Original Article

An Expeditious Approach to the Synthesis of Novel Quinolino and Diazacino Condensed Analogues of Azepino [3, 2-b] Carbazole-2-one of Medicinal Interest

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ABSTRACT

Background: Quinolino and diazacino are important heterocyclics moiety, which have been reported to possess various activities such as antiallergenic, antifungal, hypocholesterolemic, antibacterial and antiviral activities. The activities of these compounds were related to inhibition of bacterial dehydrogenase enzyme which is one of the important targets studied for designing of antimicrobial drugs. Further, molecular docking approached is used in the present study for confirming potent molecules. **Objectives:** Aim of the study is to synthesize a series of quinolino and diazacino condensed analogues and evaluation of their anti-microbial activity. **Methods:** Quinolino and diazacino condensed analogues of azepino [3, 2-b] carbazole-2-one were synthesized by Friedel-Craft acylation and Pfitzinger reaction. Structures of synthesized compounds were characterized by FTIR and HNMR and were evaluated for antimicrobial activity by *in-vitro* bacterial dehydrogenase activity agar well diffusion assay. Further, in order to determine the binding affinity, molecular docking of synthesized compounds was also performed using bacterial (3NUH of *E. coli*) and fungal proteins (1FI4). In addition, bacterial dehydrogenase inhibitory activity of most active compound was performed using MTT assay. **Results:** Synthesized compounds (3, 4 and 6) caused impressive antibacterial and antifungal activities *in-vitro* assay when compared to the ciprofloxacin and fluconazole. And molecular docking studies also revealed that the synthesized compounds 3, 4 and 6 exhibits good to excellent affinity towards target microbial proteins. **Conclusion:** Synthesized compounds (3, 4 and 6) hold substantial antibacterial potential and require further exploration to establish them as therapeutic candidates in clinical management.

Key words: Antimicrobial, Azepino [3, 2-b] carbazole-2-one, Pfitzinger reaction, Beckmann rearrangement, Organocatalyst.

INTRODUCTION

Carbazoles exhibits a variety of biological activities and because of this, their derivative form an attractive target in synthesis.[1] The proven record of impressive pharmaceutical properties shown by carbazole derivative is attributed to the planarity of its molecular framework. It is this structural feature of carbazoles that confers it the ability to undergo intercalation with DNA chain. This property is of paramount importance in manifestations of its mutagenic or anti-neoplastic behaviour.[2] Unlike to many other organic compounds, the carbazoles have an unique feature to act as building blocks for electroluminescent materials and light emitting diodes.[3,4] Owing to their innate ability and inherent potentials, they have remained in the mainstay on the chemical scene, as evergreen agents in the discovery of novel drugs. Recently, two carbazole analogues a pyridozepine-9-bromo derivative, (1)[5] and azpaullone (9-cyano-

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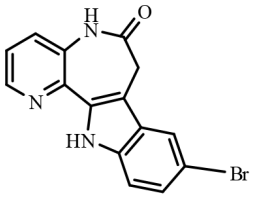
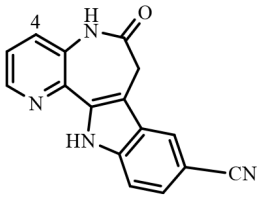
1-aza paullone (2))[6] have been reported to exhibit impressive activity in promoting β-cell protection and replication. A further study on enzymatic assays on these revealed that some 1-azapaullones showed selective GSK-3 inhibitory properties and promoted INS-1E β-cell against glucolipotoxicity induced cell death. It also stimulated the regulator of β- cell growth and development through the mRNA expression of the β-cell transcription factor Pax 4.

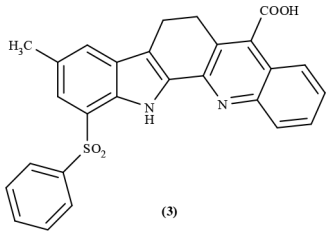


(1)



(2)

A survey of literature on carbazole and quinoline derivatives revealed that a quinoline-4-carboxylic acid derivative viz, (2'-nitrophenyl)-3-methyl-5,6,13-trihydro carbazole [1,2-b] quinoline-7-carboxy-1-sulfone(3) wherein a carbazole nucleus is fused to quinoline ring showed significant antimicrobial activity.[7] Quinoline nucleus occurs abundantly in many synthetic materials.[8] Many of these have been known to exhibit antiviral,[9] anti-fungal,[10] antibacterial,[11] Antiallergenic,[12] hypocholesterolemia[13] and hypovolemic[14] etc. activities. Besides this thieno and benzothieno quinolines show remarkable antifungal, antibacterial and anti-tumour activities.[15] It has been reported that quinoline molecular framework embellished with styryl motif show HIV integrase inhibitor activity.[16]



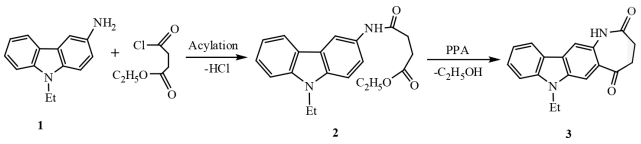
(3)

It is evident from the literature that heterocyclics that contain quinolones,[17] carbazoles,[18] azepinones[19] etc. in their molecules exhibit a wide variety of biological activities such as, anti-mycobacterial,[20] anti-leishmanicidal,[21] antitumor,[22] anti-proliferative[23] and anti-leukemic[24] etc. With a view to explore their pharmaceutical potential further, it occurred to as mind to undertake the synthesis of some such molecules which incorporated (a) quinoline-4-carboxylic acid and azepinone framework (b) diazocene pharmacophores on to the carbazole nucleus. In scheme-2 where the synthesis of these compounds have been shown, one can easily decern in compound 4 the presence of carbazole nucleus on one side of the azepinone nucleus and quinoline-4-carboxylic acid on its other side. In compound 6 one can see the presence of eight membered diazocene nucleus attached to the carbazole ring. This concept of synthesis emerged on this premise that the presence of these pharmacophores in tandem as a part of the same molecule could bring a favorable impact on the overall biological activity of carbazole nucleus. We report in this communication that the application of Pfitzinger reaction on 3 and Beckmann rearrangement on 5 provided a very convenient synthetic entry to 4 and 6 respectively.

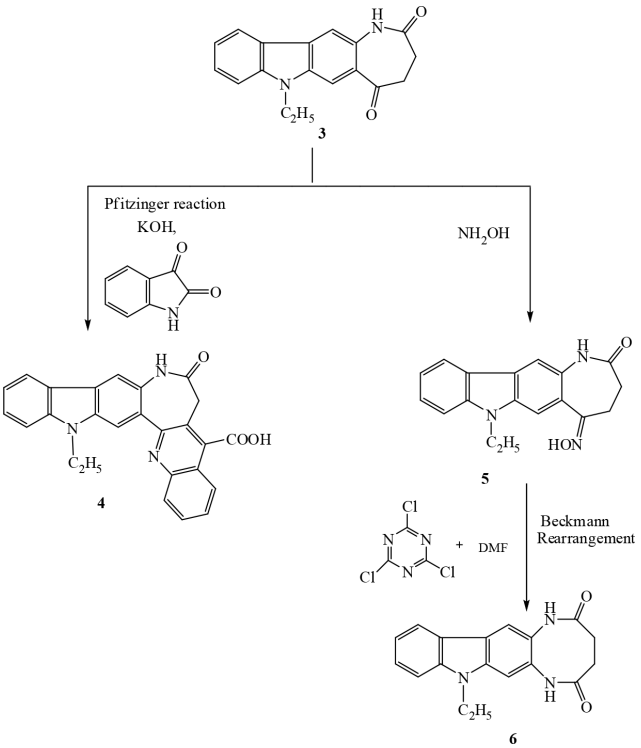
Synthetic strategy given in Scheme 1 and 2 was followed to afford the target compounds.

Experimental

Open capillary tube method was employed to record the melting point of the compounds. IR, and ^{1}HNMR spectra (in $CDCl_3$ expressed in δ ppm with tetramethylsilane (TMS) as internal reference) were recorded respectively,



Scheme 1



Scheme 2

on Schimadzu FTIR 8400S and Bruker DRX-400 MHz spectrometer.

Synthesis of ethyl 4-(9-ethyl-9H-carbazol-3-ylamino)-4-oxobutanoate (2)

Ethyl succinyl chloride (0.01 mole) and 3-Amino-9-ethyl carbazole (1) (0.01 mole) were mixed in 5ml dry pyridine and refluxed for 15 min. Resulting mixture was transferred in 200 ml ice cold water with constant stirring. The settled solid was filtered, washed with cold water. Further, the target compound 2 (yield 83%, m.p. 89-90°C) was achieved after recrystallization from hot water containing a few drops of methanol.

Synthesis of 7-ethyl-3, 4 dihydroazepino [3,2-b] carbazol-2,5 (1H,7H)-dione (3)

A mixture of ethyl 4-(9-ethyl-9H-carbazol-3-ylamino)-4-oxobutanoate (2) (0.006 mole) and PPA (5ml) was heated at 150°C for 4 h with intermittent TLC monitoring. The pH of resulting mixture was increased by adding a concentrated Na_2CO_3 solution after cooling it up to 20°C. The compound was extracted with ethyl acetate and dried over anhydrous Na_2SO_4 and concentrated in vacuo. The residue thus obtained was purified by column chromatography on silica gel with $CHCl_3$ as an eluent to give 3 (yield 60%, m.p. 158-160°C). IR (KBr) cm^{-1} 3342[NH str.], 2945[C-H str.ArH], 1702 [C=O str.], 1713 [C=O], 1454[C-H str. CH_3], 1070[C-N str.]. ^{1}H-NMR($CDCl_3$) δ, 8.0(1H,s,NH), 7.77 (1H,s,CH), 7.64(1H,s,CH), 7.56(1H,d,CH), 7.42(1H,d,CH), 7.06(1H,t,CH), 7.04 (1H,t,CH), 3.99(2H,q,CH_2), 2.90(2H,t,CH_2), 2.45(2H,t,CH_2), 1.50(3H,t,CH_3). MS: [m/z] 293 (19%); Anal. Calcd./found for $C_{18}H_{16}N_2O_2$: C, 73.99/73.63; H, 5.56/ 5.57; N, 9.55/ 9.14

Synthesis of 2-oxo-4, 6-dihydro-N-ethyl-carbazolo-1H-azepino[4,5-b]quinoline-11-carboxylic acid (4)

Equimolar quantities of 7-ethyl-3,4dihydroazepino[3,2-b] carbazol-2,5 (1H,7H)-dione (3) and isatin was mixed in 50% aq. EtOH containing KOH (3g) and heated under reflux for 20 h. Further, the mixture was diluted with 50% aq. EtOH to obtain a homogenous mixture and acidified with AcOH. The precipitate was collected, washed with 30% aq. EtOH and recystallized from MeOH to give 4, (yield 62%, m.p. 295-296°C). IR (KBr) cm^{-1} 3474[OH str.], 3312[NH str.], 2853[C-H str. ArH], 1784[C=O str.], 1718[C=O str.], 1624[C=C str. ArH], 1480[C=N str.], 1078[C-N str.], ^{1}H-NMR($CDCl_3$)δ 10.8(1H, s, OH), 9.00(1H, d, CH), 8.32(1H, s, CH). 7.78 (1H,t,CH), 7.74 (1H,t,CH), 7.60 (1H, s,CH), 7.55(1H,d,CH), 7.41(1H,d,CH), 7.10(1H,t,CH), 7.00(1H,t,CH), 3.99(2H,q,CH_2), 3.42(2H,s,CH_2), 1.54(3H,t,CH_3); MS : [m/z] 421 (20%); Anal. Calcd./ found for $C_{26}H_{19}N_3O_3$: C, 74.14/ 74.35; H, 4.58/ 4.51 ; N, 9.97/ 9.52.

Synthesis of 8-ethyl-5-(hydroxyimino)-4, 5-dihydro-1H-azocino [3, 2-b] carbazol-2,6-(3H,8H)-dione (5)

In a round bottom flask (500 ml) hydroxylamine hydrochloride (20mmol), 7-ethyl-3, 4 dihydroazepino [3,2-b] carbazol-2,5 (1H,7H)-dione (3) (10mmol), 50 ml of rectified spirit and 10 ml of water were taken. Solid sodium hydroxide (pellet form) (2.8 g) was added slowly with continuous stirring the to avoid vigorous reaction the flask was cooled in running tap water. The mixture was refluxed for 30 min when all the sodium hydroxide pellets were dissolved. The contents of the flask were cooled and poured into a concentrated HCl (7%). The desired product 5 (yield 67%, m.p. 180-182°C) was obtained after filtration followed by washing and recrystallization from methanol. IR (KBr) cm^{-1} 3340[NH str.], 2925[C-H str.ArH], 1780-1707[C=O str.], 1639[C=C str. ArH], 1432 [C=N str.], 814 [mono-sub]. ^{1}H-NMR($CDCl_3$)δ 11.11 (1H,s,OH), 8.19 (2H,s,CH), 8.3 (1H,s,NH), 7.7 (1H,d,CH), 7.9 (1H,s,CH), 7.51 (1H,d,CH),7.35 (2H,t,CH), 3.79 (2H,q,CH_2), 3.16 (2H,t,CH_2), 2.42 (2H,t,CH_2), 1.27 (3H,t,CH_3). MS: [m/z] 301 (25%); Anal. Calcd./found for $C_{18}H_{17}N_3O_2$: C, 70.34/ 70.72; H, 5.58/ 5.54; N, 13.67/ 13.96.

Preparation of 8-ethyl-3, 4-dihydro-1H-[1, 4] diazocino[2,3-b]carbazole-2,5(6H,8H)-dione (6)

2, 4, 6-Trichloro-1, 3, 5 triazine (TCT) (1.84g, 10.0mmol) was added to DMF (2ml), maintained at 25°C. After the formation of white solid, the reaction was monitored (TLC) until to the complete disappearance of TCT. Then, 8-ethyl-5-(hydroxyimino)-4, 5-dihydro-1H-azocino [3, 2-b] carbazole-2, 6(3H, 8H)-dione (5) (3.35g, 10mmol) in DMF (15ml) was added. The mixture was stirred at room temperature, monitored (TLC) until the completion of reaction (20h). Water (20ml) was added, the solid obtained was washed with 15ml of a saturated solution of Na_2CO_3, followed by 1N HCl and brine. It was then dried over anhydrous sodium sulphate and the solvent was evaporated to give 6, 2.70g (yield 87%), m.p. 155-157°C IR (KBr) cm^{-1} 3354 [NH str.], 2937 [C-H str. ArH], 1778-1705[C=O str.], 1631[C=C str. ArH], 1420[C=N str.], 810 [mono-sub]. ^{1}H-NMR($CDCl_3$) δ 9.0 (2H,s,NH), 7.45(1H,d,CH), 7.36 (2H,s,CH), 7.39(1H,d,CH), 7.06(1H,t,CH), 7.03(1H,t,CH), 3.87 (2H,q,CH_2), 2.56 (4H,s,CH_2), 1.49 (3H,t,CH_3). MS : [m/z] 307 (25%,); Anal. Calcd. /found for $C_{18}H_{17}N_3O_2$: C, 70.34/ 70.72; H, 5.58/ 5.54; N, 13.67/ 13.96.

Molecular docking

Docking of the desired heterocyclic scaffolds 3, 4 and 6 obtained from scheme-1 and 2 was carried out to determine the binding ability of the active materials in binding area of the bacterial and fungal proteins. The target proteins were retrieved from the protein databank PDB ID for bacterial protein is 3NUH of *E. coli* and for fungal protein 1FI4 as Mevalonate 5-diphosphate decarboxylase was used. The solvent molecules were removed, hydrogen was added and saved as pdb for further docking. To get a stable conformation of the protein molecule the 2D structure of the compounds were converted in 3D structure with energy minimization and saved as pdb file. All the docking studies were done by using Arguslab,[25] after completion of the docking, 2D pose was determined by discovery studio visualizer.[26] In the present study ciprofloxacin and fluconazole was used as standard for bacterial and fungal studies respectively.

In-vitro bacterial dehydrogenase activity

Bacterial dehydrogenase inhibitory testing of synthetic compounds (3, 4 and 6) was performed using [3-(4,5-dimethylthiazol-2-yl) -2,5-diphenyltetrazolium bromide] tetrazolium (MTT) for experimental reduction by specific modifications.[27]

Briefly, a 200 µL bacterial culture (prepared at 0.5 McFarland turbidity standard, approx. 10^8 CFU / ml) was inoculated in 10 ml YEMB medium and incubated at 37°C for 12 hr. 1 ml bacterial cells were harvested at 12,000 rpm for 2-3 min and washed twice with PBS buffer (pH 7.0) and resuspended with the same buffer followed by addition of 0.1 mg / ml MTT. The sample was incubated for 6h in dark at 37°C. Further, samples were withdrawn at various incubation periods and subsequently 30 µl formaldehyde was added to extinguish the reaction. Finally, the absorbance was recorded at 570 nm using a UV-Vis spectrophotometer (Labindia, India).

RESULTS AND DISCUSSION

For making our synthetic protocol depicted in scheme-2 to succeed to furnish 4, 5 and 6, a suitable strategy for the formation of the intermediate 3 was required. As shown in scheme-1, the synthesis of 3 was achieved in two steps from 9-ethyl-3-amino carbazole (1). 1 underwent a smooth interaction with ethyl succinoyl chloride to give the ester 2. Cyclocondensation of 2 under the conditions of Friedel-Craft-acylation with PPA afforded 3 in good yield.

Molecules containing a $COCH_2$ group are prone to undergo reaction with isatin in strongly alkaline medium. This reaction offers an easy convenient entry to quinoline-4-carboxylic acids.[28] This strategy was applied on 3 to form 4 in moderate to good yield.

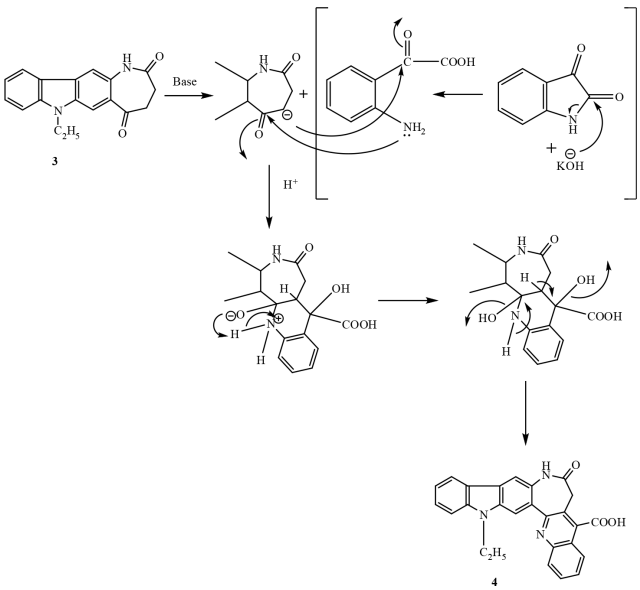
Diazocene derivative 6 was realized through a very mild Beckmann rearrangement of the ketoxime 5 with an organo catalyst derived from TCT+ DMF. In usual practice a strong acid was employed to catalyze the rearrangement of ketoximes.[29] A search for an alternate mild reagent for this rearrangement suggested the use of the above organocatalyst.[30,31] In this method, the ketoxime interacted with the product formed from DMF + TCT (2, 4, 6 trichloro-1, 3, 5-triazine) to give the amide (Scheme 4). The rearrangement with this reagent was carried out with 1 mol. equiv. of ketoxime in DMF. Treatment of ketoxime 5 under these conditions formed the diazocene derivatives 6 in an excellent yield.

The formation of the products 4 from 3 and 6 from 5 have been rationalized through the mechanistic pathways portrayed in schemes 3 and 4 respectively.

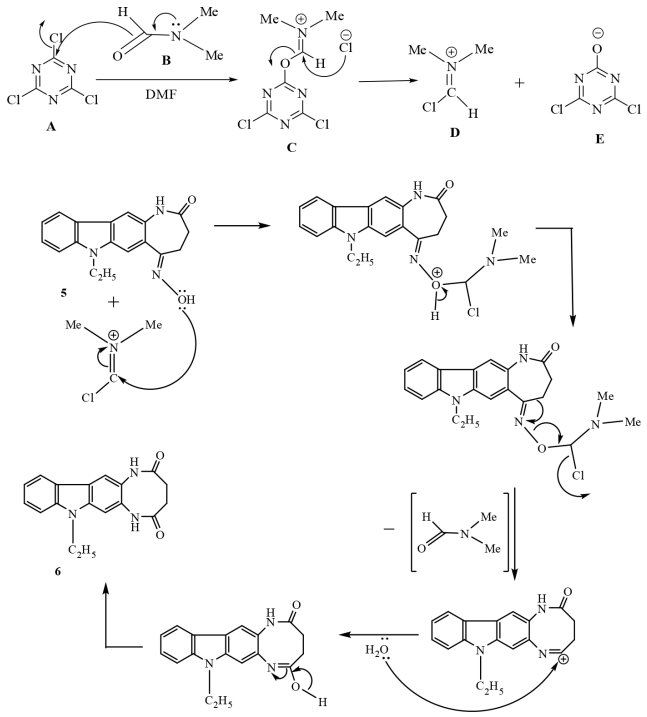
The authenticity of the products 3, 4 and 6 were corroborated from their physical data viz; (microanalytical, IR, ^{1}HNMR and MS spectral data).

Antibacterial and antifungal activity

7-Ethyl-3,4 dihydroazepino[3,2-b] carbazole-2,5 (1H,7H)-dione (3), quinoline-4-carboxylic acid derivative (4) and diazocino derivative (6) were screened for their *in-vitro* antibacterial and antifungal activities using bacterial species *(B. substilis, E. coli,* and *P. aeruginosa)* and fungal species *(F. oxysporum, M. phaseolina,* and *A. flavus)* employing disc-diffusion method. $CHCl_3$ was used



Scheme 3: Formation of 4 from 3 by Pfitzinger Reaction.



Scheme 4: Formation of 6 from the oxime 5 by Beckmann rearrangement using TCT+DMF.

as solvent for preparation of the stock solutions and further dilutions.

Compound (s) 3, 4 and 6 was examined *in vitro* for antibacterial potential against three different bacterial strains viz. *B. subtilis, E. coli*, and *P. aeruginosa*. Zone of inhibition for each compound were determined and compared with ciprofloxacin. All the three compounds were found active and exhibited impressive inhibition of targeted strains. At 400 µg/ml, compound 4 and 6 caused maximum inhibition of *E. coli* whereas compound 3 exhibited maximum inhibition of *B. subtilis*. The compound 6 showed only moderate activity (Table 1).

Similarly, in antifungal study, all the three compounds 3, 4, 6 caused impressive inhibition of targeted fungal strains. In particular, compound 4 (400 µg/ml) showed maximum inhibition of *A. flavus* followed by compound 6. Compound 3 (400 µg/ml) exhibited highest inhibitory activity against *M. phaseolina*.

Molecular docking

The affinity of compounds was presented by docking score (binding energy in kcal/mol), more the negative value suggests the better binding affinity. The standard ligand Ciprofloxacin and Fluconazole have docked efficiently with very good docking score -7.58kcal/mol and -8.41 kcal/mol (Table 2 and 3) with the target proteins (PDB Id-3NUH and 1FI4). The nitrogen and oxygen atoms of the selected compound were available

Table 1: Antibacterial and antifungal activity of compounds 3, 4, 6 and standard(s).

Comp. no.	Conc. (µg/ml)	*E. coli*		*B. subtilis*		*P. aeruginosa*		*M. phaseolina*		*F. oxysporum*		*A. flavus*	
		A	B	A	B	A	B	A	B	A	B	A	B
3	400	20±0.5	71.42	27±0.4	90.00	16±0.6	61.53	19±0.7	73.07	18±0.7	62.06	19±0.4	70.37
	200	14±0.7	63.64	18±0.7	78.26	11±0.8	55	13±0.3	65.00	13±0.5	56.52	13.5±0.6	56.25
	100	9±0.3	56.25	11.5±0.6	67.64	7±0.4	46.67	8.5±0.8	56.67	9±0.3	50.00	9±0.3	47.36
4	400	25±0.4	89.28	25±0.4	83.33	20±0.3	76.92	16.5±0.8	63.46	16±0.5	55.17	24±0.3	88.88
	200	17±0.8	77.27	17±0.7	73.91	13.5±0.6	67.5	11.5±0.7	7.50	11±0.6	47.82	18±0.6	75
	100	11.5±0.6	71.80	11±0.2	64.70	9±0.2	60	7.5±0.3	50.00	8±0.2	44.44	12±0.7	63.15
6	400	21±0.2	75.00	20±0.9	66.67	19±0.3	73.07	13.5±0.5	51.92	23±0.4	79.31	21±0.5	77.77
	200	15±0.9	68.18	13±0.3	56.52	13.5±0.7	67.5	9.5±0.7	7.50	16±0.8	69.56	16±0.6	66.66
	100	9±0.5	56.25	9±0.5	52.94	10±0.6	66.66	6.5±0.8	43.33	11±0.3	61.11	9±0.8	47.36
Ciprof loxacin	400	28±0.2	100	30±0.1	100	26±0.3	100			--	--		
	200	22±0.3	100	23±0.2	100	20±0.1	100						
	100	16±0.2	100	17±0.1	100	15±0.3	100						
Flucon azole	400	--	--	--	--			26±0.1	100	29±0.2	100	27±0.3	100
	200							20±0.2	100	23±0.1	100	24±0.2	100
	100							15±0.2	100	18±0.3	100	19±0.3	100

Table 2: Molecular docking studies of compound 3-6 and ciprofloxacin on PDB Id-3NUH.

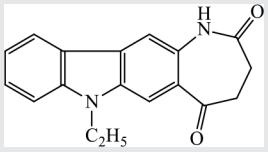
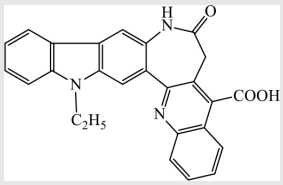
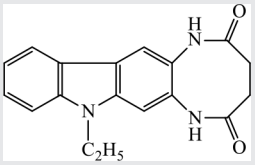
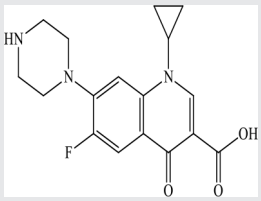
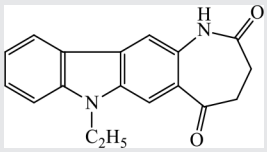
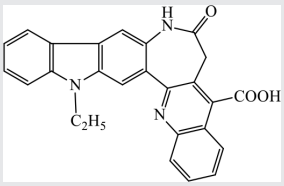
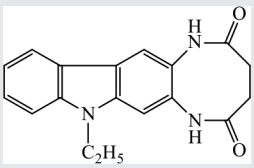
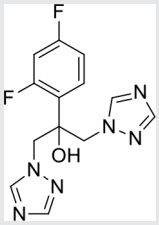
S.No	Structure	Docking score Kcal/mol	Amino Acids responsible for H bond formation	Bond distance
3		9.58	1315O-104ASP 1597N-126ARG 1600N-126ARG	2.99A 2.03A 2.65 A
4		10.168	2014N-154LYS	2.99A
6		9.10	9233O-619TYR 9233O-619TYR	2.72A 2.60A
Ciprofloxacin		7.58	500O- 50TYR	2.30A

Table 3: Molecular docking studies of compound 3-6 and fluconazole on 1FI4.

S. No	Structure	Docking score Kcal/mol	Amino Acids	Bond distance
3		-8.98	2269N-150ARG, 2270N-150ARG	2.72A 2.52A
4		-10.10	4049N-273TYR 4071N-275ASN 4118O-278SER	2.57A 2.99A 2.92A
6		-8.69	2373N-158ARG	2.99A
Fluconazole		-8.41	2373N-158ARG 3072O-208SER 152N-13ASN	2.90A 2.85A 2.4A

for the hydrogen bond formation with the different amino acids of the target protein. When the docking of synthesized compounds was performed on the selected proteins the docking score for compounds were shown the greater binding affinity as compare to ciprofloxacin (Table 2 and 3) particularly compound 4 (10.168kcal/mol) has the very good score. In the second docking study for anti- fungal activity all the compounds were comparable to the standard fluconazole and again compound no 4 has the highest docking score (-10.10 kcal/mol). Study of docking was also suggested that amino acid residues 104ASP, 126ARG, 154LYS, 619TYR, 619TYR, 126ARG, 50TYR were participates in the different bond formation like hydrogen bond, Pi-anion interaction, Pi-sigma interaction, alkyl and Pi-alkyl interactions for target protein and 158ARG, 119ALA, 155SER, 302ASP, 260PHE and 279PHS, 265LEU, 272PHE, 273TYR, 278SER are the amino acid residues play a key role for bond formation in fungal activity of the selected protein target (Figure 1-4).

In-vitro bacterial dehydrogenase activity

The ability of compounds (3, 4 and 6) to inhibit bacterial dehydrogenase was tested using the MTT trial.[32] Compounds has created a time-dependent

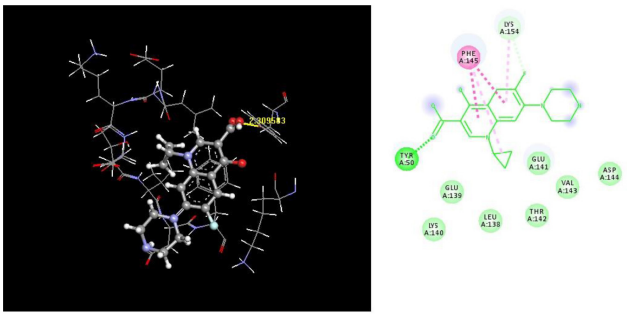


Figure 1: 3D and 2D pose view of the ciprofloxacin showing different bonds including hydrogen bonds and bond distance with different amino acid residues.

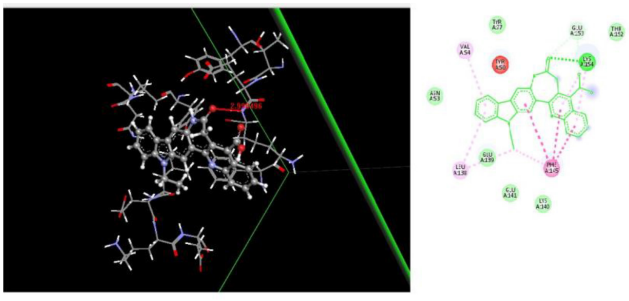


Figure 2: 3D and 2D pose view of the compound 4 showing different bonds including hydrogen bonds and bond distance with active amino acid residues.

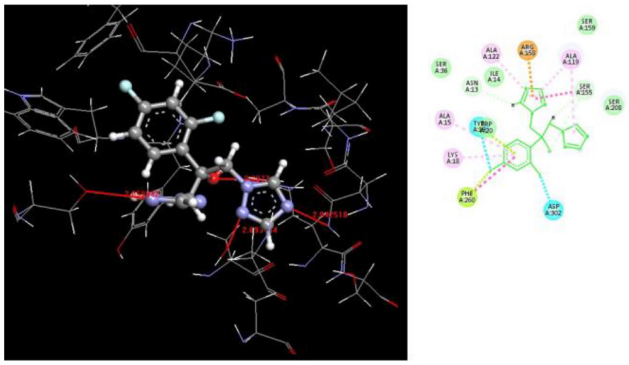


Figure 3: 3D and 2D pose view of the Fluconazole showing different bonds including hydrogen bonds and bond distance with different amino acid residues.

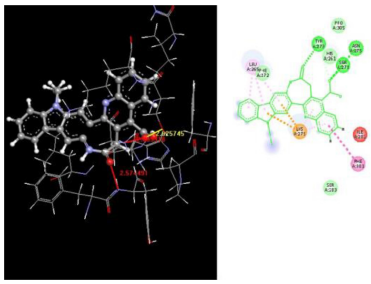


Figure 4: 3D and 2D pose view of the best active compound 4 showing different bonds including hydrogen bonds and bond distance with different amino acid residues.

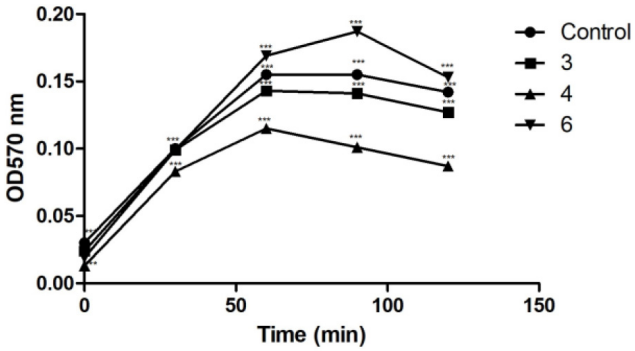


Figure 5: Enzymatic dehydrogenase activity of bacterial cell treated with different compounds (3, 4, and 6).

(The statistical significance was analyzed by two-way ANOVA test and compared with positive control in every time duration which represent $*p < 0.05$, $**p < 0.01$ and $***p < 0.001$ with corresponding standard deviation error of mean).

inhibition and concentration of bacterial dehydrogenase compared with good control. Compounds exposure (25µg/mL) has a rapid onset of bacterial dehydrogenase inhibition and is potent at 60 min as shown in Figure 5. The results suggested that compounds (3, 4 and 6) exert more bactericidal activity through the breakdown of the external and internal membranes and bacterial mitochondrial dehydrogenase inhibition.

CONCLUSION

In summary, an expeditious approach has been developed to provide an easy access to the biologically active novel azepinone and carbazolo fused quinoline-4-carboxylic acid (4) and carbazolo condensed diazocino derivative (6) respectively from 3 in high yield and purity. Synthesized compounds showed impressive antibacterial and antifungal activities in *in-vitro* assay. Molecular docking studies suggested that the synthesized compounds 3, 4 and 6 exhibits good to excellent affinity towards target microbial proteins. In addition, compound 4 also showed marked inhibition of bacterial dehydrogenase enzyme. The antimicrobial activity of synthesized compounds may be attributed to their affinity towards target microbial proteins. Synthesized compounds (3, 4 and 6) hold substantial antibacterial potential and require further exploration to establish them as therapeutic candidates in clinical management.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

ABBREVIATIONS

FTIR: Fourier-transform infrared spectroscopy; **^{1}HNMR:** Proton nuclear magnetic resonance; **E. coli:** *Escherichia coli;* ***B. subtilis*:** *Bacillus subtilis;* ***P. aeruginosa:*** *Pseudomonas aeruginosa;* ***M. phaseolina*:** *Macrophomina phaseolina;* ***F. oxysporum*:** *Fusarium oxysporum;* ***A. flavus*:** *Aspergillus flavus;* **MTT:** 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; **DNA:** Deoxyribose nucleic acid; **GSK-3:** Glycogen synthase kinase 3; **$CDCl_3$:** Deuterated chloroform; **IR:** Infrared spectroscopy; **TMS:** Tetramethylsilane; **PPA:** Phenylpropanolamine; **TLC:** Thin layer chromatography **Na_2CO_3:** Sodium carbonate; **Na_2SO_4:** Sodium sulfate; **EtOH:** Ethanol; **AcOH:** Acetic acid; **MeOH:** Methanol; **HCl:** Hydrochloric acid; **DMF:** Dimethylformamide; **PDB:** Protein data base; **TCT:** 2, 4, 6-trichloro-1, 3, 5-triazine.

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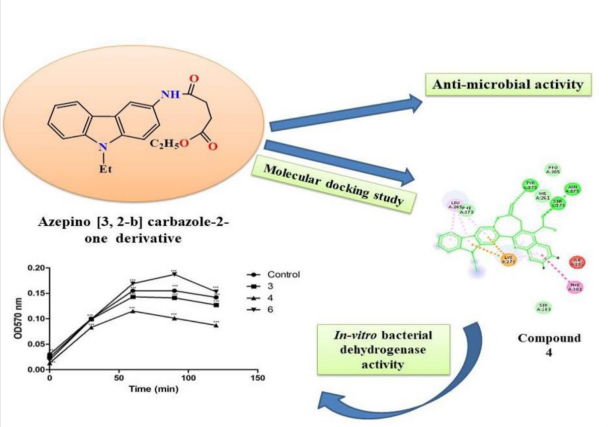
PICTORIAL ABSTRACT



SUMMARY

An expeditious procedure has been developed to achieve novel diazocino and quinolino condensed analogues employing Pfitzinger reaction and the Beckmann rearrangement. These synthetic compounds were screened for their *in-vitro* anti-bacterial and anti-fungal activities using agar well diffusion assay. Further, for determining the binding affinity, molecular docking of synthesized compounds was also performed using bacterial (3NUH of *E. coli*) and fungal proteins (IF14). In addition, bacterial dehydrogenase inhibitory activity of most active compound was performed using MTT assay. These test compounds hold substantial antibacterial potential and require further exploration to establish them as therapeutic candidates in clinical management.

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