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Research Article

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Preparation of various Schiff's bases of 9-fluorenone and its biological application

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ABSTRACT

The 9-fluorenone Schiff base derivatives have reported for the variety of diseases such as anti microbial and cancers. In this current study various Schiff bases were synthesized and characterized from 9-fluorenone.many compounds shows good in yield at specific temperature. Further the synthesized compounds were studied for anti microbial activity and molecular docking for Proteus mirabilis catalase enzyme and found that N,N'-Bis-fluoren-9-ylidene-ethane-1,2-diamine shows highest dock score of 81.947 with Internal energy (-7.169)and its microbial activity value as 17.9mm compared with other compounds

Keywords: 9-fluorenone Schiff base, anti microbial, *Proteus mirabilis*, dock score, Internal energy.

INTRODUCTION

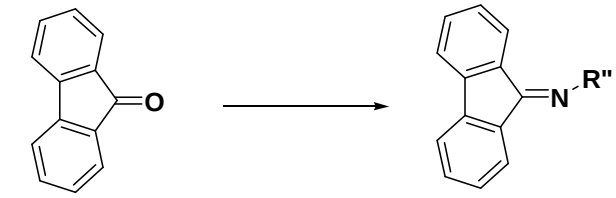
It was noted from the literature that the fluorenone schiffbase has a potential application and biological activity in therapeutic areas such as antifungal[1] and some of the metal complexes derived from thio semicarbazide has antitumor activity[2]. The bidentate Schiff's base ligands such as (9H-fluorene-9-ylidine)-thiosemicarbazide, (9H-fluorene-9-ylidine)-semicarbazide,(9H-fluorene-9-ylidine)-ethane-1,2diamine derived from the condensation of thiosemicarbazide, semicarbazide and ethylene diamine with 9-fluorenone were complexed with metal salts such as Copper, Zinc, Lanathanum and silvers were found to have antitumor activity[2]. Fluorenone thiosemicarbazone Schiff's base metal complex[2] were found to have various biological therapeutic application such as antifungal and antitumor activity other therapeutic areas. Fluorenamine Schiff base were reported for its potential anticancer[3] using different aldehydes. The preparation of 9-Fluorenone hydrazone[4], 9-Fluorenone oxime[5], and Oxime ethers[6] was reported and the study on the biological activity needs to be investigated. We have studied the various Schiff base's derived from 9-fluorenone and novel new schiffbase compounds were prepared and investigated for its microbial activity and Molecular docking.

EXPERIMENTAL SECTION

The key starting material 9-Fluorenone, various amines (Hydroxyl amine.HCl, 2,6-dimethyl aniline, semicarbazide, thiosemicarbazide, ethylene diamine, Hydrazine monohydrate and 4-hydroxy phenyl hydrazine) used for the synthesis of schiffs bases were obtained from Spectrochem. The other reagents used in the synthesis were procured

from Lab supplier. We have used Bruker Spin NMR (400MHz) for the structural elucidation. Mass spectral data was analyzed by GC-MS. The Figure 1 shows the reaction scheme for synthesizing of 9-fluorenone Schiff base derivatives

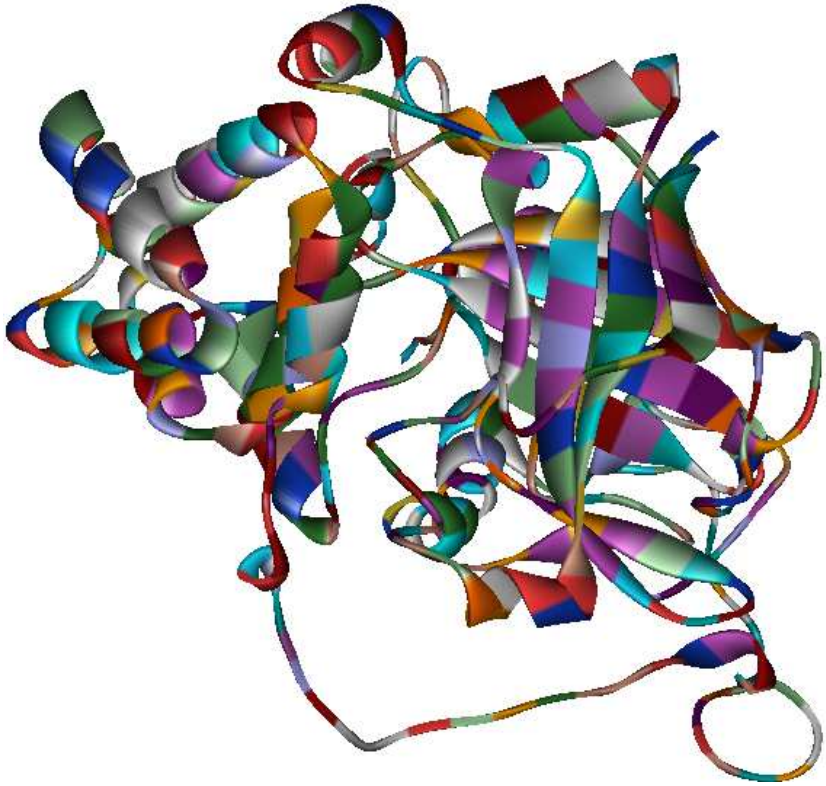
Figure 1: Reaction scheme for 9-fluorenone Schiff base derivatives



Compound-1 **Compound-2**

Where in R" is described as hydroxyl, Methoxy, 2, 6-dimethylphenyl, NH_2, 4-hydroxy phenyl amino, Urea and Thiourea.

Figure 2: Drug target protein from *Proteus mirabilis* catalase enzyme



Antibacterial activity

In determining the antimicrobial activity of the compounds was evaluated by agar well diffusion method [7]. The substrate and products (100µg) were freshly reconstituted with dimethylsulphoxide. All the microbial cultures were adjusted to 0.5 McFarland standards, which is visually comparable to a microbial suspension of approximately 1.5 $\times 10^8$ cfu/ml [8]. 20ml of Muller Hinton agar media was poured into each petriplate and plates were swabbed with 100µl inocula of the test microorganisms and kept for 15 min for adsorption. Using sterile cork borer of 8mm diameter, wells were bored into the seeded agar plates and these were loaded with a 100µl volume of extracts. All the plates were incubated at 37°C for 24 hrs for Pre-diffusion. Antimicrobial activities of samples were evaluated by measuring the zone of growth inhibition against the test organisms with zone reader (Hi Antibiotic zone scale). The medium with DMSO as solvent was used as a negative control whereas media with streptomycin (100µg) was used as positive control.

Molecular docking

In this current study *Proteus mirabilis* catalase enzyme of PDB id- 1NM0 was taken as drug target protein and docked using the ligand fit algorithm .Figure 2 shows the secondary structure of the drug target protein.

RESULTS AND DISCUSSION

General procedure-1: Preparation of Schiffs bases of fluorenone

In a 250.0mL reaction flask, ethanol (100.0mL) followed by 9-fluorenone (1.0 mole equivalent) were added. To the above clear solution, a solution of amine (4.5 mol equivalent) in ethanol was added. The reaction mixture was gradually heated to 80°C and maintained for 3h. The progress of the reaction was monitored by TLC (TLC system: Chloroform: Methanol 9:1). The reaction mixture was cooled to 0 to 5°C and stirred for 30 min. The solid precipitated was filtered, washed with chilled ethanol and dried at 55°C under vacuum (560 mm Hg). The isolated yield was found to be quantitative. The isolated Schiff's bases listed in Table-1 were characterized by ^{1}HNMR and GC-MS.

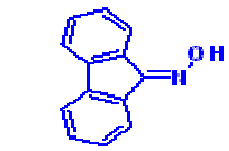
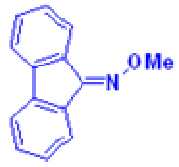
General Procedure-2: Preparation of schiffs base of Fluorenone

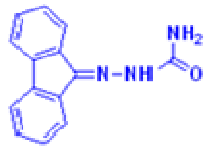
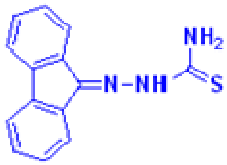
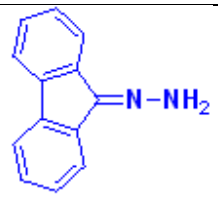
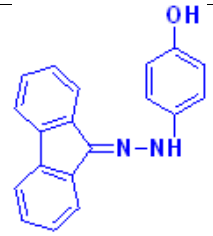
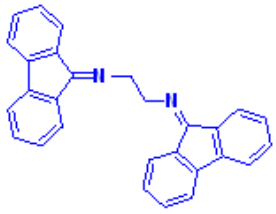
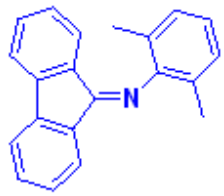
In 100mL reaction flask, toluene (50.0mL) was added followed by 9-fluorenone (1.0g, 1.0 mol equivalent) to get a clear solution at 25°C. The amine (4.5 mol equivalent) was then added followed by catalytic p-toluene sulfonic acid monohydrate (0.1 mol equivalent). The reaction mixture was heated to reflux at 110°C and water formed was distilled azeotropically from the reaction mixture using a Dean-Stark apparatus. The progress of the reaction was monitored by TLC (TLC system: Chloroform: Methanol 9:1). The reaction mixture was concentrated to remove most of the toluene. The residue was stirred with n-heptane for 30 min, filtered and dried at 55°C under vacuum and was characterized by ^{1}HNMR, and GC-MS. Compound 2g and compound2h were prepared using this procedure. Compound 2a and Compound 2c-2f were prepared using this procedure.

General Procedure-3: O-Methylation of 9-fluorenone oxime

In 100mL reaction flask, methanol (10.0mL) was added followed by 9-fluorenone oxime (1.0g, 1.0 mol equivalent) to get a suspension at 25°C.The reaction mixture was cooled to 0°C and 10% sodium hydroxide solution was added to get a clear solution at 0°C. Dimethyl sulphate was added slowly at 0°C and stirred for 1h.The progress of the reaction was monitored by TLC (TLC system: Chloroform: Methanol 9:1). The reaction mixture was quenched with ice water and extracted with dichloromethane and washed with water to remove the acidity. The organic layer was distilled to remove most of the solvent .The residue obtained after concentration was triturated with n-hexane, filtered and dried at 55°C under vacuum. The isolated product was characterized by ^{1}HNMR, and GC-MS. Compound 2b was prepared using this procedure.

Table-1: .Schiff's bases Compounds

Structure of the compound	Name of the compound	Designated compound	Temperature	Yield %
 $C_{13}H_9NO$ Mol. Wt.: 195.22	Fluoren-9-one oxime	Compound2a	80°C	95
 $C_{14}H_{11}NO$ Mol. Wt.: 209.24	Fluoren-9-one O-methyl-oxime	Compound2b	0°C	60

 $C_{14}H_{11}N_3O$ Mol. Wt.: 237.26	9-Fluorenyl semicarbazone	Compound2c	80°C	80
 $C_{14}H_{11}N_3S$ Mol. Wt.: 253.32	9-Fluorenylthio semicarbazone	Compound2d	80°C	82
 $C_{13}H_{10}N_2$ Mol. Wt.: 194.23	Fluoren-9-ylidene-hydrazine	Compound2e	80°C	85
 $C_{19}H_{14}N_2O$ Mol. Wt.: 286.33	4-(N'-Fluoren-9-ylidene-hydrazino)-phenol	Compound2f	80°C	90
 $C_{28}H_{20}N_2$ Mol. Wt.: 384.47	N,N'-Bis-fluoren-9-ylidene-ethane-1,2-diamine	Compound2g	110°C	95
 $C_{21}H_{17}N$ Mol. Wt.: 283.37	(2,6-Dimethyl-phenyl)-fluoren-9-ylidene-amine	Compound2h	110°C	75

Characterization of the compounds

The structures of the synthesized compounds were confirmed using ^{1}H NMR and MS.

Fluoren-9-one oxime: Compound2a-0.9g Yield: 95% ^{1}H NMR (DMSO-d6) δ 7.31-7.90 (m, 6H), 8.33-8.35 (m, 2H). Mass by GC-MS: 195.2 .**Fluoren-9-one O-methyl-oxime: Compound2b**-0.7g Yield: 60%, ^{1}H NMR (DMSO-d6) δ 4.34 (s, 3H), 7.33-7.49(m, 4H), 7.82-7.94 (m, 3H), 8.59-8.62 (d, 1H), Mass by GC-MS: 209.0 **Fluorenyl semicarbazone: Compound2c**-1.05g, Yield: 80%,^{1}H NMR (DMSO-d6) δ 7.3-7.5(m, 4H), 7.6-7.8(m, 4H), 8.7(s, 1H), Mass by LC-MS:238.0 (+ve mode).**Fluorenyl thio semicarbazone: Compound2d**-1.13g, Yield: 82%, ^{1}H NMR (DMSO-d6) δ 7.30-7.62(m, 7H), 7.77-8.04(m, 3H), Mass by LC-MS: 254 (+ve mode).**Fluoren-9-ylidene-hydrazine :Compound2e** -0.92g, Yield: 85%,^{1}H NMR (DMSO-d6) δ 6.42(broad s, 2H), 7.28-7.47(m, 4H), 7.64-7.90(m, 3H), 7.927-7.929 (d, 1H), Mass by LC-MS: 195 (+ve mode)

4-(N'-Fluoren-9-ylidene-hydrazino)-phenol: Compound2f -1.43g, Yield: 90%,^{1}H NMR (DMSO-d6) δ 6.92-6.94(d, 2H) 7.37-7.57 (m, 4H), 7.79-7.93(m, 5H), 8.08-8.10 (d, 1H), 10.22(broad s, 1H), 11.56(broad s, 1H)**. N, N'-Bis-fluoren-9-ylidene-ethane-1,2-diamine:Compound2g-**1.49g, Yield: 70%, ^{1}H NMR (DMSO-d6) δ 3.93(s, 2H), 7.28-7.35(m, 2H), 7.39-7.43(m, 2H), 7.57-7.59(m, 2H), 7.81-7.83(m, 2H)**.(2, 6-Dimethyl-phenyl)-Fluoren-9-ylidene-amine:**Compound2h-1.18g, Yield: 75%, ^{1}H NMR (DMSO-d6) δ 2.073 (s, 6H), 6.49-6.52(d, 1H), 6.93-7.07 (m, 4H), 7.323-7.65(m, 5H), 8.04-8.07(d, 1H), Mass by LC-MS: 285 (+ve mode)

Anti –bacterial activity

The antimicrobial activity of all products against all the test organisms had shown in the Table 2. Each compound displayed antimicrobial activity against at least one test microorganism with inhibition zones that ranged from 11.8 to 19.4mm. About 80% of all products showed exhibited activity which was comparable with standard antibiotic at a concentration of 100µg/ml. The inhibitory effect of the compound decreased in the order against: Fluoren-9-ylidene-hydrazine >Fluoren-9-one oxime> Fluoren-9-one O-methyl-oxime>9-Fluorenyl semicarbazone>9-Fluorenylthio semicarbazone>(2,6-Dimethyl-phenyl)-fluoren-9-ylidene-amine >4-(N'-Fluoren-9-ylidene-hydrazino)-phenol> N,N'-Bis-fluoren-9-ylidene-ethane-1,2-diamine

Table 2- Antibacterial activity of synthesized molecules

Name of the compound	Zone of inhibition in mm				
	E. coli	***S. aureus***	***P.aeroginosa***	***P. mirabilis***	***K. pneumonia***
Control	15.8	14.6	19.1	19.4	18.4
Fluoren-9-one oxime	14.2	12.5	14.9	14.6	15.9
Fluoren-9-one O-methyl-oxime	13.7	12.0	15.3	14.1	14.8
9-Fluorenyl semicarbazone	15.1	13.3	15.8	18.1	16.0
9-Fluorenylthio semicarbazone	14.8	13.3	16.1	17.8	16.8
Fluoren-9-ylidene-hydrazine	14.3	13.9	14.2	17.2	16.3
4-(N'-Fluoren-9-ylidene-hydrazino)-phenol	13.2	13.1	17.9	15.4	16.9
N,N'-Bis-fluoren-9-ylidene-ethane-1,2-diamine	13.1	12.2	18.2	14.6	16.5
(2,6-Dimethyl-phenyl)-fluoren-9-ylidene-amine	12.9	11.8	16.5	14.7	15.6

C-control

Table 3: The nature of interaction and its dock score with Internal energy

Sno	Dock Score	Internal Energy	Nature of Interaction	
			Pi-Pi	Hydrogen bond
Compound2a	42.98	-1.768	ARG51	ALA311,ARG344,ARG51
Compound2b	43.116	-2.571	ARG51	----------
Compound2c	46.011	-2.881	HIS54	HIS341,PHE313
Compound2d	49.795	-3.166	ARG51	SER93
Compound2e	41.43	-1.44	ARG51,ARG91	ALA311,ARG344
Compound2f	61.995	-3.906	ARG333,PHE132,PHE140,TYR337	TYR337
Compound2g	81.947	-7.169	ARG333,PHE132,PHE140, ARG51	TYR337
Compound2h	59.962	-6.391	ARG51,HIS54	----------

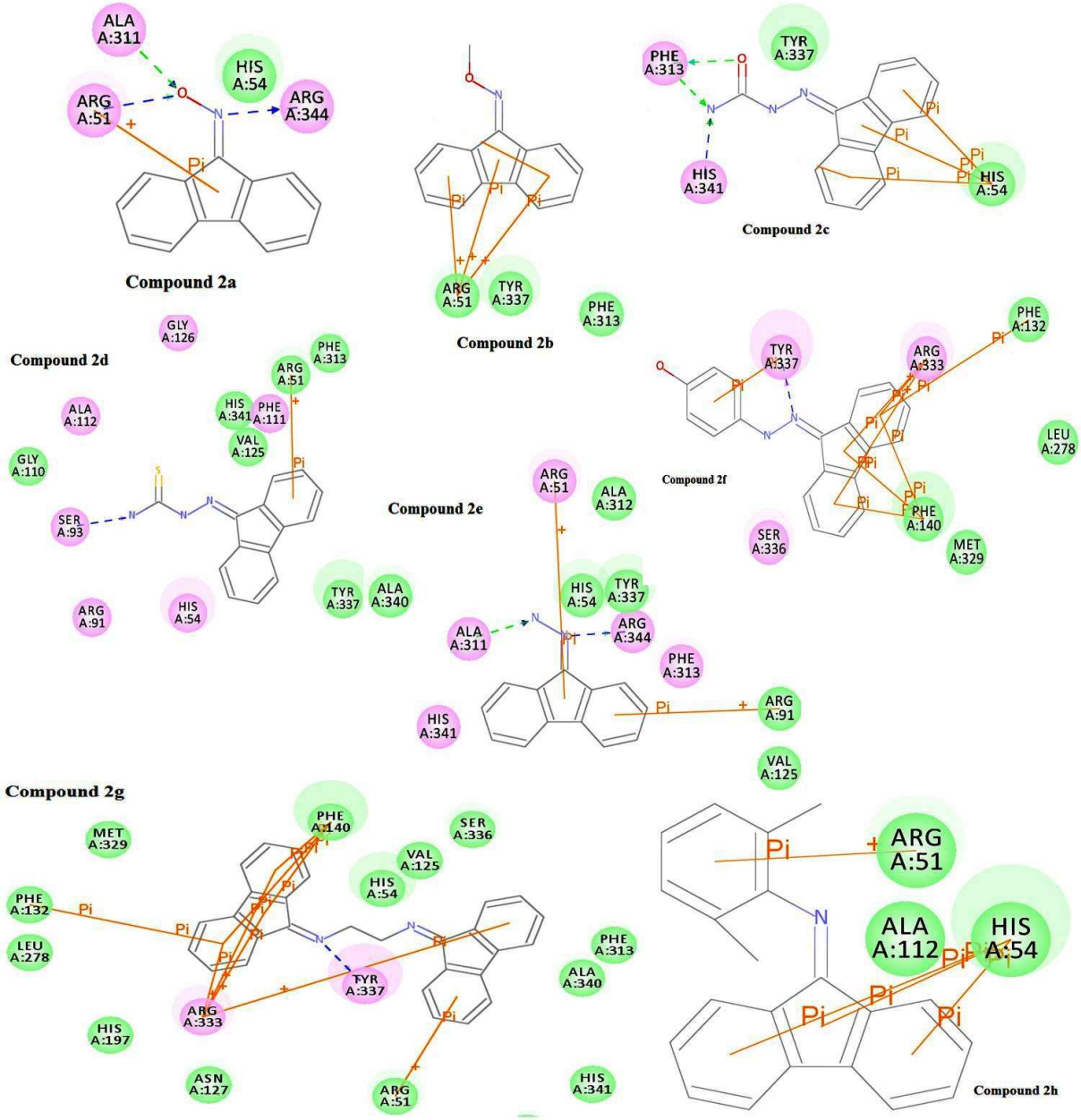
Structure based drug designing

It is iterative process to dock the lead compounds with specific site of the drug target protein. The active site of the protein of increasing volume is automatically generated using the flood filling algorithm [9].The site 1 of volume 832.75□ with partition level 3 with point count of 6662 in equal grid spacing of 0.5 (X),0.5(Y) ,0.5(Z) direction

respectively. A series of lead compounds from 2a-2h were docked with sphere site is defined as **55.37** (X), **22.595** (Y), **15.094**(Z) using "2 500 120, 4 1200 300, 6 1500 350, 10 2000 500, 25 3000 750" Number of Monte Carlo Trials. The nature of interaction and its dock score with Internal energy is tabulated in the Table 3

The ARG residues present in the active site favors for the Pi-Pi interaction in all 9-fluorenone derivatives. The compound shows dock score with decrease order of (Compound2e)> (Compound2a)> (Compound2b)> (Compound2c)> (Compound2d (Compound2h)> (Compound2f)> (Compound2g).The amino acid residues of protein active site, which as aromatic ring plays a major role in binding with the 9-fluorenone derivatives compounds. This kind of compounds shows both Pi-Pi and Hydrogen bond interaction. The interaction of compound with active site amino acid is stated in the below Figure 3

Figure 3: The interaction of compound with active site amino acid



CONCLUSION

Hence, we have synthesized various Schiff base compounds using 9-fluorenone and the same was characterized by NMR. The products were investigated for its biological activity and dock study. The other 9-fluorenone derivatives also show the activity value and good dock score but the dimer compounds favors more and lead first among eight compounds. N,N'-Bis-fluoren-9-ylidene-ethane-1,2-diamine shows highest dock score of 81.947 with Internal energy (-7.169)and its microbial activity value as 17.9mm compared with other compounds .

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