



Hydrazone Analogues: Molecular Modeling, Synthesis, *In-vivo* Anti-Nociceptive Activity and *in-vitro* Antimicrobial Activity



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M. Jebastin Sonia Jas^{1,3} G. Marimuthu^{2*} and B. Prithvirajan¹

¹Research and Development Centre, Bharathiar University, Coimbatore, 641046 India

²Department of Chemistry, Swami Dayananda College of Arts and Science, Manjakkudi, Tiruvarur District-612610

³Department of Chemistry, IFET College of Engineering, Villupuram, Tamil Nadu, 605108.

THE inevitable consequence of the widespread use of antimicrobial agents has been the emergence of antibiotic resistant pathogens, functioning an ever- increasing used for new drugs. In an effort to develop antimicrobial agents a series of hydrazones derivative (4a-e) were synthesized from chalcones. Substituted hydrazides were reacted with chalcone in the presence of acetic acid and hydrazone derivatives were synthesized. The synthesized hydrazones were characterized on the basis physical and spectroscopic data and were evaluated for their antimicrobial activity against various bacterial and fungal strains using disc diffusion method using nutrient agar media. In addition, the antinociceptive activities of the products were evaluated. Our data showed that many derivatives have promising activities as antinociceptive agents. Furthermore the assessment of structural similarity of the target compounds with various standard drugs was done. Evaluation of the compounds revealed remarkable antibacterial, antifungal and antioxidant activity. In addition, an *in silico* docking study was performed in order to explain the possible interactions and the docking scores of all the compounds into the crystal structure of DNA Gyrase (Pdb code : 3U2D) using Online docking server program.

Keywords: Chalcone, Hydrazone, Docking, DNA Gyrase, Antimicrobial activity, Antinociceptive.

Introduction

Despite an ever-growing body of knowledge about endogenous nociceptive and antinociceptive systems, clinical treatment of pain today is dominated by two main groups of analgesics: The opioids such as morphine and codeine and the non-steroidal anti-inflammatory drugs including aspirin and ibuprofen. Given the reluctance to use opiates because of their liability toward physical dependence, tolerance, respiratory depression, and constipation, and the limitations in efficacy of the peripheral analgesics associated to classical drawbacks, i.e., gastrointestinal lesions, the quest

is to develop new potent analgesic agents with the efficacy of morphine without the undesired and use-limiting side effects. Because of this, there has been a renewed interest for anti-inflammatory agents endowed with either more selective mechanisms (COX-1 vs COX-2 inhibition) or novel modes of action. Hydrazones are a class of organic compounds with the structure $R_1R_2C=NNH_2$. They are related to ketones and aldehydes by the replacement of the oxygen with the NNH_2 functional group. They are formed usually by [1] the action of hydrazine on ketones or aldehydes[2]. Hydrazone and acylhydrazone

*Corresponding author e-mail: gmarimuuthu@gmail.com

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derivatives have been the subject of considerable interest in the development of novel compounds with anti-inflammatory [3], antidepressant, analgesic [4], anticonvulsant [5], antimicrobial [6], antifungal [7], antitumor [8], antioxidant [9], antimalarial [10], antiplatelet, and antiviral, antimycobacterial and vasodilator activities [11]. The hydrazone-hydrazone derivatives are not only being considered as intermediates but they are also been a very effective organic compounds in their own right. When they are used as intermediates, coupling products can be synthesized by using the active hydrogen component of $-\text{CONHN}=\text{CH}-$ azomethine group. Hydrazones and acylhydrazones possessing an azomethine $-\text{NHN}=\text{CH}-$ and $\text{O}=\text{C}-\text{NH}-\text{N}=\text{CH}$ proton constitute an important class of compounds for new drug development [12]. These compounds contain $\text{C}=\text{N}$ bond, which is conjugated with a lone pair of electrons of the functional nitrogen atom. The nitrogen atoms of the hydrazones are nucleophilic and the carbon atom has both electrophilic and nucleophilic nature. The α -hydrogen of hydrazones is more potent than that of acidic ketones [13]. The combination of hydrazones with other functional group leads to compounds with unique physical and chemical character [14]. Owing to their biological and pharmacological properties, they are considered important for the synthesis of heterocyclic compounds [15].

Based on these facts, supported by literature and as a part of our continued research for new antimicrobial agents, we have undertaken research studies on synthesis and biological evaluation of some new substituted hydrazone derivatives (4a-4e) from 3-((E)-3-(2-amino-3,5-dibromophenyl) acryloyl)benzotrile (3), with the hope that these new molecules would exhibit enhanced biological activity, mainly due to the presence of pharmacologically active aromatic substituents. The molecules were designed based on the preliminary docking studies by choosing the appropriate pharmacophoric groups.

In this communication, we describe the synthesis of (9Z)-N'-((E)-3-(2-amino-3,5-dibromo phenyl)-1-(3 cyanophenyl) allylidene) benzohydrazide, (9Z)-N'-((E)-3-(2-amino-3,5-dibromo phenyl)-1-(3 cyanophenyl) allylidene) benzohydrazide, (9Z)-N'-((E)-3-(2-amino-3,5-dibromo phenyl)-1-(3 cyanophenyl) allylidene)-4-chloro benzohydrazide, (9Z)-N'-((E)-3-(2-amino-3,5 dibromo phenyl)-1-(3-cyano phenyl) allylidene)-4-methoxy benzohydrazide, N'-

[(1Z,2E)-3-(2 amino-3,5-dibromophenyl)-1-(3 cyanophenyl)prop-2-en-1 ylidene]benzenesulfono hydrazide derivatives and evaluation of their in vitro antibacterial activity against five strains, viz. *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Streptococcus pyogenes* and *Staphylococcus aureus*. In addition, in-vitro antifungal activity against five strains, viz. *Aspergillus flavus*, *Aspergillus niger*, *Fusarium oxysporum*, *Penicillium chryogenum* and *Trigoderma veride* were also carried out. Based on the promising in vitro antimicrobial results and by considering DNA Gyrase (PDB CODE: 3U2D) as the target receptor it was thought worthy to perform molecular docking studies and screening for supportive coordination between in silico studies with the in vitro results. Comparative and automated docking studies with newly synthesized compounds were performed to determine the best in silico conformation.

Materials and Methods

Thin layer chromatography (TLC) was used to check the progress and completion of the reaction using silica gel G as an adsorbent (stationary phase) and ethyl acetate and hexane as mobile phase. Open glass capillaries were used to determine the melting point on popular melting point apparatus and were uncorrected. ^1H and ^{13}C nuclear magnetic resonance (^1H NMR & ^{13}C NMR) spectra were recorded on Bruker Avance II 400 NMR spectrometer (400 MHz) at 298K, in appropriate deuterated solvent. Chemical shift were reported as δ (ppm) relative to tetramethyl silane (TMS) as internal standard. Infrared spectra (IR) were recorded as KBr pellet on Shimadzu FT-IR spectrometer.

General procedure for the preparation of 3-((E)-3-(2-amino-3,5-dibromophenyl)acryloyl) benzotrile (3)

To a stirred solution of NaOH (50% w/v) in water (5 mL) cooled in an ice bath, a solution of the 3-acetylbenzotrile and the 2-amino-3,5-dibromobenzaldehyde in ethanol (20 mL) was added drop wise. The reaction mixture was stirred at room temperature overnight. Then the mixture was poured into ice, adjusted to a pH of 3–4 with 1 M HCl, and then filtered. The precipitate was crystallized from ethanol. Yellow crystals. Yield: 85%, m.p.: 125°C.

General procedure for the preparation of hydrazone derivatives (4a-e)

The hydrazone derivatives of above chalcone were prepared by reacting the respective chalcone

(3) and the respective substituted hydrazides in 1:1 stoichiometric amounts in minimum amount of ethanol with a few drops of glacial acetic acid. The reaction mixture was refluxed on the water bath for 3-4 hours. Completion of the reaction was monitored by TLC using chloroform: ethanol (9:1) as the developing solvent. Product separated out when the mixture was allowed to cool. It was filtered out, washed with ether and dried in vacuum over anhydrous CaCl_2 (Fig. 1).

Molecular Docking Studies

Molecular docking studies were conducted in order to validate the obtained pharmacological data and to provide understandable evidence for the

observed antimicrobial activity of all synthesized compounds. Molecular docking study is a well-established technique to determine the interaction of two molecules and find the best orientation of ligand would form a complex with overall minimum energy. All the synthesized compounds (4a-e) were docked Structure of DNA Gyrase with a ten different orientations. The structure of the protein mentioned above [PDB:3U2D] was retrieved from the Protein Data Bank [www.rcsb.org (DOI:10.2210/pdb3b6o/pdb)] and further modified for docking calculations. The ligand molecules were drawn and analysed using Chem Draw Ultra 8.0. 3D, coordinates were

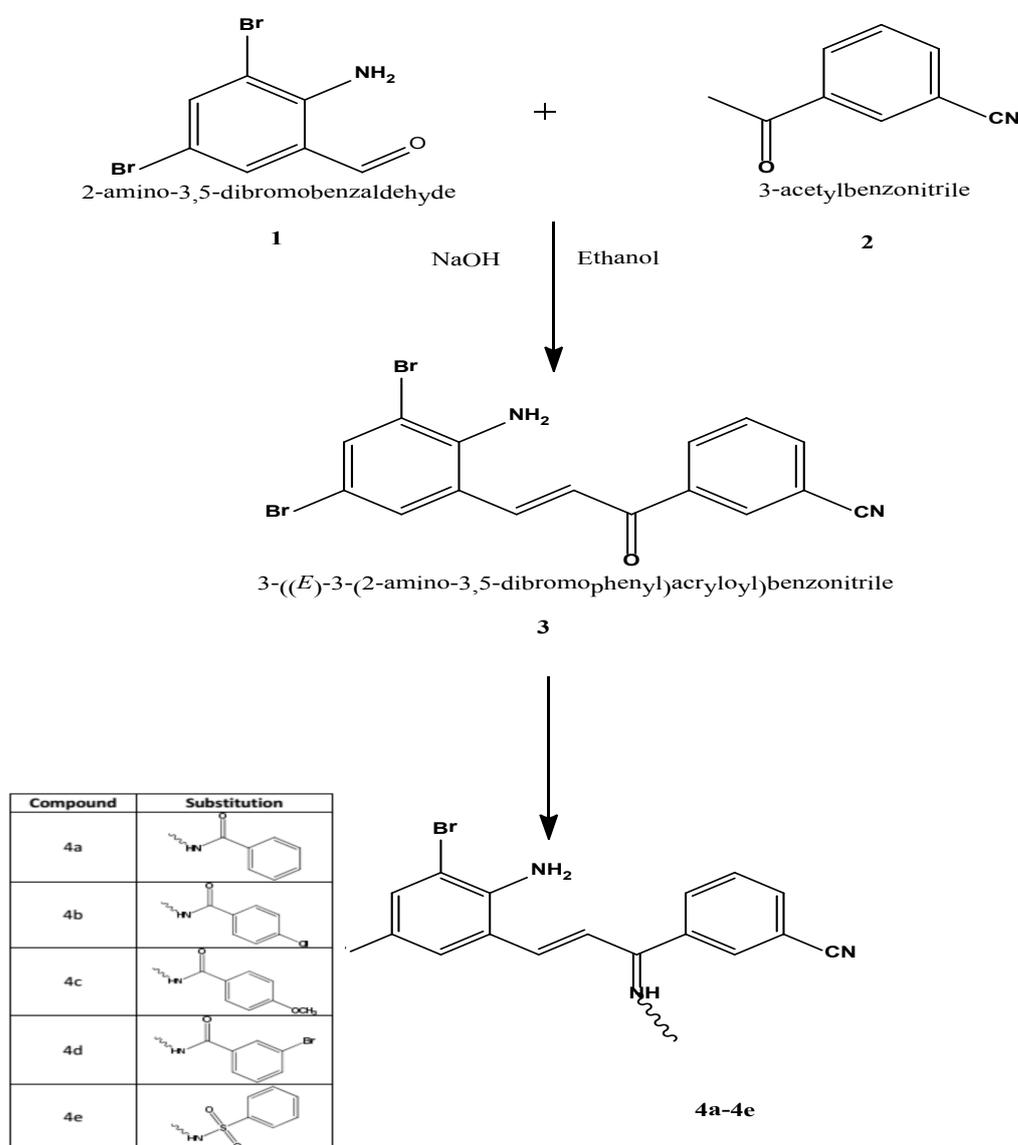


Fig. 1. Synthesis of (13Z)-N'-((E)-3-(2-amino-3,5-dibromophenyl)-1-(3-cyanophenyl)allylidene) substituted benzohydrazide analogues [4a-e].

prepared using dock server. Docking calculations were carried out using Docking Server(www.dockingserver.com) (Gasteiger partial charges were added to the ligand atoms. Non-polar hydrogen atoms were merged, and rotatable bonds were defined. Docking calculations were carried out on corresponding protein model. Essential hydrogen atoms, Kollman united atom type charges, and solvation parameters were added with the aid of AutoDock tools [18]. Affinity (grid) maps, 0.375 Å spacing were generated using the Autogrid Morris, Goodsell et al., 1998) [16]. AutoDock parameter set- and distance-dependent dielectric functions were used in the calculation of the van der Waals and the electrostatic terms, respectively.

Docking simulations were performed using the Lamarckian genetic algorithm (LGA) and the Solis & Wets local Solis and Wets, 1981)[17] Initial position, orientation, and torsions of the ligand molecules were set randomly. All rotatable torsions were released during docking. Each docking experiment was derived from 10 different runs that were set to terminate after a maximum of 250000 energy evaluations. The population size was set to 150. During the search, a translational step of 0.2 Å, and quaternion and torsion steps of 5 were applied

Anti-nociceptive activity hot plate method

All the newly synthesized compounds were tested for anti-nociceptive activity by the Eddy and Leimbach method (13). Swiss albino mice were divided into seven groups of six mice each. One group served as control and was administered 0.5 % (V/V) Tween 80 (0.5 mL) suspension. One group was administered the standard drug pentazocine (Sigma, USA) intraperitoneally at a dose of 5 mg kg⁻¹ 15 minutes before the analgesic activity evaluation. Tween 80 suspension (0.5 %, V/V) of test compounds 3a-e was administered intraperitoneally at a dose at 18 mg kg⁻¹ to the remaining groups 15 minutes before the analgesic activity evaluation. The reaction time in seconds was noted for all the groups on Eddy's hot plates before and 15 minutes after administration of test compounds and pentazocine.

Antimicrobial activity

All the synthesized compounds were screened for antimicrobial studies against antibacterial and antifungal activity by disc diffusion method. The disc diffusion method has been followed by using the Kirby-bauer method [19]. For the prepared compounds using Ciprofloxacin and

Amphotericin-B as references were subjected to preliminary antibacterial screening by disc diffusion method against bacterial strains such as *Bacillus subtilis* (*B. subtilis*), *Escherichia coli* (*E. coli*), *Pseudomonas aeruginosa* (*P. aeruginosa*), *Staphylococcus aureus* (*S. aureus*) & *Streptococcus pyogenes* (*S. pyogenes*) and fungal strains namely *Aspergillus flavus* (*A. flavus*), *Aspergillus niger* (*A. niger*), *Penicillium chrysogenum* (*P. chrysogenum*), *Trigoderma veride* (*T. veride*) & *Fusarium oxysporum* (*F. oxysporum*). The Zone of inhibition values of 4a-e along with the standard drugs for comparison is furnished in Table 7.

Statistical Analysis

Statistical analysis of the biological activity of synthesized compounds on animals was evaluated using a one-way analysis of variance (ANOVA). In all cases, posthoc comparisons of the means of individual groups were performed using Tukey's test. All values were expressed as mean ± SEM. The GraphPad Prism 3.0 version was used for statistical analysis.

Results and Discussion

The IR spectra of 3-((E)-3-(2-amino-3,5-dibromophenyl)acryloyl)benzoxazole (3) showed a carbonyl absorption at 1655 cm⁻¹ which is characteristic band of the α, β- unsaturated carbonyl group. Ethylenic double bond stretching of chalcone showed at 1581 cm⁻¹. C-Br stretching displayed at 536 cm⁻¹. A medium to strong absorption band seen at 856 cm⁻¹ is due to trans CH=CH out of plane deformation (wagging). The aromatic C-H stretching was observed at 3066 cm⁻¹.

Table 1 shows all the physical data like color, molecular formula, molecular weight, solubility, melting point, of synthesized compounds (Table 1). The IR frequencies of compounds 4a-e is showed in Table 2, in which the C=N stretching frequency appear at 1586-1667 cm⁻¹. Aromatic (CH) stretching frequencies appear at 3066-3083 cm⁻¹ and stretching frequency observed at 1622-1645cm⁻¹ C=O group present in the derivatives (Table 2).

The ¹H NMR chemical shift values of compound (4a-e) given in Table 3. The singlet observed in the range 7.18-7.20ppm is due NHproton. The singlet observed at 1.52-1.60ppm is due -NH₂ proton. The signals appearing 7.56-7.89ppm are obviously due to aromatic protons (Table 3).

The ¹³C chemical shift values of compound

TABLE 1. Physical data of various synthesized compounds.

Compound	Color	Mol. Formula	Mol. weight	Solubility	Melting point (°C)
4a	Yellow	C ₂₃ H ₁₆ Br ₂ N ₄ O	524	Ethanol	215
4b	Yellow	C ₂₃ H ₁₅ Br ₂ ClN ₄ O	558	Ethanol	223
4c	Yellow	C ₂₄ H ₁₈ Br ₂ N ₄ O ₂	554	Ethanol	286
4d	Yellow	C ₂₃ H ₁₅ Br ₃ N ₄ O	603	Ethanol	245
4e	Yellow	C ₂₃ H ₁₈ Br ₂ N ₄ O ₂ S	574	Ethanol	312

TABLE 2. Data from IR spectra of Hyrazone derivatives (4a-e).

Compounds	FREQUENCY cm ⁻¹					
	C=O	C=N	C≡N	CH=CH	N-H	ARO C-H
4a	1622	1586	2230	1470	3459	3066
4b	1645	1586	2230	1469	3436	3067
4c	1645	1589	2231	1412	3437	3070
4d	1625	1586	2231	1470	3465	3067
4e	-	1667	2351	1492	3458	3083

TABLE 3. Data from ¹H NMR spectra of Hyrazone derivatives (4a-e).

Compounds	-NH	NH ₂	Aromatic protons
4a	7.2 (1H,singlet)	1.60 (1H,singlet)	7.64-8.59 (13H, multiplet)
4b	7.18 (1H,singlet)	1.52 (1H,singlet)	7.56-8.51 (15H, multiplet)
4c	7.19 (1H,singlet)	1.57 (1H,singlet)	7.59-8.53 (15H, multiplet)
4d	7.18 (1H,singlet)	1.60 (1H,singlet)	7.58-8.51 (15H, multiplet)
4e	7.19 (1H,singlet)	1.60 (1H,singlet)	7.58-8.50 (15H, multiplet)

(32) is given in Table 4. The signal at 113.31-113.34 ppm is as signed to CN carbon. The signal observed at 155.20-155.23ppm is due to (C=N) carbon. The signal observed at 163.16-163.44ppm is due to CO carbon. The chemical shift due to aromatic carbon can be readily sorted out by their characteristic down field absorption in the region 118.70 to 143.92ppm (Table 4).

The newly designed compounds 4a-4e was docked into DNA gyrase protein to understand the binding interactions (Fig. 2). Figure 2 depicts the possible binding modes of compounds 4a. Table 5 shows the solutions of binding affinity for DNA gyrase. Molecular basis of interactions between target enzyme and synthesized ligands can be understood with the help of docking analysis and docking scores were summarized in Table 5. Compounds 4a-4e binds to the active site of DNA gyrase and makes several interactions

with nearby residues. Compound 4a has binding energy -6.66 Kcal/mol⁻¹ and show two hydrogen bonds with SER55, ASN54 . It is pertinent to note that the more active compound 4d showing nice binding energy 5.40 Kcal/mol⁻¹. Docking analysis revealed that bromine atoms in 4d are having greater binding affinity within the DNA gyrase. It can be seen clearly that compound 4d interacts with ASN54, ASP81, SER55 as hydrogen bond (Table 5).

The compound 4e exhibited binding energy -2.40 Kcal/mol⁻¹ and clearly revealed that the H-interactions with ASP81. Comparing the docking results of 4a-4e, introduction of electronegative group led to higher activity. These dry lab findings are well supported by results of in vitro antiacetal activity.

The acting force of this binding mode is mainly

TABLE 4. Data from ^{13}C NMR spectra of Hydrazone derivatives (4a-e).

Compounds	C=N	C=O	Aromatic Carbons	C \equiv N
4a	155.23	163.16	118.73-143.90	113.31
4b	155.21	163.29	118.70-143.89	113.34
4c	155.31	163.44	118.73-143.92	113.34
4d	155.21	163.24	118.73-143.88	113.31
4e	155.20	-	118.73-143.88	113.31

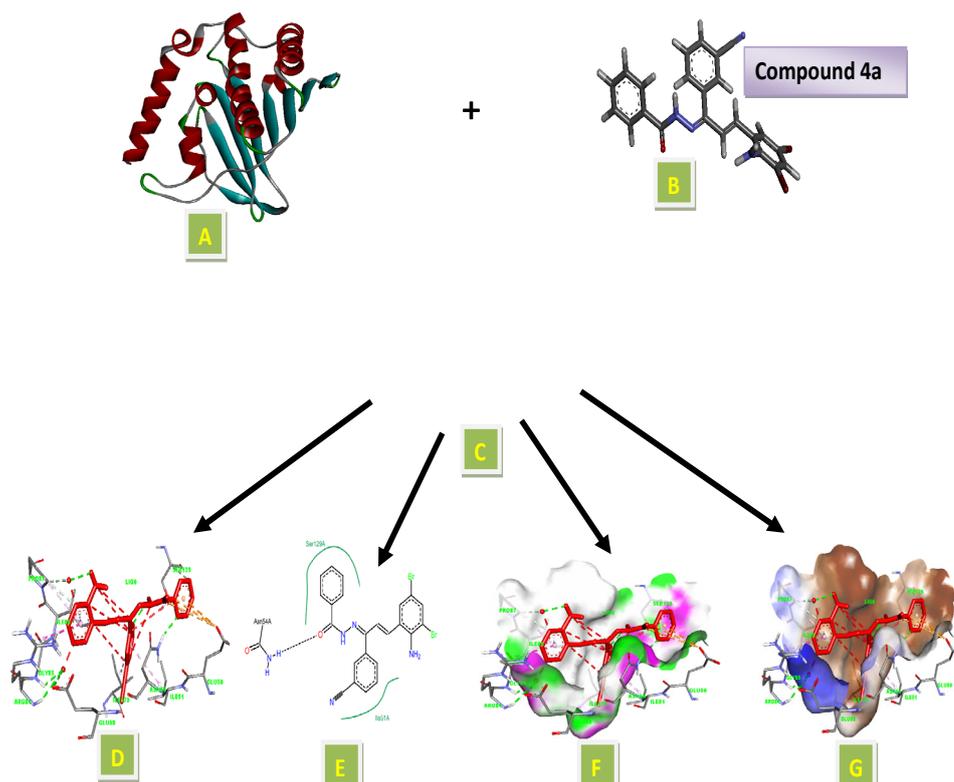


Fig. 2. (A) Receptor DNA Gyrase (PDB ID: 3U2D). (B) DFT/6-31G optimized geometry of ligand (4a). (C) Docked ligand-receptor complex (D) Binding model of compound 4a with DNA Gyrase H-bond and alkyl interactions are shown by green and red broken lines, respectively. (E) 2D view of interacting essential amino acid residues at the ligand binding site; (F) Showing hydrogen bond donor (pink) and acceptor (green) surfaces. (G) Showing hydrophobic interactions (brown) surfaces.

TABLE 5. Interaction energies of Hydrazone (4a-e) with DNA Gyrase receptor using online docking server.

Compound	Binding Energy kcal/mol	VdW+Hbond+disolv Energy kcal/mol	Inhibition Constant μM	Intermolec. Energy kcal/mol
4a	-6.66	-8.04	110.18	-7.88
4b	+5.20	+1.97	0	+2.00
4c	+2.57	-0.76	0	-0.75
4d	-5.40	-9.20	13.18	-9.05
4e	-2.44	-4.87	16.15	-4.82

hydrogen bonding, electrostatic forces, van der Waals forces, and hydrophobic interaction due to nonpolar residue interaction and water structure effect alteration. Based on studies; it supports the *in vitro* antibacterial activity. The best orientations of hydrogen bonds and hydrophobic interaction of docked molecules are given in Table 5.

In silico studies revealed that synthesized molecules 4a, 4d and 4e showed good binding energy toward the target protein ranging from -6.66 to -2.40 kcal/mol due to dipole-dipole and hydrogen bond interaction with amino acids of targeted protein. The *in vitro* antibacterial studies values are correlated well with binding energies obtained through molecular docking with DNA Gyrase (PDB 3U2D). The bacterial inhibition potency of compounds 4a and 4d are showed excellent docking energies (Table 6).

From the comparative analysis, compounds 4a and 4d showed maximum docking energy with targeted protein and the *in vitro* studies also support that compounds 4a and 4d have become active against all the tested micro organisms. So, we suggested compound 4a and 4d unsubstituted phenyl group and bromine substituted phenyl group in hydrazide moiety to exhibit better bacterial and fungal inhibition. Therefore, it is pleasing to state that the docking studies have widened the scope of developing a new class of antimicrobial agents.

The observed antibacterial data of the compounds and the reference drugs are given in Table 7. When compared with the reference drug Ciprofloxacin, most of the compounds in the series exhibited considerable anti-bacterial activity (Table 7). Compounds 4a and 4e exhibited equal antibacterial activity to ciprofloxacin against *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Streptococcus pyogenes*. Rest of them was found to be moderate active against these bacterial strains. None of the compounds performed notable growth inhibitory activity against *Staphylococcus aureus* bacterial strains (Table 8).

From the zone of inhibition values, it is clearly seen that compound 4d showed good activity against *Aspergillus niger*, *Fusarium oxysporum* and *Penicillium chrysogenum* and is comparatively half than that of the reference drug used. From Table 8 Compounds 4b and 4e were inactive against fungal strains such as *A. niger*, *T. veride* and *P. chrysogenum*. The compound 4a showed

good activity against *T. veride* and *F.oxysporum* and it is comparatively higher than that of compound 4b was moderate activity against *F.oxysporum*.

Compounds substituted with chloro, 3-bromo, and sulphonyl groups are found to be more active against the selected bacterial strains. Unsubstituted phenyl ring showed comparatively lesser activity, which proved that substitution at hydrazone moiety affects the activity. The scrutiny of the results revealed that compounds bearing a cyano group in phenyl ring B addition to a bromo group in the phenyl ring C of hydrazone nucleus are more active against chosen bacterial strains. Significant activity is shown by compounds 4b and 4d with electron withdrawing groups like chloro and bromo whereas moderate activity was shown by compounds with electron donating methoxy group 4c. Reason for these results may be due to increase of the electron density which makes compounds active against microorganisms and enhance the antimicrobial potency. Reason for these results of compound 4b and 4d may be due to the presence of strong electron withdrawing groups Cl and Br in the molecule may lead to higher polarizability of the molecule and give rise to new potential for binding the molecule to the receptor which finally enhances the van der Waals force of attraction of the molecule and in turn its diffusion rate in the cell on the whole. In particular, the positions of electron withdrawing substituents on phenyl ring appreciably influenced the antimicrobial activity (Fig. 3). Placement of electron withdrawing group in a strategic position of a molecule has materialized as a very influential and flexible tool for the development of the compounds capable with biological activities [20].

The antinociceptive activity of the tested derivatives was also evaluated by applying hot plate test [21] using Pentazocine as a standard reference. The results of analgesic testing indicated that the test compounds 4d, 4c and 4e exhibited significant analgesic activity after 15 min of reaction time (13.08, 12.18 and 9.69), tested by the hot plate method. Substitution of the electron withdrawing bromo group in 4d and methoxy group in 3c in para position of aryl moiety increased the anti-nociceptive activity. All other compounds exhibited moderate antinociceptive activity. It is worth mentioning that the potency of the antinociceptive activity of the derivatives was increasing with time giving rise to increases in both the duration and the onset of actions (Table 9).

TABLE 6. H-bond, halogen bond and the amino acids interacting in the hydrophobic pocket of hydrazone derivatives 4a-e.

Interactions	Compounds				
	4a	4b	4c	4d	4e
Hydrogen bond	SER55, ASN54	ASN54	ASN54	ASN54, ASP81, SER55	ASP81
Halogen bond	GLY85, GLU58	GLU58, SER129	GLU50, SER128	GLU58, THR173, GLY85	SER128, SER129
Polar	ASP81	THR173	ASP81, THR173	-	ASN54, SER55
Hydrophobic	ILE86, ILE51, VAL131, ILE175, PRO 87, VAL130, LEU103	ILE86, LEU103, ILE51, VAL131, ILE175	ILE86, ILE51, ILE175, ILE102, VAL131, PRO87, VAL130	ILE86, LEU103, VAL131, VAL130, ILE51, ILE175	ILE175, ILE102, ILE86
Other	GLU50, ILE102, SER129	SER55, ASP57, ARG84, ASP81, ALA61, LEU138	SER129, GLU58	ILE102, GLU50, SER129, VAL79	THR173, GLU58, ILE51, PRO87, GLU50, VAL131

TABLE 7. Antibacterial activity by disc diffusion method.

S. No.	Bacteria	Zone of inhibition (mm)						Control (DMSO)
		Ciprofloxacin	4a	4b	4c	4d	4e	
1	<i>Bacillus subtilis</i>	22	15	10	12	09	12	--
2	<i>Escherichia coli</i>	22	09	15	12	11	10	--
3	<i>Pseudomonas aeruginosa</i>	22	08	06	09	09	13	--
4	<i>Streptococcus pyogenes</i>	21	13	16	12	15	14	--
5	<i>Staphylococcus aureus</i>	18	09	09	-	08	08	--

TABLE 8. Antifungal activity by disc diffusion method.

S. No.	FUNGAL	Zone of inhibition (mm)						Control (DMSO)
		*Amphotericin-B	4a	4b	4c	4d	4e	
1	<i>Aspergillus flavus</i>	20	05	08	06	06	12	-
2	<i>Aspergillus niger</i>	20	05	--	05	12	--	-
3	<i>Fusarium oxysporum</i>	25	12	11	11	12	11	-
4	<i>Penicillium chryogenum</i>	22	--	--	12	15	--	-
5	<i>Trigoderma veride</i>	26	13	--	10	09	--	-

TABLE 9. Evaluation of anti-nociceptive activity by the hot plate method.

Compd. (dose)	Reaction time (s)	
	Before treatment ^a	After 15 minutes ^a
4a (18 mg kg ⁻¹)	3.12 ± 0.18	8.41 ± 0.17 ^b
4b (18 mg kg ⁻¹)	3.08 ± 0.29	7.15 ± 0.42 ^c
4c (18 mg kg ⁻¹)	4.11 ± 0.37	12.18 ± 0.13 ^b
4d (18 mg kg ⁻¹)	3.65 ± 0.45	13.08 ± 0.34 ^b
4e (18 mg kg ⁻¹)	4.44 ± 0.69	9.69 ± 0.22 ^b
Control	3.32 ± 0.46	3.33 ± 0.55
Pentazocine (5 mg kg ⁻¹)	4.37 ± 0.11	12.98 ± 0.18 ^b

Control – no synthesized compound and paracetamol.

^a Mean ± SEM (n = 6).

Significant difference compared with the control: b p < 0.001, c p < 0.01.

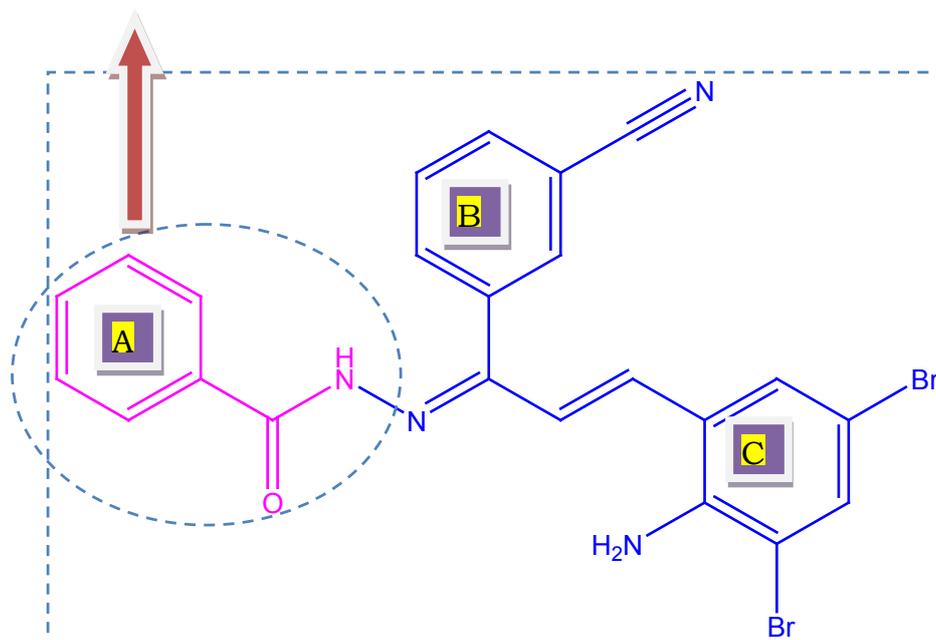


Fig. 3. Antimicrobial mechanism of synthesized hydrazone (4a-e) derivatives.

Conclusion

Five derivatives substituted hydrazone derivatives were prepared, of which compounds 4d and 4c possess potent anti-nociceptive activity by hot plate model test. Hence, the focus is on the synthesis of potent substituted hydrazone derivatives as potential drug-like scaffolds for future development. The antimicrobial activity results indicated that some of the tested compounds showed the most promising antibacterial and antifungal activities. These observations may promote a further development of our research in this field. Further development of this group of compounds may lead to compounds with better pharmacological profile than standard drugs and serve as templates for the construction of better drugs to combat bacterial and fungal infection. After studying the docking poses and binding modes of the docked compounds, the necessity of hydrogen bond formation for enhancing the activity of this class of compounds can be highly advocated.

References

- Prithivirajan B., Marimuthua G., Jebastin M. Sonia Jas Synthesis and characterization of AC-CdO-TiO₂ nanocomposite recyclable and multi-application study, *Biomedical Research* **29**(19), 3590-3598 (2018).
- Prithivirajan B., Marimuthua G., Jebastin M. Sonia Jas Synthesis, Characterization and in
- Xavier A.J., Thakur M., Marie J.M., Synthesis and spectral characterisation of hydrazone based 14-membered octaaza macrocyclic Ni (II) complexes. *J Chem Pharm Res.*; **4**, 986–90 (2012).
- Koopaei M. N., Assarzadeh M. J., Almasirad A., Ghasemi-Nirib S. F., Amini M., Kebriaeezadeh A., et al, Synthesis and Analgesic Activity of NovelHydrazide and Hydrazine Derivatives, *Iran J. Pharm. Res.*, **12**(4), 721-722 (2013).
- Kumar N., Chauhan L. S., Dashora N., Sharma C. S., Anticonvulant potential of Hydrazone derivatives: A Review., *Sch. Acad. J. Pharm.*, **3** (5), 366-373 (2014).
- Mohammad A. Anti-microbial potentials of hydrazone derivatives: A promising scaffold. *International Journal of Chemical and Applied Biological Sciences. Medknow*; **1**(1), 23 (2014).
- Wang H, Ren S-X, He Z-Y, Wang D-L, Yan X-N, Feng J-T, et al. Synthesis, Antifungal Activities and Qualitative Structure Activity Relationship of Carabrone Hydrazone Derivatives as Potential Antifungal Agents. *International Journal of Molecular Sciences [Internet]. MDPI AG*; Mar 11; **15**(3), 4257–72 (2014).

8. Ibrahim N.M., Yosef H.A.A., Ewies E.F., Mahran M.R.H., Ali M.M., Mahmoud AE. Synthesis and Antitumor Evaluation of New Heterocycles Derived from 3-Methyl-2-benzothiazolinone Hydrazone. *Journal of the Brazilian Chemical Society [Internet]. Sociedade Brasileira de Quimica (SBQ)*; **26**(6), 1086-1097 (2015).
9. Potu^oc^h kova^o E., Hrus^h kova^o K., Bures^h J., Kovar^h i^h kova^o P., S^h pirkova^o I. A., Pravdi^h kova^o K., et al, Correction: Olfactory Performance Is Predicted by Individual Sex-Atypicality, but Not Sexual Orientation, *PLoS ONE.*, **9**(11), 1-17 (2014).
10. Sharma M., Chauhan K., Srivastava R.K., Singh S.V., Srivastava K., Saxena J.K., et al. Design and Synthesis of a New Class of 4-Aminoquinolinyl- and 9-Anilinoacridinyl Schiff Base Hydrazones as Potent Antimalarial Agents. *Chemical Biology & Drug Design [Internet]. Wiley-Blackwell*; Mar 13; **84**(2), 175–81 (2014).
11. Asif M., Anti-microbial potentials of hydrazone derivatives: A promising scaffold. *International Journal of Chemical and Applied Biological Sciences.*, *Int. J. Adv. Chem.*, **2**(2), 85-103 (2014).
12. Padmini K., Preethi P. J., Divya M., Rohini P., Lohita M., Swetha K., et al, A Review on Biological Importance of Hydrazones, *Int. J. Pharma. Res. Rev.*, **2**(8), 43-58 (2013).
13. Corey E.J., Enders D. Applications of N, N-dimethylhydrazones to synthesis. Use in efficient, positionally and stereochemically selective C=C bond formation, oxidative hydrolysis of carbonyl compounds. *Tetrahedron Lett.*; **17**, 3–6 (1976).
14. Belskaya N.P., Dehaen W., Bakulev V.A. Synthesis and properties of hydrazones bearing amide, thioamide and amidine functions. *Arch Org Chem.*; **1**, 275–332 (2010).
15. Banerjee S, Mondal S, Chakraborty W, Sen S, Gachhui R, Butcher RJ, et al. Syntheses, X-ray crystal structures, DNA binding, oxidative cleavage and antimicrobial studies of two Cu (II) hydrazone complexes. *Polyhedron*; **28**, 2785–93 (2009).
16. Morris G. M., Goodsell. D. S., et al. Automated docking using a Lamarckian genetic algorithm and an empirical binding free energy function. *Journal of Computational Chemistry* **19**(14), 1639-1662 (1998).
17. Solis F. J., and Wets. R. J. B. Minimization by Random Search Techniques *Mathematics of Operations Research* **6**(1), 19-30 (1981).
18. Kuldeep Sahu and Satpal singh Bisht, virtual screening approach of drug designing for parkinson's disease, *Int J Pharm Bio Sci* **4**(1), (B) 370 – 382; Jan (2013).
19. Bauer A. W., Kirby W. M. M., Sherris J. C., Truck M., Vacuoles in Synovial Fluid Leukocytes, *Am. J. Clin. Pathol.*, **45**, 493 (1966).
20. Bhavanarushi S., Kanakaiah V., Bharath G., Gangagnirao A., Vatsala Rani J., Synthesis and antibacterial activity of 4,4'-(aryl or alkyl methylene)-bis(1H-pyrazol-5-ol) derivatives. *Medicinal Chemistry Research [Internet]. Springer Nature*; **23**(1), 158–67; May 21 (2013).
21. Woolfe G., MacDonald A.D., The evaluation of the analgesic action of pethidine hydrochloride (demerol)., *J. Pharmacol. Exp. Ther.* **80**, 300–307 (1944).