Biochemistry & Analytical Biochemistry
ISSN: 2161-1009

Ravish and Raghav, Biochem Anal Biochem 2016, 5:1
DOI: 10.4172/2161-1009.1000253

Research Article Open Access

Synthesis, Pharmacological Evaluation and Molecular Docking of Some Pyrimidinyl Hydrazones

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Abstract

The diagnostic enzymes, acid and alkaline phosphatases are used as investigative tool for the identification of various diseased conditions. It has been reported that elevated levels of acid phosphatase and alkaline phosphatase are present in different cancerous conditions. *In-vitro* enzyme related studies emphasize the potential of remedial uses of various compounds. In the present work we report the effect of hydrazones on the activities of these two clinically significant enzymes. The data revealed that most of the compounds inhibited enzymes. 2-(4,6-Dimethylpyrimidin-2-yl)-1-[1-(6-nitrobenzaldehyde)ethylidene]hydrazine has been evaluated as the most effective inhibitor of these phosphatases. Enzyme kinetic studies were conducted in order to establish the type of inhibition and K_i values. The observed data is explained on the basis of molecular modelling and *in-silico* studies.

Keywords: Acid phosphatase; Alkaline phosphatase; p-Nitrophenyl phosphate; *In-vitro* inhibition studies; Pyrimidyl hydrazones

Introduction

Enzyme inhibition studies have been a great tool in the development of newer drug molecules. The studies have been premeditated with a particular molecule and the target enzyme involved in a diseased condition. Such a group of target enzyme involved in an important biological process inside a cell is phosphatases and kinases which control the transfer of phosphoryl groups from one entity to another. Phosphorylation and dephosphorylation is a reversible and essential process that controls hundreds of pathways of cell. It is a key mechanism for regulating the activities such as cell differentiation and proliferation, gene expression, and many metabolic processes including biosynthesis of nucleic acid building blocks and control of the glycogen synthetase/phosphorylase pathway. The class of enzymes involved in the hydrolysis of phosphate-ester bonds (EC 3.1.3.), the phosphatases crucial to life include alkaline phosphatase (EC 3.1.3.1) and acid phosphatase (EC 3.1.3.2). Alkaline phosphatases (APs) membrane-bound widely distributed in nature [1], homodimeric enzymes however with few exceptions contain three metal ions i.e., two Zn and one Mg, necessary for enzymatic activity. The enzymes catalyze the hydrolysis of monoesters of phosphoric acid also involved in trans-phosphorylation reaction to phosphate acceptors. The main features of the catalytic mechanism remain conserved in APs of different origin with higher specific activity and K_m [Michalis-Menten's constant] values for mammalian APs in addition to higher optimal pH and lower heat stability. Inhibitors of APs include wide variety of compounds ranging from L-amino acids, peptides, levamisole, xanthenes and anions [2].

The alkaline phosphatases postulated to be involved in a range of processes like cell adhesion [3], vitamin B transport [4], and cell signalling [5] and drug intoxication are also engaged in metastasis [6]. High levels of serum AP are commonly correlated to the diseases of liver and bone such as Paget's disease, osteomalacia, hepatitis and obstructive jaundice [7], hepatobiliary diseases, cholecystitis, cholestasis, cholangitis, cirrhosis, fatty liver, liver tumor, liver metastasis, osteosarcoma, bone metastasis, prostatic cancer, renal osteodystrophy, fractured bone, multiple myeloma associated with fractures and others such as policythemiavera, myelofibrosis, seminoma [7-8]. Elevated levels of acid phosphatase [9], another important phosphatase catalyzing the nonspecific hydrolysis of phosphate monoesters in acidic environment, widely distributed [10-11] is also present in extra cellular fluids of prostate cancer patients, bone diseases, diseases of blood cells, or lysosomal storage diseases, such as Gaucher's disease [12] etc. Considering their role in diseased conditions and the need for identification of their inhibitors, we have previously reported the effect of some chalcones [13], bischalcones [14] and curcumin [15] having bis functionalised α,β-unsaturated moiety on these enzymes. It was observed that by altering the simple chalcones we could inhibit alkaline phosphatase. In the present work we report the effect of some pyrimidinyl hydrazones on these phosphatases where the aryl carbonyl moiety of chalcone is replaced by dimethylpyrimidine hydrazine scaffold. The potential of variety of hydrazones with diverse biological activities [16-27] has already led to the development of nifuroxazide, an intestinal antiseptic [28], dihydralazine as hypertensive, and gyromitrin, a toxin (Figure 1).

Experimental Section

Materials

The substrate p-nitrophenylphosphate (pNPP) and different substituted aldehydes were purchased from Himedia, Bombay (India). All the solutions used were prepared fresh in glass-distilled conductivity water. The source of enzymes were fresh goat liver obtained from a local slaughter house.

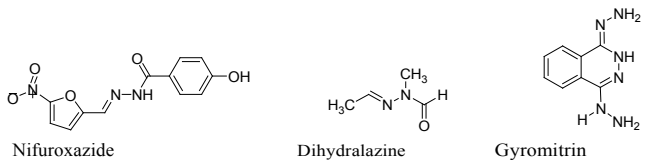

Figure 1: variety of hydrazones with diverse biological activities.

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Received: December 23, 2015; **Accepted:** February 26, 2016; **Published** February 29, 2016

Citation: Ravish I, Raghav N (2016) Synthesis, Pharmacological Evaluation and Molecular Docking of Some Pyrimidinyl Hydrazones. Biochem Anal Biochem 5: 253. doi:10.4172/2161-1009.1000253

Physical measurements

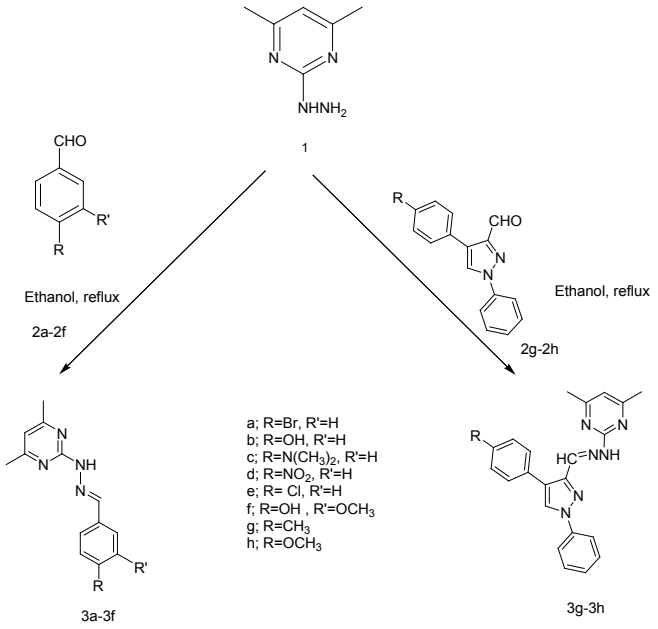
Elisa plate reader was used for measuring absorbance in the visible range. IR spectra were recorded on Horizon 300 MHz spectrometer. ^{1}H spectra were recorded on Bruker 300 MHz instrument. The chemical shifts are expressed in ppm units from an internal TMS standard. Refrigerated ultracentrifuge Remi C-24BL was used for centrifugation purpose under cold conditions.

Melting points were taken in open capillaries and are uncorrected. The progress of the reactions was monitored on silica gel G plates using iodine vapour as visualizing agent.

Synthesis

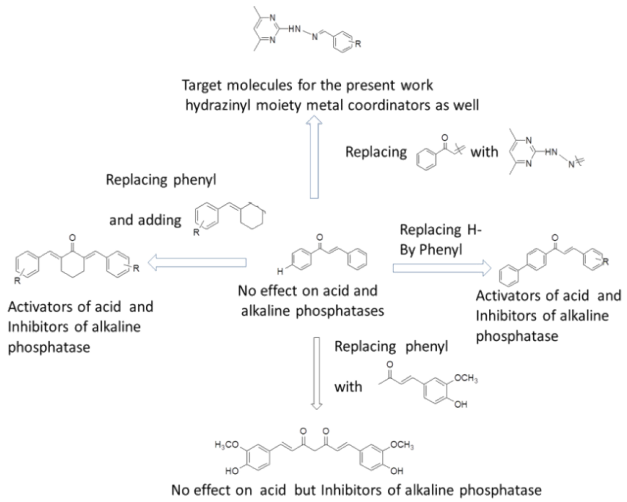
Synthesis of 1-(4,6-dimethylpyrimidin-2-yl)hydrazine (scheme 1)

Dissolved thiourea in hot ethanol and added acetyl acetone, refluxed and added concentrated HCl. Crystals thus formed were dissolved in water and neutralized with $NaHCO_3$. Ethylene glycol and hydrazine hydrate were added in prepared solid. The reaction mixture was refluxed. The product obtained after cooling was washed with water and recrystallized with ethyl alcohol. The progress of reaction and the purity of the products were confirmed through TLC. The structure was confirmed by their IR and ^{1}HNMR spectra.



Synthesis of 1,3-diphenyl-1H-pyrazole-4-carbaldehyde (scheme 2)

At 0°C $POCl_3$ (0.98 ml) was added drop wise with stirring to ice cold DMF solution over a period of 30 minute. Then, *p*-substitutedacetophenone phenylhydrazone made from p-substituted acetophenone (0.0036 mol) and phenyl hydrazine (1.0 g, 0.0036 mol) in N, N -dimethylformamide (3 ml) was added drop wise. Stirring was continued under ice cold condition for another half an hour. The reaction mixture was brought to room temperature and refluxed at 60-70°C for 4-5 hrs. The reaction mixture was then cooled and poured into crushed ice with stirring and neutralized with aq. $NaHCO_3$ solution. The solid obtained was filtered and recrystallized from ethanol. The structure elucidation of compound, 3-(4-substitutedphenyl)-1-phenyl-1*H*-pyrazole-4-carbaldehyde was based on the spectral data (IR, 1H-NMR).



Synthesis of 2-(4,6-dimethylpyrimidin-2-yl)-1-[1-benzaldehydeethylidene]hydrazine Scheme-1(3a-3h)

Hydrazones were synthesized by the reaction of 1-(4,6-dimethylpyrimidin-2-yl)hydrazine, (1) (0.03 mol) and substituted aldehyde, (2a-2h) (0.03 mol) in presence of ethanol as solvent with refluxing at room temperature. The product was washed and recrystallized with ethyl alcohol. The progress of reaction and the purity of the products were confirmed through TLC. The structures were confirmed by their IR and ^{1}HNMR spectra.

Enzymatic Studies

Isolation of enzymes

Acid phosphatase and alkaline phosphatase were isolated and purified from goat liver [29,30] by the steps including preparation of acetone powder, homogenization, acid autolysis, 30-70% a mMonium sulphate fractionation, molecular sieve chromatography on Sephadex G-100. The enzymes, thus obtained were found to have specific activity equal to 40.14 units/mg and 37.5 units/mg respectively for acid and alkaline phosphatase.

Enzyme assays

Acid and alkaline phosphatase were measured using p-nitrophenylphosphate as substrate at pH 5.3 [31] and 10.5 [32] respectively 50 µL of acid phosphatase and 100 µL of alkaline phosphatase was used for each enzyme assay. The enzyme was first equilibrated in 0.1M buffer at respective pHs at 37°C. Thereafter, 20 µL of 50 mM solution of respective hydrazone, prepared in DMSO was added in the reaction mixture, separately to effect the final concentration as 1.0×10^{-3} M. The compound was allowed to interact with respective enzyme for 30 min. Thereafter 50 µL of 50 mM substrate *p*-nitrophenyl phosphate (*p*NPP) was added to estimate the residual enzyme activities. The reaction was stopped after 10 min by adding 400 µL of 4% TCA. The released *p*-nitrophenol was estimated at 400 nm as *p*-nitrophenolate ion under alkaline conditions. Percent residual enzyme activities were estimated with respect to control in absence of any compound but in presence of 20 µL of DMSO. The experiments performed in triplicate for each concentration were averaged before further calculations. The results are presented in Table 1.

The results are the mean and S.M.D. (Standard Mean Deviation) of the experiment conducted in triplicate. % Residual activity is calculated w.r.t. control. The K_i values were calculated using Line-weaver Burk plots.

The experiments were further conducted at varying concentrations of synthesized compounds and the results are presented in Figures 2a and 2b for acid and alkaline phosphatases.

To evaluate the type of inhibition and inhibitory constant (K_i) values of individual compounds enzyme activities were estimated in presence and absence of fixed concentration of individual compound and at varying substrate concentrations as mentioned in Figures 3a and 3b. Line-weaver Burk plots were plotted between 1/ *V* (nmoles / min) and 1/S (mM).

Drug modelling studies

All docking studies were performed using iGemdock. For carrying out these studies, structures of ligands and enzyme active site structure are required. The structures of acid phosphatase and alkaline phosphatase were retrieved from Protein Data Bank as 1PHR acid-A-0.pdb file [33] and cav1B8J alkvan_SVA.pdb file [34-36] respectively assuming that the catalytic sites are conserved in enzymes of different origin [1]. The structures of compounds were prepared in Marvin sketch and were saved as MDL Mol File. The prepared ligands and the binding site were loaded in iGemdock software and docking was started at drug screening Settings. The docking was run under drug screening setting. Tables 2 and 3 represent the data of docking studies of hydrazones on alkaline phosphatase and acid phosphatase activities.

S. No.	Compound Name	Acid Phosphatase		Alkaline Phosphatase	
		Residual Activity (%)	K_i Value (10^{-4} M)	Residual Activity (%)	K_i Value (10^{-4} M)
1	**Control**	100 ± 0.00	-	100 ± 0.00	-
2	2-(4,6-dimethylpyrimidin-2-yl)-1-[1-(6-bromobenzaldehyde)ethylidene]hydrazine, 3a	45 ± 0.78	4.6	55 ± 0.82	3.3
3	2-(4,6-dimethylpyrimidin-2-yl)-1-[1-(6-hydroxybenzaldehyde)ethylidene]hydrazine, 3b	55 ± 0.45	5.4	35 ± 0.61	1.6
4	2-(4,6-dimethylpyrimidin-2-yl)-1-[1-(6-dimethylaminobenzaldehyde)ethylidene]hydrazine, 3c	58 ± 0.56	6.75	71 ± 0.59	6.8
5	2-(4,6-dimethylpyrimidin-2-yl)-1-[1-(6-nitrobenzaldehyde)ethylidene]hydrazine, 3d	10 ± 0.17	0.55	25 ± 0.32	1.0
6	2-(4,6-dimethylpyrimidin-2-yl)-1-[1-(6-chlorobenzaldehyde)ethylidene]hydrazine, 3e	40 ± 0.60	4.23	50 ± 0.65	2.8
7	2-(4,6-dimethylpyrimidin-2-yl)-1-[1-(6-hydroxy-5-methoxybenzaldehyde)ethylidene] hydrazine, 3f	50 ± 0.50	5.3	40 ± 0.25	1.9
8	2-(4,6-dimethylpyrimidin-2-yl)-1-[1-(4-methyl-1-phenyl)-1-phenyl-H-pyrazole-3-carbaldehyde)ethylidene]hydrazine, 3g	59 ± 0.33	8.34	154 ± 0.45	-
9	2-(4,6-dimethylpyrimidin-2-yl)-1-[1-(4-methoxy-1-phenyl)-1-phenyl-H-pyrazole-3-carbaldehyde)ethylidene]hydrazine, 3h	60 ± 0.65	8.6	135 ± 0.57	-
10	Levamisole	-	-	83 ± 1.05	17.5

Table 1: Percentage residual activities of acid and alkaline phosphatases in presence of 1mM concentration of different hydrazones.

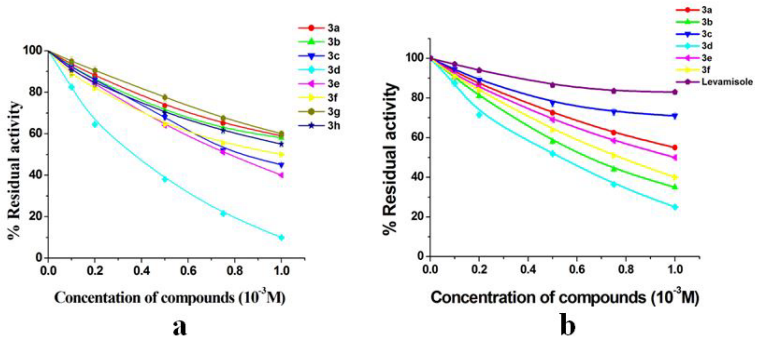


Figure 2: The results are depicted as % residual activities of acid phosphatase (a) and alkaline phosphatase (b) in presence of different concentrations (0.1 X10-3, 0.2 X 10-3, 0.5 X 10-3, 0.75 X 10-3, 1 X 10-3 M) of hydrazones after an incubation time of 30 min. The experiments were conducted in triplicate and were calculated w.r.t. the control having no compound but an equivalent amount solvent was added.

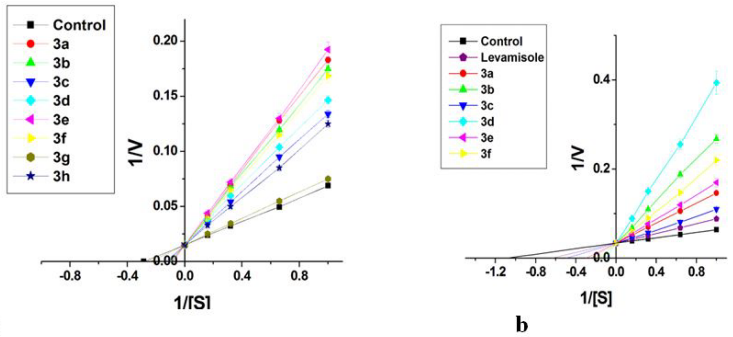


Figure 3: Line-weaver Burk plots for inhibition of hydrazones on acid (a) and alkaline (b) phosphatase at 1 × 10^{-3}M concentrations of 3a-3c, 3e-3h and 1 × 10^{-4} M of 3d, and varying concentration of substrate i.e., *p*NPP concentration (6.25, 3.125, 1.56, 1 × 10^{-3} M).

The results presented are one of the docking studies carried out using iGemdock at standard docking accuracy settings.

Results and Discussion

Involvement of phosphatases in diseased conditions emphasizes the development of their inhibitors. During literature study it was found that in addition to various anions different class of compounds have been reported as phosphatase inhibitors. With the curiosity to see a highly active biologically significant molecule, chalcone as inhibitor of phosphatases we could not obtain encouraging results. Further studies with some easy replacements in chalcone lead to alkaline phosphatase inhibitors (Scheme-2) which affected acid phosphatase differently. Focus on hydrazones can also lead to some interesting results as hydrazine moiety has the potential to chelate Zn^{+2} ions necessary for alkaline phosphatase activity. With this background the present work was undertaken and simultaneously the effect has been observed on acid phosphatase also because the binding sites of the two enzymes share a co mMon amino acid at the active site and are responsible for similar function physiologically but at different pHs. Hydrazones have been largely used as synthones for the synthesis of heterocyclic compounds [37,38] because of the presence of various reactive centres in the moiety. Both the nitrogen atoms of hydrazone are nucleophilic but the amino type nitrogen is more reactive, whereas the carbon atom possesses both characters, that is, nucleophilic and electrophilic. These reactive centres and various other features are also responsible for the variety of biological activities exhibited. In the present work we have synthesized various hydrazones with pyrimidyl scaffold to observe their effect on two diagnostic enzymes acid and alkaline phosphatases.

The synthesis of hydrazones, 3a-3h was afforded by condensing hydrazine 1 with aryl bezaldehydes, 2a-2h (Scheme 1) in ethanol as solvent. 1-(4,6-dimethylpyrimidin-2-yl)hydrazine, compound 1 was first of all synthesized by thiourea and the other compounds, 2g and 2h (Scheme 1) were synthesized by phenyl hydrazine and substituted benzaldehydes. Both the structures were confirmed from literature

#Ligand	Total Energy	VDW	H Bond	Elec
1PHR acid-PNP3-0.pdb	-106.59	-56.32	-50.26	0.093
1PHR acid-3a-0.pdb	-92.31	-64.06	-28.25	0
1PHR acid-3b-2.pdb	-92.20	-63.75	-28.44	0
1PHR acid-3c-2.pdb	-95.96	-82.32	-13.63	0
1PHR acid-3d-0.pdb	-95.09	-70.07	-24.82	-0.19
1PHR acid-3e-0.pdb	-90.74	-63.16	-27.57	0
1PHR acid-3f-1.pdb	-97.66	-76.38	-21.27	0
1PHR acid-3g-0.pdb	-109.59	-99.36	-10.22	0
1PHR acid-3h-0.pdb	-101.57	-78.60	-22.97	0

Table 2: Docking studies of acid phosphatase in presence of hydrazones.

#Ligand	Total Energy	VDW	H Bond	Elec
cav1B8J alkvan_SVA-PNP3-1.pdb	-111.16	-48.54	-52.86	-9.75
cav1B8J alkvan_SVA-3a-0.pdb	-97.02	-67.17	-29.85	0
cav1B8J alkvan_SVA-3b-1.pdb	-108.86	-79.38	-29.48	0
cav1B8J alkvan_SVA-3c-2.pdb	-86.02	-54.55	-31.47	0
cav1B8J alkvan_SVA-3d-2.pdb	-102.99	-65.48	-36.12	-1.38
cav1B8J alkvan_SVA-3e-0.pdb	-83.25	-68.54	-14.71	0
cav1B8J alkvan_SVA-3f-0.pdb	-90.57	-77.86	-12.70	0
cav1B8J alkvan_SVA-3g-1.pdb	-105.76	-88.21	-17.55	0
cav1B8J alkvan_SVA-3h-1.pdb	-120.51	-101.15	-19.35	0
Levamisole	-68.11	-56.90	-11.21	0

Table 3: Docking studies of alkaline phosphatase in presence of hydrazones.

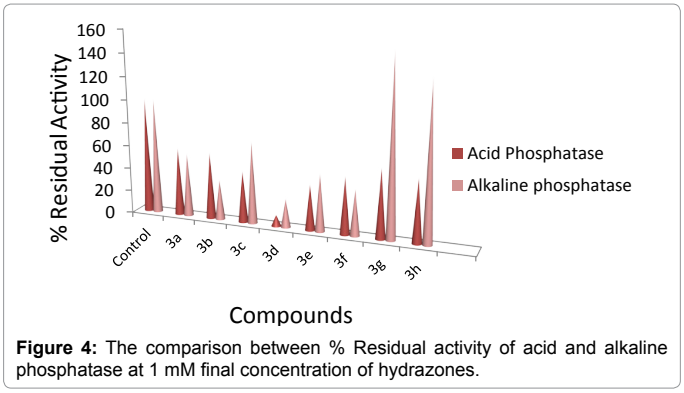


Figure 4: The comparison between % Residual activity of acid and alkaline phosphatase at 1 mM final concentration of hydrazones.

m. pt., IR & NMR spectra. Condensation of 1, with different aryl aldehydes, (2a-2h) (Scheme 1) in presence of ethanol under reflux condition resulted in desired compounds, (3a-3h). (Scheme 1). The product was formed in pure form; high yields (70-80%) and about 4-6 h were required to complete the reaction. The synthesis of compounds was confirmed with the help of their physical data, IR, ^{1}H and spectra. Synthesized compounds show a characteristic IR absorption peak at ν 3250-3150 cm^{-1} indicating the presence of a –NH as well as an olefenic C = N band in the region 1565-1550 cm^{-1}. In ^{1}H NMR the characteristic peaks of hydrazones showing two singlets were observed in the range δ 11.35-10.30 for –NH proton and δ 8.1-7.5 for CH=N proton.

Figure 4 shows the effect of hydrazones on the activity of acid and alkaline phosphatase, it can be observed that most of compounds inhibited both the enzymes at this concentration. The *in-vitro* inhibition studies show that 3d (Scheme 1) is the best inhibitor to both the enzymes. It inhibited alkaline phosphatase to maximum extent i.e., 75 ± 0.32% inhibition and acid phosphatase to maximum up to 90 ± 0.17% at 1 mM concentration. This pattern of inhibition exerted by 3d on both the enzymes can be attributed to the substrate structural features co mMon to both the enzymes. The biological substrates for both the enzymes contain an essential component, the phosphate moiety indicating that the binding site may be having some co mMon structural features. Literature study suggests the involvement of arginine for holding the substrate in the active site in alkaline phosphate, Arg-166 [39] and acid phosphatase, Arg-122 [40]. However compounds 3g and 3h exerted an activation effect 150 ± 0.45% and 135 ± 0.57 on alkaline phosphatase. The reactive centres remaining constant in 3a-3h but the binding features differing at 3g-3h molecules (Scheme 1) with the 1,3-diphenyl-1H-pyrazole-4-carbaldehyde moiety might have resulted in enzyme activation. The differential effect observed on the two enzymes is shown in Figure 4 at 1 mM concentration of each hydrazones. Though it is difficult to explain this differential behaviour, but it may be attributed to the steric crowding in the 3g and 3h molecules (Figure 4) (Scheme 1).

Once the inhibitory effect was established the experiments were also conducted at different inhibitor concentration. Figure 2a and 2b represents the effect of increasing concentration of inhibitory compounds on acid and alkaline phosphatase activities, respectively. Here again it can be observed that compound 3d (Scheme 1) is best inhibitor for acid and alkaline phosphatase. At 0.5 mM 38 ± 0.67% activity of acid phosphatase was retained whereas for alkaline phosphatase 52 ± 0.94% activity was retained at 0.5 mM concentration.

The inhibition studies were further expanded to evaluate K_i values (Table 1). After establishing the inhibitory effect of hydrazones on acid and alkaline phosphatase the K_i values and the type of inhibition were determined at varying substrate concentration in absence and presence

of a fixed concentration of inhibitor and the K_i values were calculated with the help of Line-weaver Burk plot and the result shown in Figure 3.

It was found that the K_i value for 3d, has been obtained as 1.00 ± 0.015 × 10^{-4} M for alkaline phosphatase and 5.5 ± 0.09 × 10^{-5} M for acid phosphatase. From the Figures 3a and 3b we could find out that the inhibitory hydrazones were competitive inhibitors as evidenced by Line-weaver Burk double reciprocal plots. The results were further correlated with molecular docking experiments. For authentication of the present work the results are compared with the standard inhibitor of alkaline phosphatase, levamisole. Levamisole exhibited the K_i value of 1.75 ± 0.02 × 10^{-3} M. The synthesized compound, 3d has been established as better inhibitor than levamisole.

We have previously reported that simple chalcones do not exert any effect on acid and alkaline phosphatase [14,41]. The results when compared with other molecules with similar structures suggest that there is certainly an appreciable effect of substituent on the activity of alkaline phosphatase [14,15,41]. The structure activity relationship of compounds on alkaline phosphatase suggests that bidirectional extension of conjugation increases the affinity of the inhibitor. The affinity is also enhanced when *p*-phenyl moiety is attached to the aryl group. In the present study we observed that replacing the aryl carbonyl with pyrimidyl hydrazine scaffold also resulted in increased inhibitory potential of the Ar-CH = group. The inhibition of alkaline phosphatase by the designed compounds can also be attributed to the chelating capacity hydrazine group toward Zn^{2+} ions [42].

The result obtained during *in-vitro* inhibition studies of alkaline phosphatase were further confirmed by *in-silico* docking experiments. This gave an understanding of the inhibition caused by the target compounds on structural basis, where the designed compounds were docked on one of the crystal structures of acid and alkaline phosphatase active site made available through RCSB Protein Data Bank as (entry 1 PHR acid-0-pdb) and (PDB entry cav1B8J alkvan_SVA.pdb). The decrease in total energy of hydrazones -acid phosphatase complex is in the range of ~ -92.2 to -109.6 and hydrazones -alkaline phosphatase complex is in the range of ~ -83.25 to -120.51.

Figure 5 represents the correlation between total energy v/s log K_i of designed compounds for acid and alkaline phosphatase. It can be observed from Figure 5, that most effective binding of compounds is with alkaline phosphatase as compared to acid phosphatase which is well correlated in this graph.

In alkaline phosphatase all the compounds bind to the active site and amino acids Asp-51, Thr-151, Asp-153, Asp-327 have been found to interact with the compounds through H-bonding (in green) as well as with substrate pNPP. Some amino acids, Asp101, Arg-166, His-331, His-370 and His-412 showing van der Waals interactions (in grey) can

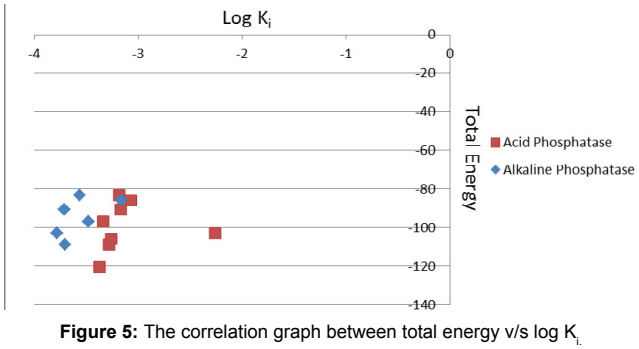


Figure 5: The correlation graph between total energy v/s log K_i.

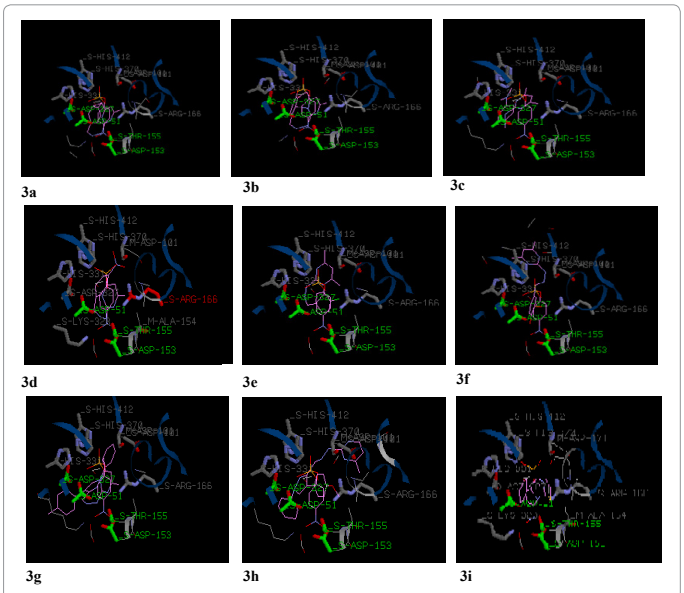


Figure 6: Docking results showing the alignment of Compounds (3a-3h) and levamisole (3i) in the active site of alkaline phosphatase (cav18J alkvan_SVA-G-0.pdb) along with the substrate pNPP respectively.

also be observed. The results are in accordance to *in-vitro* studies where compounds 3a-3h exhibited competitive inhibition as established by *in-vitro* inhibition studies. However, compounds 3g and 3h showed activating effect on alkaline phosphatase Figure 6.

Kim and Wyckoff have reported [35] that Asp-51 and Asp-327 are involved at the active site of alkaline phosphatase and interact with the two different Zn^{+2} ions. With this background we can assume that the inhibitory hydrazones hinder the involvement of Zn^{+2} ions in catalysis, however this theory needs more elaborate study and study in this direction is being pursued. A probable explanation for the differential effect can be due to the steric crowding present in the structures of 3g and 3h (Scheme 1). It can be observed in the following MM2 energy minimized structures of 3d and 3g (Scheme 1) that the active part of the molecules involved in interaction has different orientations due to greater van der Walls interactions in 3g (Scheme 1). (Table 3), as compared to 3d (Scheme 1). Compounds (3a-3f), (Scheme 1) can interact with the active site resulting in enzyme inhibition but in compounds 3g & 3h with different structural motifs may bind to a portion of alkaline phosphatase rendering the enzyme active site more susceptible to the substrate and results in enzyme activation instead of inhibition (Figure 7).

As it is known that the molecular docking is a tool to determine the binding energies and affinities of small molecules in a given active site where the computation of energy takes place. But actually the molecule can interact with the enzyme structure through various physical and chemical forces which can result in a change in native conformation of enzyme.

Molecular docking studies of acid phosphatase have not provided useful insights (detailed results in supplementary file). The differential behaviour in *in-silico* and *in-vitro* studies can be attributed to the fact that in acid phosphatase the docking experiments are accomplished using whole enzyme format and the active site has not been targeted because of its unavailability in RSCB data bank. The results further authenticate that for precise *in-silico* experiments precise docking area of enzymes should also be available be used.

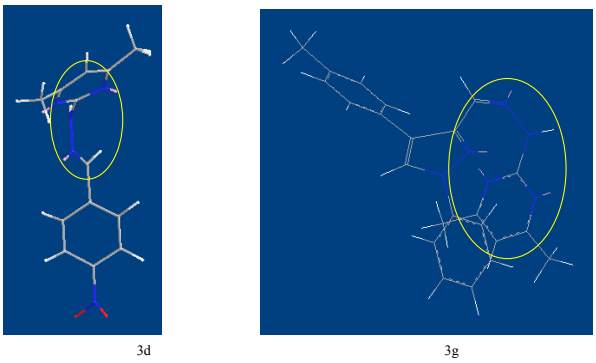


Figure 7: Orientation of hydrazinyl moiety and pyrimidinyl nitrogens in 3d and additional pyrazole nitrogens in 3g.

It has been reported by Kelleher [43] that the inhibitors of alkaline phosphatase can be lucratively used in the treatment of neurological disorders, where the inhibitors reduced degeneration of cells as well as peripheral neuropathy. The compounds have been successfully used in the treatment of Alzheimer's disease. Inhibitors of acid phosphatase can be fruitfully used in the treatment Prostate cancer, Paget's disease, multiple myeloma and Hyperparathyrodism [44]. The present study can add to the existing knowledge of this class of compounds as acid and alkaline phosphatase inhibitors and activators which is not reported earlier. These designed compounds can be of pharmacological significance as differential effect has been observed in case of alkaline phosphatase. Curcumin, bischalcones and 4'-phenylchalcones have also been found to inhibit alkaline phosphatase whereas and activating effect for bischalcones and 4'-phenylchalcones on acid phosphatase activity [14,41] and no effect on acid phosphatase activity has been observed for curcumin [15] so for the first time we are reporting organic moieties as inhibitors to acid phosphatase.

Conclusion

Hydrazones with different structural moieties have been synthesized and characterized by spectral data. The compounds exerted an inhibiting effect on the enzyme acid phosphatase whereas inhibiting and activating effects are observed for hydrazones with different type of structural motifs on alkaline phosphatase. *p*-Nitro substituted pyrimidinyl hydrazone was found to be most inhibitory to these enzymes. However, 1,3-diphenyl-1H-pyrazole carbaldehydehydrazones was evaluated as an activator to alkaline phosphatase. This novel finding of enzyme activation and inhibition having different structural features with in a class of compounds can add to the existing knowledge of enzyme ligands which may be helpful in basic as well as in applied research. The inhibitory compounds have been evaluated as competitive inhibitors.

Acknowledgements

Among the authors, Indu Ravish is thankful to UGC New Delhi, India for award of SRF and also to Kurukshetra University, Kurukshetra for providing necessary research laboratory facilities.

Conflict of Interest

The authors have declared no conflict of interest.

Supporting Information

The characterization of synthesized compounds is provided in the supplementary Figure 1.

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