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Dihydropyrimidones: As Novel Class of *β*-Glucuronidase Inhibitors

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Abstract

Dihydropyrimidones **1-37** were synthesized *via* a "one-pot" three component reaction according to well-known Biginelli reaction by utilizing $Cu(NO_3)_2.3H_2O$ as catalyst, and screened for their *in vitro β*-glucuronidase inhibitory activity. It is worth mentioning that amongst the active molecules, compounds **8** (IC_{50} = 28.16 ± .056 μM), **9** (IC_{50} = 18.16 ± 0.41 μM), **10** (IC_{50} = 22.14 ± 0.43 μM), **13** (IC_{50} = 34.16 ± 0.65 μM), **14** (IC_{50} = 17.60 ± 0.35 μM), **15** (IC_{50} = 15.19 ± 0.30 μM), **16** (IC_{50} = 27.16 ± 0.48 μM), **17** (IC_{50} = 48.16 ± 1.06 μM), **22** (IC_{50} = 40.16 ± 0.85 μM), **23** (IC_{50} = 44.16 ± 0.86 μM), **24** (IC_{50} = 47.16 ± 0.92 μM), **25** (IC_{50} = 18.19 ± 0.34 μM), **26** (IC_{50} = 33.14 ± 0.68 μM), **27** (IC_{50} = 44.16 ± 0.94 μM), **28** (IC_{50} = 24.16 ± 0.50 μM), **29** (IC_{50} = 34.24 ± 0.47 μM), **31** (IC_{50} = 14.11 ± 0.21 μM) and **32** (IC_{50} = 9.38 ± 0.15 μM) found to be more potent than the standard D-saccharic acid 1,4-lactone (IC_{50} = 48.4 ± 1.25 μM). Molecular docking study was conducted to establish the structure-activity relationship (SAR) which demonstrated that a number of structural features of dihydropyrimidone derivatives were involved to exhibit the inhibitory potential. All compounds were characterized by spectroscopic techniques such as ^{1}H-, ^{13}C-NMR, EIMS and HREI-MS.

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Keywords: Synthesis; Dihydropyrimidone; *β*-Glucuronidase; *In vitro*; Molecular docking; Structure-activity relationship.

Introduction

β-Glucuronidase (EC 3.2.1.31) enzyme exists in anaerobic *Escherichia*, *Bacteroides*, *Clostridia* and *Peptostreptococcus* genera and responsible to catalyzes the breakage of *β*-glucuronosyl-*O*-bonds [1]. It is also present in many human body fluids and organs such as bile, kidney, serum, spleen and urine [2-3]. Enhanced activity of *β*-glucuronidase leads to a variety of pathological conditions including epilepsy [4], renal diseases [5], urinary tract infection [6-9], transplantation rejection [10] and neoplasm of bladder, breast, larynx and testes [11,12].

Pyrimidine and pyrimidone are six-membered, heterocyclic, and aromatic organic compounds containing two nitrogen atoms at position 1 and 3 [13]. In 1893, the Pietro Biginelli discovered the synthesis of multi-functionalized dihydropyrimidones by a simple one-pot multicomponent reaction [14]. Pyrimidine and pyrimidine ring containing compounds possess a diverse spectrum of biological activities such as antitumor, antiviral, antibacterial, anti-inflammatory and antihypertensive activities [15-18]. Recently, pyrimidine derivatives have been reported as potent inhibitors of the enzymes responsible for diabetes, and particularly, pyrimidine-fused heterocycles, which are identified as specific *α*-glucosidase inhibitors [19]. Dihydropyrimidines derivatives also used as the calcium channel blockers, *α*-adrenergic antagonists as well as HIV gp-120-CD4 inhibitors, and *α*-1a-antagonists [20-22].

Our research group had identified a number of heterocyclic compounds for their pharmacological activities [23-29] and already published many heterocycles as potential class of *β*-glucuronidase inhibitors [30-36]. Since, we have reported the *β*-glucuronidase inhibitory activity of 2-arylquinazolin-4(3*H*)-ones [1], being structurally similar to this class, we decided to explore dihydropyrimidones as potential *β*-glucuronidase inhibitors (Figure-1). Thus, this manuscript describes the syntheses of dihydropyrimidones derivatives **1-37** and evaluation for their *β*-glucuronidase inhibition studies. To the best of our knowledge, this class of compounds never reported before for *β*-glucuronidase inhibitory activity.

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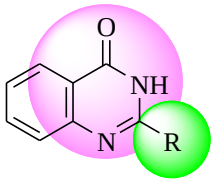
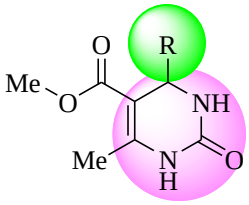


IC_{50} = 0.6-198 μM



1-37

IC_{50} = 9.38-78.16 μM

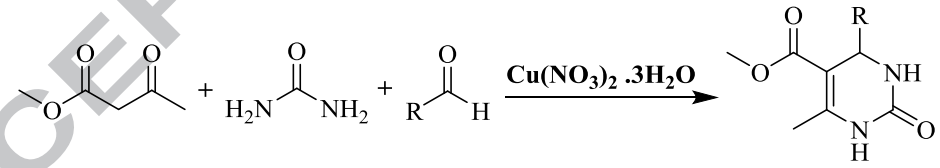
Standard = D-Saccharic acid 1,4-lactone (IC_{50} = 48.4 ± 1.25 μM)

Figure-1

Results and Discussion

Chemistry

Substituted 4-aryl dihydropyrimidones **1-37** were synthesized *via* "one pot" three component reaction by fusing urea, methyl acetoacetate and variety of aldehydes in the presence of copper nitrate trihydrate as catalyst in solvent free condition at 80-90 °C with constant stirring (Scheme-1, Table-1). Heating and stirring were continued until everything turned solidify. Reaction progress was checked by monitoring thin layer chromatography (TLC). After the completion of reaction the solid product was washed extensively with the distilled water and recrystallized from ethanol to afford the pure products. Structure of all synthetic compounds **1-37** were characterized by different spectroscopic techniques ^{1}H-, ^{13}C-NMR, EIMS and HREI-MS.



1-37

Scheme-1: "One-pot" three component syntheses of dihydropyrimidone derivatives **1-37**

Reaction starts with the chelation of copper nitrate trihydrate with the carbonyl carbon of aryl aldehyde to enhance its electrophilicity, followed by the nucleophilic attack of amino group of urea to form intermediate (**a**). Than intermediate (**a**) undergoes the E2 elimination reaction to afford iminium ion as in intermediate (**b**) which undergo the nucleophilic addition reaction by enol carbon to from intermediate (**c**). Electrophilic carbonyl carbon of acetyl group was attacked

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by the terminal amino group to give rise a cyclize intermediate (**d**) which undergo dehydration reaction to afford the dihydropyrimidone ring (Figure-2).

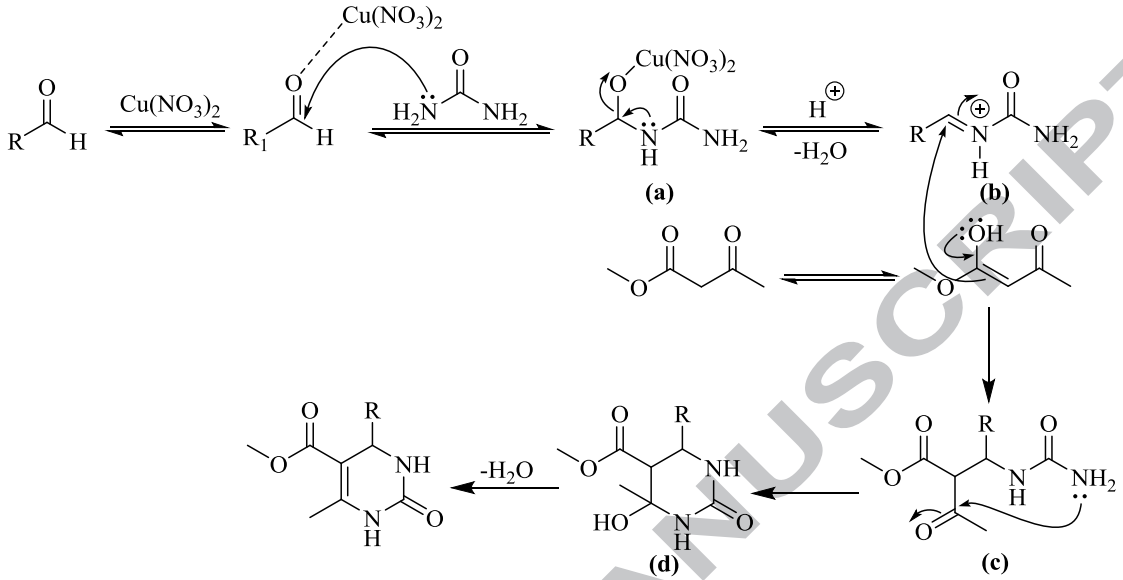

Figure-2: Mechanism for the syntheses of dihydropyrimidones

Characteristic Spectral Feature of Representative Compound (32)

^{1}H- and ^{13}C-NMR spectra of most active compound **32** against *β*-glucuronidase were recorded in deuterated DMSO. In ^{1}H-NMR spectrum, two downfield broad singlets of the acidic NH protons appeared at δ_H 9.30 and δ_H 7.83, respectively. Downfield signals was due to the extensive conjugation throughout the basic dihydropyrimidone ring system. H-3′ and H-5′ appeared at δ_H 7.70 (d, $J_{3',2/5',6'}$ = 8.0 Hz), showed *ortho* coupling with H-2' and 6'. Its downfield chemical shift was due to the electron withdrawing *ortho* CF_3 group. H-2′ and H-6′ appeared at δ_H 7.45 (d, $J_{2',3'/6',5'}$ = 8.0 Hz) and showed *ortho* coupling with H-3' and 5'. Characteristic signal of methine H-4 appeared as doublet at δ_H 5.22 (d, $J_{4,NH}$ = 3.2 Hz), and showed coupling with NH. The OCH_3 protons of methyl ester appeared as a singlet at δ_H 3.52. The singlet of allylic CH_3 appeared at δ_H 2.25 (Figure-3).

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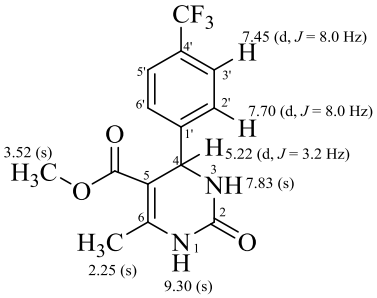

Figure-3: ^{1}H-NMR chemical shifts of compound **32**.

In ^{13}C-NMR broad-band decoupled spectrum in DMSO-d_6 showed a total of 14 carbon signals, including 2 methyl, 5 methine, and 7 quarternary carbons. The quaternary carbon of ester carbonyl, was the most downfield signal appeared at δ_C 165.6. The second most downfield signal was appeared at δ_C 151.9 corresponds to the quaternary carbon of carbonyl which is lying in between the two nitrogens. Two quaternary carbon of vinylic C-6 and phenyl C-1′ appeared at δ_C 149.3 and δ_C 149.1, respectively. Four aromatic methine carbons appeared in theusual aromatic region δ_C 125-127 while aromatic quaternary C-4′, *ipso* to triflouromethane group resonated at δ_C 125.4. CF_3 carbon and C-5 resonated at δ_C 125.5 and δ_C 98.3, respectively. Methoxy carbon of methyl ester resonated at δ_C 50.8. The most upfield allylic methyl carbon appeared at δ_C 17.9 (Figure-4).

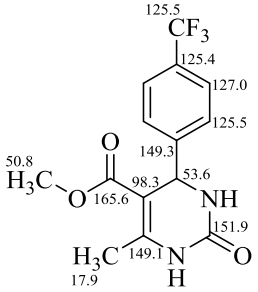

Figure-4: ^{13}C-NMR chemical shifts of compound **32**

EI-MS of compound **32** showed the molecular ion peak $[M]^+$ at *m/z* 314. High resolution EI-MS of compound **32** displayed M^+ at *m/z* = 314.0897 with a composition of $C_{14}H_{13}F_3O_3N_2$ (Calcd. 314.0878). Molecular ion undergo the loss of methyl radical to yield a cation, appeared at *m/z* 299. Similarly, loss of neutral fragment of -$PhCF_3$ from molecular ion gave a radical cation at *m/z* 169 which is also the base peak indicating its stability. A radical cation at *m/z* 145

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correspond to the $PhCF_3$ with the neutral loss of dihydropyrimidone ring. Loss of methoxy from the molecular ion yielded a peak at *m/z* 283 which was further fragmented into radical cations at *m/z* 255 and at *m/z* 240, respectively, after neutral loss of carbon monoxide and methyl radicals (Figure-5).

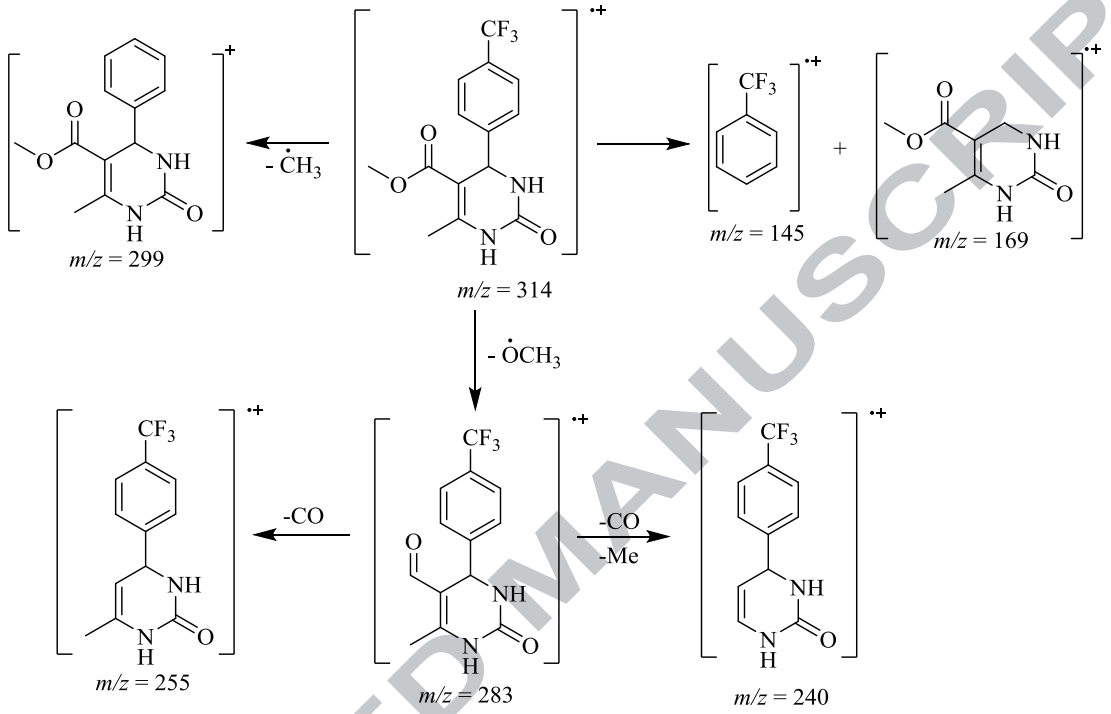
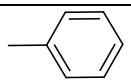
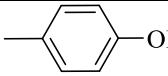
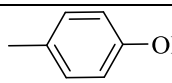


Figure-5: Key EI-MS fragmentation of compound **32**.

***In Vitro β*-Glucuronidase Inhibitory Activities**

All synthetic dihydropyrimidones **1-37** were screened to check their *in vitro β*-glucuronidase inhibitory activity. Excepts compounds **1**, **7**, **11**, **12**, **18**, **34**, **36** and **37**, all demonstrated good to moderate inhibitory potential in the range of IC_{50} = 9.38 ± 0.15-78.16 ± 1.47 μM as compared to the standard D-saccharic acid 1,4-lactone (IC_{50} = 48.4 ± 1.25 μM) (Table-1).

Table-1: *In vitro β*-glucuronidase inhibitory activity of dihydropyrimidones **1-37**

S. No.	R_1	IC_{50} ± SEM[a] [μM]
1		N.A.[b]
2		72.16 ±1.65
3		59.16 ± 1.22

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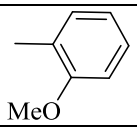
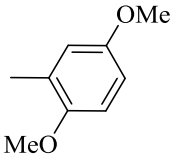
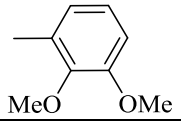
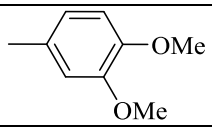
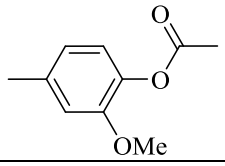
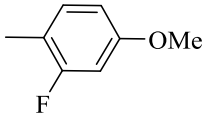
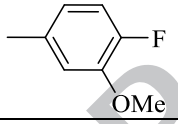
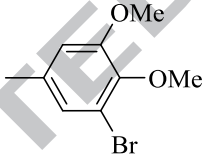
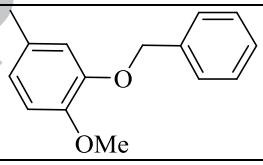
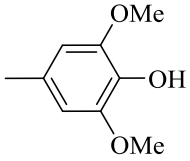
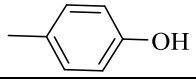
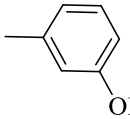
4		59.16 ± 1.25
5		61.02 ± 1.40
6		54.16 ± 1.12
7		N.A.[b]
8		28.16 ± .056
9		18.16 ± 0.41
10		22.14 ± 0.43
11		N.A.[b]
12		N.A.[b]
13		34.16 ± 0.65
14		17.60 ± 0.35
15		15.19 ± 0.30

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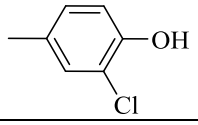
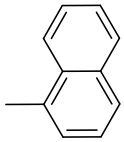
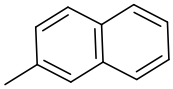
16		27.16 ± 0.48
17		48.16 ± 1.06
18		N.A.[b]
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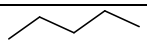
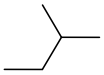
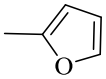
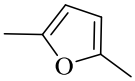
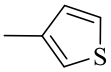
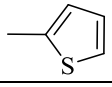
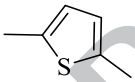
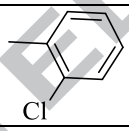
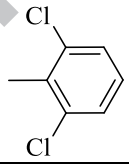
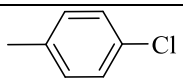
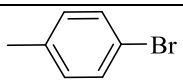
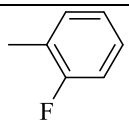
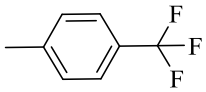
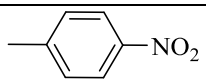
20		78.16 ± 1.47
21		55.16 ± 1.25
22		40.16 ± 0.85
23		44.16 ± 0.86
24		47.16 ± 0.92
25		18.19 ± 0.34
26		33.14 ± 0.68
27		44.16 ± 0.94
28		24.16 ± 0.50
29		34.24 ± 0.47
30		64.12 ± 1.30
31		14.11 ± 0.21
32		9.38 ± 0.15
33		49.13 ± 1.08

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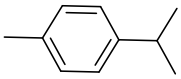
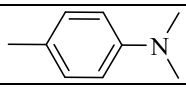
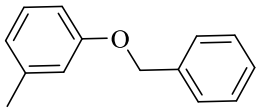
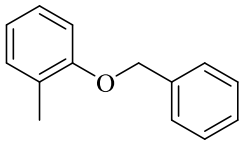
34		N.A.[b]
35		49.16 ± 1.10
36		N.A.[b]
37		N.A.[b]
Standard[c] = D-saccharic acid 1,4-lactone		48.4 ± 1.25

[a]IC_{50} (mean ± standard error of mean); N.A.[b] (Not Active); Standard[c] (Inhibitor for *β*-glucuronidase).

All persistent structural features in compounds such as dihydropyrimidone ring, methyl group, and methyl ester moiety were definitely and cordially played their role in demonstrating the inhibitory activity, however, the varying features such as the aryl ring with different substitution pattern in all compounds were also responsible for the fluctuation in activity (Figure-6).

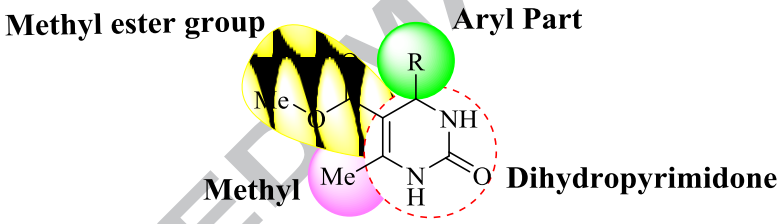


Figure-6

Limited structure-activity relationship (SAR) was rationalized by looking at the substitution pattern at the aryl part (R). Compound **32** (IC_{50} = 9.38 ± 0.15 μM) having *p*-triflouromethyl group at aryl part, found to be the five-fold more potent than the standard. It showed that being polar in nature, triflouromethyl group might interacted with the active site of *β*-glucuronidase. Another flouro group containing compound **31** (IC_{50} = 14.11 ± 0.21 μM) was found to be the second most potent of this library which verify the involvement of flouro group in activity. On comparison of the activity of **31** with the structurally similar compound **9** (IC_{50} = 18.16 ± 0.41 μM) which has an additional *p*-methoxy substituent at aryl part, a slight decreased inhibition was observed which might be due to the presence of methoxy group. Methoxy group may cause less polar interaction of flouro group or might create hindrance in interaction of flouro with the active

site. However, when methoxy come in close to flouro residue as in compound **10** (IC_{50} = 22.14 ± 0.43 μM) further decreased activity was obtained (Figure-7).

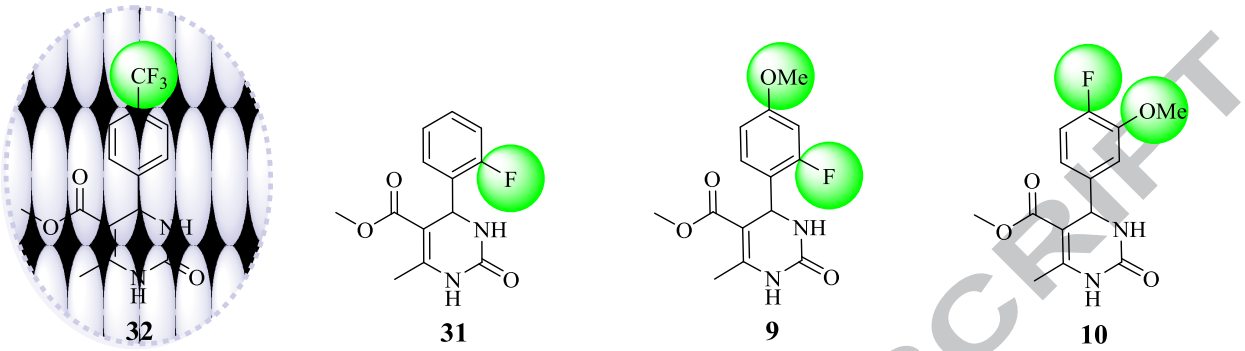


Figure-7

Compound **15** (IC_{50} = 15.19 ± 0.30 μM) having *m*-hydroxy group on aryl part, was found to be more than three-fold active than the standard. Most probably its activity was driven by the hydrogen bonding interaction made by the hydroxy group with the active site of the enzyme. Switching of hydroxy group from *m*- to *p*- as in compound **14** (IC_{50} = 17.60 ± 0.35 μM) didn't affect the activity to much extent. It was observed that incorporation of groups like chloro and methoxy as in compounds **16** (IC_{50} = 27.16 ± 0.48 μM) and **13** (IC_{50} = 34.16 ± 0.65 μM), respectively, decline in the inhibition was observed which might be due to steric hindrance to form hydrogen bond interaction by hydroxy group (Figure-8).

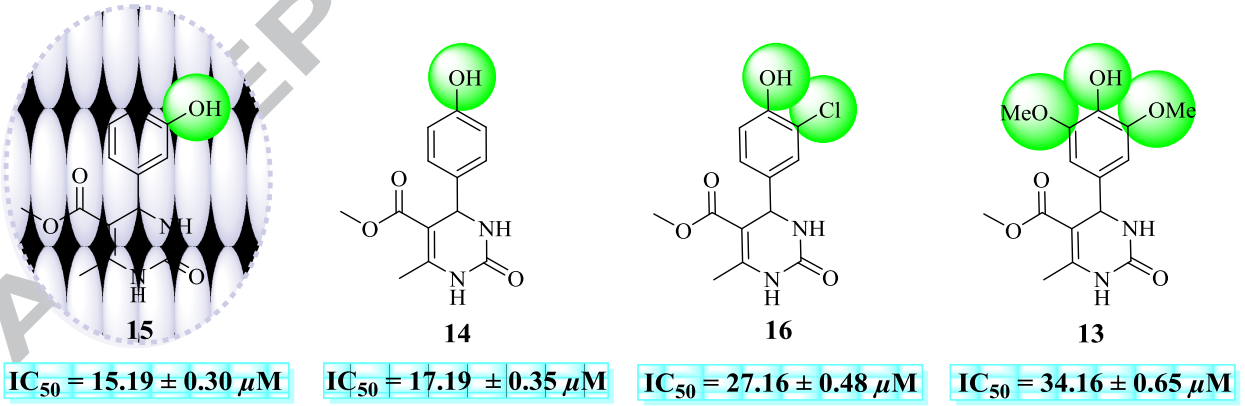


Figure-8

Amongst the potent compounds, derivatives having solely chloro group **27-29** also showed better potential than the standard. It was found that dichloro substituted compound **28** (IC_{50} = 24.16 ±

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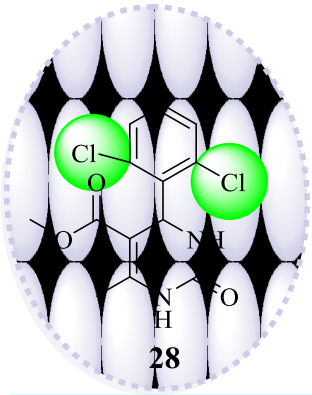
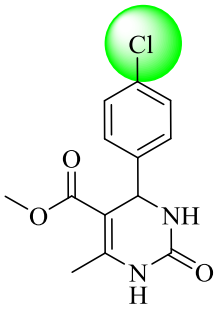
0.50 μM) was found to demonstrate two-fold more activity than the standard might be due to hydrophobic interaction made by the chloro groups with the active site. Mono chloro substituted compounds such as **29** (IC_{50} = 34.24 ± 0.47 μM) and **27** (IC_{50} = 44.16 ± 0.94 μM) demonstrated decreased activity than the dichloro substituted compound (Figure-9).



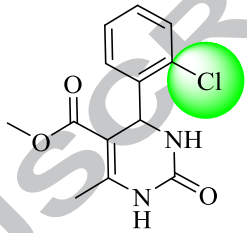
28

IC_{50} = 24.16 ± 0.50 μM



29

IC_{50} = 34.24 ± 0.47 μM



27

IC_{50} = 44.16 ± 0.94 μM

Figure-9

Compounds **22-25** have heterocyclic ring as "R" also revealed good inhibition than the standard might be due to the π-π interaction with the active site of enzyme. Amongst these, derivative **25** (IC_{50} = 18.19 ± 0.34 μM) having thiophene group substituted at position 2 was found to have superior activity. Interestingly, the structurally similar compound **24** (IC_{50} = 47.16 ± 0.92 μM) which has the only difference of position to which it is attached to the rest of the molecule, a sharp decline in the activity was observed. Activity of compound **25** can be compared with the **22** (IC_{50} = 40.16 ± 0.85 μM) which has a furan ring instead of thiophene, a reduced activity was observed might be due to lesser π-π interaction by furan ring having more electronegative oxygen atom. Similarly, compound **23** (IC_{50} = 44.16 ± 0.86 μM) also showed a comparable activity to **22** (Figure-10).

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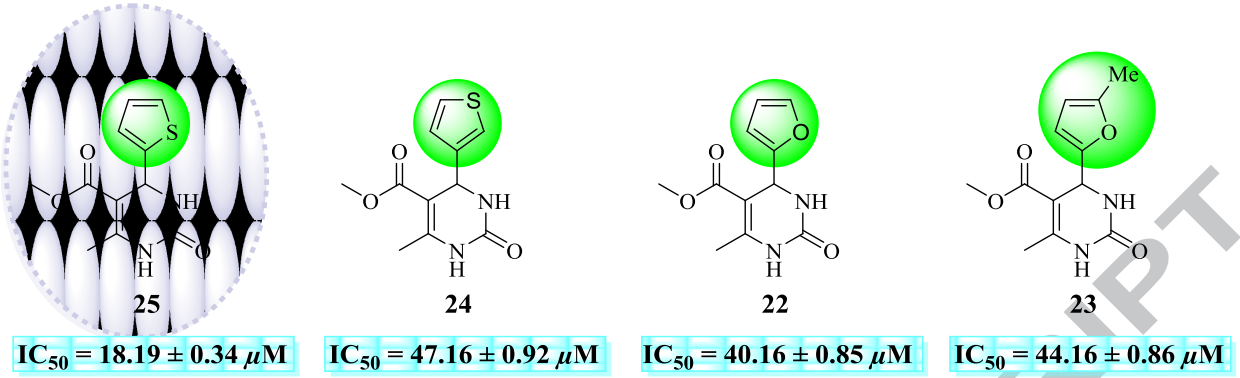

Figure-10

From the limited SAR study, it was extracted that the compounds having groups which involved to make polar interactions like hydrogen bonding, were more prone to inhibit than the compounds involved to make hydrophobic interaction with the active site of the enzyme. However, in order to get the more insights regarding the inhibition pattern by the compounds, a molecular docking studies on the most potent compounds such as **32**, **31**, **15**, **14** and **9** were carried out which is discussed below.

Molecular Docking Studies

The molecular docking studies predicted that the compounds were well fitted in the binding pocket of the enzyme. From the docking conformation of the most active compound **32** (IC_{50} = 9.38 ± 0.15 μM), it was observed that this compound forms five hydrogen bonds with the residues Phe206, Asp207, His385, Asn450, Glu451, Tyr508 and Arg600 of the binding pocket of the β-D-glucuronidase with excellent docking score (-16.2919). Asp207, Glu451, and Tyr508 were observed in creating hydrogen bonds with the hydrogen atom of the -NH moiety of the compound. Asp207 and Arg-600 interacts through active H atoms with the lone pair of the carbonyl oxygen of this compound (Figure-11**)**. This strong bonding network of the compound might be one of the reasons for good inhibitory activity of the compound against β-D-glucuronidase enzyme.

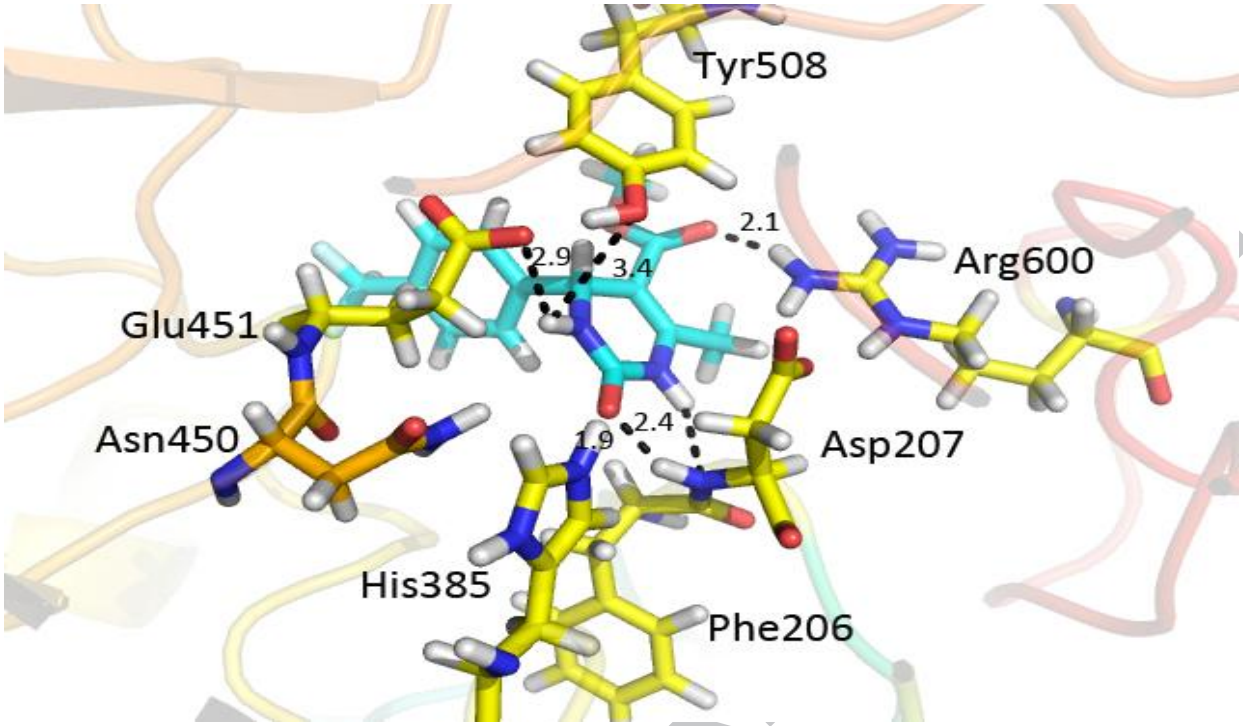

Figure-11: Binding mode of compound **32** in the active site of the β-D-glucuronidase.

The second most active compound **31** (IC_{50} = 14.11 ± 0.21 μM) also showed good docking score (-14.9956) as well as significant interactions with the active residues of the target enzyme. Phe206 displayed polar interaction with the carbonyl oxygen atom of the compound. Tyr508 demonstrated polar interaction with the -NH moiety of the compound and also forms hydrophobic interaction with the compound. Arg600 displayed hydrogen bonding with the carbonyl oxygen atom of the compound (Figure-12). The high potency of this compound may be due to the presence of electron rich groups that are exposed to polar interactions.

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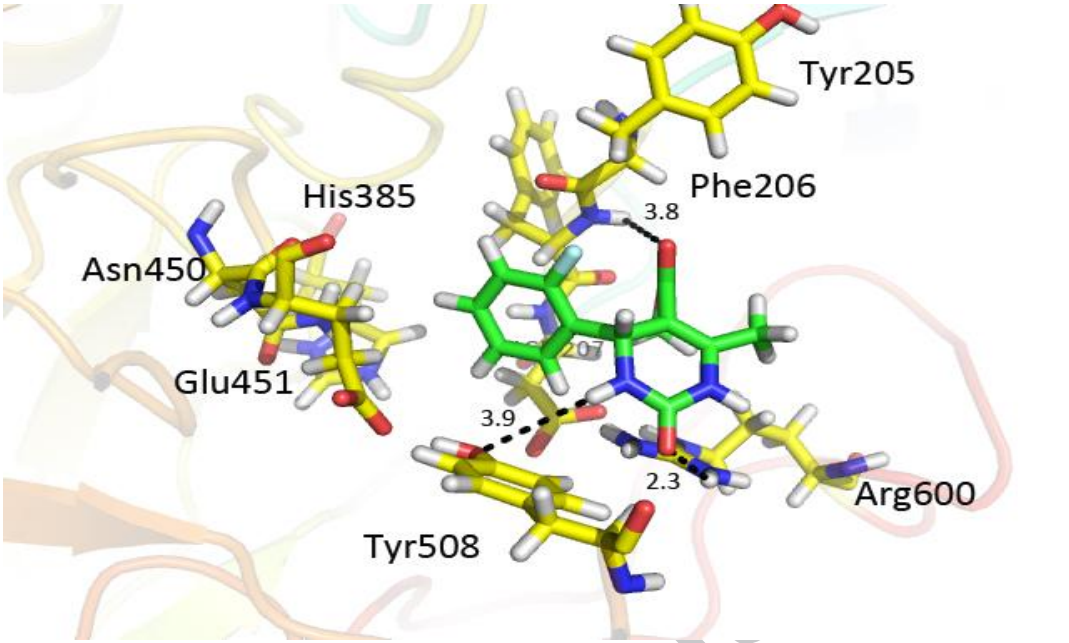

Figure-12: Binding mode of compound **31** in the active site of the *β*-D-glucuronidase.

The third most active compound **15** (IC_{50} = 15.19 ± 0.30 μM) showed high docking score (-14.5926) as well as good interactions with active site residues of the target protein. His385 and Asn450 exhibited polar interactions with hydroxyl (-OH) moiety of the compound. Arg600 demonstrated hydrogen bonding donor interaction with carbonyl oxygen atom of the same compound (Figure-13). The presence of the *meta*-OH group which is chemically more active, might be one of the reasons of its strong inhibition activity.

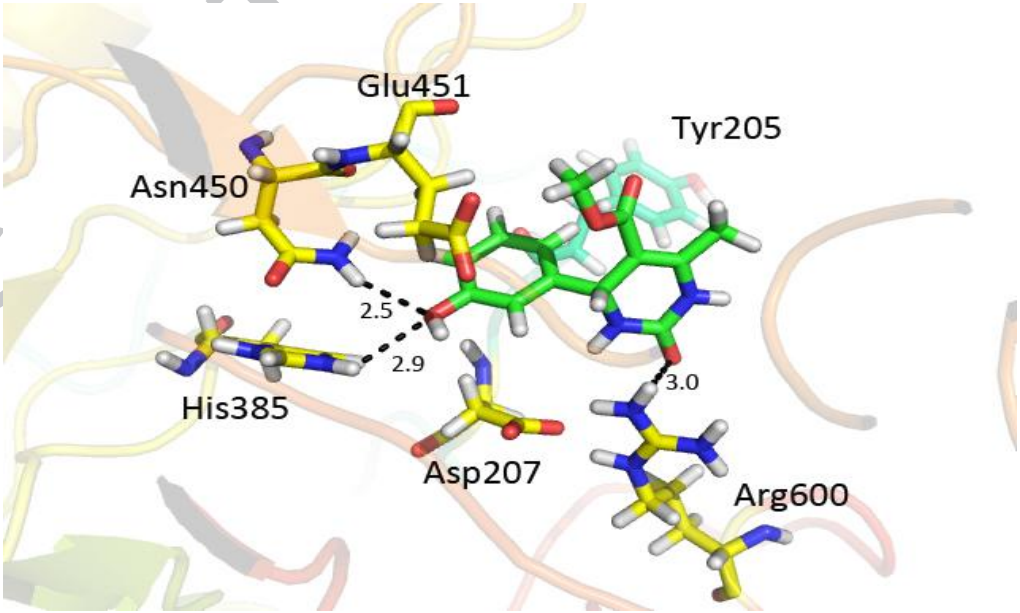

Figure-13: Binding mode of compound **15** in the active site of the *β*-D-glucuronidase.

Similarly, compound **14** (IC_{50}= 17.60 ± 0.35 μM) also has an OH group at *para* position showed the docking score (-13.7533) and important interactions with the active residues of the binding pocket (Figure-14). Asp207, His385, and Asn450 showed polar interactions with the hydroxyl group of the compound. Arg600 displayed polar interaction with the carbonyl oxygen moiety of the same compound. The position of OH group (*meta* or *para*) showed a minor role in molecular docking study.

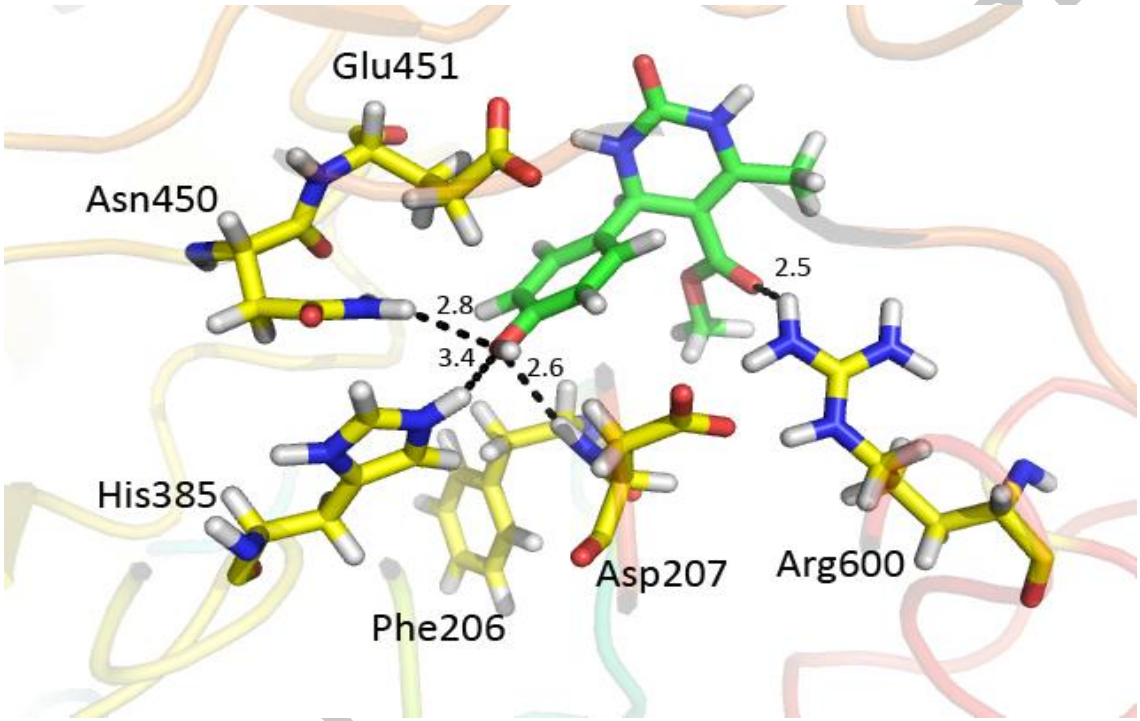

Figure-14: Binding mode of compound **14** in the active site of the β-D-glucuronidase.

The compound **9** (IC_{50} = 18.16 ± 0.41 μM) showed docking score (-13.3387). It was observed it has two polar interactions with the Asp207 and Tyr511 residues (Figure-15). Asp207 demonstrated hydrogen bonding with the oxygen atom of the compound while Tyr511 formed polar interaction with the carbonyl oxygen atom of the compound. The good inhibitory activity might be due to the high electron cloud of lone pairs of the available electronegative groups.

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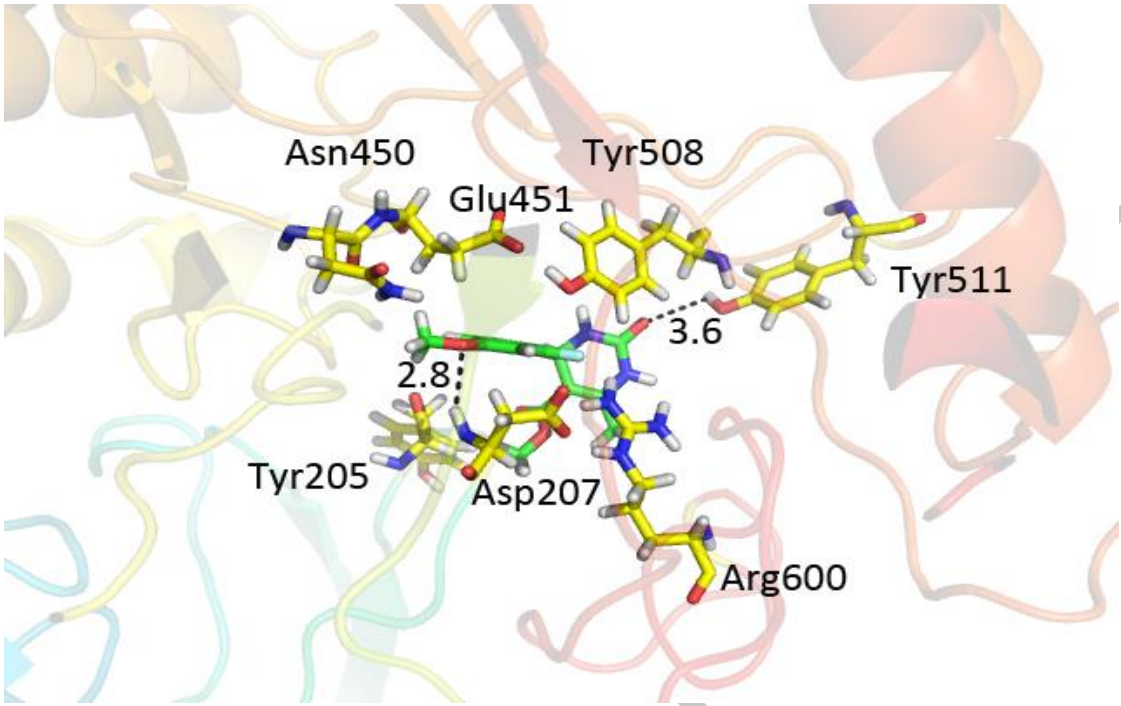

Figure-15: Binding mode of compound **9** in the active site of the *β*-D-glucuronidase.

Conclusion

Synthetic dihydropyrimidones **1-37** were first time reported to possess *in vitro β*-glucuronidase inhibitory activity and all compounds showed good inhibitory potential in the range of IC_{50} = 9.38 ± 0.15-78.16 ± 1.47 μM compared to the standard drug D-saccharic acid 1,4-lactone (IC_{50} = 48.4 ± 1.25 μM). Molecular docking study was carried out in order to establish the structure-activity relationship. As a result of this study compounds **8-10**, **13-17**, **22-29**, **31** and **32** were identified as lead molecules for further research in order to get a powerful inhibitor for *β*-glucuronidase.

Experimental

Materials and Methods

Reagents were purchased from Sigma-Aldrich, USA. All reagents and solvents were of analytical grade and used as received. Thin layer chromatography was performed on pre-coated silica gel, GF-254 (Merck, Germany). Spots were visualized under ultraviolet light at 254 and 366 nm. Melting points of the compounds were determined on BUCHI-M560 melting point apparatus. Mass spectra were recorded under on MAT 312 and MAT 113D mass spectrometers.

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The ^{1}H, ^{13}C-NMR were recorded on a Bruker AM spectrometers, operating at 300, 400 and 500 MHz. The chemical shift values are presented in ppm (δ), relative to tetramethylsilane (TMS) as an internal standard and the coupling constant (J) are in Hz.

General Experimental Procedure for the Syntheses of Dihydropyrimidones 1-37

Urea (1 mmol), ethylacetoacetate (1.2 mmol), substituted aryl aldehyde (1 mmol) and copper nitrate trihydrate (10 mol %) as catalyst were taken in a 100 mL round-bottomed flask and heated at 80-90 ºC with constant stirring. Completion of the reaction was checked by periodic TLC. After the reaction completion, the solid reaction mixture was washed with excess of distilled water and recrystallized from ethanol to afford pure products in high yields.

Bioassay

***β*-Glucuronidase Inhibition Study**

The bioassay protocol is used as reported by Taha *et al* [37]. *β*-Glucuronidase (E.C. 3.2.1.31, from bovine liver, G-0251) and *p*-nitrophenyl-*b*-D-glucuronide (N-1627) were purchased from Sigma-Aldrich USA. Anhydrous sodium carbonate and all other analytical grade reagents were obtained from Merck, Germany. Solvents and reagents were of reagent grade and used directly without purification.

β-Glucuronidase activity was determined by measuring the absorbance at 405 nm of *p*-nitrophenol formed from the substrate by the spectrophotometric method. The total reaction volume was 250 μL. DMSO (100%) was used to dissolve the compound (5 μL) which become 2% in the final assay (250 μL) and the same conditions were used for standard (D-saccharic acid 1,4-lactone). The reaction mixture contained 185 μL of 0.1 M acetate buffer, 5 μL of test compound solution, 10 μL of enzyme solution was incubated at 37 $^{\circ}$C for 30 minutes. The plates were read on a multiplate reader (SpectraMax plus 384, USA) at 405 nm after the addition of 50 μL of 0.4 mM *p*-nitrophenyl-*β*-D-glucuronide. All assays were carried in triplicate.

Molecular Docking Studies

The three dimensional (3D) structures of the dihydropyrimidones derivatives were generated by using the builder tool of the molecular operating environment (MOE) (www.chemcomp.com).

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Each compound was 3D protonated, then energy minimization was done by the default parameters of the MOE (gradient: 0.05, Force Field: MMFF94X). All compounds were then saved in separate database i-e mdb file and used for molecular docking. The 3D crystal structure of human β-D-glucuronidase (PDB code 1BHG) [38] was retrieved from the protein databank for docking studies. The B-chain of protein and hetero-atoms including cofactors were removed from the original protein data bank file. Hydrogen atoms were added to the enzyme structure by 3D protonation and then energy minimization was carried out by using the default parameters of the MOE (gradient: 0.05, Force Field: MMFF94X). The active residues of the binding pocket were found out by the site-finder module and dihydropyrimidones inhibitors were allowed to dock with the active residues of the human β-D-glucuronidase by the most default parameters. All binding interactions of the compounds in the receptor were predicted by using the Pymol software.

Methyl 6-methyl-2-oxo-4 phenyl-1,2,3,4-tetrahydropyrimidine-5-carboxylate (1)

Yield: 89%; M.P.: 210-212 $^{\circ}$C; ^{1}H-NMR (400 MHz, DMSO-d_6) δ 9.19 (s, 1H, NH), 7.73 (s, 1H, NH), 7.33 (t, $J_{3'(2',4')} = J_{5'(4',6')} = 7.6$ Hz, 2H, H-3', 5'), 7.24 (m, 3H, H-2', 4', 6'), 5.13 (d, $J_{4,NH} = 3.2$ Hz, 1H, H-4), 3.52 (s, 3H, OC$\underline{H}_3$), 2.23 (s, 3H, C$\underline{H}_3$); ^{13}C-NMR (75 MHz, DMSO-d_6):δ 165.8, 152.1, 148.6, 144.6, 128.4, 128.4, 127.2, 126.1, 126.1, 98.9, 53.7, 50.77,17.8; EIMS *m/z* (% rel. abund.): M^+ 246 (26), 231 (51), 187 (49), 169 (100); HREI-MS Calcd for $C_{13}H_{14}O_3N_2$: *m/z* = 246.1004, Found 246.0992.

Methyl 4-(4′-ethoxyphenyl)-6-methyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (2)

Yield: 85%; M.P.: 212-214 $^{\circ}$C; ^{1}H-NMR (400 MHz, DMSO-d_6) δ 9.15 (s, 1H, NH), 7.66 (s, 1H, NH), 7.12 (d, $J_{2',3'/6',5'} = 8.4$ Hz, 2H, H-2', 6'), 6.85 (d, $J_{3',2'/5',6'} = 8.4$ Hz, 2H, H-3', 5'), 5.07 (d, $J_{4,NH} = 2.8$ Hz, 1H, H-4), 3.99 (q, 2H, OC$\underline{H}_2$CH$_3$), 3.51 (s, 3H, OC$\underline{H}_3$), 2.22 (s, 3H, C$\underline{H}_3$), 1.30 (t, $J_{CH3/CH2} = 7.2$ Hz, 3H, OCH$_2$C$\underline{H}_3$); ^{13}C-NMR (75 MHz, DMSO-d_6):δ 165.8, 157.71, 152.16, 148.2, 136.6, 127.3, 127.3, 114.2, 114.2, 99.2, 62.9, 53.1, 50.7, 17.7, 14.6; EIMS *m/z* (% rel. abund.): 290 (M^+, 27), 275 (100), 230.9 (73), 169 (92); HREI-MS Calcd for $C_{15}H_{18}O_4N_2$: *m/z* = 290.1267, Found 290.1254.

Methyl 4-(4′-methoxyphenyl)-6-methyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (3)

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Yield: 86%; M.P.: 192-194 °C; ^{1}H-NMR (400 MHz, DMSO-d_6) δ 9.15 (s, 1H, NH), 7.66 (s, 1H, NH), 7.13 (d, $J_{2',3'/6',5'}$ = 8.4 Hz, 2H, H-2', 6'), 6.87 (d, $J_{3',2'/5',6'}$ = 8.4 Hz, 2H, H-3', 5'), 5.07 (d, $J_{4,NH}$ = 3.2 Hz, 1H, H-4), 3.70 (s, 3H, OCH$_3$), 3.51 (s, 3H, OCH$_3$), 2.23 (s, 3H, CH$_3$); ^{13}C-NMR (100 MHz, DMSO-d_6):δ 165.8, 158.4, 152.1, 148.2, 136.8, 127.2, 127.2, 113.7, 113.7, 99.2, 55.0, 53.1, 50.6, 17.4; EIMS *m/z* (% rel. abund.): 276 (M^+, 34), 261 (95), 217 (100), 169 (92); HREI-MS Calcd for $C_{14}H_{16}O_4N_2$: *m/z* = 276.1110, Found 276.1107.

Methyl 4-(2′-methoxyphenyl)-6-methyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (4)

Yield: 86%; M.P.: 285-287 °C; ^{1}H-NMR (400 MHz, DMSO-d_6) δ 9.12 (s, 1H, NH), 7.22 (d, $J_{6',5'}$ = 8.0 Hz, 1H, H-6'), 7.20 (s, 1H, NH), 7.02 (m, 2H, H-3', 5'), 6.86 (t, $J_{4'(3',5')}$ = 7.2 Hz, 1H, H-4'), 5.46 (d, $J_{4,NH}$ = 2.8 Hz, 1H, H-4), 3.78 (s, 3H, OCH$_3$), 3.45 (s, 3H, OCH$_3$), 2.27 (s, 3H, CH$_3$); ^{13}C-NMR (125 MHz, DMSO-d_6):δ 165.8, 156.5, 152.2, 149.2, 131.2, 128.7, 126.7, 120.1, 111.2, 97.2, 55.4, 50.7, 48.7, 17.7; EIMS *m/z* (% rel. abund.): 276 (M^+, 33), 261 (80) 169 (100); HREI-MS Calcd for $C_{14}H_{16}O_4N_2$: *m/z* = 276.1110, Found 276.1102.

Methyl 4-(2′,5′-dimethoxyphenyl)-6-methyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (5)

Yield: 80%; M.P.: 230-232 °C; ^{1}H-NMR (400 MHz, DMSO-d_6) δ 9.14 (s, 1H, NH), 7.24 (s, 1H, NH), 6.92 (d, $J_{3',4'}$ = 8.0 Hz, 1H, H-3'), 6.80 (d, $J_{4',3'}$ = 8.0 Hz, 1H, H-4'), 6.54 (s, 1H, H-6'), 5.41 (s, 1H, H-4), 3.73 (s, 3H, OCH$_3$), 3.64 (s, 3H, OCH$_3$), 3.47 (s, 3H, COOCH$_3$), 2.26 (s, 3H, CH$_3$); ^{13}C-NMR (100 MHz, DMSO-d_6):δ 165.7, 152.9, 152.2, 150.7, 149.2, 132.5, 113.8, 112.3, 111.9, 97.3, 56.0, 55.3, 50.7, 48.9, 17.7; EIMS *m/z* (% rel. abund.): 306 (M^+, 49), 169 (100), 137 (42); HREI-MS Calcd for $C_{15}H_{18}O_5N_2$: *m/z* = 306.1216, Found 306.1221.

Methyl 4-(2′,3′-dimethoxyphenyl)-6-methyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (6)

Yield: 88%; M.P.:227-229 °C; ^{1}H-NMR (400 MHz, DMSO-d_6) δ 9.13 (s, 1H, NH), 7.34 (s, 1H, NH), 6.99 (m, 2H, H-5', 6'), 6.72 (d, $J_{4',5'}$ = 6.7 Hz, 1H, H-4'), 5.46 (s, 1H, H-4), 3.78 (s, 3H, OCH$_3$), 3.75 (s, 3H, OCH$_3$), 3.45 (s, 3H, OCH$_3$), 2.25 (s, 3H, CH$_3$); ^{13}C-NMR (100 MHz, DMSO-d_6):δ 165.7, 152.3, 151.7, 148.7, 145.8, 137.4, 123.7, 119.2, 112.0, 98.1, 60.0, 55.6, 50.5,

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49.0, 17.8; EIMS *m/z* (% rel. abund.): 306 (M^+, 13), 169 (93); HREI-MS Calcd for $C_{15}H_{18}O_5N_2$: *m/z* = 306.1216, Found 306.1212.

Methyl 4-(3′,4′-dimethoxyphenyl)-6-methyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (7)

Yield: 83%; M.P.: 150-152 °C; ^{1}H-NMR (300 MHz, DMSO-d_6) δ 9.17 (s, 1H, NH), 7.68 (s, 1H, NH), 6.88 (s, 1H, H-2'), 6.86 (d, $J_{6',5'}$ = 6.0 Hz, 1H, H-6'), 6.70 (d, $J_{5',6'}$ = 6.0 Hz, 1H, H-5'), 5.09 (d, $J_{4,NH}$ = 3.0 Hz, 1H, H-4), 3.70 (s, 6H, 2OCH$_3$), 3.53 (s, 3H, OCH$_3$), 2.24 (s, 3H, CH$_3$); ^{13}C-NMR (100 MHz, DMSO-d_6):δ 165.8, 152.1, 148.5, 148.3, 148.0, 137.0, 117.7, 111.7, 110.4, 99.0, 55.5, 55.4, 53.3, 50.7, 17.7; EIMS *m/z* (% rel. abund.): 306 (M^+, 56), 291 (72), 169 (100); HREI-MS Calcd for $C_{15}H_{18}O_5N_2$: *m/z* = 306.1216, Found 306.1209.

Methyl 4-(4′-acetoxy-3′-methoxyphenyl)-6-methyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (8)

Yield: 82%; M.P.: 209-211 °C; ^{1}H-NMR (300 MHz, DMSO-$_6$) δ 9.24 (s, 1H, NH), 7.77 (s, 1H, NH), 7.02 (d, $J_{5',6'}$ = $J_{6',5'}$ = 6.0 Hz, 2H, H-5', 6'), 6.77 (s, 1H, H-2'), 5.15 (d, $J_{4,NH}$ = 3.0 Hz, 1H, H-4), 3.73 (s, 3H, OCH$_3$), 3.55 (s, 3H, OCH$_3$), 2.25 (s, 3H, OCOCH$_3$), 2.23 (s, 3H, CH$_3$); ^{13}C-NMR (75 MHz, DMSO-d_6):δ 168.5, 165.7, 152.0, 150.5, 148.8, 143.3, 138.3, 122.7, 117.5, 110.9, 98.6, 55.5, 53.4, 50.7, 20.3, 17.7; EIMS *m/z* (% rel. abund.): 334 (M^+, 11), 292 (90), 169 (100), 137 (44); HREI-MS Calcd for $C_{16}H_{18}O_6N_2$: *m/z* = 334.1165, Found 334.1175.

Methyl 4-(2′-fluoro-4′-methoxyphenyl)-6-methyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (9)

Yield: 86%; M.P.: 224-226 °C; ^{1}H-NMR (300 MHz, DMSO-d_6) δ 9.22 (s, 1H, NH), 7.62 (s, 1H, NH), 7.13 (d, $J_{5',6'}$ = 8.7 Hz, 1H, H-5'), 6.76 (d, $J_{6',5'}$ = 7.2 Hz, 1H, H-6'), 6.72 (s, 1H, H-3'), 5.34 (d, $J_{4,NH}$ = 1.8 Hz, 1H, H-4), 3.72 (s, 3H, OCH$_3$), 3.46 (s, 3H, OCH$_3$), 2.24 (s, 3H, CH$_3$); ^{13}C-NMR (100 MHz, DMSO-d_6):δ 165.5, 161.2, 159.9, 151.6, 148.8, 129.2, 123.5, 110.4, 101.5, 97.5, 55.5, 50.7, 48.2, 17.8; EIMS *m/z* (% rel. abund.): 294 (M^+, 8), 279 (71), 169 (71), 44.1 (100); HREI-MS Calcd for $C_{14}H_{15}FO_4N_2$: *m/z* = 294.1016, Found 294.0993.

Methyl 4-(4′-fluoro-3′-methoxyphenyl)-6-methyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (10)

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Yield: 80%; M.P.: 214-216 °C; ^{1}H-NMR (400 MHz, DMSO-d_6) δ 9.22 (s, 1H, NH), 7.73 (s, 1H, NH), 7.15 (d, $J_{6',5'}$ = 10.8 Hz, 1H, H-6'), 7.02 (d, $J_{5',6'}$ = 8.0 Hz, 1H, H-5'), 6.73 (br.s, 1H, H-2'), 5.12 (s, 1H, H-4), 3.79 (s, 3H, OCH$_3$), 3.53 (s, 3H, COOCH$_3$), 2.24 (s, 3H, CH$_3$); ^{13}C-NMR (75 MHz, DMSO-d_6):δ 165.7, 152.2, 149.0, 148.8, 146.8, 141.3, 117.9, 115.7, 112.1, 98.5, 55.8, 53.3, 50.7, 17.7; EIMS *m/z* (% rel. abund.): 294 (M^+, 32), 279 (41), 169 (100); HREI-MS Calcd for $C_{14}H_{15}FO_4N_2$: *m/z* = 294.1016, Found 294.1003.

Methyl 4-(3′-bromo-4′,5′-dimethoxyphenyl)-6-methyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (11)

Yield: 83%; M.P.: 223-225 °C; ^{1}H-NMR (400 MHz, DMSO-d_6) δ 9.25 (s, 1H, NH), 7.75 (s, 1H, NH), 6.92 (d, $J_{2',6'}$ = $J_{6',2'}$ = 4.8 Hz, 2H, H-2', 6'), 5.11 (d, $J_{4,NH}$ = 3.2 Hz, 1H, H-4), 3.78 (s, 3H, OCH$_3$), 3.70 (s, 3H, OCH$_3$), 3.56 (s, 3H, OCH$_3$), 2.25 (s, 3H, CH$_3$); ^{13}C-NMR (75 MHz, DMSO-d_6):δ 165.6, 153.2, 151.9, 149.1, 144.8, 141.8, 121.1, 116.4, 110.7, 98.2, 59.9, 55.9, 53.1, 50.8, 17.8; EIMS *m/z* (% rel. abund.): 384 (M^+, 40), 386 (M + 2, 38), 305 (61), 169 (100); HREI-MS Calcd for $C_{15}H_{17}BrO_5N_2$: *m/z* = 384.0321, Found 384.0337.

Methyl 4-(3′-(benzyloxy)-4′-methoxyphenyl)-6-methyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (12)

Yield: 80%; M.P.: 148-151 °C; ^{1}H-NMR (300 MHz, DMSO-d_6) δ 9.16 (s, 1H, NH), 7.67 (s, 1H, NH), 7.41 (m, 5H, H-2", 3", 4", 5", 6"), 6.92 (s, 1H, H-2'), 6.92 (d, $J_{6',5'}$ = 9.0 Hz, 1H, H-6'), 6.74 (d, $J_{5',6'}$ = 9.0 Hz, 1H, H-5'), 5.05 (s, 1H, H-4), 5.01 (s, 2H, OCH$_2$Ph), 3.72 (s, 3H, OCH$_3$), 3.49 (s, 3H, OCH$_3$), 2.22 (s, 3H, CH$_3$); ^{13}C-NMR (100 MHz, DMSO-d_6):δ 165.7, 152.1, 148.4, 147.5, 137.0, 136.9, 128.3, 128.3, 127.8, 127.8, 118.4, 112.2, 112.2, 112.0, 112.0, 98.9, 70.0, 55.6, 53.2, 50.6, 17.7; EIMS *m/z* (% rel. abund.): 382 (M^+, 27), 291 (85), 169 (61), 91 (100); HREI-MS Calcd for $C_{21}H_{22}O_5N_2$: *m/z* = 382.1529, Found 382.1512.

Methyl 4-(4′-hydroxy-3′,5′-dimethoxyphenyl)-6-methyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (13)

Yield: 82%; M.P.: 200-202 °C; ^{1}H-NMR (400 MHz, DMSO-d_6) δ 9.12 (s, 1H, OH), 8.29 (s, 1H, NH), 7.62 (s, 1H, NH), 6.46 (s, 2H, H-2', 6'), 5.06 (d, $J_{4,NH}$ = 2.4 Hz, 1H, H-4), 3.69 (s, 6H, 2OCH$_3$), 3.54 (s, 3H, OCH$_3$), 2.23 (s, 3H, CH$_3$); ^{13}C-NMR (75 MHz, DMSO-d_6):δ 165.8, 152.1,

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148.2, 148.2, 147.7, 135.0, 134.7, 103.8, 103.8, 99.0, 55.9, 55.9, 53.6, 50.6, 17.7; EIMS *m/z* (% rel. abund.): 322 (M^+, 37), 291 (27), 169 (100), 137 (95); HREI-MS Calcd for $C_{15}H_{18}O_6N_2$: *m/z* = 322.1165, Found 322.1173.

Methyl 4-(4′-hydroxyphenyl)-6-methyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (14)

Yield: 82%; M.P.: 231-233 °C; ^{1}H-NMR (300 MHz, DMSO-d_6) δ 9.32 (s, 1H, OH), 9.12 (s, 1H, NH), 7.62 (s, 1H, NH), 7.02 (d, $J_{2',3'/6',5'}$ = 8.4 Hz, 2H, H-2', 6'), 6.68 (d, $J_{3',2'/5',6'}$ = 8.4 Hz, 2H, H-3', 5'), 5.02 (d, $J_{4,NH}$ = 3.3 Hz, 1H, H-4), 3.50 (s, 3H, OCH_3), 2.22 (s, 3H, CH_3); ^{13}C-NMR (100 MHz, DMSO-d_6):δ 165.9, 156.5, 152.2, 148.1, 135.2, 127.3, 127.3, 115.0, 115.0, 99.4, 53.2, 50.7, 17.8; EIMS *m/z* (% rel. abund.): 262 (M^+, 15), 247 (72), 169 (100); HREI-MS Calcd for $C_{13}H_{14}O_4N_2$: *m/z* = 262.0954, Found 262.0948.

Methyl 4-(3′-hydroxyphenyl)-6-methyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (15)

Yield: 78%; M.P.: 220-222 °C; ^{1}H-NMR (300 MHz, DMSO-d_6) δ 9.35 (s, 1H, OH), 9.17 (s, 1H, NH), 7.68 (s, 1H, NH), 7.10 (t, $J_{5'(4',6')}$ = 8.1 Hz, 1H, H-5'), 6.66 (m, 3H, H-2', 4' ,6'), 5.04 (d, $J_{4,NH}$ = 3.0 Hz, 1H, H-4), 3.53 (s, 3H, OCH_3), 2.22 (s, 3H, CH_3); ^{13}C-NMR (100 MHz, DMSO-d_6):δ 165.8, 157.3, 152.1, 148.3, 146.0, 129.3, 116.7, 114.2, 112.9, 99.0, 53.6, 50.7, 17.7; EIMS *m/z* (% rel. abund.): 262 (M^+, 24), 230 (31), 169 (100); HREI-MS Calcd for $C_{13}H_{14}O_4N_2$: *m/z* = 262.0954, Found 262.0963.

Methyl 4-(3′-chloro-4′-hydroxyphenyl)-6-methyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (16)

Yield: 82%; M.P.: 262-264 °C; ^{1}H-NMR (300 MHz, DMSO-d_6) δ 10.12 (s, 1H, OH), 9.20 (s, 1H, NH), 7.69 (s, 1H, NH), 7.12 (s, 1H, H-2'), 6.99 (d, $J_{6',5'}$ = 9.0 Hz, 1H, H-6'), 6.90 (d, $J_{5',6'}$ = 6.0, 1H, H-5'), 5.03 (d, $J_{4,NH}$ = 3.0 Hz, 1H, H-4), 3.52 (s, 3H, OCH_3), 2.23 (s, 3H, CH_3); ^{13}C-NMR (100 MHz, DMSO-d_6):δ 165.8, 152.2, 152.0, 148.6, 136.6, 127.6, 125.8, 119.3, 116.6, 98.8, 52.9, 50.8, 17.8; EIMS *m/z* (% rel. abund.): 296 (M^+, 22), 298 (M + 2, 7), 281 (51), 169 (100), 137 (58); HREI-MS Calcd for $C_{13}H_{13}ClO_4N_2$: *m/z* = 296.0564, Found 296.0563.

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Methyl 6-methyl-4-(naphthalen-1′-yl)-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (17)

Yield: 84%; M.P.: 235-237 °C; ^{1}H-NMR (400 MHz, DMSO-d_6) δ 9.26 (s, 1H, NH), 8.29 (d, $J_{8',7'}$ = 8.4 Hz, 1H, H-8'), 7.94 (d, $J_{5',6'}$ = 8.0 Hz, 1H, H-5'), 7.83 (d, $J_{4',3'}$ = 8.0 Hz, 1H, H-4'), 7.73 (s, 1H, NH), 7.55 (m, 3H, H-3', 6', 7'), 7.37 (d, $J_{2',3'}$ = 6.8 Hz, 1H, H-2'), 6.03 (d, $J_{4,NH}$ = 3.2 Hz, 1H, H-4), 3.36 (s, 3H, OC$\underline{H_3}$), 2.35 (s, 3H, C$\underline{H_3}$); ^{13}C-NMR (100 MHz, DMSO-d_6):δ 165.7, 159.5, 151.7, 149.1, 139.8, 133.5, 130.0, 128.4, 127.9, 126.0, 125.6, 123.9, 123.5, 98.7, 50.6,49.6, 17.8; EIMS *m/z* (% rel. abund.): 296 (M^+, 58), 237 (39), 169 (100); HREI-MS Calcd for $C_{17}H_{16}O_3N_2$: *m/z* = 296.1161, Found 296.1148.

Methyl 6-methyl-4-(naphthalen-2′-yl)-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (18)

Yield: 86%; M.P.: 276-278 °C; ^{1}H-NMR (400 MHz, DMSO-d_6) δ 9.25 (s, 1H, N$\underline{H}$), 7.89 (m, 4H, H-5', 6', 7', 8'), 7.65 (s, 1H, N$\underline{H}$), 7.49 (m, 3H, H-1', 3', 4'), 5.31 (d, $J_{4,NH}$ = 2.8 Hz, 1H, H-4), 3.51 (s, 3H, OC$\underline{H_3}$), 2.28 (s, 3H, C$\underline{H_3}$); ^{13}C-NMR (100 MHz, DMSO-d_6):δ 165.8, 152.0, 148.9, 142.0, 132.7, 132.3, 128.3, 127.9, 127.4, 126.2, 125.9, 124.9, 124.4, 98.8, 54.1, 50.8, 17.9; EIMS *m/z* (% rel. abund.): 296 (M^+, 86), 237 (67), 169 (100); HREI-MS Calcd for $C_{17}H_{16}O_3N_2$: *m/z* = 296.1161, Found 276.1160.

Methyl 4-ethyl-6-methyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (19)

Yield: 80%; M.P.: 183-185 °C; ^{1}H-NMR (400 MHz, DMSO-d_6) δ 8.92 (s, 1H, NH), 7.27 (s, 1H, NH), 4.01 (d, $J_{4,NH}$ = 3.6 Hz, 1H, H-4), 3.58 (s, 3H, OC$\underline{H_3}$), 2.15 (s, 3H, C$\underline{H_3}$), 1.41 (m, 2H, $\underline{CH_2}CH_3$), 0.79 (t, $J_{CH3/CH2}$ = 7.2 Hz, $CH_2\underline{CH_3}$); ^{13}C-NMR (100 MHz, DMSO-d_6):δ 165.9, 152.7, 148.5, 98.5, 51.3, 50.6, 29.5, 17.6, 8.3; EIMS *m/z* (% rel. abund.): 199 (M^+, 1), 169 (100); HREI-MS Calcd for $C_9H_{14}O_3N_2$: *m/z* = 198.1004, Found 198.0990.

Methyl 4-butyl-6-methyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (20)

Yield: 75%; M.P.: 178-180 °C; ^{1}H-NMR (400 MHz, DMSO-d_6) δ 8.92 (s, 1H, N$\underline{H}$), 7.29 (s, 1H, N$\underline{H}$), 4.03 (d, $J_{4,NH}$ = 4.0 Hz, 1H, H-4), 3.58 (s, 3H, OC$\underline{H_3}$), 2.14 (s, 3H, C$\underline{H_3}$), 1.37 (t, 2H,$\underline{CH_2}CH_2CH_2CH_3$), 1.23 (m, 4H, $CH_2\underline{CH_2}\underline{CH_2}CH_3$), 0.83 (t, $J_{CH3/CH2}$ = 6.8 Hz, 3H, $CH_2CH_2CH_2\underline{CH_3}$); ^{13}C-NMR (100 MHz, DMSO-d_6):δ 165.9, 152.6, 148.3, 99.1, 50.6, 50.0, 36.3,

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25.8, 21.9, 17.68, 13.88; EIMS *m/z* (% rel. abund.): 226 (M^+, 2), 169 (100), 137 (70); HREI-MS Calcd for $C_{11}H_{18}O_3N_2$: *m/z* = 226.1317, Found 226.1314.

Methyl 4-isobutyl-6-methyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (21)

Yield: 80%; M.P.: 180-182 °C; ^{1}H-NMR (400 MHz, DMSO-d_6) δ 8.94 (s, 1H, NH), 7.39 (s, 1H, NH), 4.05 (d, $J_{4,NH}$ = 4.4 Hz, 1H, H-4), 3.58 (s, 3H, OCH_3), 2.14 (s, 3H, CH_3), 1.69 (m, 1H, CH(CH_3)$_2$, 1.39 (m, 1H, CH_2), 1.14 (m, 1H, CH_2), 0.85 (d, J = 6.4 Hz, 6H, CH(CH_3)$_2$); ^{13}C-NMR (75 MHz, DMSO-d_6):δ 165.8, 152.7, 148.3, 100.1, 50.7, 48.1, 45.9, 23.6, 22.8, 21.4, 17.7; EIMS *m/z* (% rel. abund.): 226 (M^+, 1), 169 (100), 137 (85); HREI-MS Calcd for $C_{11}H_{18}O_2N_3$: *m/z* = 226.1317, Found 226.1316.

Methyl 4-(furan-2′-yl)-6-methyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (22)

Yield: 86%; M.P.: 236-238 °C; ^{1}H-NMR (400 MHz, DMSO-d_6) δ 9.25 (s, 1H, NH), 7.76 (s, 1H, NH), 7.54 (s, 1H, H-5'), 6.34 (m, 1H, H-4'), 6.08 (d, $J_{3',4'}$ = 2.8 Hz, 1H, H-3'), 5.18 (d, $J_{4,NH}$ = 3.2 Hz, 1H, H-4), 3.55 (s, 3H, OCH_3), 2.22 (s, 3H, CH_3); ^{13}C-NMR (100 MHz, DMSO-d_6):δ 165.4, 155.8, 152.3, 149.6, 142.1, 110.3, 105.2, 96.5, 50.8, 47.6, 17.7; EIMS *m/z* (% rel. abund.): 236 (M^+, 60), 177 (100); HREI-MS Calcd for $C_{11}H_{12}O_4N_2$: *m/z* = 236.0797, Found 236.0811.

Methyl 6-methyl-4-(5′-methylfuran-2′-yl)-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (23)

Yield: 82%; M.P.: 198-200 °C; ^{1}H-NMR (400 MHz, DMSO-d_6) δ 9.20 (s, 1H, NH), 7.72 (s, 1H, NH), 5.92 (d, $J_{3',4'} = J_{4',3'}$ = 1.6 Hz, 2H, H-3', 4'), 5.11 (d, $J_{4,NH}$ = 3.2 Hz, 1H, H-4), 3.55 (s, 3H, OCH_3), 2.22 (s, 3H, furan-CH_3), 2.20 (s, 3H, CH_3); ^{13}C-NMR (75 MHz, DMSO-d_6):δ 165.6, 154.0, 152.3, 150.7, 149.5, 106.3, 106.0, 96.5, 50.8, 47.5, 17.7, 13.3; EIMS *m/z* (% rel. abund.): 250 (M^+, 58), 235 (75), 207 (100), 169 (52); HREI-MS Calcd for $C_{12}H_{14}O_4N_2$: *m/z* = 250.0954, Found 250.0935.

Methyl 6-methyl-2-oxo-4-(thiophen-3′-yl)-1,2,3,4-tetrahydropyrimidine-5-carboxylate (24)

Yield: 80%; M.P.: 237-239 °C; ^{1}H-NMR (400 MHz, DMSO-d_6) δ 9.18 (s, 1H, NH), 7.74 (s, 1H, NH), 7.45 (m, 1H, H-4'), 7.14 (d, $J_{2',5'}$ = 2.0 Hz, 1H, H-2'), 6.98 (dd, $J_{5',2'}$ = 0.4, $J_{5',4'}$ = 4.8 Hz, 1H, H-5'), 5.19 (d, $J_{4,NH}$ = 3.2 Hz, 1H, H-4), 3.57 (s, 3H, OCH_3), 2.21 (s, 3H, CH_3); ^{13}C-NMR (75

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MHz, DMSO-d_6):δ 165.7, 152.5, 148.7, 145.6, 126.6, 126.1, 120.7, 99.1, 50.8, 49.3, 17.7; EIMS *m/z* (% rel. abund.): 252 (M^+, 100), 193 (88), 169 (77); HREI-MS Calcd for $C_{11}H_{12}O_3N_2S$: *m/z* = 252.0569, Found 252.0572.

Methyl 6-methyl-2-oxo-4-(thiophen-2′-yl)-1,2,3,4-tetrahydropyrimidine-5-carboxylate (25)

Yield: 80%; M.P.: 226-228 °C; ^{1}H-NMR (400 MHz, DMSO-d_6) δ 9.32 (s, 1H, NH), 7.90 (s, 1H, NH), 7.34 (s, 1H, H-3'), 6.92 (s, 1H, H-5'), 6.88 (s, 1H, H-4'), 5.39 (s, 1H, H-4), 3.58 (s, 3H, OCH$_3$), 2.21 (s, 3H, CH$_3$); ^{13}C-NMR (100 MHz, DMSO-d_6):δ 165.5, 152.2, 148.9, 148.7, 126.7, 124.6, 123.5, 99.6, 50.8, 49.3, 17.7; EIMS *m/z* (% rel. abund.): 252 (M^+, 100), 193 (94), 169 (43); HREI-MS Calcd for $C_{11}H_{12}O_3N_2S$: *m/z* = 252.0569, Found 252.0561.

Methyl 6-methyl-4-(5′-methylthiophen-2′-yl)-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (26)

Yield: 80%; M.P.: 194-196 °C; ^{1}H-NMR (400 MHz, DMSO-d_6) δ 9.28 (s, 1H, NH), 7.82 (s, 1H, NH), 6.64 (s, 1H, H-3'), 6.58 (s, 1H, H-4'), 5.29 (d, $J_{4,NH}$ = 2.8, 1H, H-4), 3.58 (s, 3H, OCH$_3$), 2.34 (s, 3H, thiophene-CH$_3$), 2.19 (s, 3H, CH$_3$); ^{13}C-NMR (100 MHz, DMSO-d_6):δ 165.4, 152.1, 148.7, 146.1, 137.9, 124.6, 123.2, 99.5, 50.8, 49.3, 17.6, 14.8; EIMS *m/z* (% rel. abund.): 266 (M^+, 100), 207 (93), 169 (50); HREI-MS Calcd for $C_{12}H_{14}O_3N_2S$: *m/z* = 266.0725, Found 266.0723.

Methyl 4-(2′-chlorophenyl)-6-methyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (27)

Yield: 90%; M.P.: 226-228 °C; ^{1}H-NMR (300 MHz, DMSO-d_6) δ 9.29 (s, 1H, NH), 7.69 (s, 1H, NH), 7.40 (d, $J_{3',4'}$ = 7.2 Hz, 1H, H-3'), 7.31 (t, $J_{4'(3',5')}$ = 5.4 Hz, 1H, H-4'), 7.26 (m, 2H, H-5', 6'), 5.60 (d, $J_{4,NH}$ = 3.0 Hz, 1H, H-4), 3.44 (s, 3H, OCH$_3$), 2.28 (s, 3H, CH$_3$); ^{13}C-NMR (75 MHz, DMSO-d_6):δ 165.4, 151.3, 149.4, 141.5, 131.6, 129.4, 128.6, 127.7, 126.8, 97.6, 51.3, 50.6, 17.6; EIMS *m/z* (% rel. abund.): 279 (M^+, 6), 281 (M+2, 3), 245 (46), 169 (100); HREI-MS Calcd for $C_{13}H_{13}O_3N_2Cl$ *m/z* = 280.0615, Found 280.0602.

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Methyl 4-(2′,6′-dichlorophenyl)-6-methyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (28)

Yield: 85%; M.P.: 292-294 °C; ^{1}H-NMR (300 MHz, DMSO-d_6) δ 9.29 (s, 1H, NH), 7.57 (s, 1H, NH), 7.41 (d, $J_{3',4'} = J_{5',4'}$ = 7.8 Hz, 2H, H-3', 5'), 7.28 (t, $J_{4'(3',5')}$ = 7.8 Hz, 1H, H-4'), 6.14 (s, 1H, H-4), 3.35 (s, 3H, OCH$_3$), 2.15 (s, 3H, CH$_3$); ^{13}C-NMR (100 MHz, DMSO-d_6):δ 165.4, 150.5, 149.7, 137.5, 135.1, 130.1, 130.0, 129.3, 129.3, 93.9, 52.2, 50.2, 17.8; EIMS *m/z* (% rel. abund.): 314 (M^+, 2), 255 (37), 169 (100); HREI-MS Calcd for $C_{13}H_{12}Cl_2O_3N_2$: *m/z* = 314.0225, Found 314.0217.

Methyl 4-(4′-chlorophenyl)-6-methyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (29)

Yield: 82%; M.P.: 205-207 °C; ^{1}H-NMR (400 MHz, DMSO-d_6) δ 9.25 (s, 1H, NH), 7.77 (s, 1H, NH), 7.38 (d, $J_{3',2'/5',6'}$ = 8.0 Hz, 2H, H-3', 5'), 7.24 (d, $J_{2',3'/6',5'}$ = 8.0, 2H, H-2', 6'), 5.12 (d, $J_{4,NH}$ = 2.4 Hz, 1H, H-4), 3.51 (s, 3H, OCH$_3$), 2.23 (s, 3H, CH$_3$); ^{13}C-NMR (100 MHz, DMSO-d_6):δ 165.7, 151.9, 149.0, 143.6, 131.8, 129.7, 128.4, 128.1, 128.1, 98.6, 53.2, 50.8, 17.8; EIMS *m/z* (% rel. abund.): 280 (M^+, 17), 282 (M + 2, 5), 265 (39), 169 (100), 137 (95); HREI-MS Calcd for $C_{13}H_{13}ClO_3N_2$: *m/z* = 280.0615, Found 280.0607.

Methyl 4-(4′-bromophenyl)-6-methyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (30)

Yield: 82%; M.P.: 213-215 °C; ^{1}H-NMR (400 MHz, DMSO-d_6) δ 9.25 (s, 1H, NH), 7.77 (s, 1H, NH), 7.52 (d, $J_{3',2'/5',6'}$ = 8.0 Hz, 2H, H-3', 5'), 7.18 (d, $J_{2',3'/6',5'}$ = 8.0 Hz, 2H, H-2', 6'), 5.10 (s, 1H, H-4), 3.51 (s, 3H, OCH$_3$), 2.23 (s, 3H, CH$_3$); ^{13}C-NMR (100 MHz, DMSO-d_6):δ 165.6, 151.8, 148.9, 143.9, 131.3, 131.3, 128.4, 128.4, 120.2, 98.5, 53.2, 50.7, 17.7; EIMS *m/z* (% rel. abund.): 324 (M^+, 15), 326 (M + 2, 15), 309 (30), 169 (100), 137 (80); HREI-MS Calcd for $C_{13}H_{13}BrO_3N_2$: *m/z* = 324.0110, Found 324.0081.

Methyl 4-(2′-fluorophenyl)-6-methyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (31)

Yield: 82%; M.P.: 255-257 °C; ^{1}H−NMR (400 MHz, DMSO−d_6) δ 9.25 (s, 1H, NH), 7.68 (s, 1H, NH), 7.31 (m, 2H, H-4', 5'), 7.15 (d, $J_{3',4'} = J_{6',5'}$ = 8.0 Hz, 2H, H-3', 6'), 5.45 (d, $J_{4,NH}$ = 2.0 Hz,

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1H, H-4), 3.46 (s, 3H, OC<u>H</u>$_3$), 2.25 (s, 3H, C<u>H</u>$_3$); ^{13}C-NMR (100 MHz, DMSO-d_6):δ 165.5, 160.6, 158.1, 151.6, 149.1, 131.5, 129.4, 124.5, 115.6, 97.2, 50.7, 48.6, 17.8; EIMS *m/z* (% rel. abund.): 264 (M^+, 12), 249 (68), 169 (100), 137 (80); HREI-MS Calcd for $C_{13}H_{13}FO_3N_2$: *m/z* = 264.0910, Found 264.0905.

Methyl 6-methyl-2-oxo-4-(4′-(trifluoromethyl) phenyl)-1,2,3,4-tetrahydropyrimidine-5-carboxylate (32)

Yield: 82%; M.P.: 188-190 °C; ^{1}H-NMR (400 MHz, DMSO-d_6) δ 9.30 (s, 1H, N<u>H</u>), 7.83 (s, 1H, N<u>H</u>), 7.70 (d, $J_{3',2/5',6'}$ = 8.0 Hz, 2H, H-3', 5'), 7.45 (d, $J_{2',3'/6',5'}$ = 8.0 Hz, 2H, H-2', 6'), 5.22 (d, $J_{4,NH}$ = 3.2 Hz, 1H, H-4), 3.52 (s, 3H, OC<u>H</u>$_3$), 2.25 (s, 3H, C<u>H</u>$_3$); ^{13}C-NMR (100 MHz, DMSO-d_6):δ 165.6, 151.9, 149.3, 149.1, 127.0, 127.0, 125.5, 125.5, 125.5, 125.4, 98.3, 53.6, 50.8, 17.9; EIMS *m/z* (% rel. abund.): 314 (M^+, 26), 255 (48), 169 (100), 137 (36); HREI-MS Calcd for $C_{14}H_{13}F_3O_3N_2$: *m/z* = 314.0878, Found 314.0897.

Methyl 6-methyl-4-(4′-nitrophenyl)-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (33)

Yield: 80%; M.P.: 235-237 °C; ^{1}H-NMR (300 MHz, DMSO-d_6) δ 9.37 (s, 1H, N<u>H</u>), 8.21 (d, $J_{3',2'}$ = $J_{5',6'}$ = 8.7 Hz, 2H, H-3', 5'), 7.90 (s, 1H, N<u>H</u>), 7.50 (d, $J_{2',3'/6',5'}$ = 8.7 Hz, 2H, H-2', 6'), 5.26 (d, $J_{4,NH}$ = 3.3 Hz, 1H, H-4), 3.52 (s, 3H, OC<u>H</u>$_3$), 2.25 (s, 3H, C<u>H</u>$_3$); ^{13}C-NMR (100 MHz, DMSO-d_6):δ 165.5, 151.8, 151.8, 149.6, 146.7, 127.6, 127.6, 123.8, 123.8, 98.0, 53.5, 50.9, 17.9; EIMS *m/z* (% rel. abund.): 291 (M^+, 5), 169 (100), 137 (62); HREI-MS Calcd for $C_{13}H_{13}O_5N_3$: *m/z* = 291.0855, Found 291.0840.

Methyl 4-(4′-isopropylphenyl)-6-methyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (34)

Yield: 80%; M.P.: 200-202 °C; ^{1}H-NMR (300 MHz, DMSO-d_6) δ 9.18 (s, 1H, N<u>H</u>), 7.69 (s, 1H, N<u>H</u>), 7.19 (m, 4H, H-2', 3', 5', 6'), 5.09 (d, $J_{4,NH}$ = 3.0 Hz, 1H, H-4), 3.52 (s, 3H, OC<u>H</u>$_3$), 2.87 (m, 1H, <u>CH</u>(CH$_3$)$_2$, 2.23 (s, 3H, C<u>H</u>$_3$), 1.17 (d, J = 6.9 Hz, 6H, CH(C<u>H</u>$_3$)$_2$); ^{13}C-NMR (75 MHz, DMSO-d_6):δ 165.8, 152.2, 148.4, 147.3, 142.1, 126.2, 126.2, 126.0, 126.0, 99.1, 53.4, 50.7, 33.0, 23.8, 23.8, 17.7; EIMS *m/z* (% rel. abund.): 288 (M^+, 47), 229 (81), 169 (100); HREI-MS Calcd for $C_{16}H_{20}O_3N_2$: *m/z* = 288.1474, Found 288.1464.

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Methyl 4-(4′-(dimethyl amino) phenyl)-6-methyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (35)

Yield: 82%; M.P.: 238-240 °C; ^{1}H-NMR (300 MHz, DMSO-d_6) δ 9.17 (s, 1H, NH), 7.68 (s, 1H, NH), 6.88 (m, 2H, H-3', 5'), 6.84 (d, $J_{2',3'}$ = 6.0 Hz, 1H, H-2'), 6.71 (d, $J_{6',5'}$ = 9.0 Hz, 1H, H-6'), 5.09 (d, $J_{4,NH}$ = 3.0 Hz, 1H, H-4), 3.70 (s, 6H, N(CH$_3$)$_2$), 3.53 (s, 3H, OCH$_3$), 2.24 (s, 3H, CH$_3$); ^{13}C-NMR (75 MHz, DMSO-d_6):δ 165.9, 152.2, 149.7, 147.8, 132.4, 126.7, 126.7, 112.2, 112.2, 99.5, 53.1, 53.1, 50.6, 50.6, 17.7; EIMS *m/z* (% rel. abund.): 289 (M^+ 100), 230 (96), 169 (30), 120 (96); HREI-MS Calcd for $C_{15}H_{19}O_3N_3$: *m/z* = 289.1426, Found 289.1420.

Methyl 4-(3′-(benzyloxy) phenyl)-6-methyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (36)

Yield: 80%; M.P.: 173-175 °C; ^{1}H-NMR (400 MHz, DMSO-d_6) δ 9.18 (s, 1H, NH), 7.71 (s, 1H, NH), 7.42 (m, 5H, H-2", 3", 4", 5", 6"), 7.24 (t, $J_{5'(4',6')}$ = 7.6 Hz, 1H, H-5'), 6.90 (m, 3H, H-2', 4', 6'), 5.10 (s, 1H, H-4), 5.04 (s, 2H, OCH$_2$Ph), 3.51 (s, 3H, OCH$_3$), 2.23 (s, 3H, CH$_3$); ^{13}C-NMR (100 MHz, DMSO-d_6):δ 165.7, 158.3, 152.1, 148.7, 146.1, 136.9, 129.5, 128.3, 128.3, 127.7, 127.7, 127.7, 127.7, 118.4, 113.0, 98.7, 69.1, 53.5, 50.7, 17.7; EIMS *m/z* (% rel. abund.): 352 (M^+, 29), 293 (15), 261 (72), 169 (78), 91 (100); HREI-MS Calcd for $C_{20}H_{20}O_4N_2$: *m/z* = 352.1423, Found 352.1422.

Methyl 4-(2′-(benzyloxy) phenyl)-6-methyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (37)

Yield: 80%; M.P.: 205-207 °C; ^{1}H-NMR (400 MHz, DMSO-d_6) δ 9.13 (s, 1H, NH), 7.51 (d, $J_{2"/3"}$ = 7.2 Hz, 1H, H-2"), 7.40 (t, $J_{3"(2",6")}$ = $J_{5"(4",6")}$ = 6.8 Hz, 2H, H-3", 5"), 7.32 (t, $J_{4"(3",5")}$ = 6.8 Hz, 1H, H-4"), 7.32 (t, $J_{6",5"}$ = 7.2 Hz, 1H, H-6"), 7.22 (s, 1H, NH), 7.19 (t, $J_{4'(3',5')}$ = 7.6, 1H, H-4'), 7.09 (d, $J_{6',5'}$ = 7.6 Hz, 1H, H-6'), 7.01 (d, $J_{3',4'}$ = 8.4 Hz, 1H, H-3'), 6.88 (t, $J_{5'(4',6')}$ = 7.6 Hz, 1H, H-5'), 5.60 (s, 1H, H-4), 5.17 (s, 2H, OCH$_2$Ph), 3.43 (s, 3H, OCH$_3$), 2.26 (s, 3H, CH$_3$); ^{13}C-NMR (100 MHz, DMSO-d_6):δ 165.8, 155.2, 152.1, 149.0, 137.4, 132.0, 128.6, 128.4, 128.4, 127.6, 127.2, 127.2, 127.2, 120.5, 112.5, 97.6, 69.3, 50.7, 48.7, 17.8; EIMS *m/z* (% rel. abund.): 352 (M^+, 30), 169 (5), 91 (100); HREI-MS Calcd for $C_{20}H_{20}O_4N_2$: *m/z* = 352.1423, Found 352.1408.

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References

1. K. M. Khan, S. M. Saad, N. N. Shaikh, S. Hussain, M. I. Fakhri, S. Perveen, M. Taha, M. I. Choudhary, Bioorg. Med. Chem. **2014**, *22*, 3449.
2. M. Wakabayashi, W. H. Fishman, Ed, Academic Press: New-York. **1970**, 519.
3. K. Paigen, Prog. Nucleic Acid Res. Mol. Bio. **1989**, *37*, 155.
4. C. M. Plum, Enzymol. Bio. Clin. **1966**, *8*, 97.
5. H. C. Gonick, H. J. Kramer, A. E. Schapiro, Arch. Int. Med. **1973**, *132*, 63.
6. N. Bank, S. H. Bailine, N. Eng. J. Med. **1965**, *272*, 70.
7. A. P. Roberts, J. Frampton, S. M. M. Karim, R. W. Beard, N. Eng. J. Med. **1967**, *276*, 1468.
8. A. R. Ronald, F. Silverblatt, H. Clark, R. E. Cutler, M. Turck, Appl. Microbial. 1971, 21, 990.
9. H. A. Kallet, L. Lapco, J. Urol. **1967**, *97*, 352.
10. A. Schapiro, W. Paul, H. Gonick, J. Urol. **1968**, *100*, 146.
11. E. Hradec, R. Petrik, J. Pezlarova, J. Urol. **1965**, *94*, 430.
12. E. Boyland, J. E. Gasson, D. C. Williams, Br. J. Cancer **1957**, *11*, 120.
13. J. A. Joule, K. Mills, Heterocycl. Chem. **2000**, 194.
14. C. O. Kappe, Eur. J. Med. Chem. **2000**, *35*, 1043.
15. S. Putatunda, A. Chakraborty, *Comptes Rendus Chimie*, **2014**, *17*, 1057.
16. C. Jiang, Q. D. You, *Chinese Chem. Lett.* **2007**, *18*, 647.
17. B. K. Singh, M. Mishra, N. Saxena, G. Yadav, Eur. J. Med. Chem. **2008**, *43*, 2717.
18. M. B. Deshmukh, S. M. Salunkhe, D. R. Patil, P. V. Anbhule, Eur. J. Med. Chem. **2009**, *44*, 2651.
19. A. Barakat, M. S. Islam, A. M. Al-Majid, H. A. Ghabbour, H. K. Fun, K. Javed, A. Wadood, Bioorg. Med. Chem. **2015**, *23*, 6740.
20. S. Kang, G. Cooper, S. F. Dunne, C. H. Luan, D. J. Surmeier, R. B. Silverman, Bioorg. Med. Chem. **2013**, *21*, 4365.

ACCEPTED MANUSCRIPT

21. J. Jadhav, A. Juvekar, R. Kurane, S. Khanapure, R. Salunkhe, G. Rashinkar, Eur. J. Med. Chem. **2013**, *65*, 232.
22. A. Kamal, M. S. Malik, S. Bajee, S. Azeeza, S. Faazil, S. Ramakrishna, M. V. P. Vishnuwardhan, Eur. J. Med. Chem. **2011**, *46*, 3274.
23. K. M. Khan, Z. S. Saify, S. Hayat, M. Z. Khan, F. Noor, M. I. Choudhary, Zia Ullah, S. Perveen, J. Chem. Soc. Pak. **2002**, *24*, 226.
24. K. M. Khan, Z. S. Saify, S. Begum, F. Noor, M. Z. Khan, S. Hayat, M. I. Choudhary, S. Perveen, Zia Ullah, Nat. Prod. Res. **2003**, *17*, 115.
25. K. M. Khan, Z. S. Saify, Z. A. Khan, M. Ahmed, M. Saeed, R. J. Abdel-Jalil, G. Grubler, W. Voelter, Naturforsch. **1999**, *54b*, 1210.
26. K. M. Khan, Z. S. Saify, Z. A. Khan, M. Ahmed, M. Saeed, M. Schick, H. J. Kohlbau, W. Voelter, Arzneim Forsch./Drug Res. **2000**, *50*, 915.
27. Z. S. Saify, K. M. Khan, S. M. Haider, S. T. A. Shah, M. Saeed, M. S. Shekhani, W. Voelter, Naturforsch. **1999**, *54b*, 1327.
28. J. H. Zaidi, F. Naeem, R. Iqbal, M. I. Choudhary, K. M. Khan, S. T. A. Shah, S. Hayat, W. Z. Voelter, Naturforsch. **2001**, *56b*, 689.
29. K. M. Khan, S. Rahat, M. I. Choudhary, Atta-ur-Rahman, U. Ghani, S. Perveen, S. Khatoon, A. Dar, A. Malik, Helv. Chim. Acta, **2002**, *85*, 559.
30. U. Salar, M. Taha, N. H. Ismail, K. M. Khan, S. Imran, S. Perveen, A. Wadood, M. Riaz, Bioorg. Med. Chem. **2016**, *24*, 1909.
31. N. Khairunissa, N. A. Zawawi, M. Taha, N. Ahmat, A. Wadood, N. H. Ismail, F. Rahim, M. Ali, N. Abdullah, K. M. Khan, Bioorg. Med. Chem. **2015**, *23*, 3119.
32. K. M. Khan, M. I. Fakhri, N. N. Sheikh, S. M. Saad, S. Hussain, S. Perveen, M. I. Choudhary, Med. Chem. **2014**, *10*, 778.
33. K. M. Khan, F. Rahim, A. Wadood, M. Taha, M. Khan, S. Naureen, N. Ambreen, S. Hussain, S. Perveen, M. I. Choudhary, Bioorg. Med. Chem. Lett. **2014**, *24*, 1825.
34. K. M. Khan, A. Karim, S. Saied, N. Ambreen, X. Rustamova, S. Naureen, S. Mansoor, M. Ali, S. Perveen, M. I. Choudhary, G. A. Morales, Mol. Diver. **2014**, *18*, 295.
35. K. M. Khan, M. Khan, N. Ambreen, F. Rahim, S. Naureen, S. Perveen, M. I. Choudhary, W. Voelter, Med. Chem. **2012**, *8*, 421.

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36. K. M. Khan, F. Rahim, S. A. Halim, M. Taha, M. Khan, S. Perveen, Zaheer-ul-Haq, M. A. Mesaik, M. I. Choudhary, Bioorg. Med. Chem. **2011**, *19*, 4286.

37. a) M. Taha, N. H. Ismail, S. Imran, M. Selvaraj, H. Rashwan, F. U. Farhanah, F. Rahim, K. K. Selvarajan, M. Ali, Bioorg. Chem. **2015**, *61*, 36. b) M. Taha, N. H. Ismail, W. Jamil, K. M. Khan, U. Salar, S. M. Kashif, F. Rahim, Y. Latif, Med. Chem. Res. **2015**, *24*, 3166.

38. S. Jain, W. B. Drendel, Z. W. Chen, F. S. Mathews, W. S. Sly, J. H. Grubb, Nat. Str. Mol. Bio. **1996**, *3*, 375.

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Graphical Abstract

Dihydropyrimidones: As Novel Class of β-Glucuronidase Inhibitors

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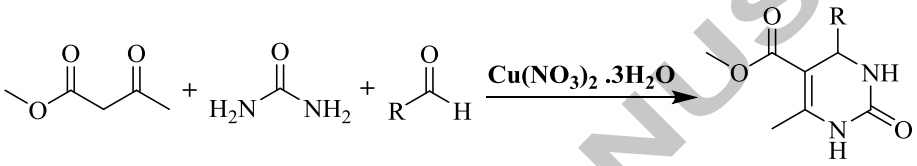

Scheme-1: "One-Pot" three Component Syntheses of Dihydropyrimidone Derivatives 1-37

Dihydropyrimidone Derivatives 1-37

IC_{50} = 0.6-198 μM **IC_{50} = 9.38-78.16 μM**

Standard = D-Saccharic acid 1,4-lactone (IC_{50} = 48.4 ± 1.25 μM)

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Research Highlights

- Dihydropyrimidones **1-37** were synthesized by "one pot" three component reaction
- *In vitro* β-glucuronidase inhibitory activity were performed
- Structure-activity relationship was established
- Molecular docking studies were carried out

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