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Insilico Design, Synthesis and Biological Evaluation of Novel Carbazole Derivatives

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Abstract:

The present work encompasses the designing of fifteen novel N–(hydrazinoacetyl)–carbazole derivatives by ACD Lab Chemsketch 12.0 and biological activity was predicted using various computational softwares such as Molinspiration, admetSAR and PASS. The designed molecules having required physicochemical properties, drug-likeness and obeying Lipinski Rule of Five were selected for docking studies. The docking studies were performed using Autodock Vina software. The compounds having better docking scores were selected for wet lab synthesis by conventional synthetic methods through a series of steps. The synthesized compounds were subjected to TLC, Melting point determination, IR, ^{1}HNMR and MASS spectroscopic studies. The newly synthesized compounds were screened for their antibacterial activity and antifungal activity by Agar Well Diffusion method respectively. The antibacterial activity was carried out both gram negative (*Escherichia coli*) and gram positive (*Staphylococcus aureus*).The antifungal activity was carried out in *Aspergillus niger* and *Candida albicans*. All the synthesized compounds shows characteristic peak in IR, ^{1}HNMR and MASS spectroscopic studies. Some of the derivatives shows ,antibacterial and antifungal activities. Hence this result is useful for further investigation in the future.

Keywords: Carbazole; *in silico* design; antibacterial activity; antifungal;IR; NMR; MASS.

INTRODUCTION

Carbazole

Carbazole is a nitrogen containing heterocyclic compounds. It has a tricyclic structure, that consists of two six membered benzene ring fused on either side with a nitrogen-containing five membered ring. Carbazole and its derivatives are very important type of nitrogen containing heterocyclic compounds that are widespread in nature.

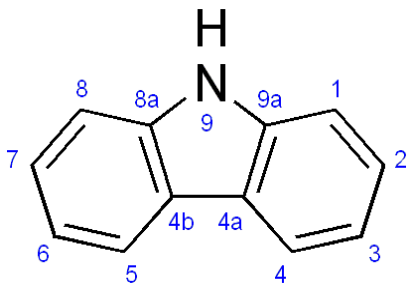


Fig 1 : Structure of carbazole

Carbazole derivatives are well known for their pharmacological activities. It is evident from the literature that the derivatives of carbazole moiety possess a wide spectrum of pharmacological activities, such as antibacterial, antifungal, antitumour, antineoplastic, anticonvulsant, antioxidant, antidiabetic, antipsychotic and larvicidal activity. The carbazole moiety is a frequent moiety of numerous drugs, such as olivacine, ondansetron, rimcazole, stauroapirone, carbazolol, carvedilol, carprofen, cacotheline, rebaccamycin, ellipticine and various naturally occurring carbazole alkaloids. Carbazole derivatives have documented consistent advances in the design of novel antipsychotic, neuroleptic and anticonvulsant agents. Furthermore, various congeners of oxadiazole, thiadiazole, azetidinone and thiazolidinone have also been reported to exhibit potential antimicrobial, anticancer, antipsychotic, antidepressant and anticonvulsant activity. In view of broad biological activity of carbazole derivatives, in this study it was planned to synthesize new carbazole derivatives and by incorporation of new pharmacophores, such as oxadiazole at position of carbazole nucleus, with the hope of obtaining better pharmacologically active drugs as anticancer and antimicrobial agents.

Carbazole and its derivatives are an important type of nitrogen containing heterocyclic compounds that are widespread in nature. The chemistry and biology of carbazole have attracted an increasing interest over the last 50 years because it possess a desirable electronic and charge transport properties, as well as large π-conjugated system so various functional groups are easily introduced into structurally rigid carbazolyl ring. These characteristics result in the extensive potential application of carbazole in the field of chemistry (photoelectrical material, dyes, supramolecular recognition) and medicinal chemistry (antitumor, antiinflammatory, antimicrobial, psychotropic, anti-oxidative). Carbazole alkaloids constitute an important class of naturally occurring heterocycles, isolated from the Rutaceae-family.

First Carbazole alkaloids were isolated as natural products from Murraya koenigii that exhibited strong antimicrobial activity. The stem bark of Murraya koenigii contains dimeric carbazole alkaloids along with six carbazole alkaloids. Traditionally, this plant is used as stimulant, stomachic, febrifuge, analgesic and for the treatment of diarrhoea, dysentery and insect bites. Along with these activities it also shows antimicrobial property. Many derivatives of the naturally occurring alkaloids elipticine and 9-methoxyelipticine which contain carbazole ring in their structure have been developed and tested for their anticancer activity. Carbazole ring are also present in a variety of naturally occurring medicinally active

substances. For example, the carbazomycins are an unprecedented class of antibiotics with a carbazole framework.

Chemistry of carbazole

Carbazole is an aromatic heterocyclic organic compound. It has a tricyclic structure, consisting of two six membered benzene ring fused on either side of a five membered nitrogen-containing ring. The structure of compound is based on the indole structure in which a second benzene ring is fused onto the five-membered ring at the 2–3 position of indole .

MATERIALS AND METHODS

Chemicals

All chemicals used were analytical or synthetic grade. Various chemicals used for the study are listed in Table 1.

Table 1: Chemicals used for the synthesis of novel analogues

Sl No:	Chemicals	Procured from
1.	Carbazole	SRL
2.	Chloroacetylchloride	Yarrow Chem Limited
3.	Acetone	Chem dyes Limited
4.	Ethanol	Yarrow Chem Limited
5.	Benzene	SRL
6.	Chloroform	Yarrow Chem Limited
7.	Hydrazine Hydrate	Yarrow Chem Limited
8.	Dioxane	SRL
9.	Methanol	SRL
10.	Isatin	Sigma Aldrich
11.	Ammonium acetate	Chem Dyes limited
12.	2- ethynyl benzaldehyde	Sigma Aldrich
13.	2 Cyano benzaldehyde	Sigma Aldrich
14.	2,4 dihydroxy benzaldehyde	Sigma Aldrich
15.	3 fluoro benzaldehyde	Sigma Aldrich
16.	2,3 dihydroxy benzaldehyde	Sigma Aldrich

Apparatus And Instruments

The apparatus and instruments used for the study are listed in the Table 2.

Table 2: Apparatus and instruments used for the study

CATEGORY	SUPPLIER
Beaker	Borosil
Test tube	Borosil
Condenser	Borosil
Round bottom flask	Borosil
Conical flask	Borosil
Funnel	Borosil
Glass rod	Borosil
Measuring cylinder	Borosil
Pipette	Borosil
Filter paper	Merck
Capillary tubes	Borosil
TLC plates	Merck
Thermometer	Merck
Magnetic stirrer	Merck
Heating mantle	Merck
IR Spectrometer	Shimadzu
NMR Spectrometer	Bruker avance 500NMR
MASS Spectrometer	Thermo Exactive Orbitrap

Softwares

- ACD Lab Chemsketch
- Molinspiration
- AdmetSAR
- PASS
- Autodock Vina

METHODS

IN SILICO DRUG DESIGN

The *in silico* modeling of all proposed compounds were carried out by using different computational software in order to predict the physico chemical parameters. The softwares used for *in silico* studies include ACD Lab Chemsketch, Molinspiration, admetSAR and PASS. Molecular docking studies carried out by Autodock Vina.

ACD Lab Chemsketch 12.0

ACD Lab Chemsketch is a chemically intelligent drawing interface that allows drawing almost all chemical structure including organics, organometallics, polymers and Markush structures. Use it to produce professional looking structures and diagrams for reports and publications.

Determination of drug likeness and Lipinski rule of five using Molinspiration software

Determination of drug likeness is an important aspects of the drug design. It is defined as a complex balance of various molecular properties and structural features which determine whether a particular molecule is similar to the known drugs. These properties mainly hydrophobicity, electronic distribution, hydrogen bonding characteristics, molecule size, flexibility and presence of various pharmacophoric features influence the behaviour of molecule in a living organism, including bioavailability, transport properties, affinity to proteins, reactivity, toxicity, metabolic stability and many others. Drug likeness score is calculated by Molinspiration software. The Lipinski Rule of five provides a measure for determining the oral bioavailability of a compound.

Determination of ADMET profile using admetSAR

In total, 22 highly predictive qualitative classification models were implemented in admetSAR software. These models includes human intestinal absorption, blood-brain barrier penetration, Caco-2 permeability, P-glycoprotein substrate and inhibitor, CYP450 substrate and inhibitor (CYP1A2, 2C9, 2D6, 2C19, and 3A4), hERG inhibitors, AMES mutagenicity, carcinogens, honey bee toxicity, and tetrahymena pyriformis toxicity. In addition, all classification models were given a probability output instead of simple binary output. In scientific community of ADMET prediction, quantitative predictions are more useful. To this a SMILES notation of the compounds taken as input. It determine the ADMET profile of the compound using different models.

Prediction of activity spectra for novel molecules using PASS software

PASS software is designed as a tool for the evaluation of general biological potential in the molecule under study. The PASS software, which predicts more than 4000 kinds of biological activity, including pharmacological effects, mechanism of action, toxic and adverse effects, interaction with metabolic enzymes and transporters, mutagenicity etc.

PASS uses input files in smiles format and results of predictions can be obtained as text format. A biological spectrum for a substance is a list of biological activity types for which the probability to be revealed (Pa) and not to be revealed (Pi) values are independent and their values ranges from zero to one. The more Pa value, the less is the probability of false positive in the set of compounds selected for study.

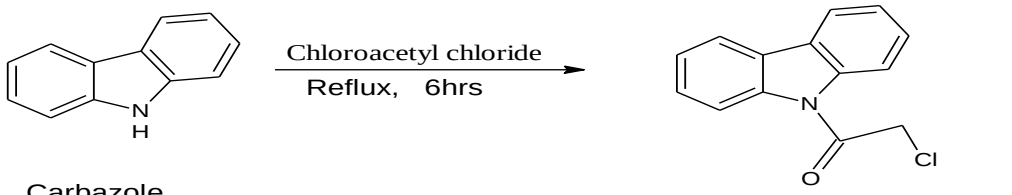
Docking methodology

Autodock Vina is an automated docking and virtual screening software for computational drug discovery that can be used to screen libraries of compounds against a potential drug target. The Autodock Vina includes docking wizard with an easy to use use interface which make it a valuable tool for computer aided drug design.The Autodock Vina software finding the best orientation of the molecues such that they have the minimum energy as scored by a predefined scoring function. It is useful in predicting the correct placement of drugs or ligands within the binding pocket of the target receptor.

SYNTHETIC METHOD

Scheme of Synthesis

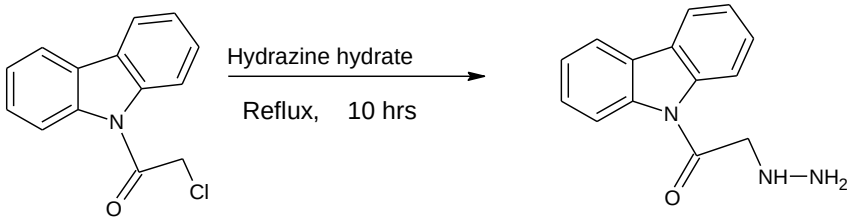
Step 1



Carbazole

N- chloroacetyl carbazole

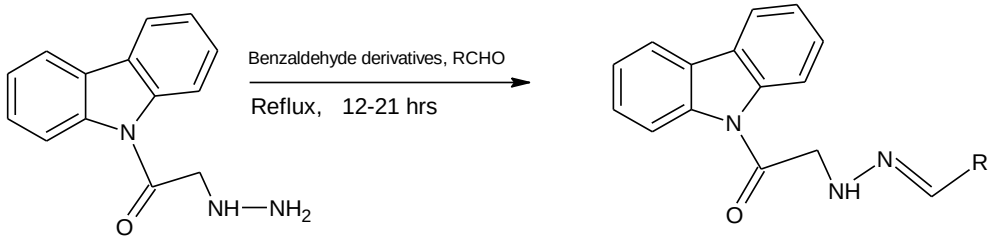
Step 2



N - chloroacetyl carbazole

N - hydrazino acetyl carbazole

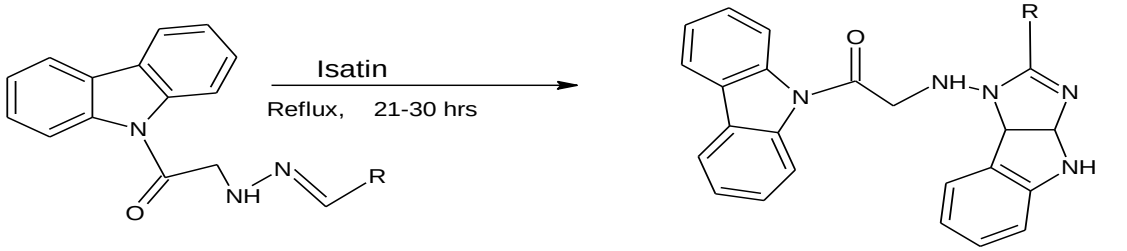
Step 3



N-hydrazino acetyl carbazole

N-(arylidene acetylhydrazino)-carbazole

Step 4



N- arylidene acetylhydrazino carbazole

1-Carbazole-9-yl-2-(substituted phenyl)-4,5-diphenyl-1*H*-imidazole-1-yl-amino)-ethanone

Procedure

Step 1: Synthesis of N 9- (chloroacetyl)-carbazole

Chloroacetyl chloride (4.9g, 0.04 mol) was added to a solution of carbazole (7g, 0.04 mol) in 60 ml acetone. Reaction mixture was refluxed on a water bath for 6 hrs. The completion of reaction was monitored by TLC. The solvent was removed and washed with water. The product was recrystallized from ethanol to obtain compound (1).

Step 2: Synthesis of N 9 -(hydrazinoacetyl)-carbazole

Hydrazine hydrate (0.6g, 0.012 mol) was added to a solution of N 9 -(chloroacetyl)-carbazole (3g, 0.012 mol) in 30 ml ethanol:dioxane (9:1v/v) Reaction mixture was refluxed on a water bath for 10 hrs. The completion of reaction was monitored by TLC. Reaction mixture was cooled, filtered and concentrated to get a solid compound and washed with water. Product was recrystallized from ethanol to obtain compound (2).

Step 3 : Synthesis of N 9 -(arylidene acetylhydrazino)-carbazole derivatives.

Equimolar amounts of compound (2) (0.007 mol) and aromatic aldehydes (0.007 mol) were transferred to a 250 ml round bottom flask containing 20 ml of ethanol: dioxane (9:1, v/v) and the mixture were refluxed for about 12-21 hours. The reaction was monitored by TLC using 5% chloroform in benzene. Products were recrystallized by methanol.

Step 4: Synthesis of 1-Carbazole-9-yl-2-(substituted phenyl)-4, 5-diphenyl-1H-imidazole-1- yl-amino)-ethanone

Equimolar amounts of N 9 -(arylidene acetylhydrazino)-carbazole (0.007 mol) and isatin (0.007 mol) was transferred to a 250 ml round bottom flask containing 30 ml of ethanol: dioxane (9:1, v/v) and excess of ammonium acetate was added to this solution. Then mixture was refluxed for about 21-30 hours with continuous stirring. The reaction was monitored by TLC using 5% chloroform in benzene. The reaction mixture was washed with 50 ml of water to remove excess of ammonium acetate and recrystallized with chloroform.

Table 3: Substituted benzaldehyde

ANALOGUES	NAME OF SUBSTITUTED BENZALDEHYDE (R)
C1	2 ethynyl benzaldehyde
C2	2 Cyano benzaldehyde
C3	2,4-dihydroxy benzaldehyde
C4	3 Fluoro benzaldehyde
C5	2,3 dihydroxy benzaldehyde

PURIFICATION

Recrystallisation

After the synthesis of individual products in each step, products were purified by recrystallisation using appropriate solvents like Ethanol Methanol Chloroform

IDENTIFICATION

Thin layer chromatography

Purity of the compounds and completion of the reactions was checked by TLC.

Procedure

Analytical chromatography was performed on TLC plates which is a product of Merck KGaA, Germany.

Preparation of plate: Precoated TLC plates made of silica gel G were used for the analysis

Stationary Phase: silica Gel

Mobile phase: Chloroform: benzene

Detection : Iodine chamber

Application of sample: The solutions of the compound were taken in small bored capillary tube and spotted at 1cm from the base end of the plate. The plates were allowed to dry at room temperature and transferred to saturation chamber containing solvent system.

Development of chromatogram: Plates were developed by ascending technique. When solvent front had reached out about three fourth of the plate, taken out and dried at room temperature.

Detection of spots: The developed spots were detected by exposing to iodine vapours and UV chamber.

Calculation of R_f value: The retention factor for each sample were calculated from the chromatogram as,

R_f = Distance travelled by solute / Distance travelled by solvent front

Melting point determination

The melting point was determined using melting point apparatus.

CHARACTERIZATION

The synthesized compounds were characterized by IR, NMR and MASS spectra.

IR spectra

IR spectra of the synthesized compounds were recorded in the range of 4000-400 cm^{-1} on Shimadzu using KBr pellet method.

NMR Spectra

NMR spectra of different compounds were recorded on a Bruker Avance 500 NMR spectrometer. The system was operated at 400MHz for proton using TMS as an internal standard. The chemical shifts are reported in the δ scale. The NMR spectra of different compounds were recorded by dissolving the sample in $CDCl_3$. Spectra were acquired at 298K temperature using a Bruker Avance 500 NMR spectrometer equipped with a magnet operating at 11.7 Tesla.

Mass Spectra

MASS spectra were recorded by Thermo Scientific Exactive instrument. The peaks were recorded as m/z value.

BIOLOGICAL EVALUATION

Antibacterial Activity (Agar well-diffusion method)

Procedure

Agar well diffusion method is widely used to evaluate the antimicrobial activity of the compound. Autoclaved 15-20 mL of Mueller-Hinton agar was poured on glass petri plates and allowed to solidify. Standardized inoculum of the test organism (E.coli and S.aureus) was uniformly spread on the surface of these plates using sterile cotton swab. Four wells with a diameter of 8 mm (20 mm apart from one another) were punched aseptically with a sterile cork borer in each plate. Compound solution (40 µL and

80µL) at desired concentration from 10mg/mL stock was added to two wells and one well with Tetracycline as positive and compound solvent as negative control. Then, the agar plates were incubated under 37°C for 24 hrs. After incubation, clear zone was observed. Inhibition of the bacterial growth was measured in mm.

Antifungal activity (Agar well- diffusion method)
Procedure
A sterile swabwas used to evenly distribute fungal cultures of *Aspergillus niger* and *Candida albicans* over the Rose Bengal agar medium. The plates were allowed to dry for 15 minutes and the stock solution (40 and 80 µL) was added into the wells at desired concentration from 10mg/ml stock. In the positive well (+) the control drug, griseofulvin was added (20µl from 10mg/ml stock) and in the negative well (-) the solvent used for the sample dilution was added. The plates were incubated at room temperature for 24 hours after which they were examined for zone of inhibition.

RESULTS AND DISCUSSION

Datas from ACD Lab Chemsketch 12.0.
Various molecular descriptors like molar volume, parachor, surface tension, polarizability, molar refractivity were computed and the results are shown in Table 4

Datas computed from Molinspiration software
The drug likeness score and Lipinski rule of five are calculated and analyzed using Molinspiration software and the results are shown in Table 5.

Datas computed from admetSAR software
The ADMET profile of novel molecules were determined using admetSAR software.

Table 4: Molecular descriptors computed from ACD Lab Chemsketch

Sample code	MR (cm^3)	MV (cm^3)	Parachor (cm^3)	Surface Tension (dyne/cm)	Polarizability (cm^3)
C1	120.22 ± 0.5	319.7 ± 7.0	892.5 ± 8.0	60.7 ± 7.0	47.66 ± 0.5 10^{-24}
C2	120.37 ±0.5	336.1 ± 7.0	897.3 ± 8.0	50.7 ± 7.0	47.72 ± 0.5 10^{-24}
C3	122.36 ± 0.5	329.6 ± 7.0	892.4 ± 8.0	54.5 ± 7.0	49.49 ± 0.5 10^{-24}
C4	136.58 ± 0.5	387.7 ± 7.0	1020.6 ±8.0	48.0 ± 7.0	54.14 ± 0.5 10^{-24}
C5	122.11 ± 0.5	327.0 ± 7.0	890.6 ± 8.0	55.0 ± 7.0	48.41 ± 0.5 10^{-24}

Table 5: Molinspiration of novel analogues

Compounds	Log P (<5)	Molecular weight (<500D)	No. of Hydrogen bond acceptors(<10)	No. of Hydrogen bond donors(<5)	nviolations
C1	4.8	481.56	6	2	0
C2	4.8	482.5	7	2	0
C3	4.58	489.5	8	3	0
C4	5.2	475.5	6	2	1
C5	4.57	489.5	8	3	0

Table 6: Drug likeness analysis of novel analogues

Sample Code	GPCR ligand	Ion channel modulator	Kinase inhibitor	Nuclear receptor Ligand	Protease inhibitor	Enzyme Inhibitor
C1	-0.46	-0.65	-0.51	-0.80	-0.77	-0.41
C2	-0.48	-0.62	-0.43	-0.86	-0.64	-0.4 5
C3	-0.44	-0.69	-0.47	-0.89	-0.71	-0.48
C4	-0.40	-0.63	-0.37	-0.78	-0.59	-0.42
C5	-0.54	-0.72	-0.46	-0.96	-0.71	-0.49

Table 7: ADMET score of novel molecules obtained from admetSAR software

Sample code	ADMET prediction				Toxicity Prediction	
	Human intestinal absorption	Subcellular localization	CYP450 1A2	Bio degradation	AMES toxicity	Carcinogen
C1	0.9236	0.7260	Inhibitor	NRB	Non AMES toxic	Non carcinogen
C2	0.9630	0.7655	Inhibitor	NRB	Non AMES toxic	Non carcinogen
C3	0.9910	0.5433	Inhibitor	NRB	Non AMES toxic	Non carcinogen
C4	0.9964	0.6569	Inhibitor	NRB	Non AMES toxic	Non carcinogen
C5	0.9825	0.6555	Inhibitor	NRB	Non AMES toxic	Non carcinogen

Data computed from PASS software

The different biological activities were predicted using PASS software

Table 8: The bioactivity score of novel molecules obtained by PASS software

Compound code	Biological activity	Pa	Pi
C1	Antibacterial Antifungal	0.582 0.512	0.009 0.010
C2	Antibacterial Antifungal	0.683 0.699	0.028 0.009
C3	Antibacterial Antifungal	0.572 0.499	0.094 0.007
C4	Antibacterial Antifungal	0.592 0.512	0.034 0.021
C5	Antibacterial Antifungal	0.690 0.614	0.071 0.015

Docking studies for antibacterial activity

Target: Dihydrodipicolinate reductase

PDB ID: 1DRU

Table 9: Docking score and interacting residues for antibacterial activity

Name of the ligand	Docking score	Interacting residues
C1	-9.2	Val, Ile
C2	-9.0	Asp
C3	-8.8	Asp,Lys
C4	-9.0	Lys, Val
C5	-9.5	Asp, Val
Std drug Tetracycline	-7.9	Val

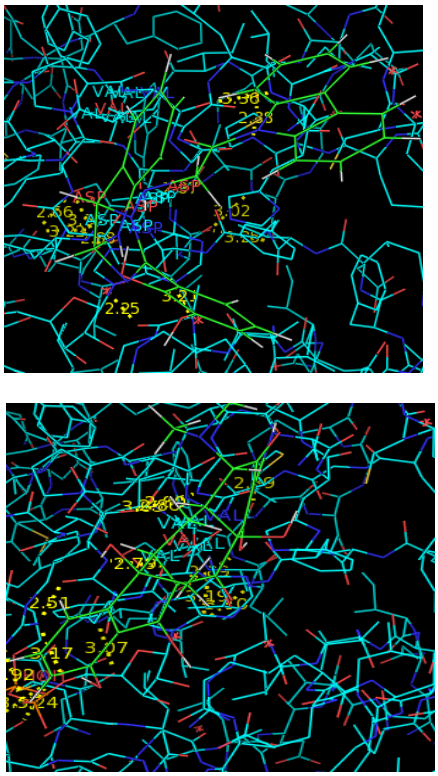


Fig 1: Hydrogen bond interaction of 1DRU with C5 and Tetracycline

Docking studies for antifungal activity

PDB ID: 4H97

The crystal structure of Candida albicans dihydrofolate reductase complexed with NADPH and 5-{3-[3-methoxy-5-(4-methylphenyl)phenyl]but-1-yn-1-yl} methylpyrimidine-2,4-diamine with PDB ID:4H97 were retrieved from PDB.

Table 10 : Docking score and interacting residues for antibacterial activity

Compound code	Docking score	Interacting Residue
C1	-8.9	Ile, Lys
C2	-9.1	Asp,Gly
C3	-8.8	Ile, Lys
C4	-8.8	Lys,Gly
C5	-8.5	Ile,Gly
Griseofulvin	-7.4	Met

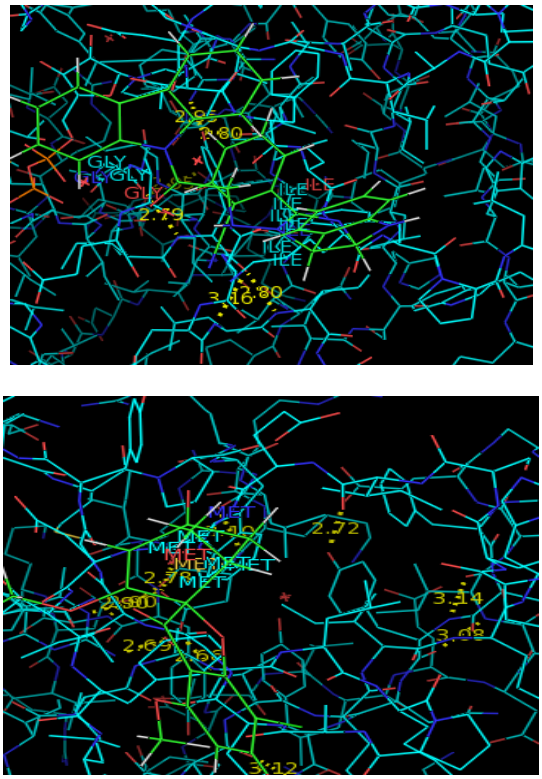


Fig 2: Hydrogen bond interaction of 4H97 with C2 and GRISEOFULVIN

Physico Chemical Data

The various physico chemical parameters like molecular formula, molecular weight, melting point and R_f value of newly synthesized compounds are shown in Table 11.

Characterization of compounds using various analytical techniques

The novel synthesized analogues were confirmed using IR, NMR and MASS spectral analysis. The characteristic values obtained are given in Table 12

Table 11: Physico chemical data of newly synthesized compounds

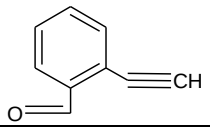
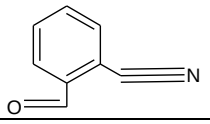
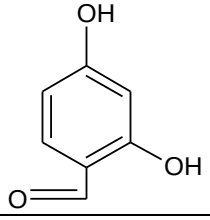
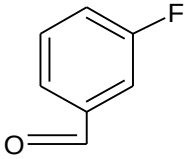
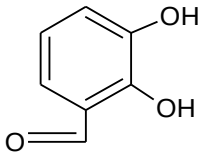
Sample Code	R	Molecular formula	Molecular weight	Melting point	R_f Value
C1		$C_{31}H_{23}N_5O$	481.54	249	0.62
C2		$C_{30}H_{22}N_6O$	482.53	254	0.64
C3		$C_{29}H_{23}N_5O_3$	489.52	250	0.60
C4		$C_{29}H_{22}FN_5O$	475.51	252	0.62
C5		$C_{29}H_{23}N_5O_3$	489.52	250	0.61

Table 12: IR, NMR, MASS spectral data

Sample Code	**IR, NMR, MASS spectral data**
C1	**IR(cm^{-1})** - Ar CH stretch(3062), NH stretch (3419), C=O stretch(1492), Ar C=C(1598), C=N(1602), CH stretch(2850),alkyne(3219) **NMR(ppm)**- Ar-H(7.4), N-H(3.25),C≡C-H (1.8). **MASS**- m/z value - 481.54 M+1 value - 482
C2	**IR(cm^{-1})** - Ar CH stretch(3051), NH stretch(3419), C=O stretch(1492),Ar C=C (1602), CN(2848), CH stretch(2850) **NMR(ppm)**- Ar-H(7.4), HC-CN(6.3), N-H(3.2) **MASS**- m/z value – 482.538 M+1 value -483 M+2 value -484
C3	**IR(cm^{-1})**- Ar CH stretch(3269), NH stretch(3481), C=O stretch(1562), Ar C=C (1647), C=N(1367), CH stretch(2995). **NMR (ppm)** - Ar-H(7.8), Ar-OH(6.6), N-H(3.2) **MASS**- m/z value – 489.512 M+1 value - 490.463
C4	**IR(cm^{-1})**- Ar CH stretch(3238), NH stretch(3487), C=O stretch(1562), Ar C=C (1654), C=N(1367),CH stretch (2910) **NMR(ppm)**- Ar-H(7.8), F-ArH(7.4), N-H(3.2) **MASS**- m/z value -475.433 M+1 value -476 M+2 value -477
C5	**IR (cm^{-1})**-Ar CH stretch(3053), C=O stretch(1492), Ar C=C(1595), C=N(1330), CH stretch(2918),OH (3838). **NMR(ppm)**- Ar-H(7.8), Ar-OH(6.6), N-H(3.2) **MASS**- m/z value - 489.512 M+1 value – 490.964

Table 13: Effects of sample C5 on antibacterial activity

SL.No	Sample	Organism	Zone of inhibition		
			+ (20µL) Tetracycline	T1(40µL)	T2(80µL)
1	C5	*Escherichia coli*	44.11±0.060	12.1±0.1872	16.2±0.2259
		Staphylococcus aureus	23.45±0.1905	8.2±0.1468	12.4±0.1848

Table 14: Effects of sample C2 on antifungal activity

Sample code	Sample	Organism	Zone of inhibition			
			Standard Griseofulvin	Negative control	T1 (40µL)	T2 (80µL)
T1	C2	*Candida albicans*	18±0.057	-	13	15
		Aspergillus niger	18±0.057	-	12	14

Antibacterial Activity

The antibacterial activity of sample C5 were evaluated using agar well diffusion assay method against *Escherichia coli* (gram negative) and *Staphylococcus aureus* (gram positive). Tetracycline was taken as standard. The zone of inhibition of sample C5 at different concentration are given in Table13 and figure 3, 4.

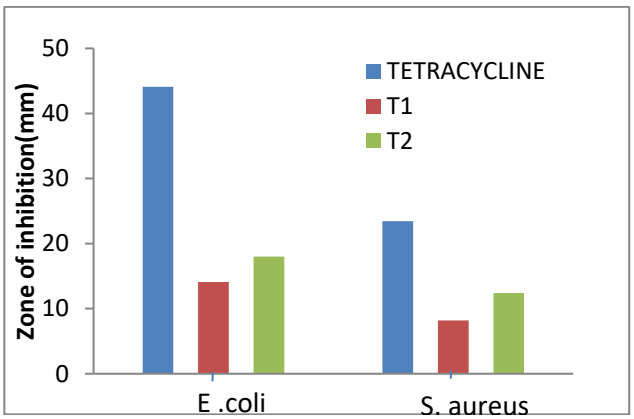


Fig 3: Effects of c5 and standard on antibacterial activity

All the data were analyzed by unpaired t test. Values are represented as mean ± SEM (n=3). Probability *P<0.05, **P<0.01, ***P<0.001 when compared.

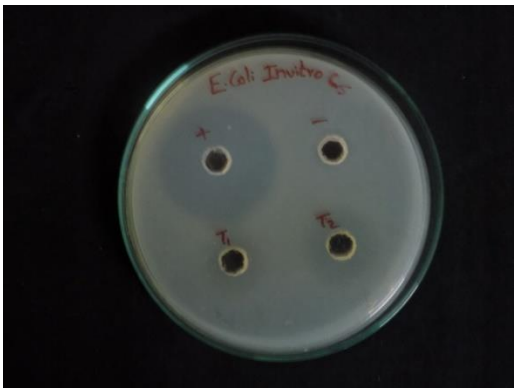


Fig 4: Effects of C5 on antibacterial activity

Antifungal Activity

The antifungal activity of sample C2 were evaluated using agar well diffusion assay method against *Aspergillus niger* and *Candida albicans*. Griseofulvin was taken as standard. The zone of inhibition of sample C2 at different concentration are given in Table 14 and figure 5,6.

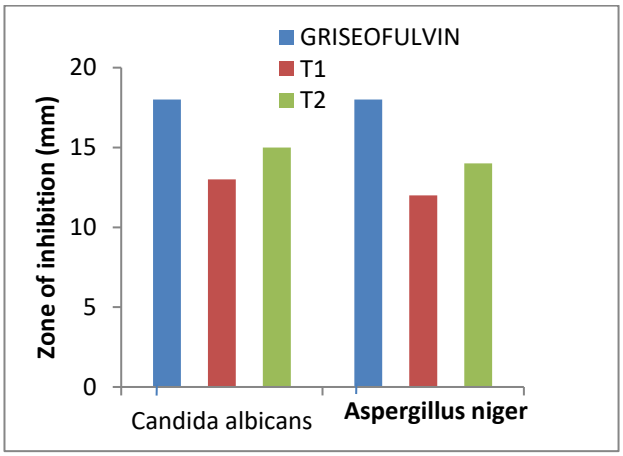


Fig 5: Effects of C2 and standard on antifungal activity

All the data were analyzed by unpaired t test. Values are represented as mean ± SEM (n=3). Probability **P<0.01 when compared to standard.

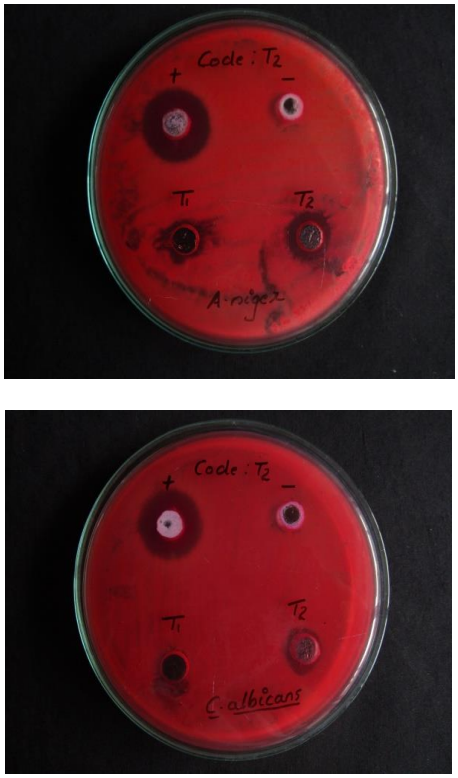


Fig 6 Effect of sample C2 on antifungal activity

DISCUSSION

Carbazole derivatives have been studied extensively because of their ready accessibility and diverse chemical activity. The chemistry of carbazole continues to draw the attention of synthetic organic chemists due to their various biological activities. Encouraged by the above reports and as a part of research, the lead compound N hydrazino acetyl carbazole has been further developed in the present study and investigations are done on their various biological activities.

In silico molecular studies

In silico molecular modification was the most important preliminary step in the rational drug design of novel compounds. In the present study, the *in silico* molecular modeling studies were carried out for the selection of suitable drug candidates prior to wet lab synthesis. *In silico* studies were performed in fifteen different analogues by means of ACD Lab Chemsketch 12.0, Molinspiration, and admetSAR softwares. Among them ten compounds were eliminated due to violation of Lipinsky Rule Five and shows AMES toxicity and carcinogenicity. The biological activities of remaining 5 compounds were predicted by PASS online software and docking software were performed using Autodock Vina software.

The docking score and hydrogen bond interactions were used for predicting the protein ligand modes. Compounds having the higher negative docking score are considered as the best one. Based on docking score, five compounds were selected for wet lab synthesis with the help of these selection parameters. They were named as C1,C2,C3,C4 and C5.

Synthesis Of Novel Analogues

The selected compounds were synthesized by conventional method through a series of steps. The steps used for the preparation of the analogues are as follows:

Step 1: Synthesis of N 9- (chloroacetyl)-carbazole

Step 2: Synthesis of N 9 -(hydrazinoacetyl)-carbazole

Step 3 : Synthesis of N 9 -(arylidene acetylhydrazino)-carbazole derivatives**.**

Step 4: Synthesis of 1-Carbazole-9-yl-2-(substituted phenyl)-4, 5-diphenyl-1H-imidazole-1- yl-amino)-ethanone

Physicochemical data

The various physicochemical parameters like molecular formula, molecular weight, melting point and R_f value of newly synthesized compounds were found out.

Analytical techniques

The synthesized compounds were subjected to TLC, IR, ^{1}H NMR and MASS spectroscopic studies. All these evaluations ensured the identity of the synthesized compounds.

Biological Evaluation

Antibacterial activity

On docking with Dihydrodipicolinatereductase (PDB ID:1DRU), C5 shows higher docking score when compared to the docking score of the standard drug, Tetracycline. So C5(1-Carbazole-9-yl-2-(2,3-dihydroxy phenyl)-4, 5-diphenyl-1H-imidazole-1- yl-amino)-ethanone) was subjected to *in vitro* antibacterial activity by agar well diffusion method and shows better activity against *Escheriehia.coli* (Gram –ve), and *Staphylococcus aureus* (Gram +ve) .

Antifungal activity

On docking with *Candida albicans* dihydrofolate reductase complexed with NADPH and 5-{3-[3-methoxy-5-(4-methylphenyl)phenyl]but-1-yn-1-yl} methylpyrimidine-2,4-diamine with PDB ID:4H97 C2 shows higher docking score when compared to the docking score of the standard drug, Griseofulvin. So C2 (1-Carbazole-9-yl-2-(2-cyano phenyl)-4, 5-diphenyl-1H-imidazole-1- yl-amino)-ethanone) was subjected to *in vitro* antifungal activity by agar well diffusion method and shows activity against *Candida albicans* and *Aspergillus niger.*

These results are useful for further investigation in future.

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