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Identifying highly effective fludarabine-based novel target cancer therapy agents by in silico studies

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ABSTRACT

Aim: To develop an alternative drug molecule design to fludarabine which is commonly used in chronic lymphocytic leukemia.

Methods: The molecular properties and biological activities of the drug molecules were determined using Molinspiration software. We investigated the biological activity and drug properties of fludarabine by changing the positions of bioisosteres on the molecular structure.

Results: In our studies of derivatives of the fludarabine drug molecule, we obtained data by adding different structures to the Y part without changing the X structure (F) of fludarabine. We have used the abbreviation 'M' to refer to the molecules in these experiments. We predict that the M6 derivative of fludarabine will have higher ion channel modulator, kinase and protease activity compared to fludarabine. We predict that the M15 derivative of fludarabine will have higher G- protein coupled receptors, ion channel modulator, kinase, and protease and enzyme inhibition activity compared to fludarabine. In our experiments with fludarabine derivatives, we have experimented by binding different molecules to both the X and Y structures of fludarabine at the same time. We have used the abbreviation 'C' to refer to the molecules in these experiments. In these experiments, we did not achieve higher biological activity than fludarabine.

Conclusions: The results suggest that this newly designed M15 derivative of fludarabine molecule may be a better antileukemic drug molecules in the future and may be useful for further drug molecule development research in medical biochemistry, chemistry and pharmacology.

Key words: Antileukemic drug, biological activities, fludarabine, in silico, pharmacokinetic.

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Introduction

Chronic lymphocytic leukemia (CLL) is a malignant lymphoproliferative disease. It is the most common leukemia in western countries and the average age of onset is around 70 years [1]. The disease does not show a very rapid course as in acute leukemias. Sometimes early and slowly progressing forms are detected only during regular check-ups and by chance. It is important to distinguish CLL from other types of diseases belonging to the lymphoid neoplasms, and some cases are still difficult to identify [2,3]. Although advances in cytogenetics and molecular biology have led to a significant improvement in the diagnosis of lymphoproliferative disorders, the successful differential diagnosis of CLL still requires blood smear and immunophenotype

evaluation [4]. Flow cytometry is the most common and practical method used in diagnosis [1,2]. The clinical course of CLL is highly variable. Most cases of CLL remain asymptomatic for a long time. In this case, therapeutic intervention is not necessary. However, a proportion of CLL cases progress rapidly, require treatment and have a reduced overall life expectancy [5]. Patients with asymptomatic, early-stage disease (Rai stage 0, Binet stage A) are kept under clinical observation without cytotoxic therapy [6,7]. In asymptomatic patients, treatment should be initiated if signs of CLL increase. Treatment regimens for CLL are rapidly changing and advancing thanks to new agents in the light of science. These agents have also improved outcomes in patients with high-risk disease [5,8]. The choice of treatment should be decided by making a risk assessment specific to each patient.

In recent years, there have been important developments in the use of new targeted agents in CLL. There are US Food and Drug Administration (FDA) approved treatment options for patients, used as single agents or in combinations [9]. Among the various agents used to treat CLL, purine analogs, Bruton's tyrosine kinase inhibitors, phosphatidylinositol 3-kinase inhibitors, B-cell lymphoma 2 inhibitors and CD20 monoclonal antibodies have shown the greatest improvements in survival in CLL patients [10-12]. Fludarabine is the most intensively investigated purine analog in the treatment of CLL [13]. Most purine analogues have low oral bioavailability, and studies aimed at increasing absorption by increasing the lipophilicity of the drug are important [14]. In bioavailability studies conducted for fludarabine, inter-individual differences in bioavailability were reported to be small, although bioavailability varied from 30% to 80% between patients [15]. Fludarabine has been repeatedly shown not to improve overall survival when used as a single agent compared with combination regimens (such as fludarabine/cyclophosphamide/rituximab) [16-18]. Fludarabine is used effectively in the treatment of CLL, but its popularity is declining due to the discovery of new drugs and the side effects of fludarabine. Side effects include myelosuppression (neutropenia, thrombocytopenia and anemia), immunosuppression, increased risk of opportunistic infections and neurotoxicity. In particular, myelosuppression is the most important dose-limiting complication [15,19]. Leukopenia is common during treatment. This decrease paves the way for secondary infections and delays further treatment. Fludarabine severely suppresses bone marrow function, causing neutropenia, thrombocytopenia and anemia. Thrombocytopenia is less common than leucopenia [20,21]. In one large study, neutropenia, thrombocytopenia and anemia were reported to occur in 19%, 14% and 7%, respectively, during treatment with fludarabine [22]. The immunosuppressive side effect of fludarabine treatment increases the risk of developing a second malignancy [23]. As the metabolite of fludarabine is excreted by the kidneys, it should be used with caution in patients with renal failure and in the elderly [24]. In addition, autoimmune hemolytic anemia may develop in CLL, especially with fludarabine treatment [25,26].

Screening for molecular, physicochemical properties facilitates the discovery and development of promising new drugs in medicinal chemistry and pharmacology. To design a drug-like molecule, Lipinski outlined five different properties. When predicting the pharmacokinetics of a prodrug, testing whether it satisfies Lipinski's Rule of 5 provides insight into bioavailability. The partition coefficient (Log P),

molecular weight (MW), number of hydrogen bond donors (NHD) and acceptors (NHA) of the precursors must each be within the ranges specified by Lipinski's Rule. Violation of any of these properties (referred to as rule 5) will lead to bioavailability problems. The ability of a drug to move from the site of application to the target area depends on its physicochemical properties. Water solubility is a useful parameter in the design of drug molecules and is expressed as LogP. A negative miLogP value indicates hydrophilicity, while a positive miLogP value indicates hydrophobicity [27]. The increase in MW is a disadvantage. There are studies linking this increase to a decrease in intestinal and blood-brain barrier permeability [28,29]. It is also reported in the literature that an excessive number of hydrogen bond donor groups disrupts permeability across the membrane bilayer [30,31]. Poor absorption/permeability occurs when the molecular weight is greater than 500, LogP is greater than 5, and the number of hydrogen bond donors and acceptors is greater than 5 and 10, respectively [32]. Similar to MW, topological polar surface area (TPSA) and molecular volume are considered as alternative key parameters for predicting permeability and oral absorption. It has been reported that low TPSA increases the ability to penetrate biological membranes and bioavailability. Another important factor in assessing receptor/channel binding efficiency and optimal bioavailability is the conformational flexibility of the molecule, defined by the number of rotatable bonds (Nrotb) [33-35].

Studies to reduce the side effects and increase the efficacy of cancer drugs are increasing daily. Nowadays, the drug development and modification of existing drugs and enzyme inhibition studies are carried out in parallel. In this study, we aimed to develop an alternative drug design to the drug fludarabine, a drug commonly used to treat CLL. In this way, we will provide preliminary information before starting the laboratory studies and guide long-term drug molecule synthesis studies.renal functional parameters, was also assessed.

Materials and metods

We optained the list of FDA approved drugs used in CLL from the official website (https://www.cancer.gov/).

Calculation of pharmacokinetic parameters*:* The molecular properties of the drug molecules (miLogP, TPSA, NHD, NHA, Nrotb, molecular volume and MW, number of heavy atoms (Nat), number of drug similarity violations) were calculated using the Molinspiration program (Molinspiration Cheminformatics, SK-900 26 Slovensky Grob, Slovak Republic, Molinspiration was founded in 1986 as a spin-off from the University of Bratislava). For the prediction of molecular lipophilicity potential, the miLogP parameter developed in-house by Molinspiration is used.

Bioactivity prediction*:* During the design study of a new drug molecule, various functional groups are functionalised with R groups. In drug molecule design, drug candidates are designed using different R groups. In our drug molecule design study, molecular physicochemical properties such as lipophilicity, polar surface area, hydrogen bond donor and receptor number, molecular weight and volume were used for computational evaluation of drug molecule similarity assessment. The determination of the biological activity of drug molecules (such as GPCRs, kinase inhibitors, nuclear receptors, ion channel modulators (CM), protease and enzyme inhibitors) was also determined using the Molinspiration programme.

Bioisosteric replacement*:* Bioisosterism is a concept often used in the design of drug

molecules. It is a modification that facilitates the enhancement of drug activity, elimination of undesirable effects and target selectivity. In our study, we aimed to design new molecules that can reduce toxicity and side effects and increase target selectivity by making modifications to Fludarabine drug used in CLL patients. We investigated the biological activity and drug properties of fludarabine by changing the positions of bioisosteres (with monovalent groups -OH, benzene, p-xylene, m-xylene, o-xylene, 1-bromo-4-methylbenzene, tert-butyl, 2-methoxypropanal, 1-methyl-2-(4-((5-methyltetrahydrofuran-2-yl)methyl)phenyl)-1H-pyrrole, ethanesulfonamide, 4-methyl-1,1'-biphenyl, 1-(methylthio)urea, 1-(4-(ethylsulfonyl)phenyl)urea, 1-(ethylthio)pyrrolidine, ethanethiol, ethanamine, 4-ethylbenzoic acid, 1-methylurea, propionic acid, 1-methyl-2-(p-tolyl)-1H-pyrrole) on the molecular structure.

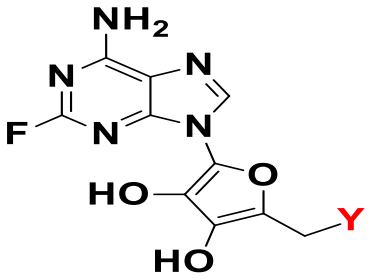
Results and Discussion

In our studies of fludarabine derivatives, we obtained data by adding various structures to the Y part without changing the X structure (F) of fludarabine, and we present these data in Table 1. The data in Table 1 show the relationship between fludarabine derivatives as protease inhibitors, kinase inhibitors, enzyme inhibitors, etc.

The bioactivity scores of all proposed fludarabine derivatives are shown in Table 1. In general, the higher the bioactivity score, the higher the probability that the fludarabine derivatives under investigation are active. According to the prediction of the software's, scores of 0.5 or higher are considered as good activity, while scores between 0.2 and 0.5 are interpreted as moderate activity [36]. G protein-coupled receptors (GPCRs) are the largest class of membrane proteins [37]. They activate intracellular signalling pathways by sensing extracellular signals such as hormones, neurotransmitters and local mediators. The GPCR protein family, which plays a role in many diseases such as diabetes, obesity and Alzheimer's, is considered one of the most important pharmacological targets. Another important pharmacological target is that GPCRs undergo significant changes in their receptor shape upon activation, meaning that they have a very dynamic structure [38-40]. The work of by Eryılmaz E in 2019 is similar to our work and inspired us. Their study was based on the drug nelarabine, which is used to treat acute lymphoblastic leukaemia. In their study, they found that the C19 compound they designed fulfilled all the Lipinski rules. They also highlighted that the C19 compound had higher GPCR, ion CM and protease activity compared to nelarabine, and that it had the potential to be a good anti-leukaemic drug [41]. We compared our fludarabine derivatives with fludarabine in terms of their bioactivity performance. M6 derivative of fludarabine showed higher ion CM, kinase and protease activity compared to fludarabine. The M15 derivative of fludarabine (2-(6-amino-2-fluoro-9H-purin-9-yl)-5-(2-aminoethyl)furan-3,4-diol) showed higher GPCR, ion CM, kinase, protease and enzyme inhibition activity compared to fludarabine. In addition, the GPCR, ion CM, kinase and enzyme inhibition activity values of this molecule were greater than 0.5.

In our experiments with fludarabine-derived drug molecules, we experimented by binding different molecules to both the X and Y structures of fludarabine at the same time. We use the abbreviation 'C' to refer to the molecules in these trials. In these experiments, we did not achieve higher biological activity than fludarabine (Table 2). In Table 2, we only found a higher value for Kinase activity in the C1 molecule compared to fludarabine.

Table 1. Modification of the Y-moiety of fludarabine with different functional groups.



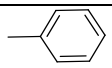
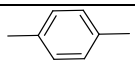
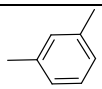
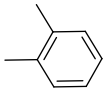
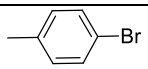
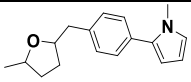
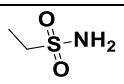
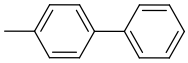
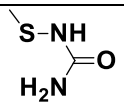
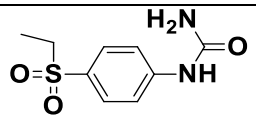
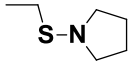
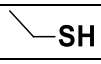
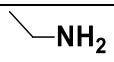
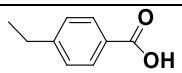
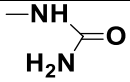
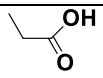
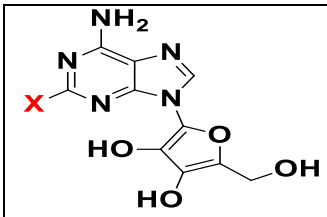
Drug Molecule	Y	GPCR	Ion CM	Kinase	Nuclear Receptor Ligand	Protease	Enzyme
Fludarabine	-OH	1.30	0.44	0.53	-0.91	0.15	1.33
M1		0.53	-0.01	0.33	-0.19	**0.17**	0.91
M2		1.02	0.38	0.51	-0.59	**0.24**	0.97
M3		0.78	0.36	0.25	-0.27	0.15	0.75
M4		0.32	-0.01	0.11	-0.18	0.11	0.50
M5		0.97	0.38	0.50	-0.67	**0.19**	0.95
M6	-CMe_3	1.22	**0.47**	**0.56**	-0.65	**0.32**	1.23
M7	-C(C=O) OMe	1.17	0.38	0.50	-0.66	**0.28**	1.18
M8		0.76	0.16	**0.57**	**0.30**	0.09	0.88
M9		1.26	0.32	0.46	-0.62	**0.55**	1.28
M10		0.93	0.41	0.52	-0.42	**0.28**	0.89
M11		1.22	0.29	**0.64**	-0.86	**0.29**	0.64
M12		0.40	-0.15	0.08	-0.34	**0.32**	0.57
M13		1.18	0.26	0.52	-0.77	**0.34**	1.15
M14		1.28	0.39	0.60	-0.95	**0.43**	1.59
M15		**1.40**	**0.58**	**0.78**	-1.04	**0.34**	**1.57**
M16		0.98	0.39	0.46	-0.44	**0.27**	0.97
M 17		1.05	0.22	**0.56**	-0.77	**0.31**	1.19
M18		0.52	0.16	0.06	-0.23	0.09	0.65

Table 2. Modification of the X and Y moieties of fludarabine with different functional groups.



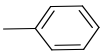
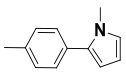
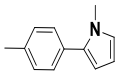
Drug Molecule	Y	X	GPCR	Ion CM	Kinase	Nuclear Receptor Ligand	Protease	Enzyme
Fludarabine	**-OH**	**F**	**1.30**	**0.44**	**0.53**	**-0.91**	**0.15**	**1.33**
C1	-OH		0.86	0.34	**0.68**	-0.65	0.12	0.94
C2			0.56	-0.09	0.30	-0.55	0.14	0.49

Table 3. Physicochemical properties and drug molecule similarity parameters for fludarabine and itsderivatives.

Drug Molecule	Mi LogP	MW	Volume	TPSA	NHD	NHA	Nrotb	Nat
Fludarabine	-0.37	285.24	223.46	139.55	9	5	2	20
M6	2.51	339.37	298.43	119.32	8	4	4	24
M15	-0.93	284.25	226.73	145.35	9	6	2	20

Logarith of partition coefficient between n-octanol and water (miLogP); Molecular weight (MW); Topological polar surface area (TPSA); Number of hydrogen bond donors (NHD); Number of hydrogen bond acceptors (NHA); Number of rotatable bond (Nrotb); Number of heavy atoms (Nat).

Basic indicators such as LogP, molecular weight, topological polar surface area, number of rotatable bonds, hydrogen bond donors and acceptors are used to determine whether a drug has good bioavailability [42]. A negative miLogP value indicates hydrophilicity, while a positive miLogP value indicates hydrophobicity. In their study, Bade et al. reported that synthetic drugs in particular exceeded the Lipinski LogP value, while natural drugs had acceptable LogP values and emphasised the importance of drug lipophilicity [43]. Khan et al. reported in their study of drug similarity and toxicity calculations that the LogP values, which indicate molar lipophilicity, were within the desired limits for all complexes and that these complexes may have good permeability across cell membranes [44]. Fludarabine and our M15 derivative have a negative LogP value, wereas our molecule M6 derivative has a positive LogP value. The M15 derivative of fludarabine is much more hydrophilic as it has a negative LogP value.

If we compare in terms of MW; according to Ghose's [45] and Lipinski's [46] criteria, a MW range of 160 to 480 g/mol and <500 g/mol respectively is preferred, Khan et al. reported in their study that the molecular weights of all the complexes were <500 and therefore these complexes could be easily transported, dispersed and absorbed [44]. These criteria (MW<500 g/mol) are fulfilled by our derivative molecules M6 and M15. Comparing the molecular volumes,

the presence of tertiary butyl groups in our M6 derivative increases the volume and surface area of our molecule. For this reason, the volume value of the M6 molecule is high. The volume values of fludarabine and the M15 derivative molecule are close to each other (Table 3).

TPSA is associated with the hydrogen bonding of a molecule and is used as a good indicator of the bioavailability of molecules. Muegge et al. [47] emphasized that TPSA≤150 is appropriate. In their study, Khan et al. found that the TPSA values of the molecules ranged from 41.05 to 98.60. They also reported that the oral bioavailability of doxorubicin HCl was not good, with a TPSA value of 206.08 [44]. While fludarabine has a TPSA value of 139.55, we found that our M15 derivative of fludarabine has an increased polar surface area effect compared to fludarabine with a TPSA value of 145.35. It has been reported that hydrogen bond donors >5 and hydrogen bond acceptors >10 have a negative effect on cell membrane permeability and absorption [46,47]. In our fludarabine derivatives (M6: 8, M15: 9, respectively) we found the number of hydrogen bond donors to be >5 (as in fludarabine (NHD: 9), while the hydrogen bond acceptor value was in agreement with the references for fludarabine (NHA: 5) and our fludarabine derivatives (M6: 4, M15: 6).

Nrotb is important for the conformational flexibility of the molecule and should be ≤ 10 or ≤ 15 [45,48]. Fludarabine and our fludarabine derivatives meet these criteria. The higher number of rotatable bonds in M6 (n: 4) gives it an advantage over fludarabine (n: 2) and M15 (n: 2) (Table 3). However, the disadvantage of the M6 derivatives is that the number of rotatable bonds has hydrophobic properties.

Conclusions

A priori knowledge is generated using computational methods prior to experimental drug discovery studies. In this way, drug molecule development studies are accelerated without spending much time on the experimental process. In drug molecule synthesis development studies, we have designed new therapeutic molecules by making bioisocentric modifications in Fludarabine drug to increase the potential effect and reduce side effects. Biological activity studies of Fludarabine drug derivatised using different functional groups were carried out (shown in Table 1,2. -CMe3, ethanamine; etc.). From the series of bioisosteric analogues of the commercially available drug molecule Fludarabine (M1-M18) - (C1-C2), our derivative of Fludarabine with -CMe3, ethanamine added at the Y-position, showed good pharmacokinetic property and biological activity. Of these, especially our M15 derivative molecule in particular showed higher bioactivity spectra in five of the six drug molecule targets. The results show higher pharmacokinetic and biological activity potential compared to clinically used leukemia drugs. This original derivative molecule (M15) designed by us is useful for further research in drug molecule development in medical biochemistry, chemistry and pharmacology. Our results suggest that this new derivative of fludarabine may be a better anti-leukemic drug molecule in the future.

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