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Acetohydroxyacid Synthase (AHAS) Inhibitor-Based Commercial Sulfonylurea Herbicides as Glutathione Reductase Inhibitors: in Vitro and in Silico Studies

Sedat Sel,[a] Turgay Tunç,[b] Ahmet Buğra Ortaakarsu,[c] Serhat Mamaş,[c] Nurcan Karacan,[c] and Mehmet Sayım Karacan*[c]

In this study, *in vitro* inhibitory effect of AHAS inhibiting-based commercial sulfonylurea herbicides on human GR and *S. cerevisiae* GR was determined by electrochemical method. Our findings, the first report in literature, show that the four commercial herbicides were found to be the inhibitors in the range of 4.90–9.75 μM for ScGR, and in the range of 8.54–18.84 μM for hGR. Global reactivity descriptors (energy gaps, electronegativity, hardness and electrophilicity index) of the herbicides were calculated by DFT/B3LYP/6-31G(d,p) method in gas phase. The electrochemical behavior of the herbicides was studied by cyclic voltammetry. Single-electron half-wave reduction potentials and global reactivity descriptors were correlated with the IC_{50} values of the herbicides. Molecular docking analysis using Schrödinger Suite was applied to examine the interaction between the herbicides and human GR (PDB ID:1XAN and 2GH5), *S. cerevisiae* GR (PDB ID:2HQM), *P. falciparum* GR (PDB ID:1ONF), *C. albicans AHAS* (PDB ID:6DEL) and ScAHAS (PDB ID: 5FEM. Based on the docking results, it can be predicted that (a) herbicides have similar binding potential to two different binding sites of hGRs, (b) herbicides may have antimalarial potential against *P. falciparum* (c) herbicides may have antifungal potential against *C. albicans.*

Introduction

Inhibitors of AHAS are widely used for weed control in agricultural industry due to their potent activity, low dosage, good selectivity, broad spectra of herbs, and low animal toxicity.[1] AHAS plays a critical role in valine, isoleucine, and lysine biosynthesis.[2] This enzyme is found in plants, bacteria, and fungi but not in animals. Thus, it is the target for more than 50 commercial herbicides.[3] Three AHAS isozymes have been characterized in bacteria, whereas only one isozyme is known in fungi and plants. In recent years, there has been a growing interest in targeting AHAS for the discovery of new antimicrobial agents against pathogenic bacteria and fungi.[4] Chlorimuron ethyl (CE), a sulfonylurea herbicide, has previously been reported to inhibit the growth of *C. albicans*[5] and *C. neoformans.*[6] Previous work had presented that sulfosulfuron has the ability to break through the barrier of the cell membrane and damage cellular DNA.[7]

A cellular imbalance between ROS production and ROS-regulating antioxidant defense system lead to oxidative stress, and increases the likelihood of cell death or promotes cancer through DNA-mutation accumulation.[8] Antioxidant molecules (such as ascorbic acid and glutathione) and antioxidant enzymes (such as catalase, superoxide dismutase, ascorbate peroxidase, glutathione reductase, NADH-oxidase) are found in living organisms to prevent oxidative stress and regulate cellular redox homeostasis.[9] Glutathione reductase is the main protection system for detoxification of ROS, catalyzing the reduction of glutathione disulfide (GSSG) to glutathione (GSH) to maintain a high GSH/GSSG ratio and reduce oxidative stress.[10]

It is well known that herbicides that inhibit PSI and PSII such as paraquat, atrazine and diuron, act mainly through the production of ROS, causing an imbalance in the redox state of the cell, leading to oxidative damage and ultimately to cell death.[11] However, Averina *et al.* reported plants grown in the presence of methsulfuron methyl, a sulfonylurea herbicide, suppressed ascorbate peroxidase activity, but increased glutathione reductase activity in winter rape (Brassica napus L.).[12] Zabalza et al. analyzed that AHAS-inhibiting imidazolinone herbicide caused enhancement in guaiacol peroxidase activity in leaves and glutathione content in roots, suggesting that

Table 1. In vitro activity (IC_{50}) of the herbicides on ScGR and hGR.

Herbicides	ScGR IC_{50} μM ± SD	hGR IC_{50} μM ± SD
MSM	6.78 ± 0.36	12.82 ± 0.29
TBM	4.90 ± 0.22	8.54 ± 0.28
TRS	8.30 ± 0.57	16.69 ± 0.27
TFS	9.75 ± 0.13	18.84 ± 0.31

[a] *S. Sel*
İstanbul University Pharmacy Faculty,
Analytic Chemistry, 34116, Beyazıt
İstanbul, Turkey
[b] *Prof. Dr. T. Tunç*
Department of Chemistry Engineering,
Faculty of Engineering University of Kırşehir Ahi Evran,
Kırsehir, 40100, Turkey
[c] *A. B. Ortaakarsu, S. Mamaş, Prof. Dr. N. Karacan, Prof. Dr. M. S. Karacan*
Gazi University, Science Faculty,
Chemistry Department, 06500,
Ankara, Turkey
E-mail: mkaracan@gazi.edu.tr

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oxidative stress is not related to the mode of action of AHAS-inhibitors.[13] In addition, broad-spectrum systemic herbicide glyphosate, was found to increase in GSH content and antioxidant enzyme activity (catalase, glycolate oxidase and glutathione reductase) of *Arabidopsis thaliana* seeds.[14]

However, to our knowledge, there are no reports on whether sulfonylurea herbicides can inhibit human GR (hGR) and *Saccharomyces cerevisiae* GR (ScGR) activity. This is the first study to examine the *in vitro* activities of hGR and ScGR of commercial herbicides (Figure 1) such as tribenuron methyl (TBM), metsulfuron methyl(MSM), thifensulfuron(TFS), tritosulfuron (TRS) using the square-wave voltammetric method. The theoretical calculation of the herbicides were performed using DFT/B3LYP/6-31 G (d,p) method *in vacuo*. The optimized structural parameters, Frontier molecular orbitals and global reactivity descriptors such as HOMO-LUMO gap, hardness, absolute electronegativity, electrophilicity index were determined. Structure-activity relationship was further discussed to evaluated the reactivity of sulfonylureas. We also docked the herbicides into the receptor of two human GR having different

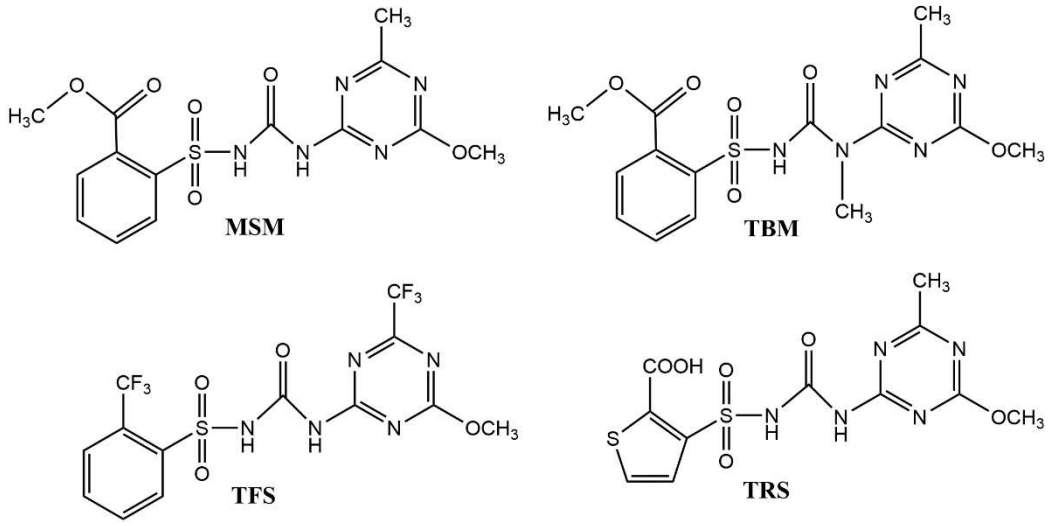

Figure 1. Commercial sulfonylurea herbicides used in this study.

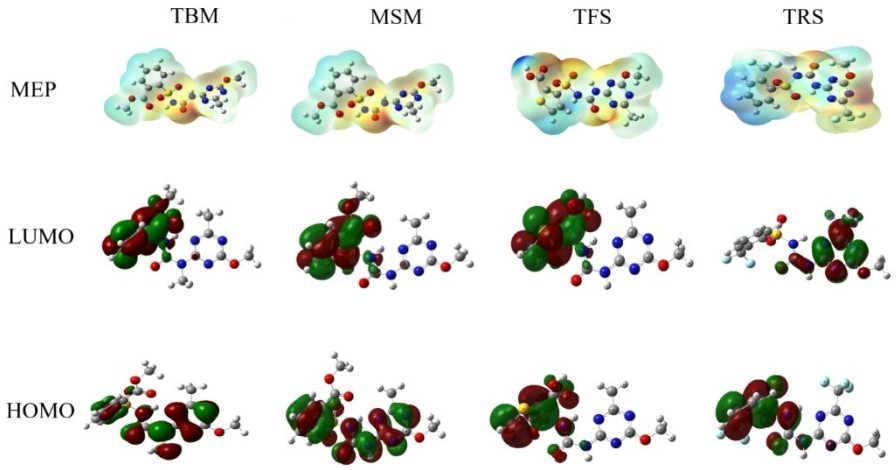

Figure 2. 3D-plots of HOMO, LUMO and MEP of the herbicides.

Table 2. The global chemical reactivity descriptor of the herbicides.

	HOMO (eV)	LUMO (eV)	ΔE (eV)	η (eV)	χ (eV)	ω (eV)	pK_a
TBM	−0.252	−0.0662	0.186	0.093	0.159	0.136	5.2
MSM	−0.259	−0.0678	0.191	0.096	0.163	0.139	4.1
TRS	−0.280	−0.0688	0.211	0.106	0.174	0.144	3.2
TFS	−0.271	−0.0801	0.191	0.095	0.176	0.161	2.9/4.2

Table 3. Some electrochemical parameters of the herbicides at scan rate 0.1 V/s.

Parameters	TBM	MSM	TRS	TFS
E_{pc} (V)	−2.011	−1.981	−1.942	−1.738
i_{pc} (V)	7.04	10.417	18.74	22.40
$E_{1/2}$ (V)	−1.981	−1.893	−1.866	−1.701
Number of transferred electron	1.23	1.26	1.24	1.25

E_{pa} = anodic peak potential, V; E_{pc} = cathodic peak potential V; i_{pc} = cathodic peak current μA.

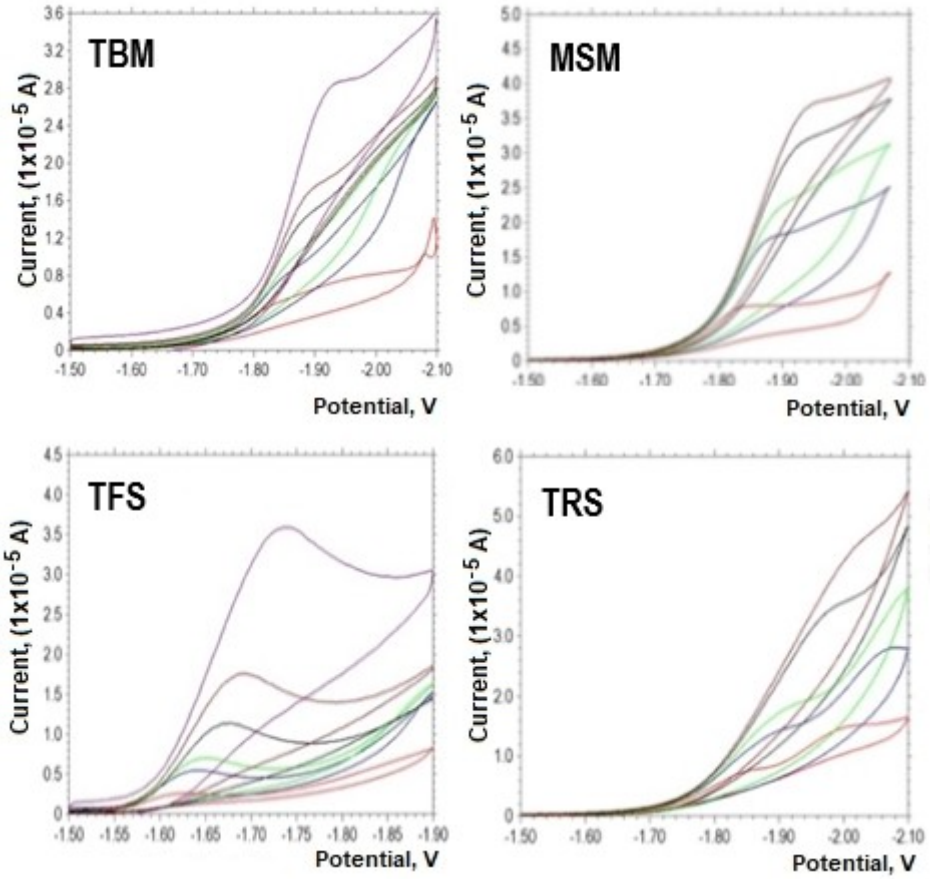

Figure 3. Cyclic voltammograms obtained different scanning speed of the herbicides.(0.01 V/s (red); 0.05 V/s (dark blue); 0.1 V/s (green); 0.5 V/s (brown); 1 V/s (purple) V/s).

Table 4. Glide/IFD binding score (kcal/mol) of the herbicides with human GR (PDB ID:2GH5 and 1XAN).

Herbicides	IFD Docking Score (kcal/mol)	
	2GH5	1XAN
MSM	−6.185	−5.370
TBM	−6.554	−6.270
TRS	−6.404	−5.303
TFS	−5.995	−4.500
ELI	−10.485	–
HXP	–	−6.188

Table 5. Glide/IFD binding score (kcal/mol) of the herbicides with ScGR and PfGR.

herbicides	ScGR (kcal/mol)		PfGR (kcal/mol)	
	Site-1	Site-3	Site-1	Site-3
MSM	−5.982	−4.988	−5.087	−6.232
TBM	−6.779	−5.716	−6.746	−6.824
TFS	−6.570	−5.889	−6.294	−6.970
TRS	−5.477	−4.676	−5.957	−6.312

binding sites (PDB:1XAN and 2GH5), *S. cerevisiae* GR (PDB 2HQM), *P. falciparum* GR (PDB:1ONF), *S. cerevisiae* AHAS (PDB ID:5WKC), *C. albicans AHAS* (PDB ID:6DEL) and *T. afroharzianum AHAS* (PDB ID:7EGV) to predict their antimalarial and antifungal activities using molecular docking analysis with Schrödinger Suite.

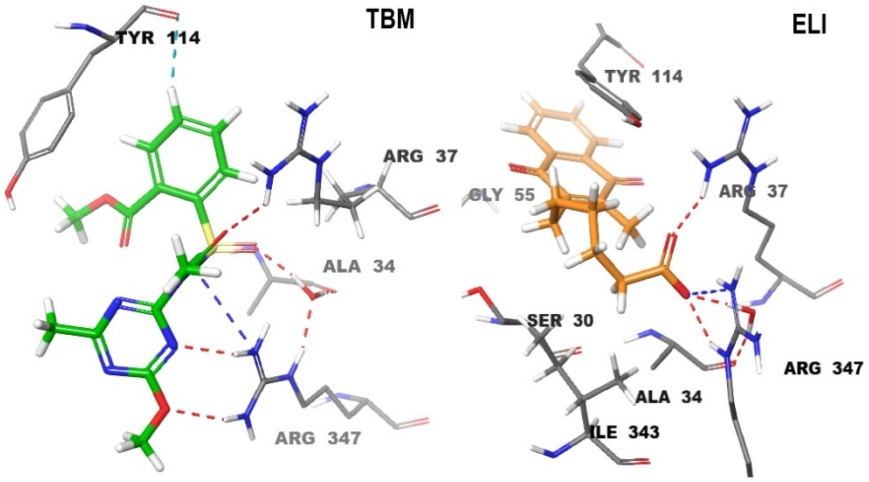

Figure 4. The best pose of the cognate (ELI) and TBM for hGR (PDB ID:2GH5).

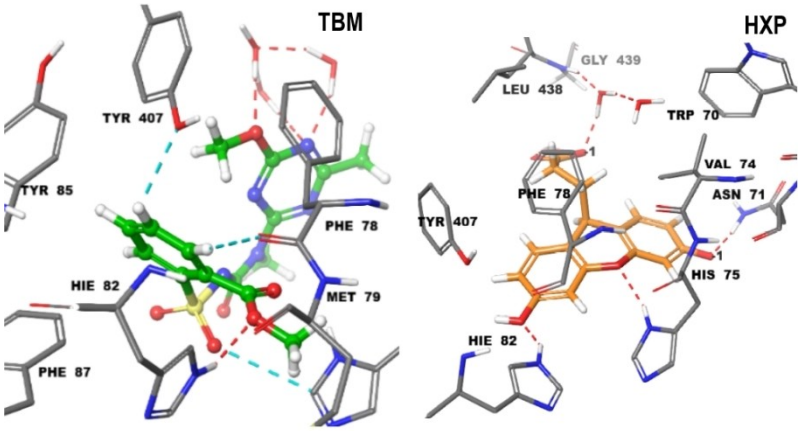

Figure 5. The best pose of the cognate (HXP) and TBM for hGR (PDB ID:1XAN).

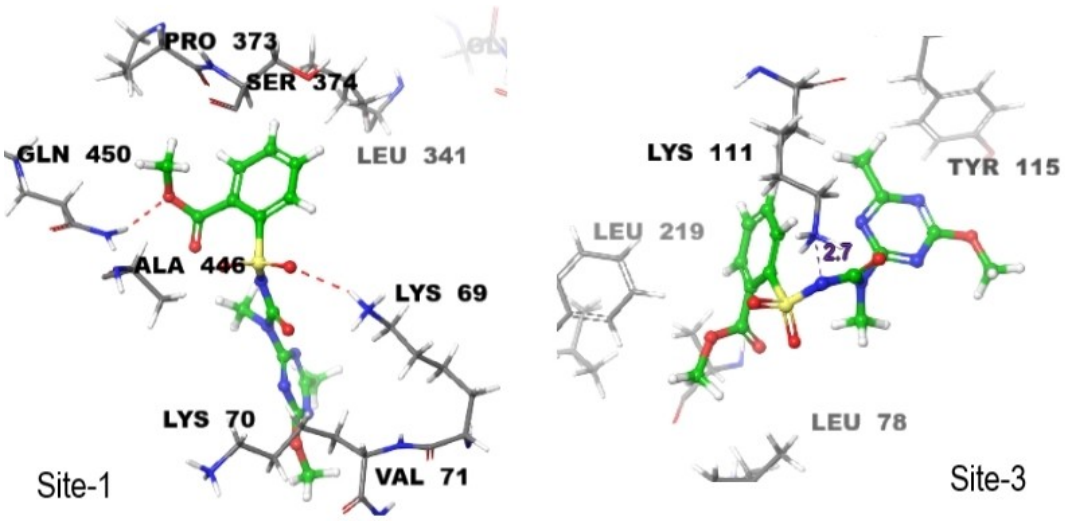

Figure 6. The best pose of the TBM at site-1and site-3 for ScGR.

Table 6. Glide/IFD binding score (kcal/mol) of herbicides for *Ca*AHAS (PDB ID:6DEL).

CaAHAS	HB	Pi-pi stacking	Salt bridge	Hydrophobic
MSM	Trp582	Trp582		Met350, Met578, Val579, Phe586, Met650, Val651, Pro652, Ala653
TBM	Arg376 Try587	Trp582		Met350, Met498, Met578, Val579, Met650, Phe586
TFS	Arg380 Trp705	Trp586	Arg380 Trp705	Met354, Val497, Leu522, Met582, Met654
TRS	Arg376	Trp582	Arg376	Met350, Ile381, Met578, Leu585, Phe586, Met650, Val651, Pro652, Ala653
CE	Arg376 Gln581 Ala653	Trp582		Met350, Met498, Met578, Trp582, Leu585 Phe586, Try587, Val647, Met650, Val651, Pro652
BSM	Arg376	Trp582	Arg376	Met350, Met578, Val579, Met650, Val651, Pro652, Ala653
ScAHAS				
MSM	Arg380 Trp705 Trp586	Trp586	Arg380	Met354, Met582, Val583, Phe590, Ala656
TBM	Arg380 Trp586	–		Met354, Met582, Val583, Phe590, Met654, Ala656
TFS	Trp705	Trp586	Arg380	Met354, Val497, Leu522, Met582, Met654,
TRS	Try591 Arg380	Trp586	Arg380 Trp706	Met354,Val497, Leu522,Met582, Val583
CE	Arg380 Trp705			Met354, Met654, Val497, Met582, Val583, Trp586
BSM	Arg380 Trp586			Met354, Met582, Val583, Phe590, Met654, Ala656

Table 7. Interactions of herbicides at the active site of the CaAHAS and ScAHAS.

Herbicides	IFD Docking Score (kcal/mol)		MIC_{50}* (μM)	
	*Ca*AHAS	*Sc*AHAS	*Ca*AHAS	*Sc*AHAS
MSM	−4.995	−6.008	–	–
TBM	−6.037	−5.710	NI	NI
TFS	−5.731	−5.897	–	–
TRS	−6.589	−5.778	–	–
CE	−7.822	−5.438	2	19.5
BSM	−5.467	−6.346	62	20.8

*Values taken from ref[5]. NI = no inhibition.

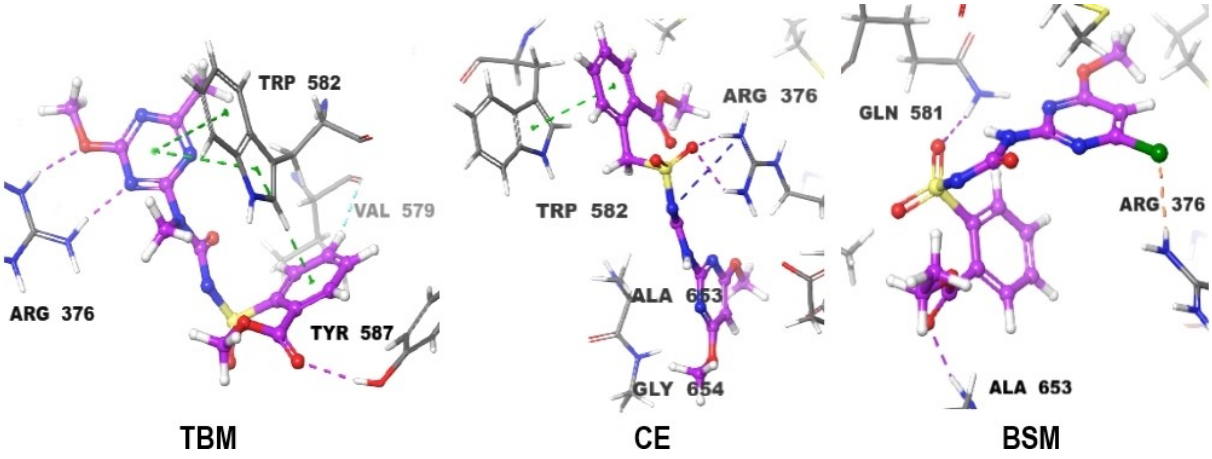

Figure 7. The best poses of the TBM, CE and BSM at CaAHAS (PDB ID:6DEL).

Results and Discussion

Glutathione reductase activity

GR activity (GR; $GSSG + NADPH + H^+ \rightarrow GSH + NADP^+$) of the herbicides was determined by square-wave voltammetric procedure described previously.[15] Reduction peak potential of GSH at optimized conditions was found at −0.44 V (Figure S1, left side). Square wave voltammogram was recorded at increasing GSH concentrations at phosphate buffer (pH 7.2) with hanging mercury drop electrode versus Ag/AgCl. The calibration graphs of the peak current versus GSH concentration were found to be linear in the working condition, which is directly proportional to the GR enzymatic reaction rate (Figure S1, right side). IC_{50} values obtained from graph plotting percent inhibition against herbicide concentration were listed in Table 1.

The commercial sulfonylurea herbicides showed significant effect in inhibiting both *S. cerevisiae* GR (*Sc*GR) and human GR (hGR) activity. They exhibit better inhibitory effect on the ScGR. TBM has the highest GR inhibitory activity, showing that the addition of CH_3 group instead of NH proton increases the inhibitory activity compared to MSM. TFS showed the lowest GR inhibitory activity. Decreasing order of GR inhibitory activity is as follows: TBM > MSM > TRS > TFS.

Dissociation plays an important role in receptor binding processes of drugs. So, we calculated the macroscopic pKa value of the herbicides using Jaguar pK_a module (Table 2). pK_a values of SO_2-NH proton of the herbicides was calculated in the range of 2.9–5.2, implying that they are weak organic acids. Thifensulfuron (TFS) with two acidic proton $-SO_2.$**NH** (pKa 2.9) and COO**H** (pKa 4.2)- act as diprotic acid at physiological pH. The computed pK_a values correlate well with the experimental pK_a values found in the literature.[16] As the pKa values of the herbicides increases tendency towards proton donation decreases, and inhibitory activity increases. This finding explains why diprotic TFS shows the lowest activity.

Global reactivity descriptor has been greatly used to explain biological activity. On the basis of Koopman's theorem, Global reactivity descriptors such as hardness(η) electronegativity(χ), absolute electronegativity and electrophilicity index(ω) are calculated by B3LYP/6-31G(d,p) level in the gas phase on the basis of frontier molecular orbitals, E_{HOMO} and E_{LUMO} (Table 2). The ΔE energy gap and the chemical hardness are very important terms of stability and reactivity.[17] They are the measure of the resistance to change in the electronic distribution. Higher-gap is more stable than smaller gap. In our case, the ΔE energy gaps and the hardness orders of the herbicides were determined as **TBM < MSM < TRS TFS**. From Table 2, it was observed that TBM exhibits low value of chemical hardness and high value of global softness, therefore, it is chemically more reactive and less stable than all other compounds. Absolute electronegativity which evaluates Lewis acidity was obtained the following order: TBM < MSM < TRS < TFS. As seen in Table 2, As the Lewis acidic character of herbicides increases, their GR inhibitory activity values decrease. Electrophilicity index (ω) is a measure of stabilization in energy when a system acquires an additional amount of electronic charge from the surrounding. The electrophilicity index was obtained in the following order: TBM < MSM < TRS < TFS. Except for TFS, inhibitory activity decreases as the energy gap and hardness increases. HOMOs, LUMOs and the MEPs of the herbicides were displayed in Figure 2.

Molecular Electrostatic Potential (MEP) is a very powerful tool utilized to determine the nucleophilic and electrophilic regions on a molecular system. The negative electrostatic potential regions (red) are mainly concentered over the O atom of the SO_2 and CO group of TBM, MSM and TRS, showing that these sites can be involved in interactions with hydrogen bonds with the amino acid residues of protein receptors. The positive electrostatic potential regions (blue) are over the benzene ring and indicate that the regions are the preferred sites for nucleophilic attack for TBM, MSM and TFS. However, the positive electrostatic potential regions are concentered over NH protons of the bridge and phenyl ring, indicating that the regions are the preferred sites for nucleophilic attack for TRS. As seen in Figure 2, HOMO is mainly located on the triazine rings of TBM and MSM, on the phenyl ring of TRS, and over the entire molecule of MSM. However, LUMO is mainly localized on the phenyl ring of TBM, MSM and TFS, on the triazine ring of TRS. Electron-withdrawing substituents on the phenyl and triazine ring of TRS reduce its pi-pi interaction potential.

The redox potentials of the herbicides were studied by cyclic voltammetry in order to reveal a possible correlation between the binding strength of the herbicides and their reduction potential. From the recorded cyclic voltammogram of the herbicides (Figure 3), some voltammetric quantities including the peak potentials E_{pc} and half-wave reduction potential $E_{1/2}$ were evaluated and listed in Table 3. Negative single-electron half-wave reduction potentials ($E_{1/2}$) and negative cathodic peak potential of the herbicides were determined as in the following order: **TBM > MSM > TRS > TFS**. As seen, the inhibitory activity of the herbicides increases with shifting the single-electron reduction midpoint potential to more negative values. As the Lewis acidic property increases, the reduction potentials shift towards less negative values. There is a linear relationship the $E_{1/2}$ values of the herbicides and their LUMO energies, absolute electronegativity and electrophilicity index. Electron-withdrawing substituents on phenyl and triazine ring shifts the potentials to more positive values.

Molecular Docking Analysis

Human GR has multiple binding sites. Firstly, we have docked the herbicides into the human GRs with two different binding sites (PDB ID: 2GH5 and 1XAN). The most negative Glide/IFD docking scores of the herbicides were listed in Table 4.

ELI, co-crystal ligand, is covalently linked to Cys58 via a sulfur-carbon covalent bond in the active site (GSSG) of the human GR (PDB ID:2GH5). It was redocked at the active site of the hGR to compare with the herbicides to be docked. ELI forms a hydrogen bond between the O atom of carbonyl group and Arg37 at a distances of 2.0 Å. There is an electrostatic

interaction (salt bridge) at a distance of 3.0 Å between guanidine group of Arg347 and carboxylate anion of ELI, also hydrophobic interaction with Leu33, Ala34, Leu54, Val59, Try114, Gln115, Leu118 and Ile343. We docked the herbicides into the same active site, they exhibited similar interaction with ELI. TBM forms a hydrogen bond at the distance 2.3 Å between carbonyl oxygen atom of TBM and NH_2 group of Arg37. There are three hydrogen atoms between SO_2-N anion, N atom of triazol ring and methoxy group of TBM and guanidine group of Arg347, in addition to hydrophobic interaction with Leu33, Ala34, Leu54, Val59, Try114, Leu118 and Ile343 (Figure 4).

Then, we have docked herbicides into the human GR (PDB ID: 1XAN). Its cognate ligand (HXP) is surrounded in the binding cavity by hydrogen bonding with Asn71, His 75, His82 and hydrophobic interaction with Trp70, Val74, Phe78, Met79, Leu438 and Tyr407.[18] Molecular docking analysis indicates that TBM forms hydrogen bonding at the distances of 2.1 Å between methoxy group of TBM and imidazole group of His82, also hydrophobic interaction with Val74, Phe78, Tyr85, Phe87, Met79 and Tyr407 (Figure 5). Herbicides have the potential to bind the two different binding sites of hGRs.

Next, we have docked herbicides to *Saccharomyces cerevisiae* GR (PDB 2HQM)[19] and *Plasmodium falciparum* GR (PDB:1ONF).[20] However, they contain no cognate ligand. Therefore, SiteMap analysis was applied to ScGR and PfGR to predict their binding sites.[21] Results for top five binding sites were given in Table S1, and the binding sites of the ScGR were illustrated in Figure S2. The herbicides were docked to these binding sites of ScGR and PfGR. Two binding sites with the most negative Glide/IFD docking scores also had the highest druggability score (Dscore): Site-1 and Site-3 (Table 5).

Interestingly, if we superimpose the images of ScGR (PDB ID:2HQM)/hGR (PDB ID 2GH5) and PfGR(PDB ID:1ONF) /hGR (PDB ID:1XAN), we found that Site-1 pocket of *Sc*GR and site-3 pocket of *Pf*GR are located near the ELI and HXP, respectively (Figure S3.)

At the site-1 of ScGR, TBM forms hydrogen bonding with Lys69 and Gln450, and hydrophobic interaction with Val62, Cys66, Val67, Lys70, Val71, Try115, Leu341, Pro343, Pro373, Ser374, Val372, Val375, Phe377, Leu449, Ala446. At the site-3 of ScGR, TBM has a salt bridge at a distance of 2.7 Å between cationic ammonium of Lys 111 and anionic N atom of TBM, and hydrophobic interaction with Val71, Met72, Ala75, Leu78, Phe103, Leu219, Try115, Val181 (Figure 6). According to docking results, TBM has the potential to bind to both Site-1 and Site-3.

Some sulfonylurea derivatives have antimalarial activities against *Plasmodium falciparum* as hemozoin inhibitors.[22] Li pan et.al also reported that some monosubstitued sulfonylureas exhibited good antituberculosis activities against *Mycobacterium tuberculosis* H37Rv in vitro, which were comparable with that of the sulfometuron methyl (SM) herbicides with 10 mg/L values..[23] Our molecular docking results indicate that herbicides may have potential inhibitor for PfGR via binding to site-1 and site-3 (Table 5). Binding poses of TFS at site-1 and site-3 was given in Figure S4. These predicting encourage further study of the antituberculosis activity against *P. falciparum* of these herbicides.

The sulfonylurea herbicides such as chlorimuron ethyl(CE), bensulfuron methyl (BSM), ethoxysulfuron, chlorosulfuron and sulfometuron methyl have previously been shown to be good inhibitors of *C. albicans, C. neoformans* and *S. cerevisiae AHAS* enzymes.[5] In this study, molecular docking analysis was also performed to predict whether herbicides have antifungal activity on *C. albicans AHAS* (*PDB ID:6DEL*) and *S. cerevisiae* AHAS (*PDB ID:5FEM*). Intermolecular interactions of herbicides at the active site of the *Ca*AHAS and *Sc*AHAS were given in Table 6. Experimental *C. albicans* antifungal activity values taken from literature[5] of CE, BSM and TBM were added to Table 7 The best poses of the TBM, CE and BSM were illustrated for *Ca*AHAS in Figure 7 and for *Sc*AHAS in Figure S5.

CE and BSM are the cognate ligands of CaAHAS (*PDB ID:6DEL*) and *Sc*AHAS (*PDB ID:6DEL*), respectively. Docking results show that the herbicides bind to the active site of AHAS enzymes. Intermolecular interactions (Table 6) indicate that herbicides have similar interaction types to each other. Accordingly, we can predict that the herbicides may have antifungal potential against *C. albicans* and *S. cerevisiae*. However, experimental MIC values do not correlate with docking scores of both CaAHAS and ScAHAS enzymes. Although the docking scores and interaction types of CE, BSM and TBM are similar, CE has good activity, but TBM does not. As mentioned earlier,[24] docking analysis alone is not adequate tool to estimate binding affinity.

Conclusion

in vitro GR inhibitory effect of commercial herbicides such as tribenuron methyl (TBM), metsulfuron methyl(MSM), thifensulfuron(TFS), tritosulfuron (TRS) on human GR and *S. cerevisiase* GR was determined for the first time using square-wave voltammetric method. The order of GR inhibitory activity for hGR and ScGR was found as follows: TBM > MSM > TRS > TFS. IC_{50} value of the TBM was observed at 6.78 ± 0.36 μM for ScGR, and at 12.82 ± 0.29 μM for hGR. The inhibitory activity of herbicides correlates with pKa values calculated using Jaguar pK_a module, as the pKa value increases, the inhibitory activity increases

Global reactivity descriptors such as HOMO-LUMO gaps, hardness, absolute electronegativity, electrophilicity index of the herbicides were calculated to obtain an insight into the activity. As the electrophilicity index (ω) and absolute electronegativity values decrease, the softness and inhibitory activities of the herbicides increase. GR activity is also correlate with experimental negative half-wave reduction potential ($E_{1/2}$) of herbicides As the activity increases, half-wave reduction potentials shift towards more negative values.

Herbicides docked to the active site of human GR (PDB ID: 2GH5 and 1XAN) which they have different binding sites. Docking results indicated that herbicides have equal binding potential to different binding sites of hGRs.

Herbicides were also docked to *S. cerevisiae* GR (PDB ID: 2HQM) and *P. falciparum* GR(PDB ID:1ONF) containing no

cognate ligand. Sitemap analysis exhibited that two sites (Site-1 and Site-3) had more drugability scores, and also showed the highest docking scores. According to the analysis results, we predicted that herbicides have binding potential to these two different binding sites of *S. cerevisiae* GR, and they may have antimalarial effect against P. *falciparum*. These predictions encourage further study of the experimental antituberculosis activity of these herbicides against *P. falciparum*.

Based on the docking analysis showing that the herbicides bind to the active site of *C. albicans* AHAS (PDB ID:6DEL) and *S.cerevisiae* AHAS (PDB ID:5FEM), and have similar interaction types at the active sites, we predicted they have similar antifungal activity against *C.albicans*, and *S. cerevisiae* .However, experimental antifungal MIC_{50} values of the CE, BSM, and TBM taken from the literature data were not correlated with the docking scores. CE had good activity, while TBM was inactive.

Supporting Information Summary

Glutathione reductase inhibitory activity protocol, computational details, and molecular docking study are described in detail.

Conflict of Interest

The authors declare no conflict of interest.

Data Availability Statement

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

Keywords: AHAS inhibitors · glutathione reductase · herbicides · molecular docking · sulphonylurea

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