Computational Study of Novel Natural Inhibitors Targeting Kirsten Rat Sarcoma Viral Oncogene Homolog (KRAS) G12C

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Abstract

Lung adenocarcinoma is one of the most aggressive and rapidly fatal type of malignant lung tumor. Molecular docking and virtual screening were effectively and systematically used to identify specific targets in malignant tumors and screen potential drugs. Here, we screen perfect leading compounds from a medicate library (ZINC15 database) and analyze their properties (conveyance, absorption, metabolism, and excretion and harmless forecasts) with potential inhibition of Kirsten Rat Sarcoma Viral Oncogene Homolog G12C. Further results demonstrated that ZINC000013817014 and ZINC000004098458 were screened out from the ZINC15 database and were identified to have a much better binding affinity and more favorable interaction vitality binding with KRAS G12C and less rat carcinogenicity, Ames mutagenicity, way better dissolvability in water and non-inhibition with cytochrome P-450 2D6. Molecular dynamics simulation analysis indicated that the binding capacity of these two compounds and KRAS G12C, ZINC000013817014-KRAS G12C and ZINC000004098458-KRAS G12C is stable in the natural environment. Our findings reveal that ZINC000013817014 and ZINC000004098458 were perfect leading compounds to be inhibitions binding with KRAS G12C, which were selected as safe drug candidates and a cornerstone for KRAS G12C related medicine plan and improvement. It establishes a solid framework for systematic anticancer medication research and development.

Introduction

Lung adenocarcinoma is one of the most aggressive and rapidly fatal non-small cell lung cancer types which is often diagnosed at advanced stages with hematogenous metastasis and lymphatic metastasis. (Denisenko, Budkevich, & Zhivotovsky, 2018) Surgery, chemotherapy, immunotherapy, targeted therapy, hormone therapy, and radiation therapy have all become part of the treatment. (Bodor, Kasireddy, & Borghaei, 2018; Hsu, Chu, & Kao, 2017; Scafoglio et al., 2018; Sharpnack et al., 2018; Skříčková, Kadlec, Venclíček, & Merta, 2018) There are different treatment approaches have different mechanisms of action and achievements in understanding the pathogenesis of this disease and the development of new approaches in its treatment, and their common goal is to stop cells from proliferating indefinitely. (Skříčková et al., 2018) The therapeutic target at the heart of this is some genes encode related enzymes playing a role in certain mechanism were driver oncogenes of lung adenocarcinoma. (El Osta et al., 2019)

Kirsten rat sarcoma viral oncogene homolog G12C (KRAS G12C) is a GTP/GDP-binding protein that is encoded by the KRAS G12C gene. It is a member of the Ras family of small GTPases. KRAS G12C is one of the most common mutants in KRAS, which is the most frequently mutated oncogene in many types of cancers. More importantly, KRAS G12C have a significant influence in RAS signal transduction pathway. (Canon et al., 2019; Downward, 2003) The KRAS G12C mutant has a cysteine residue that binding to the GDP to the switch-II pocket (S-IIP), which is a allosteric combination pocket. (Westover, Jänne, & Gray, 2016) KRAS G12C can keep the GTP-bound active state of KRAS active and activate its downstream effects like differentiation and proliferation. (Nagasaka et al., 2020; Ostrem, Peters, Sos, Wells, & Shokat, 2013; Xue et al., 2020) KRAS G12C can over-activate KRAS-driven signaling containing RAS signal transduction pathway, and leads to the excessive growth of the tumor. It is indicated that the nucleotide state of KRAS G12C is in a state of dynamic flux, which can generate a 'hyperexcitable' state activate downstream signaling pathway. Finally,

it affected the development and progression of carcinomas. Moreover, some recent studies reported that KRAS G12C may inhibit the apoptosis of cells. (Masliah-Planchon, Garinet, & Pasmant, 2016; Murugan, Grieco, & Tsuchida, 2019; Patricelli et al., 2016; Spaargaren, Bischoff, & McCormick, 1995)

In conclusion, KRAS G12C is linked to the intracellular signaling pathways, cell proliferation and cell apoptosis, all of which are correlated with carcinogenesis. Recently, researchers have found that directly occupying cysteine residues can inhibit its activity, which is a guanine nucleotide (GN)-binding pocket can dictate the signaling state of K-Ras. (Hunter et al., 2014) There are enormous studies have been found that KRAS G12C (glycine 12 to cysteine) mutation has been identified as an oncogenic driver of tumorigenesis and is common in lung adenocarcinoma, non-small cell lung carcinoma, colon adenocarcinoma and colorectal adenocarcinoma, etc. (Aredo et al., 2019; Hayama et al., 2019; Molina-Arcas et al., 2019; Roa, Sánchez, Majlis, & Schalper, 2013) Therefore, many studies are devoted to identify effective compounds inhibiting KRAS G12C. ARS-1620 is a KRAS G12C-specific inhibitor to treat lung adenocarcinoma (belong to non-small cell lung cancer) and has been already marketed, which achieves in vivo target occupancy to induce tumor regression. (Janes et al., 2018; Molina-Arcas et al., 2019) However, it still has disadvantages that the reversible binding affinity is weak, and the patient usually has a poor prognosis. (Hansen, Peters, et al., 2018) This study aims to discover a few other characteristic compound inhibitors from a natural medicate library more successful than ARS-1620 in cancer treatment.

At present, natural items and related compounds play a more pivotal part in pharmacologic showcase. They get much attention because of the growing appreciation of functional assays and phenotypic screens, and we should realize the importance of the natural medicine's rational development and clinical application of precision medicine. (Harvey, Edrada-Ebel, & Quinn, 2015; Rao et al., 2019) Nowadays, it is identified that KRAS G12C is related with mechanisms implicated in limiting antitumor activity. (Hallin et al., 2020; Ostrem et al., 2013) We used a variety of auxiliary biologic and chemical methodologies, including virtual screening, molecular docking, and risk-free forecasting, to screen, identify, and characterize compounds with putative inhibitory properties for KRAS G12C. An important outcome of our research was the prediction of selected chemicals' absorption/transport/metabolism/excretion/harmfulness. Finding records of possible compounds in the ZINC15 database provided a framework for advancing KRAS G12C inhibitor research, and we anticipated their pharmacological properties. The goal of this research is to find possible KRAS G12C inhibitors in order to put up the foundation for pharmaceutical advancement and compound alteration in cancer treatment.

Results

Virtual screening of natural products database against KRAS G12C

KRAS G12C could cause KRAS dependent signaling activated because of an important regulatory site of KRAS G12C, the ligand-binding pocket, which was confirmed to be as GDP binding site, and then its downstream effect such as excessive cell proliferation was activated. KRAS G12C resulted in an GDP-binding active state in cancer cells, and the ligand-binding pocket had chances to bind with other ligands, which can provide more possibility to perform RAS signal transduction pathway of cancer cells. Therefore, the research can be conducted by targeting the ligand-binding pocket. A total of 17,799 biogenic-for-sale-named-item

particles were retrieved from the ZINC15 database. We used the KRAS G12C receptor protein's chemical structure to assess the pharmacological effects of various drugs. A KRAS G12C-specific inhibitor, ARS-1620, was used as a standard. 7579 compounds were chosen from the database as being eligible to bind to KRAS G12C in a stable manner, with 20325 binding poses. There were 14590 compounds were chosen since of their higher LibDock scores, which are shown in Supplementary Table 1. The best 20 positions compounds are recorded in Table 1.

Table 1
Top 20 Ranked Compounds with Higher LibDock

Number	Compound	Libdock Score
1	ZINC000049784088	173.17
2	ZINC000013130933	165.442
3	ZINC000004098695	165.347
4	ZINC000004098631	164.718
5	ZINC000044086691	164.477
6	ZINC000038143594	160.978
7	ZINC000013130935	160.767
8	ZINC000042805482	159.709
9	ZINC000004098302	159.58
10	ZINC000085826837	157.672
11	ZINC000008214697	156.982
12	ZINC000014614772	155.848
13	ZINC000026990660	155.619
14	ZINC000004098458	155.357
15	ZINC000038143593	155.254
16	ZINC000004098466	154.246
17	ZINC000014811844	153.994
18	ZINC000003827362	153.06
19	ZINC000013817014	152.969
20	ZINC000011616633	152.944
21	ARS-1620	100.673

ADME and toxicity prediction

In order to investigate the pharmacological properties of all 20 ligands with ARS-1620, the ADME module of Discovery Studio 4.5 was used, which included watery solvency, blood-brain boundary level, CYP2D6 binding, hepatotoxicity, amount of absorption by the human digestive system, and plasma protein binding qualities (Table 2). According to our fluid dissolvability expectation evaluated at 25°C, all the compounds were soluble, with the majority being very soluble. Furthermore, the ability to cross blood-brain barrier of these compounds in Table 2 gets the feature of indefiniteness except ZINC000014614772 and ZINC000004098302. As it were two compounds, ZINC000014614772 and ZINC000004098302, were anticipated to be inhibitors with CYP2D6, which were basic chemicals in sedate digestion system. In terms of hepatotoxicity, half of these compounds were determined to be hazardous, similar to ARS-1620. Other ligands had higher retention levels than ARS-1620 in human intestinal retention, except two molecules, ZINC000003827362 and ZINC000014614772. The plasma protein binding properties revealed that 8 compounds had good absorption levels comparable to ARS-1620, while the remaining 12 compounds had poor absorption.

Table 2
Adsorption, Distribution, Metabolism, and Excretion Properties of Compounds

Number	Compounds	Solubility Level	BBB Level	CYP2D6	Hepatotoxicity	Absorption Level	PPB Level
1	ZINC000049784088	4	4	0	0	3	0
2	ZINC000004098458	3	4	0	0	3	0
3	ZINC000004098631	1	4	0	1	3	1
4	ZINC000026990660	3	4	0	1	3	0
5	ZINC000014811844	3	4	0	1	2	0
6	ZINC000004098695	3	4	0	1	3	0
7	ZINC000011616633	2	4	0	0	3	0
8	ZINC000008214697	2	4	0	0	3	1
9	ZINC000014614772	1	1	1	1	0	1
10	ZINC000003827362	3	4	0	1	0	0
11	ZINC000038143593	3	4	0	0	3	0
12	ZINC000013817014	2	4	0	1	2	0
13	ZINC000038143594	3	4	0	0	3	0
14	ZINC000044086691	1	4	0	0	3	1
15	ZINC000004098302	1	0	1	1	1	1
16	ZINC000004098466	2	4	0	0	1	1
17	ZINC000085826837	2	4	0	0	2	0
18	ZINC000013130935	1	4	0	1	2	1
19	ZINC000013130933	1	4	0	1	2	1
20	ZINC000042805482	2	4	0	0	2	0

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.

BBB level: 0, very high penetrant; 1, high; 2, medium; 3, low; 4, undefined.

CYP2D6 level: 0, noninhibitor; 1, inhibitor.

Hepatotoxicity: 0, nontoxic; 1, toxic.

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

PPB: 0, absorbent weak; 1, absorbent strong.

Number	Compounds	Solubility Level	BBB Level	CYP2D6	Hepatotoxicity	Absorption Level	PPB Level
21	ARS-1620	2	1	0	1	0	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.							
BBB level: 0, very high penetrant; 1, high; 2, medium; 3, low; 4, undefined.							
CYP2D6 level: 0, noninhibitor; 1, inhibitor.							
Hepatotoxicity: 0, nontoxic; 1, toxic.							
Human-intestinal absorption level: 0, good; 1, moderate; 2, poor; 3, very poor.							
PPB: 0, absorbent weak; 1, absorbent strong.							

Security and poisonous quality are be completely considered as pivotal variables in this study. We used a computational strategy in the TOPKAT module of Discovery Studio 4.5 to verify the safety and various toxicity indicators of the top 20 compounds and ARS-1620, including rat carcinogenicity (based on the US National Toxicology Program dataset), developmental toxicity potential properties, and Ames mutagenicity (Table 3). There were no mutagenic findings for 11 compounds and no developmental toxicity findings for 6 other compounds in light of the data (DTP). No carcinogenicity in rats was expected from the reference ARS-1620. Non-CYP2D6 inhibitors, high aqueous solubility, enhanced plasma protein binding, reduced carcinogenicity in rodents, and formative toxic quality potential distinguish ZINC000013817014 and ZINC000004098458 from the competition. In summary, ZINC000013817014 and ZINC000004098458 were affirmed as secure sedate candidates and were chosen for the consequent investigate. (Fig. 2).

Table 3
Toxicities of Compounds

Number	Compounds	Mouse NTP		Rat NTP		Ames	DTP
		Female	Male	Female	Male		
1	ZINC000049784088	0.995	0	0	0.008	1	1
2	ZINC000004098458	0.005	0	0.988	0.003	0	1
3	ZINC000004098631	0	0.117	1	1	0	1
4	ZINC000026990660	0	0.966	1	0.012	1	1
5	ZINC000014811844	0.656	1	1	1	0.002	1
6	ZINC000004098695	0	1	1	0.932	1	1
7	ZINC000011616633	0	1	1	1	1	1
8	ZINC000008214697	0	0.003	0	1	0	0
9	ZINC000014614772	0	1	1	1	0	0
10	ZINC000003827362	0.702	1	0.878	1	1	0
11	ZINC000038143593	0.061	0	0.274	0.088	0	1
12	ZINC000013817014	0	1	0.211	1	1	1
13	ZINC000038143594	0.061	0	0.274	0.088	0	1
14	ZINC000044086691	1	0	0.987	0.998	0.004	1
15	ZINC000004098302	0	1	1	1	0	0
16	ZINC000004098466	0.09	1	1	1	1	1
17	ZINC000085826837	0.186	1	1	0.998	0	1
18	ZINC000013130935	0	1	1	0.011	0	0
19	ZINC000013130933	0	1	1	0.011	0	0
20	ZINC000042805482	0.186	1	1	0.998	0	1
21	ARS-1620	1	1	0	0.003	0	0.017
NTP, U.S. National Toxicology Program; DTP, developmental toxicity potential.							
NTP < 0.3 (noncarcinogen); >0.8 (carcinogen).							
Ames < 0.3 (nonmutagen); >0.8 (mutagen).							
DTP < 0.3 (nontoxic); >0.8 (toxic).							

We used the CDOCKER module to dock ZINC000013817014 and ZINC000004098458 into the molecular structure of KRAS G12C to identify and investigate ligand binding processes of these chemicals and ARS-1620. The CDOCKER potential energy calculation is shown in Table 4. Is is seen in Table 5 that ZINC000013817014 and ZINC000004098458 had much lower CDOCKER potential vitality than the reference ligand ARS-1620, indicating that these two compounds had a significantly stronger affinity for KRAS G12C binding than ARS-1620. Using a basic computation research, hydrogen bonding and associated interactions were also investigated (Figs. 3 and 4). It is appeared that ZINC000013817014 shaped 3 sets of hydrogen bonds with KRAS G12C, which compound with GLU62:OE1, HIS95 and TYR96 of KRAS G12C(Table 6). Apart from that, the complex fashioned four-related intelligence, including the -Orbitals between ZINC000013817014 and the HIS95 of KRAS G12C. ZINC000004098458 produced 6 pairs of hydrogen bonds with KRAS G12C, which compound with ARG68:NH2 of KRAS G12C, GLU62:OE1 of KRAS G12C, HIS95 of KRAS G12C, TYR96 of KRAS G12C, CYS12 of KRAS G12C, and VAL9 of KRAS G12C. It also shaped five sets π -related interactions within the complex, including two sets between GLU62 of KRAS G12C and the compound, HIS95 of KRAS G12C with the compound, two sets between TYR96 of KRAS G12C with the compound and VAL9 of KRAS G12C, and CYS12 of KRAS G12C with the compound. By the C119 of the compound with HIS95 of KRAS G12C, it formed four hydrogen bonds with KRAS G12C, compound with two kinds of TYR96 and HIS95, and compound with two kinds of TYR96 and HIS95. What's more, four π -related interactions shaped between ARS-1620 and KRAS G12C, including ALA11, GLU62, HIS95 and MET72 of KRAS G12C with ARS-1620.

Table 4
CDOCKER Potential Energy of Compounds with KRAS G12C

Compound	CDOCKER Potential Energy (kcal/mol)
ZINC000013817014	-53.6005
ZINC000004098458	-58.7358
ARS-1620	-42.7724

Table 5. Hydrogen Bond Interaction Parameters for Each Compound with KRAS G12C

Receptor	Compound	Donor Atom	Receptor Atom	Distances (Å)
KRAS G12C	ZINC000013817014	A:ARG68:NH2	ZINC000013817014:O14	2.63
		ZINC000013817014:H66	A:GLU62:OE1	1.91
		ZINC000013817014:H58	A:GLY10:O	2.71
		ZINC000013817014:H65	A:TYR96:OH	2.65
		ZINC000013817014:H65	ZINC000013817014:O30	2.65
		A:TYR96:OH	ZINC000013817014	2.96
		A:ARG102:NH2	ZINC000004098458:O8	2.14
		ZINC000004098458:O8	A:GLN99:OE1	2.86
		ZINC000004098458:C10	A:ASP69:OD1	3.54
		A:ARG68:CD	ZINC000004098458:O12	3.5
	ARS-1620	ZINC000004098458:O6	ZINC000004098458:O1	2.75
		A:CYS12:N	ARS-1620:F21	3.55
		A:CYS12:SG	ARS-1620:F21	3.31
		A:ARG68:NH2	ARS-1620:N12	2.95
		A:TYR96:OH	ARS-1620:F27	3.12
		A:TYR64:CA	ARS-1620:O1	3.69
		A:ARG68:CD	ARS-1620:O1	3.18
		A:TYR96:OH	ARS-1620	3.55

Table 6. p-Related Interaction Parameters for Each Compound with KRAS G12C

Receptor	Compound	Donor Atom	Receptor Atom	Distances (Å)
KRAS G12C	ZINC000013817014	A:GLU62:OE1	ZINC000013817014	4.01
		A:HIS95	ZINC000013817014	3.75
		A:TYR96	ZINC000013817014	5.16
		A:ARG68:NH2	ZINC000004098458	3.99
		A:GLU62:OE1	ZINC000004098458	3.55
	ZINC000004098458	A:HIS95	ZINC000004098458	4.33
		A:TYR96	ZINC000004098458	4.84
		A:CYS12	ZINC000004098458	3.96
		ZINC000004098458	A:VAL9	5.36
		A:TYR96	ARS-1620	5.24
	ARS-1620	A:TYR96	ARS-1620	5.06
		A:HIS95	ARS-1620	5.41
		A:HIS95	ARS-1620:Cl19	4.81

Molecular dynamics simulation

The simulation module test for molecular dynamics was used to evaluate the stability of the ligand-KRAS G12C complexes in realistic environments. RMSD bending and potential vitality charts for each complex, corresponding to the initial conformations obtained from the molecular docking experiment using the CDocker module, are depicted in Fig. 5. After 25ps, the directions of all complexes had achieved harmony, and the RMSD and potential vitality of the two complexes had stabilized. The results of molecular dynamics simulations revealed that the compound's hydrogen bonds and related interactions with KRAS G12C contributed to the complexes' soundness. ZINC000013817014 and ZINC000004098458 appear to be connected with KRAS G12C, and their complexes with KRAS G12C appear to be found in a natural setting consistently, acting regulatory and more outstanding to KRAS G12C, as did the reference ARS-1620.

Discussion

At the present time the occurrence of cancer is increasing and cause an enormous burden on society. It's imperative for human to suppress tumor growth and find more effective treatment. We should do more studies and develop more new drugs realized that it had to be settled urgently to resist drug resistance. (Hanahan & Weinberg, 2011; Torre et al., 2015) Past researches has proved that the overaction of signal transduction pathways in the cancer cells was the major reason of cancerization of tumors, and the main way for activating tumorigenesis.(Haigis, 2017) On the one hand KRAS G12C can activate GTP-loading, holding an GDP-binding active state in cancer cells, on the other hand it can activate downstream KRAS-

dependent signaling, which have the mutant cysteine residue in GDP-bound site.(Christensen, Olson, Briere, Wiel, & Bergo, 2020) As a result, developing a KRAS G12C inhibitor to stifle its migration could be a critical step toward fighting cancer. The advancement of KRAS G12C inhibitors was recently conducted to be potentially combinative by allele-specific covalent targeting of Cys-12 in vicinity to the switch II pocket (S-IIP). (Lito, Solomon, Li, Hansen, & Rosen, 2016) In these compounds was found as inhibitors with KRAS G12C, ARS-1620, the ligand chosen as the reference sedate in this study, has been generally developed in abundant studies until presently.(Hansen, Firdaus, et al., 2018; Janes et al., 2018) Nevertheless, it still have limitations in mechanism of action and resistance.

Despite the fact that KRAS G12C inhibitors have therapeutic potential in cancer treatment, ARS-1620, which is now being evaluated as a possible anticarcinogen, has significant drawbacks. There was a research that a panel of KRAS G12C cell lines were treated with ARS-1620 to assess effects on signaling and viability. As a result, we find that prompt adaptive RAS pathway feedback reactivation following KRAS G12C inhibition in the majority of KRAS G12C models, which cannot be inhibited by KRAS G12C-specific inhibitors.(Ryan et al., 2020) The study elucidated that ARS-1620 can develop adaptive resistance. This fact is certain to cause issues with the use of clinically specific inhibitors. Subsequently, it is direly fundamental to create an unused inhibitor of KRAS G12C.

17799 biogenic-for-sale-named product chemicals were selected from the ZINC15 database for broad analog screening in this work, which was followed by ADME, TOPKAT, CDOCKER, and molecular dynamics modeling. According to the degree of energy optimization and uniformity of configuration, LibDock gives out a score. In comparison to compounds with lower LibDock scores, those with higher scores demonstrated superior vitality optimization and consistency. 7579 compounds were chosen as competent to connect stably with KRAS G12C after the LibDock module was calculated. Furthermore, it is indicated that 14590 compounds appear to form a more stable complex with KRAS G12C and have improved vigor enhancement than ARS-1620, which have an advantage in LibDock score than ARS-1620 (LibDock score: 100.673). The finest 20 natural chemicals were picked based on the LibDock score and advanced consideration was given to give more potential openings for clinical applications and chemotherapy. The 20 medicines that were selected to be evaluated for their pharmacological qualities, ADME and harmfulness projections were performed. Because of their strong dissolvability in water and fairly high intestine retention level, the results suggested that ZINC000013817014 and ZINC000004098458 can be recognized as perfect candidacy compounds. Furthermore, compared to other compounds, ZINC000013817014 and ZINC000004098458 were expected to have no CYP2D6 inhibitors, no mutagenicity in Ames, and less rat carcinogenicity and formative harmfulness potential. This data recommended that the two compounds possess prevalent security, so they are qualified to be potential perfect driving compounds. Thus, ZINC000013817014 and ZINC000004098458 were sensibly recognized as moderately high-quality medicate candidacies. As a result, ZINC000013817014 and ZINC000004098458 could be considered relatively high-quality drug candidacies. The above-mentioned findings revealed their potential for use in medical planning. Sedate progression may still be possible with the remaining drugs, despite their toxicity or bad effects on records, by adding, deleting, or changing connected functional groups and atoms. ZINC000013817014 and ZINC000004098458 were identified as perfect driving compounds as previously stated, and further analysis was carried out. KRAS G12C's ligand binding and chemical interactions with prospective drugs have been studied further. ZINC000013817014 and

ZINC000004098458 may have a higher affinity for KRAS G12C than the reference ligand ARS-1620, according to the CDOCKER module's calculations. Molecular structure inspection was used to examine the chemical structures of ZINC000013817014 and ZINC000004098458, two compounds that, when combined with KRAS G12C, formed more chemical and firm bonds than ARS-1620. These two drugs are thought to bind more stably to KRAS G12C at a dynamic region in the ligand-binding pocket, contributing to competitive suppression of KRAS G12C activity and improving the impact of cancer therapy.

As a result, molecular dynamics simulations were used to evaluate their stability in the natural world. The RMSD and potential vitality of these ligand-KRAS G12C complexes were computed, and the orientations of the complexes were found to be balanced after 25ps. The graph of RMSD and potential vitality of these complexes have a tendency to be consistent throughout time, implying that these two complexes were stable in nature. Further debate, medication assignment and development, as well as subsequent revising and modifying, may be planned to create a more stable and dynamic ligand-receptor combination. Furthermore, the structure of agonist and inhibitor is unquestionably similar, and therefore adding or erasing different bunches or molecules would result in the opposite actions. Considering what has been discovered, such as their significant pharmacologic properties, profoundly binding affinity, and stabilization when combined with KRAS G12C, natural compounds discovered in this study could point out a possible direction for KRAS G12C-related inhibitors development. It also prompted us to discover and develop other new characteristic compounds with potential anti-cancer properties and progress chemicals for cancer treatment.

In the field of anticancer, searching for new drugs has become a hot topic in lots of studies due to the emergence of drug resistance. However, the overall process that anti-cancer drugs developed is nearly development-restricted in experimental studies and clinical application. This study articulated forcefully that the screen and discovery of ideal leading compounds was the primary and the foremost significant step in sedate assignment and advancement. According to the study, five modules of Discovery Studio 4.5 were used to screen, identify, and assess the biochemical structural features of novel prospective compounds, including LibDock, ADME, TOPKAT, CDOCKER, and molecular dynamics simulation. It is comprehensively tested and calculated each chosen compound in molecule conformation, pharmacological characteristics, binding affinity, and stability. Then, in comparison to the standard compound, ARS-1620, we determined and took into account all focal points. According to lots of computational analyses, these two chemicals may have a potential impact on the treatment of a variety of cancers. The research and development of natural drugs is the foundation. Nevertheless, the further designment, refinement and improvement are necessary for marketing and applying extensively in the future. Further research could focus on the advancement and improvement of these two main candidate molecules, based on their identification. In addition, our research has the value of expansion that we can use these high-tech methods and means to screen and identify more leading compounds, which could widely discover new drugs and increase efficiency in medication development.

This study had high application value in drug research and development. However, there are some limitations such as lacking of animal experiments, further testing, repeating and confirming the experimental results. There are also other parameters to consider, such as the half-maximal inhibitory concentration and the half-maximal effective concentration, which are both measured in half-maximal inhibitory concentration.

Materials And Methods

Discovery Studio Software and Ligand Library

There has been an advancement in the field of molecular modeling and environmental simulation with the release of BIOVIA's Discovery Studio 4.5 computer application (San Diego, CA, USA). Programs for modeling the dynamics of both small and large molecules are included. By combining protein structure and supplementary biologic computation, protein modeling, optimization, and sedate plan devices are the main goals of the project. This method found and refined several driving molecules and drug possibilities. The ADME module looked at pharmacologic characteristics whereas the Discovery Studio LibDock module looked into virtual screening. STING agonists were found in the Natural Products database of the ZINC15 database. The ZINC15 database, which is maintained by the Irwin and Shoichet Laboratories of the Department of Pharmaceutical Chemistry at the University of California, San Francisco, is a free database of commercially accessible compounds that can be searched (San Francisco, California, USA).

Structure-based virtual screening using LibDock

The KRAS G12C ligand-binding pocket area was chosen as the at a specific spot to screen and discover compounds with the ability to be KRAS G12C inhibitors. The LibDock module of Discovery Studio 4.5 was used to conduct virtual screening. It is a docking module that uses a grid at the binding site and probes that are both polar and nonpolar in order to identify the most active regions of the protein. The hotspots are also used to tweak the ligands for a more pleasant intuitive experience. An algorithm and a force field from Harvard University, Cambridge, Massachusetts, were utilized for ligand reduction and all ligand positions were placed in accordance with their score. The 1.9-crystal structure of human KRAS G12 was obtained from the Protein Data Bank, and 17799 ligands, including the inhibitor ARS-1620, were obtained from the Zinc15 database and integrated into the LibDock working environment. KRAS G12C's chemical structure is shown in Fig. 1. After removing crystal water and other heteroatoms around it, hydrogen addition, protonation, ionization and vitality minimization were utilized to arrange the protein. The CHARMM force field and the Smart Minimiser approach were utilized to reduce vitality. (Rodriguez-Brenes, Komarova, & Wodarz, 2013) The root mean square gradient tolerance was 12.277, and the final root mean square gradient was 0.690. Prepared proteins were made in order to identify their binding sites. The docking active site was built utilizing the binding locations of the ligands (17799 ligands and ARS-1620). In order to conduct a virtual screening, All of the produced ligands were docked to the active site using LibDock. The LibDock score was used to rate all compounds, and docked poses were sorted and categorized accordingly.

Absorption, distribution, metabolism, and excretion and toxicity prediction

This study used Discovery Studio 4.5's ADME module to figure out the dispersion, absorption and metabolism and excretion (ADME) of various compounds, including the solubility in water, blood-brain-barrier penetration, cytochrome P-450 2D6 (CYP2D6) inhibition, liver toxicity and plasma protein binding levels of the tested compounds under study. The TOPKAT module of Discovery Studio 4.5 was used to calculate the poisonous quality and other attributes of all possible substances, including 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 features were taken into account extensively when looking for potential sedative candidates for KRAS G12C.

Molecule docking

The molecular docking investigation was carried out using the CDOCKER module of Discovery Studio 4.5. CDOCKER is a high-precision molecular docking approach on the premise of the CHARMM constrain field, which was used to study receptors and ligands. In the course of docking, the receptor has a limited field and can deliver high-precision docking. The CHARMM vitality and the interaction vitality, which showed ligand binding affinity, were determined for each complex posture. In a stiff and semi-flexible docking method, the crystal structure of KRAS G12C molecules was generally eliminated, leading the fixed water molecules to potentially influence the conformation of the receptor-ligand complex. After water molecules were removed from the protein, hydrogen bonds were added. To demonstrate the unwavering quality of the examination result, the initial inhibitor compound ARS-1620 was found from the Zinc15 database, which was identical to the Natural Products screened, and then docked into the crystal structure of KRAS G12C to compare the root-mean-square deviation (RMSD) between these two conformations. The locations within a 5-radius radius of the geometric centroid of the ligand ARS-1620 were identified as the binding site sphere of KRAS G12C. The ligands were allowed to connect with the residues of protein groups within the binding site sphere during the docking procedure. The detected hits' structures were constructed and docked into the KRAS G12C binding site. (Kessler and colleagues, 2019) Based on CDOCKER interaction liveliness, several poses of each ligand-KRAS G12C complex were created and studied.

Molecular dynamics simulation

Among the postures predicted by the particle docking program, the leading binding conformations of the ligand-KRAS G12C complexes were chosen and prepared for molecular dynamics modeling. An explicit periodic boundary solvation water model was used to solve the ligand-receptor complex in an orthorhombic box. The system was supplemented with sodium chloride of ionic strength 0.145 to mimic the physiological environment. The system was then de-stressed using a root mean square gradient of 0.227 after 500 steps of steepest descent and 500 steps of conjugated gradient. For 2 ps, the system was forced from 296 K to 302 K, followed by 5 ps of equilibration simulations to achieve equilibrium. The molecular dynamics simulation was ran for 25 ps with a 1 fs time step in the production module. The average pressure and temperature system was kept at a temperature of about 300 K throughout the simulation. Using a particle mesh Ewald technique, long-range electrostatics were estimated and all hydrogen bonds were repaired. The Discovery Studio 4.5 analysis trajectory technique was applied to calculate the RMSD, potential energy, and structural parameters of a complex system using the initial complex configuration as a starting point.

Conclusions

This work used a high-tech method that included virtual screening, molecular docking, ADME, toxicity prediction, and other computer-aided auxiliary and chemical analysis technology to screen, identify, and analyze the optimum driving compounds capable of inhibiting KRAS G12C. Two compounds, ZINC000013817014 and ZINC000004098458 were chosen and were competent with secure candidacies

since they had better dissolvability in water and great intestinal absorption level. Moreover, they also bind with KRAS G12C more firmly and can exist in natural environment stably. They have a leading role in the development of new inhibitors of KRAS G12C. The findings of the current study also included a list of medication candidates and their pharmacological properties that could aid in the design and modification of KRAS G12C or other proteins in medicine, and a new screening method for anti-cancer chemicals.

Declarations

Competing interest: The authors declared that there are no conflicts of interest in this research. We declare that we do not have any commercial or associative stakes in this research submitted that would create a conflict of interest.

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Authors' contributions

Junliang Ge designed experiments; Junliang Ge and Sheng Zhong performed experiments and did data analysis; Meng Yuan wrote the original draft and the main manuscript text; Jialin Li and Meng Yuan participated in writing manuscript. Gaojing Dou and Laiyu Yang prepared figures 1-5; Junan Ren, Yang Zhou and Haoqun Xie prepared table 1-6. Xinhui Wang wrote the cover letter. Junliang Ge and Yuan Meng contributed equally to this manuscript.

Conflict of Interest

The authors have declared that they have no competing interests in this study. A conflict of interest would be created if we had any commercial or associative interests in the material you have contributed.

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Figures

Figure 1

Molecular structure of Kirsten Rat Sarcoma Viral Oncogene Homolog G12C(KRAS G12C)

- A. Initial molecular structure.
- B. Surface of binding area added. Blue represents positive charge, and red represents negative charge.

Figure 2. ARS-1620 and novel compounds selected from virtual screening.

Figure 2

ARS-1620 and novel compounds selected from virtual screening.

Figure 3

Schematic drawing of interactions between ligands and KRAS G12C. The surface of binding areas was added. Blue represents positive charge; red represents negative charge; and ligands are shown in sticks, with the structure around the ligand-receptor junction shown in thinner sticks.

(A) ZINC000013817014-KRAS G12C complex

(B) ZINC000004098458-KRAS G12C complex

Figure 4. Schematic of intermolecular interaction of the predicted binding modes of (A) ZINC000013817014 with KRAS G12C; (B) ZINC000004098458 with KRAS G12C, and (C) ARS-1620 with KRAS G12C.

Figure 4

Schematic of intermolecular interactions of the predicted binding modes of (A)ZINC000013817014 with KRAS G12C; (B)ZINC000004098458 with KRAS G12C, and (C)ARS-1620 with KRAS G12C

Figure 5

Results of molecular dynamics simulation of the compounds ZINC000013817014 and ZINC000004098458.

(A) Average backbone root-mean-square deviation.

(B) Potential energy. RMSD root-mean-square deviation.

Supplementary Files

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