In-Silico Evaluations of Some α -Methylene-γ-Butyrolactone Analogues

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ABSTRACT

The efficacy along with proper pharmacokinetics and toxicity profiling is the major determinants for the present scenario of successful drug development. The toxicity prospect accompanying weak ADMET (absorption, distribution, metabolism, elimination, and toxicity) profile are the vital justifications of late costly jeopardy of drug development. To predict the ADMET idiosyncrasy, *in-silico* inspection of some α-methylene-γ-butyrolactones (**2-7**) was induced on the foundation of several physico-chemical criteria to predict their pharmacokinetic, pharmacodynamic, drug-likeness, bioactivity and molecular docking profile exploiting several computational tactics.

Key Words: *lactones, α-methylene-γ butyrolactones, in-silico molecular docking, ADMET, Drug likeness, bioactivity.*

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INTRODUCTION

Pharmaceutical consultation is an integral part of the retail sale of drugs to the public [1]. The liposomal formulations are targeted to deliver the important drug combinations to the body [2-4]. Lactones are compounds that are extensively dispersed in nature and several of them have been elucidated to date with influential bioactivity against a variety of human cancer cell lines, bacteria, and fungi. They exhibit interesting and useful biological activities. There is an antibacterial and antifungal property of lactones described in the literature. Much research, which appears understandable, refers to antitumor and cytotoxic activity lactones. The factual "hunt" for the presence of lactose in plants began at the end of the 60s of the preceding century [5, 6]

Sesquiterpene lactones were estimated as one of the largest classes of natural compounds [7]. The α-methylene-γ-lactone ring is a prime structural skeleton in several natural products, majorly the sesquiterpene lactones (Figures 1a and 1b) [8, 9]. It was gauged in 1985, that almost 10% of the familiar 30,000 natural products comprises of this α-methylene γ-lactone functionality [10].

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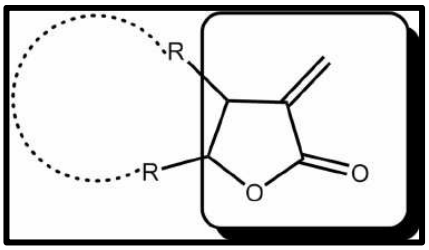


Figure 1a: General structure of α-methylene-γ-lactones

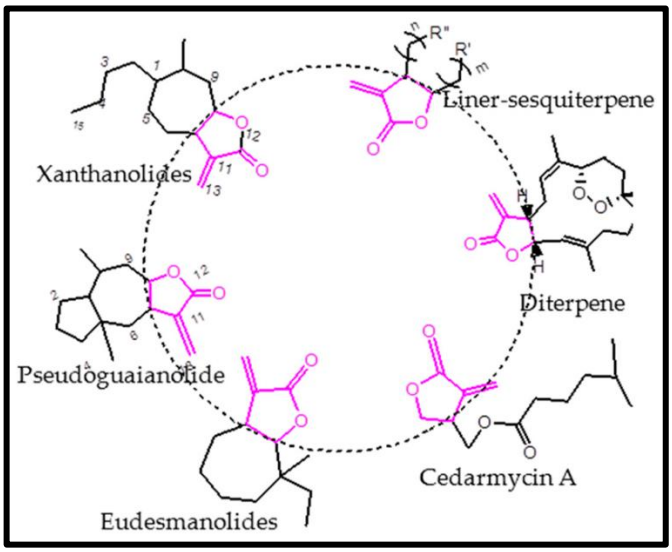


Figure 1b: Some sesquiterpene lactones.

This class of compounds has gain attraction due to their unique biological traits and in various cases, this high potency is reported to be due to the existence of the electrophilic exocyclic enoate moiety, which is responsible for trapping nucleophilic residues existing in the active site of target enzymes. They have been reported as DNA polymerase inhibitors, cellular steroidal inhibitors, nuclear vitamin D receptor inhibitors, blockers of tumor necrosis factor-α production, etc [11, 12]. The potency of these drug candidates is due to their cytotoxic, anti-inflammatory, antiallergenic, phytotoxic, anti-tumor, and antimicrobial properties [13-16].

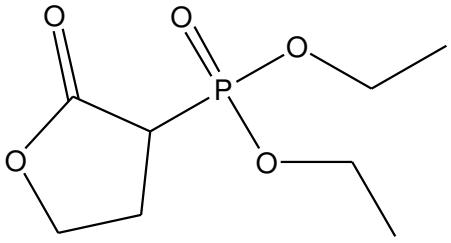
It is computationally and economically unfeasible to develop and sieve candidates with anti-microbial activity from among innumerable compounds. The development of Computer-aided drug designing (CADD) or *in silico* notion is a promising shortcut to resolve the cost and time issues [17]. The computational approach legalizes the calculations of the copious number of quantitative descriptors on the foundation of molecular structural particulars and is very effective in optimizing important details such as biological activity or toxicity. Meanwhile, virtual screenings are useful in providing additional guidance for the design and development of new potent anti-microbial agents [18, 19].

To evaluate our previously reported α-methylene-γ-butyrolactone derivatives [20] as anti-microbial agents virtually we exploited *in-silico* computational tools to virtually screen for their pharmacodynamic, pharmacokinetic, drug-likeness, bioactivity, and molecular docking traits.

EXPERIMENTAL

Ligand identification

The ligands to be screened in the present study i.e. α-methylene-γ-butyrolactones analogs have been synthesized and characterized previously [20].



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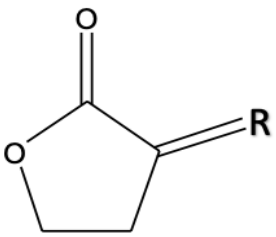

R= CH_2, C_2H_4, C_3H_6, C_4H_8, C_5H_{10}, C_6H_{12}.

2-7

Figure 1: Structures of the selected compounds 1-7 for *in-silico* studies.

In-silico predictive studies

Effective and efficient integration of pharmacokinetics/pharmacodynamics (PK/PD) traits of compounds in initial stages of development will not only assist with the compound selection but also guides the devising of systematic clinical development tactics [21]. SwissADME [22] and ProTox [23] were utilized to predict

the PK/PD properties of the target ligands **1-7**. For the prediction of the drug-like properties, drug-likeness is considered to be one of the qualitative ideas employed and it plays a pivotal role in demonstrating whether the studied drug examinees are alike the known drugs or not. The targeted ligands **1-7** were assessed for predicting their drug-likeness on the motive of five distinct filters namely Egan [24], Ghose [25], Muegge [26], Veber [27] and Lipinski [28] rules which were further escorted by bioavailability and drug-likeness scores using the Molsoft software (https://www.molsoft.com).

Also, these compounds were evaluated for *in-silico* bioactivity prediction using the software from Molinspiration Cheminformatics server, which screens the compounds against six important classes of drugs, i.e. G protein-coupled receptors ligand (GPCR ligands), ion channel blockers/modulators, kinase inhibitors, nuclear receptor ligands, protease inhibitors, and enzyme inhibitors.

In-silico molecular docking screenings were engaged to understand the interaction between the drug and the chosen receptor. While crafting the drug molecules to be effective antimicrobial agents, the supreme targets considered as a prime choice are those enzymes that are associated with the biosynthetic procedure of the microbe cell wall. To achieve this goal, the enzyme glucosamine-6-phosphate synthase (GlmS, GlcN-6-P synthase, L-glutamine: D-fructose-6P amido-transferase, EC 2.6.1.16) was contemplated for performing the docking studies [29]. The earlier mentioned enzyme is required both in the fungal and bacterial cell walls in the outset of the main building block viz. N-acetyl Glucosamine (the core amino sugar) [30, 31]. ACD/Labs-Chemsketch program was engaged in crafting 3D atomic coordinates of the ligands. The PDB ID, 2VF5 was considered for docking studies and it was obtained from the protein data bank (PDB) (Source: www.rcsb.org/pdb/). Utilization of the Dundee PRODRG2 server endeavored to do energy minimization [32]. Autodock4 program having a graphical user interface from "Auto-Dock Tools (ADT, 1.5.6)" was approached for the docking studies [33] and was implemented to recognize torsion angles in ligands, add the solvent model and allocate the Gasteiger charges to both the ligand and the protein. A 60x60x60 point grid was constructed representing X, Y, Z-axis. The values of RMSD (root mean square deviation) was utilized for cluster investigation. Furthermore, the cluster obtaining the lowest value for energy was appraised to be the most authentic solution. UCSF Chimera 1.11.2 and mcule, a web interface was employed for 3D visualization of the ligand-protein alliance.

RESULT AND DISCUSSION

The research work outlined here was escorted with the motive to foretell the physicochemical hallmarks, pharmacokinetic/ADME and toxicity traits, drug-likeness, and molecular docking screenings of our previously reported compounds **1-7** using *in-silico* computational tools.

Assorted physicochemical attributes like some rotatable bonds, count of specific atom types, lipophilicity, water solubility, and molecular refractivity were itemized. Topological Polar Surface Area (TPSA) is a fundamental physiochemical trait appraised for gauging drug transport characteristics. Calculations were attempted to forecast *in-silico* % absorption for all the aimed compounds by the reported formula (%ABS = 109-(0.345 X TPSA) [33]. All the targeted ligands revealed excellent *in-silico* % absorption with the highest being 99.93%. These physicochemical traits are given in Table 1.

Table 1: Physicochemical properties of the selected compounds 1-7.

Comp. No.	Fraction Csp3[a]	No. of rotatable bonds	HBA[b]	HBD[c]	iLogP[d]	M.R[e]	Log *S*[f]	TPSA[g]	In-silico % absorption
1	0.88	5	5	0	1.92	50.57	S	71.64	84.28
2	0.40	0	2	0	1.31	24.85	S	26.30	99.93
3	0.50	0	2	0	1.57	29.65	S	26.30	99.93
4	0.57	1	2	0	1.79	34.46	S	26.30	99.93
5	0.62	2	2	0	2.02	39.27	S	26.30	99.93
6	0.67	2	2	0	2.23	44.07	S	26.30	99.93
7	0.70	4	2	0	2.49	48.88	S	26.30	99.93

[a]The ratio of sp^3 hybridized carbons over the total carbon count of the molecule; [b]number of hydrogen bond acceptors; [c]number of hydrogen bond donors; [d] lipophilicity; [e]Molar refractivity, [f]Water solubility (SILICOS-IT [S=Soluble]); [g]topological polar surface area (Å^2).

Predictions of the pharmacokinetic/ADME and toxicity properties of the tested compounds **1-7** are given in Table 2. All the screened compounds demonstrated high gastrointestinal (GI) absorption and emerged as P-gp (p-glycoprotein) non-inhibitors. All the tested molecules were efficient to move through the blood-brain barrier (BBB) except compound **2**. The forecast for the HIA (human gastrointestinal absorption), passive BBB permeation, and P-gp substrates is revealed jointly in the BOILED-Egg diagram as displayed in Figure 2. None of the tested ligands impede the Cytochrome P450 isomers and emerged to be low on skin permeability. Compound 1,2

and 7 are predicted to be non-toxic (class 6) in nature while the rest of the compounds fall in class 5, that it may be harmful if swallowed.

Table 2. Pharmacokinetic/ADME and toxicity properties of the selected compounds 1-7.

Comp. No	Pharmacokinetic/ADME properties									Toxicity
	GI abs[a]	BBB permeant[b]	P-gp substrate[c]	CYP1A2 inhibitor[d]	CYP2C19 inhibitor[e]	CYP2C9 inhibitor[f]	CYP2D6 inhibitor[g]	CYP3A4 inhibitor[h]	Log K_p[i]	LD_{50} (mg/kg)
1	High	Yes	No	No	No	No	No	No	-7.45	25000
2	High	No	No	No	No	No	No	No	-6.37	11990
3	High	Yes	No	No	No	No	No	No	-6.29	3700
4	High	Yes	No	No	No	No	No	No	-6.07	5000
5	High	Yes	No	No	No	No	No	No	-5.76	5000
6	High	Yes	No	No	No	No	No	No	-5.67	5000
7	High	Yes	No	No	No	No	No	No	-5.17	8000

[a]Gastro Intestinal absorption, [b]Blood Brain Barrier permeant, [c]P-glycoprotein substrate, [d] CYP1A2: Cytochrome P450 family 1 subfamily A member 2 (PDB:2HI4), [e] CYP2C19: Cytochrome P450 family 2 subfamily C member 19 (PDB:4GQS), [f]CYP2C9: Cytochrome P450 family 2 subfamily C member 9 (PDB:1OG2), [g] CYP2D6: Cytochrome P450 family 2 subfamily D member 6 (PDB:5TFT), [h] CYP3A4: Cytochrome P450 family 3 subfamilies A member 4 (PDB:4K9T), [i]Skin permeation in cm/s.

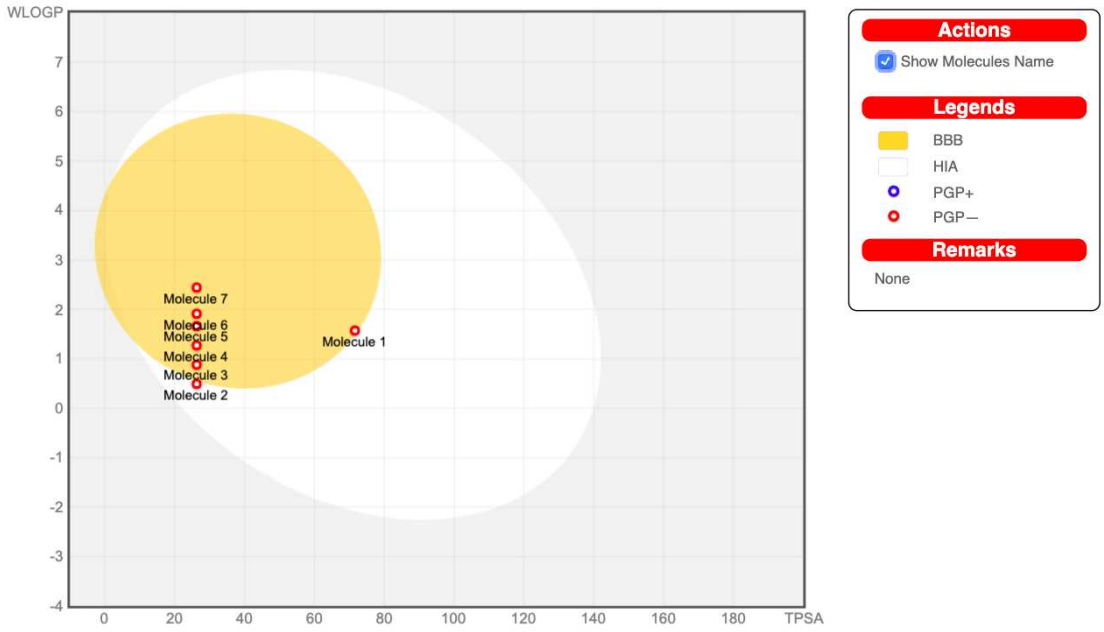

Figure 2: BOILED-Egg diagram of the selected compounds 1-7.

Drug likeness is explored as an essential element that furnishes the base for the candidates to be an influential drug aspirant. Several rules namely Lipinski, Ghose, Veber, Egan, and Muegge were contemplated to predict drug-likeness of the candidate molecules (**1-7**) to realize whether they can be potential drug candidates as per some acute benchmarks like molecular weight, LogP, number of hydrogen bond donors and acceptors. The number of breaches to the above-revealed rules jointly with bioavailability and drug-likeness scores is revealed in Table 3. None of the compounds **1-7** violated Lipinski, Veber, and Egan rule. All the compounds except **1** and **7** breached Ghose rule with 1-3 violations. All the compounds except for compound **1**, discarded to be drug-like with one violation as per Muegge rule. All the tested compounds exhibited bioavailability scores around 0.55 and flaunted moderate drug-likeness scores ranged from -1.67 to -0.84. The bioavailability radar of the compounds **1-7** is showcased in figure 3. Forecast of bioactivity scores of all the tested ligands against six different protein structures namely GPCR ligand, ion channel modulator, a kinase inhibitor, nuclear receptor ligand, protease inhibitor, and enzyme inhibitor is given in Table 4. All these integers stipulate binding affinity of the targeted ligands (**1-7**) to the declared receptors and enzymes. Most of the tested compounds have displayed negative bioactivity while compounds **1** and **7** displayed affinities towards some of the selected protein structures. The prime *in-silico* conformation was endorsed by employing molecular docking studies of the compounds **1-7** on GlcN-

6-P synthase (PDB ID: 2VF5) as the target protein receptor. To achieve this objective AutoDock 4.0 was appointed. The docking pose of the ligand **6** is manifested in Figure 4 and their docking scores are shown in Table 3. Docking scores range from -3.6 to -5.1.

Table 3. Drug likeness predictions and docking scores of the selected compounds 1-7.

Comp. No.	Lipinski violations	Ghose violations	Veber violations	Egan violations	Muegge violations	Bioavailability Score	Drug Likeness score	Docking score
1	0	0	0	0	0	0.55	-0.88	-5.1
2	0	3	0	0	1	0.55	-1.67	-3.6
3	0	3	0	0	1	0.55	-1.35	-4.0
4	0	3	0	0	1	0.55	-1.11	-4.1
5	0	2	0	0	1	0.55	-1.08	-4.3
6	0	1	0	0	1	0.55	-0.84	-4.7
7	0	0	0	0	1	0.55	-0.96	-4.6

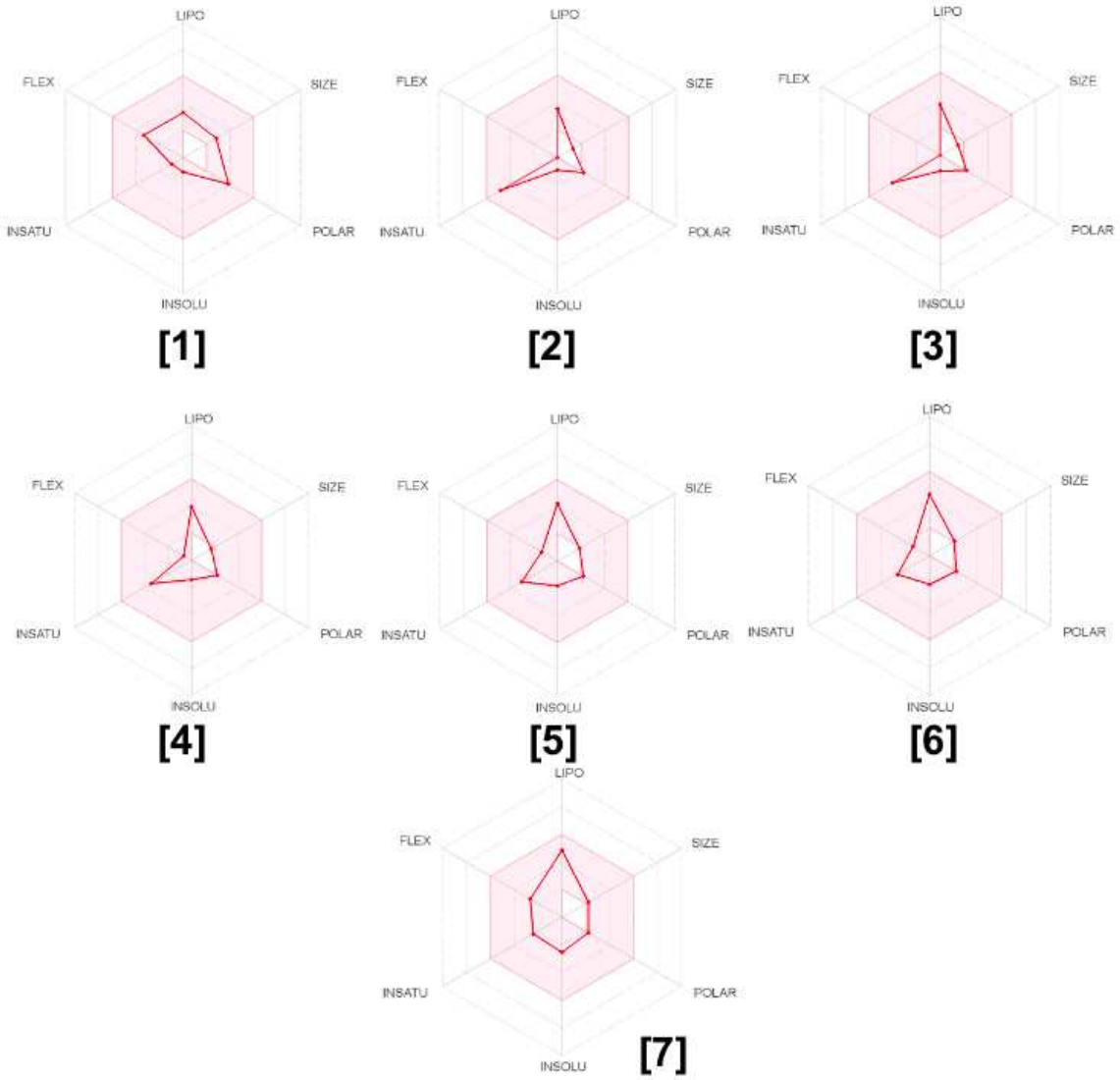

Figure 3: Bioavailability Radar of the tested compounds (1-7) The pink area represents the optimal range for each property (lipophilicity: XLOGP3 between − 0.7 and + 5.0, size: MW between 150 and 500 g/mol, polarity: TPSA between 20 and 130 (Å^2)., solubility: log S not higher than 6, saturation: the fraction of carbons in the sp^3 hybridization not less than 0.25, and flexibility: no more than 9 rotatable bonds. In this example, the compound is predicted not orally bioavailable, because too flexible and too polar.

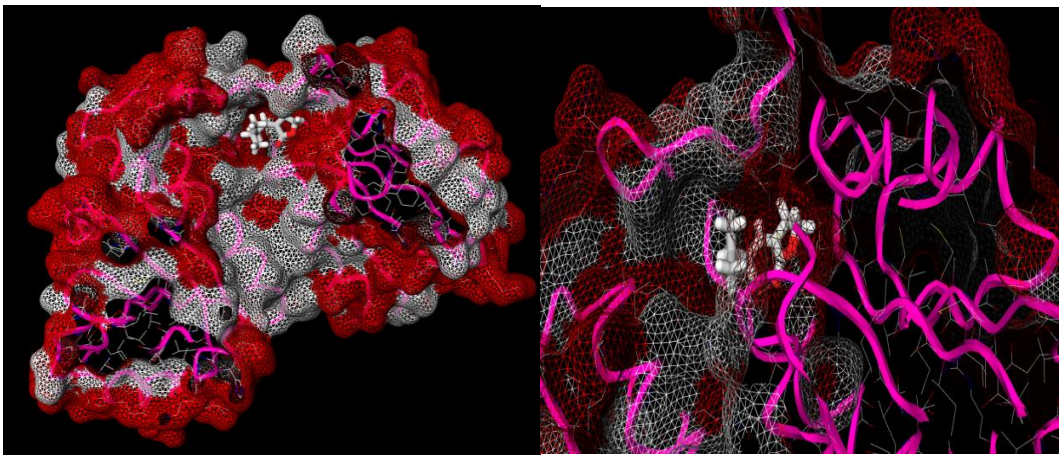

Figure 4: Docking of compound 6 into the active site of GlcN-6-P synthase (PDB ID: 2VF5).

Table 4: Bioactivity of the selected compounds 1-7.

Comp. No.	GPCR Ligand	Ion channel modulator	Kinase inhibitor	Nuclear receptor ligand	Protease inhibitor	Enzyme inhibitor
1	-0.55	0.24	-0.70	-0.71	-0.18	0.31
2	-3.22	-3.34	-3.51	-2.24	-3.43	-2.92
3	-2.94	-2.62	-2.96	-2.07	-3.26	-2.13
4	-1.89	-1.46	-2.00	-1.38	-2.24	-1.16
5	-0.76	-0.46	-0.79	-0.32	-1.10	-0.09
6	-0.73	-0.38	-0.76	-0.27	-0.86	-0.11
7	-0.53	-0.28	-0.54	-0.12	-0.80	0.06

CONCLUSION

The research work consolidated here is related to the *in-silico* forecasting of the physicochemical and ADMET features, drug-likeness, and molecular docking of some previously reported α-methylene-γ-lactone derivatives utilizing online available servers. The anticipated study revealed that all the screened compounds **1-7** displayed excellent *in-silico* % absorption where the highest obtained value was 99.93%. The screened compounds have showcased high GI absorption and are forecasted to be CNS active candidates as they can easily cross the blood-brain barrier (BBB) except compound **2** which is considered to be CNS inactive. All the tested compounds emerged to be non-inhibitors of P-gp and Cytochrome P450 isomers and possess low skin permeability. Compounds **1,2** and **7** are non-toxic as their LD_{50} is more than 5000 mg/kg while the rest of the compounds emerged to, may be harmful if swallowed. All the screened compounds were like a drug according to Lipinski, Veber, and Egan rule while except compound **1** and **7**, all the candidates breached Ghose rule. Also, most of the compounds except compound **1**, rejected to be drug-like with 1 violation according to the Muegge rule. Their bioavailability score is 0.55 and their drug-likeness and docking scores range from -1.67 to -0.84 and -3.6 to -5.1 respectively. The compound **6** has emerged to be the best compound out of the α-methylene-γ-lactone derivatives in the *in-silico* predictions with a drug-likeness score of -0.84 and docking score of -4.7. Most of the tested compounds have displayed low bioactivity scores while compound **1** and **7** flaunted affinity towards some of the selected protein structures. In conclusion, these forecasted evaluations give the information about the physico-chemical as well as ADMET attributes along with illuminating the drug-likeness, bioactivity, and binding/affinity fashion on the target protein of the tested ligands thus aiding the lead for the drugs of the future with additional efficacy.

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