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Discovery of Novel Cathepsin D Inhibitors by High-Throughput Virtual Screening

Abhijit Debnath [1,*], Hema Chaudhary [2], Rajesh Kumar [3], Ankit Shokeen [4], Riya Khurana [5]

[1] Noida Institute of Engineering and Technology (Pharmacy Institute), 19 Knowledge Park-II, Institutional Area, Greater Noida-201306, Uttar Pradesh, India; theabhijitdebnath@gmail.com (A.D.);
[2] School of Medical & Allied Sciences, K R Mangalam University, Gurugram, India; hemamanannitin@gmail.com (H.C.);
[3] Institute of Medical Sciences, Banaras Hindu University, Varanasi, India; rkszoology@gmail.com (R.K.);
[4] Faculty of Pharmaceutical Sciences, PDM University, Delhi, India; ankitshokeen51@gmail.com (A.S.);
[5] Faculty of Pharmaceutical Sciences, PDM University, Delhi, India; anushkakhuranark@gmail.com (R.K.);
* Correspondence: theabhijitdebnath@gmail.com; abhijit.debnath@niet.co.in (A.D.);
Scopus Author ID 57666377100

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Abstract: Cathepsin D (CatD) is a cancer-associated inflammation protein that plays an important role in cancer-associated inflammation, osteoarthritis, and various neurodegenerative diseases. CatD is genetically stable and does not easily develop drug resistance. Targeting CatD is an interesting strategy for both cancer therapy and cancer prevention. Thus, we have tried working on CatD as a therapeutic target in this research. To identify potent CatD inhibitors, we have screened the Million Molecules Database, Natural Product Database, and NCI Database. Top hits have been filtered based on various drug-likeness rules, followed by ADME profiling, toxicity prediction, and Consensus Molecular docking. Our research work resulted in three molecules: ZINC12198861 (-9.6 Kcal/mol), ZINC2438311 (-9.5 kcal/Mol), and ZINC14533276 (-8.9 kcal/mol) are stability-binding at the active site of CatD with strong binding affinity, drug-like properties, effective ADME properties, low toxicity, and high stability. Inhibiting CatD with these identified molecules will promote apoptosis and cure cancer-associated inflammation and osteoarthritis.

Keywords: Cathepsin D; CatD; Cathepsin D inhibitors; CatD inhibitors; osteoarthritis; cancer; inflammation; Alzheimer's disease; cancer-associated inflammation; molecular docking.

1. Introduction

Cathepsin D (CatD) is mostly found in lysosomes [1]. It belongs to the extensive family of aspartic proteases [2]. Numerous biological activities, such as controlling cell death, breaking down intracellular and extracellular proteins, and inducing inflammatory responses in cells, are performed by CatD [3]. Protein turnover is attributed to CatD, which favors apoptosis when proteostasis is disturbed [4]. Various health issues can result from the disruption in its regulation [5]. Thus, CatD is becoming a favorable therapeutic target for cancer drug targets [7], inflammation [8], osteoarthritis [6], Alzheimer's disease, and other neurodegenerative diseases [9,10].

CatD is prominently overexpressed in cancer cells [7]. CatD promotes angiogenesis, metastasis, and invasion of cancer. Gastric and breast cancer cells need CatD to migrate and invade [11,12]. Many times the development and spread of cancer are often correlated with inflammation, such as hepatocellular carcinoma, lung, cutaneous, esophageal, gastric, colorectal, and pancreatic cancer [8,13]. Inflammation is proven to facilitate the establishment of a tumor microenvironment, which promotes cancer start and progression [14]. An environment rich in inflammatory cells, active stroma, growth factors, and DNA- damage-promoting chemicals undoubtedly increases and boosts the probability of developing cancer [15]. A pro-inflammatory tumor milieu is produced because various immune cells invade the area where the tumor is developing. But the tumor cells use the inflammatory reactions' capacity by releasing chemokines and cytokines (Interleukin-1, IL-6, tumor necrosis factor, and interferon) to attract immune cells and promote the growth of malignancy[16–18]. In 1863, Rudolf Virchow initially proposed the link between cancer and inflammation based on the abundance of leukocytes in malignant tissue [19,20]. Anti-inflammatory drugs have the potential not only to enhance the efficacy of conventional and next-generation immunotherapies but also to slow the progression of cancer. [21].

As various scientific reports suggested that the cells that cancer-associated inflammation-related proteins do not easily develop drug resistance; targeting inflammation-related proteins are an interesting strategy for both cancer therapy and cancer prevention [8]. Thus, in this research, we have tried to target CatD. To identify potent CatD inhibitors, we have employed RASPD-based virtual screening of the Million Molecules Database, Natural Product Database, and NCI Database, followed by filtering based on various drug-likeness rules, ADME, toxicity, and Molecular docking. Our research work resulted in three molecules that have shown strong binding affinity with CatD, effective ADME profile, low toxicity, and high stability. Inhibiting the CatD with these identified molecules will promote apoptosis.

2. Materials and Methods

2.1. Identification of hits by virtual screening.

Virtual screening was conducted by utilizing the RASPD tool [22]. Three databases available at RASPD [22] (Million Molecules Database, the Natural Product Database, and the NCI Database) were screened by following RASPD Protocol. A limit was set for Binding energy at -7.0 Kcal/mol, and the molecules successfully passed the threshold; they were subjected to further studies.

2.2. Filtering hits based on drug-likeness properties.

Using the SwissADME server [23], the drug-like characteristics were examined. To eliminate compounds that are incompatible with drug-like properties: Lipinski's rule of five [24], the Ghose rule [25], the Veber rule [26], and the Muegge rule [27] based filters were applied. The compounds that m*et al*l these criteria and had "drug-like" characteristics were selected for further lead optimization.

2.3. Lead Optimization.

2.3.1. Molecular Docking.

To make all the compounds ready for docking, energy minimization, charge, and H-bond addition has been done by Avogadro and Dock Prep (Chimera). Dock Prep was also used in target Protein preparation after retrieving the Crystal structure of CatD from the RCSB-Protein Data Bank [28] [PDB id: 6QBH, resolution: 1.85 Å [3]. Water molecules, Co-Factors, ions, and covalent ligands present in the protein structure have been cleaned [29]. After calculating charges, polar hydrogen atoms were inserted. The grid box centered in (X = 9.207889, Y = 16.524528, Z = 6 .945556) based onthe active sites of the protein (Chain A: VAL31, TYR78, and Chain B: THR125, PHE131, ASP231, MET309) [2,3,30]. The center points and dimensions of the grid boxes were adjusted to target the substrate-binding pocket of the protein. Auto Dock Tools 4.2 implemented in AMDock was used for [31,32] Molecular Docking. The Lamarckian Genetic Algorithm was chosen with 1000 iterations as the docking parameter. Based on its binding energy score and significant interactions in Active sites, the optimal docked conformation was chosen. The best result was evaluated for ADME and toxicity based on the ΔG.

2.3.2. ADME.

SwissADME was used to examine the Pharmacokinetic profile [23], Pre-ADMET [33], and vnnadmet [34]. ADME properties such as Solubility Class, GI Absorption, Human intestinal absorption (HIA %), Partition Coefficent (LogP), BBB, Pgp Inhibition, P-gp Substrate, CYP1A2 inhibitor, CYP3A4 inhibitor, CYP3 A4 Substrate, CYP2D6 inhibitor, CYP2D6 substrate, CYP2C9 inhibitor, CYP2C19 inhibitor were predicted. The compounds that have a suitable pharmacokinetic profile were taken for further research.

2.3.3. Toxicity.

Toxicity causes 30% of lead candidates to fail. The toxicity study was carried out by using Pre-ADMET [33], vnnadmet [34], and lazar [35]. Toxicity parameters such as Liver Toxicity: Cyto- toxicity, Mitochondrial Toxicity (MMP), Carcinogenicity (Mouse), Carcinogenicity (Rat), Carcinogenicity (Rodent), Ames TA100 (-S9), Ames TA1535 (+S9), Ames TA1535 (-S9) were predicted. Nontoxic compounds were taken for further studies.

2.3.4. Consensus molecular docking and molecular interaction analysis.

Docking software can accurately forecast the docking pose can be impacted by system-bias effects resulting from parameter training or overfitting [36]. To circumvent this constraint, exponential consensus approaches combine the results of multiple docking algorithms by averaging the score or position of each molecule received from various programs. Auto Dock Tools, Autodock Vina, idock, and SwissDock were utilized to conduct Consensus Molecular Docking [32,37–39]. All complexes were analyzed to discover residue-by-residue interactions between ligand and receptor, as well as chemical bonds and other chemical interactions, to comprehend the interaction at the molecular level better.

3. **Results and Discussion**

3.1. Identification of Hits.

Following the screening and elimination of duplicate molecules, 4198 unique hit molecules that bind to the receptor with an affinity of less than -7.0 Kcal/mol were identified and selected for further study.

3.2. Filtering hits based on drug-likeness properties

To obtain a molecule resembling lead, the SwissADME server was utilized to calculate all the hits in numerous batches. Microsoft Excel was used for processing and analyzing

SwissADME-generated data. Only 62 out of 4198 found molecules were Molecules. Obeyed multiple drug-likeness rules such as the rule of 5 given by Lipinski rule, Ghose's rule, Veber's rule, and Muegge's rule.2860 molecules obeyed the Lipinski Rule of 5, while 2474 molecules obeyed the Veber rule, 2374 molecules obeyed the Egan rule, 2067 molecules obeyed the Muegge rule, and 63 molecules obeyed the Ghose rule.

3.3. Lead optimization.

3.3.1. Molecular docking.

Docking was used to identify the best appropriate Confirmation molecule that can bind to CatD with the lowest binding energy. The top 27 of the 62 drug-like compounds were selected for future research based on their binding energy and chemical interactions. Table 1 contains the molecular docking results of all 91 compounds, along with their SMILEs and binding energies.

Table 1. Results of Molecular docking (Binding Energy in Kcal/Mol)

Molecule ID	SMILE	Binding Energy
ZINC12301853	c1ccc[cc1]c2ccc[cc2]c3nnc[o3]CCC[=O]NCCc4cn5cccnc5n4	-9.4
ZINC12149963	c1cc-2c[c[c1]C[=O]NC[C@@H]3Cc4cc[ccc4O3]c5cncnc5]C[=O]c6c2ccc[c6]Cl	-8.9
ZINC12283149	Cc1c2cc[ccc2nc[n1]N3CCC[CC3]C[=O]NCCSc4nnnn4C]C(c)C	-8.9
ZINC11840098	Cc1cc[n[n1]c2cccc[c2]C[=O]NC[C@H]3Cc4cc[ccc4O3]c5ccc[nn5]OC]C	-8.8
ZINC02438311	Cc1c2ccccc2c3nnc[n3n1]COc4ccc[cc4]NC[=O]COc5ccccc5	-8.7
ZINC12198861	c1ccc[cc1][C@@H]2CC[NH+][C2]CCNC[=O]c3cc[on3]COc4cccc5c4ccnc5	-8.7
ZINC12134416	COc1ccc[cc1]C[=O]NCCc2nnc3n2CC[NH+][CC3]Cc4ccc5c[c4]CCO5	-8.4
ZINC14533276	CN(c)C[=O]c1c2c[n[n1]Cc3ccccc3]CCN[C2]C[=O]Cn4cc5ccccc5n4	-8.4
ZINC12038620	c1ccc-2c[c1]Cc3c2ccc[c3]C[NH+]4CCC[C@@H][C4]n5cc[nn5]C[=O]NCCCO	-8.3
ZINC02504256	c1ccc[cc1]N2CCN[CC2]C[=O]c3cnn4c3nc[cc4C[F][F]F]c5ccco5	-8.2
ZINC11786358	Cc1cc[n[n1]Cc2cccc[c2]C[=O]NCCc3nnc[n3C]SCc4ccccn4]C	-8.2
ZINC12190457	c1ccc[cc1]OCC[=O]Nc2cnn[c2]CC[=O]N3CCC4[CC3]C=Cc5c4cccc5	-8.2
ZINC12039623	c1ccc[cc1]c2nc[on2]CCNC[=O]c3ccc[cc3]n4cc[cn4]NC[=O]c5ccoc5	-8.1
ZINC12126821	Cc1c[sc[n1]NCc2cc3c[c[c2]OC]OCO3]c4ccn[n4]c5ccc[cc5]OC	-8.1
ZINC01070635	Cn1c2c[c[=O]n[c1=O]C][nH]c[n2]c3ccc[cc3]OCC[=O]NCCc4ccccc4	-8
ZINC11972241	CCCc1cc[no1]C[=O]NC[C@H]2Cc3cc[ccc3O2]c4ccc[cc4]S[=O][=O]C	-8
ZINC00945916	Cn1c2ccccc2nc1SCC[=O]N/N=C/c3ccc[cc3]OCc4ccccc4	-7.9
ZINC02881932	CCCCn1cc[cn1]NC[=O]c2cc3n[n2][C@H][C[C@H][N3]c4ccc5c[c4]OCO5]C[F][F]F	-7.9
ZINC12152120	c1cc[ccc1CNC[=O]Cn2cc[cn2]NC[=O]c3ccc4c[c3]OCO4]N5CCOCC5	-7.9
ZINC14885414	Cc1ccc[nc1]c2ccc3c[c2]C[C@H][O3]CNC[=O]CCN(c)[C@@H]4CCS[=O][=O]C4	-7.9
ZINC14987901	c1ccc[cc1]c2ccc[cc2]C[NH+]3CCC[C@H][C3]n4cc[nn4]C[=O]NCCCO	-7.9
ZINC00793735	c1ccc[cc1]C[NH+]2CCN[CC2]C[=O]c3nc4nc[cc[n4n3]C[F][F]F]c5cccs5	-7.8
ZINC01216760	c1ccc[cc1]C[NH+]2CCN[CC2]C[=O]c3cnn4c3nc[cc4C[F][F]F]c5ccco5	-7.8
ZINC02859380	CCOc1ccc[cc1]NC[=O]CSc2nnc[n2C]COc3ccc4c[c3]CCCC4	-7.8
ZINC11872831	CN[Cc1cc2ccccc2s1]C[=O]c3ccc[cc3]n4cc[cn4]NC[=O]c5ccoc5	-7.8
ZINC14755609	Cc1c[nc2cc[ccc2n1]C[=O]N3CCc4c[c[nn4C]C[=O]NCCCOC]C3]C	-7.8
ZINC19774479	c1ccc[cc1]CN2CCN[CC2]C[=O]c3cnn4c3nc[cc4C[F][F]F]c5ccco5	-7.8
ZINC11789487	CS[=O][=O]c1ccc[cc1]c2ccc3c[c2]C[C@@H][O3]CNC[=O]Cc4cn5ccsc5n4	-7.7
ZINC11996261	Cc1c[cnn1c2nccc[n2]c3cc[ccc3OC]OC]C[=O]N(c)CCc4ccccn4	-7.7

Molecule ID	SMILE	Binding Energy
ZINC12152048	Cn1c2ccccc2c3c1ccc[c3]C[=O]NCCc4nnc[n4C]SCCOC	-7.7
ZINC14884412	C[NH+][CCn1cc[nn1]C[=O]NCc2ccncc2]Cc3ccc[cc3]c4ccccc4	-7.7
ZINC08680620	c1ccc[cc1]C[NH+]2CCN[CC2]C[=O]c3cnn4c3nc[cc4C[F][F]F]c5cccs5	-7.6
ZINC11972231	CCCc1cc[no1]C[=O]NC[C@@H]2Cc3cc[ccc3O2]c4ccc[cc4]S[=O][=O]C	-7.6
ZINC12100287	Cc1c[sc[n1]NCc2ccc[cc2]C[=O]OC]c3ccn[n3]C[C@@H]4COc5ccccc5O4	-7.6
ZINC12707752	Cc1ccc[cc1]CCC[=O]Nc2ccc3c[c2]CN[CC3]C[=O]CCCn4cncn4	-7.6
ZINC01058046	Cn1c[nnc1SCC[=O]Nc2ccc[cc2]OCc3ccccc3]Cc4cccs4	-7.5
ZINC12228980	COc1cccc[c1]OC[C@@H][CNC[=O]c2ccc3c[c2]oc[n3]C4CCCCC4]O	-7.5
ZINC12100288	Cc1c[sc[n1]NCc2ccc[cc2]C[=O]OC]c3ccn[n3]C[C@H]4COc5ccccc5O4	-7.4
ZINC13644479	c1ccc[cc1]Sc2ccccc2NC[=O]c3cnn4c3nccc4c5ccco5	-7.3
ZINC00955034	CS[=O][=O]c1ccc2c[c1]sc[n2]NC[=O]/C=C/c3ccc[cc3]OCc4ccccc4	-7.2
ZINC01886291	CCCCOC[=O]c1ccc[cc1]NC[=O]COc2ccc3c[cc[=O]oc3c2]C	-7.2
ZINC11783096	CCOCCCNC[=O]c1cnn[c1c2cccs2]c3ncc[c[n3]c4ccc[cc4]F]C	-7.2
ZINC11936621	Cc1nccn1c2ccc[cc2]NC[=O][C@H]3CCC[NH+][C3]Cc4cnc[nc4]CCOC	-7.2
ZINC02237046	CCCCCCOc1ccc[cc1]N2C[=O]C[C@@H][C2=O][NH2+]Cc3ccc4c[c3]OCO4	-7.1
ZINC11812816	CCN[CCn1cccn1]C[=O]C2CCN[CC2]c3nc[c4cc[ccc4n3]OCC]C	-7.1
ZINC12050264	c1ccc[cc1]Oc2cccc[c2]C[NH+]3CCC[C@H][C3]n4cc[nn4]C[=O]NCCCO	-7.1
ZINC12333933	CCOC[=O]CCNC[=O]c1cnn[c1C]c2nccc[n2]c3ccc[c[c3]F]OC	-7.1
ZINC12456992	COCCc1ncc[cn1]C[NH+]2CCC[C@H][C2]C[=O]Nc3cccc[c3]n4cccn4	-7.1
ZINC03000298	c1ccc[cc1]COC[=O]NCCC[=O]Oc2ccc3c[c2]occ[c3=O]c4ccc[cc4]Cl	-7
ZINC11936620	Cc1nccn1c2ccc[cc2]NC[=O][C@@H]3CCC[NH+][C3]Cc4cnc[nc4]CCOC	-7
ZINC11998134	CC(c)OCCCNC[=O]c1cnn[c1C2CC2]c3nccc[n3]c4ccc5c[c4]OCO5	-7
ZINC12156575	CCOC[=O]CCNC[=O]c1cnn[c1C2CC2]c3ncc4c[n3]-c5cc[ccc5CC4]OC	-7
ZINC14988056	CCOCCCNC[=O]c1cnn[c1C2CC2]c3nccc[n3]c4ccc5c[c4]OCO5	-7
ZINC18250336	CCCCCCOc1ccc[cc1]N2C[=O]C[C@H][C2=O]NCc3ccc4c[c3]OCO4	-7
ZINC12050268	c1ccc[cc1]Oc2cccc[c2]C[NH+]3CCC[C@@H][C3]n4cc[nn4]C[=O]NCCCO	-7
ZINC19143677	Cn1ccnc1SCCNC[=O]COc2ccc[nn2]N3CCN[CC3]c4ccccn4	-7
ZINC11784547	COc1ccc2c[c1][nH]c[n2]CCNC[=O]CC[C@@]3[CCC[=O]N3]Cc4ccc[cc4]Cl	-7
ZINC12219151	Cc1cc[on1]C[NH2+]C2CCN[CC2]c3ccc[cc3]CC[=O]N4CCC[C@H]4C[=O]OC	-7
ZINC12038301	CCOCCC[NH2+][C@@H]1CCc2c[sc3c2c[=O]n[cn3]C4Cc5ccccc5C4]C1	-7
ZINC12041387	CCOCCC[NH2+][C@@H]1CCc2c[sc3c2c[=O]n[cn3]CCc4ccccc4OC]C1	-7
ZINC12455545	CCOCCC[NH2+][C@@H]1CCc2c[sc3c2c[=O]n[cn3]CCc4ccc[cc4]OC]C1	-7
ZINC11878770	c1ccc2c[c1]CC[NH+][C2]C[C@H][COc3ccc[cc3]C[NH2+]CCc4cccs4]O	-7

3.3.2. ADME.

Lead compounds fail in clinical trials due to their poor ADME characteristics. To exclude compounds with a poor pharmacokinetic profile, Insilco pharmacokinetics properties of the molecules have been predicted. Those molecules were found as water-soluble, with high GI Absorption, high human intestinal absorption (HIA%), and Partition Coefficient (LogP) ranges from 1 to 4, No Blood Brain Barrier permeability was taken for further studies. Additionally, P-gp Inhibition, P-gp Substrate, a CYP1A2 inhibitor, a CYP3A4 inhibitor, CYP3A4 Substrate, a CYP2D6 inhibitor, a CYP2D6 substrate, CYP2C9 inhibitor, CYP2C19 inhibitor were predictedto understand about the journey of the drug after oral administration. Only 12 compounds out of 27 have m*et al*l ADME Profile requirements. Detail view is displayed in Table 2.

Table 2. Results ADME prediction

Molecule ID	**Solubility Class**	**GI Absorption**	**Human intestinal absorption**	**Partition Coefficent (LogP)**	**BBB**	**Pgp Inhibition**	**P-gp Substrate**	**CYP1A2 inhibitor**	**CYP3A4 inhibitor**	**CYP3A4 Substrate**	**CYP2D6 inhibitor**	**CYP2D6 substrate**	**CYP2C9 inhibitor**	**CYP2C19 inhibitor**
ZINC00793735	Soluble	High	94.238774	2.47	No	Non	Yes	No	No	Non	No	Non	Yes	No
ZINC00945916	Poorly soluble	High	96.871525	4.07	No	Inhibitor	No	Yes	Yes	Non	Yes	Non	Yes	Yes
ZINC01070635	Soluble	High	91.777448	2.52	No	Non	No	No	Yes	Non	No	Non	Yes	Yes
ZINC01216760	Soluble	High	93.448984	2.27	Yes	Inhibitor	Yes	No	No	Non	No	Inhibitor	Yes	No
ZINC02438311	Soluble	High	96.751341	3.59	No	Non	Yes	Yes	Yes	Non	Yes	Non	Yes	Yes
ZINC02504256	Soluble	High	97.586951	3.09	Yes	Inhibitor	Yes	No	Yes	Non	Yes	Non	Yes	Yes
ZINC02859380	Poorly soluble	High	97.179052	3.92	No	Inhibitor	No	No	Yes	Non	Yes	Non	Yes	Yes
ZINC02881932	Soluble	High	94.761109	3.25	No	Non	Yes	No	Yes	Non	Yes	Non	Yes	Yes
ZINC11786358	Soluble	High	97.776806	3	No	Non	Yes	No	Yes	Non	Yes	Non	Yes	Yes
ZINC11840098	Soluble	High	96.712977	3.64	No	Non	Yes	No	Yes	Non	Yes	Non	Yes	Yes
ZINC11872831	Soluble	High	96.92794	3.77	No	Non	Yes	No	Yes	Non	Yes	Non	Yes	Yes
ZINC11972241	Soluble	High	97.774668	3.66	No	Non	No	Yes	Yes	Non	Yes	Non	Yes	Yes
ZINC12038620	Moderately soluble	High	89.753368	1.84	No	Non	Yes	No	No	Non	No	Inhibitor	No	No
ZINC12039623	Soluble	High	96.10391	2.78	No	Non	Yes	No	Yes	Non	Yes	Non	Yes	Yes
ZINC12126821	Poorly soluble	High	97.733943	3.99	No	Non	Yes	No	Yes	Non	Yes	Non	Yes	Yes
ZINC12134416	Soluble	High	91.192956	1.54	No	Non	Yes	No	Yes	Non	No	Non	Yes	No
ZINC12149963	Poorly soluble	High	97.007733	4.21	No	Inhibitor	Yes	Yes	Yes	Non	No	Non	Yes	Yes
ZINC12152120	Soluble	High	95.165682	1.66	No	Non	Yes	No	Yes	Non	Yes	Non	Yes	Yes
ZINC12190457	Moderately soluble	High	96.4773	2.84	Yes	Non	Yes	No	Yes	Non	Yes	Non	Yes	Yes
ZINC12198861	Soluble	High	92.496402	2.27	No	Non	Yes	No	No	Non	No	Inhibitor	Yes	Yes
ZINC12283149	Soluble	High	98.310751	3.05	No	Non	No	Yes	Yes	Non	No	Non	Yes	Yes
ZINC12301853	Soluble	High	96.926871	3.36	No	Non	Yes	No	Yes	Non	Yes	Non	Yes	Yes
ZINC14533276	Soluble	High	97.589159	2.34	No	Non	Yes	No	Yes	Non	Yes	Non	Yes	Yes
ZINC14755609	Soluble	High	97.714108	2.02	No	Non	Yes	No	Yes	Non	Yes	Non	Yes	No

Molecule ID	Solubility Class	GI Absorption	Human intestinal absorption	Partition Coefficent (LogP)	BBB	Pgp Inhibition	P-gp Substrate	CYP1A2 inhibitor	CYP3A4 inhibitor	CYP3A4 Substrate	CYP2D6 inhibitor	CYP2D6 substrate	CYP2C9 inhibitor	CYP2C19 inhibitor
ZINC14885414	Soluble	High	97.30467	2.4	No	Non	Yes	No	Yes	Non	Yes	Non	No	No
ZINC14987901	Moderately soluble	High	89.32498	1.72	No	Non	Yes	No	No	Non	No	Inhibitor	No	No
ZINC19774479	Soluble	High	97.585841	3.29	Yes	Inhibitor	Yes	No	Yes	Non	Yes	Inhibitor	Yes	Yes

3.3.3. Toxicity.

Toxicity causes 30% of lead candidates to fail. To eliminate the molecules having toxic properties toxicity properties of all the molecules have been predicted. The toxicity study was carried out by using Pre-ADMET [33], vnnadmet [34], and lazar [35].

Table 3. Results of toxicity prediction.

Molecule id	Liver Toxicity: Cyto- toxicity	Mitochondrial Toxicity (MMP)	Carcinogenicity (Mouse)	Carcinogenicity (Rat)	Carcinogenicity (Rodent)	Ames TA100 (-S9)	Ames TA1535 (+S9)	Ames TA1535 (-S9)
ZINC12283149	No	No	negative	Positive	non-carcinogenic	negative	negative	negative
ZINC11786358	No	No	negative	positive	non-carcinogenic	negative	positive	negative
ZINC11972241	No	No	negative	negative	non-carcinogenic	negative	negative	negative
ZINC14755609	No	No	negative	positive	carcinogenic	positive	negative	negative
ZINC14533276	No	No	negative	negative	non-carcinogenic	negative	negative	negative
ZINC14885414	No	No	negative	negative	non-carcinogenic	negative	negative	negative
ZINC11872831	No	No	negative	positive	non-carcinogenic	positive	negative	negative
ZINC12301853	No	No	negative	positive	non-carcinogenic	negative	negative	negative
ZINC02438311	No	No	negative	negative	non-carcinogenic	negative	negative	negative
ZINC11840098	No	No	negative	positive	non-carcinogenic	negative	negative	negative
ZINC12039623	No	No	negative	positive	carcinogenic	positive	positive	negative
ZINC12152120	No	No	negative	positive	carcinogenic	negative	negative	negative
ZINC02881932	No	No	negative	positive	carcinogenic	negative	negative	negative
ZINC00793735	No	No	negative	positive	non-carcinogenic	negative	negative	negative
ZINC12198861	No	No	negative	negative	non-carcinogenic	positive	negative	negative
ZINC01070635	No	No	negative	negative	non-carcinogenic	negative	negative	negative
ZINC12134416	No	No	negative	positive	non-carcinogenic	negative	negative	negative

Those molecules having no Liver Toxicity: Cyto- toxicity, No Mitochondrial Toxicity (MMP), negative Carcinogenicity (Mouse), negative Carcinogenicity (Rat), non-carcinogenic

(Rodent), negative Ames TA100 (-S9), negative Ames TA1535 (+S9), and negative Ames TA1535 (-S9) were taken for further studies. The nontoxic compounds were reported as CatD inhibitors with therapeutic promise. Only 6 compounds out of 12 have met all nontoxic properties. Table 3 lists the Toxicity prediction for all 12 compounds.

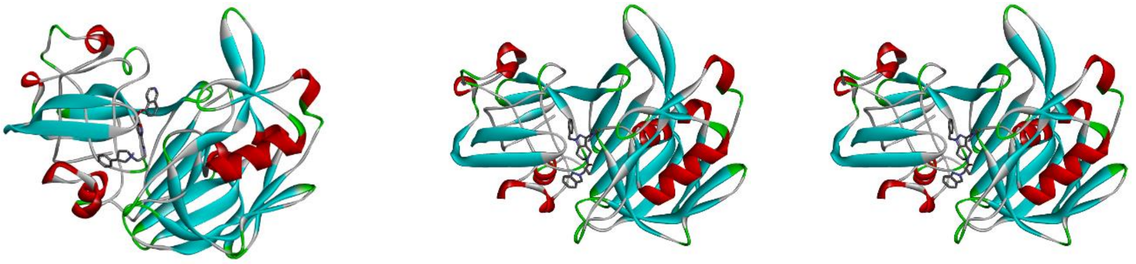
3.3.4. Consensus molecular docking and molecular interaction analysis.

Compared to single docking methods, consensus docking enhanced the quality of docking and virtual screening results [40,41]. Pose prediction accuracy was qualitatively improved by emphasizing ligand-binding poses generated by a wide number of docking techniques. High hit rates were obtained in virtual screening studies by picking compounds demonstrating a high level of pose consensus.

Table 4. Results of consensus molecular docking (binding energy in Kcal/Mol).

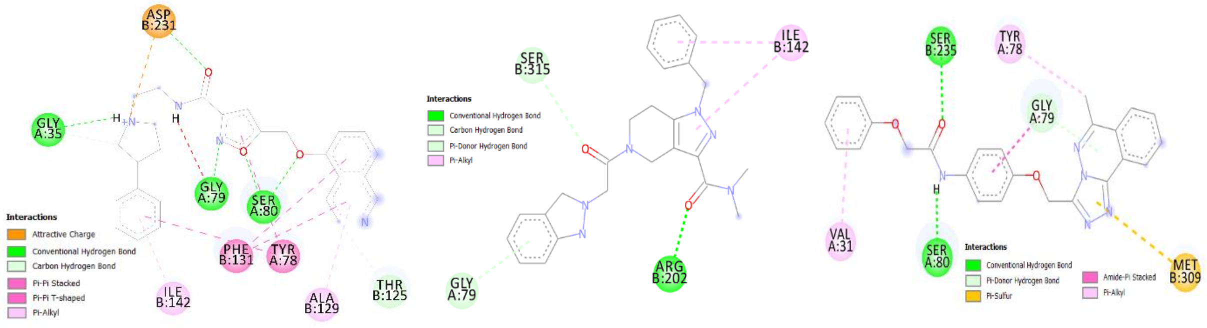
Molecule id	**Auto Dock Vina**	**Auto Dock Tools 4.2**	**idock**	**SwissDock**
ZINC1070635	-8.6	-8.2	-8.75	-8.4
ZINC11972241	-8.7	-8.94	-8.41	-8
ZINC12198861	-9.6	-9.39	-9.14	-9.58
ZINC14533276	-8.9	-8.77	-8.72	-8.54
ZINC14885414	-8.6	-9.29	-8.69	-8.22
ZINC2438311	-9.5	-8.67	-9.5	-8.47

The interaction details of CatD all the molecules have been reported in ribbon representation and 2D Depiction in Figures no. 1 and 2.



(a) (b) (c)

Figure 1. Ribbon representation of CatD with ligands: **(a)** CatD-ZINC12198861 Complex; **(b)** CatD-ZINC14533276 Complex; **(c)** CatD- ZINC2438311Complex.



(a) (b) (c)

Figure 2. 2D representation of CatD with ligands: **(a)** CatD-ZINC12198861 Complex; **(b)** CatD-ZINC14533276 Complex; **(c)** CatD-ZINC2438311 Complex.

To comprehend the molecular level interaction, the top six molecules (ZINC11972241, ZINC14533276, ZINC14885414, ZINC02438311, ZINC12198861, ZINC01070635) that have passed all Drug Likeness, ADME, and Toxicity studies have been subjected to Consensus Molecular Docking. Detailed results of Consensus Molecular Docking have been shown in Table 4.

All compounds have been discovered to efficiently bind to the same amino acids located in the active site of CatD (Chain A: VAL31, TYR78, and Chain B: THR125, PHE131, ASP231, MET309) with a strong binding affinity and have generated sufficient hydrogen bonds to form a stable complex. All the poses generated by multiple docking software have been manually evaluated to get the optimized conformer. The best conformer with the lowest binding energy stably interacting with hydrogen bonds has been optimized after 1000 iterations in Auto dock Tools 4.2. Residue-wise hydrogen bond interaction between the target and ligands has been given in Table 5.

Table 5. Details of Hydrogen bond interaction between the target and ligands

Molecule id	Interaction Name	Distance	Hydrogen bond Type
ZINC12198861	CatD:GLY79:HN - ZINC12198861:N2	2.53401 Å	Conventional Hydrogen Bond
	CatD:SER80:HG - ZINC12198861:O2	2.25458 Å	Conventional Hydrogen Bond
	CatD:SER80:HG - ZINC12198861:O3	2.23423 Å	Conventional Hydrogen Bond
	CatD:ASP231:HD2 - ZINC12198861:O1	2.21593 Å	Conventional Hydrogen Bond
	ZINC12198861:H27 - CatD:GLY35:O	2.91173 Å	Conventional Hydrogen Bond
	ZINC12198861:C25 - CatD:THR125:O	2.97203 Å	Carbon Hydrogen Bond
	ZINC12198861:C10 - CatD:GLY35:O	2.51834 Å	Carbon Hydrogen Bond
ZINC14533276	CatD:ARG202:HH11 - ZINC14533276:O1	2.3372 Å	Conventional Hydrogen Bond
	CatD:ARG202:HH21 - ZINC14533276:O1	2.1635 Å	Conventional Hydrogen Bond
	CatD:SER315:HB2 - ZINC14533276:O2	3.08942 Å	Carbon Hydrogen Bond
	CatD:GLY79:HN - ZINC14533276:	3.23506 Å	Pi-Donor Hydrogen Bond
ZINC2438311	CatD:SER235:HN - ZINC2438311:O2	2.13062	Conventional Hydrogen Bond
	ZINC2438311:H14 - CatD:SER80:OG	2.4086	Conventional Hydrogen Bond

3.4. Discussion.

As CatD promotes angiogenesis, invasion, and metastasis of cancer, numerous attempts have been made to develop potent CatD inhibitors in recent times. Goyal *et al.* havetried to develop a small molecule-based Cathepsin D inhibitor [42]. Whereas Ibrahim *et al.* tried to evaluate the biological activity of thiophenes [plant metabolite] as a CatD inhibitor [43]. Hu *et al.* have successfully delivered pepstatin A as cathepsin D inhibitor to cartilage using the scaffold in combination with the type II collagen-targeting peptide WYRGRL [44,45]. Jantas *et al.* have inhibited CatD by using Kempferol derivatives from *Maesa membranacea* [46]. Each study has merits of its own. The main obstacle in drug discovery is ADME and toxicity.

Many of the lead molecules get failed during pre-clinical and clinical trials. Orally administered drugs are widely accepted. Recently conducted studies on CatD have overlooked the drug's ADME, Toxicity, and oral administration. To overcome these issues, we have employed a comprehensive *in silico* study. After getting the hits, we filtered our orally administrable hit molecules that obeyed all the established drug-likeness rules. We also have conducted ADME and toxicity studies by employing multiple web servers to get reliable results. Consensus docking has been conducted by utilizing multiple docking software to improve the quality of docking and virtual screening findings compared to single docking methods. To get the best conformer, all the top conformers predicted by multiple docking software have been evaluated. Ultimately, we have found that all three molecules efficiently bind to the active site of CatD. The identified molecules have the propensity to construct stable hydrogen bonds well with the amino acids present in the active site. With drug-like properties, these molecules have made oral formulation development easier, as the oral route is the standard route for drug delivery due to its patient-convenient platform. With effective ADME properties, the molecules will be able to get absorbed easily through the gastrointestinal tract and be able to distribute to the site of action. Due to their nontoxic properties, these molecules could be a safe, suitable, and effective alternative to the currently available therapies.

4. Conclusions

The compounds ZINC12198861, ZINC14533276, and ZINC2438311 have significant binding affinities with CatD and drug-like characteristics, ADME, and non-toxicity. As a result, we believe that our work will advance the discovery of new CatD inhibitors with the potential to be therapeutic. The three identified molecules could serve as lead molecules for developing novel CatD inhibitors with therapeutic promise.

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Conflicts of Interest

The authors declare no conflict of interest.

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