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RESEARCH ARTICLE

ONE-POT SYNTHESIS, ANTIBACTERIAL, DNA PHOTOCLEAVAGE AND SAR STUDIES OF SOME FUSED 1,4-DIHYDROPYRIDINES *VIA* SYMMETRICAL HANTZSCH CONDENSATION

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ABSTRACT

Fused carbo/heterocyclic 1,4-dihydropyridine (1,4-DHPs) derivatives have been synthesized using *β*-cyclodextrin an efficient catalyst in alcohol with high yields and purity under one-pot method. The structures of synthesized compound were established using ^{1}H, ^{13}C-NMR, IR, ESI-MS and elemental analysis. The synthesized fused heterocyclic 1,4-DHPs were found to exhibit potent antimicrobial activity as compared to carbocyclic 1,4-DHPs and these results were further supported by percentage structure similarity using Chem 3D software with the standard antibiotic drug (Cefixime). Moreover, heterocyclic 1,4-DHPs shows excellent DNA photocleavage potential using agarose gel electrophoresis.

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INTRODUCTION

The chemistry of 1,4-dihydropyridines (1,4-DHPs) found birth in 1882 with Hantzsch condensation (Hantzsch, 1882). After Hantzsch, multifarious new methods have been nurtured for the synthesis of original compounds (Sohal, 2014; Paul, 202). 1,4-DHPs have attracted more attention due to their presence in the coenzyme, diphosphopyridine nucleotide (DPNH) (Hutton, 1958), and identification as bio-active material. In the present scenario many representatives have been commercialised such as nifedipine (Rahway, 1996), felodipine (Boström, 1981), nicardipine (Iwanami, 1979), amlodipine (Arrowsmith, 1986) and even more have made their presence felt in the market (Goldmann, 1991) in the treatment of angina and hypertension. The 1,4-DHPs were found to possess wide range of pharmacological activities such as anti-tumor (Boer, 1995), anti-inflammatory (Briukhanov, 1994), anticonvulsant (Tusell, 1993), antitubercular (Wachter, 1998; Desai, 2001) cerebral antischemic in the treatment of Alzheimer's disease, PAF-acether antagonists (Sunkel, 1990). In the last decade, many heterocycles are evaluated for their DNA photocleavage potential (Kumar, 2015; Sharma, 2014). It has been observed that some azoles like oxadiazoles (Kulkarni, 2011; Hanumanagoud, 2012; Taj, 2012 and Kumar, 2015), triazoles (Kumar, 2015 and Sharma, 2014) and heteroaryl-linked hydrazones (Gowda, 2013) show good DNA photocleavage potential may be due to their interacting/binding ability with the DNA structure. Therefore, such nitrogen containing heterocyclic compounds could be used as probes for DNA structure, potential chemotherapeutic and diagnostic agents (Kurdekar, 2011). Some of the chemotherapeutic drugs bind with DNA and that result photocleavage of DNA or death of cells which are mainly responsible for cancer (Raman, 2007). Cyclodextrins are composed of 6-8 glucopyranoside units and produced by the treatment of ordinary starch with a set of easily available enzymes (Biwer, 2002). In 1891, A. Villiers (Villiers, 1891), first describes cyclodextrins and soon after, F. Schardinger identified the three naturally occurring cyclodextrins -*α*, -*β*, and -*γ*. Cyclodextrins have been widely used in organic syntheses, which can bind substrates and catalyze chemical reactions with high selectivity as well as transfer hydrophobic molecules into environmental friendly medium by supramolecular interaction through reversible formation of host-guest complexes (Zhou, 2010). Not a long time ago, *β*-cyclodextrin is widely used in various other reactions (Ghanem, 2000). Understanding the importance of cyclodextrin and persisting of our research on 1,4-DHPs (Sohal, 2014), Antibacterial (Yusuf, 2012; Kumar, 2014), SAR studies (Kumar, 2014) and DNA (Kumar, 2015; Kumar, 2015 and Sharma, 2014) photocleavage study, a new β-cyclodextrin catalysed one pot protocol has been described for the synthesis

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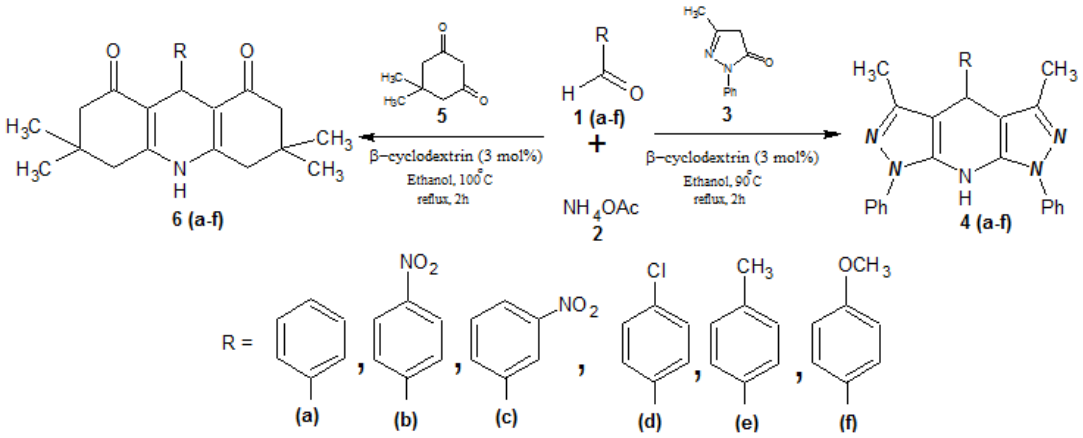
of fused 1,4-dihydropyridines by the aim of generating good antibacterials and DNA photocleavage agents. This present protocol is efficacious over other in terms of rate, yields and use of harsh reaction conditions scheme is not a pre-requisite to it (Scheme-1).

analysis of their spectral data (IR, ^{1}H-NMR & ^{13}C-NMR) and were found fully in accordance with their proposed structure. ESI-MS of selected compounds was performed. The elemental analysis was also carried out to confirm the purity of these products.



Scheme 1. Synthesis of carbocyclic and heterocyclic fused 1,4-DHP derivatives

Table 1. Effect of catalyst on the synthesis of 4a and 6a at 100°C

Entry	Amount of catalyst (mol %)	Compound	Time (hr)	Yield[a] (%)	Compound	Time (hr)	Yield (%)
1	1	4a	10	73	6a	9	60
2	2	4a	7	77	6a	5	68
3	3	4a	2	94	6a	2	92
4	4	4a	2	94	6a	2	92
5	5	4a	2	94	6a	2	92

[a]Yield refer to combined amounts of different crops.

Table 2. Effect of temperature on the synthesis 4a and 6a

Entry	Temperature[a](°C)	Compound	Time (hr)	Yield[b] (%)	Compound	Time (hr)	Yield[b] (%)
1	70	4a	18	40	6a	13	57
2	80	4a	11	59	6a	6	78
3	90	4a	5	88	6a	1	91
4	100	4a	2	94	6a	1	91
5	110	4a	2	94	6a	1	90
6	120	4a	2	93	6a	1	89

[a]Reaction carried out in oil bath and temperature is controlled with thermometer.
[a]Yield refer to combined amounts of different crops.

RESULT AND DISCUSSION

Chemistry

Condensation of 5-methyl-2-phenyl-2,4-dihydro-3*H*-pyrazol-3-one, benzaldehyde and ammonium acetate were carried out in ethanol at different temperatures (80-120°C) using varying amount of *β*-cyclodextrin. It was observed that 100°C temperature and 3 mol% of *β*-cyclodextrin (Table-1, Entry-3) is the optimal condition for the synthesis of pyrazolo-1,4-dihydropyridines. Silimarly, condensation of dimedone, benzaldehyde and ammonium acetate were carried out in ethanol at different temperatures (80-120°C) using varying amount of *β*-cyclodextrin. 3 mol% *β*-cyclodextrin at 90 °C gives the maximum yield (Table-2, Entry-3). It was found that decrease in the amount of catalyst will decrease the reaction yield and increase the reaction time but on increasing the amount of catalyst will not much affect both reaction time and yield. After catalyst optimization, the reaction was carried out at different temperatures (70-120) and it was found that 90°C is the optimal temperature for the synthesis of heterocyclic fused 1,4-DHPs. Further, rise in temperature results in the decomposition of the reaction mixture. The structures of all the prepared compounds were confirmed from the rigorous

IR spectra of 4a exhibited strong absorptions at 3360 and 1596 cm^{-1} which clearly indicated the presence of N-H and C=N group respectively. Another stretching peaks at 3063 and 2975 cm^{-1} shows the presence of aromatic sp^2 and sp^3 hybridized C-H group. ^{1}H-NMR spectrum (400MHz, DMSO-d_6) of 4a was quite informative where a doublet and triplet centered at δ 7.70 and δ 7.36 could be easily assignable to H-2', 6', 2", 6" and H-4', 4" respectively. Aromatic protons were found to be resonating at δ 7.24, 7.19, 7.14 and 7.12 could be ascribed to H-3', 5', 3", 5", H-2"', 5"', H-4"' and H-2"', 6"' protons. Further, a broad singlet at δ 13.78 for N*H* and a sharp singlet at δ 4.88 for H-4 confirms the presence of 1,4-dihydropyridine ring. A singlet in the most upfield region appeared at δ 2.31 which could be given by six protons of two methyl groups. ^{13}C-NMR (100 MHz, DMSO-d_6) spectrum of 4a proved very instrumental to corroborate its proposed structure. The signals corresponding to C-7a, 8a, C-3, 5 and C-3a, 4a appeared at δ 149.1, 146.3 and 111.0 respectively. Aromatic carbons gave characteristic resonances at δ 132.3 for C-1"', 129.1 for 2"', 6"', 128.8 for 3"', 5"' and 128.6 for 4"'. The carbon atoms C-4 & *C*H$_3$ showed suitable resonances at δ 35.2 and δ 11.7 respectively in the spectrum. In the IR spectrum of 6a, a strong absorption at 1705 cm^{-1} clearly indicates the presence of C=O

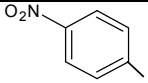
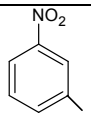
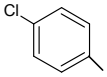
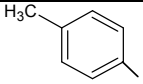
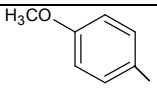
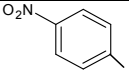
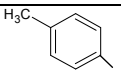
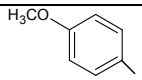
groups. Here significant bands were observed at 3420 (N-H), 3014 (aromatic C-H), 2928 cm^{-1} (methylene C-H) respectively. In the ^{1}H-NMR spectrum (400 MHz, DMSO-d_6), a doublet at δ 7.30 appeared in most downfield region could be assigned to H-2', 6' protons. A multiplet due to overlapping of signals at δ 7.21-7.14 could be ascribed to H-3', 4', 5'. Two singlets at δ 8.47 for N*H* and 5.42 for H-6 confirms the presence of 1,4-dihydropyridine ring. A singlet for two CH_3 groups was observed at δ 1.12 and 1.03, multiplets at δ 2.33-2.19 were observed for CH_2 groups.

general and affords the resultant products in excellent yield (88-95%) and products are obtained by simple work up.

Antimicrobial Activity

It is evident from table-4 that all the synthesized 4a-f were found to be potent antibacterial agents against the tested strains. Compound 4b & 4c carrying electron withdrawing nitro group

Table 3. Synthesis of carbocyclic and heterocyclic fused 1,4-DHPs

Entry	R	Time (hr)	Yield [b] (%)	Melting Point (°C)	R_f value
4a		2	94	189-191	0.63
4b		2	95	245	0.72
4c		2	92	247-249	0.71
4d		2	91	225-227	0.66
4e		2	89	205-207	0.52
4f		2	91	197-199	0.61
6a		1	92	201-203	0.50
6b		1	93	281	0.63
6c		1	91	277-278	0.64
6d		1	90	226-228	0.59
6e		1	90	207-209	0.49
6f		1	88	210-213	0.52

[a] Products were characterized with latest spectral techniques.
[b] Yield refer to combined amounts of different crops.

In ^{13}C-NMR spectrum (100 MHz, DMSO-d_6), presence of carbonyl group was confirmed by its downfield appearance at δ 188.9 (C=O). Resonances present at δ 149.5 and δ 111.9 given to the carbons belong to double bonds of the 1,4-DHP ring C-1a, 10a and C-5a, 6a, respectively. Signals for aromatic carbons can be easily assigned at δ 119.6 for C-1', 129.1 for 2', 6', 128.7 for 3', 5' and 125.4 for 4', respectively. Signal for C-6 was appeared at δ 40.2 and free *C*H_3 group was appeared at δ 27.2. Further, Mass spectrum and elemental analysis (C, H & N) also support the formation of the compound 6a. The condensation of other aldehydes 1b-f with 5-methyl-2-phenyl-2,4-dihydro-3*H*-pyrazol-3-one/dimedone and sodium acetate have been carried out and the results are summarized in Table-3. This method endures various functionalities like nitro, ether, halogen etc. on the aldehydes. Efficacy of this method is fairly

are found to exhibit significant antibacterial potency as compared to 4d, 4e and 4f bearing electron donating groups chloro, methyl, methoxy respectively. Compound 4b was found to most active against *Pseudomonas aeruginosa, Escherichia coli*, *Klebsellia pneumonia, Staphylococcus aureus, Bacillus subtilis, and Streptococcus pyogenes* with MIC-4 & 8 µg/mL. Compound 4c showed MIC-8 µg/mL against *Escherichia coli*, *Klebsellia pneumonia, Bacillus subtilis* and *Streptococcus pyogenes.* Compounds 4d & 4e were found to inhibit the growth of *Pseudomonas aeruginosa, Streptococcus pyogenes and Escherichia coli, Staphylococcus aureus* effectively at MIC-8 µg/mL respectively. The compounds fused with carbocyclic ring 6a-f are not much active against the tested bacterial strains and show MIC of 32-128 µg/mL (Table-5). Only compound 6b is active to small extent which inhibits growth of *Escherichia coli* and *Straphylococcus aureus* at

Table 4. Minimal inhibitory concentration (MIC, µg/mL) of heterocyclic fused 1,4-DHPs

Entry	Gram (-ve) bacteria			Gram (+ve) bacteria		
	E. Coli	*K. Pneumonia*	*P. Aeruginosa*	*S. aureus*	*B. subtilis*	*S. pyogenes*
4a	16	16	32	16	32	16
4b	8	8	4	8	8	4
4c	8	8	16	16	8	8
4d	16	32	8	16	16	8
4e	8	16	32	8	64	16
4f	16	32	16	16	16	32
Cefixime	4	4	4	4	4	4

Table 5. Minimal inhibitory concentration (MIC, µg/mL) of carbocyclic fused 1,4-DHPs

Entry	Gram (-ve) bacteria			Gram (+ve) bacteria		
	E. Coli	*K. Pneumonia*	*P. Aeruginosa*	*S. aureus*	*B. subtilis*	*S. pyogenes*
6a	64	32	32	128	64	64
6b	16	32	64	16	32	32
6c	32	64	64	128	32	32
6d	64	32	64	32	64	64
6e	128	64	32	64	64	128
6f	16	32	64	128	128	32
Cefixime	4	4	4	4	4	4

Table 6. Calculation of various steric and physico-chemical parameters of the compounds 4a–f and 6a–f and the standard drug, Cefixime

Compound	Log P	MR (cm^3/mol)	MW	TotE (eV) (-)	ElcE (eV) (-)	HOMO (-)	LUMO (-)	BIndx	TIndx	WIndx	% similarity with Cefixime
4a	6.803	12.7529	417.516	4711.34	39197.7	5.38427	4.69244	855873	20086	2558	84.78
4b	-	13.3644	462.514	5531.31	43191.9	7.52643	6.14043	1324523	24942	3352	90.86
4c	-	13.3644	462.514	5534.24	42646.3	7.72919	2.39737	1264722	23984	3196	91.27
4d	7.4185	13.2443	451.961	5071.09	41421.6	5.3861	4.7076	991795	21261	2800	88.41
4e	7.3474	13.2167	431.543	4860.23	40267.5	5.31153	4.09344	991795	21990	2800	87.56
4f	6.7339	13.3798	447.542	4880.33	41831.3	5.83106	4.41012	1150982	23663	3075	89.38
6a	2.0038	10.4163	349.475	4041.68	31069.7	6.21825	1.67756	421888	10960	1412	67.45
6b	3.783	11.0278	394.473	4834.61	34593.4	5.19843	4.47321	730500	14458	1990	81.29
6c	3.783	11.0278	394.473	4859.99	33702.3	5.50258	4.79124	687831	13728	1870	79.78
6d	2.562	10.9077	383.92	4375.06	33332.8	6.3359	1.58387	508717	11795	1586	72.57
6e	2.4909	10.8801	363.502	4162.35	31980	4.71297	3.62476	508717	12320	1586	71.42
6f	1.8774	11.0332	379.502	4182.44	33393.4	4.7764	3.81704	613635	13537	1787	75.63
Cefixime	0.64	10.84	453.45	5902.1	43943.6	8.85	1.76	1216151	16510	2560	

MIC- 16 µg/mL. Therefore, it is evident from Table-4 & 5 that 1,4-DHPs fused with heterocyclic nucleus shows significant antibacterial activity as compared to 1,4-DHPs fused with carbocyclic nucleus.

Computational study: Structural Similarity Assessment

The biological activity assessment of all compounds was also predicted on the basis of computational study using Chem 3D software. In this part of investigation, the compounds were assessed for their percentage similarity with the standard drug on the basis of some important molecular parameters. The sets of parameters used in an equation to calculate the distance di of titled compounds are given in Table 6.

The equation can be expressed as:

$$d_i^2 = \frac{1}{n}\sum(1 - \frac{X_{i,j}}{X_{i,standard}})^2$$

In Xi,j, the i denotes the value of physico-chemical parameter for synthesized compound j, and Xi,standard is the value of same parameters calculated against standard. n is the total number of considered molecular parameter for standard compound. The similarity of the compounds can be calculated as (Table):

% Similarity = (1-R) x 100

Where, R is quadratic mean also known as the root mean square and can be calculated as:

$$R = \sqrt{d_i^2}$$

DNA Photocleavage Study

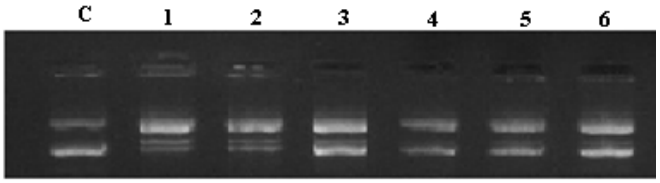


Figure 2. DNA Photocleavage Study of heterocyclic fused 1,4-DHPs

Lane-C: Control plasmid DNA + UV + DMSO, Lane-1: DNA + 40 µg 4a, Lane-2: DNA + 40 µg 4b, Lane-3: DNA + 40 µg 4c, Lane-4: DNA + 40 µg 4d, Lane-5: DNA + 40 µg 4e, Lane-6: DNA + 40 µg 4f, respectively.

The DNA photocleavage study was performed using agarose gel electrophoresis and the overall pattern is shown in figure-2 and 3. A significant change in intensity of plasmid DNA in case of **4a-f** as well as 6a-f was indicated some kinds of fragmentations or interactions caused by the compounds. In case of heterocyclic fused 1,4-DHPs, 4d and 4e (Lane-4 & 5, respectively), the intensity of Form I of *p*BR3225 DNA was found to be decreased whereas, compound **4a** and **4b** (Lane-1 & 2) decreased the intensity of supercoiled (Form I) and

increased Form II, III (Open circular and linear) of DNA in comparison to control. However, the intensity of DNA (Form I and II) was found to be increased in case of 4c and 4f (Lane 3 & 6, respectively).

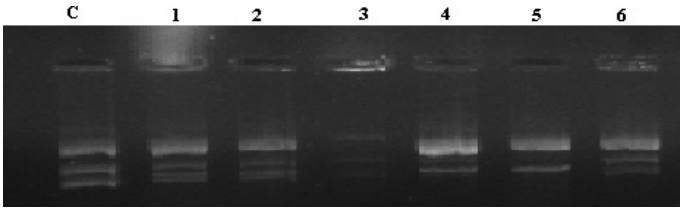


Figure 3. DNA Photocleavage Study of carbocyclic fused 1,4-DHPs

Lane C: Control plasmid DNA + UV + DMSO, Lane 1: DNA + 40 µg 6a, Lane 2: DNA + 40 µg 6b, Lane 3: DNA + 40 µg 6c, Lane 4: DNA + 40 µg 6d, Lane 5: DNA + 40 µg 6e, Lane 6: DNA + 40 µg 6f, respectively. On UV-irradiation the compounds 6d and 6e (Lane-4 & 5, respectively) were found responsible for the conversion of Form III into Form II to a large extent. In case of 1,4-dihydropyridine (6c) where nitro group is present at *meta*-position of phenyl ring, both the forms of DNA (supercoiled and open circular) were appeared diminished as comparison to control. In lane 6 the compound 6f showed no cleavage of DNA. It has been observed the intensity of plasmid DNA was decreased to a great extent in case of 1,4-DHPs fused with heterocycle ring as compared to carbocycle ring.

Conclusion

In the present procedure, *β*-cyclodextrin is an efficient catalyst for production of fused 1,4-DHPs from readily available starting materials under one-pot method with inherent flexibility and diversity. This method was efficacious to reduce labor, cost, waste production and also devoid of harsh reaction conditions. The target compounds were obtained in excellent yields. The synthesized heterocyclic fused 1,4-DHPs exhibit potent antibacterial activity as compared to carbocyclic fused 1,4-DHPs and shows 85-91% structure similarity with the reference drug, Cefixime. Further, It has been observed that synthesized compounds showed a significant level of DNA photocleavage activity. Heterocyclic fused 1,4-DHP (4b) was found to be an effective agent due to the presence of nitro group at *para*-position of phenyl ring attached to position-4 of 1,4-DHP on the basis three studies. Further modification in the basic structure may lead to construct some potential chemotherapeutic agents in future.

Experimental

Chemistry

Material and Methods

Materials were obtained from commercial suppliers and were used without further purifications. Melting points were recorded in open end capillaries and are uncorrected. ^{1}H-NMR spectra were recorded in DMSO-d_6 on a Bruker Avance II 400 MHz spectrometer; chemical shifts are reported in ppm relative to TMS as internal standard. The mass spectrum and IR spectra were recorded at LC-MS Spectrometer Model Q-ToF Micro Waters and Perkin-Elmer Spectrum II infra-red spectrophotometer, respectively. Elemental analyses (C, H, and N) were performed using a Thermo Scientific elemental analyser. Chemicals were purchased from local suppliers and used without further purification. The reactions were monitored on thin layer chromatography (TLC) using silica gel-G. The spots were visualized by using iodine vapours.

Synthesis of 3,5-dimethyl-1,4,7-triphenyl-1,4,7,8-tetrahydrodipyrazolo[3,4-b:4',3'-e]pyridine (4a)

In a conical flask, benzaldehyde (1.06 mL, 0.01 mol), 5-methyl-2-phenyl-2,4-dihydro-3*H*-pyrazol-3-one (3.48g, 0.02 mol) and ammonium acetate (1.155 g, 0.015 mol) were taken in a ethanol (10 mL) containing 3 mol% *β*-cyclodextrin and refluxed at 100^{o}C for the stipulated time **Table-1**. After the completion of reaction (*vide* TLC), reaction mixture was cooled to room temperature. After the addition of 50 mL ice-cold water, a solid was separated out. Wash the product with excess of ice-cold water and recrystalized from ethanol to afford 4a (**Table-3**, Entry-1).

4a: Yield: 94%; off white solid; m.p.: 189-191^{o}C; R_f = 0.63 [benzene: ethylacetate (8:2)]; IR (KBr): ν_{max} (cm^{-1}) 3360 (N-H), 3063 (aromatic C-H), 2975 (methyl C-H), 1596 (C=N), 1500 (C=C);^{1}H-NMR (400 MHz, DMSO-d_6): δ 13.78 (1H, brs, N***H***), 7.70 (4H, d, J_o = 7.6 Hz, H-2', 6', 2", 6"), 7.36 (2H, t, J_o = 7.7 Hz, H-4', 4"), 7.24 (4H, dt, $J_{o,m}$ = 7.7, 2.6 Hz, H-3', 5', 3", 5"), 7.19 (2H, t, J_o = 7.4 Hz, H-2"', 5"'), 7.14 (1H, t, J_o = 7.1 Hz, H-4"'), 7.12 (2H, d, J_o = 7.0 Hz, H-2"', 6"'), 4.88 (1H, s, H-4), 2.31(6H, s, C***H$_3$***); ^{13}C-NMR (100 MHz, DMSO-d_6): δ 149.1 (C-7a, 8a), 146.3 (C-3, 5), 139.6 (C-1', 1"), 132.3 (C-1"'), 129.4 (C-3', 5', 3", 5"), 129.1 (C-2"', 6"'), 128.8 (C-3"', 5"'), 128.6 (C-4"'), 126.2 (C-4', 4"), 120.7 (C-2', 6', 2", 6"), 111.0 (C-3a, 4a), 35.2 (C-4), 11.7 (***C***H$_3$); Anal. Calcd for $C_{27}H_{33}N_5$: C, 77.67; H, 5.55; N, 16.77. Found: C, 77.64; H, 5.52; N, 16.75; MS (EI) m/z 418 (M+1).

Synthesis of 3,5-dimethyl-1,7-diphenyl-4-(4-nitrophenyl)-1,4,7,8-tetrahydro dipyrazolo[3,4-b:4',3'-e]pyridine (4b)

The compound 4b was obtained by the condensation of 4-nitrobenzaldehyde (1.51g, 0.01 mol), 5-methyl-2-phenyl-2,4-dihydro-3*H*-pyrazol-3-one (3.48g, 0.02 mol) and ammonium acetate (1.155 g, 0.015 mol) under the same conditions as used earlier for 4a.

4b: Yield: 95%; yellow solid; m.p.: 245^{o}C; R_f = 0.72 [benzene: ethylacetate (8:2)]; IR (KBr): ν_{max} (cm^{-1}) 3447 (N-H), 3080 (aromatic C-H), 2950 (methyl C-H), 1599 (C=N), 1533 (C=C); ^{1}H-NMR (400 MHz, DMSO-d_6): 13. 77 (1H, s, N***H***), 8.05 (2H, d, J_o = 7.3 Hz, H-3"', 5"'), 7.92 (2H, d, J_o = 7.1 Hz, H-2"', 6"'), 7.63 (4H, d, J_o = 7.6 Hz, H-2', 6', 2", 6"), 7.50 (6H, m, H-3', 4', 5', 3", 4", 5"), 5.01 (1H, s, H-4), 2.42 (6H, s, C***H$_3$***); ^{13}C-NMR (100 MHz, DMSO-d_6): δ 149.8 (C-7a, 8a), 146.6 (C-3, 5), 145. 4 (C-4"'), 137.1 (C-1"'), 139.7 (C-1', 1"), 126.5 (C-2"', 6"'), 129.4 (C-3', 5', 3", 5"), 126.3 (C-4', 4"), 123.9 (C-3"', 5"'), 120.2 (C-2', 6', 2", 6"), 112.6 (C-3a, 4a), 37.1 (C-4), 11.9 (***C***H$_3$); Anal. Calcd for $C_{27}H_{22}N_6O_2$: C, 70.12; H, 4.79; N, 18.17. Found: C, 70.08; H, 4.75; N, 18.11; MS (EI) m/z 463 (M+1).

Synthesis of 3,5-dimethyl-1,7-diphenyl-4-(3-nitrophenyl)-1,4,7,8-tetrahydro dipyrazolo[3,4-b:4',3'-e]pyridine (4c)

The compound **4c** was obtained by the condensation of 3-nitrobenzaldehyde (1.51g, 0.01 mol), 5-methyl-2-phenyl-2,4-

dihydro-3*H*-pyrazol-3-one (3.48g, 0.02 mol) and ammonium acetate (1.155 g, 0.015 mol) under the same conditions as used earlier for **4a**.

4c: Yield: 92%; light yellow solid; m.p.: 247-249°C; R_f = 0.71 [benzene: ethylacetate (8:2)]; IR (KBr): ν_{max} (cm^{-1}) 3436 (N-H), 3075 (aromatic C-H), 2920 (methyl C-H), 1601 (C=N), 1527 (C=C); ^{1}H-NMR (400 MHz, DMSO-d_6): 13.66 (1H, brs, N***H***), 8.06 (1H, s, H-2"'), 8.00 (1H, d, J_o = 7.8 Hz, H-4"'), 7.69 (5H, m, H-2', 6', 2", 6", 6"'), 7.48 (1H, s, J_o = 8.0 Hz, H-5"'), 7.37 (4H, t, J_o = 7.8 Hz, H-2', 6', 2", 6"), 7.19 (2H, t, J_o = 7.2 Hz, H-4', 4"), 4.98 (1H, s, H-4), 2.34 (6H, s, C***H***$_3$); ^{13}C-NMR (100 MHz, DMSO-d_6): δ 149.9 (C-7a, 8a), 148.3 (C-3"'), 146.6 (C-3, 5), 139.6 (C-1', 1"), 139.0 (C-1"'), 135.2 (C-6"'), 129.6 (C-5"'), 129.3 (C-3', 5', 3", 5"), 126.7 (C-4', 4"), 124.3 (C-2"'), 120.3 (C-2', 6', 2", 6"), 118.1 (C-4"'), 112.7 (C-3a, 4a), 36.5 (C-4), 11.9 (***C***H$_3$); Anal. Calcd for $C_{27}H_{22}N_6O_2$: C, 70.12; H, 4.79; N, 18.17. Found: C, 70.11; H, 4.78; N, 18.16; MS (EI) m/z 463 (M+1).

Synthesis of 3,5-dimethyl-1,7-diphenyl-4-(4-chlorophenyl)-1,4,7,8-tetrahydro dipyrazolo[3,4-b:4',3'-e] pyridine (4d)

The compound **4d** was obtained by the condensation of 4-chlorobenzaldehyde (1.40g, 0.01 mol), 5-methyl-2-phenyl-2,4-dihydro-3*H*-pyrazol-3-one (3.48g, 0.02 mol) and ammonium acetate (1.155 g, 0.015 mol) under the same conditions as used earlier for **4a**.

4d: Yield: 91%; off white solid; m.p.: 225-227°C; R_f = 0.66 [benzene: ethylacetate (8:2)]; IR (KBr): ν_{max} (cm^{-1}) 3369 (N-H), 3053 (aromatic C-H), 2920 (methyl C-H), 1600 (C=N), 1501 (C=C); ^{1}H-NMR (400 MHz, DMSO-d_6): 13.66 (1H, brs, N***H***), 7.67 (4H, d, J_o = 7.7 Hz, 2', 6', 2", 6"), 7.35 (4H, t, J_o = 7.6 Hz, 3', 5', 3", 5"), 7.21 (6H, m, H-4', 4", 2"', 3"', 5"', 6"'), 4.84 (1H, s, H-4), 2.30 (6H, s, C***H***$_3$); ^{13}C-NMR (100 MHz, DMSO-d_6): δ 149.4 (C-7a, 8a), 146.1 (C-3, 5), 133.6 (C-4"'), 130.4 (C-2"', 6"'), 130.1 (C-1"'), 129.4 (C-3', 5', 3", 5"), 128. 9 (C-3"', 5"'), 126.8 (4', 4"), 120.4 (C-2', 6', 2", 6"), 111.6 (C-3a, 4a), 35.3 (C-4), 11.9 (***C***H$_3$); Anal. Calcd for $C_{27}H_{22}N_5Cl$: C, 71.75; H, 4.91; N, 15.50. Found: C, 71.71; H, 4.90; N, 15.49; MS (EI) m/z 453 (M+1).

Synthesis of 3,5-dimethyl-1,7-diphenyl-4-(4-methylphenyl)-1,4,7,8-tetrahydro dipyrazolo[3,4-b:4',3'-e] pyridine (4e)

The compound 4e was obtained by the condensation of 4-methylbenzaldehyde (1.18mL, 0.01 mol), 5-methyl-2-phenyl-2,4-dihydro-3*H*-pyrazol-3-one (3.48g, 0.02 mol) and ammonium acetate (1.155 g, 0.015 mol) under the same conditions as used earlier for 4a.

4e: Yield: 89%; yellow solid; m.p.: 205-207°C; R_f = 0.52 [benzene: ethylacetate (8:2)]; IR (KBr): ν_{max} (cm^{-1}) 3399 (N-H), 2928 (aromatic C-H), 2879 (methyl C-H), 1598 (C=N), 1516 (C=C); ^{1}H-NMR (400 MHz, DMSO-d_6): 13.60 (1H, brs, N***H***), 7.52 (4H, d, J_o = 7.4 Hz, H-2', 6' 2", 6"), 7.30 (4H, t, J_o = 7.3 Hz, H-3', 5', 3", 5"), 7.24 (2H, d, J_o = 7.0 Hz, 4', 4"), 6.94 (4H, m, H-2"', 3"', 5"', 6"'), 4.75 (1H, s, H-4), 2.51(3H, s, C***H***$_3$), 2.29 (6H, s, C***H***$_3$); ^{13}C-NMR (100 MHz, DMSO-d_6): δ 149.03 (C-7a, 8a), 146.42 (C-3, 5), 139.10 (C-1', 1"), 138.2 (C-4"'), 129.4 (C-3', 5', 3", 5"), 129.3 (C-2"', 6"'), 129.0 (C-1"'), 128.9 (C-3"', 5"'), 126.7 (C-4', 4"), 121.10 (C-2', 6', 2", 6"), 110.07 (C-3a, 4a), 34.8 (C-4), 23.1 (***C***H$_3$), 11.1 (***C***H$_3$); Anal. Calcd for $C_{28}H_{25}N_5$: C, 77.93; H, 5.84; N, 16.23. Found: C, 77.88; H, 5.81; N, 16.19; MS (EI) m/z 432 (M+1).

Synthesis of 3,5-dimethyl-1,7-diphenyl-4-(4-methoxyphenyl)-1,4,7,8-tetrahydro dipyrazolo[3,4-b:4',3'-e] pyridine (4f)

The compound **4f** was obtained by the condensation of 4-methoxybenzaldehyde (1.21mL, 0.01 mol), 5-methyl-2-phenyl-2,4-dihydro-3*H*-pyrazol-3-one (3.48g, 0.02 mol) and ammonium acetate (1.155 g, 0.015 mol) under the same conditions as used earlier for **4a**.

4f: Yield: 91%; pale yellow solid; m.p.: 197-199°C; R_f = 0.61 [benzene: ethylacetate (8:2)]; IR (KBr): ν_{max} (cm^{-1}) 3412 (N-H), 2919 (aromatic C-H), 2839 (methyl C-H), 1600 (C=N), 1507 (C=C); ^{1}H-NMR (400 MHz, DMSO-d_6): 13.74 (1H, brs, N***H***), 7.69 (4H, d, J_o = 8.3 Hz, 2', 6', 2", 6"), 7.35 (4H, t, J_o = 7.6 Hz, H-3', 5', 3", 5"), 7.17 (4H, t, J_o = 7.0 Hz, H-4', 4", 2"', 6"'), 6.75(2H, d, J_o = 8.2 Hz, 3"', 5"'), 4.82 (1H, s, H-4), 3.69 (3H, s, OC***H***$_3$), 2.30 (6H, s, C***H***$_3$); ^{13}C-NMR (100 MHz, DMSO-d_6): δ 157.8 (C-4"'), 148.3 (C-7a, 8a), 144.3 (C-3, 5), 139.6 (C-1', 1"), 129.76 (C-3', 5', 3", 5"), 126.52 (C- 4', 4"), 127.6 (C-2"', 6"'), 120.8 (2', 6', 2", 6"), 120.5 (C-1"'), 114.3 (C-3"', 5"'), 110.1 (C-3a, 4a), 56.1 (O***C***H$_3$), 34.6 (C-4), 11.7 (***C***H$_3$); Anal. Calcd for $C_{28}H_{25}N_5O$: C, 75.15; H, 5.63; N, 15.65. Found: C, 75.11; H, 5.59; N, 15.64; MS (EI) m/z 448 (M+1).

Synthesis of 9-(phenyl)-3,3,6,6-tetramethyl-3,4,6,7,9,10-hexahydroacridine-1,8(2H,5H)-dione (6a)

In a conical flask benzaldehyde (1.06 mL, 0.01 mol), dimedone (2.80g, 0.02 mol) and ammonium acetate (1.155g, 0.015 mol) were taken in a ethanol (10 mL) containing 3 mol% *β*-cyclodextrin and reflux at 90°C for the stipulated time (Table-1). After the completion of reaction (*vide* TLC), reaction mixture was cooled to room temperature and added 50 mL ice-cold water. A solid was separated out which was washed with excess of ice-cold water. The crude product was filtered, dried and recrystallized from ethanol to afford compound **6a**. (Table-3, Entry-7). Similarly, other aldehydes were reacted with dimedone and ammonium acetate to afford various carbocyclic fused 1,4-DHP derivatives **6b-f** (**Table-3**). Data obtained using advanced spectral techniques have been summarized below.

6a: Yield: 92%; off white solid; m.p.: 201-203°C; R_f = 0.50 [benzene: ethylacetate (7:3)]; IR (KBr): ν_{max} (cm^{-1}) 3420 (N-H), 3014 (aromatic C-H), 2928 (methyl C-H), 1705 (C=O); ^{1}H-NMR (400 MHz, DMSO-d_6): δ 8.47 (1H, brs, N***H***), 7.30 (2H, d, J_o = 7.2 Hz, H-2', 6'), 7.21 (3H, m, J_o = 7.3 Hz, H- 3', 4', 5'), 5.42 (1H, s, H-6), 2.33-2.17 (8H, m, H-2, 4, 8, 10), 1.12 (6H, s, C***H***$_3$), 1.03 (6H, s, C***H***$_3$); ^{13}C-NMR (100 MHz, DMSO-d_6): δ 188.9 (C=O), 149.5 (C-1a, 10a), 129.1 (C-2', 6'), 128.7 (C-3', 5'), 125.4 (C-4'), 119.6 (C-1'), 111.9 (C-5a, 6a), 51.3 (C-4, 8), 43.5 (C-2, 10), 40.2 (C-6), 31.7 (C-3, 9), 27.2 (***C***H$_3$); Anal. Calcd for $C_{23}H_{27}NO_2$: C, 79.05; H, 7.79; N, 4.01. Found: C, 79.01; H, 7.75; N, 3.99; MS (EI) m/z 350 (M+1).

Synthesis of 3,3,6,6-tetramethyl-9-(4-nitrophenyl)-3,4,6,7,9,10-hexahydroacridine- 1,8(2H,5H)-dione (6b)

The compound 6b was obtained by the condensation of 4-nitrobenzaldehyde (1.51g, 0.01 mol), dimedone (2.80g, 0.02 mol) and ammonium acetate (1.155 g, 0.015 mol) under the same conditions as used earlier for 6a.

6b: Yield: 93%; orange solid; m.p.: 281°C; R_f = 0.63 [benzene: ethylacetate (7:3)]; IR (KBr): ν_{max} (cm^{-1}) 3460 (N-H), 3052 (aromatic C-H), 2970 (methyl C-H), 1710 (C=O); ^{1}H-NMR (400 MHz, DMSO-d_6): δ 8.65 (1H, s, N***H***), 8.15 (2H, d, J_o = 7.8 Hz, H-3', 5'), 7.87 (2H, d, J_o = 7.9 Hz, H-2', 6'), 5.61 (1H, s, C***H***), 2.35 -2.19 (8H, m, H-2, 4, 8, 10), 1.17 (6H, s, C$\boldsymbol{H_3}$), 1.12 (6H, s, C$\boldsymbol{H_3}$); ^{13}C-NMR (100 MHz, DMSO-d_6): δ 192.6 (C=O), 149.7 (C-1a, 10a), 145.2 (C-4'), 130.0 (C-2', 6'), 122.5 (C-1'), 121.0 (C-3', 5'), 119.7 (C-5a, 6a), 54.6 (C-4, 8), 44.8 (C-6), 44.5 (C-2, 10), 33.8 (C-3, 9), 27.8 (***C***H_3); Anal. Calcd for $C_{23}H_{26}N_2O_4$: C, 70.03; H, 6.64; N, 7.10. Found: C, 70.01; H, 6.62; N, 7.07; MS (EI) m/z 395 (M+1).

Synthesis of 3,3,6,6-tetramethyl-9-(3-nitrophenyl)-3,4,6,7,9,10-hexahydroacridine- 1,8(2H,5H)-dione (6c)

The compound **6c** was obtained by the condensation of 3-nitrobenzaldehyde (1.51g, 0.01 mol), dimedone (2.80g, 0.02 mol) and ammonium acetate (1.155 g, 0.015 mol) under the same conditions as used earlier for **6a**.

6c: Yield: 91%; off white solid; m.p.: 277-278°C; R_f = 0.64 [benzene: ethylacetate (7:3)]; IR (KBr): ν_{max} (cm^{-1}) 3449 (N-H), 3049 (aromatic C-H), 2966 (methyl C-H), 1707 (C=O); ^{1}H-NMR (400 MHz, DMSO-d_6): δ 8.62 (1H, s, N***H***), 8.10 (1H, d, J_o = 7.8 Hz, H-4'), 8.01 (1H, s, H-2'), 7.66 (1H, d, J_o = 7.7 Hz, H-6'), 7.62 (1H, t, J_o = 7.8 Hz, H-5'), 5.59 (1H, s, C***H***), 2.35-2.20 (8H, m, H-2, 4, 8, 10), 1.18 (6H, s, C$\boldsymbol{H_3}$), 1.12 (6H, s, C$\boldsymbol{H_3}$); ^{13}C-NMR (100 MHz, DMSO-d_6): δ 191.7 (C=O), 149.6 (C-1a, 10a), 148.3 (C-3'), 135.2 (C-6'), 130.1 (C-1'), 129.6 (C-5'), 128.1 (C-4'), 118.6 (C-5a, 6a), 54.4 (C-4, 8), 44.6 (C-6), 44.3 (C-2, 10), 32.9 (C-3, 9), 27.8 (***C***H_3); Anal. Calcd for $C_{23}H_{26}N_2O_4$: C, 70.03; H, 6.64; N, 7.10. Found: C, 70.00; H, 6.61; N, 7.09; MS (EI) m/z 395 (M+1).

Synthesis of 3,3,6,6-tetramethyl-9-(4-chlorophenyl)-3,4,6,7,9,10-hexahydroacridine- 1,8(2H,5H)-dione (6d)

The compound **6d** was obtained by the condensation of 4-chlorobenzaldehyde (1.40g, 0.01 mol), dimedone (2.80g, 0.02 mol) and ammonium acetate (1.155 g, 0.015 mol) under the same conditions as used earlier for **6a**.

6d: Yield: 90%; yellow solid; m.p.: 226-228°C; R_f = 0.59 [benzene: ethylacetate (7:3)]; IR (KBr): ν_{max} (cm^{-1}) 3441 (N-H), 3023 (aromatic C-H), 2959 (methyl C-H), 1702 (C=O); ^{1}H-NMR (400 MHz, DMSO-d_6): δ 8.50 (1H, s, N***H***), 7.27 (2H, d, J_o = 6.6 Hz, H-3', 5'), 7.14 (2H, d, J_o = 6.7 Hz, H-2', 6'), 5.50 (1H, s, C***H***), 2.35-2.18 (8H, m, H-2, 4, 8, 10), 1.13 (6H, s, C$\boldsymbol{H_3}$), 1.03 (6H, s, C$\boldsymbol{H_3}$); ^{13}C-NMR (100 MHz, DMSO-d_6): δ 190.1 (C=O), 149.5 (C-1a, 10a), 131.3 (C-4'), 130.5 (C-2', 6'), 128.8 (C-3', 5'), 121.4 (C-1'), 116.3 (C-5a, 6a), 53.6 (C-4, 8), 44.0 (C-2, 10), 43.3 (C-6), 32.1 (C-3, 9), 27.5 (***C***H_3); Calcd for $C_{23}H_{26}ClNO_2$: C, 71.96; H, 6.83; N, 3.65. Found: C, 71.92; H, 6.79; N, 3.64; MS (EI) m/z 384 (M+1).

Synthesis of 3,3,6,6-tetramethyl-9-(4-methylphenyl)-3,4,6,7,9,10-hexahydroacridine- 1,8(2H,5H)-dione (6e)

The compound **6e** was obtained by the condensation of 4-methylbenzaldehyde (1.18mL g, 0.01 mol), dimedone (2.80g, 0.02 mol) and ammonium acetate (1.155 g, 0.015 mol) under the same conditions as used earlier for **6a**.

6e: Yield: 90%; yellow solid; m.p.: 207-209°C; R_f = 0.49 [benzene: ethylacetate (7:3)]; IR (KBr): ν_{max} (cm^{-1}) 3399 (N-H), 2997 (aromatic C-H), 2905 (methyl C-H), 1700 (C=O); ^{1}H-NMR (400 MHz, DMSO-d_6): δ 8.39 (1H, brs, N***H***), 7.08 (2H, d, J_o = 6.8 Hz, H-2', 6'),), 6.89 (2H, d, J_o = 7.2 Hz, H-3', 5'), 5.39 (1H, s, C***H***), 2.31–2.17 (8H, m, H-2, 4, 8, 10), 2.12 (3H, s, C$\boldsymbol{H_3}$), 1.13 (6H, s, C$\boldsymbol{H_3}$), 1.03 (6H, s, $\boldsymbol{H_3}$); ^{13}C-NMR (100 MHz, DMSO-d_6): δ 188.6 (C=O), 148.9 (C-1a, 10a), 135.4 (C-4'), 129.0 (C-3', 5'), 128.2 (C-2', 6'), 118.4 (C-1'), 111.5 (C-5a, 6a), 51.0 (C-4, 8), 43.2 (2, 10), 40.8 (C-6), 31.5 (C-3, 9), 26.9 (***C***H_3); Anal. Calcd for $C_{24}H_{29}NO_3$: C, 79.30; H, 8.04; N, 3.85. Found: C, 77.64; H, 5.52; N, 16.75; MS (EI) m/z 364 (M+1).

Synthesis of 3,3,6,6-tetramethyl-9-(4-methoxyphenyl)-3,4,6,7,9,10-hexahydroacridine- 1,8(2H,5H)-dione (6f)

The compound **6f** was obtained by the condensation of 4-methoxybenzaldehyde (1.21mL, 0.01 mol), dimedone (2.80g, 0.02 mol) and ammonium acetate (1.155 g, 0.015 mol) under the same conditions as used earlier for **6a**.

6f: Yield: 88%; pale yellow solid; m.p.: 210-213°C; R_f = 0.52 [benzene: ethylacetate (7:3)]; IR (KBr): ν_{max} (cm^{-1}) 3407 (N-H), 3003 (aromatic C-H), 2916 (methyl C-H), 1703 (C=O); ^{1}H-NMR (400 MHz, DMSO-d_6): δ 8.44 (1H, brs, N***H***), 7.09 (2H, d, J_o = 6.9 Hz, H-2', 6'),), 6.84 (2H, d, J_o = 7.6 Hz, H-3', 5'), 5.40 (1H, s, C*H*), 3.74 (3H, s, OC$\boldsymbol{H_3}$), 2.33–2.19 (8H, m, H-2, 4, 8, 10), 1.12 (6H, s, C$\boldsymbol{H_3}$), 1.03 (6H, s, C$\boldsymbol{H_3}$); ^{13}C-NMR (100 MHz, DMSO-d_6): δ 188.6 (C=O), 157.7 (C-4'), 148.8 (C-1a, 10a), 130.1 (C-2', 6'), 118.7 (C-1'), 114.2 (C-3', 5'), 111.5 (C-5a, 6a), 50.7 (C-4, 8), 43.1 (C-2, 10), 40.3 (C-6), 31.5 (C-3, 9), 26.8 (***C***H_3); Anal. Calcd for $C_{24}H_{29}NO_3$: C, 75.96; H, 7.70; N, 3.69. Found: C, 77.64; H, 5.52; N, 16.75; MS (EI) m/z 380 (M+1).

Antimicrobial evaluation

Synthesized compounds 4a-f, 6a-f were screened for their *in vitro* antibacterial activity against three gram negative bacterial species namely *Escherichia coli* (MTCC 443), *Klebsellia pneumonia* (MTCC 3384), *Pseudomonas aeruginosa* (MTCC 424) and three gram positive bacterial species namely *Staphylococcus aureus* (MTCC 96), *Bacillus subtilis* (MTCC 441), *Streptococcus pyogenes* (MTCC 442). Cefixime was used as the standard drug as positive control while the DMSO was used as negative control. The minimum inhibitory concentrations of the newly prepared compounds (4a-f, 6a-f) were determined by using Serial tube dilution method at the concentration of 128, 64, 32, 16, 8, 4, 2 and 1 µg/mL against above said microorganisms. The bacterial strains susceptibility to the studied compounds was determined by the appearance of turbidity after 24 h of incubation at 37°C. The observed MIC values (µg/mL) for the compounds 4a-f & 6a-f are represented in Table-4 and 5.

Computational study: Structural Similarity Assessment

Success of SAR studies depends on the selection of appropriate molecular descriptors to explain the biological activity. It has already been found that the topological index signifies the degree of branching, connectivity of atoms and unsaturation in the molecule that accounts for variation in activity. Topological parameter, balaban topological index coupled with electronic parameter, and electronic energy resulted in a significant improvement to assess the structural similarity. In this study,

we considered a number of molecular parameters such as Molar refractivity (MR), Molecular weight (MW), Total energy (TotE), Electronic energy (ElcE), HOMO energy (Homo), LUMO energy (Lumo), Balaban index (BIndx), Molecular topological index (TIndx), Wiener index (WIndx) of compounds, and standard drug, amoxicillin and fluconazole using Chem3D (Kumar, 2014; Nikolova, 2004). The values of theses parameters for synthesized compounds were compared with amoxicillin and fluconazole to assess the structural similarity (**Table-6**).

DNA Photocleavage Study

DNA photocleavage experiment was performed by taking 10 µL solution containing pBR322 DNA in TE (Tris 10 mM, EDTA 0.01 mM, pH 8.0) buffer in the presence of 40 µg of synthesized compounds. The sample solution held in caps of polyethylene microcentrifuge tubes were placed directly on the surface of a transilluminator (8000 mW/cm) at 360 nm and were irradiated for 30 min at room temperature. After irradiation, samples were further incubated at 37 ^{o}C for 1 h. Irradiated samples were mixed with 6X loading dye containing 0.25 % bromophenol blue and 30 % glycerol. The samples were then analyzed by electrophoresis on a 0.8 % agarose horizontal slab gel in Tris–acetate EDTA buffer (40 mM Tris, 20 mM acetic acid, 1 mM EDTA, pH: 8.0). Untreated plasmid DNA was maintained as a control in each run of gel electrophoresis which was carried out at 5 V/cm for 2 h. Gel was stained with ethidium bromide (1 µg/mL) and photographed under UV light. To account the effect of synthesized compounds on DNA, the band intensities were analyzed using the GelQuant.NET software provided by biochemlabsolutions.com.

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