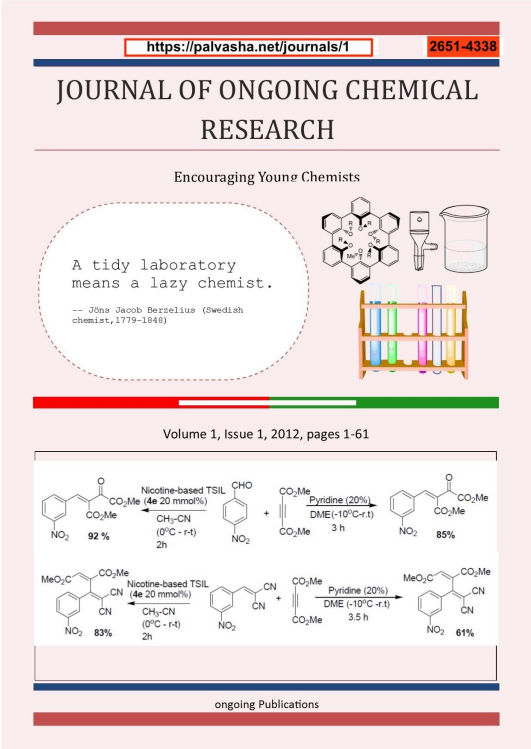
SYNTHESIS, ALPHA-GLUCOSIDASE, THYMIDINE PHOSPHORYLASE, LIPOXYGENASE INHIBITORY POTENTIALS OF THIOBARBITURIC ACID ANALOGUES AND THEIR MOLECULAR DOCKING STUDIES



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Research Article

Synthesis, Alpha-Glucosidase, Thymidine Phosphorylase, Lipoxygenase Inhibitory Potentials of Thiobarbituric Acid Analogues and Their Molecular Docking Studies

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Abstract

We have reacted 2-dioxo-dihydropyrimidine-2,4(1*H*, 5*H*)-dione (thiobarbituric acid) with a different aromatic aldehyde in the presence of NaOH to give thiobarbituric acids analogs (**1-29**) which were characterized by EI-MS and ^{1}H-NMR and subjected for *α*-glucosidase, thymidine phosphorylase and lipoxygenase inhibition studies. Analog **19** showed good *α*-glucosidase inhibitory potential with IC_{50} values of 37.61 ± 0.08 µM, while analogs **3, 6, 7, 9, 13, 15, 16, 18, 21-27** showed good to moderate *α*-glucosidase inhibitory potential when compared with standard acarbose having IC_{50} value 38.25±0.12 µM. Three analogs such as analog **20, 21** and **28** showed outstanding thymidine phosphorylase inhibitory potential with IC_{50} values 18.8, 40 and 6.9 µM respectively when compared with standard 7-deazaxanthine having IC_{50} value 41.0 ± 1.63 µM. In the case of lipoxygenase all the analogs were found inactive. Molecular docking studies were carried out to understand the binding interaction of the most active compounds.

Keywords: Synthesis, Molecular Docking, α-glucosidase, Lipoxygenase, Thiobarbituric Acids, Thymidine Phosphorylase

INTRODUCTION

α-Glucosidase (EC 3.2.1.20) enzyme is involved in the digestion of carbohydrates significantly decrease the postprandial increase of blood glucose after a mixed carbohydrate diet and therefore can be an important strategy in the management of postprandial blood glucose level in type-2 diabetic patients and borderline patients [1]. The intestinal a-glucosidase hydrolyzes complex carbohydrates to glucose and monosaccharide in the small intestine. Inhibition of this enzyme systems helps to reduce the rate of digestion of carbohydrates [2]. In diabetics the short term effect of enzyme inhibitor drug therapies is to decrease high blood glucose levels [3]. *α*-Glucosidase has drawn a special interest of the pharmaceutical research community because in earlier studies it shows the inhibition of its catalytic activity resulted in the retardation of glucose absorption and decrease in postprandial blood glucose level [4]. *α*-Glucosidase inhibitors are expected to cause no hypoglycemic events or other life-threatening events, even at overdoses, and cause no weight gain [5]. Glucosidase inhibitors are potential bio-tools and highly useful for medical therapies, such as diabetes, obesity, hyperlipoproteinemia, cancer, and HIV [6]. *α*-Glucosidase inhibitors have also been observed to block viral infections and proliferation in HIV-infections [7].

Thymidine phosphorylase (EC 2.4.2.4) is an angiogenic factor that exerts its effect by stimulating endothelial cell migration [8, 9]. Thymidine phosphorylase (TP) enzyme similar to platelet-derived endothelial cell growth factor catalyzes the reversible phosphorolysis of thymidine to thymine and 2-deoxy-a-Dribofuranosylphosphate [10-12]. The inhibition of TP may result in the reduction of tumor growth and metastasis [13, 14].

Lipoxygenase (LOX, EC 1.13.11) is a non-heme iron-containing dioxygenase, which catalyzes the oxidation of polyunsaturated fatty acids (PUFAs) with one or several (1*Z*, 4*Z*)-pentadiene moieties to produce specific *E, Z*-conjugated-hydroperoxy PUFAs and their corresponding reduced hydroxy fatty acids (HFAs) [15, 16]. These fatty acids are subsequently metabolized into important bioactive signaling compounds, such as leukotrienes and lipoxins in animals [17] and jasmonic acids in plants [18].

Thiobarbiturates are valuable compounds for different pharmacological applications, such as general anesthesia, sedation, and anticonvulsant effects [19]. Thiobarbituric acid derivatives also exhibited antimicrobial [20], antifungal [21], antiviral [22], antitumor [23], antidiabetic and antibacterial activities [24]. They show diverse biological activities, such as antituberculosis [25], anticancer with anti-inflammatory activities [26]. The biological activity of the compounds depends on the acid-base properties

[27].

Our research group is continuously doing effort in search of biologically potent scaffolds, which are easy to synthesize and have no tedious chemistry. Previously our group has reported thiazole [28], triazinoindole [29] and isatin based Schiff bases analogs as α-glucosidase inhibitors [30].

Due to the wide range application of thiobarbiturates, here in this study, we are going to report various biological activities and molecular docking analysis of thiobarbituric acid derivatives.

RESULTS AND DISCUSSION

Chemistry

The reaction of thiobarbituric acid with different aromatic aldehyde in the presence of 10ml of 20% NaOH gives the product which will precipitates as a solid, filter, dried, and recrystallized from ethanol [31, 32].

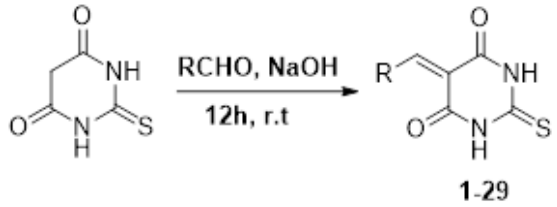

Figure 1. Synthesis of various thiobarbituric acid derivatives **1-29**

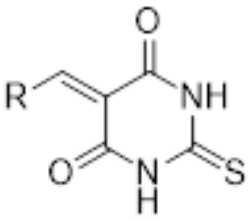

Figure 2. General structure of the synthesized thiobarbituric acid analogs **1-29**

α-Glucosidase Inhibitory Potential

Thiobarbituric acid analogs (**1-29**) were evaluated for α-glucosidase inhibition and most of the analogs showed a variable potential when compared with standard acarbose having IC_{50} value 38.25±0.12 µM. Among the series, analog **19** showed excellent inhibitory potential with IC_{50} values of 37.61±0.08 µM. Analogs **3, 6, 7, 9, 13, 15, 16, 18, 21-27** with IC_{50} values 45.46±0.04, 112.15±0.17, 73.96±0.12, 41.75±0.05, 322.78±0.35, 43.95±0.08, 118.75±0.17, 39.19±0.09, 172.59±0.15, 318.75±0.29, 363.52±0.16, 41.95±0.03, 46.76±0.04, 118.71±0.15 and 71.62±0.17µM respectively, showed good to moderate inhibitory potential. All other analogs were found inactive.

Table 1. Different Substituents of thiobarbituric acid analogs 1-29

S. No	R	S. No	R	S. No	R
1	2,6-Dimethoxyphenol	11	4-Methoxy-3-Phenol	21	2-Methylbenezene
2	2-Bromo-4,5-dimethoxybenzene	12	3,4-Dimethoxybenzene	22	4-Ethoxybenzene
3	Napthanlen-2-ol	13	2-Iodo-6-methoxyphenol	23	Benezene-1,3-diol
4	Thiazolyl	14	Anthracene	24	3-Methoxyphenol
5	3,4,5-Trimethoxybenzene	15	3-Methoxyphenol	25	5-methylfuran
6	*Para*-thiotolyl benzene	16	2,4-Dichlorophenol	26	Benzene-1,2-diol
7	6-Bromo-4-chloro-2H-chromen-2-one	17	Aniline	27	4-Methoxyphenol
8	Pyridine	18	5-benzylidene-2-thioxodihydropyrimidine-4,6(1H,5H)-dione	28	2,4-Di(*tert*butyl) benezene
9	6-Methylpyridine	19	2,6-Dibromophenol	29	Benzene-1,2,3,-triol
10	4-Bromo-2,5-dimethoxybenzene	20	*N,N*-dimethylaniline		

The structure-activity relationship has been established, which was mainly based on the variation of substitution on phenyl ring. Among the series of compounds, analog **19**, a 3, 5-dibromo-4-hydroxyl analog was found to the most active. The greater inhibitory potential of this compound among the series it might be due to substituents like hydroxyl and bromo groups. The hydroxyl group might be involved in hydrogen bonding. If we compare it with other mono hydroxyl analogs like **1**, **15**, **11,** and **24**, analog **19** is superior. One possible reason is that it possesses EWG along with hydroxyl while the other compounds possess EDG along with hydroxyl group. The second most active compound **18** has only phenyl moiety. The third most active compound **9** having pyridine ring only. Other active compounds **24, 15, 3, 25, 27** and **7** having various substituents like methyl, methoxy and hydroxy moiety on the phenyl part.

If we compare analog **15** having IC_{50} value 43.95±0.08 µM with analog **11** inactive and analog **24** having IC_{50} value 41.95±0.03, all the three analogs have one hydroxy and one methoxy groups but the arrangement of groups are different. In analog **15** the hydroxy group is present at *ortho* position but methoxy group is present at *para* position, in analog **11** the hydroxy group is present at *meta* position but methoxy group is present at *para* position while in analog **24** the hydroxy group is present at *ortho* position but methoxy group is present at *meta* position. Here we observed that the position of substituents on the aromatic ring also affects the inhibitory potential of compounds.

We observed in this study that all those analogs having

only methoxy functionality like **5** and **12** are completely inactive, while those having methoxy group along with other substituents like **1**, **2**, **10**, **13** (completely inactive), except **15**, **24** and **27** which have hydroxyl group at *ortho* position. The binding interactions of these compounds were confirmed through molecular docking studies.

Table 2. α-glucosidase activity of thiobarbituric acid analogs 1-29

S. No	Inhibition (%) at 0.5 mM	IC_{50} (μM)
1	9.12±0.58	NA
2	2.43±0.33	NA
3	98.56±0.49	45.46±0.04
4	9.24±0.11	NA
5	12.56±0.28	NA
6	91.13±0.32	112.15±0.17
7	97.11±0.23	73.96±0.12
8	7.88±0.11	NA
9	91.51±0.36	41.75±0.05
10	5.75±0.19	NA
11	1.56±0.17	NA
12	4.69±0.25	NA
13	82.38±0.59	322.78±0.35
14	12.31±0.16	NA
15	94.25±0.29	43.95±0.08
16	95.83±0.35	118.75±0.17
17	9.76±0.29	NA
18	95.25±0.13	39.19±0.09
19	98.83±0.26	37.61±0.08
20	6.72±0.39	NA
21	98.45±0.41	172.59±0.15
22	96.67±0.48	318.75±0.29
23	97.55±0.31	363.52±0.16
24	98.23±0.12	41.95±0.03
25	92.34±0.25	46.76±0.04
26	87.16±0.24	118.71±0.15
27	98.17±0.31	71.62±0.17
28	Nil	Nil
29	Nil	Nil
Acarbose	92.23±0.14	38.25±0.12

Docking study of alpha-glucosidase activity

Molecular docking of thiobarbituric acid analogs against α-glucosidase enzyme was performed to investigate their binding modes. From the docking simulation, it was observed that all the compounds fitted well in the binding cavity of α-glucosidase.

The binding mode of the most active compound **19** (IC_{50} = 37.61±0.08) with dibromophenol substituent showed three interactions with the active site residues Asp 349, Asp 214 and Arg 439 of the enzyme (**Figure 3a**). Two hydrogen bonds were formed between the NH and OH moieties of the compound with Asp 439 and Asp 214 respectively. Whereas an arene-cation interaction between the phenyl ring of the compound and active site residue Arg 439 was observed. Additionally, several hydrophobic interactions were observed between the compound and the active residues (Phe 300, Phe 158 and Phe 177 etc.) of the enzyme. The high potency of the compound might be due to the strong binding interaction with the active site residues.

The binding mode of the second most active compound **18** (IC_{50} = 39.19±0.09) showed three interactions with the active site residues Asp 214, Asp 349, and Arg 212 of the enzyme. Two hydrogen bonds were formed between the NH moieties of the compound with the active residue Asp 214 and Asp 349 whereas the third hydrogen interaction was observed between the carbonyl oxygen with active site residue Arg 212 (**Figure 3b**).

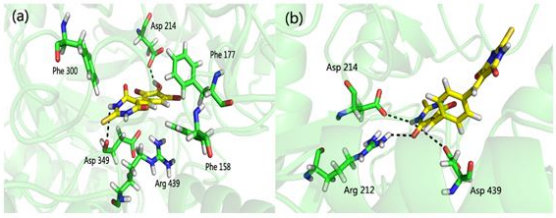

Figure 3. Docking conformation of compounds a) **19** and b) **18** in active site of the α-glucosidase enzyme.

In the case of methoxyphenol substituted compounds (Compound **15**, **24,** and **27**), it was observed that the position of the methoxy group plays a key role in the biological activity of the compound. For example, in compound **15**, the methoxy group present at *para* position showed good biological activity as well as good interaction as compared to compound **27** where the methoxy group present at *meta* position. The top-ranked docking conformation of compound **15** established two hydrogen bonds between active side residue Glu 276 and Asp 214 with the hydroxyl group of methoxyphenol moiety of the compound (**Figure 4a**). In the case of compound **27,** only a single hydrogen bond was observed between the carbonyl oxygen of the compound with active site residue Arg 212 (**Figure 4b**).

In the case of dihydroxy substituted compounds (Compound **23** and **26**), the position of the OH moiety

plays a key role in biological activities. For example, when the hydroxyl group present at adjacent carbon on the phenyl ring of the compound (Compound **26**), showed good inhibitory activity (IC_{50} = 118.71±0.15) and good interaction (**Figure 5a**).

Table 3. Thymidine phosphorylase activity of thiobarbituric acid analogs 1-29

S. No	IC_{50} (µM±SEM)
1	318.6+2.0
2	188.73+1.32
3	192.55+3.07
4	226.8+1.06
5	190.4+2.58
6	189.83+2.0
7	74.6+2.5
8	281.6+2.12
9	215.1+1.63
10	337.9+2.53
11	234.1+1.57
12	211.625+1.93
13	328.96+4.0
14	NA
15	375.8+3.28
16	Nil
17	174.9+.70
18	162.6+2.57
19	263.96+2.17
20	18.8
21	40
22	383.4+1.27
23	379+3.68
24	356.75+4.0
25	44
26	320.5+2.16
27	NA
28	6.9
29	251.63+3.2
7-Deazaxanthine	41.0 ± 1.63 µM

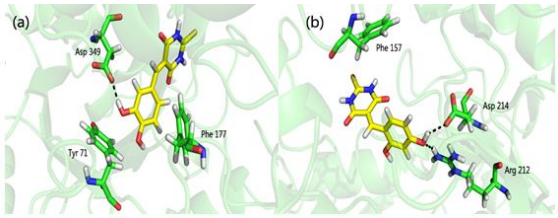

Figure 4. Docking conformation of compounds a) **26** and b) **23** in active site of the α-glucosidase enzyme.

Whereas the separation of two hydroxyl group over the phenyl ring of the compound (Compound **23**) decrease the inhibitory activity (IC_{50} = 363.52±0.16) as well as interaction with the active site residues (**Figure 5b**).

In the case of Compound **21** (IC_{50} = 172.59 ± 0.15), where the methyl group present at *ortho* position over the benzene ring showed an arene-arene interaction between Phe 177 and toluene moiety of the compound (**Figure 6a**). In the case of compound **22** (IC_{50} = 318.75 ± 0.29), a slightly bulky group (methoxy) is present as compare to compound **21** in which methyl moiety is present. This bulky moiety might be responsible for the less activity and poor interaction of compound **22** with the active site residues as compared to compound **21**. For example, the docking conformation of compound **22** showed that only arene-cation interaction between Arg 312 and the benzene ring of the compound was observed (Figure **6b**).

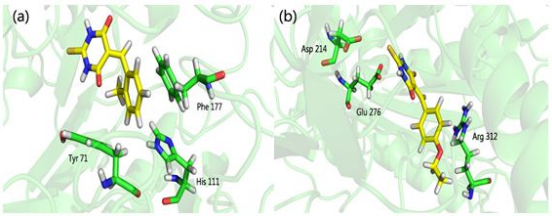

Figure 5. Docking conformation of compounds a) **21** and b) **22** in active site of the α-glucosidase enzyme.

Overall, the docking result showed that the steric hindrance plays a key role in the inhibitory activity and interaction pattern of the compound with the active site residues of the enzyme.

Thymidine phosphorylase inhibitory Potential:

Thiobarbituric acid analogs (**1-29**) were also evaluated for thymidine phosphorylase inhibitory potential and most of the analogs showed a variable potential ranging from 6.9 to 383.4 + 1.27 µM when compared with standard 7-deazaxanthine having IC_{50} value 41.0 ± 1.63 µM. Among the series, three analogs such as analog **20, 21,** and **28** showed outstanding inhibitory potential with IC_{50} values 18.8, 40, and 6.9 µM. Two analogs such as analog **7** and **25** showed excellent inhibitory potential with IC_{50} values 74.6 + 2.5 and 44 µM respectively. Twenty-one analogs **1-6, 8-13, 15, 17-19, 22-24, 26,** and **29** showed good to moderate inhibitory potential while three analogs **14, 16,** and **27** remain inactive.

Docking study of thymidine phosphorylase activity

In order to explore the binding mode of these newly synthesized compounds, molecular docking was performed. The docking results showed that all the compounds well accommodated in the active site of thymidine phosphorylase.

From the docking conformation of the most active compound, compound **28 (IC_{50} = 6.9)**, it was observed that this compound established seven polar interactions and one H-pi interaction with active site residues and good docking score (**-10.3210**) as compared to the reference compound having docking score of **-7.5093** and biological activity with IC_{50} of **41.0 ± 1.63**. The two carbonyl oxygen moieties present at the phenyl ring of the compound formed four H-acceptor bonds with Ser 86, Thr 87, Tyr 168, and Arg 171 respectively. Furthermore, sulfur atom of the compound formed H-acceptor bonds with active site residue Thr 87 and Phe 210 whereas the phenyl ring of the compound formed H-pi interaction with phenyl ring of the Tyr 168. Met 211 formed H-donor interaction with the sulfur atom of the compound (**Figure-7A**). This strong bonding network might be one of the reasons for this compound to show good biological activity. The docking conformation of the second most active compound, compound **20** (**IC_{50} = 18.8**), showed that this compound formed six bonds with the active site residues His 85, Ser 86, Tyr 168, Arg 171 and Lys 190 respectively (**Figure-7B**) and good docking score **(-9.9765).** The two carbonyl oxygen moieties present at the phenyl ring of the compound formed four H-acceptor bonds with His 85, Ser 86, and Arg 171 respectively. Tyr 168 and Lys 190 were observed making H-pi and pi-cation linkages with the compound respectively. The potency of this compound might be due to the presence of the electron-withdrawing group (carbonyl oxygen) and electron-donating group (-NH). The third most active compound **21 (IC_{50} = 40)** was observed with a docking score of **-8.7301** and good interactions with the binding pocket residues (**Figure-7C**). His 85 and Arg 171 established polar interactions with the carbonyl oxygen atoms while Ser 186 formed a polar bond with the –NH moiety of the ligand. The electronic cloud system of this compound might be the reason of its high potency. Compound **25** also showed good biological inhibitory activity **(IC_{50} = 44)** as well as a good docking score **(-8.4012).** From the docking conformation of compound **25**, it was observed that this compound formed four H-bonds with the active site residues of the target enzyme (**Figure-7D**). His 85 formed hydrogen bond with the carbonyl oxygen atom while Arg 171 established H-bonds with another carbonyl oxygen atom of the same ligand. The hydroxyl group of the Ser 186 residue formed H-acceptor interaction with the –NH moiety of the same compound. Some of the compounds in the series of the compounds e.g., Compound **14**, **16,** and **27** were observed having no biological activities as well as poor docking scores. The steric hindrance or low polarizability might be one of the reasons for these compounds to show no biological activity.

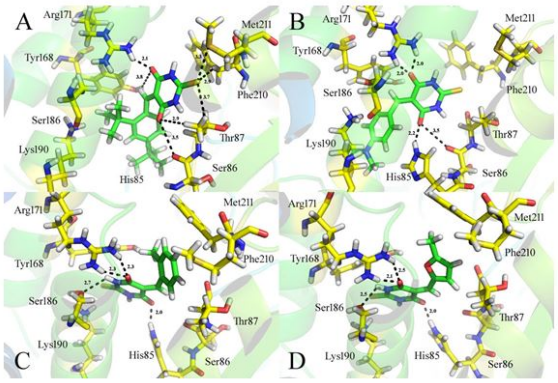

Figure 6. Docking conformations of compounds on thymidine phosphorylase enzyme. **A.** 3D binding mode of compound **28** as an inhibitor of thymidine phosphorylase enzyme. **B.** 3D binding mode of compound **20**. **C.** 3D binding mode of compound **21**. **D.** 3D binding mode of compound **25** in the binding cavity of thymidine phosphorylase enzyme. Ligands are shown green color.

Overall a good correlation was observed between the docking scores and biological activities of these compounds (**Figure-8**).

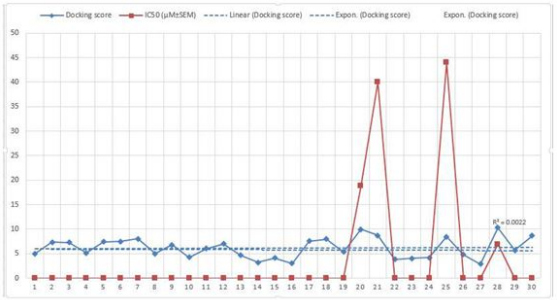

Figure 7. Correlation between the docking scores and biological activities

Lipoxygenase inhibitory Potential:

Thiobarbituric acid analogs (**1-29**) were also evaluated for Lipoxygenase inhibitory potential. All the analogs were found inactive.

EXPERIMENTAL

In this research work all the chemicals, such as solvents and reagents were purchased from foreign companies (Sigma/Aldrich, Alpha Aesar and Merck), and were used as such without any purification and distillation. These chemicals were 98-99.9% pure.

^{1}H NMR spectra were recorded in DMSO on Avance Bruker AM 300-500 MHz Instrument and TMS was used as an external standard. Chemical shifts are given in δ (ppm). Electron impact mass spectra (EI-MS) experimented on a Finnigan MAT-311A, Germany. Thin-layer chromatography (TLC) was performed on pre-coated silica gel aluminum plates (Kieselgel 60, 254, E. Merck, Germany). Chromatograms developed were visualized by UV at 254 and 365 nm.

General Procedure for the Synthesis of Thiobarbituric Acid Derivatives

By the reaction of 2-dioxo-dihydropyrimidine-2,4(1H, 5H)-dione (thiobarbituric acid 0.01 mmol) with different aromatic aldehyde (0.01 mmol) in the presence of 10 ml of 1M NaOH. Typically, these reactions were carried out in 95% ethanol about 12 hrs stirring at room temperature, after this the reaction mixture was poured into crushed ice followed by acidification with 2 ml dil. HCl, the product will precipitate as a solid, filter, dried, and recrystallized from ethanol [31, 32].

Characterization of the Representative Compound 5-(4-Hydroxy-3,5-dimethoxybenzylidene)-2-thioxodihydro-4,6(1H,5H)-pyrimidinedione (1)

Yield: 0.88 g (93%); Yellow Solid; m.p 263 ^{o}C; Chemical Formula: $C_{13}H_{12}N_2O_5S$: 1H NMR: (DMSO-d_6, 300 MHz): δ 12.3 (s, 2H, NH), 11.1 (s, 1H, OH), 8.3 (s, 1H, CH aldehydic), 7.3 (s, 2H, H-2/6), 3.7 (s, 6H, OCH_3); EI-MS: m/z (rel. int. %): 308 (M+, 100), 280 (69), 224 (44), 190 (35), 165 (30). Anal. Cald: C, 50.64; H, 3.92; N, 9.09; Found C, 50.63; H, 3.91; N, 9.08.

In vitro α-Glucosidase inhibitory assay

The α-glucosidase inhibition activity was performed with slight modifications as given by Pierre et al., [33]. The total volume of 100 µL reaction mixture contained 70 µL 50 mM phosphate buffer pH 6.8, 10 µL test compound (0.5 mM in methanol) followed by the addition of 10 µL enzyme solution (0.057 units, Sigma Inc.) in the buffer. The contents were mixed, pre-incubated for 10 min at 37 ºC and pre-read at 400 nm. The reaction was initiated by the addition of 10 µL of 0.5 mM substrate (*p*-nitrophenyl glucopyranoside, Sigma Inc.). After 30 min of incubation at 37 ºC, the absorbance of *p*-nitrophenol released was measured using Synergy HT 96-well plate reader, Bio Tek, USA. Acarbose was used as a positive control. All experiments were carried out in triplicates. The percent inhibition was calculated by the following equation:

$$Inhibition(\%) = (\frac{Abs_{control} - Abs_{test}}{Abs_{control}}) \times 100$$

Active compound solutions were suitably diluted and their inhibition studies were determined. Data obtained was used for the determination of IC_{50} values (concentration at which there is 50% enzyme inhibition) using EZ-Fit Enzyme Kinetics Software (Perrella Scientific Inc. Amherst, USA).

Assay Protocol of Thymidine phosphorylase

Thymidine phosphorylase activity was determined by measuring the absorbance at 290 nm spectrophotometrically. The original method described by Krenitsky and Bush by (1979) was modified [34, 35]. In brief, the total reaction mixture of 200 µL contained 145 µL of potassium phosphate buffer (pH 7.4, 50 mM), 30 µL of the enzyme (human and E. coli) at concentration 0.05 and 0.002 U, respectively, was incubated with 5 µL of test materials (0.5 mM) for10 min at 25 °C in a microplate reader. After incubation, pre-read at 290 nm was taken to deduce the absorbance of substrate particles. Substrate "Thymidine" (20 µL, 1.5 mM), was dissolved in potassium phosphate buffer, was immediately added to the plate and continuously read after 10, 20, and 30 min in a microplate reader (spectra max, molecular devices, CA, USA). 7-Deazaxanthine was used as a positive control. All assays were performed in triplicate.

Molecular docking of α-glucosidase

The study was designed to dock thiobarbituric acid analogs against α-glucosidase enzyme using the Molecular Operating Environment (MOE 2010.11) software package. The same protocol was used for the protein, ligand preparations, and molecular docking as described in our previous papers [36, 37].

Molecular docking of thymidine phosphorylase

The synthesized compounds were docked into the active site of the target enzyme in MOE

(www.chemcomp.com) by the default parameters i.e., Placement: Triangle Matcher, rescoring 1: London dG, Refinement: Force field, Rescoring 2: London dG. In order to validate the docking protocol, the 3D crystal structure of the thymidine phosphorylase complex with an analog of DIDEOXYURIDINE was retrieved from the protein databank PDB ID: 4EAD and re-docked in the active site of the enzyme. For each ligand ten conformations were generated and the top-ranked confirmation on the basis of docking score was selected for further studies in molecular docking. The RMSD between docked and co-crystallized ligand was found to be 1.89 Å indicates that the docking method is reliable [38]. The superposition of docked and co-crystallized ligands is shown in **Figure-9**. After the molecular docking, we analyzed the best poses having polar, H-pi, and pi-H interactions by Pymol software.

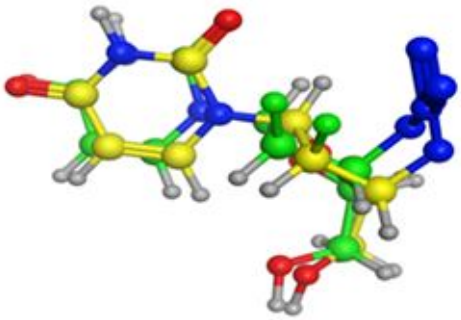

Figure 8. Superposition of Docked and co-crystallized ligand, green represents the co-crystallized ligand and yellow the re-docked conformation of the ligand.

CONCLUSION

New α-glucosidase, thymidine phosphorylase and lipoxygenase inhibitors of thiobarbituric acid have been discovered in this study, including molecule active at low micro molar concentrations. Among the series analog **19** was identified as best α-glucosidase inhibitor while three analogs such as **20, 21** and **28** was identified as best thymidine phosphorylase inhibitor for further studies.

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