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Biology-oriented drug synthesis (BIODS): *In vitro* β-glucuronidase inhibitory and *in silico* studies on 2-(2-methyl-5-nitro-1*H*-imidazol-1-yl)ethyl aryl carboxylate derivatives

Uzma Salar, Khalid Mohammed Khan, Muhammad Taha, Nor Hadiani Ismail, Basharat Ali, Qurat-ul-Ain, Shahnaz Perveen, Mehreen Ghufran, Abdul Wadood

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Graphical Abstract

Biology-Oriented Drug Synthesis (BIODS): *In Vitro* β-Glucuronidase Inhibitory and *In Silico* Studies on 2-(2-Methyl-5-nitro-1*H*-imidazol-1-yl)ethyl aryl carboxylate Derivatives

Uzma Salar,[a] Khalid Mohammed Khan,[a] Muhammad Taha,[b,c] Nor Hadiani Ismail,[b] Basharat Ali,[a] Qurat-ul-Ain,[a] Shahnaz Perveen,[d] Mehreen Ghufran[e], Abdul Wadood[e]

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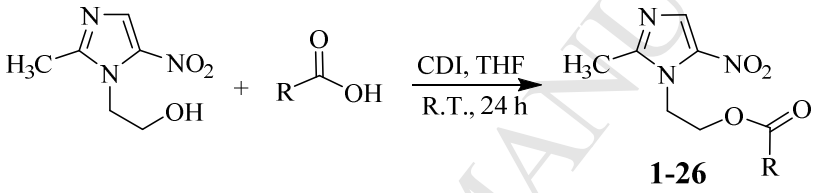
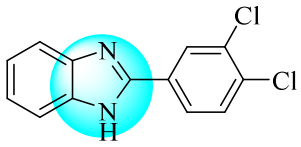
[c]*Faculty of Applied Science, Universiti Teknologi MARA, Shah Alam 40450, Selangor D. E., Malaysia*

[d]*PCSIR Laboratories Complex, Karachi, Shahrah-e-Dr. Salimuzzaman Siddiqui, Karachi-75280, Pakistan*

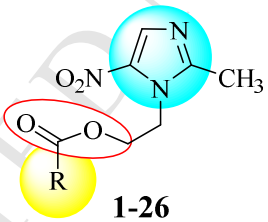
[e]*Department of Biochemistry, Computational Medicinal Chemistry Laboratory, UCSS, Abdul Wali Khan University, Mardan, Pakistan*



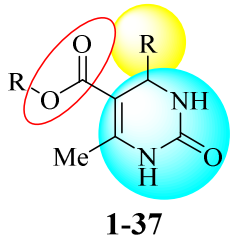
Scheme-1: Syntheses of 2-(2-methyl-5-nitro-1*H*-imidazol-1-yl)ethyl aryl carboxylate derivatives 1-26



IC_{50} = 6.33 ± 0.40 μM



IC_{50} = 1.20 ± 0.01-60.30 ± 1.40 μM



IC_{50} = 9.38-78.16 μM

Standard D-saccharic acid 1,4-lactone (IC_{50} = 48.38 ± 1.05 μM)

Biology-Oriented Drug Synthesis (BIODS): *In Vitro* β-Glucuronidase Inhibitory and *In Silico* Studies on 2-(2-Methyl-5-nitro-1*H*-imidazol-1-yl)ethyl aryl carboxylate Derivatives

Uzma Salar,[a] Khalid Mohammed Khan,[*a] Muhammad Taha,[b,c] Nor Hadiani Ismail,[b] Basharat Ali,[a] Qurat-ul-Ain,[a] Shahnaz Perveen,[d] Mehreen Ghufran[e], Abdul Wadood[e]

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[b]*Atta-ur-Rahman Institute for Natural Product Discovery, Universiti Teknologi MARA (UiTM), Puncak Alam Campus, 42300 Bandar Puncak Alam, Selangor D. E., Malaysia*

[c]*Faculty of Applied Science, Universiti Teknologi MARA, Shah Alam 40450, Selangor D. E., Malaysia*

[d]*PCSIR Laboratories Complex, Karachi, Shahrah-e-Dr. Salimuzzaman Siddiqui, Karachi-75280, Pakistan*

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Abstract: Current study is based on the biology-oriented drug synthesis (BIODS) of 2-(2-methyl-5-nitro-1*H*-imidazol-1-yl)ethyl aryl carboxylate derivatives **1**-**26**, by treating metronidazole with different aryl and hetero-aryl carboxylic acids in the presence of 1,1'-carbonyl diimidazole (CDI) as a coupling agent. Structures of all synthetic derivatives were confirmed with the help of various spectroscopic techniques such as EI-MS, ^{1}H -NMR and ^{13}C-NMR. CHN elemental analyses were also found in agreement with the calculated values. Synthetic derivatives were evaluated to check their β-glucuronidase inhibitory activity which revealed that except few derivatives, all demonstrated good inhibition in the range of IC_{50} = 1.20 ± 0.01-60.30 ± 1.40 μM as compared to the standard D-saccharic acid 1,4-lactone (IC_{50} = 48.38 ± 1.05 μM). Compounds **1**, **3**, **4**, **6**, **9**-**19**, and **21**-**24** were found to be potent analogs and showed superior activity than standard. Limited structure-activity relationship is suggested that the molecules having electron withdrawing groups like NO_2, F, Cl, and Br, were displayed better activity than the compounds with electron donating groups such as Me, OMe and BuO. To verify these interpretations, *in silico* study was also performed, a good correlation was observed between bioactivities and docking studies.

Keywords: Synthesis; *in vitro*; β-glucuronidase; *in silico*; structure-activity relationship.

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Introduction

Nitroimidazole derived compounds have a diverse spectrum of biological activities [1]. Amongst them, 5-nitroimidazole is the privileged scaffold of many marketed drugs such as secnidazole, ketoconazole, miconazole, and metronidazole (Flagyl®) which are being clinically used to treat the bacterial infections since many years (Figure-1). Toxicology and metabolism of nitroimidazoles have been well characterized and documented [2-4]. Metronidazole is one of the most important nitroimidazole derivatives which has been commonly used as antimicrobial prescription [4-10].

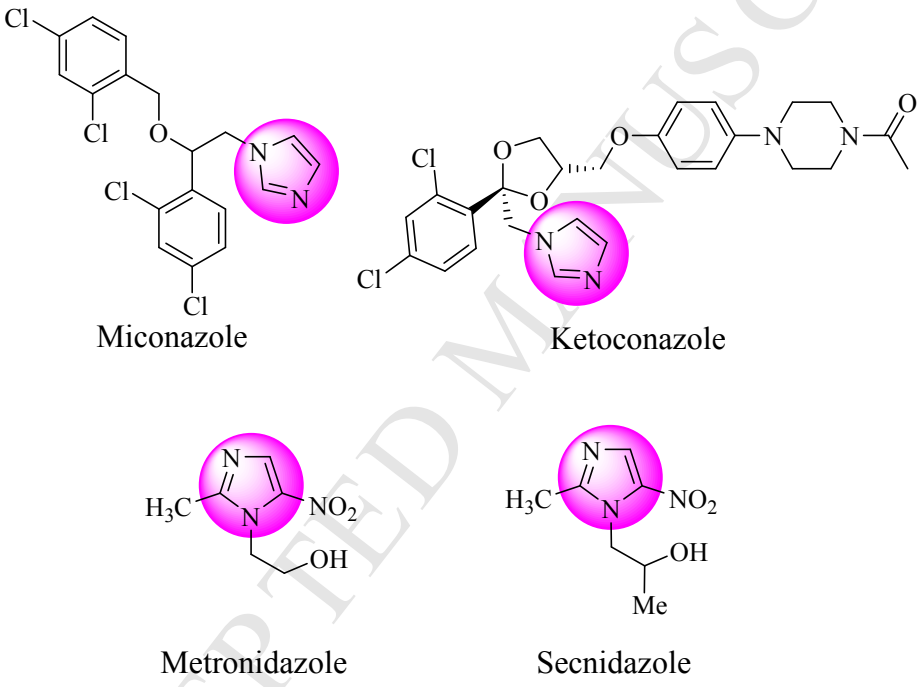


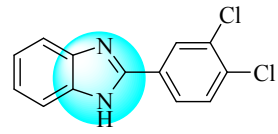
Figure-1: Marketed Analogs of Imidazole

β-Glucuronidase enzyme (EC 3.2.1.31) is commonly found in anaerobic bacteroides, *Clostridia*, *Escherichia*, and *Peptostreptococcus*. It is also found in human body fluids, serum, blood cells, gastric juice, bile, urine, spleen, and in organs like kidney, liver, lung, and muscles [11-13]. This enzyme catalyzes the breaking of *β*-glucuronosyl-*O*-bonds [14]. Insufficiency of this enzyme causes Sly syndrome in humans which is related to the increase level of glycosaminoglycans in cells [15,16]. Similarly, over-expression is associated with many disorders such as urinary tract infections [17-20], active pyelonephritis and acute renal necrosis [21]. Certain pathological

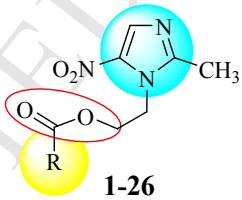
conditions including epilepsy, cancer, acquired immune deficiency syndrome (AIDS), renal diseases, neoplasm of breast, larynx, bladder, and testes, and inflammation in joints and hepatic [22-24], also leads to over-expression of *β*-glucuronidase. Hence, it is the topmost task to inhibit this enzyme for curing several pathologies.

Based on our previously introduced terminology biology-oriented drug synthesis (BIODS) [25] which aims to design and synthesis of libraries of compounds on the skeleton of authentic drugs (marketed drugs) with more and diversified biological activities.

We have already identified many heterocycles as potential class of *β*-glucuronidase inhibitors [26-33]. Since, we have reported the *β*-glucuronidase inhibitory activity of benzimidazoles [32] and recently published the ester derivatives of dihydropyrimidone as potent inhibitors of *β*-glucuronidase enzyme [26], therefore, we decided to screen metronidazole esters for their *β*-glucuronidase inhibitory activity due to their appreciable structural similarity with benzimidazole as well as the esters of dihydropyrimidones (Figure-2). Herein, this manuscript describes the BIODS of 2-(2-methyl-5-nitro-1*H*-imidazol-1-yl)ethyl aryl carboxylate derivatives **1-26** and evaluation of their *β*-glucuronidase inhibition studies. To the best of our information, these compounds have never been reported for *β*-glucuronidase inhibitory activity.

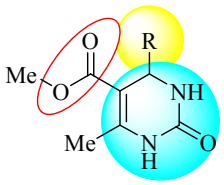


IC_{50} = 6.33 ± 0.40 *μ*M



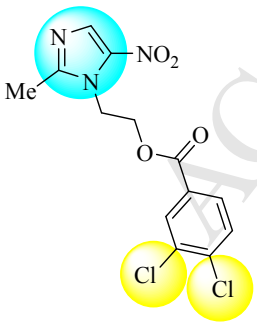
1-26

IC_{50} = 1.20 ± 0.01-60.30 ± 1.40 *μ*M



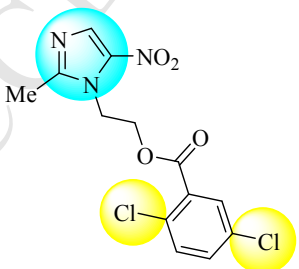
1-37

IC_{50} = 9.38-78.16 *μ*M



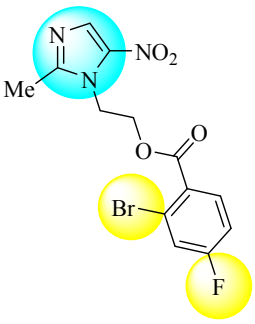
11

IC_{50} = 3.60 ± 0.10 *μ*M



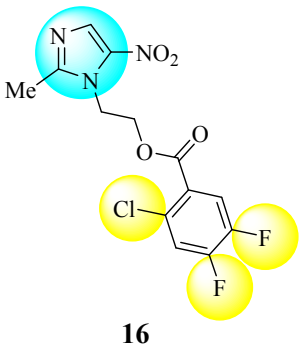
12

IC_{50} = 6.20 ± 0.10 *μ*M



15

IC_{50} = 6.30 ± 0.1 *μ*M



16

IC_{50} = 1.20 ± 0.01 *μ*M

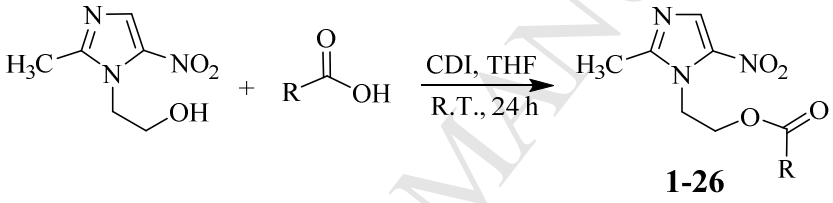
Standard = D-Saccharic acid 1,4-lactone (IC_{50} = 48.38 ± 1.05 *μ*M)

Figure-2: Rationale of the Current Study

Results and Discussion

Chemistry

All derivatives **1-26** were synthesized by reacting metronidazole with different aryl and hetero-aryl carboxylic acid in the presence of 1,1'-carbonyl diimidazole (CDI) as a coupling agent. Reactions were performed in tetrahydrofuran (THF) at room temperature. CDI was readily reacted with the carboxylic acid derivative to form acid imidazole intermediate which was immediately coupled with metronidazole to afford products **1-26**. All synthetic molecules were structurally characterized by spectroscopic techniques such as, EI-MS, ^{1}H-NMR and ^{13}C-NMR. CHN analysis was also performed and found satisfactory.

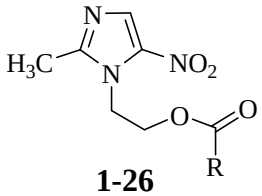


Scheme-1: Syntheses of 2-(2-methyl-5-nitro-1*H*-imidazol-1-yl)ethyl aryl carboxylate derivatives **1-26**

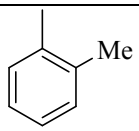
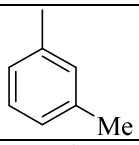
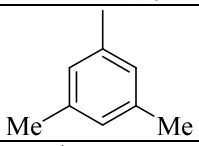
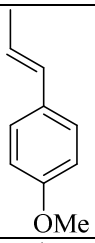
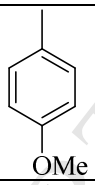
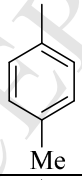
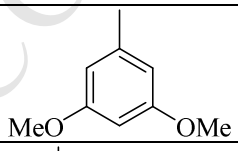
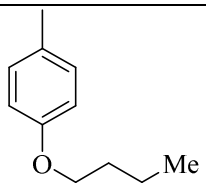
In Vitro β-Glucuronidase Inhibitory Activity

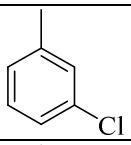
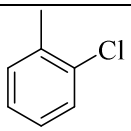
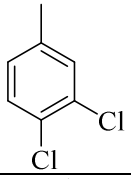
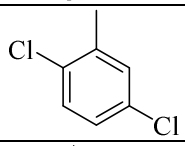
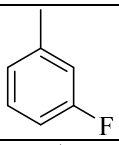
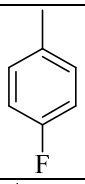
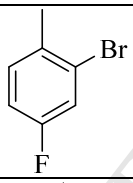
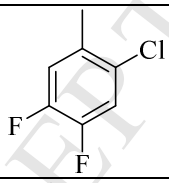
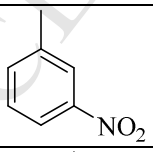
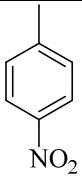
Synthetic compounds **1-26** were screened for their *in vitro β*-glucuronidase inhibitory activity. It is worth mentioning that except compounds **2**, **8**, **25**, and **26**, all compounds were demonstrated good inhibitory activity in the range of IC_{50} = 1.20 ± 0.01-60.30 ± 1.40 μM as compared to the standard D-saccharic acid 1,4-lactone (IC_{50} = 48.38 ± 1.05 μM) (Table-1). Compounds **1**, **3**, **4**, **6**, **9-19** and **21-24** were exhibited superior *β*-glucuronidase inhibitory activity than standard as given in Table-1.

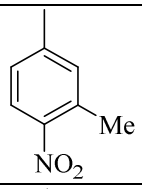
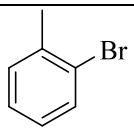
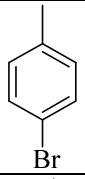
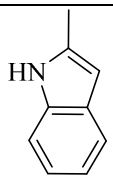
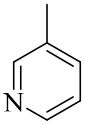
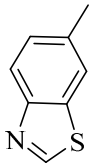
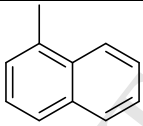
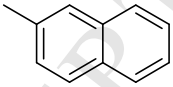
Table-1: *In vitro* β-glucuronidase inhibitory activity and docking scores of synthetic derivatives **1-26**



1-26

Comp. No.	R	IC_{50} ± SEM[a] [μM]	Docking Score (S)
1		44.16 ± 0.90	-7.1356
2		NA[b]	-6.7176
3		35.50 ± 0.80	-7.5236
4		31.10 ± 0.75	-7.6320
5		60.16 ± 1.40	-7.7753
6		24.20 ± 0.50	-8.0149
7		60.30 ± 1.40	-7.7583
8		NA[b]	-6.5987

9		16.30 ± 0.20	-7.9033
10		19.30 ± 0.30	-7.9216
11		3.60 ± 0.10	-9.3028
12		6.20 ± 0.10	-8.6108
13		8.10 ± 0.10	-8.4793
14		12.40 ± 0.20	-7.9357
15		6.30 ± 0.1	-9.0313
16		1.20 ± 0.01	-9.7108
17		27.60 ± 0.70	-7.6861
18		8.90 ± 0.20	-8.2156

19		7.30 ± 0.10	-8.4376
20		48.50 ± 1.10	-7.5368
21		42.61 ± 0.90	-7.5297
22		2.10 ± 0.10	-9.6250
23		32.60 ± 0.80	-7.5639
24		8.40 ± 0.10	-8.6232
25		NA[b]	-7.3328
26		NA[b]	-7.2938
Standard[c] = D-saccharic acid 1,4-lactone		**48.38 ± 1.05**	

[a]IC_{50} values (mean ± standard error of mean); [b]NA (Not active); [c]Standard inhibitor for β-glucuronidase.

Structure-Activity Relationship (SAR)

Limited structure-activity relationship (SAR) is suggested that apparently all structural features of compounds **1-26** as shown in Figure-3 are taking part in the inhibitory activity, however, the variation in the activity of different compound was due to the nature and position of substituents at ring "R".

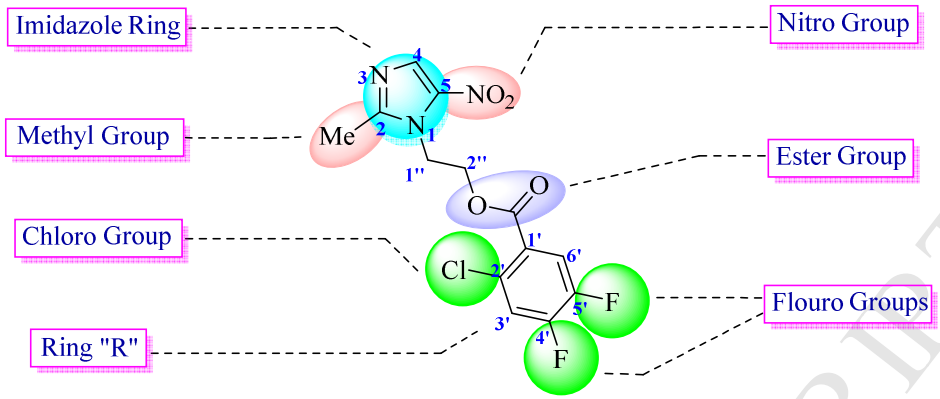


Figure-3: Structure of compound **16** with numbering

As shown in table-1, compound **20** (IC_{50} = 48.50 ± 1.10 μM) with *ortho* bromo substitution at ring "R", showed inhibitory activity comparable to standard D-saccharic acid 1,4-lactone (IC_{50} = 48.38 ± 1.05 μM). Switching of bromo residue from *ortho* to *para* as in compound **21** (IC_{50} = 42.61 ± 0.9 μM), a slight increased activity was observed. It proves our hypothesis that the position of a substituent also influence the activity. Structure of compound **15** (IC_{50} = 6.30 ± 0.1 μM) is closely resembles to compound **20** (IC_{50} = 48.50 ± 1.10 μM), only the addition of fluoro group at *para* position of **15**, displayed an eight-fold better inhibitory activity (Figure-4).

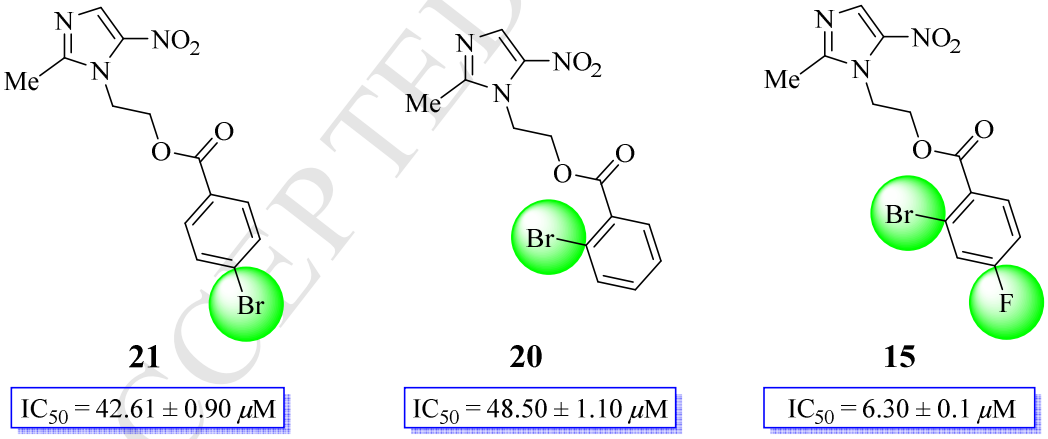


Figure-4: Comparison of structure-activity relationship of compound **21** with **20** and **15**.

Amongst the chloro substituted derivatives, compounds **11** (IC_{50} = 3.60 ± 0.10 μM) with dichloro substituents *ortho* to each other, was found to be the third most potent and fifteen-fold more active compound than the standard D-saccharic acid 1,4-lactone. Compound **12** (IC_{50} = 6.20 ± 0.10 μM) is also a dichloro substituted derivative, but both chloro substitutions are *para* to each

other, showed almost half inhibitory activity than compound **11** (Figure-5). It verifies that the position of substituents highly effects the inhibitory potential. Mono chloro substituted compounds such as **9** (IC_{50} = 16.30 ± 0.20 μM) and **10** (IC_{50} = 19.30 ± 0.30 μM) showed lesser inhibitory activity than dichloro substitution **11** and **12** as shown in Figure-5.

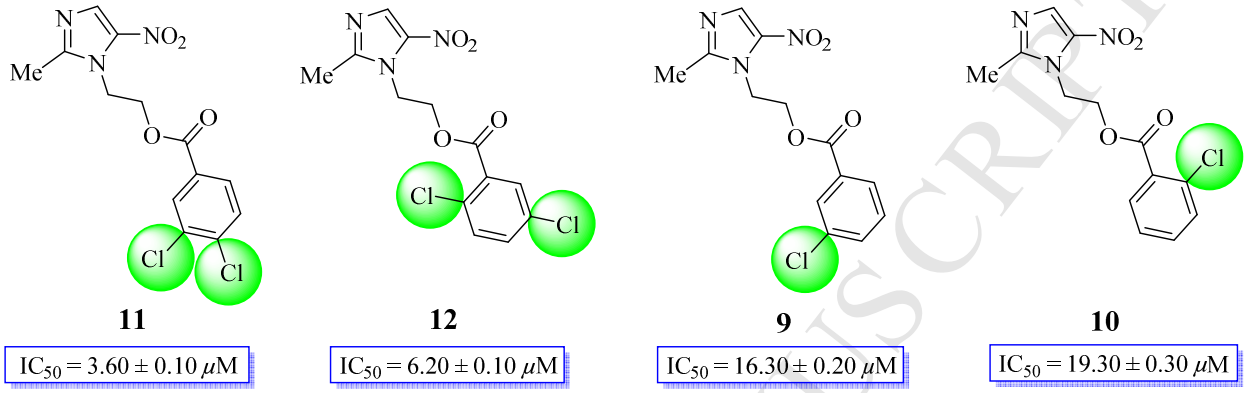


Figure-5: Comparison of structure-activity relationship of compound **11** with **12**, **9**, and **10**.

It is worth mentioning that compound **16** (IC_{50} = 1.20 ± 0.01 μM), the most potent compound of this series and distinctly similar in structure to compound **10** (IC_{50} = 19.30 ± 0.30 μM), but the presence of two fluoro substituents were greatly enhanced the activity of compound **16**. Amongst the mono-fluorinated compounds, compound **13** (IC_{50} = 8.10 ± 0.10 μM) which has fluoro at *meta* position was more active than its *para* counterpart **14** (IC_{50} = 12.40 ± 0.20 μM) (Figure-6).

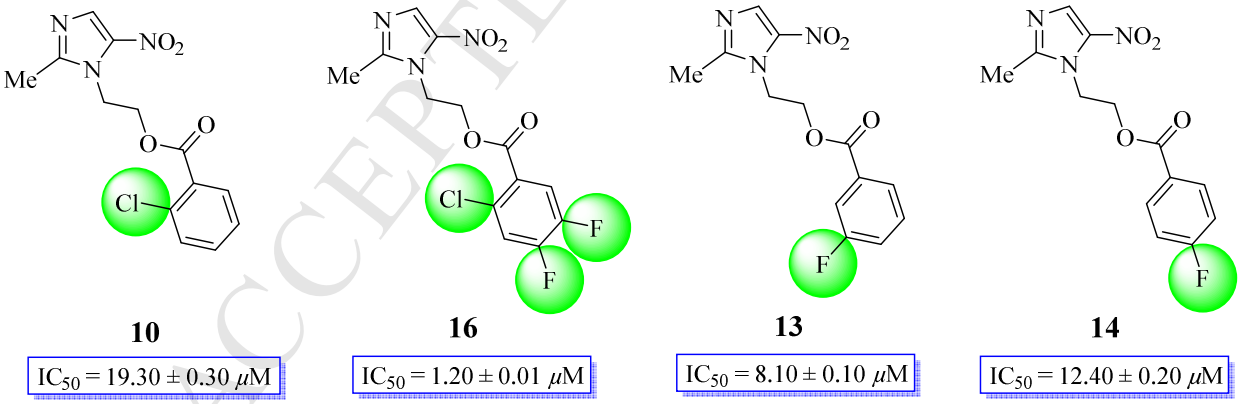


Figure-6: Comparison of structure-activity relationship of compound **16** with **15**, **14**, and **13**.

More critically, it was monitored that halogens were played vital roles in the inhibitory potential, especially, fluoro groups were enhanced the inhibitory activity.

Compounds containing strong electron withdrawing nitro group such as **17-19** were found to be potent inhibitors too. Amongst them, compound **19** (IC_{50} = 7.30 ± 0.10 μM) having 3-methyl-4-nitro substitutions at "R" was found to be one of the most potent compounds, about seven fold more active than standard. Absence of methyl group as in analog **18** (IC_{50} = 8.90 ± 0.20 μM), displayed slightly lowered activity. Similarly, switching of nitro group from *para* to *meta* as in compound **17** (IC_{50} = 27.60 ± 0.70 μM) sharply declined the activity. It showed that nitro group at *para* position played a vital role in enhancing the inhibitory activity (Figure-7).

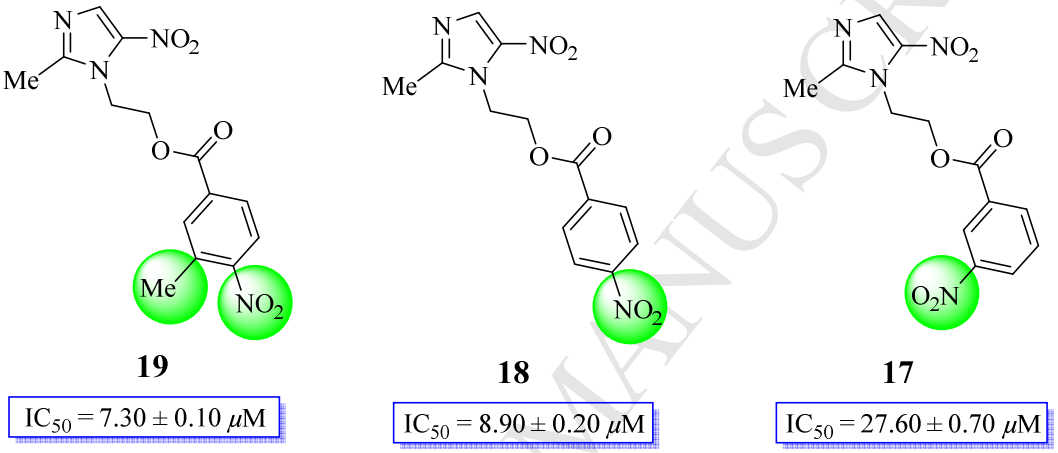


Figure-7: Comparison of structure-activity relationship of compound **19** with **18**, and **17**.

Compounds **22-24** having heterocyclic ring as "R" were displayed potent inhibition than standard. Out of them, compound **22** (IC_{50} = 2.10 ± 0.10 μM) having indole ring was found to be the potent derivative and demonstrated twenty-four times more activity than standard. Compound **24** (IC_{50} = 8.40 ± 0.10 μM) having benzothiazole ring as "R", was exhibited six times better potential than standard. It was observed that both compounds **22** and **24** have bicyclic aromatic rings as "R", which might involve in strong π-π interactions with the active site of enzyme, however, the lesser activity of compound **23** (IC_{50} = 32.60 ± 0.80 μM) with pyridine ring due to comparatively weak π-π interactions with active site as compared to compounds **22** and **24** (Figure-8).

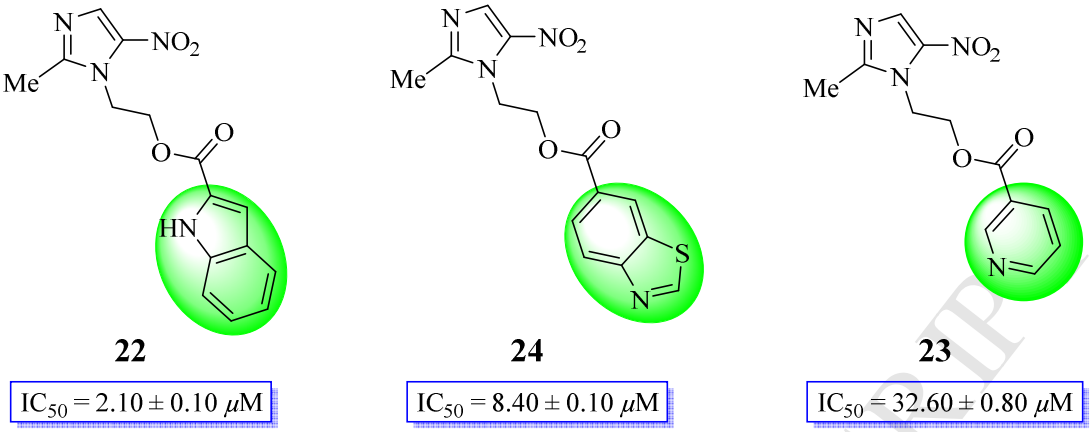


Figure-8: Comparison of structure-activity relationship of compound **22** with **24**, and **23**.

Compounds **1**, **3**, and **6** having electron donating methyl substituents were also demonstrated better activity, but lesser than the compounds having electron withdrawing groups. Out of them, compound **1** (IC_{50} = 44.16 ± 0.90 μM) having methyl at *ortho* position showed good inhibition. Switching of methyl group from *ortho* to *meta* as in compound **2**, leads to complete loss of activity, however, from *ortho* to *para* as in compound **3** (IC_{50} = 35.50 ± 0.80 μM), brings further better inhibitory activity. Compound **6** (IC_{50} = 24.20 ± 0.50 μM) having dimethyl substituents at *meta* positions, showed much better activity than mono substituted compounds (Figure-9).

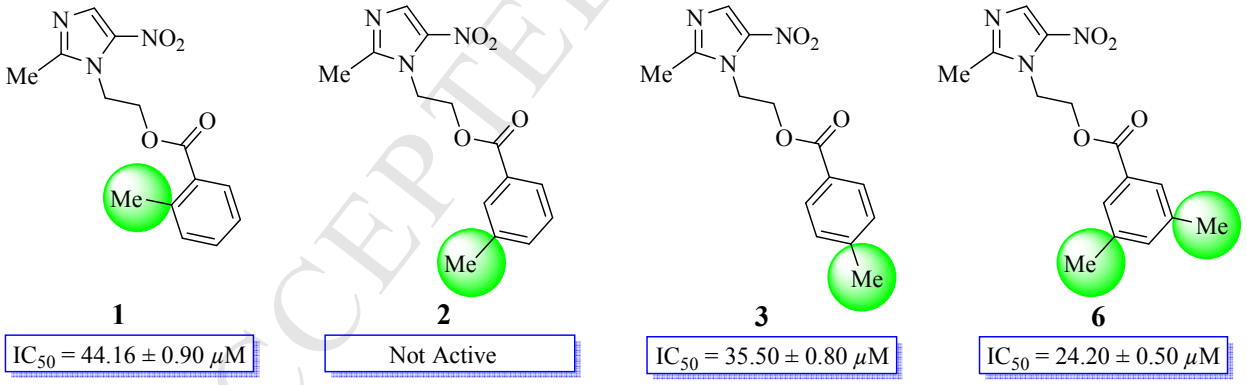


Figure-9: Comparison of structure-activity relationship of compound **1** with **2**, **3**, and **6**.

Methoxy containing derivatives **4**, **5**, and **7** demonstrated good to moderate inhibition. Such as compound **4** (IC_{50} = 31.10 ± 0.75 μM) was found to be good inhibitor as compared to standard. Its activity can compare with compound **5** (IC_{50} = 60.16 ± 1.40 μM) which only lacks the olefin moiety, a sharp decline in the activity was observed. Decreased activity of compound **5** was

might be due to the lesser π-π interactions. Compound **7** (IC_{50} = 60.30 ± 1.40 μM) having dimethoxy groups at *meta* positions, also demonstrated moderate inhibition, however, *para*-butoxy derivative **8** was found to be completely inactive (Figure-10).

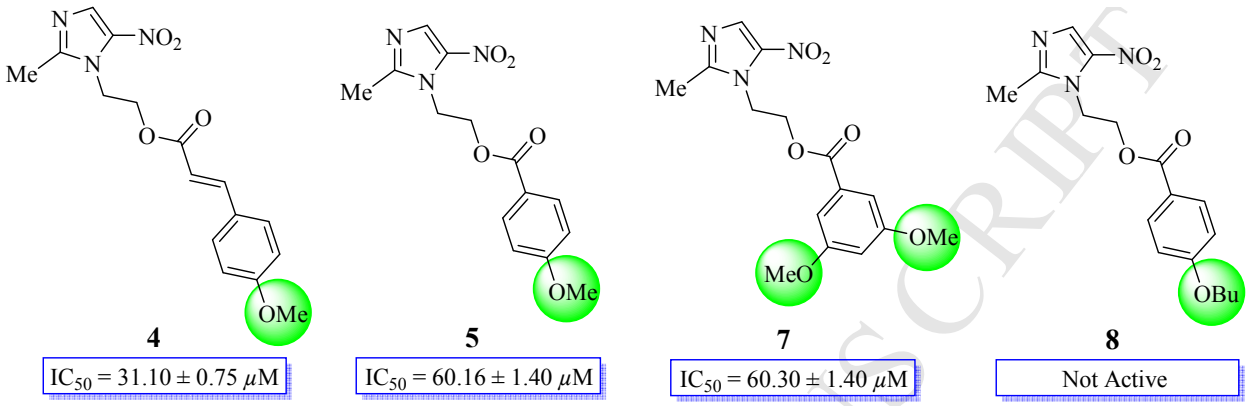


Figure-10: Comparison of structure-activity relationship of compound **4** with **5**, **7**, and **8**.

In a nut shell, careful assessment of the influence of groups on activity is revealed that compounds having electron withdrawing groups were found to be more potent than the compounds having electron donating groups except few (Table-1). However, in order to get in-depth insights regarding the SAR as well as molecular interactions of compounds with the active sides of β-glucuronidase enzyme, *in silico* studies were also performed on all compounds and discussed in the next section.

In Silico Studies

p-Nitro phenyl β-D-glucuronide, the known substrate molecule [34], was docked into the active site of the enzyme using MOE-Dock program prior to docking of the synthetic molecules. Similar to our previous results [27,33], the modeled three dimensional substrate-bound structure of human β-D-glucuronidase was displayed that the glycoside bond of *p*-nitrophenyl β-D-glucuronide was properly oriented towards the catalytic residues such as Glu540, Glu451, and Tyr504 (Figure-11). The substrate molecule was demonstrated appropriate binding with the active site residues; Asp207, Glu451, Glu540, His385, Tyr508, Tyr504 and Arg600 [30]. This 3D protein structure was used in the assessment of the binding mode of the synthetic derivatives.

The docking of the compounds was carried out *via* MOE with the parameters *i.e.*, Placement: Triangle Matcher, Rescoring: London dG. For each ligand, ten conformations were generated

and on the basis of docking score (S) the top-ranked conformation of each compound was used for further analysis. The docking score is the binding free energy calculated by the GBVI/WSA scoring function and the lower score indicates more favorable poses. The unit for all scoring functions is kcal/mol [35].

The docking study predicted that the compounds with electron withdrawing substituents *i.e.* F, Cl, Br, NO_2 showed good *in silico* inhibition. It was observed that the number of these substituents and their respective positions may affect the orientation and binding pattern of the compound in the active site of the enzyme. For example, compound **22** with 1*H*-indole group showed the best interactions mode (Figure-12) among these synthetic compounds. Asp207, His385, and Asn450 were interacted through a polar bond with the imidazole moiety. Arg600 was showed arene-cation contacts with 2-methyl-1*H*-indole residue.

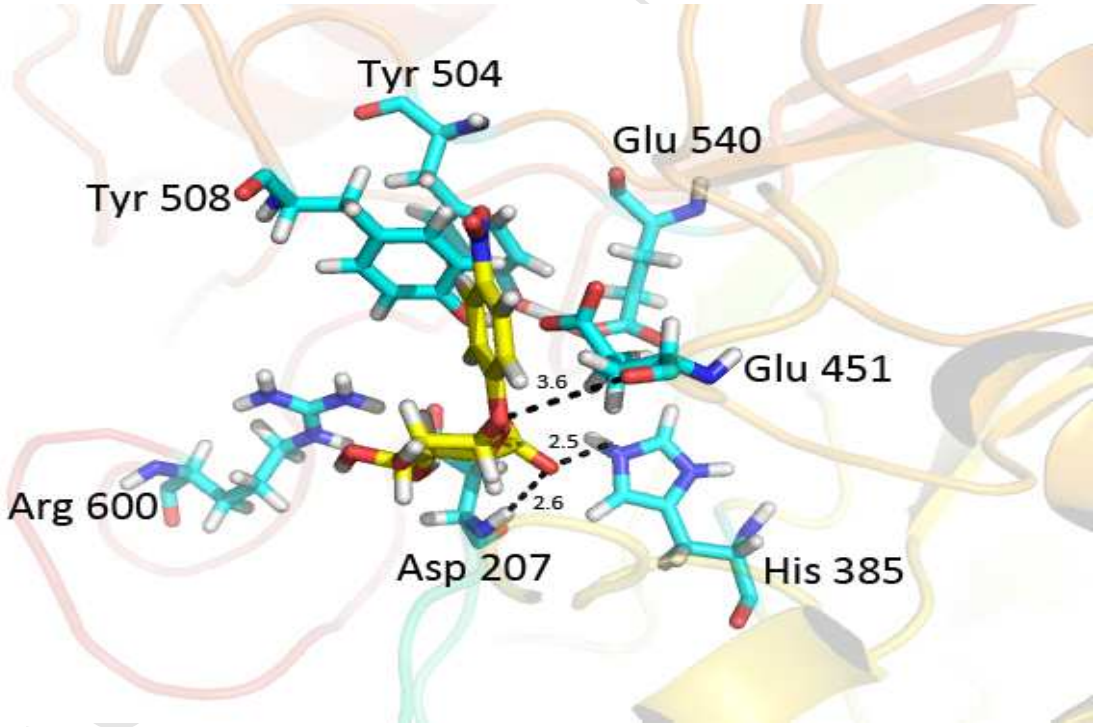


Figure-11: Molecular interactions of substrate with human *β*-glucuronidase.

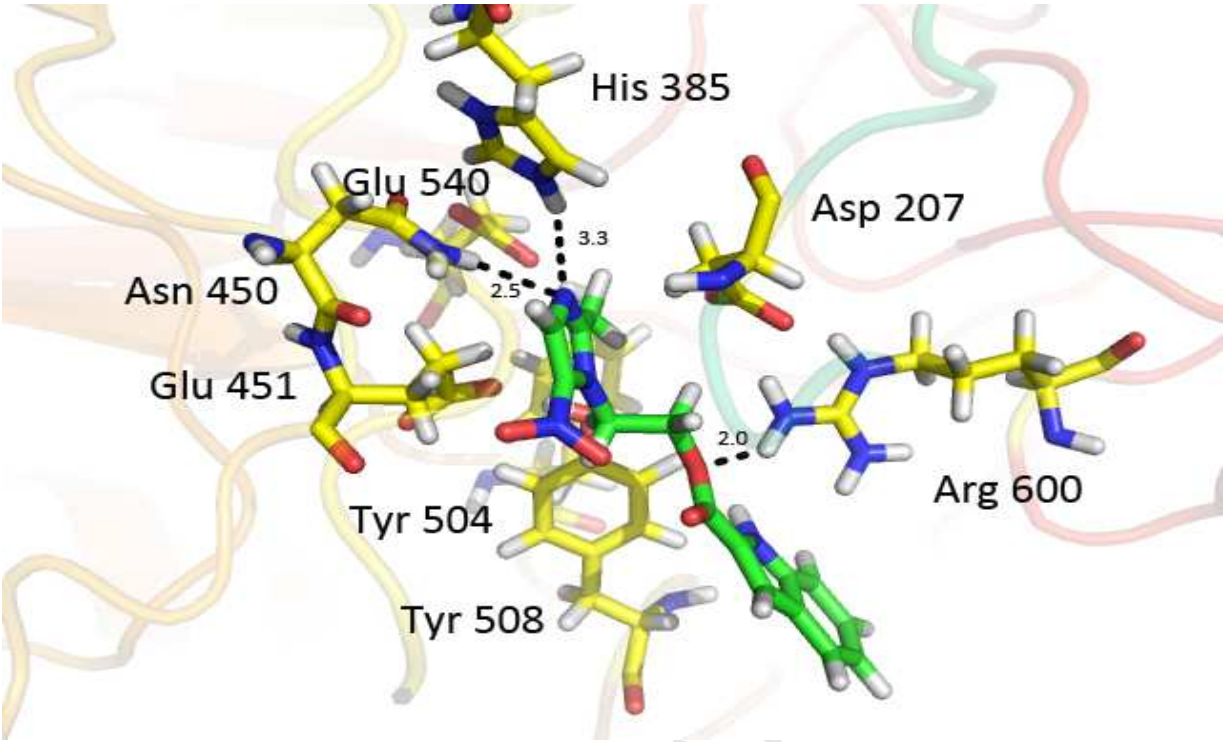


Figure-12: Molecular interactions of compound **22**.

Similarly, compound **11** with dichlorobenzene (*meta-para* dichloro) showed three interactions with the active site residues Tyr508 and Arg600 as shown in Figure-13. Changing the positions of chlorine groups in compound **12** *i.e. ortho* and opposite *meta*, resulted in mild interaction mode which was in accordance to its biological activity. The docking study was predicted almost the same behavior for the different halogen groups as experienced in biological evaluations. However, some contrast behavior was observed for the most active compound **16** (IC_{50} = 1.20 ± 0.01 μM) in the docking study. This compound was unpredictably showed two hydrogen bonding with Thr599 and Arg600 (Figure-14). The contact *i.e.* hydrogen bonding with Arg600 is shown by compounds **14**, **15** and **19**. Whereas, compound **24** showed arene-cation interaction with Arg600.

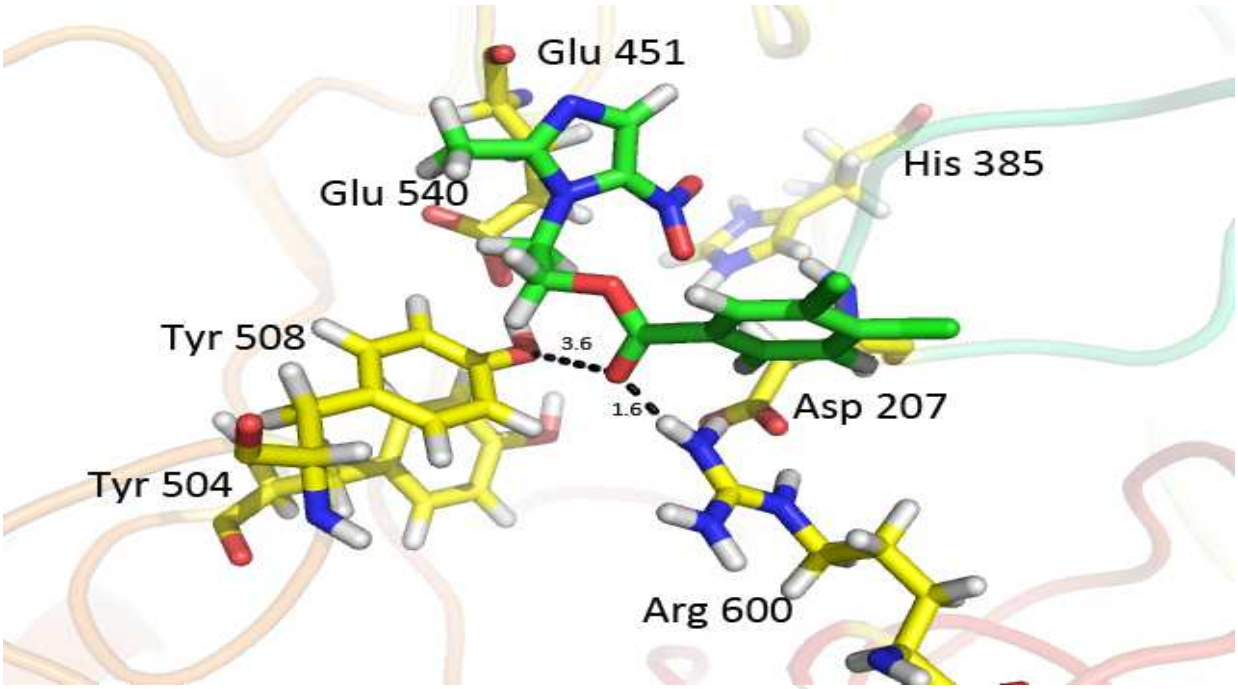


Figure-13: Molecular interactions of compound **11**.

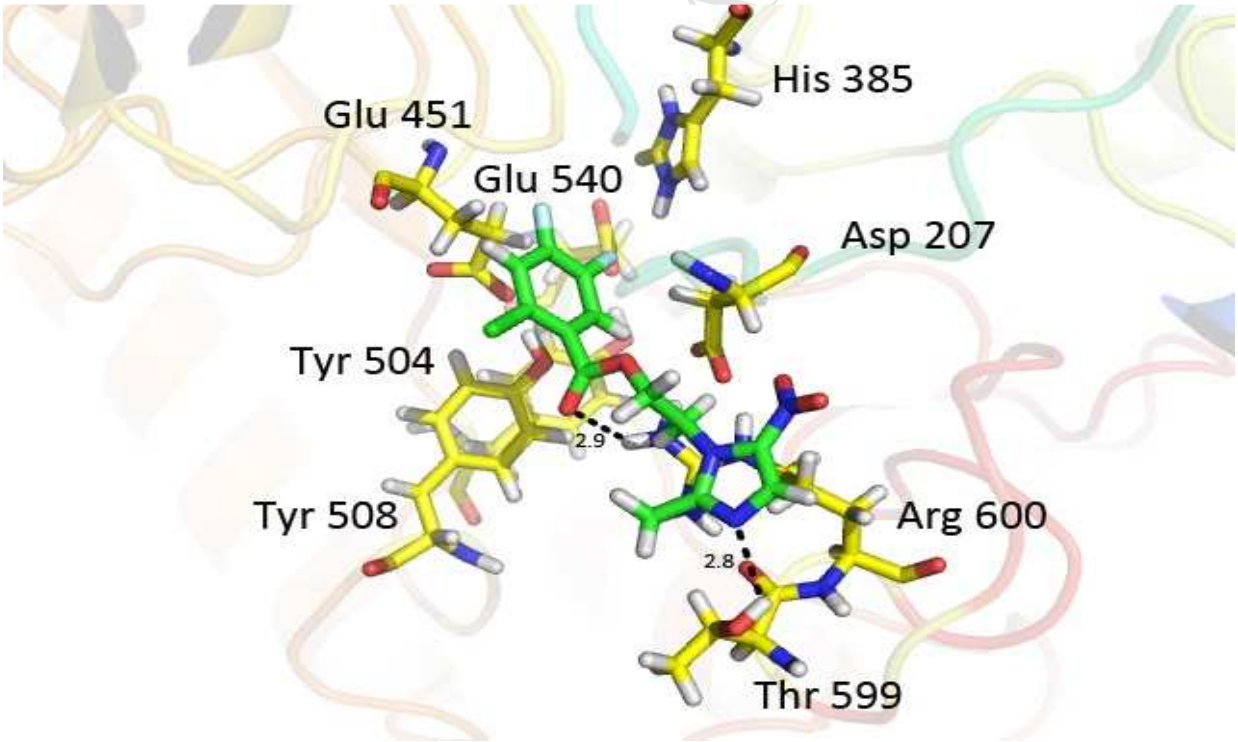


Figure-14: Molecular interactions of compound **16**.

Compound **13** with fluorine at *meta* position of ring "R" showed good mode of interactions with Asn484 and Arg600. Asn484 was formed hydrogen bonding with the N of imidazole group and basic Arg600 made hydrogen bonding with the carbonyl oxygen (Figure-15). Nitro substituted

compound **18** was also showed a single arene-arene contact with Tyr508 through nitrobenzene substituent. In case of non-active/poor-active compounds and the compounds with methyl (Me) or methoxy (MeO) substituents, no valuable binding network with the enzyme was observed.

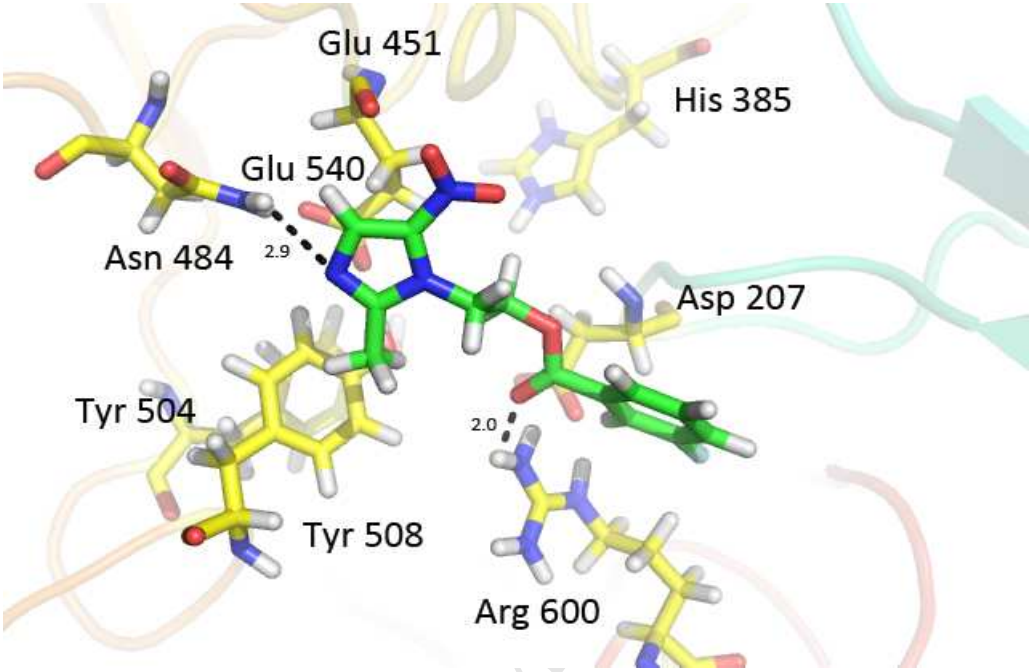


Figure-15: Molecular interaction of compound **13**.

Overall a good correlation was observed between the docking study and biological evaluation of active compounds. The correlation graph and the correlation coefficient value are given in Figure-16.

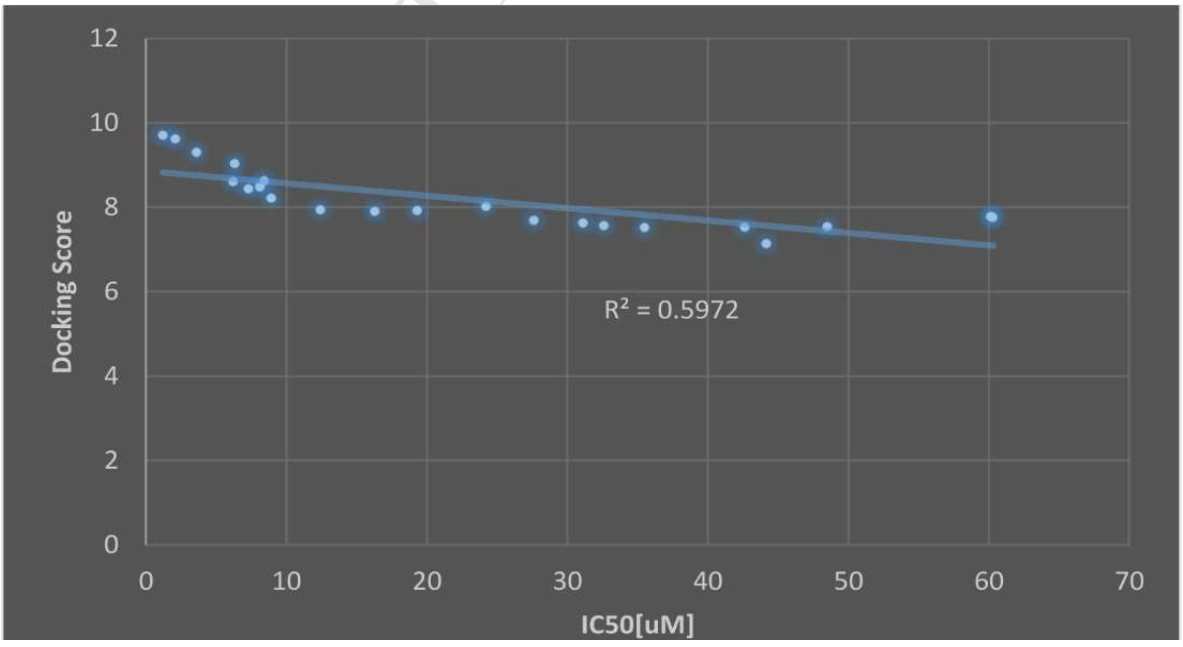


Figure-16: A correlation graph for docking predicted activity and IC_{50} values.

Conclusion

β-Glucuronidase inhibitory activity of 2-(2-methyl-5-nitro-1*H*-imidazol-1-yl)ethyl aryl carboxylate derivatives **1-26** was examined which revealed that almost all compounds were demonstrated potent inhibition except few. Limited structure-activity relationship and *in silico* study rationalized that compounds having electron withdrawing groups such as NO_2, F, Cl, and Br were more prone to inhibit the *β*-glucuronidase enzyme as compared to the compounds having electron donating groups *e.g.* Me, OMe and BuO. This study identified a set of lead compounds for further research in order to get good *β*-glucuronidase inhibitors.

Experimental

Material 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 or iodine vapors. Mass spectra were recorded under electron impact (EI) on MAT 312 and MAT 113D mass spectrometers. The ^{1}H, ^{13}CNMR were recorded on Bruker AM spectrometers, operating at 300, 400 and 500 MHz. The chemical shift are presented in ppm (*δ*), relative to tetramethylsilane (TMS) as an internal standard and the coupling constant (*J*) are in Hz. Elemental analysis was performed on a Carlo Erba Strumentazione-Mod-1106, Italy.

General Experimental Procedure for the Synthesis of Compounds 1-26

Carboxylic acids (1 mmol) and 1,1'-carbonyl diimidazole (1 mmol) were taken in THF (15 mL) in a round-bottommed flask (100 mL) and stirred for 30 minutes in order to activate the carboxylic acids. Then metronidazole (1 mmol) was added into the reaction mixture with constant stirring for 24 h. Reaction progress was monitored by TLC (6:4 = EtOAc:Hexane). Reaction mixture was poured onto crushed ice (100 mL), precipitates appeared immediately which were filtered and dried in air. The precipitates were crystallized from ethanol. Products were characterized by spectroscopic techniques such as EIMS, ^{1}H-NMR and ^{13}C-NMR. CHN analysis was also performed.

β-Glucuronidase Inhibitory Assay

Bioassay protocol was used according to literature report [36]. *β*-Glucuronidase enzyme (E.C. 3.2.1.31), from bovine liver (G-0251), and *p*-nitrophenyl-*β*-D-glucuronide (N-1627) were obtained from Sigma-Aldrich USA.

β-Glucuronidase activity was determined spectrophotometrically by quantifying the absorbance at 405 nm of *p*-nitrophenol formed during the reaction. 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 0.1 M acetate buffer (185 μL), test compound solution (5 μL), and enzyme solution (10 μL), 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 run in triplicate.

Molecular Docking Study

The molecular docking program is widely used to predict the binding interaction of the compounds in the binding pocket of the enzyme. The crystal structure of the human *β*-glucuronidase was obtained from the protein databank (PDB ID: 1BHG) [37] having 80% sequence similarity with bovine *β*-D-glucuronidase [30] as the 3D structure of bovine *β*-D-glucuronidase is not reported yet. From the original protein data bank file, the B-chain of protein and hetero-atoms including cofactors were removed. Using MOE (Molecular Operating Environment) software (www.chemcomp.com) the retrieved protein molecule was 3D protonated and the energy minimization was carried out using the default parameters of MOE [the Amber99 force field and gradient: 0.05]. The structures of the synthetic compounds **1-26** were built in MOE and were energy minimized.

Spectral Analysis of Compounds (1-26)

2-(2-Methyl-5-nitro-1*H*-imidazol-1-yl)ethyl 2-methylbenzoate (1)

Solid; Yield: 73%; ^{1}H-NMR: (400 MHz, DMSO-d_6): δ 8.04 (s, 1H, H-4), 7.67 (d, $J_{6',5'}$ = 7.6 Hz, 1H, H-6′), 7.49 (t, $J_{5'(4',6')}$ = 7.2 Hz, 1H, H-5′), 7.31 (m, 2H, H-3′, H-4′), 4.72 (t, $J_{2'',1''}$ = 5.2 Hz, 2H, CH_2-2″), 4.60 (t, $J_{1'',2''}$ = 5.2 Hz, 2H, CH_2-1″), 2.41 (s, 3H, CH_3), 2.40 (s, 3H, CH_3); ^{13}C

NMR (75 MHz, DMSO-d_6): δ 167.6, 151.6, 139.5, 138.3, 132.7, 132.3, 131.1, 129.9, 129.7, 125.5, 62.4, 40.6, 21.6, 12.8; EI-MS: *m/z* (rel. abund. %), 288 (M^+, 18), 272 (25), 260 (7), 243 (15), 199 (4), 177 (19), 163 (33), 149 (15), 138 (21), 119 (100); Anal. Calcd for $C_{14}H_{15}N_3O_4$: C = 58.13, H = 5.23, N = 14.53; Found: C = 58.16, H = 5.25, N = 14.51.

2-(2-Methyl-5-nitro-1*H*-imidazol-1-yl)ethyl 3-methylbenzoate (2)

Solid; Yield: 78%; ^{1}H-NMR: (300 MHz, DMSO-d_6): δ 8.04 (s, 1H, H-4), 7.63 (s, 1H, H-2′), 7.63 (d, $J_{6',5'}$ = 8.1 Hz, 1H, H-6′), 7.47 (d, $J_{4',5'}$ = 7.2 Hz, 1H, H-4′), 7.41 (t, $J_{5'(4',6')}$ = 7.2 Hz, 1H, H-5′), 4.74 (t, $J_{2'',1''}$ = 4.8 Hz, 2H, CH_2-2″), 4.62 (t, $J_{1'',2''}$ = 5.1 Hz, 2H, CH_2-1″), 2.44 (s, 3H, CH_3), 2.34 (s, 3H, CH_3); ^{13}C NMR (75 MHz, DMSO-d_6): δ 165.9, 151.5, 138.6, 138.4, 133.4, 132.2, 130.4, 130.0, 128.6, 126.8, 62.4, 40.7, 21.4, 12.7; EI-MS: *m/z* (rel. abund. %), 289 (M^+, 5), 243 (23), 163 (35), 136 (5), 119 (100), 91 (69); Anal. Calcd for $C_{14}H_{15}N_3O_4$: C = 58.13, H = 5.23, N = 14.53; Found: C = 58.16, H = 5.25, N = 14.51.

2-(2-Methyl-5-nitro-1*H*-imidazol-1-yl)ethyl 3,5-dimethylbenzoate (3)

Solid; Yield: 79%; ^{1}H-NMR: (400 MHz, DMSO-d_6): δ 8.03 (s, 1H, H-4), 7.43 (s, 2H, H-2′, H-6′), 7.28 (s, 1H, H-4′), 4.73 (t, $J_{2'',1''}$ = 4.8 Hz, 2H, CH_2-2″), 4.60 (t, $J_{1'',2''}$ = 4.8 Hz, 2H, CH_2-1″), 2.44 (s, 3H, CH_3), 2.30 (s, 6H, $2CH_3$); ^{13}C NMR (75 MHz, DMSO-d_6): δ 165.8, 151.6, 138.5, 138.3, 138.3, 135.4, 132.1, 129.7, 127.0, 127.0, 62.4, 40.7, 21.5, 21.5, 12.8; EI-MS: *m/z* (rel. abund. %), 303 (M^+, 8), 257 (17), 191 (3), 176 (21), 149 (3), 133 (100); Anal. Calcd for $C_{15}H_{17}N_3O_4$: C = 59.40, H = 5.65, N = 13.85; Found: C = 59.43, H = 5.62, N = 13.88.

(*E*)-2-(2-Methyl-5-nitro-1*H*-imidazol-1-yl)ethyl 3-(4-methoxyphenyl)acrylate (4)

Solid; Yield: 78%; ^{1}H-NMR: (400 MHz, DMSO-d_6): δ 8.03 (s, 1H, H-4), 7.66 (d, $J_{2',3'/6',5'}$ = 8.8 Hz, 2H, H-2′, H-6′), 7.54 (d, $J_{1''',2'''}$ = 16.0 Hz, 1H, H-1‴), 6.98 (d, $J_{3',2'/5',6'}$ = 8.8 Hz, 2H, H-3′, H-5′), 6.42 (d, $J_{2''',1'''}$ = 16.0 Hz, 1H, H-2‴), 4.64 (t, $J_{2'',1''}$ = 5.2 Hz, 2H, CH_2-2″), 4.49 (t, $J_{1'',2''}$ = 5.2 Hz, 2H, CH_2-1″), 3.79 (s, 3H, OCH_3), 2.47 (s, 3H, CH_3); ^{13}C NMR (75 MHz, DMSO-d_6): δ 166.4, 159.7, 151.6, 145.2, 138.5, 132.3, 130.3, 130.3, 127.6, 116.3, 114.0, 114.0, 62.5, 55.7, 40.6, 12.7; EI-MS: *m/z* (rel. abund. %), 331 (M^+, 32), 285 (48), 244 (15), 216 (4), 205 (21), 161 (100); Anal. Calcd for $C_{16}H_{17}N_3O_5$: C = 58.00, H = 5.17, N = 12.68; Found: C = 58.02, H = 5.15, N = 12.65.

2-(2-Methyl-5-nitro-1*H*-imidazol-1-yl)ethyl 4-methoxybenzoate (5)

Solid; Yield: 82%; ^{1}H-NMR: (400 MHz, DMSO-d_6): δ 8.02 (s, 1H, H-4), 7.79 (d, $J_{2',3'/6',5'}$ = 8.4 Hz, 2H, H-2′, H-6′), 7.03 (d, $J_{3',2'/5',6'}$ = 7.2 Hz, 2H, H-3′, H-5′), 4.71 (t, $J_{2'',1''}$ = 4.8 Hz, 2H, CH_2-2″), 4.60 (t, $J_{1'',2''}$ = 4.8 Hz, 2H, CH_2-1″), 3.82 (s, 3H, OCH_3), 2.44 (s, 3H, CH_3); ^{13}C NMR (75 MHz, DMSO-d_6): δ 165.8, 164.6, 151.5, 138.5, 132.3, 130.8, 130.8, 122.3, 114.4, 114.4, 62.5, 55.7, 40.7, 12.6; EI-MS: *m/z* (rel. abund. %), 305 (M^+, 25), 259 (100), 218 (8), 179 (68), 152 (32), 135 (100); Anal. Calcd for $C_{14}H_{15}N_3O_5$: C = 55.08, H = 4.95, N = 13.76; Found: C = 55.05, H = 4.97, N = 13.79.

2-(2-Methyl-5-nitro-1*H*-imidazol-1-yl)ethyl 4-methylbenzoate (6)

Solid; Yield: 76%; ^{1}H-NMR: (400 MHz, DMSO-d_6): δ 8.03 (s, 1H, H-4), 7.73 (d, $J_{2',3'/6',5'}$ = 8.0 Hz, 2H, H-2′, H-6′), 7.32 (d, $J_{3',2'/5',6'}$ = 7.6 Hz, 2H, H-3′, H-5′), 4.73 (t, $J_{2'',1''}$ = 4.8 Hz, 2H, CH_2-2″), 4.62 (t, $J_{1'',2''}$ = 4.8 Hz, 2H, CH_2-1″), 2.44 (s, 3H, CH_3), 2.36 (s, 3H, CH_3); ^{13}C NMR (75 MHz, DMSO-d_6): δ 165.9, 151.6, 142.9, 138.6, 132.3, 129.7, 129.7, 128.6, 128.6, 127.2, 62.4, 40.6, 21.4, 12.8; EI-MS: *m/z* (rel. abund. %), 289 (M^+, 8), 243 (79), 217 (11), 173 (32), 163 (39), 119 (100), 91 (41); Anal. Calcd for $C_{14}H_{15}N_3O_4$: C = 58.13, H = 5.23, N = 14.53; Found: C = 58.16, H = 5.25, N = 14.51.

2-(2-Methyl-5-nitro-1*H*-imidazol-1-yl)ethyl 3,5-dimethoxybenzoate (7)

Solid; Yield: 80%; ^{1}H-NMR: (400 MHz, DMSO-d_6): δ 8.04 (s, 1H, H-4), 6.92 (d, $J_{2',4'/6',4'}$ = 2.4 Hz, 2H, H-2′, H-6′), 6.76 (s, 1H, H-4′), 4.75 (t, $J_{2'',1''}$ = 5.2 Hz, 2H, CH_2-2″), 4.61 (t, $J_{1'',2''}$ = 5.2 Hz, 2H, CH_2-1″), 3.77 (s, 6H, $2OCH_3$), 2.49 (s, 3H, CH_3); ^{13}C NMR (75 MHz, DMSO-d_6): δ 165.8, 161.4, 161.4, 151.8, 138.5, 132.3, 108.8, 106.4, 106.4, 104.5, 62.5, 55.6, 55.6, 40.7, 12.7; EI-MS: *m/z* (rel. abund. %), 335 (M^+, 42), 318 (45), 305 (4), 289 (8), 209 (30), 165 (100), 151 (20), 137 (48); Anal. Calcd for $C_{15}H_{17}N_3O_6$: C = 53.73, H = 5.11, N = 12.53; Found: C = 53.76, H = 5.14, N = 12.55.

2-(2-Methyl-5-nitro-1*H*-imidazol-1-yl)ethyl 4-butoxybenzoate (8)

Solid; Yield: 77%; ^{1}H-NMR: (400 MHz, DMSO-d_6): δ 8.03 (s, 1H, H-4), 7.78 (d, $J_{2',3'/6',5'}$ = 8.8 Hz, 2H, H-2′, H-6′), 7.02 (d, $J_{3',2'/5',6'}$ = 8.8 Hz, 2H, H-3′, H-5′), 4.70 (t, $J_{2'',1''}$ = 5.2 Hz, 2H, CH_2-2″), 4.60 (t, $J_{1'',2''}$ = 6.4 Hz, 2H, CH_2-1″), 4.05 (t, 2H, CH_2), 2.44 (s, 3H, CH_3), 1.71 (m, 2H, CH_2),

1.45 (m, 2H, CH_2), 0.94 (t, 3H, CH_3); ^{13}C NMR (75 MHz, DMSO-d_6): δ 165.8, 163.6, 151.8, 138.3, 132.3, 130.6, 130.6, 121.8, 114.4, 114.4, 68.3, 62.5, 40.7, 31.9, 19.2, 12.7, 14.0; EI-MS: *m/z* (rel. abund. %), 347 (M^+, 6), 301 (91), 245 (23), 221 (10), 177 (100), 165 (33), 138 (20), 121 (100); Anal. Calcd for $C_{17}H_{21}N_3O_5$: C = 58.78, H = 6.09, N = 12.10; Found: C = 58.76, H = 6.07, N = 12.13.

2-(2-Methyl-5-nitro-1*H*-imidazol-1-yl)ethyl 3-chlorobenzoate (9)

Solid; Yield: 82%; ^{1}H-NMR: (400 MHz, DMSO-d_6): δ 8.03 (s, 1H, H-4), 7.79 (s, 1H, H-2′), 7.79 (d, $J_{4',5'}$ = 6.8 Hz, 1H, H-4′), 7.75 (d, $J_{6',5'}$ = 8.0 Hz, 1H, H-6′), 7.58 (t, $J_{5'(4',6')}$ = 8.0 Hz, 1H, H-5′), 4.75 (t, $J_{2'',1''}$ = 5.2 Hz, 2H, CH_2-2″), 4.64 (t, $J_{1'',2''}$ = 5.2 Hz, 2H, CH_2-1″), 2.49 (s, 3H, CH_3); ^{13}C NMR (75 MHz, DMSO-d_6): δ 165.7, 151.8, 138.3, 134.6, 134.3, 133.2, 132.0, 130.2, 129.7, 128.1, 62.5, 40.6, 12.8; EI-MS: *m/z* (rel. abund. %), 309 (M^+, 4), 311 (M+2, 2), 263 (19), 183 (31), 139 (100), 111 (19); Anal. Calcd for $C_{13}H_{12}ClN_3O_4$: C = 50.42, H = 3.91, N = 13.57; Found: C = 50.45, H = 3.93, N = 13.54.

2-(2-Methyl-5-nitro-1*H*-imidazol-1-yl)ethyl 2-chlorobenzoate (10)

Solid; Yield: 78%; ^{1}H-NMR: (400 MHz, DMSO-d_6): δ 8.04 (s, 1H, H-4), 7.68 (d, $J_{6',5'}$ = 7.6 Hz, 1H, H-6′), 7.58 (m, 1H, H-4′, H-5′), 7.47 (m, 1H, H-3′), 4.73 (t, $J_{2'',1''}$ = 4.8 Hz, 2H, CH_2-2″), 4.63 (t, $J_{1'',2''}$ = 4.8 Hz, 2H, CH_2-1″), 2.42 (s, 3H, CH_3); ^{13}C NMR (75 MHz, DMSO-d_6): δ 165.7, 151.8, 138.3, 134.3, 133.4, 133.2, 132.1, 131.2, 129.8, 126.6, 62.4, 40.7, 12.9; EI-MS: *m/z* (rel. abund. %), 309 (M^+, 1), 311 (M+2), 274 (8), 263 (9), 183 (28), 153 (3), 139 (100), 111 (20); Anal. Calcd for $C_{13}H_{12}ClN_3O_4$: C = 50.42, H = 3.91, N = 13.57; Found: C = 50.43, H = 3.91, N = 13.52.

2-(2-Methyl-5-nitro-1*H*-imidazol-1-yl)ethyl 3,4-dichlorobenzoate (11)

Solid; Yield: 86%; ^{1}H-NMR: (400 MHz, DMSO-d_6): δ 8.03 (s, 1H, H-4), 7.96 (d, $J_{2',6'}$ = 1.6 Hz, 1H, H-2′), 7.82 (m, 2H, H-5′, H-6′), 4.74 (t, $J_{2'',1''}$ = 4.8 Hz, 2H, CH_2-2″), 4.65 (t, $J_{1'',2''}$ = 4.8 Hz, 2H, CH_2-1″), 2.47 (s, 3H, CH_3); ^{13}C NMR (75 MHz, DMSO-d_6): δ 165.8, 151.6, 138.5, 137.8, 132.4, 132.1, 130.0, 129.8, 129.5, 129.3, 62.4, 40.6, 12.8; EI-MS: *m/z* (rel. abund. %), 343 (M^+, 5), 345 (M+2, 3), 217 (38), 190 (2), 173 (100), 145 (18); Anal. Calcd for $C_{13}H_{11}Cl_2N_3O_4$: C = 45.37, H = 3.22, N = 12.21; Found: C = 45.34, H = 3.25, N = 12.23.

2-(2-Methyl-5-nitro-1*H*-imidazol-1-yl)ethyl 2,5-dichlorobenzoate (12)

Solid; Yield: 88%; ^{1}H-NMR: (400 MHz, DMSO-d_6): δ 8.04 (s, 1H, H-4), 7.72 (d, $J_{6',4'}$ = 2.4 Hz, 1H, H-6′), 7.68 (dd, $J_{4',6'}$ = 2.4, $J_{4',3'}$ = 8.4 Hz, 1H, H-4′), 7.61 (d, $J_{3',4'}$ = 8.4 Hz, 1H, H-3′), 4.73 (t, $J_{2'',1''}$ = 5.2 Hz, 2H, CH_2-2″), 4.62 (t, $J_{1'',2''}$ = 5.2 Hz, 2H, CH_2-1″), 2.43 (s, 3H, CH_3); ^{13}C NMR (75 MHz, DMSO-d_6): δ 165.7, 151.6, 138.5, 133.2, 132.4, 132.0, 131.5, 130.4, 129.8, 128.6, 62.4, 40.7, 12.7; EI-MS: *m/z* (rel. abund. %), 343 (M^+, 55), 345 (M+2, 37), 308 (81), 297 (73), 217 (100), 173 (78); Anal. Calcd for $C_{13}H_{11}Cl_2N_3O_4$: C = 45.37, H = 3.22, N = 12.21; Found: C = 45.35, H = 3.20, N = 12.23.

2-(2-Methyl-5-nitro-1*H*-imidazol-1-yl)ethyl 3-fluorobenzoate (13)

Solid; Yield: 79%; ^{1}H-NMR: (400 MHz, DMSO-d_6): δ 8.03 (s, 1H, H-4), 7.68 (d, $J_{6',5'}$ = 7.6 Hz, 1H, H-6′), 7.59 (m, 3H, H-2′, H-4′, H-5′), 4.74 (t, $J_{2'',1''}$ = 4.8 Hz, 2H, CH_2-2″), 4.65 (t, $J_{1'',2''}$ = 4.8 Hz, 2H, CH_2-1″), 2.45 (s, 3H, CH_3); ^{13}C NMR (75 MHz, DMSO-d_6): δ 165.8, 162.7, 151.8, 138.5, 132.3, 131.8, 130.3, 125.6, 119.7, 114.6, 62.4, 40.7, 12.8; EI-MS: *m/z* (rel. abund. %), 293 (M^+, 11), 247 (48), 167 (72), 154 (4), 123 (100), 95 (47); Anal. Calcd for $C_{13}H_{12}FN_3O_4$: C = 53.24, H = 4.12, N = 14.33; Found: C = 53.26, H = 4.14, N = 14.30.

2-(2-Methyl-5-nitro-1*H*-imidazol-1-yl)ethyl 4-fluorobenzoate (14)

Solid; Yield: 82%; ^{1}H-NMR: (400 MHz, DMSO-d_6): δ 8.03 (s, 1H, H-4), 7.92 (m, 2H, H-2′, H-6′), 7.38 (m, 2H, H-3′, H-5′), 4.72 (t, $J_{2'',1''}$ = 4.5 Hz, 2H, CH_2-2″), 4.64 (t, $J_{1'',2''}$ = 5.4 Hz, 2H, CH_2-1″), 2.44 (s, 3H, CH_3); ^{13}C NMR (75 MHz, DMSO-d_6): δ 167.3, 165.8, 151.6, 138.5, 132.3, 131.4, 131.4, 125.6, 115.3, 115.3, 62.4, 40.6, 12.7; EI-MS: *m/z* (rel. abund. %), 293 (M^+, 16), 247 (64), 167 (58), 123 (100), 95 (43); Anal. Calcd for $C_{13}H_{12}FN_3O_4$: C = 53.24, H = 4.12, N = 14.33; Found: C = 53.21, H = 4.15, N = 14.30.

2-(2-Methyl-5-nitro-1*H*-imidazol-1-yl)ethyl 2-bromo-4-fluorobenzoate (15)

Solid; Yield: 90%; ^{1}H-NMR: (400 MHz, DMSO-d_6): δ 8.03 (s, 1H, H-4), 7.76 (m, 2H, H-5′, H-6′), 7.42 (m, 1H, H-3′), 4.72 (t, $J_{2'',1''}$ = 4.8 Hz, 2H, CH_2-2″), 4.62 (t, $J_{1'',2''}$ = 4.8 Hz, 2H, CH_2-1″), 2.42 (s, 3H, CH_3); ^{13}C NMR (75 MHz, DMSO-d_6): δ 169.5, 165.8, 151.6, 138.3, 133.6, 132.3, 127.4, 123.6, 120.4, 114.5, 62.5, 40.7, 12.8; EI-MS: *m/z* (rel. abund. %), 371 (M^+, 15), 373

(M+2, 14), 325 (16), 292 (16), 245 (29), 203 (100), 173 (14); Anal. Calcd for $C_{13}H_{11}BrFN_3O_4$: C = 41.96, H = 2.98, N = 11.29; Found: C = 41.94, H = 2.96, N = 11.27.

2-(2-Methyl-5-nitro-1*H*-imidazol-1-yl)ethyl 2-chloro-4,5-difluorobenzoate (16)

Solid; Yield: 78%; ^{1}H-NMR: (400 MHz, DMSO-d_6): δ 8.04 (s, 1H, H-4), 7.90 (m, 1H, H-6′), 7.82 (m, 1H, H-3′), 4.72 (t, $J_{2'',1''}$ = 5.2 Hz, 2H, CH_2-2′′), 4.62 (t, $J_{1'',2''}$ = 5.2 Hz, 2H, CH_2-1′′), 2.42 (s, 3H, CH_3); ^{13}C NMR (75 MHz, DMSO-d_6): δ 165.7, 155.3, 151.6, 147.4, 138.5, 132.3, 131.6, 128.7, 119.4, 117.8, 62.5, 40.7, 12.8; EI-MS: *m/z* (rel. abund. %), 345 (M^+, 5), 347 (M+2, 2), 310 (9), 299 (19), 219 (45), 175 (100), 147 (20); Anal. Calcd for $C_{13}H_{10}ClF_2N_3O_4$: C = 45.17, H = 2.92, N = 12.16; Found: C = 45.15, H = 2.95, N = 12.14.

2-(2-Methyl-5-nitro-1*H*-imidazol-1-yl)ethyl 3-nitrobenzoate (17)

Solid; Yield: 88%; ^{1}H-NMR: (300 MHz, DMSO-d_6): δ 8.52 (s, 1H, H-2′), 8.50 (d, $J_{4',5'}$ = 8.0 Hz, 1H, H-4′), 8.24 (d, $J_{6',5'}$ = 8.0 Hz, 1H, H-6′), 8.04 (s, 1H, H-4), 7.85 (t, $J_{5'(4',6')}$ = 8.0 Hz, 1H, H-5′), 4.77 (t, $J_{2'',1''}$ = 4.8 Hz, 2H, CH_2-2′′), 4.71 (t, $J_{1'',2''}$ = 5.2 Hz, 2H, CH_2-1′′), 2.48 (s, 3H, CH_3); ^{13}C NMR (75 MHz, DMSO-d_6): δ 165.8, 151.6, 147.9, 138.5, 136.1, 132.3, 129.4, 129.4, 128.3, 123.4, 62.4, 40.7, 12.8; EI-MS: *m/z* (rel. abund. %), 320 (M^+, 6), 274 (50), 194 (83), 150 (100), 123 (5), 104 (17); Anal. Calcd for $C_{13}H_{12}N_4O_6$: C = 48.75, H = 3.78, N = 17.49; Found: C = 48.73, H = 3.75, N = 17.47.

2-(2-Methyl-5-nitro-1*H*-imidazol-1-yl)ethyl 4-nitrobenzoate (18)

Solid; Yield: 85%; ^{1}H-NMR: (400 MHz, DMSO-d_6): δ 8.35 (d, $J_{2',3'/6',5'}$ = 9.0 Hz, 2H, H-2′, H-6′), 8.08 (d, $J_{3',2'/5',6'}$ = 9.0 Hz, 2H, H-3′, H-5′), 8.04 (s, 1H, H-4), 4.74 (t, $J_{2'',1''}$ = 4.2 Hz, 2H, CH_2-2′′), 4.70 (t, $J_{1'',2''}$ = 4.2 Hz, 2H, CH_2-1′′), 2.46 (s, 3H, CH_3); ^{13}C NMR (75 MHz, DMSO-d_6): δ 165.8, 152.3, 151.6, 138.3, 136.3, 132.1, 130.7, 130.7, 123.9, 123.9, 62.5, 40.7, 12.7; EI-MS: *m/z* (rel. abund. %), 320 (M^+, 28), 274 (48), 194 (68), 164 (7), 150 (100), 104 (58); Anal. Calcd for $C_{13}H_{12}N_4O_6$: C = 48.75, H = 3.78, N = 17.49; Found: C = 48.73, H = 3.80, N = 17.47.

2-(2-Methyl-5-nitro-1*H*-imidazol-1-yl)ethyl 3-methyl-4-nitrobenzoate (19)

Solid; Yield: 82%; ^{1}H-NMR: (400 MHz, DMSO-d_6): δ 8.07 (d, $J_{5',6'}$ = 8.4 Hz, 1H, H-5′), 8.04 (s, 1H, H-4), 7.91 (s, 1H, H-2′), 7.85 (d, $J_{6',5'}$ = 8.0 Hz, 1H, H-6′), 4.74 (t, $J_{2'',1''}$ = 4.4 Hz, 2H, CH_2-

2″), 4.67 (t, $J_{1″,2″}$ = 4.4 Hz, 2H, CH_2-1″), 2.52 (s, 3H, CH_3), 2.46 (s, 3H, CH_3); ^{13}C NMR (75 MHz, DMSO-d_6): δ 165.7, 153.5, 151.6, 138.3, 136.2, 132.3, 132.1, 131.0, 127.7, 123.6, 62.4, 40.7, 18.4, 12.7; EI-MS: *m/z* (rel. abund. %), 334 (M^+, 12), 288 (41), 208 (68), 164 (100), 135 (7), 118 (17); Anal. Calcd for $C_{14}H_{14}N_4O_6$: C = 50.30, H = 4.22, N = 16.76; Found: C = 50.32, H = 4.20, N = 16.79.

2-(2-Methyl-5-nitro-1*H*-imidazol-1-yl)ethyl 2-bromobenzoate (20)

Solid; Yield: 72; ^{1}H-NMR: (400 MHz, DMSO-d_6): δ 8.04 (s, 1H, H-4), 7.75 (m, 1H, H-4′), 7.64 (m, 1H, H-5′), 7.49 (m, 2H, H-3′, H-6′), 4.72 (t, $J_{2″,1″}$ = 4.8 Hz, 2H, CH_2-2″), 4.63 (t, $J_{1″,2″}$ = 4.8 Hz, 2H, CH_2-1″), 2.42 (s, 3H, CH_3); ^{13}C NMR (75 MHz, DMSO-d_6): δ 165.8, 151.6, 138.5, 132.7, 132.3, 132.0, 131.8, 127.5, 126.4, 121.2, 62.5, 40.7, 12.7; EI-MS: *m/z* (rel. abund. %), 353 (M^+, 4), 355 (M+2, 5), 307 (13), 274 (37), 227 (35), 183 (17); Anal. Calcd for $C_{13}H_{12}BrN_3O_4$: C = 44.09, H = 3.42, N = 11.86; Found: C = 44.06, H = 3.45, N = 11.83.

2-(2-Methyl-5-nitro-1*H*-imidazol-1-yl)ethyl 4-bromobenzoate (21)

Solid; Yield: 79%; ^{1}H-NMR: (400 MHz, DMSO-d_6): δ 8.02 (s, 1H, H-4), 7.74 (s, 4H, H-2′, H-3′, H-5′, H-6′), 4.73 (t, $J_{2″,1″}$ = 5.1 Hz, 2H, CH_2-2″), 4.65 (t, $J_{1″,2″}$ = 5.1 Hz, 2H, CH_2-1″), 2.44 (s, 3H, CH_3); ^{13}C NMR (75 MHz, DMSO-d_6): δ 165.7, 151.8, 138.5, 132.3, 132.2, 132.2, 131.4, 131.4, 129.0, 127.3, 62.4, 40.7, 12.8; EI-MS: *m/z* (rel. abund. %), 353 (M^+, 4), 355 (M+2, 4), 307 (21), 229 (20), 183 (100), 155 (13); Anal. Calcd for $C_{13}H_{12}BrN_3O_4$: C = 44.09, H = 3.42, N = 11.86; Found: C = 44.07, H = 3.45, N = 11.82.

2-(2-Methyl-5-nitro-1*H*-imidazol-1-yl)ethyl 1*H*-indole-2-carboxylate (22)

Solid; Yield: 80%; ^{1}H-NMR: (400 MHz, DMSO-d_6): δ 11.89 (s, 1H, NH), 8.04 (s, 1H, H-4), 7.66 (d, $J_{7′,6′}$ = 8.0 Hz, 1H, H-7′), 7.43 (d, $J_{4′,5′}$ = 8.4 Hz,1H, H-4′), 7.27 (t, $J_{5′(4′,6′)}$ = 7.6 Hz, 1H, H-5′), 7.09 (t, $J_{6′(5′,7′)}$ = 7.6 Hz, 1H, H-6′), 7.03 (s, 1H, H-3′), 4.71 (t, $J_{2″,1″}$ = 4.4 Hz, 2H, CH_2-2″), 4.68 (t, $J_{1″,2″}$ = 4.4 Hz, 2H, CH_2-1″), 2.47 (s, 3H, CH_3); ^{13}C NMR (75 MHz, DMSO-d_6): δ 158.3, 151.6, 138.5, 132.3, 131.5, 130.2, 125.2, 121.8, 120.6, 119.7, 112.4, 111.0, 62.4, 40.7, 12.8; EI-MS: *m/z* (rel. abund. %), 314 (M^+, 67), 297 (5), 286 (28), 268 (8), 187 (53), 154 (26), 144 (100), 115 (44); Anal. Calcd for $C_{15}H_{14}N_4O_4$: C = 57.32, H = 4.49, N = 17.83, Found: C = 57.30, H = 4.46, N = 17.85.

2-(2-Methyl-5-nitro-1*H*-imidazol-1-yl)ethyl nicotinate (23)

Solid; Yield: 83%; ^{1}H-NMR: (300 MHz, DMSO-d_6): δ 8.95 (d, $J_{2',4'}$ = 1.6 Hz, 1H, H-2′), 8.81 (d, $J_{4',5'}$ = 3.6 Hz, 1H, H-4′), 8.17 (d, $J_{6',5'}$ = 8.0 Hz, 1H, H-6′), 8.04 (s, 1H, H-4), 7.58 (m, 1H, H-5′), 4.75 (t, $J_{2'',1''}$ = 4.8 Hz, 2H, CH_2-2″), 4.67 (t, $J_{1'',2''}$ = 4.8 Hz, 2H, CH_2-1″), 2.46 (s, 3H, CH_3); ^{13}C NMR (75 MHz, DMSO-d_6): δ 165.8, 151.6, 151.3, 150.5, 138.6, 136.3, 132.1, 126.2, 122.0, 62.5, 40.7, 12.7; EI-MS: *m/z* (rel. abund. %), 276 (M^+, 4), 230 (17), 150 (49), 137 (4), 124 (8), 106 (100), 85 (20); Anal. Calcd for $C_{12}H_{12}N_4O_4$: C = 52.17, H = 4.38, N = 20.28; Found: C = 52.14, H = 4.36, N = 20.25.

2-(2-Methyl-5-nitro-1*H*-imidazol-1-yl)ethyl benzo[d]thiazole-6-carboxylate (24)

Solid; Yield: 76%; ^{1}H-NMR: (400 MHz, DMSO-d_6): δ 9.60 (s, 1H, H-2′), 8.73 (s, 1H, H-7′), 8.19 (d, $J_{4',5'}$ = 8.0 Hz, 1H, H-4′), 8.04 (s, 1H, H-4), 7.98 (d, $J_{5',4'}$ = 8.4 Hz,1H, H-5′), 4.75 (t, $J_{2'',1''}$ = 4.8 Hz, 2H, CH_2-2″), 4.70 (t, $J_{1'',2''}$ = 4.8 Hz, 2H, CH_2-1″), 2.49 (s, 3H, CH_3); ^{13}C NMR (75 MHz, DMSO-d_6): δ 165.7, 155.9, 154.7, 151.8, 138.5, 133.7, 132.0, 126.7, 126.2, 123.5, 121.8, 62.4, 40.9, 12.8; EI-MS: *m/z* (rel. abund. %), 332 (M^+, 28), 286 (74), 206 (63), 179 (10), 162 (100), 143 (2), 134 (93); Anal. Calcd for $C_{14}H_{12}N_4O_4S$: C = 50.60, H = 3.64, N = 16.86; Found: C = 50.63, H = 3.61, N = 16.89.

2-(2-Methyl-5-nitro-1*H*-imidazol-1-yl)ethyl 1-naphthoate (25)

Solid; Yield: 82%; ^{1}H-NMR: (400 MHz, DMSO-d_6): δ 8.57 (d, $J_{2',3'}$ = 8.4 Hz, 1H, H-2′), 8.20 (d, $J_{8',7'}$ = 8.4 Hz,1H, H-8′), 8.05 (s, 1H, H-4), 8.03 (d, $J_{4',3'}$ = 8.8 Hz, 1H, H-4′), 8.00 (d, $J_{5',6'}$ = 7.2 Hz, 1H, H-5′), 7.61 (m, 3H, H-3′, H-6′, H-7′), 4.78 (t, $J_{2'',1''}$ = 4.8 Hz, 2H, CH_2-2″), 4.73 (t, $J_{1'',2''}$ = 4.4 Hz, 2H, CH_2-1″), 2.41 (s, 3H, CH_3); ^{13}C NMR (75 MHz, DMSO-d_6): δ 167.8, 151.8, 138.5, 134.4, 133.6, 133.4, 132.2, 129.8, 129.1, 128.3, 127.7, 126.9, 126.7, 126.4, 62.4, 40.7, 12.8; EI-MS: *m/z* (rel. abund. %), 325 (M^+, 53), 308 (3), 279 (26), 199 (17), 172 (10), 155 (100), 139 (5), 127 (58); Anal. Calcd for $C_{17}H_{15}N_3O_4$: C = 62.76, H = 4.65, N = 12.92; Found: C = 62.74, H = 4.67, N = 12.95.

2-(2-Methyl-5-nitro-1*H*-imidazol-1-yl)ethyl 2-naphthoate (26)

Solid; Yield: 78%; ^{1}H-NMR: (400 MHz, DMSO-d_6): δ 8.49 (s, 1H, H-1′), 8.09 (d, $J_{4',3'}$ = 8.4 Hz, 1H, H-4′), 8.05 (s, 1H, H-4), 8.03 (d, $J_{8',7'}$ = 8.4 Hz,1H, H-8′), 8.01 (d, $J_{5',6'}$ = 8.4 Hz, 1H, H-5′),

7.86 (dd, $J_{3',1'}$ = 1.6, $J_{3',2'}$ = 8.4, 1H, H-3′), 7.70 (t, $J_{6'(5',7')}$ = 8.0 Hz, 1H, H-6′), 7.64 (t, $J_{7'(6',8')}$ = 7.6 Hz, 1H, H-7′), 4.79 (t, $J_{2'',1''}$ = 4.8 Hz, 2H, CH_2-2″), 4.70 (t, $J_{1'',2''}$ = 4.8 Hz, 2H, CH_2-1″), 2.49 (s, 3H, CH_3); ^{13}C NMR (75 MHz, DMSO-d_6): δ 165.8, 151.6, 138.5, 135.6, 132.7, 132.3, 130.6, 129.4, 128.7, 128.4, 128.2, 128.2, 127.2, 125.8, 62.4, 40.7, 12.8; EI-MS: *m/z* (rel. abund. %), 325 (M^+, 31), 279 (30), 199 (21), 172 (12), 155 (100), 127 (63); Anal. Calcd for $C_{17}H_{15}N_3O_4$: C = 62.76, H = 4.65, N = 12.92; Found: C = 62.79, H = 4.67, N = 12.95.

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Highlights:

- Biology-oriented drug synthesis (BIODS) of 2-(2-methyl-5-nitro-1*H*-imidazol-1-yl)ethyl aryl carboxylate derivatives **1-26**
- All compounds were evaluated for *in vitro* β-glucuronidase inhibitory activity.
- Except few derivatives, all molecules were demonstrated good inhibition.
- Molecular docking studies were carried out to verify the structure-activity relationship.