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Synthesis and Biological Evaluation of Schiff base of Dapsone and their derivative as Antimicrobial agents

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Abstract:
A series of Schiff base and 2-azetidinones of 4,4'-diaminodiphenylsulphone have been synthesized. 4,4'-diaminodiphenylsulphone was condensed with various aromatic or heterocyclic aldehyde in ethanol in the presence of concentrated sulphuric acid as a catalyst to yield the Schiff base **(Ia-e)**. These Schiff's bases on treatment with chloroacetylchloride in the presence of triethylamine gave substituted 2-azetidinone **(IIa-e)**. The structure of synthesized compounds has been established on the basis of their spectral (IR, ^{1}H NMR and Mass) data. The purity of the compounds was confirmed by TLC. A number of molecular docking experiments were carried out to identify potential inhibitor of AmpC enzyme of E. Coli HKY28. All these compounds were evaluated for their *in vitro* activity against several microbes. Compound **Ic**, **Ie**, **IIb** and **IIc** exhibited potent antibacterial activity with the reference standard ciprofloxacin and fluconazol.
Key Words: Dapsone, Schiff base, Azetidinone.

Introduction

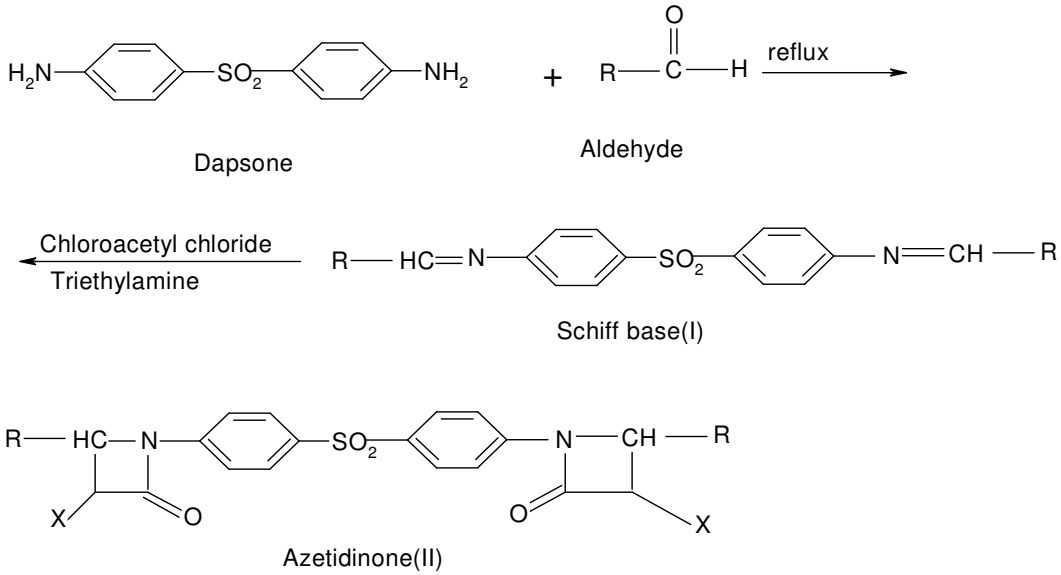
Dapsone (4, 4'-diaminodiphenylsulphone), a sulphone analog, has been proved to be a powerful antimicrobial agent.[1] Schiff base are associated with antibacterial, antifungal and antitubercular activities and have diverse biological activities.[2] Literature revealed that 2-azetidinone derivatives occupy an important place in medicinal chemistry as they show a variety of microbiological activity.[3,4] Therefore, an attempt was made to synthesize the Schiff base and 2-azetidinone of dapsone and to study the antibacterial and antifungal activity of synthesized Schiff base and 2-azetidinone. It was also planned to employ the structure base CADD on Schiff base and 2-azetidinone of dapsone. Employing the structure based CADD techniques, we have evaluated a series of virtual Schiff base and 2-azetidinone of dapsone using Ampc enzyme of E. Coli HKY28

enzyme. Based on these studies, we have taken up the compounds for synthesis and evaluated for antibacterial activity. The structural assignment of the products was based on their IR, NMR and Mass spectral data. The title compounds were screened for their antibacterial and antifungal activity.

Materials and Methods

Melting points were determined in a DBK programmed melting point apparatus and are uncorrected. The TLC of the compounds was performed on silica gel G coated glass plate with chloroform : ethanol (9:1) as solvent. Iodine vapour was used as detecting agent. The absorbance maxima (λ max) were recorded on Shimadzu 2401 UV-Visible spectrophotometer. ^{1}H NMR was recorded on Bruker DRX-300 (300 MHz FT NMR), using DMSO, IR spectra was recorded on Shimadzu 8000S and Mass spectra were recorded on Joel SX-120 mass spectrophotometer.

Scheme 1



Scheme 1: Synthesis of Schiff base and azetidinone, R= aromatic aldehyde.

Molecular modeling

A number of molecular docking experiments were carried out to identify potential inhibitor of AmpC enzyme of E. Coli HKY28. The structure was downloaded from PDB, active site characterization, energy minimization of molecules and docking (binding energy of molecules with enzymes express kcal/mol) have been done. The standard dock score is –96. The score –35 to –55 dock score means there is future scope to improve dock score. Binding energy improve by doing structure base drug design. Docking gives path to synthesis. The methodology adopted by software,

- Potential mean force[PMF] and genetic algorytham

The results of docking score of Schiff base and Azetidinone are given in Table 1.

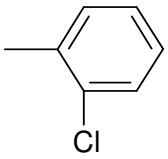
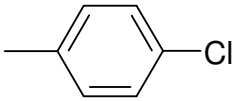
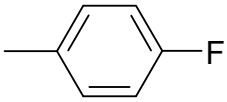
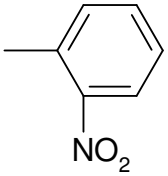
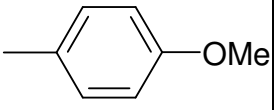
Table 1:Docking Analysis of Schiff Base and Azetidinone of Dapsone

Sr. No	Compound	Dock score
1	Dapsone	-39.421
2	Compound **Ia**	-41.421
3	Compound **Ib**	-49.152
4	Compound **Ic**	-52.214
5	Compound **Id**	-48.152
6	Compound **Ie**	-51.321
7	Compound **IIa**	-42.165
8	Compound **IIb**	-54.241
9	Compound **IIc**	-51.874
10	Compound **IId**	-48.241
11	Compound **IIe**	-47.154

Preparation of Schiff base from dapsone[5] (I)

To a mixture of 4,4'-diaminodiphenylsulphone (2.48 g, 0.01 mol) and o-chlorobenzaldehyde (2.81g, 0.02 mol) dissolved in ethanol, one drop of concentrated sulphuric acid was added. The reaction mixture was refluxed for 1 h. The reaction mixture was then poured into crushed ice. Separated solid was filtered, dried and re-crystallized from ethanol and water to gave 4,4'-bis (4-chlorobenzylidene amine) diphenyl sulphone. The reaction was monitored by TLC. The physico-chemical data for synthesized Schiff base are given in Table 2.

Table 2 Physico-Chemical data of synthesized Schiff Base of Dapsone

Compound	R	M.P. (^{0}C)	Yield (%)	R_f Value	λ max
Ia		**185-190**	**70**	**0.68**	**305**
Ib		**170-175**	**78**	**0.71**	**321**
Ic		**215-220**	**65**	**0.62**	**315**
Id		**165-170**	**80**	**0.74**	**327**
Ie		**245-250**	**74**	**0.69**	**305**

The physicochemical and spectral data:

Chemical name: 4,4'-bis (2-chlorobenzylidine amine) diphenyl sulphone **(Ia)**

White crystals, m.p. 185-190 ^{O}C; yield 70 %, IR (KBr) cm^{-1}: IR (KBr, cm^{-1}): 3000 –3100 (Ar C-H stretch), 1498.59-1581 (Ar C=C stretch), 1298 (asymmetric –SO2- stretch), 1155.28 (symmetric –SO2- stretch), 833.19 (2 adjacent H on aromatic ring), 551 (-SO- scissoring), N=C (1608.4), ^{1}H NMR (DMSO) δ ppm: 8.9 (s, ArH), 8.0 (s, ArH), 7.0-7.7 (d, -CH-Ar). MASS m/z: 492 calculated for $C_{26}H_{18}Cl_2N_2O_2S$ found 493.

Chemical name: 4,4'-bis (4-chlorobenzylidine amine) diphenyl sulphone **(Ib)**

White crystals, m.p. 170-175 ^{O}C; yield 78 %, IR (KBr) cm^{-1}: IR (KBr, cm^{-1}): 3000 –3100 (Ar C-H stretch), 1498.59-1581 (Ar C=C stretch), 1298 (asymmetric –SO2- stretch), 1155.28 (symmetric –SO2- stretch), 833.19 (2 adjacent H on aromatic ring), 551 (-SO- scissoring), N=C (1608.4), ^{1}H NMR (DMSO) δ ppm: 8.9 (s, ArH), 8.0 (s, ArH), 7.0-7.7 (d, -CH-Ar). MASS m/z: 492 calculated for $C_{26}H_{18}Cl_2N_2O_2S$ found 493.

Chemical name: 4,4'-bis (4-flurobenzylidine amine) diphenyl sulphone **(Ic)**

White crystals, m.p. 215-220 ^{O}C; yield 65 %, IR (KBr) cm^{-1}: IR (KBr, cm^{-1}): 3000 –3100 (Ar C-H stretch), 1498.59-1581 (Ar C=C stretch), 1298 (asymmetric -SO_2- stretch), 1155.28 (symmetric –SO_2- stretch), 833.19 (2 adjacent H on aromatic ring), 551 (-SO- scissoring), 1608.2 (N=C), ^{1}H NMR (DMSO) δ ppm: 8.9(s, ArH), 8.0 (m, ArH), 7.0-7.7 (m, ArH). MASS m/z: 460 calculated for $C_{26}H_{18}F_2N_2O_2S$, found 462.

Chemical name: 4,4'-bis (4-nitrobenzylidine amine) diphenyl sulphone **(Id)**

White crystals, m.p. 165-170 ^{O}C; yield 80 %, IR (KBr) cm^{-1}: IR (KBr, cm^{-1}): 3000 –3100 (Ar C-H stretch), 1498.59-1581 (Ar C=C stretch), 1298 (asymmetric -SO_2- stretch), 1155.28 (symmetric –SO_2- stretch), 833.19 (2 adjacent H on aromatic ring), 551 (-SO- scissoring), 1608.4 (N=C).^{1}H NMR (DMSO) δ ppm: 8.9 (s, ArH), 8.0 (m, ArH), 7.0-7.7(m, ArH). MASS m/z: 514 calculated for $C_{26}H_{18}N_4O_6S$, found 515.

Chemical name: 4,4'-bis (4-methoxybenzylidine amine) diphenyl sulphone **(Ie).**

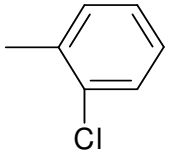
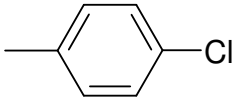
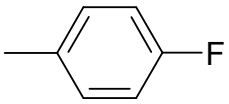
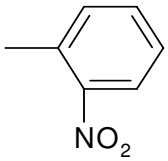
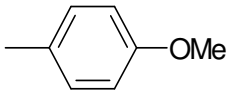
White crystals, m.p. 245-250 ^{O}C; yield 74 %, IR (KBr) cm^{-1}: IR (KBr, cm^{-1}): 3000 –3100 (Ar C-H stretch), 1498.59-1581 (Ar C=C stretch), 1298 (asymmetric -SO_2- stretch), 1155.28 (symmetric –SO_2- stretch), 833.19 (2 adjacent H on aromatic ring), 551 (-SO- scissoring), 1608.2 (N=C). ^{1}H NMR (DMSO) δ ppm: 8.9 (m, ArH), 8.0 (m, ArH), 7.0-7.7 (m, ArH), 3.7 (s,CH3). MASS m/z: 470 calculated for $C_{27}H_{23}N_2O_4S$, found 472.

Preparation of azetidinone from Schiff base (II)[7,8]

To a mixture of compound **I** (4.9 g, 0.01 mol) in dioxane (10 ml), triethylamine (3.49 ml, 0.025 mol), was added chloroacetyl chloride (1.99 ml, 0.025 mol) drop-wise at 5-10 ^{0}C.

The reaction mixture was then poured into crushed ice. The solid separated was dried and recrystallized from ethanol and water to give 4,4'-bis (3-chloro-4-(2-chloro phenyl)-2-oxo azetidin-1-yl) diphenyl sulphone. The reaction was monitored by TLC.[5] The physicochemical data for synthesized Schiff bases are given in Table 3.

Table 3
Physico-Chemical data of synthesized Azetidinone of Schiff Base of Dapsone

Compound	R	X	M.P. (^{0}C)	Yield (%)	R_f Value	λ max
IIa		Cl	240-245	78	0.55	306
IIb		Cl	189-193	74	0.64	307
IIc		Cl	205-210	75	0.69	311
IId		Cl	265-270	67	0.63	314
IIe		Cl	320-325	81	0.65	331

Chemical name: 4,4'-bis (3-chloro-4-(2-chloro phenyl)-2-oxo azetidin-1-yl) diphenyl sulphone **(IIa)**

White crystals, m.p. 240-245 ^{O}C; yield 78 %, IR (KBr) cm^{-1}: IR (KBr, cm^{-1}): 1681.2 (C=O), 1340 (SO_2, asymmetric str.), 1150 (SO_2, symmetric str.), 745 (C-S-C).^{1}H NMR (DMSO) δ ppm:8.1 (s, ArH), 7.4-7.8 (m, ArH), 5.0-5.6 (m, ArH). MASS m/z: 643 calculated for $C_{30}H_{20}Cl_4N_2O_4S$, found 644.

Chemical name: 4,4'-bis (3-chloro-4-(4-chloro phenyl)-2-oxo azetidin-1-yl) diphenyl sulphone **(IIb).**

White crystals, m.p. 189-193 ^{O}C; yield 74 %, IR (KBr) cm^{-1}: IR (KBr, cm^{-1}): 1681.8 (C=O), 1340 (SO_2, asymmetric str.), 1150 (SO_2, symmetric str.), 745 (C-S-C). ^{1}H NMR (DMSO) δ ppm:8.1 (s, ArH), 7.4-7.8 (m, ArH), 5.0-5.6 (m, ArH). MASS m/z: 643 calculated for $C_{30}H_{20}Cl_4N_2O_4S$, found 644.

Chemical name: 4,4'-bis (3-chloro-4-(4-fluro phenyl)-2-oxo azetidin-1-yl) diphenyl sulphone **(IIc).**

White crystals, m.p. 205-210 ^{O}C; yield 75 %, IR (KBr) cm^{-1}: IR (KBr, cm^{-1}): 1681.5 (C=O), 1340 (SO_2, asymmetric str.), 1150 (SO_2, symmetric str.), 745 (C-S-C). ^{1}H NMR (DMSO) δ ppm: 8.1 (s, ArH), 7.4-7.8 (m, ArH), 5.0-5.6 (m, ArH). MASS m/z: 612 calculated for $C_{30}H_{20}Cl_2F_2N_2O_4S$, found 614.

Chemical name: 4,4'-bis (3-chloro-4-(4-nitro phenyl)-2-oxo azetidin-1-yl) diphenyl sulphone **(IId).**

White crystals, m.p. 265-270 ^{O}C; yield 67 %, IR (KBr) cm^{-1}: IR (KBr, cm^{-1}): 1681.7 (C=O), 1340 (SO_2, asymmetric str.), 1150 (SO_2, symmetric str.), 745 (C-S-C). ^{1}H NMR (DMSO) δ ppm: 8.0-8.5 (s, ArH), 7.0-7.8 (m, ArH), 5.0-5.6 (m, ArH). MASS m/z: 665 calculated for $C_{30}H_{20}Cl_2N_4O_8S$, found 666.

Chemical name: 4,4'-bis (3-chloro-4-(4-methoxy phenyl)-2-oxo azetidin-1-yl) diphenyl sulphone **(IIe)**.

White crystals, m.p. 320-325 ^{O}C; yield 81 %, IR (KBr) cm^{-1}: IR (KBr, cm^{-1}): 1681.2 (C=O), 1340 (SO_2, asymmetric str.), 1150 (SO_2, symmetric str.), 745 (C-S-C). ^{1}H NMR (DMSO) δ ppm:3.4 (s, CH3), 5.0-5.4 (m, ArH), 6.8-7.0 (m, ArH), 7.5 -8.0 (m, ArH). MASS m/z: 635 calculated for $C_{32}H_{26}Cl_2N_2O_6S$, found 637.

In vitro antimicrobial activity[6]

The synthesized compounds were screened for their antibacterial activity using *Staphylococcus aureous* and *E. coli.* Control experiment was carried out under similar condition by using ciprofloxacin as standard. The inhibition zone measure in mm showed

that compound **Ic** and **Ie** were more active than other compounds tested against the above microbes.

The antifungal activity was tested against the fungal species *Aspergilus niger* and *Candida albicans* at 100 µg concentration. The antifungal data revealed that the compound **IIb** and **IIc** were more active than other compounds tested against the above microbes.

Table 4 : Antimicrobial activity data of synthesized 1 Compounds

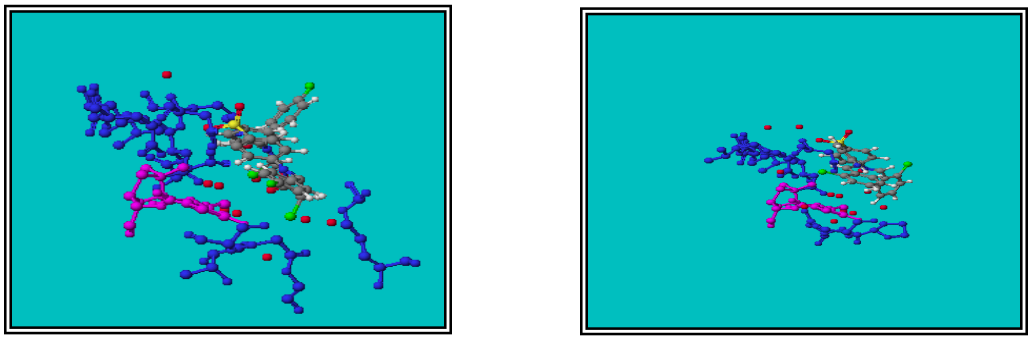
Compound	**Bacteria and fungi along with zone of inhibition (mm)**			
	S. Aureus	***E. Colic***	***C. Albicans***	***A. Niger***
Dapsone	16.8	17.2	24	23.6
Ia	17.5	18.6	24.9	24.7
Ib	19.4	21.4	24.8	25.1
Ic	20.1	20.4	24.9	25.2
Id	19.2	20.2	24.7	25.1
Ie	20.2	20.3	24.7	14.9
IIa	16.9	17.3	24.4	23.9
IIb	20.3	20.4	24.7	25.3
IIc	20.1	20.4	24.7	25.4
IId	18.4	20.1	24.2	24.1
IIe	17.9	19.8	23.6	23.2
Std-1	18.6	19.2	-	-
Std-2	-	-	24	23.6

Results and Discussion

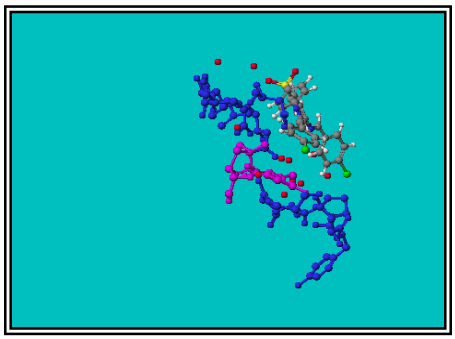
Schiff base from dapsone and their Azetidinone derivative were synthesized. Thin layer chromatography was performed on pre-coated silica gel G, glass plates using chloroform: ethanol (9:1) solvent systems to ascertain the purity of these compounds. The compounds gave single spots. The structure of synthesized compounds was confirmed by infrared spectroscopy, ^{1}H NMR spectroscopy and mass spectroscopy. Infrared spectroscopy showed the characteristic absorption bands of C=N stretching and C=O vibration of these compounds. The ^{1}H NMR spectra of the synthesized compounds show chemical shifts, which are characteristics of the anticipated structure of compounds. The mass spectra of the synthesized compounds showed the parent peak confirming the molecular weight of the compounds. Employing the structure based CADD techniques, we have evaluated a series of virtual Schiff base of dapsone using Ampc enzyme of E. Coli HKY28 enzyme. Based on these studies, we have taken up the compounds for synthesis and evaluated for antibacterial activity. Antibacterial screening of newly synthesized compounds was carried out against *E. coli, S. aureus* and antifungal activity against *C. albicans and A. niger* according to cup-plate method. The synthesized compounds have found to be better antimicrobial activity than parent compound. All the synthesised compounds have shown mild to good activity against the pathogenic bacteria and fungi. Compounds **Ic, Ie, IIb, IIc** have shown to be more potent than ciprofloxacin and compound **IIb** and **IIc** are more potent than fluconazol and other were near about equipotent in antibacterial and antifungal activity**.** The present studies are model for application of structure based CADD in development of novel molecules.

Figure 1 : Docking Results

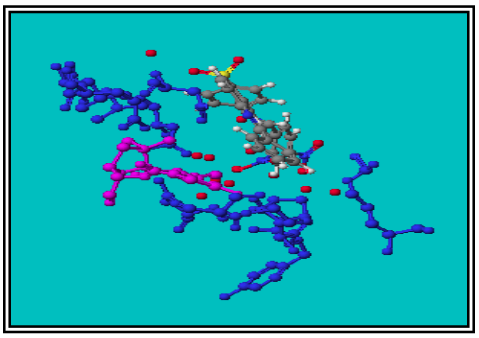
Dapsone **Compound Ia**



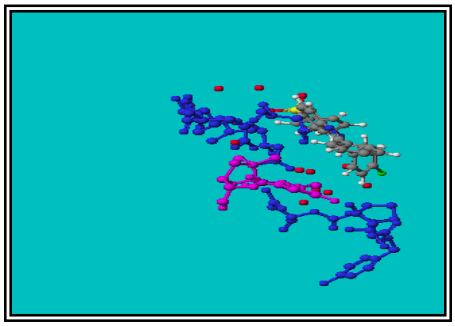
Compound Ib



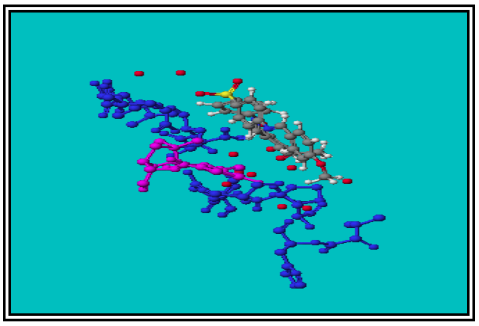
Compound Ic



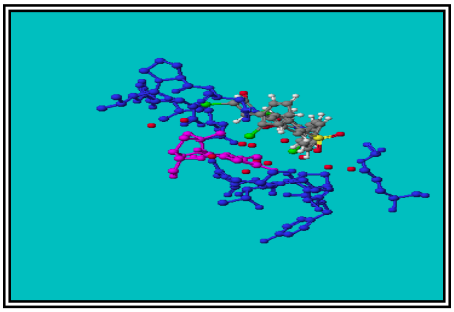
Compound Id



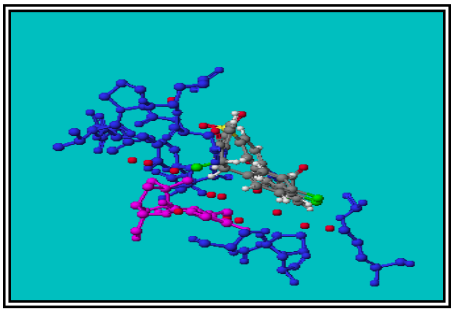
Compound Ie



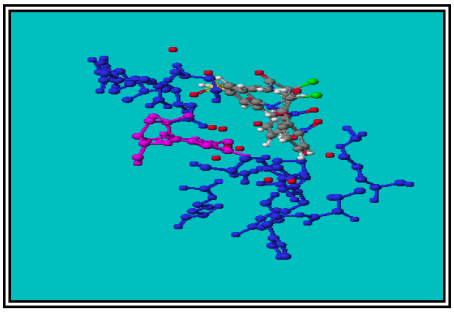
Compound IIa



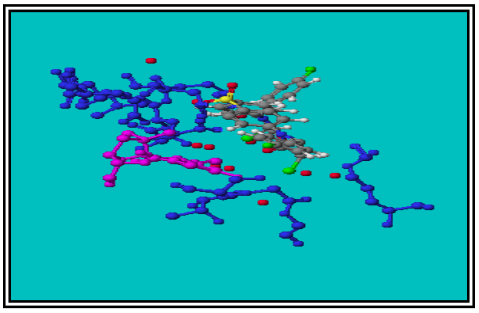
Compound IIb



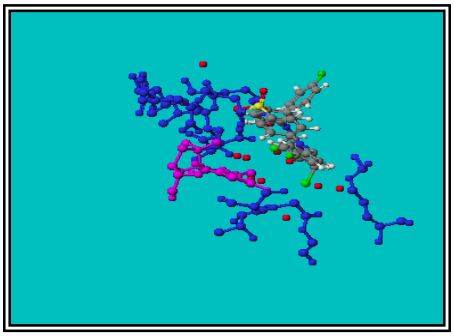
Compound IIc



Compound IId



Compound IIe



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