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Novel Amide Derivatives of Pyrimidinediones: Design, Synthesis, Characterization, Biological Assessment, ADMET and *in silico* Docking Studies

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In an attempt to synthesize new chemical entities with promising biological activity, a set of six novel pyrimidinedione analogues (**6a-f**) was prepared. Compound **4** was coupled with different amines by using HBTU as a coupling reagent. The derivatives were characterized by ^{1}H NMR, ^{13}C NMR and HRMS. The analogues were evaluated for their antibacterial and antioxidant activity. The DPPH and ABTS methods were used to test the antioxidant activity of the moieties. Among the six pyrimidinedione analogues, **6b**, **6d** and **6f** exhibited significant antioxidant effects. The analogues **6b**, **6c** and **6e** exhibited potency against Gram-negative and Gram-positive pathogens. Further, to evaluate the binding abilities of the analogues to the protein active sites, molecular docking studies were performed. The results of the docking studies were found to be consistent with the antibacterial potential of the analogues. The efficacy of these analogues provides insight into their use as novel antioxidant and antibacterial agents.

Keywords: Pyrimidinediones, Antioxidant activity, Disc diffusion method, Molecular docking, ADMET.

INTRODUCTION

Infectious ailments spurred on by fungi, bacteria, parasites and viruses keep posing an imminent threat to public health, regardless of the great advancements in medical science. Due to relative medical scarcity and the advent of widespread drug resistance, the impact is more acute in underdeveloped nations [1]. In past two decades, the emergence of drug resistance and the unfavourable side effects of a few antibiotics have prompted researchers to look for new antibiotics in an effort to identify novel chemical structures that can overcome the drawbacks [2,3].

Pyrimidine-containing heterocycles are of great importance in organic chemistry and the field of medicinal chemistry [4,5]. Living organisms contain substituted purines and pyrimidines and research into these substances has aided in the development of new drugs and a better understanding of biological processes [6]. Pyrimidinediones have unquestionably shown great promise in a variety of medical applications. These substances demonstrated therapeutic uses as anticancer, antiviral, hypoglycemic, anticonvulsive, analgesic and anti-inflammatory drugs [7-12].

Antioxidant compounds have grown in popularity because oxidative stress damages cells and accelerates diseases like Alzheimer's [13] and Parkinson's [14,15]. When reactive oxygen species accumulate in the cells without being detoxified, it leads to oxidative stress in the cells [16]. Studies have shown that antioxidant supplementation may reverse the damage caused by oxidative stress [17,18]. Investigations on the antioxidant potential of the synthesized heterocyclic compounds showed that all samples were able to scavenge free radicals, which was assessed by both 2,2-diphenyl-1-picrylhydrazyl (DPPH)

and 2,2′-azino-*bis*(3-ethylbenzothiazoline-6-sulfonic acid (ABTS) assays. Compounds with antioxidant potential have gained attraction in contemporary research as cell damage by oxidative stress and reactive oxygen species leads to various disease progressions [19,20]. Studies have shown that various biomolecules, including DNA and RNA also get damaged by oxidative stress [21-23]. As oxidative stress is associated with deleterious cellular effects, compounds with antioxidant potential are under exploration. Previous studies have shown that pyrimidine-containing heterocyclic [24,25] compounds have very good antioxidant potential. *In vitro* anticancer and antioxidant potentials of pyrimidinedione derivatives produced by one-pot multicomponent reactions were demonstrated in studies by Gouda *et al.* [26]. The antioxidant properties of pyrimidine depend on the electron density of the ring system [27]. Sharma *et al*. [28] revealed that among the diones, OBP-05 exhibited both nitric oxide and ferric ion scavenging abilities.

Owing to their biological significance, in the present work, six novel pyrimidinedione derivatives have been synthesized. The compounds were characterized by ^{1}H NMR, ^{13}C NMR and high resolution mass spectrometry (HRMS) techniques. To investigate further the biological efficacy of the targets, the antibacterial and antioxidant potentials were analyzed. A molecular docking investigation further revealed that three of the target molecules exhibited good antibacterial properties.

EXPERIMENTAL

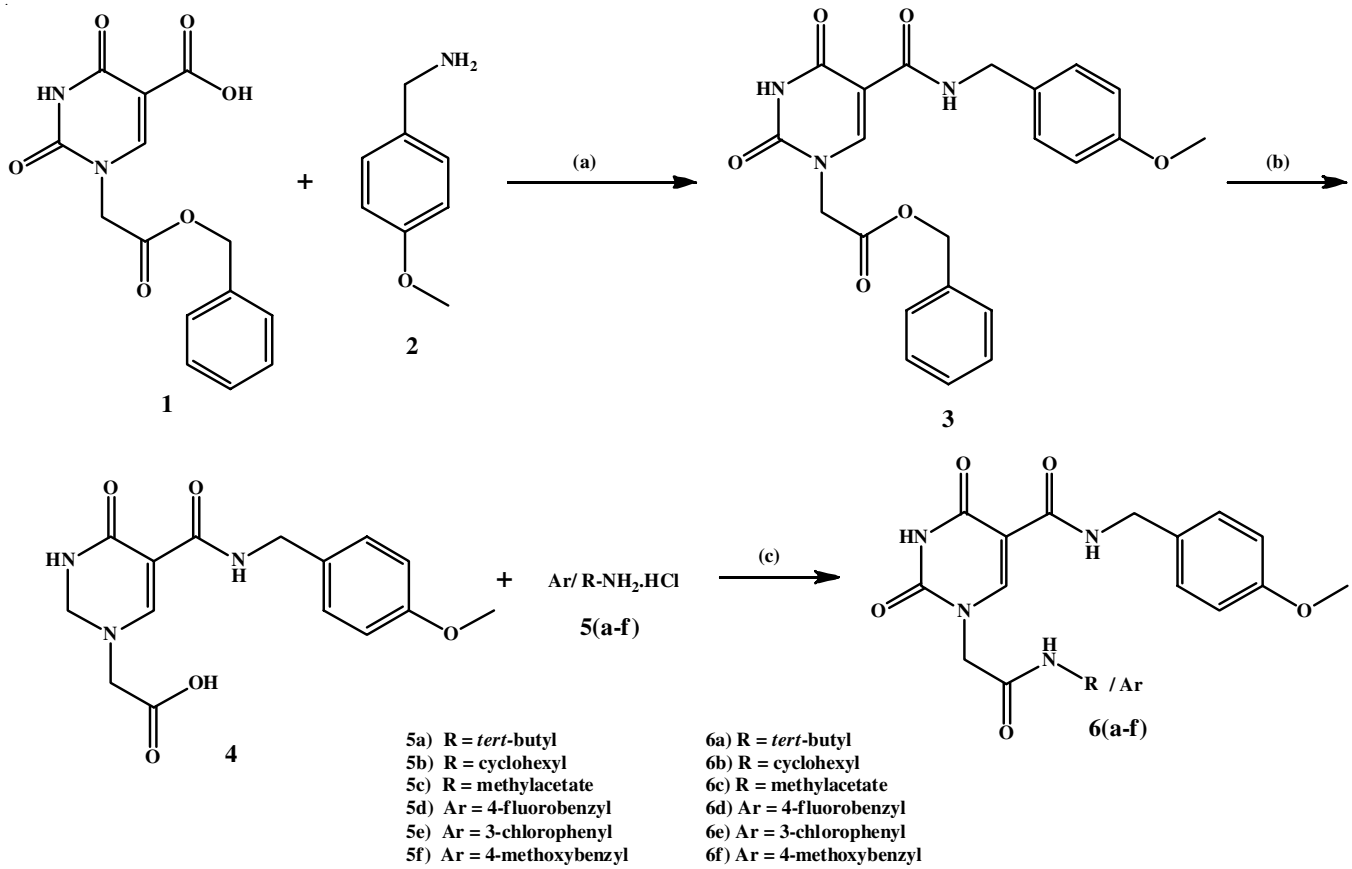
Silica gel plates having 60 F_{254} stationary phase was used for the thin-layer chromatography (TLC) technique. The visualization of TLC spots was achieved using either an ultraviolet (UV) lamp or an iodine indicator. Proton NMR spectral studies were performed using JEOL JNM-ECZR (600 MHz) and Bruker Biospin (400 MHz) instruments. ^{13}C spectral studies were performed using 125 MHz and 100 MHz. Chemical shift information was expressed in ppm and coupling constant values in Hz. An API-3000 LC-MS spectrometer was employed for mass spectral data collection. A Waters G2-XS QT mass spectrometer was used for high resolution mass spectral studies. The synthesized compounds melting points were recorded using an electro thermal device. The DPPH and ABTS compounds were purchased from Himedia, Mumbai, India and ascorbic acid from SRL Mumbai, India. UV-visible spectrophotometer (Systronic India Ltd) was used for analyzing radical scavenging activity.

Synthesis of benzyl 2-(5-(4-methoxybenzylcarbamoyl)-3,4-dihydro-2,4-dioxopyrimidin-1(2*H*)-yl)acetate (3): Compound **1** (4.0 g, 13.157 mmol) was placed in a clean and dry round bottom flask and then 60 mL of DMF was added slowly. The contents of the reaction mixture were thoroughly mixed, then HBTU (9.9 g, 26.315 mmol) was cautiously added at room temperature followed by the addition of DIPEA (5.0 g, 39.473 mmol). For 30 min at room temperature, the contents in flask were stirred continuously and *p*-methoxybenzylamine (2.7 g, 19.736 mmol) was added and the contents were stirred again for 16 h. The completion of the reaction was confirmed using the TLC technique. The resulting residue was transferred to ice-cold water taken in a beaker to ensure complete precipitation. The contents were further stirred on a stirrer for another 10 min till a pale yellow coloured solid was formed. After filtering the crude sample, it was subjected to thorough washing with a mixture of water and hexane. The obtained solid material was dried under vacuum to attain the final product. To purify compound **3** obtained, column chromatography was employed, using silica gel with 100-200 mesh. The solvent used was 1% methanol in DCM. After the purification compound **3** was obtained. White solid, 74% yield. m.p.: 196-198 ºC. ^{1}H NMR (DMSO-d_6, 600 MHz): δ = 12.05 (bs, 1H, NH-Py), 8.99-8.88 (m, 1H, C=CH), 8.76-8.61 (m, 1H, CONHCH$_2$), 7.34-7.18 (m, 7H, Ar-H), 6.91-6.84 (m, 2H, Ar-H), 5.17-5.13 (m, 2H, CH$_2$-Ar), 4.86-4.66 (m, 2H, CH$_2$COO), 4.38-4.24 (m, 2H. NCH$_2$CO), 3.69 (bs, 3H).^{13}C NMR (DMSO-d_6, 125 MHz): δ 158.86, 152.11, 136.0, 129.37, 129.25, 129.0, 128.77, 128.50, 114.55, 114.36, 67.23, 67.13, 55.60, 42.34, 42.14.

Synthesis of 2-(5-(4-methoxybenzylcarbamoyl)-3,4-dihydro-2,4-dioxopyrimidin-1(2*H*)-yl)acetic acid (4): In a dry 250 mL single necked round bottom flask, intermediate **3** (3.0 g, 7.092 mmol) was placed along with methanol and water in a ratio of 8:2. After cooling the contents to 0 ºC, LiOH (1.48 g, 35.461 mmol) was added to the above solution and then stirred for 1 h at 0 ºC. The completion of the reaction was confirmed by performing thin-layer chromatography. Rotary evaporator was used to distil the resulting contents to remove the solvent, methanol. The impure product obtained was cooled to 0 ºC and later acidified with 20% citric acid solution to maintain a pH of 3-4. The precipitate formed was later filtered and thoroughly washed with 75 mL of EtOAc. A column (silica gel 100-200 mesh grade) was used to purify the product. White solid, 80% yield. m.p.: 194-196 ºC. ^{1}H NMR (DMSO-d_6, 600 MHz): δ 11.88 (s, 1H, COOH), 9.27-9.25 (t, 1H, *J* = 12 Hz, NH-Py), 8.47 (s, 1H, C=CH-Py), 7.42- 7.40 (d, 2H, *J* = 12 Hz, Ar-H), 7.08-7.06 (m, 2H, Ar-H), 4.60 (bs, 2H, CONHCH$_2$), 4.06 (s, 2H, NCH$_2$COOH), 3.91 (bs, 3H, OCH$_3$). ^{13}C NMR (DMSO-d_6, 125 MHz): δ 169.05, 164.34, 162.50, 158.81, 153.26, 150.80, 131.75, 129.22, 114.34, 103.77, 55.58, 52.13, 42.03.

General preparation method for the analogues 6(a-f): The amide compounds **6a-f** were synthesized by adding appropriate amine, 0.04 g (0.47 mmol) to a solution of scaffold **4** (0.15 g, 0.45 mmol) dissolved in 3 mL of DMF. To the reaction contents, HATU (0.26 g, 0.68 mmol) was added and the solution was cooled and stirred at 0 ºC for 10 min. After the addition of *N*-methylmorpholine (0.1 mL) dropwise, stirring was continued for 3 h at room temperature. Using thin layer chromatography, the completion of the reaction was monitored. The resultant product was poured onto ice and stirred for 30 min (**Scheme-I**). A mixture of hexane and water was used to wash the solid, which was then filtered. The solid was vacuum dried and then passed through a column (silica gel of 100-200 mesh) to purify the crude product. The elution was carried out using 1% methanol in DCM solvent.

1-(2-*tert*-Butylamino)-2-oxoethyl)-N-(4-methoxybenzyl)-2,4-dioxo-1,2,3,4-tetrahydropyrimidine-5-carboxamide (6a): Pale white solid, 72% yield. m.p.: 198-200 ºC. ^{1}H NMR (DMSO-d_6, 600 MHz): δ 12.04 (s,1H, NH-py), 9.21-9.19 (t, 1H, C=CH), 8.61 (s, 1H, CONHCH$_2$), 8.04 (s, 1H, CONH-*t*-Bu),



Scheme-I: Reagents and conditions: (a) HBTU, DIPEA, DMF, 16 h, room temperature, (b) LiOH, MeOH:H_2O (3:2), 1 h, 0 °C, (c) 5a, HATU, NMM, DMF, 3 h, room temperature

7.39 (d, 2H, *J* = 6 Hz, H-2′ and H-6′ of Ph-OCH_3), 7.05 (d, 2H, *J* = 6 Hz, H-3′ and H-5′ of Ph-OCH_3), 4.53 (s, 2H, CH_2CONH), 4.58 (d, 2H, *J* = 6 Hz, NCH_2CO), 3.83 (s, 3H, OCH_3), 1.41 (s, 9H, *t*-Bu). ^{13}C NMR (DMSO-d_6, 125 MHz): δ 166.0, 164.21, 162.19, 158.84, 153.10, 150.58, 131.67, 129.21, 114.35, 104.50, 55.58, 51.01, 50.79, 42.08, 29.00. HRMS (ES+) *m/z* (M+) calculated for $C_{19}H_{24}N_4O_5$ = 388.1747; found = 389.1916. Purity of compound **6a** given by HPLC was 90.86.

1-(2-Cyclohexyl)-2-oxoethyl)-N-(4-methoxybenzyl)-2,4-dioxo-1,2,3,4-tetrahydropyrimidine-5-carboxamide (6b): White solid, 85%. m.p.: 256-258 ºC. ^{1}H NMR (DMSO-d_6, 600 MHz): δ 11.91 (s, 1H, NH-py), 9.05 (s, 1H, C=CH), 8.48 (s, 1H, *J* = 6 Hz, CONHCH$_2$), 8.11 (d, 1H, *J* = 6 Hz, CONH-cyclohexyl), 7.23 (d, 2H, *J* = 6 Hz, H-2′and H-6′ of Ph-OCH_3), 6.89 (d, 2H, *J* = 6 Hz, H-3′ and H-5′ of Ph-OCH_3), 4.90 (s, 2H, CH_2CONH), 4.42 (d,2H, *J* = 6 Hz, NCH_2CO), 3.72 (s, 3H, OCH_3), 1.74-1.66 (m, 4H, H-2″ and H-6″ of cyclohexyl), 1.54-1.52 (m, 1H, H-1″ of cyclohexyl), 1.26-1.14 (m, 6H, H-3″, H-4″ and H-5″ of cyclohexyl).^{13}C NMR (DMSO-d_6, 125 MHz): δ 165.73, 164.24, 162.15, 158.84, 153.0, 150.60, 131.66, 129.22, 114.35, 104.65, 55.57, 50.66, 48.41, 42.09, 32.86, 25.68, 24.94. HRMS (ES+) *m/z* (M+) calculated for $C_{21}H_{26}N_4O_5$ = 414.1903; found = 415.2105. Purity of compound **6b** given by HPLC was 91.30.

Methyl(2-(5-((4-methoxybenzyl)carbamoyl)-2,4-dioxo-3,4-dihydropyrimidin-1-(2*H*)-yl)acetyl)glycinate (6c): White solid, 80%. m.p.: 212-214 ºC. ^{1}H NMR (DMSO-d_6, 600 MHz): δ 11.94 (s,1H, NH-py), 9.03 (s, 1H, C=CH), 8.72 (s, 1H, CONHCH$_2$), 8.51 (s, 1H, CONH-R), 7.23 (d, 2H, *J* = 8 Hz, H-2′ and H-6′ of Ph-OCH_3), 6.89 (d, 2H, *J* = 8 Hz, H-3′ and H-5′ of Ph-OCH_3), 4.62 (s, 2H, CH_2CONH), 4.42 (d, 2H, *J* = 4 Hz, NCH_2CO), 3.91 (d, 2H, *J* = 4 Hz, NHCH$_2$ of R), 3.73 (s, 3H, PhOCH_3), 3.64 (s, 3H, COOCH_3).^{13}C NMR (DMSO-d_6, 125 MHz): δ 170.51, 167.63, 164.22, 162.09, 158.84, 152.72, 150.55, 131.65, 129.23, 114.35, 104.95, 55.58, 52.31, 50.40, 42.10, 41.15. HRMS (ES+) *m/z* (M+) calculated for $C_{18}H_{20}N_4O_7$ = 404.1332 found = 405.1495. Purity of compound **6c** given by HPLC was 96.03.

1-(2-((4-Fluorobenzyl)amino)-2-oxoethyl)-*N*-(4-methoxybenzyl)-2,4-dioxo-1,2,3,4-tetrahydropyrimidine-5-carboxamide (6d): Pale white, 70%. m.p.: 245-247 ºC. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 11.83 (s, 1H, NH-py), 9.06 (t, 1H, *J* = 4 Hz, C=CH), 8.75 (t, 1H, *J* = 4 Hz, CONH-R), 8.35 (s, 1H, CONH-R), 7.32-7.29 (m, 2H, H-2″ and H-6″ of Ar-F), 7.23 (d, 2H, *J* = 8 Hz, H-2′ and H-6′ of PhOCH_3), 7.15 (t, 2H, *J* = 4 Hz, H-3″ and H-5″ of Ar-F), 6.89 (d, 2H, *J* = 8Hz, H-3′ and H-5′ of PhOCH_3), 4.60 (s, 2H, NHCH$_2$R), 4.43 (d, 2H, *J* = 4 Hz, NHCH$_2$), 4.30 (d, 2H, *J* = 8 Hz, NCH_2), 3.73 (s, 3H, OCH_3).^{13}C NMR (DMSO-d_6, 125 MHz): δ 167.05, 164.30, 162.6, 162.2, 160.9, 158.8, 152.8, 150.69, 135.70, 131.66, 129.73, 129.67, 129.22, 115.61, 115.46, 114.35, 104.93, 55.58, 50.96, 42.10, 42.01. HRMS (ES+) *m/z* (M+) calculated for $C_{22}H_{21}FN_4O_5$ = 440.1496 found = 441.1585. Purity of compound **6d** given by HPLC was 91.76.

1-(2-((3-Chlorophenyl)amino)-2-oxoethyl)-*N*-(4-methoxybenzyl)-2,4-dioxo-1,2,3,4-tetrahydropyrimidine-5-carboxamide (6e): White solid, 75%. m.p.: 250-252 ºC. ^{1}H NMR (DMSO-d_6, 600 MHz): δ 11.98 (s, 1H, NH-py), 10.52 (s,1H, C=CH), 9.00 (s, 1H, CONHCH$_2$), 8.56 (s, 1H, CONH-R), 7.71 (s, 1H), 7.38-7.34 (m, 2H), 7.20 (d, 2H, *J* = 12 Hz), 7.11-7.09 (m, 1H), 6.85 (d, 2H, *J* = 6 Hz), 4.72 (s, 2H, NHCH$_2$), 4.40 (s, 2H, NCH$_2$), 3.69 (s, 3H, OCH$_3$).^{13}C NMR (DMSO-d_6, 125 MHz): δ 166.21, 164.21, 162.08, 158.85, 152.81, 150.70, 140.42, 133.74, 131.64, 131.18, 129.23, 123.92, 119.13, 118.02, 114.36, 105.03, 55.58, 51.44, 42.13. HRMS (ES+) *m/z* (M+) calculated for $C_{21}H_{19}ClN_4O_5$ = 442.1044; found = 443.1193. Purity of compound **6e** given by HPLC was 95.79.

***N*-(4-Methoxybenzyl)-1-(2-((4-methoxybenzyl)amino)-2-oxoethyl)-2,4-dioxo-1,2,3,4-tetrahydropyrimidine-5-carboxamide (6f):** White solid, 85%. m.p.: 218-220 ºC. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 11.99 (s,1H, NH-py), 9.12-9.09 (t, 1H, C=CH), 8.73-8.71 (t, 1H, CONHCH$_2$), 8.58 (s, 1H, CONH-R), 7.30-7.23 (m, 4H, C$_6$H$_4$-OCH$_3$), 6.96-6.92 (m, 4H, C$_6$H$_4$-OCH$_3$), 4.53 (s, 2H, NHCH$_2$), 4.48-4.46 (d, 2H, *J* = 8 Hz, NCH$_2$R), 4.30 (d, 2H, *J* = 4 Hz, NCH$_2$), 3.78 (s, 6H, OCH$_3$). ^{13}C NMR (DMSO-d_6, 125 MHz): δ 166.83, 164.28, 162.15, 158.84, 152.86, 150.65, 131.66, 131.37, 129.23, 129.12, 114.35, 114.23, 104.88, 55.59, 50.90, 42.22, 42.10. HRMS (ES+) *m/z* (M+) calculated for $C_{23}H_{24}N_4O_6$ = 452.1696; found = 453.1849. Purity of compound **6f** given by HPLC was 90.94.

Antibacterial activity: The paper disc diffusion method is a qualitative technique for assessing the antibiotic susceptibility of bacteria. Various test concentrations of chemicals (1.0, 0.5, 0.25, 0.125, 0.0625, 0.0315 and 0.01575 mg/mL) were dissolved in DMSO and then deposited onto sterile filter paper discs [29]. The two test bacterial isolates, *Staphylococcus aureus* and *Pseudomonas putida* were employed to test the antimicrobial potential of compounds. Filter paper discs were individually inoculated with bacterial cultures and then placed on the nutritional agar plates using sterile forceps. As a positive control, ciprofloxacin, a common antibiotic, was utilized, while DMSO solvent was used as a negative control. The MICs of the samples under test were then ascertained visually by viewing the zone of inhibition encircling the filter paper discs, after the plates were incubated for 24 h at 37 ºC. The sample with the lowest concentration that displayed an inhibitory zone was observed as MIC value. The experiment was conducted in three batches to ensure accuracy and repeatability.

Antioxidant activity

DPPH assay: The antioxidant potentials of the target molecules were screened using the DPPH assay. The samples at a concentration of 50 μg mL^{-1} were allowed to react with a methanolic DPPH solution and maintained at room temperature for 30 min under dark conditions. Ascorbic acid as a positive control and DPPH as a negative control was used for the antioxidant assay. The radical scavenging activity was analyzed in a UV-visible spectrophotometer at 517 nm. The following equation was used for calculating the antioxidant capacity of the samples.

$$\text{Scavenging activity (\%)} = \frac{Ab - Aa}{Ab} \times 100$$

wherein, Ab = optical density of DPPH control and Aa = optical density of samples/ascorbic acid [30].

ABTS assay: The ABTS assay was employed to screen the free radical scavenging capacity of the synthesized compounds. ABTS in water (7.4 mM) and potassium persulfate (2.45 mM) were combined (1:1 ratio), to form the ABTS$^{\bullet+}$ cation radical. The mixture was allowed to stand in dark for 12 h at room temperature. Samples were then made to react with ABTS solution (2.8 mL) for 1 h under dark conditions. An appropriate ABTS blank with no sample was also taken as a control in each assay. Ascorbic acid with known antioxidant activity was used as a standard. The assay was performed in triplicate to ensure the reliability of the results. The radical scavenging activity was measured in a UV-visible spectrophotometer at 734 nm and calculated using the formula:

$$\text{ABTS}^{\bullet+}\text{ scavenging effect (\%)} = \frac{Ab - Aa}{Ab} \times 100$$

wherein, Ab = optical density of ABTS radical + methanol; Aa = optical density of ABTS radical + samples/standard [30].

RESULTS AND DISCUSSION

Compounds **1** and **2** were made to react in the presence of HBTU and DIPEA, respectively in DMF solvent for 16 h at room temperature to give amide **3**. Spectroscopic analysis was used to characterize the synthesized targets. LCMS was employed to analyze the purity and mass spectral data of the compounds. The characterization of compound **3** was done by ^{1}H NMR and ^{13}C NMR. ^{1}H NMR indicated the presence of methylene hydrogens of the -NCH$_2$ group of the amide linkage at δ 4.99-4.45 ppm. The -NCH$_2$ group of pyrimidinedione ring appeared at δ 5.07-4.86 ppm. The -OCH$_2$ group peak appeared at δ 5.39-5.34 ppm. The appearance of a singlet at δ 3.89 ppm with three protons confirms the methoxy moiety.

Compound **3** was ester hydrolyzed using aqueous LiOH in MeOH for 1 h at 0 ºC to yield compound **4**. The ^{1}H NMR of compound **4** confirmed the disappearance of benzylic protons at δ 7.55-7.39 ppm and the appearance of the acidic proton as a singlet at δ 11.88 ppm. Scaffold **4** was treated with *tert*-butylamine 5**a** in the presence of HATU, NMM and DMF for 3 h at room temperature to produce the derivative **6a**. The structural elucidation of compound **6a** was confirmed by the presence of hydrogen of amide of pyrimidine ring at δ 12.04 ppm as a singlet. The appearance of nine protons at δ 1.41 ppm as singlet confirms the *tert*-butyl group of compound **6a**. The rest of the protons are in accordance with the proposed structure. The ^{13}C NMR spectrum obtained for the synthesized compounds was found to be consistent with the expected target structure. HRMS (ES+) indicated the presence of (M+) at *m/z* 389.1916.

Analogues **6a-f** were synthesized following the general procedure outlined in the study. The structural elucidation of the newly synthesized pyrimidinediones was executed using HRMS and NMR analysis. Amide NH protons have been confirmed by D_2O exchange solvent.

Antibacterial activity: Antibacterial analysis by the paper disc diffusion method showed that three of the six examined compounds **6b**, **6c** and **6e** were found to be effective against

both test pathogens. All these compounds exhibited strong inhibition of Gram-negative bacteria, *Pseudomonas putida* with low MIC values recorded against this bacterium. The MIC values of the synthesized samples are given in Table-1. It was observed that compound **6e** emerged as one of the compounds with the highest activity suggests that it has the potential to be a leading molecule for more optimization and development as a new antibacterial agent. The high antimicrobial potential of the compound **6e** may be attributed to the presence of electron withdrawing chloro group in their structure.

TABLE-1
ANTIBACTERIAL ACTIVITY OF COMPOUNDS **6a-f**

Analogues	*Staphylococcous* (mic) mg/mL	*Pseudomonas putida* (mic) mg/mL
6a	0.000	0.000
6b	1.000	0.500
6c	1.000	0.500
6d	0.000	0.000
6e	0.500	0.125
6f	0.000	0.000
Ciprofloxacin	0.0625	0.0625

Antioxidant activity: The antioxidant efficacy revealed that the samples were able to scavenge the free radicals produced by DPPH. The results of the ABTS assay also showed the same pattern of antioxidant efficacy of samples. At the tested concentrations (50 µg/mL), all samples were able to exhibit an antioxidant effect. Among the samples, **6b**, **6d** and **6f** showed the highest antioxidant activity, which was comparable with ascorbic acid and compound **6e** showed the lowest activity (Table-2).

Thus, in present study, the synthesized pyrimidinedione compounds showed antioxidant potential. Among them, three compounds **6b**, **6d** and **6f** exhibited excellent antioxidant activity, which was comparable to that of standard ascorbic acid.

TABLE-2
DPPH AND ABTS ASSAY OF COMPOUNDS **6a-f**

Compound	% Inhibition (DPPH)	% Inhibition (ABTS)
6a	60	59
6b	91	89
6c	71	76
6d	89	87
6e	50	49
6f	89	90
Ascorbic acid	92	93

Molecular docking studies: In this study, molecular docking studies was performed by using DNA gyrase protein (PDB ID 6z1a) [31] for compounds **6b**, **6c**, **6e** and ciprofloxacin used as a control. The targets showed good binding affinity with DNA gyrase protein (Table-3).

TABLE-3
BINDING ENERGY VALUES OF PYRIMIDINEDIONE DERIVATIVES-ANTIBACTERIAL ACTIVITY

Compounds	Binding affinity (Kcal/mol)
6b	-7.5
6c	-7.3
6e	-8.1
Ciprofloxacin	-7.6

Molecule **6b** shows van der Waals interactions, hydrogen bond and alkyl interactions with amino acids like ARG D 1092, SER D 1098, GLY D 1115, VAL D 1091 and ALA D 1118. Binding orientation of compound **6b** to DNA gyrase at its active site represent the molecular interactions of the ligand in 2D and 3D diagrams (Fig. 1). The binding energy value of compound was -7.5 kcal/mol.

Molecule **6c** shows van der Waals interactions, hydrogen bond, alkyl interactions with amino acids like ARG D 1092, ARG D 1033, HIS D 1046, LYS D 1043, VAL D 1045, ALA D 1089, GLY D 1082, ARG B 1122 and GLU D 435. Binding

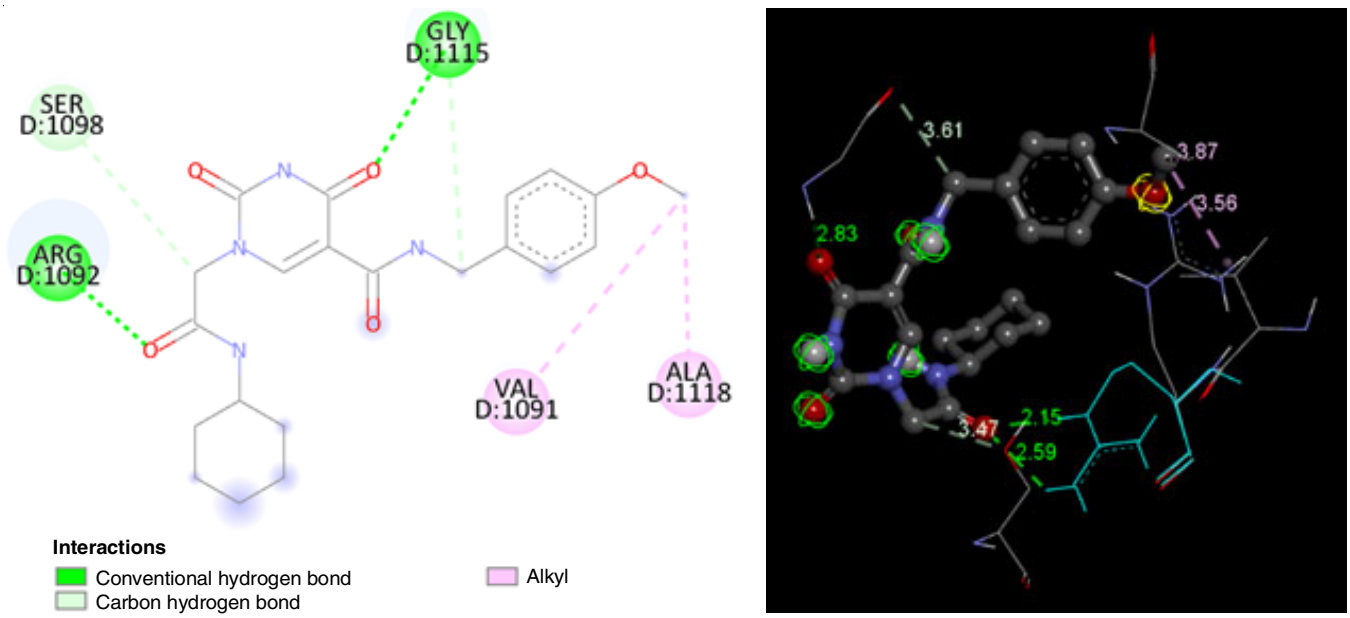


Fig. 1. Binding packet 2D and 3D diagrams of **6b** with DNA gyrase

orientation of compound **6c** to DNA gyrase at its active site represent the molecular interactions of the ligand in 2D and 3D diagrams (Fig. 2). The binding energy value of compound was -7.3 kcal/mol.

Molecule **6e** shows van der Waals interactions, hydrogen bond, alkyl interactions with amino acids like TYR D 1087, VAL D 1091, ALA D 1118, GLU D 1088, PHE D 1097 and MET D 1113. Binding orientation of compound **6e** to DNA gyrase at its active site represent the molecular interactions of the ligand in 2D and 3D representation is shown in Fig. 3. The binding energy value of compound was -8.1 kcal/mol.

Ciprofloxacin molecule shows van der Waals interactions, hydrogen bond and alkyl interactions with amino acids like GLN D 1267, MET D 1113, ASP D 1116, GLY D 1115, ARG D 1092 and PHE D 1097. Binding orientation of ciprofloxacin to DNA gyrase at its active site present the molecular interactions of ligand in 2D and 3D images in Fig. 4. The binding energy value of ciprofloxacin was -7.6 kcal/mol.

ADMET properties: Tables 4 and 5 show the computational results of the synthesized compounds (**6a-f**) using pkCSM from the Biosig Lab University of Melbourne ADMET prediction servers [32,33]. The ADMET characteristics of the pyrimidinedione analogues (**6a-f**) revealed that they have strong solubility, which indicates their efficient absorption and enhanced elimination.

Conclusion

Six novel pyrimidinedione analogues (**6a-f**) were synthesized by coupling reactions with different amines. The targets compounds were characterized by ^{1}H NMR, ^{13}C NMR and HRMS techniques. The synthesized analogues were further explored for their antioxidant and antibacterial potentials. The *in vitro* studies exhibited that compounds **6b, 6d** and **6f** have significant antioxidant activity. Compound **6e** on the other hand, demonstrates more potent antibacterial activity compared to other compounds. *In silico* molecular docking studies between the

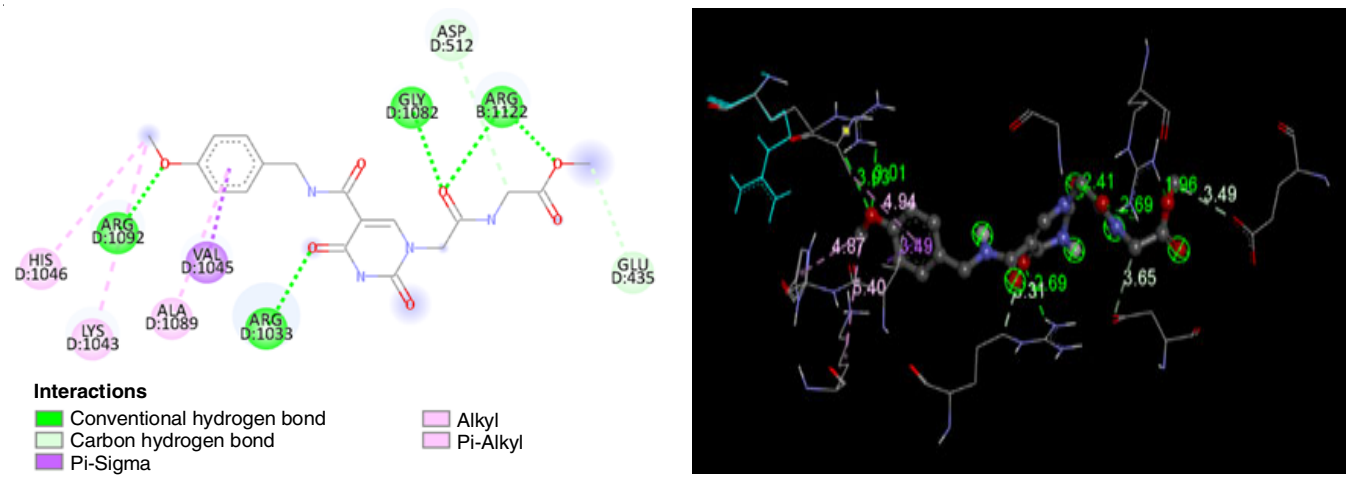


Fig. 2. Binding packet 2D and 3D diagrams of **6c** with DNA gyrase

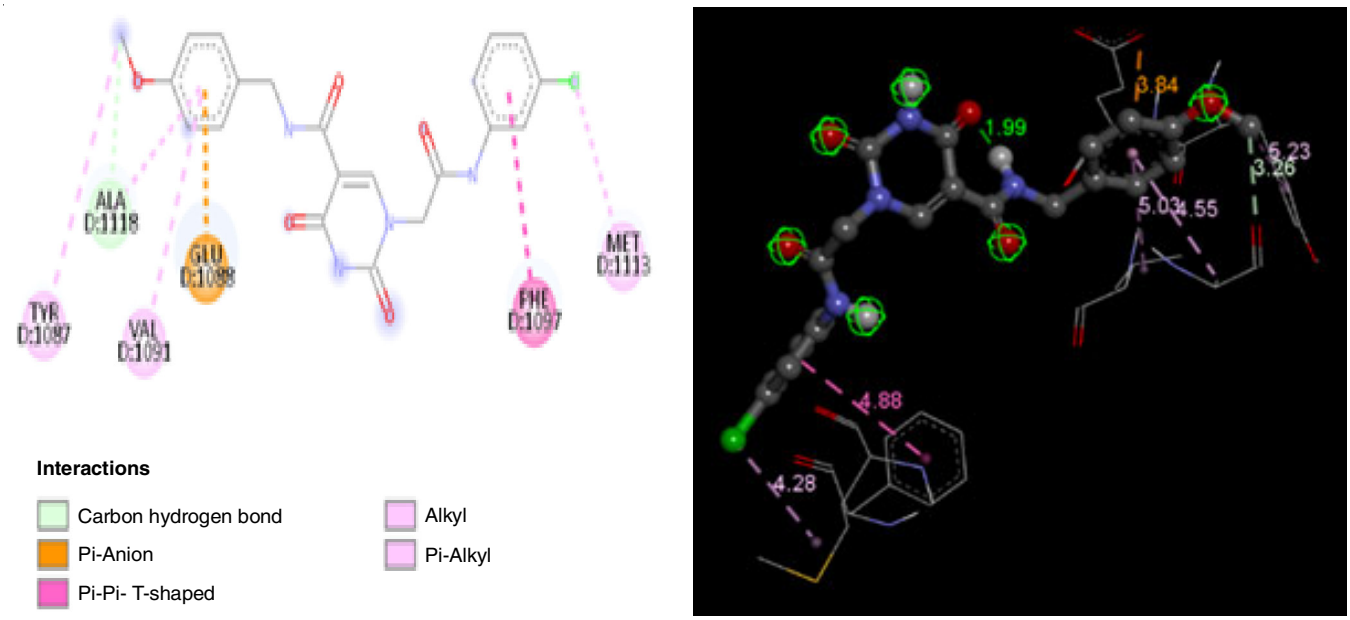


Fig. 3. Binding packet 2D and 3D diagrams of **6e** with DNA gyrase

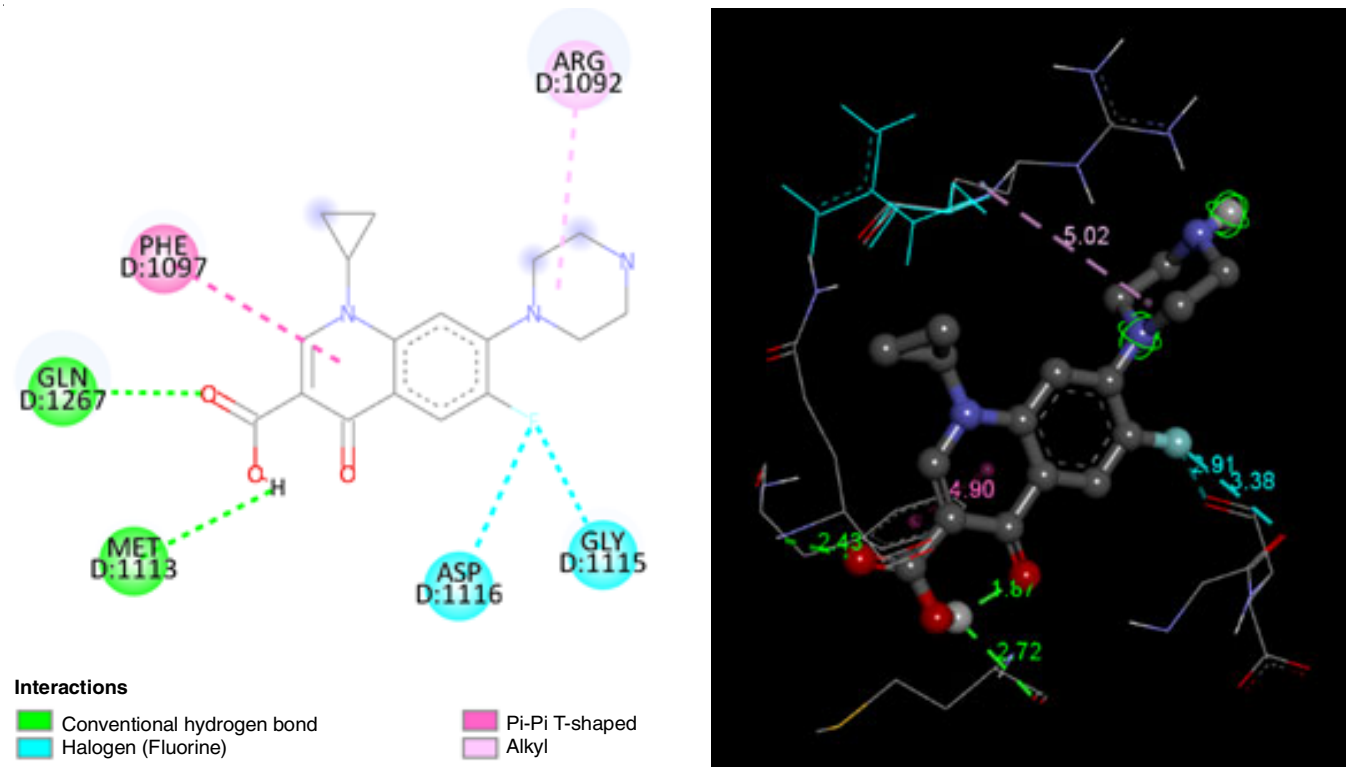


Fig. 4. Binding packet 2D and 3D diagrams of ciprofloxacin with DNA gyrase

TABLE-4
In silico CALCULATED PHYSICO-CHEMICAL PROPERTIES OF **6a-f**

Compound	MW	HBD	HBA	Log P	NRB	PSA	Violations
6a	388.424	3	6	0.3899	6	161.282	0
6b	414.462	3	6	0.9241	7	173.006	0
6c	404.379	3	8	-1.2356	8	164.192	0
6d	440.431	3	6	0.9307	8	181.409	0
6e	442.859	3	6	1.7673	7	181.182	0
6f	452.467	3	7	0.8002	9	188.722	0

TABLE-5
PHARMACOKINETIC PROFILE AND TOXICITY PREDICTION OF **6a-f**

Parameter	**6a**	**6b**	**6c**	**6d**	**6e**	**6f**
Absorption						
Water solubility (log mol/L)	-3.618	-3.471	-2.972	-3.643	-3.822	-3.629
Caco-2 permeability (log Papp, cm/s)	0.012	0.053	0.241	0.222	0.296	0.213
HIA (%)	59.483	65.375	45.266	62.395	67.818	60.488
Skin permeability (log Kp) (cm/s)	-2.879	-2.813	-2.767	-2.763	-2.748	-2.759
Distribution						
VDSs (human) (log L/kg)	0.457	-0.565	-0.963	-0.734	-0.752	-0.652
BBB permeability (log BB)	-1.542	-1.662	-1.887	-1.782	-1.806	-1.776
CNS permeability (log PS)	-3.32	-3.901	-4.066	-3.661	-3.066	-3.727
Metabolism						
CYP2D6	Yes	Yes	No	Yes	Yes	Yes
CYP3A4	Yes	Yes	Yes	Yes	Yes	Yes
Excretion						
Total clearance	0.72	1.074	0.361	0.296	-0.427	0.426
Renal OCT2 substrate	No	No	No	No	No	No
hERG I inhibitor	No	No	No	No	No	No
hERG II inhibitor	Yes	Yes	No	Yes	Yes	Yes
Toxicity						
AMES test	No	No	No	No	No	No
Oral rat acute toxicity (LD_{50}, mol/kg)	2.201	2.586	2.182	2.666	2.614	2.663

antibacterial target-PDB: 6z1a and compounds **6b**, **6c** and **6e** were examined with Autodock. Compound **6e** revealed better inhibition against DNA gyrase protein than compounds **6b** and **6c**. The existence of an electron-withdrawing chloro substitution on the ring might have played a vital role in the high docking scores for compound **6e** (8.1 kcal/mol). Thus, the *in vitro* antibacterial activity and *in silico* docking studies can be correlated. The *in silico* ADMET attributes predicted that the synthesized targets have good solubility, absorption and non-toxic properties.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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