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Research article

Synthesis, characterization, molecular docking and antimicrobial evaluation of azo coupled – 3, 4-dihydropyrimidine-2(1h)-one derivatives

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ABSTRACT

A series of 5-acetyl-4-[(4-substituted phenyl) diazinyl) phenyl]- 6-methyl-3, 4-dihydropyrimidine-2(1H)-one derivatives were synthesised via the coupling reaction between 4- substituted-3, 4- dihydropyrimidine-2(1H)-one and diazonium salt of p-nitro aniline. All the synthesised compounds were structurally elucidated by elemental analysis and IR, ^{1}H NMR and Mass spectral techniques. Investigation of antibacterial and antifungal activity was done on titled compounds using disc diffusion method. Moderate inhibitory activity were displayed by compounds IIa, IId against bacterial strains such as *Escherichia coli*, *Staphylococcus aureus* and fungal strains *Candida albicans, Aspergillus niger* at a concentration range from 400-800µg/ml. Antimicrobial activity of the compounds may be due to the presence of electron donating substituent on phenyl ring attached at fourth position of dihydropyrimidinones. Molecular docking investigation of synthesised compounds performed with bacterial DNA gyrase B which showed that the compound IIa had the binding affinity of -8.3kcal/mol and the compound IId had the binding energy of -9.1 kcal/mol against fungal sterol-14α-demethylase which further supported the biological activity.

Keywords: : Dihydropyrimidinones, Antimicrobial, Disc diffusion, Docking.

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INTRODUCTION

Nitrogen containing heterocycles constitute the significant scaffold for drug design. Dihydropyrimidinone (DHPM) are one of the eminent heterocyclic compounds which attracted attention on account of its outstanding broad spectrum of pharmacological and therapeutic properties [1,2]. DHPM is considered as the important pharmacophore in the field of organic and medicinal chemistry due to their versatile range of biological activities. DHPM exhibits the biological activities including the microbicidal against bacteria, fungi[3-7], preventing inflammation[8], suppressing the cancer[9, 10], activity against virus[11], inhibition of HIV gp-120 CD4 cells[12, 13], blocking calcium channel[14, 15], lowering hypertension[16], prevention of convulsion[17, 18], effective on Mtb[19], effectively inhibit the pathogenic *P. falciparum*[20], scavenging free radical[21], inhibition of ulcer by antisecretory and gastroprotective activity[22], blood glucose lowering property[23]. Marine alkaloid Batzelladine A and B contain DHPM core is known to block the HIV-gp-120 binding with CD4 cells [24]. Monastrol is the most important analogy of dihydropyrimidinone class which have the specific inhibitory activity on Eg5 human kinesin protein causes the arrest of mitosis and as a result of apoptosis in cancer cells [25].

Antimicrobial resistance occurs when the pathogen develop resistant to medicines. This arises as a result of inappropriate use of antimicrobials. As a consequence of drug resistance, the ineffectiveness of the antimicrobial compounds against infections and making the infection more difficult to treat. Emergence of drug resistant pathogens continues to a major threat to the public health. It is imperative that the development of new antimicrobial agent alleviate the dissemination of antimicrobial resistance. In order to tackle the drug resistant microbes, alternate antimicrobial agents are necessary. Many researchers involved in searching for new entity and revamping the existing drugs for treating infection. Nowadays, in antimicrobial drug discovery, the research aspect toward the development of more active therapeutic antimicrobial agent that can selectively targeting the specific genome of pathogen. Recent focus in development of compounds toward the inhibition of target enzyme involved in crucial role in replication of pathogen.

3,4- dihydropyrimidin-2(1H)-one were firstly reported by Pietro Biginelli in 1983. The common way of synthesising Biginelli compounds through three component one pot cyclocondensation reaction of β-ketoester, aromatic aldehyde and

diamide in presence of strong acidic medium[26]. Moreover, several compounds commercially available found to have DHPM nucleus eg: 5- fluorouracil for treatment of neoplasm, thiouracil for hyperthyroidism and 5- flucytocine for candida infection[27].

On account of great medicinal and commercial applications of dihydropyrimidinones scaffold, considerable attention put forth on this scaffold in order to design valuable derivatives contain dihydropyrimidinone nucleus in search for treatment of serious illness caused by infectious agents. Our ultimate aim of this work is to develop better efficacious antimicrobial agent with spectrum of antimicrobial action and also effective against drug resistant microbes

MATERIAL AND METHOD

All the materials used in this synthesis were purchased from reputed chemical suppliers and it was used without further purification. Melting point of all synthesized compounds were determined by using digital melting point apparatus and presented uncorrected. Thin layer chromatography was performed for synthesized compounds for testing its purity. Silica gel G coated plates were used as stationary phase and n- hexane and ethyl acetate in the ratio of 6:4 was used as mobile phase. The spot was visualized using iodine vapour. The synthesized compounds were characterized by means of spectroscopic techniques such as IR, ^{1}H NMR and mass spectrometry. Elemental analysis was also carried out on synthesized compounds. Schimadzu Fourier transform infrared spectrophotometer was utilized for recording IR spectra of all synthesized compounds in the frequency range of 4000 cm^{-1} to 400 cm^{-1}, using KBr pellet method. Proton NMR Spectra were recorded using BRUKER Advance 400MHz NMR Spectrometer using the solvent deuterated DMSO. Chemical shifts were recorded in parts per million and Trimethyl silane as an internal standard. Mass spectrum was taken using JEOL GC mate.

Step 1 Synthesis of 3, 4-dihydropyrimidin (1H)-2-one derivatives (Ia-Ie)

0.025 mol of acetyl acetone, 0.021 mol of various substituted aromatic aldehyde, 0.025 mol of urea, 10 drops of conc.HCl and 20 ml of ethanol were refluxed in a round bottom flask for 4 hours. The reaction mixture was cooled and poured in ice cold water. Solid product separated which was filtered, washed with water and dried. Dihydropyrimidinones were recrystallized from ethanol.

Step 2 Preparation of diazonium salt of p-nitro aniline

0.005 mol of p-nitro aniline was dissolved in solution of 2ml conc. HCl and 10 ml water. To this added the solution of 0.005 mol of sodium nitrite in 10 ml of water.

Synthesis of 5-acetyl-6-methyl-4(substituted phenyl) diazenyl phenyl-3, 4-dihydro pyrimidine (1H)-2-ones (IIa-IIe)

0.005 mol of 3,4 dihydropyrimidine-(1H)-2-one derivatives (step 1 product) were dissolved in ethanol and 10ml of 10% sodium hydroxide solution was added to it. Then diazonium salt of p-nitro aniline (step 2 product) was added drop wise to the dihydropyrimidinone solution. The reaction mixture was stirred using magnetic stirrer for 1 hour. The crystallized product was separated by filtration, washed with ice water and dried. The compounds were recrystallized from ethanol.

Spectral data of synthesised compounds5-acetyl-4-(2-hydroxyphenyl)-6-methyl-3,4-dihydropyrimidin-2(1*H*)-one (Ia).

Brown crystals, yield: 72%, mp (°C): 110. IR (γ cm^{-1}): 3394 (NH str of amide), 3240 (OH str phenol), 3103 (CH str of alkene), 2991(CH str of methyl), 1681 (C=O str), 1595, 1517, 1504, 1458 (C=C str of aromatic). ^{1}H NMR (γ ppm): 1.68 (3H, s), 2.43 (3H, s), 4.8 (1H, s), 5.45 (1H, s), 5.97 (2H, s), 6.63-6.85 (4H, m). MS (m/z): 245.8 (M$^+$).

5-acetyl-4-(4-methoxyphenyl)-6-methyl-3,4-dihydropyrimidin-2(1*H*)-one (Ib).

Red crystals, yield: 75%, mp (°C): 108. IR (γ cm^{-1}): 3562 (NH str of amide), 3228 (OH str of phenol), 2952, 2935 (CH stretch of alkene), 2837 (CH stretch of methyl), 1681 (C=O str) 1458, 1512 (C=C str of aromatic). ^{1}H NMR (γ ppm): 1.72 (3H, s), 2.33 (3H, s), 3.69 (3H, s), 5.51 (1H, s), 5.86 (2H, s), 6.56-6.95 (4H, m). MS (m/z): 260.37 (M$^+$).

5-acetyl-4-(4-hydroxy-3-methoxyphenyl)-6-methyl-3,4-dihydrop yrimidin-2(1*H*)-one (Ic).

Dark brown crystals, yield: 73%, mp (°C): 120. IR (γ cm^{-1}): 3481 (NH str of amide), 3361 (OH str of phenol), 3217 (CH str of alkene), 2966 (CH str of methyl), 1631 (C=O str), 1596, 1575, 1492, 1469 (C=C str of aromatic). ^{1}H NMR (γ ppm): 1.78 (3H, s), 2.23 (3H, s), 3.79 (3H, s), 4.94 (1H, s), 5.73 (1H, s), 6.09 (2H, s), 7.15-7.79 (3H, m). MS (m/z): 275.73 (M$^+$).

5-acetyl-6-methyl-4-phenyl-3,4-dihydropyrimidin-2(1*H*)-one (Id).

Brown crystals, yield: 76%, mp (°C): 115. IR (γ cm^{-1}): 3649, 3614, 3566 (NH str of amide), 3095 (CH str of alkene), 2902 (CH str of methyl), 1660 (C=O str), 1552, 1525 (C=C str of aromatic), 1335 (CN str). ^{1}H NMR (γ ppm): 1.63 (3H, s), 2.52 (3H, s), 5.64 (1H, s), 6.06 (2H, s), 7.24-7.95 (5H, m). MS (m/z): 230.44 (M$^+$).

5-acetyl-4-(4-hydroxyphenyl)-6-methyl-3,4-dihydropyrimidin-2(1*H*)-one (Ie).

Red crystals, yield: 79%, mp (°C): 125. IR (γ cm^{-1}): 3566 (NH str of amide), 3110 (CH str of alkene), 2947 (CH str of methyl), 1676 (C=O str), 1598, 1558 (C=C str of aromatic). ^{1}H NMR (γ ppm): 1.73 (3H, s), 2.21 (3H, s), 4.97 (1H, s), 5.76 (1H, s), 6.18 (2H, s), 7.18-7.87 (4H, m). MS (m/z): 245.75 (M$^+$).

5-acetyl-4-{2-hydroxy-5-[(*E*)-(4-nitrophenyl) diazenyl] phenyl}-6-methyl-3, 4-dihydropyrimidin-2(1*H*)-one (IIa)

Red crystals, yield: 78%, mp (°C): 128. **IR (γ cm^{-1}):** 3305 (NH str of amide), 3010 (OH str of phenol), 3240 (CH str of alkene), 2979 (CH str of methyl), 1608 (N=N str), 1585, 1502 (C=C str), 1323 (aromatic nitro group). ^{1}H NMR **(γ ppm):** 2.1 (3H, s), 2.3 (3H, s), 5

(1H, s), 5.4 (1H, s), 6.1 (2H, s), 6.83-7.57 (3H, m), 8.20-8.59 (4H, m). MS (m/z): 394.10 (M^+), 246.9, 333.85, 233.04. Elemental analysis: C (58.24), H (7.04), N (19.71), O (20.23).

5-acetyl-4-{4-methoxy-3-[(*E*)-(4-nitrophenyl) diazenyl] phenyl}-6-methyl-3, 4-dihydropyrimidin-2(1H)-one (IIb)

Brown crystals yield: 80%, mp (°C): 123. **IR (γ cm^{-1}):** 3217 (NH str of amide), 2952 (CH str of alkene), 1596 (N=N str), 1512 (C=C str), 1357 (aromatic nitro group). **^{1}H NMR (γ ppm):** 1.1 (3H, s), 2.2 (3H, s), 3.2 (3H, s), 5.5 (1H, s), 5.9 (2H, s), 6.75-7.86 (3H, m), 8.0-8.49 (4H, m). **MS**: 409.37 (M^+), 365.36, 334.61, 319.6, 307.6. Elemental analysis: C (57.22), H (5.04), N (18.021), O (19.54).

5-acetyl-4-{4-hydroxy-3-methoxy-5-[(*E*)-(4-nitrophenyl) diazenyl] phenyl}-6-methyl-3, 4-dihydropyrimidin-2(1*H*)-one (IIc)

Black crystals, yield: 75%, mp (°C): 131. **IR γ (cm^{-1}):** 3213 (NH str of amide), 3068 (OH str of phenol), 2958 (CH str of methyl), 1620 (N=N str), 1365 (aromatic nitro). **^{1}H NMR (γ ppm):** 1.1 (3H, s), 2.2 (3H, s), 3 (3H, s), 5.1 (1H, s), 5.5 (1H, s), 5.8 (2H, s), 6.4-8.39 (2H, 4H). MS: 425 (M^+), 317.76, 210.86. Elemental analysis: C (58.01), H (4.99), N (18.05), O (22.23).

5-acetyl-6-methyl-4-{4-[(*Z*)-(4-nitrophenyl) diazenyl] phenyl}-3, 4-dihydropyrimidin-2(1H)-one (IId)

Yellow crystals, yield: 73%, mp (°C): 127. **IR γ (cm^{-1}):** 3419 (NH str of amide), 3294 (CN str), 2817 (CH str of methyl), 1660 (N=N str), 1313 (aromatic nitro). ^{1}H NMR **(γ ppm):** 1.8 (3H, s), 2.4 (3H, s), 5.7 (1H, s), 6.3 (2H, s), 7.37-8.4 (8H, m). MS: 378.8 (M^+), 234.5, 220.48. Elemental analysis: C (62.24), H (5.08), N (19.41), O (16.87).

5-acetyl-4-{4-hydroxy-3-[(*E*)-(4-nitrophenyl) diazenyl] phenyl}-6-methyl-3, 4-dihydropyrimidin-2(1H)-one (IIe)

Brown crystals, yield: 71%, mp (°C): 125. **IR γ (cm^{-1}):** 3591 (NH str of amide), 3120 (CH str of alkene), 1676 (N=N str), 1328 (aromatic nitro). ^{1}H NMR **(γ ppm):** 2.1 (3H, s), 2.2 (3H, s), 4.7 (1H, s), 5.4 (1H, s), 6 (2H, s), 6.75-7.57 (3H, m), 8.23-8.59 (4H, m). MS: 395.4 (M^+), 329.7, 240.78. Elemental analysis: C (59.04), H (5.12), N (18.06), O (20.23).

Molecular docking study

The interaction of the synthesised compound with protein was found out using Auto Dock Vina 1.1.2 with MGL tools 1.5.6[28-31].

Protein preparation before docking

X-ray crystal structures of fungal sterol-14-α-demethylase (PDB ID: 5TZ1), fungal dihydrofolate reductase (PDB ID: 4HOF), E. coli dihydrofolate reductase (PDB ID: 4DFR), E.coli DNA gyrase (PDB ID: 5L3J) were retrieved as PDB file from RCSB Protein Data Bank. Protein was refined by removing the water molecules, bound ligand and other hetero atoms using the Discovery Studio Visualizer. The resulting protein further optimized by adding polar hydrogen using Auto Dock tool and the file was saved as 'pdbqt' files.

Ligand preparation before docking

Structure of synthesised compounds was drawn using Chemsketch and SMILES were generated. PDB file of compounds were obtained on copying the SMILES notation to Online SMILES translator. 'pdbqt' files of the compound was prepared using Auto Dock tools.

Configuration file

Input configuration file was prepared as a 'text' file which contain the information of parameters required for docking including the 'pdbqt' file of both protein and ligand, centre 'X, Y, Z' Coordinates, radius of binding site sphere.

Docking

For the docking of compounds with Auto Dock Vina, 'pdbqt' files of protein and ligand, configuration file necessary which were prepared as aforementioned process? Input command was given in command prompt and enters all the necessary parameters. log file was generated after successful docking. Vina output 'log' file consists of the poses generated along with binding affinities and RMSD score values. More binding affinity without any RMSD value was considered as the best pose.

Visualization of interaction

Binding and the interaction of ligand with active residue of protein was visualized in Discovery Studio Visualizer 4.1 and shown as 2D diagram.

Assessment of Drug-likeness properties

Physiochemical properties of the synthesised compounds (IIa-IIe) were calculated using Mo inspiration online web tool.

Biological property evaluation

Antibacterial activity

Well diffusion method was used for estimation of *in-vitro* antibacterial activity of synthesized compounds against Gram positive bacteria such as *Staphylococcus aureus*, *Bacillus subtilis* and Gram negative bacteria such as *Escherichia coli*, *Pseudomonas aeruginosa*. Melted agar medium was poured in to sterilized petri plates and inoculated with the above said bacterial strains. Well was made on plates and 0.1ml of tested compounds in DMSO at a concentration of 100 µg/ml, 200 µg/ml, 400 µg/ml, 800 µg/ml were placed in well. Petri plates were incubated at 37°C for 24hrs. DMSO was used as negative control and gentamycin was a standard. Zone of inhibition was produced by each compound were measured and compared with control.

Antifungal activity

Agar well diffusion method was followed for evaluating antifungal activity against *Candida albicans*, *Aspergillus niger*. The synthesized samples were prepared in the concentration of 100µg/ml, 200µg/ml, 400µg/ml, 800µg/ml respectively in DMSO and tested for antifungal activity. Potato dextrose agar media was prepared and the petri plates were sterilized by autoclaving at 121°C for about 30 minutes at 15 1bs pressure. Under aseptic conditions in the laminar

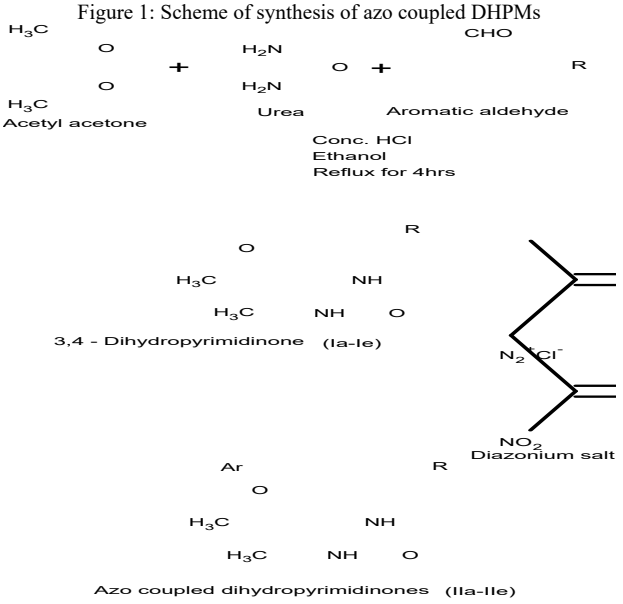
air flow chamber, about 20ml of the agar medium was dispensed into each petri plate to yield a uniform depth of 4mm. After solidification of the media, 18hours culture of fungi was swabbed on the surface of the agar plate. Well was prepared by using cork borer followed with loading of 100 µl of each sample to the distinct well and with DMSO as negative control and fluconazole as positive control. The sample loaded plates were then incubated at 37°C for 48hours to observe the zone of inhibition.

RESULT AND DISCUSSION

Chemistry

3,4-dihydropyrimidinones were synthesized by one pot synthesis of cyclocondensation between acetyl acetone, urea, aromatic aldehydes in presence of conc. Hydrochloric acid. Aryl diazonium salt of p-nitro aniline was prepared and coupled with dihydropyrimidinone to get target compound. Figure 1 represents the synthetic scheme.

Figure 1: Scheme of synthesis of azo coupled DHPMs



Compound IIa: Ar = 5-N=N-C_6H_4-NO_2, R = 2-OH
Compound IIb: Ar = 3-N=N-C_6H_4-NO_2, R = 4-OCH_3
Compound IIc: Ar = 5-N=N-C_6H_3-NO_2, R = 4-OH, 3-OCH_3
Compound IId: Ar = 4-N=N-C_6H_5-NO_2, R = -H
Compound IIe: Ar = 3-N=N-C_6H_4-NO_2, R = 4-OH

Synthesized new azo coupled dihydropyrimidinone derivatives were obtained in moderate yield. Melting point of the compounds were determined using open capillary method and presented uncorrected. Purity of the synthesized compounds were tested by thin layer chromatography. This was performed using silica gel G coated plate as stationary phase and hexane and ethyl acetate as mobile phase. All the compounds were obtained in pure state. Further the compounds were characterized by spectral methods. The first step products dihydropyrimidinones were confirmed by the appearance of common stretching vibrations for all the compounds at 3394 – 3371 cm^{-1}(NH- str of amides), 1681cm^{-1} (C=O str of ketone), 1594, 1517, 1504, 1458 cm^{-1} (C=C str of Ar), 2991, 2941cm^{-1} (CH str of methyl). Azo coupled dihydropyrimidinones were confirmed by the presence new stretching vibration appeared at 1608 cm^{-1} along with the above said stretching vibration. The proton NMR spectral data were showed that the aromatic protons appeared at the range of 6.8 – 8.39 ppm, methyl protons at 1.7 – 2.3 ppm, phenolic hydroxyl proton at 5ppm and amide protons at 5.8-6.2 ppm. All the synthesized compounds were shown their molecular ion peaks as per their molecular weight and fragmentation peaks in their respective m/z values.

Antimicrobial evaluation

In vitro antibacterial and Antifungal activity

The azo coupled dihydropyrimidinone compounds were tested for antibacterial action against Gram positive bacterial strains *Staphylococcus aureus*, *Bacillus subtilis* and Gram negative bacteria such as *Escherichia coli*, *Pseudomonas aeruginosa* and fungal strains C*andida albicans*, *Aspergillus niger*. Antibacterial and antifungal actions of the synthesised compounds were indicated by the zone of inhibition which was shown in Table 1.

Table 1. Zone of inhibition in mm±SEM

Compound	Concentration (µg/ml)	Zone of inhibition (mm diameter)					
		Gram positive bacteria		Gram negative bacteria		Fungal strains	
		S.aureus	B.subtilis	E. coli	P.aeuriginosa	C.albicans	A.niger
IIa	100	-	10±0.5	-	-	10±0.5	-
	200	11±0.17	-	11±0.5	10+0.5	12±0.3	12±0.3
	400	13±0.6	12±0.3	16±0.5	13±0.6	13±0.6	14±0.5
	800	16±0.5	15	20±0.5	14	16±0.5	17±0.5
IIb	100	-	-	10±0.5	-	13±0.6	10±0.5
	200	-	10±0.5	12±0.3	11±0.5	15±0.5	12±0.3
	400	12±0.3	14±0.3	15±0.5	14±0.3	16±0.6	15±0.5
	800	13±0.6	16±0.5	17±0.3	15±0.5	19±0.3	18±0.3
IIc	100	-	-	11±0.5	-	-	12±0.3
	200	-	12±0.5	14±0.6	14±0.5	11±0.5	13±0.6
	400	14±0.6	14±0.6	16±0.5	17±0.3	13±0.3	15±0.5
	800	16±0.3	15±0.5	17±0.3	18±0.5	18±0.3	16±0.6
IId	100	-	-	13±0.5	11±0.6	13±0.5	12±0.3
	200	14±0.6	11±0.5	15±0.6	12±0.6	15±0.6	15±0.6
	400	16±0.6	12±0.3	16±0.5	15±0.3	19±0.3	15±0.6
	800	16±0.5	15±0.6	18±0.3	17.7±0.11	21±0.5	16±0.5
IIe	100	-	10±0.5	14±0.3	-	12±0.6	-
	200	13±0.3	11±0.5	16±0.6	-	14±0.6	14±0.6
	400	14±0.3	13±0.6	17±0.6	12±0.6	16±0.5	15±0.5
	800	16±0.5	17±0.3	19±0.5	16±0.5	17±0.6	16±0.5
Control	-	-	-	-	-	-	-
Fluconazole	10	-	-	-	-	26±0.3	24±0.5
Gentamicin	10	23±0.3	22±0.5	24±0.3	23±0.6	-	-

From the above result, it was found that all the compounds inhibit the growth of bacteria and fungi. The maximum activity was observed at higher concentration of 400 and 800µg/ml. Antimicrobial activity exhibited by the synthesised azo coupled compounds may be due to the presence of electron donating group present on aromatic ring attached at fourth position of dihydropyrimidinone scaffold coupled with phenylazo group bearing electron withdrawing substituent. Compound IIa, IIe and IId, IIb displayed more activity against bacteria and fungi respectively compared to that of positive control.

Molecular docking

Microbial proteins such as bacterial DNA gyrase and dihydrofolate reductase, fungal sterol-14α-demethylase and dihydrofolate reductase was performed using AutoDock Vina and the interaction of the molecules with microbial proteins were visualized by means of Discovery Studio Visualizer. Compound IIa displayed the interaction of amino acid residue Glu 50, Asp 73 through conventional hydrogen bond and Ile 78, Val 120 Val 167 as Pi-alkyl interaction. IIe displayed the interaction on amino acid residues such as Gly 77, Asp 73, Thr 165 and Ala 53 as hydrogen bonding and pi-alkyl interaction respectively. IId had the interaction with fungal sterol-14α-demethylase aminoacid residues on His 377, Ser 378, His 468 through H-bond interaction and Leu 376, Met 508 as Pi-alkyl interaction. Compounds IIa and IIe had higher binding energy of -8.3kcal/mol and -8.0kcal/mol respectively on bacterial DNA gyrase. Compound IId, IIb exhibited higher binding energy -9.1kcal/mol and -9.0 kcal/mol respectively on fungal sterol-14-alpha-demthylase. The interaction is shown in Figure 2-5.

Based on the docking of the synthesised compounds (IIa-IIe) as a new antimicrobial agent against bacterial and fungal proteins, it was suggested that the compounds may act through inhibition of bacterial growth by inhibiting DNA gyrase and fungal cell wall synthesis by inhibiting sterol-14α-demethylase. Molecular docking investigation of synthesised compounds performed with bacterial DNA gyrase B which showed the binding affinity of -8.3kcal/mol and -9.1 kcal/mol against fungal sterol-14α-demethylase which further supported the biological activity. From this docking study, it may be found that the compound with hydroxyl substitution on aromatic ring at fourth position of dihydropyrimidinone core may displayed more activity on fungi and methoxy substituted compound may exhibited more growth inhibitory activity on bacteria. Molecular docking also performed against bacterial and fungal dihydrofolate reductase protein which displayed the lesser binding energy compared with that of DNA gyrase and sterol demthylase. Binding energy shown in the Table 2.

Table 2. Binding energy of synthesized compounds

Compound code	Bacterial proteins		Fungal proteins	
	DNA gyrase B (5L3J)	DHFR (4DFR)	Sterol-14-α-demethylase (5TZ1)	DHFR (4HOF)
IIa	-8.3	-7.4	-8.8	-8.6
IIb	-7.3	-7.0	-9.0	-7.7
IIc	-7.8	-6.6	-8.6	-8.1
IId	-7.5	-7.4	-9.1	-8.9
IIe	-8.0	-7.0	-8.9	-8.3

Figure 2. 2D Interaction of IIa docking with active site of DNA gyrase (PDB ID: 5L3J).

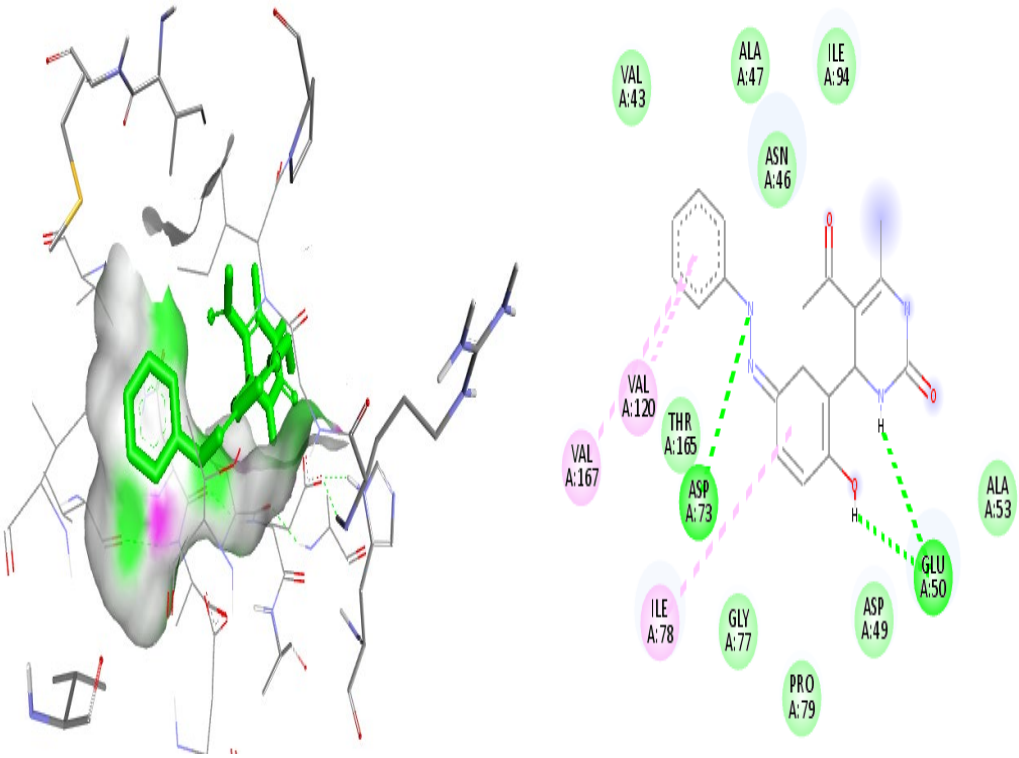


Figure 3 2D Interaction of IIe docking with active site of DNA gyrase (PDB ID: 5L3J).

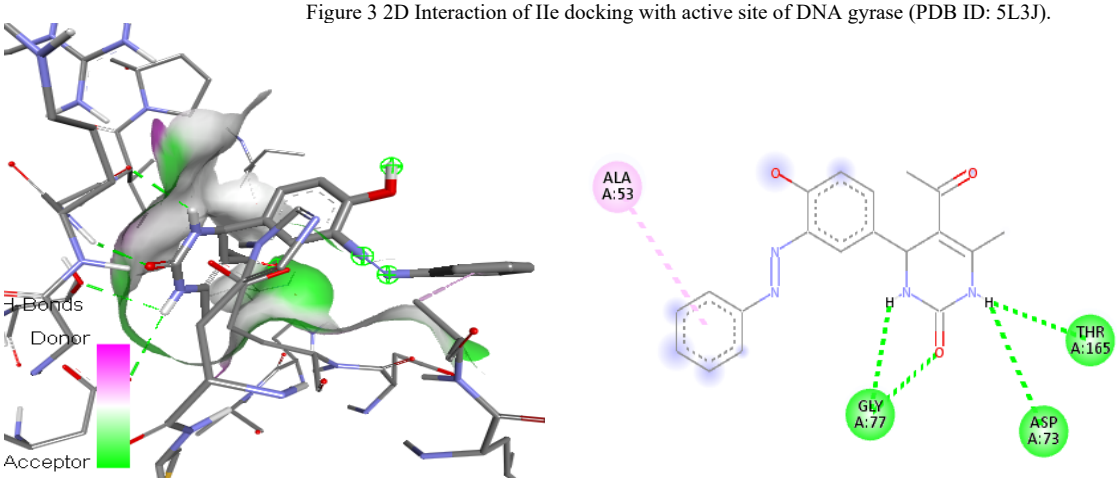


Figure 4 2D Interaction of IId docking with active site of sterol-14α-demethylase (PDB ID: 5TZ1).

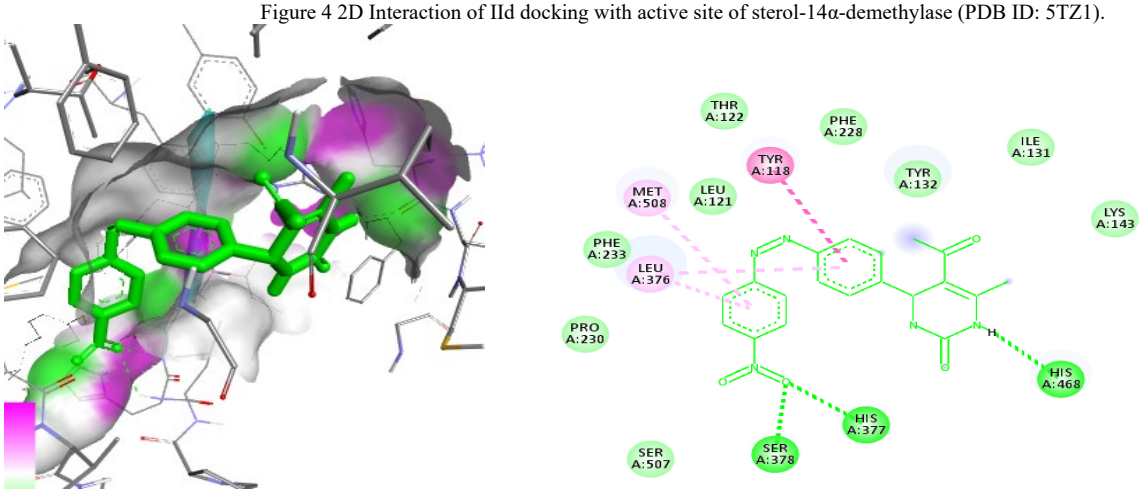
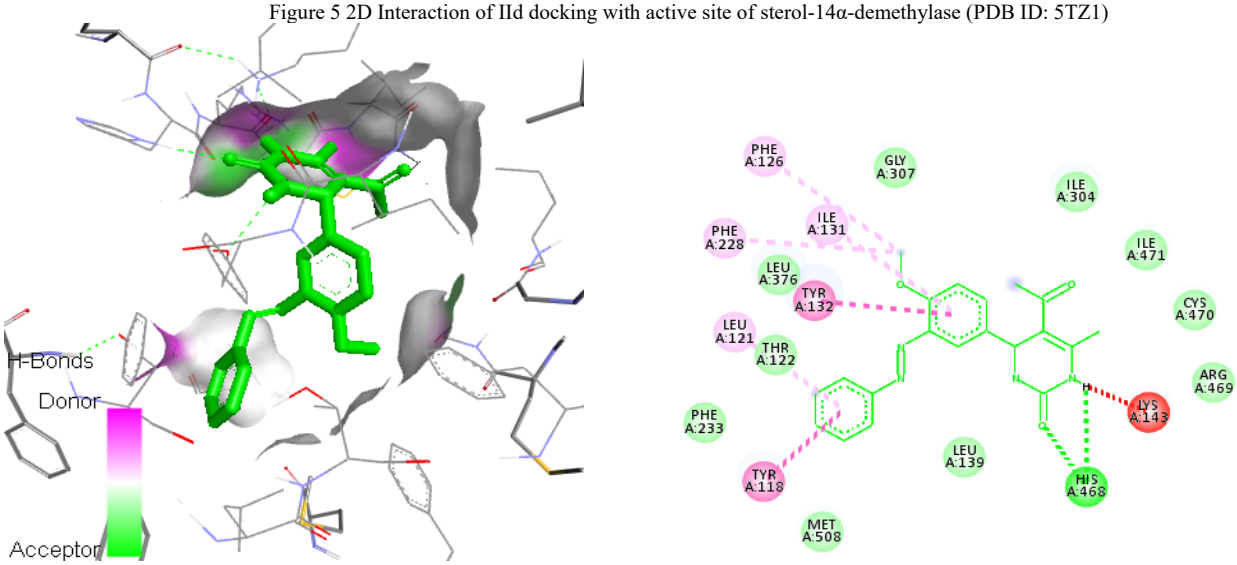


Figure 5 2D Interaction of IId docking with active site of sterol-14α-demethylase (PDB ID: 5TZ1)



Molecular property prediction

Determination of physiochemical properties of the synthesised compounds were done on Molinspiration online tool. The calculated properties presented in Table 3. All the synthesised compounds were shown the total polar surface area with in the limit range of less than 140A˚ which displayed direct correlation of the compounds transported across cell membranes. Hydrophobicity was determined as logP value with in 5 shows good permeability and bioavailability. Other physiochemical properties such as hydrogen

bond acceptor/donor within range of 10 and 5 and number of rotatable bonds with in 6 indicated that all the compounds follow the Lipinski rule of five. Insilco evaluation of molecular properties of synthesised compounds showed the drug-likeness property.

Table 3. Molecular properties of synthesised compounds

Compound	MW	logP	TPSA	natoms	nON	nOHON	nrotb	Volume	nViolat
IIa	395.38	3.75	148.98	29	10	3	5	335.20	0
IIb	409.40	3.81	137.98	30	10	2	6	352.73	0
IIc	425.40	3.33	158.21	31	11	3	6	360.75	1
IId	379.38	3.83	128.75	28	9	2	5	327.19	0
IIe	395.38	3.54	148.98	29	10	3	5	335.20	0

MW= Molecular weight; TPSA= Total polar surface area; nON=hydrogen bond acceptor; nOHON= Hydrogen bond donor; rotb = number of rotatable bonds

CONCLUSION

The present study investigated on the synthesis of azo-coupled 3, 4-dihydropyrimidine-2-one derivatives from the reaction between 3, 4-dihydropyrimidinones and diazonium salt of p-nitroaniline. All the synthesised compounds were exhibited the moderate antibacterial and antifungal action against bacterial and fungal strains. The present study was concluded that the electron donating substituents such as hydroxyl group on aromatic ring at fourth position of dihydropyrimidinones bearing azo-linked derivatives and methoxy substituted azo-coupled derivatives possess antimicrobial activity. Physiochemical properties of compounds follow the Lipinski rule of five and possess drug-likeness property. Molecular docking studies revealed that the compounds may acts by inhibiting the bacterial and fungal growth. From this study, it was concluded that the compound may act as lead for further development of drug as antimicrobial agent..

CONFLICT OF INTEREST

The authors declare no conflict of interest

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