RSC Advances

PAPER

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Cite this: *RSC Adv.*, 2024, **14**, 29288

Synthesis, *in vitro* and *in silico* study of novel 1,3-diphenylurea derived Schiff bases as competitive α-glucosidase inhibitors†

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Received 8th August 2024
Accepted 6th September 2024

DOI: 10.1039/d4ra05767h

rsc.li/rsc-advances

Diabetes mellitus has become a major global health burden because of several related consequences, including heart disease, retinopathy, cataracts, metabolic syndrome, collapsed renal function, and blindness. In the recent study, thirty Schiff base derivatives of 1,3-diphenylurea were synthesized and their anti-diabetic activity was evaluated by targeting α-glucosidase. The compounds exhibited an overwhelming inhibitory potential for α-glucosidase with higher potency ranging from 2.49–37.16 μM. The most effective compound, **5h**, showed competitive inhibition of α-glucosidase ($K_i = 3.96 \pm 0.0048$ μM) in the kinetic analysis and strong binding interactions with key residues α-glucosidase in docking analysis, indicating its potential for better glycemic control in diabetes patients.

1. Introduction

Diabetes mellitus is a chronic condition that has become a major global health concern because of various complications associated with it including heart disease, retinopathy, cataracts, metabolic syndrome, collapsed renal function, and blindness. Globally, >1.31 billion (1.22–1.39) people are estimated to have diabetes by 2050. Age-standardized global diabetes prevalence rates more than 10% are expected for two super-regions: North Africa and the Middle East, at 16.8% (16.1–17.6), and Latin America and the Caribbean, at 11.3% (10.8–11.9).[1,2] The primary risk factor for type 2 diabetes (T2DM) is postprandial hyperglycemia, which is linked to the deficiency of insulin or defect in the function of insulin.[3,4] The key carbohydrate metabolic enzyme, α-glucosidase (EC 3.2.1.20) is present in brush boarder of small intestine, and converts non-absorbable complex carbohydrates into absorbable monosaccharides, such as glucose molecules. Inhibiting α-glucosidase is a crucial approach to manage conditions linked to the absorption of carbohydrates, such as diabetes, obesity, dental caries, and periodontal illnesses. The α-glucosidase inhibitors block its catalytic activity, thereby slow down carbohydrates digestion and control blood glucose level.[5] Therefore, in the current study, new Schiff base of 1,3-dipheny urea derivatives were synthesized and their antidiabetic potential was evaluated by particularly inhibiting α-glucosidase enzyme.

Urea scaffold is embedded in a variety of important bioactive compounds and FDA approved drugs like regorafenib and sorafenib which shows its therapeutic importance.[6,7] Diarylurea core has gained noteworthy pharmacological interest due to the presence of NH–CO scaffold which binds with diverse range of biological targets[8] and consequently produces broad spectrum of biological activities like anti-viral, antitumor, antimalarial[9–11] activities. Furthermore, urea derivatives are well known α-glucosidase inhibitors and various studies have explored their potential as antidiabetic agents (Fig. 1).[12–17]

Azomethine functionality has shown anticancer, antioxidant, antifungal, antibacterial, antiviral and antidiabetic activities[14,18–24] which makes it a promising pharmacophore to develop new drug candidates. Salicylaldehyde and its derivatives are well known synthetic precursor for the preparation of different drugs like Aspirin, Warfarin, and Salsalate.[25] Salicylaldehyde nucleus linked with Schiff bases, have exhibited potential biological activities like antibacterial,[26] anticancer,[27,28] antiviral,[29] tyrosinase,[30] antimicrobial,[31] antioxidant,[32] and antidiabetic[33] activities. Notably, the presence of halogen

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† Electronic supplementary information (ESI) available. See DOI: https://doi.org/10.1039/d4ra05767h

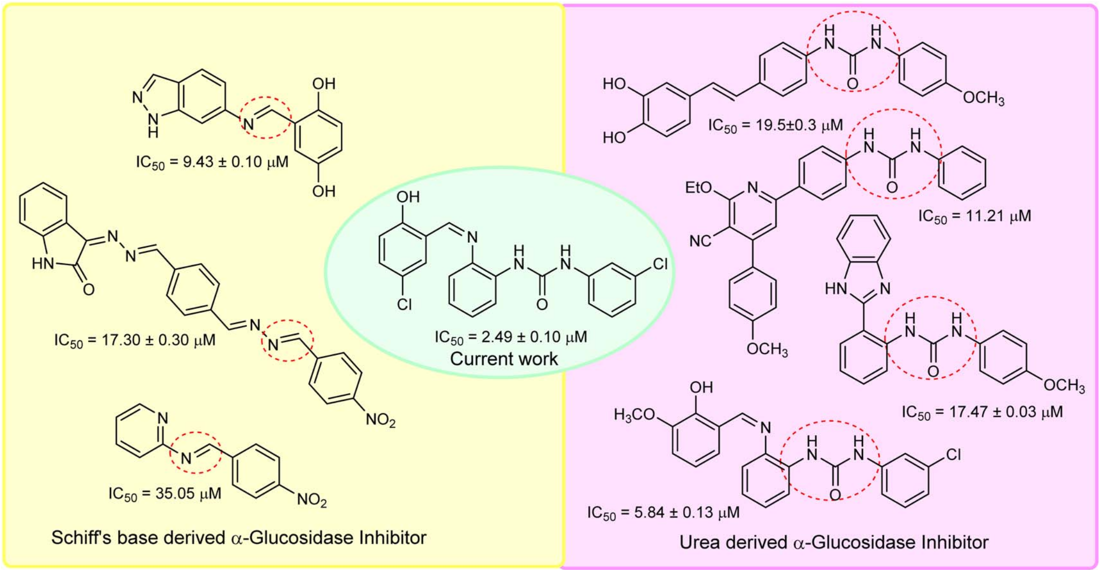


Fig. 1 The chemical structures of some reported α-glucosidase inhibitors are shown.[12–18]

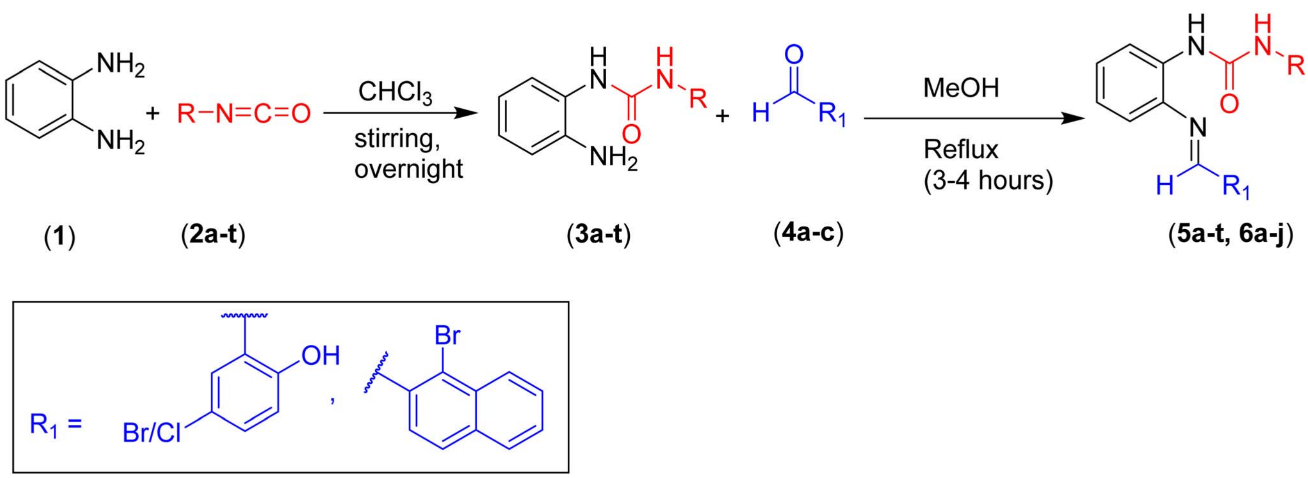
moiety (chloro or bromo) in salicylaldehyde Schiff base nucleus have shown significant biological activity due to more facilitated interactions with the binding sites of biological targets.[34]

Naphthalene moiety is a typical fluorophore, present in different naturally occurring bioactive phytoconstituents like Patentiflorin A, and Rifampicin. Naphthalene is a crucial building block in the design of new drugs because of its antifungal, antitumor,[35] antibacterial,[36] and antidiabetic,[37] activities. Moreover, various molecules with naphthalene moiety are available as FDA approved drugs.[38] Because of the high significance of these moieties, we aimed to join the urea and Schiff base pharmacophore in one molecule along with the salicylaldehyde or naphthaldehyde core to explore the pharmacological profile of urea clubbed imines. For this purpose, 1,3-diphenyl urea analogues were reacted with 5-chlorosalicylaldehyde, 5-bromosalicylaldehyde and 1-bromo-2-naphthaldehyde and Schiff base derivatives of 1,3-dipheny urea were synthesized and screened those analogues against α-glucosidase.

2. Results and discussion

2.1. Chemistry

o-Phenylenediamine (**1**) was reacted with equimolar amount of different substituted isocyanates (**2a–t**) by constant stirring at room temperature overnight and the resulting mono substituted 1,3-diphenyl ureas (**3a–t**) were refluxed for 3–4 hours with substituted aldehyde (**4**) *via* condensation in methanol to obtain the final products (**5a–t**) and (**6a–j**). The scope of reaction was broadened by using a variety of different mono substituted 1,3-diphenyl ureas. The targeted compounds (**5a–t**) and (**6a–j**) were obtained in good to excellent yield (Scheme 1).



Scheme 1 Synthesis of Schiff bases of 1,3-dipheny urea derivatives.

Table 1 *In vitro* α-glucosidase inhibition results of Schiff base of 1,3-diphenyl urea analogues are summarized with their R and R_1 moieties

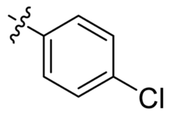
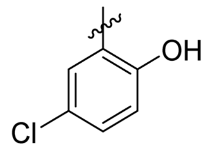
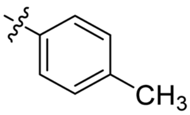
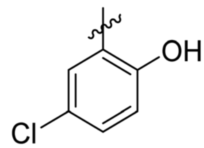
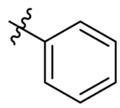
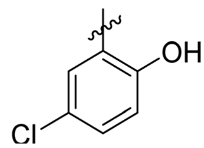
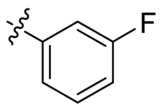
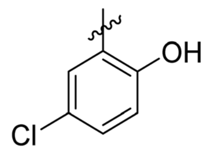
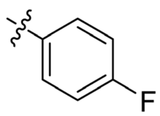
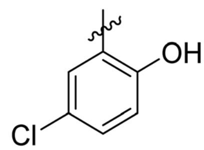
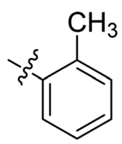
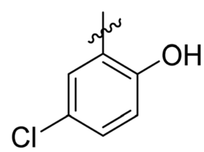
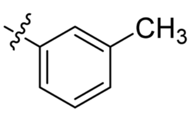
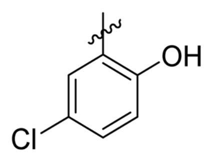
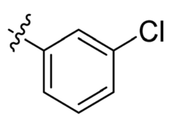
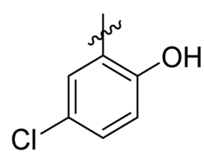
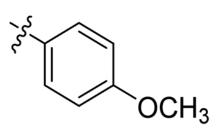
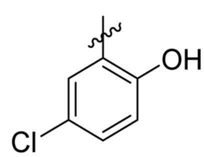
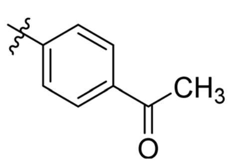
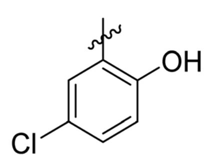
S. no.	Compounds	R	R_1	Percent inhibition (0.5 mM)	$IC_{50} \pm \mu M$ (SEM)
1	**5a**			92.38	3.26 ± 0.10
2	**5b**			90.62	20.10 ± 0.51
3	**5c**			90.11	14.20 ± 0.30
4	**5d**			86.18	25.16 ± 0.57
5	**5e**			90.46	3.76 ± 0.11
6	**5f**			90.36	20.18 ± 0.36
7	**5g**			92.52	4.03 ± 0.12
8	**5h**			92.60	2.49 ± 0.10
9	**5i**			90.63	18.35 ± 0.47
10	**5j**			90.24	16.35 ± 0.28

Table 1 (*Contd.*)

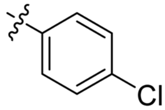
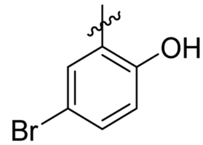
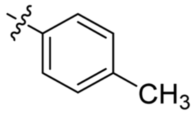
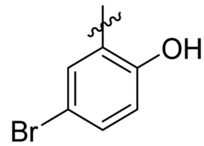
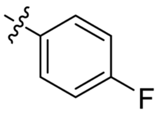
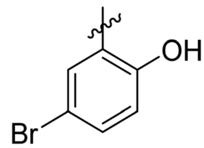
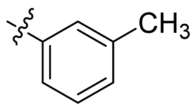
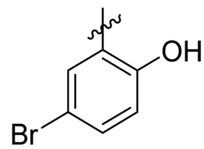
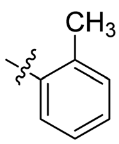
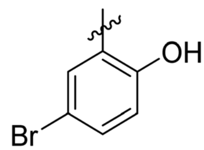
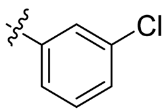
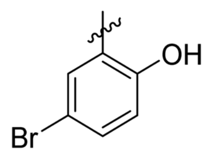
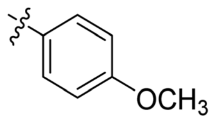
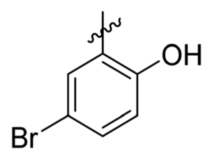
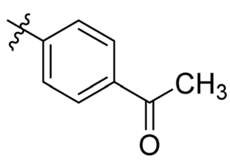
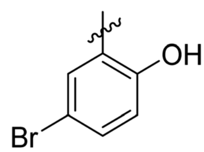
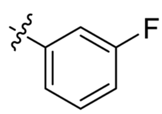
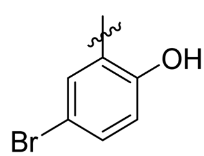
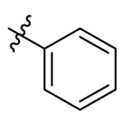
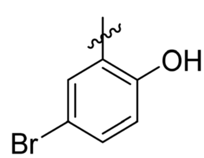
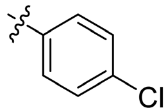
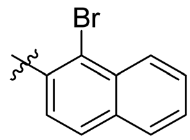
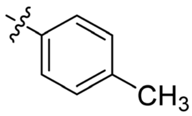
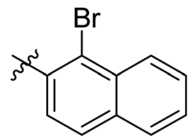
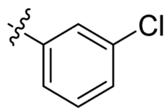
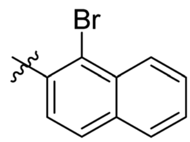
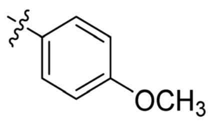
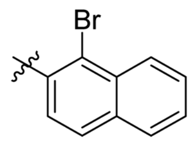
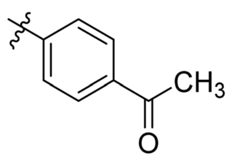
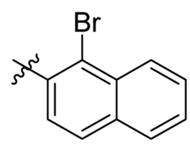
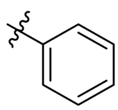
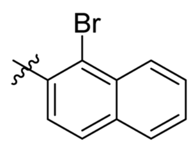
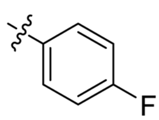
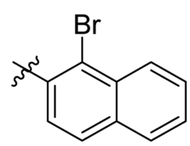
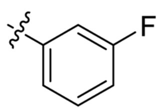
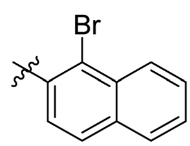
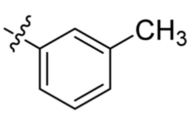
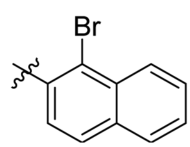
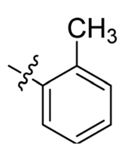
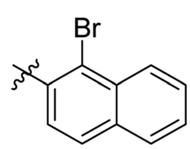
S. no.	Compounds	R	R_1	Percent inhibition (0.5 mM)	$IC_{50} \pm \mu M$ (SEM)
11	**5k**			89.73	16.38 ± 0.40
12	**5l**			92.48	5.10 ± 0.13
13	**5m**			91.74	6.30 ± 0.19
14	**5n**			91.38	7.05 ± 0.13
15	**5o**			90.85	9.15 ± 0.25
16	**5p**			90.82	8.24 ± 0.17
17	**5q**			90.72	10.60 ± 0.31
18	**5r**			90.85	15.20 ± 0.36
19	**5s**			92.26	4.10 ± 0.11
20	**5t**			91.41	12.22 ± 0.30

Table 1 (*Contd.*)

S. no.	Compounds	R	R_1	Percent inhibition (0.5 mM)	$IC_{50} \pm \mu M$ (SEM)
21	**6a**			91.11	20.24 ± 0.38
22	**6b**			90.16	29.42 ± 0.40
23	**6c**			91.57	18.35 ± 0.40
24	**6d**			89.63	27.29 ± 0.37
25	**6e**			90.48	26.17 ± 0.43
26	**6f**			88.95	31.48 ± 0.64
27	**6g**			91.26	17.00 ± 0.42
28	**6h**			90.24	22.39 ± 0.54
29	**6i**			87.39	34.62 ± 0.85
30	**6j**			88.40	37.16 ± 0.75

Standard: acarbose ($IC_{50} = 873.34 \pm 1.67$ μM)

The structures of Schiff base 1,3-dipheny urea derivatives were established using microanalysis (CHN) and spectral data *i.e.*, ^{1}H NMR and ^{13}C NMR. In ^{1}H NMR, phenolic –OH was observed between 11.62–11.91 ppm while Ph–NH–CO proton appeared in the range of 9.99–8.89 ppm as broad singlet. Second NH–R was observed in the range of 8.85–9.22 ppm whereas the imine C═N proton showed singlet ranging from 8.23–8.63 ppm. The spectral data of other aromatic and aliphatic protons were in accordance with the structures of anticipated compounds which supports the proposed structure of 1,3-diphenylurea derived Schiff based derivatives. ^{13}C NMR also supported the structure of the synthesized compounds and the carbon peaks were in complete agreement with the structures. CHN analysis corresponds to the molecular formula of the synthesized compounds. In order to determine the purity of compounds, HPLC analysis was carried out using $CH_3CN:H_2O = 80:20$ eluent system with 263 nm wavelength. All the compounds exhibited great than 95% purity. QTOF MS was also carried out to find out the molecular mass of the compounds which further supports characterization of our target compounds.

The synthesized molecules were *in vitro* tested against α-glucosidase to reveal their potential in the treatment of diabetes mellitus. All the compounds exhibited potent inhibition of α-glucosidase with IC_{50} values in the range of 2.49–37.16 μM (Table 1), as compared to the available marketed drug, acarbose ($IC_{50} = 873.34 \pm 1.67$ μM). The structure activity relationship of these compounds was established by segregating them into three groups based on their R-substituents, namely A, B, and group C.

Group A comprises of ten molecules (**5a–5j**) with similar R_1 (5-chloro 2-hydroxy phenyl) with diverse R-group moieties. Group B contains compounds **5k–5t** with similar R_1 (5-bromo 2-hydroxy phenyl) and different R-substituents. Due to different R_1 and R moieties, groups A and B exhibited varied α-glucosidase inhibitory capability. For instance, compound **5a** exhibited potent inhibitory capability ($IC_{50} = 3.26 \pm 0.10$ μM), while **5k** with similar R-substituent exhibited decreased α-glucosidase inhibition ($IC_{50} = 16.38 \pm 0.40$ μM) as compared to **5a**. In contrast, **5b** with *p*-methyl phenyl R group exhibited low inhibitory activity ($IC_{50} = 20.10 \pm 0.51$ μM) as compared to **5l** ($IC_{50} = 5.10 \pm 0.13$ μM). Compound **5c** with phenyl substituent R-group exhibited less potent inhibition ($IC_{50} = 14.20 \pm 0.30$ μM) as compared to **5m** ($IC_{50} = 6.30 \pm 0.19$ μM). Similarly, compound **5d** with *m*-flouro phenyl R-group displayed less potent inhibitory activity ($IC_{50} = 25.16 \pm 0.57$ μM), as compared to **5n** ($IC_{50} = 7.05 \pm 0.13$ μM) with similar R moiety. While compound **5e** with *p*-flouro phenyl substituent exhibited higher potent inhibition ($IC_{50} = 3.76 \pm 0.11$ μM) as compared to **5o** ($IC_{50} = 9.15 \pm 0.25$ μM) with similar substituent (*p*-flouro phenyl). Compound **5f** ($IC_{50} = 20.18 \pm 0.36$ μM) with *o*-methyl phenyl substituent exhibited less potent inhibitory activity as compared to **5p** ($IC_{50} = 8.24 \pm 0.17$ μM) with *m*-chloro phenyl group. Whereas compound **5g** ($IC_{50} = 4.03 \pm 0.12$ μM) with *m*-methyl phenyl substituent exhibited higher inhibitory activity as compared to **5q** ($IC_{50} = 10.60 \pm 0.31$ μM) with *p*-methoxy phenyl substitution. Interestingly, compound **5h** ($IC_{50} = 2.49 \pm 0.10$ μM) with *m*-chloro phenyl substitution exhibited the higher potent inhibitory activity against α-glucosidase as compared to **5r** ($IC_{50} = 15.20 \pm 0.36$ μM) with *p*-keto phenyl moiety. Compound **5i** ($IC_{50} = 18.35 \pm 0.47$ μM) with *p*-methoxy phenyl substitution exhibited less potent inhibition as compared to **5s** ($IC_{50} = 4.10 \pm 0.11$ μM) with *m*-flouro phenyl substitution. Compound **5j** ($IC_{50} = 16.35 \pm 0.28$ μM) with acetophenyl R-substituent exhibited less potent inhibition of α-glucosidase as compared to **5t** ($IC_{50} = 12.22 \pm 0.30$ μM) with phenyl substitution.

Group C is comprising of compounds **6a–6j** with similar R_1 group (2 bromo naphthyl) and diverse R-substituents, these molecules displayed slight variation in the α-glucosidase inhibition. Like compounds **6a** and **6c** with *para* and *m*-chloro phenyl substituents exhibited almost similar potency against α-glucosidase with IC_{50} values of 20.24 ± 0.38 μM and 18.35 ± 0.40 μM, respectively. Compound **6b** ($IC_{50} = 29.42 \pm 0.40$ μM) with *p*-methyl phenyl substituent displayed slightly higher

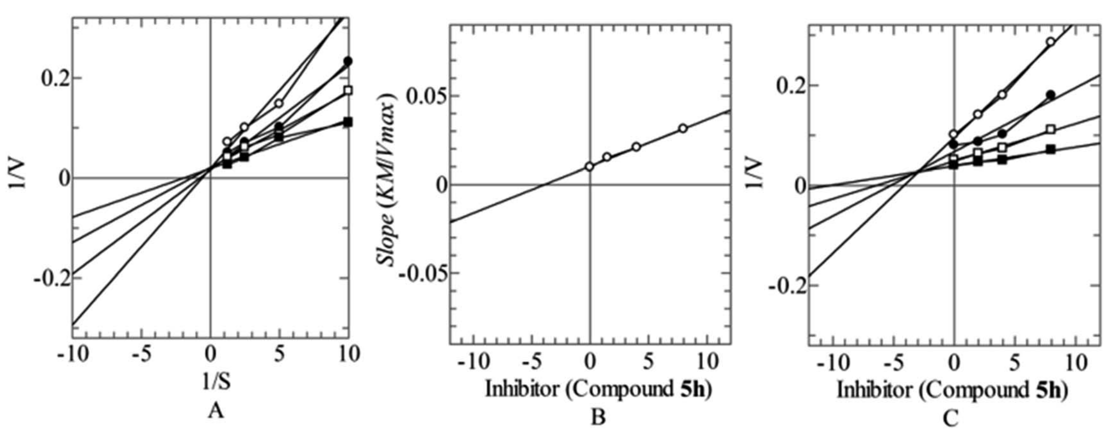
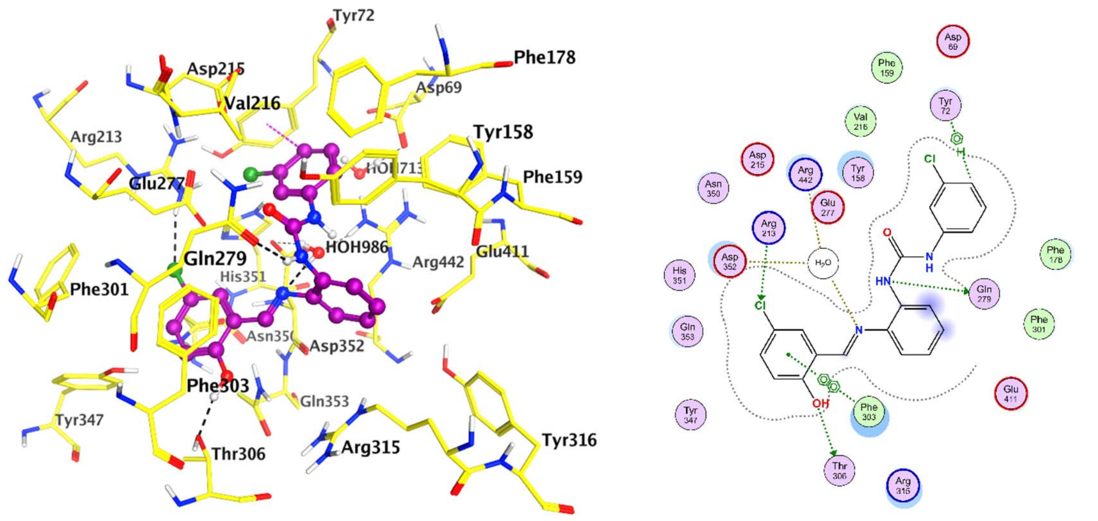


Fig. 2 Mode of inhibition of **5h** against α-glucosidase (A) Lineweaver–Burk plot of reciprocal of rate of reaction (V) *vs.* reciprocal of substrate (*p*-nitro phenyl α-D-glucopyranoside) in the absence of (■), and in the presence of 8.00 μM (○), 4.00 μM (●), and 1.50 μM (□) of **5h** (B) secondary replot of Line Weaver–Burk plot between the slopes of each line on Line Weaver–Burk plot *vs.* different concentrations of **5h** (C) dixon plot of reciprocal of rate of reaction (*V*) *vs.* different concentrations of **5h**.



(a) (b)

Fig. 3 (a) The binding mode of **5h** is shown in 3D-format. The active site residues (yellow sticks), **5h** (magenta ball and stick model), H-bonds (black dashed lines) and hydrophobic interactions (magenta dashed lines) are shown. (b). 2D-interactions including hydrogen bonds (green dotted arrows), hydrophobic interactions (green dotted lines) and solvent bridges (beige dotted lines) are shown between binding residues and **5h**.

inhibition as compared to **6i** ($IC_{50} = 34.62 \pm 0.85$ μM) and **6j** ($IC_{50} = 37.16 \pm 0.75$ μM) with *m*- and *o*-methyl phenyl substituents, respectively. Compound **6d** ($IC_{50} = 27.29 \pm 0.37$ μM) with *p*-methoxy phenyl and **6e** ($IC_{50} = 26.17 \pm 0.43$ μM) with *p*-aceto phenyl substituents exhibited very close inhibition of α-glucosidase. While **6f** ($IC_{50} = 31.48 \pm 0.64$ μM) with phenyl R group exhibited less inhibition compared to **6d** and **6e**. The flouro substituted compounds, **6g** ($IC_{50} = 17.00 \pm 0.42$ μM) with *p*-flouro phenyl substituent exhibited significantly higher potent inhibition as compared to **6h** ($IC_{50} = 22.39 \pm 0.54$ μM) with *m*-flouro phenyl substituent. Overall, compounds in group A and B displayed higher potency than compounds in group C. This favorable inhibitory effect might be due to the R_1 and R substituents.

2.2. Kinetic study

The mechanism of action of the identified inhibitors was deduced *in vitro* by kinetic analysis of the most potent compound, **5h** which showed concentration dependent type of inhibition with K_i value 3.96 ± 0.0048 μM. The mechanistic analysis indicates that **5h** binds at the active site of α-glucosidase. Thus, increases K_m of the enzyme while V_{max} of the enzyme remains constant (Fig. 2).

2.3. Molecular docking

Docking was performed to elucidate the mode of binding of **5h** at the active site of α-glucosidase, which reflects excellent binding of **5h** with active site residues. The urea moiety of **5h** mediates a hydrogen bond with Gln279 (2.06 Å) which is one of the residues in catalytic triad. While the hydroxyl group of **5h** exhibited a hydrogen bond with the –OH of Thr306 (1.79 Å), and substituted chlorine at phenyl ring forms halogen bond with the side chain of Arg213 (2.47 Å). Moreover, a solvent molecule provided a hydrogen bond to the amino group of **5h** (1.96 Å) and Tyr72 creates a hydrophobic interaction with the chloro-substituted phenyl ring of **5h** (4.31 Å). These interactions help in fitting of **5h** at the active site of enzyme with a highly negative docking score (-6.15 kcal mol^{-1}). The binding mode of **5h** is shown in Fig. 3 in 3D and 2D-mode.

3. Conclusion

Schiff base diphenyl urea derivatives play a crucial role in medicinal chemistry. The current study demonstrates the synthesis of novel Schiff bases of 1,3-diphenyl urea derivates their evaluation against α-glucosidase to explore their therapeutic potential for diabetes mellitus. Fortunately, all the compounds exhibited several fold potent inhibition of α-glucosidase in the range of 2.49–37.16 μM, as compared to standard drug. In the kinetic analysis, the most potent inhibitor, compound **5h** reflected competitive mode of inhibition and demonstrates favorable interactions with the active site residues of α-glucosidase in the molecular docking investigation. The nature of these interactions, such as hydrogen bonds, and hydrophobic interactions provides insight into the molecular basis for the high inhibitory activity of **5h**, observed in kinetic studies. These findings highlight the therapeutic potential of the identified inhibitors for the treatment of diabetes mellitus. α-Glucosidase hydrolysis carbohydrates into glucose and subsequently increases blood glucose levels. By inhibiting α-glucosidase, **5h** can slow down the absorption of glucose in the intestine, thereby control postprandial blood glucose spikes and reduces the risk of diabetes associated long-term complications. These promising results warrant further preclinical evaluation of **5h** for the development of a new class of antidiabetic agent with better glycemic control.

4. Materials and method

All the starting materials employed in the synthesis were purchased from Sigma-Aldrich Co. (Germany) and used without purification. Methanol, absolute ethanol, and other solvents were also purchased from different commercial sources in adequate purity and used without purification in the reaction media. To monitor the reaction, thin layer chromatography (TLC) was performed with silica gel 60 aluminum backed plates with suitable solvent system. Spotson TLC plates were visualized by using UV light with 254 nm. The ^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectra were recorded using DMSO-d^{6} as solvents *via* Bruker spectrophotometer 600 MHz and 151 MHz as dilute solution at 25 °C. Chemical shifts were reported in parts per million (δ = ppm) and coupling constants (J) were expressed in Hertz (Hz). The signals were described as singlet (s), doublet (d), triplet (t) multiplet (m). HPLC was carried out on Agilent, Germany (Liquid Chromatographic Column 150 mm × 4.6 mm (id) packed with 5-micron C18; 263 nm). Mass spectra (ESI-MS), were recorded by means of Agilent QTOF MS 6530 WITH 1260 HPLC. Thermo Scientific FLASH 2000 CHNS/O analyzer. Melting points were determined using MPS10 melting point apparatus.

4.1. Chemistry: general procedure for the synthesis of Schiff base 1,3-dipheny urea derivatives

o-Phenylenediamine (**1**) (5 mmol) was dissolved in 15–20 mL of chloroform by constant stirring at room temperature. Then equimolar amount of different substituted isocyanates (**2a–t**) were added carefully dropwise with the help of dropping funnel into this diamine solution. Immediately, solid product precipitated out at stirring that was filtered followed by washing with *n*-hexane and dried under vacuum. The resulting mono substituted 1,3-diphenyl urea (**3a–t**) (1 mmol) were refluxed for 3–4 hours with substituted aldehyde (**4**) (1 mmol) in 8–10 mL of methanol to obtain the final products (**5a–t**) and (**6a–j**) that were filtered, washed with cold ethanol, and dried under vacuum.

4.2. Experimental data

1-(2-{[(5-Chloro-2-hydroxybenzylidene)amino]}phenyl)-3-(4-chlorophenyl)urea (5a). Cream yellow solid; yield: 62%, m. p.: 239–241 °C; ^{1}H-NMR (DMSO-d^{6}) δ ppm; 7.03 (d, 1H, J = 9 Hz), 7.64 (td, 1H, J = 7.8, 1.2 Hz), 7.23–7.25 (m, 2H), 7.30–7.33 (m, 2H), 7.44 (dd, 1H, J = 9, 3 Hz), 7.47–7.50 (m, 2H), 7.99 (d, 1H, J = 2.4 Hz), 8.10 (d, 1H, J = 7.8 Hz), 8.35 (s, 1H), 8.88 (s, 1H), 9.51 (s, 1H), 11.63 (s, 1H); ^{13}C-NMR ppm; 118.4, 118.6, 119.8, 119.9, 122.3, 122.9, 123.0, 125.4, 127.3, 128.7, 129.3, 132.9, 133.6, 138.7, 138.9, 152.2, 158.0, 159.0; HPLC purity: t_R = 2.234 min, 96% ($CH_3CN : H_2O$ = 80 : 20); anal. calcd for $C_{20}H_{15}Cl_2N_3O_2$: C, 60.02; H, 3.78; N, 10.50; found: C, 60.06; H, 3.81; N, 10.45; QTOF MS ES+ (m/z): $[M + H]^+$, calcd: 400.0619, found: 400.0590.

1-(2-{[(5-Chloro-2-hydroxybenzylidene)amino]}phenyl)-3-(*p*-tolyl)urea (5b). Off white solid; yield: 64%, m. p.: 235–237 °C; ^{1}H-NMR (DMSO-d^{6}) δ ppm; 2.23 (s, 3H, CH_3), 7.00–7.05 (m, 2H), 7.07 (d, 2H, J = 7.8 Hz), 7.22–7.24 (m, 2H), 7.34 (d, 2H, J = 8.4 Hz), 7.44 (dd, 1H, J = 9, 3 Hz), 7.99 (d, 1H, J = 3 Hz), 8.10 (dd, 1H, J = 8.4, 1.2 Hz), 8.28 (s, 1H), 8.88 (s, 1H), 9.27 (s, 1H), 11.64 (s, 1H); ^{13}C-NMR ppm; 20.3, 118.3, 118.4, 118.5, 119.7, 122.2, 122.5, 123.0, 127.2, 129.2, 129.3, 130.7, 132.8, 133.8, 137.1, 138.7, 152.3, 158.0, 159.0; HPLC purity: t_R = 2.767 min, 100% ($CH_3CN : H_2O$ = 80 : 20); anal. calcd for $C_{21}H_{18}ClN_3O_2$: C, 66.40; H, 4.78; N, 11.06; found: C, 60.36; H, 3.88; N, 10.55; QTOF MS ES+ (m/z): $[M + H]^+$, calcd: 380.1165, found: 380.1158.

1-(2-{[(5-Chloro-2-hydroxybenzylidene)amino]}phenyl)-3-phenylurea (5c). Off white solid; yield: 66%, m. p.: 232–234 °C; ^{1}H-NMR (DMSO-d^{6}) δ ppm; 6.96 (t, 1H, J = 7.2 Hz), 7.01 (d, 1H, J = 9 Hz), 7.05 (ddd, 1H, J = 15.6, 9, 1.8 Hz), 7.22–7.25 (m, 2H), 7.26–7.29 (m, 2H), 7.44 (d, 1H, J = 3 Hz), 7.45–7.47 (m, 2H), 7.99 (d, 1H, J = 3 Hz), 8.13 (dd, 1H, J = 9, 1.2 Hz), 8.23 (s, 1H), 8.89 (s, 1H), 9.37 (s, 1H), 11.65 (s, 1H); ^{13}C-NMR ppm; 118.2, 118.3, 118.5, 119.8, 121.9, 122.2, 122.6, 123.0, 127.3, 128.8, 129.3, 132.8, 133.7, 138.8, 139.6, 152.3, 158.0, 159.0; HPLC purity: t_R = 2.212 min, 99% ($CH_3CN : H_2O$ = 80 : 20); anal. calcd for $C_{20}H_{16}ClN_3O_2$: C, 65.67; H, 4.41; N, 11.49; found: C, 65.76; H, 4.39; N, 11.54; QTOF MS ES+ (m/z): $[M + H]^+$, calcd: 366.1009, found: 366.0992.

1-(2-{[(5-Chloro-2-hydroxybenzylidene)amino]}phenyl)-3-(3-fluorophenyl)urea (5d). Light yellow solid; yield: 78%, m. p.: 237–239 °C; ^{1}H-NMR (DMSO-d^{6}) δ ppm; 7.03 (td, 1H, J = 3 Hz), 6.98 (d, 1H, J = 8.4 Hz), 7.02–7.06 (m, 2H), 7.20–7.28 (m, 3H), 7.40 (dd, 1H, J = 9, 3 Hz), 7.48 (dt, 1H, J = 12, 2.4 Hz), 7.95 (d, 1H, J = 2.4 Hz), 8.06–8.08 (m, 1H), 8.35 (s, 1H), 8.85 (s, 1H), 9.57 (s, 1H), 11.61 (s, 1H); ^{13}C-NMR ppm; 104.8, 104.9, 108.1, 108.2, 113.9, 118.3, 118.5, 119.9, 122.2, 122.9, 123.0, 127.2, 129.2, 130.3, 130.3, 132.8, 133.3, 139.0, 141.5, 141.5, 152.1, 158.0, 159.0, 161.6, 163.2; HPLC purity: t_R = 2.401 min, 100% ($CH_3CN : H_2O$ = 80 : 20); anal. calcd for $C_{20}H_{15}ClFN_3O_2$: C, 62.59; H, 3.94; N, 10.95; found: C, 62.76; H, 3.89; N, 10.99; N, 11.54; QTOF MS ES+ (m/z): $[M + H]^+$, calcd: 384.0915, found: 384.0900.

1-(2-{[(5-Chloro-2-hydroxybenzylidene)amino]}phenyl)-3-(4-fluorophenyl)urea (5e). Light yellow solid; yield: 79%, m. p.: 231–233 °C; ^{1}H-NMR (DMSO-d^{6}) δ ppm; 7.00 (d, 1H, J = 8.4 Hz), 7.05 (td, 1H, J = 7.8, 1.2 Hz), 7.11 (ddd, 2H, J = 15, 9, 2.4 Hz), 7.22–7.25 (m, 2H), 7.43–7.48 (m, 3H), 7.98 (d, 1H, J = 3 Hz), 8.11–8.12 (m, 1H), 8.30 (s, 1H), 8.89 (s, 1H), 9.40 (s, 1H), 11.64 (s, 1H); ^{13}C-NMR ppm; 115.3, 115.4, 118.3, 118.5, 119.8, 119.9, 120.0, 122.2, 122.6, 123.0, 127.3, 129.3, 132.8, 133.7, 136.0, 136.0, 138.8, 152.3, 156.6, 158.0, 158.1, 159.0; HPLC purity: t_R = 2.268 min, 100% ($CH_3CN : H_2O$ = 80 : 20); anal. calcd for $C_{20}H_{15}ClFN_3O_2$: C, 62.59; H, 3.94; N, 10.95; found: C, 62.66; H, 3.85; N, 11.02; QTOF MS ES+ (m/z): $[M + H]^+$, calcd: 384.0915, found: 384.0901.

1-(2-{[(5-Chloro-2-hydroxybenzylidene)amino]}phenyl)-3-(*o*-tolyl)urea (5f). Off white solid; yield: 64%, m. p.: 247–249 °C; ^{1}H-NMR (DMSO-d^{6}) δ ppm; 2.24 (s, 3H, CH_3), 6.97 (td, 1H, J = 7.2, 0.6 Hz), 7.00 (d, 1H, J = 9 Hz), 7.07 (td, 1H, J = 7.8, 1.2 Hz), 7.13 (t, 1H, J = 7.8 Hz), 7.17 (d, 1H, J = 7.2 Hz), 7.22–7.27 (m, 2H), 7.44 (dd, 1H, J = 9, 2.4 Hz), 7.65 (d, 1H, J = 7.8 Hz), 7.99 (d, 1H, J = 3 Hz), 8.03 (dd, 1H, J = 8.4, 1.2 Hz), 8.53 (s, 1H), 8.56 (s, 1H), 8.90 (s, 1H), 11.87 (s, 1H); ^{13}C-NMR ppm; 18.0, 118.4, 118.5, 120.7, 122.0, 122.6, 122.8, 122.9, 123.3, 126.1, 127.2, 129.0, 129.6, 130.2, 132.8, 133.7, 137.1, 139.1, 152.7, 158.2, 159.5; HPLC purity: t_R = 2.526 min, 100% ($CH_3CN : H_2O$ = 80 : 20); anal. calcd for $C_{21}H_{18}ClN_3O_2$: C, 66.40; H, 4.78; N, 11.06; found: C, 66.36; H, 4.84; N, 11.11; QTOF MS ES+ (*m*/*z*): [M + H]$^+$, calcd: 380.1165, found: 380.1206.

1-(2-{[(5-Chloro-2-hydroxybenzylidene)amino]}phenyl)-3-(*m*-tolyl)urea (5g). Cream yellow solid; yield: 65%, m. p.: 220–222 °C; ^{1}H-NMR (DMSO-d^6) δ ppm; 2.26 (s, 3H, CH_3), 6.78 (d, 1H, J = 7.2 Hz), 7.00 (d, 1H, J = 8.4 Hz), 7.05 (td, 1H, J = 8.4, 1.8 Hz), 7.15 (t, 1H, J = 7.8 Hz), 7.22–7.24 (m, 3H), 7.30 (s, 1H), 7.44 (dd, 1H, J = 8.4, 2.4 Hz), 7.99 (d, 1H, J = 2.4 Hz), 8.13 (dd, 1H, J = 8.4, 1.2 Hz), 8.30 (s, 1H), 8.88 (s, 1H), 9.03 (s, 1H), 11.65 (s, 1H); ^{13}C-NMR ppm; 21.2, 115.4, 118.3, 118.5, 118.8, 119.7, 122.2, 122.6, 122.6, 123.0, 127.2, 128.7, 129.3, 132.8, 133.7, 138.0, 138.7, 139.6, 152.3, 158.0, 159.0; HPLC purity: t_R = 2.528 min, 100% ($CH_3CN : H_2O$ = 80 : 20); anal. calcd for $C_{21}H_{18}ClN_3O_2$: C, 66.40; H, 4.78; N, 11.06; found: C, 66.45; H, 4.87; N, 11.21; QTOF MS ES+ (*m*/*z*): [M + H]$^+$, calcd: 380.1165, found: 380.1091.

1-(2-{[(5-Chloro-2-hydroxybenzylidene)amino]}phenyl)-3-(3-chlorophenyl)urea (5h). Light yellow solid; yield: 75%, m. p.: 230–232 °C; ^{1}H-NMR (DMSO-d^6) δ ppm; 7.00–7.02 (m, 2H), 7.07 (ddd, 1H, J = 15, 8.4, 1.2 Hz), 7.23–7.28 (m, 3H), 7.29 (t, 1H, J = 7.8 Hz), 7.44 (dd, 1H, J = 9, 3 Hz), 7.74 (t, 1H, J = 1.8 Hz), 7.99 (d, 1H, J = 2.4 Hz), 8.10–8.11 (m, 1H), 8.38 (s, 1H), 8.89 (s, 1H), 9.58 (s, 1H), 11.63 (s, 1H); ^{13}C-NMR ppm; 116.6, 117.6, 118.4, 118.5, 120.0, 121.5, 122.2, 123.0, 123.0, 127.3, 129.3, 130.4, 132.9, 133.2, 133.4, 139.0, 141.2, 152.1, 158.0, 159.1; HPLC purity: t_R = 2.270 min, 99% ($CH_3CN : H_2O$ = 80 : 20); anal. calcd for $C_{20}H_{15}Cl_2N_3O_2$: C, 60.02; H, 3.78; N, 10.50; found: C, 60.10; H, 3.88; N, 10.45; QTOF MS ES+ (*m*/*z*): [M + H]$^+$, calcd: 400.0619, found: 400.0574.

1-(2-{[(5-Chloro-2-hydroxybenzylidene)amino]}phenyl)-3-(4-methoxyphenyl)urea (5i). Light yellow solid; yield: 86%, m. p.: 223–225 °C; ^{1}H-NMR (DMSO-d^6) δ ppm; 3.70 (s, 3H, CH_3), 6.86 (d, 2H, J = 9 Hz), 7.02 (dd, 2H, J = 13.8, 7.8 Hz), 7.21–7.23 (m, 2H), 7.35 (d, 2H, J = 9 Hz), 7.44 (dd, 1H, J = 9, 3 Hz), 7.98 (d, 1H, J = 3 Hz), 8.13 (d, 1H, J = 8.4 Hz), 8.23 (s, 1H), 8.88 (s, 1H), 9.19 (s, 1H), 11.64 (s, 1H); ^{13}C-NMR ppm; 55.1, 114.0, 118.3, 118.5, 119.6, 120.1, 122.2, 122.4, 123.0, 127.2, 129.3, 132.6, 132.8, 133.9, 138.6, 152.4, 154.5, 158.0, 159.0; HPLC purity: t_R = 2.672 min, 99% ($CH_3CN : H_2O$ = 80 : 20); anal. calcd for $C_{21}H_{18}Cl\ N_3O_3$: C, 63.72; H, 4.58; N, 10.62; found: C, 63.79; H, 4.68; N, 10.55; QTOF MS ES+ (*m*/*z*): [M + H]$^+$, calcd: 396.1114, found: 396.1089.

1-(4-Acetylphenyl)-3-(2-{[(5-chloro-2-hydroxybenzylidene) amino]}phenyl)urea (5j). Light yellow solid; yield: 69%, m. p.: 239–241 °C; ^{1}H-NMR (DMSO-d^6) δ ppm; 3.32 (s, 3H, CH_3), 7.00 (d, 1H, J = 8.4 Hz), 7.44 (dd, 1H, J = 8.4, 2.4 Hz), 7.24–7.27 (m, 2H), 7.44 (dd, 1H, J = 8.4, 2.4 Hz), 7.60 (d, 2H, J = 9 Hz), 7.90 (d, 2H, J = 9 Hz), 8.00 (d, 1H, J = 2.4 Hz), 8.12–8.13 (m, 1H), 8.46 (s, 1H), 8.89 (s, 1H), 9.79 (s, 1H), 11.62 (s, 1H); ^{13}C-NMR ppm; 26.3, 117.2, 118.4, 118.5, 120.0, 122.2, 123.0, 123.1, 127.3, 129.3, 129.7, 130.5, 132.9, 133.3, 139.1, 144.3, 152.0, 158.0, 159.1, 196.3; HPLC purity: t_R = 2.282 min, 98% ($CH_3CN : H_2O$ = 80 : 20); anal. calcd for $C_{22}H_{18}ClN_3O_3$: C, 64.79; H, 4.45; N, 10.30; found: C, 64.88; H, 4.38; N, 10.45.

1-(2-{[(5-Bromo-2-hydroxybenzylidene)amino]}phenyl)-3-(4-chlorophenyl)urea (5k). Cream yellow solid; yield: 82%, m. p.: 235–237 °C; ^{1}H-NMR (DMSO-d^6) δ ppm; 6.95 (d, 1H, J = 9 Hz), 7.06 (ddd, 1H, J = 15.6, 9, 1.2 Hz), 7.23–7.25 (m, 2H), 7.31 (dd, 2H, J = 6.6, 1.8 Hz), 7.48 (dd, 2H, J = 7.2, 2.4 Hz), 7.55 (dd, 1H, J = 9, 3 Hz), 8.09–8.11 (m, 2H), 8.34 (s, 1H), 8.87 (s, 1H), 9.51 (s, 1H), 11.67 (s, 1H); ^{13}C-NMR ppm; 110.5, 118.4, 118.9, 119.7, 119.9, 122.8, 122.8, 125.4, 127.3, 128.7, 132.2, 133.5, 135.6, 138.7, 138.9, 152.2, 158.4, 159.0; HPLC purity: t_R = 2.349 min, 97% ($CH_3CN : H_2O$ = 80 : 20); anal. calcd for $C_{20}H_{15}BrClN_3O_2$: C, 54.02; H, 3.40; N, 9.45; found: C, 54.11; H, 3.48; N, 9.55; QTOF MS ES+ (*m*/*z*): [M + 2H]$^+$, calcd: 444.0114, found: 445.9910.

1-(2-{[(5-Bromo-2-hydroxybenzylidene)amino]}phenyl)-3-(*p*-tolyl)urea (5l). Light yellow solid; yield: 76%, m. p.: 232–234 °C; ^{1}H-NMR (DMSO-d^6) δ ppm; 2.23 (s, 3H, CH_3), 6.95 (d, 1H, J = 8.4 Hz), 7.04 (td, 1H, J = 7.8, 1.2 Hz), 7.07 (d, 2H, J = 8.4 Hz) 7.21–7.24 (m, 2H), 7.34 (d, 2H, J = 8.4 Hz), 7.55 (dd, 1H, J = 8.4, 2.4 Hz), 8.09 (d, 1H, J = 2.4 Hz), 8.12 (dd, 1H, J = 8.4, 1.2 Hz), 8.27 (s, 1H), 8.87 (s, 1H), 9.27 (s, 1H), 11.69 (s, 1H); ^{13}C-NMR ppm; 20.3, 110.5, 118.3, 118.9, 119.7, 122.5, 122.8, 127.2, 129.2, 130.7, 132.3, 133.8, 135.6, 137.1, 138.7, 152.3, 158.4, 159.0; HPLC purity: t_R = 2.657 min, 100% ($CH_3CN : H_2O$ = 80 : 20); anal. calcd for $C_{21}H_{18}BrN_3O_2$: C, 59.45; H, 4.28; N, 9.90; found: C, 59.40; H, 4.38; N, 9.95; QTOF MS ES+ (*m*/*z*): [M + H]$^+$, calcd: 424.0660, found: 424.0620.

1-(2-{[(5-Bromo-2-hydroxybenzylidene)amino]}phenyl)-3-(4-fluorophenyl)urea (5m). Off white solid; yield: 76%, m. p.: 238–240 °C; ^{1}H-NMR (DMSO-d^6) δ ppm; 6.95 (d, 1H, J = 8.4 Hz), 7.05 (td, 1H, J = 9, 1.2 Hz), 7.11 (t, 2H, J = 9 Hz), 7.22–7.24 (m, 2H), 7.45–7.47 (m, 2H), 7.55 (dd, 1H, J = 9, 3 Hz), 8.10 (dd, 1H, J = 4.2, 1.8 Hz), 8.12 (d, 1H, J = 0.6 Hz), 8.29 (s, 1H), 8.87 (s, 1H), 9.40 (s, 1H), 11.68 (s, 1H); ^{13}C-NMR ppm; 110.5, 115.3, 115.4, 118.4, 118.9, 119.8, 119.9, 120.0, 122.7, 122.8, 127.3, 132.3, 133.6, 135.6, 136.0, 138.9, 152.3, 156.6, 158.1, 158.4, 159.1; HPLC purity: t_R = 2.347 min, 100% ($CH_3CN : H_2O$ = 80 : 20); anal. calcd for $C_{20}H_{15}BrFN_3O_2$: C, 56.09; H, 3.53; N, 9.81; found: C, 56.22; H, 3.58; N, 9.85; QTOF MS ES+ (*m*/*z*): [M + H]$^+$, calcd: 428.0409, found: 428.0278.

1-(2-{[(5-Bromo-2-hydroxybenzylidene)amino]}phenyl)-3-(*m*-tolyl)urea (5n). Cream yellow solid; yield: 67%, m. p.: 219–221 °C; ^{1}H-NMR (DMSO-d^6) δ ppm; 2.26 (s, 3H, CH_3), 6.78 (d, 1H, J = 7.2 Hz), 6.95 (d, 1H, J = 8.4 Hz), 7.05 (td, 1H, J = 7.8, 1.2 Hz), 7.15 (t, 1H, J = 7.8 Hz), 7.22–7.24 (m, 3H), 7.30 (s, 1H), 7.55 (dd, 1H, J = 10.2, 3 Hz), 8.09 (d, 1H, J = 2.4 Hz), 8.12 (dd, 1H, J = 8.4, 1.2 Hz), 8.30 (s, 1H), 8.87 (s, 1H), 9.30 (s, 1H), 11.69 (s, 1H); ^{13}C-NMR ppm; 21.2, 110.5, 115.4, 118.4, 118.8, 118.9, 119.8, 122.6, 122.6, 122.8, 127.2, 128.7, 132.3, 133.7, 135.6, 138.0, 138.8, 139.6, 152.2, 158.4, 159.1; HPLC purity: t_R = 2.671 min, 100% ($CH_3CN : H_2O$ = 80 : 20); anal. calcd for $C_{21}H_{18}BrN_3O_2$: C, 59.45; H, 4.28; N, 9.90; found: C, 59.51; H, 4.31; N, 9.85; QTOF MS ES+ (*m*/*z*): [M + 2H]$^+$, calcd: 424.0660, found: 426.0672.

1-(2-{[(5-Bromo-2-hydroxybenzylidene)amino]}phenyl)-3-(*o*-tolyl)urea (5o). Cream yellow solid; yield: 76%, m. p.: 234–236 °C; ^{1}H-NMR (DMSO-d^6) δ ppm 2.24 (s, 3H, CH_3), 6.95–6.98 (m, 2H), 7.07 (td, 1H, J = 7.8, 1.2 Hz), 7.13 (t, 1H, J = 7.8 Hz), 7.17 (d, 1H, J = 7.2 Hz), 7.22–7.27 (m, 2H), 7.54 (dd, 1H, J = 8.4, 2.4 Hz), 7.66 (d, 1H, $J = 7.2$ Hz), 8.01 (dd, 1H, $J = 8.4, 1.2$ Hz), 8.05 (d, 1H, $J = 2.4$ Hz), 8.53 (s, 1H), 8.56 (s, 1H), 8.89 (s, 1H), 11.91 (s, 1H); ^{13}C-NMR ppm; 18.0, 110.3, 118.5, 118.9, 120.8, 122.6, 122.6, 122.9, 123.3, 126.1, 127.2, 128.9, 130.2, 132.6, 133.7, 135.6, 137.1, 139.1, 152.7, 158.6, 159.5; HPLC purity: $t_R = 2.391$ min, 100% ($CH_3CN : H_2O = 80 : 20$); anal. calcd for $C_{21}H_{18}BrN_3O_2$: C, 59.45; H, 4.28; N, 9.90; found: C, 59.50; H, 4.37; N, 9.98; QTOF MS ES+ (m/z): $[M + H]^+$, calcd: 424.0660, found: 424.0556.

1-(2-{[(5-Bromo-2-hydroxybenzylidene)amino]}phenyl)-3-(3-chlorophenyl)urea (5p). Off white solid; yield: 90%, m. p.: 228–230 °C; ^{1}H-NMR (DMSO-d^6) δ ppm; 7.00 (dd, 1H, $J = 1.8, 0.6$ Hz), 7.01 (dd, 1H, $J = 1.8, 0.6$ Hz), 7.07 (td, 1H, $J = 8.4, 1.2$ Hz), 7.23–7.27 (m, 3H), 7.29 (t, 1H, $J = 7.8$ Hz), 7.55 (dd, 1H, $J = 9, 3$ Hz), 7.74 (t, 1H, $J = 1.8$ Hz), 8.10 (dd, 2H, $J = 7.8, 1.2$ Hz), 8.37 (s, 1H), 8.87 (s, 1H), 9.58 (s, 1H), 11.68 (s, 1H); ^{13}C-NMR ppm; 110.5, 116.6, 117.6, 118.4, 118.9, 120.0, 121.5, 22.8, 123.0, 127.3, 130.4, 132.3, 133.2, 133.3, 135.7, 139.1, 141.2, 152.1, 158.4, 159.1; HPLC purity: $t_R = 2.777$ min, 100% ($CH_3CN : H_2O = 80 : 20$); anal. calcd for $C_{20}H_{15}BrClN_3O_2$: C, 54.02; H, 3.40; N, 9.45; found: C, 54.21; H, 3.38; N, 9.51; QTOF MS ES+ (m/z): $[M + 2H]^+$, calcd: 444.0114, found: 445.9989.

1-(2-{[(5-Bromo-2-hydroxybenzylidene)amino]}phenyl)-3-(4-methoxyphenyl)urea (5q). Off white solid; yield: 80%, m. p.: 224–226 °C; ^{1}H-NMR (DMSO-d^6) δ ppm; 3.70 (s, 3H, CH_3), 6.86 (d, 2H, $J = 9$ Hz), 6.95 (d, 1H, $J = 9$ Hz), 7.03–7.05 (m, 1H), 7.21–7.23 (m, 2H), 7.35 (d, 2H, $J = 9$ Hz), 7.54 (dd, 1H, $J = 9, 2.4$ Hz), 8.09 (d, 1H, $J = 3$ Hz), 8.12 (d, 1H, $J = 7.8$ Hz), 8.23 (s, 1H), 8.86 (s, 1H), 9.19 (s, 1H), 11.69 (s, 1H); ^{13}C-NMR ppm; 55.6, 110.9, 114.5, 118.8, 119.4, 120.1, 120.5, 122.8, 123.2, 127.7, 132.7, 133.1, 134.3, 136.0, 139.1, 152.9, 154.9, 158.9, 159.5; HPLC purity: $t_R = 2.350$ min, 96% ($CH_3CN : H_2O = 80 : 20$); anal. calcd for $C_{21}H_{18}BrN_3O_3$: C, 57.29; H, 4.12; N, 9.54; found: C, 57.41; H, 4.08; N, 9.58.

1-(4-Acetylphenyl)-3-(2-{[(5-bromo-2-hydroxybenzylidene) amino]}phenyl)urea (5r). Yellow solid; yield: 82%, m. p.: 239–241 °C; ^{1}H-NMR (DMSO-d^6) δ ppm; 3.50 (s, 3H, CH_3), 6.95 (d, 1H, $J = 9$ Hz), 7.08 (ddd, 1H, $J = 15, 8.4, 1.2$ Hz), 7.24–7.27 (m, 2H), 7.55 (dd, 1H, $J = 9, 3$ Hz), 7.60 (d, 2H, $J = 9$ Hz), 7.90 (d, 2H, $J = 9$ Hz), 8.10–8.13 (m, 2H), 8.46 (s, 1H), 8.88 (s, 1H), 9.79 (s, 1H), 11.67 (s, 1H); ^{13}C-NMR ppm; 26.3, 110.5, 117.2, 118.4, 118.9, 120.0, 122.8, 123.1, 127.3, 129.7, 130.5, 132.2, 133.3, 135.7, 139.1, 144.3, 152.0, 158.4, 159.1, 196.3; HPLC purity: $t_R = 2.229$ min, 95% ($CH_3CN : H_2O = 80 : 20$); anal. calcd for $C_{22}H_{18}BrN_3O_3$: C, 58.42; H, 4.01; N, 9.29; found: C, 58.55; H, 4.08; N, 9.35; QTOF MS ES+ (m/z): $[M + 2H]^+$, calcd: 452.0609, found: 454.0568.

1-(2-{[(5-Bromo-2-hydroxybenzylidene)amino]}phenyl)-3-(3-fluorophenyl)urea (5s). Off white solid; yield: 74%, m. p.: 222–224 °C; ^{1}H-NMR (DMSO-d^6) δ ppm; 6.93–6.96 (m, 1H), 7.04 (td, 1H, $J = 8.4, 1.2$ Hz), 7.21–7.24 (m, 2H), 7.26 (t, 2H, $J = 7.8$ Hz), 7.44 (d, 2H, $J = 7.8$ Hz), 7.54 (dd, 1H, $J = 12, 2.4$ Hz), 8.08 (d, 1H, $J = 2.4$ Hz), 8.10 (dd, 1H, $J = 8.4, 4.2$ Hz), 8.31 (s, 1H), 8.86 (s, 1H), 9.36 (s, 1H), 11.69 (s, 1H); ^{13}C-NMR ppm; 104.8, 104.9, 108.1, 108.2, 110.5, 113.9, 118.4, 118.9, 120.0, 122.8, 122.9, 127.2, 130.3, 130.4, 132.3, 133.3, 135.6, 139.0, 141.5, 141.6, 152.1, 158.4, 159.1, 161.6, 163.2; HPLC purity: $t_R = 2.232$ min, 96% ($CH_3CN : H_2O = 80 : 20$); anal. calcd for $C_{20}H_{15}BrFN_3O_2$: C, 56.09; H, 3.53; N, 9.81; found: C, 56.19; H, 3.65; N, 9.95; QTOF MS ES+ (m/z): $[M + H]^+$, calcd: 428.0409, found: 428.0403.

1-(2-{[(5-Bromo-2-hydroxybenzylidene)amino]}phenyl)-3-phenylurea (5t). Cream yellow solid; yield: 79%, m. p.: 234–236 °C; ^{1}H-NMR (DMSO-d^6) δ ppm; 6.77 (td, 1H, $J = 8.4, 2.4$ Hz), 6.95 (d, 1H, $J = 9$ Hz), 7.05–7.08 (m, 2H), 7.23–7.25 (m, 2H), 7.28 (dd, 1H, $J = 15.6, 3$ Hz), 7.50 (dt, 1H, $J = 9.9, 1.8$ Hz), 7.54 (dd, 1H, $J = 9, 3$ Hz), 8.09 (dd, 2H, $J = 6.6, 1.2$ Hz), 8.36 (s, 1H), 8.86 (s, 1H), 9.59 (s, 1H), 11.68 (s, 1H); ^{13}C-NMR ppm; 110.5, 118.2, 118.4, 118.9, 119.8, 121.9, 122.6, 122.8, 127.2, 128.8, 132.3, 133.6, 135.6, 138.8, 139.6, 152.3, 158.4, 159.1; HPLC purity: $t_R = 2.526$ min, 99% ($CH_3CN : H_2O = 80 : 20$); anal. calcd for $C_{20}H_{16}Br\ N_3O_2$: C, 58.55; H, 3.93; N, 10.24; found: C, 58.70; H, 3.88; N, 10.35; QTOF MS ES+ (m/z): $[M + H]^+$, calcd: 410.0504, found: 410.0533.

1-(2-{[((1-Bromonaphthalen-2-yl)methylene)amino]}phenyl)-3-(4-chlorophenyl)urea (6a). Greenish yellow solid; yield: 90%, m. p.: 240–242 °C; ^{1}H-NMR (DMSO-d^6) δ ppm; 7.05–7.08 (m, 1H), 7.26–7.28 (m, 1H), 7.30–7.34 (m, 2H), 7.37 (dd, 1H, $J = 8.4, 1.2$ Hz), 7.49–7.52 (m, 2H), 7.71–7.77 (m, 2H), 8.08 (d, 1H, $J = 7.2$ Hz), 8.12 (d, 1H, $J = 9$ Hz), 8.27 (dd, 1H, $J = 8.4, 1.2$ Hz), 8.38 (d, 1H, $J = 8.4$ Hz), 8.48–8.50 (m, 2H), 9.22 (s, 1H), 9.71 (s, 1H); ^{13}C-NMR ppm; 117.5, 118.9, 119.8, 122.4, 125.5, 127.2, 127.7, 127.9, 128.2, 128.6, 128.7, 128.8, 131.6, 132.2, 134.6, 135.6, 138.3, 138.7, 152.1, 158.8; HPLC purity: $t_R = 8.169$ min, 98% ($CH_3CN : H_2O = 80 : 20$); anal. calcd for $C_{24}H_{17}BrClN_3O$: C, 60.21; H, 3.58; N, 8.78; found: C, 60.30; H, 3.50; N, 8.90; QTOF MS ES+ (m/z): $[M + 2H]^+$, calcd: 478.0321, found: 480.0301.

1-(2-{[((1-Bromonaphthalen-2-yl)methylene)amino]}phenyl)-3-(*p*-tolyl)urea (6b). Greenish off-white solid; yield: 74%, m. p.: 237–238 °C; ^{1}H-NMR (DMSO-d^6) δ ppm; 2.23 (s, 1H), 7.05 (td, 1H, $J = 7.8, 1.8$ Hz), 7.08 (d, 2H, $J = 8.4$ Hz), 7.26–7.29 (m, 1H), 7.36 (d, 3H, $J = 8.4$ Hz), 7.71–7.77 (m, 2H), 8.08 (d, 1H, $J = 7.8$ Hz), 8.08 (d, 1H, $J = 8.4$ Hz), 8.11 (d, 1H, $J = 8.4$ Hz), 8.29 (dd, 1H, $J = 8.4, 1.2$ Hz), 8.38 (d, 1H, $J = 9$ Hz), 8.44 (s, 1H), 8.48 (d, 1H, $J = 9$ Hz), 9.21 (s, 1H), 9.46 (s, 1H); ^{13}C-NMR ppm; 20.39, 117.47, 118.58, 118.80, 122.05, 125.47, 127.23, 127.62, 127.92, 128.16, 128.60, 128.70, 128.81, 129.27, 130.86, 131.58, 132.25, 134.92, 135.58, 137.09, 138.14, 152.28, 158.62; HPLC purity: $t_R = 6.836$ min, 95% ($CH_3CN : H_2O = 80 : 20$); anal. calcd for $C_{25}H_{20}BrN_3O$: C, 65.51; H, 4.40; N, 9.17; found: C, 65.66; H, 4.48; N, 9.09; QTOF MS ES+ (m/z): $[M + 2H]^+$, calcd: 458.0867, found: 460.1058.

1-(2-{[(1-Bromonaphthalen-2-yl)methylene]amino}phenyl)-3-(3-chlorophenyl)urea (6c). Greenish off-white solid; yield: 83%, m. p.: 225–227 °C; ^{1}H-NMR (DMSO-d^6) δ ppm; 7.01–7.03 (m, 1H), 7.08 (td, 1H, $J = 7.8, 1.2$ Hz), 7.25–7.31 (m, 3H), 7.38 (dd, 1H, $J = 8.4, 1.2$ Hz), 7.72–7.78 (m, 3H), 8.08 (d, 1H, $J = 7.8$ Hz), 8.12 (d, 1H, $J = 9.0$ Hz), 8.27 (dd, 1H, $J = 8.4, 1.2$ Hz), 8.38 (d, 1H, $J = 8.4$ Hz), 8.48 (d, 1H, $J = 8.4$ Hz), 8.52 (s, 1H), 9.22 (s, 1H), 9.78 (s, 1H); ^{13}C-NMR ppm; 116.7, 117.6, 117.7, 119.0, 121.6, 122.5, 125.4, 127.2, 127.7, 127.9, 128.2, 128.6, 128.7, 128.8, 130.5, 131.6, 132.2, 133.3, 134.5, 135.6, 138.4, 141.3, 152.1, 158.9; HPLC purity: $t_R = 8.528$ min, 100% ($CH_3CN : H_2O = 80 : 20$); anal. calcd for $C_{24}H_{17}BrClN_3O$: C, 60.21; H, 3.58; N, 8.78; Found; C, 60.32; H, 3.71; N, 8.90; QTOF MS ES+ (m/z): $[M + 2H]^+$, calcd: 478.0321, found: 480.0355.

1-(2-{[(1-Bromonaphthalen-2-yl)methylene]amino}phenyl)-3-(4-methoxyphenyl)urea (6d). Greenish off-white solid; yield: 84%, m. p.: 220–222 °C; ^{1}H-NMR (DMSO-d^6) δ ppm 3.70 (s, 3H, CH_3), 6.87 (dd, 1H, J = 8.4, 1.8 Hz), 7.04 (td, 1H, J = 7.8, 1.2 Hz), 7.27 (ddd, 1H, J = 15.6, 8.4, 1.2 Hz), 7.35–7.38 (m, 3H), 7.71–7.78 (m, 2H), 8.07–8.11 (m, 2H), 8.29 (dd, 1H, J = 8.4, 1.8 Hz), 8.37–8.40 (m, 2H), 8.45 (d, 1H, J = 8.4 Hz), 9.21 (s, 1H), 9.37 (s, 1H); ^{13}C-NMR ppm; 55.2, 114.1, 117.4, 118.7, 120.4, 121.9, 125.4, 127.2, 127.6, 127.9, 128.2, 128.6, 128.7, 128.8, 131.6, 132.2, 132.6, 135.0, 135.6, 138.0, 152.4, 154.7, 158.5; HPLC purity: t_R = 8.145 min, 100% ($CH_3CN : H_2O$ = 80 : 20); anal. calcd for $C_{25}H_{20}BrN_3O_2$: C, 63.30; H, 4.25; N, 8.86; found: C, 63.39; H, 4.28; N, 8.95; QTOF MS ES+ (m/z): $[M + 2H]^+$, calcd: 474.0817, found: 476.1053.

1-(4-Acetylphenyl)-3-(2-{[((1-bromonaphthalen-2-yl) methylene)amino]}phenyl)urea (6e). Greenish off-white solid; yield: 74%, m. p.: 223–235 °C; ^{1}H-NMR (DMSO-d^6) δ ppm; 7.07–7.10 (m, 1H), 7.30 (ddd, 1H, J = 16.8, 8.4, 1.2 Hz), 7.39 (dd, 1H, J = 7.8, 1.2 Hz), 7.61–7.63 (m, 2H), 7.72–7.78 (m, 2H), 7.90 (d, 2H, J = 9 Hz), 8.08 (d, 1H, J = 7.2 Hz), 8.12 (d, 1H, J = 8.4 Hz), 8.29 (dd, 1H, J = 7.8, 0.6 Hz), 8.37 (d, 1H, J = 8.4 Hz), 8.50 (d, 1H, J = 8.4 Hz), 8.62 (s, 1H), 9.22 (s, 1H), 9.99 (s, 1H); ^{13}CNMR ppm; 26.4, 117.3, 117.6, 119.0, 122.6, 125.5, 127.2, 127.7, 127.9, 128.2, 128.6, 128.7, 128.8, 129.7, 130.5, 131.6, 132.2, 134.4, 135.6, 138.5, 144.4, 151.9, 158.9, 196.3; HPLC purity: t_R = 6.560 min, 98% ($CH_3CN : H_2O$ = 80 : 20); anal. calcd for $C_{26}H_{20}BrN_3O_2$: C, 64.21; H, 4.15; N, 8.64; found: C, 64.30; H, 4.18; N, 8.55; QTOF MS ES+ (m/z): $[M + 2H]^+$, calcd: 486.0817, found: 488.0739.

1-(2-{[((1-Bromonaphthalen-2-yl)methylene)amino]}phenyl)-3-phenylurea (6f). Greenish off-white solid; yield: 89%, m. p.: 230–232 °C; ^{1}H-NMR (DMSO-d^6) δ ppm; 6.97 (t, 1H, J = 7.2 Hz), 7.06 (td, 1H, J = 7.2, 1.2 Hz), 7.26–7.30 (m, 3H), 7.37 (dd, 1H, J = 7.8, 1.2 Hz), 7.49 (d, 2H, J = 7.2 Hz), 7.72–7.78 (m, 2H), 8.08 (d, 1H, J = 7.8 Hz), 8.12 (d, 1H, J = 9 Hz), 8.29 (dd, 1H, J = 8.4, 1.2 Hz), 8.37 (d, 1H, J = 8.4 Hz), 8.48–8.50 (m, 2H), 9.22 (s, 1H), 9.57 (s, 1H); ^{13}C-NMR ppm; 117.5, 118.4, 118.9, 122.0, 122.2, 125.5, 127.2, 127.6, 127.9, 128.2, 128.6, 128.7, 128.8, 128.9, 131.6, 132.3, 134.8, 135.6, 138.2, 139.7, 152.2, 158.7; HPLC purity: t_R = 5.660 min, 100% ($CH_3CN : H_2O$ = 80 : 20); anal. calcd for $C_{24}H_{18}BrN_3O$: C, 64.88; H, 4.08; N, 9.46; found: C, 66.79; H, 4.18; N, 9.54; QTOF MS ES+ (m/z): $[M + 2H]^+$, calcd: 444.0711, found: 446.0777.

1-(2-{[((1-Bromonaphthalen-2-yl)methylene)amino]}phenyl)-3-(4-fluorophenyl)urea (6g). Greenish off-white solid; yield: 87%, m. p.: 236–238 °C; ^{1}H-NMR (DMSO-d^6) δ ppm; 7.05–7.14 (m, 3H), 7.28 (ddd, 1H, J = 15.6, 8.4, 1.2 Hz), 7.37 (dd, 1H, J = 7.8, 1.2 Hz), 7.47–7.49 (m, 2H), 7.72–7.78 (m, 2H), 8.10 (d, 1H, J = 7.8 Hz), 8.12 (d, 1H, J = 9 Hz), 8.27 (dd, 1H, J = 8.4, 1.2 Hz), 8.38 (d, 1H, J = 8.4 Hz), 8.46–8.49 (m, 2H), 9.22 (s, 1H), 9.60 (s, 1H); ^{13}C-NMR ppm; 115.3, 115.5, 117.5, 118.8, 120.1, 120.2, 122.2, 125.4, 127.2, 127.6, 127.9, 128.2, 128.6, 128.7, 128.8, 131.6, 132.2, 134.8, 135.6, 136.0, 138.2, 152.3, 156.7, 158.2, 158.7; HPLC purity: t_R = 8.216 min, 96% ($CH_3CN : H_2O$ = 80 : 20); anal. calcd for $C_{24}H_{17}BrFN_3O$: C, 62.35; H, 3.71; N, 9.09; found: C, 62.44; H, 3.68; N, 9.21; QTOF MS ES+ (m/z): $[M + 2H]^+$, calcd: 462.0617, found: 464.0687.

1-(2-{[((1-Bromonaphthalen-2-yl)methylene)amino]}phenyl)-3-(3-fluorophenyl)urea (6h). Greenish off-white solid; yield: 86%, m. p.: 227–230 °C; ^{1}H-NMR (DMSO-d^6) δ ppm; 7.07–7.12 (m, 3H), 7.27–7.32 (m, 2H), 7.38 (dd, 1H, J = 7.8, 1.2 Hz), 7.36 (dt, 1H, J = 11.4, 2.4 Hz), 7.71–7.77 (m, 2H), 8.08 (d, 1H, J = 7.2 Hz), 8.12 (d, 1H, J = 8.4 Hz), 8.27 (dd, 1H, J = 8.4, 1.2 Hz), 8.37 (d, 1H, J = 8.4 Hz), 8.48 (d, 1H, J = 9 Hz), 8.53 (s, 1H), 9.22 (s, 1H), 9.80 (s, 1H); ^{13}C-NMR ppm; 104.9, 105.1, 108.2, 108.4, 114.0, 117.6, 119.0, 122.5, 125.4, 127.2, 127.7, 127.9, 128.2, 128.6, 128.7, 128.8, 130.4, 130.4, 131.6, 132.2, 134.5, 135.6, 138.4, 141.5, 141.6, 152.1, 158.9, 161.7, 163.3; HPLC purity: t_R = 6.560 min, 100% ($CH_3CN : H_2O$ = 80 : 20); anal. calcd for $C_{24}H_{17}BrFN_3O$: C, 62.35; H, 3.71; N, 9.09; found: C, 62.44; H, 3.69; N, 9.18; QTOF MS ES+ (m/z): $[M + 2H]^+$, calcd: 462.0617, found: 464.0687.

1-(2-{[((1-Bromonaphthalen-2-yl)methylene)amino]}phenyl)-3-(*m*-tolyl)urea (6i). Greenish off-white solid; yield: 87%, m. p.: 219–221 °C; ^{1}H-NMR (DMSO-d^6) δ ppm; 2.25 (s, 1H), 6.79 (d, 1H, J = 7.8 Hz), 7.05 (td, 1H, J = 7.8, 1.8 Hz), 7.16 (t, 1H, J = 7.2 Hz), 7.26–7.29 (m, 2H), 7.32 (s, 1H), 7.36 (dd, 1H, J = 7.8, 1.2 Hz), 7.71–7.77 (m, 2H), 8.08 (d, 1H, J = 7.2 Hz), 8.11 (d, 1H, J = 8.4 Hz), 8.29 (dd, 1H, J = 8.4, 1.2 Hz), 8.37 (d, 1H, J = 8.4 Hz), 8.47–8.49 (m, 2H), 9.21 (s, 1H), 9.50 (s, 1H); ^{13}C-NMR ppm; 21.3, 115.6, 117.5, 118.8, 119.0, 122.1, 122.8, 125.5, 127.2, 127.6, 127.9, 128.2, 128.6, 128.7, 128.8, 131.6, 132.2, 134.8, 135.6, 138.0, 138.2, 139.6, 152.2, 158.7; HPLC purity: t_R = 6.824 min, 100% ($CH_3CN : H_2O$ = 80 : 20); anal. calcd for $C_{25}H_{20}BrN_3O$: C, 65.51; H, 4.40; N, 9.17; found: C, 65.55; H, 4.38; N, 9.25; QTOF MS ES+ (m/z): $[M + 2H]^+$, calcd: 458.0867, found: 460.1104.

1-(2-{[((1-Bromonaphthalen-2-yl)methylene)amino]}phenyl)-3-(*o*-tolyl)urea (6j). Greenish off-white solid; yield: 89%, m. p.: 235–237 °C; ^{1}H-NMR (DMSO-d^6) δ ppm; 2.25 (s, 1H), 7.05–7.07 (m, 2H), 7.16 (t, 1H, J = 7.8 Hz), 7.20 (d, 1H, J = 7.8 Hz), 7.26 (ddd, 1H, J = 15.6, 8.4, 1.2 Hz), 7.36 (dd, 1H, J = 8.4, 1.2 Hz), 7.60 (d, 1H, J = 7.8 Hz), 7.71–7.77 (m, 2H), 8.07–8.10 (m, 2H), 8.24 (dd, 1H, J = 7.8, 0.6 Hz), 8.37 (t, 2H, J = 8.4 Hz), 8.63 (s, 1H), 8.76 (s, 1H), 9.21 (s, 1H); ^{13}C-NMR ppm; 18.1, 117.5, 119.3, 122.2, 123.6, 123.9, 125.4, 126.2, 127.2, 127.6, 127.9, 128.2, 128.6, 128.7, 128.8, 130.4, 131.6, 132.2, 134.9, 135.6, 136.9, 138.4, 152.8, 158.6; HPLC purity: t_R = 5.204 min, 96% ($CH_3CN : H_2O$ = 80 : 20); anal. calcd for $C_{25}H_{20}BrN_3O$: C, 65.51; H, 4.40; N, 9.17; found: C, 65.57; H, 4.37; N, 9.21; QTOF MS ES+ (m/z): $[M + H]^+$, calcd: 458.0867, found: 460.0945.

4.3. *In vitro* α-glucosidase inhibitory assay and statistical analysis

The α-glucosidase inhibitory activity was performed by spectrophotometric assay as published earlier.[2] Briefly, in a total of 200 μL per well reaction volume, 135 μL of sodium phosphate buffer (50 mM, pH 7.0), 20 μL of test compounds (0.5 mM in DMSO), and 20 μL of α-glucosidase solution (0.02 U per well) were added into 96-well plate. Blank contained 20 μL 7% DMSO only, while acarbose was used as the positive control. The reaction mixture was incubated at 37 °C for 15 min. 25 μL of substrate solution, *p*-nitro phenyl α-D-glucopyranoside (0.7 mM) was added and change in absorbance was recorded continuously at 400 nm for 30 min through 96-well plate reader (xMark™ Microplate Spectrophotometer, BIO-RAD). All the reactions were performed in triplicates in 96-well microplates. The kinetic study was performed for **5h** through a similar experimental procedure with one modification (*i.e.*, four different concentrations of the substrate were used including 0.1, 0.2, 0.4 and 0.8 mM).

SoftMax Pro package and Excel were utilized to analyze the results of biological activity. Grafit 7 software was used for kinetics analysis. The percent inhibition was calculated using the formula given below:

$$\%\ \text{inhibition} = 100 - \left(\frac{\text{OD}_{\text{test compound}}}{\text{OD}_{\text{control}}}\right) \times 100 \tag{1}$$

EZ-FIT (Perrella Scientific, Inc., USA) was used to calculate IC_{50} of all compounds. To overcome the expected errors, all experiments were performed in triplicate, and variations in the results are reported in Standard Error of Mean values (SEM).

$$\text{SE} = \frac{\sigma}{\sqrt{n}} \tag{2}$$

4.4. Docking analysis

The docking of **5h** was performed in 3A4A by Molecular Operating Environment (MOE v2022.02). The enzyme file was prepared by MOE's protonate-3D that added hydrogen atoms in the enzyme and calculated partial charges with Amber12:EHT force field. The chemical structure of **5h** was drawn on MOE, AM1-BCC charges were added on it and the structure was energy minimized with MMFF94x force field (RMS gradient = 0.5 kcal mol^{-1} Å^{-1}). The Triangle-Matcher docking algorithm of MOE and London dG scoring function was scrutinized initially by re-docking of co-crystallized ligand in the active of 3A4A, which showed RMSD of 0.17 Å and docking score of −7.30 kcal mol^{-1}. Therefore, the same parameters were used in the docking of **5h** and thirty conformations of **5h** were saved. Later, each conformation was analyzed by conformational sampling and the best pose was selected based on the docking score and optimal binding interactions.

Data availability

The data supporting this article have been included as part of the ESI.†

Conflicts of interest

The authors have declared no conflict of interest.

Acknowledgements

The research was funded by Taif University, Saudi Arabia through project number TU-DSPP-2024-19. Z. S. is thankful to the Alexander von Humboldt Foundation for the award of Return Fellowship.

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