Quality Improvement Study

Medicine®

OPEN

Computational study on natural compounds inhibitor of c-Myc

Junan Ren, MBBS[b], Yuhao Huangfu, MD[c], Junliang Ge, MM[b], Bo Wu, MD[b,d], Weihang Li, MM[f], Xinhui Wang, MD[b,e], Liyan Zhao, MD[a,*]

Abstract

To screen and identify ideal leading compounds from a drug library (ZINC15 database) with potential inhibition effect against c-Myc to contribute to medication design and development.

A series of computer-aided virtual screening techniques were performed to identify potential inhibitors of c-Myc. LibDock from the software Discovery Studio was used to do a structure-based screening after ADME (absorption, distribution, metabolism, excretion) and toxicity prediction. Molecular docking was utilized to show the binding affinity and potential mechanism between ligands and c-Myc. Stability of the ligand-receptor complex was analyzed by molecular dynamic simulation at the end of the research.

Compounds with more interactive energy which are confirmed to be the potential inhibitors for c-Myc were identified from the ZINC15 databases. Additionally, those compounds are also anticipated with fewer ames mutagenicity, rodent carcinogenicity, nondevelopmental toxic potential, and tolerant with cytochrome p450 2D6(CYP2D6). Dynamic simulation analysis also revealed that the very compounds had more favorable potential energy compared with 10058-F4(ZINC12406714). Furthermore, we prove that those compounds are stable and can exist in natural conditions.

This study demonstrates that the compounds are potential therapeutic inhibitors for c-Myc. These compounds are safe and stable for drug candidates and may play a critical role in c-Myc inhibitor development.

Abbreviations: BBB = blood–brain barrier, CYP2D6 = cytochrome P-450 2D6, DTP = Developmental Toxicity Potential, NTP = National Toxicology Program, PPB = plasma protein-binding.

Keywords: c-Myc inhibitor, virtual screening

1. Introduction

Oncogene Myc plays an essential part in oncogenic tumors such as breast, prostate, colon, and cervical cancers. It also contributes to lymphomas, myeloid leukaemia, small-cell lung carcinomas, and neuroblastoma.[1] The very gene has been discovered for 40 years, and its regulation and structural function are well understood. Furthermore, C-MYC, MYCN, and MYCL belong to the Myc oncogene family. Tightly got under controlled under normal circumstances, the *Myc* gene is highly upregulated and expressed in many cancers.[2–4]

The 100-amino acids-long C- terminal region of Myc proteins comprises the basic, helix-loop-helix, leucine zipper (bHLHLZ) dimerization, and DNA-binding (DBD) domains.[5] Max, a protein interacts with the C-terminal region, is a required step for Myc transcriptional activity.[6] In details, the Myc/Max heterodimer recruits a chromatin-modifying complex which consists of TRRAP, GCN5, TIP60, and TIP48. This specific complex activates transcription by binding to the conserved E-box DNA sequence (CACGTG) located in the transcriptional regulatory region of target genes.[7–9] Additionally, there are several other interactors with Myc's C terminus, including Miz1(Myc-interacting Zn-finger protein 1), ARF, and SKP2.[10–12]

Previously, there are few compounds which were identified for the direct inhibition of Myc-Max protein–protein interactions. In 2002, Berg et al[13] used a combinatorial library to discover IIA6B17. In the following year, Yin et al[14] employed yeast 2-hybrid system from the Chembridge DiverSet combinatorial library and identified 3 compounds—10058-F4, 10074-G5, and 10074-A4—which have complete specificity toward Myc-Max. Wang et al in 2007[15] also used the same system to try to develop a more selective analogue of 10058-F4 drug but failed to do so due to the lack of improvement from his selected analogues. Previous studies have found that benzamide can cause c-Myc loss in HL-60 cell line. Moreover, noninhibitory analogues of benzamide did not induce loss of MYC. There are few reports in this area in recent years. So benzamide was selected as negative

Editor: Eric Bush.

This study was supported by Special Project of Medical and Health Talents in Jilin Province (2019SRCJ018): Study on the role of PDGF-D high expression in promoting epithelial-mesenchymal transition in glioma cells and the regulation mechanism of Notch1 signaling pathway.

The authors report no conflicts of interest.

Supplemental Digital Content is available for this article.

All data generated or analyzed during this study are included in this published article (and its supplementary information files).

[a] Department of Clinical Laboratory, The Second Hospital of Jilin University, [b] Clinical College, Jilin University, Changchun, [c] Department of Medicine, Boston University, [d] Department of Orthopedics, [e] Department of Oncology, The First Hospital of Jilin University, Changchun, [f] Department of Orthopaedics, Xijing Hospital, The Fourth Military Medical University, Xian, China.

[*] Correspondence: Liyan Zhao, Department of Clinical Laboratory, The Second Hospital of Jilin University (e-mail: zhaoliy@jlu.edu.cn).

How to cite this article: Ren J, Huangfu Y, Ge J, Wu B, Li W, Wang X, Zhao L. Computational study on natural compounds inhibitor of c-Myc. Medicine 2020;99:50(e23342).

Received: 28 November 2019 / Received in final form: 9 April 2020 / Accepted: 23 October 2020

http://dx.doi.org/10.1097/MD.0000000000023342

control.[16] The major issues for the compounds that were identified were low potency, lack of selectivity, poor pharmacokinetic behavior, which hardly enable them to accumulate sufficient concentration to block Myc-Max concentration.[17] Therefore, it is urgent to develop new compounds which have high specificity as well as pharmacokinetics.

In the significant pharmaceutical market,[18,19] the natural products and their derivatives still play the major part in it. The very chemicals not only possess properties like unique chemical structures and potential biological functions, but they also contribute to the design and refinement of medication. For the past few years, several publications showed that small molecular compounds have the potential inhibitory effect of c-Myc. The goal of this study is to find lead compounds of c-Myc inhibitor for the development and medication of the drugs. A series of structural biologic and chemical methods were deployed and identify the lead compounds, and the study also predicts their absorption, distribution, metabolism, excretion, and toxicity. Furthermore, a list of candidates for the drugs as well as their pharmacological properties were shown to provide the basis for the development of c-Myc inhibitor research.

2. Methods and materials

2.1. Structure-based virtual screening using LibDock

Ligand-binding pocket region of c-Myc was chosen as the binding site to screen compounds that could potentially inhibit c-Myc. Virtual screening was performed with the aid of LibDock module of Discovery Studio 4.5.22. LibDock is a strictly based docking module which calculates hotspots, which are further aligned to create optimum interactions, for the protein using a grid placed into the binding site and polar and apolar probes. The Smart Minimizer algorithm and CHARMM force field (Harvard University, Cambridge, MA) was carried out to minimize ligand. Once minimization was completed, all ligand poses were ranked by the ligands score. The 2.0-Å crystal structure of human c-Myc (Protein Data Bank identifier: 5vhe) and the inhibitor 10058-F4 (ZINC15 database identifier: ZINC12406714) were downloaded and imported to the working circumstance of LibDock. Figure 1 shows the chemical structure of c-Myc. Crystal water and other heteroatoms around were removed to prepare for the protein. Additional hydrogen was added later followed by protonation, ionization, and energy minimization of the protein. The CHARMM force field and the Smart Minimizer algorithm were executed for energy minimization.[20] The 2000 steps minimization had a root mean square gradient tolerance of 10, and the final root means square gradient was 0.690. The prepared protein was applied to define the binding site. Using the ligands 10058-F4 binding position, the active site for docking was created. Docking all the prepared ligands was carried for the virtual screening at the defined active site using LibDock and generated individual LibDock score. All the docked poses were grouped based on the LibDock score, and all compounds were ranked by LibDock score accordingly.

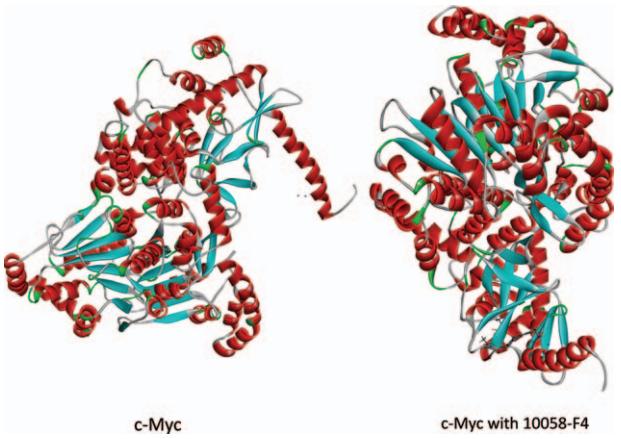

Figure 1. The molecular structure of c-Myc. The red represents negative charge, whereas the blue represents the positive charge. (A) The initial structure of c-Myc with the addition of surface binding area. (B) The structure when c-Myc binds with 10058-F4(ZINC000012406714).

2.2. Absorption, distribution, metabolism, and excretion and toxicity prediction

Calculation of absorption, distribution, metabolism, and excretion (ADME) of selected compounds, including their aqueous solubility, blood–brain barrier (BBB) penetration, cytochrome P-450 2D6 (CYP2D6) inhibition, hepatotoxicity, human intestinal absorption, and plasma protein-binding (PPB) level was employed by ADME module of Discovery Studio 4.5. TOPKAT module of Discovery Studio 4.5 was also employed to calculate the toxicity and other properties of all the potential compounds, such as US National Toxicology Program rodent carcinogenicity, Ames mutagenicity, developmental toxicity potential, and rat oral median lethal dose (LD50) and chronic oral lowest observed adverse effect level (LOAEL). These pharmacologic characteristics were all considered when filtering proper drug candidates.

2.3. Molecule docking and pharmacophore prediction

The module of Discovery Studio 4.5 was utilized for the analysis of molecular docking. CDOCKER is a molecular docking method based on CHARMM force field, which can produce high-precision docking results. The very force field was used for both receptors and ligands. The Receptor was firmly fixed, whereas ligands are permitted to flex around during the docking process. Discovery Studio calculated the CHARMM energy and the interaction energy, which indicates ligand-binding affinity in each complex. Crystal structure of c-Myc was extracted from the protein data bank. A rigid and semiflexible docking process was deployed to remove the crystal water molecules, causing the fixed water molecules to possibly affect the conformation of the receptor-ligand complex.[21,22] Hydrogen atoms were added to the protein after completed the above procedure. To confirm the reliability of the combination mode, the initial inhibitor compound 10058-F4 was obtained from the binding site and then redocked into the crystal structure of c-Myc to compare the root-mean-square deviation (RMSD) between the 2 conformations. The binding site sphere of c-Myc was defined as the regions that come within around a 3-Å radius from the geometric centroid of the ligand 10058-F4. During the docking procedure, the ligands were acceptable to bind with the residues of protein groups within the binding site sphere. The structures of identified hits were then created and docked into the binding pocket of c-Myc. Different poses of the respective ligand-c-Myc complex were generated and analyzed according to CDOCKER interaction energy. Pharmacophore of compounds was created and

visualized using 3D-QSAR pharmacophore generation module, which is a conformational space that produces up to 255 conformations per molecule to simulate a series of the small molecule of the same kind. Conformations with energy values within the energy threshold of 10 kcal/mol were allowed to be preserved.

2.4. Molecular dynamics simulation

The fittest binding conformations of the ligand-c-Myc complexes among the poses picked by the molecule docking program were prepared for the process of molecular dynamics simulation. The orthorhombic box was used to contain the ligand-complexes and solvated with an explicit periodic boundary solvation water model. Sodium chloride was added with the ionic strength of 0.145 to mimic the physiological environment. Then the system was exposed to the CHARMM force field, and its intensity is reduced by energy minimization (500 steps of steepest descent and 500 steps of the conjugated gradient), with the final root mean square gradient of 0.227. The system was driven from an initial temperature of 296 K to the target temperature of 302 K for 2 ps in a slow-motion following an equilibration simulation for 5 ps. Molecular dynamics simulation (production module) was carried out for 25 ps with a time step of 1 fs. The whole simulation was performed within a standard pressure as well as a temperature system with a constant temperature of nearly 300 K. The particle mesh Ewald algorithm was used to calculate long-range electrostatics, and the linear constraint solver algorithm was incorporated to fix all bonds involving hydrogen. Discovery Studio 4.5 analysis trajectory protocol determined the trajectory for RMSD, potential energy, and structural characteristics through using the initial complex setting as a reference.

3. Results

3.1. Virtual screening of natural products database on the inhibitor of c-Myc

Ligand-binding pocket was always an essential site of c-Myc. 10058-F4 has previously been selected as a potential inhibitor. And benzamide is confirmed as invalid inhibitors. Therefore, the pocket that 10058-F4 binds with c-Myc is selected to be the reference site. A total of 17,799 purchasable natural compounds and benzamide were extracted from ZINC15 database for the analysis.

Furthermore, the molecular structure of c-Myc was chosen as the receptor protein. A total of 6085 compounds successfully docked with protein c-Myc. A total of 1168 compounds had a higher score than 10058-F4 (LibDock score: 86.2782) after the initial analysis. And benzamide (LibDock score: 70.5673) has a lower fraction than 10058-F4. The top 20 compounds ranked according to LibDock score were listed in Table 1.

The top 20 high score LibDock compounds are listed in Table 1.

(All score LibDock compounds are listed in supplement Table 1, http://links.lww.com/MD/F222.)

Table 1

Top 20 ranked compounds with high Libdock scores.

No.	Compounds	Libdock Score
1	ZINC000002526389	141.675
2	ZINC000002526388	141.442
3	ZINC000004654971	140.38
4	ZINC000004098749	137.816
5	ZINC000038143594	137.436
6	ZINC000015122022	135.661
7	ZINC000004654958	135.292
8	ZINC000027646625	135.287
9	ZINC000013485434	132.397
10	ZINC000012495776	132.132
11	ZINC000015212183	131.763
12	ZINC000012495616	129.722
13	ZINC000008214697	129.359
14	ZINC000008689961	128.382
15	ZINC000027646625	127.866
16	ZINC000031165470	127.265
17	ZINC000229763735	126.773
18	ZINC000030731451	126.488
19	ZINC000003791929	124.933
20	ZINC000013485432	124.831

3.2. ADME and toxicity prediction

After the selection, pharmacological properties of all ligands from the compounds and 10058-F4 were predicted by ADME module of Discovery Studio 4.5. Human intestinal absorption, aqueous solubility, BBB, and cytochrome p450 2D6-binding, hepatotoxicity, and PPB properties (Table 2).

Defined in the water at 25°C, the aqueous solubility prediction demonstrates the solubility in water for all compounds. Fifteen compounds are found to have an ideal absorption level, and the rest of the 5 have poor to very poor absorption level in accordance with the intestinal absorption level listed. Nine compounds, in terms of binding affinity, have a strong absorbency with the plasma protein, whereas the other 11 are proved to have a poor binding affinity. Among the 20 compounds, 5 compounds are found to be hepatoxic. Sixteen of the 20 compounds do not inhibit the important metabolic enzyme cytochrome p450 2D6 (CYP 2D6) with the exceptions of ZINC000002526389, ZINC000002526388, ZINC000015122022, and ZINC000012495616.

In terms of the safety investigation, Ames Mutagenicity (AMES), Rodent carcinogenicity (US National Toxicology Program (NTP), as well as developmental toxicity potential properties, are analyzed using a module named TOP-KAT from the discovery studio 4.5 (Table 3). Eleven compounds are potentially not developmental toxic accordingly. Three compounds ZINC000004654958, ZINC000004654971, and ZINC000008689961 after filtration are considered to be ideal compounds for they share the properties of low Ames mutagenicity, rodent carcinogenicity, and developmental toxicity potential. They are also non-CYP2D6 inhibitors with lower hepatotoxicity than 10058-F4. In summary, ZINC000004654958, ZINC000004654971, and ZINC000008689961 were identified as safe drug candidates and were selected for the subsequent research (Fig. 2).

3.3. Ligand-binding analysis

The CDOCKER module applied in the study was proved to be reliable as the RMSD between the docked position and the chemical structure of the complex was 10. Under the force field CHARMm36, compounds ZINC000004654958, ZINC000004654971, and ZINC000008689961 were docked into the molecule structure of c-Myc via CDOCKER module. Table 4 is listed to show respective

Table 2

Adsorption, distribution, metabolism, and excretion properties of compounds.

Number	Compounds	Solubility level*	BBB Level†	CYP2D6‡	Hepatotoxicity§	Absorption‖ level	PPB level¶
1	ZINC000002526389	2	4	1	1	0	1
2	ZINC000002526388	2	4	1	1	0	1
3	ZINC000004654971	3	4	0	0	1	0
4	ZINC000004098749	3	4	0	1	1	0
5	ZINC000038143594	3	4	0	0	3	0
6	ZINC000015122022	2	4	1	0	2	1
7	ZINC000004654958	3	4	0	0	1	0
8	ZINC000027646625	3	1	0	0	0	0
9	ZINC000013485434	1	4	1	1	1	1
10	ZINC000012495776	4	3	0	0	0	1
11	ZINC000015212183	3	3	0	0	0	0
12	ZINC000012495616	3	4	0	1	3	0
13	ZINC000008214697	2	4	0	0	3	1
14	ZINC000008689961	3	0	0	0	0	0
15	ZINC000013485432	1	4	1	1	1	1
16	ZINC000027646625	3	1	0	0	0	0
17	ZINC000031165470	4	4	0	0	3	0
18	ZINC000229763735	3	4	0	0	0	1
19	ZINC000030731451	3	4	0	0	0	0
20	ZINC000003791929	0	4	0	0	3	1

BBB = blood–brain barrier, CYP2D6 = cytochrome P-450 2D6, PPB = plasma protein binding

* Aqueous-solubility level: 0 (extremely low); 1 (very low, but possible); 2 (low); 3 (good).

† Blood–brain barrier level: 0 (Very high penetrant); 1 (high); 2 (medium); 3 (low); 4 (undefined).

‡ Cytochrome P450 2D6 level: 0 (noninhibitor); 1 (inhibitor).

§ Hepatotoxicity: 0 (nontoxic); 1 (toxic).

‖ Human-intestinal absorption level: 0 (good); 1 (moderate); 2 (poor); 3 (very poor).

¶ Plasma protein-binding: 0 (absorbent weak); 1 (absorbent strong).

calculated CDOCKER potential energy, MM/GBSA-binding free energy. Those compounds show the characteristic to have a significant lower MM/GABA binding energy comparing to the reference compounds 10058-F4 (25.5844 kcal/mol). Another reason it would serve to be a better c-Myc inhibitor due to its lower energy. Structural computation was employed to analyze the hydrogen bonds and Pi-Pi interactions of ligands-c-Myc complex (Figs. 3 and 4, Table 5). The reference compound

Table 3

Toxicities of compounds.

		Mouse NTP*		Rat NTP†			
No. compounds		Female	Male	Female	Male	Ames‡	DTP
1	ZINC000002526389	0.999	0.036	0	0.999	0.999	0.769
2	ZINC000002526388	0.999	0.041	0	0.999	0.999	0.745
3	ZINC000004654971	0	0	0	0.001	0	0
4	ZINC000004098749	0	0	1	0	0	1
5	ZINC000038143594	0.061	0	0.274	0.088	0	1
6	ZINC000015122022	0	1	1	0	1	1
7	ZINC000004654958	0	0	0	0	0	0
8	ZINC000027646625	0	0	0	0	1	0
9	ZINC000013485434	0	0.014	1	0	1	0.821
10	ZINC000012495776	0.891	0.056	0	0	0.009	0
11	ZINC000015212183	0.003	0.261	1	1	0	0
12	ZINC000012495616	0.998	1	1	1	0.016	1
13	ZINC000008214697	0	0.003	0	1	0	0
14	ZINC000008689961	0	0	0	0	0	0
15	ZINC000013485432	0	0.014	1	0	1	0.821
16	ZINC000027646625	0	0	0	0	1	0
17	ZINC000031165470	1	0	0	0.004	0	0.999
18	ZINC000229763735	0.314	0	0.065	0.016	0.001	0.151
19	ZINC000030731451	1	0	0	0.816	0	0
20	ZINC000003791929	0	1	1	0	1	1

* <0.3 (noncarcinogen); >0.7 (carcinogen).

† <0.3 (nonmutagen); >0.7 (mutagen).

‡ <0.3 (nontoxic); >0.7 (toxic).

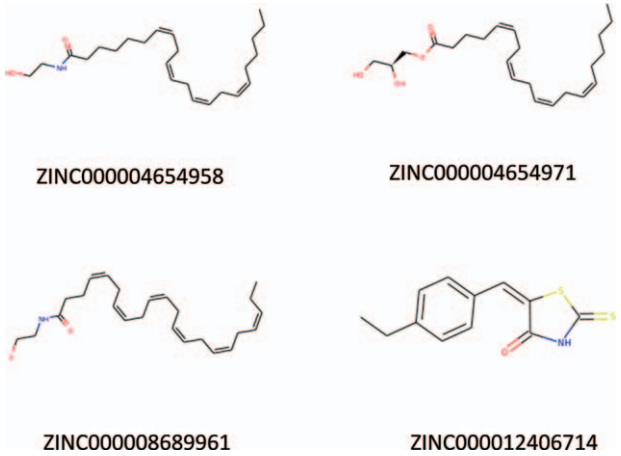

Figure 2. The structure of ZINC00012406714(10058-F4) and new compounds selected from virtual screening.

ZINC000012406714 (10058-F4) formed 2 pairs of hydrogen bonds with c-Myc, which includes O14 of the compound to A: ARG849: HA of c-Myc and the compound to A:LEU850: HN. As for Pi-Pi interaction, the compound ZINC000012406714 includes the compound to A: TRP887. It is shown that compound ZINC000004654971 formed 4 pairs of hydrogen bonds with c-Myc, which includes O21 of the compound to A:LEU850:HN of c-Myc, O22 of the compound to A:ASN886:HD21 of c-Myc, O22 of the compound to A:ASN886:HD22 of c-Myc, O25 of the compound to A:ASN886:HD22 of c-Myc. ZINC000004654958 only shares 1 hydrogen bond with c-Myc which its O23 binds to A: ASN886: HD22 of c-Myc. ZINC000008689961 has 2 extra hydrogen bonds compared to the former drug candidate (A:LYS847:HZ1: ZINC000008689961:O27, A:LYS847:HE1: ZINC000008689961:O23, A:TRP935:O: ZINC000008689961: H60). Worth mentioning is that the selected 3 compounds were failed to see any pi-pi interaction with m-Myc protein.

Table 4

CDOCKER potential energy of compounds with c-Myc under CHARMm36 force field.

Compound	CDOCKER potential energy, kcal/mol
ZINC000004654971	−18.004
ZINC000004654958	−19.9436
ZINC000008689961	−54.544
ZINC000012406714	25.5844

3.4. Molecular dynamics simulation

Evaluation of the stabilities of ligand-c-Myc complexes under natural environment was employed via molecular dynamics simulation. The RMSD curves and potential energy statistics are displayed in Figure 5. The complexes trajectories at 50 ps reached equilibrium. It was also known that the potential energy of respective complexes was stabilized with time. The simulation also validates the stabilization between c-Myc and the potential

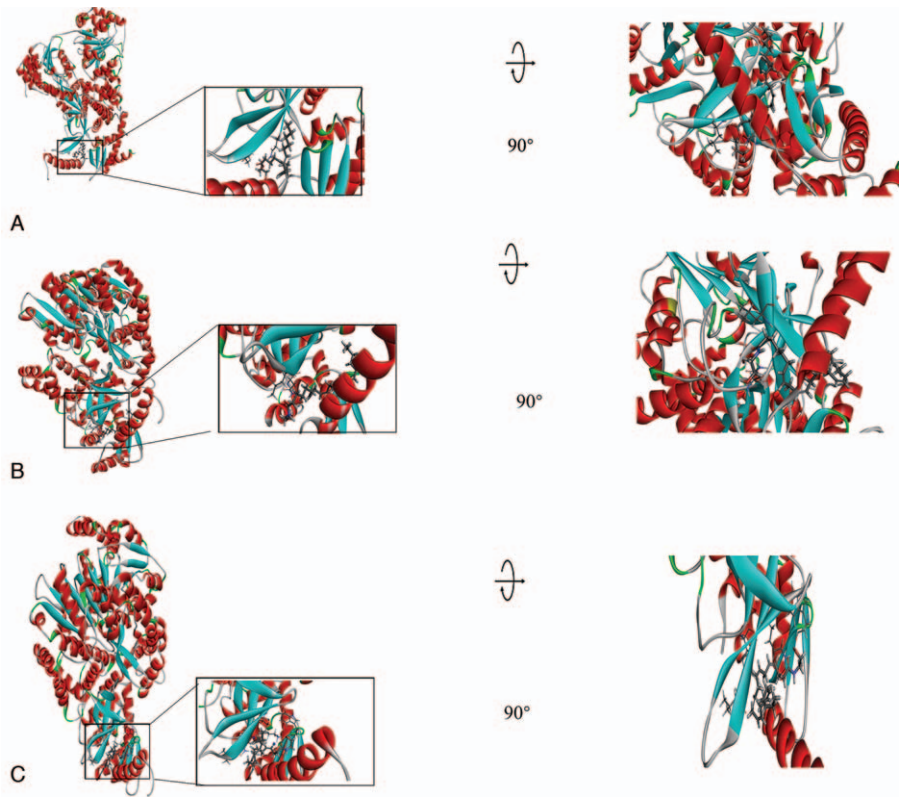

Figure 3. Schematic drawing of interaction between ligands and c-Myc; the color blue represented positive charge, whereas the red represented negative charge. Thicker sticks are used to represent ligand, whereas thinner sticks were used to show the structure around the ligand-receptor junction. (A) ZINC000004654971-c-Myc complex; (B) ZINC000004654958-c-Myc complex; (C) ZINC000008689961-c-Myc complex.

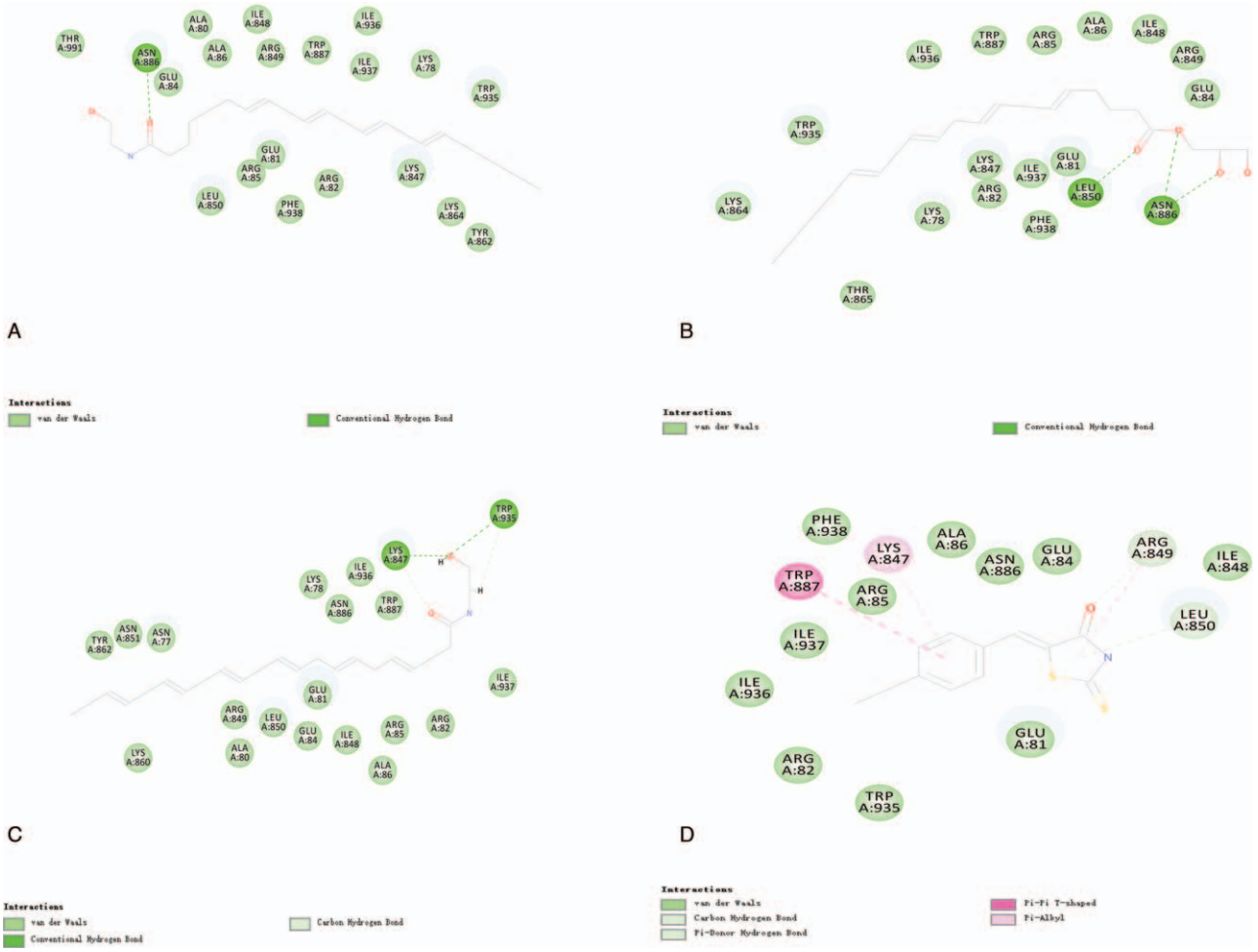

Figure 4. The intermolecular interaction of the predicted binding modes of (A) ZINC000004654958, (B) ZINC000004654971, (C) ZINC000008689961 (D), 10058-F4.

drug candidates was supported by the interaction of the hydrogen bonds. Conclusively, the results prove that the 3 compounds can steadily exist in nature condition and could potential modulatory effect on c-Myc.

4. Discussion

c-Myc was and still now a popular target for human cancer research. The very oncogene has proved to be related to numerous tumor suppressor and other oncogenes. Ma in 2014 demonstrates that c-Myc represses tumor suppressor let-7, which correlates well with another oncogene Lin28's induction.[23] Wu et al in 2018 also shows that c-Myc can be enhanced for transcription to promote cancer progression under the effect of protein Menin.[24] Consequently, urgent it is for scientist and clinician to screen and select optimal drug candidates for the inhibition of c-Myc.

Table 5

Hydrogen bond interaction parameters for each compound and c-Myc.

Receptor	Compound	Donor atom	Receptor atom	Distances (Å)
C-Myc	ZINC000004654971	A:LEU850:HN	ZINC000004654971:021	2.14
		A:ASN886:HD21	ZINC000004654971:022	2.79
		A:ASN886:HD22	ZINC000004654971:022	2.61
		A:ASN886:HD22	ZINC000004654971:025	1.95
	ZINC000004654958	A:ASN886:HD22	ZINC000004654958:023	2.10
	ZINC000008689961	A:LYS847:HZ1	ZINC000008689961:027	1.74
		A:LYS847:HE1	ZINC000008689961:023	2.50
		A:TRP935:O	ZINC000008689961:H60	2.57
	ZINC000012406714	A:ARG849:HA	ZINC000012406714:014	2.46
		A:LEU850:HN	ZINC000012406714	3.06
		A:TRP887	ZINC000012406714	5.49

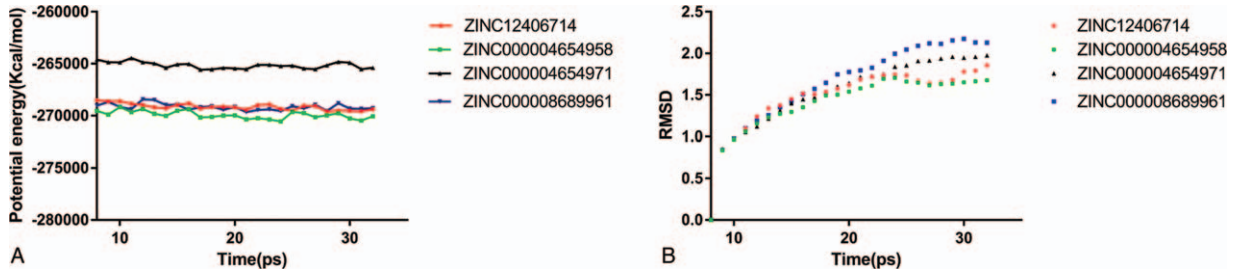

Figure 5. Results of molecular dynamics simulation of complexes. (A) Potential Energy (B) Average backbone RMSD. Ethical approval was not necessary; this is just a computer simulation of drug research.

LibDock, ADME, TOPKAT, ligand binding analysis, and molecular dynamics simulation were utilized to screen and analyze the structural biological characteristics in this study individually. Molecular conformation, pharmacological properties, the affinity of binding, and stability were investigated to prove the foremost characteristic of the selected compounds. Seventeen thousand nine hundred thirty-one of the natural, market available, named product molecules were analyzed from the ZINC15 database for virtual screening. LibDock score represented a degree of energy optimization and stability of the conformation. A high LibDock score showed that it had a relatively good energy optimization and stabilization. Six thousand one hundred thirty-three of the compounds are discovered to have a high binding affinity with c-Myc whose LibDock score is relatively higher than 10058-F4 and could be considered to be an energy optimum stable conformation with c-Myc. The top 20 compounds selected via LibDock score could be very useful in stabilization for future research.

ADME, as well as toxicology of the compounds, were evaluated for the pharmacological properties accordingly. ZINC000004654958, ZINC000004654971, and ZINC000008689961, from the results, were believed to be ideal lead compounds for further commercially available research. Soluble in the water, these compounds are proved to be nonhepatoxic and do not inhibit the liver enzyme CYP2D6. Other drugs could have the chance to be modified for further research since the modification of the very organic group could decrease the potential toxicity.

CDOCKER module is also used to investigate the binding mechanism and chemical bonds. ZINC000004654958, ZINC000004654971, and ZINC000008689961 are also proved to possess low CDOCKER interaction energy. They also show to have relatively low MM/GBSA-binding energy, indicating the high binding affinity with c-Myc. For the following analysis, chemical structures are examined and shows that the 3 compounds are axisymmetric, which are similar to the structures of 10058-F4. Besides the resemblance, the 3 selected to overcome the weakness of the 10058-F4, which has low potency, lack of selectivity, poor pharmacokinetic behavior mentioned previously.

Molecular dynamic simulation is carried out to estimate the stabilities of the compounds. RMSD and potential energy of the ligand-c-Myc complexes were calculated and analyzed. Trajectories of the complexes reach the equilibrium after 50 ps. RMSD value and complexes' potential energy got stabilized with time, indicating that the compounds which are selected previously can exist stably in environmental condition. The advent of modification and refinement could be useful to make the compounds bind more firmly to the c-Myc judging from the current results.

There is still some limitation to our study, although be elaborately designed and precisely measured. Drug structure may affect the binding of chemical bonds and energy, but this is unavoidable at present because the current positive drugs, such as 10074-G5 and 10074-A4, are not available in the Zinc library and therefore cannot be compared. In our study and Yu's study, pocket sites were established on the protein model, and virtual screening was conducted by computer to obtain drug inhibitors. However, Yu's study aims to screen and modify the structure of c-Myc inhibitors through physical and chemical methods based on the classification of c-Myc protein inhibitors currently used, so as to obtain more effective drugs.[25] However, our study only uses computers to screen out more promising drugs than those currently used in clinical studies from a more comprehensive ZINC library, and provides research directions for other drug developers. There are essential differences between the 2 in the method and the end goal. More experiments shall be carried to validate our results, and it is always good to have more indicators for the drug safety test, such as maximum tolerated dosage, aerobic biodegradability. It would also be helpful to carry out an animal experiment to validate the study.

5. Conclusions

This study used a series of methods based on biology and chemistry (virtual screening, molecule docking, ligand binding analysis, and molecular dynamics simulation) to screen and identify the top-notched compounds which could potentially be the inhibitor of c-Myc. In a nutshell, ZINC000004654958, ZINC000004654971, and ZINC000008689961 were filtered from 6133 natural products to be an ideal candidate for the c-Myc inhibitor, which could be marketable shortly. They are not only proved to be relatively safe, but they also had a great significance in the development of c-Myc protein inhibitor. Some extra credit the study has provided is a list of drug candidates along with their pharmacological properties, which is beneficial for future scientists to investigate and develop more ideal c-Myc inhibitors.

Author contributions

Conceptualization: Junan Ren, Liyan Zhao.
Methodology: Bo Wu, Junliang Ge.
Writing – original draft: Yuhao Huangfu.
Writing – review & editing: Junan Ren.

References

[1] Kalkat M, De Melo J, Hickman KA, et al. MYC deregulation in primary human cancers. Genes 2017;8:151.
[2] Adhikary S, Eilers M. Transcriptional regulation and transformation by Myc proteins. Nat Rev Mol Cell Biol 2005;6:635–45.
[3] Pelengaris S, Khan M, Evan G. c-MYC: more than just a matter of life and death. Nat Rev Cancer 2002;2:764–76.
[4] Dang CV. MYC on the path to cancer. Cell 149 2012;149:22–35.
[5] Conacci-Sorrell M, McFerrin L, Eisenman RN. An overview of MYC and its interactome. Cold Spring Harbor Perspect Med 2014;4:a014357.
[6] Wierstra I, Alves J. The c-myc promoter: still myster Y and challenge. Adv Cancer Res 2008;99:113–333.
[7] Grandori C, Cowley SM, James LP, et al. The Myc/Max/Mad network and the transcriptional control of cell behavior. Annu Rev Cell Dev Biol 2000;16:653–99.
[8] Luscher B. Function and regulation of the transcription factors of the Myc/Max/Mad network. Gene 2001;277:1–4.
[9] Carroll PA, Freie BW, Mathsyaraja H, et al. The MYC transcription factor network: Balancing metabolism, proliferation and oncogenesis. Front Med 2018;12:412–25.
[10] Zhang Q, Spears E, Boone DN, et al. Domain-specific c-Myc ubiquitylation controls c-Myc transcriptional and apoptotic activity. Proc Natl Acad Sci USA 2013;110:978–83.
[11] Hann SR. MYC cofactors: molecular switches controlling diverse biological outcomes. Cold Spring Harbor Perspect Med 2014;4:a014399.
[12] Tu WB, Helander S, Pilstal R, et al. Myc and its interactors take shape. Biochim Biophys Acta 2014;1849:469–83.
[13] Berg T, Cohen SB, Desharnais J, et al. Small-molecule antagonists of Myc/Max dimerization inhibit Myc-induced transformation of chicken embryo fibroblasts. Proc Natl Acad Sci USA 2002;99:3830–5.
[14] Yin X, Giap C, Lazo JS, et al. Low molecular weight inhibitors of Myc-Max interaction and function. Oncogene 2003;22:6151–9.
[15] Wang H, Hammoudeh DI, Follis AV, et al. Improved low molecular weight Myc-Max inhibitors. Mol Cancer Ther 2007;6:2399–408.
[16] Shima H, Nakayasu M, Aonuma S, et al. Loss of the MYC gene amplified in human HL-60 cells after treatment with inhibitors of poly(ADP-ribose) polymerase or with dimethyl sulfoxide. Proc Natl Acad Sci U S A 1989;86:7442–5.
[17] Wang H, Chauhan J, Hu A, et al. Disruption of Myc-Max heterodimerization with improved cell-penetrating analogs of the small molecule 10074-G5. Oncotarget 2013;4:936–47.
[18] Newman D. Developing natural product drugs: supply problems and how they have been overcome. Pharmacol Ther 2009;162:1–9.
[19] Li JW, Vederas JC. Drug discovery and natural products: end of an era or an endless frontier. Science 2009;325:161–5.
[20] Brooks BR, Brooks CL3rd, Mackerell ADJr, et al. CHARMM: the biomolecular simulation program. J Computat Chem 2009;30:1545–614.
[21] Garay-Canales CA, Licona-Limón I, Ortega E. Distinct epitopes on CD13 mediate opposite consequences for cell adhesion. BioMed Res Int 2018;2018:4093435.
[22] Resheq YJ, Menzner AK, Bosch J, et al. Impaired transmigration of myeloid-derived suppressor cells across human sinusoidal endothelium is associated with decreased expression of CD13. J Immunol (Baltimore, Md : 1950) 2017;199:1672–81.
[23] Ma X, Li CC. Lin28/let-7 axis regulates aerobic glycolysis and cancer progression via PDK1. Nat Commun 2014;5:Article number: 5212.
[24] Wu GW, et al. Menin enhances c-Myc-mediated transcription to promote cancer progression. Nat Commun 2017;8:15278.
[25] Yu C, Niu X, Jin F, et al. Structure-based Inhibitor Design for the Intrinsically Disordered Protein c-Myc. Sci Rep 2016;6:22298.