IRSTI 31.23.00 https://doi.org/10.26577/IJBCh2024v17.i2.8

F. Islamoğlu

Recep Tayyip Erdoğan University, Rize, Turkey
e-mail: fatih.islamoglu@erdogan.edu.tr
(Received 22 August 2024; received in revised form 24 September 2024; accepted 10 October 2024)

Theoretical determination of the biological activities of some benzimidazole derivative compounds with potential as active pharmaceutical agents

Abstract. The biological activities of twelve different benzimidazole derivative compounds synthesized and registered in the literature were theoretically calculated with Way2Drug PASS software. Seven different biological activities, including acute rat toxicity, adverse drug effects, antibacterial activity, antifungal activity, anti-HIV activity, antiviral activity, and cell line cytotoxicity, were calculated for each benzimidazole derivative compound examined here. Rat acute toxicity was calculated in four different ways. These are Rat IP (intraperitoneal administration route) LD_{50}, Rat IV (intravenous administration route) LD_{50}, Rat Oral (oral administration route) LD_{50}, and Rat SC (subcutaneous administration route) LD_{50}. According to the results, a classification was also made for each method. Adverse effects that the molecules may show were determined with the help of the calculated Pa (probability of activity) and Pi (probability of inactivity) values. The antibacterial effect of each molecule against which bacteria was determined, and the confidence value of this effect was calculated. Likewise, it was determined whether the molecules showed antifungal properties. It was determined against which fungus the molecules showing antifungal properties showed this effect, and the confidence value was calculated. The anti-HIV properties of the molecules were studied for five different targets (protease (HIV-1), reverse transcriptase (HIV-1), integrase (HIV-1), REV (regulator of virion) (HIV-1), and TAT (trans-activator of transcription) (HIV-1)) and the p function of the IC_{50} (half maximal inhibitory concentration) values obtained were analyzed. Anti-viral effects of molecules examined. Here, the viruses against which they show this effect were determined, and the confidence value was calculated together with the target protein. Finally, cancer cell line and non-tumor cell line properties of the molecules were determined by Pa and Pi values as well as tissue and tumor type.
Key words: biological activities, benzimidazole derivatives, antibacterial, antifungal, anti-HIV, antiviral activity.

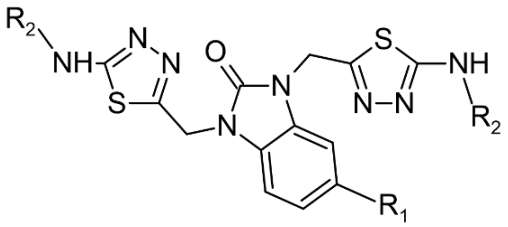
Introduction

A family of chemical compounds called benzimidazole derivatives is made up of molecules that have fused benzene and imidazole rings to form a benzimidazole moiety. These substances have been thoroughly researched and used in a variety of sectors, such as material science, medical chemistry, and agriculture, due to the wide range of biological activities [1]. Benzimidazole derivatives are known to have various biological activities. Many benzimidazole derivatives exhibit potent antibacterial and antifungal activities. They can inhibit the growth of a wide variety of pathogens by interfering with vital cellular processes [2]. Some benzimidazole derivatives are effective against parasites. For example, albendazole and mebendazole are used to treat parasitic worm infections by inhibiting microtubule synthesis [3]. Some derivatives exhibit antiviral properties, including activity against HIV and hepatitis viruses. They may inhibit viral replication by targeting viral enzymes or proteins [4]. Benzimidazole derivatives have shown potential as anticancer agents. They can induce apoptosis in cancer cells, inhibit cell proliferation, and disrupt cancer cell signaling pathways [5]. Some derivatives have been found to have anti-inflammatory and analgesic properties, making them potential candidates for the treatment of inflammatory diseases and pain management [6]. Benzimidazole derivatives are used in various therapeutic applications [7]. Drugs such as albendazole, mebendazole, and thiabendazole are used to treat helminth infections by inhibiting tubulin polymerization in the parasites [8]. Compounds such as omeprazole, lansoprazole, and pantoprazole are benzimidazole derivatives used

to treat gastroesophageal reflux disease (GERD) by inhibiting the gastric H^+/K^+ ATPase enzyme [9]. Various benzimidazole derivatives are being investigated as potential anticancer agents due to their ability to inhibit cell proliferation and induce apoptosis in cancer cells [10]. Some derivatives are used as antifungal agents in agriculture and medicine to control fungal infections [11]. Continuous research is being conducted to develop new benzimidazole derivatives with increased efficacy, reduced toxicity, and a broader spectrum of activity [12]. The studies aim to understand the molecular mechanisms by which benzimidazole derivatives exert their biological effects, which may aid in the design of more potent and selective compounds. The biological actions of benzoimidazole derivatives are diverse and include antibacterial, antiparasitic, antiviral, anticancer, anti-inflammatory, and analgesic properties. They continue to be the subject of study for the creation of novel medications and treatments. They are utilized in a variety of therapeutic applications, including anthelmintics and proton pump inhibitors [13-15].

Way2Drug PASS is a powerful computational tool for predicting the biological activity spectra of chemical compounds. Based on the structural formula of compounds, it provides predictions of therapeutic effects, modes of action, toxicities, and other features by utilizing a large database of known activities [16]. PASS facilitates the early stages of drug development by directing the validation of experiments and identifying promising activities. Notwithstanding its drawbacks, PASS is an invaluable tool for scientists studying chemical biology, pharmaceutical development, and related subjects [17].

Studied molecules. In this study, twelve different benzimidazole derivatives synthesized and registered [18] in the literature were studied. These benzimidazole derivatives are 1,3-Bis((5-(ethylamino)-1,3,4-thiadiazol-2-yl) methyl)-1,3-dihydro-2H-benzimidazol-2-one (1), 1,3-Bis((5-(phenylamino)-1,3,4-thiadiazol-2-yl) methyl)-1,3-dihydro-2H-benzimidazol-2-one (2), 1,3-Bis((5-((4-nitro phenyl) amino)-1,3,4-thiadiazol-2-yl)methyl)-1,3-dihydro-2H-benzimidazol-2-one (3), 1,3-Bis((5-((4-fluoro phenyl) amino)-1,3,4-thiadiazol-2-yl)methyl)-1,3-dihydro-2H-benzimidazol-2-one (4), 1,3-Bis((5-(ethylamino)-1,3,4-thiadiazol-2-yl)methyl)-5-methyl-1,3-dihydro-2H-benzimidazol-2-one (5), 5-methyl-1,3-bis((5-(phenylamino)-1,3,4-thiadiazol-2-yl)methyl)-1,3-dihydro-2H-benzimidazol-2-one (6), 5-methyl-1,3-bis((5-((4-nitrophenyl) amino)-1,3,4-thia diazol-2-yl)methyl)-1,3-dihydro-2H-benzimidazol-2-one (7), 1,3-Bis((5-((4-fluorophenyl)amino)-1,3,4-thiadiazol-2-yl) methyl)-5-methyl-1,3-dihydro-2H-benzimidazol-2-one (8), 1,3 -Bis((5-(ethylamino)-1,3,4-thiadiazol -2-yl) methyl)-5-nitro-1,3-dihydro-2H-benzimidazol-2-one (9), 5-nitro-1,3-bis((5-(phenylamino)-1, 3,4-thiadiazol-2-yl)methyl)-1,3-dihydro-2H-benzimidazol-2-one (10), 5-nitro-1,3-bis((5-((4-nitro phenyl)amino)-1,3,4-thiadiazol-2-yl) methyl)-1,3-dihydro-2H-benzimidazol-2-one (11), and 1,3-Bis ((5-((4-fluorophenyl)amino)-1,3,4-thiadiazol-2-yl) methyl)-5-nitro-1,3-dihydro-2H-benzimidazol-2-one (12) The open structures of these molecules are given in Figure 1.



Mol.	R_1	R_2	Mol.	R_1	R_2
1	-H	$-C_2H_5$	7	$-CH_3$	*-(4)*NO_2-C_6H_4
2	-H	$-C_6H_5$	8	$-CH_3$	*-(4)*F-C_6H_4
3	-H	*-(4)*NO_2-C_6H_4	9	$-NO_2$	$-C_2H_5$
4	-H	*-(4)*F-C_6H_4	10	$-NO_2$	$-C_6H_5$
5	$-CH_3$	$-C_2H_5$	11	$-NO_2$	*-(4)*NO_2-C_6H_4
6	$-CH_3$	$-C_6H_5$	12	$-NO_2$	*-(4)*F-C_6H_4

Figure 1 – Molecular formulas of studied benzimidazole derivatives

Determination of acute rat toxicity. The term "acute rat toxicity" describes the harmful consequences that occur in rats following a single or brief exposure to a chemical. Toxicology uses this kind of testing frequently to assess the possible health risks associated with chemicals, medications, and other substances. For humans and other animals, the outcomes of acute toxicity testing are utilized to establish safe dosage ranges [19,20]. There are numerous recorded instances of using the GUSAR online application to forecast acute rat toxicity [21-23]. We calculated acute rat toxicity for all molecules as four administration methods using Way2Drug PASS software. The methods we calculated here are Rat IP LD_{50} (intraperitoneal administration toxicity measure), Rat IV LD_{50} (intravenous administration toxicity measure), Rat Oral LD_{50} (oral administration toxicity measure), and Rat SC LD_{50} (subcutaneous

administration toxicity measure). Calculations for each method were made as LD_{50} log10 (mmol/kg), LD_{50} (mg/kg), and LD_{50} Classification, and the obtained data were tabulated in this form. The classification recorded in the literature [24] is given in Table 1, and the data obtained is provided in Table 2.

Table 1 – Oral toxicity, dermal toxicity, and inhalation toxicity (for gases, vapors, and dusts/mists) classification and values recorded in the literatüre [24]

Category	Oral Toxicity	Dermal Toxicity	**Inhalation Toxicity (for gases, vapors, and dusts/mists)**
1	$LD_{50} \leq 5$ mg/kg	$LD_{50} \leq 50$ mg/kg	Gases: ≤ 100 ppm Vapors: ≤ 0.5 mg/L Dusts/Mists: ≤ 0.05 mg/L
2	5 mg/kg < LD_{50} ≤ 50 mg/kg	50 mg/kg < LD_{50} ≤ 200 mg/kg	Gases: 100 ppm < $LC_{50} \leq 500$ ppm Vapors: 0.5 mg/L < $LC_{50} \leq 2$ mg/L Dusts/Mists: 0.05 mg/L < $LC_{50} \leq 0.5$ mg/L
3	50 mg/kg < LD_{50} ≤ 300 mg/kg	200 mg/kg < LD_{50} ≤ 1000 mg/kg	Gases: 500 ppm < $LC_{50} \leq 2500$ ppm Vapors: 2 mg/L < $LC_{50} \leq 10$ mg/L Dusts/Mists: 0.5 mg/L < $LC_{50} \leq 1$ mg/L
4	300 mg/kg < LD_{50} ≤ 2000 mg/kg	1000 mg/kg < LD_{50} ≤ 2000 mg/kg	Gases: 2500 ppm < $LC_{50} \leq 5000$ ppm Vapors: 10 mg/L < $LC_{50} \leq 20$ mg/L Dusts/Mists: 1 mg/L < $LC_{50} \leq 5$ mg/L
5	2000 mg/kg < LD_{50} ≤ 5000 mg/kg	2000 mg/kg < LD_{50} ≤ 5000 mg/kg	Gases: 5000 ppm < $LC_{50} \leq 20000$ ppm Vapors: 20 mg/L < $LC_{50} \leq 50$ mg/L Dusts/Mists: 5 mg/L < $LC_{50} \leq 10$ mg/L

Determination of adverse effect. The Way2Drug platform offers a tool called adverse effect that is intended to help predict the possible negative effects of chemical compounds. This tool provides information about potential toxicological and pharmacological side effects that a substance may show using cheminformatics techniques [25].

Adverse effect forecasts a broad spectrum of unfavorable outcomes that may result from coming into contact with a chemical substance. Numerous pharmacological and toxicological outcomes may be among these consequences [26]. The adverse effect values (Pa and Pi) calculated for all molecules and the side effect made by the adverse effect values are given in Table 3.

Determination of antibacterial activity. The term "antibacterial activity" describes a substance's capacity to either stop or eradicate bacterial growth. This quality is essential in many industries, including food safety, agriculture, and healthcare [27]. In microbiology, biochemistry, and medicine, antibacterial activity is a major field of study that focuses on the identification of novel antibacterial agents, comprehension of their modes of action, and development of strategies to counter antibiotic resistance [28]. The antibacterial properties of the molecules we studied against which bacteria and their confidence values (high confidence (> 0.7, there's a good chance the substance will have antibacterial properties. These substances are typically given priority for additional experimental investigation and advancement), medium confidence, (0.5-0.7, the chemical may have antibacterial activity, but more research is needed to confirm this claim), low confidence (<0.5, there is little chance that the substance will exhibit antibacterial activity. These kinds of chemicals are typically regarded as less important)) and MIC(μg/mL) values are given in Table 4.

Table 2 – Acute rat toxicity values and classification of molecules

Molecule	Rat Acute Toxicity											
	Rat IP LD_{50} log10 (mmol/kg)	Rat IP LD_{50} (mg/kg)	Rat IP LD_{50} Classification	Rat IV LD_{50} log10 (mmol/kg)	Rat IV LD_{50} (mg/kg)	Rat IV LD_{50} Classification	Rat Oral LD_{50} log10 (mmol/kg)	Rat Oral LD_{50} (mg/kg)	Rat Oral LD_{50} Classification	Rat SC LD_{50} log10 (mmol/kg)	Rat SC LD_{50} (mg/kg)	Rat SC LD_{50} Classification
1	0.341 out of AD	913.000 out of AD	Class 5 out of AD	-0.192 in AD	267.900 in AD	Class 4 in AD	0.665 in AD	1928.000 in AD	Class 4 in AD	0.533 in AD	1421.000 in AD	Class 5 in AD
2	-0.006 in AD	505.100 in AD	Class 5 in AD	-0.139 in AD	371.900 in AD	Class 5 in AD	0.538 in AD	1769.000 in AD	Class 4 in AD	0.542 in AD	1786.000 in AD	Class 5 in AD
3	0.573 out of AD	2255.000 out of AD	Non Toxic out of AD	-0.473 in AD	202.700 in AD	Class 4 in AD	0.484 in AD	1838.000 in AD	Class 4 in AD	0.548 out of AD	2130.000 out of AD	Class 5 out of AD
4	0.324 out of AD	1157.000 out of AD	Class 5 out of AD	-0.288 in AD	282.600 in AD	Class 4 in AD	0.701 in AD	2757.000 in AD	Class 5 in AD	0.520 out of AD	1819.000 out of AD	Class 5 out of AD
5	0.085 in AD	523.900 in AD	Class 5 in AD	-0.436 in AD	157.600 in AD	Class 4 in AD	0.699 in AD	2153.000 in AD	Class 5 in AD	0.563 out of AD	1574.000 out of AD	Class 5 out of AD
6	0.156 in AD	754.000 in AD	Class 5 in AD	-0.379 in AD	219.900 in AD	Class 4 in AD	0.613 out of AD	2159.000 out of AD	Class 5 out of AD	0.959 in AD	4788.000 in AD	Non Toxic in AD
7	0.174 in AD	921.000 in AD	Class 5 in AD	-0.481 in AD	203.800 in AD	Class 4 in AD	-0.160 out of AD	426.200 out of AD	Class 4 out of AD	0.419 out of AD	1617.000 out of AD	Class 5 out of AD
8	0.187 out of AD	865.800 out of AD	Class 5 out of AD	-0.483 in AD	185.000 in AD	Class 4 in AD	0.637 out of AD	2438.000 out of AD	Class 5 out of AD	0.425 out of AD	1497.000 out of AD	Class 5 out of AD
9	0.414 out of AD	1197.000 out of AD	Class 5 out of AD	-0.348 in AD	207.200 in AD	Class 4 in AD	0.625 in AD	1947.000 in AD	Class 4 in AD	0.422 out of AD	1219.000 out of AD	Class 5 out of AD
10	0.261 in AD	1016.000 in AD	Class 5 in AD	-0.464 in AD	191.400 in AD	Class 4 in AD	0.809 in AD	3592.000 in AD	Class 5 in AD	0.389 in AD	1365.000 in AD	Class 5 in AD
11	0.158 in AD	932.100 in AD	Class 5 in AD	-0.330 in AD	302.800 in AD	Class 5 in AD	0.493 in AD	2013.000 in AD	Class 5 in AD	0.074 in AD	767.800 in AD	Class 4 in AD
12	0.223 in AD	991.700 in AD	Class 5 in AD	-0.385 in AD	244.600 in AD	Class 4 in AD	-0.043 out of AD	537.400 out of AD	Class 4 out of AD	0.198 out of AD	937.500 out of AD	Class 4 out of AD

*: In AD (Inside Applicability Domain), Out of AD (Outside Applicability Domain)

Table 3 – Adverse effect properties realized by molecules

Molecule	Pa	Pi	Side Effect
1	-	-	-
2	0.273	0.187	Nephrotoxicity
3	0.419	0.245	Hepatotoxicity
4	0.410 0.357 0.324	0.252 0.110 0.266	Hepatotoxicity Nephrotoxicity Arrhythmia
5	-	-	-
6	-	-	-
7	0.403	0.257	Hepatotoxicity
8	0.394 0.306	0.263 0.150	Hepatotoxicity Nephrotoxicity
9	-	-	-
10	0.422	0.243	Hepatotoxicity
11	0.422	0.243	Hepatotoxicity
12	0.641	0.124	Hepatotoxicity

Table 4 – Antibacterial activity properties of molecules

Molecule	Name	Confidence	MIC(μg/mL)
1	Shigella sp.	0.2737	0.73073
	Pseudomonas sp.	0.0280	7.14286
	Providencia rettgeri	0.1150	1.73913
	Morganella morganii	0.0069	28.98551
	Haemophilus parainfluenzae	0.0280	7.14286
	Citrobacter koseri	0.0280	7.14286
	Acinetobacter calcoaceticus	0.1521	1.31492
2	Shigella sp.	0.2067	0.96759
3	Shigella sp.	0.0801	2.49688
	Clostridium difficile	0.0655	3.05344
	Bacillus cereus	0.0315	6.34921
	Bacteroides stercoris	0.0010	200.00000
4	Shigella sp.	0.1973	1.01368
	Bacillus sphaericus	0.0099	20.20202
	Yersinia pseudotuberculosis	0.0087	22.98851
5	Shigella sp.	0.2288	0.87413
	Acinetobacter calcoaceticus	0.1059	1.88857
	Providencia rettgeri	0.0940	2.12766
	Bacteroides stercoris	0.0441	4.53515
	Citrobacter koseri	0.0133	15.03759
	Haemophilus parainfluenzae	0.0133	15.03759
	Pseudomonas sp.	0.0133	15.03759
6	Shigella sp.	0.1730	1.15607
	Yersinia pseudotuberculosis	0.0062	32.25806

Continuation of the table

Molecule	Name	Confidence	MIC(μg/mL)
7	Bacteroides stercoris	0.1473	1.35777
	Shigella sp.	0.0590	3.38983
	Bacillus cereus	0.0384	5.20833
	Clostridium difficile	0.0198	10.10101
8	Shigella sp.	0.1696	1.17925
	Yersinia pseudotuberculosis	0.0345	5.79710
	Bacillus sphaericus	0.0246	8.13008
9	Bacteroides stercoris	0.1355	1.47601
	Shigella sp.	0.1327	1.50716
	Dialister invisus	0.0895	2.23464
	Providencia rettgeri	0.0812	2.46305
	Clostridium difficile	0.0717	2.78940
	Acinetobacter calcoaceticus	0.0250	8.00000
	Actinomyces meyeri	0.0070	28.57143
	Helicobacter pylori	0.0004	500.00000
	Citrobacter koseri	0.0001	2000.00000
	Haemophilus parainfluenzae	0.0001	2000.00000
	Pseudomonas sp.	0.0001	2000.00000
10	Shigella sp.	0.0516	3.87597
	Clostridium difficile	0.0457	4.37637
	Mycobacterium tuberculosis H37Rv	0.0441	4.53515
	Bacillus cereus	0.0038	52.63158
11	Shigella sp.	0.0496	4.03226
	Mycobacterium tuberculosis H37Rv	0.0469	4.26439
	Clostridium difficile	0.0427	4.68384
	Bacillus cereus	0.0048	41.66667
12	Mycobacterium tuberculosis H37Rv	0.0755	2.64901
	Shigella sp.	0.0608	3.28947
	Clostridium difficile	0.0453	4.41501
	Bacillus sphaericus	0.0403	4.96278

Determination of antifungal activity. Antifungal activity refers to the ability of a substance to inhibit the growth of or kill fungi. Treating fungal infections in people, animals, and plants requires this characteristic. Research on antifungal action is essential in the fields of pharmacy, agriculture, and medical mycology [29]. Research is still being done to find new antifungal medicines, comprehend how they work, and deal with the problem of antifungal resistance [30]. Antifungal activity is divided into four classes according to the MIC (μg/mL) value. These are; highly active (MIC ≤ 1 μg/mL, indicates strong antifungal activity and the agent is effective at very low concentrations), moderately active (MIC 1-10 μg/mL, shows good antifungal activity at moderate concentrations), weakly active (MIC 10-50 μg/mL, exhibits some antifungal activity but requires higher concentrations to be effective), and inactive (MIC > 50 μg/mL, indicates little to no antifungal activity, even at high concentrations). The fungi for which the molecules show antifungal effect, and the confidence and MIC(μg/mL) values of this effect are given in Table 5.

Table 5 – Antifungal activity properties of molecules

Molecule	Name	Confidence	MIC (µg/mL)
1	Cryptococcus bacillisporus	0.0050	20.00000
2	-	-	-
3	-	-	-
4	Cryptococcus bacillisporus	0.1019	0.98135
	Cryptococcus albidus	0.0574	1.74216
5	-	-	-
6	-	-	-
7	-	-	-
8	Cryptococcus bacillisporus	0.0582	1.71821
	Cryptococcus albidus	0.0217	4.60829
9	-	-	-
10	-	-	-
11	-	-	-
12	-	-	-

Determination of HIV targets. The term "HIV targets" refers to the process of locating and forecasting possible biological targets that may be utilized in the development or improvement of HIV-related medications and treatments [31]. This is essential for creating successful therapies that can change the host's immune response, stop the virus from replicating, or stop it from infecting new cells. All things considered, HIV targets prediction is an important field of study in the continuous endeavor to manage and ultimately eradicate HIV/AIDS [32]. When it comes to predicting HIV targets, pIC_{50} (pIC_{50} = -log (IC_{50}, half maximal inhibitory concentration)) is a metric that expresses how well a substance inhibits a particular biological target-like an enzyme or receptor-that is essential to the HIV life cycle. The potency of various compounds in blocking important targets implicated in the HIV life cycle may be evaluated and compared in a straightforward and scalable manner using pIC_{50}, a crucial parameter in the prediction of HIV targets [33]. HIV targets and pIC_{50} values determined for the molecules are given in Table 6.

Table 6 – HIV targets and prediction pIC_{50} value of molecules

Molecule	Target	pIC_{50}	IC_{50} (µM)
1	Protease (HIV-1)	5.014	9.68278E-06
	Reverse transcriptase (HIV-1)	5.163	6.87068E-06
	Integrase (HIV-1)	4.776	1.67494E-05
	REV (regulator of expression of virion proteins) (HIV-1)	4.697	2.00909E-05
	TAT (trans-activator of transcription) (HIV-1)	inactive	inactive
2	Protease (HIV-1)	5.510	3.09030E-06
	Reverse transcriptase (HIV-1)	4.615	2.42661E-05
	Integrase (HIV-1)	4.551	2.81190E-05
	REV (HIV-1)	4.731	1.85780E-05
	TAT (HIV-1)	inactive	inactive

Continuation of the table

Molecule	Target	pIC_{50}	IC_{50} (μM)
3	Protease (HIV-1)	5.835	1.46218E-06
	Reverse transcriptase (HIV-1)	4.815	1.53109E-05
	Integrase (HIV-1)	4.677	2.10378E-05
	REV (HIV-1)	4.895	1.27350E-05
	TAT (HIV-1)	active	active
4	Protease (HIV-1)	6.259	5.50808E-07
	Reverse transcriptase (HIV-1)	5.143	7.19449E-06
	Integrase (HIV-1)	4.889	1.29122E-05
	REV (HIV-1)	4.464	3.43558E-05
	TAT (HIV-1)	inactive	inactive
5	Protease (HIV-1)	5.471	3.38065E-06
	Reverse transcriptase (HIV-1)	5.373	4.23643E-06
	Integrase (HIV-1)	4.894	1.27644E-05
	REV (HIV-1)	4.577	2.64850E-05
	TAT (HIV-1)	inactive	inactive
6	Protease (HIV-1)	6.190	6.45654E-07
	Reverse transcriptase (HIV-1)	4.829	1.48252E-05
	Integrase (HIV-1)	4.415	3.84592E-05
	REV (HIV-1)	4.616	2.42103E-05
	TAT (HIV-1)	inactive	inactive
7	Protease (HIV-1)	6.125	7.49894E-07
	Reverse transcriptase (HIV-1)	5.084	8.24138E-06
	Integrase (HIV-1)	4.666	2.15774E-05
	REV (HIV-1)	4.775	1.67880E-05
	TAT (HIV-1)	active	active
8	Protease (HIV-1)	6.662	2.17771E-07
	Reverse transcriptase (HIV-1)	5.337	4.60257E-06
	Integrase (HIV-1)	4.817	1.52405E-05
	REV (HIV-1)	4.400	3.98107E-05
	TAT (HIV-1)	inactive	inactive
9	Protease (HIV-1)	5.038	9.16220E-06
	Reverse transcriptase (HIV-1)	5.470	3.38844E-06
	Integrase (HIV-1)	5.135	7.32825E-06
	REV (HIV-1)	4.935	1.16145E-05
	TAT (HIV-1)	inactive	inactive
10	Protease (HIV-1)	5.776	1.67494E-06
	Reverse transcriptase (HIV-1)	4.840	1.44544E-05
	Integrase (HIV-1)	4.649	2.24388E-05
	REV (HIV-1)	4.932	1.16950E-05
	TAT (HIV-1)	inactive	inactive

Continuation of the table

Molecule	Target	pIC_{50}	IC_{50} (μM)
11	Protease (HIV-1)	6.120	7.58578E-07
	Reverse transcriptase (HIV-1)	4.866	1.36144E-05
	Integrase (HIV-1)	4.687	2.05589E-05
	REV (HIV-1)	4.970	1.07152E-05
	TAT (HIV-1)	active	active
12	Protease (HIV-1)	6.224	5.97035E-07
	Reverse transcriptase (HIV-1)	5.355	4.41570E-06
	Integrase (HIV-1)	4.972	1.06660E-05
	REV (HIV-1)	4.677	2.10378E-05
	TAT (HIV-1)	inactive	inactive

Determination of antiviral properties. The ability of a material or molecule to prevent or treat viral infections by blocking the replication or activity of viruses is known as its antiviral capabilities. These characteristics are crucial for the creation of antiviral medications, which fight viral illnesses as COVID-19, hepatitis, HIV, and influenza [34]. A substance's ability to impede a virus at any point in its life cycle-from entry through replication, assembly, and release is referred to as its antiviral property [35]. Effective antiviral therapy depends on these qualities, with selectivity, resistance, and clinical use being key factors in the development and use of antiviral medications that can cure or prevent viral infections [36]. Confidence values closer to 1 indicate a higher probability that the compound has the predicted antiviral activity. The viruses, target proteins and confidence values calculated for the antiviral effect of the molecules are given in Table 7.

Determination of cancer line cell. In general, the phrase "cancer cell line value" describes how active a substance is in relation to a particular cancer cell line. A population of cells that can be cultivated and maintained in a laboratory environment that are derived from a specific cancer is known as a cancer cell line. A549 (lung cancer), MCF-7 (breast cancer), and the HeLa cell line (cervical cancer) are a few examples [37]. These cell lines are used by researchers to examine how substances affect cancer cells, evaluate possible anticancer medications, and comprehend the biology of cancer [38]. Pa and Pi values are crucial in drug discovery and development, as they provide a quantitative measure of a compound's efficacy against specific cancer types [39]. To determine which substances are the most effective anticancer medicines, researchers evaluate the Pa and Pi values of various chemicals and cell lines, and these values are used in predictive models to estimate the potential clinical effectiveness of new compounds [40]. The cell-line, non-tumor cell line, cell-line full name, tissue, and tumor type we determined for the molecules and the Pa and Pi values we calculated based on these are given in Table 8.

Table 7 – Antiviral activity properties of molecules

Molecule	Virus	Protein target	Confidence
1	Dengue virus 2	Genome polyprotein	0.5092
	Vaccinia virus (strain Western Reserve) (VACV) (Vaccinia virus (strainWR))	DNA polymerase	0.1266
	Varicella-zoster virus (strain Dumas) (HHV-3) (Human herpesvirus 3)	DNA polymerase	0.1015
	Herpes simplex virus (type 1 / strain 17)	Human herpesvirus 1 DNA polymerase	0.1015
	Severe acute respiratory syndrome coronavirus 2	Replicase polyprotein 1ab	0.0846
	Middle East respiratory syndrome-related coronavirus (isolate UnitedKingdom/ H123990006/2012) (Betacoronavirus England 1) (Humancoronavirus EMC)	Replicase polyprotein 1ab	0.0475
	Human herpesvirus 6A (strain Uganda-1102) (HHV-6 variant A) (Human Blymphotropic virus)	Human herpesvirus 6 DNA polymerase	0.0263

Continuation of the table

Molecule	Virus	Protein target	Confidence
2	Dengue virus 2	Genome polyprotein	0.3627
	Severe acute respiratory syndrome coronavirus 2	Replicase polyprotein 1ab	0.3075
	Varicella-zoster virus (strain Dumas) (HHV-3) (Human herpesvirus 3)	DNA polymerase	0.1398
	Herpes simplex virus (type 1 / strain 17)	Human herpesvirus 1 DNA polymerase	0.1398
	Dengue virus type 2	Genome polyprotein	0.1253
	Vaccinia virus (strain Western Reserve) (VACV) (Vaccinia virus (strainWR))	DNA polymerase	0.1083
	Macacine herpesvirus 1	Thymidine kinase	0.0362
	Human herpesvirus 6A (strain Uganda-1102) (HHV-6 variant A) (Human Blymphotropic virus)	Human herpesvirus 6 DNA polymerase	0.0240
	Middle East respiratory syndrome-related coronavirus (isolate UnitedKingdom/H123990006/2012) (Betacoronavirus England 1) (Humancoronavirus EMC)	Replicase polyprotein 1ab	0.0177
3	Severe acute respiratory syndrome coronavirus 2	Replicase polyprotein 1ab	0.3104
	Dengue virus 2	Genome polyprotein	0.3050
	Human herpesvirus 6A (strain Uganda-1102) (HHV-6 variant A) (Human Blymphotropic virus)	Human herpesvirus 6 DNA polymerase	0.0700
	Infectious bronchitis virus	3C-like protease	0.0242
	SARS coronavirus	SARS coronavirus 3C-like proteinase	0.0060
4	Dengue virus 2	Genome polyprotein	0.3262
	Severe acute respiratory syndrome coronavirus 2	Replicase polyprotein 1ab	0.1594
5	Dengue virus 2	Genome polyprotein	0.4442
	Vaccinia virus (strain Western Reserve) (VACV) (Vaccinia virus (strainWR))	DNA polymerase	0.0899
	Severe acute respiratory syndrome coronavirus 2	Replicase polyprotein 1ab	0.0729
	Varicella-zoster virus (strain Dumas) (HHV-3) (Human herpesvirus 3)	DNA polymerase	0.0642
	Herpes simplex virus (type 1 / strain 17)	Human herpesvirus 1 DNA polymerase	0.0642
6	Dengue virus 2	Genome polyprotein	0.3082
	Severe acute respiratory syndrome coronavirus 2	Replicase polyprotein 1ab	0.2992
	Dengue virus type 2	Genome polyprotein	0.1655
	Vaccinia virus (strain Western Reserve) (VACV) (Vaccinia virus (strainWR))	DNA polymerase	0.0842
	Varicella-zoster virus (strain Dumas) (HHV-3) (Human herpesvirus 3)	DNA polymerase	0.0642
	Herpes simplex virus (type 1 / strain 17)	Human herpesvirus 1 DNA polymerase	0.0642
7	Severe acute respiratory syndrome coronavirus 2	Replicase polyprotein 1ab	0.2983
	Dengue virus 2	Genome polyprotein	0.2671
	Infectious bronchitis virus	3C-like protease	0.0304
	Human herpesvirus 6A (strain Uganda-1102) (HHV-6 variant A) (Human Blymphotropic virus)	Human herpesvirus 6 DNA polymerase	0.0284
8	Dengue virus 2	Genome polyprotein	0.2862
	Severe acute respiratory syndrome coronavirus 2	Replicase polyprotein 1ab	0.1463

Continuation of the table

Molecule	Virus	Protein target	Confidence
9	Dengue virus 2	Genome polyprotein	0.4178
	Severe acute respiratory syndrome coronavirus 2	Replicase polyprotein 1ab	0.1287
	Human herpesvirus 6A (strain Uganda-1102) (HHV-6 variant A) (Human Blymphotropic virus)	Human herpesvirus 6 DNA polymerase	0.0668
	Infectious bronchitis virus	3C-like protease	0.0526
10	Severe acute respiratory syndrome coronavirus 2	Replicase polyprotein 1ab	0.3533
	Dengue virus 2	Genome polyprotein	0.2952
	Human herpesvirus 6A (strain Uganda-1102) (HHV-6 variant A) (Human Blymphotropic virus)	Human herpesvirus 6 DNA polymerase	0.0598
	Infectious bronchitis virus	3C-like protease	0.0482
11	Severe acute respiratory syndrome coronavirus 2	Replicase polyprotein 1ab	0.3489
	Dengue virus 2	Genome polyprotein	0.2982
	Human herpesvirus 6A (strain Uganda-1102) (HHV-6 variant A) (Human Blymphotropic virus)	Human herpesvirus 6 DNA polymerase	0.0608
	Infectious bronchitis virus	3C-like protease	0.0437
12	Dengue virus 2	Genome polyprotein	0.2735
	Severe acute respiratory syndrome coronavirus 2	Replicase polyprotein 1ab	0.1983
	Human herpesvirus 6A (strain Uganda-1102) (HHV-6 variant A) (Human Blymphotropic virus)	Human herpesvirus 6 DNA polymerase	0.0009

Table 8 – Cancer cell line and non-tumor cell line types of molecules and calculated Pa/Pi values

Molecule	Pa	Pi	Cell-line	Cell-line full name	Tissue	Tumor type
1	*Cancer cell line prediction*					
	0.145	0.063	HOS-TE85	Osteosarcoma	Bone	Sarcoma
	0.116	0.104	Melanoma cells	Melanoma	Skin	Melanoma
	0.354	0.003	SK-MEL	Melanoma	Skin	Melanoma
	0.070	0.048	MV4-11	Myeloid leukemia	Haematopoietic and lymphoid tissue	Leukemia
	0.171	0.079	Ramos	Burkitts lymhoma B-cells	Blood	Leukemia
	0.093	0.087	RT-4	Bladder carcinoma	Urinary tract	Carcinoma
	0.142	0.046	LNCaP	Prostate carcinoma	Prostate	Carcinoma
	0.160	0.148	MIA PaCa-2	Pancreatic carcinoma	Pancreas	Carcinoma
	0.372	0.041	SK-MES-1	Squamous cell lung carcinoma	Lung	Carcinoma
	0.377	0.134	YAPC	Pancreatic carcinoma	Pancreas	Carcinoma
	0.072	0.038	SISO	Uterine cervical adenocarcinoma	Cervix	Adenocarcinoma
	0.265	0.097	AGS	Gastric adenocarcinoma	Stomach	Adenocarcinoma
	0.347	0.045	OVCAR-3	Ovarian adenocarcinoma	Ovarium	Adenocarcinoma
	0.420	0.045	MDA-MB-231	Breast adenocarcinoma	Breast	Adenocarcinoma
	0.421	0.031	PC-9	Lung adenocarcinoma	Lung	Adenocarcinoma
	Non-tumor cell line prediction					
	0.364	0.040	HEK293	Embryonic kidney fibroblast	Kidney	-
	0.086	0.049	PBMC	Peripheral blood mononuclear cell	Blood	-

Continuation of the table

Molecule	Pa	Pi	Cell-line	Cell-line full name	Tissue	Tumor type
2	*Cancer cell line prediction*					
	0.731	0.005	MDA-MB-231	Breast adenocarcinoma	Breast	Adenocarcinoma
	0.590	0.013	OVCAR-3	Ovarian adenocarcinoma	Ovarium	Adenocarcinoma
	0.420	0.014	SK-MES-1	Squamous cell lung carcinoma	Lung	Carcinoma
	0.410	0.034	PC-9	Lung adenocarcinoma	Lung	Adenocarcinoma
	0.394	0.108	YAPC	Pancreatic carcinoma	Pancreas	Carcinoma
	0.309	0.033	AGS	Gastric adenocarcinoma	Stomach	Adenocarcinoma
	0.258	0.005	SK-MEL	Melanoma	Skin	Melanoma
	0.259	0.010	LNCaP	Prostate carcinoma	Prostate	Carcinoma
	0.180	0.019	HOS-TE85	Osteosarcoma	Bone	Sarcoma
	0.201	0.118	J82	Bladder carcinoma	Urinary tract	Carcinoma
	0.085	0.018	SISO	Uterine cervical adenocarcinoma	Cervix	Adenocarcinoma
	0.100	0.039	DAN-G	Human pancreas adenocarcinoma cell line	Pancreas	Adenocarcinoma
	0.072	0.030	SK-ES1	Ewing sarcoma	Bone	Sarcoma
	0.100	0.059	RT-4	Bladder carcinoma	Urinary tract	Carcinoma
	0.165	0.135	MIA PaCa-2	Pancreatic carcinoma	Pancreas	Carcinoma
	0.197	0.174	PC-3	Prostate carcinoma	Prostate	Carcinoma
	0.154	0.144	Ramos	Burkitts lymhoma B-cells	Blood	Leukemia
	0.114	0.110	Melanoma cells	Melanoma	Skin	Melanoma
	Non-tumor cell line prediction					
	0.346	*0.044*	*HEK293*	*Embryonic kidney fibroblast*	*Kidney*	-
3	*Cancer cell line prediction*					
	0.631	0.011	MDA-MB-231	Breast adenocarcinoma	Breast	Adenocarcinoma
	0.568	0.015	OVCAR-3	Ovarian adenocarcinoma	Ovarium	Adenocarcinoma
	0.454	0.029	YAPC	Pancreatic carcinoma	Pancreas	Carcinoma
	0.400	0.021	SK-MES-1	Squamous cell lung carcinoma	Lung	Carcinoma
	0.313	0.031	AGS	Gastric adenocarcinoma	Stomach	Adenocarcinoma
	0.338	0.068	PC-9	Lung adenocarcinoma	Lung	Adenocarcinoma
	0.219	0.015	LNCaP	Prostate carcinoma	Prostate	Carcinoma
	0.291	0.091	5637	Urothelial bladder carcinoma	Urinary tract	Carcinoma
	0.178	0.020	HOS-TE85	Osteosarcoma	Bone	Sarcoma
	0.158	0.009	SK-MEL	Melanoma	Skin	Melanoma
	0.142	0.013	DAN-G	Human pancreas adenocarcinoma cell line	Pancreas	Adenocarcinoma
	0.133	0.013	RT-4	Bladder carcinoma	Urinary tract	Carcinoma
	0.106	0.005	SISO	Uterine cervical adenocarcinoma	Cervix	Adenocarcinoma
	0.204	0.108	J82	Bladder carcinoma	Urinary tract	Carcinoma
	0.275	0.190	U-266	Plasma cell myeloma	Blood	Myeloma
	0.268	0.207	RKO	Colon carcinoma	Colon	Carcinoma
	0.270	0.210	T98G	Glioblastoma	Brain	Carcinoma
	0.079	0.020	SK-ES1	Ewing sarcoma	Bone	Sarcoma
	0.166	0.132	MIA PaCa-2	Pancreatic carcinoma	Pancreas	Carcinoma
	0.109	0.102	NSCLC	Non-small cell lung carcinoma	Lung	Carcinoma
	Non-tumor cell line prediction					
	0.416	0.033	HEK293	Embryonic kidney fibroblast	Kidney	-

Continuation of the table

Molecule	Pa	Pi	Cell-line	Cell-line full name	Tissue	Tumor type
4	*Cancer cell line prediction*					
	0.650	0.009	MDA-MB-231	Breast adenocarcinoma	Breast	Adenocarcinoma
	0.522	0.019	OVCAR-3	Ovarian adenocarcinoma	Ovarium	Adenocarcinoma
	0.413	0.016	SK-MES-1	Squamous cell lung carcinoma	Lung	Carcinoma
	0.387	0.043	PC-9	Lung adenocarcinoma	Lung	Adenocarcinoma
	0.298	0.042	AGS	Gastric adenocarcinoma	Stomach	Adenocarcinoma
	0.374	0.139	YAPC	Pancreatic carcinoma	Pancreas	Carcinoma
	0.240	0.012	LNCaP	Prostate carcinoma	Prostate	Carcinoma
	0.205	0.007	SK-MEL	Melanoma	Skin	Melanoma
	0.181	0.019	HOS-TE85	Osteosarcoma	Bone	Sarcoma
	0.179	0.067	NCI-N87	gastric carcinoma	Stomach	Carcinoma
	0.282	0.184	RKO	Colon carcinoma	Colon	Carcinoma
	0.212	0.129	MDA-MB-468	Breast adenocarcinoma	Breast	Adenocarcinoma
	0.081	0.017	SK-ES1	Ewing sarcoma	Bone	Sarcoma
	0.195	0.141	J82	Bladder carcinoma	Urinary tract	Carcinoma
	0.235	0.204	HuP-T3	Pancreatic adenocarcinoma	Pancreas	Adenocarcinoma
	0.164	0.137	MIA PaCa-2	Pancreatic carcinoma	Pancreas	Carcinoma
	0.272	0.253	Hs 683	Oligodendroglioma	Brain	Glioma
	Non-tumor cell line prediction					
	0.260	0.075	HEK293	Embryonic kidney fibroblast	Kidney	-
5	*Cancer cell line prediction*					
	0.391	0.041	PC-9	Lung adenocarcinoma	Lung	Adenocarcinoma
	0.393	0.052	MDA-MB-231	Breast adenocarcinoma	Breast	Adenocarcinoma
	0.327	0.004	SK-MEL	Melanoma	Skin	Melanoma
	0.366	0.047	SK-MES-1	Squamous cell lung carcinoma	Lung	Carcinoma
	0.314	0.056	OVCAR-3	Ovarian adenocarcinoma	Ovarium	Adenocarcinoma
	0.366	0.153	YAPC	Pancreatic carcinoma	Pancreas	Carcinoma
	0.172	0.075	Ramos	Burkitts lymhoma B-cells	Blood	Leukemia
	0.138	0.050	LNCaP	Prostate carcinoma	Prostate	Carcinoma
	0.237	0.154	AGS	Gastric adenocarcinoma	Stomach	Adenocarcinoma
	0.132	0.108	HOS-TE85	Osteosarcoma	Bone	Sarcoma
	0.163	0.140	MIA PaCa-2	Pancreatic carcinoma	Pancreas	Carcinoma
	0.244	0.227	C8166	Leukemic T-cells	Blood	Leukemia
	0.065	0.064	SISO	Uterine cervical adenocarcinoma	Cervix	Adenocarcinoma
	Non-tumor cell line prediction					
	0.337	0.046	HEK293	Embryonic kidney fibroblast	Kidney	-
	0.090	0.045	PBMC	Peripheral blood mononuclear cell	Blood	-

Continuation of the table

Molecule	Pa	Pi	Cell-line	Cell-line full name	Tissue	Tumor type
6	*Cancer cell line prediction*					
	0.650	0.009	MDA-MB-231	Breast adenocarcinoma	Breast	Adenocarcinoma
	0.515	0.020	OVCAR-3	Ovarian adenocarcinoma	Ovarium	Adenocarcinoma
	0.405	0.019	SK-MES-1	Squamous cell lung carcinoma	Lung	Carcinoma
	0.364	0.053	PC-9	Lung adenocarcinoma	Lung	Adenocarcinoma
	0.386	0.120	YAPC	Pancreatic carcinoma	Pancreas	Carcinoma
	0.227	0.014	LNCaP	Prostate carcinoma	Prostate	Carcinoma
	0.279	0.068	AGS	Gastric adenocarcinoma	Stomach	Adenocarcinoma
	0.197	0.007	SK-MEL	Melanoma	Skin	Melanoma
	0.162	0.033	HOS-TE85	Osteosarcoma	Bone	Sarcoma
	0.257	0.184	C8166	Leukemic T-cells	Blood	Leukemia
	0.160	0.115	Ramos	Burkitts lymhoma B-cells	Blood	Leukemia
	0.075	0.032	SISO	Uterine cervical adenocarcinoma	Cervix	Adenocarcinoma
	0.191	0.155	MDA-MB-468	Breast adenocarcinoma	Breast	Adenocarcinoma
	0.248	0.214	5637	Urothelial bladder carcinoma	Urinary tract	Carcinoma
	0.166	0.132	MIA PaCa-2	Pancreatic carcinoma	Pancreas	Carcinoma
	0.098	0.065	RT-4	Bladder carcinoma	Urinary tract	Carcinoma
	0.085	0.064	DAN-G	Human pancreas adenocarcinoma cell line	Pancreas	Adenocarcinoma
	0.186	0.182	J82	Bladder carcinoma	Urinary tract	Carcinoma
	0.187	0.183	PC-3	Prostate carcinoma	Prostate	Carcinoma
	Non-tumor cell line prediction					
	0.316	0.053	HEK293	Embryonic kidney fibroblast	Kidney	-
7	*Cancer cell line prediction*					
	0.584	0.016	MDA-MB-231	Breast adenocarcinoma	Breast	Adenocarcinoma
	0.505	0.021	OVCAR-3	Ovarian adenocarcinoma	Ovarium	Adenocarcinoma
	0.443	0.039	YAPC	Pancreatic carcinoma	Pancreas	Carcinoma
	0.391	0.026	SK-MES-1	Squamous cell lung carcinoma	Lung	Carcinoma
	0.285	0.058	AGS	Gastric adenocarcinoma	Stomach	Adenocarcinoma
	0.307	0.089	PC-9	Lung adenocarcinoma	Lung	Adenocarcinoma
	0.295	0.081	5637	Urothelial bladder carcinoma	Urinary tract	Carcinoma
	0.208	0.018	LNCaP	Prostate carcinoma	Prostate	Carcinoma
	0.272	0.141	C8166	Leukemic T-cells	Blood	Leukemia
	0.162	0.032	HOS-TE85	Osteosarcoma	Bone	Sarcoma
	0.137	0.010	SK-MEL	Melanoma	Skin	Melanoma
	0.126	0.017	RT-4	Bladder carcinoma	Urinary tract	Carcinoma
	0.114	0.026	DAN-G	Human pancreas adenocarcinoma cell line	Pancreas	Adenocarcinoma
	0.092	0.011	SISO	Uterine cervical adenocarcinoma	Cervix	Adenocarcinoma
	0.266	0.212	RKO	Colon carcinoma	Colon	Carcinoma
	0.168	0.129	MIA PaCa-2	Pancreatic carcinoma	Pancreas	Carcinoma
	0.258	0.227	U-266	Plasma cell myeloma	Blood	Myeloma
	0.190	0.162	J82	Bladder carcinoma	Urinary tract	Carcinoma
	0.262	0.236	T98G	Glioblastoma	Brain	Carcinoma
	0.066	0.051	CCRF-SB	Childhood T acute lymphoblastic leukemia	Blood	Leukemia
	0.061	0.057	SK-ES1	Ewing sarcoma	Bone	Sarcoma
	Non-tumor cell line prediction					
	0.373	0.039	HEK293	Embryonic kidney fibroblast	Kidney	-

Continuation of the table

Molecule	Pa	Pi	Cell-line	Cell-line full name	Tissue	Tumor type
8	*Cancer cell line prediction*					
	0.602	0.014	MDA-MB-231	Breast adenocarcinoma	Breast	Adenocarcinoma
	0.456	0.025	OVCAR-3	Ovarian adenocarcinoma	Ovarium	Adenocarcinoma
	0.402	0.020	SK-MES-1	Squamous cell lung carcinoma	Lung	Carcinoma
	0.353	0.059	PC-9	Lung adenocarcinoma	Lung	Adenocarcinoma
	0.222	0.015	LNCaP	Prostate carcinoma	Prostate	Carcinoma
	0.364	0.157	YAPC	Pancreatic carcinoma	Pancreas	Carcinoma
	0.274	0.078	AGS	Gastric adenocarcinoma	Stomach	Adenocarcinoma
	0.166	0.008	SK-MEL	Melanoma	Skin	Melanoma
	0.165	0.029	HOS-TE85	Osteosarcoma	Bone	Sarcoma
	0.230	0.109	MDA-MB-468	Breast adenocarcinoma	Breast	Adenocarcinoma
	0.278	0.191	RKO	Colon carcinoma	Colon	Carcinoma
	0.167	0.095	NCI-N87	gastric carcinoma	Stomach	Carcinoma
	0.166	0.134	MIA PaCa-2	Pancreatic carcinoma	Pancreas	Carcinoma
	0.244	0.227	C8166	Leukemic T-cells	Blood	Leukemia
	0.063	0.050	SK-ES1	Ewing sarcoma	Bone	Sarcoma
	0.242	0.230	5637	Urothelial bladder carcinoma	Urinary tract	Carcinoma
	Non-tumor cell line prediction					
	0.250	0.079	HEK293	Embryonic kidney fibroblast	Kidney	-
9	*Cancer cell line prediction*					
	0.436	0.046	YAPC	Pancreatic carcinoma	Pancreas	Carcinoma
	0.354	0.043	OVCAR-3	Ovarian adenocarcinoma	Ovarium	Adenocarcinoma
	0.355	0.058	SK-MES-1	Squamous cell lung carcinoma	Lung	Carcinoma
	0.298	0.004	SK-MEL	Melanoma	Skin	Melanoma
	0.351	0.060	PC-9	Lung adenocarcinoma	Lung	Adenocarcinoma
	0.310	0.081	MDA-MB-231	Breast adenocarcinoma	Breast	Adenocarcinoma
	0.278	0.071	AGS	Gastric adenocarcinoma	Stomach	Adenocarcinoma
	0.165	0.030	HOS-TE85	Osteosarcoma	Bone	Sarcoma
	0.265	0.166	5637	Urothelial bladder carcinoma	Urinary tract	Carcinoma
	0.120	0.022	RT-4	Bladder carcinoma	Urinary tract	Carcinoma
	0.083	0.019	SISO	Uterine cervical adenocarcinoma	Cervix	Adenocarcinoma
	0.123	0.076	LNCaP	Prostate carcinoma	Prostate	Carcinoma
	0.093	0.048	DAN-G	Human pancreas adenocarcinoma cell line	Pancreas	Adenocarcinoma
	0.165	0.135	MIA PaCa-2	Pancreatic carcinoma	Pancreas	Carcinoma
	0.070	0.049	MV4-11	Myeloid leukemia	Haematopoietic and lymphoid tissue	Leukemia
	Non-tumor cell line prediction					
	0.402	0.035	HEK293	Embryonic kidney fibroblast	Kidney	-
	0.099	0.085	WI-38	Embryonic lung fibroblast	Lung	-

Continuation of the table

Molecule	Pa	Pi	Cell-line	Cell-line full name	Tissue	Tumor type
10	*Cancer cell line prediction*					
	0.615	0.013	MDA-MB-231	Breast adenocarcinoma	Breast	Adenocarcinoma
	0.566	0.015	OVCAR-3	Ovarian adenocarcinoma	Ovarium	Adenocarcinoma
	0.448	0.034	YAPC	Pancreatic carcinoma	Pancreas	Carcinoma
	0.396	0.023	SK-MES-1	Squamous cell lung carcinoma	Lung	Carcinoma
	0.314	0.030	AGS	Gastric adenocarcinoma	Stomach	Adenocarcinoma
	0.324	0.077	PC-9	Lung adenocarcinoma	Lung	Adenocarcinoma
	0.213	0.017	LNCaP	Prostate carcinoma	Prostate	Carcinoma
	0.290	0.095	5637	Urothelial bladder carcinoma	Urinary tract	Carcinoma
	0.192	0.015	HOS-TE85	Osteosarcoma	Bone	Sarcoma
	0.160	0.009	SK-MEL	Melanoma	Skin	Melanoma
	0.129	0.015	RT-4	Bladder carcinoma	Urinary tract	Carcinoma
	0.126	0.019	DAN-G	Human pancreas adenocarcinoma cell line	Pancreas	Adenocarcinoma
	0.100	0.008	SISO	Uterine cervical adenocarcinoma	Cervix	Adenocarcinoma
	0.202	0.115	J82	Bladder carcinoma	Urinary tract	Carcinoma
	0.074	0.027	SK-ES1	Ewing sarcoma	Bone	Sarcoma
	0.267	0.221	T98G	Glioblastoma	Brain	Carcinoma
	0.167	0.132	MIA PaCa-2	Pancreatic carcinoma	Pancreas	Carcinoma
	0.117	0.086	NCI-H647	Adenosquamous lung carcinoma	Lung	Carcinoma
	0.254	0.236	U-266	Plasma cell myeloma	Blood	Myeloma
	Non-tumor cell line prediction					
	0.392	0.036	HEK293	Embryonic kidney fibroblast	Kidney	-
11	*Cancer cell line prediction*					
	0.624	0.012	MDA-MB-231	Breast adenocarcinoma	Breast	Adenocarcinoma
	0.572	0.015	OVCAR-3	Ovarian adenocarcinoma	Ovarium	Adenocarcinoma
	0.447	0.035	YAPC	Pancreatic carcinoma	Pancreas	Carcinoma
	0.397	0.022	SK-MES-1	Squamous cell lung carcinoma	Lung	Carcinoma
	0.314	0.030	AGS	Gastric adenocarcinoma	Stomach	Adenocarcinoma
	0.329	0.073	PC-9	Lung adenocarcinoma	Lung	Adenocarcinoma
	0.216	0.016	LNCaP	Prostate carcinoma	Prostate	Carcinoma
	0.288	0.100	5637	Urothelial bladder carcinoma	Urinary tract	Carcinoma
	0.193	0.015	HOS-TE85	Osteosarcoma	Bone	Sarcoma
	0.164	0.008	SK-MEL	Melanoma	Skin	Melanoma
	0.129	0.015	RT-4	Bladder carcinoma	Urinary tract	Carcinoma
	0.122	0.021	DAN-G	Human pancreas adenocarcinoma cell line	Pancreas	Adenocarcinoma
	0.098	0.008	SISO	Uterine cervical adenocarcinoma	Cervix	Adenocarcinoma
	0.202	0.116	J82	Bladder carcinoma	Urinary tract	Carcinoma
	0.072	0.029	SK-ES1	Ewing sarcoma	Bone	Sarcoma
	0.264	0.228	T98G	Glioblastoma	Brain	Carcinoma
	0.167	0.131	MIA PaCa-2	Pancreatic carcinoma	Pancreas	Carcinoma
	0.114	0.096	NCI-H647	Adenosquamous lung carcinoma	Lung	Carcinoma
	Non-tumor cell line prediction					
	0.397	0.036	HEK293	Embryonic kidney fibroblast	Kidney	-

Continuation of the table

Molecule	Pa	Pi	Cell-line	Cell-line full name	Tissue	Tumor type
12	*Cancer cell line prediction*					
	0.563	0.019	MDA-MB-231	Breast adenocarcinoma	Breast	Adenocarcinoma
	0.508	0.020	OVCAR-3	Ovarian adenocarcinoma	Ovarium	Adenocarcinoma
	0.434	0.049	YAPC	Pancreatic carcinoma	Pancreas	Carcinoma
	0.394	0.024	SK-MES-1	Squamous cell lung carcinoma	Lung	Carcinoma
	0.304	0.037	AGS	Gastric adenocarcinoma	Stomach	Adenocarcinoma
	0.315	0.083	PC-9	Lung adenocarcinoma	Lung	Adenocarcinoma
	0.210	0.018	LNCaP	Prostate carcinoma	Prostate	Carcinoma
	0.192	0.015	HOS-TE85	Osteosarcoma	Bone	Sarcoma
	0.283	0.113	5637	Urothelial bladder carcinoma	Urinary tract	Carcinoma
	0.140	0.010	SK-MEL	Melanoma	Skin	Melanoma
	0.112	0.033	RT-4	Bladder carcinoma	Urinary tract	Carcinoma
	0.273	0.198	T98G	Glioblastoma	Brain	Carcinoma
	0.273	0.198	RKO	Colon carcinoma	Colon	Carcinoma
	0.082	0.017	SK-ES1	Ewing sarcoma	Bone	Sarcoma
	0.196	0.136	J82	Bladder carcinoma	Urinary tract	Carcinoma
	0.161	0.114	NCI-N87	gastric carcinoma	Stomach	Carcinoma
	0.094	0.047	DAN-G	Human pancreas adenocarcinoma cell line	Pancreas	Adenocarcinoma
	0.072	0.038	SISO	Uterine cervical adenocarcinoma	Cervix	Adenocarcinoma
	0.154	0.121	SAOS-2	Osteosarcoma	Bone	Sarcoma
	0.166	0.133	MIA PaCa-2	Pancreatic carcinoma	Pancreas	Carcinoma
	0.257	0.229	U-266	Plasma cell myeloma	Blood	Myeloma
	0.108	0.107	NSCLC	Non-small cell lung carcinoma	Lung	Carcinoma
	Non-tumor cell line prediction					
	0.304	0.057	HEK293	Embryonic kidney fibroblast	Kidney	-

Results and discussion

In this study, we have considered twelve benzimidazole derivative compounds because we are still conducting important studies on these molecules for their potential use as active pharmaceutical ingredients for the treatment of MS. Therefore, in this study, we thought it was necessary to examine these molecules in terms of biological activity. It was determined that the most toxic molecule was molecule 5 at 523.900 mg/kg in class 5 and molecule 3 at 2255.000 mg/kg was non-toxic when we examined all molecules in terms of Rat IP LD_{50} (intraperitoneal administration route). The reason for the very high toxicity of molecule 5 is likely to be the $-C_6H_5$ bound at the $-R_2$ point. It is seen that a ranking is formed as 2>5>6>8>1>7> 11>12>10>4>9>3 when we rank the toxicity in molecules from the strongest to the lowest. When we analyze the results in terms of Rat IV LD_{50}, it is seen that the most toxic molecule is molecule 5 with 157.600 mg/kg in class 4. Molecule 2 was found to have the lowest toxicity among all molecules as 371.900 mg/kg in class 5. The reason for the very high toxicity of molecule 5 is likely to be the $-CH_3$ bound at the $-R_1$ point. When we rank the Toxicity in molecules from the strongest to the lowest, it is seen that a ranking is formed as 5>8>10>3>7>9>6>12>1>4> 11>2. It is seen that the most toxic molecule is molecule 7 with 426.200 mg/kg in class 4 when we examined the results in terms of Rat Oral LD_{50}. Molecule 10 was found to have the lowest toxicity among all molecules at 3592.000 mg/kg in class 5. The reason for the very high toxicity of molecule 7 is likely to be the $-CH_3$ bound at the $-R_1$ point. When we rank the toxicity in molecules from the strongest to the lowest, it is seen that a ranking

is formed as 7>12>2>3>1>9>11>5>6>8>4>10. Finally, when we examine the results in terms of Rat SC LD_{50}, it is seen that the most toxic molecule is molecule 11 as 767.800 mg/kg in class 4. Molecule 6 was found to have no toxicity among all molecules as 4788.000 mg/kg. Graphical representation of the data obtained in Table 2 for rat acrute toxicity is demonstrated on Figure 2.

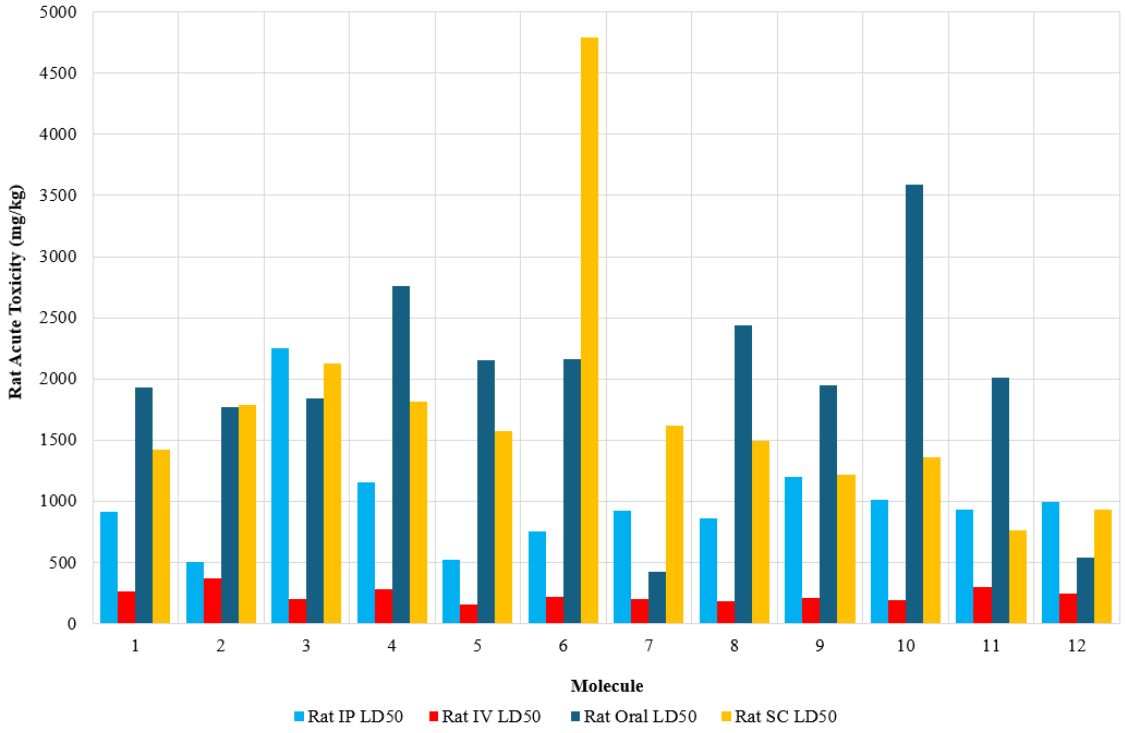


Figure 2 – Acute rat toxicity values of molecules

It was determined that molecules 1, 5, 6, and 9 did not show any adverse effect when we examined the molecules in terms of the adverse effect they showed. Among the other molecules, hepatotoxicity was the most adverse effect, followed by nephrotoxicity. The term "hepatotoxicity" describes a substance's capacity to harm the liver, including medications, chemicals, and natural compounds. The liver is an essential organ that produces crucial proteins, breaks down medications, and detoxifies blood. Hepatotoxic substances have the potential to cause liver harm, which can vary in severity from slight increases in liver enzymes to complete liver failure [41]. Seven different molecules showed hepatotoxicity effect (molecules 3, 4, 7, 8, 10, 11, and 12), and three different molecules (molecules 2, 4, and 8) showed nephrotoxicity effect. The term "nephrotoxicity" describes a substance's capacity to harm the kidneys. The kidneys are vital organs that filter waste materials out of the blood, control fluid balance, and preserve electrolyte levels. Nephrotoxicity is the ability of a chemical to damage kidney function, resulting in either chronic kidney disease (CKD) or acute kidney injury (AKI) [42]. It was determined that only molecule 4 showed an arrhythmia effect. Any irregularity in the heart's rhythm, such as beating too quickly, too slowly, or irregularly, is referred to as an arrhythmia [43]. Molecule 4 showed three different effects, molecule 8 showed two different effects, while molecules 2, 3, 4, 7, 8, 10, 11, and 12 showed only one effect. The highest Pa value was 0.422 for molecules 10 and 11, and the lowest Pa value was 0.273 for molecule 2. The highest Pi value was 0.266 in molecule 4, and the lowest Pi value was 0.110 in molecule 4. Graphical representation of the data obtained in Table 3 for adverse effects is presented on Figure 3.

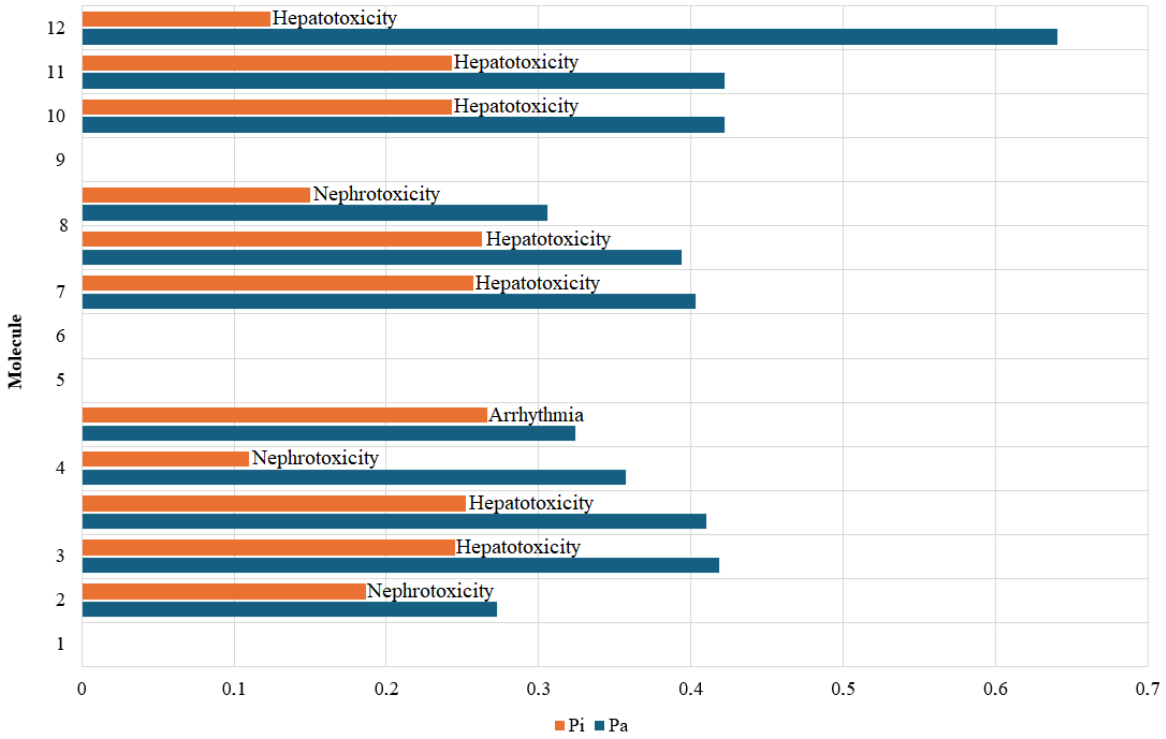


Figure 3 – Adverse effect values of molecules

Classifying antibacterial activity according to MIC (minimum inhibitory concentration) values is a widely used method for assessing the effectiveness of a substance on a bacterial species. MIC refers to the lowest concentration of antibiotic or antimicrobial agent required to stop the growth of a particular bacterium. The lower the MIC value, the stronger the antibacterial agent. The efficacy of an agent can be assessed by classifying MIC values according to certain ranges. This classification is usually done as follows: strong antibacterial activity (MIC≤1 µg/mL, this range indicates that the substance is effective even at very low concentrations, indicating a high antibacterial potential), moderate antibacterial activity (MIC>1 µg/mL and ≤10 µg/mL, this range indicates that the substance is still effective but at higher concentrations), weak antibacterial activity (MIC>10 µg/mL and≤100 µg/mL, this range indicates that the antibacterial effect of the substance is weak and much higher concentrations are needed to be clinically effective), and very weak or no inhibitory effect (MIC>100 µg/mL, in this case, the substance is considered to have no or a very weak inhibitory effect on bacterial growth). When we examined the Antibacterial activity MIC values given in Table 4, it was found that molecule 2 showed the strongest antibacterial effect against Shigella sp. (MIC 0.96759 µg/mL), molecule 5 against Shigella sp. (MIC 0.87413 µg/mL), and molecule 1 against Shigella sp. (MIC 0.73073 µg/mL). Antibacterial effect of other molecules was moderate, weak, and very weak or there was no inhibitory effect. Information on strong, moderate, and weak antibacterial activity and MIC (µg/mL) values shown by the molecules is presented on Figure 4.

The term “antifungal activity” describes a substance’s capacity to stop fungus growth or eradicate fungal organisms. A vast variety of organisms, including molds like *Aspergillus* species and yeasts like *Candida* species, are classified as fungi. These organisms can cause a variety of illnesses, especially in those with impaired immune systems [44]. Since fungal infections can range from minor skin infections to serious systemic disorders, antifungal activity is an essential part of treating fungal infections. Treatment plans are determined by the type of infection, the fungus present, and the general health of the patient. The efficiency of an antifungal medication is frequently gauged by its minimum inhibitory concentration (MIC) against particular fungi [45]. It is seen that most of the molecules we studied do not have antifungal activity. Molecules 1, 4 and 8 appear to have antifungal activity. Among these, molecule 4 showed the highest antifungal

activity against *Cryptococcus bacillisporus* with a value of 0.98135 µg/mL. The weakest antifungal activity was shown in molecule 1 with 20.00000 µg/mL against *Cryptococcus bacillisporus*. A visual representation of the data on antifungal activity is given in Figure 5.

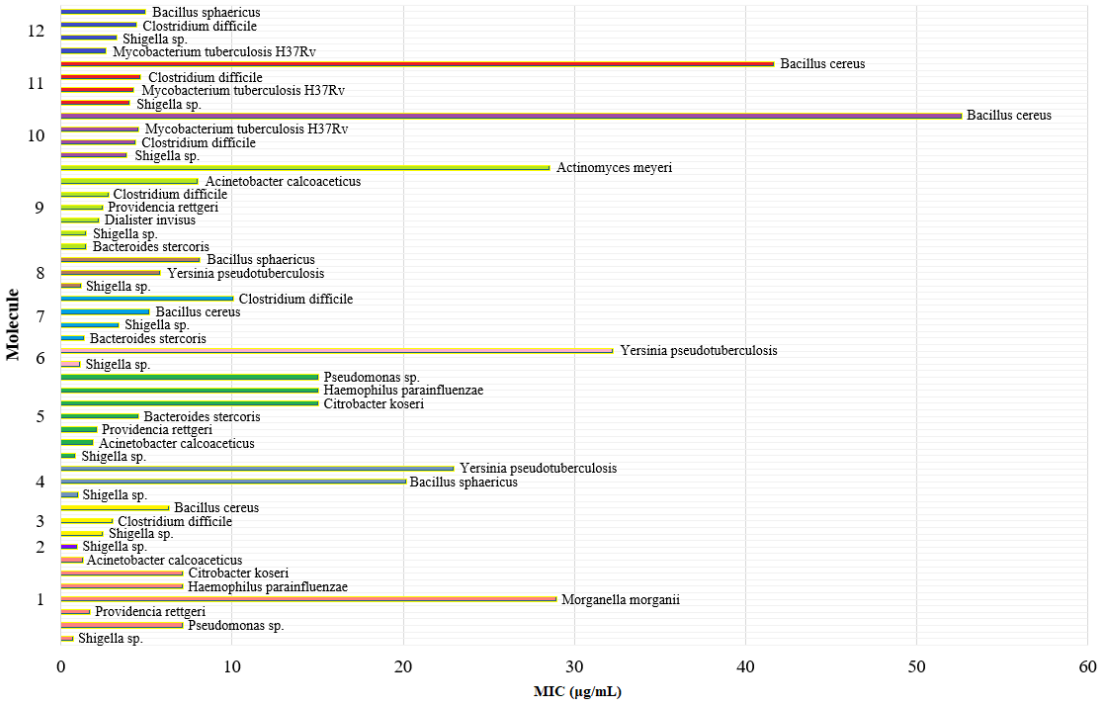


Figure 4 – Strong, moderate, and weak antibacterial activity and MIC values by the molecules

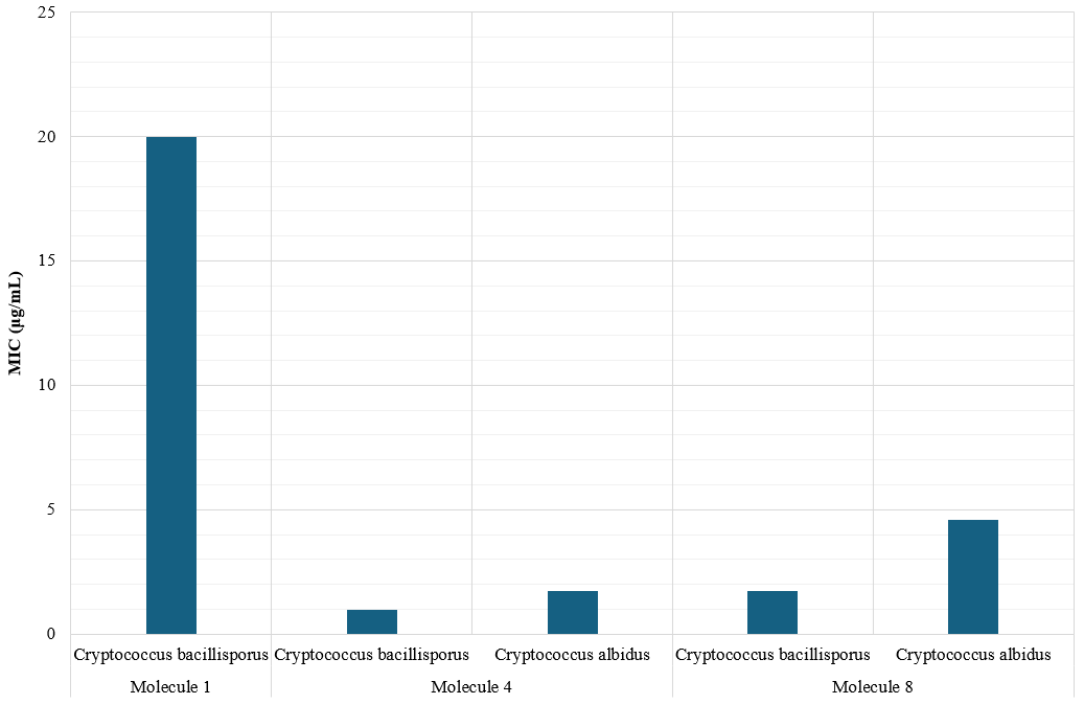


Figure 5 – Antifungal activity values of molecules

HIV Targets Prediction is the computational prediction of a compound's capacity to interact with particular HIV (human immunodeficiency virus) targets in PASS. Researchers working on drug discovery and development might benefit greatly from HIV Targets Prediction in PASS Online, which offers insights into possible interactions between chemical compounds and important HIV-related targets. This may hasten the discovery and development of novel antiviral medications intended to cure or prevent HIV infection [44]. The pIC_{50} value is a measure of the potency of a compound to inhibit a specific target, such as an HIV-associated protein or enzyme. Based on its size, which is correlated with the compound's potency in blocking HIV targets, pIC_{50} values are categorized (high potency (strong inhibitor) : pIC_{50} > 8, moderate potency (moderate inhibitor) : pIC_{50} between 6 and 8, low potency (weak inhibitor) : pIC_{50} between 4 and 6, and very low potency or inactive : pIC_{50} < 4). Stronger inhibition and more potential as an antiviral agent are indicated by higher pIC_{50} values, which makes these compounds more desirable candidates for HIV medication development. As HIV targets, the molecules were found to play an active role in five different targets. Molecules 4, 6, 7, 8, 11, and 12 have moderate potency in protease (HIV-1) target, molecules 1, 2, 3, 5, 9, and 10 have moderate potency in protease (HIV-1), reverse transcriptase (HIV-1), integrase (HIV-1), and REV (HIV-1)), molecules 4, 6, 7, 8, 11, and 12 were found to have low potency in reverse transcriptase (HIV-1), integrase (HIV-1), and REV (HIV-1). Finally, it was concluded that molecules 1, 2, 4, 5, 6, 8, 9, 10, and 11 were inactive, while molecules 3, 7, and 11 were active in the target TAT (HIV-1). Visual representation of the data given in Table 6 is given in Figure 6.

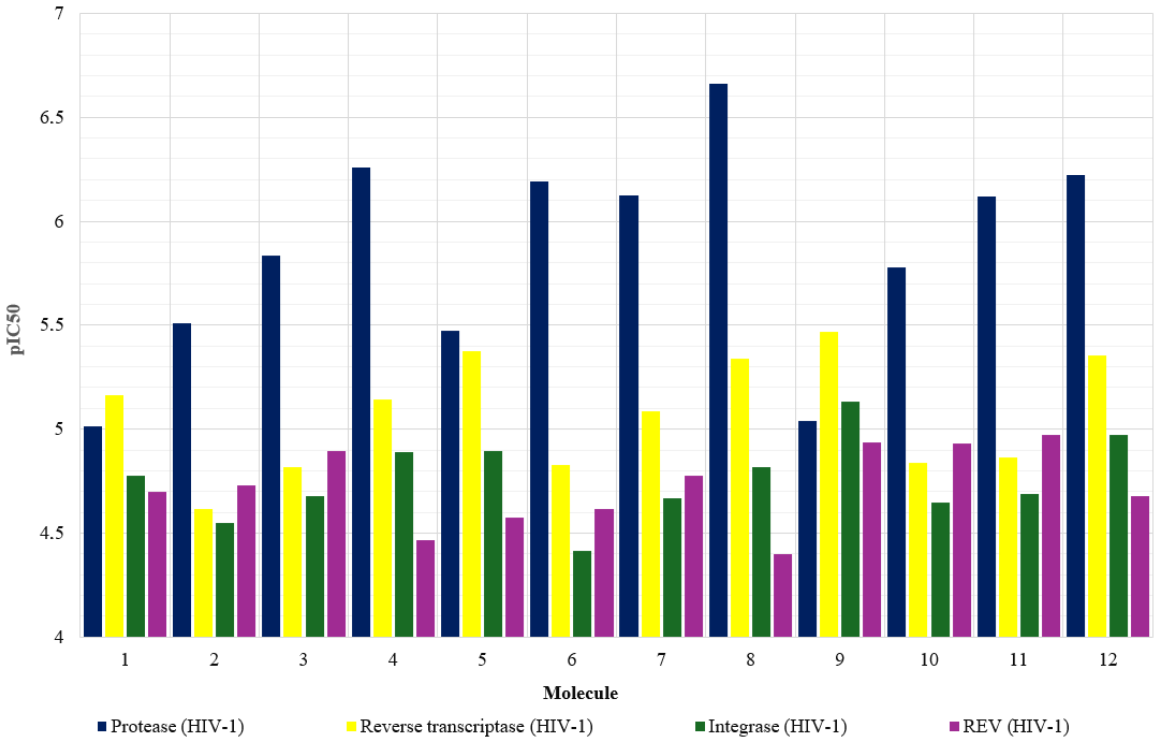


Figure 6 – HIV targets and pIC50 values of molecules

The ability of a material, usually a medication or natural molecule, to prevent viruses from replicating and spreading within a host organism is known as the antiviral effect. A crucial characteristic of substances or medications that prevent viral replication and aid in the management or eradication of viral illnesses is their antiviral effect [45]. This impact can be attained via a number of strategies that target distinct phases of the viral life cycle, ultimately stopping the virus's replication and lessening the infection's severity or spread [46]. As can be seen in Table 7, all the molecules studied showed antiviral effects on specific viruses and proteins. The quantitative magnitude of this effect is given in this table with confidence

values. The antiviral effect is classified according to the confidence value as strong activity (confidence value > 0.7), moderate activity (confidence value between 0.5 and 0.7), low activity, (confidence value between 0.3 and 0.5), and very low activity (confidence value < 0.3). As can be seen from the confidence values given in Table 7, almost all of the molecules show low and very low antiviral activity. The highest antiviral effect was obtained in molecule 1 against dengue virus 2 with a confidence value of 0.5092 in the target protein genome polyprotein. The lowest antiviral effect was obtained in molecule 12 against human herpesvirus 6A (strain Uganda-1102) (HHV-6 variant A) (human blymphotropic virus) with a confidence value of 0.0009 in human herpesvirus 6 DNA polymerase target protein. A visual of the confidence values of the molecules with the target protein they show antiviral effect is given in Figure 7.

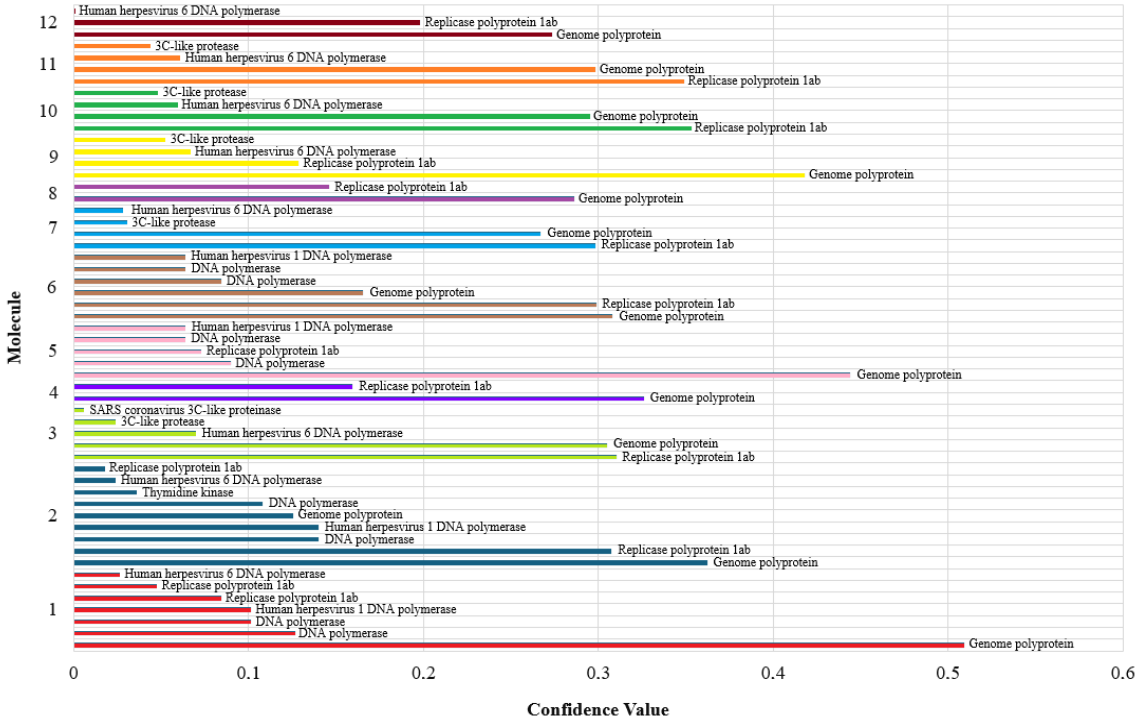


Figure 7 – Target proteins and confidence values of molecules with antiviral effects

Cancer cell lines are cells derived from human or animal tumors that can be grown in the laboratory [47]. The practice of forecasting how a specific substance or medication would influence different cancer cell lines is known as "cancer cell line prediction." This is a crucial component of oncology research and medication development since it enables researchers to assess a compound's possible effectiveness against various cancer cell types [48]. PASS (Prediction of Activity Spectra for Substances) Online is one tool that may be used to predict the activity of chemicals against different cancer cell lines. This tool offers probability scores for the chance that a substance would be effective against particular cancer cell lines, which are comparable to Pa and Pi values. Pa and Pi values give insight into the likelihood that a compound will be effective against a specific cancer cell line [49]. If Pa is considerably greater than Pi (Pa > 0.7 and Pi < 0.3, for example), there is a good chance that the drug will be effective against the cancer cell line. These substances are seen to be excellent candidates for additional experimental confirmation. The prediction is less certain when Pa and Pi values are near to one another (Pa = 0.5 and Pi ≈ 0.5, for example). Although the forecast does not support the compound's candidacy, it may have some activity. To fully understand its potential, more testing might be necessary. There is less chance that the drug will be effective against the cancer cell line when Pa is low and Pi is high (e.g., Pa < 0.3 and Pi > 0.7). Generally speaking, these substances are not given as much priority for more research about

that particular activity [50]. As can be seen from the data given in Table 8, it is seen that the molecules have certain Pa and Pi values, although not very high, on the tumor types that the tissue has in many cell-line lines. These show us that the molecules have the potential to be effective on many cancer types.

Conclusion

As a result, seven different biological activities, including acute rat toxicity, adverse drug effects, antibacterial activity, antifungal activity, anti-HIV activity, antiviral activity, and cell line cytotoxicity, were calculated for twelve benzimidazole derivative compound examined here. Rat IP LD_{50} (intraperitoneal administration toxicity measure), Rat IV LD_{50} (intravenous administration toxicity measure), Rat Oral LD_{50} (oral administration toxicity measure), and Rat SC LD_{50} (subcutaneous administration toxicity measure) The toxicities of the molecules were generally not very low. It was determined that some of the molecules had side effects while others had no side effects. In terms of antibacterial activity, it was observed that generally the molecules had moderate antibacterial activity and very few had high antibacterial activity. While some of the molecules had antifungal activity, this effect was not observed in some of them. In terms of HIV targets, it was observed that they showed different qualities of activity. In terms of antiviral activity, they did not exhibit very strong activity, but all molecules showed a certain antiviral activity. Finally, it was observed that the molecules were active on many tumor types.

Conflict of interest

The author is aware of the article's content and declares no conflict of interest.

References

1. Barot P.K., Nikolova S., Ivanov I., Ghate M.D. (2013) Novel research strategies of benzimidazole derivatives: a review. *Mini Rev. Med. Chem*., 13(10), pp. 1421-1447.

2. Al-rifaie D.A., Rasheed M.K., Meri M.A., Mustafa M.A. (2023) Synthesis and characterization of some benzimidazole derivatives derived from pharmaceutical compounds, and evaluation of their antibacterial and antifungal activity. *Lat. Am. J. Pharm*., 42, pp. 229-233.

3. Francesconi V., Rizzo M., Schenone S., Carbone A., Tonelli M. (2024) State-of-the-art review on the antiparasitic activity of benzimidazole-based derivatives: Facing malaria, leishmaniasis, and trypanosomiasis. *Curr. Med. Chem.*, 31(15), pp. 1955-1982.

4. Chen M., Su S.J., Zhou Q., Tang X.M., Liu T.T., Peng F., He M., Luo H., Xue W. (2021) Antibacterial and antiviral activities and action mechanism of flavonoid derivatives with a benzimidazole moiety. *J. Saudi. Chem. Soc.*, 25(2), pp. 1-14. https://doi.org/10.1016/j.jscs.2020.101194.

5. Divaeva L.N., Zubenko A.A., Morkovnik A.S., Sochnev V.S., Svyatogorova A.E., Klimenko A.I. (2024) Synthesis of new n-[β-(hetero)arylethyl]benzimidazole-2-carbothioamides and their analogues as anti-Infective agents and compounds with possible neuro(psycho)tropic and anticancer activity. *Russ. J. Gen. Chem.*, 94(2), pp. 341-351. https://doi.org/10.1134/S1070363224020105.

6. Chikkula K.V., Sundararajan R. (2017) Analgesic, anti-inflammatory, and antimicrobial activities of novel isoxazole/pyrimidine/pyrazole substituted benzimidazole analogs. *Med. Chem. Res.*, 26(11), pp. 3026-3037. https://doi.org/10.1007/s00044-017-2000-0.

7. Sagnou M., Mavroidi B., Shegani A., Paravatou-Petsotas M., Raptopoulou C., Psycharis V., Pirmettis I., Papadopoulos M.S., Pelecanou M. (2019) Remarkable brain penetration of cyclopentadienyl $M(Co)^{3+}$ (m=99mtc, re) derivatives of benzothiazole and benzimidazole paves the way for their application as diagnostic, with single-photon-emission computed tomography (spect), and therapeutic agents for alzheimer's disease. *J. Med. Chem.*, 62(5), pp. 2638-2650. https://doi.org/10.1021/acs.jmedchem.8b01949.

8. Mishra G.P., Tripathy S., Pattanayak P. (2024) Novel urea substituted benzimidazole derivatives as anthelmintics: in silico and in vitro approaches. *Russ. J. Bioorg. Chem.*, 50(3), pp. 962-973. https://doi.org/10.1134/S1068162024030221.

9. Van Oosten, M.J., Silletti S., Guida G., Cirillo V., Di Stasio E., Carillo P., Woodrow P., Maggio A., Raimondi G. (2017) A benzimidazole proton pump inhibitor increases growth and tolerance to salt stress in tomato. *Front. Plant Sci*., 8, pp. 1-14. https://doi.org/10.3389/fpls.2017.01220.

10. Shabana K., Salahuddin S., Mazumder A., Kumar R., Datt V., Tyagi S., Yar M.S., Ahsan M.J., Sarafroz M. (2024) Review on the discovery of new benzimidazole derivatives as anticancer agents: synthesis and structure-activity relationship (2010-2022). *Lett. Drug Des. Discov.*, 21(3), pp. 451-479.

11. Güzel E., Çevik U.A., Evren A.E., Bostanci H.E., Gül U.D., Kayis U., Özkay Y., Kaplancikli Z.A. (2023) Synthesis of benzimidazole-1,2,4-triazole derivatives as potential antifungal agents targeting 14α-demethylase. *ACS Omega*, 8(4), pp. 4369-4384. https://doi.org/10.1021/acsomega.2c07755.

12. Rostami H., Haddadi M.H. (2022) Benzimidazole derivatives: a versatile scaffold for drug development against helicobacter pylori-related diseases. *Fundam. Clin. Pharmacol*., 36(6), pp. 930-943. https://doi.org/10.1111/fcp.12810.

13. Krstulovic L., Rastija V., De Carvalho L.P., Held J., Rajic Z., Zivkovic Z., Bajic M., Glavas-Obrovac L. (2024) Design, synthesis, antitumor, and antiplasmodial evaluation of new 7-chloroquinoline-benzimidazole hybrids. *Molecules*, 29(13), pp. 1-20. https://doi.org/10.3390/molecules29132997.

14. Abdelhafiz A.H.A., Serya R.A.T., Lasheen D.S., Wang N., Sobeh M., Wink M., Abouzid K.A.M. (2022) Molecular design, synthesis and biological evaluation of novel 1,2,5-trisubstituted benzimidazole derivatives as cytotoxic agents endowed with ABCB1 inhibitory action to overcome multidrug resistance in cancer cells. *J. Enzyme Inhib. Med. Chem.*, 37(1), pp. 2710-2724.

15. Chintakunta R., Meka G. (2020) Synthesis, in silico studies and antibacterial activity of some novel 2-substituted benzimidazole derivatives. *Future J. Pharm. Sci.*, 6(1), pp. 1-6. https://doi.org/10.1186/s43094-020-00144-9.

16. Druzhilovskiy D.S., Rudik A.V., Filimonov D.A., Gloriozova T.A., Lagunin A.A., Dmitriev A.V., Pogodin P.V., Dubovskaya V.I., Ivanov S.M., Tarasova O.A., Bezhentsev V.M., Murtazalieva K.A., Semin M.I., Maiorov I.S., Gaur A.S., Sastry G.N., Poroikov V.V. (2017) Computational platform Way2Drug: from the prediction of biological activity to drug repurposing. *Russ. Chem. Bull.*, 66(10), pp. 1832-1841. https://doi.org/10.1007/s11172-017-1954-x.

17. Druzhilovskiy D., Rudik A., Filimonov D., Sastry G.N., Poroikov V. (2018) Way2drug platform – ligand-based approach to drug repurposing. *Abstr. Pap. Am. Chem. Soc.*, 256, pp. 452.

18. Güven O., Menteşe E., Emirik M., Sökmen B.B., Akyüz G. (2023) Benzimidazolone-piperazine/triazole/thiadiazole/furan/thiophene conjugates: Synthesis, in vitro urease inhibition, and in silico molecular docking studies. *Arch. Pharm.*, 356(11), pp. 1-14. https://doi.org/10.1002/ardp.202300336.

19. Chu I., Secours V.E., Valli V.E. (1982) Acute and sub-acute toxicity of octachlorostyrene in the rat. *J. Toxicol. Environ Health*, 10(2), pp. 285-296. https://doi.org/10.1080/15287398209530251.

20. Gebbers J.O., Lotscher M., Kobel W., Portmann R., Laissue J.A. (1986) Acute toxicity of pyridostigmine in rats – histological-findings. *Arch. Toxicol.*, 58(4), pp. 271-275.

21. Liu Z.Y., Dang K., Gao J.H., Fan P., Li C.Z., Wang H., Li H., Deng X.N., Gao Y.C., Qian A.R. (2022) Toxicity prediction of 1,2,4-triazoles compounds by QSTR and interspecies QSTTR models. *Ecotoxicol. Environ. Saf.*, 242, pp. 1-12. https://doi.org/10.1016/j.ecoenv.2022.113839.

22. Unnissa S.H., Rajan D. (2016) Drug design, development and biological screening of pyridazine derivatives. *J. Chem. Pharm. Res.*, 8(8), pp. 999-1004.

23. Fedorova E.V., Buryakina A.V., Zakharov A.V., Filimonov D.A., Lagunin A.A., Poroikov V.V. (2014) Design, synthesis and pharmacological evaluation of novel vanadium-containing complexes as antidiabetic agents. *PLoS One*, 9(7), pp. 1-11. https://doi.org/10.1371/journal.pone.0100386.

24. Wexler P. (2014) Encyclopedia of toxicology (3rd ed.), Cambridge, Massachusetts, ABD, Academic Press., pp. 638-642, ISBN: 978-0-12-386455-0.

25. Poroikov V.V., Filimonov D.A., Gloriozova T.A., Lagunin A.A., Druzhilovskiy D.S., Rudik A.V., Stolbov L.A., Dmitriev A.V., Tarasova O.A., Ivanov S.M., Pogodin P.V. (2019) Computer-aided prediction of biological activity spectra for organic compounds: the possibilities and limitations. *Russ. Chem. Bull.*, 68(12), pp. 2143-2154. https://doi.org/10.1007/s11172-019-2683-0.

26. Ivanov S.M., Lagunin A.A., Rudik A.V., Filimonov D.A., Poroikov V.V. (2018) Adverpred-web service for prediction of adverse effects of drugs. *J. Chem. Inf. Model.*, 58(1), pp. 8-11.

27. Lasure P.P., Munot N.M., Lawande S.S. (2012) Determination of antibacterial activity of punica granatum fruit. *Int. J. Pharm. Sci. Res.*, 3(11), pp. 4421-4424.

28. Li Y., Chen L., Han G.J., Zhou J.H., Zhao Y. (2014) Determination of antibacterial activity of aucubigenin and aucubin. *Asian J. Chem.*, 26(2), pp. 559-561.

29. Judd W.R., Martin C.A. (2009) Antifungal activity of nontraditional antifungal agents. *Curr. Fungal Infect. Rep.*, 3(2), pp. 86-95. https://doi.org/10.1007/s12281-009-0012-z.

30. Campoy S., Adrio J.L. (2017) Antifungals. *Biochem. Pharmacol.*, 133, pp. 86-96.

31. Meadows D.C., Gervay-Hague J. (2006) Source, Targeting HIV. *ChemMedChem.*, 1(1), pp. 16-29. https://doi.org/10.1002/cmdc.200500026.

32. Martrus G., Altfeld M. (2016) Immunological strategies to target HIV persistence. *Curr. Opin. HIV AIDS*, 11(4), pp. 402-408. https://doi.org/10.1097/COH.0000000000000289.

33. Puhl A.C., Demo A.G., Makarov V.A., Ekins S. (2019) New targets for HIV drug discovery. *Drug Discov. Today*, 24(5), pp. 1139-1147. https://doi.org/10.1016/j.drudis.2019.03.013.

34. Amvrosyeva T.V., Votyakov V.I., Vladyko G.V., Andreeva O.T., Vervetchenko S.G., Goretskaya I.S., Klimashevskaya L.M. (1992) On antiviral properties of official drugs. *Antibiot Khimioter*, 37(11), pp. 5-8.

35. Al-Khikani F.H.O., Almosawey H.A.S., Abdullah Y.J., Al-Asadi A.A., Hameed R.M., Hasan N.F., Al-Ibraheemi M.K.M. (2020) Potential antiviral properties of antifungal drugs. *J. Egypt Women Dermatol. Soc.*, 17(3), pp. 185-186. https://doi.org/10.4103/JEWD.JEWD_40_20.

36. Meghana S.K.V.S. (2023) Comparative analysis of antiviral properties of carbohydrates derivatives over commercial antiviral drugs. *Cardiometry*, 25, pp. 1577-1583.

37. Furuyama H., Arii S., Mori A., Imamura M. (2000) Role of E-cadherin in peritoneal dissemination of the pancreatic cancer cell line, Panc-1, through regulation of cell to cell contact. *Cancer Lett.*, 157(2), pp. 201-209. https://doi.org/10.1016/S0304-3835(00)00488-2.

38. Timoumi R., Amara I., Ayed Y., Ben Salem I., Abid-Essefi S. (2020) Triflumuron induces genotoxicity in both mice bone marrow cells and human colon cancer cell line. *Toxicol. Mech. Methods*, 30(6), pp. 438-449. https://doi.org/10.1080/15376516.2020.1758981.

39. Leonessa F., Coialbu T., Toma S. (1986) Different techniques for drug cytotoxicity evaluation on mcf-7 human-breast cancer cell-line. *Anticancer Res.*, 6(6), pp. 1291-1296.

40. Doki Y., Shiozaki H., Tahara H., Inoue M., Oka H., Iihara K., Kadowaki T., Takeichi M., Mori T. (1993) Correlation between e-cadherin expression and invasiveness *in-vitro* in a human esophageal cancer cell-line. *Cancer Res*., 53(14), pp. 3421-3426.

41. Timbrell J.A. (1983) Drug hepatotoxicity. *Br. J. Clin. Pharmacol.*, 15(1), pp. 3-14. https://doi.org/10.1111/j.1365-2125.1983.tb01456.x.

42. Walker R.J., Duggin G.G. (1988) Drug nephrotoxicity. *Annu. Rev. Pharmacol. Toxicol.*, 28, pp. 331-345.

43. Mandel W.J. (1992) Arrhythmias. *Curr. Opin. Cardiol.*, 7(1), pp. 1-2.

44. De Clercq E. (2002) New anti-HIV agents and targets. *Med. Res. Rev.*, 22(6), pp. 531-565.

45. James J.P., Jyothi D., Priya S. (2021) *In silico* screening of phytoconstituents with antiviral activities against sars-cov-2 main protease, nsp12 polymerase, and nsp13 helicase proteins. *Lett Drug. Des. Discov.*, 18(8), pp. 841-857. https://doi.org/10.2174/1570180818666210317162502.

46. Ng T., Cheung R., Wong J., Wang Y., Ip D., Wan D.C.C, Xia J. (2017) Antiviral activities of whey proteins. *Appl. Microbiol. Biotechnol.*, 99(17), pp. 6997-7008. https://doi.org/10.1007/s00253-015-6818-4.

47. Radicchi D.C., Melo A.S., Lima A.P.B., Almeida T.C., De Souza G.H.B., Da Silva G.N. (2022) Naringin: antitumor potential in silico and in vitro on bladder cancer cells. *Ars Pharm.*, 63(2), pp. 132-143. https://doi.org/10.30827/ars.v63i2.22430.

48. Suliman R.S., Alghamdi S.S., Ali R., Rahman I., Alqahtani T., Frah I.K., Aljatli D.A., Huwaizi S., Algheribe S., Alehaideb Z., Islam I. (2022) Distinct mechanisms of cytotoxicity in novel nitrogenous heterocycles: future directions for a new anti-cancer agent. *Molecules*, 27(8), pp. 1-22. https://doi.org/10.3390/molecules27082409.

49. Hosseini M.S., Hadadzadeh H., Mirahmadi-Zare S.Z., Farrokhpour H., Aboutalebi F., Morshedi D. (2023) A curcumin-nicotinoyl derivative and its transition metal complexes: synthesis, characterization, and in silico and *in vitro* biological behaviors. *Dalton Trans.*, 52(40), pp. 14477-14490. https://doi.org/10.1039/d3dt01351k.

50. Tarai S.K., Tarai A., Mandal S., Nath B., Som I., Bhaduri R., Bagchi A., Sarkar S., Biswas A., Moi S.C. (2023) Cytotoxic behavior and DNA/BSA binding activity of thiosemicarbazone based Ni(II) complex: bio-physical, molecular docking and DFT study. *J. Mol. Liq.*, 383, pp. 1-16. https://doi.org/10.1016/j.molliq.2023.121921.

Information about the author:

Fatih İslamoğlu – (corresponding author) – Professor, Recep Tayyip Erdoğan University, Faculty of Science and Arts, Department of Chemistry, 53100 Rize/Turkey, e-mail: fatih.islamoglu@erdogan.edu.tr