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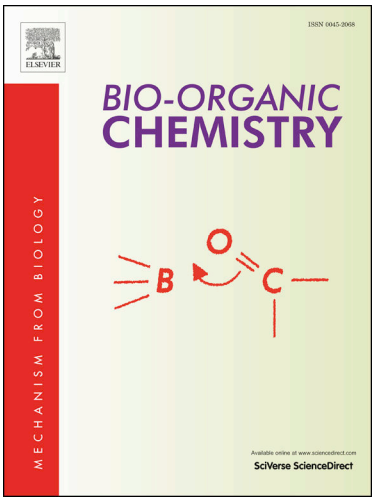

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Novel Schiff bases derived from N-aryl maleimide derivatives as an effective antimicrobial agent: Theoretical and experimental approach

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ABSTRACT:

A set of new Schiff bases of N-aryl 3- and 4-substituted maleimides has been prepared via condensation of N-aryl 3- and 4- substituted maleimides with p-toluene sulfonyl hydrazide in acidic medium at room temperature. The structures of synthesized compounds were characterized by IR, ^{1}H NMR, ^{13}C NMR, MS spectral data, and further confirmed by single-crystal x-ray crystallography for 5c. The computational study was carried out using Gaussian 09 software by using the B3LYP/6-311+G(d,p) basis set. Single-crystal study results showed much closeness with computational study results. These novel compounds were screened for their antimicrobial activity against two pathogenic bacteria such as *Escherichia coli* (ATCC 8739) and *Staphylococcus aureus* (ATCC25923) and two pathogenic fungi such as *Aspergillus niger* (MCIM10231) and *Candida albicans* (MTCC6275). The investigation of antimicrobial screening data showed that the most of tested compounds are moderate to good microbial inhibitors.

Keywords: Schiff base, N-aryl 3- and 4-substituted maleimide, p-toluene sulfonyl hydrazide, antibacterial, antifungal activity

1. Introduction

This is the era in which various invading microorganisms threaten to mankind by their adverse effects. Antimicrobial resistance by this microbial organism is a new challenge for the pharmaceutical industry and clinicians. The Multi-Drug-Resistance (MDR) bacteria and fungi are some of the important factors for the failure of chemotherapy in the number of infectious disease[1,2]. Heterocyclic compounds are widely used in the pharmaceutical industry due to their physicochemical properties, selective affinity towards biomolecules, and constructive properties

as therapeutics agents. Moreover, synthetic drugs like diazepam, chlorpromazine, metronidazole, antipyrine, isoniazid, etc. are well-known heterocycles[3,4].They are also an efficient antifungal[5], anti HIVactivity[6], antimalarial[7], antimycobacterial[8], antibacterial[9], analgesic, and anti-inflammatory agent[10]. Therefore, nowadays synthesis of new heterocyclic compounds and their variety of derivatives became a thrust area for the organic chemist. Hence, every year lot of efforts were made to synthesize new organic heterocyclic compounds.

Recently many researchers reported that maleimide derivatives can be used as selective inhibitors for monoglyceridelipase[11], Cdc25B[12], GSK-3α[13], Bfl-1[14]and DNMT-1[15]. Moreover, Maleimides are one of the promising group of heterocyclic compounds containing -CO-N(R)-CO-chain. Due to their hydrophobic and neutral nature, it can easily penetrate through biological membrane[16]. Hence widely applied for various biological applications like antibacterial[17], antimicrobial[18], antiprotozoal[19], analgesics[20], antitiangiogenic[21], antistressagents[22], cytotoxic, DNA binding and apoptotic inducing activity[23]. Maleimides are the emerging class of heterocyclic compounds and showed wide biological applications. Therefore many researches are motivated to synthesize maleimide derivatives.

S. Zacchino and co-workers reported the antimicrobial activity of N- substituted maleimide moiety against *Candida* spp. They observed that the maleimide framework is playing a vital role in enhancing the biological activity of thesecompounds[24].N. Salewskaet.al was reported the structure-dependent antifungal activity of N- substituted maleimides[25]. N.S. Patil and group were synthesized α-hydroxy phosphonates clubbed derivative of N-aryl maleimides and investigated their antimicrobial activity towards *Bacillus subtilis*, *Escherichia coli* bacteria and *Candida Albicans, Candida tropicalis, Aspergillusniger* and *Aspergillus clavatus* fungi[26].

The compounds containing azomethine (CH=N-) groups are generally known as Schiff bases. They are synthesized by condensation of carbonyl compounds with primary amines.Such compounds possess good biological properties due to the presence of the CH=N group. They are good intermediates in enzyme-catalyzed reactions of amino or carbonyl group substrates[27]. They also possess good excellent biological activities such as antibacterial[28], antifungal[29], antimalarial[30], antitumor[31], antiviral[32], antioxidants[33], analgesic, and anti-inflammatory[34]. However, clubbing of both maleimide derivative and Schiff base may enhance their biological property due to the synergistic effect.

In the present study, we have reported the facile synthesis of new Schiff base derivatives of N-aryl 3- and4- substituted maleimides by condensation with p-toluene sulfonyl hydrazide. Most of the research group reported synthesis at 50-70 ^{0}C [35,36]. But here we have performed synthesis at room temperature. Moreover, synthesized derivatives have good yield and crystallinity. The synthesized derivative was characterized by different analytical techniques such as ^{1}H NMR, ^{13}C NMR, IR, mass spectroscopy, and single-crystal x-ray diffractometer. The experimental results were supported by density functional theory (DFT) calculation with the help of the Gaussian 09 software package. The antibacterial activity of synthesized derivatives was evaluated against both gram-positive and gram-negative bacteria *viz Escherichia coli (E. coli), Staphylococcus aureus (S. aureus).* The antifungal activity of the synthesized derivative was also evaluated against fungi *viz*. *Aspergillus Niger (A. niger)* and *Candida albicans (C. albicans)*. The results showed enhanced activity than standard Fluconazole. Moreover, we observed those substituents are playing a major role in biological activity. Thus present work may provide a new direction to synthesize modified derivative of maleimides. Furthermore, it can be applied as a promising candidate for various biological applications.

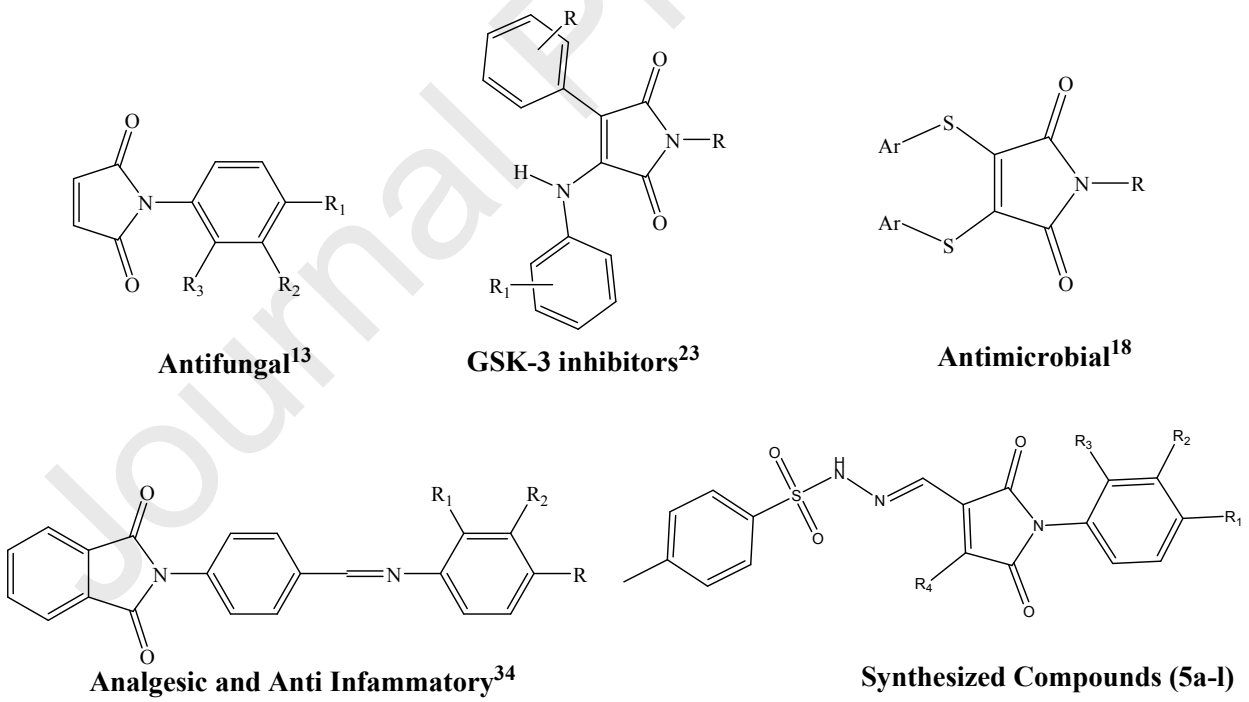
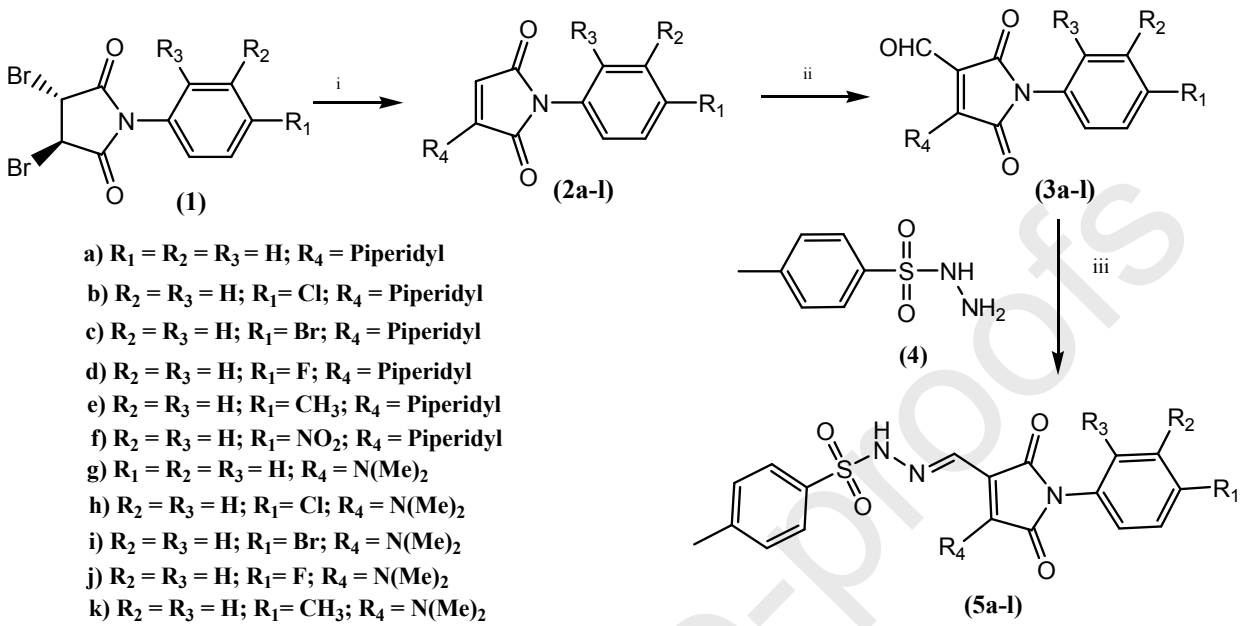


Figure 1. Chemical structures of some biologically active maleimide derivatives and Schiff base scaffold



a) $R_1 = R_2 = R_3 = H$; R_4 = Piperidyl
b) $R_2 = R_3 = H$; R_1= Cl; R_4 = Piperidyl
c) $R_2 = R_3 = H$; R_1= Br; R_4 = Piperidyl
d) $R_2 = R_3 = H$; R_1= F; R_4 = Piperidyl
e) $R_2 = R_3 = H$; R_1= CH_3; R_4 = Piperidyl
f) $R_2 = R_3 = H$; R_1= NO_2; R_4 = Piperidyl
g) $R_1 = R_2 = R_3 = H$; R_4 = $N(Me)_2$
h) $R_2 = R_3 = H$; R_1= Cl; R_4 = $N(Me)_2$
i) $R_2 = R_3 = H$; R_1= Br; R_4 = $N(Me)_2$
j) $R_2 = R_3 = H$; R_1= F; R_4 = $N(Me)_2$
k) $R_2 = R_3 = H$; R_1= CH_3; R_4 = $N(Me)_2$
l) $R_1 = R_3 = H$; R_2= NO_2; R_4 = $N(Me)_2$

Scheme 1: Reagent and conditions; (i) N,N-dimethylamine / Piperidine, DMF,0-10 ^oC,30 min.; (ii) DMF, $POCl_3$,0-5 ^oC, 30 min.; (iii) Ethanol, Acetic acid,30 min.

Table 1: Melting point, practical yield and approximate IR frequencies of the compounds (5a-l)

Entry	R_1	R_2	R_3	R_4	M.P.(^oC)	Yield (%)	υ/cm^{-1}		
							C=O	C=N	N-H
5a	H	H	H	Piperidyl	170-172	92	1752, 1686	1602	3432
5b	Cl	H	H	Piperidyl	168-170	94	1752, 1692	1607	3465
5c	Br	H	H	Piperidyl	161-163	92	1757, 1689	1602	3439
5d	F	H	H	Piperidyl	177-179	90	1754, 1701	1612	3193
5e	CH_3	H	H	Piperidyl	163-165	95	1748, 1688	1601	3435
5f	NO_2	H	H	Piperidyl	191-193	90	1756, 1698	1605	3455
5g	H	H	H	$N(Me)_2$	177-180	94	1761, 1690	1609	3387
5h	Cl	H	H	$N(Me)_2$	202-204	93	1755, 1701	1612	3472
5i	Br	H	H	$N(Me)_2$	194-196	94	1753, 1694	1605	3421
5j	F	H	H	$N(Me)_2$	192-194	91	1744, 1688	1604	3265
5k	CH_3	H	H	$N(Me)_2$	178-180	95	1753, 1696	1607	3415
5l	H	NO_2	H	$N(Me)_2$	201-203	89	1759, 1701	1612	3460

2. Result and Discussion

2.1 Spectroscopic analysis

IR spectrum of the synthesized compounds (5a-l) showed the stretching frequencies for the carbonyl group at 1743-1761 cm^{-1} and 1687-1701 cm^{-1}. The IR spectra show a strong band at 1602-1612 cm^{-1} which is attributed to the presence of azomethine (C=N) group. IR spectrum also shows N-H stretching frequency at 3387-3439 cm^{-1}. The ^{1}H NMR spectrum ($DMSOd_6$) of synthesized compound(5a-f) showed broad singlet at the range δ 1.53-1.54 ppm (J= 4 Hz) for four protons of two $–CH_2$- groups, broad singlet at the range δ 1.58-1.59 ppm (J= 4-5 Hz) for two protons of one $–CH_2$ group and a broad singlet at the range δ 3.68-3.69 ppm for four protons of two $–CH_2$ group of piperidine nucleus whereas for compounds (5g-l) showed broad singlet at the range δ 3.21-3.24 ppm for six protons of $–N(CH_3)_2$ group. The ^{1}H NMR peaks singlet at 7.809-7.842 ppm attributed the presence of -CH=N- group. The ^{1}HNMR spectrum showed peaks singlet at 11.252-11.281 ppm are due to the presence of –NH- proton whereas singlet at 2.382-2.389 ppm is due to $–CH_3$ group of toluene side chain. The peaks in the range 7.275-7.833 ppm are attributed to the aromatic protons.

The ^{13}C NMR spectrum of synthesized compounds (5a-e) showed peaks in the range of 22.94-23.54 ppm for one carbon, 26.25-26.85 ppm for two carbons and 51.17-51.84 ppm for two carbon of piperidine framework while for compounds (5g-l) showed characteristic peaks in the range 43.10-43.92 ppm for six carbons of dimethylamine group. The ^{13}C NMR spectrum showed peaks in the range 161-169 ppm for carbonyl (C=O) groups and 144.31-145.98 ppm for the –C=N- group. The peaks at the range 93-94 ppm attributed to the presence of -CH= group whereas the peaks at the range of 20-26 ppm showed the presence of –CH3 group of Toluene nucleus.

2.2 Crystal Structure of 5c

The molecular structure of the compound **5c** with the numbering scheme is given in Figure (2). The technical data collection report and some selected parameters of the refinement of compound **5c** are tabulated in Table 2 and some selected bond angles and the bond length also shown in Table 3. The compound **5c** possesses a triclinic crystal system with a P-1 space group.

The molecular density of compound **5c** was found to be 1.515 mg $m^{-3}$and which is in agreement with the theoretical expected value 1.53±0.1 mg m^{-3}. The crystal structure of **5c** shows C8-N2 distance 1.268 Å for the C=N double bond. The Schiff base molecule adopted E-configuration in connection with the C=N bond with N1-N2-C8 bond angle 117.8° and N2-C8-C9 bond angle 124.3°. Crystal packing visualization of compound 5c along different axis is shown in Figure 3 and Figure 4.

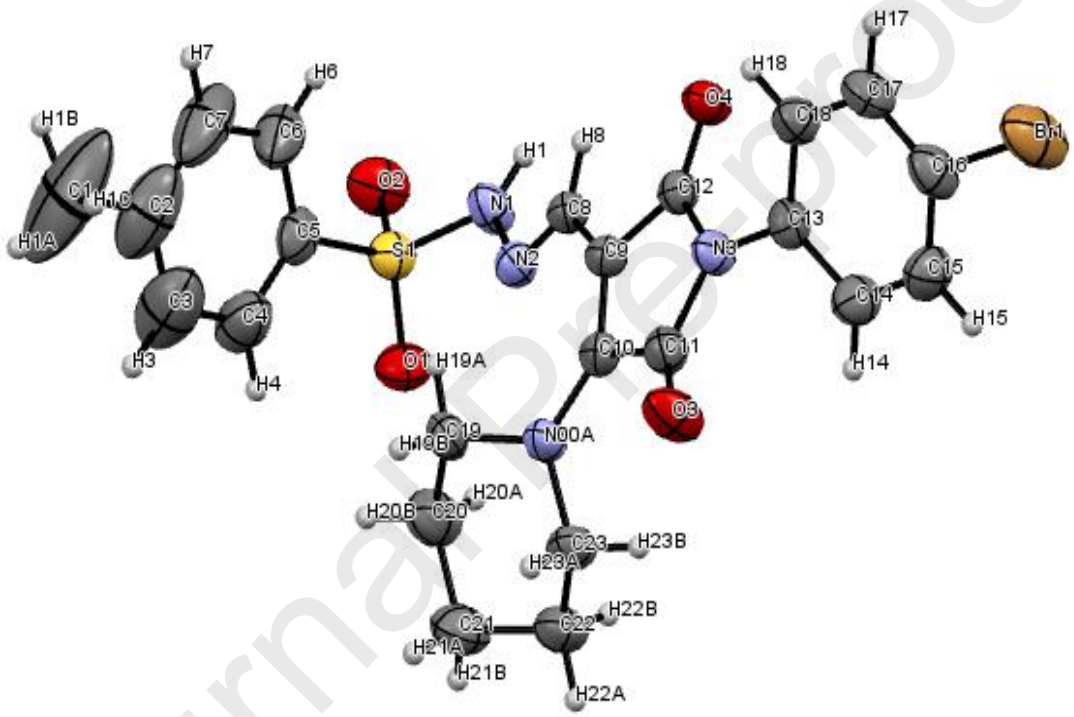


Figure 2: ORTEP diagram of compound 5c

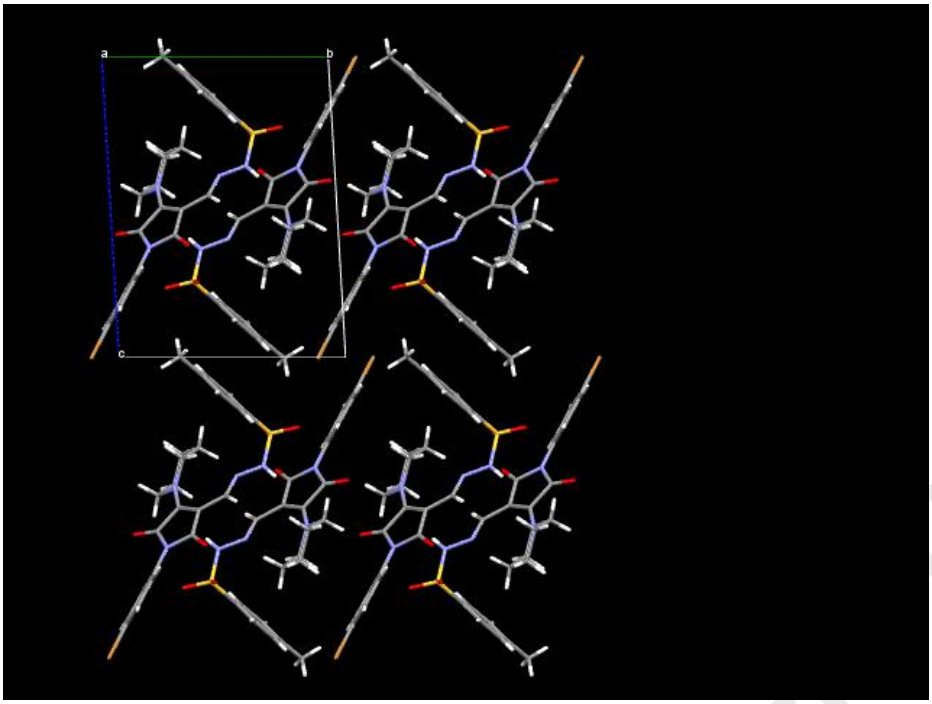


Figure 3: Crystal packing of the 5c viewed along the axis

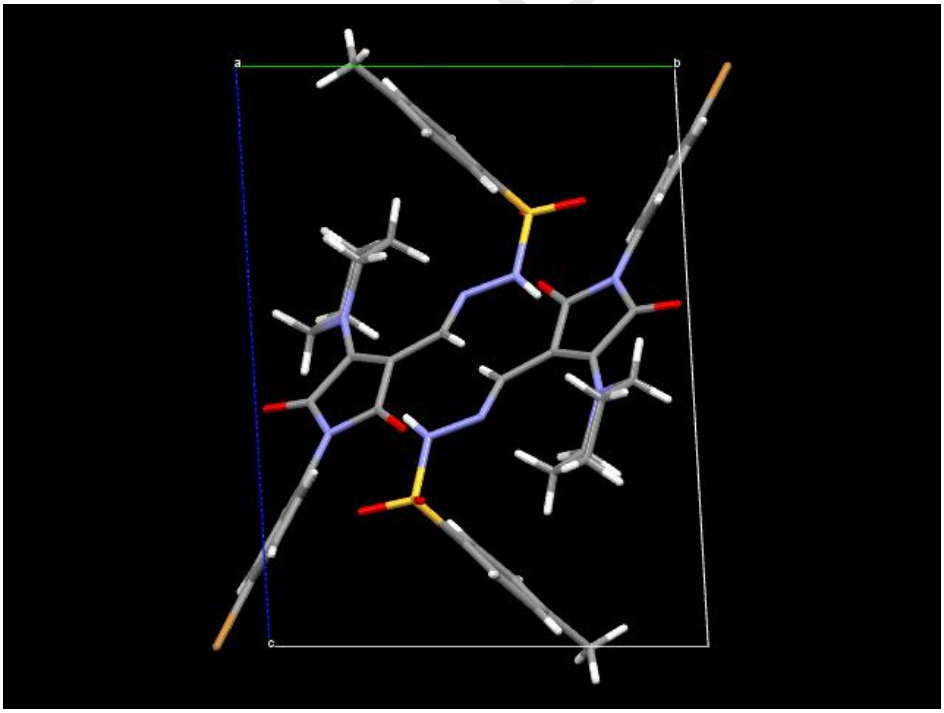


Figure 4: Crystal packing of compound 5c along the A-axis

Table 2: Crystal data and structure refinement for compound 5c

Empirical formula	C23 H23 Br N4 O4 S	
Formula weight	531.42	
Temperature	273(2) K	
Wavelength	71.073 pm	
Crystal system	Triclinic	
Space group	P -1	
Unit cell dimensions	a = 785.57(6) pm	α= 86.521(2)°.
	b = 1092.13(6) pm	β= 88.989(2)°.
	c = 1402.32(8) pm	γ = 75.993(2)°.
Volume	1.16518(13) nm^3	
Z	2	
Density (calculated)	1.515 Mg/m^3	
Absorption coefficient	1.891 mm^{-1}	
F(000)	544	
Crystal size	0.350 x 0.250 x 0.122 mm^3	
Theta range for data collection	2.911 to 28.422°.	
Index ranges	-10<=h<=10, -14<=k<=14, -18<=l<=18	
Reflections collected	15418	
Independent reflections	5833 [R(int) = 0.0303]	
Completeness to theta = 25.242°	99.3 %	
Refinement method	Full-matrix least-squares on F^2	
Data / restraints / parameters	5833 / 0 / 300	
Goodness-of-fit on F^2	1.024	
Final R indices [I>2sigma(I)]	R1 = 0.0564, wR2 = 0.1282	
R indices (all data)	R1 = 0.1020, wR2 = 0.1505	
Extinction coefficient	0.018(2)	
Largest diff. peak and hole	1.088 and -1.338 $e.Å^{-3}$	

Table 3: Comparison of bond lengths (Å) for compound 5c

Bond Lengths(Å)	B3LYP/6-311+G(d,p)	Experimental	Bond Lengths(Å)	B3LYP/6-311+G(d,p)	Experimental
C1-C2	1.3966	1.3860	C18-H23	1.0955	0.9700
C1-C6	1.3966	1.3780	C18-H49	1.0946	0.9700
C1-N11	1.425	1.4270	C20-C22	1.5329	1.5080
C2-C3	1.3916	1.3860	C20-H24	1.095	0.9700
C2-H7	1.0811	0.9300	C20-H47	1.0942	0.9700
C3-C4	1.3909	1.3650	C22-H25	1.0936	0.9700
C3-H8	1.0821	0.9300	C22-H48	1.0978	0.9700
C4-C5	1.3907	1.3790	C26-H27	1.0961	0.9300
C4-Br56	1.9178	1.8950	C26-N38	1.2815	1.2680
C5-C6	1.3919	1.3770	C28-C29	1.3914	1.3680
C5-H9	1.0821	0.9300	C28-C30	1.3948	1.3720
C6-H10	1.0808	0.9300	C28-S51	1.7938	1.7500
N11-C12	1.4382	1.4070	C29-C31	1.3935	1.3780
N11-C13	1.3834	1.3850	C29-H32	1.0829	0.9300
C12-C14	1.4805	1.4620	C30-C33	1.389	1.4030
C12-O44	1.2017	1.2130	C30-H34	1.083	0.9300
C13-C15	1.5262	1.5140	C31-C35	1.3984	1.3420
C13-O43	1.2118	1.2020	C31-H36	1.0847	0.9300
C14-C15	1.3754	1.3800	C33-C35	1.4027	1.3760
C14-C26	1.4514	1.4460	C33-H37	1.0849	0.9300
C15-N42	1.3552	1.3390	C35-C52	1.5081	1.5290
C16-C18	1.5347	1.5070	N38-N39	1.3726	1.4040
C16-H19	1.0881	0.9700	N39-H40	1.0254	0.8600
C16-N42	1.4688	1.4630	N39-S51	1.7425	1.6480
C16-H45	1.0999	0.9700	O41-S51	1.4619	1.4180
C17-C20	1.529	1.5070	O50-S51	1.4519	1.4300
C17-H21	1.1008	0.9700	C52-H53	1.0926	0.9600
C17-N42	1.4766	1.4770	C52-H54	1.0952	0.9600
C17-H46	1.0843	0.9700	C52-H55	1.0916	0.9600
C18-C22	1.532	1.5080			

Table 4: Comparison between theoretical and experimental bond angles of compound 5c

Bond angles(°)	B3LYP/6-311+G(d,p)	Experimental	Bond angles(°)	B3LYP/6-311+G(d,p)	Experimental
C2-C1-C6	120.0	120.0	C17-C20-H24	108.8	109.2
C2-C1-N11	120.2	120.8	C17-C20-H47	108.7	109.2
C6-C1-N11	119.9	119.2	C22-C20-H24	110.2	109.2
C1-C2-C3	120.2	119.4	C22-C20-H47	110.8	109.2
C1-C2-H7	120.1	120.3	H24-C20-H47	107.2	107.9
C3-C2-H7	119.7	120.3	C18-C22-C20	110.4	110.0
C2-C3-C4	119.4	120.0	C18-C22-H25	110.3	109.7
C2-C3-H8	120.1	120.0	C18-C22-H48	109.4	109.7
C4-C3-H8	120.5	120.0	C20-C22-H25	110.6	109.7
C3-C4-C5	121.0	120.9	C20-C22-H48	109.5	109.7
C3-C4-Br56	119.5	118.7	H25-C22-H48	106.6	108.2
C5-C4- Br 56	119.5	120.4	C14-C26-H27	116.9	117.8
C4-C5-C6	119.4	119.4	C14-C26-N38	122.7	124.3
C4-C5-H9	120.5	120.3	H27-C26-N38	120.4	117.8
C6-C5-H9	120.0	120.3	C29-C28-C30	121.2	119.9
C1-C6-C5	120.1	120.3	C29-C28-S51	119.5	120.7
C1-C6-H10	120.1	119.8	C30-C28-S51	119.3	119.4
C5-C6-H10	119.8	119.8	C28-C29-C31	119.0	118.6
C1-N11-C12	124.6	123.2	C28-C29-H32	119.8	120.7
C1-N11-C13	125.1	126.8	C31-C29-H32	121.2	120.7
C12-N11-C13	110.4	109.9	C28-C30-C33	118.9	120.0
N11-C12-C14	106.8	108.0	C28-C30-H34	120.1	120.0
N11-C12-O44	123.5	128.2	C33-C30-H34	121.0	120.0
C14-C12-O44	129.7	123.8	C29-C31-C35	121.6	121.1
N11-C13-C15	106.4	106.6	C29-C31-H36	119.2	119.4
N11-C13-O43	126.3	125.4	H35-C31-H36	119.6	119.4
C15-C13-O43	127.2	127.9	C30-C33-C35	121.2	121.9
C12-C14-C15	108.4	108.1	C30-C33-H37	119.3	119.1
C12-C14-C26	122.8	116.8	H35-C33-H37	119.5	119.1
C15-C14-C26	128.4	134.5	C31-C35-C33	118.5	118.5

C13-C15-C14	108.0	107.3	C31-C35-C52	121.0	119.3
C13-C15-N42	120.5	120.0	C33-C35-C52	120.5	122.2
C14-C15-N42	131.2	132.6	C26-N38-N39	116.6	115.2
C18-C16-H19	109.7	109.2	N38-N39-H40	115.4	123.4
C18-C16-N42	110.6	112.0	N38-N39-S51	113.7	113.1
C18-C16-H45	109.8	109.7	H40-N39-S51	109.0	123.4
H19-C16-N42	108.8	109.2	C15-N42-C16	122.3	123.6
H19-C16-H45	108.0	107.9	C15-N42-C17	122.8	122.4
N42-C16-H45	109.9	109.2	C16-N42-C17	114.1	113.5
C20-C17-H21	109.6	109.6	C28-S51-N39	98.2	105.7
C20-C17-N42	111.5	110.4	C28-S51-O41	108.0	108.5
C20-C17-H46	110.9	109.6	C28-S51-O50	110.1	109.6
H21-C17-N42	107.3	109.6	N39-S51-O41	109.9	108.6
H21-C17-H46	108.4	108.1	N39-S51-O50	106.8	103.8
N42-C17-H46	109.1	109.6	O41-S51-O50	121.4	119.7
C16-C18-C22	111.9	111.5	C35-C52-H53	111.4	109.5
C16-C18-H23	108.6	109.3	C35-C52-H54	110.6	109.5
C16-C18-H49	108.4	109.3	C35-C52-H55	111.5	109.5
C22-C18-H23	109.8	109.3	H53-C52-H54	107.3	109.5
C22-C18-H49	110.9	109.5	H53-C52-H55	108.3	109.5
H23-C18-H49	107.1	108.0	H54-C52-H55	107.6	109.5
C17-C20-C22	111.0	112.3			

2.3 Computational Study

The computational study of compound 5c was carried out by using the Gaussian 09 software by using the B3LYP/6-311+G(d,p) basis set. The optimized structure of compound 5c is shown in Figure 5. The optimized parameters obtained by the theoretical study were compared with the experimental parameters of single-crystal study results. The values of the parameters like bond angle, bond length are much closer to each other, and as shown in Table 3 and Table 4. Thus theoretical results corroborate very well with the experimental results.

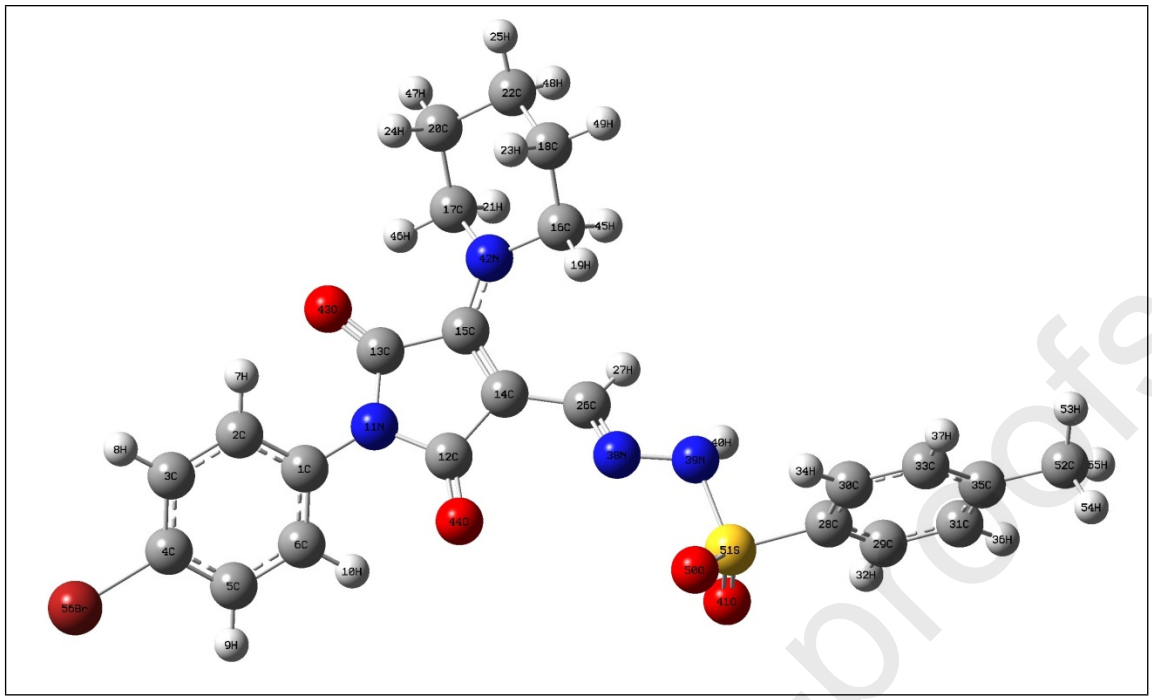


Figure 5: Optimized structure of the compound 5c

Here, the charge distribution has been characterized by Molecular electrostatic surface potentials (MESP) mapped topography as illustrated in Figure. 6.

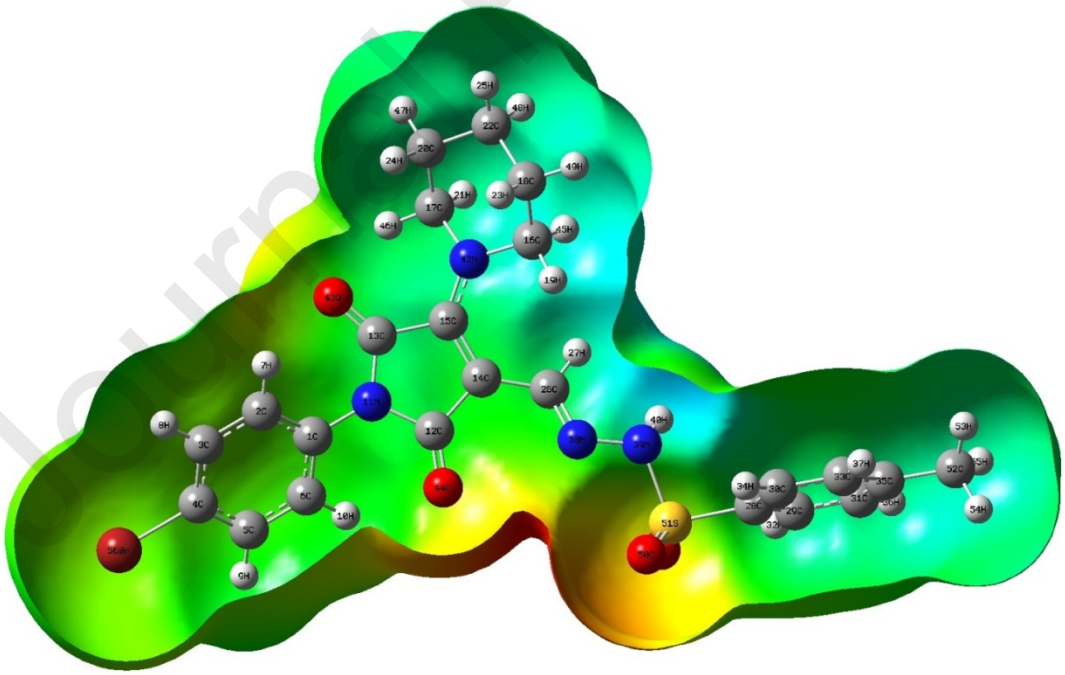
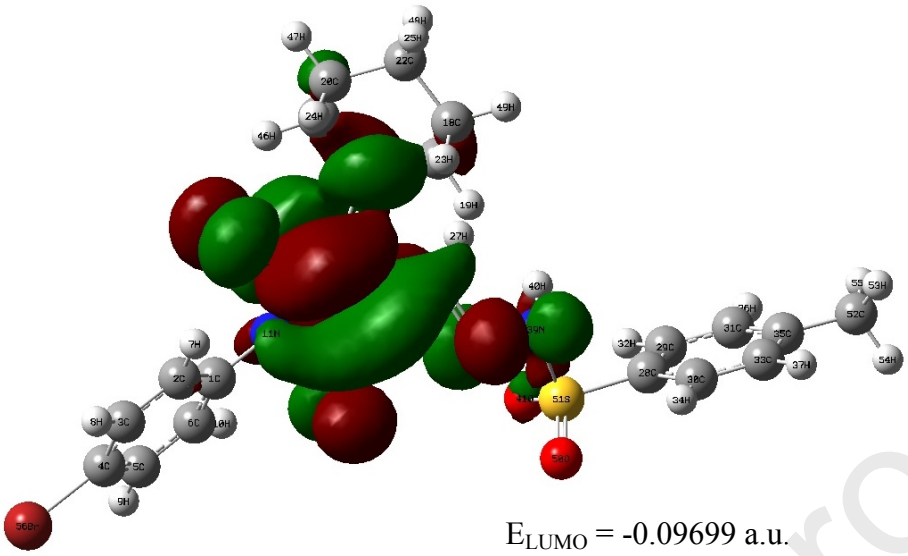


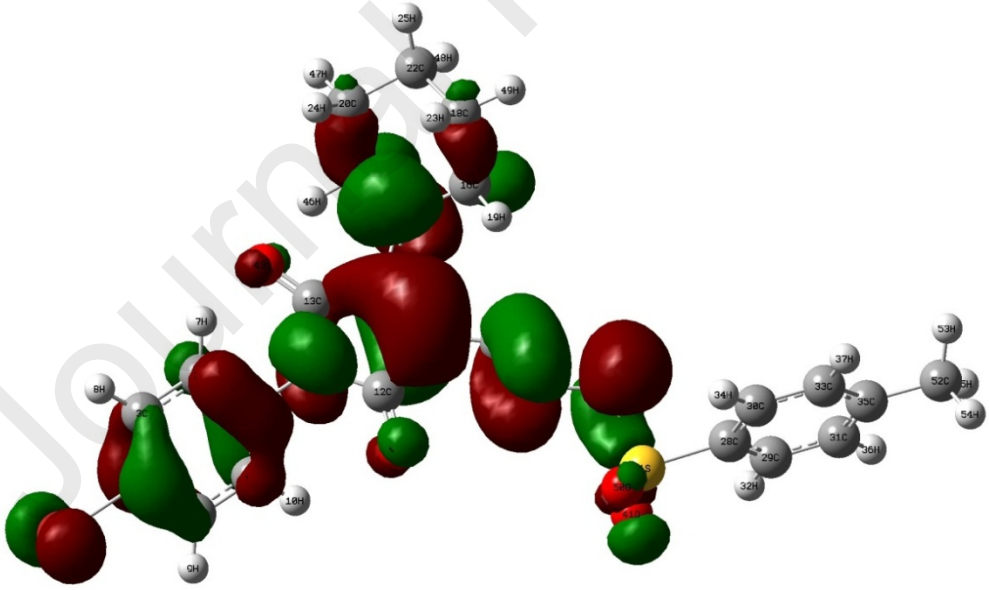
Figure 6: MESP plot by the DFT/B3LYP method with the 6-311+G(d,p) as a basis set

The effective electron-rich regions (red) are observed in the vicinity of the lone pair residing on oxygen atom centers. Whereas, more electron-deficient region was identified near the bromine groups than the aromatic hydrogens. The net Mulliken charges on each atom were obtained from population analysis and given in Table S3, which further corroborates these inferences. The gradual variation of electron-rich regions with varying functional groups on 5c comprising bromine atom is evident from Figure. 6. Moreover, the intense aromatic C=O stretching of the imide group was assigned to a band near ~1698 cm^{-1} which is consistent with experimental values. The intense vibration of aromatic C-N is observed 1347 cm^{-1}. Furthermore to substantiate the stability and reactivity of the proposed molecule frontier molecular orbital analysis was performed. Figure 7 represents the HOMO-LUMO plots of compound 5c. The frontier orbital's LUMO resides near the central five-membered ring consisting of imide functionality. On the other hand frontier orbital's HOMO is mostly located over bromobenzene and piperidine ring of the synthesized compound 5c. The thermodynamic parameters (Table S1) and Cartesian coordinates (Table S5) are given separately in supporting the information table.



E_{LUMO} = -0.09699 a.u.

ΔE = 0.12531 a. u.



E_{HOMO} = -0.22230 a.u.

Figure 7: The HOMO and LUMO plots by the DFT/B3LYP method with 6-311+G(d,p)

2.4 Antimicrobial Activity/ screening

Maleimide derivatives are showing efficient antimicrobial activity due to their hydrophobic and neutral nature. Moreover, Schiff bases are also known as excellent antimicrobial agents. To study the synergic effect due to clubbing of N-aryl 3- and 4-substituted maleimides with Schiff base, the antimicrobial activity of the synthesized compounds was examined against bacteria (*E. coli S. aureus) and* fungi *(A. niger*, *C. albicans)*. To choose a suitable concentration for antimicrobial study, concentration-dependent activity was examined against pathogenic bacteria and fungi. Initially, antimicrobial activity was checked for the one of the synthesized derivative (5c) at different concentrations *viz.* 10, 20, and 30 μg mL^{-1}. We noticed that at 20 and 30 μg mL^{-1} concentration results are almost similar whereas, at 10 μg mL^{-1} concentration zone of inhibition was relatively less than other concentrations (20,30 μg mL^{-1}). Therefore, the antimicrobial activity of other derivatives was examined at 20 μg mL^{-1} concentration.

Table 5: Antimicrobial screening of synthesized compounds 5a-l (zone diameter of growth inhibition in mm)

Entry	*Escherichia coli* ATCC 8739	*Staphylococcus aureus* ATCC25923	*Aspergillusniger* MCIM10231	*Candida albicans* MTCC6275
5a	15 ±0.8	10 ±0.2	**22 ±0.8**	15 ±0.8
5b	15 ±0.5	12 ±0.5	17 ±0.9	14 ±0.2
5c	14 ±0.3	11 ±0.3	16 ±0.3	14 ±0.4
5d	14 ±0.2	12 ±0.7	**26 ±0.5**	15 ±1.2
5e	13 ±0.5	11 ±0.8	**24 ±0.6**	14 ±0.6
5f	10 ±0.4	10 ±0.2	16 ±0.2	15 ±0.4
5g	12 ±0.7	13 ±0.8	**16 ±0.4**	13 ±0.2
5h	13 ±0.3	14 ±0.4	**17 ±0.2**	13 ±0.5
5i	13 ±0.5	15 ±0.3	**18 ±0.7**	14 ±0.7
5j	12 ±0.8	12 ±0.6	15 ±0.5	12 ±0.3
5k	11 ±0.4	11 ±0.2	14 ±0.8	11 ±0.4
5l	11 ±0.5	12 ±0.7	16 ±1.1	11 ±0.6
DMSO	8 ±0.4	8 ±0.4	9 ±0.3	9 ±0.6
Gentamicin (20μg mL^{-1})	22 ±0.3	23 ±0.5	-	-
Fluconazole (20μg mL^{-1})	-	-	24 ±0.2	22 ±0.4

The antibacterial activity was examined against the gram-negative *E. coli* while gram-positive *S. aureus* and observed results were summarised in Table 5. The inhibition zone diameter of

dimethylamine substituted derivatives (5g-l) against *E. coli* was found to be 12±0.7, 13±0.3, 13±0.5, 12 ±0.8,11 ±0.4 and 11 ±0.5 mm respectively whereas, for piperidine substituted derivative (5a-f), it was 14 ±0.3, 15 ±0.5, 14 ±0.2, 15 ±0.8, 13 ±0.4 and 10 ±0.4 mm respectively. The diameter of inhibition zone against *S. aureus* bacteria in dimethylamine substituted derivatives (5g-l) was observed to be 13 ±0.8, 14 ±0.4, 15 ±0.3, 12 ±0.6, 11 ±0.2 and 12 ±0.7 mm and for piperidine substituted compounds(5a-f), it was 11 ±0.3, 12 ±0.5, 12 ±0.7, 10 ±0.2, 11 ±0.8 and 10±0.2 mm respectively. The bacterial study indicate that the dimethylamine substituted compounds (5g-l) showed higher activity against gram-positive bacteria (*S.aureus*) whereas, piperidine substituted compounds (5a-e) showed better activity against gram-negative bacteria (*E. coli*).

The antifungal activity of synthesized derivatives (5a-l) was also tested against *A. niger* and *C. albicans* fungi and the observed results were summarized in table 5.The inhibition zone diameter due to piperidine substituted derivatives (5a-f) against *A. niger* was found to be 16 ±0.3, 17 ±0.9, 26 ±0.5, 22 ±0.8, 24 ±0.6 and 16 ±0.3 mm respectively. Similarly, the inhibition zone against *C. albicans* was observed 14 ±0.4, 14 ±0.2, 15±0.2, 15±0.6, 14 ±0.6, and 15±0.4 mm respectively. The inhibition zone diameter of dimethylamine substituted compounds (5g-l) towards *A. niger* was observed to be 16 ±0.4, 17 ±0.2, 18 ±0.7, 15±0.5, 14±0.8, 16±0.1 mm respectively. Against *C. albicans,* it was observed to be 13 ±0.2, 13±0.5, 14 ±0.7, 12 ±0.4 and 11 ±0.6 mm respectively.

For antimicrobial study, the solution of the synthesized derivative was prepared in DMSO. Therefore the activity of the DMSO was also investigated as a control. The control sample showed 8 ±0.4 mm inhibition zones against *E. coli* and *S. aureus* bacteria and 9 ±0.6 mm against *A. niger* and *C. albicans* fungi.

From our present antimicrobial study, it was noticed that the substituent playing an important role. The antifungal activity of dimethylamine substituted derivatives **5g, 5h, 5i** was found to be 16 ±0.7, 15 ±0.5, and 14±0.8 mm however for piperidine substituted derivatives **5a, 5d, 5e** it is observed 22 ±0.8, 26 ±0.8 and 24 ±0.6 mm respectively. The improvement in the antifungal activity may be due to piperidine substituent or better interaction of the fungi with piperidine substituted derivatives. A similar observation was also reported by N.Salewska and co-workers[24]. They were synthesized several N-substituted maleimides and tested for antimicrobial and

cytostatic activity. They were observed that neutral maleimides displayed relatively strong antifungal effects and their antibacterial activity was structure-dependent. The slightly enhanced antibacterial activity of piperidine substituted derivatives was also observed against *E.coli.* From antimicrobial study, it was noticed that substituents have more effect on antifungal activity than antibacterial activity.

To compare our result with the standard antibacterial agent gentamicin, its antibacterial activity was also recorded under identical conditions. It was found to be 22 ±0.3 and 23±0.5 mm against *E. coli* and *S. aureus* bacteria respectively. Similarly, antifungal activity of fluconazole was also recorded against *A. niger* and *C. albicans* and it was observed 24 ±0.2 and 24 ±0.4 mm respectively. The most important outcome from our present study is that the some of the piperidine substituted derivatives **5a, 5d, 5e** showed equal / greater antifungal activity than the standard fluconazole. The observed difference in antibacterial activity may be due to the different interaction of the bacteria/fungi with the standard compounds or the different molecular composition of the gram-positive and gram-negative bacteria. A detailed study will require elucidating the mechanism of antibacterial activity. From our data, it is clear that synthesized derivatives can be used as an antimicrobial agent for both gram-positive and gram-negative bacteria and fungi.

3. Conclusions

A series of new Schiff bases of N-aryl 3- and 4- substituted maleimides (5a-l) were successfully synthesized by condensation of N-aryl 3- and 4- substituted maleimides with p-toluene sulphonyl hydrazide in ethanol in the acidic medium at room temperature. The novelty of this work is the synthesis was performed at mild condition i.e. room temperature than other reports. The synthesized compounds have excellent practical yield and good crystallinity. The purified compound was characterized by different spectroscopic techniques.The single-crystal X-ray diffraction study reveals a triclinic crystal structure with a P-1 space group. The ^{1}H NMR and ^{13}C NMR results confirm the formation of the proposed compound. Moreover, they also support the high purity of the compound. The molecular weight obtained from mass spectroscopy was in very good agreement with those obtained from theoretical (calculated). The synthesized compound showed good antibacterial activity against *Escherichia coli* and *Staphylococcus aureus* bacteria. Moreover, they also showed efficient antifungal activity towards *Aspergillus*

niger and *Candida albicans* fungi. From antimicrobial evaluation, it was observed that substituent has some effect on activity. It worth mentioning here that some of the derivative(**5a, 5d, 5e**) showed equal / greater antifungal activity than those of standard Fluconazole.We assume that due to good yield, high purity and efficient antimicrobial activity of synthesized compounds can be used for different biological applications. Furthermore, our study may provide a new direction to chemists (researchers) for the synthesis of newly substituted maleimide derivatives.

4. Experimental Section

4.1. Materials

All the reagents and chemicals used for the synthesis were analytical grade (AR). P-toluene sulfonyl hydrazide was purchased from Spectrochem, India. N, N-Dimethyl formamide (DMF), phosphorus oxychloride, acetic acid, maleic anhydride and dimethyl sulphoxide (DMSO) were purchased from Merck chemicals India. Different substituted aniline was purchased from SD-fine chemicals, India. Thin-layer chromatography (TLC) plate of 0.2 mm thin silica layer was purchased from Merck Chemicals, India. All the solvents were used after purification by standard literature procedures. Bacterial and fungal cultures were purchased from NCIM, NCL Pune India. Muller Hinton agar and Czapek Dox agar were purchased from Hi-media, India.

4.2. Synthetic method

General procedure for the synthesis of compound (1)

The solutions of various substituted anilines (0.01 mol) were prepared in 10 mL acetic acid. Then maleic anhydride (0.01 mol) was added with constant magnetic stirring. To this reaction mixture bromine (0.011mol) was added dropwise. Further in the same pot sulfuric acid (0.025 mmol) was added and the reaction mixture was stirred for 30-45 minutes. Then the reaction mixture was cooled at room temperature and poured onto ice-cold water. The solid was separated, filtered, and washed with sodium bicarbonate followed by water and recrystallized by aqeous1:1 ethyl alcohol[37].

General procedure for the synthesis of compound (2a-l)

The solution of compound I (0.01 mol) was prepared in 10 mL purified DMF. In this solution 0.031 mol dimethylamine / piperidine was added dropwise at 0-10 ^{o}C with constant magnetic stirring. After completion of the reaction, the reaction mixture was poured into cold water. The bright yellow solid appeared, then filtered and recrystallized by using ethanol[26].

General procedure for the synthesis of compound (3a-l)

The compound 2a-l (0.01 mol) was dissolved in 2 mL DMF. Vilsmeier Haack adduct (DMF (0.012 mol) and $POCl_3$ (0.05 mol)) were added slowly dropwise at 0 ^{o}C, then the reaction mixture was stirred for 30 minutes at 0-5 ^{o}C. The reaction mixture was poured into crushed ice and neutralized by 10% $NaHCO_3$. The yellow compound was obtained, which was filtered and washed with cold water. The product was purified by recrystallization by using ethanol[26].

General procedure for the synthesis of compound (5a-l)

5 mL ethanolic solution of synthesized compound **3a-l** (0.01 mol) was prepared with the addition of a small amount of acetic acid (0.005 mol). To the above solution 0.01 mol p-toluene, sulfonyl hydrazide (**4)** was added slowly under constant stirring at room temperature. The initial yellowish color of solution changes to transparent reddish. After ~20 minutes orange-red crystals were obtained. The product was separated by filtration at the suction pump using Buchner funnel. To remove impurity, the product was washed with purified cold ethanol. The shiny orange crystals of compound 5a-l were purified by recrystallization using ethanol. The reaction was monitored by TLC method using solvent n-hexane/ethyl acetate (1:1). The synthetic procedure is schematically represented in Scheme 1.

4.3. Characterization

Synthesized compounds were purified and characterized by different analytical techniques. Chemical composition information was obtained by recording the Infra-red (IR) spectrum on Shimadzu Fourier transform infrared spectrophotometer. Nuclear magnetic resonance (NMR) spectra were recorded for purification and structural information. The ^{1}H-NMR and ^{13}C-NMR spectra were recorded on 500 MHz NMR spectrometer (BRUKER ADVANCE NEO, SAIF, Panjab University, India). The values of chemical shifts (δ) were given in ppm relative to

tetramethylsilane (TMS) as standard. The multiplicities are abbreviated as s (singlet), brs (broad singlet), d (doublet), t (triplet), q (quartet), or m (multiplet). Molecular weight and possible fragmentation was estimated from Mass spectra (MS). MS was recorded on a mass spectrometer (WATERS, Q-TOF MICROMASS (LC-MS)), SAIF, Punjab University, India). The crystal structure was determined by Single Crystal- X-Ray Diffractometer (SCXRD), (Bruker, D8 VENTURE, S. P. P. U, Pune).

N'-{(*E*)-[4-(piperidin-1-yl)-2, 5-dioxo-1-phenyl-2,5-dihydro-1*H*-pyrrol-3-yl]methylidene}-4-methylbenzene-1-sulfonohydrazide (5a)

^{1}H NMR(500 MHz.DMSOd_6) (δ-ppm) 1.53(br,4H, 2xCH_2),1.59(br,2H, J=4.6Hz,CH_2), 2.36 (s,3H, CH_3), 3.68 (br,4H, 2xCH_2), 7.26-7.43(m,4H, Ar-H), 7.64-7.81(m,5H, Ar-H), 7.82(s,1H, CH=N), 11.29(s,1H,NH),^{13}C NMR (500MHz, DMSOd_6) 20.86 (CH_3), 22.95 (1C,CH_2), 26.26(2C's, 2xCH_2), 51.23(2C's,2xCH_2), 94.18(CH=), 120.21(Ar-C), 126.87(Ar-C), 127.21 (J=170Hz, (Ar-C)), 128.53(Ar-C),128.73 (J=100Hz, Ar-C),129.51(Ar-C),131.98(Ar-C),131.51(Ar-C),136.16(Ar-C),139.73(Ar-C),139.84(J=55Hz,Ar-C),143.22(Ar-C),144.40(=C-N),164.41()C=O), 168.76 (C=O), MS m/z : 452(M+),453 (M+1).

N'-{(*E*)-[1-(4-chlorophenyl)-4-(piperidin-1-yl)-2,5-dioxo-2,5-dihydro-1*H*-pyrrol-3-yl]methylidene}-4-methylbenzene-1-sulfonohydrazide (5b)

^{1}H NMR(500 MHz. DMSOd_6) (δ-ppm) 1.53(br,4H, J=4Hz,2xCH_2),1.59(br,2H, J=4.5Hz,CH_2), 2.38(s,3H, CH_3), 3.68(br,4H, 2xCH_2), 7.32-7.35(m,2H, Ar-H), 7.42(d,2H,J=8Hz,Ar-H), 7.51-7.53(m,2H,Ar-H)7.71(t,2H,J=6.5Hz,Ar-H), 7.82(s,1H, CH=N), 11.28(s,1H,NH),^{13}C NMR (500MHz, DMSOd_6) 20.87(CH_3), 22.95 (1C,CH_2), 26.27(2C's, 2xCH_2), 51.50(2C's,2xCH_2), 94.21(CH=), 127.22(Ar-C),128.51(J=65Hz,Ar-C), 129.51(Ar-C), 130.56(Ar-C), 131.78(Ar-C), 136.19(Ar-C), 139.75(Ar-C), 143.22(Ar-C), 144.39(=C-N),164.48(C=O), 168.81(C=O), MS m/z : 487(M+),488(M+1).

N'-{(*E*)-[1-(4-bromophenyl)-4-(piperidin-1-yl)-2,5-dioxo-2,5-dihydro-1*H*-pyrrol-3-yl]methylidene}-4-methylbenzene-1-sulfonohydrazide (5c)

^{1}H NMR(500 MHz. DMSOd_6) (δ-ppm) 1.54(br,4H, J=4Hz,2xCH_2), 1.59(br,2H, J=5Hz,CH_2), 2.38(s,3H, CH_3), 3.69(br,4H, 2xCH_2), 7.29(t,2H,J=2Hz,Ar-H), 7.43(d,2H,J=8Hz, Ar-H),

7.66(t,2H,J=1.5Hz,Ar-H),7.71(d,2H,J=8.5Hz,Ar-H), 7.82(s,1H, CH=N), 11.28(s,1H,NH), ^{13}C NMR (500MHz, DMSOd_6) 21.45(CH_3), 23.54 (1C,CH_2), 26.85 (2C's,2xCH_2),51.84 (2C's,2xCH_2),94.83(CH=),120.80(Ar-C),127.81(Ar-C),129.32(Ar-C),130.09(Ar-C),132.11(Ar-C),136.79(Ar-C),140.35(Ar-C),143.82(Ar-C),144.99(=C-N),165.01(C=O), 169.34(C=O), MS m/z : 531(M+), 532(M+1).

N'-{(*E*)-[4-(piperidin-1-yl)-1-(4-fluorophenyl)-2, 5-dioxo-2,5-dihydro-1*H*-pyrrol-3-yl]methylidene}-4-methylbenzene-1-sulfonohydrazide (5d)

^{1}H NMR(500 MHz. DMSOd_6) (δ-ppm) 1.53(br,4H, J=4Hz,2xCH_2), 1.59(br,2H, J=4.5Hz,CH_2), 2.36(s,3H, CH_3), 3.69(br,4H, 2xCH_2), 7.27-7.35(m,4H, Ar-H), 7.42(d,2H,J=8Hz, Ar-H),7.70(t,2H,J=7Hz,Ar-H), 7.81(s,1H, CH=N), 11.27(s,1H,NH), ^{13}C NMR (500MHz, DMSOd_6) 20.86 (CH_3), 22.95 (1C,CH_2), 26.26 (2C's, 2xCH_2), 51.20 (2C's,2xCH_2), 94.13(CH=),115.44 (J=90Hz,Ar-C), 127.21(Ar-C), 127.88(Ar-C), 129 (J=35Hz,Ar-C),129.49 (Ar-C),136.19 (Ar-C),139.81 (Ar-C),143.21(Ar-C), 144.34 (=C-N),164.68 (C=O), 169.04 (C=O), MS m/z : 470(M+), 471(M+1).

4-methyl-*N*'-{(*E*)-[4-(piperidin-1-yl)-1-(4-methylphenyl)-2, 5-dioxo-2, 5-dihydro-1*H*-pyrrol-3-yl]methylidene}benzene-1-sulfonohydrazide (5e)

^{1}H NMR(500 MHz. DMSOd_6) (δ-ppm) 1.53(br,4H, J=4Hz,2xCH_2), 1.58(br,2H,J=5Hz,CH_2), 2.32(s,3H, CH_3),2.36(s,3H,CH_3) 3.69(bs,4H, 2xCH_2), 7.15(q,2H,J_a=2Hz and J_b=6.5Hz,Ar-H), 7.24(d,2H,J=8.5Hz,Ar-H), 7.42(d,2H,J=7Hz,Ar-H), 7.71(d,2H,J=8Hz,Ar-H),7.81(s,1H, CH=N), 11.25(s,1H,NH),^{13}CNMR(500MHz,DMSOd_6)20.70(J=155Hz,CH_3),22.97(1C,CH_2),26.25(2C's,2xCH_2),51.17(2C's,2xCH_2),94.07(CH=),126.71(Ar-C),127.21(Ar-C),129.01(Ar-C),129.49(Ar-C),136.20(Ar-C),1363.93(Ar-C),139.88(Ar-C),143.20(Ar-C),144.31(=C-N),164.79(C=O), 169.21(C=O), MS m/z : 466(M+), 467(M+1).

N'-{(E)-[4-(piperidin-1-yl)-1-(4-nitrophenyl)-2,5-dioxo-2,5-dihydro-1H-pyrrol-3-yl]methylidene}-4-methylbenzene-1-sulfonohydrazide (5f)

^{1}H NMR(500 MHz. DMSOd_6) (δ-ppm) 1.60(br,4H, J=5Hz,2xCH_2), 2.38(s,3H, CH_3), 3.32(d,2H,J=12Hz, CH_2), 3.69 (br,4H,2xCH_2), 7.43 (d,2H,J=8Hz, Ar-H), 7.63 (q,2H,J_a=2HzandJ$_b$=7Hz, Ar-H),7.71(d,2H,J=8Hz,Ar-H), 7.83(s,1H, CH=N),8.33(q,2H,J_a=2Hz

and J_b= 7Hz,Ar-H), 11.33(s,1H,NH), ^{13}C NMR (500MHz, $DMSOd_6$) 20.88 (CH_3),22.94 (1C,CH_2),26.28 (2C's,2xCH_2),51.37 (2C's,2xCH_2),94.59 (CH=),123.92 (Ar-C),126.74 (Ar-C),127.24 (Ar-C),127.92 (Ar-C),129.54 (Ar-C),136.18 (Ar-C),137.55 (Ar-C),139.57 (Ar-C),143.28 (Ar-C),144.53 (Ar-C),145.43 (=C-N),164.12 (C=O), 168.30 (C=O), MS m/z : 497(M+), 498(M+1).

N'-{(*E*)-[4-(dimethylamino)-2,5-dioxo-1-phenyl-2,5-dihydro-1*H*-pyrrol-3-yl]methylidene}-4-methylbenzene-1-sulfonohydrazide (5g)

^{1}H NMR(500 MHz. $DMSOd_6$) (δ-ppm) 2.38(s,3H,-CH_3), 3.23(s,6H,-N(CH_3)$_2$), 7.28(q,2H,J_a=1Hz and J_b=7.5Hz,Ar-H), 7.41-7.45(m,5H,Ar-H), 7.72(d,2H,J=8Hz,Ar-H), 7.84(s,1H,-CH=N), 11.27(s,1H,-NH-),^{13}C NMR (500MHz, $DMSOd_6$) 21.48(CH_3),43.92(2C's, N(CH_3)$_2$),94.31(CH=),120.81(Ar-C),127.45(Ar-C),127.76(Ar-C),128.04 (Ar-C), 129.20 (d.J=75Hz,Ar-C), 130.12 (Ar-C), 131.55 (Ar-C),132.18 (d,J=45Hz, Ar-C), 136.77 (Ar-C),140.35 (d,J=60Hz,Ar-C),143.85 (Ar-C), 145.98 (=C-N), 165.08 (C=O), 169.61 C=O), MS m/z : 412 (M+),413 (M+1),414 (M+2).

N'-{(*E*)-[1-(4-chlorophenyl)-4-(dimethylamino)-2,5-dioxo-2,5-dihydro-1*H*-pyrrol-3-yl]methylidene}-4-methylbenzene-1-sulfonohydrazide (5h)

^{1}H NMR(500 MHz. $DMSOd_6$) (δ-ppm) 2.38(s,3H,-CH_3), 3.22(s,6H,-N(CH_3)$_2$), 7.32(q,2H,J_a=2Hz and J_b=6.5Hz,Ar-H), 7.40(d,2H,J=8Hz,Ar-H), 7.52(q,2H,J_a=2.5Hz and J_b=7Hz,Ar-H),7.71(q,2H,J_a=1.5Hz and J_b=6.5Hz,Ar-H) 7.82(s,1H,-CH=N),11.27(s,1H,-NH-),^{13}CNMR(500MHz,$DMSOd_6$)20.87(CH_3),43.31(2C's,N(CH_3)$_2$),93.12(CH=),127.14 (Ar-C),128.42 (Ar-C),128.58 (Ar-C),129.51 (Ar-C),130.50 (Ar-C),131.77 (Ar-C),136.13 (Ar-C),139.66 (Ar-C),143.25 (Ar-C),145.36 (=C-N), 164.22 (C=O), 168.70 (C=O), MS m/z : 446 (M+),447 (M+1).

N'-{(E)-[1-(4-bromophenyl)-4-(dimethylamino)-2,5-dioxo-2,5-dihydro-1H-pyrrol-3-yl]methylidene}-4-methylbenzene-1-sulfonohydrazide (5i)

^{1}H NMR(500 MHz. $DMSOd_6$) (δ-ppm) 2.38(s,3H,-CH_3), 3.21(s,6H,-N(CH_3)$_2$), 7.26(q,2H,J_a=2Hz and J_b=6.5Hz,Ar-H), 7.41(d,2H,J=8Hz,Ar-H), 7.65(q,2H,J_a=2Hz and J_b=6.5Hz,Ar-H),7.71(q,2H,J_a=1.5Hz and J_b=6.5Hz,Ar-H) 7.82(s,1H,-CH=N), 11.28(s,1H,-NH-

),13C NMR(500MHz,DMSOd_6)20.89(CH_3),43.50(2C's, N(CH_3)$_2$),93.75(CH=),120.22(Ar-C),127.17(Ar-C),128.72(Ar-C),129.53(Ar-C),130.95(Ar-C),131.54(Ar-C),136.15(Ar-C),139.68(Ar-C),143.27(Ar-C),145.39(=C-N),164.19(C=O), 168.66(C=O), MS m/z : 490(M+),491(M+1).

N'-{(*E*)-[4-(dimethylamino)-1-(4-fluorophenyl)-2,5-dioxo-2,5-dihydro-1*H*-pyrrol-3-yl]methylidene}-4-methylbenzene-1-sulfonohydrazide (5j)

^{1}H NMR(500 MHz. DMSOd_6) (δ-ppm) 2.38(s,3H,-CH_3), 3.21(s,6H,-N(CH_3)$_2$), 7.26(q,2H,J_a=2Hz and J_b=6.5Hz,Ar-H), 7.41(d,2H,J=8Hz,Ar-H), 7.65(q,2H,J_a=2Hz and J_b=6.5Hz,Ar-H),7.70(t,2H,J=7Hz ,Ar-H) 7.82(s,1H,-CH=N), 11.27(s,1H,-NH-), ^{13}C NMR (500MHz,DMSOd_6)20.87(CH_3),43.20(2C's,N(CH_3)$_2$),93.73(CH=),120.20(Ar-C),127.15(Ar-C),128.70(Ar-C),129.51(Ar-C),130.94(Ar-C),131.52(Ar-C),136.13(Ar-C),139.66(Ar-C),143.25,145.36(=C-N),164.17(C=O),168.65(C=O), MS m/z : 490(M+),491(M+1).

4-methyl-*N*'-{(*E*)-[4-(dimethylamino)-1-(4-methylphenyl)-2,5-dioxo-2,5-dihydro-1*H*-pyrrol-3-yl]methylidene}benzene-1-sulfonohydrazide (5k)

^{1}H NMR(500 MHz. DMSOd_6) (δ-ppm) 2.32(s,3H,-CH_3),2.38(s,3H,CH_3) 3.21(br,6H,-N(CH_3)$_2$), 7.14(q,2H,J_a=2Hz and J_b=6.5Hz,Ar-H), 7.24(d,2H,J=8Hz,Ar-H), 7.41(d,2H,J=8Hz,Ar-H),7.71(d,2H,J=8Hz ,Ar-H) 7.82(s,1H,-CH=N), 11.25(s,1H,-NH-),^{13}C NMR (500MHz,DMSOd_6)20.54(CH_3),20.87(CH_3),43.10(2C's, N(CH_3)$_2$),93.61(CH=),126.69(Ar-C),127.15(Ar-C),129.01(d,J=15Hz),129.51(Ar-C),136.15(Ar-C),136.94(Ar-C),139.80(Ar-C),143.23(Ar-C),145.26(=C-N),164.55(C=O), 169.12 (C=O), MS m/z : 426(M+),427(M+1).

N'-{(E)-[4-(dimethylamino)-1-(3-nitrophenyl)-2,5-dioxo-2,5-dihydro-1H-pyrrol-3-yl]methylidene}-4-methylbenzene-1-sulfonohydrazide (5l)

^{1}H NMR(500 MHz. DMSOd_6) (δ-ppm) 2.39(s,3H,-CH_3), 3.24(s,6H,-N(CH_3)$_2$), 7.41(d,2H,J=8.05Hz,Ar-H), 7.72(d,2H,J=8.25Hz,Ar-H), 7.76(t,1H,J=7.95Hz,Ar-H), 7.80(q,1H,J_a=1.4Hz andJ_b=3Hz,Ar-H), 7.84(s,1H,-CH=N), 8.23(m,2H,Ar-H), 11.33(s,1H,-NH-),^{13}C NMR (500MHz, DMSOd_6) 20.86(CH_3),43.12(2C's,N(CH_3)$_2$), 93.85(CH=), 120.21(Ar-C),126.87(Ar-C),127.21(Ar-C),128.73(d, J=100Hz,Ar-C), 130.98(Ar-C), 131.51(Ar-C),

136.16(Ar-C), 139.80(d,J=55Hz, Ar-C), 143.22, 144.40(=C-N), 164.41(C=O), 168.76(C=O), MS m/z : 457(M+),458 (M+1).

4.4 Computational Details

Density functional theory calculation was carried out on Intel ® Core™ i7 computer using Gaussian® 09W, Rev: A.02 Front: 6. 1 version software. Geometry of the synthesized molecule 5c was optimized by the B3LYP functional and 6-311+G(d,P) basis set. The geometrical parameter, thermodynamic properties, molecular electrostatic potential [MEP], and Mulliken charges were estimated using the same basis set. Vibrational frequencies were assigned with the help of Gauss View 5.0 molecular visualization program

4.5. Biological evaluation

The antimicrobial activity of the synthesized derivative was examined by the well diffusion method. The antibacterial activity was investigated against gram-negative *E. coli* (ATCC8739) and gram-positive *S. aureus* (ATCC25923) bacteria. The antifungal activity was tested for *A. niger* (ATCC10231) and *C. albicans* (ATCC6275). The 0.1 mL suspension of each bacterial culture (10^8CFUs/mL) and 0.1 mL spore suspension (10^8spore/mL) of each fungus was spread on the sterile Muller Hinton agar plates and Czapek Dox plates respectively. The plates were allowed to dry for 30 minutes in the laminar airflow and wells were made aseptically using sterile 6 mm diameter cork borer. The solution of the synthesized derivative was prepared in DMSO at a concentration of 20 μg mL^{-1}. The wells were filled with 0.3 mL solution of the derivative. For comparison, the same volume of DMSO was also loaded as a control on a separate plate. Bacterial plates were incubated for 24 h at 37 °C in the incubator. The fungal plate was incubated for 48 h at 30 ^oC. The experiments were performed in triplicate manner and zone inhibition was measured by applying a standard deviation method.

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HIGHLIGHTS

- Maleimides (5a-l) were successfully synthesized by condensation of N-aryl 3- and 4-substituted maleimides with p-toluene sulphonyl hydrazide in ethanol in the acidic medium at room temperature.
- The novelty of this work is the synthesis was performed at mild condition i.e. room temperature than other reports.
- The synthesized compound showed good antibacterial activity against *Escherichia coli* and *Staphylococcus aureus* bacteria.
- They also showed efficient antifungal activity towards *Aspergillus niger* and *Candida albicans* fungi.
- We assume that due to good yield, high purity and efficient antimicrobial activity of synthesized compounds can be used for different biological applications.

- The values of densities, viscosities and relative viscosities of 6-(4-chlorophenyl)-1, 2, 3, 4-tetrahydro-4-oxo-2-thioxopyrimidine-5-carbonitrile in 60% DMSO in the temperature range 298 to 313 K are studies
- From relative velocity change in Gibb's free energy, enthalpy and entropy is evaluated.

- The magnitude of Gibb's free energy, enthalpy and entropy determines the spontaneity of solution.

Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Graphical Abstract: