Research Article

Synthesis, Antimicrobial Evaluation and Docking Studies of Novel Quinoline Carboxamide analogs.

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ABSTRACT

A series of twenty novel quinoline carboxamide analogues have been synthesized. Treatment of different anilines with quinoline carboxylic acid derivative was carried out to yield the desired product. A series of compounds from ***1a-1t*** were synthesized, characterized by physical and spectral data and evaluated for their antimicrobial activity using disc plate method at 200µg/ml concentration using ciprofloxacin as standard which had initial zone of inhibition of 23mm. Compounds ***1a***, ***1b***, ***1k*** to ***1o*** gave promising activity against *Aeromonas* and compounds ***1f*** to ***1q*** *and* ***1s*** *and* ***1t*** were active against *Enterococcus* species. Docking studies were carried out to study the interaction of these analogues with the target *Aeromonas hydrophila*.

Keywords: Quinoline, carboxamide, aniline, disc-plate method, antimicrobial, ciprofloxacin, *Aeromonas*, *Anterococcus*.

INTRODUCTION

A Number of the compounds based on the quinoline backbone has shown antibacterial, antimycobacterial, antifungal, anti-protozoal, molluscicidal as well as anti-inflammatory and antineoplastic activities. Recently, a number of organic carbamate derivatives have also been found to be as potential antibacterial, antimycobacterial and antiviral agents. It has been reported that the incorporation of the carbamate moiety present in the different molecules contributed towards the improvement of its pharmacodynamic and pharmacokinetic properties. Quinoline carboxamides are an important class of aromatic compounds with a wide range of pharmacological activities[1]. Quinoline-3-carboxamides and their analogues synthesized using indole ring have found to be exhibited effective anti nephritic as well as antiobiotic activity against methicillin resisitant strain [2]. Chinifur, a well-known marketed quinoline carboxamide based drug, is a selective inhibitor of trypanosome trypanothione reductase, acting as a competitive inhibitor of NADPH. 2-aryl-4-carboxamide quinoline has been assessed for prolyl-tRNA synthetase inhibitory action with preferred specificity towards significant bacterial enzymes. These compounds have also inhibited calpain, a calcium dependant proteolytic enzyme. Towards achieving the same, we have chosen the quinoline carboxamide analogues for the assessment of antibacterial studies with the incorporation of the trifluoryl group, hitherto unreported. The incorporation of trifluoryl group was decided on account of effect on the biological efficacy of the whole moiety[2].

There has been a growing interest in researching and developing new antimicrobial agents from various sources to combat microbial resistance. The existing compounds are in the danger of losing their efficacy because of the increase in microbial resistance. Its impact is so much that the treatment has failed due to multidrug-resistant bacteria and it has become a global concern to public health Therefore, a greater attention should be paid to antimicrobial activity screening. Antimicrobial susceptibility testing can be used for drug discovery, epidemiology and prediction of therapeutic outcome. For this reason, discovery of new antimicrobials is an exclusively important objective. Hence we have screened our synthesized compounds for antimicrobial activity.

MATERIALS AND METHODS

Synthesis

The Solvents, reagents and chemicals used in the present work were purchased from Aldrich, E. Merck, Spectrochem, and S. D. Fine Chem., HI-MEDIA and used without further purification. The purity of the synthesized compounds was checked by TLC on silica gel 60 F254 (E. Merck) aluminum plates. Melting points were determined using laboratory melting point apparatus (Toshniwal P. Ltd.) and were uncorrected. IR spectra of the synthesized compounds were recorded on FT-IRAffinity-1 (Shimadzu) IR Spectrometer. Mass spectra were recorded on GCMS-QP5050A (Shimadzu). NMR spectra were recorded on Bruker 400 MHz spectrometer using DMSO-d6 as the solvent.

Procedure for the synthesis of Quinoline Carboxamide analogues [3,4]

HOBT (Hydroxybenzotriazole), EDC.HCl (1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide) and Dimethylformamide were taken in 100ml round bottom flask. This mixture was then stirred for five minutes and

unimolar quantity of the 4-hydroxy-7-trifluoromethylquinoline-3-carboxylic acid was added in it and stirred for 15min. Further, substituted aniline added followed by DIPEA(Diisopropylethyl amine) and stirred continuously for 24h (Figure 1). Later, small quantity of water was added and carboxamidewas precipitated out. The product was filtered, dried, washed and recrystallized from methanol[5-10].Twenty analogues were synthesized with various substituted anilines.Various R substitution, % Yield, Melting point, logP and antibacterial activity are listed in Table 1.

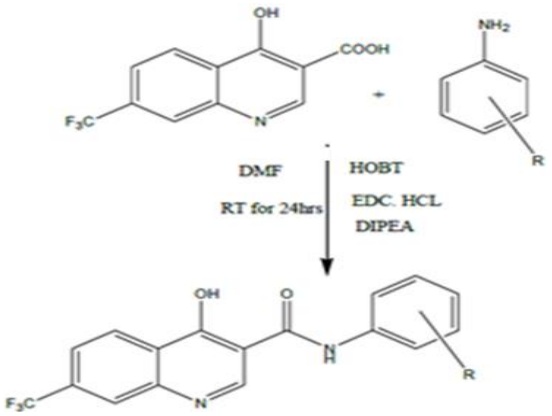


Figure 1: Scheme for the proposed synthesis of Quinoline carboxamide analogues

Table 1: Physical data of synthesized quinoline carboxamide analogues

S. No.	Code	R	Melting point ^{0}C	% yield	logP	Antibacterial Activity for Aeromonas at 200µg /ml
1	1a	4-bromo-aniline	318	31.76	4.57	+
2	1b	2,3-dimethyl-aniline	256	46.29	4.82	+
3	1c	2,4-dichloro-aniline	308	29.9	5.01	-
4	1d	4-fluoro-aniline	293	38.47	3.94	-
5	1e	2-bromo-aniline	300	44.77	4.57	-
6	1f	3-bromo-aniline	310	66.61	4.53	-
7	1g	4-methoxy-aniline	391	24.33	3.64	-
8	1h	2-fluoro-aniline	340	40	3.94	-
9	1i	3,4-difluoro-aniline	260	28.94	4.08	-
10	1j	3-chloro-2- methyl-aniline	260	14.91	4.91	-
11	1k	2,4-dibromo-aniline	261	20.9	5.33	+
12	1l	3,4-dichloro-aniline	320	43.33	4.94	+
13	1m	3-chloro-4- fluoro-aniline	291	48.44	4.54	+
14	1n	2,4-dimethyl-aniline	280	33.33	4.80	+
15	1o	2,5-dimethyl-aniline	246	33.33	4.71	+
16	1p	3- trifluoromethyl-aniline	272	30	4.67	-
17	1q	2,4-difluoro-aniline	340	47.10	4.02	-
18	1r	2-methoxy-aniline	275	27.62	3.67	-
19	1s	3-chloro-aniline	292	34.54	4.40	-
20	1t	2,6-dimethyl-aniline	282	51.85	4.63	-

(-) represents no inhibition of growth – Resistant, (+) represents inhibition of growth - Susceptible.

Physical and Spectral data of analogues

4-Hydroxy-7- trifluoromethyl-quinoline-3- carboxylic acid (2-bromo-phenyl)-amide, *(1e)*: White crystalline solid; 44% yield;M.P 300 ^{o}C ; *m/z* 411 **FTIR: KBr cm^{-1} (neat):** 3388 (N-H str); 1658 (C=O str); 1172 (C-N str); 1533 (N-H bending);1124(C-F str); 680(C-Br-Str) **^{1}H NMR 400 Hz,DMSO-d$_6$** 3.17 (s);7.08(s); 7.42(m); 7.82(dd);7.84(dd);8.53(dd)

4-Hydroxy-7- trifluoromethyl-quinoline-3- carboxylic acid (3-bromo-phenyl)-amide, *(1f)*: White crystalline solid; 66% yield; M.P 310 ^{o}C; *m/z*411; **FTIR: KBr cm^{-1}(neat):**3234(N-H str); 1672(C=O str); 1176(C-N str); 1120 (C-F str); 623 (C-Br str); 1531(N-H bend), **^{1}H NMR400 Hz, DMSO-d^6**: 7.31(s); 7.55(s); 8.46(dd); 8.51(dd); 8.12(dd);8.18(dd);9.03(s);13.90(m);12.39(s)

4-Hydroxy-7- trifluoromethyl-quinoline-3- carboxylic acid (3-chloro-4- fluoro- phenyl)- amide, *(1m)*: White crystalline solid; 48% yield; M.P 291 ^{o}C; *m/z*384; **FTIR: KBr cm^{-1}(neat):** 3223(N-H str); 3637(O-Hstr); 1676(C=O str); 1176(C-N str);1139 (C-F str);692 (C-Cl str);1535(N-H bend); **^{1}H NMR 400 Hz,DMSO-d$_6$:** 3.17(s); 7.42(s); 7.59(dd);7.85(dd);8.14(dd);8.52(dd);9.04(s);13.19(m).

4-Hydroxy-7- trifluoromethyl-quinoline-3- carboxylic acid (2,5-dimethyl-phenyl)-amide, ***(1o)*** **:** White crystalline solid;33% yield; M.P 246; *m/z*: 360; **FTIR: KBr cm^{-1}(neat):** 3230 (N-H str); 3678(O-H str); 1666(C=O str); 1176(C-N str); 1120(C-F str); 1541(N-H Bend); **^{1}H NMR 400 Hz,DMSO-d$_6$:** 2.29(s);2.36(s); 3.17(s); 6.85(s); 8.13(dd); 8.20(dd); 8.53(dd); 8.55(dd); 9.03(dd); 13.15(m)

Antibacterial activity[11, 12]

All the compounds exhibited significant antibacterial and moderate antifungal activities. The activity was carried out using Disc diffusion plate method using Mueller Hinton agar medium and the compounds were at a concentration of 200µg/ml. The compounds were found to be active against some of the microorganism against standard drug ciprofloxacin. They were tested against *S. aureus, Aeuromonas, Entero bacteriaceae, E. coli, Klebsiella, salmonella, Shigella, Pseudomonas Vibrio (cholera), Candida albicans* and *Candida tropicalis* microorganisms.

Ciprofloxacin was used as the standard with inhibition diameter of 23mm. Compounds **1a**, ***1b*** and ***1k*** to ***1o*** showed good zone of inhibition against *Aeuromonas* as shown in Table 1 whereas compounds ***1f*** to ***1q and 1s-1t*** showed good zone of inhibition against *Enterococcus*. The compounds were found to be inactive against other microorganisms used.

Docking studies[13]

Docking was done by GRIP batch docking method with the help of Vlife MDS 4.2 software. Three crystal structure of *Aeromonas hydrophila* were obtained from pdb domain with the code given (PDB ID: 1PRE,3C0N and 5JZH) for antimicrobial docking studies and were obtained from the protein data bank. These were chosen as the compounds showed good activity against *Aeromonas hydrophila*. As not much work has been done towards the docking of ligands against these targets we chose all three to see which target would give good docking score. The parameter fixed for docking simulation was number of placements: 50, rotation angle: 10°, ligand flexible, exhaustive method, scoring function: dock score. The ligand forming most stable drug-receptor complex was the one which was having minimum dock score. After docking simulation, the best docked conformer of each ligand was checked for various interactions with receptor like hydrogen bonding and hydrophobic bonding interaction and results are shown in the Table 2 and Table 3. Compound ***1k*** showed the best dock score of **-52.60** for 1PRE and **-93.46** for 5JZH targets indicating that this ligand has good antibacterial activity and as well as good dock score. Molecular docking was done to predict the mechanism of action of the synthesised compounds with the target. As our compounds showed good activity against *Aeromonas hydrophila,* the microorganism which is responsible for causing gastro enteritis, we selected this target and obtained the structure of the target from protein data bank and selected the target with pdb code mentioned above. We carried out this study to predict the mechanism of action of the synthesized compounds and correlate it with the activity and to study the forces involved in the binding of the compounds with the target. The compounds which showed good activity against the microorganism *Aeromonas* have also shown good docking score indicating that the compounds bind well to the chosen target. The highest dock score with 1PRE was **-52.60** for compound ***1k*** , with 3CON was **-66.10** for compound ***1p*** and for 5jzh was **-93.46** for compound ***1k*** whose Hydrogen bonding and hydrophobic bonding poses have been shown in the Table 3 below. And Table 4 indicates the various interaction studies between the protein residues of the target with the ligands.

RESULTS AND DISCUSSION

Among the tested compounds, 4-Hydroxy-7-trifluoro methyl quinoline-3-carboxylic acid (4-bromophenyl)-amide ***(1a),*** 4-Hydroxy-7-trifluoromethylquinoline-3-carboxylic acid (2,3-dimethylphenyl)-amide ***(1b)*** and 4-Hydroxy-7-trifluoromethylquinoline-3-carboxylic acid (2,4-dibromophenyl)-amide ***(1k),*** 4-Hydroxy-7-trifluoro methylquinoline-3-carboxylicacid(3,4-dichlorophenyl)-amide **(1l),** 4-Hydroxy-7-trifluoro methylquinoline-3-carboxylicacid (3-chloro-4-fluoro-phenyl)-amide ***(1m),***4-Hydroxy-7-trifluoro methylquinoline-3-carboxylic acid (2,4-dimethylphenyl)-amide ***(1n)*** and 4-Hydroxy-7-trifluoro methylquinoline-3-carboxylic acid(2,5-dimethylphenyl)-amide ***(1o)*** *have* shown good antimicrobial activity against *aeromonas*.

The substitution of electron withdrawing groups such as chloro, bromo and fluoro and also electron donating group such as methyl on the phenyl ring attached to the quinoline carboxamide have exhibited antimicrobial activity. The activity was further confirmed by the docking score of these compounds where the best docked compound ***1k,*** with the target pdb code 1PRE and 5JZH also exhibited antimicrobial activity against *aeromonas whereas the compound* ***1p*** though showed highest docking score with the target pdb code 3CON didn't possess any antimicrobial activity against *aeromonas*. The ligand which possessed good antibacterial activity with *aeromonas* as well as good dock score was with dibromo substitution. To carry out the docking studies for *Enterococcus* target will be our future aim.

Table 2: Docking score of the twenty compounds

Compound code	Dock score with 1PRE	Dock score with 3C0N	Dock score with 5JZH
1a	-43.43	-47.59	-73.01
1b	-45.67	-41.73	-71.17
1c	-42.96	-30.43	-73.80
1d	-45.67	-55.27	-73.55
1e	-42.53	-37.45	-73.99
1f	-43.65	-62.38	-75.76
1g	-42.23	-63.11	-74.24
1h	-42.59	-40.15	-83.91
1i	-46.02	-60.87	-81.25
1j	-40.70	-43.91	-77.77
1k	-52.60	-36.46	-93.46
1l	-42.66	-52.26	-71.84
1m	-41.55	-56.23	-74.79
1n	-45.42	-39.13	-72.15
1o	-43.54	-24.43	-80.20
1p	-45.71	-66.10	-80.96
1q	-45.45	-46.33	-77.86
1r	-40.85	-21.47	-75.54
1s	-42.51	-56.97	-74.55
1t	-36.60	-20.54	-62.79

Table 3: Hydrogen and hydrophobic interaction of ligands with receptors

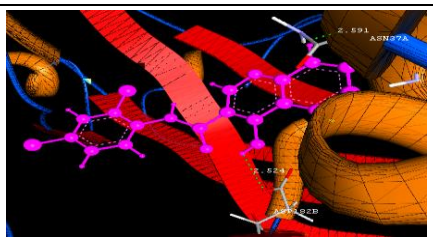
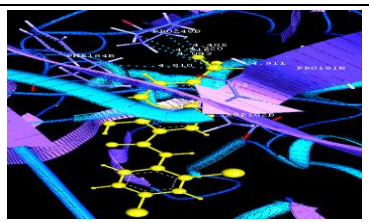
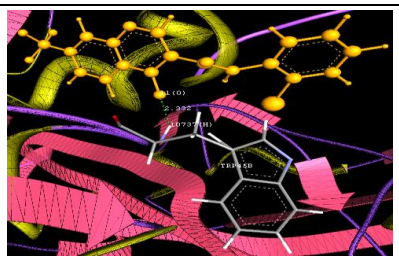
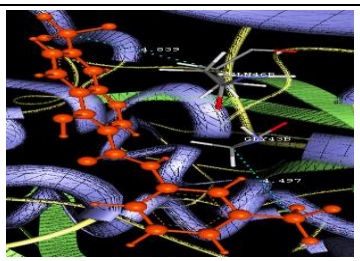
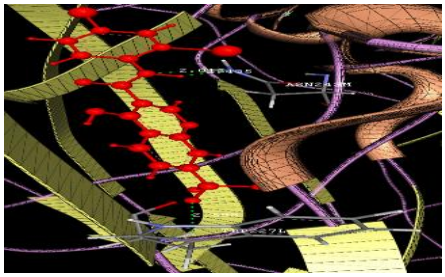
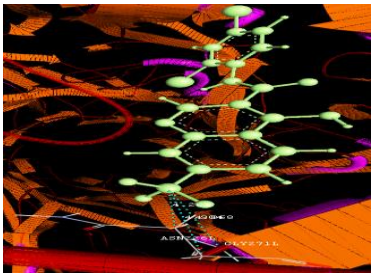
Ligands	Receptors/ Target	Hydrogen bonding	Hydrophobic bonding
1k	**1PRE**		
1p	**3C0N**		
1k	**5JZH**		

Table 4: Interaction studies between the protein and ligands

Ligands	Receptors	Hydrogen bonding with Residues	Hydrophobic bonding with Residues
1k	1PRE	ASN37A, ASP182B	PRO181B, ASP182B, PHE184B, PRO248B
1p	3C0N	TRP45B	GLN46B, GLY43B
1k	5JZH	TRP227L, ASN243M	ASN226L, GLY271L

CONCLUSION

Quinoline carboxamide analogues were synthesized by single step reaction which involves coupling of quinoline-3-carboxylic acid and substituted anilines in presence of HOBT and EDCl. All the compounds were obtained in good yield (20-60%) and were confirmed by physical data and spectral data. The synthesized compounds were found to be more active than the corresponding quinoline precursors. The overall antimicrobial activity of the synthesized quinoline carboxamides were found to be at moderate extent. Good activity was shown towards A*eromonas* organism and this was also supported by docking studies especially for compound ***1k*** [4-Hydroxy-7-trifluoro methylquinoline-3-carboxylic acid (2, 4-dibromophenyl)-amide]. And the target with pdb code 5JZH can be further used to study the docking of quinoline carboxamide derivatives. So quinoline carboxamide derivatives can be further explored to design drugs for gastro enteritis.

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REFERENCES

1. Wang, K.; Herdtweck, E.; Dömling, A, One-pot synthesis of 2- amino-indole-3-carboxamide and analogous, ACS Comb. Sci. 13, 2010, 140–146.
2. Canuto.C.V.B.S. Gomes .C.R.B., Marques .I.P., Faro L.V., Santos .F.C., Frugulhetti. I.C.P.P, Souza.T.M.L., Romeiro.G.A., Ferreira.V.F., Souza. M.C.B.V., Synthesis and Anti-HSV-1 Activity of 1,4-dihydro-4-oxoquinoline Ribonucleosides Lett. Drug Des. Discov. 4 , 2007, 404-409.
3. Jiang, J.; Hoang, M.; Young, J. R.; Chaung, D.; Eid, R.; Turner, C.; Lin, P.; Tong, X.; Wang, J.; Tan, C.; Feighner, S.; Palyha, O.; Hreniuk, D. L.; Pan, J.; Sailer, A. W.; MacNeil, D. L.; Howard, A.; Shearman, L.; Stribling, S.; Camacho, R.; Strack, A.; Van der Ploeg, L. H. T.; Goulet, M. T.; DeVita, R. J.2-Aminoquinoline melaninconcentrating hormone (MCH)1R antagonists., Bioorg. Med. Chem. Let., 16, 2006, 5270–5274.
4. Mistry, S. N.; Valant, C.; Sexton, P. M.; Capuano, B.; Christopoulos, A.; Scammells, P. J. Synthesis and Pharmacological Profiling of Analogues of Benzyl Quinolone Carboxylic Acid (BQCA) as Allosteric Modulators of the M1 Muscarinic Receptor. J. Med. Chem., 56, 2013, 5151–5172.
5. Yang, F. V.; Shipe, W. D.; Bunda, J. L.; Nolt, M. B.; Wisnoski, D. D.; Zhao, Z.; Barrow, J. C.; Ray, W. J.; Ma, L.; Wittman, M.; Seager, M. A.; Koeplinger, K. A.; Hartman, G. D.; Lindsley, C. W.,Parallel Synthesis of N-Biaryl Quinolone Carboxylic Acids as Selective M1 Positive Allosteric Modulators.Bioorg. Med. Chem. Lett., 20, 2010,531–536.
6. Yoneda J.D, Albuquerque M. G, Leal K Z, Santos FC, Batalha P N,Brozeguinnia L, Seidl. P. R.,Docking of anti-HIV-1 Oxoquinoline Acylhydrazone Derivatives as Potential HSV-1 DNA Polymerase Inhibitors.Journal of Molecular Structure ,2014, Volume 1074, 263-270.
7. Costi R, Metifiot M, Chung S, Crucitti G C, Maddali K,Pescatori L, Messore A, Basic Quinolinonyl Diketo Acid Derivatives as Inhibitors of HIV Integrase and their Activity against RNase H Function of Reverse Transcriptase J. Med. Chem., 57,2014, 3223–3234.
8. Horchler. C. L, McCauley J. P., Jr., Hall J. E., Synthesis of novel quinolone and quinoline-2-carboxylic acid (4-morpholin-4-yl-phenyl) amides. Bioorganic & Medicinal Chemistry, 15 , 2007, 939–950.
9. Hua .Yi, Emme C.K. Lin a, Lan M. Pham a, Julia Cajica a, Christopher M. Amantea a, Eric Okerberg,, Amides of 4-hydroxy-8-methanesulfonylamino-quinoline-2-carboxylic acid as zinc-dependent inhibitors of Lp-PLA2, Bioorganic &Medicinal Chemistry Letters 23 ,2013, 1553–1556.
10. Peet, N. P; Baugh, L. E.; Sunder, S.; Lewis, J. E.,Synthesis and antiallergic activity of some quinolinones and imidazoquinolinones. J. Med.Chem., 28, 1985,298.
11. Gillespie, S.H. Medical Microbiology/Illustrated, Butterworth Heinemann Ltd, United Kingdom, 1994, 234-247.
12. Fukumoto L.R., Mazza G., Assessing antioxidant and prooxidant activities of phenolic compounds. J. Agric. Food Chem., 48, 2000, 3597-3604.
13. VLife MDS 4.3 documentation, tutorial. Molecular Docking using MDS, 1-26.

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