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Identification of Bisindolylmethane-Hydrazone Hybrids as Novel Inhibitors of β- Glucuronidase, DFT and In Silico SAR Intimations

Muhammad Taha[*a,b], Nor Hadiani Ismail[a,b], Syahrul Imran[a,b], El Hassane Anouar[c], Muhammad Ali[d,e], Waqas Jamil[f], Nizam Uddin[g], Syed Muhammad Kashif[f]

[a]*Atta-ur-Rahman Institute for Natural Product Discovery, Universiti Teknologi MARA (UiTM), Puncak Alam Campus, 42300, Bandar Puncak Alam, Selangor, Malaysia*
[b]*Faculty of Applied Science Universiti Teknologi MARA (UiTM), 40450, Shah Alam, Selangor, Malaysia*
[c]*Chemistry Department, College of Sciences and Humanities,Prince Sattam bin Abdulaziz University, P.O. Box 83, Al-Kharij 11942, Saudi Arabia.*
[d]*Center for Advanced Drug Research, COMSATS Institute of Information Technology, University Road, Abbottabad-22060, KPK, Pakistan*
[e]*Department of Chemistry, COMSATS Institute of Information Technology, University Road, Abbottabad-22060, KPK, Pakistan*
[f]*Institute of Advance Research Studies in Chemical Sciences, University of Sindh Jamshoro, Hyderabad 76080, Pakistan,*
[g]*Batterje Medical College for Science & Technology, Jeddah-21442, Kingdom of Saudi Arabia*

Abstract:

Current study involves the synthesis of bisindolylmethane-hydrazone hybrids **1-30** in a three-step reaction sequence followed by the evaluation against β-glucuronidase enzyme. The IC_{50} values for potent compounds were in the range of 0.10 to 83.50 μM. Compound **16**, a trihydroxy analog was found to be the most potent derivative having IC_{50} value of 0.10 ± 0.001 μM. Molecular docking revealed that active compounds could fit perfectly into binding groove of β-D-glucuronidase. The presence of hydroxyl groups on aromatic side chain proved to be the single most important factor that contributed towards inhibitory potential of these compounds. On the other hand, imino group of hydrazone linkage displayed interactions with the side chain carboxyl oxygen (Oε2) of Asp207. The high inhibitory potential of these copounds could be associated with these strong hydrogen bonds. Structures of all the synthesized compounds were confirmed using modern spectroscopic methods .

Keywords: bisindolylmethanes, benzohydrazones, β-Glucuronidase, SAR, Molecular Modeling, DFT

* Correspondence and reprints
E-mail: taha_hej@yahoo.com and muhamm9000@puncakalam.uitm.edu.my, Tel: 0060193098141

1. Introduction

A number of indole alkaloids were found naturally from marine and terrestrial sources with unique chemical structures and diverse biological potentials like antibacterial, cytotoxic [1], and protection against DNA damage in human colon cell lines [2]. Some commercial drugs contain indole moiety as either a fundamental unit or an attached part to invoke biological activities [3]. Bisindole is a pompous class of indole family applauded as an important scaffold in pharmaceutical chemistry [4-9]. Bisindole and its derivatives have been widely identified as a pharmacophore to exhibit antiviral [10], anticancer [11], antitubercular [12], antihypertension [13], free radical induced lipid peroxidation [14], antioxidant [15], Alzheimer disease and antihyperlipidemic potentials [16]. A number of bis(indolyl)methanes were reported to have antioxidant potentials [17], anti-inflammatory with analgesic properties and ulcerogenic activities in mice and rats models [18]. The interesting biological profiles, distinctive chemical structures and low availability, made the bisindole alkaloids as attractive target for drug discovery.

β-glucuronides are the final metabolites of hydrophobic xenobiotics [19] and their hydrolysis is catalyzed by *β*-glucuronidase enzyme that is commonly found in animals, plants, and bacteria [20]. In animals, low *β*-glucuronidase activity is maintained through localization in lysosomes [21]. However, *β*-glucuronidase produced by intestinal bacteria hydrolyzes glucuronide to liberate xenobiotics. These xenobiotics exhibit toxicity in the intestine and lower the rate of excretion of xenobiotic by reabsorption. Thus to decrease toxicity, inhibition of bacterial *β*-glucuronidase in the intestine is required to promote excretion of xenobiotics. A number of *β*-glucuronidase inhibitors have been isolated from nature and categorized as terpenoids and their glucuronides [22-25], flavonoids and their glucuronides [26-27], and pseudo-sugars that contains nitrogen [28], while various synthetic compounds were also found as potent *β*-glucuronidase inhibitors [26].

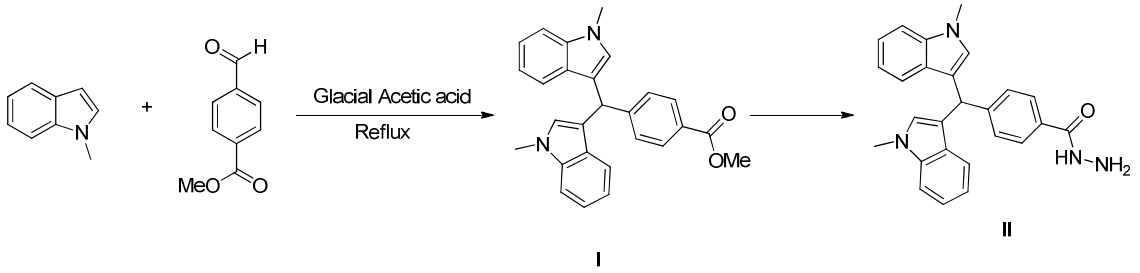
In continuation of our work on the development of new enzyme inhibitors for drug discovery, we have shown relentless work on synthesis and biological evaluation of potent heterocyclic scaffolds [27-29]. Recently we have published bisindolylmethane analogs as potent inhibitor of *β*-D-glucuronidase enzyme. This effort resulted in the identification of some novel inhibitors of *β*-D-glucuronidase enzyme [30]. However, these bisindolylmethane derivatives seriousy lacked the sites for further modifications for the identification of more potential leads. For more potent analogue identification, we have adopted the technique of

hybrid molecules in which we have combined two or more biologically active scaffolds. This methodology proved extremely successful and lead us in identifying some distinctly potent inhibitors, the bisindolylmethane-hydrazone hybrids **1-30**, of *β*-D-glucuronidase enzyme in the current study. Synthesized hybrids were evaluated *in vitro* against *β*-D-glucuronidase enzyme, an SAR was developed while the mode of binding action was evaluated by molecular modeling and DFT studies.

2. Results and Discussion

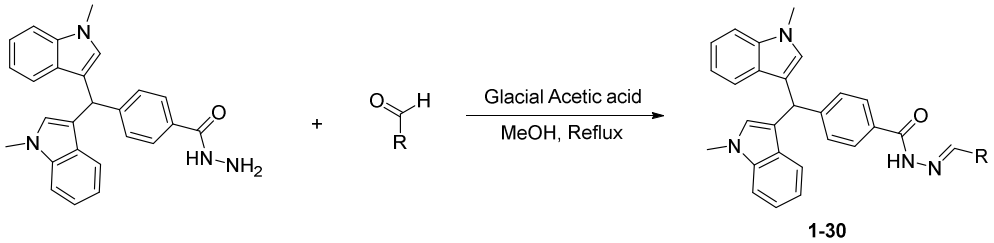
2.1 Chemistry

Bisindolylmethane ester **I** was obtained by reacting 1-methyl-1*H*-indole and methyl 4-formylbenzoate in refluxing acetic acid. Intermediate **I** was transformed into an advanced hydrazide intermediate **II** by refluxing it in ethanolic hydrazine hydrate solution (Scheme-1). In this study, various methods were used (I: $NaBrO_3/NaHSO_3$, EAN, and $BF_3{\cdot}Et_2O$) to synthesize the starting material methyl 4-(bis(1-methyl-1H-indol-3-yl)methyl)benzoate (I) [31] but in very low. When the same reaction was carried out in refluxing acetic acid, we could manage to recover up to 93% yield by handling the reaction carefully. The other conversions from ester to hydrazide and hydrazide to hydrazone are well documented with yields up to 70-85%.



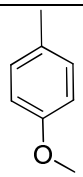
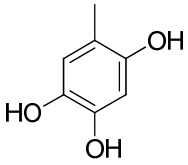
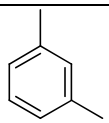
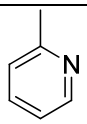
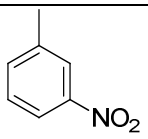
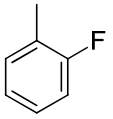
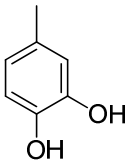
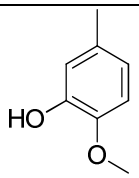
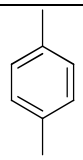
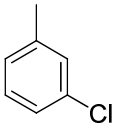
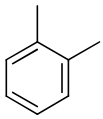
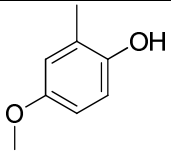
Scheme-1. Synthesis of hydrazide **II**

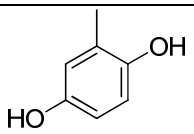
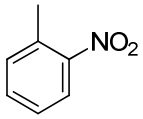
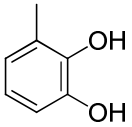
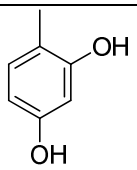
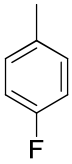
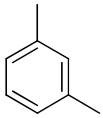
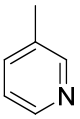
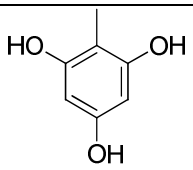
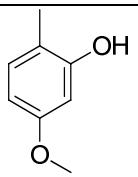
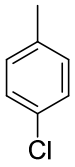
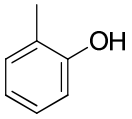
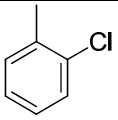
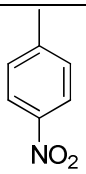
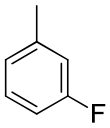
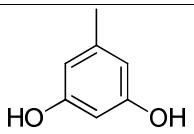
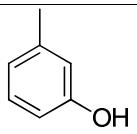
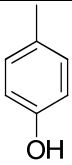
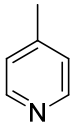
The advanced intermediate hydrazide II was then reacted with various aryl aldehydes in the presence of acetic acid to yield targeted bisindolylmethane-hydrazone hybrids **1-30** (Scheme 2; Table 1) in good to excellent yields. Structures of all the synthesized compounds **1-30** were confirmed using various spectroscopic techniques such as NMR, EIMS and were further confirmed through CHN analysis.



Scheme-2. Synthesis of hydrazone derivatives **1-30**

Table-1 Bisindolylmethane-hydrazone hybrids **1-30** and their β-D-glucuronidase inhibitory potentials

S. No.	R	IC_{50} (μM ± SEM[a])	S. No.	R	IC_{50} (μM ± SEM[a])
1		83.50 ± 1.70	16		0.10 ± 0.001
2		N. A.[b]	17		18.60 ± 0.30
3		73.50 ± 1.40	18		9.60 ± 0.16
4		0.20 ± 0.01	19		32.36 ± 0.64
5		51.10 ± 1.29	20		16.10 ± 0.29
6		23.12 ± .45	21		11.28 ± 0.23

7		3.50 ± 0.05	22		13.38 ± 0.22
8		0.50 ± 0.3	23		6.30 ± 0.10
9		3.80 ± 0.08	24		N. A.[b]
10		88.56 ± 1.88	25		2.20 ± 0.06
11		13.60 ± 0.26	26		26.63 ± 0.38
12		12.10 ± 0.24	27		15.20 ± 0.28
13		N. A.[b]	28		26.20 ± 0.39
14		22.18 ± 0.45	29		16.25 ± 0.29
15		15.26 ± 0.26	30		N. A.[b]
D-Saccharic acid 1,4-lactone			48.4 ± 1.25		

[a]SEM is the standard error of the mean, [b]N.A. No activity

2.2 *β*-Glucuronidase Inhibition Studies

In this study, we have evaluated bis-indole bearing hydrazones **1-30** for their *β*–glucuronidase inhibitory potential according to the protocol reported in literature [30]. These compounds demonstrated varying degree of inhibitory potentials while the IC_{50} values for potent compounds were found to be in the range of 0.10 to 83.50 μM (Table-1) when compared with standard *D*-Saccharic acid 1,4-lactone (IC_{50} value 48.4 ± 1.25 μM). In addition to the determination of inhibitory potentials, molecular dockings and DFT calculations were also performed to estimate the binding modes of the current series of compounds. The results are shown in Table-1. These compounds do not show toxicity that may be due to higher carbon content and are expected to have lower toxicity level as compared to compounds with small molecules as reported [32].

Compound **16**, a trihydroxy derivative, with IC_{50} value of 0.10 ± 0.001, was found to be ~ 500 times more active than the standard *D*-Saccharic acid 1,4-lactone. Compounds **6** (IC_{50} = 18.46 ± 0.65 μM), **7** (IC_{50} = 34.46 ± 0.85 μM), **8** (IC_{50} = 29.15 ± 0.75 μM) **9** (IC_{50} = 15.14 ± 0.55 μM) and **18** (IC_{50} = 21.14 ± 1.05 μM) were found more potent than the standard also. Compounds **25** (IC_{50} = 42.26 ± 1.16 μM) and **11** (IC_{50} = 44.16 ± 1.20 μM displayed inhibitory potentials comparable to the standard. It was shown that presence of hydroxyl and halogen groups, in general, proved to be the decisive factor that resulted in the enhanced inhibition of *β*-glucuronidase as depicted by our previous studies [33, 34]. Furthermore, some stunning similarities in inhibition potential between fluoro-, chloro-, hydroxyl and and nitro-substituted derivatives were observed. For example, all ortho analogues, **12** with OH group, **27** with chloro group, **18** with fluoro substitution and finally compound **22** with a nitro group, displayed a similar trend in their inhibitory potential (9.60 ± 0.16 to 13.38 ± 0.22 μM) towards *β*–glucuronidase. Similarly, meta- hydroxy and meta- chloro analogues, **29** and **20**, showed similar potential of inhibition. These findings suggest that electronic factors are also operating and playing their role in inhibition beside hydrogen bonding affects of hydroxyl groups. However, it is difficult to argue the strong inhibitory potentials of *di*- and *tri*-hydroxy substituted analogues and their interactions with receptor binding sites. To better understand the inhibition mechanisms and binding modes of the compounds **1-30** with *β*-glucuronidase, molecular modeling studies became imperative to understand the underlying phenomenon.

2.3 Molecular Docking Analysis

In order to obtain more insights into the binding mode of bisindolylmethane derivatives within the active site of *β*-D-glucuronidase and to obtain additional validations for experimental results, molecular docking studies were performed. In this study, X-ray crystal structure of human *β*-glucuronidase enzyme at 2.6 Å resolution (PDB ID: 1BHG) [36] was employed to further identify binding modes involved in the inhibition activity. Human *β*-D-glucuronidase 3D structure was used for our structure-activity relationship (SAR) studies due to the absence of bovine *β*-D-glucuronidase structure.

Prior to the docking of bisindolylmethane derivatives, the known substrate molecule *p*-nitrophenyl *β*-D-glucuronide was first docked into the active site of *β*-D-glucuronidase using the docking program AutoDock 4.2 [37]. The modeled substrate-bound structure of human *β* -D-glucuronidase showed that the glycoside bond of *p*-nitrophenyl *β*-D-glucuronide was properly oriented towards the catalytic residues Glu451 and Glu540 (**Fig. 1**). It has been proposed for human *β* -D-glucuronidase that during catalysis, Glu451 acts as the acid/base catalyst while Glu540 serves as the nucleophilic residue [36]. As shown in **Figure 1**, the substrate neatly fits in the active site making various hydrogen bonding interactions with the active site residues including Asp207, His385, Glu451, Tyr507, and Glu540.

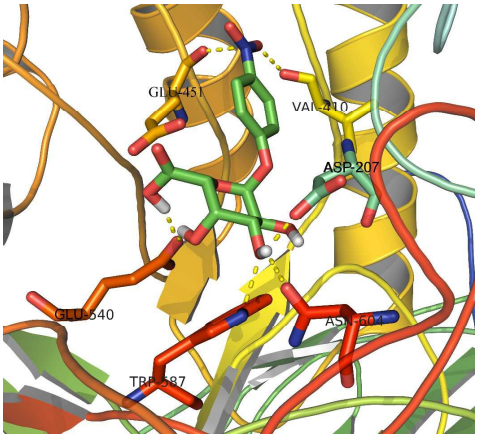


Figure 1. Native *β*-D-glucuronide in active site of *β*-glucuronidase surrounded by amino acid residues Asp207, His385, Glu451, Tyr507, and Glu540.

All the residues play an important role in binding of the substrate molecule in the active site of *β*-D-glucuronidase [36]. This modeled protein structure was used in the prediction of a favorable binding mode of newly synthesized bisindolylmethane derivatives. The predicted

binding models of the biologically active compounds are shown in **figure 2**. Docking studies showed that active compounds are well accommodated in the binding cavity of the enzyme. Analysis of the predicted binding conformations of our most active compound **16** (IC_{50} = 0.10 ± 0.001 μM) revealed that compound **16** can fit straight into the binding groove of β-D-glucuronidase. Visually inspecting the best binding position for compound **16** showed that the hydroxyl group at C-3 position formed a strong hydrogen bond interaction with the side chain carboxyl oxygen (Oε1) of Glu451 (1.94 Å) while hydroxyl group at C-5 position formed hydrogen bond with the side chain nitrogen (Nε2) of His385 (2.43 Å). However, the hydroxyl group at C-4 position does not form any interaction with the surrounding amino acid residues. Amino group of hydrazone linkage is involved in strong hydrogen bonding interaction with the side chain carboxyl oxygen (OD2) of Asp207 (1.82 Å). The high inhibition activity for compound **16** towards β-D-glucuronidase can be explained using these strong hydrogen bonds. The predicted binding mode of compound **16** is shown in **figure 2c**. His385, Asn450, Asn484, Glu451, and Tyr508, are involved in stabilizing the binding of compound in the active site of β-D-glucuronidase through π-sigma, π-anion, and T-shaped π-π interactions.

In contrast, the binding mode of compound **4** (IC_{50} = 0.20 ± 0.01 μM), which is the second most active compound among the series revealed that the hydroxyl group at *meta* position is involved in hydrogen bond interaction with the side chain carboxyl oxygen (Oε1) of Glu540 (1.98 Å)(**Figure 2a**). Another hydroxyl group for compound **4** at *para* position was observed to form hydrogen bond with Asn450 (2.68 Å). The binding mode of compound **8** (IC_{50} = 0.50 ± 0.3 μM) revealed that the hydroxyl group at *ortho* position makes hydrogen bond with the side chain carboxyl of Glu451 (1.93 Å), while the hydroxyl at para position did not adopt favorable conformation for binding with the surrounding residues (**Fig. 2b**). The observed binding mode of compound **25** showed that it possesses three hydroxyl groups at *ortho* and *para* position. Docking study showed that the hydroxyl at *para* position is able to interacts well with Glu540 (2.03 Å), Asn450 (2.94 Å), and His385 (2.59 Å). Both hydroxyl groups at *ortho* position for compound **25** formed strong interactions with the side chain hydroxyl oxygen (OD2) of both Asp207 (1.67Å) and Glu451 (1.56 Å) (**Fig. 2d**). Hydrazone linkage formed weak hydrogen bonding with some important residues like Arg600 (3.03 Å), Lys606 (2.81 Å), and Tyr504 (2.36 Å).

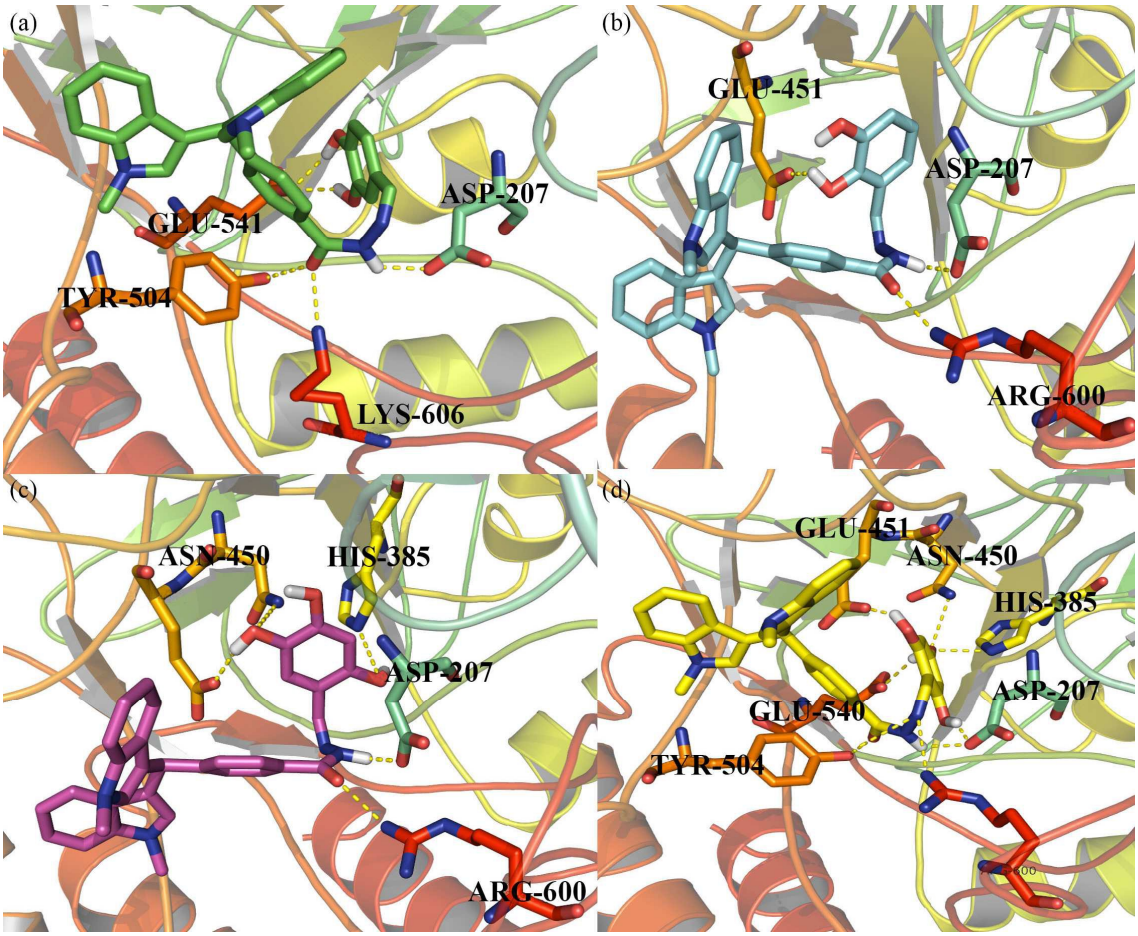


Figure-2. Binding position for (a) compounds **4**, (b) compounds **8**, (c) compounds **16**, and (d) compounds **25** in the same binding cavity occupied by native *β*-D-glucuronide surrounded by amino acid residues Asp207, His385, Glu451, Tyr507, and Glu540.

In this study, it was observed that the compounds are positioned in a way that enabled *meta* hydroxyl substituent to form better interaction with the important residues like Glu451 and Glu540 than to hydroxyls being at *ortho* and *para* positions.

Docking studies on inactive compounds **1** (IC_{50} = 83.50 ± 1.70 μM), **3** (IC_{50} = 73.50 ± 1.40 μM), and **10** (IC_{50} = 88.56 ± 1.88 μM) (**Fig. 3**) has clearly demonstrated the reason for inactivity against the enzyme. These compounds are unable to form any noteworthy hydrogen bond interactions with the surrounding amino acid residues. It was observed that their hydrazone linkage generally formed hydrogen bond interactions with Asn207 and Arg600 with the distance between 1.81-2.65 Å. Hence, the activity of compounds largely depends on the maximum hydrophilic interactions with the active site amino acids of the *β*-D-glucuronidase.

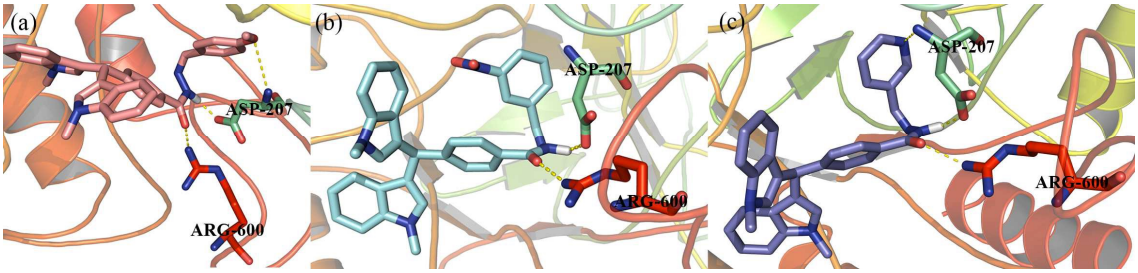


Figure 3. Binding mode of inactive compounds **1** (a), compounds **3** (b), and compounds **10** (c) in the same binding cavity occupied by native *β*-D-glucuronide surrounded by amino acid residues Asp207, His385, Glu451, Tyr507, and Glu540.

2.4 Density Functional Predictions for Hydrogen Bonding

In order to emphasize the role of hydrogen bonds (intramolecular or intermolecular) of the synthesized bisindolylmethane-hyrdazone hybrids in *β*-Glucuronidase inhibition, DFT calculations were performed at the B3LYP/6-31+G(d,p) level. The potency of β-glucuronidase inhibition of synthesized bisindolylmethane derivatives can be related to the number of intramolecular hydrogen bonds occur in the substrates (bisindolylmethane derivatives), and to the number of intermolecular hydrogen bonds that occur at substrate-receptor complex. Here, we focussed on the stability and effects of the number of intramolecular hydrogen bonds of bisindolylmethane derivatives in *β*-glucuronidase inhibition. A high number of hydrogen bonds mean that bisindolylmethane is more stable, and the inhibition is more profound (low IC_{50}). The gap energies (eV), electronic energies, number of electronic donor groups, number of withdrawing groups, number of intramolecular hydrogen bonds, number of intramolecular hydrogen bonds of the bisindolylmethane hybrids are reported in Table 2. Bisindolylmethane hybrids showing high *β*-glucuronidase inhibition ($0.1 \leq IC_{50} \leq 10$ μM) are those with high number of electron donating groups. For instance, bisindolylmethane derivative **16**, with two intramolecular hydrogen bonds (Table 1s), and three electron donating groups (*i.e.,* ability to form three intermolecular hydrogen bonds) showed a potent β-glucuronidase inhibition (IC_{50} =0.1 μM) compared with bisindolylmethane derivative **7**, which possesses one intramolecular hydrogen bond (Table 1S, supporting information), and two electron donor groups (IC_{50} =3.50 μM). The bisindolylmethane derivative **16** is relatively stable compared to **7** (ΔE = 4.7 kcal/mol), which confirms the role of hydrogen bonding on the stability of the substrate and its role (i.e., hydrogen bond) in inhibiting *β*-glucuronidase. The 3D-structure of the optimized

bisindolylmethane-hydrazone hybrids are reported in Figure 1S in supporting information. Based on results from docking and density functional theory studies, some discussion on the ability of these compounds to mainly interact with the target enzyme through hydrogen bonding or van der waals interactions had been included. As the results showed that the compounds are interacting quite well through these interactions, which involves no chemical interaction, these compounds could possibly be reversible inhibitors.

Table 2 Number of electron donating groups (EDG), Number of electron withdrawing groups (EDG), number of intramolecular hydrogen bonds (HB), number of possible intermolecular hydrogen bonds (HB'), electronic energies E (kcal/mol), and gap energies of active bisindolylmethane derivatives.

Compounds	EDG	EWG	HBs	HB'	OH	Electronic energy, E	Gap Energy	IC_{50} (μM ± SEM[a])
1	1	0	0	1	0	-1056229.52	3.69	83.5 ± 1.70
2	1	0	0	0	0	-1009033.76	3.60	N. A.[b]
3	0	1	0	0	0	-1112695.66	2.43	73.5 ± 1.40
4	2	0	1	2	2	-1078770.62	3.67	0.2 ± 0.01
5	1	0	0	0	0	-1009034.12	3.62	51.1 ± 1.29
6	1	0	0	0	0	-1009032.00	3.55	23.12 ± 0.45
7	2	0	1	2	2	-1078773.53	3.52	3.5 ± 0.05
8	2	0	1	2	2	-1078768.97	3.57	0.5 ± 0.3
9	0	1	1	0	0	-1046636.60	3.56	3.8 ± 0.08
10	0	0	0	0	0	-994424.90	3.42	88.56 ± 1.88
11	2	0	1	2	1	-1103439.57	3.67	13.6 ± 0.26
12	1	0	1	1	1	-1031570.87	3.55	12.1 ± 0.24
13	0	1	0	0	0	-1112696.32	2.31	N. A.[b]
14	2	0	1	2	2	-1078773.53	3.52	22.18 ± 0.45
15	1	0	1	1	1	-1031566.53	3.69	15.26 ± 0.26
16	3	0	2	3	3	-1125978.56	3.65	0.1 ± 0.008
17	0	0	0	0	0	-994424.73	3.36	18.6 ± 0.30
18	0	1	0	0	0	-1046636.25	3.46	9.6 ± 0.16
19	2	0	1	2	1	-1103434.15	3.69	32.36 ± 0.64
20	0	1	0	0	0	-1272761.43	3.44	16.1 ± 0.29
21	2	0	1	2	1	-1103436.46	3.54	11.28 ± 0.23

22	0	1	0	0	0	-1112690.60	2.44	13.38 ± 0.22
23	2	0	1	2	2	-1078776.66	3.67	6.3 ± 0.10
24	1	0	0	0	0	-1009033.85	3.59	N. A.[b]
25	3	0	1	3	3	-1125980.82	3.69	2.2 ± 0.06
26	0	1	0	0	0	-1272761.84	3.47	26.63 ± 0.38
27	0	1	0	0	0	-1272757.00	3.47	15.2 ± 0.28
28	0	1	0	0	0	-1046636.45	3.45	26.2 ± 0.39
29	1	0	1	1	1	-1031565.89	3.53	16.25 ± 0.29
30	0	0	0	0	0	-994425.20	3.24	N. A.[b]

[a]SEM is the standard error of the mean, [b]N.A. No activity

In conclusion, the binding of active compounds with the target site is mainly dependent upon the nature and position of polar groups such as hydroxyl and halogen groups. Besides identification of hydroxyl and halogen substituted analogues as the most potent compounds, it was also observed that the compounds in which hydroxyl groups were positioned adjacently were found to interact exclusively with the important residues like Glu451 and Glu540 as compared to hydroxyl groups that were far apart from each other. Further to this, DFT studies confirmed the role of hydrogen bonding along with the role of electronic nature of the substitutents; these factors played pivotal role in the inhibition potential of *β*-glucuronidase.

3. Experimental

3.1 General

NMR experiments were performed on UltraShield Bruker FT NMR 500 MHz; CHN analysis was performed on a Carlo Erba Strumentazion-Mod-1106, Italy. Electron impact mass spectra (EI-MS) were recorded on a Finnegan MAT-311A, Germany. Thin layer chromatography (TLC) was performed on pre-coated silica gel aluminum plates (Kieselgel 60, 254, E. Merck, Germany). Chromatograms were visualized by UV at 254 and 365 nm.

3.2 *β*-glucuronidase inhibition activity

β-glucuronidase (E.C. 3.2.1.31 from bovine liver, G-0251) inhibition activity of the compounds had been evaluated by using method as reported in [30, 37]. In this study, *D*-saccharic acid 1,4-lactone had been used as a standard drug [38].

3.3 Synthesis of methyl 4-(bis(1-methyl-1H-indol-3-yl)methyl)benzoate (I)

Methyl 2-hydroxybenzoate (**1**) (7.60 g, 53 mmol) and 20 ml of hydrazine hydrate were mixed in methanol (50 mL). The mixture was refluxed for 6 hours. Methanol was then evaporated and the product formed was being rinsed with plenty of water to remove excess hydrazine hydrate. The product formed was left to dry at room temperature and yielded. Yield is 9.68 g (94.9 %). White solid, m.p. 239-241°C; IR (cm-1, ATR): 3082, 2975, 1738, 1627, 1546, 1468, 1126; ^{1}H NMR (500 MHz, DMSO-d_6): δ 7.97 (d, J = 7.5 Hz, 2H), 7.83 (dd, J = 7.5, 1.6 Hz, 2H), 7.76 (dd, J = 7.4, 1.5 Hz, 2H), 7.63 (s, 2H), 7.44 (d, J = 7.5 Hz, 2H), 7.32 (dtd, J = 29.3, 7.5, 1.5 Hz, 4H), 5.74 (s, 1H), 3.92 (s, 3H), 3.78 (s, 6H); ^{13}C-NMR (DMSO-d_6, 125 MHz): δ 167.45, 141.55, 141.55, 140.27, 130.69, 130.37, 130.37, 128.65, 128.65, 128.49,

128.49, 128.20, 128.20, 124.69, 124.69, 123.42, 123.42, 122.72, 122.72, 111.63, 111.63, 111.08, 111.08, 52.18, 38.43, 35.19, 35.19; HREI-MS: m/z calcd for C27H24N2O2 [M]+ 408.1838; Found 408.1838; Anal. Calcd for C27H24N2O2, C, 79.39; H, 5.92; N, 6.86; O, 7.83, found C, 79.39; H, 5.92; N, 6.86; O, 7.83.

3.4 Synthesis of 4-(bis(1-methyl-1H-indol-3-yl)methyl)benzohydrazide (II)

A mixture of compound **2** (7.00 g, 46 mmol), methyl 4-formylbenzoate (7.56 g, 46 mmol) and catalytic amount of acetic acid in methanol (50 mL) was refluxed for 3 hours. The solvent was evaporated and the residue (**3**) was washed with diethyl ether, filtered, dried, and then crystallized from ethanol and gives brownish solid, (5.69 g, 93.0 %). m.p. 269-271 °C; IR (cm-1, ATR): 3423, 3352, 3137, 2997, 1655, 1612, 1558, 1469, 1341, 1268; 1H NMR (500 MHz, DMSO-d6): δ 8.33 (s, 1H), 7.94 – 7.84 (m, 4H), 7.76 (dd, J = 7.5, 1.4 Hz, 2H), 7.49 (d, J = 7.5 Hz, 2H), 7.42 (s, 2H), 7.34 (td, J = 7.4, 1.5 Hz, 2H), 7.26 (td, J = 7.4, 1.5 Hz, 2H), 5.73 (s, 1H), 4.08 (s, 2H), 3.76 (s, 6H); ^{13}C-NMR (150 MHz, DMSO-d6,): δ 167.32, 142.28, 141.57, 141.57, 135.10, 135.10, 130.34, 130.34, 129.66, 129.66, 128.20, 128.20, 126.76, 126.76, 124.79, 124.79, 123.56, 123.56, 122.85, 122.75, 111.78, 111.78, 111.28, 111.28, 38.48, 35.99, 35.99; HREI-MS: m/z calcd for C26H24N4O [M]+ 408.1950; Found 408.1950; Anal. Calcd for C26H24N4O, C, 76.45; H, 5.92; N, 13.72;, found C, 76.45; H, 5.92; N, 13.72;

3.4.1 General procedure for synthesis of oxadiazole benzohydrazones (1-30)

Equimolar quantities (1 mmol) of compound **5** and substituted benzaldehydes (1 mmol) in methanol (25 mL) were refluxed for 3 h, in the presence of catalytic amount of glacial acetic acid. The resulting solid was filtered and recrystallized from methanol in good yields.

3.4.1.1.(E)-4-(bis(1-methyl-1H-indol-3-yl)methyl)-N'-(4-methoxybenzylidene)benzohydrazide (1)

Brown solid. Yield: 75.2%. m.p.: 259-261 °C. IR (cm-1, ATR): 3425, 3339, 3143, 1643, 1609, 1254. 1H NMR (500 MHz, DMSO-d6): δ 11.62 (s, 1H), 8.36 (s, 1H), 7.81 (d, J = 8.2 Hz, 2H), 7.66 (d, J = 8.3 Hz, 2H), 7.50 (d, J = 8.0 Hz, 2H), 7.40 (d, J = 8.2 Hz, 2H), 7.33 (d, J = 7.9 Hz, 2H), 7.13 (t, J = 7.6 Hz, 2H), 7.02 (d, J = 8.4 Hz, 2H), 6.93 (t, J = 7.5 Hz, 2H), 6.88 (s, 2H), 5.94 (s, 1H), 3.81 (s, 3H), 3.72 (s, 6H); ^{13}C NMR (150 MHz, DMSO-d6): δ 164.32, 160.20, 149.54, 142.36, 141.70, 141.70, 134.31, 130.25, 130.25, 129.63, 129.63, 129.22, 129.22, 128.19, 128.19, 128.09, 128.09, 127.15, 124.79, 124.79, 123.42, 123.42, 122.71,

122.71, 114.42, 114.42, 111.78, 111.78, 111.02, 111.02, 56.13, 38.32, 35.92, 35.92; HREI-MS: m/z calcd for $C_{34}H_{30}N_4O_2$ [M]+ 526.2369; Found 526.2373; Anal. Calcd for $C_{34}H_{30}N_4O_2$, C, 77.54; H, 5.74; N, 10.64; found C, 77.56; H, 5.73; N, 10.65;

3.4.1.2.*(E)-4-(bis(1-methyl-1H-indol-3-yl)methyl)-N'-(3-methylbenzylidene)benzohydrazide (2)*

Pale yellow solid. Yield: 82.9%. m.p.: 262-263 °C. IR (cm-1, ATR): 3324, 3260, 3064, 1656, 1590, 1539, 1469, 1357, 1277, 1180, 752; ^{1}H NMR (500 MHz, DMSO-d6): δ 11.73 (s, 1H), 8.38 (s, 1H), 7.82 (d, J = 7.9 Hz, 2H), 7.55 (s, 1H), 7.50 (d, J = 8.0 Hz, 2H), 7.40 (d, J = 8.2 Hz, 2H), 7.33 (d, J = 7.9 Hz, 2H), 7.25 (d, J = 8.1 Hz, 1H), 7.13 (t, J = 7.6 Hz, 2H), 6.93 (t, J = 7.5 Hz, 2H), 6.88 (s, 2H), 5.95 (s, 1H), 3.72 (s, 6H); ^{13}C NMR (150 MHz, DMSO-d6): *δ* 164.38, 148.78, 142.56, 141.40, 141.40, 137.86, 136.61, 134.31, 130.45, 130.45, 129.81, 129.63, 128.74, 128.74, 128.59, 128.31, 128.09, 128.09, 124.79, 124.79, 123.51, 123.51, 123.49, 123.49, 122.73, 122.73, 111.78, 111.78, 111.15, 111.15, 38.48, 35.69, 35.69, 21.35; HREI-MS: m/z calcd for $C_{34}H_{30}N_4O$ [M]+ 510.2420; Found 510.2424; Anal. Calcd for $C_{34}H_{30}N_4O$, C, 79.97; H, 5.92; N, 10.97; Found C, 79.98; H, 5.93; N, 10.98;

3.4.1.3. ***(E)-4-(bis(1-methyl-1H-indol-3-yl)methyl)-N'-(3-nitrobenzylidene)benzohydrazide (3)***

Red solid. Yield: 71.3%. m.p.: 262-264 °C. IR (cm-1, ATR): 3335, 3260, 1560, 3138, 1657, 1609, 1275, 1125; 1H NMR (500 MHz, DMSO-d6): δ 12.03 (s, 1H), 8.55 (s, 2H), 8.26 (d, J = 7.4 Hz, 1H), 8.15 (d, J = 7.0 Hz, 1H), 7.84 (d, J = 7.8 Hz, 2H), 7.76 (t, J = 7.8 Hz, 1H), 7.52 (d, J = 8.1 Hz, 2H), 7.40 (d, J = 8.2 Hz, 2H), 7.34 (d, J = 7.9 Hz, 2H), 7.13 (t, J = 7.5 Hz, 2H), 6.94 (t, J = 7.5 Hz, 2H), 6.89 (s, 2H), 5.96 (s, 1H), 3.73 (s, 6H); ^{13}C NMR (150 MHz, DMSO-d6): δ 164.52, 148.78, 147.38, 142.71, 141.47, 141.47, 137.78, 134.11, 132.69, 130.31, 130.31, 129.96, 129.51, 129.51, 128.25, 128.25, 128.20, 128.20, 124.79, 124.79, 124.12, 123.44, 123.44, 122.72, 122.72, 122.43, 111.78, 111.78, 111.26, 111.26, 38.37, 35.92, 35.92; HREI-MS: m/z calcd for $C_{33}H_{27}N_5O_3$ [M]+ 541.2114; Found 541.2118;Anal. Calcd for $C_{33}H_{27}N_5O_3$, C, 73.18; H, 5.02; N, 12.93; Found C, 73.19; H, 5.00; N, 12.94;

3.4.1.4.*(E)-4-(bis(1-methyl-1H-indol-3-yl)methyl)-N'-(3,4-dihydroxybenzylidene)benzohydrazide (4)*

Red solid. Yield: 78.2%. m.p.: 278-280 °C. IR (cm-1, ATR): 3423, 3299, 3072, 1636, 1617, 1545, 1277, 1180; 1H NMR (500 MHz, DMSO-d6): δ 11.50 (s, 1H), 9.35 (s, 1H), 9.23 (s, 1H), 8.23 (s, 1H), 7.80 (d, J = 8.2 Hz, 2H), 7.49 (d, J = 8.2 Hz, 2H), 7.39 (d, J = 8.3 Hz, 2H), 7.33 (d, J = 7.9 Hz, 2H), 7.23 (s, 1H), 7.13 (t, J = 7.6 Hz, 2H), 6.91-6.95 (m, 3H), 6.88 (s, 1H), 6.78 (d, J = 8.1 Hz, 1H), 5.94 (s, 1H), 3.72 (s, 6H); ^{13}C NMR (150 MHz, DMSO-d_6): δ 164.38, 148.69, 148.09, 145.47, 142.76, 141.48, 141.48, 134.18, 130.32, 130.32, 129.73, 129.73, 128.49, 128.49, 128.20, 128.20, 127.87, 124.69, 124.69, 123.36, 123.36, 122.65, 122.65, 121.12, 116.73, 115.93, 111.58, 111.58, 111.18, 111.18, 38.52, 35.92, 35.92; HREI-MS: m/z calcd for $C_{33}H_{28}N_4O_3$ [M]+ 528.2161; Found 528.2161; Anal. Calcd for $C_{33}H_{28}N_4O_3$, C, 74.98; H, 5.34; N, 10.60; Found C, 75.01; H, 5.32; N, 10.58;

3.4.1.5.*(E)-4-(bis(1-methyl-1H-indol-3-yl)methyl)-N'-(4-methylbenzylidene)benzohydrazide (5)*

Dark brown solid. Yield: 79.4%. m.p.: 246-248 °C. IR (cm-1, ATR): 3347, 3352, 3043, 1631, 1591, 1555, 1357, 1270, 1143, 752; 1H NMR (500 MHz, DMSO-d_6): δ 11.68 (s, 1H), 8.38 (s, 1H), 7.81 (d, J = 8.1 Hz, 2H), 7.61 (d, J = 7.4 Hz, 2H), 7.50 (d, J = 7.9 Hz, 2H), 7.40 (d, J = 8.2 Hz, 2H), 7.33 (d, J = 7.6 Hz, 2H), 7.27 (d, J = 7.5 Hz, 2H), 7.13 (t, J = 7.6 Hz, 2H), 6.93 (t, J = 7.5 Hz, 2H), 6.88 (s, 2H), 5.95 (s, 1H), 3.72 (s, 6H), 2.35 (s, 3H); ^{13}C NMR (150 MHz, DMSO-d6): δ 164.38, 149.24, 142.56, 141.47, 141.47, 138.49, 134.15, 131.96, 130.38, 130.38, 129.43, 129.43, 129.25, 129.25, 128.28, 128.28, 128.07, 128.07, 127.30, 127.30, 124.82, 124.82, 123.36, 123.36, 122.63, 122.63, 111.67, 111.67, 111.04, 111.04, 38.38, 35.72, 35.72, 21.09; HREI-MS: m/z calcd for $C_{34}H_{30}N_4O$ [M]+ 510.2420; Found 510.2425; Anal. Calcd for $C_{34}H_{30}N_4O$, C, 79.97; H, 5.92; N, 10.97; Found C, 79.98; H, 5.90; N, 10.95;

3.4.1.6.*(E)-4-(bis(1-methyl-1H-indol-3-yl)methyl)-N'-(2-methylbenzylidene)benzohydrazide (6)*

Light brown solid. Yield: 89.7%. m.p.: 246-248 °C. IR (cm-1, ATR): 3325, 3299, 3043, 1631, 1568, 1357, 1277, 1180, 752; 1H NMR (500 MHz, DMSO-d6): δ 11.73 (s, 1H), 8.72 (s, 1H), 7.83 (t, J = 7.7 Hz, 2H), 7.51 (d, J = 7.9 Hz, 2H), 7.40 (d, J = 8.2 Hz, 2H), 7.33 (d, J = 7.9 Hz, 2H), 7.32 – 7.23 (m, 3H), 7.13 (t, J = 7.6 Hz, 2H), 6.94 (t, J = 7.5 Hz, 2H), 6.88 (s, 2H), 5.95 (s, 1H), 3.72 (s, 6H), 2.43 (s, 3H); ^{13}C NMR (150 MHz, DMSO-d_6): δ 164.28, 145.62, 142.69, 141.45, 141.45, 136.27, 134.26, 133.49, 130.51, 130.51, 130.31, 129.50, 129.50, 128.49, 128.31, 128.31, 128.12, 128.12, 126.79, 126.27, 124.69, 124.69, 123.48,

123.48, 122.79, 122.79, 111.58, 111.58, 111.18, 111.18, 38.32, 35.91, 35.91, 20.15; HREI-MS: m/z calcd for $C_{34}H_{30}N_4O$ [M]+ 510.2420; Found 510.2415; Anal. Calcd for $C_{34}H_{30}N_4O$, C, 79.97; H, 5.92; N, 10.97; Found C, 79.99; H, 5.90; N, 10.96;

3.4.1.7.*(E)-4-(bis(1-methyl-1H-indol-3-yl)methyl)-N'-(2,5-dihydroxybenzylidene)benzohydrazide (7)*

Red solid. Yield: 92.6%. m.p.: 284-285 °C. IR (cm-1, ATR): 3353, 3139, 2994, 1657, 1611, 1556, 1495, 1466, 1259, 1166, 749; 1H NMR (500 MHz, DMSO-d6): δ 11.90 (s, 1H), 10.40 (s, 1H), 8.95 (s, 1H), 8.53 (s, 1H), 7.84 (d, J = 8.0 Hz, 2H), 7.51 (d, J = 8.1 Hz, 2H), 7.40 (d, J = 8.2 Hz, 2H), 7.33 (d, J = 7.9 Hz, 2H), 7.13 (t, J = 7.6 Hz, 2H), 6.97 – 6.91 (m, 3H), 6.88 (s, 2H), 5.95 (s, 1H), 3.72 (s, 6H); ^{13}C NMR (150 MHz, DMSO-d_6): δ 164.46, 152.63, 151.39, 149.69, 142.52, 141.47, 141.47, 134.18, 130.34, 130.34, 129.57, 129.57, 128.16, 128.16, 128.09, 128.09, 124.76, 124.76, 123.26, 123.26, 122.65, 122.65, 122.16, 121.52, 118.28, 116.47, 111.78, 111.78, 111.04, 111.04, 38.47, 35.93, 35.93; HREI-MS: m/z calcd for $C_{33}H_{28}N_4O_3$ [M]+ 528.2161; Found 528.2157; Anal. Calcd for $C_{33}H_{28}N_4O_3$, C, 74.98; H, 5.34; N, 10.60; Found C, 74.99; H, 5.32; N, 10.58;

3.4.1.8.*(E)-4-(bis(1-methyl-1H-indol-3-yl)methyl)-N'-(2,3-dihydroxybenzylidene)benzohydrazide (8)*

Orange solid, Yield: 86.5%. m.p.: 257-259 °C. IR (cm-1, ATR): 3381, 3062, 1613, 1611, 1269, 1205, 750; 1H NMR (500 MHz, DMSO-d6): δ 12.02 (s, 1H), 11.18 (s, 1H), 9.17 (s, 1H), 8.56 (s, 1H), 7.85 (d, J = 6.4 Hz, 2H), 7.52 (d, J = 6.6 Hz, 2H), 7.40 (d, J = 8.1 Hz, 2H), 7.34 (d, J = 7.7 Hz, 2H), 7.13 (t, J = 6.5 Hz, 2H), 6.94 (t, J = 7.3 Hz, 2H), 6.89 (s, 1H), 6.86 (d, J = 7.1 Hz, 1H), 6.74 (t, J = 6.6 Hz, 1H), 5.96 (s, 1H), 3.73 (s, 6H); ^{13}C NMR (150 MHz, DMSO-d_6): δ 164.37, 149.97, 146.93, 144.72, 142.68, 141.57, 141.57, 134.19, 130.34, 130.34, 129.52, 129.52, 128.18, 128.18, 128.08, 128.08, 124.91, 124.91, 123.49, 123.49, 122.85, 122.85, 122.09, 121.42, 119.90, 119.73, 111.61, 111.61, 111.18, 111.18, 38.39, 35.94, 35.94; HREI-MS: m/z calcd for $C_{33}H_{28}N_4O_3$ [M]+ 528.2161; Found 528.2165; Anal. Calcd for $C_{33}H_{28}N_4O_3$, C, 74.98; H, 5.34; N, 10.60; Found C, 74.99; H, 5.35; N, 10.58;

3.4.1.9.*(E)-4-(bis(1-methyl-1H-indol-3-yl)methyl)-N'-(4-fluorobenzylidene)benzohydrazide (9)*

Brown solid. Yield: 85.8%. m.p.: 248-250 °C. IR (cm-1, ATR): 3354, 3212, 3021, 1626, 1606, 1519, 1470, 1372, 1269, 1186, 760; 1H NMR (500 MHz, DMSO-d6): δ 11.77 (s, 1H), 8.42 (s, 1H), 7.86 – 7.73 (m, 4H), 7.50 (d, J = 7.9 Hz, 2H), 7.40 (d, J = 8.2 Hz, 2H), 7.33 (d, J = 7.9 Hz, 2H), 7.32 – 7.26 (m, 2H), 7.13 (t, J = 7.6 Hz, 2H), 6.94 (t, J = 7.4 Hz, 2H), 6.88 (s, 2H), 5.95 (s, 1H), 3.73 (s, 6H); ^{13}C NMR (150 MHz, DMSO-d_6): δ 164.52, 160.29 (d, J = 261.5 Hz), 149.43, 142.51, 141.62, 141.62, 134.74, 130.69, 130.40, 130.40, 130.25, 130.25, 129.43, 129.43, 128.31, 128.31, 128.15, 128.15, 124.82, 124.82, 123.36, 123.36, 122.72, 122.72, 115.51, 115.51, 111.78, 111.78, 111.18, 111.18, 38.25, 35.69, 35.69; HREI-MS: m/z calcd for $C_{33}H_{27}FN_4O$ [M]+ 514.2169; Found 514.2172; Anal. Calcd for $C_{33}H_{27}FN_4O$, C, 77.02; H, 5.29; N, 10.89; Found C, 77.01; H, 5.31; N, 10.90;

3.4.1.10.*(E)-4-(bis(1-methyl-1H-indol-3-yl)methyl)-N'-(pyridin-3-ylmethylene)benzohydrazide (10)*

Light brown solid. Yield: 89.6%. m.p.: 258-260 °C. IR (cm-1, ATR): 3373, 3159, 2835, 1609, 1558, 1470, 1445, 1257, 1186, 754; 1H NMR (500 MHz, DMSO-d6): δ 11.93 (s, 1H), 8.85 (s, 1H), 8.61 (s, 1H), 8.48 (s, 1H), 8.13 (s, 1H), 7.83 (d, J = 7.3 Hz, 2H), 7.51 (d, J = 7.8 Hz, 2H), 7.40 (d, J = 8.0 Hz, 2H), 7.34 (d, J = 7.7 Hz, 2H), 7.13 (t, J = 7.3 Hz, 2H), 6.94 (t, J = 7.5 Hz, 2H), 6.89 (s, 2H), 5.95 (s, 1H), 3.72 (s, 6H); ^{13}C NMR (150 MHz, DMSO-d_6): δ 165.32, 150.15, 146.51, 145.72, 142.46, 141.70, 141.70, 135.59, 134.17, 133.82, 130.45, 130.45, 129.33, 129.33, 128.18, 128.18, 128.07, 128.07, 124.91, 124.91, 124.31, 123.45, 123.45, 122.85, 122.85, 111.72, 111.72, 111.02, 111.02, 38.52, 35.79, 35.79; HREI-MS: m/z calcd for $C_{32}H_{27}N_5O$ [M]+ 497.2216; Found 497.2213; Anal. Calcd for $C_{32}H_{27}N_5O$, C, 77.24; H, 5.47; N, 14.07; Found C, 77.25; H, 5.48; N, 14.05;

3.4.1.11.*(E)-4-(bis(1-methyl-1H-indol-3-yl)methyl)-N'-(2-hydroxy-4-methoxybenzylidene)benzohydrazide (11)*

Red solid. Yield: 89.6%. m.p.: 255-257 °C. IR (cm-1, ATR): 3389, 3249, 3002, 1684, 1652, 1585, 1467, 1496, 1282, 1205, 750; 1H NMR (500 MHz, DMSO-d6): δ 11.90 (s, 1H), 11.64 (s, 1H), 8.51 (s, 1H), 7.83 (d, J = 8.1 Hz, 2H), 7.51 (d, J = 8.2 Hz, 2H), 7.40 (t, J = 4.2 Hz, 2H), 7.39 (s, 1H), 7.33 (d, J = 7.9 Hz, 2H), 7.13 (t, J = 7.6 Hz, 2H), 6.93 (t, J = 7.5 Hz, 2H), 6.88 (s, 2H), 6.52 (d, J = 8.6 Hz, 1H), 6.50 (s, 1H), 5.95 (s, 1H), 3.78 (s, 3H), 3.72 (s, 6H); ^{13}C NMR (150 MHz, DMSO-d_6): δ 164.41, 162.10, 161.25, 151.29, 142.69, 141.72, 141.72, 134.31, 130.92, 130.32, 130.32, 129.58, 129.58, 128.26, 128.26, 128.18, 128.18, 124.79,

124.79, 123.36, 123.36, 122.62, 122.62, 113.87, 111.69, 111.69, 111.18, 111.18, 107.39, 102.25, 56.23, 38.39, 35.92, 35.92; HREI-MS: m/z calcd for $C_{34}H_{30}N_4O_3$ [M]+ 542.2318; Found 542.2323; Anal. Calcd for $C_{34}H_{30}N_4O_3$, C, 75.26; H, 5.57; N, 10.33; Found C, 75.27; H, 5.56; N, 10.35;

3.4.1.12.*(E)-4-(bis(1-methyl-1H-indol-3-yl)methyl)-N'-(2-hydroxybenzylidene)benzohydrazide (12)*

Brown solid. Yield: 79.7%. m.p.: 272-274 °C. IR (cm-1, ATR): 3347, 3140, 3054, 1650, 1603, 1489, 1465, 1260, 1184, 750; 1H NMR (500 MHz, DMSO-d6): δ 12.02 (s, 1H), 11.30 (s, 1H), 8.60 (s, 1H), 7.85 (d, J = 8.0 Hz, 2H), 7.52 (d, J = 7.9 Hz, 2H), 7.40 (d, J = 8.2 Hz, 2H), 7.33 (d, J = 7.9 Hz, 2H), 7.13 (t, J = 7.6 Hz, 2H), 6.96 – 6.91 (m, 3H), 6.89 (s, 2H), 5.96 (s, 1H), 3.73 (s, 6H); ^{13}C NMR (150 MHz, DMSO-d_6): δ 164.51, 158.09, 151.32, 142.50, 141.66, 141.66, 134.34, 130.20, 130.20, 129.86, 129.43, 129.43, 128.51, 128.32, 128.32, 128.08, 128.08, 124.79, 124.79, 123.35, 123.35, 122.86, 122.86, 121.23, 120.42, 117.31, 111.64, 111.64, 111.28, 111.28, 38.45, 35.69, 35.69; HREI-MS: m/z calcd for $C_{33}H_{28}N_4O_2$ [M]+ 512.2212; Found 512.2209; Anal. Calcd for $C_{33}H_{28}N_4O_2$, C, 77.32; H, 5.51; N, 10.93; Found C, 77.33; H, 5.53; N, 10.95;

3.4.1.13. *(E)-4-(bis(1-methyl-1H-indol-3-yl)methyl)-N'-(4-nitrobenzylidene)benzohydrazide (13)*

Yellow solid. Yield: 91.7%. m.p.: 284-285 °C. IR (cm-1, ATR): 3429, 3254, 2835, 1642, 1614, 1584, 1561, 1520, 1470, 1440, 1275, 1236, 1180, 755; 1H NMR (500 MHz, DMSO-d6): δ 12.06 (s, 1H), 8.52 (s, 1H), 8.30 (d, J = 8.2 Hz, 2H), 7.99 (d, J = 6.7 Hz, 2H), 7.84 (d, J = 7.9 Hz, 2H), 7.52 (d, J = 8.0 Hz, 2H), 7.40 (d, J = 8.2 Hz, 2H), 7.34 (d, J = 7.9 Hz, 2H), 7.13 (t, J = 7.6 Hz, 2H), 6.94 (t, J = 7.5 Hz, 2H), 6.89 (s, 2H), 5.96 (s, 1H), 3.73 (s, 6H); ^{13}C NMR (150 MHz, DMSO-d_6): δ 164.34, 149.22, 148.66, 142.14, 141.95, 141.95, 139.64, 134.35, 130.26, 130.26, 129.21, 129.21, 128.52, 128.52, 128.12, 128.12, 127.87, 127.87, 124.95, 124.95, 124.58, 124.58, 123.47, 123.47, 122.65, 122.65, 111.78, 111.78, 111.06, 111.06, 38.52, 35.78, 35.78; HREI-MS: m/z calcd for $C_{33}H_{27}N_5O_3$ [M]+ 541.2114; Found 541.2117; Anal. Calcd for $C_{33}H_{27}N_5O_3$, C, 73.18; H, 5.02; N, 12.93; Found C, 73.19; H, 5.01; N, 12.95;

3.4.1.14.*(E)-4-(bis(1-methyl-1H-indol-3-yl)methyl)-N'-(3,5-dihydroxybenzylidene)benzohydrazide (14)*

Brown solid. Yield: 86.2%. m.p.: 262-263 °C. IR (cm-1, ATR): 3143, 2962, 3062, 1603, 1583, 1490, 1462, 1266, 1164, 756; 1H NMR (500 MHz, DMSO-d6): δ 11.61 (s, 1H), 9.41 (s, 2H), 7.81 (d, J = 8.3 Hz, 2H), 7.50 (d, J = 8.3 Hz, 2H), 7.40 (s, 1H), 7.40 – 7.27 (m, 4H), 7.13 (t, J = 7.3 Hz, 2H), 6.93 (t, J = 7.3 Hz, 2H), 6.88 (s, 2H), 6.59 (s, 2H), 6.26 (s, 1H), 5.95 (s, 1H), 3.72 (s, 6H); ^{13}C NMR (150 MHz, DMSO-d_6): δ 164.30, 158.28, 158.28, 147.66, 142.91, 141.52, 141.52, 138.42, 134.29, 130.25, 130.25, 129.52, 129.52, 128.29, 128.29, 128.12, 128.12, 124.89, 124.89, 123.46, 123.46, 122.45, 122.45, 111.78, 111.78, 111.28, 111.28, 107.45, 107.45, 104.27, 38.32, 35.49, 35.49; HREI-MS: m/z calcd for $C_{33}H_{28}N_4O_3$ [M]+ 528.2161; Found 528.2158; Anal. Calcd for $C_{33}H_{28}N_4O_3$, C, 74.98; H, 5.34; N, 10.60; Found C, 75.01; H, 5.32; N, 10.58;

3.4.1.15.*(E)-4-(bis(1-methyl-1H-indol-3-yl)methyl)-N'-(4-hydroxybenzylidene)benzohydrazide (15)*

Dark brown solid. Yield: 86.2%. m.p.: 276-278 °C. IR (cm-1, ATR): 3394, 3296, 3064, 1607, 1587, 1507, 1462, 1266, 1205, 1157, 736; 1H NMR (500 MHz, DMSO-d6): δ 11.53 (s, 1H), 9.88 (s, 1H), 8.31 (s, 1H), 7.80 (d, J = 8.2 Hz, 2H), 7.54 (d, J = 8.3 Hz, 2H), 7.49 (d, J = 8.1 Hz, 2H), 7.39 (d, J = 8.2 Hz, 2H), 7.33 (d, J = 7.9 Hz, 2H), 7.13 (t, J = 7.6 Hz, 2H), 6.93 (t, J = 7.4 Hz, 2H), 6.88 (s, 2H), 6.83 (d, J = 8.4 Hz, 2H), 5.94 (s, 1H), 3.72 (s, 6H); ^{13}C NMR (150 MHz, DMSO-d_6): δ 164.28, 158.34, 149.44, 142.56, 141.32, 141.32, 134.41, 130.25, 130.25, 129.72, 129.72, 129.43, 129.43, 128.25, 128.25, 128.18, 128.18, 126.72, 124.69, 124.69, 123.75, 123.75, 122.46, 122.46, 115.68, 115.68, 111.59, 111.59, 111.23, 111.23, 38.89, 35.42, 35.42. HREI-MS: m/z calcd for $C_{33}H_{28}N_4O_2$ [M]+ 512.2212; Found 512.2215; Anal. Calcd for $C_{33}H_{28}N_4O_2$, C, 77.32; H, 5.51; N, 10.93; Found C, 77.34; H, 5.48; N, 10.90;

3.4.1.16.*(E)-4-(bis(1-methyl-1H-indol-3-yl)methyl)-N'-(2,4,5-trihydroxybenzylidene)benzohydrazide (16)*

Light brown solid. Yield: 85.3%. m.p.: 291-293 °C. IR (cm-1, ATR): 3352, 3182, 3075, 1633, 1591, 1276, 1246, 1171, 752; 1H NMR (500 MHz, DMSO-d6): δ 11.70 (s, 1H), 10.65 (s, 1H), 9.51 (s, 1H), 8.52 (s, 1H), 8.41 (s, 1H), 7.82 (d, J = 8.2 Hz, 2H), 7.50 (d, J = 8.1 Hz, 2H), 7.40 (d, J = 8.2 Hz, 2H), 7.33 (d, J = 7.9 Hz, 2H), 7.13 (t, J = 7.6 Hz, 2H), 6.93 (t, J = 7.4 Hz, 2H), 6.88 (s, 2H), 6.85 (s, 1H), 6.33 (s, 1H), 5.94 (s, 1H), 3.72 (s, 6H); ^{13}C NMR (150 MHz, DMSO-d_6): δ 164.51, 153.78, 150.93, 149.71, 142.51, 141.78, 141.78, 140.25, 134.35,

130.53, 130.53, 129.32, 129.32, 128.72, 128.72, 128.18, 128.18, 124.83, 124.83, 123.31, 123.31, 122.85, 122.85, 117.22, 112.87, 111.64, 111.64, 111.06, 111.06, 102.76, 38.37, 35.86, 35.86; HREI-MS: m/z calcd for $C_{33}H_{28}N_4O_4$ [M]+ 544.2111; Found 544.2107; Anal. Calcd for $C_{33}H_{28}N_4O_4$, C, 72.78; H, 5.18; N, 10.29; Found C, 72.79; H, 5.20; N, 10.31;

3.4.1.17.*(E)-4-(bis(1-methyl-1H-indol-3-yl)methyl)-N'-(pyridin-2-ylmethylene)benzohydrazide (17)*

Light red solid. Yield: 86.3%. m.p.: 251-252 °C. IR (cm-1, ATR): 3433, 3253, 2915, 1660, 1557, 1466, 1277, 755; 1H NMR (500 MHz, DMSO-d6): δ 11.94 (s, 1H), 8.61 (d, J = 4.3 Hz, 1H), 8.45 (s, 1H), 7.98 (d, J = 8.9 Hz, 1H), 7.86 (T, J = 8.9 Hz, 1H), 7.83 (d, J = 8.2 Hz, 2H), 7.52 (d, J = 8.2 Hz, 2H), 7.40 (d, J = 8.2 Hz, 2H), 7.34 (d, J = 7.9 Hz, 2H), 7.13 (t, J = 7.6 Hz, 2H), 6.94 (t, J = 7.4 Hz, 2H), 6.89 (s, 2H), 5.96 (s, 1H), 3.73 (s, 6H); ^{13}C NMR (150 MHz, DMSO-d_6): δ 164.32, 154.70, 148.07, 146.05, 142.66, 141.50, 141.50, 137.60, 134.21, 130.35, 130.35, 129.53, 129.53, 128.21, 128.21, 128.10, 128.10, 124.89, 124.89, 123.46, 123.46, 123.00, 122.75, 122.75, 119.09, 111.68, 111.68, 111.08, 111.08, 38.42, 35.89, 35.89; HREI-MS: m/z calcd for $C_{32}H_{27}N_5O$ [M]+ 497.2216; Found 497.2221; Anal. Calcd for $C_{32}H_{27}N_5O$, C = 77.24; H = 5.47; N = 14.07; Found C = 77.25; H = 5.49; N = 14.05;

3.4.1.18.*(E)-4-(bis(1-methyl-1H-indol-3-yl)methyl)-N'-(2-fluorobenzylidene)benzohydrazide (18)*

Light pink solid, Yield: 80.7%. m.p.: 243-245 °C. IR (cm-1, ATR): 3432, 3183, 3065, 1625, 1542, 1469, 1253, 743; 1H NMR (500 MHz, DMSO-d6): δ 11.88 (s, 1H), 8.68 (s, 1H), 7.95 (s, 1H), 7.83 (d, J = 7.9 Hz, 2H), 7.50 (t, J = 8.7 Hz, 3H), 7.40 (d, J = 8.2 Hz, 2H), 7.34 (d, J = 7.9 Hz, 2H), 7.32 – 7.26 (m, 2H), 7.13 (t, J = 7.6 Hz, 2H), 6.94 (t, J = 7.5 Hz, 2H), 6.89 (s, 2H), 5.95 (s, 1H), 3.73 (s, 6H); ^{13}C NMR (150 MHz, DMSO-d_6): δ 164.41, 161.35, 149.37, 142.76, 141.41, 141.41, 134.27, 130.83, 130.55, 130.55, 129.63, 129.32, 129.32, 128.18, 128.18, 128.09, 128.09, 125.35, 124.75, 124.62, 124.54, 123.41, 123.41, 122.85, 122.85, 117.38, 111.52, 111.52, 111.16, 111.16, 38.38, 35.86, 35.86; HREI-MS: m/z calcd for $C_{33}H_{27}FN_4O$ [M]+ 514.2169; Found 514.2172; Anal. Calcd for $C_{33}H_{27}FN_4O$, C, 77.02; H, 5.29; N, 10.89; Found C, 77.03; H, 5.26; N, 10.86;

3.4.1.19.*(E)-4-(bis(1-methyl-1H-indol-3-yl)methyl)-N'-(3-hydroxy-4-methoxybenzylidene)benzohydrazide (19)*

Dark brown solid. Yield: 86.4%. m.p.: 279-281 °C. IR (cm-1, ATR): 3427, 3126, 2947, 1609, 1464, 1270, 749; 1H NMR (500 MHz, DMSO-d6): δ 11.56 (s, 1H), 9.26 (s, 1H), 8.27 (s, 1H), 7.80 (d, J = 8.2 Hz, 2H), 7.49 (d, J = 8.2 Hz, 2H), 7.40 (d, J = 8.3 Hz, 2H), 7.33 (d, J = 7.9 Hz, 2H), 7.26 (s, 1H), 7.13 (t, J = 7.3 Hz, 2H), 7.03 (t, J = 8.4 Hz, 1H), 6.97 (d, J = 8.5 Hz, 1H), 6.93 (t, J = 7.5 Hz, 2H), 6.88 (s, 2H), 5.94 (s, 1H), 3.81 (s, 3H), 3.72 (s, 6H); ^{13}C NMR (150 MHz, DMSO-d_6): δ 164.48, 149.80, 148.58, 146.65, 142.46, 141.32, 141.32, 134.28, 130.25, 130.25, 129.76, 129.31, 129.31, 128.34, 128.34, 128.09, 128.09, 124.93, 124.93, 123.41, 123.41, 122.62, 122.62, 120.75, 115.34, 115.21, 111.78, 111.78, 111.26, 111.26, 56.69, 38.46, 35.79, 35.79; HREI-MS: m/z calcd for $C_{34}H_{30}N_4O_3$ [M]+ 542.2318; Found 542.2321; Anal. Calcd for $C_{34}H_{30}N_4O_3$, C, 75.26; H, 5.57; N, 10.33; Found C, 75.27; H, 5.56; N, 10.31;

3.4.1.20.*(E)-4-(bis(1-methyl-1H-indol-3-yl)methyl)-N'-(3-chlorobenzylidene)benzohydrazide (20)*

Red solid. Yield: 81.7%. m.p.: 275-277 °C. IR (cm-1, ATR): 3406, 3293, 3153, 1623, 1543, 1467, 1280, 757; 1H NMR (500 MHz, DMSO-d6): δ 11.89 (s, 1H), 8.41 (s, 1H), 7.82 (d, J = 7.7 Hz, 2H), 7.77 (s, 1H), 7.68 (s, 1H), 7.54 – 7.47 (m, 4H), 7.40 (d, J = 8.2 Hz, 2H), 7.33 (d, J = 7.9 Hz, 1H), 7.13 (t, J = 7.2 Hz, 2H), 6.93 (t, J = 7.2 Hz, 2H), 6.88 (s, 2H), 5.95 (s, 1H), 3.72 (s, 6H); ^{13}C NMR (150 MHz, DMSO-d_6): δ 164.31, 148.58, 142.54, 141.68, 141.68, 135.32, 134.18, 134.14, 130.45, 130.45, 130.26, 129.57, 129.57, 128.65, 128.29, 128.29, 128.15, 128.15, 127.68, 125.23, 124.91, 124.91, 123.59, 123.59, 122.65, 122.65, 111.51, 111.51, 111.16, 111.16, 38.46, 35.72, 35.72; HREI-MS: m/z calcd for $C_{33}H_{27}ClN_4O$ [M]+ 530.1873; Found 530.1877; Anal. Calcd for $C_{33}H_{27}ClN_4O$, C, 74.64; H, 5.12; N, 10.55; Found C, 74.65; H, 5.09; N, 10.53;

3.4.1.21.*(E)-4-(bis(1-methyl-1H-indol-3-yl)methyl)-N'-(2-hydroxy-5-methoxybenzylidene)benzohydrazide (21)*

Brown solid. Yield: 91.4%. m.p.: 263-265 °C. IR (cm-1, ATR): 3273, 3120, 3021, 1608, 1589, 1309, 1256, 747; 1H NMR (500 MHz, DMSO-d6): δ 11.99 (s, 1H), 10.71 (s, 1H), 8.59 (s, 1H), 7.84 (d, J = 7.9 Hz, 2H), 7.52 (d, J = 8.0 Hz, 2H), 7.40 (d, J = 8.2 Hz, 2H), 7.34 (d, J = 7.9 Hz, 2H), 7.15 – 7.11 (m, 3H), 6.95 – 6.92 (m, 2H), 6.89 (s, 2H), 6.86 (d, J = 9.0 Hz, 1H), 5.95 (s, 1H), 3.74 (s, 3H), 3.73 (s, 6H); ^{13}C NMR (150 MHz, DMSO-d_6): δ 164.26, 154.31, 154.34, 149.71, 142.56, 141.13, 134.25, 130.53, 129.35, 128.47, 128.21, 124.96,

123.56, 122.43, 121.78, 116.67, 116.23, 112.86, 111.38, 111.18, 56.25, 38.48, 35.94; HREI-MS: m/z calcd for $C_{34}H_{30}N_4O_3$ [M]+ 542.2318; Found 542.2314; Anal. Calcd for $C_{34}H_{30}N_4O_3$, C, 75.26; H, 5.57; N, 10.33; Found C, 75.27; H, 5.58; N, 10.31;

3.4.1.22. ***(E)-4-(bis(1-methyl-1H-indol-3-yl)methyl)-N'-(2-nitrobenzylidene)benzohydrazide (22)***

Light yellow solid. Yield: 72.5%. m.p.: 232-233 °C. IR (cm-1, ATR): 3422, 3382, 3242, 1609, 1546, 1466, 1273, 752; 1H NMR (500 MHz, DMSO-d6): δ 12.12 (s, 1H), 8.85 (s, 1H), 8.14 (d, J = 6.9 Hz, 1H), 8.08 (d, J = 8.2 Hz, 1H), 7.84 (d, J = 7.8 Hz, 2H), 7.71 – 7.64 (m, 1H), 7.52 (d, J = 8.1 Hz, 2H), 7.40 (d, J = 8.2 Hz, 2H), 7.34 (d, J = 7.9 Hz, 2H), 7.13 (t, J = 7.4 Hz, 2H), 6.94 (t, J = 7.4 Hz, 2H), 6.89 (s, 1H), 5.96 (s, 1H), 3.72 (s, 6H); ^{13}C NMR (150 MHz, DMSO-d_6): δ 164.39, 148.03, 142.84, 142.62, 141.47, 141.47, 134.18, 133.79, 130.49, 130.31, 130.31, 129.43, 129.43, 129.38, 129.38, 128.16, 128.16, 128.07, 128.07, 125.16, 124.69, 124.69, 123.42, 123.42, 122.71, 122.71, 111.69, 111.69, 111.18, 111.18, 38.32, 35.81, 35.81; HREI-MS: m/z calcd for $C_{33}H_{27}N_5O_3$ [M]+ 541.2114; Found 541.2110; Anal. Calcd for $C_{33}H_{27}N_5O_3$, C, 73.18; H, 5.02; N, 12.93; Found C, 73.17; H, 5.03; N, 12.91;

3.4.1.23.***(E)-4-(bis(1-methyl-1H-indol-3-yl)methyl)-N'-(2,4-dihydroxybenzylidene)benzohydrazide (23)***

Brown solid. Yield: 72.5%. m.p.: 262-264 °C. IR (cm-1, ATR): 3431, 3255, 3023, 1613, 1589, 1543, 1519, 1468, 1274, 755; 1H NMR (500 MHz, DMSO-d6): δ 11.82 (s, 1H), 11.49 (s, 1H), 9.93 (s, 1H), 8.47 (s, 1H), 7.82 (d, J = 7.9 Hz, 2H), 7.51 (d, J = 8.0 Hz, 2H), 7.40 (d, J = 8.2 Hz, 2H), 7.33 (d, J = 7.9 Hz, 2H), 7.28 (d, J = 8.5 Hz, 1H), 7.13 (t, J = 7.6 Hz, 2H), 6.93 (t, J = 7.5 Hz, 2H), 6.88 (s, 2H), 6.36 (d, J = 8.3 Hz, 1H), 6.32 (s, 1H), 5.95 (s, 1H), 3.72 (s, 6H); ^{13}C NMR (150 MHz, DMSO-d_6): 164.36, 160.58, 160.52, 151.66, 142.09, 141.53, 141.53, 134.86, 130.35, 130.21, 130.21, 129.21, 129.21, 128.53, 128.53, 128.34, 128.34, 124.46, 124.46, 123.89, 123.89, 122.85, 122.85, 113.19, 111.58, 111.58, 111.18, 111.18, 109.25, 103.14, 38.43, 35.94, 35.94; HREI-MS: m/z calcd for $C_{33}H_{28}N_4O_3$ [M]+ 528.2161; Found 528.2161; Anal. Calcd for $C_{33}H_{28}N_4O_3$, C, 74.98; H, 5.34; N, 10.60; Found C, 74.98; H, 5.34; N, 10.60;

3.4.1.24.***(E)-4-(bis(1-methyl-1H-indol-3-yl)methyl)-N'-(3-methylbenzylidene)benzohydrazide (24)***

Dark brown solid. Yield: 84.8%. m.p.: 254-256 °C. IR (cm-1, ATR): 3539, 3252, 3075, 3143, 1640, 1564, 1469, 1342, 1274, 1145, 732; 1H NMR (500 MHz, DMSO-d6): δ 11.76 (s, 1H), 8.40 (s, 1H), 7.82 (d, J = 7.9 Hz, 2H), 7.51 (d, J = 7.9 Hz, 2H), 7.40 (d, J = 8.2 Hz, 2H), 7.34 (d, J = 7.9 Hz, 2H), 7.28 (s, 1H), 7.13 (t, J = 7.6 Hz, 2H), 6.94 (t, J = 7.5 Hz, 2H), 6.89 (s, 1H), 5.95 (s, 1H), 3.81 (s, 3H), 3.73 (s, 6H); 13C-NMR (150 MHz, DMSO-d_6): δ 164.52, 148.78, 142.56, 141.52, 141.52, 137.89, 136.76, 134.31, 130.28, 130.28, 129.61, 129.33, 129.33, 128.47, 128.47, 128.13, 128.13, 128.09, 127.89, 124.53, 124.53, 123.51, 123.51, 123.26, 122.71, 122.71, 111.58, 111.58, 111.04, 111.04, 38.39, 35.86, 35.86, 21.18; HREI-MS: m/z calcd for $C_{34}H_{30}N_4O$ [M]+ 510.2420; Found 510.2423; Anal. Calcd for $C_{34}H_{30}N_4O$, C = 79.97; H = 5.92; N = 10.97; Found C = 79.98; H = 5.94; N = 10.96;

3.4.1.25.*(E)-4-(bis(1-methyl-1H-indol-3-yl)methyl)-N'-(2,4,6-trihydroxybenzylidene)benzohydrazide (25)*

Dark red solid. Yield: 89.4%; m.p.: 313-315 °C, IR (cm-1, ATR): 3413, 1466, 1341, 3051, 1657, 1519, 1269, 1113, 756; 1H NMR (500 MHz, DMSO-d6): δ 11.77 (s, 1H), 11.07 (s, 1H), 9.77 (s, 1H), 8.77 (s, 1H), 7.83 (d, J = 7.8 Hz, 2H), 7.50 (d, J = 8.0 Hz, 2H), 7.39 (d, J = 8.3 Hz, 2H), 7.33 (d, J = 8.0 Hz, 2H), 7.13 (t, J = 7.6 Hz, 2H), 6.93 (t, J = 7.4 Hz, 2H), 6.88 (s, 1H), 5.94 (s, 1H), 5.84 (s, 1H), 3.72 (s, 6H); 13C-NMR (150 MHz, DMSO-d_6): δ 163.92, 163.12, 161.47, 161.47, 144.68, 142.35, 141.47, 141.47, 134.18, 130.25, 130.25, 129.50, 129.50, 128.38, 128.38, 128.09, 128.09, 124.69, 124.69, 123.56, 123.56, 122.85, 122.85, 111.69, 111.69, 111.18, 111.18, 106.29, 95.87, 95.87, 38.32, 35.86, 35.86; HREI-MS: m/z calcd for $C_{33}H_{28}N_4O_4$ [M]+ 544.2111; Found 544.2108; Anal. Calcd for $C_{33}H_{28}N_4O_4$, C = 72.78; H = 5.18; N = 10.29; Found C = 72.79; H = 5.15; N = 10.26;

3.4.1.26.*(E)-4-(bis(1-methyl-1H-indol-3-yl)methyl)-N'-(4-chlorobenzylidene)benzohydrazide (26)*

Red solid. Yield: 88.3%; m.p.: 237-239 °C, IR (cm-1, ATR): 3434, 3232, 3019, 1633, 1514, 1469, 1233, 1187, 752; 1H NMR (500 MHz, DMSO-d6): δ 11.82 (s, 1H), 8.41 (s, 1H), 7.82 (d, J = 8.1 Hz, 2H), 7.74 (d, J = 7.2 Hz, 2H), 7.51 (t, J = 8.0 Hz, 4H), 7.40 (d, J = 8.3 Hz, 2H), 7.33 (d, J = 7.9 Hz, 2H), 7.13 (t, J = 7.6 Hz, 2H), 6.93 (t, J = 7.4 Hz, 2H), 6.88 (s, 2H), 5.95 (s, 1H), 3.72 (s, 6H); 13C-NMR (150 MHz, DMSO-d_6): δ 164.42, 149.37, 142.56, 141.51, 141.51, 135.23, 134.82, 134.20, 130.37, 130.37, 129.51, 129.51, 129.48, 129.48, 129.16, 129.16, 128.31, 128.31, 128.13, 128.13, 124.97, 124.97, 123.47, 123.47, 122.85, 122.85,

111.58, 111.58, 111.09, 111.09, 38.43, 35.83, 35.83; HREI-MS: m/z calcd for $C_{33}H_{27}ClN_4O$ [M]+ 530.1873; Found 530.1877; Anal. Calcd for $C_{33}H_{27}ClN_4O$, C, 74.64; H, 5.12; N, 10.55; Found C, 74.66; H, 5.13; N, 10.57;

3.4.1.27.*(E)-4-(bis(1-methyl-1H-indol-3-yl)methyl)-N'-(2-chlorobenzylidene)benzohydrazide (27)*

Red solid. Yield: 79.4%; m.p.: 247-249 °C, IR (cm-1, ATR): 3119, 3026, 2828, 1658, 1539, 1449, 1269, 745; 1H NMR (500 MHz, DMSO-d6): δ 11.99 (s, 1H), 8.84 (s, 1H), 8.02 (s, 1H), 7.84 (d, J = 7.7 Hz, 2H), 7.52 (t, J = 7.3 Hz, 2H), 7.45 (d, J = 4.0 Hz, 2H), 7.40 (d, J = 8.2 Hz, 2H), 7.33 (d, J = 7.9 Hz, 2H), 7.13 (t, J = 7.4 Hz, 2H), 6.94 (t, J = 7.4 Hz, 2H), 6.89 (s, 2H), 5.95 (s, 1H), 3.72 (s, 6H); 13C-NMR (150 MHz, DMSO-d_6): δ 164.48, 149.92, 142.32, 141.58, 141.58, 134.31, 132.59, 131.20, 130.93, 130.37, 130.37, 129.73, 129.73, 128.69, 128.69, 128.41, 128.41, 128.20, 128.20, 127.73, 124.92, 124.92, 123.51, 123.51, 122.79, 122.79, 111.58, 111.58, 111.28, 111.28, 38.38, 35.72, 35.72; HREI-MS: m/z calcd for $C_{33}H_{27}ClN_4O$ [M]+ 530.1873; Found 530.1871; Anal. Calcd for $C_{33}H_{27}ClN_4O$, C, 74.64; H, 5.12; N, 10.55; Found C, 74.62; H, 5.10; N, 10.56;

3.4.1.28.*(E)-4-(bis(1-methyl-1H-indol-3-yl)methyl)-N'-(3-fluorobenzylidene)benzohydrazide (28)*

Light orange solid. Yield: 81.9%; m.p.: 263-264 °C, IR (cm-1, ATR): 3421, 3282, 3061, 1660, 1608, 1519, 1466, 1360, 1272, 752; 1H NMR (500 MHz, DMSO-d6): δ 11.86 (s, 1H), 8.43 (s, 1H), 7.82 (d, J = 7.5 Hz, 2H), 7.52 (t, J = 13.1 Hz, 3H), 7.40 (d, J = 8.2 Hz, 2H), 7.33 (d, J = 7.8 Hz, 2H), 7.13 (t, J = 7.6 Hz, 2H), 6.93 (t, J = 7.5 Hz, 2H), 6.88 (s, 2H), 5.95 (s, 1H), 3.72 (s, 6H); 13C-NMR (150 MHz, DMSO-d_6): δ 164.51, 162.78 (d, J = 261.5 Hz), 148.79, 142.46, 141.51, 141.51, 138.63, 134.31, 130.37, 130.37, 129.87, 129.43, 129.43, 128.31, 128.31, 128.12, 128.12, 124.91, 123.51, 123.51, 122.74, 122.05, 117.14, 116.92, 114.35, 114.19, 111.58, 111.58, 111.09, 111.09, 38.54, 35.91, 35.91; HREI-MS: m/z calcd for $C_{33}H_{27}FN_4O$ [M]+ 530.1873; Found 530.1869; Anal. Calcd for $C_{33}H_{27}FN_4O$, C = 77.02; H = 5.29; N = 10.89; Found C = 77.01; H = 5.31; N = 10.90;

3.4.1.29.*(E)-4-(bis(1-methyl-1H-indol-3-yl)methyl)-N'-(3-hydroxybenzylidene)benzohydrazide (29)*

Brown solid. Yield: 72.4%; m.p.: 233-234 °C, IR (cm-1, ATR): 3312, 3179, 2833, 1633, 1609, 1505, 1467, 1253, 1141, 747; 1H NMR (500 MHz, DMSO-d6): δ 11.69 (s, 1H), 9.92 (s, 1H), 9.60 (s, 1H), 8.33 (s, 1H), 7.81 (d, J = 8.0 Hz, 2H), 7.50 (d, J = 8.1 Hz, 2H), 7.40 (d, J = 8.3 Hz, 2H), 7.33 (d, J = 7.9 Hz, 2H), 7.25 (t, J = 7.8 Hz, 2H), 7.19 (s, 1H), 7.16-7.09 (m, 3H), 6.93 (t, J = 7.5 Hz, 2H), 6.88 (s, 2H), 5.95 (s, 1H), 3.72 (s, 6H); 13C-NMR (150 MHz, DMSO-d_6): δ 164.52, 156.37, 148.69, 142.51, 141.40, 141.40, 137.07, 134.31, 130.72, 130.38, 130.38, 129.52, 129.52, 128.31, 128.31, 128.15, 128.15, 124.91, 124.91, 123.56, 123.56, 122.73, 122.73, 120.07, 119.35, 114.82, 111.48, 111.48, 111.18, 111.18, 38.40, 35.92, 35.92; HREI-MS: m/z calcd for $C_{33}H_{28}N_4O_2$ [M]+ 512.2212; Found 512.2216; Anal. Calcd for $C_{33}H_{28}N_4O_2$, C = 77.32; H = 5.51; N = 10.93; Found C = 77.30; H = 5.52; N = 10.94.

3.4.1.30.*(E)-4-(bis(1-methyl-1H-indol-3-yl)methyl)-N'-(pyridin-4-ylmethylene)benzohydrazide (30)*

Red solid. Yield: 82.6%; m.p.: 253-254 °C, IR (cm-1, ATR): 3423, 3352, 3138, 1657, 1611, 1269; 1H NMR (500 MHz, DMSO-d6): δ 12.02 (s, 1H), 8.65 (d, J = 4.2 Hz, 2H), 8.42 (s, 1H), 7.83 (d, J = 8.0 Hz, 2H), 7.66 (s, 2H), 7.52 (d, J = 8.1 Hz, 2H), 7.40 (d, J = 8.2 Hz, 2H), 7.34 (d, J = 7.9 Hz, 2H), 7.13 (t, J = 7.6 Hz, 2H), 6.94 (t, J = 7.5 Hz, 2H), 6.89 (s, 2H), 5.96 (s, 1H), 3.72 (s, 6H); 13C-NMR (150 MHz, DMSO-d_6): δ 164.52, 150.19, 150.19, 149.46, 142.58, 141.79, 141.79, 140.75, 134.31, 130.38, 130.38, 129.63, 129.63, 128.32, 128.32, 128.20, 128.20, 124.93, 124.93, 123.56, 123.56, 122.71, 122.71, 122.45, 122.45, 111.98, 111.98, 111.18, 111.18, 38.52, 35.79, 35.79; HREI-MS: m/z calcd for $C_{32}H_{27}N_5O$ [M]+ 497.2216; Found 497.2221; Anal. Calcd for $C_{32}H_{27}N_5O$, C = 77.24; H = 5.47; N = 14.07; Found C = 77.25; H = 5.45; N = 14.06;

3.5. Cytotoxicity assays using 3T3-L1 and CC-1 cell-lines

In vitro cytotoxicity assays were performed as described in Taha *et al*. 2015 [35], using the 3T3-L1 mouse embryo fibroblast cell line (American Type Culture Collection 'ATCC', Manassas, VA 20108, USA), and CC-1 cells, a rat Wistar hepatocyte cell line (European Collection of Cell Cultures, Salisbury, UK). The CC-1 cells were suspended in Minimum Essential Medium Eagle (MEM) supplemented with 10% FBS, 2 mM glutamine, 1% non-

essential amino acids and, 20 mM HEPES. While the 3T3-L1 cells were suspended in Dulbecco's Modified Eagle's Medium (DMEM) formulated with 10% FBS. Using flat bottomed plates, both cell-lines were plated at a concentration of 6 × 104 cells/mL and incubated for 24 h at 37 ℃ and 5% CO2 environment. After removal of media, cells were challenged with three different concentrations (1.0, 5.0, and 20 μg/mL) of compounds in triplicates and were then further incubated for 48 h at 37 ℃ in CO2 incubator. Following exposure to each compound, cells viability was assessed by using 0.5 mg/mL of MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) for 4 h followed by removal of supernatant and addition of DMSO to solubilize the formazan complex. Plates were read at 540 nm after one minute shaking and readings were processed using MS Excel software. Results were expressed as means ± SD of triplicate readings.

3.6. Docking studies

The structure of all compounds were prepared using Chem3D by CambridgeSoft. The human *β*-D-glucuronidase crystal structure was first retrieved from the protein data bank (PDB code: 1BHG) [36]. Docking had been carried out using AutoDock 4.2 [37]. In Genetic Algorithm (GA) search parameter, number of runs was set at 150, while the other settings were left as default. Docking parameter was also left with its default settings. The 3D docking results was visualized using Discovery Studio visualizer 4.1.

3.7. DFT studies

The geometry optimization and frequency calculations of the synthesized novel bisindolylmethane derivatives have been carried out at the B3LYP/6-31+G(d,p) level of theory [40] as implemented in Gaussian09 package [41] . The optimized minima were confirmed by the absence of imaginary frequencies. The solvent effects were taken into account implicitly using an integral equation formalism (IEF) version of the polarizable continuum model (PCM). In PCM, the molecule (e.g., bisindolylmethane derivative) is embedded into a cavity surrounded by solvent, which is described by its dielectric constant ε [42].

Conclusions

We synthesized novel bisindolylmethane derivatives consisting of benzohydrazone moiety. In conclusion, molecular docking studies have evidently demonstrated the interaction pattern of the synthetic compounds in correspondence with biological inhibitory assay. Analysis of the binding mode clearly shows the presence of hydrophilic group i.e. hydroxyl moiety plays a key role in the activity profile. It was also observed that the compounds are position in the binding site in a way that two or more hydroxyl groups have to be substituted on carbon adjacent to each other for good interaction to take place with important residues like Glu451 and Glu540 as compared to hydroxyls that are substituted far apart.

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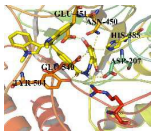
Identification of Bisindolylmethane-Hydrazone Hybrids as Novel Inhibitors of *β*-Glucuronidase, DFT and In Silico SAR Intimations

Muhammad Taha, Nor Hadiani Ismail, Syahrul Imran, El Hassane Anouar, Muhammad Ali, Waqas Jamil, Nizam Uddin, Syed Muhammad Kashif



Synthesis and *β*-Glucuronidase Inhibition Activity of Novel Bisindolylmethane Derivatives (**1-30**)