntheses produces a large volume of unwanted toxic waste, large amount of toxic organic solvents like N,N-dimethylformamide, 1-methyl-2-pyrrolidone and dichloromethane etc., Hence, searching of an alternate environmentally friendly methods of amide synthesis are critical. Over the past few decades, employing water as a environmentally benign solvent increased considerable interest in the field[8]. Employing water as a reaction solvent offers lots of advantages because it is non-toxic, non-flammable, and eliminates or reduces the organic solvent waste in chemical industry.[9] In addition, the microwave offers rapid and greener alternative process compared to other conventional heating[10]. Considering green chemistry, we have developed a suitable eco-friendly process in neat water and this protocol proceeds smoothly under very mild reaction conditions in short reaction times.

By realizing these observations, we anticipated that the introduction of novel pyridyl moiety and the amide group to pyridine molecules might generate a new group of biologically active compounds. We have already synthesised and reported few novel 5-substituted-pyridine-2-carboxamides. Also, the synthesized compounds were evaluated for their in vitro antimicrobial activity. 2D and 3D structural features of the synthesized derivatives were recognized by the 3D-QSAR[11]. In continuation, we report the synthesis of few novel carboxamide derivatives containing a pyridyl moiety (**2a**-**2h**). To the best of our knowledge, compound **2a**-**2h** is unreported.

Experimental Details

All the reagents and solvents used were of reagent grade and used without purification. Microwave

heating was performed employing CEM Discover® microwave reactor. Reaction completion was monitored by TLC on silica gel coated aluminum sheets (using Type 60 GF254). ^{1}H NMR spectra of all the compounds were recorded on a Bruker Avance 400 spectrometer at 400 MHz for ^{1}H NMR and ^{13}C NMR in DMSO (dimethyl sulfoxide) with tetramethylsilane as an internal standard. Chemical shift values (d) were provided in ppm. The mass spectra recorded on the Agilent 6140 quadrupole LC/MS using 1200 series spectrometer. All the reagents employed were analytical grade reagent and chemically pure. All the NMR spectra of the synthesized amide compounds are provided in the Supplementary Information (Fig. S1-S8).

General procedure for the synthesis of amide using TBTU (2a-2h)

To a solution of 5-(4-chloro-2-fluoro-3-methoxyphenyl)pyridine-2-carboxylic acid (4.0 mmol), aromatic amine (R-NH_2) (4.4 mmol) and DMF (10.0 mL) were added TBTU (6.0 mmol) and DIPEA (12.0 mmol). The reaction mixture was stirred at 25-30°C for 12 - 16 h under the nitrogen atmosphere. The resulting reaction mss were diluted with water (50.0 mL) and then extracted with dichloromethane (2 × 30.0 mL). The resulting organic layer was washed with brine solution (5.0 mL) and further dried with anhydrous sodium sulfate. The solvent were removed under reduced pressure to get the target compounds **2a**-**2h**.

General procedure for the synthesis of amide using HATU (2a-2h)

To a solution of 5-(4-chloro-2-fluoro-3-methoxyphenyl)pyridine-2-carboxylic acid (4.0 mmol), aromatic amine (R-NH2) (4.4 mmol) and DMF (10.0 mL) were added HATU (6.0 mmol) and DIPEA (12.0 mmol). The reaction mixture was stirred at 25-30°C for 12 - 16 h under the nitrogen atmosphere. The resulting reaction mss were diluted with water (50.0 mL) and then extracted with dichloromethane (2 × 30.0 mL). The resulting organic layer was washed with brine solution (5.0 mL) and further dried with anhydrous sodium sulfate. The solvent were removed under reduced pressure to get the target compounds **2a**-**2h**.

General procedure for the preparation of amide using TBTU under MW irradiation (2a-2h)

In a 10 mL microwave vial set with a magnetic stir bar, 5-(4-chloro-2-fluoro-3-methoxyphenyl)pyridine-2-carboxylic acid (1.0 mmol), aromatic amine (R-NH_2) (1.1 mmol), DIPEA (3.0 mmol), water (2.5 mL) and TBTU (1.5 mmol) were added. The resulting reaction mixture were subjected to MW irradiation (employing CEM Discover® microwave reactor) with gas cooling (pressure of 40 psi were maintained during the irradiation) for 30 min at 40 W along with magnetic stirring with temperature limit of 60°C (reaction time refers to hold time at the preferred set temperature). Water was evaporated, the reaction mass were purified with 1:2 ethyl acetate : hexanes and isolated by filtration to obtain wet solids. The wet solids were dried under vacuum at below 40°C for 3 h to get the target compounds **2a**-**2h**.

5-(4-chloro-2-fluoro-3-methoxyphenyl)-N-(1-(4-nitrophenyl)ethyl)picolinamide (2a): Pale brown solid, yield 81%, ^{1}H NMR (400 MHz, DMSO-d6): d 1.59 (d, J = 6.80 Hz, 3H, CH3), 3.96 (s, 3H, CH3), 5.31 (m, J = 6.80 Hz, 1H, CH), 7.43 (t, J = 8.00 Hz, 1H, Ar-H), 7.54 (q, J = 8.40 Hz, 1H, Ar-H), 7.72 (d, J = 8.40 Hz, 2H, Ar-H), 8.11 (d, J = 8.00 Hz, 1H, Py-H), 8.21 (d, J = 8.80 Hz, 3H, Py-H & Ar-H), 8.87 (s, 1H, Py-H), 9.44 (d, J = 8.00 Hz, 1H, NH). ^{13}C NMR (400 MHz, DMSO-*d*$_6$): 25.83, 43.65, 62.14, 123.26, 123.30, 126.28, 126.42, 128.82, 128.97, 129.14, 129.17, 129.20, 135.67, 135.74, 142.18, 142.24, 148.45, 152.38**,** 152.43, 154.90, 164.77. Exact mass calcd. (M+H) for $C_{21}H_{17}ClFN_3O_4$: 430.09; found: 430.1.

5-(4-chloro-2-fluoro-3-methoxyphenyl)-N-(5-chloro-2-methylphenyl)picolinamide (2b): Pale brown solid, yield 87%. ^{1}H NMR (400 MHz, DMSO-d6): d 2.33 (s, 3H, CH3), 3.97 (s, 3H, CH3), 7.22 (d, J = 7.60 Hz, 1H, Ar-H), 7.34 (d, J = 8.00 Hz, 1H, Ar-H), 7.50 (m, J = 8.00 Hz, 2H, Ar-H), 7.98 (s, 1H, Ar-H), 8.29 (s, 2H, Py-H), 8.95 (s, 1H, Py-H). ^{13}C NMR (400 MHz, DMSO-*d*$_6$): 18.18, 62.14, 119.90, 123.44, 126.04, 126.33, 128.82, 128.96, 129.04, 129.07, 134.42, 135.64, 135.76, 136.84, 136.98, 148.11, 152.26, 152.33, 154.84, 164.74. Exact mass calcd. (M+H) for $C_{20}H_{15}Cl_2FN_2O_2$: 406.05; found: 407.1.

5-(4-chloro-2-fluoro-3-methoxyphenyl)-N-(1-phenylethyl)picolinamide (2c): Brown solid, yield 89%. ^{1}H NMR (400 MHz, DMSO-d6): d 1.55 (d, J = 6.80 Hz, 3H, CH_3), 3.96 (s, 3H, CH3), 5.20 (t, J = 7.60 Hz, 1H, CH), 7.24 (t, J = 7.60 Hz, 1H, Ar-H), 7.34 (t, J = 8.00 Hz, 2H, Ar-H), 7.45 (t, J = 5.20 Hz, 3H, Ar-H), 7.51 (d, J = 8.80 Hz, 1H, Ar-H), 8.12 (d, J = 8.00 Hz, 1H, Py-H), 8.20 (d, J = 8.00 Hz, 1H, Py-

H), 8.85 (s, 1H, Py-H), 9.15 (d, J = 8.80 Hz, 1H, NH). ^{13}C NMR (400 MHz, DMSO-*d_6)*: 44.15, 25.73, 62.12, 122.82, 122.86, 124.33, 125.49, 125.56, 125.63, 128.49, 128.54, 128.63, 128.67, 135.34, 136.44, 138.53, 146.32, 151.96, 151.96, 154.42, 164.43. Exact mass calcd. (M+H) for $C_{21}H_{18}ClFN_2O_2$: 385.11; found: 385.1

5-(4-chloro-2-fluoro-3-methoxyphenyl)-N-(isoxazol-3-yl)picolinamide (2d): Pale brown solid, yield 82%. ^{1}H NMR (400 MHz, DMSO-d6): d 3.97 (s, 3H, CH_3), 6.19 (s, J = 7.60 Hz, 1H, CH), 7.07 (s, J = 7.60 Hz, 1H, CH), 7.57 (d, J = 60.4 Hz, 2H, Ar-H), 8.28 (s, J = 8.00 Hz, 1H, Py-H), 8.54 (s, J = 8.00 Hz, 1H, Py-H), 8.94 (d, J = 0 Hz, 1H, Py-H), 11.30 (s, J = 8.80 Hz, 1H, NH). ^{13}C NMR (400 MHz, DMSO-*d_6)*: 62.14, 123.40, 123.44, 126.04, 126.33, 128.82, 128.96, 129.04, 129.07, 134.42, 135.64, 135.76, 136.84, 136.98, 148.11, 152.26, 152.33, 154.84, 158.4, 164.74. 166.2. Exact mass calcd. (M+H) for $C_{16}H_{11}ClFN_3O_3$: 348.05; found: 348.1.

5-(4-chloro-2-fluoro-3-methoxyphenyl)-N-(2-isopropylphenyl)picolinamide (2e): Brown solid, yield 86 %. ^{1}H NMR (400 MHz, DMSO-d6): d 1.22 (d, J = 8.80 Hz, 6H, CH_3), 3.19 (t, J = 9.20 Hz, 1H, CH), 3.97 (s, 3H, CH3), 7.26 (d, J = 5.20 Hz, 2H, Ar-H), 7. 46 (m, J = 4.40 Hz, 3H, Ar-H), 7.66 (d, J = 5.60 Hz, 1H, Ar-H), 8.27 (s, 2H, Py-H), 8.93 (s, 1H, Py-H), 10.39 (s, 1H, NH). ^{13}C NMR (400 MHz, DMSO-*d_6)*: 25.6, 25.6, 43.6, 62.14, 123.26, 123.30, 126.28, 126.42, 128.82, 128.97, 129.14, 129.17, 129.20, 135.67, 135.74, 136.81, 136.94, 148.44, 152.28, 152.33, 154.80, 164.78. Exact mass calcd. (M+H) for $C_{22}H_{20}ClFN_2O_2$: 399.12; found: 399.1.

5-(4-chloro-2-fluoro-3-methoxyphenyl)-N-(2-isopropyl-6-methylphenyl)picolinamide (2f): Pale brown solid, yield 89 %. ^{1}H NMR (400 MHz, DMSO-d6): d 1.13 (d, J = 8.80 Hz, 6H, CH3), 2.17 (s, J = 9.20 Hz, 3H, CH_3), 3.12 (s, 1H, CH), 3.97 (s, 3H, CH_3), 7.21(m, J = 111.6 Hz, 5H, Ar-H), 8.23 (s, J = 4.40 Hz, 2H, Py-H), 8.91 (s, 1H, Py-H), 10.27 (s, 1H, NH). ^{13}C NMR (400 MHz, DMSO-*d_6)*: 19.2, 25.6, 25.6, 43.6, 62.14, 123.26, 123.30, 126.28, 126.42, 128.82, 128.97, 129.14, 129.17, 129.20, 135.67, 135.74, 136.81, 136.94, 148.44, 152.28, 152.33, 154.80, 164.78. Exact mass calcd. (M+H) for $C_{23}H_{22}ClFN_2O_2$: 413.14; found: 413.1.

5-(4-chloro-2-fluoro-3-methoxyphenyl)-N-(2-chloropyridin-4-yl)picolinamide (2g): Brown solid, yield 82%. ^{1}H NMR (400 MHz, DMSO-d6): d 3.95 (s, 3H, CH_3), 7.47 (m, J = 12.40 Hz, 3H, Ar-H), 7. 60 (t, J = 10.0 Hz, 1H, Ar-H), 8.20 (d, J = 9.60 Hz, 1H, Ar-H), 8.27 (s, 1H, Py-H), 8.45 (s, 1H, Py-H), 8.92 (s, 1H, Py-H), 11.09 (s, 1H, NH). ^{13}C NMR (400 MHz, DMSO-*d_6)*: 62.15, 109.9, 118.86, 122.37, 122.37, 123.01, 128.49, 128.54, 135.33, 136.41, 137.54, 146.33, 151.91, 151.91, 154.42, 161.8, 161.8, 164.33. Exact mass calcd. (M+H) for $C_{18}H_{12}Cl_2FN_3O_2$: 392.03; found: 392.0.

5-(4-chloro-2-fluoro-3-methoxyphenyl)-N-(4-(trifluoromethyl)phenyl)picolinamide (2h): Brown solid, yield 85 %. ^{1}H NMR (400 MHz, DMSO-d6): d 3.95 (s, 3H, CH_3), 7.55 (t, J = 44.4 Hz, 6H, Ar-H), 8.27 (s, J = 9.60 Hz, 1H, Py-H), 8.45 (s, 1H, Py-H), 8.91 (s, 1H, Py-H), 11.08 (s, 1H, NH). ^{13}C NMR (400 MHz, DMSO-*d_6)*: 62.14, 117.24, 117.24, 122.1, 122.2, 123.26, 123.30, 126.28, 126.42, 128.82, 128.97, 129.14, 135.67, 135.74, 139.82, 148.44, 152.28, 152.33, 154.80, 164.78. Exact mass calcd. (M+H) for $C_{20}H_{13}ClF_4N_2O_2$: 425.06; found: 425.1.

Determination of antibacterial activity

The antibacterial activity was performed by disc diffusion method followed by NCCLS (1993) and Awoyinka et al., (2007). Nutrient Agar (NA-Himedia) (composition: Animal's tissue= 5.00 g, Sodium chloride = 5.00 g, Beef extract= 1.50 g, Yeast extract = 1.50 g, Agar = 15.0 g) was used as the media for bacteria. 28.0 g of nutrient agar was suspended in 1000 ml distilled water, heated to boiling to dissolve the medium completely. It was sterilized by autoclaving at 15 Ibs pressure (121℃) for 15 min, mixed well and poured into sterile Petri plates. The microbial strains employed in the biological assay were Gram–negative bacteria: *Escherichia coli (E. coli)* (MTCC 732), obtained from Microbial type culture collection (MTCC) at the Institute of Microbial Technology (IMTECH), Chandigarh, India.

A loop full of each of the microorganisms was suspended in about 10 mL of physiological saline in a Roux bottle. Each of these was streaked on to the appropriate culture slants and was incubated at 37ºC for 24 h. After completion of incubation period, when growth was observed the tubes were kept into 2-8℃ until use.

The samples were weighed (10 mg/10 mL) and dissolved in ethanol to prepare appropriate dilution to get required concentrations of about 50 µL (50 µg), 100 µL (100 µg) and 150 µL (150 µg). The standard solution was Chloramphenicol for bacteria

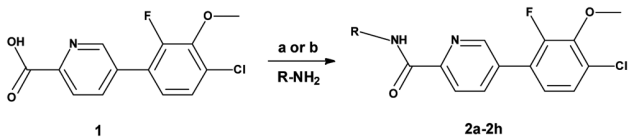
(25 mg/mL distilled water). They were kept under refrigerated condition unless they were used for the experiment.

Whattman filter paper (No:1) was used to prepare four discs approximately 6 mm in diameter, which are placed in hot air for sterilization. After sterilization, discs were loaded with 50 µL, 100 µL and 150 µL of each sample, Chloramphenicol 30 µL as standard solution was used to compare the test solution. They were kept under refrigerated condition unless they were used for the experiment.

Antibiogram was done by disc diffusion method using samples. Petri plates were prepared by pouring 30 mL of Nutrient agar medium. The bent glass rod was sterilized and used to spread the microbe-containing liquid uniformly on the Nutrient agar plates using 24 h culture of respective bacteria. Briefly, inoculums containing *E. coli* was spread on Nutrient agar plates for bacteria strains. Using sterile forceps, the sterile filter papers (6 mm diameter) containing the crude samples (50 µL, 100 µL and 150 µL) were laid down on the surface of inoculated agar plate. The plates were incubated at 37± 2°C for 24 h for bacterial strains. Each sample was tested in triplicate. The antimicrobial potential of test compounds was determined on the basis of mean diameter of zone of inhibition around the disc in millimeters. The zones of inhibition of the tested microorganisms by the samples were measured using a millimeter scale.

Results and Discussion

The key intermediate and the target compounds were synthesized by the reactions illustrated in Scheme 1. We have initially synthesized the compound **2a**-**2h** by coupling 5-(4-chloro-2-fluoro-3-methoxyphenyl)pyridine-2-carboxylic acid (compound **1**)[11] with aromatic amines (R-NH_2) using TBTU {O-(benzotriazol-1yl)-N,N,N',N'-tetramethyluronium tetrafluoroborate} followed by HATU {1-[Bis(dimethylamino)methylene]-1H-1,2,3-triazolo[4,5-b]pyridinium 3-oxid hexafluorophosphate} as an uranium coupling reagent and DIPEA (N,N-diisopropyl ethyl amine) as base in dimethylformamide as reaction solvent to afford corresponding amides (**2a**-**2h**). Later, we have synthesized the compound **2a**-**2h** by microwave assisted amide synthesis by coupling compound **1** with aromatic amines (R-NH_2) employing TBTU {O-(benzotriazol-1yl)-N,N,N',N'-tetramethyluronium tetrafluoroborate} as uranium coupling reagent and DIPEA as base in water to afford corresponding amides **2a**-**2h**. This environment friendly microwave assisted amide synthesis employing neat water as reaction solvent offers many advantages due to its non-toxic and non-flammable nature and. also this method effectively reduces organic solvent waste in chemical industry.



Scheme 1 — Synthesis of compounds **2a**-**2h**; (a) TBTU or HATU, DIPEA, DMF, 25-30°C, 12-16 h; (b) TBTU, DIPEA, water, Microwave, 60°C, 30 min

The amide synthesis proceeded smoothly in both TBTU as well as in HATU as coupling regent and gave the expected amide products **2a**-**2h** in moderate yields (65-80%). However, the microwave assisted synthesis of amides in neat water employing TBTU as reagent proceeds effectively and provides good yields (81-89%), the results are tabulated in Table 1. All the title compounds have been characterized using ^{13}C NMR, ^{1}H NMR and LCMS spectroscopic data. ^{1}H NMR of compound **2a**-**2h** showed one proton at 9 to 11 ppm due to amide proton and confirmed the presence of amide group. Also, ^{13}C NMR showed peak at 163 - 165 ppm due to carbonyl (amide) confirmed the presence of amide group.

Antibacterial activity

The antibacterial activity was performed by disc diffusion method followed by NCCLS (1993)[12] and Awoyinka et al., (2007)[13] The in vitro antimicrobial activity of the amide compounds **2a**-**2h** were investigated against bacteria *E. coli* and qualitatively assessed by presence of inhibition zones represented in the photographic Fig. S9 in Supplementary Information. The inhibitory activities in culture media of the amide compounds 2a-2h are tabulated in Table 2. Compounds **2a**-**2h** were comparable with standard antimicrobiotic viz. chloromphenical. All the compounds showed inhibitory effect on *E. coli* with varying degrees of zone of inhibition. The maximum zone of inhibition was seen with the standard drug chloromphenical. Amongst the various doses (50 µL, 100 µL and 150 µL) of amide compounds, the highest dose (150 µL) of all amide compound **2a**-**2h** has greatest activity against bacteria *E. coli*.

ADMET and protein-compounds interaction analysis

Molecule retrieval and ADMET analysis: The ligand molecules were sketched using the ACD/

Table 1 — Comparison of reaction yields of **2a-2h**

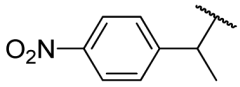
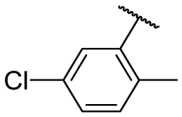
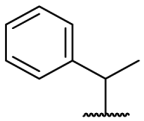
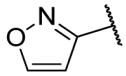
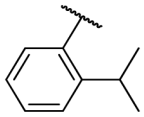
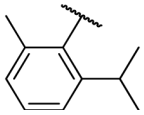
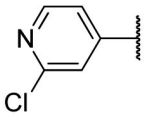
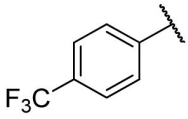
Compound	R	Isolated yield (%)		
		TBTU (Microwave)	TBTU	HATU
2a		81	67	72
2b		87	73	79
2c		89	75	78
2d		82	70	74
2e		86	78	76
2f		89	77	80
2g		82	68	73
2h		85	73	70

Table 2 — Antibacterial activity of samples **2a-2h**

Compound	*Escherichia coli* (mm)				
	50 µL	100 µL	150 µL	Standard (30 µL)	Control
2a	1.80±0.12	4.40±0.30	6.10±0.42	7.00±0.49	0.10±0.01
2b	1.50±0.10	4.10±0.28	5.90±0.41	7.10±0.50	0.10±0.01
2c	1.40±0.09	3.80±0.26	5.60±0.39	6.90±0.48	0.10±0.01
2d	1.00±0.07	3.10±0.21	5.20±0.36	6.80±0.47	0.10±0.01
2e	1.70±0.11	4.20±0.29	6.00±0.42	6.90±0.48	0.10±0.01
2f	1.20±0.08	3.60±0.25	5.40±0.37	6.80±0.33	0.10±0.01
2g	2.00±0.14	4.70±0.32	6.30±0.44	7.20±0.50	0.10±0.01
2h	1.10±0.07	3.30±0.23	5.40±0.37	6.70±0.46	0.10±0.01

Values were expressed as Mean ± SD, Bacterial standard – Chloromphenical, Control (Solvent): Ethanol

ChemSketch freeware. The SMILES were generated from the software, and they were converted to a 3D PDB structure using the OpenBabel software. The SMILES were given as the input for the ADMET analysis using the SwissADME server. The 3D structure of the protein molecule was retrieved from the Protein Data Bank with PDB ID 1USQ (Complex of *E. Coli* DraE adhesin with Chloramphenicol). This structure is co-crystallized with the Chloramphenicol interacting with ILE111, PRO43, PRO40, TYR115, and GLY113. Thus, these interacting amino acids were taken as the active site for the interaction analysis.

ADMET results: The ADMET analysis was performed using the online Swiss ADME server. From the output, we observed that all the eight compounds taken for the investigation had been shown to satisfy Lipinski's rule of 5. This elucidates that these compounds can be further taken for drug analysis. The detailed results from the SwissADME server are tabulated in Supplementary Information (Table S10).

Protein-compounds interaction analysis: The first step is the preparation of the protein and ligand file for AutoDock in the docking process. Further, polar hydrogens were added, then the charges of Kollman United Atom assigned, the charges of Gasteiger measured, and the protein PDB file were updated. The total per-residue energy was an integer. ADT attaches the charges to the ligand register. The file was further altered by selecting and detecting the torsion root, and the number of torsions is set. These modifications built the PDBQT file of the macromolecule and the ligand. The preparation of the Grid parameter file was followed by the collection of Grid-Set Map Types-Choose Ligand. The GPF file instructs AutoGrid to compute maps and their position and identify potential energy parameters in pairs. To understand the ligand-binding mode, the active site residues are identified. A grid is set by defining grid points in x, y, and z dimensions from the Grid Options widget. To allow free rotation of the ligand, the volume of the grid must be sufficiently large. In our analysis, in all three dimensions, the points are set to 60, and the modifications are saved. To run Auto Grid, a grid parameter file created with an extension of. gpf in the previous step is used. Finally, by defining the map files, the number of torsions, the docking algorithm, and the number of runs, the docking parameter file is prepared to run AutoDock. This is accomplished by setting the rigid filename macromolecule, followed by setting the ligand's parameters by selecting the ligand. When the number of GA runs is set to 10, the genetic algorithm parameters are modified, and the maximum number of energy evaluations is set to 2500000 evaluations (medium). The random number generator docking parameters, energy parameters, phase size parameters, and output format parameters are set to normal. The DPF file includes the docking parameters and the docking instructions for the Lamarckian Genetic Algorithm (LGA). To estimate binding energies, LGA uses a free-energy parameter scoring technique. Autodock is being launched now. A docking log file with an extension. dlg that is further analyzed is created after its completion. For further analysis, the best binding pose based on the binding energy was considered.

Protein-compounds interaction results: The molecular interaction study was performed using the AutoDock software. All the 8 test compounds were found to have higher binding affinity than the control compound chloramphenicol to the *E. Coli* DraE adhesin. The control Chloramphenicol was found to have binding energy of -5.2 kcal/mol. Compound 3 was found to have the highest binding affinity of -7 kcal/mol. The detailed binding affinity of the compounds are tabulated in Table 3. The number of interacting amino acids with the compounds are tabulated in the Table 4. The 2D representation are showed in Fig. 1.

Table 3 — Binding affinity of the compounds **2a** – **2h**

Protein ID	Ligand	Binding affinity (kcal/mol)
1usq	Comp-3	-7
1usq	Comp-6	-6.9
1usq	Comp-1	-6.6
1usq	Comp-5	-6.6
1usq	Comp-2	-6.5
1usq	Comp-7	-6.5
1usq	Comp-8	-6.4
1usq	Comp-4	-6.1
1usq	Chloramphenicol	-5.2

Table 4

Ligand	Interacting Residues	No. of interacting residues
1	VAL28, THR10, THR45, GLY134, GLN47	5
2	GLN47, VAL46, LEU58, THR45, THR10, PRO43, THR11	7
3	GLN47, VAL46, THR45, THR7, VAL44	5
4	THR45, THR10, PRO43	3
5	THR7, THR45, VAL44	3
6	LEU58, THR45, THR10, PRO43, THR11	5
7	GLN47, VAL46, THR45, THR10, PRO43, THR11	6
8	THR7, GLN47, VAL46, THR11, THR10, VAL28, LYS12, THR45	8
Control	THR45, GLN47, GLY134	3

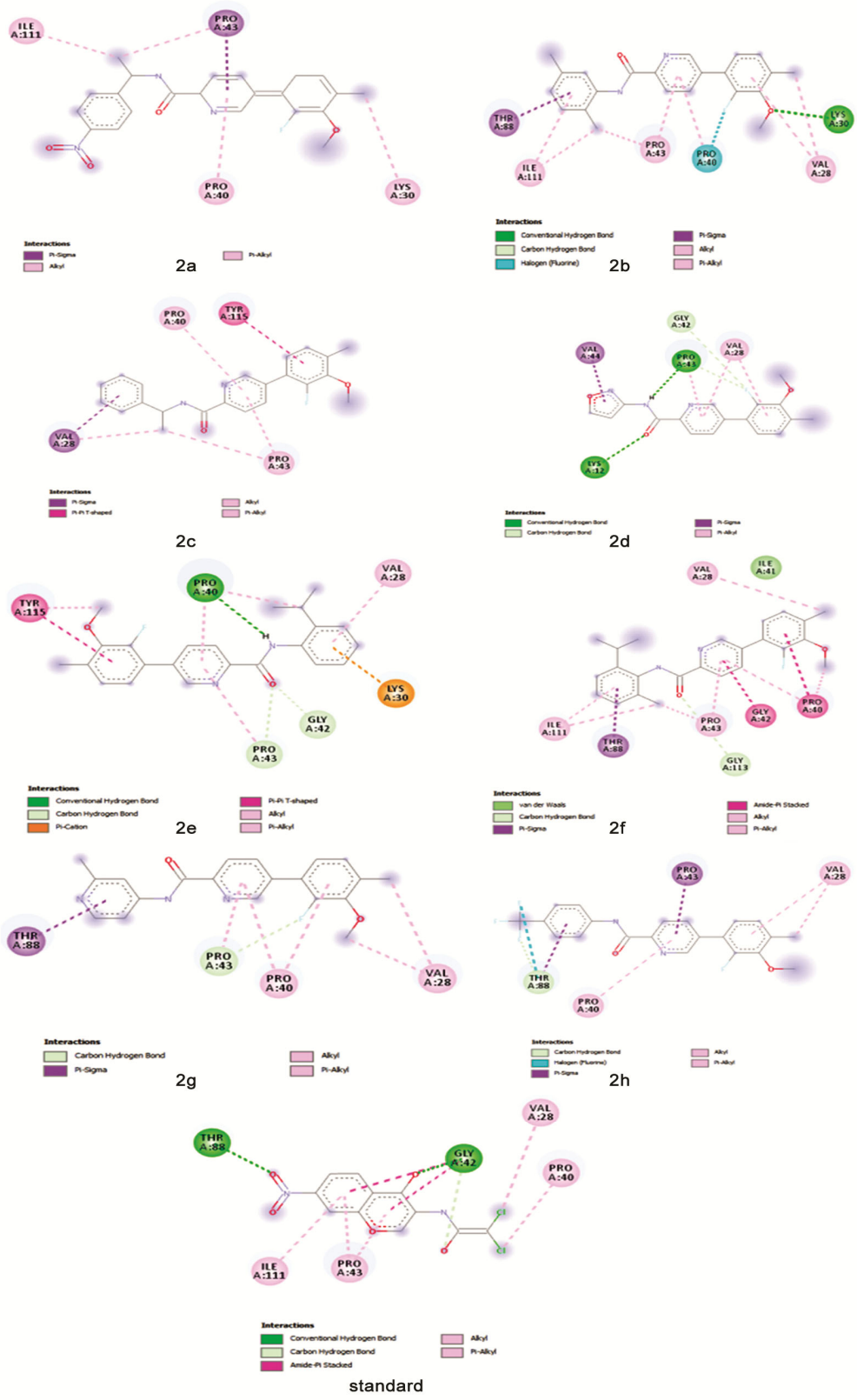


Fig. 1 — 2D representation of compounds **2a**-**2h** and standard; conditions: TBTU, DIPEA, water, Microwave, 60°C, 30 min

Conclusion

The present work explains a simple and capable green synthesis of novel 5-substituted-pyridine-2-carboxamides (**2a**-**2h**) in good yield under the mild reaction condition. It can also be useful synthons in the production of novel heterocycles. The eco friendly microwave assisted synthesis of amides in neat water tender several benefits like mild reaction conditions, short reaction times and eliminates the usage of toxic organic solvents like N,N-dimethylformamide, 1-methyl-2-pyrrolidone etc., This procedure would be very useful in the synthesis of amides. The study proved that all the eight amide compounds had been shown satisfy results of Lipinski's rule of 5. The molecular interaction studies of all the eight test compounds were found to have higher binding affinity than the control compound chloramphenicol to the *E. Coli* DraE adhesion. In addition antibacterial activity of all the 8 compounds has been determined and shown good activity. This elucidates that these compounds can be further taken for drug analysis.

Supplementary Information

Supplementary information is available in the website http://nopr.niscpr.res.in/handle/123456789/58776.

Acknowledgement

This research was supported by Bharathiar University, Research & Development Centre, Coimbatore, India.

References

1 Valverde M G & Torroba T, *Molecules*, 10 (2005) 318.
2 Sachin K, Pramod K S, Rupesh D & Nitin K, *J Chronotherapy Drug Deliv*, 2 (2011) 71.
3 Nehal A H & Amira M G, *Eur J Med Chem*, 44 (2009) 454.
4 Miyazawa S, Harada H, Fujisaki H, Kubota A, Kodama K, Nagakawa J, Watanabe N & Oketani K W O, Patent 103 049 A1, 2005. (https://patents.google.com/patent/WO2005103049A1/und)
5 Andrew S, Richard D, Jean H, Shuliang L, Donald M, David P, Dennis F, Michael F, Matthew W, Penny L, Paul L, Anne G, Chris B, John M, Donald T, Dennis S & Tesfaye B, *Eur J Med Chem*, 42 (2007) 1334.
6 Gui-Bai L, Xiaoxia O, Dennis F, Michael F, Christine M B, Anne G, Penny S L, Paul A L, Andrew S M, Tamas T, Dennis M S, Matthew W & Tesfaye B, *Bioorg Med Chem Lett*, 17 (2007) 3558.
7 Tesfaye B, Dennis F, Michael F, Gui-Bai L, Xiaoxia O, Andrew S, Richard D, Shuliand L, Paul A L, Chris B, Anne G, Penny S L, Donald T, John M, Andrew M, Samantha S, Tamas T, Joseph F S, Kathleen A M, Crystal G M, Dennis M S & Matthew W, *Bioorg Med Chem Lett*, 16 (2006) 2479.
8 (a) Manabe K, Iimura S, Sun X M & Kobayashi S, *J American Chem Soc*, 124 (2002) 11971; (b) Röttger S, Sjöberg P J & Larhed M, *J Comb Chem*, 9 (2007) 204; (c) Gawande M B & Branco P S, *Green Chem*, 13 (2011) 3355; (d) Marziale A N, Jantke D, Faul S H, Reiner T, Herdtweck E & Eppinger J, *Green Chem*, 13 (2011) 169.
9 (a) Lindström U M, *Chem Rev*, 102 (2002) 2751; (b) Li C J, Acc Chem Res, 35 (2002) 533; (c) Venkatraman S, Huang T & Li C J, *Adv Synth Catal*, 344 (2002) 399; (d) Klijn J E & Engberts J B, *Nature*, 435 (2005) 746.
10 (a) Varma R S, *Green Chem*, 1 (1999) 43; (b) Nuchter M, Ondruschka B, Bonrath W & Gum A, *Green Chem*, 6 (2004) 128; (c) Li C J, *Chem Rev*, 105 (2005) 3095; (d) Kappe C O, Pieber B & Dallinger D, *Angew Chem Int Ed Engl*, 52 (2013) 1088.
11 Dharman P, Babu V & Basha K A, *J Chin Chem Soc*, 66 (2019) 415.
12 Awoyinka O A, Balogun I O & Ogunnow A A, *J Med Plant Res*, 1 (2007) 63.
13 NCCLS, *Performance standards for antimicrobial disc susceptibility tests*, (National Committee for Clinical Laboratory Standards, P A, Publications, U S A) 1993.