Novel dihydropyrimidine derivatives as potential HDAC inhibitors: in silico study

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Abstract Dihydropyrimidine derivatives possess many biological activities due to presence of pyrimidine ring structure in various nucleic acids, vitamins, coenzymes, uric acid and their derivatives. They have possessed broad spectrum actions like antibacterial, antifungal, antiviral, anticancer and antihypertensive etc. Before synthesis of compounds, it is good to predict biological activity using in silico methods. Here, we have selected some of N (**3a–f**) and O (**4a–f**) mannich bases of dihydro pyrimidine derivatives emphasized on histone deacetylase 4 (HDAC-4) inhibitions activity. We have used the different software tools like Lipinski's rule of five; pass online; osiris property explorer and docking studies to predict anti cancer activity. All the selected compounds exhibited potential drug like molecule with anti cancer activity. Among all compound the substitution with methoxy group (**3c**) exhibited more drugs like property and substation with hydrogens (**4a**) showed high anti neoplastic activity; whereas substitution with dichloro groups (**4e**) showed more drug docking scores. These were compared with standard drugs tamoxifen and 5-flourouracil. The approach of predicting anticancer activity using in silico method may be more useful to select and synthesis novel compounds in research as well as in industry.

Keywords Dihydropyrimidines · In Silico · Lipinski's rule · Pass online · Osiris property explorer · HDAC-4 enzyme

Introduction

In recent years a paradigm shift was observed from normal traditional drug design strategies to the Computer Aided Drug Design (CADD) as potential tool to predict biological activity. To facilitate the discovery of novel therapeutic agents, rational drug design methods in combination with structural biology offer great potential (Dutta et al. 2010). Computational drug discovery is an effective strategy for accelerating and economizing drug discovery and development process. Because of the dramatic increase in the availability of biological information at macro and micro level, the applicability of computational drug discovery has been extended and broadly applied to nearly every stage in the drug discovery and development workflow, including target identification and validation, lead discovery and optimization and preclinical tests. Over the past decades, computational drug discovery methods such as molecular docking, pharmacophore modeling and mapping, de novo design, molecular similarity calculation and sequence-based virtual screening have been greatly improved (Ou-Yang et al. 2012). This move resulted as advances in the fields of pharmacy, biochemistry, molecular biology and cell biology, facilitated by developments in genomics and proteomics are producing a large number of novel biological targets that might be exploited for therapeutic interventions.

Here, we have selected some of novel N (**3a–f**) and O (**4a–f**) mannich bases of dihydropyrimidine derivatives (Fig. 1) and checked their potential as anti cancer activity using human histone deacetylase (HDAC-4) enzyme inhibition as target site. Literature also revealed that these pyrimidine pharmacophore derivatives are playing a vital role in many biological processes due to ring system being present in nucleic acids, several vitamins, coenzymes, uric acid and other purines (Sahu

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N-Mannich Bases

Code	R ₁	R ₂	Mol.formula
3a	H	H	C ₁₉ H ₁₈ N ₆ O
3b	H	Cl	C ₁₉ H ₁₇ ClN ₆ O
3c	H	OCH ₃	C ₂₀ H ₂₀ N ₆ O ₂
3d	Cl	H	C ₁₉ H ₁₇ ClN ₆ O
3e	Cl	Cl	C ₁₉ H ₁₆ Cl ₂ N ₆ O
3f	Cl	OCH ₃	C ₂₀ H ₁₉ ClN ₆ O ₂

O-Mannich Bases

Code	R ₁	R ₂	Mol.formula
4a	H	H	C ₁₉ H ₁₈ N ₆ O
4b	H	Cl	C ₁₉ H ₁₇ ClN ₆ O
4c	H	OCH ₃	C ₂₀ H ₂₀ N ₆ O ₂
4d	Cl	H	C ₁₉ H ₁₇ ClN ₆ O
4e	Cl	Cl	C ₁₉ H ₁₆ Cl ₂ N ₆ O
4f	Cl	OCH ₃	C ₂₀ H ₁₉ ClN ₆ O ₂

Fig. 1 Selected dihydropyrimidine derivatives of N (3a–f) and O (4a–f) mannich base series compounds were represented for in silico biological activity

and Siddiqui 2016), and abundant occurrence in nature (Mayer et al. 1999). The synthetic and natural dihydropyrimidine derivatives possessed various biological activities like antimicrobial (El-Sayed et al. 2009; Shah et al. 2010), anti-inflammatory (Nofal et al. 2011; Bahekar and Shinde 2003), anti-malarial (Narayanaswamy et al. 2014), antitubercular (Narayanaswamy et al. 2013),

anticonvulsant (Shah et al. 2010), analgesic (Nofal et al. 2011), anticancer (Mayer et al. 1999; Abadi et al. 2009), anti HIV (Giffin et al. 2008), antifungal (Zou et al. 2012), Antihypertensive (Atwal et al. 1991) activities. Here, we have assessed some of N and O mannich bases of dihydropyrimidine derivatives through in silico methods like lipinski rule, pass online, oris and docking studies have

been applied and identified and predicted their potency and toxicity scores.

Some of selected selected dihydropyrimidine derivatives

Pyrimidine is a basic moiety present in DNA or RNA of several basic structure components, its derivatives of dihydropyrimidines possessed various biological activities (Kumarachari et al. 2016). Before synthesis of compounds it would be better approach to apply available In silico methods to predict probable biological activities. Here, we have selected some of N-Mannich (**3a–f**) and O-Mannich (**4a–f**) bases of dihydropyrimidines derivatives have been considered for In Silico studies to know their drug likeness, to predict the biological activity spectrum, to assess toxicity and to identify protein–ligand interactions. These molecule were designed against HDAC inhibition activity through their maldock score has been obtained. These structures were used further to predict the anti cancer activity.

Methods

In silico tools

Lipinski's rule of five

Lipinski rule of five is help to distinguishing between drug like and nondrug like molecules. It predicts the probability of success or failure of drug likeness of a molecule. If a molecule contains molecular mass less than 500 Da, lipophilicity score less than 5, hydrogen bond donors score less than 5, hydrogen bond acceptors score less than 10 and molar refractivity score between 40 and 130, then it is considered as a drug-like molecule (Lipinski 2004).

PASS online

PASS (Prediction of Activity Spectra for Substances) is a software product designed as a tool for evaluating the general biological potential of an organic drug-like molecule. PASS provides simultaneous predictions of many types of biological activity based on the structure of compounds. Thus, PASS can be used to estimate the biological activity profiles for virtual molecules, prior to their chemical synthesis and biological testing (Filimonov et al. 2014). The biological activity spectrum of a chemical compound is the set of different types of biological activity that reflect the results of the compound's interaction with various biological entities. Biological activity is defined qualitatively (yes/none) suggesting that the biological activity spectrum represents the “intrinsic” property of a substance depending only on its structure and physical–chemical characteristics. Though this may be a generalization, it provides the possibility for combining information from many different sources in the same training set, which is necessary because no one particular publication comprehensively covers all the various facets of the biological action of a compound.

Osiris property explorer

Prediction of toxicity, drug likeness and drug score can be done by the computer programmer. OSIRIS provides the basis to avoid the experimental study of potentially harmful substances. In Osiris toxicity risk assessment predicts mutagenicity, tumorigenicity, irritating and reproductive effects. The Osiris property explorer is an integral part of Acetlione's in-house substance registration system developed by Thomas sander at Acetlione's pharmaceuticals limited, Switzerland (Molecular Properties Prediction 2017). It lets us draw chemical structures and calculates on-the-fly various

Table 1 Lipinski five rules applied on some of dihydropyrimidine derivatives

Compounds	MF	MW < 500	Log P < 5	MR [40–130]	HBD < 5	HBA < 10	PSA < 140o	Violations
3a	C19H18N6O	346.3858	2.34	102.58	3	4	85.94	0
3b	C19H17CIN6O	380.831	2.95	107.38	3	4	85.94	0
3c	C20H20N6O2	376.4118	2.18	109.04	3	5	95.17	0
3d	C19H17CIN6O	380.831	2.95	107.38	3	4	85.94	0
3e	C19H16Cl2N6O	415.276	3.55	112.19	3	4	85.94	0
3f	C20H19CIN6O2	410.857	2.79	113.85	3	5	95.17	0
4a	C19H18N6O	346.3858	3.16	102.95	3	6	87.22	0
4b	C19H17CIN6O	380.831	3.77	107.76	3	6	87.22	0
4c	C20H20N6O2	376.4118	3.00	109.42	3	7	96.45	0
4d	C19H17CIN6O	380.831	3.77	107.76	3	6	87.22	0
4e	C19H16Cl2N6O	415.276	4.37	112.56	3	6	87.22	0
4f	C20H19CIN6O2	410.857	3.61	114.22	3	7	96.45	0
Tamoxifen	C25H27NO	357.4880	5.91	123.83	0	2	12.47	1

Fig. 2 In Silico anti mitotic activity and drug toxicity in selected dihydropyrimidine derivatives

Table 2 Anti cancer activity of selected dihydropyrimidine derivatives with Osiris and pass online scores

Compounds	Predictive activity	Pass online score
3a	Antimitotic activity	0.534
3b	Antimitotic activity	0.456
3c	Antimitotic activity	0.515
3d	Antimitotic activity	0.456
3e	Antimitotic activity	0.463
3f	Antimitotic activity	0.456
4a	Antineoplastic activity	0.669
4b	Antineoplastic activity	0.545
4c	Antineoplastic activity	0.651
4d	Antineoplastic activity	0.545
4e	Antineoplastic activity	0.561
4f	Anti neoplastic activity	0.557
Tamoxifen	Antineoplastic activity	0.821

drug-relevant properties whenever a structure is valid. Prediction results will be valued and colour coded. Properties with high risks of undesired effects like mutagenicity or a poor intestinal absorption will be shown in red colour. Whereas a green colour indicates drug-confirm behavior.

Docking studies

Docking studies are useful for the prediction of protein–ligand interactions. The ability of a ligand to bind to a specific protein is related to molecular structure, orientation and conformation. During binding process there are enthalpy and entropy changes in the protein–ligand system, associated with alteration of both intra- and inter-molecular

structures of protein and ligand. These conformational changes allow the ligand to bind to the receptor active site in a more stable manner. In general, protein–ligand interactions of pharmaceutical interest principally involve non-covalent interactions, including hydrogen bonds, ionic interactions, hydrophobic interactions, π – π interactions and cation– π interactions.

Results

Lipinski's rule of five

The Lipinski's rule of five is used for predicting whether selected and designed compounds have the property of drug like molecule or not. This is based on the selected compound satisfy the five rules of drug remain small (Lipinski et al. 2001), hydrophobic ring system hydrophobic packets on protein surface activity (Bondensgaard et al. 2004; Hajduk et al. 2000), structure homology for biological for endogenous substances (Sangameswaran et al. 1986) and biological activities (DeSimone et al. 2004). However, as per Lipinski's rule, we have analyzed all selected dihydropyrimidine derivatives (3a–e) and (4a–e) series molecular weight of the compounds were less than < 500 kd and molecular surface activity within the range (40–130), hydrogen bond donor (HBD) capacity < 5, Hydrogen bond acceptor < 10 and polar surface area should be < 140° were desirable to act as drug like molecule. Among all compounds were within the limit, but the standard drug tamoxifen is violated the Lipinski rule with higher log P value that more than > 5. All selected compounds passed the Lipinski's filters (Table 1), and hence all are considered to be drug-like molecules.

Fig. 3 Dihydropyrimidine derivatives interact with HDAC-4 enzyme inhibition activity with docking studies

PASS online

The PASS online software generates the pass online scores were estimated for selected compounds on basis of online program PASS. Its application of wide varieties pharmacological properties could be confirmed by different experimental studies. Comparative biological activity based on structural formula of the chemical compounds is necessary to obtain PASS prediction at the earliest stage of the Investigation. The structure of the chemical compounds was

drowning through ACD lab Chem Skech software, which predicts the possible mechanism of actions as good as biological activities (Filimonov et al. 2014). We have predicted biological activity of N-mannich (**3a–f**) and O-mannich bases (**4a–F**). Based on calculated pass online scores of N and O-Mannich bases predicted the anticancer activity. Here, the compounds N mannich (**3a–f**) possess more antimitotic activity, among all compounds compound 3a showed more antimetabolic activity with high score with 0.534, whereas Omannich (**4a–F**) compounds were showed antineoplastic

Fig. 3 (continued)

activity, among all compound **4a** showed more activity with 0.669, however, these were less score than selected standard drug tamoxifen estimated as 0.821.

Osiris property explorer

Osiris property explorer is use full to calculate the drug relevant property on valid structure (Molecular Properties Prediction 2017). This software predicts mutagenicity and poor intestinal absorption with red colour, where as green

colour indicates drug confirms behaviour. It also predicts the ADME toxicity studies (Taj et al. 2011). This OSIRIS property explorer is known estimate harmful substances, mutagenesis and toxicity for the compounds to be tested. Here all the selected compounds were found to be drug like and non toxic candidates by toxicity assessment studies. The cLog P values should be < 5 for better activity. Here, we have analyzed some of dihydropyrimidine (**3a–e**) and (**4a–f**)

Fig. 3 (continued)

have shown better activity than standard drug tamoxifen and 5-flouro uracil (Fig. 2).

Docking studies

Docking studies were designed to study the hybrid compound formulated to predict the estimated of biological activity based on docking engine of targeted enzymes (Thomsen and Christensen 2006). These were predicted based on pre stored data of more than 200 structurally

diverse complexes with known PDB binding activity (Wang et al. 2004). Here we have selected human histone deacetylase 4 (HDAC4) (PDB ID: 4CBT) was employed to identify the HDAC inhibitory potential of the molecules. Molegro virtual docker was used for docking. All the compounds showed more docking score than that of co-crystal ligand and **4e** shows the highest score. **3e**, **3f**, **4a**, **4b**, **4e**, **4e** showed significant H-bond interactions whereas tamoxifen showed no hydrogen bond interactions. **4e**, **4a** showed highest interactions.

Fig. 4 Hypothetical representation of advantages of in silico approaches over conventional drug development process

Discussion

In silico pharmacological activity prediction has been increased in pharmaceutical industry and employed number increased to deal with modern molecular modeling (Hughes et al. 2011). It is a process to estimate the biological actions by using available computational based software's methods provided intricate aspects of molecular recognition (Weigelt 2010). Before design and synthesis of new compounds, it is better to predict the pharmacological properties of final products. Here, we have selected N-mannich (3a–f) and O Mannich (4a–f) dihydropyrimidine derivatives (Fig. 1) by considering their simple synthetic possibilities and expected potential HDAC-4 enzyme anti cancer activity of compounds (Fig. 1). We have selected tamoxifen drug as standard compound for comparison. However, Lipinsky rule 5 is a well known in silico method (Yusof and Segall 2013; Congreve et al. 2003; Nakashima et al. 2013) to estimate drug likeness property, which was optimized considering

different parameters of absorption, distribution, metabolism, excretion and toxicity parameters as integral part of drug discovery studies (Di et al. 2009). We have observed all our compounds were satisfied the properties of drug like molecules of Lipinski rule, among all compound 3c has been exhibited with least log P value and other parameters were indicated in Table 1.

Another important method has been used is orisis toxicity prediction software (Molecular Properties Prediction 2017). Where, it is important to measure the safety parameters and side effects of compounds were based on structural orientation. Here, we have assessed the mutagenic and toxicity of all selected dihydropyrimidine derivatives was represented in Fig. 2. All compounds were exhibited less toxicity and mutagenic properties based on their less cLog P values were presented in Fig. 2. Among all the compound 3c has been exhibited 2.49 value which was comparable with tamoxifen indicated is 4.72. The pharmacological activity prediction is very important to select the compounds. Here, we have used pass online software to estimate anti cancer activity and ACD lab chemskeh software. We have used this software to predict anti cancer activity. Among all compounds N manich series (3a–f) has been predicted with antimitotic activity, where as O mannich series (4a–f) has been exhibited anti neoplastic activity. The compound 4a has been showed more score of 0.669, but it was less than tamoxifen exhibited pass online score 0.821 (Table 2).

Another important property of docking studies was based on target site of enzyme binding site involvement in the hybrid of selected compounds. Here, we have selected the targeted site id HDAC-4 enzyme inhibition is for docking to predict anti cancer activity. All compounds were exhibited high score of docking score than co crystal ligand indicates that these may be exhibit better biological activity of the compounds (Fig. 3). However, the approaches for drug discovery have the option to screen through conventional and in silico methods. Here, we have hypothetically represented merits of in silico drug screening general approaches over conventional methods (Fig. 4). This approach may save lots of time and financial burden for designing the new molecule and predict the pharmacological actions of drug. This may help to identify the potential compounds from the bulk groups.

Conclusion

In summary, we conclude that it is a best method to screen the compounds with some of available in silico methods to predict desired the biological activities. Here, we have selected some of dihydropyrimidine derivatives and processed for in silico biological activity. All the compounds exhibited good anti cancer activity. Whereas, compound

substitution with methoxy group (**3c**) exhibited more drugs like property and substitution with hydrogens (**4a**) showed high anti neoplastic activity; whereas substitution with dichloro groups (**4e**) showed more drug docking scores. The approach may predict the potential compounds and save time and money.

References

- Abadi AH, Ibrahim TM, Abouzid KM, Lehmann J, Tinsley HN, Gary BD et al (2009) Design, synthesis and biological evaluation of novel pyridine derivatives as anticancer agents and phosphodiesterase 3 inhibitors. *Bioorg Med Chem* 17:5974–5982
- Atwal KS, Swanson BN, Unger SE, Floyd DM, Moreland S, Hedberg A et al (1991) Dihydropyrimidine calcium channel blockers. 3, 3-carbamoyl-4-aryl-1, 2, 3, 4-tetrahydro-6-methyl-5-pyrimidine-carboxylic acid esters as orally effective antihypertensive agents. *J Med Chem* 34:806–811
- Bahekar SS, Shinde DB (2003) Synthesis and anti-inflammatory activity of some [2-amino-6-(4-substituted aryl)-4-(4-substituted phenyl)-1, 6-dihydropyrimidine-5-yl]-acetic acid derivatives. *Acta Pharm* 53:223–229
- Bondensgaard K, Ankersen M, Thogersen H, Hansen BS, Wulff BS, Bywater RP (2004) Recognition of privileged structures by G-protein coupled receptors. *J Med Chem* 47:888–899
- Congreve M, Carr R, Murray C, Jhoti H (2003) A 'rule of three' for fragment-based lead discovery? *Drug Discov Today* 8:876–877
- DeSimone RW, Currie KS, Mitchell SA, Darrow JW, Pippin DA (2004) Privileged structures: applications in drug discovery. *Comb Chem High Throughput Screen* 7:473–494
- Di L, Kerns EH, Carter GT (2009) Drug-like property concepts in pharmaceutical design. *Curr Pharm Des* 15:2184–2194
- Dutta S, Sutradhar S, Sachan K (2010) Computer-aided drug design—a new approach in drug design and discovery. *Computer* 4:025
- El-Sayed WA, Nassar IF, Adel A-H (2009) C-Furyl glycosides, II: synthesis and antimicrobial evaluation of C-furyl glycosides bearing pyrazolines, isoxazolines, and 5, 6-dihydropyrimidine-2 (1H)-thiones. *Monatshefte für Chem Chem Mon* 140:365–370
- Filimonov DA, Lagunin AA, Gloriovova TA, Rudik AV, Druzhilovskii DS, Pogodin PV et al (2014) Prediction of the biological activity spectra of organic compounds using the pass online web resource. *Chem Heterocycl Compd* 50:444–457
- Giffin MJ, Heaslet H, Brik A, Lin YC, Cauvi G, Wong CH et al (2008) A copper(I)-catalyzed 1,2,3-triazole azide-alkyne click compound is a potent inhibitor of a multidrug-resistant HIV-1 protease variant. *J Med Chem* 51:6263–6270
- Hajduk PJ, Bures M, Praestgaard J, Fesik SW (2000) Privileged molecules for protein binding identified from NMR-based screening. *J Med Chem* 43:3443–3447
- Hughes JP, Rees S, Kalindjian SB, Philpott KL (2011) Principles of early drug discovery. *Br J Pharmacol* 162:1239–1249
- Kumarachari RK, Peta S, Surur AS, Mekonnen YT (2016) Synthesis, characterization and in silico biological activity of some 2-(N, N-dimethyl guanidiny)-4,6-diaryl pyrimidines. *J Pharm Bioallied Sci* 8:181–187
- Lipinski CA (2004) Lead- and drug-like compounds: the rule-of-five revolution. *Drug Discov Today Technol* 1:337–341
- Lipinski CA, Lombardo F, Dominy BW, Feeney PJ (2001) Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. *Adv Drug Deliv Rev* 46:3–26
- Mayer TU, Kapoor TM, Haggarty SJ, King RW, Schreiber SL, Mitchison TJ (1999) Small molecule inhibitor of mitotic spindle bipolarity identified in a phenotype-based screen. *Science* 286:971–974
- Molecular Properties Prediction (2017) OSIRIS property explorer. <http://www.organic-chemistry.org/prog/peo/>. Accessed 14 Apr 2017
- Nakashima S, Yamamoto K, Arai Y, Ikeda Y (2013) Impact of physico-chemical profiling for rational approach on drug discovery. *Chem Pharm Bull* 61:1228–1238
- Narayanaswamy VK, Nayak SK, Pillay M, Prasanna R, Coovadia YM, Odhav B (2013) Synthesis and antitubercular activity of 2-(substituted phenyl/benzyl-amino)-6-(4-chlorophenyl)-5-(methoxycarbonyl)-4-methyl-3, 6-dihydropyrimidin-1-ium chlorides. *Chem Biol Drug Des* 81:219–227
- Narayanaswamy VK, Gleiser RM, Chalannavar RK, Odhav B (2014) Antimosquito properties of 2-substituted phenyl/benzylamino-6-(4-chlorophenyl)-5-methoxycarbonyl-4-methyl-3,6-dihydropyrimidin-ium chlorides against *Anopheles arabiensis*. *Med Chem* 10:211–219
- Nofal ZM, Fahmy HH, Zarea ES, El-Eraky W (2011) Synthesis of new pyrimidine derivatives with evaluation of their anti-inflammatory and analgesic activities. *Acta Pol Pharm* 68:507–517
- Ou-Yang SS, Lu JY, Kong XQ, Liang ZJ, Luo C, Jiang H (2012) Computational drug discovery. *Acta Pharmacol Sin* 33:1131–1140
- Sahu M, Siddiqui N (2016) A review on biological importance of pyrimidines in the new era. *Int J Pharm Pharm Sci* 2016:14
- Sangameswaran L, Fales HM, Friedrich P, De Blas AL (1986) Purification of a benzodiazepine from bovine brain and detection of benzodiazepine-like immunoreactivity in human brain. *Proc Natl Acad Sci USA* 83:9236–9240
- Shah T, Gupte A, Patel M, Chaudhari V, Patel H, Patel V (2010) Synthesis and in vitro study of biological activity of heterocyclic N-Mannich bases of 3,4-dihydro-pyrimidine-2 (1H)-thiones. *Indian J Chem Sect B Org Incl Med* 49:578
- Taj T, Kamble RR, Gireesh TM, Hunnur RK, Margankop SB (2011) One-pot synthesis of pyrazoline derivatised carbazoles as antitubercular, anticancer agents, their DNA cleavage and antioxidant activities. *Eur J Med Chem* 46:4366–4373
- Thomsen R, Christensen MH (2006) MolDock: a new technique for high-accuracy molecular docking. *J Med Chem* 49:3315–3321
- Wang R, Fang X, Lu Y, Wang S (2004) The PDBbind database: collection of binding affinities for protein–ligand complexes with known three-dimensional structures. *J Med Chem* 47:2977–2980
- Weigelt J (2010) Structural genomics-impact on biomedicine and drug discovery. *Exp Cell Res* 316:1332–1338
- Yusof I, Segall MD (2013) Considering the impact drug-like properties have on the chance of success. *Drug Discov Today* 18:659–666
- Zou Y, Zhao Q, Liao J, Hu H, Yu S, Chai X et al (2012) New triazole derivatives as antifungal agents: synthesis via click reaction, in vitro evaluation and molecular docking studies. *Bioorg Med Chem Lett* 22:2959–2962