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Design, synthesis, molecular docking studies and anti-microbial activity of novel 1,2,3,4-tetrahydrocarbazole derivatives

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ABSTRACT
The heterocyclic compounds naming tetrahydrocarbazoles having the significant biological properties. The newly substituted tetrahydrocarbazole derivatives were prepared from substituted phenylhydrazine and cyclohexanone in glacial acetic acid under reflux. These intermediates on reaction with substituted aromatic acid chlorides in alkaline media finally converted to N-Substituted tetrahydrocarbazoles. Fifteen compounds are synthesized and characterized by their melting point (MP), IR, NMR, MS and elemental analysis. All the compounds were subjected to molecular docking studies for Gln-6-p enzyme (1XFF) inhibition. The results of *in silico* molecular docking showed that all the derivates have significant binding energies, good affinity with active pocket and it may be reflected as a good inhibitor of GlcN-6-P synthase. The anti-microbial activity was assessed by agar cup plate method and the result showed 8 compounds having the better anti-microbial response against the bacterial and fungal strains. In conclusion, the study helps to give the greater scope of developing these tetrahydrocarbazoles derivatives which help to promote the effective anti-bacterial agents.

***Key Words*:** Tetrahydrocarbazoles, fisher indole synthesis, molecular docking, anti-microbial activity.

INTRODUCTION

In general, the microbes of mesophiles causes the most of the pathogenic diseases in the animals include human beings. In the present situation most of the pathogens getting more resistant to anti-microbials so, it is considered as a major issue all around the world. Hence, there is a significant need to develop new anti-microbial agents to fight against life threatening invasive infections. As per the new concepts of drug design, docking studies help to understand in the better way about the interactions of drugs with receptor. (Hughes *et al.*, 2011, Kapetanovic 2008, Meng *et al.*, 2013). The enzyme- GlcN-6-P synthase or glucosamine-6-phosphate synthase, is a new target for the drugs of anti-microbials (Vijesh *et al.*, 2013). It is a protein synthesis inhibitor in bacteria. It binds to a small 16s rRNA of the 30s subunit of the ribosome in bacteria which interferes the binding of formyl–methionyl-tRNA to the 30s subunit.

The tetrahydrocarbazoles (figure 1) represents leading group of the ring system and have attracted a great deal of activites. It mainly acts up on the peptide cyclin dependent kinases inhibitors (CDKs) (Zhu *et al.*, 2004), check point kinase inhibitors (Chk1) (Conchon *et al.*, 2008), and peroxisome proliferation activated receptors (PPAR α, β, and γ) (Kumar *et al.*, 2005). In addition, tetrahydrocarbazoles possess more active to treat the neoplastic and cardiovascular disorders (Kaushik *et al.*, 2012). Literature survey revealed that tetrahydrocarbazoles having the nitrogen atom having the rigid aromatic moiety helps to the electron transfer in the π-conjugated system (Chakraborty *et al.*, 2014, Zhang *et al.*, 2010) possess the wide range of pharmacological activities include anti-microbial activity, antipyretics, anti-inflammatory, antiproliferative, serum lipid lowering agent (Subramanyam *et al.*, 2014, Ya-ching *et al.*, 2005, Crosby *et al.*, 1947).

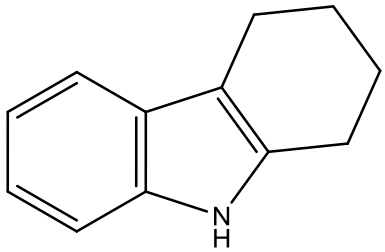

Figure 1: Structure of Tetrahydrocarbazole.

In the present study of tetrahydrocarbazoles, we are aimed to synthesize the tetrahydrocarbazole derivatives in an efficient manner. The objective of the study was to synthesize, characterize the N-substituted tetrahydrocarbazole compounds along with molecular docking studies of the synthesized compounds in associated with the evaluation of anti-bacterial activity by agar cup method.

MATERIALS AND METHODS

All the chemicals for the synthetic work were used of analytical grade from SD Fine Chem Limited, Merck, Loba Chemie Private limited and Sigma Aldrich. The residue has been dried in vacuum desiccator and recrystallised from ethanol. The melting points of the compounds were determined in open capillaries using thiel's tube. Precoated silicagel- G plates are activated at 110°C for 30 minutes were used for thin layer chromatography and the spots were developed in iodine chamber. The mobile phase for the analysis of TLC was n-hexane: EtoAc (7:3 v/v). The IR spectra of the compounds were recorded using pressed pellet technique using KBr on FTIR-8400 Spectrophotometer Shimadzu. ^{1}H –NMR and ^{13}C Spectra ($CDCl_3$, DMSO) were recorded on bruker advance-11, 400 Spectrophotometer on 400 MHz at Loyola index laboratories using TMS as internal standard. The chemical shifts are reported in parts

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per million. Mass spectras were recorded using Waters Instrument having Q-TOF-MS. Elemental analysis was carried out SAIF Punjab University, Chandigarh.

General Procedure

Synthesis of tetrahydrocarbazole

The synthesis of the tetrehydrocarbazole was carried out based up on the Fischer indole synthesis (Colin *et al.*, 2006) and the scheme was showed in figure 2.

Scheme 1: Synthesis of substituted tetrahydrocarbazole

Dissolve 8.8 gm (0.08mol) of cyclohexanone in 50 ml of glacial acetic acid add 8.8 gm (0.08mol) of substituted phenyl hydrazine and boil the solution under reflux for 15 min, cooled the solution, where the product was crystallised out. Filtered it with vacuum pump, then drained well and recrystallised using Ethanol.

Scheme 2: Synthesis of N-substituted Tetrahydrocarbazoles

Suspended 1 gm (0.07 mol) of substituted tetrahydrocarbazole in 20 mL of 10% NaOH solution into a well corked conical flask and add 2 mL of substituted acid chloride with constant shaking for 10 minutes until the odour has disappeared and then it was cooled. Filtered and washed the synthesized N-Substituted derivative using water and recrystallized with ethanol.

Computational Methods

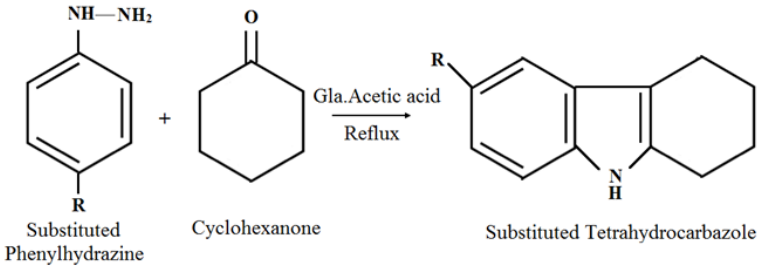
Molecular docking studies were carried out to explore binding modes of our derivatives with the target enzyme L-Glutamine: D -fructose-6–phosphate amido transferase. The molecular docking simulation studies were carried out by biopredicta tool of V Life MDS software version 4.2. The receptor employed here was Gln-6-p (PDB code 1XFF) obtained from RCSB protein data bank. The initial crystal structure consists of bound ligands it was removed and missing loops were added with help of homology modeling from the same software.

Anti-bacterial activity

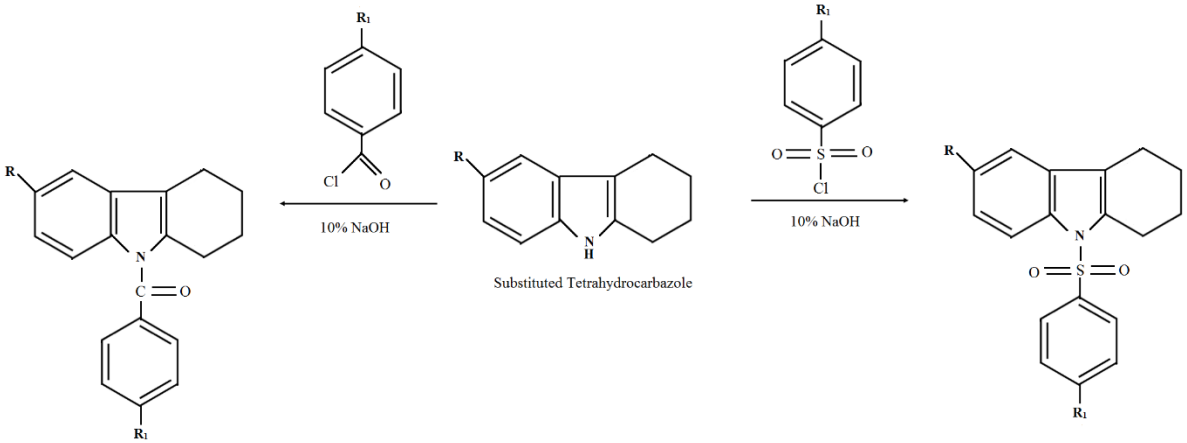
Determination by Agar cup method

The antibacterial activity of 1,2,3,4-tetrahydrocarbazolederivatives was studied by agar cup method. The nutrient broth culture media was chosen as basal medium for testing the microbe. The nutrient broth medium (Hi media M0001) was plated into Petri dishes, allowed to solidification and then the microbe was inoculated into broth medium and allowed for incubation for a period of 24 hours at 25°C. Bacterial culture was spread evenly over the entire surface to avoid the aggregation and left undisturbed for few minutes to permeate the culture. The wells/holes (4 mm) were drawn using a sterile borer into the solidified nutrient medium. The compounds of substituted tetrhydrocarbazoles were added to each well (100µL) at peripheral of the petridish and the reference compounds (ciprofloxacin for bacterial, flucanazole for fungal) was added at the centre and then the plates are incubated for 24

Scheme 1:



Scheme 2:



	1a	1b	1c	1d	2a	2b	2c	2d	3a	3b	3c	3d	3e	3f	3g
R	H	Cl	F	CH_3	H	Cl	F	CH_3	H	Cl	F	CH_3	Cl	F	NO_2
R_1	H	H	H	H	CH_3	CH_3	CH_3	CH_3	Cl	Cl	Cl	Cl	NO_2	NO_2	NO_2

Figure 2: Scheme of 1,2,3,4 substituted Tetrahydrocarbazoles.

hrs at 25°C. The plates were collected and analyze the zone of inhibition with respect to millimeters (mm) (Biswanath *et al.*, 2014, Rajesh *et al.*, 2015).

RESULTS AND DISCUSSION

By adopting above methodology the following mono, di-substituted tetrahydracarbazole were synthesized and it was showed in the scheme 1 and 2.

The synthesized tetrahydracarbazoles were analyzed using spectroscopic techniques. In the IR spectra the aromatic skeleton of the tetrahydrocarbazole appears at region of 1432-1630 cm^{-1} and characteristic –NH streching 3413-3410 cm^{-1}, C=O adsorption at 1730-1750 cm^{-1} and S=O adsorption peak at 1350-1405 cm^{-1}. The proton magnetic resonance spectra all the signals are at the respective positions. All the synthesized compounds give corresponding sharp M+1 peak in the Mass spectroscopy. The details of the spectral data are given below:

Spectral data:

1a.*2,3,4,9-Tetrahydro-1H-carbazole*

Yield 91 %; mp 117 – 119 ºC (ethanol); R_f0.39 (n – Hexane: EtoAC ,7:3); IR (KBr) cm^{-1}:3314.12(N-H stretching),2954.8 (C-H Stretching aliphatic),1623 (C=C Stretching),1513.7 (C-N stretching) ; ^{1}H NMR ($CDCl_3$, 400 MHz):2.08 (m, 2H, ABq, CH_2), 2.20 (m, 2H, ABq, CH_2), 2.28(m,4H,CH_2),3.90 (s, 1H, NH), 6.73 (m, 4H, Ar); ^{13}CNMR: 115.76, 118.50, 129 (Ar), 168.16, 169.10, 170.12 (Tetrahydrocarbazole); Em (Es, Positive mode) m/z 171.165; Anal. Calcd % for ($C_{12}H_{13}N$); C,84.59; H,9.68; N,5.80; Found: C,84.59; H,9.69; N,5.81.

1b.*6-Chloro-2,3,4,9-tetrahydro-1H-carbazole*

Yield 61.28 %; mp 121-123 ºC (ethanol); Rf 0.67 (n – hexane: EtoAc,7:3); IR KBr cm^{-1}: 3315.4 (N-H stretching) ,2948.4 (C-H Stretching aliphatic) ,1521.9 (C=C stretching),680.13 (C-Cl stretching); ^{1}HNMR($CDCl_3$,400MHz): 2.01(d.2H,CH_2), 2.18(m,2H,ABq,CH_2), 2.29 (m,4H,CH_2), 3.12 (s,1H,NH), 6.83 (m,3H,Ar): ^{13}CNMR:116.75, 118.50, 119.40 (Ar), 169.00, 169.14, 170.22 (Tetrahydrocarbazole, Ar): Em, (Es, Positive mode) m/z 205.02 (m+H): Anal. Calcd % for ($C_{12}H_{12}ClN$); C,70.67; H,5.83; N,6.80, Found: C,70.68; H,5.84; N,6.81.

1c.*6-Fluoro-2,3,4,9-tetrahydro-1H-carbazole*

Yield 65.38 %; mp142 -143 ºC (ethanol); Rf 0.68 (n – hexane : EtoAc,7:3); IR KBr cm^{-1}:317.12 (N-H Stretching),2924.4 (C-H stretching aliphatic), 1622.14(C=C stretching),1052.12(C-F stretching); ^{1}H NMR ($CDCl_3$,400 MHz): 2.09 (m, 2H, ABq, CH_2), 2.30 (m, 2H ABq, CH_2), 2.79(m,4H,CH_2),3.90 (s,1H, NH),6.43(m,3H,Ar);^{13}C NMR: 118.75, 119.50, 121.40 (Ar), 168.01, 169.14, 172.12 (TetrahydrocarbazoleAr); EM (ES, Positive mode): m/z 189.126 (m+H)$^+$; Anal. Calcd % for: ($C_{12}H_{12}FN$); C,76.17; H,6.34; N,7.39; F,10.04: Found: C,76.18: H, 6.35; N,7.38.

1d.*6-Methyl-2,3,4,9-tetrahydro-1H-carbazole*

Yield 55%; mp 212-284 ºC (ethanol); Rf 0.76 (n-hexane: EtoAC, 7:3); IR KBr cm^{-1}:3329.12 (N-H stretching),2884.4 (C-H stretching aliphatic),1622,67 (C=C stretching),738.78 (C-CH_3) ; ^{1}H NMR ($CDCl_3$, 400 MHz): 1.90(m, 4H, ABq, CH_2), 2.05 (m, 2H, ABq, CH_2), 1.35(m, 3H, CH_3), 3.92(s, H, NH), 2.30(m, 2H, ABq, CH_2), 6.53(m, 3H , Ar); ^{13}C NMR ($CDCl_3$,125 M Hz): 18.30(CH_3), 22.30, 30.15 (aliphatic), 114.76, 119.89, 127.10 (Ar), 168.13, 170.12, 171.14 (TetrahydracarbazoleAr); EM(ES positive mode): m/z185.124 $(m+H)^+$; Anal. calcd % for: ($C_{12}H_{15}N$); C,84.28; H,8.63; N,7.55: Found: C, 84.29; H, 8.64; N, 7.56.

2a: 9-[(4-methylphenyl)sulfonyl]-2,3,4,9-tetrahydro-1*H*-carbazole

Yield 61%;mp 322- 323 ºC (ethanol); Rf 0.86 (n-hexane EtoAC, 7:3) ; IR KBrcm^{-1}:3018.45(C-H aromatic Stretching) ,2856.78 (C-H aliphatic stretching),1601.78 (C=C aromatic stretching),1346.67(O =S = O Stretching) ,^{1}H NMR ($CDCl_3$, 400 MHz);2.28 (m, 4H, CH_2), 2.08 (m, 2H, ABq, CH_2), 1.35 (t, 3H, CH_3), 2.20 (m, 2H, ABq, CH_2), 6.83 (m, 4H, Ar), 7.95(m, 4H, Ar); ^{13}CNMR ($CDCl_3$, 125MHz); 17.30, 18.90, (CH_3), 21.96, 22.30, 30.15 (aliphatic), 114.76, 117.89, 127.10 (Ar), 167.13, 170.12, 171.14 (Tetrahydracarbazole Ar). EM (ES positive mode); m/z 328.76 (m+H) $^+$.Anal. calcd for($C_{19}H_{19}NO_2S$); C,70.70; H, 5.83; N, 4.30: Found: C,70.29; H,5.64; N, 4.56.

2b: 6-chloro-9-[(4-methylphenyl)sulfonyl]-2,3,4,9-tetrahydro-1*H*-carbazole

Yield 61%; mp : 338-340 ºC (ethanol); Rf 0.91 (n-hexane EtoAC,7:3) IR KBr cm^{-1}: 2950.68 (C-Haromatic stretching), 2876.98 (C-H aliphatic stretching), 1627.34 (C=C aromatic stretching), 1334.74 (O=S=O Stretching), 735.78(C-Cl stretching) : ^{1}H NMR ($CDCl_3$, 400 M Hz);2.21(m,4H,CH_2), 2.08 (m,2H, ABq ,CH_2) 1. 25 (t,3 H, CH_3), 2.10 (m,2H,ABq,CH_2), 6.23 (m 3H , Ar), 7.85 (m, 4H,Ar).^{13}C NMR ($CDCl_3$,125 M Hz)16.90,17.56, 18.30 (CH_3), 22.30,30.15(aliphatic), 114.96, 116.89, 128.10 (Ar) 166.13, 169.12, 171.15 (Tetrahydracarbazole Ar). EM (ES positive mode); m/z 358.76 (m+H)$^+$.Anal. calcd for($C_{19}H_{18}ClNO_2S$); C,63.70; H,5.00;N,3.30.Found: C, 63.79; H,5.04; N,4.91.

2c: 6-fluoro-9-[(4-methylphenyl) sulfonyl]-2,3,4,9-tetrahydro-1*H*-carbazole

Yield 63%;mp 342- 343ºC (ethanol); Rf 0.98 (n-hexane EtoAC,7:3); IR KBrcm^{-1}:3097.56 C-H aromatic stretching),2987.56 (C-H aliphatic stretching),1464.12(O =S = O Stretching),1598.35(C=C aromaticstretching) ,1143.79(C-F stretching) : ^{1} H NMR ($CDCl_3$, 400 MHz);2.16(m,4H,CH_2), 2.18 (m,2H, ABq , CH_2) 1. 35 (t,3H, CH_3), 2.60 (m, 2H, ABq, CH_2), 6.78 (m 3H , Ar), 7.25 (m, 4H,Ar);^{13}C NMR ($CDCl_3$, 125 M Hz) 16.30.17.89 (CH_3), 22.30,23.67, 30.15(aliphatic), 114.76, 119.89, 128.10 (Ar) 165.13, 169.12, 171.84 (Tetrahydracarbazole Ar). EM (ES positive mode); m/z 344.76 $(m+H)^+$.Anal. calcd for ($C_{19}H_{18}FNO_2S$); C, 66.45 ; H, 5.23; N, 4.07; Found: C, 66.49; H,5.24;N,4.06.

2d: 6-methyl-9-[(4-methylphenyl)sulfonyl]-2,3,4,9-tetrahydro-1*H*-carbazole

Yield 61%; mp 346- 348ºC (ethanol); Rf 0.91(n-hexane EtoAC 7:3); IR KBrcm^{-1}:3111.18(C-H aromatic Stretching), 2956.13 (C-H aliphatic stretching), 1537.98 (C=C aromatic stretching),1426.87(O=S=OStretching),924.24(C-CH_3Stretching); ^{1}HNMR ($CDCl_3$, 400MHz); 2.35(m,4H,CH_2), 2.38(m,2H,ABq ,CH_2)1.72(t,3H,CH_3),1. 25 (t,3 H, CH_3) , 2.20 (m, 2H, ABq, CH_2), 6.73 (m 3H , Ar), 7.35 (m, 4H,Ar) ^{13}C NMR ($CDCl_3$, 125 M Hz)17.34,19.30 (CH_3), 22.30,24.37, 30.15(aliphatic), 114.76, 124.89, 127.10 (Ar), 168.13, 169.12, 171.14 (Tetrahydracarbazole Ar). EM (ES positive mode); m/z 339.76 (m+H) $^+$.Anal. calcd for($C_{20}H_{21}FNO_2S$); C, 70.45; H, 6.23; N,4.12: Found: C, 70.49; H,6.24; N,4.13.

3a.(4-chlorophenyl)(1,2,3,4-tetrahydro-9*H*-carbazol-9-yl)methanone

Yield : 64.12 % : mp : 256-258ºC (Ethanol): R_f : 0.54 (n- hexanol : EtoAC 7:3): IR KBrcm^{-1} :3052.67 (C-H aromatic Stretching) ,2967.35(C-H aliphatic strectching),1702.78 (C=O stretching) ,1623.89 (C=C stretching Ar) ,739.38 (C-Cl stretching): ^{1} H NMR ($CDCl_3$,

Table 1: Docking scores of the synthesized compounds.

Sl. No.	Ligands	Docking scores	Hydrogen bond interactions
1	ciprofloxacine	-3.5399	Met28A-Pro29A-Leu30A-Thr36A-VAL31A
2	1a	-4.4808	Pro29A-LEU30A-Val31A-Thr36A-ILE37A-SER35A
3	1b	-4.6162	Leu30A-val31A-Ser35A-Thr36A-Ile37A
4	1c	-4.5488	Met28A-Pro29A-Val31A-Cys38A
5	1d	-4.2528	Met28A-Pro29A-Leu30A
6	2a	-4.2017	Leu30A-val31A-Ser35A-Thr36A-Ile37A
7	2b	-4.3146	Pro29A-LEU30A-Val31A-Thr36A-ILE37A
8	2c	-0.7244	Met28A-Ser35A
9	2d	-4.8527	Met28A-Pro29A-Val31A-Cys38A
10	3a	0.9109	Met28A-Ser35A
11	3b	-4.3018	Pro29A-LEU30A-Val31A-Thr36A-ILE37A
12	3c	-2.1018	Met28A-Ser35A-Ile37A
13	3d	-2.2002	Pro29A-LEU30A-Val31A-Thr36A-ILE37A
14	3e	-1.7063	LEU30A-Val31A-Thr36A-ILE37A
15	3f	-0.9294	LEU30A-Val31A-Thr36A-ILE37A
16	3g	-1.8973	Met28A-Ser35A

Table 2: Anti-microbial activity of synthesized compounds.

Sl. No.	Ligands	Zone of inhibitions in mm.					
		E. coli ATCC 25922	*S. aureus* ATCC 29213	*P. aeruginosa* ATCC27953	*B. subtilis* ATCC 6633	*C. albucans* NRRC477	*A. niger*
1	1a	18	16	13	11	10	13
2	1b	17	18	14	13	11	16
3	1c	16	17	16	14	10	15
4	1d	15	16	12	12	13	10
5	2a	16	17	11	11	9	12
6	2b	14	16	14	8	14	9
7	2c	15	13	10	13	12	10
8	2d	18	14	12	10	14	11
9	3a	14	16	11	8	10	13
10	3b	13	14	10	9	13	12
11	3c	15	8	12	11	11	9
12	3d	14	16	14	9	12	10
13	3e	18	14	11	10	11	11
14	3f	11	15	13	8	11	10
15	3g	12	14	11	16	10	
16	solvent	-	-	-	-	-	-
17	Ciprofloxacin	23	22	21	23	-	-
18	Fluconazole	-	-	-	-	22	21

400 MHz) : 2.09 (m, 2H, CH_2) , 2.21 (m, 2H , CH_2) , 2.98 (m.4H, CH_2) , 6.73 (m. 3H,Ar) , 7.36 (m, 5H, Ar) : ^{13}C NMR ($CDCl_3$,125 MHz) :119.76, 120.80, 121.70, 129.13, 133.82 (Ar), 167.13, 169.14, 171.13 (tetrahydrocarbazole (Ar) , 192 .90 (C=O): Em (Es, Positive mode) m/z 309.31: Anal, Calc for($C_{19}H_{16}NO$): C,73.58; H,5.16; N,7.99; F,4.41: Found: C,72.57; H,5.43; N,7.98.

3b.(4-chlorophenyl)(6-chloro-1,2,3,4-tetrahydro-9*H*-carbazol-9-yl)methanone

Yield : 55.23 % : mp : 260-262^{0}C (Ethanol): R_f : 0.58 (n- hexanol : EtoAC 7:3): IR KBrcm^{-1}
:3109.78 (C-H aromatic Stretching) ,2993.01(C-H aliphatic stretching),1748.12 (C=O stretching) ,1615.34 (C=C stretching Ar) ,780.15,736.12 (C-Cl stretching): ^{1}H NMR ($CDCl_3$, 400 MHz) : 2.10 (m, 2H, CH_2) , 2.32 (m, 2H , CH_2) ,3.78(m,4H,CH_2), 6.24 (m. 3H,Ar) , 7.89 (m, 4H, Ar) : ^{13}C NMR ($CDCl_3$,125 MHz) :119.71, 120.89, 121.74, 129.19, 133.84 (Ar), 167.16, 169.19, 171.15 (tetrahydrocarbazole (Ar) , 192 .98 (C=O): Em (Es, Positive mode) m/z 345.34: Anal, Calc for($C_{19}H_{15}Cl_2NO$): C, 66.28; H,4.35; N,4.06: Found: C,66.89; H,4.78; N,4.89.

3c.(4-chlorophenyl)(6-fluoro-1,2,3,4-tetrahydro-9*H*-carbazol-9-yl)methanone

Yield : 59.67 % : mp : 264-266^{0}C (Ethanol): R_f : 0.60 (n- hexanol : EtoAC 7:3): IR KBrcm^{-1}
:3111.18 (C-H aromatic Stretching) ,2943.37(C-H aliphatic stretching),1729.65 (C=O stretching) ,1615.36 (C=C stretching Ar) ,1130.29 (C-F stretching),745.23 (C-Cl stretching): ^{1}H NMR ($CDCl_3$, 400 MHz) : 2.08 (m, 2H, CH_2) , 2.32 (m, 2H , CH_2) ,2.86(m,4H, CH_2), 6.24 (m. 3H,Ar) , 7.89 (m, 4H, Ar) : ^{13}C NMR ($CDCl_3$,125 MHz) :119.78, 120.84, 121.754, 129.23, 133.67 (Ar), 167.45, 169.87, 171.23 (tetrahydrocarbazole (Ar) , 192 .13 (C=O): Em (Es, Positive mode) m/z 328.45: Anal, Calc for($C_{19}H_{15}ClFNO$): C, 69.61; H,5.49; N,4.32: Found: C,69.78; H,5.89; N,4.69.

3d. (4-chlorophenyl)(6-methyl-1,2,3,4-tetrahydro-9*H*-carbazol-9-yl)methanone

Yield: 54.78 %: mp: 272-274^{0}C (Ethanol): R_f : 0.59 (n- hexanol : EtoAC 7:3): IR KBrcm^{-1}
:3052.41 (C-H aromatic Stretching) ,2927.56(C-H aliphatic stretching),1742.56 (C=O stretching) ,1617.98 (C=C stretching Ar) ,1423.56 (C-CH_3 stretching),765.23 (C-Cl stretching):

^{1}H NMR ($CDCl_3$, 400 MHz) : 2.45 (m, 2H, CH_2) , 2.57 (m, 2H , CH_2) ,2.89(m,4H, CH_2), 6.16 (m. 3H,Ar) , 7.45 (m, 4H, Ar) : ^{13}C NMR ($CDCl_3$,125 MHz) :119.68, 120.48, 121.28, 129.89, 133.689 (Ar), 167.29, 169.20, 171.219 (tetrahydrocarbazole (Ar) , 192 .78 (C=O): Em (Es, Positive mode) m/z 324.89: Anal, Calc for($C_{20}H_{18}NClO$): C, 74.17; H,5.55; N,4.31: Found: C,75.89; H,5.78; N,4.79.

3e. (6-chloro-1,2,3,4-tetrahydro-9*H*-carbazol-9-yl)(4-nitrophenyl)methanone

Yield : 60.15 % : mp : 278-280^{0}C (Ethanol): R_f : 0.76 (n- hexanol : EtoAC 7:3): IR KBrcm^{-1}
:3167.45 (C-H aromatic Stretching) ,2978.56(C-H aliphatic stretching),1726.89 (C=O stretching) ,1660.57 (C=C stretching Ar) ,1356.23 (C-NO_2 stretching),756.46 (C-Cl stretching) : ^{1}H NMR ($CDCl_3$, 400 MHz) : 2.56 (m, 2H, CH_2) , 2.78 (m, 2H , CH_2) ,2.89(m,4H, CH_2), 6.54 (m. 3H,Ar) , 7.16 (m, 4H, Ar) : ^{13}C NMR ($CDCl_3$,125 MHz) :119.18, 120.78, 121.24, 129.78, 133.94 (Ar), 167.25, 169.67, 171.79 (tetrahydrocarbazole (Ar) , 192 .74 (C=O): Em (Es, Positive mode) m/z 355.68s: Anal, Calc for($C_{19}H_{15}ClN_2O_3$): C, 64.31; H,4.22; N,7.89: Found: C,65.34; H,4.89; N,7.43.

3f. (6-fluoro-1,2,3,4-tetrahydro-9*H*-carbazol-9-yl)(4-nitrophenyl)methanone

Yield: 54.98 % : mp : 280-282^{0}C (Ethanol): R_f : 0.78 (n- hexanol : EtoAC 7:3): IR KBrcm^{-1}
:3121.89 (C-H aromatic Stretching) ,2987.47(C-H aliphatic stretching),1710.14 (C=O stretching) ,1635.76 (C=C stretching Ar) ,1335.67 (C-NO_2 stretching),746.35 (C-Cl stretching) : ^{1}H NMR ($CDCl_3$, 400 MHz) : 2.34 (m, 2H, CH_2) , 2.57 (m, 2H , CH_2) ,2.92(m,4H, CH_2), 6.16 (m. 3H,Ar) , 7.27 (m, 4H, Ar) : ^{13}C NMR ($CDCl_3$,125 MHz) :119.19, 120.28, 121.24, 129.39, 133.29 (Ar), 167.36, 169.28, 171.98 (tetrahydrocarbazole (Ar) , 192 .89 (C=O): Em (Es, Positive mode) m/z 339.45: Anal, Calc for($C_{19}H_{15}FN_2O_3$): C, 67.44; H,4.43; N,8.27: Found: C,67.34; H,4.80; N,8.49.

3g. (6-nitro-1,2,3,4-tetrahydro-9*H*-carbazol-9-yl)(4-nitrophenyl)methanone

Yield : 57.45 % : mp : 282-284^{0}C (Ethanol): R_f : 0.80 (n- hexanol : EtoAC 7:3): IR KBrcm^{-1}
:3135.35 (C-H aromatic Stretching) ,2927.46(C-H aliphatic stretching),1708.34 (C=O stretching) ,1618.34 (C=C stretching Ar) ,1435.24,1337.89. (C-NO_2 stretching),746.35 : ^{1}H NMR ($CDCl_3$, 400 MHz) : 2.16 (m, 2H, CH_2) , 2.35 (m, 2H , CH_2) ,2.39(m,4H, CH_2), 6.25 (m. 3H,Ar) , 7.78 (m, 4H, Ar) : ^{13}C NMR ($CDCl_3$,125 MHz) :119.89, 120.57, 121.45, 129.47, 133.69 (Ar), 167.37, 169.69, 171.35 (tetrahydrocarbazole (Ar) , 192 .25 (C=O): Em (Es, Positive mode) m/z 366.89: Anal, Calc for($C_{19}H_{15}N_3O_5$): C, 62.46; H,4.10; N,11.49: Found: C,63.67; H,4.56; N,11.78.

Docking studies

Substituted 1,2,3,4- tetrahydrocarbazoles were subjected to molecular docking studies to the target enzyme GlcN-6-P, Code 1XFF by using V life MDS software. The compounds shown good interaction with the enzyme. The enzyme 1XFF is having 9 active pockets and the ligands showed good interaction with 2nd active pocket. The structure of GlcN-6-P was showed in figure 3. The interaction of ligands and selective inhibition in terms of docking scores (binding energies) is depicted in the table 1. The ligands showed hydrogen bonding, Van der Waals forces, Charge, hydrophobic interactions with the enzyme. The amino acids that interact with GlcN-6-P in most of the ligands are MET28A, PRO29A, LEU 30A, VAL31A, SER35A, THR36A, ILE37A, CYS38. The hydrogen bond between receptor and ligand is observed to be 3.425-4.135Å.

Anti-microbial activity

All the newly synthesized compounds were screened for their anti-bacterial activity against *S. aureus, E. coli, B. subtilis, P. aeruginosa and Antifungal activity* for *C. albicans*, *A. niger*. The compounds showed marked zone of inhibition. The different spectrum of activity by the test compounds against the tested microorganisms may be due to different

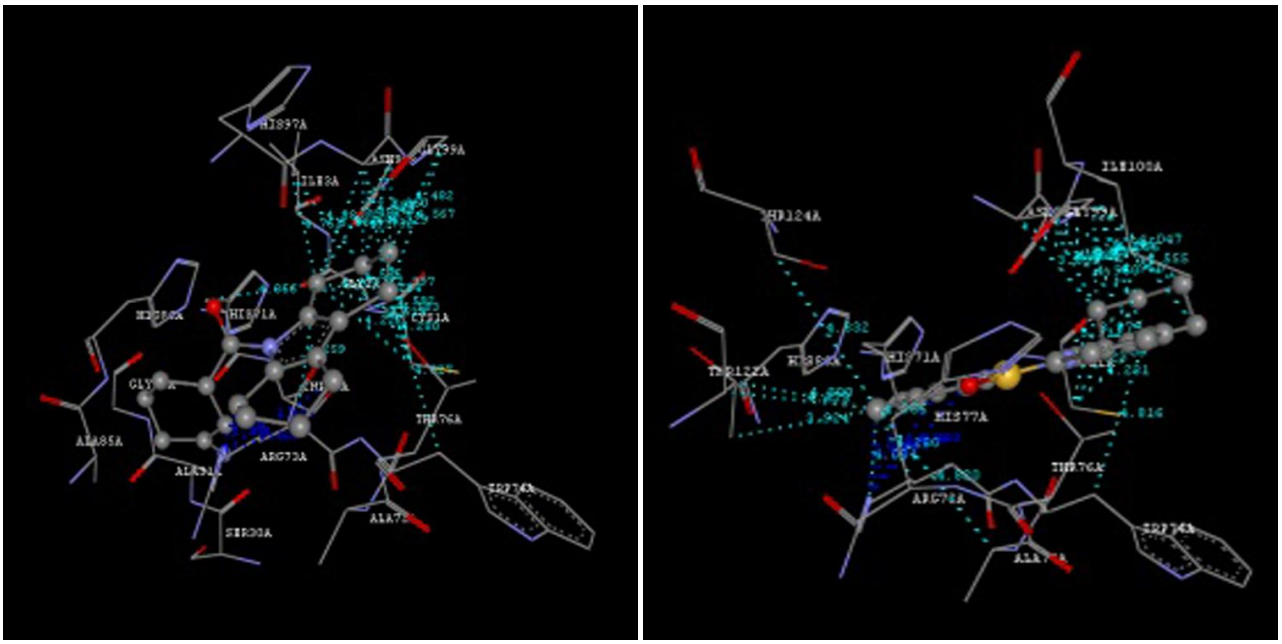


Figure 3: Docked complex with the Gln-6p Enzyme.

substituents present in the substituted tetrahydrocarbazoles. The results of the anti-microbial activity were showed in table 2.

A series of substituted tetrahydrocarbazole derivatives were synthesized in well manner with respect to percentage yield and all the compounds are subjected for anti-microbial studies. The synthesized tetrahydrocarbazole compounds was projected to analytical techniques of FT-IR, ^{1}H-NMR, ^{13}C NMR, Mass Spectrometry for confirming their chemical structure. The studies of the docking were carried out using V-Life MDS software for all the derivatives. The derivatives compounds were docked with target enzyme GlcN-6-P synthase. The results of docking scores were shown in the Table 1 among all the docked compounds 1a, 1b, 1c, 1d, 2a, 2b, 2d, 3b showed good binding affinity and interaction with enzyme with reference to ciprofloxacin and flucanazole. The docking results suggests that the parameters for docking simulation are optimum in producing experimental orientation of these compounds. The pharmacological study was undertaken to evaluate the effect of substituent on the anti-microbial activity. All the synthesized tetrahydrocarbazoles showed prominent anti-microbial activity.

CONCLUSION

Fifteen new tetrahydrocarbazole derivatives were synthesized and were characterized by ^{1}H NMR, ^{13}C NMR, Mass spectrometry, FT-IR studies and elemental analysis. The newly synthesized tetrahydrocarbazole derivatives were studied for anti-microbial activity using agar cup method. *In silico* studies exposed that all the synthesized compounds have relatively better binding affinity as compared to the standard drug. So, it may be considered as a good inhibitor of GlcN-6-P. The compounds are subjected to antimicrobial activity, out of fifteen compounds, eight compounds are showed good zone of inhibition. Hence, this study has widened the scope of developing these tetrahydrocarbazoles derivatives as promising anti-microbial agents.

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