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Synthesis and Antimicrobial Activity Evaluation of 2, 2, 2-trifluoro-1-(6-substituted-4-(thiophen-2-yl) cinnolin-3-yl) ethanone Derivatives

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ABSTRACT
The research work depicts the synthesis and development of cinnoline candidates by following the synthetic approach. The obtained compounds were characterized and investigated for anti-microbial activity. The synthesized cinnoline molecules were allowed to subject for molecular docking in moldock file to attain the docking score for protein using DNA Gyrase B (PDB lD: 4BAE). Molecular docking studies of antibacterial activity is having high inhibition constant with good binding energy. All cinnoline candidates obey Lipinski's rule of five and afforded the best docking score.The newly synthesized fourteen compounds were analyzed by FT-lR, H l NMR and also mass spectrum. The activity of synthesized compounds is screened against the panel of selected gram positive and gram negative species with hehelp of disc plate method.The newly synthesized Compound- 11was considered to be potent with minimum inhibitory concentration (MIC) value 12.5 µg/ml against E.coli.
KEYWORDS: *Click synthesis, Antimicrobial activity,In silico study, Molecular docking,Molegro virtual docker,Cinnolines.*

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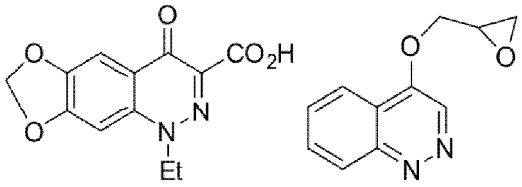
INTRODUCTION

The utmost need of antimicrobial resistant drug leads to the discovery of new potential drugs of science and it was part of the most dangerous health issues around the world. Now a day's bacterial is developing resistance continuously against anti-microbial compounds so it is a gigantic task which is a challenge for inventing novel antimicrobial compounds. Sometimes bacterial infections may occur which have been fatal [1-3]. The immediate need of drug-resistant strains of bacteria is a dangerous threat to society.The evolution of Resistance to the bacterial strains leads to ineffective antibiotics in the treatment of bacterial infection [4,5]. These findings envisaged to develop a novel molecular structure with better antibacterial activity.Cinnoline analogs have been accounted for various bio activities such as anti-inflarnrnatory[6], antimicrobial [7], anti-tubercular[8], anticancer [9] and antiplatelet activity [10]. The acceptance of cinnoline derivatives as biologically active molecules has aroused interest in need for productive ways to make this heterocyclic lead. Cinoxacin is the compound which posse's cinnoline as its basic structure(Fig-1).



Cinoxacin **4-(Oxiran-2-ylmethoxy) cinnoline**

Provoked by the significance of cinnoline moiety, our research work was focused on synthesize Cinnoline derivatives (CN1- 14) with good antibacterial activity against a panel of bacterial strain.

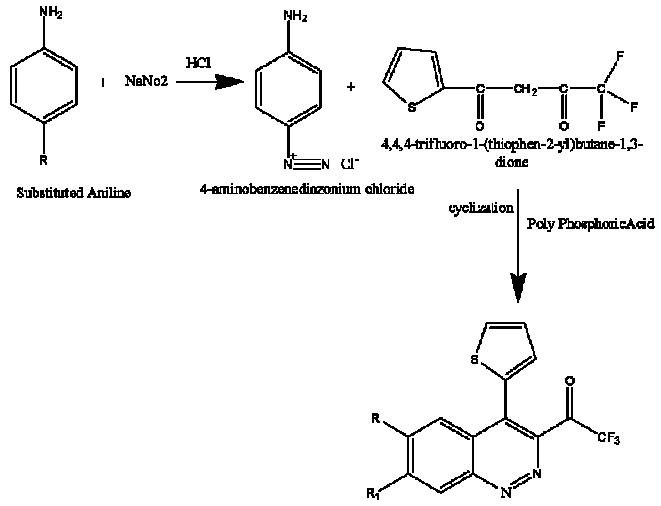


Figure 1: Scheme for synthesis of cinnoline derivatives

Table-1: Substituent groups at 6th and 7th positions R &R 1

S.No	R	R_1
1	NO_2	-
2	NH_2	-
3	CH_3	-
4	Cl	-
5	Br	-
6	I	-
7	COOH	-
8	OH	-
9	HSO_3	-
10	NH_2SO_2	-
11	Cl	NO_2
12	CH_3O	Cl
13	F	Cl
14	Cl	CF3

Influenced by synthesis of cinoxacin by Helen Giarnarellou in (1975) and evaluation of its antibacterial activity driven our research proposal to synthesize new compounds of Scheme-I (Figure 2) with high yield and better potency [11].In our present work,synthesis of novice title compounds by rearrangement reaction of phenyl hydrazone followed by cyclization attributes substituted new potent cinnoline candidates.Over the past 10 years, the research studies have revealed the importance of cinnolines with special interest in anti-microbial activity. Therefore, there is an emerging need for developing novel antibacterial agents. Hence, we described the synthesis of cinnoline derivatives bearing different substituent groups in the 6th and the 7thposition by cyclization of diazonium salt with substituted diketone like thenoyltrifluoro acetone to yield potent novice Cinnoline derivatives that can help in the interaction of DNA, resulting in a wider biological screening [12]and selecting of definite binding site algorithm and no of runs, maximum interaction, maximum size of population, energy threshold, maximum steps, neighbor distance factor and pose clustering was done and then followed by grid generation for co-crystallized structure of ligand around its binding pocket and submitted for visual docking [13,14].

MOLECULAR DOCKING STUDIES

Designing of drugs based on structural, molecular properties including Ligand virtual docking were performed by Molegro Virtual Docker (MolegromApS, Aarhus C, and Denmark). Fourteen newly synthesized compounds were particularly picked in the search of novel ligand for GyrB ATPase (a domain of DNA Gyrase) inhibitor as a novel antibacterial drug like compounds. The given structures were neatly drawn by using software ChemDraw version 12.0 and also saved in mol format after energy minimization. The proteins targeted for docking studies are DNA Gyrase Subunit B (PDB lD: 4BAE).The main 3D structures of targeted proteins were downloaded as pdb format from.The Selection of chain from the target protein was and importment was done into the workspace. Prediction of binding pocket and surface area creation was done and imported to workspace for docking .The adjustment of parameters like choosing suitable ligand to dock, selecting score function.

MATERIAL AND METHODS

The various chemical substances used for synthesis were purchased from respective vendors like sodium nitrate (Merck, Hyderabad, India), para nitro aniline (LobaChemie, Mumbai, India), polyphosphoric acid (Otto Chem, Mumbai, India), sulphuric acid (LobaChemie, Mumbai, India), agar, bees wax, trigacanth gum (LobaChemie, Mumbai, India). All other required chemicals and necessary reagents used were analytical grades.

Method of preparation Synthesis of 2, 2, 2-trifluoro- 1-(6- Nitro-4-(thiophen-2-yl) cinnolin-3 -yl) ethanone (CN 1- 14):

Substituted aniline (0.1 moles) was added to 5ml of HCl (200ml) in cooled condition. To this, sodium nitrite solution was added by stirring while the temperature is maintained below 5°c. To the diazonium salt (0.1 mol) of 2-thenoyl trifluoro acetone and of 2g of phosphoric acid was allowed to condense for 1 hr.The progress of reaction in each step was observed through TLC and then allowed to recrystalize by using ethanol, and then finally, the contents are poured into the ice cold water then filtered and dried. Compounds CN (1-14) were synthesized by substituting different R alkyl groups mentioned in (Table-1).The basic structures of the synthesized compounds (CN -1 to CN -14) were confirmed by IR, 1H NMR and Massdata. Physical Properties of synthesized compounds (CN-1 to CN-14) and IUPAC names of compounds are depicted in (Table 2 and 3).

Table 2: physical data of Cinnoline compounds

S.No	Name of of Compound	Molecular Formula	Molecular Weight	Melting point	Perc yield
1.	CN-1	$C_{14}H_6F_3N_3O_3S$	353.28	123-125	78
2	CN-2	$C_{14}H_8F_3N_3OS$	323.29	105-108	76
3	CN-3	$C_{15}H_9F_3N_2OS$	322.30	115-118	71
4	CN-4	$C_{14}H_6ClF_3N_2OS$	387.17	130-132	69
5	CN-5	$C_{14}H_6BrF_3N_2OS$	385.17	100-103	67
6	CN-6	$C_{14}H_6F_3IN_2OS$	433.92	106-108	68
7	CN-7	$C_{15}H_7F_3N_2O_3S$	352.29	112-114	77
8	CN-8	$C_{14}H_7F_3N_2O_2S$	324.29	125-126	72
9	CN-9	$C_{14}H_7F_3N_2O_4S_2$	387.72	121-124	75
10	CN-10	$C_{14}H_8F_3N_3O_3S_2$	385.0	208-210	78
11	CN-11	$C_{14}H_5ClF_3N_3O_3S$	387.73	211-213	79
12	CN-12	$C_{15}H_8ClF_3N2O_2S$	371.98	120-123	77
13	CN-13	$C_{14}H_5ClF_4N_2OS$	360.72	156-159	76
14	CN-14	$C_{15}H_5ClF_6N_2OS$	410.72	120-122	75

S.No	IUPAC name of the compounds
1	2,2,2-trifluoro-1-(6-nitro-4-(thiophen-2-yl)cinnolin-3-yl)ethanone
2	1-(6-amino-4-(thiophen-2-yl)cinnolin-3-yl)-2,2,2-trifluoroethanone
3	2,2,2-trifluoro-1-(6-methyl-4-(thiophen-2-yl)cinnolin-3-yl)ethanone
4	1-(6-chloro-4-(thiophen-3-yl)cinnolin-3-yl)-2,2,2-trifluoroethanone
5	1-(6-bromo-4-(thiophen-2-yl)cinnolin-3-yl)-2,2,2-trifluoroethanone
6	2,2,2-trifluoro-1-(6-iodo-4-(thiophen-2-yl)cinnolin-3-yl)ethanone
7	4-(thiophen-2-yl)-3-(2,2,2-trifluoroacetyl)cinnoline-6-carboxylic acid
8	2,2,2-trifluoro-1-(6-hydroxy-4-(thiophen-2-yl)cinnolin-3-yl)ethanone
9	4-(thiophen-2-yl)-3-(2,2,2-trifluoroacetyl)cinnoline-6-sulfonic acid
10	4-(thiophen-2-yl)-3-(2,2,2-trifluoroacetyl)cinnoline-6-sulfonamide
11	1-(6-chloro-7-nitro-4-(thiophen-2-yl)cinnolin-3-yl)-2,2,2-trifluoroethanone
12	1-(7-chloro-6-methoxy-4-(thiophen-3-yl)cinnolin-3-yl)-2,2,2-trifluoroethanone
13	1-(7-chloro-6-fluoro-4-(thiophen-3-yl)cinnolin-3-yl)-2,2,2-trifluoroethanone
14	1-(6-chloro-4-(thiophen-3-yl)-7-(trifluoromethyl)cinnolin-3-yl)-2,2,2-trifluoroethanone

Table-3: IUPAC naming of synthesized compounds

FT-IR, H1 NMR of 2, 2, 2-trifluoro-1-(6-subsitued-4-(thiophen-3-yl) cinnolin-3-yl)-ethanone.

2, 2, 2-trifluoro-1-(6-nitro-4-(thiophen-2-yl) cinnolin-3-yl) ethanone (CN-1) Yield: 78%, MP:123-125 IR (KBr) Kmax in (cm) IR (KBr, cm-1) 3199.31 (NH stretching), 801 (C-S),1251(C-F),1609.31 (C=N is stretching), 1353.71 (NO2 stretching), 1248.69 (OH bending), 1021.11 (N-N stretching), 758.85 (DI subs benzene), 1393.32 (NO2 stretching),1601 (C=O),1535(N=N),1H NMR (DMSO-d6, 300 MHz, d ppm) 8.80

(s,1H.Ar H), 8.53 (t,1H, Ar H), 8.27 (d,1H,Ar H), 7.68 (d,1H,thenoyl ring), 7.41 (t,1H,thenoyl ring),7.16 (t,1H,thenoyl ring) MS, m/z (%), 353(M+) Anal. Calcd. For C14H6F3N3O3S: C, 47.60; H, 1.71; N, 11.89 %.

1-(6-amino-4-(thiophen-2-yl) cinnolin-3-yl)-2, 2, 2-trifluoroethanol (CN-2) Yield: 76%, MP:105-108 IR (KBr) Kmax in (cm) IR (KBr, cm-1) 3199.31 (NH stretching), 1535(N=N), 800 (C-S),1251(C-F), 1601 (C=O),(1609.31 (C=N is stretching),1601 (C=O),(1243.62 (OH bending), 1024.11(N-N stretching), 758.85 (DI subs benzene), 3199.33 (NH2 stretching) ,1H NMR (DMSO-d6, 300 MHz, d ppm) 8.80 (s, 1H.Ar H), 8.53 (t,1H, Ar H),8.27 (d,1H,Ar H),7.68 (d,1H,thenoyl ring), 7.41 (t,1H,thenoyl ring), 7.16 (t,1H,thenoyl ring), 6.26 (d,2H,NH2)MS, m/z (%), 32 3(M+) Anal. Calcd. For C14H8F3N3OS: C, 52.01; H, 2.41; N, 13.00%; O, 4.95; S, 9.92 %.

2, 2, 2-trifluoro-1-(6-methyl-4-(thiophen-2-yl) cinnolin-3-yl) ethanone (CN-3) Yield: 71% MP; 115-118;IR (KBr, cm-1) 3198.31 (NH stretching), 1535(N=N),800 (C-S), 1609.31 (C=N stretching), 1601(C=O),2862(CH3),1021.12(N-N stretching), 758.85 (DI subs benzene), 3199.33(NH2 stretching) 1H NMR (DMSO-d6, 300 MHz, d ppm), 2.33 (Ss,3H,CH3),7.16 (t,1H,thenoyl), 7.41 (d,1H, thenoyl), 7.52 (s,1H,Ar H),8.03 (d,1H,Ar H), 7.58 (m,1H,Ar H). MS, m/z (%), 341.98 (M+) Anal. Calcd. For C14H6ClF3N2OS: C, 49.06; H, 1.71; Cl, 10.34%; O, 4.67; S, 9.36 %.

1-(6-chloro-4-(thiophen-3-yl) cinnolin-3-yl)-2, 2, 2-trifluoroethanol (CN-4) Yield: 69%; MP; 130-132IR (KBr) Kmax in (cm-1) 3198.33 (NH stretching), 801 (C-S),1252 (C-F), 1601 (C=O), 1535 (N=N), (1609.31 (C=N stretching), 1248.61 (OH bending), 1021.1 1(N-N stretching), 758.85 (DI subs benzene),850 (C-Cl), 1H NMR (DMSO-d6, 300 MHz, d ppm),8.03 (d,1H,Ar),7.68-7.773 (3H,m,Ar),7.41 (d,1H,thenoyl),7.6 (t,1H, thenoyl).

MS, m/z (%), 322 (M+) Anal. Calcd. For C15H9F3N2OS: C, 55.90; H, 2.81; N, 8.69%; O, 4.96; S, 9.95%

1-(6-bromo-4-(thiophen-2-yl) cinnolin-3-yl)-2, 2, 2-trifluoroethanol (CN-5) Yield:67%; MP:100-103;IR (KBr) Kmax in (cm-1) 691 (Br),800 (C-S), 1535(N=N), 1609.31 (C=N stretching), 1601(C=O),1021.12(N-N stretching), 758.85 (DI subs benzene), 3199.33(NH2 stretching), 1H NMR (DMSO-d6, 300 MHz, d ppm), 7.16 (t,1H,Ar),7.68-7.873 (2H,m,Ar), 7.41 (d,1H,thenoyl),7.68 (d,1H,thenoyl). MS, m/z (%), 322(M+) Anal. Calcd. For C14H6BrF3N2OS: C, 43.43; H, 1.56; Br, 20.64, F, 14.74, N, 7.24%; O, 4.13; S, 8.28 %.

2, 2, 2-trifluoro-1-(6-iodo-4-(thiophen-2-yl) cinnolin-3-yl) ethanone (CN-6) Yield:68%; MP: 106-108;IR (KBr) Kmax in (cm-1) 680.01(C-S), 1251 (C-F), 1601 (C=O), 1609.31 (C=N is stretching), 1535 (N=N), 1021.12 (N-N Stretching), 758.85 (DI subs benzene), 1H NMR (DMSO-d6, 300 MHz, d ppm),8.10 (m,2H,Ar H),7.85 (d,1H,Ar H),7.68 (d,1H,thenoyl),7.40 (d,1H,thenoyl),7.16 (t,1H,thenoyl), MS, m/z (%), 433.92 (M+) Anal. Calcd. For C14H6F3IN2OS: C, 38.73; H, 1.39, F, 13.13, I, 29.23, N, 6.45%; O, 3, 69; S, 7.39 %.

4-(thiophen-2-yl)-3-(2, 2, 2-trifluoroacetyl) cinnoline-6-carboxylic acid (CN-7) Yield:77 %; MP:112-114; IR (KBr) Kmax in (cm-1),690 (C-Br), 1252 (C=S), 1609.31 (C=N stretching), 1535 (N=N),1359.79 (NO2 stretching), 1248.69 (OH bending), 1021.12 (N-N stretching), 758.85 (DI subs benzene), 1393.32 (NO2 stretching), 3199.33 (NH2 stretching), 1H NMR (DMSO-d6, 300 MHz, d ppm),7.16 (t,1H,Ar), 7.68-7.873 (2H,m,Ar), 7.41 (d,1H,thenoyl),7.68 (d,1H,thenoyl). MS, m/z (%), 322 (M+) Anal. Calced. For C14H6BrF3N2OS: C, 43.43; H, 1.56; Br, 20.64, F, 14.74, N, 7.24%; O, 4.13; S, 8.28 %.

2, 2, 2-trifluoro-1-(6-hydroxy-4-(thiophen-2-yl) cinnolin-3-yl) ethanone (CN-8) Yield:72%; MP:125-126; IR (KBr) Kmax in (cm-1) 1248.69 (OH bending), 1535 (N=N),800 (C-S), 1609.31 (C=N stretching), 1359.79 (NO2 stretching), 1244.68 (OH), 1022.11 (N-Nstretching), cinnoline ring (889),758.85 (DI subs benzene), 1393.32 (NO2 stretching), 3199.33 (NH2 stretching), 1H NMR (DMSO-d6, 300 MHz, d ppm), 7.03 (S,1H,Ar),7.16 (t,1H,Ar),7.31-7.43 (2H,m,Ar), 8.04 (d,1H,Ar), 7.68 (d,1H, thenoyl). MS, m/z (%), 324 (M+) Anal. Calcd. For C14H7F3N2O2S: C, 51.85.43; H, 2.18; Br, 20.64, F, 17.58, N, 8.64%; O, 9.87; S, 9.89 %.

4-(thiophen-2-yl)-3-(2,2,2-trifluoroacetyl)cinnoline-6-sulfonic acid (CN-9) Yield:75%; MP; 121-124; IR (KBr) Kmax in (cm-1) 460 (SO3), 800 (C-S), 1609.31 (C=N stretching), 1535 (N=N), 1359.79 (NO2 stretching), 1242.64 (OH bending), 1029.12 (N-N stretching), cinnoline ring (889),758.85 (DI subs benzene), 1393.32 (NO2 stretching), 3199.33 (NH2 stretching) 1H NMR (DMSO-d6, 300 MHz, d ppm), 8.36-8.41 (m,3H,Ar), 7.68 (d,1H,thenoyl), 7.41 (1H,t,thenoyl), 7.16 (t,1H, thenoyl). MS, m/z (%), 387(M+) Anal. Calcd. For C14H7F3N2O4S2: C, 43.30; H, 1.82; F, 14.68, N, 7.21%; O, 16.48; S, 16.51%.

4-(thiophen-2-yl)-3-(2, 2, 2-trifluoroacetyl) cinnoline-6-sulfonamide (CN-10) Yield:78%; MP:208-210; IR (KBr) Kmax in (cm-1) 1166 (SO2NH2), 800 (C-S), 1608.33 (C=N is stretching), 1535 (N=N), 1359.79 (NO2 stretching), 1248.65 (OH bending), 1027.13 (N-N stretching), cinnoline ring (889),758.85 (DI subs benzene), 1393.32 (NO2 stretching), 3199.33(NH2,stretching)1HNMR(DMSO-d6,300MHz,dppm), 8.368.41 (m,3H,Ar), 7.68 (d,1H,thenoyl), 7.41 (1H,t,thenoyl),7.16 (t,1H,thenoyl), 2.01(S,1H,NH2)MS, m/z (%), 387(M+) Anal. Calcd. For C14H8F3N3O3S2: C, 43.41; H, 2.08; F, 14.71, N, 10.85%; O, 12.39; S, 16.56 %.

1-(6-chloro-7-nitro-4-(thiophen-2-yl) cinnolin-3-yl)-2, 2, 2-trifluoroethanone (CN-11) Yield: 79%; MP:211-213;IR (KBr) Kmax in (cm-1) 800 (C-S), 1607.35 (C=N is stretching), 1535 (N=N), 1600.21

(C=OStr), 1359.79(NO2 stretching), 1245.68 (OH bending), 1021.12 (N-N stretching), cinnoline ring (889),758.85 (DI subs benzene), 1393.32 (NO2 stretching), 3199.33 (NH2 stretching) 1H NMR (DMSO-d6, 300 MHz, d ppm), 8.36-8.41 (m,3H,Ar),7.68 (d,1H,thenoyl),7.41 (1H,t,thenoyl), 7.16 (t,1H,thenoyl), 2.01 (s,2H,NH2) MS, m/z (%), 387(M+) Anal. Calcd. For C14H5ClF3N3O3S: C, 43.37; H, 1.30; Cl, 9.14; F, 14.71, N, 10.85%; O, 12.39; S, 8.27 %.

1-(7-chloro-6-methoxy-4-(thiophen-3-yl) cinnolin-3-yl)-2, 2, 2-trifluoroethanone (CN-12) Yield:77%; MP:120-123; IR (KBr) Kmax in (cm-1) Cl,CH 30-800 (C-S), 601 (C-Cl), 1535 (N=N), 1609.31 (C=N stretching), 1359.79 (NO2 stretching), 1243.67 (OH bending), 1022.11 (N-N stretching), cinnoline ring (889), 758.85 (DI subs benzene), 1393.32 (NO2 stretching), 3199.33 (NH2 stretching) 1H NMR (DMSO-d6, 300 MHz, d ppm), 8.36 (s,1H, Ar), 7.68 (d,1H,thenoyl), 7.41 (1H,d, thenoyl), 6.96 (s,1H,Ar), 7.16 (t,1H,thenoyl), 3.82.0 (s,3H,CH3) MS, m/z (%), 371(M+) Anal. Calcd. For C15H8ClF3N2O2S: C, 48.33; H, 2.16; Cl, 9.51; F, 15.29, N, 7.92%; O, 8.58; S, 8.60 %.

1-(7-chloro-6-fluoro-4-(thiophen-3-yl) cinnolin-3-yl)-2, 2, 2-trifluoroethanone (CN-13) Yield:76%; MP:156-159; IR (KBr) Kmax in (cm-1) 601 (C-Cl), 800 (C-S), 1251 (C-F), 1535 (N=N), 1609.32 (C=N is stretching), 1021.12(N-N stretching), cinnoline ring (889), 758.85 (DI subs benzene), 1H NMR (DMSO-d6, 300 MHz, d ppm),8.36 (d,1H,Ar),7.68 (d,1H, thenoyl),7.37-7.39 (t,2H,Ar), 7.16 (t,1H, thenoyl), MS, m/z (%), 371(M+) Anal. Calcd. For C14H5ClF4N2OS: C, 46.62; H, 1.40; Cl, 9.83; F, 21.07, N %.

1-(6-chloro-4-(thiophen-3-yl)-7-(trifluoromethyl)cinnolin-3-yl)-2,2,2-trifluoroethanone(CN-14)
Yield:75%; MP:120122;IR (KBr) Kmax in (cm-1) 711.45,60 (C-Cl), 800(C-S), 1251(C-F), 1535(N=N),1609.31 (C=N is stretching),1021.12(N-N Stretching), cinnoline ring (889),758.85 (DI subs benzene),1H NMR (DMSO-d6),8.75(s,1H,Ar),7.95-7.98(d.2H,Ar),7.72 (t,1H,Ar),7.22(d,1H,Ar). m/z (%),409.97(M+) Anal. Calcd. For C15H5ClF6N2OS: C, 43.86; H, 1.23; Cl, 8.63; F, 27.75; N, 6.82; O, 3.90; S, 7.81 %.

Molecular virtual docker is used for structural drug design and molecular drug design. In this process of docking, structures of compounds are neatly drawn by using Draw version 12.0 and were allowed to save in Mol format. Designed fourteen potent compounds were selected as a ligand for GyrB ATPase (a domine of DNA Gyrase) and Ligand based docking studies were Performed by Molegro Virtual Docker (MolegromApS, Aarhus C, and Denmark).Fourteen compounds were selected from the search of new ligand for GyrB ATPase (a domine of DNA Gyrase) inhibitor as a novice antibacterial drug like candidates. The Main structures were neatly drawn using Chem. Draw version 12.0 and files are saved in Mol format after energy minimization. The selected targeted protein for docking studies is DNA gyrase subunit B (PDB lD: 4BAE) .The 3D structure of target proteins was first downloaded from the website protein data bank pdb format. The Selected chain in the target protein tabled in the workspace. Prediction of binding pocket and surface area creation was done and imported to workspace for docking. The adjustment of parameters like choosing suitable ligand to dock, selecting score function and selecting of definite binding site algorithm and no of runs, maximum interaction, maximum size of population, energy threshold, maximum steps neighbor distance factor and pose clustering was done and then followed by grid generation for co-crystallized structure of ligand around its binding pocket and submitted for virtual docking.Docking score (mol dock) of the ligands is compared with co-crystallized ligand of the respective protein ciprofloxacin[l5,16]. In silico pharmacokinetics (ADME) properties of desired compounds are well predicted by online software's like Swiss ADME [17].ln our body, pharmacokinetics properties of receptor are mostly dependent on molecular properties of compounds. Lipinski rule is used to predict some physical and chemical properties and also bioavailability.

Studies of desired compounds. Compounds obeying Lipinski rule should posses Clog P (1 .92 to+5.31), molecular weight (321 .73-434.l7D), H-bond donors (Not more than 2), HBA (not more than 9), rotatable bonds (4 or fewer) polar surface area (equal to or <I 30A).Drug candidate can crosses the BBB barrier easily between log p values 1.5 and 2.5.According to in silico ADME studies, all the synthesized cinnoline compounds passed Lipinski's rule of five allowing compounds and to reach its site of target by crossing the BBB through oral absorption (Table 4 and5).lt was restricted only to absorb by diffusion (passive mode) of the cinnoline compounds through the cell membranes, but not for the drugs which are actively transported (active mode).Anti-bacterial evaluation was done by disk plate method .

All the novel synthesized cinnoline compounds were investigated against a panel of antibacterial activity against *Bacillus subtilis* MTCC 441, *S.aureus* ATCC 96, *E.coli* ATCC 8739,*K.pneumoniae* MTCC 109.Minimum inhibitory concentration (MIC) was determined by disk plate method according to standard procedure. The standard drug used was ciprofloxacin19.Experimental result reveals that all the cinnoline candidates have shown activity between ranges l 2.5µg/ml- 100µg/ml (Table 6 and 7).

Table-4: Molecular docking reports for compounds CN(1-14)against protein DNA Gyrase B

S.No	PDB code	Compounds	Binding energy (Kcal/mol)	Residue involving H-bond
1	4BAE DNAGyrase B	CN -1	-136.11	Thr-169, Ile-84, Asn-52
2	4BAE DNAGyrase B	CN -2	-130.01	Asp-79
3	4BAE DNAGyrase B	CN -3	-128.12	-
4	4BAE DNAGyrase B	CN -4	-112.23	-
5	4BAE DNAGyrase B	CN -5	-118.43	-
6	4BAE DNAGyrase B	CN -6	-109.31	-
7	4BAE DNAGyrase B	CN -7	-138.229	Ile-171
8	4BAE DNAGyrase B	CN -8	-105.21	-
9	4BAE DNAGyrase B	CN -9	-129.13	Ile-171
10	4BAE DNAGyrase B	CN-10	-128.81	Val-77
11	4BAE DNAGyrase B	CN -11	-139.597	Ile-171, Thr169
12	4BAE DNAGyrase B	CN-12	-121.01	-
13	4BAE DNAGyrase B	CN-13	-111.21	-
14	4BAE DNAGyrase B	CN-14	-129.12	Thr169,Ile84,Gly-83,Arg 82,Arg 82

Table-5:*insilico* ADME properties of cinnoline compounds

Sl.No	M.wt	Clog p	HBD	HBA	Nrotb	TpsA
1.	353.28	3.1	0	8	4	116.91 A^2
2.	323.2	3.21	1	6	3	97.11
3.	322.30	4.2	0	6	3	71.09
4.	342.72	4.31	0	6	3	71.09
5.	387.17	4.40	0	6	3	71.09 A^2
6.	434.17	4.44	0	6	3	71.09
7.	352.29	3.34	1	8	4	108.39
8.	324.28	3.37	1	7	7	91.32
9.	405.37	1.92	2	10	4	137.08
10.	387.36	2.62	1	9	4	139.63
11.	387.72	3.64	0	8	4	116.91
12.	344.74	4.49	0	6	3	63.25
13.	362.73	4.77	0	7	3	70.03
14.	410.72	5.31	0	9	4	7.09 A^2

Table-6: Antibacterial Activity of compounds using disk plate method Zone of Inhibition

	Zone of Inhibition value (mm)			
	Gram-positive bacteria		**Gram-negative bacteria**	
Compounds	***B.s***	***S.a***	***E.c***	***k.p***
CN-1	12	9	13	12
CN-2	10	11	13	14
CN-3	12	13	12	13
CN-4	10	13	15	13
CN-5	12	10	11	12
CN-6	11	12	14	10
CN-7	10	11	18	15
CN-8	12	13	12	13
CN-9	12	11	14	11
CN-10	10	12	12	13
CN-11	13	11	17	16
CN-12	10	12	16	15
CN-13	11	12	14	11
CN-14	12	13	12	13
Ciprofloxacin:	22	20	25	23

Gram positive*: Bacillus subtilis, Staphylococcus aureus,*
Gram negative: *Escherichia coli, Klebsiella pneumoniae*

Table-7: Antibacterial Activity MIC data µg/ml

CN	*B.s*	*S.a*	*E.c*	*k.p*
CN-1	25	50	25	50
CN-2	50	25	50	100
CN-3	100	-	25	100
CN-4	25	100	50	25
CN-5	100	25	-	50
CN-6	50	50	25	100
CN-7	25	50	12.5	50
CN-8	25	100	50	100
CN-9	100	50	25	50
CN-10	50	25	100	25
CN-11	25	50	12.5	25
CN-12	50	25	12.5	25
CN-13	-	100	50	25
CN-14	50	25	100	50
Ciprofloxacin	3.12	3.10	3.50	3.23

RESULTS AND DISCUSSION

The substituted diketone(thenoyltrifluoro acetone) is one of the best component for facile synthesis of various heterocyclic compounds. The titled cinnoline compounds are well synthesized by following the schematic procedure reported in the literature 20-22.Schematic strategy was depicted in (Fig2). Calculated moles of substituted aniline is well reacted with sodium nitrate in the cooling conditions for diazotization by treating with strong acids like I N HCl to get Benzene diazonium salt, on further reaction of thenoyltrifluoro acetone with strong acid like Polyphosphoric acid at high temperature cinnoline was yielded.CN 1-14 were afforded by substituting different anilines to afford new cinnoline candidates.The signal observed at 7.26ppm corresponds to one proton is due to thiophenemethine proton at C15.The two doublet observed at 7.62 ppm and 7.77ppm were due thiophene in ethine proton at C16 and C14 respectively. The singlet observed at 8. 1 8 ppm and 8.20 ppm are due to the aromatic protons at C3 and C6 respectively.

Molecular docking studies

Here we reported the results of docking of compound selectively based on their interaction with a protein enzyme namely DNA gyrase presented in (Table 4).The oxygen atom in nitro group of Compound- 11 has interacted with IIe 171 and also nitrogen of cinnoline core interacted with Thr 169 and afforded best docking score. In the Compound-7 oxygen atom of carboxylic group interacted with IIe 171as best fit and noted as best potent compound and the 3D plotting of ligand protein interaction profile was done by MVD. Visuals are given for of hydrogen bond interaction for selected compounds (a) (CN- I : Tyr- 1 69, lle 84, Asn 52) (b) (CN-2: Asp 79) (c) (CN -7: lle l7 I , Thr1 69) and (d) (CN-11: lle 17 I (Fig-2). *ln silico* pharmacokinetics (ADME) properties The movement of drug into the target site and its action is based on its physical and chemical properties at its limited range. This investigation driven us to calculate properties of both physical and chemical properties molecular weight, partition coefficient, n-no of hydrogen bond donors, n-no of hydrogen bond acceptors, n-no of rotatable bonds and total polar surface area screened using Swiss ADME predictor and were tabulated in (Table 5).Surprisingly, all the cinnoline compounds obey Lipinski's rule of five.

In antibacterial studies, the minimal inhibitory concentration (MIC) was well determined for all the synthesized compounds with efficient antibacterial profile in the preliminary screening against *E. coli* and *K.pneumonia* (a Gram-negative and amp;facultative anaerobic bacteria), *S. aureus* and *Bacillus subtilis* (a gram-positive and amp; facultative anaerobic bacterium).The measured zone of inhibition and MIC was noted in (Table 6 and 7).Surprisingly, almost all of the synthesized cinnoline compounds were observed to be potent against *E.coli* and few out of total synthesized compounds has exhibited appreciable antibacterial activity against *K. pneurnoniae*. Compounds CN- 11 and CN-7, CN-2 designed as the best newer antibacterial drugs Against *E. coli* with MIC 12.5µg/ml.The potency of anti-bacterial activity was attained due to the attachment of the active group 6-chloro-7-nitro substituent groups of cinnoline ring system. Electro negativity of chlorine atom is responsible for efficient activity.lt was very interesting to see that compound-7 with carboxylic group at 6 the position, showed significant Antibacterial activity against *E.coli* and *K.pneumoniae*. lt was asserted that liphophilict y causes increased activity(Fig-3). The result is also proven that the Tested compounds exhibited better activity against *E.coli* and *K.pneumonia* than *S.aureus*. Cinoxacin, a cinnoline derivative exerts its antibacterial action through the inhibitory action of the bacterial enzyme, DNA-gyrase.

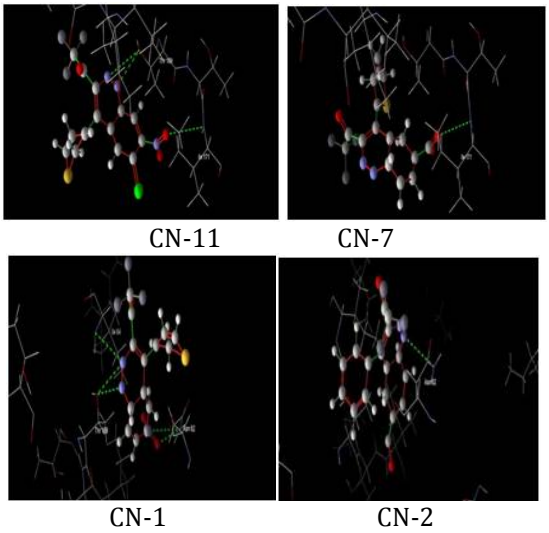


Figure 3: 2D plot of ligands protein interaction profile by MVD. Visualization of hydrogen bond interaction between 1a and (a) DNA Gyrase B Receptor (Ile 171, Thr 169), (b) (Ile 171), (c) (Tyr-169, Ile 84, Asn 52) (d)(Asp 79).

CONCLUSION

The present work depicts the significance of synthesized compounds with profound activity against bacterial strains when compared over the standard drug with good percentage of yield. These derivatives had proven to be best potent drug for fighting against microbes. The target selected for docking was DNA gyrase .TheMol Dock scores of the fourteen tested compounds range between - 108 and - 139.Docking analysis supports antibacterial result. Lead compound identification can be better possible with development of cinnoline molecule by optimization of pharmacodynamic and pharmacokinetic properties. All the synthesized compounds had show potent activity on lesser concentration because of presence of cinnoline ring system.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

REFERENCES

1. Yang, Z., Kitano, Y., Chiba, K., Shibata, N., Kurokawa, H., Doi, Y., ... & Tada, M. (2001). Synthesis of variously oxidized abietanediterpenes and their antibacterial activities against MRSA and VRE. *Bioorganic & medicinal chemistry*, *9*(2), 347-356.
2. Chandiran, P. R., Premnath, D., & Kumar, S. V. Design, Synthesis, Molecular Docking And Antibacterial Evaluation of Novel N-(6, 11-Dioxo-Dihydro-5h-Benzo [B] Carbazol-2yl) Benzamide Derivatives As Potent Antibacterial Agents. International Journal of Pharmacy and Pharmaceutical Sciences6(6): 244-249
3. Lewgowd, W., &Stanczak, A. (2007). Cinnoline derivatives with biological activity. *Archiv der Pharmazie: An International Journal Pharmaceutical and Medicinal Chemistry*, *340*(2), 65-80.
4. Wiederhold, N. P. (2017). Antifungal resistance: current trends and future strategies to combat. *Infection and drug resistance*, *10*, 249.
5. Ventola, C. L. (2015). The antibiotic resistance crisis: part 1: causes and threats. *Pharmacy and therapeutics*, *40*(4), 277.
6. Bantick, J., Hirst, S., Perry, M., & Phillips, E. (1998). Preparation of Fused Pyridazines as Allergy Inhibitors and Antiinflammatories. PCT Int. Appl. WO 9809969, 1998. In *Chem. Abstr* (Vol. 128, p. 217375).
7. Awad, E. D., El-Abadelah, M. M., Matar, S., Zihlif, M. A., Naffa, R. G., Al-Momani, E. Q., & Mubarak, M. S. (2012). Synthesis and Biological Activity of Some 3-(4-(Substituted)-piperazin-1-yl) cinnolines. *Molecules*, *17*(1), 227-239..
8. Ramalingam, P., Ganapaty, S., Rao, C. B., & Ravi, T. K. (2006). Synthesis of some new coumarino [4, 3-b] pyrido [6, 5-c] cinnolines as potent antitubercular agents. *Indian Journal of Heterocyclic Chemistry*, *15*(4), 359-362.
9. Yu, Y., Singh, S. K., Liu, A., Li, T. K., Liu, L. F., &LaVoie, E. J. (2003). Substituted dibenzo [c, h] cinnolines: topoisomerase I-targeting anticancer agents. *Bioorganic & medicinal chemistry*, *11*(7), 1475-1491.
10. Rehse, K., &Gonska, H. (2005). New Pyrimido [5, 4-c] cinnolines with Antiplatelet Activities. *Archiv der Pharmazie: An International Journal Pharmaceutical and Medicinal Chemistry*, *338*(12), 590-597.
11. Giamarellou, H., & Jackson, G. G. (1975). Antibacterial activity of cinoxacin in vitro. *Antimicrobial agents and chemotherapy*, *7*(5), 688-692.
12. Wakelin, L. P. G., & Waring, M. J. (1990). DNA intercalating agents. *Comprehensive medicinal chemistry*, *2*, 703-724.

13. McDonnell, J. R., Reynolds, R. G., &Fogel, D. B. (1995). Docking conformationally flexible small molecules into a protein binding site through evolutionary programming.
14. Kannan, V., Sitty, M. B., &Periyannan, M. (2018). Design synthesis in silico in vitro and in vivo evaluation of novel l-cysteine derivatives as multi-target-directed ligands for the treatment of neurodegenerative diseases. *Beni-Suef University journal of basic and applied sciences*, *7*(4), 452-460..
15. Gautam, N., &Chourasia, O. P. (2010). Synthesis, antimicrobial and insecticidal activity of some new cinnoline based chalcones and cinnoline based pyrazoline derivatives.
16. Thomsen, R., & Christensen, M. H. (2006). MolDock: a new technique for high-accuracy molecular docking. *Journal of medicinal chemistry*, *49*(11), 3315-3321..
17. Daina, A., Michielin, O., &Zoete, V. (2017). SwissADME: a free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules. *Scientific reports*, *7*, 42717.
18. Gfeller, D., Grosdidier, A., Wirth, M., Daina, A., Michielin, O., &Zoete, V. (2014). SwissTargetPrediction: a web server for target prediction of bioactive small molecules. *Nucleic acids research*, *42*(W1), W32-W38..
19. Gopal, J., Chun, S., Anthonydhason, V., Jung, S., Mwang'ombe, B. N., Muthu, M., &Sivanesan, I. (2018). Assays evaluating antimicrobial activity of nanoparticles: a myth buster. *Journal of Cluster Science*, *29*(2), 207-213.
20. Awad, E. D., El-Abadelah, M. M., Matar, S., Zihlif, M. A., Naffa, R. G., Al-Momani, E. Q., & Mubarak, M. S. (2012). Synthesis and Biological Activity of Some 3-(4-(Substituted)-piperazin-1-yl) cinnolines. *Molecules*, *17*(1), 227-239.
21. Gautam, N., &Chourasia, O. P. (2010). Synthesis, antimicrobial and insecticidal activity of some new cinnoline based chalcones and cinnoline based pyrazoline derivatives. IJCB 49 B, 830-835
22. Narayana, B., Raj, K. K., Ashalatha, B. V., &Kumari, N. S. (2006). Antibacterial and antifungal studies on some new acetylcinnolines and cinnolinylthiazole derivatives. Indian J. Chem 45, 1704-1709

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