



In Silico Studies of New Telomerase Inhibitors

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Abstract

Many studies have demonstrated that treatment with NSAIDs reduces the incidence and mortality of a wide range of tumors. The anticarcinogenic effects of NSAIDs are primarily attributed to their COX-2 inhibitory activity. However, increasing evidence suggests the involvement of molecular targets other than COX in the anti-proliferative effects and induction of apoptosis of selective COX-2 inhibitors such as telomerase enzyme. Telomerase has been found to be activated in more than 80% of human cancers and, therefore, can be considered as a potential marker for tumorigenesis. In this work, the drug-likeness of 14 reported 5,5-diphenylimidazolidine-2,4-diones, as potential COX-2 inhibitors, was calculated using Molsoft program and 10 of the tested compounds showed acceptable drug-likeness scores. Compound **3** which showed good COX-2 inhibitory activity is not expected to show acceptable pharmacokinetic profile according to the low drug-likeness score obtained (-0.11). However, the less potent compounds **10** and **13** showed the best drug-likeness scores 0.70 and 0.86, respectively. Docking of compound **13** into telomerase enzyme active site, showed three strong hydrogen bonds with the conserved aminoacids (LYS 406, & LYS 189 and ASP 343) and hence, can provide proper telomerase inhibition and consequently promising antitumor activity.

Keywords: *Telomerase; Antitumor activity; Docking; Drug-likeness*

1. Introduction

Cancer is caused by changes (mutations) to the DNA within cells. The DNA inside a cell is packaged into a large number of individual genes, each of which contains a set of instructions telling the cell what functions to perform, as well as how to grow and divide. Errors in the instructions can cause the cell to stop its normal function and may allow a cell to become cancerous. These genetic alterations may be either inherited or caused by carcinogenic agents (Yokota, 2000). These carcinogenic agents include Viruses (Ziegler et al., 2001; Cardiff, 1977), chemicals (Loli et al., 2004, Fisher et al., 2005, Benigi et al., 2000, and Jackson and Geopman, 1999) and radiation (Little, 2000, Yazzie et al., 2003). The main strategy in treatment of cancer is to find a way to specifically act on cancer cells without affecting normal cells to avoid undesirable side effects. The major clinical methods are cancer surgery, chemotherapy and radiotherapy which are considered as the most traditional treatments. New targeted strategies are developed based on technological advances that allow manipulation of nucleic acids and proteins such as monoclonal antibodies-targeted therapy. The telomere is a region of repetitive nucleotide sequences at each end of a chromosome (Figure

1). For vertebrates, the sequence of nucleotides in telomeres is TTAGGG with the complementary DNA strand being AATCCC, with a single-stranded TTAGGG overhang (Figure 2, Stewart et al., 2010). This sequence of TTAGGG is repeated approximately 2,500 times in humans and the average telomere length declines from about 11 kilobases at birth to less than 4 kilobases in old age, with the average rate of decline being greater in men than in women (Corey, 2009). During chromosome replication, the DNA polymerase that duplicates DNA cannot continue their duplication all the way to the end of a chromosome, so in each duplication the end of the chromosome is shortened (Stewart et al., 2010).

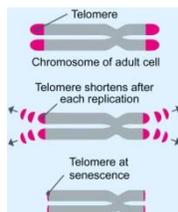


Figure 1: Chromosome and telomere

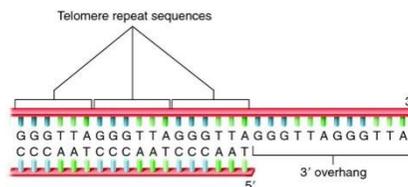


Figure 2: Telomere repeat sequence

The telomeres are disposable buffers at the ends of chromosomes which are truncated during cell division; their presence protects the genes before them on the chromosome from being truncated instead. Also, they maintain chromosomal stability by preventing attrition and end to end fusion. Telomeres become shorter with each cell division and can reach a point where they are no longer able to protect the chromosomes, leaving them open to deterioration. So, they are replenished by an enzyme, telomerase reverse transcriptase (Corey, 2009). Telomerase is a ribonucleoprotein complex that helps to stabilize telomere length in human stem cells, reproductive cells and cancer cells. It contains several protein subunits and one RNA Component (Figure 3, Popli et al., 2017). The human telomerase RNA component (hTERC) provides AAUCCC template to code for the G-rich telomeric DNA strand (TTAGGG). In addition to catalytic telomerase subunit hTERT (human telomerase reverse transcriptase) (Zhang and Wang, 2017).

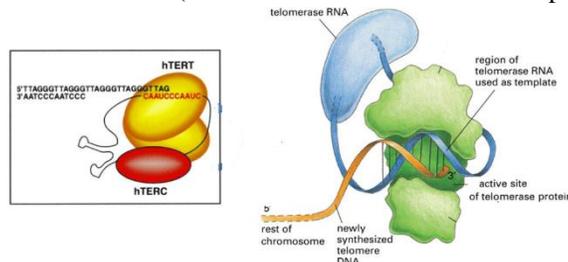
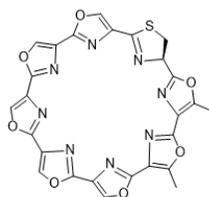


Figure 3: Telomerase structure

Telomerase is expressed in germline and embryonic stem cells as well as most somatic stem cells but is barely detectable in the great majority of adult somatic cells. Telomerase is up-regulated in the vast majority of cancer cells that are dependent upon this enzyme for maintaining their telomere lengths thereby conferring unlimited proliferative capacity or cellular immortality. Depending on the previous data, telomere length can be preserved by the telomerase enzyme, which synthesizes the new telomeric DNA from a RNA template, but it is normally restricted to cells needing a high proliferative capacity, such as stem cells. Consequently, telomerase-based therapies to elongate short telomeres were developed, some of which have successfully reached the stage I in clinical trials. However, Telomerase has been found to be activated in more than 80% of human cancers and, therefore, can be considered as a potential marker for tumorigenesis. Telomerase provides a good target not only for cancer diagnosis, but also for the development of novel therapeutic agents (Parkinson, 2003). Accordingly, the observation that the enzyme telomerase is up-regulated in 80–90% of all cancer cells isolated from primary human tumors but is absent in neighboring cells of healthy tissue has resulted in significant efforts to validate telomerase as an anticancer drug target and to develop effective approaches toward its inhibition (Stephanie et al., 2019).

Telomerase inhibitors, commonly derived from natural plant materials, include secondary metabolites such as polyphenols, alkaloids, terpenoids, xanthenes, and macrocyclic chemical compounds. They target telomerase

including hTERT and hTERC, telomerase substrates, and their associated proteins. Strategies to inhibit telomerase generally target either TERT or TERC, although indirect inhibitors targeting telomerase substrates and regulators are also used to a lesser extent, such as G-quadruplex binders (Martin et al., 1999). The utility of telomerase inhibition in combination with other chemotherapeutic agents was reported to enhance anticancer effects. For example, telomestatin was combined with imatinib, daunorubicin, mitoxantrone, or vincristine and was shown to enhance the sensitivity of these chemotherapeutic agents (Lucy and Trygve, 2008).



Telomestatin

In 2016, a series of 5,5-diphenylimidazolidine-2,4-dione was developed and evaluated for their anti-inflammatory and COX-2 inhibitory activities (Alaa et al., 2016). Selective COX-2 inhibitors appeared to be sufficiently safe to permit large-scale clinical testing and numerous clinical trials are currently underway to determine whether selective inhibitors of COX-2 are effective in the prevention and treatment of cancer (Alaa et al., 2016). Many studies have demonstrated that treatment with NSAIDs reduces the incidence and mortality of a wide range of tumors. The anticarcinogenic effects of NSAIDs are primarily attributed to their COX-2 inhibitory activity.

However, increasing evidence suggests the involvement of molecular targets other than COX in the anti-proliferative effects and induction of apoptosis of selective COX inhibitors such as telomerase enzyme (Hua et al., 2006).

Based on these studies, *in silico* studies including drug-likeness study of the reported 5,5-diphenylimidazolidine-2,4-diones (Figure 4), as potential antitumor agents was calculated in this work using Molsoft program as a qualitative concept used to estimate the bioavailability of molecules from the molecular structure before the substance is even synthesized and tested to aid the search for molecules with potential ability to be drug candidates. In addition, molecular docking was performed on the compound that showed the best drug-likeness score to get insight in its degree of recognition with the conserved aminoacids of the telomerase enzyme active site (code: 3DU6) as promising lead in the cancer cure era (Kim et al., 1994).

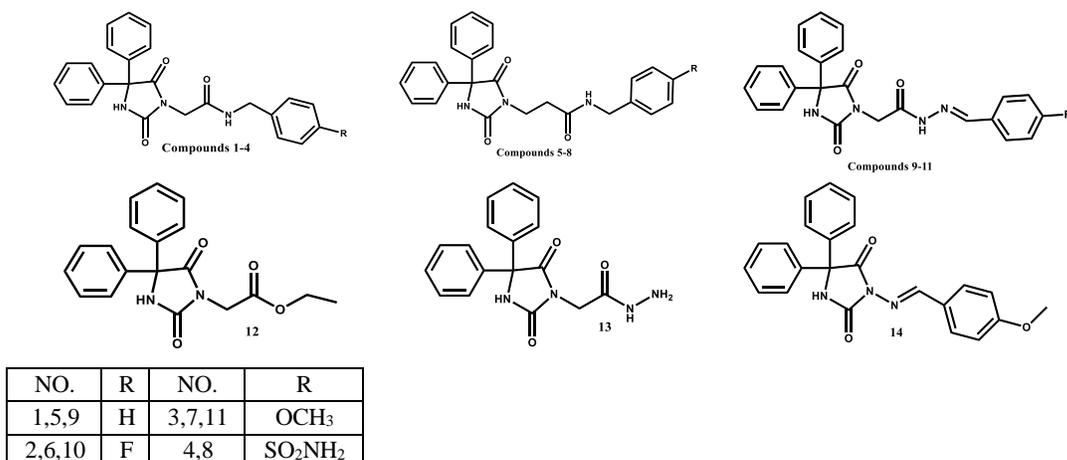


Figure 4: Reported 5,5-diphenylimidazolidine-2,4-diones

2. Materials and methods

2.1. Drug-likeness study

The drug-likeness study has been performed using Molsoft molecules *in-silico* drug likeness and molecular property prediction tool. Chemdraw software was used for sketching the structures of some reported 5-diphenylimidazolidine-

2,4-diones. Molecular properties, Lipinski's rule of 5 (RO5) and drug likeness score were predicted using the software from Molsoft server (<http://www.molsoft.com>) in which we can draw or import the ligand file from chemdraw. All molecular property predictors are calculated using fragment-based contributions like LogP (octanol/water partition coefficient) and LogS (water solubility). Molecular Polar Surface Area (PSA) was also calculated based as a sum of fragment contributions. PSA is defined as sum of surfaces of oxygens, nitrogens and attached hydrogens and it is a very useful parameter for prediction of drug transport properties. Drug-likeness score Predicts an overall drug-likeness score using and Molsoft's chemical fingerprints.

Lipinski's rule states that, in general, an orally active drug has no more than one violation of the following criteria¹⁶:

- ❖ No more than 5 hydrogen bond donors (the total number of nitrogen–hydrogen and oxygen–hydrogen bonds)
- ❖ No more than 10 hydrogen bond acceptors (all nitrogen or oxygen atoms)
- ❖ A molecular mass less than 500 daltons
- ❖ An octanol-water partition coefficient (log P) not greater than 5

2.2. Molecular docking study

Moreover, molecular docking of compound 13 into the binding site of telomerase was carried out using the MOE software package (version 2007). The three-dimensional structures of the aforementioned compounds were constructed using Chem. 3D Ultra 11.0 software, and Gasteiger–Hückel charges of ligands were assigned. They were energetically minimized by using AMBER with 100 iterations and minimum RMS gradient of 0.10. The template (PDB code: 3DU6) was retrieved from the RCSB Protein Data Bank (**Figure 5**). In addition, docking of compound 13 was also performed using both Molegro and Schrodinger programs.

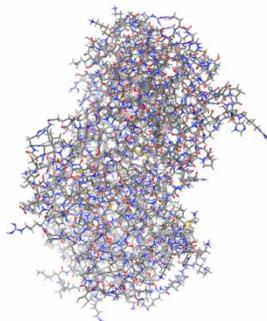


Figure 5: Telomerase enzyme (3DU6) obtained from PDB

3. Results and discussion

All the tested compound showed no violation and exhibited complete agreement with the Lipinski's rule and also showed good polar surface area (less than 140) which indicated good membrane permeability of these compounds (**Table 1**). Regarding the drug-likeness scores, compounds **1,2,4,6 and 8-14** showed good scores ranging from 0.09 to 0.86 and expected to have favorable pharmacokinetic properties (**Figure 6**).

However, only four compounds among the tested ones (**3,5,7 and 12**) have weak drug-likeness scores ranging from -0.11 to -0.26 and hence they do not match ideal requirements to be best drug candidate.

No.	HBD	HBA	Mollogp	MollogS	MolPSA	M. Wt.	Drug likeness score
1	2	3	3.01	-4.17 (in Log (moles/L)) 26.72 (in mg/L)	66.05	399.45	0.09
2	2	3	3.28	-4.69 (in Log (moles/L)) 8.49 (in mg/L)	66.05	417.44	0.42
3	2	4	3.10	-4.43 (in Log (moles/L)) 15.79 (in mg/L)	73.59	429.48	-0.11
4	4	6	1.82	-4.70 (in Log (moles/L)) 9.57 (in mg/L)	116.65	478.52	0.17
5	2	3	3.29	-3.94 (in Log (moles/L)) 47.86 (in mg/L)	66.03	413.48	-0.24
6	2	3	3.56	-4.45 (in Log (moles/L)) 15.18 (in mg/L)	66.03	431.47	0.09
7	2	4	3.38	-4.20 (in Log (moles/L)) 28.22 (in mg/L)	73.57	443.50	-0.34
8	4	7	2.17	-4.48 (in Log (moles/L)) 16.93 (in mg/L)	111.13	492.55	0.01
9	2	4	3.39	-4.92(in Log (moles\L)) 4.90 (in mg/L)	76.95	412.45	0.51
10	2	4	3.66	-5.44 (in Log (moles/L)) 1.55(in mg/L)	76.95	430.44	0.70
11	2	5	3.48	-5.18 (in Log (moles/L)) 2.89 (in mg/L)	84.50	442.48	0.12
12	1	4	2.36	(in Log (moles/L))-2.83 (in mg/L)498.79	62.01	338.36	-0.26
13	4	4	0.25	-3.18 (in Log (moles/L)) 450.52 (in mg/L)	90.25	324.34	0.86
14	1	4	4.37	-6.51 (in Log (moles/L)) 0.12 (in mg/L)	59.25	399.16	0.06

Table 1: Predicted drug likeness scores for diphenylimidazolidine compounds

HBD: Number of hydrogen bond donors

HBA: Number of hydrogen bond acceptors

Mollogp: LogP (octanol/water partition coefficient)

MollogS: Water solubility

MolPSA: Molecular Polar Surface Area

M. Wt.: Molecular Weight

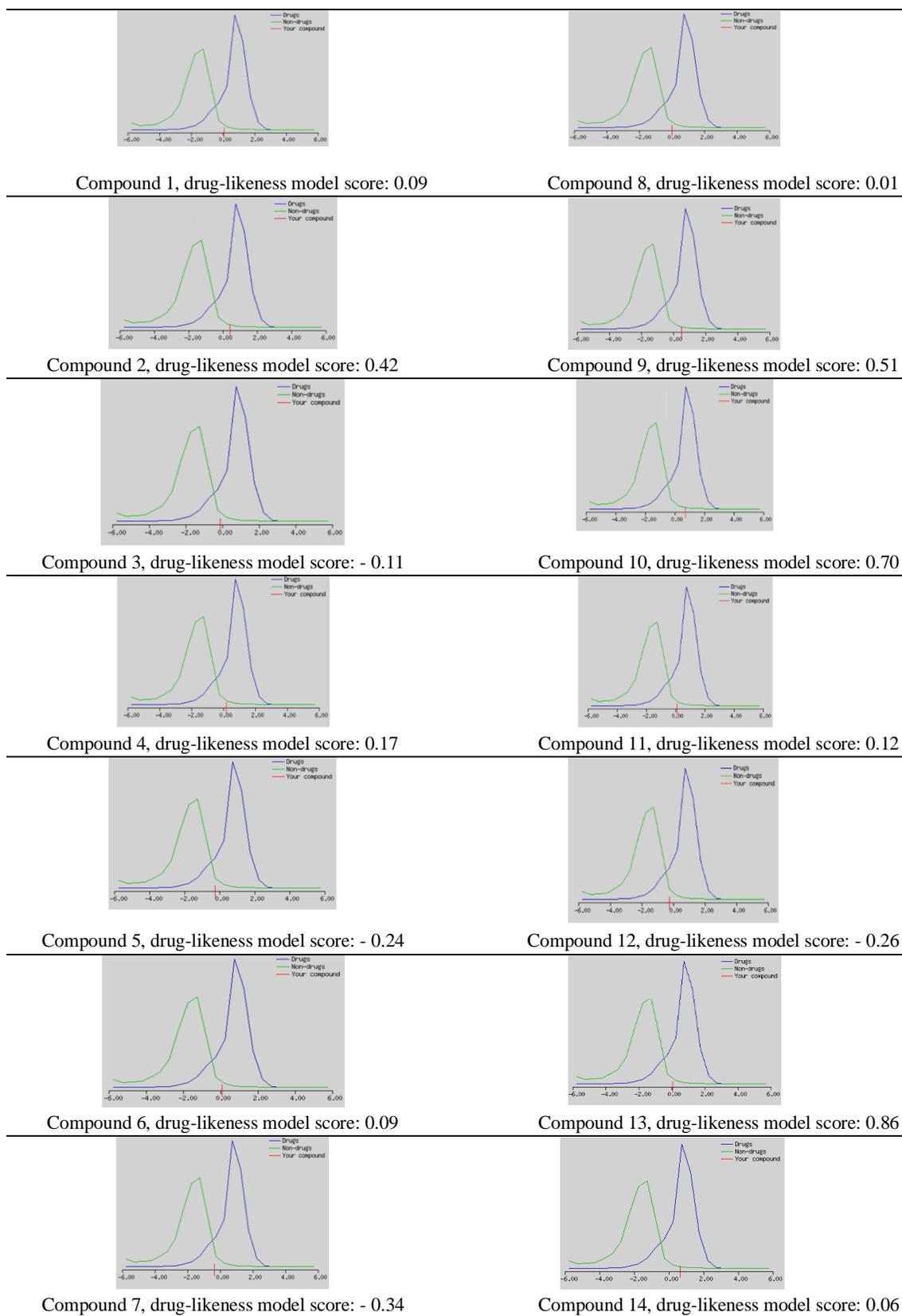


Figure 6: Drug-likeness model scores of compounds 1-14

Docking of compound **13** which showed the best drug-likeness score (0.86) into the binding site of telomerase enzyme showed that, the two crucial key amino acids namely LYS 406 (Lysine) & LYS 189 afford proper recognition with the two carbonyl active functions one of the imidazole moiety and the other of the terminal hydrazine branched. The carboxylic carbonyl group of ASP 343 (aspartic acid) showed proper hydrogen bonding with the corresponding hydrazine amino group. The three hydrogen bonds are strong enough to provide proper telomerase inhibition and consequently promising antitumor activity and these crucial bonds appeared with the three docking softwares (**Figures 7-9**).

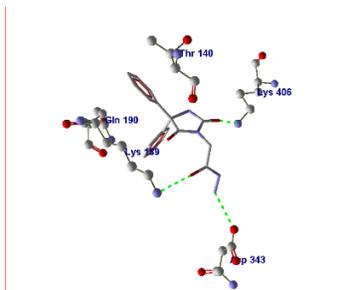


Figure 7: Molecular docking of compound **13** at the ATP binding site of 3DU6 using Molegro software.

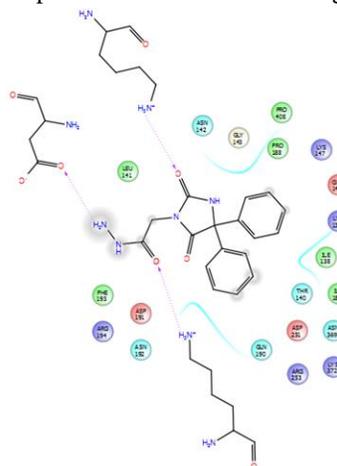
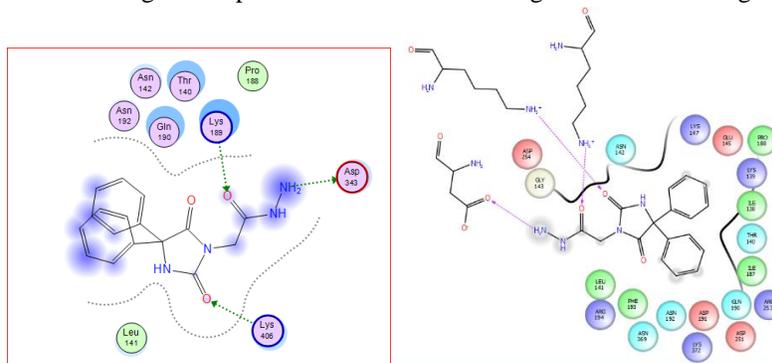


Figure 8: Molecular docking of compound **13** at the ATP binding site of 3DU6 using Schrodinger software.



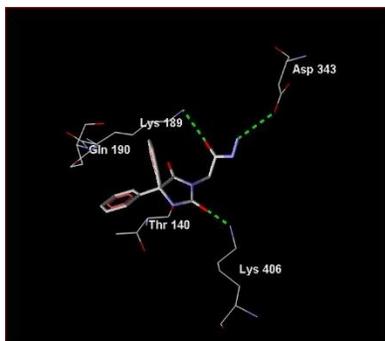


Figure 9: Molecular docking of compound 13 at the ATP binding site of 3DU6 using MOE software.

4. Conclusion

Compounds **2**, **4** and **6** which reported to have good experimental bioactivity as COX-2 inhibitors can be orally bioavailable drug candidates according to the high drug-likeness scores (0.09 to 0.42) obtained from the calculations using Molsoft program. However, the reported compound **3** which showed good COX-2 inhibitory activity is not expected to show acceptable pharmacokinetic profile according to the low drug-likeness score obtained (-0.11). So, highly potent COX-2 inhibitors may not be the most pharmacokinetically favorable agents. Therefore, it can be advantageous to choose less potent, but more orally bioavailable candidates for further studies such as compounds **10** and **13** with the best drug-likeness scores 0.70 and 0.86, respectively. So, the rational design of new drugs provides useful tools for synthesis of promising drug candidates, thus saving time and costs during drug development.

Disclosure

This research did not receive any specific grant from funding agencies. There is no Conflict of Interest

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