Synthesis and Bioactivity of Dihydropyrimidinone Derivatives Containing Phthalimide Moiety

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Abstract **- A simple and precise synthesis method that have been used to synthesis of hybrid compounds containing dihydropyrimidinone and phthalimide moieties. The 2-(4-acetyl-phenyl)-1H-isoindole-1,3 (2H) dione.(III) derivatives were synthesized (Scheme-I) by refluxing phthalic anhydride (I) and para-aminoacetophenone (II) in glacial acetic acid for 2 hr. Newly synthesized 2-(4-acetylphenyl)-1H-isoindole-1,3 (2H)-dione (III), by refluxing with dimethylformamide-dimethylacetal (DMF-DMA) without solvent for 12 h. gives enaminone, 2-{4-[(2*E*)-3-(dimethylamino)prop-2-enoyl]phenyl}-1H-isoindole-1,3 (2H)-dione(IV), The enaminone, 2-{4-[(2*E*)-3-(dimethylamino) prop-2-enoyl]phenyl}-1H-isoindole-1,3 (2H)-dione (IV), used for synthesis of new series of novel Biginelli compounds,5-benzoyl-substituted phenyl-3,4-dihydropyrimidin-2(1H) -one - 1H - isoindole- 1, 3 (2H) -dione (Va-Vb), The compounds (V-a to V-d) obtained were identified using physical and spectroscopic ^{1}HNMR, ^{13}CNMR and Mass) A series of novel dihydropyrimidinone derivatives containing phthalimide moiety were synthesized with good yield, at high purity, and in efficient manner from the enaminone, which was derived from phthalimide by simple and solvent-free method. Compound Va-Vd was tested for antibacterial strain Escherichia coli (E. coli) by Agar well diffusion method. It has been observed that tested compounds Vb and Vc shows good antibacterial activities against Escherichia coli (E.coli) bacterial strains.**

Index Terms **- DHPMs, Biginelli Synthesis, Antibacterial analysis, Escherichia coli (E.coli)**

I.INTRODUCTION

3,4-Dihydropyrimidinone and their sulphur analogue 3,4-Dihydropyrimidinthiones[1] are classified as hetero-cycles compound[2] and containing pyrimidine ring which is containing two nitrogen atoms in the six-member ring. The structure of dihydropyrimidinones and their derivatives (DHPMs) illustrated below: R= aliphatic substituent, aromatic substituent, aroma to aliphatic substituent or heterocyclic substituent. X= O or S. The (DHPMs) have attracted great attention recently in synthetic organic chemistry due to their applications in the field of drug research and pharmacological and therapeutic properties such as antibacterial[3,4], anti-inflammatory[4], antiviral[5], antitumor[6], antimalarial agents[7], hypnotics, anticonvulsant, antithyroid, antihistaminic agents, antibiotics[2] and in addition, 4-aryldihy-dropyrimidines have emerged antihypertensive activity as well as behaving as calcium channelblockers[8,9],αantagonistsandneuropeptideY(NPY)antagonists[10].Synthesisofdihydropyrimidinone and their thioanalogue is increasing tremendously in current years and also synthesized earlier a series of dihydropyrimidinone/thione by three component condensation of urea/thiourea [2]. The simplest and the most straightforward procedure, originally reported by Biginelli in 1893 involve three-component one-pot condensation of an aldehyde, β-ketoester and urea or thiourea [11] in ethanol under strong acidic condensation HCl [12]. Biginelli reaction is acid catalyzed cyclo-condensation reaction of ethyl acetoacetate, benzaldehyde and urea given as under. The three components reaction was carried out by simply heating a mixture in ethanol with a catalytic amount of hydrochloric acid at reflux temperature. The product of this novel one-pot, three components synthesis that precipitated on cooling of the reaction mixture was identified as 3,4-dihydropyrimidin-2*(1H)* one. When the synthesis involves three-component one-pot condensation of an aldehyde, β-ketoester and thiourea will give derivative (Dihydropyrimidinthiones) act as antitumor, fungicidal, bactericidal, anti-inflammatory and antiviral activities[13]. The hybrid compounds containing these two important moieties (dihydropyrimidinone and phthalimide) may have a

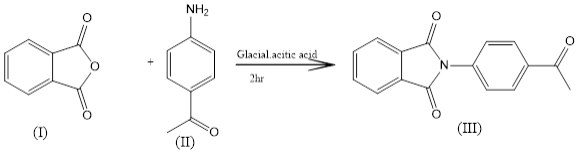
substantial therapeutic potential. In continuation of our work on dihydropyrimidinones, a series of novel phthalimide dihydropyrimidinone hybrids were synthesized by simple method and fully characterized by elemental analysis and spectral data [14,15]. In 1893, the synthesis of functionalized 3, 4-dihydropyrimidin-2(1H)-ones (DHPMs) via three-component condensation reaction of an aromatic aldehyde, urea and ethyl acetoacetate were reported for the first time by P. Biginelli [16]. In the past decades, such Biginelli-type dihydropyrimidones have received a considerable amount of attention due to the interesting pharmacological properties.

II.EXPERIMENTAL METHODS

The physical testing like melting points were determined on a Gallenkamp melting point apparatus. UV, IR, 1H NMR, and GC-MS spectra were recorded on Shimadzu-1800UV Spectrophotometer, Shimadzu8400s FT-IR Spectrophotometer, and 500MHz Brucker Spectrometer and Shimadzu GC-MS QP 2010 GC respectively.

2.1: The dihydropyrimidinone derivatives were synthesized by refluxing phthalic anhydride (I) (0.01 mol) and para-aminoacetophenone (II) (0.01 mol) in glacial acetic acid for (2 hr) reaction mixture was added to the ice-cold water. The white product was precipitated and was filtered by vacuum filtration with repeated washing of cold water. The compound 2-(4-acetyl-phenyl)-1H-isoindole-1, 3(2H) dione. (III) was obtained (Scheme-I), which was recrystalised by ethanol.

Scheme-I Synthesis of 2-(4-acetyl-phenyl)-1H-isoindole-1, 3 (2H) dione. (III)



2.2: A mixture of 2-(4-acetyl-phenyl)-1H-isoindole-1, 3 (2H) dione. (III), (0.02 mol) and dimethyl formamide (DMF) or dimethyl acetal (0.023 mol) was refluxed for 12 hrs. Without solvent on a sand bath. After the completion of reaction, the reaction mixture was cooled to room temperature. Diethyl ether was added to precipitate the reaction mixture and vacuum filtration was performed. The obtained product (Scheme-II) enaminone2-(4-(3-(dimethylamino) prop-2 enoyl) phenyl)-1H-isoindole-1, 3(2H)-dione (IV) was recrystallized from absolute ethanol.

Scheme-II enaminone2-(4-(3-(dimethylamino) prop-2 enoyl) phenyl)-1H-isoindole-1, 3(2H)-dione (IV)

2.3: Synthesis of hybrid compounds containing dihydropyrimidinone and phthalimide moieties. (Va-Vd)

2.3.1. Synthesis of 5-Benzoyl-3nitrophenyl-3-4-dihydropyrimidine-2(1H)-one-1H-isoindole-1,3(2H)-dione. (Va) A mixture of enaminone2-(4-(3-(dimethylamino) prop-2-enoyl) phenyl)-1H-isoindole-1, 3(2H)-dione (IV) (0.01 mol), differently substituted 3-nitro-benzaldehyde (0.01 mol), urea (0.01 mol), and glacial acetic acid (10 mL), was refluxed for 3 hr. The precipitates were obtained by adding ice cold water to the reaction mixture. The products were obtained filtered by vacuum filtration (V-a to V-d). The products were washed several times with water and purified by recrystallization from glacial acetic acid and ethanol mixture.

2.3.2. Synthesis of 5-Benzoyl-3nitrophenyl-3-4-dihydropyrimidine-2(1H)-one-1H-isoindole-1,3(2H)-dione. (Vb) A mixture of enaminone2-(4-(3-(dimethylamino) prop-2-enoyl) phenyl)-1H-isoindole-1, 3(2H)-dione (IV) (0.01 mol), differently substituted 4-nitro-benzaldehyde (0.01 mol), urea (0.01 mol), and glacial acetic acid (10 mL), was refluxed for 3 hr. The precipitates were obtained by adding ice cold water to the reaction mixture. The products were obtained filtered by vacuum filtration (V-a to V-d). The products were washed several times with water and purified by recrystallization from glacial acetic acid and ethanol mixture.

2.3.3. Synthesis of 5-Benzoyl-2,3dimethoxyphenyl-3-4-dihydropyrimidine-2(1H)-one-1H-isoindole-1,3 (2H)-dione. (Vc) A mixture of enaminone2-(4-(3-(dimethylamino) prop-2-enoyl) phenyl)-1H-isoindole-1, 3(2H)-dione (IV) (0.01 mol), 2,3 dimethoxy benzaldehyde (0.01 mol), urea (0.01 mol), and glacial acetic acid (10 mL), was refluxed for 3 hr. The precipitates were obtained by adding ice cold water to

the reaction mixture. The products were obtained filtered by vacuum filtration (V-a to V-d). The products were washed several times with water and purified by recrystallization from glacial acetic acid and ethanol mixture.

2.3.4. Synthesis of 5-Benzoyl-2,3dimethoxyphenyl-3-4-dihydropyrimidine-2(1H)-one-1H-isoindole-1,3(2H)-dione. (Vd), A mixture of enaminone2-(4-(3-(dimethylamino) prop-2-enoyl) phenyl)-1H-isoindole-1, 3(2H)-dione (IV) (0.01 mol), 3,4 dimethoxy benzaldehyde (0.01 mol), urea (0.01 mol), and glacial acetic acid (10 mL), was refluxed for 3 hr. The precipitates were obtained by adding ice cold water to the reaction mixture. The products were obtained filtered by vacuum filtration (V-a to V-d). The products were washed several times with water and purified by recrystallization from glacial acetic acid and ethanol mixture. To obtain compounds (V-a to V-d). The Products obtained were identified using physical and spectroscopic IR, ^{1}HNMR, GC. Mass, UV, techniques. All products were tested for E. coli bacterial strains.

Scheme –III Biginelli Synthesis of Dihydropyrimidinone Derivatives Containing Phthalimide Moiety by (Va-Vd)

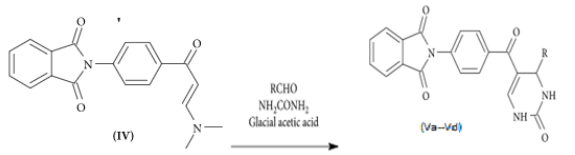


Table-1: Physico- Chemical Characterization of compounds (Va-Vd)

Compound	*R*	% yield	Solvent used for Crystalization	Melting points ^{0}C	Mol.Formula	MS: *m/z*
Va	3-NO_2-C_6H_4	75	Glacial acetic acid and ethanol	239 – 242° C	$C_{25}H_{16}N_4O_6$	468.12
Vb	4-NO_2-C_6H_4	78	Glacial acetic acid and ethanol	176 – 178° C	$C_{25}H_{16}N_4O_6$	468.12
Vc	2,3-(OCH_3)$_2$-C_6H_3	70	Glacial acetic acid and ethanol	191 – 193° C	$C_{27}H_{21}N_3O_6$	483.47
Vd	3,4-(OCH_3)$_2$-C_6H_3	72	Glacial acetic acid and ethanol	151 – 153° C	$C_{27}H_{21}N_3O_6$	483.47

III. RESULTS AND DISCUSSIONS

3.0 Spectral Analysis:

3.1.1.(Va): 5-Benzoyl-3-nitrophenyl-3, 4-dihydropyrimidin-2(1H)-one-1H-isoindole-1, 3(2H)-dione

m.p.: 239–242°C; ^{1}H NMR (500 MHz, DMSO–d_6): δ = 5.64 (1H, s, H-4), 7.22–8.22 (13H, m, Ar-H), 9.60 (1H, s, CONH, D_2O exchg.); ^{13}C NMR (125.76 MHz, DMSO–d_6): δ = 111.6, 121.7, 123.0, 124.0, 127.4, 129.1, 130.7, 131.9, 133.7, 134.6, 135.2, 138.0, 146.5, 148.3, 151.3, 167.2, 191.2; MS: m/z = 468.12

3.1.2. ((Vb): 5-Benzoyl-4-nitrophenyl-3, 4-dihydropyrimidin-2(1H)-one-1H-isoindole-1, 3(2H)-dione

m.p.: 176–178°C; ^{1}H NMR (500 MHz, DMSO–d_6): δ = 5.62 (1H, s, H-4), 7.30–8.42 (13H, m, Ar-H), 9.59 (1H, s, CONH, D2O exchg.), 10.18 (1H, s, NH, D_2O exchg.); ^{13}C NMR (125.76 MHz, DMSO–d_6): δ = 112.7, 124.0, 124.7, 128.4, 129.0, 131.0, 131.8, 134.7, 134.9, 138.0, 140.4, 143.3, 147.2, 151.4, 167.2, 190.9, 192.7, 208.0; MS: m/z = 468.12

3.2.3. (Vc): 5-Benzoyl-2, 3-dimethoxyphenyl-3, 4-dihydropyrimidin-2(1H)-one-1H-isoindole-1, 3(2H)-dione

m.p.: 191–193°C; ^{1}H NMR (500 MHz, DMSO–d_6): δ = 3.81 (3H, s, -OCH3), 3.89 (3H, s, OCH3), 5.76 (1H, s, H-4), 6.94–7.85 (12H, m, Ar-H), 9.34 (1H, s, CONH, D2O exchg.), 10.29 (1H, s, NH, D2O exchg.); ^{13}C NMR (125.76 MHz, DMSO–d_6): δ = 50.8, 55.5, 56.6, 59.6, 61.4, 65.4, 113.3, 113.6, 119.7, 120.2, 123.9, 124.4, 127.4, 129.0, 131.9, 133.4, 136.2, 137.3, 138.4, 145.5, 146.5, 150.4, 152.9, 168.2, 190.2, 191.1; MS: m/z = 483.47

3.2.4. (Vd): 5-Benzoyl-3, 4-dimethoxyphenyl-3, 4-dihydropyrimidin-2(1H)-one-1H-isoindole-1, 3(2H)-dione

m.p.: 151–153°C; ^{1}H NMR (500 MHz, DMSO–d_6): δ = 3.74 (3H, s, -OCH_3), 3.76 (3H, s, OCH_3), 5.41 (1H, s, H-4), 6.98–8.00 (12H, m, Ar-H), 9.40 (1H, s, CONH, D_2O exchg.); ^{13}C NMR (1255.43 MHz, DMSO–d_6): δ = 54.3, 54.9, 66.4, 111.9, 112.1, 112.6, 118.6, 124.0, 127.5, 129.1, 132.0, 134.8, 135.4, 136.7, 138.8, 142.5, 148.8, 151.7, 167.2, 191.3; MS: m/z = 483.47

IV. STUDY OF BIOACTIVITY

4.1 Antimicrobial activity

The antibacterial activities of all the synthesized compounds (Vb and Vc) were studied in dimethyl sulphoxide using Agar well diffusion method. The solvent DMSO. The synthesized compounds were dissolved in DMSO at a concentration of 1mg/µl.

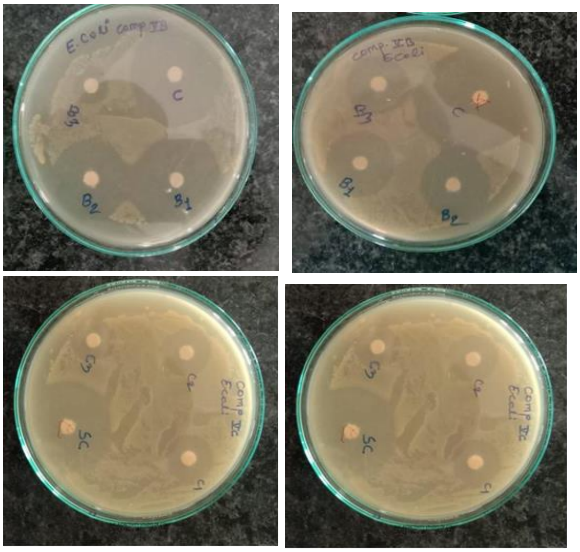
Test microorganisms: The synthesized compounds were tested for its antibacterial activity against bacteria Escherichia coli (E. coli) These microorganisms were maintained at 4℃ using nutrient agar slants.

4.2 Agar well diffusion method: The antimicrobial studies of synthesized compounds were done by agar well diffusion method. The bacterial strains were activated by inoculating in 25 ml of N-broth. This mixture was incubated for 24h in an incubator at 35℃. 0.2 ml of the activated strain was incubated in Mueller Hinton Agar. The nutrient medium Mueller Hinton agar was kept at 45℃ and was then poured in Petri dishes. These dishes are then left without disturbance so that the medium should be solidified. Using a sterile cork borer, 0.85 cm well was made in the Petri dishes and in each well, 0.1 ml of test solution was filled. These dishes were then incubated for 24h at 35℃. For each bacterial strain, three experiments were done for each compound and mean value was used as zone of inhibition for each sample. The inhibition was also measured for pure DMSO for each strain, which was considered as control. The zone of inhibition for each compound for strain is given after subtraction of zone of control.

Table -2 Parameters used for antibacterial E.coli testing by disc diffusion methods

Methods	Microorganism	Growth medium	Zone against E.coli	Incubation temperature (^{0}C)	Incubation time(h)
Agar well diffusion	Bacteria E.coli	MHA	Standard control (Sc)	35±2	24±2
Compound Vb	Bacteria E.coli	MHA	(Sc) C1=1.8cm 2.5cm C2=1.4cm 2.5cm C3=1.2cm 2.5cm	35	24
Compound Vc	Bacteria E.coli	MHA	(Sc) C1=1.5cm, 1.9cm C2=1.2cm 1.9cm C3=1.0cm 1.9cm	35	24

Fig-1-4. Picture indicating antibacterial E.coli testing by Agar well diffusion method



V. CONCLUSION

Physical and chemical properties of the prepared Dihydropyrimidinone Derivatives Containing Phthalimide Moiety are given in Table 1. The adopted method provided an excellent yield ranging from 78 to 91 % for aromatic, aroma to aliphatic, and heterocyclic aldehydes in shorter reaction time of around 2-4 hours and purity of the products as determined by TLC techniques. A series of novel dihydropyrimidinone derivatives Scheme I -III containing phthalimide moiety were synthesized in good yield, at high level of purity, and in efficient manner from the enaminone, which was derived from phthalimide by simple and solvent-free method. As shown in Scheme I, The dihydropyrimidinone derivatives were synthesized by refluxing phthalic anhydride (I) (0.01 mol) and para-aminoacetophenone (II) (0.01 mol) in glacial acetic acid for (2 hr) gives 2-(4-acetylphenyl)-1H-isoindole-1,3(2H)-dione (III). The enaminone, 2-{4-[(2E)-3-(dimethylamino) prop-2-enoyl] phenyl}-1H-isoindole-1, 3(2H)-dione (IV), was synthesized by refluxing 2-(4-acetylphenyl)-1H-isoindole-1, 3(2H)-dione (III) with dimethylformamide-dimethylacetal (DMF-DMA) under solvent-free conditions for 12 h. The novel final dihydropyrimidinone derivatives were

synthesized by using Biginelli Synthesis, a mixture of substituted benzaldehyde (0.01 mol), enaminone (IV) (0.01 mol), urea (0.01 mol), and glacial acetic acid (10 mL) was heated under reflux for 3 h. The H-4 protons of dihydropyrimidinone moiety and aromatic protons were observed at δ 5.30–6.01 and δ 6.48–8.29 ppm, respectively [17]. The ethylenic protons indicate that the enaminone existed in the E-configuration [18]. Compounds presented the D_2O exchangeable broad singlet at δ 9.33–9.62 ppm and δ 10.12–10.31 ppm corresponding to the two NH protons. ^{13}C NMR spectra confirmed all the carbon atoms for compounds (Va-Vd). The possible reaction mechanism involves the acid catalyzed formation of iminium ion intermediate from the substituted aryl aldehydes and urea. Reaction of phthalimide enaminone by iminium ion yields ureidenone, which forms hexahydropyrimidine by cyclization. Final dihydropyrimidinone derivatives (Va-Vd) were obtained by elimination of NH $(CH_3)_2$ groups from hexahydropyrimidine in presence of glacial acetic acid Molecular weights of the compounds were confirmed by mass spectral data. Molecular ion peaks were observed in all compounds respective to their molecular weights. The composition of the synthesized compounds (Va-Vd) was confirmed by spectral and elemental data. The compound obtained in scheme-III from compound Va-Vd was tested for antibacterial strain Escherichia coli (E. coli) by Agar well diffusion method. It has been observed that tested compounds Vb and Vc shows good antibacterial activities against Escherichia coli (E.coli) bacterial strains.

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