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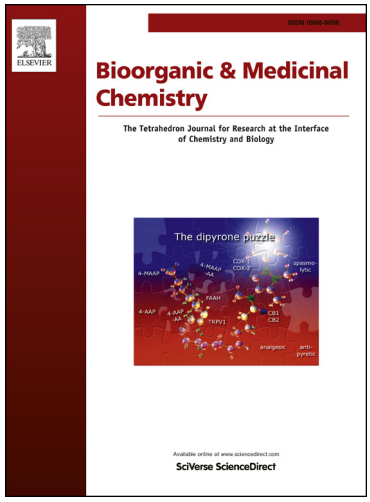

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Synthesis, *In vitro* Biological Activities and *In Silico* Study of Dihydropyrimidines Derivatives

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Abstract

We describe here the synthesis of dihydropyrimidines derivatives **3a-p**, and evaluation of their α-glucosidase enzyme inhibition activities. Compounds **3b** (IC_{50} = 62.4±1.5 μM), **3c** (IC_{50} = 25.3±1.26 μM), **3d** (IC_{50} = 12.4±0.15 μM), **3e** (IC_{50} = 22.9±0.25 μM), **3g** (IC_{50} = 23.8±0.17 μM), **3h** (IC_{50} = 163.3±5.1 μM), **3i** (IC_{50} = 30.6±0.6 μM), **3m** (IC_{50} = 26.4±0.34 μM), and **3o** (IC_{50} = 136.1±6.63 μM) were found to be potent α-glucosidase inhibitors in comparison to the standard drug acarbose (IC_{50} = 840±1.73 μM). The compounds were also evaluated for their *in vitro* cytotoxic activity against PC-3, HeLa, and MCF-3 cancer cell lines, and 3T3 mouse fibroblast cell line. All compounds were found to be non cytotoxic, except compounds **3f** and **3m** (IC_{50} = 17.79±0.66 μM - 20.44±0.30 μM), which showed a weak cytotoxic activity against the HeLa, and 3T3 cell lines. In molecular docking simulation study, all the compounds were docked into the active site of the predicted homology model of α-glucosidase enzyme. From the docking result, it was observed that most of the synthesized compounds showed interaction through carbonyl oxygen atom and polar phenyl ring with active site residues of the enzyme.

Keywords: Spiro heterocycles; pyrimidines; α-glucosidase inhibitors; cytotoxicity; cancer cell lines; molecular docking

1. Introduction

α-Glucosidase (EC 3.2.1.20) enzyme catalyzes the hydrolysis of complex carbohydrates into α-glucose. Type 2 diabetes mellitus (DM), known as non insulin-dependent DM, is a common disorder of glucose and fat metabolism which causes immense sufferings to patients [1]. Diabetes is characterized by high levels of blood sugar, which ultimately can cause serious complications in the kidneys, eyes and cardiovascular system.

Therefore, the treatment of diabetes primarily focuses on reducing fluctuations of sugar levels in blood and controlling subsequent complications [2]. α-Glucosidase is an important mediator of glucose release in the small intestine [3]. Therefore, inhibition of α-glucosidase can significantly decrease the postprandial hyperglycemia as a key strategy in the control of type 2 DM. Recently, α-glucosidase inhibitors have been reported to retard the absorption of glucose and improve postprandial hyperglycemia [4]. Consequently, it was found useful to develop new glucosidase inhibitors [5].

Several types of α-glucosidase inhibitors have been reported, including natural products, such as anthocyanins, curcuminoids, triterpenoids, flavonoids, and xanthones, and synthetic compounds [6–11]. However, the role of these inhibitors in the treatment of diabetes, through delaying the absorption of sugar, has not been validated through clinical studies. Since 1980, the prevalence of diabetes has increased from 153 million to 347 million in 2008 [12]. Based on WHO projection, diabetes will become the seventh leading cause of death globally by 2030. One therapeutic approach for diabetes is to suppress the postprandial hyperglycemia by inhibition of carbohydrate-hydrolyzing enzymes, such as α-glucosidase [13,14]. α-Glucosidase inhibitors reversibly inhibit digestive α-glucosidase, which retards the liberation of glucose from dietary complex carbohydrate and starch, delaying absorption of glucose into the bloodstream and, thus reducing the plasma glucose levels. Inhibition of α-glucosidase has drawn a special interest by the pharmaceutical research community as a remedy for the treatment of carbohydrate mediated diseases, such as cancer, viral infections, diabetics and hepatitis [15–17]. The inhibitors of α-glucosidase are known to possess antiviral, antitumor, antidiabetic, and immunoregulatory activities [18–20]. In addition, α-glucosidase inhibitors, such as *N*-butyl-deoxynojirimycin (NB-DNJ), deoxynojirimycin (DNJ), and castanospermine are potent inhibitors of the HIV replication and HIV mediated

syncytium formation *in vitro* [20]. Currently, glucosidic based α-glucosidase inhibitors, such as nojirimycin [21], miglitol [22], acrabose [23], and voglibose [24] are clinically used to control blood glucose levels in diabetic patients (Fig. 1). Although effective, these inhibitors exhibit adverse side effects such as abdominal distension, flatulence, meteorism and possibly diarrhea [25]. In order to search for effective and safer α-glucosidase inhibitors, efforts are now focused on the development of non-glucosidic based inhibitors [26, 27].

Dihydropyrimidines (DHPMs) are pharmacologically important molecules due to their diverse biological activities, such as antibacterial, antihypertensive, antiviral, and antagonistic properties [28–32]. Pyrimidines also occupy a unique position in the medicinal chemistry, as they are part of nucleic acids [29]. Recently, pyrimidine derivatives have been reported as potent inhibitors of the enzymes responsible for diabetes [33], and particularly, pyrimidine-fused heterocycles, which are identified as specific α-glucosidase inhibitors (Fig. 1) [34].

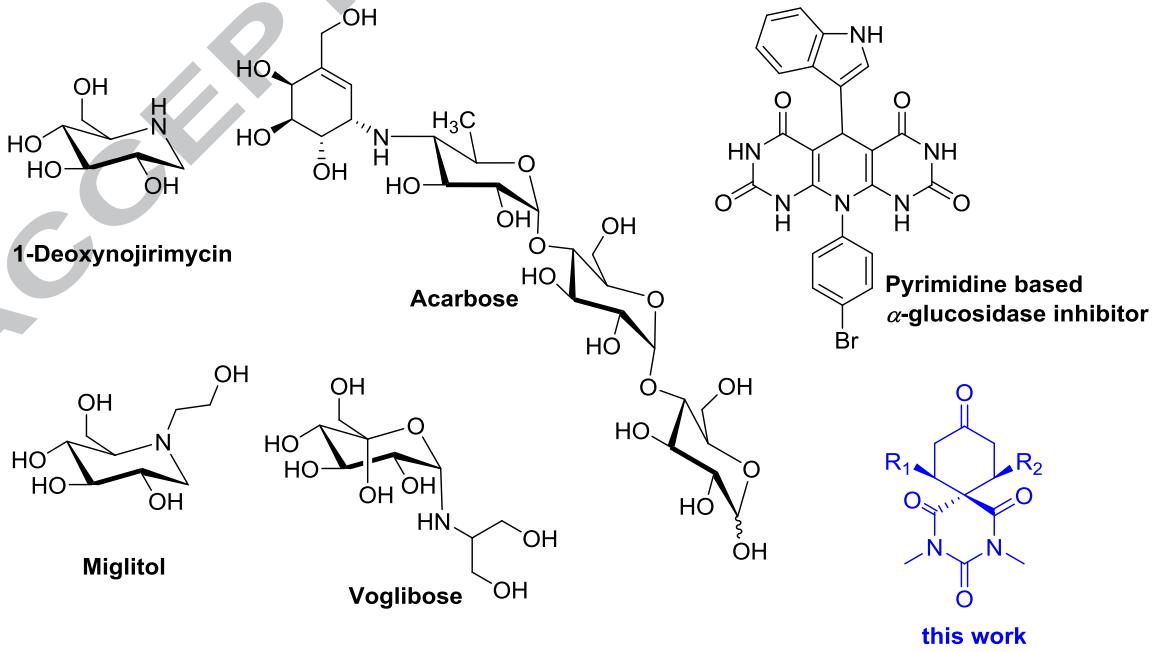


Figure. 1. Known *α*-glucosidase inhibitors

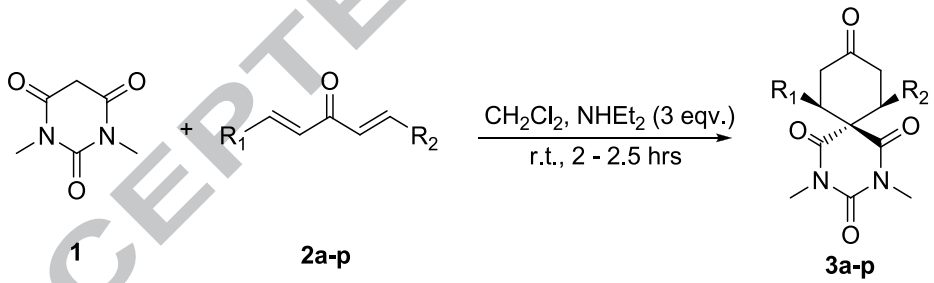
2. Results and discussion

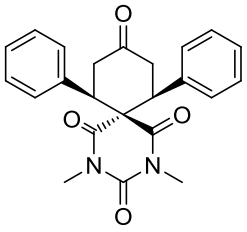
2.1. Chemistry

*2.1.1. Synthesis of the target compounds **3a-p***

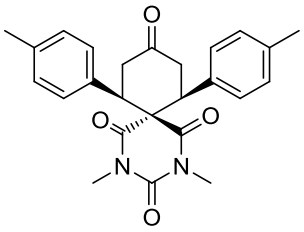
The desired diazaspiro[5.5]undecane-1,3,5,9-tetraone derivatives **3a-p** [35], bearing different substituents, were prepared in excellent yields (up to 99%), as shown in Scheme-1. The preparation of **3a-p** was carried out *via* double Michael addition of *N*,*N*-dimethyl barbituric acid **1** into diaryledine **2a-p**, mediated by $NHEt_2$. Notably, a variety of functional groups, such as hydroxyl, methoxy and chloro were tolerated under our new reaction protocol. The chemical structures of all the synthesized compounds were deduced with the help of physical and spectroscopic methods.

Scheme 1. Double Michael addition reaction as key step in the synthesis of spirocyclic products **3a-p**.

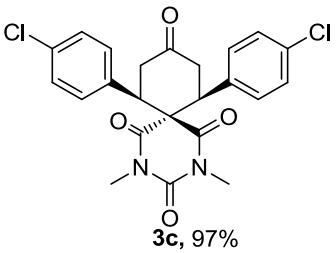




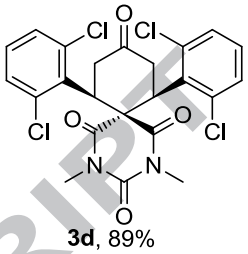
3a, 98%



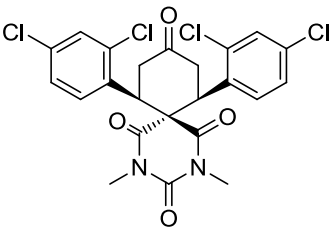
3b, 96%



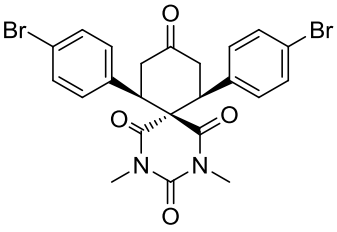
3c, 97%



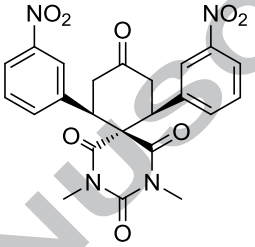
3d, 89%



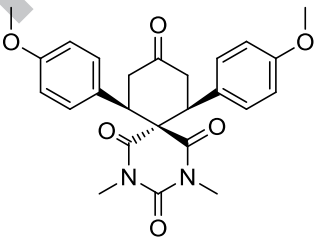
3e, 91%



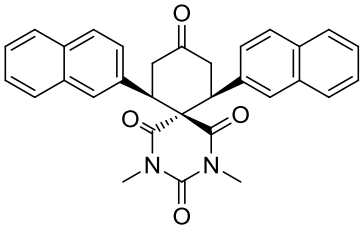
3f, 93%



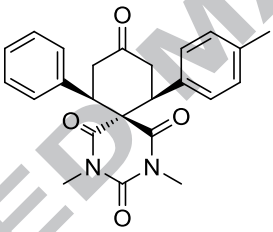
3g, 95%



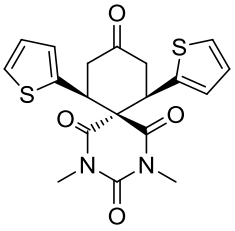
3h, 97%



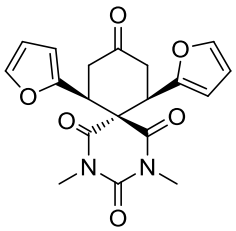
3i, 96%



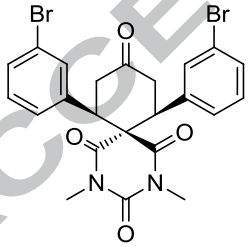
3j,96%



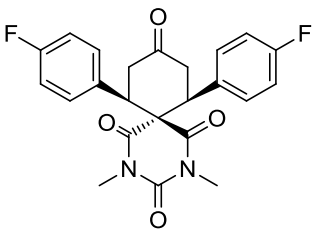
3k, 98%



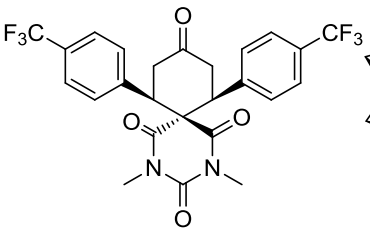
3l, 98%



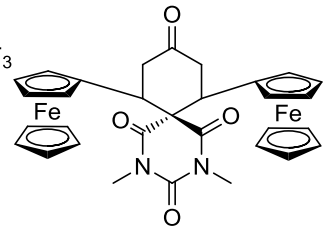
3m, 95%



3n, 98%



3o, 94%

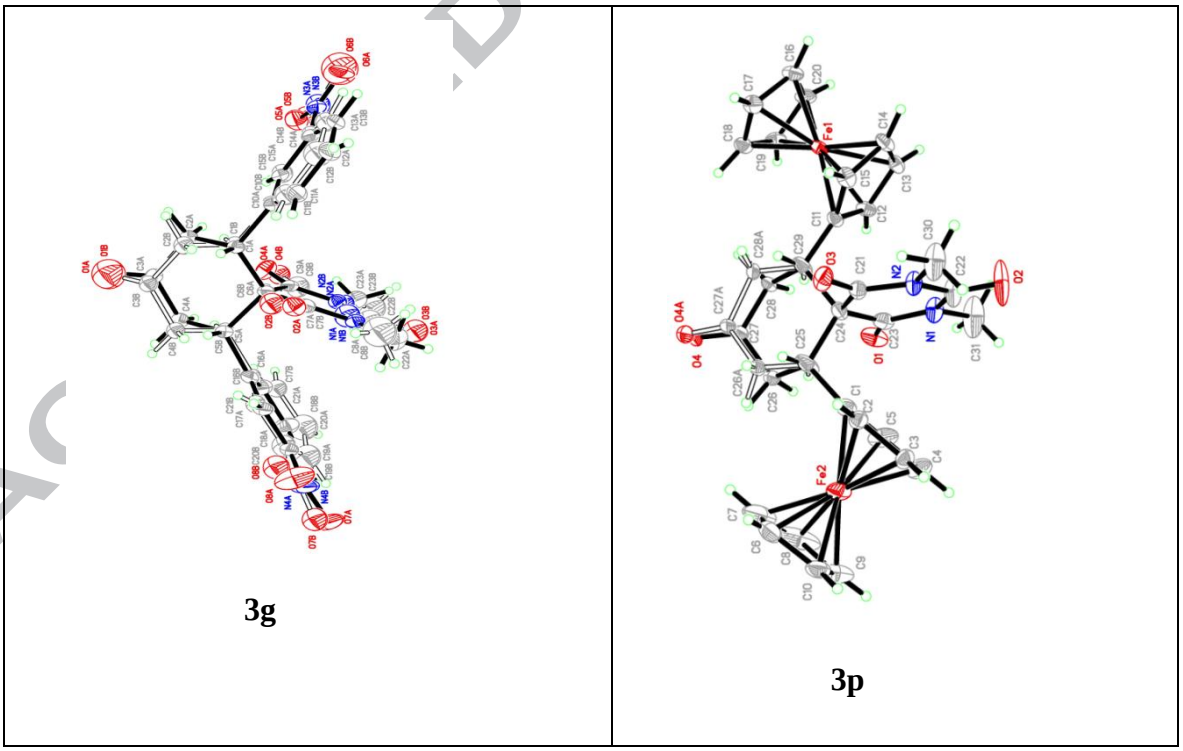


3p, 97%

2.1.2. Single-Crystal X-Ray Crystal Diffraction Studies

The chemical structures of **3a**, **3h**, **3i**, **3n**, and **3o** were unambiguously deduced with the aid of single-crystal X-ray diffraction techniques, and have been reported before [35e,f].

Compounds **3g** and **3p** were obtained as single-crystals by slow diffusion with diethyl ether, in dichloromethane at room temperature for 2 days. Data was collected on a Bruker APEX-II D8 Venture area diffractometer, equipped with graphite monochromatic Mo Kα radiation at 100 (2) K was used. Cell refinement and data reduction was carried out by Bruker SAINT. SHELXS-97 [36, 37] was used to solve the structures. The final refinement was carried out by full-matrix least-squares techniques with anisotropic thermal data for non-hydrogen atoms on F2. All the hydrogen atoms were placed in calculated positions (Table S1-S6, supplementary material). Similarity and simulation restraints were applied for the disordered atoms. The crystal structures of **3g,** and **3p** are shown in Fig. 2. The structures of **3g,** and **3p** were confirmed by single-crystal X-ray diffraction analysis (Bruker AXS GmbH). CCDC- 1042003; and CCDC-1042005 contain the supplementary crystallographic data for these compounds.



3g **3p**

Figure. 2. The ORTEP diagram of the final X-ray model of compounds **3g** and **3p** with displacement ellipsoids drawn at 30% probability level.

2.2. Biological Activity

2.2.1. α-Glucosidase inhibition assay

Compounds **3a-3p** were evaluated for their *in vitro* α-glucosidase inhibitory activity, in comparison to acarbose as a standard inhibitor (IC_{50} = 840±1.73 μM). Compounds **3b** (IC_{50} = 62.4±1.5 μM), **3c** (IC_{50} = 25.3±1.26 μM), **3d** (IC_{50} = 12.4±0.15 μM), **3e** (IC_{50} = 22.9±0.25 μM), **3g** (IC_{50} = 23.8±0.17 μM), **3h** (IC_{50} = 163.3±5.1 μM), **3i** (IC_{50} = 30.6±0.6 μM), **3m** (IC_{50} = 26.4±0.34 μM), and **3o** (IC_{50} = 136.1±6.63 μM) were found to be more potent α-glucosidase inhibitors than the standard α-glucosidase inhibitors drug acarbose (IC_{50} = 840±1.73 μM). Among all tested compounds, *o,o*-dichloro substituted phenyl ring containing compound **3d** (IC_{50} = 12.4±0.15 μM) was found to be the most active member of the series. A decrease in activity due to positional effects of chloro groups on phenyl ring was observed for compound **3e** (IC_{50} = 22.9±0.25 μM), having *o,p* dichloro substituted phenyl ring instead of *o,o*-dichloro substituted phenyl ring. Further decrease in activity was observed for compound **3c** (IC_{50} = 25.3±1.26 μM) containing a mono chloro susbtituant, instead of dichloro group, attached to the benzene ring of the basic 2,4-dimethyl-7,11-diphenyl-2,4-diazaspiro[5.5]-undecane-1,3,5,9-tetraone moiety. The aforementioned observation of the change in α-glucosidase inhibition activity due to substitution pattern of halogen on phenyl ring was further supported by comparing the inhibition activity of *meta* bromo substituted phenyl ring containing **3m** (IC_{50} = 26.4±0.34 μM) with that of *para* bromo substituted phenyl ring containing **3f**, which found to be inactive. However potent activity was observed for **3g** (IC_{50} = 23.8±0.17 μM) which has electron withdrawing nitro group at *meta* position instead of electronegative bromine, as observed in compound **3m**. Several fold decrease in activity was observed for compound **3h** (IC_{50} = 163.3±5.1 μM), as compared to **3b** (IC_{50} = 62.4±1.5 μM). The decrease in activity may be due to the

presence of *para* methoxy (–OCH_3) substituted phenyl ring of **3h**, instead of toluene moiety of **3b**. A decrease in activity was also observed when methyl on the phenyl ring of **3b** (IC_{50} = 62.4±1.5 μM) was replaced with a bulky trifloro methyl group in **3o** (IC_{50} = 136.1±6.63 μM). All other compounds were found to be inactive. The results are summarized in Table 1.

2.2.2. Cytotoxic activity

Compounds **3a-3p** were also tested for their cytotoxic activity against PC-3, Hela, MCF-7 and 3T3 cell lines. Compound **3m** showed a weak cytotoxic effect against HeLa (IC_{50} = 17.79±0.66 μM) and 3T3 (IC_{50} = 19.81±0.18 μM)) cell lines, whereas, compound **3f** showed a weak cytotxicity against 3T3 (IC_{50} = 20.44±0.30 μM) cell line only. Doxorubicine was used as the standard drug to compare the activities against Hela (IC_{50} = 0.51±0.15 μM), and MCF-3 (IC_{50} = 0.924±0.01 μM) cell lines, while cycloheximide (IC_{50} = 0.26±0.12 μM) was used as standard drug against 3T3 cell line. The cytotoxic activities of these compounds may be due to the presence of bromo substituted phenyl ring of the basic 2,4-dimethyl-7,11-diphenyl-2,4- diazaspiro[5.5] undecane- 1,3,5,9-tetraone moiety . All tested compounds were found to be non cytotoxic against PC-3 cell line. The results are summarized in Table 1.

Table 1. Results of various biological assays on compounds **3a-3p**.

Compounds	**Cytotoxicity Assay**				***α*-Glucosidase Inhibition (IC_{50} = μM)**
	PC3 (IC_{50} = μM)	**Hela (IC_{50} = μM)**	**MCF-7 (IC_{50} = μM)**	**3T3 (IC_{50} = μM)**	
3a	>30	>30	NA	>30	NA
3b	>30	>30	>30	>30	62.4±1.5

3c	>30	>30	NA	>30	25.3±1.26
3d	>30	>30	>30	>30	12.4±0.15
3e	>30	>30	NA	>30	22.9±0.25
3f	>30	>30	>30	20.44±0.30	NA
3g	>30	>30	NA	>30	23.8±0.17
3h	>30	>30	NA	>30	163.3±5.1
3i	>30	>30	NA	>30	30.6±0.6
3j	>30	>30	>30	>30	NA
3k	>30	>30	NA	>30	NA
3l	>30	>30	NA	>30	NA
3m	>30	17.79±0.66	>30	19.81±0.18	26.4±0.34
3n	>30	>30	NA	>30	NA
3o	>30	>30	NA	>30	136.1±6.63
3p	>30	>30	NA	>30	NA
STD.	Doxorubicin 1.69±0.29	Doxorubicin 0.51±0.15 μM	Doxorubicin 0.92±0.01	Cycloheximide 0.26±0.12	Acarboses 840±1.73

2.3. Computational studies

2.3.1. Homology Model and Molecular Docking study of α-glucosidase enzyme

The crystallographic structure of *α*-glucosidase enzyme has not been solved yet. However, since a few homology models have been reported [37-40], we attempted to build the 3D structure for *α*-glucosidase by comparative homology modeling technique using the same propriety as described by Burke *et al* [41]. The sequence in fasta format of *α*-glucosidase was retrieved from UniProt (access code P53341). Template selection

search was performed by means of MOE-Search tools against the PDB-database implemented in MOE 2010.11. The 1.30 Å resolving crystallographic structure of *Saccharomyces cerevisiae* isomaltase (SCI) (PDB code: 3AJ7) [42] with 72.4% was selected as the template for modeling. The 3D structure was built by means of MOE homology modeling tools. The predicted 3D model was subjected to energy minimization up to 0.05 gradients. Before docking simulation, ligands and protein were prepared by means of MOE 2010.11 software. 3D structure of all synthesized compounds was built in MOE of Molecular Builder program. Finally, a database was created in which all the ligands were converted into their particular 3D structures and this database was used as input file MOE-docking. Subsequently, the energy of compounds was minimized up to 0.05 gradient using MMFF94x force field. Energy minimization of the database was followed by the preparation of protein for docking purposes. Most macromolecular crystal structures contain little or no hydrogen coordinate data due to limited resolution, and thus protonation was accomplished prior to docking using Protonate 3D tools implemented in MOE. Protonation was followed by energy minimization up to 0.05 gradient, using Amber99 force field. The database was docked into the active site of protein using the Triangular Matching docking method, and 30 conformations of each ligand protein complex were generated with docking score (S). Each complex was analyzed for interactions and their 3D pose was taken.

2.3.2. Molecular docking simulation

From the molecular docking simulation study, it was observed that the top ranked conformation of all the compounds were well accommodated inside the active site of the predicted homology model of *α*-glucosidase enzyme. From the docking result, it was observed that most of the synthesized compounds showed interaction through carbonyl oxygen atom and polar phenyl ring with active site residues of the enzyme.

In case of the most active compound, **3d**, in the series of sixteen synthesized compounds, two hydrogen bonds, one arene-arene and one arene-cation interactions were found with the catalytically active site residues Arg439, Arg212, Asp214, and Asp349, respectively, as shown in Figure 3a. It was observed that both Arg439 and Arg212 established hydrogen bonds (donor) with the carbonyl oxygen atoms of pyrimidine and hexanone ring of compound **3d**. Asp214 and Asp349 were found in making *phi*-bonds with the phenyl ring of the compound. The highest activity (IC_{50} value 2.46 ± 0.008) of compound **3d** in the series might be attributed to the delocalization effect of electrons, which is possible up to some extent, or might be due to the position of polar group (Cl) over the benzene ring, i.e., *ortho* with respect to the hexanone moiety of the compound. As a result of this effect compound **3d** showed good interactions with the active site residues of the enzyme (Fig. 3a). The activity of compound **3d** is more as compared to compounds **3c** and **3e**, although there is little difference in their structures. In case of compound **3c** the chlorine atoms are attached at para position of the phenyl ring, whereas in case of compound **3e** the chlorine moiety is present at *ortho* and *para* positions of the phenyl ring of the compound. The position of chlorine atoms attached to the phenyl ring might play a very important role in the activities of these compounds. For example, when chlorine atoms present at *meta* or *para* positions of the compounds (compound **3c** and **3d**) the activity decreases. From the docking results it was observed that these compounds show less interaction (Fig. 3b) as compared to compound **3d**.

The docking conformation of compound **3m** showed that the carbonyl oxygen of pyrimidine ring established hydrogen bond with the active site residue Arg312, and

phenyl ring of the compound formed arene-cation interaction with Arg212. Furthermore, the compound showed hydrophobic interaction (Fig. 3c) with the active site residues, Phe157 and Phe300, respectively. In case of compound **3f**, poor interactions (Fig. 3d) with active site residues were observed. These poor interactions might be one of reasons for the lack of activity for this compound. Overall, the docking results showed that when halogen moiety is present at *ortho* and *meta* positions (**3d**, **3e** and **3m**) showed good interactions (Fig. 3a) and good biological activity (Table 1) whereas poor interactions (Fig. 3c) and poor biological activity (Table 1) were observed when present at *para* positions of phenyl ring (compounds **3c**, **3f** and **3n**).

Over all the docking results showed that halogens moiety present at *para* position or hydrophobic groups attached to the phenyl ring of the compounds (**3c**, **3f**, **3i** and **3j**) showed less interaction. These poor interactions might be one of the reasons for poor biological activities of these compounds.

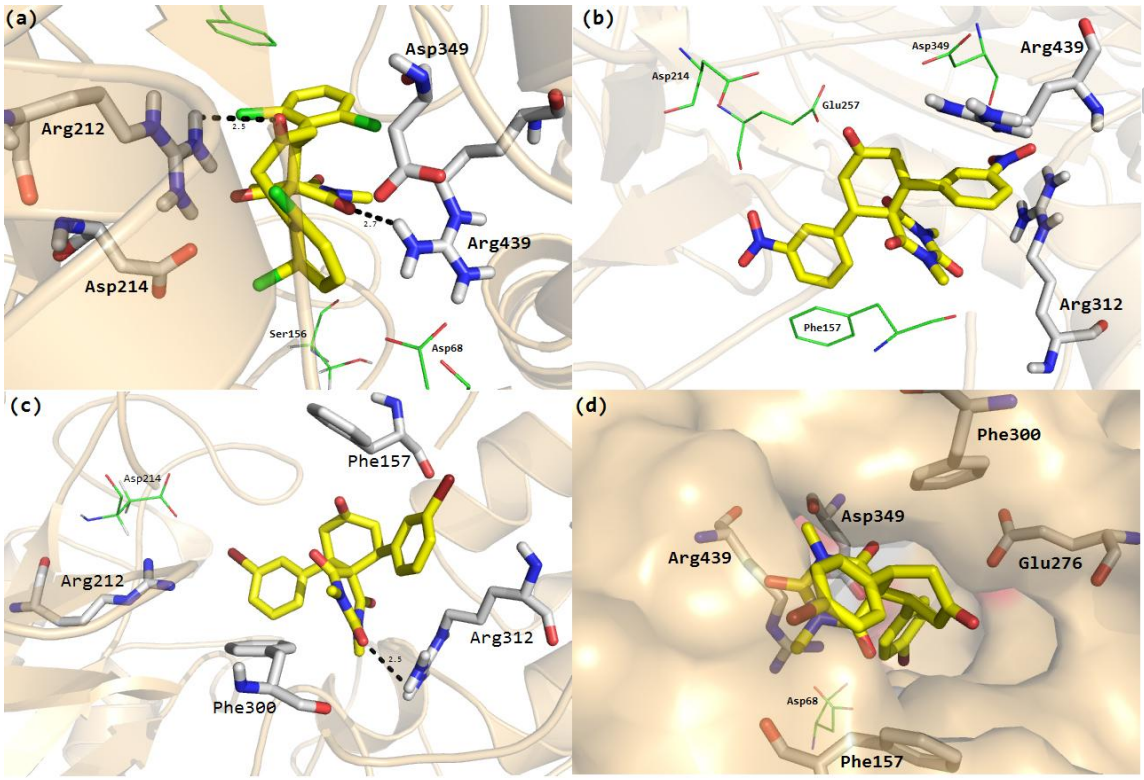


Figure. 3. Molecular docking conformations of compounds **3d** (a), **3g** (b), **3m** (c) and **3f** (d) in the active site of the α-glucosidase enzyme.

3. Conclusion

In conclusion, we have synthesized spiro heterocyclic molecules by double Michael addition reaction in excellent yield. The synthesized compounds were evaluated *in vitro* against their cytotoxities including anti-cancer, and α-glucosiade enzyme inhibition assay. Moreover, the molecular docking study was performed and the docking results showed that halogen moiety present at the *ortho* and *meta* positions showed good interactions and good biological activity as compared to *para* position. In addition hydrophobic groups attached to the phenyl ring of the compounds (**3c**, **3f**, **3i** and **3j**) showed less interaction. The results showed promising activities and can be considered for further studies.

4. Experimental

4.1. General remarks

All glassware was oven–dried before use and the reactions were conducted under inert atmosphere. Progress of the reaction was monitored by TLC (Merck Silica Gel 60 F–254 thin layer plates; Merck, Schwalbach; Hessen, Germany). The chemicals were purchased from Aldrich (Gillingham, Dorset, UK), and Fluka Chemie GmbH (Buchs, Switzerland), etc, and were used without further purification, unless otherwise stated. Petroleum ether (PE), hexane and ethyl acetate were distilled prior to use, especially for column chromatography. All the major solvents were dried by using slandered drying techniques mentioned in the literature. Melting points were measured on a Gallen-kamp melting point apparatus in open glass capillaries, and are uncorrected. IR Spectra were measured as KBr pellets on a Nicolet 6700 FT-IR spectrophotometer (Thermo Fisher Scientific, Madison,WI, USA). The NMR spectra were recorded on a Jeol-400 NMR spectrometer (Peabody, MA, USA). ^{1}H-NMR (400 MHz), and ^{13}C-NMR (100 MHz) were run in deuterated chloroform ($CDCl_3$). Chemical shifts (δ) are referred in *ppm* and *J* -coupling constants are given in *Hz*. Mass spectrometric analysis was conducted by using ESI mode on AGILENT Technologies 6410–triple quad LC/MS instrument (Santa Clara, CA, USA). Elemental analysis was carried out on Elmer 2400 Elemental Analyzer, CHN mode (Waltham, MA, USA).

General procedure of double Michael addition reaction for the synthesis of spiro-compounds 3a-m (GP1).

A solution of *N*,*N*-dimethyl barbituric acid (**1**) (2 mmol) and diarylidene acetone derivatives (**2a-p**) (2 mmol) in 10 mL of dry CH_2Cl_2 were charged into a 50 mL round bottom flask under inert atmsopher. Et_2NH (2.5 mmol) was then added to the reaction mixture and stirred at room temperature for up to 1.5 – 2 hours, until TLC showed

complete consumption of both the reactants. After completion of the reaction, the crude product was directly subjected to column chromatography, using 100 – 200 mesh silica gel and ethyl acetate/*n*-hexane (2:8, v/v) as an eluent to afford the pure products **3a-p**. The solid products were further crystallized from a mixture of $CHCl_3$/*n*-heptane.

4.2.1. 2,4-Dimethyl-7,11-diphenyl-2,4-diazaspiro[5.5]undecane-1,3,5,9-tetraone (**3a**) [35e, 42]

Diarylidene acetone **2a** (468.2 mg, 2 mmol) reacted with compound 1 (312.1 mg, 2 mmol) according to GP1 yielded white solid spiro-product **3a** (765 mg, 1.96 mmol, 98%); m.p. 125–127 ^{o}C; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 2.59 & 2.63 (dd, 2H, J =15.36 Hz, 4.40 Hz, C$\mathbf{H}_{2(e)}$), 2.85 (s, 3H, –NC$\mathbf{H}_3$), 3.01 (s, 3H, –NC$\mathbf{H}_3$), 3.72 (t, 2H, J =14.7 Hz, C$\mathbf{H}_{2(a)}$), 3.99 & 4.03 (dd, 2H, J = 14.7 Hz, 4.40 Hz, C**H**), 7.06–7.08 (m, 4H, Ar-**H**), 7.21–7.26 (m, 6H, Ar-**H**); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 27.98, 28.39, 42.99, 50.55, 60.95, 127.56, 128.69, 128.94, 137.17, 149.70, 169.04, 170.71, 208.29; IR (KBr, cm^{-1}) ν_{max} = 2959, 2925, 1716, 1675, 1484, 1422, 1381, 1125, 755, 706; [Anal. Calcd. for $C_{23}H_{22}N_2O_4$: C, 70.75; H, 5.68; N, 7.17; Found: C, 70.69; H, 5.65; N, 7.01]; LC/MS (ESI, *m/z*): [M+], calculated 390.21, $C_{23}H_{22}N_2O_4$ found 390.16. CCDC-1007513

4.2.2. 2,4-Dimethyl-7,11-di-*p*-tolyl-2,4-diazaspiro[5.5]undecane-1,3,5,9-tetraone (**3b**)

Diarylidene acetone **2b** (524.3 mg, 2 mmol) reacted with compound **1** (312.1 mg, 2 mmol) according to **GP1** yielded white solid spiro-product **3b** (802 mg, 1.92 mmol, 96%); m.p. 122 – 124 ^{o}C; ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 2.25 (s, 3H, C$\mathbf{H}_3$), 2.55 & 2.58 (dd, 2H, J = 14.7 Hz, 4.40 Hz, C$\mathbf{H}_{2(e)}$), 2.87 (s, 3H, –NC$\mathbf{H}_3$), 3.01 (s, 3H, –NC$\mathbf{H}_3$), 3.68 (t, 2H, J = 14.7 Hz, C$\mathbf{H}_{2(a)}$), 3.94 & 3.98 (dd, 2H, J = 13.9 Hz, 4.4 Hz, C**H**), 6.93 (d, 4H, J = 8.0 Hz, Ar-**H**), 7.01 (d, 4H, J = 8.0 Hz, Ar-**H**); ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 21.13 27.94, 28.41, 43.15, 50.19, 61.08, 127.39, 129.58, 134.19, 138.39, 149.65, 169.29, 170.88, 208.63; IR (KBr, cm^{-1}) ν_{max} = 3019, 2970, 1740, 1678, 1441, 1370, 1221, 902,

672, 520; [Anal. Calcd. for $C_{25}H_{26}N_2O_4$: C, 71.75; H, 6.26; N, 6.69; Found: C, 71.58; H, 6.37; N, 6.81]; LC/MS (ESI, m/z): [M^+], calculated 418.3, $C_{25}H_{26}N_2O_4$ found 418.19.

4.2.3.7,11-*Bis*(4-chlorophenyl)-2,4-dimethyl-2,4-diazaspiro[5.5]undecane-1,3,5,9-tetrao ne (**3c**)

Diarylidene acetone **2c** (604.1 mg, 2 mmol) reacted with compound **1** (312.1 mg, 2 mmol) according to **GP1** yielded white solid spiro-product **3c** (889 mg, 1.92 mmol, 97%); m.p. 211 – 213 ^oC; ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 2.55 & 2.58 (dd, 2H, J = 14.7 Hz, 4.40 Hz, C**H**$_{2(e)}$), 2.89 (s, 3H, –NC**H**$_3$), 3.04 (s, 3H, –NC**H**$_3$), 3.64 (t, 2H, J = 14.7 Hz, C**H**$_{2(a)}$), 3.95 & 3.98 (dd, 2H, J = 14.7 Hz, 4.40 Hz, C**H**), 6.99 (d, 4H, J = 8.80 Hz, Ar-**H**), 7.22 (d, 4H, J = 8.80 Hz, Ar-**H**); ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 28.49, 28.68, 42.94, 49.87, 60.58, 128.86, 129.01, 135.25, 135.52, 149.41, 168.75, 170.44, 207.20; IR (KBr, cm^{-1}) ν_{max} = 3015, 2970, 1740, 1678, 1437, 1369, 1218, 904, 672, 521; [Anal. Calcd. for $C_{23}H_{20}Cl_2N_2O_4$: C, 60.14; H, 4.39; N, 6.10; Found: C, 59.97; H, 4.46; N, 6.19]; LC/MS (ESI, *m/z*): [M^+], calculated 458.1, $C_{23}H_{20}Cl_2N_2O_4$ found 458.08.

4.2.4.7,11-*Bis*(2,6-dichlorophenyl)-2,4-dimethyl-2,4-diazaspiro[5.5]undecane-1,3,5,9-te traone (**3d**)

Diarylidene acetone **2d** (734 mg, 2 mmol) reacted with compound **1** (312.1 mg, 2 mmol) according to **GP1** yielded white solid spiro-product **3d** (936 mg, 1.78 mmol, 89%); m.p. 149 – 151 ^oC; ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 2.58 & 2.62 (dd, 2H, J = 16.1 Hz, 4.40 Hz, C**H**$_{2(e)}$), 2.93 (s, 3H, –NC**H**$_3$), 3.26 (s, 3H, –NC**H**$_3$), 3.42 (t, 2H, J = 15.4 Hz, C**H**$_{2(a)}$), 4.67 & 4.71 (dd, 2H, J = 13.9 Hz, 4.40 Hz, C**H**), 7.04 (d, 2H, J = 8.8 Hz, Ar-**H**), 7.15 & 7.17 (dd, 2H, J = 8.8 Hz, 2.20 Hz, Ar-**H**), 7.38 (d, 2H, J = 2.2 Hz, Ar-**H**); ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 28.51, 29.10, 43.55, 45.20, 57.36, 128.28, 130.55, 133.99, 134.98, 149.55, 167.90, 169.91, 205.63; IR (KBr, cm^{-1}) ν_{max} = 3015, 2970, 2030, 1977, 1722, 1776, 1585, 1470, 1446, 1376, 1223, 1106, 1050, 528, 751,

521, 469; [Anal. Calcd. for $C_{23}H_{18}Cl_4N_2O_4$: C, 52.30; H, 3.43; N, 5.30; Found: C, 52.41; H, 3.45; N, 5.37]; LC/MS (ESI, *m/z*): [M^+], calculated 526.10.1, $C_{23}H_{18}Cl_4N_2O_4$ found 526.00.

4.2.5.7,11-*Bis*(2,4-dichlorophenyl)-2,4-dimethyl-2,4-diazaspiro[5.5]undecane-1,3,5,9-tetraone (**3e**)

Diarylidene acetone **2e** (734 mg, 2 mmol) reacted with compound **1** (312.1 mg, 2 mmol) according to **GP1** yielded white solid spiro-product **3e** (957 mg, 1.82 mmol, 91%); m.p. 185 – 187 ^oC; ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 2.57 & 2.61 (dd, 2H, *J* = 16.1 Hz, 4.40 Hz, C**H**$_{2(e)}$), 2.96 (s, 3H, –NC**H**$_3$), 3.26 (s, 3H, –NC**H**$_3$), 3.42 (t, 2H, *J* = 15.4 Hz, C**H**$_{2(a)}$), 4.67 & 4.71 (dd, 2H, *J* = 15.4 Hz, 4.4 Hz, C**H**), 7.11 – 7.17 (m, 2H, Ar-**H**), 7.21 (s, 2H, Ar-**H**), 7.11 – 7.38 (m, 2H, Ar-**H**); ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 28.70, 29.14, 43.57, 45.24, 57.36, 127.75, 129.86, 130.50, 131.91, 133.02, 134.00, 149.55, 169.43, 169.91, 205.60; IR (KBr, cm^{-1}) ν_{max} = 2920, 1718, 1673, 1444, 1375, 1108, 1045, 828, 747, 465; [Anal. Calcd. for $C_{23}H_{18}Cl_4N_2O_4$: C, 52.30; H, 3.43; N, 5.30; Found: C, 52.41; H, 3.45; N, 5.37]; LC/MS (ESI, *m/z*): [M^+], calculated 526.10.1, $C_{23}H_{18}Cl_4N_2O_4$ found 526.00.

4.2.6.7,11-*Bis*(4-bromophenyl)-2,4-dimethyl-2,4-diazaspiro[5.5]undecane-1,3,5,9-tetraone (**3f**)

Diarylidene acetone **2f** (780 mg, 2 mmol) reacted with compound **1** (312.1 mg, 2 mmol) according to **GP1** yielded white solid spiro-product **3f** (1.0 g, 1.86 mmol, 93%); m.p. 205 – 207 ^oC; ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 2.55 & 2.58 (dd, 2H, *J* = 14.7 Hz, 4.4 Hz, C**H**$_{2(e)}$), 2.90 (s, 3H, –NC**H**$_3$), 3.02 (s, 3H, –NC**H**$_3$), 3.59 (t, 2H, *J* = 14.7 Hz, C**H**$_{2(a)}$), 3.94 & 3.97 (dd, 2H, *J* = 14.7 Hz, 4.40 Hz, C**H**), 6.92 (d, 4H, *J* = 8.80 Hz, Ar-**H**), 7.37(d, 4H, *J* = 8.8 Hz, Ar-**H**); ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 28.16, 28.59, 42.82, 49.95, 60.40, 122.90, 129.25, 129.86, 132.22, 132.42, 149.90, 169.53, 170.48, 207.15;

IR (KBr, cm^{-1}) ν_{max} = 3020, 1712, 1675, 1640, 1415, 1376, 1284, 1177, 1067, 979, 806, 547, 443; [Anal. Calcd. for $C_{23}H_{20}Br_2N_2O_4$: C, 50.39; H, 3.68; N, 5.11; Found: C, 50.51; H, 3.73; N, 5.17]; LC/MS (ESI, *m/z*): [M^+], calculated 546.11, $C_{23}H_{20}Br_2N_2O_4$ found 545.98.

4.2.7.2,4-Dimethyl-7,11-*bis*(3-nitrophenyl)-2,4-diazaspiro[5.5]undecane-1,3,5,9-tetraone (**3g**)

Diarylidene acetone **2g** (648 mg, 2 mmol) reacted with compound **1** (312.1 mg, 2 mmol) according to **GP1** yielded white solid spiro-product **3g** (912 mg, 1.9 mmol, 95%); m.p. 232 - 234 ^oC; ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 2.66 & 2.69 (dd, 2H, *J* = 15.4 Hz, 4.4 Hz, C$\mathbf{H}_{2(e)}$), 2.87 (s, 3H, –NC$\mathbf{H}_3$), 3.06 (s, 3H, –NC$\mathbf{H}_3$), 3.75 (t, 2H, *J* = 14.7 Hz, C$\mathbf{H}_{2(a)}$), 4.14 & 4.18 (dd, 2H, *J* = 13.9 Hz, 4.4 Hz, C**H**), 7.42 – 7.51 (m, 4H, Ar-**H**), 7.91(s, 2H, Ar-**H**), 8.13 (d, 2H, *J* = 8.0 Hz, Ar-**H**); ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 28.36, 28.71, 42.63, 49.98, 60.09, 122.40, 124.01, 130.28, 133.95, 138.95, 148.64, 168.26, 169.84, 205.46; IR (KBr, cm^{-1}) ν_{max} = 2953, 1715, 1673, 1527, 1420, 1381, 901, 808, 731, 681, 451; [Anal. Calcd. for $C_{23}H_{20}N_4O_8$: C, 57.50; H, 4.20; N, 11.66: Found: C, 57.56; H, 4.32; N, 11.43]; LC/MS (ESI, m/z): [M^+], calculated 480.03, $C_{23}H_{20}N_4O_8$ found 480.13; CCDC-1042003.

4.2.8.7,11-*Bis*(4-methoxyphenyl)-2,4-dimethyl-2,4-diazaspiro[5.5]undecane-1,3,5,9-tetraone (**3h**)

Diarylidene acetone **2h** (588 mg, 2 mmol) reacted with compound **1** (312.1 mg, 2 mmol) according to **GP1** yielded white solid spiro-product **3h** (873 mg, 1.94 mmol, 97%); m.p. 131 - 133 ^oC; ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 2.53 & 2.56 (dd, 2H, *J* = 14.7Hz, 4.4 Hz, C$\mathbf{H}_{2(e)}$), 2.88 (s, 3H, –NC$\mathbf{H}_3$), 3.01 (s, 3H, –NC$\mathbf{H}_3$), 3.68 (t, 2H, *J* = 14.7 Hz, C$\mathbf{H}_{2(a)}$), 3.72 (s, 3H, OC$\mathbf{H}_3$), 3.91 & 3.94 (dd, 2H, *J* = 13.9 Hz, 4.4 Hz, C**H**), 6.73 (d, 4H, *J* = 8.8 Hz, Ar-**H**), 6.96 (d, 4H, *J* = 8.8 Hz, Ar-**H**); ^{13}C-NMR (100 MHz,

$CDCl_3$) δ: 28.01, 28.45, 43.29, 49.73, 55.26, 61.39, 114.17, 128.64, 129.21, 149.98, 159.51, 169.25, 171.79, 208.54; IR (KBr, cm^{-1}) ν_{max} = 2957, 2838, 1713, 1670, 1609, 1510, 1449, 1420, 1248, 1031, 831, 729, 530, 452; [Anal. Calcd. for $C_{25}H_{26}N_2O_6$: C, 66.65; H, 5.82; N, 6.22; Found: C, 66.81; H, 5.71; N, 6.34]; LC/MS (ESI, *m/z*): [M^+], calculated 450.15, $C_{25}H_{26}N_2O_6$ found 450.18; CCDC-1004326.

4.2.9.2,4-Dimethyl-7,11-di(naphthalen-1-yl)-2,4-diazaspiro[5.5]undecane-1,3,5,9-tetrao ne (**3i**)

Diarylidene acetone **2i** (668 mg, 2 mmol) reacted with compound **1** (312.1 mg, 2 mmol) according to **GP1** yielded white solid spiro-product **3i** (941 mg, 1.92 mmol, 96%); m.p. 218 - 220 ^oC; ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 1.98 (s, 3H, –NC**H**$_3$), 2.75 & 2.79 (dd, 2H, *J* = 15.4 Hz, 4.4 Hz, C**H**$_{2(e)}$), 3.22 (s, 3H, –NC**H**$_3$), 3.89 (t, 2H, *J* = 15.0 Hz, C**H**$_{2(a)}$), 5.11 & 5.14 (dd, 2H, *J* = 13.9 Hz, 4.4 Hz, C**H**), 7.33 – 7.39 (m, 4H, Ar-**H**), 7.47 (t, 2H, *J* = 8.0 Hz, Ar-**H**), 7.57 (t, 2H, *J* = 8.0 Hz, Ar-**H**), 7.73 & 7.74 (dd, 2H, *J* = 7.6 Hz, 2.40 Hz, Ar-**H**), 7.80 (d, 2H, *J* = 8.1 Hz, Ar-**H**), 8.22 (d, 2H, *J* = 8.8 Hz, Ar-**H**); ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 28.03, 28.32, 44.37, 44.86, 59.34, 123.16, 124.20, 124.95, 126.19, 126.74, 128.94, 129.23, 130.91, 134.05, 134.22, 149.61, 169.47, 170.38, 208.33; IR (KBr, cm^{-1}) ν_{max} = 3049, 2919, 1711, 1666, 1421, 1374, 1266, 1241, 1018, 773, 467; [Anal. Calcd. for $C_{31}H_{26}N_2O_4$: C, 75.90; H, 5.34; N, 5.71; Found: C, 76.13; H, 5.41; N, 5.83]; LC/MS (ESI, *m/z*): [M^+], calculated 490.23, $C_{31}H_{26}N_2O_4$ found 490.19; CCDC-1004327.

4.2.10.2,4-Dimethyl-7-phenyl-11-(p-tolyl)-2,4-diazaspiro[5.5]undecane-1,3,5,9-tetraone (**3j**)

Diarylidene acetone **2j** (496 mg, 2 mmol) reacted with compound **1** (312 mg, 2 mmol) according to **GP1** yielded white solid spiro-product **3j** (776 mg, 1.92 mmol, 96%); m p. 110 - 112 ^oC; ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 2.25 (s, 3H, C**H**$_3$), 2.55 & 2.59 (dd, 2H, *J*

= 14.7 Hz, 4.4Hz, C**H**$_{2(e)}$), 3.87 (s, 3H, –NC**H**$_3$), 3.01 (s, 3H, –NC**H**$_3$), 3.72 (t, 2H, *J* = 14.7 Hz, C**H**$_{2(a)}$), 3.94 & 3.98 (dd, 2H, *J* = 14.7 Hz, 4.40 Hz, C**H**), 6.92 (d, 4H, *J* = 8.1 Hz, Ar-**H**), 7.01 (d, 4H, *J* = 8.1 Hz, Ar-**H**), 7.21 – 7.25 (m, 1H, Ar-**H**); ^{13}C-NMR (100 MHz, $CDCl_3$) *δ*: 21.12, 27.97, 28.40, 44.17, 43.15, 50.19, 61.8, 127.39, 128.92, 129.57, 134.18, 138.39, 149.98, 168.18, 170.89, 208.66; IR (KBr, cm^{-1}) ν_{max} = 29.57, 2924, 1717, 1672, 1446, 1419, 1377, 1285, 814, 730, 560, 509; [Anal. Calcd. for $C_{24}H_{24}N_2O_4$: C, 71.27; H, 5.98; N, 6.93; Found: C, 71.36; H, 6.07; N, 7.01]; LC/MS (ESI, *m/z*): [M$^+$], calculated 404.11, $C_{24}H_{24}N_2O_4$ found 404.17.

4.2.11.2,4-Dimethyl-7,11-di(thiophen-2-yl)-2,4-diazaspiro[5.5]undecane-1,3,5,9-tetraon e (**3k**)

Diarylidene acetone **2k** (520 mg, 2 mmol) reacted with compound **1** (312.1 mg, 2 mmol) according to **GP1** yielded white solid spiro-product **3k** (788 mg, 1.96 mmol, 98%); m.p. 136 – 138 ^{o}C; ^{1}H-NMR (400 MHz, $CDCl_3$) *δ*: 2.80 & 2.83 (dd, 2H, *J* = 15.4 Hz, 4.4 Hz, C**H**$_{2(e)}$), 3.11 (s, 3H, –NC**H**$_3$), 3.14 (s, 3H, –NC**H**$_3$), 3.66 (t, 2H, *J* = 14.6 Hz, C**H**$_{2(a)}$), 4.34 & 4.37 (dd, 2H, *J* = 13.9 Hz, 4.4 Hz, C**H**), 6.84 (d, 2H, *J* = 3.7 Hz, Ar-**H**), 6.93 – 6.95 (m, 2H, Ar-**H**), 7.23 (d, 2H, *J* = 5.1 Hz, Ar-**H**); ^{13}C-NMR (100 MHz, $CDCl_3$) *δ*: 28.19, 28.61, 44.16, 45.44, 61.50, 125.48, 125.87, 126.96, 139.84, 149.83, 168.60, 170.90, 205.77; IR (KBr, cm^{-1}) ν_{max} = 2959, 2921, 1715, 1668, 1420, 1373, 1260, 1036, 799, 702, 501, 444; [Anal. Calcd. for $C_{19}H_{18}N_2O_4S_2$: C, 56.70; H, 4.51; N, 6.96; Found: C, 56.76; H, 4.43; N, 7.03]; LC/MS (ESI, *m/z*): [M$^+$], calculated 402.11, $C_{19}H_{18}N_2O_4S_2$ found 402.07

4.2.12. 7,11-Di(furan-2-yl)-2,4-dimethyl-2,4-diazaspiro[5.5]undecane-1,3,5,9-tetraone (**3l**)

Diarylidene acetone **2l** (456 mg, 2 mmol) reacted with compound **1** (312.1 mg, 2 mmol) according to **GP1** yielded white solid spiro-product **3l** (725 mg, 1.96 mmol, 98%); m.p.

114 - 116 ^{o}C; ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 2.67 & 2.71 (dd, 2H, J = 15.4 Hz, 4.4 Hz, C**H**$_{2(e)}$), 3.08 (s, 3H, –NC**H**$_3$), 3.10 (s, 3H, –NC**H**$_3$), 3.49 (t, 2H, J = 14.7 Hz, C**H**$_{2(a)}$), 4.08 & 4.11 (dd, 2H, J = 14.0 Hz, 4.4 Hz, C**H**), 6.05 (d, 2H, J = 3.6 Hz, Ar-**H**), 6.25 – 6.26 (m, 2H, Ar-**H**), 7.23 (d, 2H, J = 1.40 Hz, Ar-**H**); ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 28.18, 28.80, 40.90, 43.32, 57.09, 107.39, 110.56, 142.56, 151.20, 151.32, 168.01, 170.75, 206.10; IR (KBr, cm^{-1}) ν_{max} = 3115, 1959, 1721, 1670, 1446, 1420, 1377, 1011, 922, 738, 465; [Anal. Calcd. for $C_{19}H_{18}N_2O_6$: C, 61.62; H, 4.90; N, 7.56; Found: C, 61.49; H, 5.11; N, 7.43]; LC/MS (ESI, *m/z*): [M$^+$], calculated 370.18, $C_{19}H_{18}N_2O_6$ found 370.12.

4.2.13.7,11-*Bis*(3-bromophenyl)-2,4-dimethyl-2,4-diazaspiro[5.5]undecane-1,3,5,9-tetra one (**3m**)

Diarylidene acetone **2m** (780 mg, 2 mmol) reacted with compound **1** (312.1 mg, 2 mmol) according to **GP1** yielded white solid spiro-product **3m** (1037 mg, 1.90 mmol, 95%); m.p. 118 - 120 ^{o}C; ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 2.57 & 2.61 (dd, 2H, J = 14.7 Hz, 4.4 Hz, C**H**$_{2(e)}$), 2.91 (s, 3H, –NC**H**$_3$), 3.06 (s, 3H, –NC**H**$_3$), 3.64 (t, 2H, J = 14.7 Hz, C**H**$_{2(a)}$), 3.92 & 3.96 (dd, 2H, J = 14.7 Hz, 4.4 Hz, C**H**), 6.98 (d, 2H, J = 8.1Hz, Ar-**H**), 7.11 (t, 2H, J = 8.1Hz, Ar-**H**), 7.22, (s, 2H, Ar-**H**), 7.37 (d, 2H, J = 7.4 Hz, Ar-**H**); ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 28.09, 28.57, 42.67, 50.01, 60.52, 123.11, 126.25, 130.53, 130.69, 131.97, 139.22, 149.44, 168.66, 170.29, 206.89; IR (KBr, cm^{-1}) ν_{max} = 2959, 2921, 1710, 1667, 1423, 1349, 1285, 1256, 1070, 787, 749, 695, 443; [Anal. Calcd. for $C_{23}H_{20}Br_2N_2O_4$: C, 50.39; H, 3.68; N, 5.11; Found: C, 50.51; H, 3.73; N, 5.17]; LC/MS (ESI, *m/z*): [M$^+$], calculated 546.11, $C_{23}H_{20}Br_2N_2O_4$ found 545.98.

4.2.14.7,11-*Bis*(4-fluorophenyl)-2,4-dimethyl-2,4-diazaspiro[5.5]undecane-1,3,5,9-tetra one (**3n**)

Diarylideneacetone **2n** (540 mg, 2 mmol) was reacted with compound **1** (312.1 mg, 2 mmol) according to GP1 to yield a white solid of spiro-product **3n** (835 mg, 1.96 mmol, 98%); m.p. 175–177 °C; UV-Vis: 204 nm, 218 nm (sh) and 233 nm (sh) (in Dichloromethane); ^{1}H-NMR ($CDCl_3$) δ: 2.55 and 2.59 (dd, 2H, J = 15.4 Hz, J = 4.4 Hz, C**H**$_{2(e)}$), 2.88 (s, 3H, -NC**H**$_3$), 3.02 (s, 3H, –NC**H**$_3$), 3.64 (t, 2H, J = 14.7 Hz, C**H**$_{2(a)}$), 3.95 & 3.99 (dd, 2H, J = 14.0 Hz, J = 4.4Hz, C**H**), 6.89–6.94 (m, 4H, Ar-**H**), 7.01–7.04 (m, 4H, Ar-**H**): ^{13}C-NMR ($CDCl_3$) δ: 27.92, 28.34, 42.93, 49.56, 60.82, 115.73 and 115.95 (d, J^2 = 21.4 Hz), 115.10 and 129.18 (d, J^3 = 7.65 Hz), 132.78 and 132.81 (d, J^4 = 3.06 Hz), 149.33, 161.20 & 163.67 (d, J^1 = 147 Hz), [ArC_1, C_2, C_3& C_4 are split into doublets due to ^{19}F], 168.74, 170.48, 207.27; IR (KBr, cm^{-1}) ν_{max} = 2920, 1718, 1618, 1507, 1418, 1378, 1223, 1159, 828, 753, 510, 466; [Anal. Calcd. for $C_{23}H_{20}F_2N_2O_4$: C, 50.39; H, 3.68; N, 5.11; Found: C, 50.51; H, 3.73; N, 5.17]; LC/MS (ESI, *m/z*): [M$^+$], calculated 426.21, $C_{23}H_{20}F_2N_2O_4$ found 426.14.

4.2.15.(7R,11S)-2,4-Dimethyl-7,11-bis(4-(trifluoromethyl)phenyl)-2,4-diazaspiro[5.5]u

ndecane-1,3,5,9-tetraone (**3o**)

Diarylideneacetone **2o** (684 mg, 2 mmol) was reacted with compound **1** (312.1 mg, 2 mmol) according to GP1 to yield the white solid spiro-product **3o** (936 mg, 1.88 mmol, 94%); m p. 180–182 °C; UV-Vis: (in ethanol): 204nm and 232nm; ^{1}H-NMR ($CDCl_3$) δ: 2.70 and 2.74 (dd, 2H, J = 15.4 Hz, J = 4.4 Hz, C**H**$_{2(e)}$), 3.02 (s, 3H, -NC**H**$_3$), 3.04 (s, 3H, –NC**H**$_3$), 3.56 (t, 2H, J = 14.7 Hz, C**H**$_{2(e)}$), 4.25 and 4.28 (dd, 2H, J = 14.0 Hz, Jae = 4.4 Hz, C**H**), 6.75–7.14 (m, 4H, Ar-**H**); ^{13}C-NMR ($CDCl_3$) δ: 28.21, 28.63, 44.17, 45.44, 61.51, 125.48, 125.87, 126.96, 139.84, 149.93, 168.60, 170.91, 205.78; IR (KBr, cm^{-1}) ν_{max}

= 2956, 2919, 1715, 1669, 1419, 1373, 1280, 702, 500, 444; [Anal. Calcd. for $C_{23}H_{16}F_6N_2O_4$: C, 55.43; H, 3.24; N, 5.62; Found: C, 55.29; H, 3.17; N, 5.75]; LC/MS (ESI, *m/z*): [M^+], calculated 498.19, $C_{23}H_{16}F_6N_2O_4$ found 498.10.

Biological activities:

In vitro anti-cancer and a-glucosidase inhibition assays were performed to assess the biological activity of synthesized compounds. Results are presented here as means ± standard error from triplicate (n=3) observation. IC_{50} values were calculated by using EZ-FIT, Enzyme kinetics software by Perrella Scientific.

***In Vitro α*-Glucosidase Inhibition Assay:**

α-Glucosidase inhibition assay was performed spectrophotometrically. α-Glucosidase from *Saccharomyces cerevisiae* (G0660-750UN, Sigma Aldrich), was dissolved in phosphate buffer (pH 6.8., 50 mM). Test compounds were dissolved in 70% DMSO. In 96-well plates, 20µL of test sample, 20 µL of enzyme and 135 µL of buffer were added and incubated for 15 minutes at 37°C. After incubation, 25 µL of p-nitrophenyl- α -D-glucopyranoside (0.7 mM, Sigma Aldrich) was added and change in absorbance was monitored for 30 minutes at 400 nm. Test compound was replaced by DMSO (7.5% final) as control. Acarbose (Acarbose, Sigma Aldrich) was used as a standard inhibitor [43].

Cytotoxic activity:

Cytotoxic activity of compounds was evaluated in 96-well flat-bottomed microplates by using the standard MTT (3-[4, 5-dimethylthiazole-2-yl]-2, 5-diphenyl-tetrazolium bromide, MP) colorimetric assay [39]. For this purpose, PC3 cells (Prostrate Cancer) were cultured in Dulbecco's Modified Eagle Medium, supplemented with 10% of fetal bovine serum (FBS, PAA), 100 IU/mL of penicillin and 100 µg/mL of streptomycin in 75 cm^2 flasks, and kept in 5% CO_2 incubator at 37^o C. Exponentially growing cells were

harvested, counted with haemocytometer and diluted with a particular medium with 5% FBS. Cell culture with the concentration of $1x10^5$ cells/mL was prepared and introduced (100 µL/well) into 96-well plates. After overnight incubation, medium was removed and 200 µL of fresh medium was added with different concentrations of compounds (1-30 µM). Stock solution, 20 mM of compounds were prepared in 100% DMSO and final concentration of DMSO at 30 µM is 0.15%. After 48 hrs, 200 µL MTT (0.5 mg/mL) was added to each well and incubated further for 4 hrs. Subsequently, 100 µL of DMSO was added to each well. The extent of MTT reduction to formazan within cells was calculated by measuring the absorbance at 570 nm, using a micro plate reader (Spectra Max plus, Molecular Devices, CA, USA). The cytotoxicity was recorded as concentration causing 50% growth inhibition (IC_{50}) for PC3 cells. The percent inhibition was calculated by using the following formula:

% inhibition = 100-((mean of O.D of test compound – mean of O.D of negative control)/ (mean of O.D of positive control – mean of O.D of negative control)*100).

The results (% inhibition) were processed by using Soft- Max Pro software (Molecular Device, USA).

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Graphical abstract