 doi:10.20944/preprints202009.0689.v1

Molecular Docking Studies of α-Pyrone Derivatives isolated from *Alternaria phragmospora* as CRM1 Inhibitors

Ahmed M. Metwaly[1]*, Ahmad E. Mostafa[1], Ahmed Ibrahim H. Eissa[2]*

[1]Department of Pharmacognosy, Faculty of Pharmacy, Al-Azhar University, Cairo, Egypt.

[2]Department of Pharmaceutical chemistry, Faculty of Pharmacy, Al-Azhar University, Cairo, Egypt.

Corresponding authors *

Ibrahim H. Eissa, Department of Pharmaceutical chemistry, Faculty of Pharmacy, Al-Azhar University, Cairo, Egypt ; E-mail: ibrahimeissa@azhar.edu.eg

Ahmed M. Metwaly, Department of Pharmacognosy, Faculty of Pharmacy, Al-Azhar University, Cairo, Egypt ; E-mail: ametwaly@azhar.edu.eg

 doi:10.20944/preprints202009.0689.v1

Abstract

Some α-Pyrone derivatives isolated from *Alternaria phragmospora* fungus showed promising anti leukemic activities, while others were inactive. CRM1/XPO1 (chromosome region maintenance 1 protein, also called exportin1 or PO1 in humans) has been chosen as a target for antileukemic molecular docking study for those compounds to understand their modes of interaction and structure activity relationships. The results showed that two (**2** and **4**), out of six, natural α-Pyrone derivatives exhibited well-established interactions with the amino acids of the receptor, which was in agreement with the experimental anti-leukemic results of these compounds. Moreover, twenty hypothetical chemically modified α-Pyrone derivatives (**7**-**27**) have been designed. Compounds **7**, **8**, **22** and **24** showed more efficient docking properties than the previously considered natural compounds.

Keywords: Molecular docking, α-Pyrone, antileukemic, CRM1inhibitors

 doi:10.20944/preprints202009.0689.v1

1. INTRODUCTION:

Endophytic fungi can be considered as an untapped reservoir for biologically active secondary metabolites belonging to different chemical classes such as alkaloids(1), steroids(2), terpenoids(3), pyranones(4), quinones(5), isochromenes(6) α-pyrones(7, 8) and benzopyran derivatives(9)

Molecular docking of a drug molecule with a receptor (target) gives important information about drug receptor interaction and commonly used to find out the binding orientation of drug candidates to their protein targets in order to predict the affinity and activity(10).

Computer-Aided Drug Design (CADD) is a specialized discipline that uses computational methods to stimulate drug-protein interaction(11).

The major nuclear exporter protein CRM1 (chromosome region maintenance 1 protein, also called exportin1 or XPO1 in humans) is one of seven exportins mediate the transport of 220 proteins and several mRNAs. Interestingly, CRM1 is the sole nuclear exporter of the major tumour suppressor and growth regulatory Proteins.(12)

CRM1/XPO1 has been found to be a target in chronic lymphocytic leukemia and the selective CRM1/XPO1 inhibitors have been showed efficacy with an acceptable therapeutic index(13).

The natural product Leptomycin B (LMB**)** act as an efficient selective inhibitor of nuclear export mediated by the chromosomal region maintenance 1 protein (CRM1). (14) .

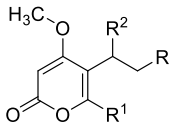
Keeping in view the therapeutic importance of α-pyrone derivatives as antitumor (15),(16), and in continuation of our work on separation of biologically active α-pyrone derivatives, we herein report the molecular docking of six previously reported α-pyrone derivatives. These compounds were screened for their antileukemic activity before(8). The

 doi:10.20944/preprints202009.0689.v1

molecular docking study of all the compounds has been done for the better understanding of the drug-receptor interaction. Moreover, we suggested hypothetical chemical modifications for the examined compounds to identify their antileukemic activity. This step may be an important step in the way to discover new antileukemic agents.

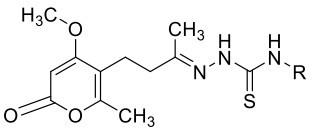
Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 28 September 2020 doi:10.20944/preprints202009.0689.v1

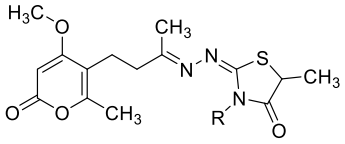
A

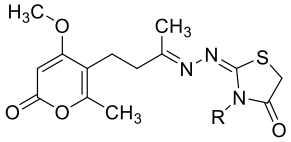


Compound	R	R^1	R^2
1	CH_2CH_3	CH_3	H
2	CH_2CH_3	CH_2OH	H
3	CH_2CH_3	CH_3	αOH
4	$COCH_3$	CH_3	H
5	OH	CH_3	H
6	$=CHCH_3$	CH_3	H

B

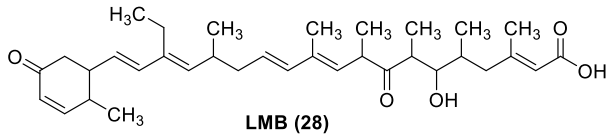






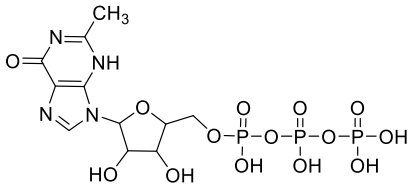
Compound	R	Compound	R	Compound	R
7	H	14	H	21	H
8	CH_3	15	CH_3	22	CH_3
9	CH_2CH_3	16	CH_2CH_3	23	CH_2CH_3
10	$CH_2CH_2CH_3$	17	$CH_2CH_2CH_3$	24	$CH_2CH_2CH_3$
11	$CH_2CH_2CH_2CH_3$	18	$CH_2CH_2CH_2CH_3$	25	$CH_2CH_2CH_2CH_3$
12	$CH_2CH_2CH_2CH_2CH_3$	19	$CH_2CH_2CH_2CH_2CH_3$	26	$CH_2CH_2CH_2CH_2CH_3$
13	$CH_2CH_2CH_2CH_2CH_2CH_3$	20	$CH_2CH_2CH_2CH_2CH_2CH_3$	27	$CH_2CH_2CH_2CH_2CH_2CH_3$

C



LMB (28)

D



Guanosine-5'- triphosphate(29)

Fig. 1: A) structures of the isolated compounds (**1-6**). B**)** Suggested hypothetical compounds (**7-27**). C) Leptomycin B (LMB). D) Guanosine-5'- triphosphate

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 28 September 2020 doi:10.20944/preprints202009.0689.v1

2. Materials and methods

2.1. Compounds for docking study

Six compounds (**1-6**) isolated from *Alternaria phragmospora* (Fig. 1A) and 21 (**7-27**) suggested hypothetical compounds (Fig. 1B) were chosen for binding analysis with CRM1/XPO1.

2.2. Ligand preparation

Ligand and the designed compounds 2D structures were sketched using ChemBioDraw Ultra 14.0 and saved in .sdf format. Sdf file opened, 3D structures are protonated and energy minimized by applying CHARMM force fields for charge, and MMFF94 force field for partial charge, then prepared for docking by optimization the parameters.

2.3. Protein preparation

The 3D crystal structure of CRM1/XPO1 was downloaded from the protein data bank. Before docking, water molecules were removed from protein file. Crystallographic disorders and unfilled valence atoms were corrected using alternate conformations and valence monitor options. Protein was subjected to energy minimization by applying CHARMM (Chemistry at HARvard Macromolecular Mechanics) force fields for charge, and MMFF94 (Merck Molecular force field) force field for partial charge. Inflexibility of structure is obtained by creating fixed atom constraint.

2.4. Docking studies

Molecular docking was performed using Discovery the Dock ligands (CDOCKER) protocol which is an implementation of the CDOCKER algorithm. CDOCKER is a grid-based molecular docking method that employs CHARMm-based molecular dynamics (MD) scheme to dock ligands into a receptor binding site. The receptor is held rigid while the ligands are allowed to flex during the refinement.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 28 September 2020 doi:10.20944/preprints202009.0689.v1

3. Result and discussion

The compounds were docked against CRM1 protein (PDB code: 3M1I, resolution: 2.00A) using discovery studio 2.5 to evaluate the free energy (ΔG) and mode of binding with the active site of CRM1 protein.

All molecules (**1-27**) were docked against the generated target grid. The Guanosine-5'-triphosphate -bound CRM1 (Fig. 1D) and LMB (Fig. 1C) were used as reference ligands. The active pocket of CRM1consists of amino acid residues as Gly22, Lys39, Lys125, Lys40, Ile42, Thr26, Thr44, Lys25, Thr27 (Fig. 2).

3.1. Docking of reference ligands

The proposed binding mode of Guanosine-5'- triphosphate revealed an affinity value of -72.15 kcal/mol. The tri phosphate group formed hydrogen bonds with Gly22, Lys25, Lys40, Ile42, Thr26, Thr44, Lys25 and Thr27. These are key amino acids acting as a gate for ligand entrance to the CRM1. The Guanine moiety formed hydrogen bonds with The27, Lys40, and Lys39 and occupied Lys25, Lys125, Lys154, Glu38, and Phe37 in the hydrophobic pocket of CRM1. The ribose sugar moiety formed hydrogen bonds with Gly22, Lys125, Lys39, Lys40 (Fig. 2).

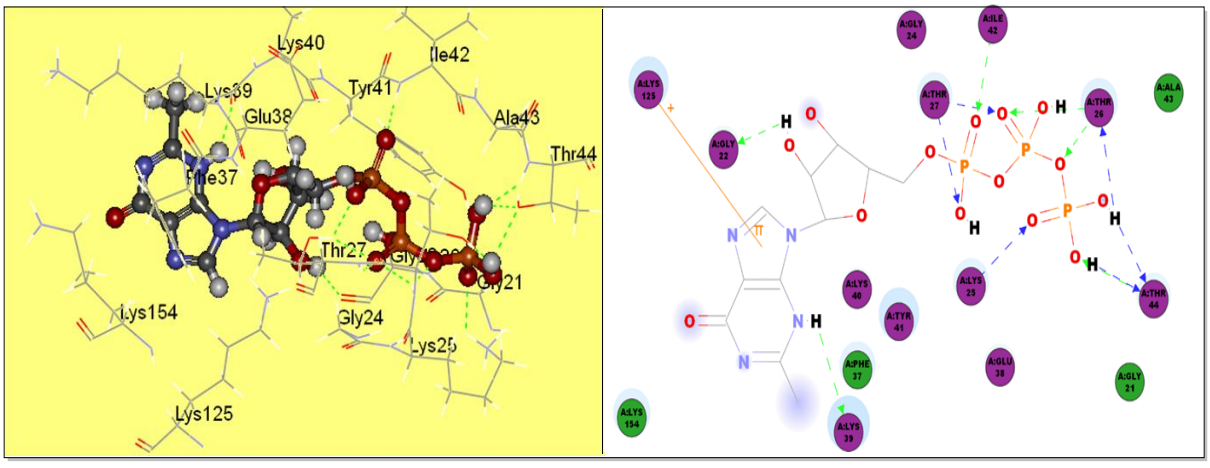


Fig. 2: 3D crystal structure of Guanosine-5'- triphosphate in binding pocket of CRM1

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 28 September 2020 doi:10.20944/preprints202009.0689.v1

The proposed binding mode of LMB revealed an affinity value of -70.98 kcal/mol. LMB contains a β-hydroxy-ketone moiety formed a hydrogen bond with Ile42, a terminal carboxylate formed two hydrogen bonds with Lys25 and Asp20 and an α,β-unsaturated δ-lactone moiety binded to the hydrophobic groove of CRM1 which formed of Lys25, Lys125, Lys154, Glu38, and Phe37 (14) (Fig. 3).

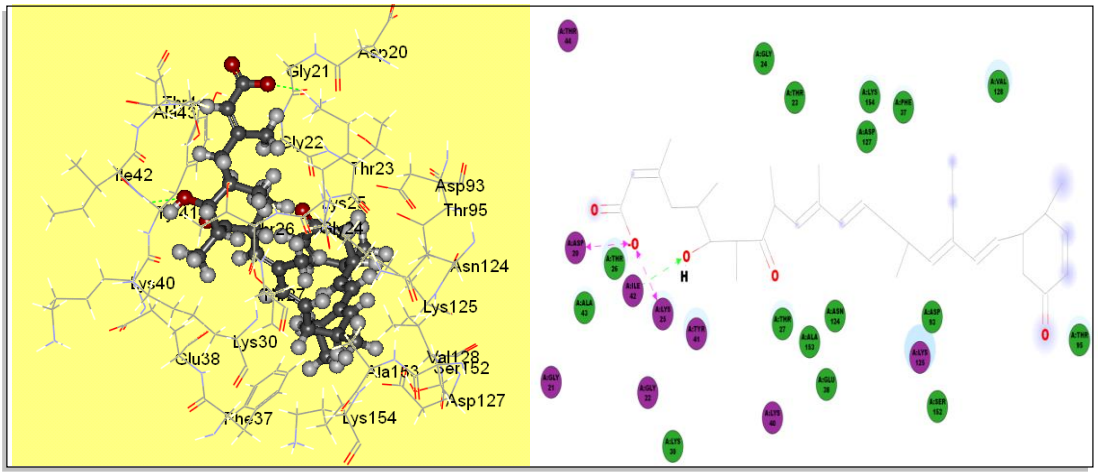


Fig. 3: Binding interaction of LMB in binding pocket of CRM1.

3.2. Docking of the isolated compounds (1-6)

Six α-Pyrone derivatives (**1-6**) isolated from *Alternaria phragmospora* fungus have been docked with CRM1. The obtained results of the free energy of binding (ΔG) explained that only two compounds had moderate binding affinity toward the receptor and the computed values reflected the overall trend (Table 1). Compounds **2** and **4** exhibited well established bonds with the amino acids of the receptor.

The proposed binding mode of compound **2** revealed a binding affinity value of -32.82 kcal/mol. The hydroxyl group formed two hydrogen bonds with Lys125 with 1.85 A ˚ and Gly22 with 1.86 A ˚, while the methoxy group formed another hydrogen bond with Thr27 with

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 28 September 2020 doi:10.20944/preprints202009.0689.v1

2.24 A ˚. The α-pyrone moiety occupied the hydrophobic pocket formed by Lys25, Lys125, Lys154, Glu38, and Phe37 (Fig. 4A).

The proposed binding mode of compound **4** revealed an affinity value of - 35.27 kcal/mol, where the carbonyl group formed two hydrogen bonds with Lys125 with 2.22 A ˚ and 1.32 A ˚. The α-pyrone moiety occupied the hydrophobic pocket formed by Lys25, Lys125 Glu38, and Phe37 (Fig. 4B).

The proposed binding mode of compounds **1**, **3**, **5** and **6** revealed affinity values of -20.18, -22.13, -23.23 and -21.26 kcal/mol respectively. Compounds **1**, **5** and **6** did not form any hydrogen bond with the target protein while compound **3** formed one hydrogen bond (Fig. 5).

The interactions of compounds **2** and **4** exhibit weak hydrogen bond interaction compared to Guanosine-5'- triphosphate and LMB. These modes of interactions of compounds **2** and **4** may explain the moderate binding free energy and antileukemic activity. The interactions of compounds **1**, **5** and **6** did not form any hydrogen bond interaction while compound **3** formed only one hydrogen bond interaction. The modes of interactions of compounds **1**, **3**, **5** and **6** may explain the weak binding free energy and antileukemic activity. These data found to be in agreement with the published antileukemic data for compounds (**1-6**) before(8).

The weak hydrogen bond interaction of separated compounds (**1-6**) may be due to the absence of hydrophilic tail in the chemical structure. This explanation drove us to suggest hypothetical chemical modifications for the separated compounds and identify their modes of interactions with the target protein and hence antileukemic activity.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 28 September 2020 doi:10.20944/preprints202009.0689.v1

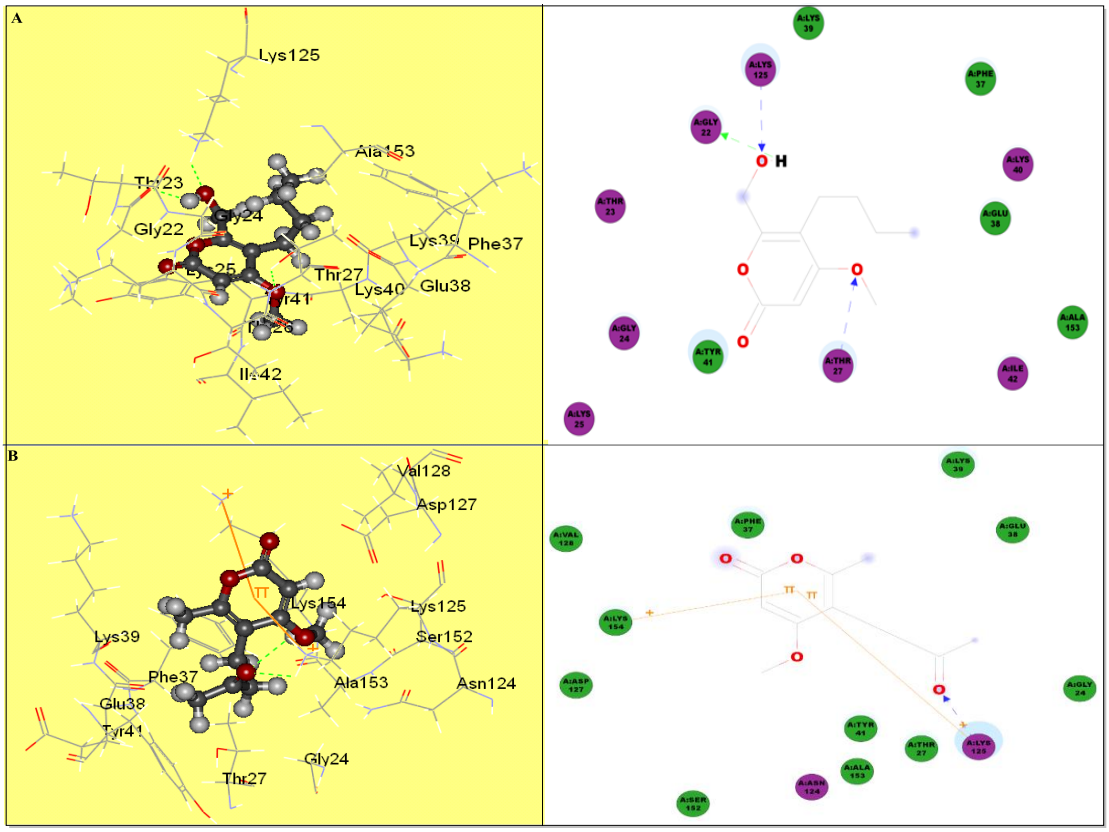
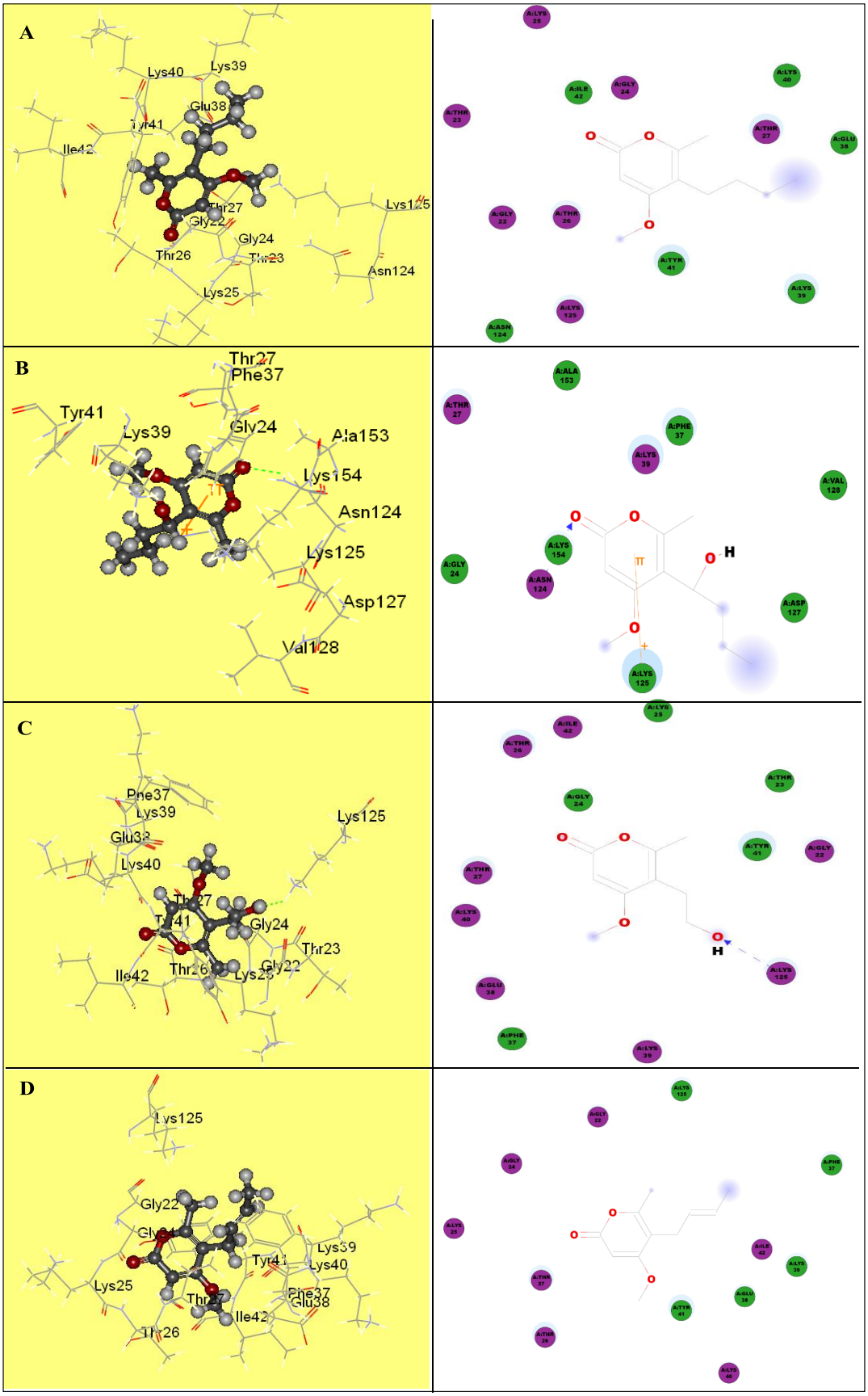


Fig.4: A) Binding interaction of compound **2** in binding pocket of CRM1.

B) Binding interaction of compound **4** in binding pocket of CRM1.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 28 September 2020 doi:10.20944/preprints202009.0689.v1



Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 28 September 2020 doi:10.20944/preprints202009.0689.v1

Fig. 5: Binding poses of highly ranked conformers for compounds **1**, **3**, **5** and **6** inside the binding pocket of CRM1

3.3. Docking of suggested hypothetical compounds (7-27)

All compounds possessed the required binding energy to dock itself with the binding pocket of CRM1 ranging from - 41.97 to -65.82 kcal/mol. (Table 1).

Compounds **7**, **8**, **22** and **24** exhibited the most correct binding mode and binding free energy (ΔG).

The proposed binding mode of compound **7** (affinity value of - 55.23 kcal/mol and three hydrogen bonds) (Fig. 6A) where the oxygen atom of pyrone moiety formed a hydrogen bond with Lys125 with a distance of 2.42 A ˚, the sulfur atom formed another hydrogen bond with Thr23 with a distance of 2.16 A ˚, the amino group of side chain formed a hydrogen bond with Thr26 with a distance of 1.95 A ˚ and the,α- pyrone moiety occupied the hydrophobic pocket formed by Lys25, Lys125, Lys154, Glu38, and Phe37.

The proposed binding mode of compound **8** (affinity value of – 61.76 kcal/mol and three hydrogen bonds) (Fig. 4B) where the oxygen atom of pyrone moiety formed a hydrogen bond with Lys125 with a distance of 2.45 A ˚, the sulfur atom formed another hydrogen bond with Gly24 with a distance of 2.39 A ˚, the methyamino group of side chain formed a hydrogen bond withThr26 a distance of 2.23 A ˚ and the α- pyrone moiety occupied the hydrophobic pocket formed by Lys25, Lys125, Lys154, Glu38, and Phe37 (Fig. 6B).

The proposed binding mode of compound **22** (affinity value of – 60.33 kcal/mol and two hydrogen bonds) (Fig. 4C) where sulfur atom of methylthiazolidin-4-one moiety formed a hydrogen bond with Gly24 with a distance of 2.16 A˚, the carbonyl group of methylthiazolidin-4-one moiety formed another hydrogen bond with Thr44 with a distance of 1.96 A˚ and the α - pyrone moiety occupied the hydrophobic pocket formed by Lys25, Lys125, Lys154, Glu38, and Phe37 (Fig. 6C).

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 28 September 2020 doi:10.20944/preprints202009.0689.v1

The proposed binding mode of compound **24** (affinity value of –65.82kcal/mol and two hydrogen bonds) (Fig. 4D) where sulfur atom of methylthiazolidin-4-one moiety formed a hydrogen bond with Gly24 with a distance of 2.27 A˚, the carbonyl group of methylthiazolidin-4-one moiety formed another hydrogen bond with Thr44 with a distance of 2.23 A˚ and the α - pyrone moiety occupied the hydrophobic pocket formed by Lys25, Lys125, Lys154, Glu38, and Phe37 (Fig. 6A).

The chemical modification by insertion of hydrophilic tail such as thiosemicarbazide and methylthiazolidin-4-one moieties may be responsible for the increased binding free energy. The docking results of the suggested hypothetical compounds may lead to discovery a new promising antileukemic drug.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 28 September 2020 doi:10.20944/preprints202009.0689.v1

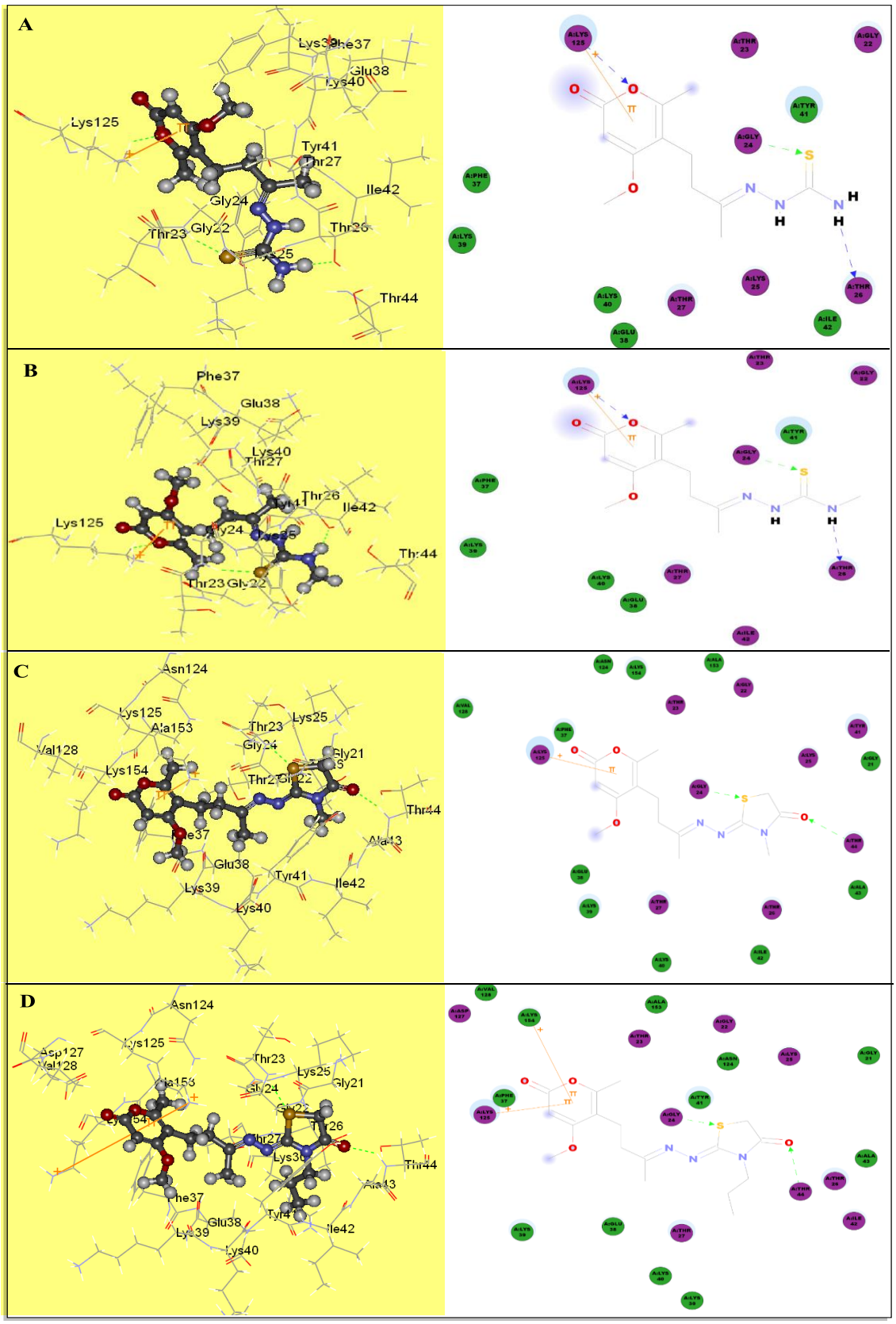


Fig. 6: Binding poses of highly ranked conformers for compounds number **7**, **8**, **22** and **24** inside the binding pocket of CRM

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 28 September 2020 doi:10.20944/preprints202009.0689.v1

Table 1: The docking binding free energies of compounds (1-29)

Comp. No.	Binding free energy (kcal/mol)	Comp. No	Binding free energy (kcal/mol)
1	-20.18	16	-49.83
2	-32.82	17	-50.46
3	-22.13	18	-51.64
4	-35.27	19	-46.31
5	-23.23	20	-51.72
6	-21.26	21	-46.91
7	-55.23	22	-60.23
8	-61.76	23	51.18
9	-60.11	24	-65.82
10	-58.18	25	-41.97
11	-58.22	26	-52.25
12	-49.63	27	-49.64
13	-51.23	LMB	-70.98
14	-47.17	Guanosine-5'-triphosphate	-72.15
15	-51.57		

4. Conclusion

Six α-pyrone derivatives have been isolated before from the endophytic fungus *Alternaria phragmospora*. Two of them showed promising antileukemic activities. Chromosome region

maintenance 1 protein (CRM1/XPO1) (PDB ID: 3M1I) was used to find the best inhibitors by studying the interaction between 3M1I protein receptor and the isolated compounds. Based on the docking score, compound **2** and **4** showed moderate CDOCKER energy. These data found to be in agreement with the published antileukemic data for compounds (**1**-**6**) before(8). Suggested hypothetical chemical modifications for the isolated compounds has been proceeded and resulted in obtaining compounds (**7**-**27**). Compounds **7**, **8**, **22** and **24** showed better binding activities with protein receptor. This study could be utilized for the designing and synthesis of effective drugs for the treatment of leukemia.

References

1. Ahmed M. Metwaly MMG, Arafa Musa. Two New Antileishmanial Diketopiperazine Alkaloids from the Endophytic Fungus Trichosporum sp. Derpharmachemica. 2015;7(11):322-7.
2. Gu W. Bioactive metabolites from Alternaria brassicicola ML-P08, an endophytic fungus residing in Malus halliana. World Journal of Microbiology and Biotechnology. 2009;25(9):1677-83.
3. Liebermann B, Nussbaum R-P, Günther W. Bicycloalternarenes produced by the phytopathogenic fungus< i> Alternaria alternata</i>. Phytochemistry. 2000;55(8):987-92.
4. Sheridan H, Canning A-M. Novel radicinol derivatives from long-term cultures of Alternaria chrysanthemi. Journal of natural products. 1999;62(11):1568-9.
5. Suemitsu R, YAMADA Y, SANO T, YAMASHITA K. Phytotoxic activities of altersolanol A, B and dactylariol, and activities of altersolanol A against some microorganisms. Agricultural and biological chemistry. 1984;48(9):2383-4.
6. Metwaly AM, Kadry HA, Atef A, Mohammad A-EI, Ma G, Cutler SJ, et al. Nigrosphaerin A a new isochromene derivative from the endophytic fungus Nigrospora sphaerica. Phytochemistry letters. 2014;7:1-5.
7. Metwaly AM, Wanas AS, Radwan MM, Ross SA, ElSohly MA. New α-Pyrone derivatives from the endophytic fungus Embellisia sp. Medicinal Chemistry Research. 2017;26(8):1796-800.
8. Metwaly AM, Fronczek FR, Ma G, Kadry HA, Atef A, Mohammad A-EI, et al. Antileukemic α-pyrone derivatives from the endophytic fungus Alternaria phragmospora. Tetrahedron letters. 2014;55(24):3478-81.
9. Metwaly A, Kadry H, El-Hela A, Elsalam A, Ross S. New antimalarial benzopyran derivatives from the endophytic fungus Alternaria phragmospora. Planta Medica. 2014;80(10):PC11.
10. Bano S, Alam MS, Javed K, Dudeja M, Das AK, Dhulap A. Synthesis, biological evaluation and molecular docking of some substituted pyrazolines and isoxazolines as potential antimicrobial agents. European journal of medicinal chemistry. 2015;95:96-103.
11. Chen GS, Chern J-W. Computer-aided drug design: Wiley, NY, USA; 2007.
12. Etchin J, Sanda T, Mansour MR, Kentsis A, Montero J, Le BT, et al. KPT-330 inhibitor of CRM1 (XPO1)-mediated nuclear export has selective anti-leukaemic activity in preclinical models of T-cell acute lymphoblastic leukaemia and acute myeloid leukaemia. British journal of haematology. 2013;161(1):117-27.

 doi:10.20944/preprints202009.0689.v1

13. Lapalombella R, Sun Q, Williams K, Tangeman L, Jha S, Zhong Y, et al. Selective inhibitors of nuclear export show that CRM1/XPO1 is a target in chronic lymphocytic leukemia. Blood. 2012;120(23):4621-34.
14. Sun Q, Carrasco YP, Hu Y, Guo X, Mirzaei H, MacMillan J, et al. Nuclear export inhibition through covalent conjugation and hydrolysis of Leptomycin B by CRM1. Proceedings of the National Academy of Sciences. 2013;110(4):1303-8.
15. KOYAMA K, OMINATO K, NATORI S, TASHIRO T, TSURUO T. Cytotoxicity and antitumor activities of fungal bis (naphtho-. GAMMA.-pyrone) derivatives. Journal of pharmacobio-dynamics. 1988;11(9):630-5.
16. KOYAMA K, NATORI S. Chaetochromins B, C and D, bis (naphtho-. GAMMA.-pyrone) derivatives from Chaetomium gracile. Chemical and pharmaceutical bulletin. 1987;35(2):578-84.