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Original Article

Design, Synthesis, Characterization, and Study of *in vitro* Antioxidant Activity of Some Substituted Biginelli Derivatives

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ABSTRACT

The Biginelli class of 3,4-dihydropyrimidinone (DHPM) or its thione (DHPMT) derivatives can act as an exciting pharmacophoric moiety, which in turn has attracted the extensive attention of medicinal chemists in the last few decades. Similarly, imidazolidin-4-one also occupies an inevitable place in drug discovery chemistry. Despite numerous diverse pharmacologic effects ascribed to these two derivatives together being reported, there are few reports on the antioxidant evaluation of the Biginelli class of pyrimidinone derivatives annexed to imidazolidinone by an amide bond. In this study, the synthesis of 20 novel 3,4-dihydropyrimidinones with imidazolidin-4-one derivatives is described. The physical characteristics of the synthesized 3,4-DHPM and DHPMT derivatives were determined and analytically characterized by various spectral tools like FT-IR, Lc-Ms/Ms, and proton and carbon NMR. The scavenging radical potential for the synthesized 20 novel Biginelli derivatives was assessed using the DPPH assay. The results indicated that all the tested compounds had good to excellent antioxidant potency in comparison to the standard drug ascorbic acid. The synthesized compounds **1**, **6**, **8**, **9**, **14**, **15**, **16**, **17**, **18**, and **19** showed a good degree of scavenging potency. Interestingly, remarkable activity was observed with compounds **16**, **17**, and **19**. The present study reveals that not only the compounds with an OH group can exhibit a high degree of scavenging potency, but other groups like -OCH_3, –CH_3, and -Cl can also exhibit activity effectively. In addition, the radical scavenging potential for the studied compounds could be due to either the presence of more than one labile hydrogen atom attached to a nitrogen atom or the conjugated system, i.e. the 3,4-dihydropyrimidinone ring attached to an amide linkage, present in these compounds, which would have further sponsored the compound to get stabilized when these compounds become radicals by donating an electron to ROS.

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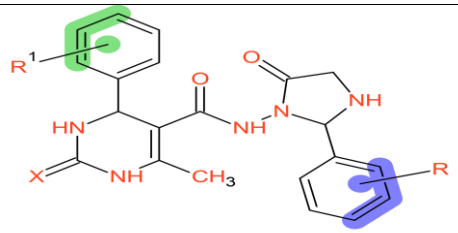
GRAPHICAL ABSTRACT



1 - X=O, R=OH (p), R^1=H
6 - X=S, R=Cl (m), R^1=OCH_3(m) OH(p)
8 - X=S, R=Cl (m), R^1=Cl(o)
9 - X=O, R=H, R^1=Cl(m)
14- X=O,R=NO_2(o), R^1=Cl(p)
15 - X=O, R=OH(o), R^1=NO_2(o)
16 - X=O, R=H, R^1=CH_3(p)
17 - X=O, R=OCH_3(p), R^1=CH_3(p)
18 - X=O, R=OH(o), R^1=CH_3(p)
19 -X=O, R=Cl(p), R^1=CH_3(p)

Introduction

Pyrimidine is chemically a 1,3-diazines, contributing as an imperative pharmacophore in medicinal chemistry and also one of the significant heterocyclic rings that occur extensively in the biological system [1]. 3,4-Dihydropyrimidin-2(1H)-one (DHPM) or thione (DHPMT) are otherwise called Biginelli compounds derived by the cyclic nucleophilic condensation reaction of aromatic aldehydes with urea and β-keto ester [2]. Biginelli compounds occupy a substantial place in medicinal chemistry, as these compounds demonstrate versatile biological activities including antitumor, anti-inflammatory, antihypertensive, antibacterial, antidiabetic, antimalarial, antiviral activities, etc. Besides pyrimidine, imidazole is another widely studied pharmacophore and explored for its inordinate spectrum of biological activities [3].

Free radicals (FRs) are oxidants in nature and unstable entities with an unpaired electron in their atomic orbitals that are generated through a number of metabolic oxidation reactions in living organisms [4]. They play a pivotal role in oxidative stress, mitochondrial dysfunction, protein aggregation, and are followed by silent-chronic inflammation, which involves them in the pathogenesis of numerous diseases and disorders such as cancer, hyperthyroidism, atherosclerosis, neurodegenerative diseases, diabetes, Alzheimer's, Parkinson's, and other diseases related to the aging progressive process [5, 6]. FRs, like the superoxide type of reactive oxygen species (ROS), can trigger a chain initiation reaction in microseconds that leads to oxidative stress and is followed by programmed apoptosis to cause cell death and necrosis, leading to the mass production of FRs in living cells [7]. The human body has a natural inbuild antioxidative mechanism to combat FRs and the generated FRs level in the body is elevated than the inbuild antioxidant system resulting in oxidative stress [8]. Thus, the key role played by FRs has attracted the attention of the scientific community to use antioxidants as they have the ability to scavenge free radicals in preventing and treating various illnesses [6]. Due to increased exposure to radiation and lifestyle changes, nowadays there are overproduction of free radicals in the body [9]. Hence, it is essential to find out potent and safe antioxidant molecules. This accounts for continuing effort and curious in exploring and developing novel antioxidants.

Despite the widespread studies on the pharmacology of Biginelli derivatives were done and reported, there are few reports registered on their antioxidant efficiency [10]. In 2006, Stefani *et al*. synthesized novel ester derivatives of Biginelli dihydropyrimidines consisting of either an unsubstituted or nitro substituted aromatic ring at the C_4 position of the dihydropyrimidine and reported that the synthesised compounds possessed mild to moderate antioxidant activity against lipid peroxidation using DCHF-DA and the thiol-peroxidase assay, displayed in Figure 1 [11]. Another study in the same year was reported by Magerramov *et al*. on the antioxidant potency of the synthesised ester derivatives of Biginelli pyrimidines substituted at the C_4 position with various groups such as methyl, phenyl, naphthyl, hydroxy phenyl, and hydroxy bromo phenyl,

depicted in Figure 2 and these compounds were found to be potent in terminating free radical chain reactions by reacting with cumyl peroxyl radicals [12]. Biginelli dihydropyrimidines adducted with quinoline derivatives; namely, hexahydropyrimido quinoline-2,5-diones and 2-thioxohexahydropyrimido quinoline-5-ones, Figure 3 were synthesized by Lhassane Ismaili *et al*. and evaluated their antioxidant potential in two ways; namely, DPPH and hydroxyl radical scavenging. The study revealed that the thio-adducts showed better activity than the *oxo*-adducts [13].

A series of Biginelli adducts synthesised and studied by de Vasconcelos *et al.* revealed that the *thio*-adduct of Biginelli compounds exhibited better scavenging potency towards the DPPH radical than its *oxo*-Biginelli adduct, and also shown activity against lipid peroxidation at similar extents [14]. Mansouri *et al*. have synthesized some novel methyl, ethyl, isopropyl, tert-butyl, and benzyl esters of 6-methyl-2-thioxo-1,2,3,4-tetrahydropyrimidine -5-carboxylate with a five-membered heterocyclic ring furan at the C_4 position of the pyrimidine ring and determined the antioxidant activity of those reported compounds. Among the reported compounds, the isopropyl substituted adduct has shown the most potency in reducing the DPPH radical, given in Figure 4 [15].

A series of ethyl ester derivatives of Biginelli adducts depicted in Figure 5, were prepared by da Silva *et al*. and compared the scavenging ability of *oxo* and *thio*-derivatives against RNS and ROS radicals. It was reported that, comparatively, *thio*-derivatives had shown promising RNS scavengers [16].

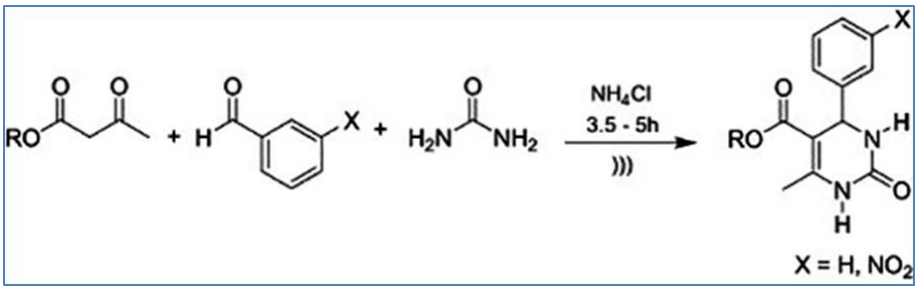


Figure 1: Novel ester derivatives of Biginelli dihydropyrimidines

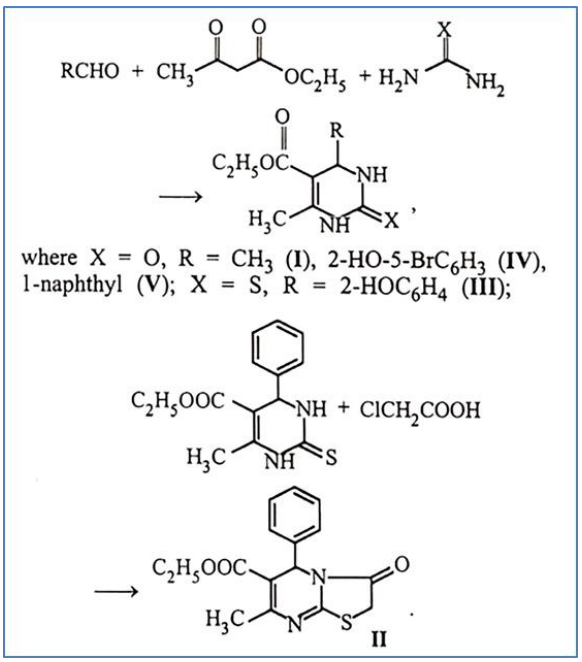


Figure 2: Ester derivatives of Biginelli dihydropyrimidines substituted at the C_4 position with various groups

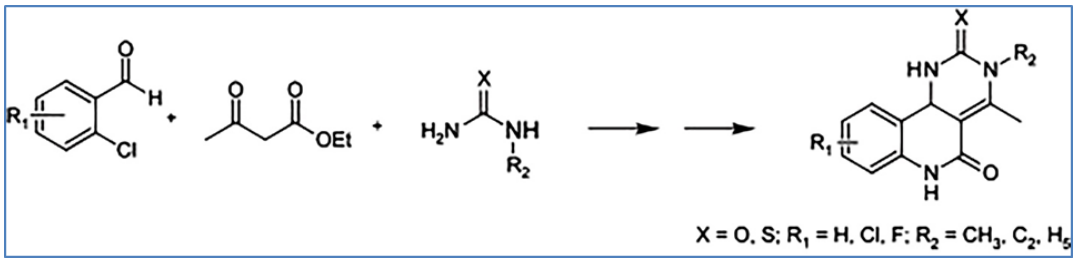


Figure 3: Biginelli dihydropyrimidines adducted with quinoline derivatives

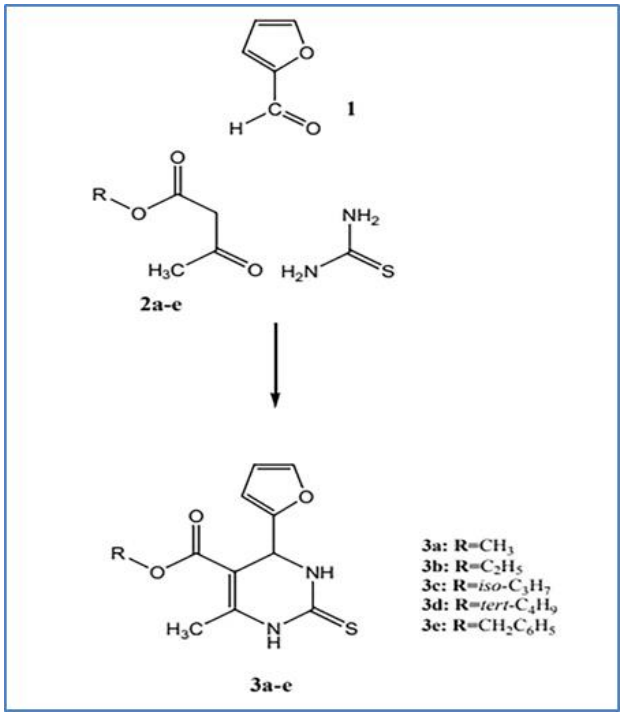


Figure 4: Novel esters derivatives of 6-methyl-2-thioxo-1,2,3,4-tetrahydropyrimidine -5- carboxylate with a five-membered heterocyclic ring furan

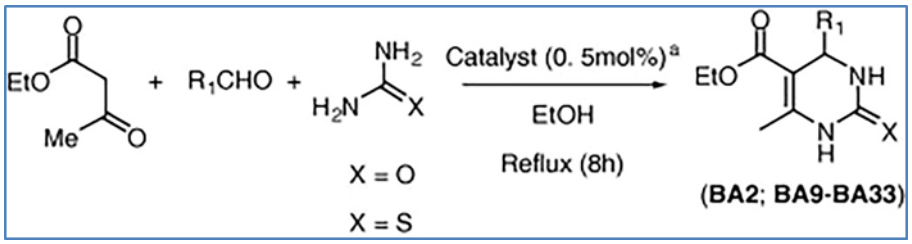
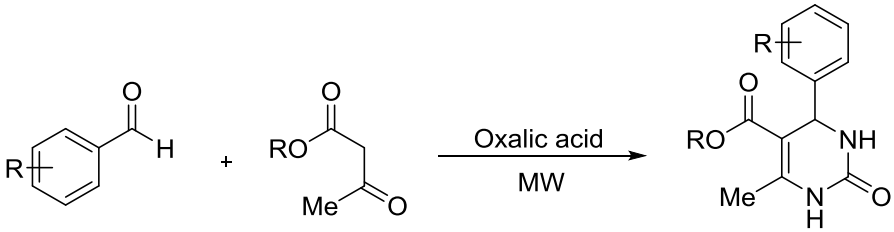


Figure 5: Ethyl ester derivatives of Biginelli adducts



R1=H, 4-NO2, 3-NO2, 4-OMe, 4-OH, 3-OMe, 4-OH
R2= Et, Me

Figure 6: Methyl ester derivatives of *oxo*-Biginelli dihydropyrimidines

A study described by Gangwar *et al.* about the synthesis of methyl ester derivatives of *oxo*-Biginelli dihydropyrimidines and their scavenging activity. It was observed that Biginelli adducts made from hydroxy aromatic aldehydes had higher antioxidant properties when evaluated by DPPH radicals, reducing power, and Fe^{2+} chelating methods than from other aromatic aldehydes, as displayed in Figure 6 [17]. A novel amide-derived Biginelli 3,4-dihydropyrimidine, indicated in Figure 7, was prepared using dihydroxy aromatic aldehydes by Shanmugam *et al*. and evaluated for its antioxidant activity by both DPPH and ABTS assay methods. The results showed that the presence of two hydroxyl groups on the phenyl moiety attached to the 3,4-dihydropyrimidine ring possessed good radical scavenging ability [18].

Malek R. *et al.* synthesized benzyl piperidine, N-substituted *oxo*- and *thio*-Biginelli derivatives, shown in Figure 8 and evaluated their oxygen radical absorbance capacity by the ORAC-FL assay; the synthesised compounds showed a good degree of antioxidant activity [19]. A series of amides of phthalyl and naphthyl-derived Biginelli compounds were synthesised and studied by Totawar *et al*. for their hydrogen peroxide scavenging activity. The synthesized adducts had shown moderate potency, and the structures are demonstrated in Figures 9 and 10 [20].

In this present study, substituted imidazolidinone linked with Biginelli compounds through amide bond were designed as derivatives of Biginelli [21]. A library of 200 ligands was designed and created and from which 20 compounds were synthesized after screening them for their anticancer potency by molecular docking. The general structure of DHPMs and DHPMs / Biginelli derivatives is given in Figure 11. The synthesized Biginelli derivatives were characterized by various spectroscopical studies and evaluated for their radical scavenging antioxidant potency by DPPH assay [22].

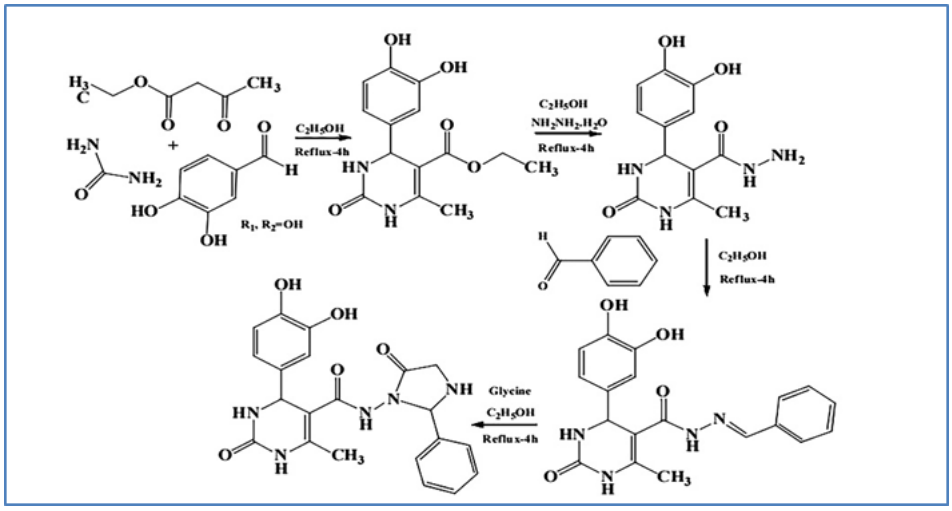


Figure 7: Novel amide-derived Biginelli 3,4-dihydropyrimidine

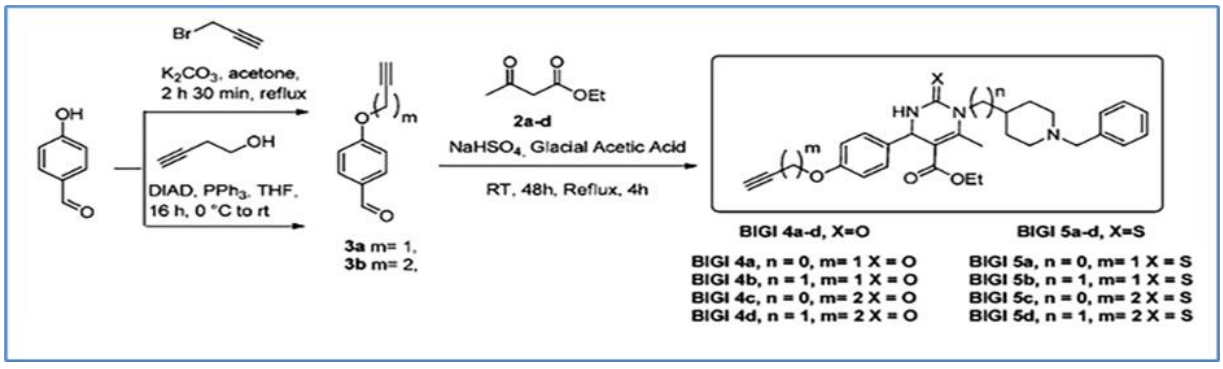


Figure 8: Benzyl piperidine-N-substituted *oxo*- and *thio*-Biginelli derivatives

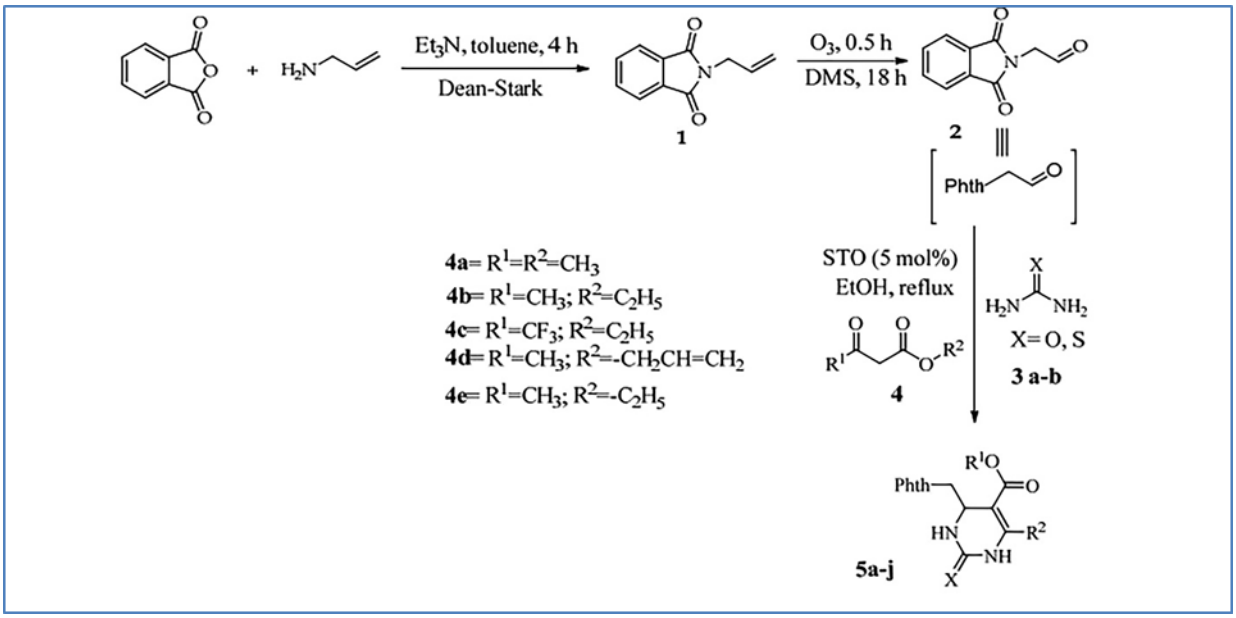


Figure 9: Phthalyl derived Biginelli compounds

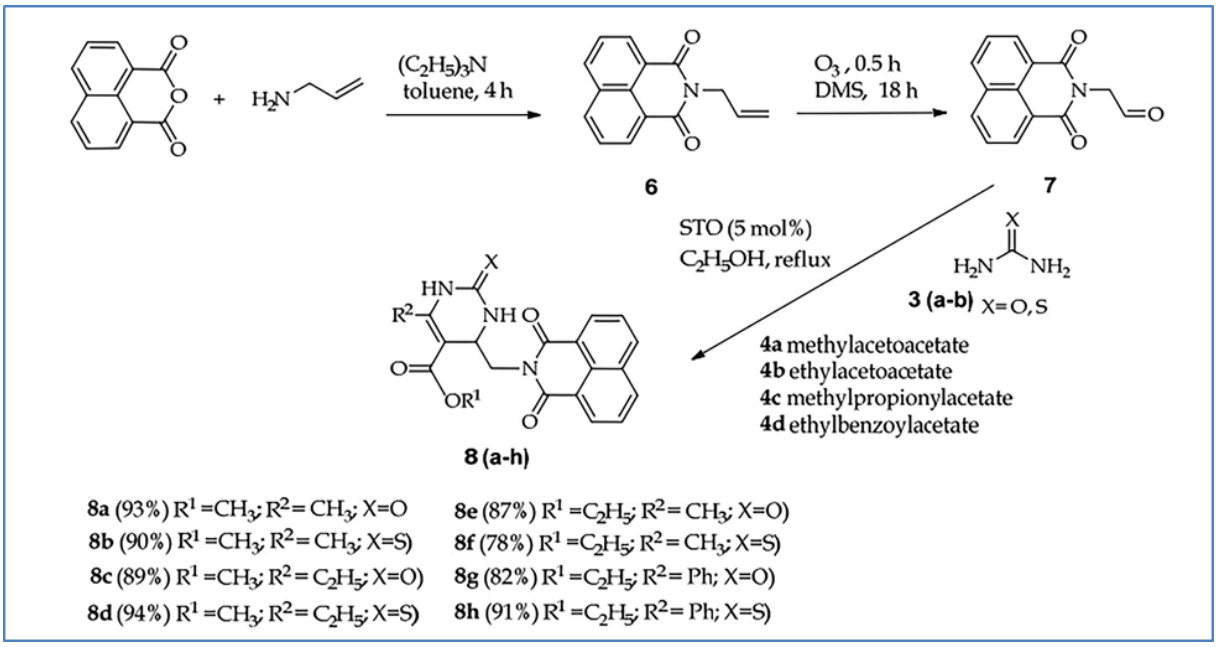


Figure 10: Naphthyl derived Biginelli compounds

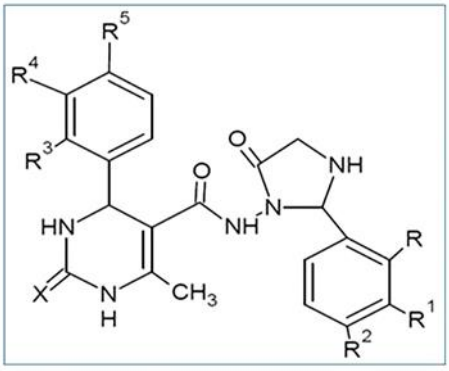


Figure 11: General structure of Biginelli derivatives

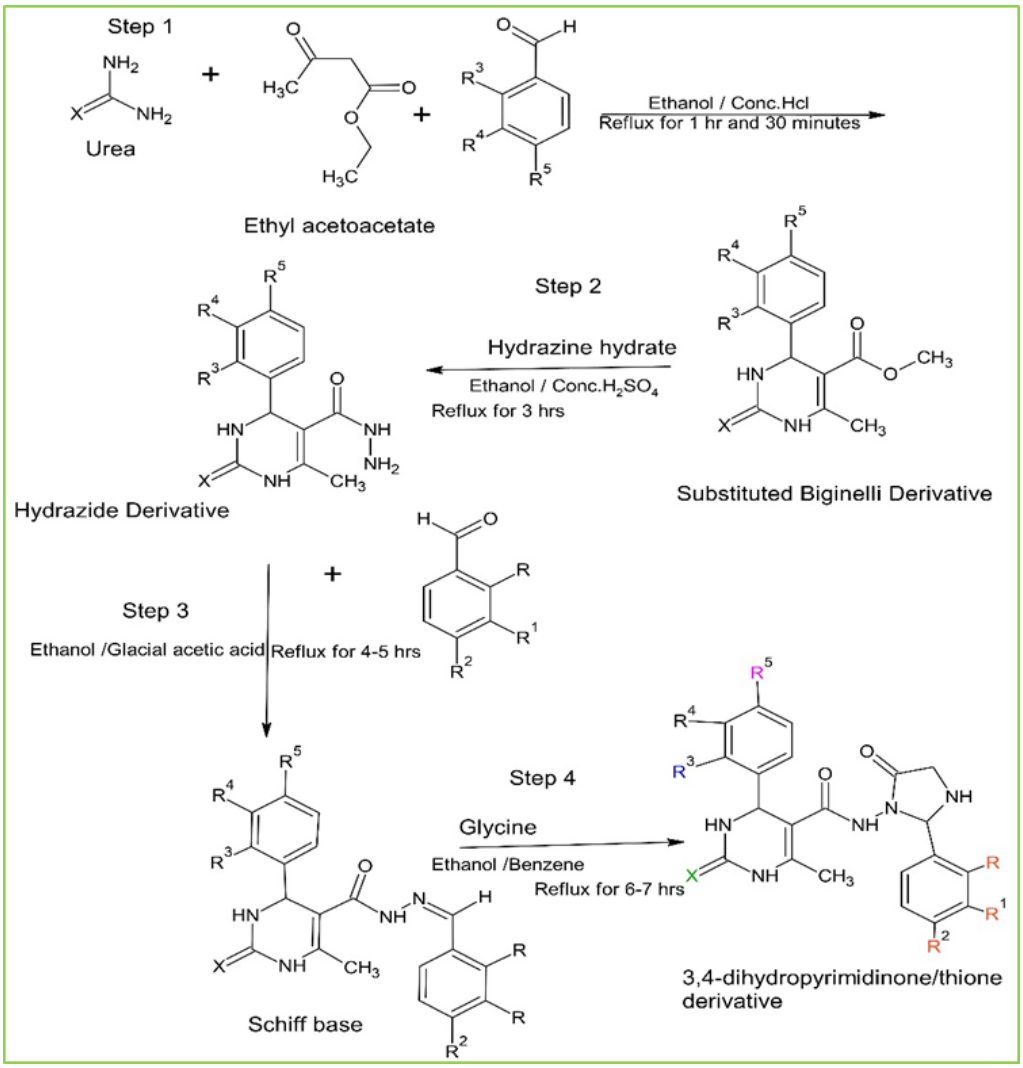


Figure 12: Scheme for the synthesis of Biginelli derivatives

Material and methods

All the chemical and biological reactions were carried out using oven-dried Borosil glassware. The chemicals used for all the studies were of analytical grade and obtained from Merck (India) Ltd., Sigma-Aldrich (India), and Loba Chemie (India). The melting point was recorded using the Veego VMP-1 melting point apparatus by the open glass capillary tube method, and the values are uncorrected. The spectral analysis was done using different techniques, such as Perkin-Elmer to study FT-IR spectra, Bruker DRX-300 (300 MHz FT-NMR) to obtain ^{1}H-NMR (DMSO and TMS as solvents and internal standards, respectively), Bruker for ^{13}C-NMR (DMSO as solvent), Shimadzu for Lc-Ms/Ms for the synthesized compounds, and EuroVector EuroEA3000 CHNS-O Elemental Analyser for the determination of mass fraction of carbon, hydrogen, nitrogen, oxygen, and sulphur in the synthesized compounds.

Synthesis of (3,4-DHPM or DHPMT) or Biginelli derivatives from aromatic aldehydes [27]

The synthesis of 3,4-DHPM and DHPMT derivatives (**1-20**) was performed by the one-pot method, as per the scheme shown in Figure 12 [27]. It is a four-step reaction in which the Biginelli compound was synthesized from an aromatic aldehyde (step1), followed by the conversion of the step 1 product into its hydrazide derivatives using hydrazine hydrate (step 2), and then the resultant compound from step 2 was treated with an aromatic aldehyde to convert it into its Schiff base (step 3) [28]. Finally, 3,4-dihydropyrimidinone (its thione analog) or Biginelli derivatives were obtained by treating

the Schiff base with the amino acid glycine (step 4) [29].

Step 1

Aromatic aldehydes (0.1 *mol*) and ethyl acetoacetate (β-keto ester) (0.1 *mol*) were placed in a 500 ml round-bottom flask and dissolved in 25 ml of ethyl alcohol, which contained 0.15 *mol* of urea or thiourea and 3-4 drops of concentrated hydrochloric acid [30]. This was refluxed for one hour and thirty minutes on an electric water bath. After cooling to room temperature, the liquid mixture was poured into a 500 ml beaker with 100-150 ml of ice-cold water while being shaken intermittently. Finally, the mixture was kept overnight at room temperature, and then it was subjected to filtering and drying to obtain the Biginelli compound. Recrystallization was done using alcohol, and the product was confirmed by TLC.

Step 2

The synthesized Biginelli compound (0.1 *mol*) was transferred to a 500 ml round-bottom flask, added to hydrazine hydrate (0.1 *mol),* and dissolved with 20 ml of ethyl alcohol and 3-4 drops of concentrated sulfuric acid. It was then refluxed for about three hours on an electric water bath, followed by evaporation to obtain the product. Finally, it was recrystallized using alcohol and confirmed by TLC.

Step 3

The synthesized carbohydrazide derivative (0.01 *mol*) and aromatic aldehyde (0.01 *mol*) were placed in a round-bottom flask containing 20 ml of ethyl alcohol and 5 ml of glacial acetic acid. This mixture was refluxed for about 4-5 hours on an electric water bath and cooled, and then it was transferred into a 500-ml beaker with 100-150 ml of ice-cold water while stirring. Later, it was filtered and dried to obtain the product. Recrystallization was done using alcohol, and the product was confirmed by TLC.

Step 4

The synthesized Schiff base (0.01 *mol*) from the above step and glycine (0.01 *mol*) were dissolved in a mixture of benzene and ethyl alcohol placed in a 500 ml RBF. It was refluxed for about 6-7 hours on an electric water bath. After cooling the reaction mixture, it was transferred to a 500 ml beaker with 100-150 ml of ice-cold water, stirring constantly. The product was obtained after it was filtered and dried. The recrystallization was done using alcohol, and the TLC plates were used to confirm the product. The physical characterization was performed for the synthesized compounds, and the structure of the compounds was studied by different spectral interpretation tools such as FT-IR, Lc-Ms/Ms, ^{1}H-NMR, and ^{13}C- NMR.

Characterization of Biginelli derivatives

Compound **6** (2-(3-chlorophenyl)-3-(((4-(4-hydroxy-3-methoxyphenyl)-6-methyl-2-thioxo-1,2,3,4-tetrahydropyrimidin-5-yl) carbonyl) amino) imidazolidin-4-one)

$C_{22}H_{22}ClN_5O_4S$, orange coloured product with Mol. weight 487.96, Yield: 66%, M.P.: 291-293 °C, R_f Value: 0.93, FTIR (ATR, cm-^{1}): 3318.69 (C aro -OH stret), 2192.74 (=C-C=O stret),1596.78 (C=C alipha stret),1594.90 (C=C, alipha stret), 1503.60 (C=C alipha stret), 1462.37 (C-N stret), 1109.38 (C-O- stret), 1035.89 (C-O- stret), 933.74(C=C bend), 805.84 (C=C bend), 759.18 (C-Cl stret), ^{1}H-NMR (400MHz, DMSO-d_6, δ ppm): 1.17 (s, 3H, CH_3), 3.40, 3.90 (s, 3H, OCH_3), 4.48 (s, 2H, CH_2), 6.17, 6.74, 6.96 (m, 7H, C aro -H), 7.35, 7.22 (s, 1H, O=C-NH), 9.70 (s, 1H, NH), 10.25,10.64 (s, 1H, OH), ^{13}C-NMR (101MHz, DMSO-d_6, δppm): 19.03 (2^{o} carbon-CH_3), 44.85 (C-Cl), 47.42 (N-CH_2-R), 53.74, (O-CH_3), 59.16 (CH_2 in imidazolidinone ring), 80.46 (C-O-), 126.08, 127.51, 129.23(C in aromatic ring), 132.09 (C-phenyl ring), 159.65 (C-OH), 169.67, 175.38, 179.98 (O=C-NH), m/z: 487.40 (100.0%), Calculated : C 53.93, H 4.94, Cl 7.24, N 14.29, O 13.06, S, 6.54, and Observed: C 52.86, H 4.82, Cl 7.56, N 14.32, O 13.12, S, 6.72.

Compound **9** (4-(3-chlorophenyl)-6-methyl-5-((5-oxo-2-phenylimidazolidin-1-yl) carbamoyl)-3,4-dihydropyrimidin-2(1H)-one)

$C_{21}H_{20}$ Cl N_5O_3, red coloured product with Mol. weight 425.87, Yield: 76%, m.p 247-249 °C, R_f Value: 0.91, FTIR (ATR, cm-^{1}): 3348 (N-H stret),

3060.6 (C aro-H stret), 2885 (C-H stret), 2198.3 (=C-C=O stret), 1628.8 (C=O stret in amide), 1585 (C=C alipha stret), 819.76 (C=C bend),746.24 (C-Cl stret), ^{1}H-NMR (400 MHz, DMSO-d_6, δppm): 1.89 (s, 3H, CH_3), 4.05 (s, 2H, CH_2), 5.37, 6.09 (m, 9H, C aro -H), 7.39, 7.44, 7.48 (s, 1H, O=C-NH), 7.80, 7.87 (s, 1H, NH), ^{13}C NMR (126 MHz, DMSO-d_6, δppm): 18.59 (2^o carbon-CH_3), 49.62 (C-Cl), 64.87 (CH_2 in imidazolidinone ring), 67.61 (-C-NH-), 75.19 (C-O-), 119.01, 119.40 (-CH), 123.71, 124.45, 128.02, 129.99 (C in aromatic ring), 134.50,134.70 (C-phenyl ring), 155.97 (C=O), 170.31, 179.24 (O=C-NH), m/z: 425.00 (100.0%), 428.90 (32.0%), Calculated: C 59.23, H 4.73, Cl 8.32, N 16.45, O 11.27, and Observed: C 60.14, H 4.56, Cl 7.89, N 16.58, O 12.01.

Compound **16** (6-methyl-4-(4-methylphenyl)-5-((5-oxo-2-phenylimidazolidin-1-yl) carbamoyl)-3,4-dihydropyrimidin-2(1H)-one)

$C_{22}H_{23}N_5O_3$, dark orange coloured product with Mol. weight 405.45, Yield: 55%, M.P.: 147-149 °C, R_f Value: 0.98, FTIR (ATR, cm^{-1}): 3360.11 (N-H stret), 2905.76 (C aro-H stret), 2400.94 (O=C-N stret), 1735.99 (C=O stret), 1669.46 (C=O stret in amide), 1647.26 (C=O stret in amide), 1546.0 (C=C aliphatic stret), 1429.30 (C-N stret), 1278.85 (C-O- stret), 1119.71 (C-O- stret), 991.47 (C=C bend), 846.79 (C-H bend), ^{1}H NMR (500MHz, DMSO-d_6, δppm): 2.27. 2.50 (s, 3H, CH_3), 3.09 (s, 2H, CH_2), 5.40, 6.02 (m, 9H, C aro -H), 7.18, 7.39, 7.80, 7.87 (s, 1H, O=C-NH), 9.67 (s, 1H, NH), ^{13}C NMR (126 MHz, DMSO-d_6, δppm): 17.17, 20.08 (2^o carbon-CH_3), 64.86 (Ar-CH_3), 108.40(C=C in heterocyclic ring) 119.01(-CH), 124.45, 128.03 (C in aromatic ring), 134.50 (C-phenyl ring), 170.30, 172.98 (O=C-NH), m/z: 405 (100.0%), 408 (23.8%), 407.19 (2.7%), 406.18 (1.8%), and Calculated: C 65.17, H 5.72, N 17.27, and O 11.84, and Observed: C 65.44, H 5.48, N 17.56, O 11.76. The Lc-Ms/Ms spectra of compound **16** are given in Figure 13 and other spectra are given in the supporting information.

Compound **17** (5-((2-(4-methoxyphenyl)-5-oxoimidazolidin-1-yl) carbamoyl)-6-methyl-4-(4-methylphenyl)-3,4-dihydropyrimidin-2(1*H*)-one) $C_{23}H_{25}N_5O_4$, orange yellow coloured product with Mol. weight 435.48, Yield: 53%, M.P.: 141-143 °C, R_f Value: 0.99, FTIR (ATR, cm^{-1}): 3359.14, 2878.85 (N-H stret), 2743.00 (C aro-H stret), 1737.92,1669.45 (O=C-N stret), 1647.26 (C=O stret in amide), 1518.03 (C=C stret), 1279.81 (C-O- stret), 991.44 (C=C bend), 845.81 (C-H bend), ^{1}H-NMR (500 MHz, DMSO-d_6, δppm): 2.27, 2.50 (s, 3H, CH_3), 3.09, 3.33 (s, 3H, OCH_3), 5.40, 6.02 (m, 8H, C aro -H), 7.18, 7.39, 7.87 (s, 1H, O=C-NH), 9.67 (s, 1H, NH), ^{13}C-NMR (126 MHz, DMSO-d_6, δppm): 17.17, 20.08 (2^o carbon-CH_3), 53.05, 54.76 (Ar-CH_3), 108.40 (C=C in heterocyclic ring), 119.01(-CH), 127.03, 128.03, 130.00 (C in aromatic ring), 134.50 (C-phenyl ring), 170.30, 172.98 (O=C-NH), m/z: 437 (100.0%), 335.9 (24.9%), 357.05 (2.7%), 329.00 (1.8%), 217.90 (2.7%), Calculated: C 63.44, H 5.79, N 16.08, O 14.7, and Observed: C 63.8, H 5.9, N 15.65, O 14.98. The Lc-Ms/Ms spectra of compound **17** are given in Figure 14 and other spectra are given in the supporting information.

Compound **19** (5-((2-(4-chlorophenyl)-5-oxoimidazolidin-1-yl) carbamoyl)-6-methyl-4-(4-methylphenyl)-3,4-dihydropyrimidin-2(1H)-one) $C_{22}H_{22}$ Cl N_5O_3, whitish orange coloured product with Mol. weight 439.89, Yield: 55%, M.P.: 145-148 °C, R_f Value: 0.95, FTIR (ATR, cm^{-1}): 3010.98 (C aro-OH stret), 2916.47 (N-H stret), 2750.58 (C aro-H stret), 2484.04 (O=C-N stret), 1581.68 (C=O stret in amide), 1564.32 (C=C stret), 1456.30 (C-N stret), 1380.04 (C-O- stret), 1128.04 (C-O- stret), 864.14 (C-Cl stret), ^{1}H-NMR (500MHz, DMSO-d_6, δppm): 2.27, 2.50 (s, 3H, CH_3), 5.40, 6.02 (m, 8H, C aro -H), 7.39, 7.87 (s, 1H, O=C-NH), 9.67, 9.86 (s, 1H, NH), ^{13}C-NMR (126 MHz, DMSO-d6, δppm): 22.31, 23.14 (2^o carbon-CH_3), 53.05, 64.68 (Ar-CH_3), 108.40 (C=C in heterocyclic ring), 119.01(-CH), 124.45, 127.03, 128.03, 129.31, 130.00 (C in aromatic ring), 134.50 (C-phenyl ring), 170.30, 172.98 (O=C-NH), m/z: 439.10 (100.0%), 441.10 (32.0%), 440.14 (23.8%), 442.14 (7.6%), 441.15 (2.7%), 440.14 (1.8%), Calculated: C 60.07, H 5.04, Cl 8.06, N 15.92, O 10.91 and Observed: C 59.65, H 5.84, Cl 8.18, N 15.48, and O 11.2. The Lc-Ms/Ms spectra of compound **19** are given in Figure 15 and other spectra are given in the supporting Information.

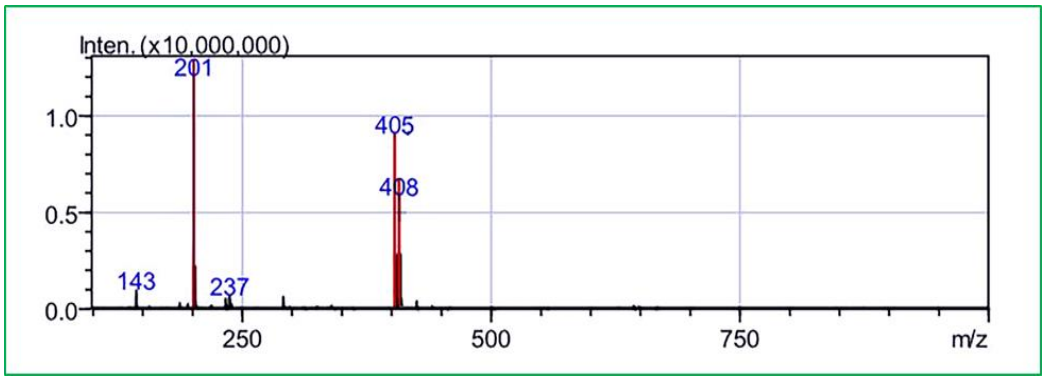


Figure 13: Lc-Ms/Ms spectra of compound **16**

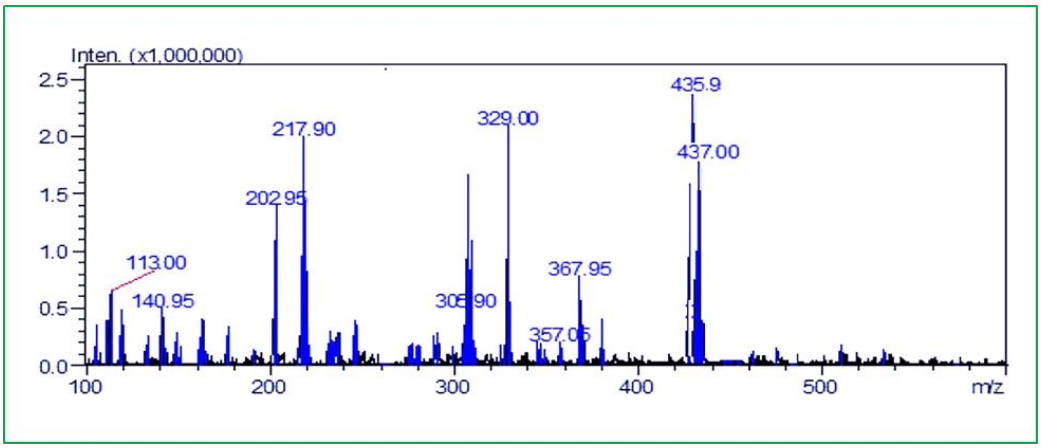


Figure 14: Lc-Ms/Ms spectra of compound **17**

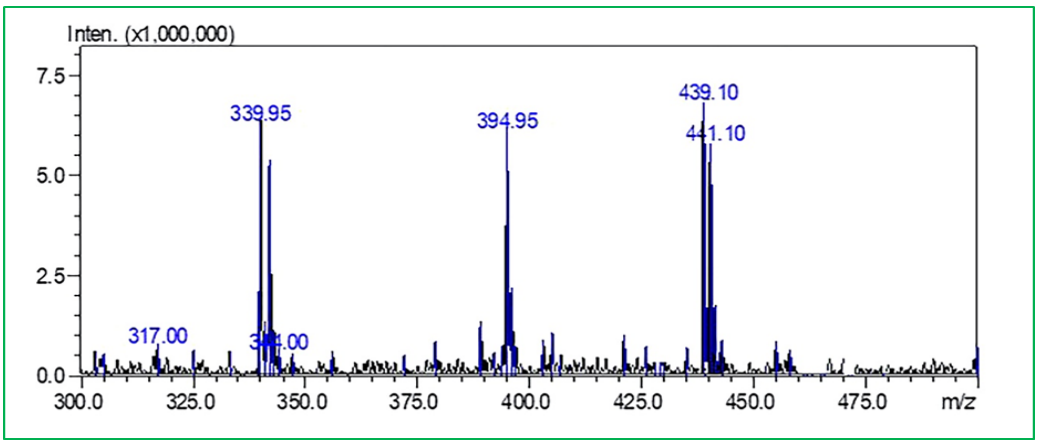


Figure 15: Lc-Ms/Ms spectra of compound **19**

In vitro DPPH radical scavenging capacity

The *in vitro* scavenging capability of the synthesized derivatives was assessed by following the protocols and procedures given in the DSSA assay. It was determined by the ability of the compound to donate either a hydrogen atom or a single electron to the stable free radical 2,2-diphenyl-1-picryl hydrazyl in a model system and thereby scavenge the radical. The stable radical DPPH in ethanol is a purple solution and becomes pale yellow when it gets a single electron from any compound. This chemical reaction occurs after 30 minutes of incubation in the dark, and the intensity of purple color reduction is measured in terms of absorbance at 517 nm. The working solution of the sample compounds (Biginelli derivatives) was prepared using ethanol and DMSO (1:1) in a concentration range of 25, 50, 100, 250, and 500 µg/ml. The

standard drug solution (ascorbic acid, 100 µg/ml) and the control solution (DPPH, 0.1 mM) were also prepared, and then 200 µl of the working sample solution was pipetted out and thoroughly mixed with 1 ml of DPPH solution and 800 µl of Trios-HCl buffer and incubated for 30 minutes. The intensity (in terms of absorbance) of color of the sample solution mixed with DPPH solution was measured at 517 nm against the control DPPH solution. The radical scavenging potency of the compounds was expressed in percentage of inhibition and calculated using the equation:

The percentage radical scavenging activity (% RSA) = ((Absorbance of control-Absorbance of sample) / Absorbance of control) × 100

The antioxidant potency of the compounds is expressed as $IC_{50,}$ (IC_{50}- the minimum concentration of substance that can scavenge 50% of DPPH radical in the DPPH assay).

Results and Discussion

In this study, a variety of substituted aromatic aldehydes, β-keto esters, and carbamide (urea) / thio-carbamide (thiourea) were allowed to react chemically to undergo various nucleophilic addition reactions to obtain the corresponding 3,4-dihydropyrimidinone/thione derivatives via forming Biginelli compound as described in the Scheme Figure 12 [23]. The 20 best-scored ligands in molecular docking (compounds 1-20) from the designed library were synthesized involving four steps and recrystallized, as indicated in Table 1. Analytical TLC plates were used to confirm the product formed. The physical parameters of the synthesized derivatives such as molecular weight, color, and appearance, melting point, yield, and R_f value were noted and are presented in Table 2. As the conventional method of preparation was employed to prepare the compounds, the yield obtained from 20 compounds was comparatively not more than 75%. The solubility of DHPM/DHPMT derivatives was determined and found to be the mixture of organic solvents i.e. ethanol and DMSO in 1:1. The synthesized derivatives of DHPM/DHPMT were analytically characterized using various analytical tools like FT-IR, Lc-Ms/Ms, proton, and carbon NMR.

In-vitro antioxidant activity

The oxidants (FRs) scavenging potency of the synthesized compounds was determined using the DPPH assay [23]. Since this assay is simple, accurate, and sensitive, it is the most commonly employed method to evaluate the antioxidant potential of the compounds [23]. Hence, the scavenging radical activities of test compounds have been determined using this method. As a newly made 2,2-diphenyl-1-picryl hydrazyl (DPPH) solution has a deep purple colour with λ_{max} at 517 nm and any substance or compound has the ability to donate an electron to the DPPH radical is added with DPPH solution, the purple colour intensity decreases with the time that gives rise to a reduction in the absorbance [24]. Usually, a hydrogen atom attached with hetero atoms like NH-, OH-, or SH- groups scavenge DPPH radicals by donating a hydrogen atom to them, and also hetero atoms without hydrogen attached can donate an electron by getting converted into free radicals which is resonance stabilized [25, 26].

Table 3 demonstrates that the potential radical scavenging activity was manifested by all the compounds, primarily compounds such as 6-methyl-4-(4-methyl phenyl) -5-((5-oxo-2-phenyl imidazolidin-1-yl) carbamoyl)-3, 4 - dihydropyrimidin-2 (1H) - one (**16**), 5-((2-(4-chloro phenyl)-5-oxoimidazolidin-1-yl) carbamoyl)-6-methyl-4-(4-methyl phenyl)-3,4-dihydropyrimidin-2(1H)-one (**19**), 5-((2-(4-methoxy phenyl)-5-oxoimidazolidin-1-yl)carbamoyl)-6-methyl-4-(4-methyl phenyl)-3,4-dihydropyrimidin-2(1*H*)-one (**17**) and 4-(4-chloro phenyl)-6-methyl-5-((2-(2-nitro phenyl)-5-oxoimidazolidin-1-yl) carbamoyl)-3,4-dihydropyrimidin-2(1*H*)-one (**14**), as illustrated by the IC_{50} values of these compounds, 64.26, 123.11, 128.37, and 129.55 µg/ml, respectively, and the IC_{50}, which are quite low compared to the standard drug (350.56 µg/ml). Besides the reported compounds, the radical inhibition percentage of the other test compounds showed a concentration-dependent pattern. The top 10 compounds with best scavenging potency are illustrated in Figures 16 and 17.

Table 1: The list of the synthesized compounds

The List of Compounds Synthesized								
Compound #	Ligand #	X	R	R^1	R^2	R^3	R^4	R^5
1	5	O	H	H	OH	H	H	H
2	8	O	H	H	Cl	H	H	H
3	29	O	NO_2	H	H	H	H	OCH_3
4	47	O	H	Cl	H	OH	H	H
5	48	O	H	H	Cl	OH	H	H
6	77	S	H	Cl	H	H	OCH_3	OH
7	83	O	OH	H	H	H	H	OH
8	117	S	H	Cl	H	Cl	H	H
9	121	O	H	H	H	H	Cl	H
10	125	O	H	H	OH	H	Cl	H
11	126	O	Cl	H	H	H	Cl	H
12	127	O	H	Cl	H	H	Cl	H
13	135	S	H	H	OH	H	Cl	H
14	149	O	NO_2	H	H	H	H	Cl
15	163	O	OH	H	H	NO_2	H	H
16	181	O	H	H	H	H	H	CH_3
17	182	O	H	H	OCH_3	H	H	CH_3
18	183	O	OH	H	H	H	H	CH_3
19	188	O	H	H	Cl	H	H	CH_3
20	192	S	H	H	OCH_3	H	H	CH_3

** Ligand # is the No of ligands designed for molecular docking (1-200).*

Compound # is the No of top 20 compounds synthesized from 200 ligands (1-20).

Table 2: Physical parameters of the synthesized compounds

Physical parameters of the synthesized compounds						
Compound #	Ligand #	Molecular Weight	Colour and appearance	% Yield	Melting Point	R_f Value
1	5	407.42	Reddish brown	56	290-292	0.97
2	8	425.86	Light orange	54	186-188	0.91
3	29	466.44	Yellow	54	177-181	0.93
4	47	441.87	Dark yellow	61	176-180	0.97
5	48	441.87	Light brown	63	287-288	0.97
6	77	487.96	Orange	66	291-293	0.93
7	83	423.42	Brownish red	71	280-283	0.96
8	117	476.38	Whitish yellow	68	151-155	0.99
9	121	425.87	Red	76	247-249	0.91
10	125	441.87	Dark red	71	271-273	0.91
11	126	460.31	Yellowish orange	70	181-183	0.88
12	127	460.31	Black	71	183-184	0.89
13	135	457.93	Reddish orange	72	185-187	0.93
14	149	470.87	Dark brown	72	181-185	0.95
15	163	452.42	Brown	60	194-198	0.91
16	181	405.45	Dark orange	55	147-149	0.98
17	182	435.48	Orangish yellow	53	141-143	0.99
18	183	421.5	Light reddish brown	55	151-153	0.97
19	188	439.89	Whitish orange	55	145-148	0.95
20	192	451.54	Dark brownish red	53	167-169	0.9

Table 3: IC_{50} value of the synthesized compounds

Compound #	Ligand #	% RSA					IC_{50} value
		25 µg/ml	50 µg/ml	100 µg/ml	250 µg/ml	500 µg/ml	
1	5	23.46	34.73	48.90	72.01	92.68	153.44
2	8	19.05	23.58	30.66	48.78	65.62	328.59
3	29	20.09	22.76	35.89	61.56	67.83	265.87
4	47	19.40	34.61	46.69	61.32	85.02	189.85
5	48	18.12	24.51	40.19	51.45	72.71	265.03
6	77	24.74	35.54	49.01	70.73	81.53	142.48
7	83	20.44	23.34	32.52	47.50	66.67	225.61
8	117	21.14	22.07	34.49	60.63	68.41	161.26
9	121	20.44	35.19	48.20	59.58	86.41	185.31
10	125	16.72	25.90	40.88	52.15	73.98	226.16
11	126	24.04	35.08	48.90	71.31	92.92	218.95
12	127	19.74	23.46	31.59	48.08	66.09	324.83
13	135	20.56	22.42	35.19	61.09	68.06	274.19
14	149	40.30	44.60	49.94	61.79	70.38	129.55
15	163	19.86	34.84	47.39	60.39	85.71	187.91
16	181	30.20	47.15	63.76	71.54	75.38	64.266
17	182	23.11	48.55	57.49	68.29	80.14	128.37
18	183	29.04	36.47	50.99	58.77	75.73	182.89
19	188	26.60	46.34	54.59	70.38	87.80	123.11
20	192	17.42	25.20	40.53	51.80	73.29	261.83
Std				19.05			350.56

The structure of best 3 compounds **16, 17,** and **19** exhibited a good spectrum of scavenging potency on DPPH assay is shown in Figure 18.

A high degree of activity for the compounds **16**, **19**, and **17** was observed, though groups like -OH were not present. It could be attributed to the presence of electron donating methyl groups on the aromatic rings of these compounds in addition to NH, OCH_3, and Cl. The difference in scavenging potency between **16** (IC_{50} 64.26 µg /ml) and **19** (IC50 123.11 µg /ml), despite the presence of -CH_3 groups in both, may be due to the presence of -Cl group at para position in the compound **19**. However, in general, it has been observed that not only compounds having an OH group alone can exhibit a high degree of inhibition of radicals, but other groups like OCH_3, CH_3, and Cl can have further the potency to scavenge the FRs.

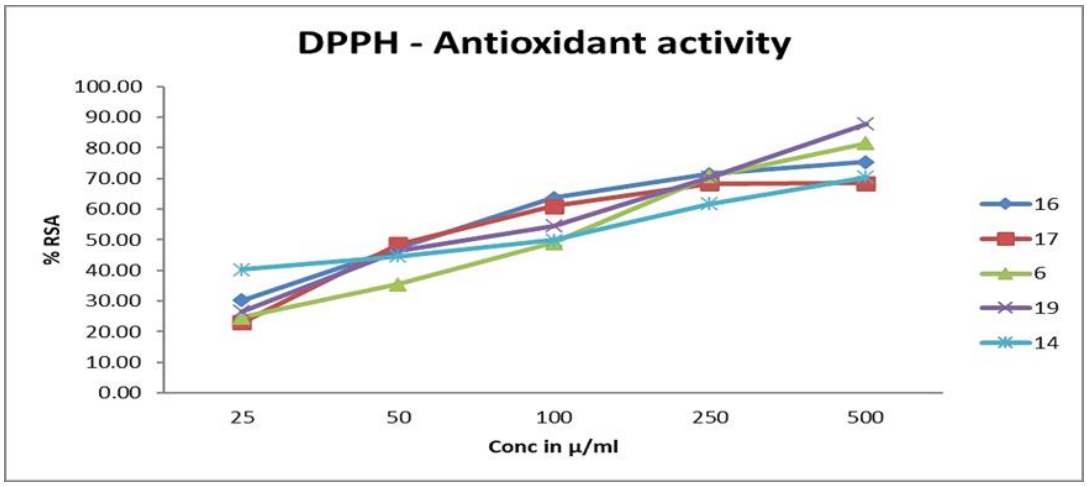


Figure 16. Radical scavenging potency of the Compounds **6**, **14**, **16**, **17**, and **19**

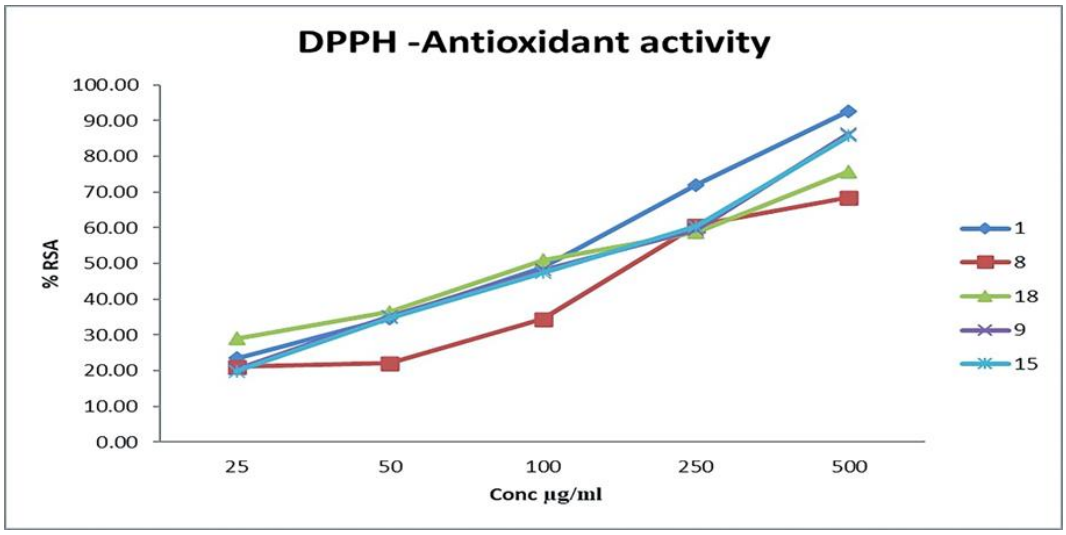


Figure 17. Radical scavenging potency of the compounds **1**, **8**, **9**, **15,** and **18**

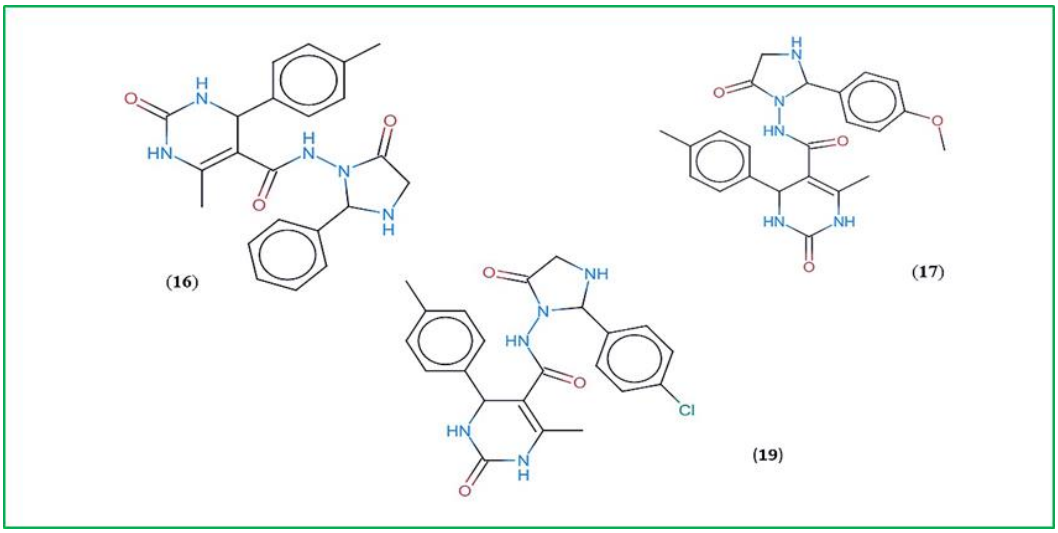


Figure 18. The **s**tructures of the best 3 compounds **16**, **17**, and **19** exhibited a good spectrum of scavenging potency on DPPH assay

Conclusion

A scaffold-based library consisting of 20 Biginelli derivatives was synthesized using substituted aromatic aldehydes with β-keto esters and carbamides (urea/thiourea), followed by the incorporation of hydrazine hydrate and glycine in subsequent steps involving various nucleophilic addition reactions. The physical parameters for the synthesized 3,4-DHPM/DHPMT derivatives such as molecular weight, color, and appearance, melting point, yield and R_f value were determined and reported. The synthesized derivatives of 3,4-DHPM/DHPMT were analytically characterized by various spectral tools like FT-IR, Lc-Ms/Ms, proton, and carbon NMR. The scavenging radical potential of all the test compounds were evaluated using DPPH assay. The results indicated that all the tested compounds were found to exhibit a good to better antioxidant potency in comparison to the standard drug ascorbic acid. The compounds **1**, **6**, **8**, **9**, **14**, **15, 16**, **17**, **18**, and **19** showed good degree of scavenging potency, of these the compounds **16**, **17**, and **19** exhibited a high grade of activity, though they do not have -OH group in their structure shown in Figure 14. The present study revealed that compounds with electron donating groups like $-OCH_3$, $-CH_3$, and -Cl can also participate effectively in scavenging the free radicals like OH group does. Likewise, it revealed

that apart from those electron donating groups on the core structure, presence of both labile hydrogen atom attached with nitrogen atom and the conjugated system in the Biginelli derivatives as well could contribute efficiently in scavenging the free radicals.

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Authors' Contributions

All authors have contributed equally while analyzing the date, drafting, and reviewing the manuscript and agreed to be responsible for all the features of this work.

Conflict of interest

The authors have no conflicts of interest to disclose and declare.

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