UDC 54.057:547.83:547.79
DOI: 10.15587/2706-5448.2025.328968

APPLICATION OF CLUSTERING ALGORITHMS AND PHARMACOPHORE SCREENING FOR IDENTIFICATION OF THIAZOLIDINONE AND PYRAZOLINE DERIVATIVES WITH DUAL ANTIPARASITIC AND ANTICANCER ACTIVITY

Anna Kryshchyshyn-Dylevych, Roman Lesyk

Thiazolidinones and related heterocycles exhibiting antimicrobial, antiparasitic, anticancer, antidiabetic, and anti-inflammatory activities are considered privileged scaffolds for the development of novel, drug-like molecules. 4-Thiazolidinone-based hybrids with alkanecarboxylic acid moieties, pyrazoline, phenylindole or imidazothiadiazole fragments have been thoroughly investigated as potential antiparasitic agents. Along with numerous studies that proved their high anticancer potential, this class of compounds is attractive for the promising strategy of redirecting antiparasitic drugs for cancer treatment.

The aim of the study. *We aimed to investigate the correlation between the antileukemic and antiparasitic properties of various thiazolidinone and pyrazoline derivatives.*

Materials and methods. *The anticancer activity of a data set of 31 compounds against five Leukemic cell lines was studied at a single concentration (10^{-5} M). The antitrypanosomal activity data has been collected under the same assay protocol against Trypanosoma brucei brucei (Tbb). The clustering algorithms were implemented in Python using the NumPy, Pandas, Scikit-learn, Matplotlib, and Plotly libraries. LigandScout 4.4 software was used for the 3D-pharmacophore design.*

Results. *The compounds with antitrypanosomal activity were divided into 3 classes according to the IC50 values calculated in the growth inhibition assay against Tbb. The percentage of cell growth in the in vitro assay of studied compounds on five Leukemic cell lines was used for the machine learning study. Applying both the K-means and Agglomerative hierarchical clustering algorithms, compounds from class 1 were grouped into one cluster. The pharmacophore screening using merged pharmacophore derived from BCL-2-venetoclax complexes showed good pharmacophore-fit scores for the compounds selected in one cluster by both algorithms. The same pharmacophore model, when applied to a dataset of thiazolidinone/thiazole-indole/imidazothiadiazole hybrid molecules with high antitrypanosomal activity in vitro, assigned them as active.*

Conclusions. *The findings of the study suggest that thiazolidine derivatives and related compounds exhibit dual anti-parasitic and anticancer properties, which may help to identify their antiproliferative mechanism of action in parasitic and cancer cells*

Keywords: *machine learning, thiazolidinone, pyrazoline, antitrypanosomal acivity, anticancer activity, pharmacophore design*

How to cite:
Kryshchyshyn-Dylevych, A., Lesyk, R. (2025). Application of clustering algorithms and pharmacophore screening for identification of thiazolidinone and pyrazoline derivatives with dual antiparasitic and anticancer activity. ScienceRise: Pharmaceutical Science, 3 (55), 17–29. http://doi.org/10.15587/2706-5448.2025.328968

1. Introduction

Thiazolidinones and related cores are treated as privileged heterocycles in the modern medicinal chemistry. Among different thiazolidinones, a class of derivatives with aryl(heteryl)idene moieties in the C5 position are characterized by antimicrobial, antiparasitic, anticancer, antidiabetic, anti-inflammatory activity, etc. [1]. A group of 4-thiazolidinone derivatives, such as 5-ene-4-thiazolidinones with alkanecarboxylic acid moieties in the N3 position, thiazolidinone hybrids with pyrazoline, phenylindole, or imidazothiadiazole fragments designed within a hybrid pharmacophore approach [2], as well as 4-thiazolidinone-based fused heterocycles, especially thiopyrano[2,3–*d*]thiazoles [3] are the most discussed as potential antiparasitic agents. Moreover, the thiazolidine core is considered a synthetically feasible and pharmacologically attractive scaffold, making it widely utilised as a building block for the development of novel drug-like molecules. Among various pharmacological profiles being studied for thiazole and thiazolidinone derivatives, their anticancer potential deserves special attention due to their capabilities to bind to numerous cancer-specific bio-targets [2, 4, 5].

The development of hybrid molecules that combine thiazole/thiazolidinone rings with other heterocycles is the approach that allows their antiproliferative properties to increase, e.g., imidazopyridine-linked thiazolidinones [6], 4-thiazolidinone-phenylaminopyrimidine [7], indole-thiazolidinone hybrid structures [8], and pyridine-thiazolidinone derivatives [9]. Thiosemicarbazone moeity with thiazolidinedione core in hybrid molecules contributes not only to anticancer activity [10, 11] but is a well-known

fragment used for the search for novel compounds with antirypanosomal, antimalarial activity [12], and antileishmanial properties [13, 14].

Human African trypanosomiasis (HAT) and Chagas disease belong to the world's neglected tropical diseases. However, fewer than 1000 cases of HAT were reported in 2022 [15]; it is mainly seen in rural communities and impoverished areas. Therefore, HAT occurrence remains often underreported. A protozoan parasite, *Trypanosoma cruzi*, is the causative agent of Chagas disease (also known as American trypanosomiasis), a fatal human disease that affects approximately 6-7 million people worldwide. Although the disease was previously mostly found in Latin American countries, in recent decades, due to population growth and migration, the infection has spread to other countries, including the U.S., Europe, and Canada. When a blood-sucking triatomine bug bites a human, the parasite enters the body through cracks in the skin or mucous membranes and spreads to other people through contaminated food. Inside the human host's cells, the parasite divides in the amastigote form before differentiating into the infectious trypomastigote form, which is then released when the host-cell ruptures, resulting in inflammatory reactions that cause megaesophagus, megacolon, and abnormalities in cardiac conduction. As Chagas disease is transmitted by the triatomine bug and there is no vaccine, vector control remains the primary and most effective way to prevent its spread in endemic regions of South America. Only two drugs are currently used for the treatment of American trypanosomiasis, namely Benznidazole and Nifurtimox. These medicines are effective in the acute stages, while the treatment of the chronic stages has been mostly nonethiologic [16]. Therefore, the absence of the vaccine, the ineffectiveness of the treatment with available antichagasic drugs in the chronic stages and the high frequency of the adverse reactions [17] urge the necessity of the development of novel agents for the Chagas disease treatment.

The repurposing strategy has been a potentially attractive strategy to combat the lack of anticancer agents as well as antiparasitic drugs. Eflornithine being originally developed as a potential anticancer agent in the 1970s and further used for the treatment of human African trypanosomiasis, recently had been approved by the FDA under the brand name Iwilfin to reduce the risk of relapse in adults and children with high-risk neuroblastoma [18, 19]. Moreover, a great number of drugs investigated for repurposing in oncology are antiparasitics [20]. The studies have proven that some of these medications exhibit antitumor properties, either killing cancer cells via mechanisms similar to those of parasites or involving novel molecular pathways. Among the latter, ferroptosis has been discovered as a new way to induce cancer cell death; for example, artemisinin derivatives are agents that target this process [21]. Among other mechanisms of antitumor action uncovered for antiparasitic agents, there are autophagy regulating effects, mitochondria disrupting effects, immunoregulating, and metabolic disrupting effects [22].

Therefore, a strategy of redirecting antiparasitic drugs for oncology, as well as investigating highly active antiparasitic agents as molecules with potent anticancer properties, has been a fruitful and promising direction in medicinal chemistry. In this article, we aimed to investigate the correlation between the antiparasitic and antileukemic properties of various thiazolidinone and pyrazoline derivatives.

2. Planning (methodology) of research

The analysis of sub-libraries of thiazolidinone- and pyrazoline-based derivatives from "in-house" library of the Department of Pharmaceutical, Organic and Bioorganic Chemistry allowed us to identify a series of substances with established dual antiparasitic activity against *Trypanosoma brucei brucei* (*Tbb*) and anticancer activity. Clustering algorithms were applied to cluster the active anticancer agents and find possible correlations between the anticancer and antiparasitic activity of the same molecules. The IC_{50} values calculated from the growth inhibition assay against *Tbb* were used to define the classification labels for compounds' activity.

The study included pharmacophore screening of the compounds grouped in a single cluster from one class as dual antileukemic/antiparasitic agents using a pharmacophore model derived from three BCL-2/venetoclax complexes (PDB IDs: 6O0K, 6O0M, 6O0P). Then, the same pharmacophore model was applied to several thiazolidinone/thiazole-indole/imidazothiadiazole hybrid molecules with high antitrypanosomal activity identified in our earlier studies [2] in order to test the hypothesis of their potent dual antiparasitic/anticancer action (Fig. 1).

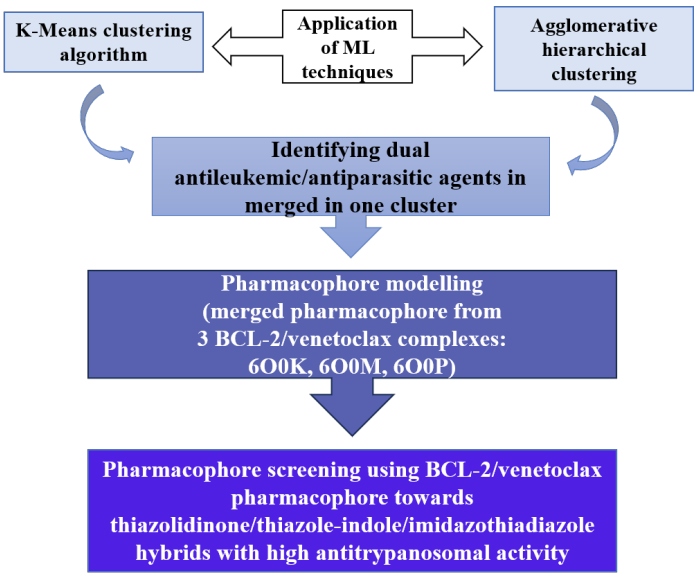


Fig. 1. Study algorithm

3. Materials and methods

Biological assays.

31 Heterocyclic compounds from our "in-house" library, namely isothiochromenothiazoles and thiopyranothiazoles [3], 5-enamine-4-thiazolidinones [23], 2-amino(imino)- and 2-hydrazynilidene-thiazolidine-4-ones, and other thiazolidinone- and pyrazoline-related hetero-

cyclic derivatives [24, 25] with confirmed activity against *Tbb* were included into the study. All antitrypanosomal activity data have been collected under the same assay protocol from a single laboratory at the National Museum of Natural History (Paris, France). Each experiment was performed in triplicate and reported as mean_SD. Biological activity data was represented as concentration inhibiting 50% of parasite growth (IC_{50}) and was calculated as the mean±the standard deviation of three independent experiments. For some of the tested compounds, the biological activity data was represented as the percentage of growth inhibition of *Trypanosoma* bloodstream forms under the effect of 10 mg/mL of assayed substance. The description of the assays can be found elsewhere [26, 27].

The primary anticancer assay was performed on a panel of approximately sixty human tumour cell lines derived from nine neoplastic diseases following the protocol of the Drug Evaluation Branch, National Cancer Institute (Bethesda, USA) [28, 29]. Tested compounds were added to the culture at a single concentration (10^{-5} M), and the cultures were incubated for 48 h. End-point determinations were made with a protein-binding dye, sulforhodamine B (SRB). Results for each tested compound were reported as the percent of growth of the treated cells when compared to the untreated control cells. The percentage growth was evaluated spectrophotometrically versus controls not treated with test agents. The results of the action of tested compounds against five *Leukemic cell lines – CCRF-CEM, HL*-60 (*TB*)*, K*-562*, MOLT*-4*, and RPMI*-8226 – were selected for statistical modelling.

Computational methods.

The machine learning models were built in Python. The K-means clustering algorithm was adopted to cluster these candidate drugs. A K-means clustering algorithm is a known and popular method for cluster analysis in machine learning and data mining [30, 31]. Cluster analysis was performed using the cluster.hierarchy module (hierarchical clustering method) from the SciPy python library and the cluster K-means module (K-means clustering method) from the Scikit-learn library. As part of the data preprocessing, the MinMaxScaler module from the Scikit-learn library was used to normalize (standardize, reduce to a single scale) the data. The MinMaxScaler is a popular data normalization technique used in machine learning to transform features so that they fit within a specific range. This helps machine learning models train more effectively. It ensures that all features contribute equally to the model by scaling them to the same range. The formula to scale a feature

$$x_{scaled} = \frac{X - X_{\min}}{X_{\max} - X_{\min}}.$$

The first step of K-means clustering is to create c new observations among the unlabelled data and locate them randomly, called centroids. The number of centroids represents the number of output classes. The first step of the iterative process for each centroid is to find the nearest point (in terms of Euclidean distance) and assign it to its category. Next, for each category, the average of all the points attributed to that class is computed. The output is the new centroid of the class. With every iteration, the observations can be redirected to another centroid. After several reiterations, the centroid's change in location is less critical as the initial random centroids converge with the real ones – the process ends when there is no change in the centroids' position. Within the K-means clustering method, the default settings for clustering into 3 clusters were used.

Within the hierarchical clustering method, the complete, average and single linkage methods were applied to construct dendrograms using Euclidean distance to estimate the distances between individual clustering objects (linkages). Agglomerative hierarchical clustering is used to group similar data points and organize data in a tree-like structure [32]. The key part of this process is a linkage, which calculates the distance between clusters before they are merged or divided. Different types of linkage measure this distance differently. For two clusters R and S the single linkage returns the minimum distance between two points. This method creates long, chain-like clusters because it is sensitive to outliers and can connect clusters based on a very small number of close points. For two clusters R and S the complete linkage returns the maximum distance between two points. It tends to create compact and spherical clusters because it is more sensitive to outliers and attempts to ensure that the clusters are not too far apart.

The average linkage method returns the average distance between all pairs of points from two clusters. This method maintains a balance between single and complete linkage by considering all pairs of points, rather than just the closest or farthest points. It usually results in moderately compact clusters. Based on the dendrogram analysis, robustness between different linkage methods was found, so the average linkage method was chosen to segment compounds into 2 clusters.

The K-means and Agglomerative hierarchical clustering algorithms were implemented in Python using the NumPy, Pandas, Scikit-learn, Plotly, and Matplotlib libraries.

Pharmacophore design was performed in LigandScout 4.4 software [33].

4. Results

The analysis of sub-libraries of compounds based on the synthesized various thiazolidinones from the "in-house" library of Danylo Halytsky Lviv National Medical University Department of Pharmaceutical, Organic and Bioorganic chemistry with the anticancer activity against five *Leukemic cell lines* tested according to the NCI protocol [28, 29] (Table 1) and *in vitro* anti-parasitic activity against *Tbb* were chosen for the machine learning (ML) studies. Several isothiochromenothiazoles and thiopyranothiazoles [3], 5-enamine-4-thiazolidinones [23], 2-amino(imino)- and 2-hydrazynilidene-thiazolidine-4-ones, and other thiazolidinone- and pyrazoline-related heterocyclic derivatives [24, 25] were included into the studied data set (Fig. 2).

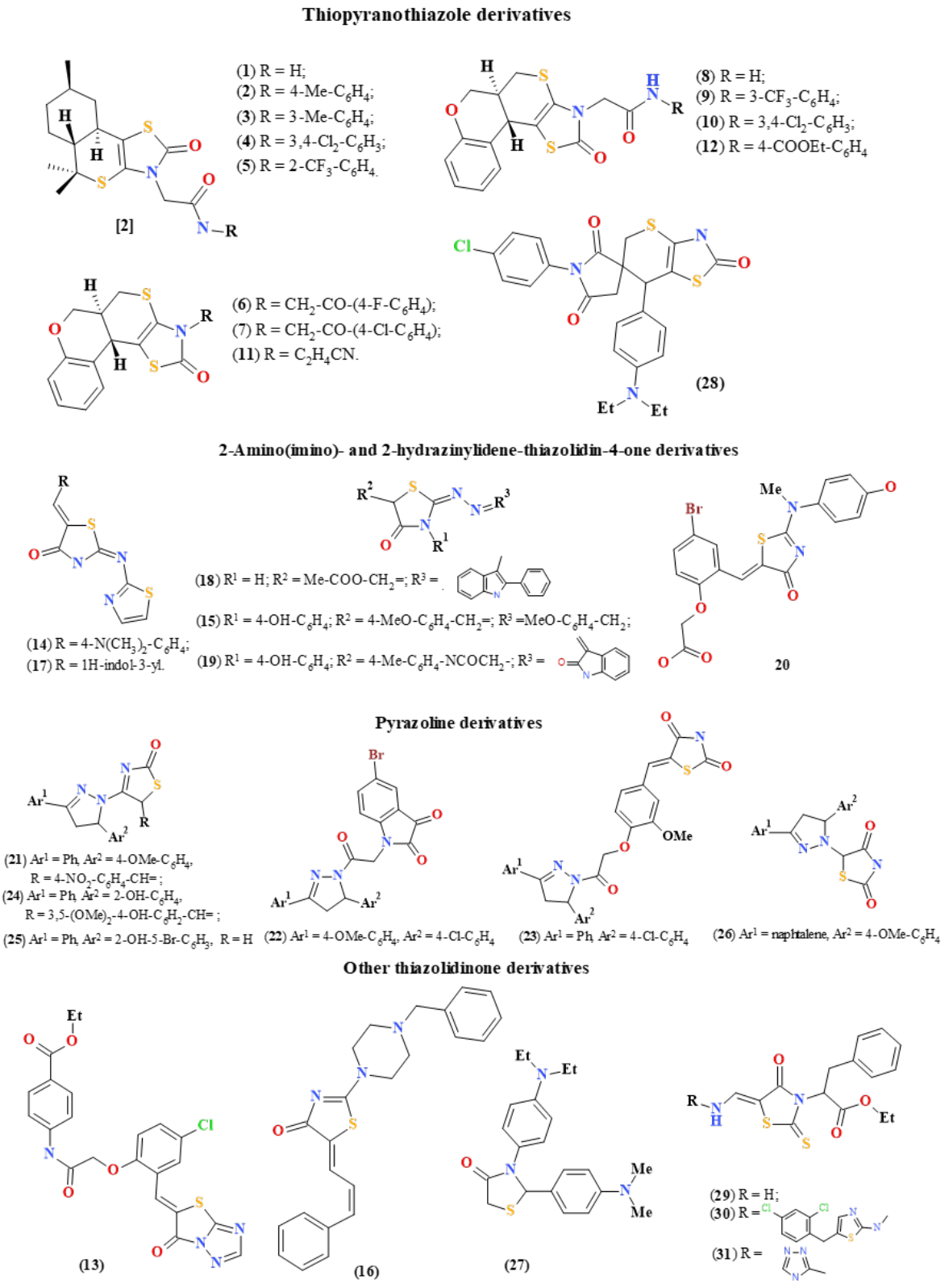


Fig. 2. Structures of different groups of thiazolidinone and pyrazoline derivatives included in the studied data set

The IC_{50} value calculated in the growth inhibition assay against *Tbb* was used to define the classification labels for the compound's activity. The classes were divided as follows:

I) class 1: $IC_{50} < 5$ μM or inhibition > 90% at 10 mg/mL;

II) class 2: $IC_{50} = 5–10$ μM or inhibition < 90% > 50% at 10 mg/mL;

III) class 3: $IC_{50} > 10$ μM or inhibition < 50% at 10 mg/mL (Table 1).

The first machine learning method applied to the heterogeneous set of studied molecules was an unsupervised K-means clustering algorithm, which aims to group more similar compounds into one cluster and to minimise the sum of distances between the points and their respective cluster centroids.

Table 1

Classes of antitrypanosomals and their anticancer activity studied at single concentration 10^{-5}M

Comp.#	Antitrypan. Class	Percentage of cell growth, *Leukemic cell lines*				
		CCRF-CEM	*HL-60(TB)*	*K-562*	*MOLT-4*	*RPMI-8226*
1	2	–71	60	16.75	38	40
2	1	60.78	89.44	66.59	58.86	46.87
3	2	81.57	85.35	89.04	36.02	102.26
4	2	88.48	79.38	60.88	61.09	72.46
5	3	103.83	107.25	98.37	108.11	101.42
6	1	66.75	73.37	67.75	72.02	69.55
7	1	86.2	101.16	104.08	93.06	90.44
8	3	108.81	117.16	92.68	90.68	107.79
9	1	34	31	14	22	13
10	3	8	4	7	22	22
11	3	106.2	100.08	108.48	109.87	95.99
12	1	0.43	72.84	54.83	61.88	35.25
13	3	60.25	58	68	79	36
14	1	–39	–42	–7	–22	–45
15	1	48	71	51	40	36
16	1	98.8	101.27	86.9	82.83	105.18
17	2	2	13	41	8	14
18	2	93.44	112.61	106.36	112.96	103.13
19	2	–27	38	11	–37	–13
20	1	–15	–60	–33	–38	–2
21	2	–26	13	–14	3	–46
22	1	4	4	8	–8	–3
23	1	53.89	76.91	35.98	61.78	72.61
24	2	–27	38	11	–37	–13
25	1	–19	–35	10	–41	22
26	3	103.18	110.34	94.69	105.82	101.85
27	3	90.34	97.09	88.67	87.18	91.5
28	1	83.8	99.24	62.85	94.54	97.55
29	3	113.35	85.27	85.48	93.15	81.81
30	1	2.77	–35.22	2.17	–7.05	–7.55
31	3	90.02	84.93	75.13	84.45	103.33

The K-means clustering algorithm was chosen as an unsupervised learning approach due to its simplicity, interpretability, and robust performance with small data sets. Although, Fuzzy C-means algorithm is basically similar to K-Means algorithm, in this method each data point belongs to a cluster to the degree that is specified by a membership grade. We aimed to identify clear and distinct clusters of compounds based on their cytotoxicity profiles against several *Leukemic cell lines*, rather than degrees of partial membership across clusters. The K-Means algorithm also requires defining the number of final clusters beforehand. Therefore, three clusters were applied according to the distinct number of antitrypanosomal activity profiles and data set size.

The patterns of compound separation into biological groups based on their cytotoxicity profiles against five *Leukemic cell lines* (CCRF-CEM, HL-60(TB), K-562, MOLT-4, and RPMI-8226) are shown in Fig. 3. Cluster 2 marked in blue is repeatedly formed across different *Leukemic cell line* pairings by a group of compounds that mostly belong to the first (highly active) and partly to the second class of antitrypanosomals in the data set. K-Means clustering algorithm showed that out of 12 compounds from Cluster 2 (**1**, **9**, **10**, **12**, **14**, **17**, **19-22**, **24**, **25**, **30**) 5 compounds are from class 2 (Fig. 1, *a*), and there is only 1 outlier – compound **10** that belongs to class 3 (Fig. 1, *b*).

Each cluster is initially treated as a singleton cluster by the Agglomerative hierarchical clustering or bottom-up algorithms, which then progressively agglomerate pairings of clusters until all clusters have been combined into a single cluster that contains all data. This method is based on the development of dendograms. Agglomerative hierarchical clustering was performed using three methods of complete clustering (*a*), average clustering (*b*), and single clustering (*c*) (Fig. 4), yielding dendograms that divided tested compounds into two clusters. Further analysis was performed for the average linkage. Out of 12 compounds from Cluster 0 (**1, 9, 10, 14, 17, 19-22, 24, 25, 30),** 6 compounds are from class 1 and 5 compounds are from class 2, and compound **10** belongs to class 3 (Fig. 5).

The next step of our study involved pharmacophore screening that was carried out for 5 compounds (from the class 1) identified in both **Cluster 2 (**K-Means clustering algorithm) **and Cluster 0 (A**gglomerative hierarchical clustering): (2E,5Z)-5-[[4–(dimethylamino)phenyl]methylene]-2-thiazol-2-ylimino-thiazolidin-4-one **14**, 2-[4–bro-

mo–2–[(Z)–[2–(4–hydroxy–*N*–methyl–anilino)–4–oxo–thiazol–5–ylidene]methyl]phenoxy]acetic acid **20**, 5-bromo-1-[2–[3–(4–chlorophenyl)–5–(4–methoxyphenyl)–3,4–dihydropyrazol–2–yl]-2-oxo-ethyl]indoline-2,3-dione **22**, 4-[3–(5–bromo–2–hydroxy–phenyl)–5–phenyl–3,4–dihydropyrazol–2–yl]-5*H*-thiazol-2-one **25**, and 2-(5-{[5–(2,4–dichlorobenzyl)–thiazol–2–ylamino]-methylene}-4-oxo-2-thioxothiazolidin-3-yl)-3-phenyl-propionic acid ethyl ester (**30**). Although, there were two more compounds in Cluster 2 (**9**, **12**) and only compound **9** in Cluster 0, the *rel*-(5aR,11bR)-3,5a,6,11b-tetrahydro-2*H*,5*H*-chromeno[4',3':4,5]thiopyrano[2,3–*d*][1,3]thiazol-2-one derivatives **9** and **12** were not included in the set as the sample was limited to non-fused molecules. The study aimed to find a correlation between the antileukemic and antitrypanosomal properties of thiazolidinone/pyrazoline-related heterocyclic molecules. Consequently, pharmacophore modelling was performed using a pharmacophore derived from a number of BCL-2 complexes with the antagonist venetoclax (PDB IDs: 6O0K, 6O0M, 6O0P) [34]. The results of pharmacophore modelling carried out using LigandScout 4.4 Software [33] characterized all studied molecules as active with pharmacophore-fit scores within 37.95-58.74 (Table 2). The overlaying of compound **25** with the merged pharmacophore model is shown in Fig. 6.

Based on the obtained pharmacophore fit scores, we hypothesised that thiazolidinone-based hybrid molecules might possess dual antiparasitic and anticancer activity. So, the structure-based pharmacophore model (Fig. 4) was used for the screening of several hybrid molecules bearing a combination of 4-thiazolidinone or thiazole core and phenyl-indole or 6-phenyl-imidazo[2,1–*b*][1,3,4]thiadiazole fragments. These molecules had previously shown submicromolar levels of IC_{50} *in vitro* against *Tbb* and *Trypanosoma brucei gambiense* (*Tbg*) [2]. The hit compounds were selected based on their antitrypanosomal activity, with the IC_{50} values ranging from 0.03 to 0.17 μM in the *Tbb* assay and from 0.14 to 1.0 μM in the *Tbg* assay. All 18 tested compounds fit the pharmacophore model, with pharmacophore-fit scores ranging from 36.01 to 55.55 (Table 3).

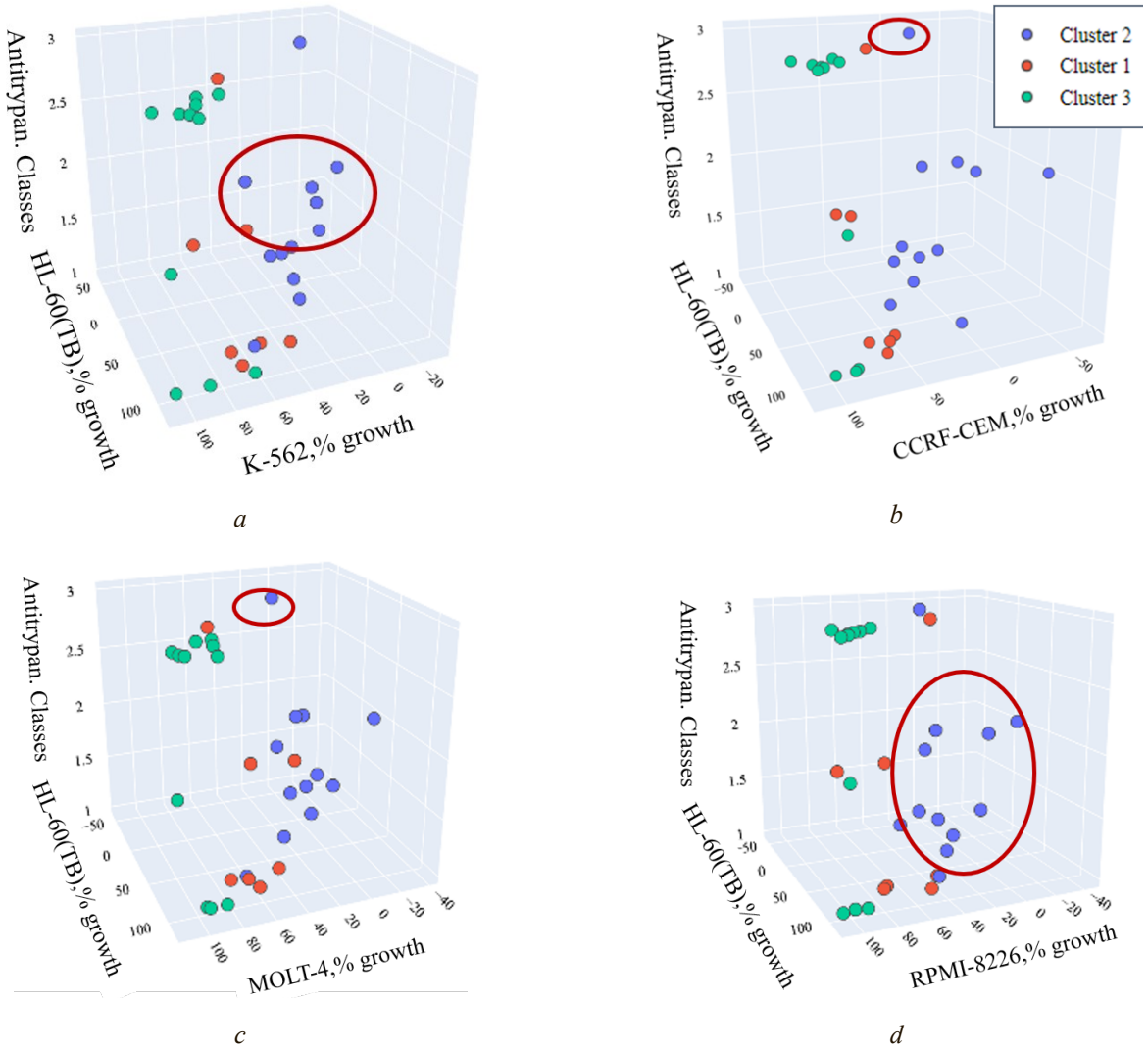
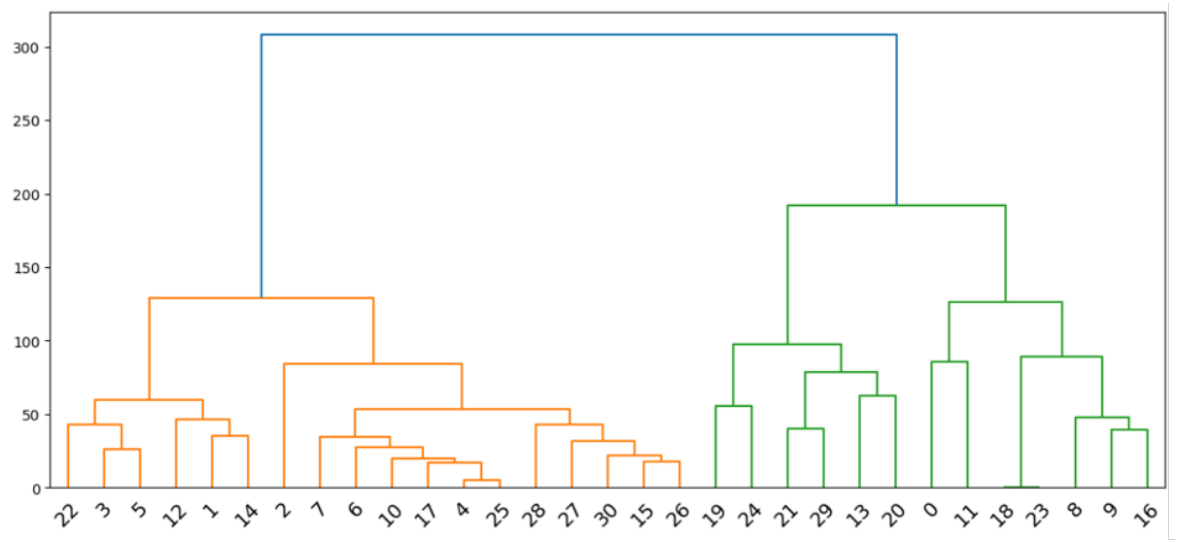
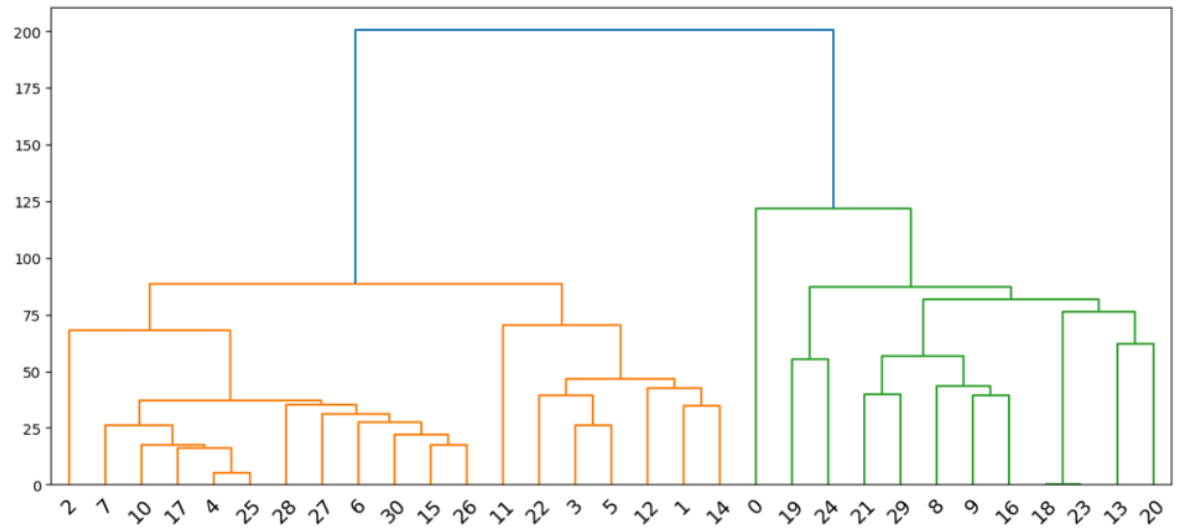


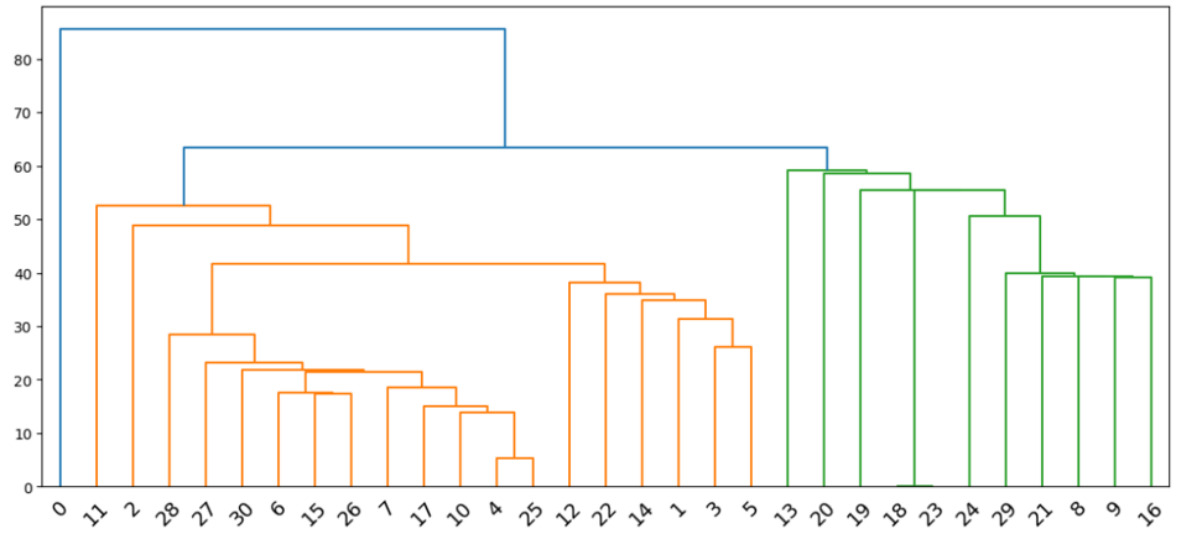
Fig. 3. 3*D* Visualization of K-Means clustering algorithm based on growth inhibition of five *Leukemic cell lines* and classes of antitrypanosomals, namely correlation between percentage of *Leukemic cell HL*-60(*TB*) *line* growth, antitrypanosomal activity and next *Leukemic cell lines*: *a* – K-562; *b* – CCRF-CEM; *c* – MOLT-4; *d* – RPMI-8226



a



b



c

Fig. 4. Hierarchical clustering of the compounds using three linkage methods: *a* – complete linkage; *b* – average linkage; *c* – single linkage

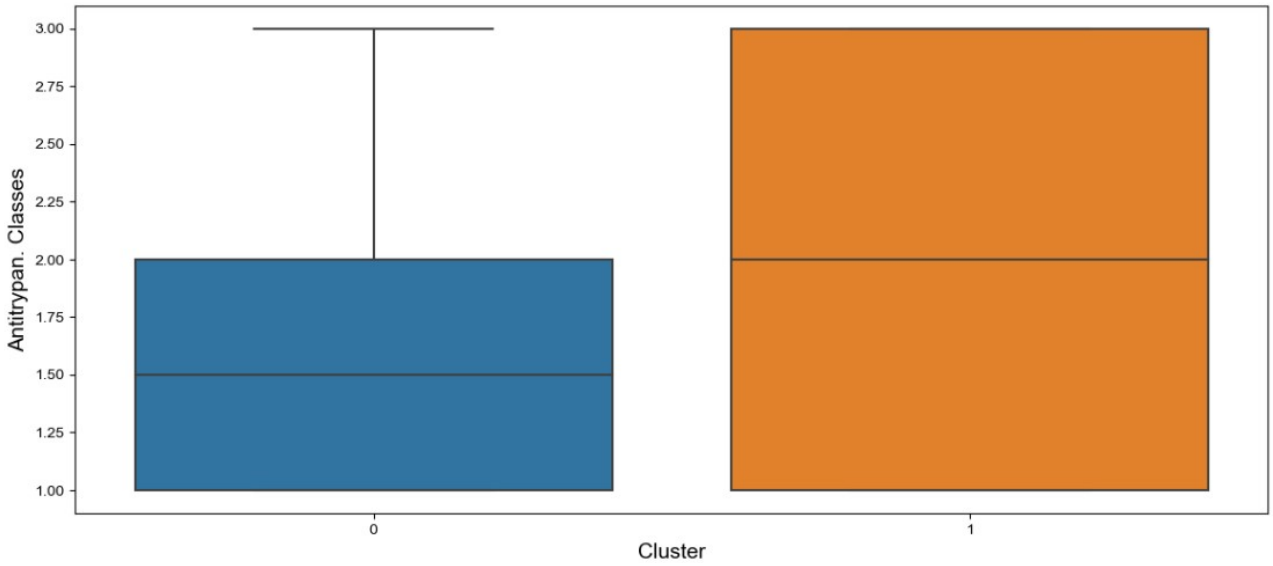


Fig. 5. The Agglomerative hierarchical clustering, average clustering method; 12 compounds in a Cluster 0: **1, 9, 10, 14, 17, 19-22, 24, 25, 30**

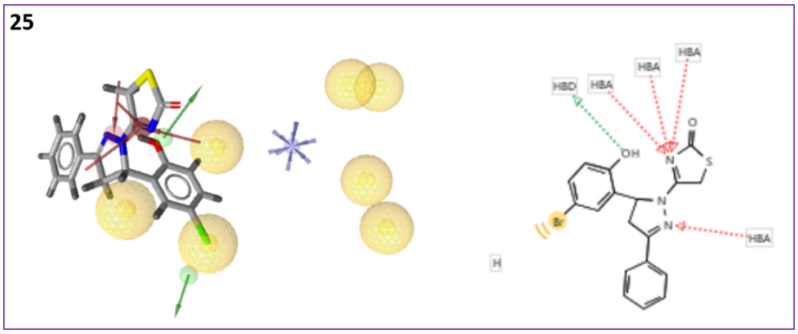


Fig. 6. Overlaying of the compound **25** with merged pharmacophore model (derived from BCL-2 complexes with venetoclax (PDB IDs: 6O0K, 6O0M, 6O0P)

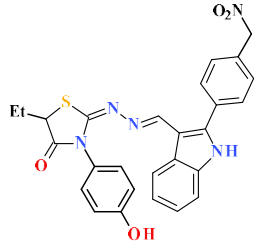
Table 2

Pharmacophore-fit scores calculated for the compounds identified in Cluster 2 and Cluster 0 that belong to class 1

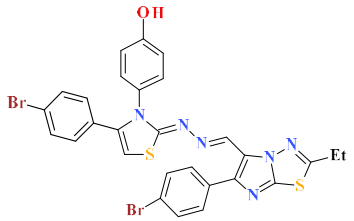
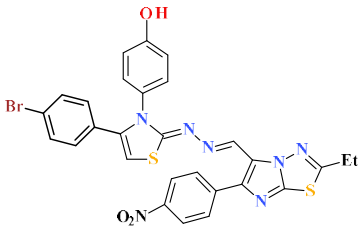
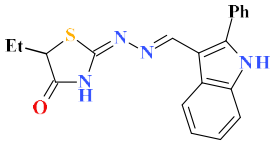
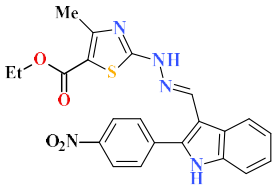
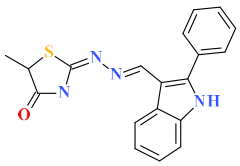
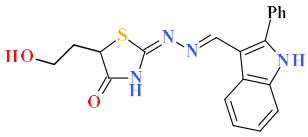
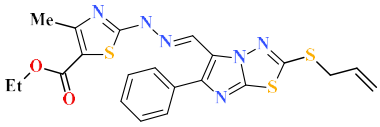
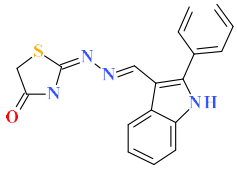
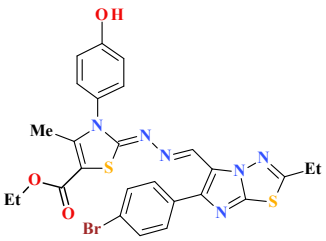
Merged pharmacophore from 3 complexes (PDB codes: 6O0K, 6O0M, 6O0P)	
# Comp	Pharmacophore fit-score
14	58.74
25	56.77
20	55.94
22	47.47
30	37.95

Table 3

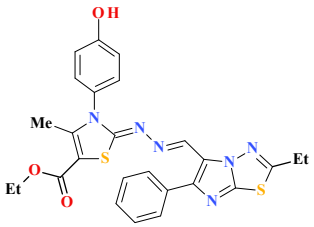
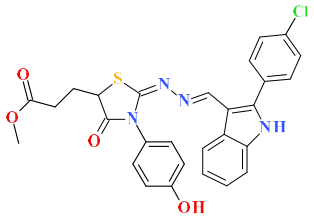
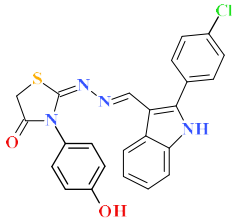
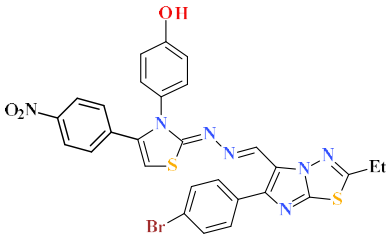
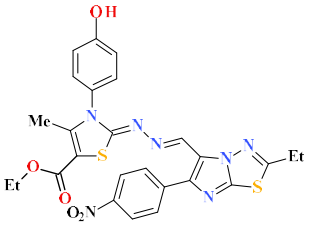
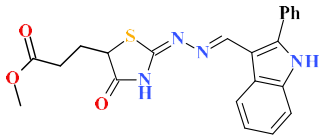
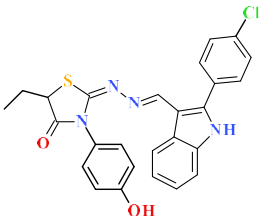
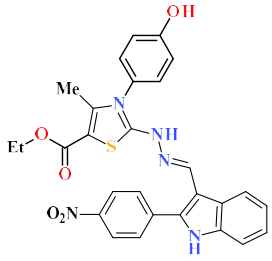
Structures of antitrypanosomal thiazolidinone/thiazole-indole/imidazothiadiazole hybrids and pharmacophore-fit scores calculated for several hit-compounds identified in the *in vitro* test against *Tbb* and *Tbg*

# Comp.	Structure	IC_{50} (μM), *Tbb*	IC_{50} (μM), *Tbg*	Pharmacophore fit score
1	2	3	4	5
1		*nt	0.14 ± 0.010	55.55

Continuation of Table 3

1	2	3	4	5
2		nt	0.476 ± 0.066	55.45
3		nt	0.594 ± 0.036	55.43
4		0.03 ± 0.004	nt	55.12
5		nt	0.74 ± 0.068	54.79
6		nt	0.21± 0.014	46.54
7		0.17 ± 0.004	nt	46.44
8		nt	0.652 ± 0.113	45.64
9		nt	0.23 ± 0.009	45.61
10		nt	1.0 ± 0.10	45.56

Continuation of Table 3

1	2	3	4	5
11		nt	0.745 ± 0.098	45.56
12		nt	0.38 ± 0.080	45.38
13		nt	0.82 ± 0.111	45.38
14		nt	0.510 ± 0.110	37.29
15		nt	0.639 ± 0.074	37.28
16		0.06 ± 0.003	nt	37.11
17		nt	0.86 ± 0.076	36.22
18		nt	0.66 ±0.030	36.01

Note: nt – not tested.

5. Discussion

The application of different clustering algorithms resulted in the unification of compounds belonging to the same classes of antitrypanosomals within a single cluster. Notably, compounds from class 1 with the highest inhibition rates against parasites were grouped together in one cluster by both the K-Means and Agglomerative hierarchical clustering methods. We can assume that compounds characterised by the highest antitrypanosomal activity from classes 1 and 2 were grouped into the same clusters: Cluster 2 in the K-Means clustering algorithm and Cluster 0 in the Agglomerative hierarchical clustering, when applying the two aforementioned machine learning methods, which demonstrate the robustness of the algorithms.

The next step of our study involved structure-based pharmacophore modelling. The BCL-2-venetoclax complexes were selected as numerous studies have suggested the pro-apoptotic potential of thiazolidine derivatives [35, 36]. The choice of venetoclax was further supported by growth inhibition assay results of thiazolidine/pyrazoline derivatives against *Leukemia cell lines*. Venetoclax is used as a cancer therapy for chronic lymphocytic leukemia, acting via the selective inhibition of BCL-2, which promotes apoptosis through the release of cytochrome C and subsequent cell death [37]. Furthermore, the apoptotic cell death pathway in some parasites has been recently characterised and found to share similarities with that in humans [38]. Molecules **14**, **20**, **22**, **25**, and **30** that underwent pharmacophore screening matched multipoint 3D merged pharmacophore from BCL-2-venetoclax complex via 3-4 or more chemical features like hydrogen bond donors, acceptors or lipophilic fragments (Fig. 4) that describe their interaction with the surrounding binding site of a BCL-2 and can assert their possible mechanism of action.

Application of the same structure-based pharmacophore model (Fig. 4) in LigandScout 4.4 to screen a set of highly active antitrypanosomal thiazolidinone/thiazole-indole/imidazothiadiazole hybrid molecules resulted in all compounds being classified as actives. This may indirectly support the initial hypothesis regarding the similarity in the antiproliferative mechanism of action of thiazolidine-related compounds in both cancer and parasitic cells, thereby suggesting a potential dual antiparasitic and antitumor effect of these compound classes.

Practical relevance. The obtained results are intended to help in the search for dual anticancer/anti-parasitic properties in various classes of thiazole/thiazolidinone derivatives and related heterocycles.

Research limitations. The main limitation of the research lies in a small sample size in a set and is related to the limit ability to study simultaneously anticancer and anti-parasitic properties of the target compounds.

Prospects for further research. The obtained result should serve as a basis for further in-depth *in vitro* and *in-vivo* research of the mechanism of thiazolidine/pyrazoline-related compounds action as potent anticancer and/or antiparasitic agents.

6. Conclusions

A set of thiazolidine/pyrazoline derivatives with studied dual antitrypanosomal and anticancer activity was analysed using machine learning methods, namely K-means and Agglomerative Hierarchical Clustering algorithms, which resulted in the selection of a group of highly active antitrypanosomals with good anticancer properties. The latter underwent pharmacophore screening using a merged pharmacophore derived from BCL-2-venetoclax complexes and showed good pharmacophore-fit scores. The same pharmacophore model, when applied to a data set of highly active antitrypanosomal thiazolidinone/thiazole-indole/imidazothiadiazole hybrids discovered in *in vitro* assays against *Tbb* and *Tbg*, had also shown high pharmacophore-fit scores, suggesting they might possess antiproliferative properties as well.

Conflict of interest

The authors declare that they have no conflicts of interest concerning this research, whether financial, personal, or authorship-related, that could affect the research and its results presented in this article.

Funding

The study was performed without financial support.

Data availability

The manuscript has no associated data. Codes are publicly available on GitHub: https://github.com/AnnDylevych/K-MeansCluster.Anticancer_Antitrypanos.; https://github.com/AnnDylevych/Hierarch.Cluster._Anticanser_Antitrypanos

Use of artificial intelligence

The authors confirm that they did not use artificial intelligence technologies when creating the current work.

Acknowledgements

We thank Prof. Thierry Langer for giving us the opportunity to utilize the LigandScout 4.4 suite.

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Received 08.05.2025
Received in revised form 23.05.2025
Accepted 10.06.2025
Published 30.06.2025

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