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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Synthesis, docking, and in vitro studies of some substituted bischalcones on acid and alkaline phosphatases

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Abstract A series of bischalcones was synthesized and screened for their effect on acid and alkaline phosphatases. In addition, molecular modeling and docking of these compounds into alkaline phosphatase using iGemdock was performed in order to predict the affinity and orientation of the designed compounds at the active site and was compared with the established inhibitor levamisole. The iGemdock docking fitness resulted in decrease in total energy (ranging from −75.50 to −100.84) for all the synthesized compounds which were less than levamisole (−50.69) revealing higher binding with the enzyme. The compounds were synthesized by Clasien–Schimdt condensation and their effect was observed on the activity of acid and alkaline phosphatases. The results showed that synthesized bischalcones were inhibitory to alkaline phosphatases, whereas an activating effect was observed on the activity of acid phosphatase. The type of inhibition and K_i values of bischalcones on alkaline phosphatase were also determined.

Keywords Acid phosphatase · Alkaline phosphatase · Bischalcones · *p*-Nitrophenyl phosphatase

Introduction

The pharmaceutical importance of bischalcone derivatives lies in the fact that they can be effectively utilized as ameliorative (Sarojini *et al.*, 2011), nitric oxide production inhibitors, and cytotoxic agents (Reddy *et al.*, 2012), antimicrobial (Asiri and Khan, 2011; Husain *et al.*, 2011, 2012, 2013; Nagaraj and Reddy, 2007), anticancer (Modzelewska *et al.*, 2006), antimalarial (Ram *et al.*, 2000; Srivastava *et al.*, 2008), radioprotective and antiviral agents (Raj *et al.*, 2012a), in-vivo peritoneal antiangiogenesis, and in vitro antiproliferative agents (Raj *et al.*, 2012b). Acid and Alkaline phosphatase are enzymes found in all living tissues, and are concerned with the removal of the phosphate from protein and other molecules. These enzymes are used as diagnostic tools in various diseased conditions. Their altered concentration in various pathological disorders necessitates the evaluation of various classes of natural and synthetic compounds on the activities of these physiologically important enzymes. Acid phosphatases (EC 3.1.3.2) catalyze the nonspecific hydrolysis of phosphate monoesters and oxygen exchange from water into inorganic phosphate in an acidic environment (Hollander and Boyer 1971). These enzymes are widely distributed in mammalian serum (Nakanishi *et al.*, 2000), plants (Yam *et al.*, 1971), and in microorganisms (Nakanishi *et al.*, 2000). The highest levels of acid phosphatase are found in the circulation of prostate cancer patients, bone diseases, diseases of blood cells, or lysosomal storage diseases, such as Gaucher's disease (Nair and Johnson, 2008) etc. Alkaline phosphatases (ALP, EC 3.1.3.1) hydrolyze various monophosphate esters at a high pH optimum (McComb *et al.*, 1979). These enzymes, which are widely distributed in nature, exist as membrane-bound glycoproteins in higher organisms. These enzymes consist of a group of isoenzymes that are encoded by at least four gene loci: tissue nonspecific, intestinal, placental, and germ cell ALP (Weiss *et al.*, 1986, 1988; Millan 1986, 1988). Elevated alkaline phosphatase levels have been used as diagnostic tool in a number of diseases, including those associated with liver, bile duct, and bone. Elevated alkaline phosphatase

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levels are found in cholecystitis, cholestasis, cholangitis, cirrhosis, hepatitis, fatty liver, liver tumor, liver metastasis, drug intoxication, Paget's disease, osteosarcoma, bone metastasis, prostatic cancer, renal osteodystrophy, fractured bone, multiple myeloma associated with fractures, osteomalacia, and others such as policythemiavera, myelofibrosis, seminoma (Gennari *et al.*, 2005; Lange *et al.*,1982; Schiele *et al.*, 1998). We are involved in the synthesis of different class of compounds and their evaluation as phosphatase inhibitors. In the present work, we report the synthesis of various bischalcones and their effect has been investigated on the activity of acid and alkaline phosphatase of mammalian origin.

Experimental

Materials and methods

General remarks

Melting points were determined in open capillary tubes and are uncorrected. All the chemicals and solvents used were of laboratory grade. IR spectra (KBr, cm^{-1}) were recorded on a Horizon 300 MHz spectrometer. ^1H NMR spectra was recorded on Brucker 300 MHz NMR spectrometer (chemical shifts in δ ppm) using TMS as an internal standard. The purity of the compounds was ascertained by thin layer chromatography on aluminium plates per coated with silica gel G (Merck) in various solvent systems using iodine vapors as detecting agent or by irradiation with ultraviolet lights (254 nm). ELISA plate reader was used for measuring absorbance in the visible range. Refrigerated ultracentrifuge Remi C-24BL was used for centrifugation purpose under cold conditions.

Phosphatase activity

Acid phosphatase

Isolation Fresh goat liver purchased from local slaughter house was washed with cold isotonic saline solution and was cut into small pieces and disintegrated in a mixer-cum-blender with chilled acetone. The crude extract was filtered through Buchner funnel and washed 2–3 times with 10 volumes of chilled acetone. A stream of N_2 gas was flushed through it and dried over concentrated H_2SO_4 and stored over anhydrous $CaCl_2$. 10 % homogenate was prepared in 0.1 M acetate buffer pH 5.3 containing 0.2 M NaCl for acid phosphatase activity. The homogenate was centrifuged at 10,000 rpm for 10 min at 4 °C to obtain a clear supernatant solution which was further used as enzyme source.

Assay for acid phosphatase Enzyme homogenate having specific activity 42.2 n moles/min/mg was incubated with 0.1 M acetate buffer pH 5.3. Enzyme activities were measured using *p*-nitrophenyl phosphate as substrate (Plummer 1987).

Determination of acid phosphatase activity in presence of synthesized bischalcones Enzyme homogenate (50 µl) was incubated with 0.1 M acetate buffer pH 5.3 containing 1 mM final concentration of compounds (**1a–1i**). The solution of all compounds was prepared in DMSO. After half an hour, the residual enzyme activities were measured using *p*-nitrophenyl phosphate as substrate. The concentration of liberated *p*-nitrophenol was determined from the absorbance at 410 nm. The results were compared with the controls run along with the experiments. The reaction was terminated by the addition of 100 µl of 0.1 M NaOH. Table 1 represent the percentage residual activity left in solution after the interaction of acid phosphatase with the individual compound for 30 min.

Alkaline phosphatase

Isolation Fresh goat liver purchased from local slaughter house was washed with cold isotonic saline solution and was disintegrated in a mixer-cum-blender and 10 % homogenate was prepared in 0.1 M glycine–NaOH buffer pH 10.5 containing 0.2 M NaCl for alkaline phosphatase activity. The homogenate was centrifuged at 10,000 rpm for 10 min at 4 °C to obtain a clear solution which was further used as enzyme source.

Assay for liver alkaline phosphatase Enzyme homogenate having specific activity 41.7 n moles/min/mg was incubated with 0.1 M glycine–NaOH buffer pH 10.5. Enzyme activities were measured using *p*-nitrophenyl phosphate as substrate (Sahney and Singh, 2001).

Determination of alkaline phosphatase activity in presence of synthesized bischalcones Enzyme homogenate (150 µl) was incubated with 0.1 M glycine–NaOH buffer pH 10.5 containing 1 mM concentration of compounds (**1a–1i**). After half an hour, the residual enzyme activities were measured using *p*-nitrophenyl phosphate as substrate. The concentration of liberated *p*-nitrophenol was determined from the absorbance at 410 nm. The results were compared with the controls run along with the experiments. The reaction was terminated by the addition of 100 µl ml of 0.1 M NaOH. Table 1 represents the percentage residual activity left in solution after the interaction of alkaline phosphatase with the individual compound for 30 min.

Table 1 Percentage residual activities of acid and alkaline phosphatases in presence of bischalcones

S. no.	Compound name	Acid phosphatase		Alkaline phosphatase	
		M.D. ± S.M.D.	Percentage residual activity	M.D. ± S.M.D.	Percentage residual activity
	Control	0.609 ± 0.0095	100	0.600 ± 0.102	100
1.	2,6-Bis(4′-fluoro benzylidene) cyclohexanone (**1a**)	0.855 ± 0.0145	140.39 ± 2.38	0.347 ± 0.0390	57.83 ± 6.50
2.	2,6-Bis(4′-chloro benzylidene) cyclohexanone (**1b**)	0.784 ± 0.0085	128.73 ± 1.39	0.277 ± 0.0062	46.16 ± 1.03
3.	2,6-Bis(4′-bromo benzylidene) cyclohexanone (**1c**)	0.771 ± 0.0104	126.60 ± 1.71	0.261 ± 0.0023	43.50 ± 0.38
4.	2,6-Bis(4′-methyl benzylidene) cyclohexanone (**1d**)	0.905 ± 0.0120	148.60 ± 1.97	0.279 ± 0.0106	46.00 ± 1.77
5.	2,6-Bis(4′-methoxy benzylidene) cyclohexanone (**1e**)	0.963 ± 0.0134	158.13 ± 2.20	0.190 ± 0.0032	31.67 ± 0.53
6.	2,6-Bis(4′-nitro benzylidene) cyclohexanone (**1f**)	0.809 ± 0.0118	132.84 ± 1.94	0.156 ± 0.0012	26.00 ± 0.20
7.	2,6-Bis(3′-phenylallylidene) cyclohexanone (**1g**)	0.788 ± 0.0147	129.39 ± 2.41	0.185 ± 0.0034	30.83 ± 0.57
8.	2,6-Bis(4′- (dimethyl amino) benzylidene)cyclo hexanone (**1h**)	0.688 ± 0.0088	112.97 ± 1.44	0.241 ± 0.0072	40.17 ± 1.20
9.	2,6-Di benzylidene cyclo hexanone (**1i**)	0.734 ± 0.0103	120.52 ± 1.69	0.247 ± 0.0069	41.17 ± 1.15
10.	Levamisole			0.528 ± 0.0075	88.00 ± 1.25

The results are the mean and S.M.D. of the experiment conducted in triplicate and is calculated as phosphatase activity in nmol/min/ml enzyme homogenate. The enzyme activity was determined at 1 mM concentration of each compound and % residual activity is calculated w.r.t. control where no compound was added but an equivalent amount of solvent was present. The specific activities of the acid phosphatase and alkaline phosphatase were 42.2 and 41.7 nmol/min/mg, respectively

General procedure for the synthesis of bischalcones Mixture of cyclohexanone (0.01 mol) and aromatic aldehyde (0.02 mol) in methanol (25 ml) was cooled for 10–15 °C in ice bath. To this cold solution 40 % sodium hydroxide (5 ml) was added drop wise, with continuous stirring for 30 min using magnetic stirrer and was then left overnight. The reaction mixture was poured into crushed ice and acidified carefully using dilute hydrochloric acid. The solid obtained was filtered, washed with ice-cold water, dried and recrystallized from ethanol to give compounds.

*2,6-Bis(4′-fluoro benzylidene) cyclohexanone (**1a**)* Yield 84.76 %; m.p. 152–154 °C; IR (KBr, cm^{-1}): 1,651(>C=O str), 1,489–1,612(−C=C− str), 648(−C–F str); ^{1}H-NMR (300 MHz, $CDCl_3$, δ ppm): 1.78–1.82(s, 2H, $>CH_2$), 2.90–2.93(s, 4H, $>CH_2$), 6.94–7.71(m, 8H, arom. H), 7.99(s, 2H, =CH−); ^{13}C-NMR (300 MHz, $CDCl_3$, δ ppm): 189.85, 136.54, 135.83, 134.75, 131.79, 131.65, 122.94, 77.43, 77.00, 76.58, 28.37, 22.80.

*2,6-Bis(4′-chloro benzylidene) cyclohexanone (**1b**)* Yield 86.57 %; m.p. 148-150 °C; IR (KBr, cm^{-1}): 1,666(>C=O str), 1,427–1,605(−C=C− str), 774(−C–Cl str);^{1}H-NMR (300 MHz, $CDCl_3$, δ ppm): 1.69–1.73(s, 2H, $>CH_2$), 2.85–2.88(s, 4H, $>CH_2$), 7.49–7.59(m, 8H, arom. H), 7.55(s, 2H, =CH−); ^{13}C-NMR (300 MHz, $CDCl_3$, δ ppm): 189.85, 136.43, 135.77, 134.62, 134.32, 131.57, 128.69, 77.44, 77.01, 76.59, 28.38, 22.82.

*2,6-Bis(4′-bromo benzylidene) cyclohexanone (**1c**)* Yield 78.25 %; m.p. 162–164 °C; IR (KBr, cm^{-1}): 1,659(>C=O str), 1,489–1,574(−C=C− str), 825(−C–Br str); ^{1}H-NMR (300 MHz, $CDCl_3$, δ ppm): 1.71–1.73(s, 2H, $>CH_2$), 2.85–2.92(s, 4H, $>CH_2$), 7.47–7.65(m,8H, arom. H), 7.56(s, 2H, =CH−); ^{13}C-NMR (300 MHz, $CDCl_3$, δ ppm): 190.05, 164.34, 161.02, 135.87, 135.74, 132.30, 132.19, 132.09, 115.67, 115.38, 77.43, 77.01, 76.58, 28.35, 22.91.

*2,6-Bis(4′-methyl benzylidene) cyclohexanone (**1d**)* Yield 83.00 %; m.p. 166–168 °C; IR (KBr, cm^{-1}): 1,659(>C=O str), 1,412–1,597(−C=C− str), 2,916 (−C–H− str); ^{1}H-NMR (300 MHz, $CDCl_3$, δ ppm): 1.77–1.85(s, 2H, $>CH_2$), 2.40(s, 3H, $-CH_3$), 2.92–2.96 (s, 4H, $>CH_2$), 7.22–7.41(m, 8H, arom. H), 7.79(s, 2H, =CH−); ^{13}C-NMR (300 MHz, $CDCl_3$, δ ppm): 190.41, 138.80, 136.87, 135.51, 133.23, 130.48, 129.14, 77.45, 77.03, 76.60, 28.54, 23.04, 21.40.

*2,6-Bis(4-methoxy benzylidene) cyclohexanone (**1e**)* Yield 74.54 %; m.p. 156–158 °C; IR (KBr, cm^{-1}): 1,659(>C=O str), 1,489–1,574 (−C= C− str), 2,932(−C–H− str), 1,250(−C−OCH_3 str); ^{1}H-NMR (300 MHz, $CDCl_3$, δ ppm): 1.74–1.76(s, 2H, $>CH_2$), 2.46(3H, s, $-COCH_3$), 2.88–2.92(s, 4H, $>CH_2$), 7.32–7.55(m, 8H, arom. H), 7.65(s, 2H, =CH−); ^{13}C-NMR (300 MHz, $CDCl_3$, δ ppm): 190.41, 138.79, 136.87, 135.51, 133.24, 130.47, 129.13, 77.44, 77.02, 76.59, 28.53, 23.04, 21.39.

*2,6-Bis(4′-nitro benzylidene) cyclohexanone (**1f**)* Yield 88.26 %; m.p. 200–202 °C; IR (KBr, cm^{-1}): 1,674(>C=O str), 1,435–1,589(−C=C− str), 1,342, 1,512 (−C−NO_2 str); ^{1}H-NMR (300 MHz, $CDCl_3$, δ ppm): 1.75–1.77(s, 2H, >CH_2), 2.93–2.99(s, 4H, >CH_2), 7.79–7.89(m, 8H, arom. H), 7.68(s, 2H, =CH−); ^{13}C-NMR (300 MHz, $CDCl_3$, δ ppm): 190.85, 147.40, 142.13, 138.66, 134.88, 130.79, 123.68, 77.42, 77.00, 76.58, 28.35, 22.54.

*2,6-Bis(3′-phenyl allylidene) cyclohexanone (**1g**)* Yield 71.34 %; m.p. 182–184 °C; IR (KBr, cm^{-1}): 1,651(>C=O str), 1,450–1,612(−C=C−str); ^{1}H-NMR (300 MHz, $CDCl_3$, δ ppm): 1.77−1.81(s, 2H, >CH_2), 2.81–2.84(s, 4H, >CH_2), 7.30–7.66(m, 10H, arom. H), 7.09(dd, 2H), 7.21(dd, 2H), 7.30(s, 2H, =CH−); ^{13}C-NMR (300 MHz, $CDCl_3$, δ ppm): 189.85, 140.76, 136.80, 136.29, 135.40, 128.68, 123.69, 121.17, 77.42, 77.00, 76.58, 26.65, 22.09.

*2,6-Bis(4′-(dimethyl amino)benzylidene)cyclohexanone (**1h**)* Yield 72.45 %; m.p. 240–242 °C; IR (KBr, cm^{-1}): 1,659(>C=O str), 1,427–1,574(−C=C− str), 2,916(−C–H− str); ^{1}H-NMR (300 MHz, $CDCl_3$, δ ppm): 1.70–1.76(s, 2H, >CH_2), 2.88–2.92(s, 4H, >CH_2), 7.31–7.55(m, 8H, arom. H), 7.63(s, 2H, =CH−); ^{13}C-NMR (300 MHz, $CDCl_3$, δ ppm): 190.02, 150.36, 136.98, 132.39, 124.21, 111.64, 77.51, 77.29, 77.08, 76.66, 40.63, 40.35, 40.12, 39.79, 28.70, 23.17.

*2,6-Bis benzylidene cyclohexanone (**1i**)* Yield 83.58 %; m.p. 116–118 °C; IR (KBr, cm^{-1}): 1,659(>C=O str), 1,489–1,605(−C=C− str); ^{1}H-NMR (300 MHz, $CDCl_3$, δ ppm): 1.70–1.76(s, 2H, >CH_2), 2.88–2.92(s, 4H, >CH_2), 7.31–7.55(m, 10H, arom. H), 7.63(s, 2H, =CH−); ^{13}C-NMR (300 MHz, $CDCl_3$, δ ppm): 190.38, 136.94, 136.21, 136.01, 130.37, 128.58, 128.39, 77.45, 77.05, 76.61, 28.47, 23.04.

Determination of K_i values and type of inhibition of alkaline phosphatase activity in presence of bischalcones The alkaline phosphatase activity was estimated in presence and absence of inhibiting bischalcones at varing concentration of substrate. The assays were performed as described earlier and the K_i values were calculated by plotting Lineweaver–Burk plot.

Drug modeling studies

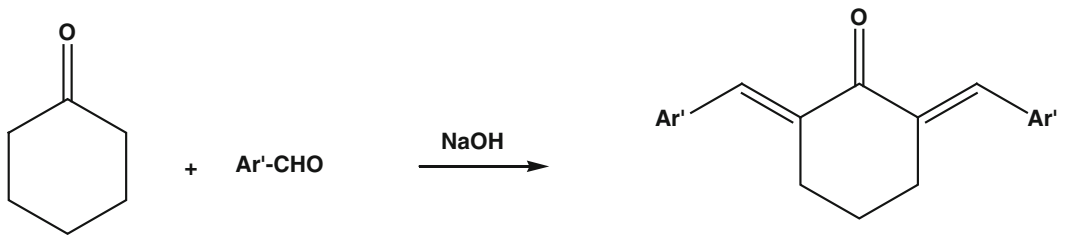
All docking studies were performed using iGemdock. For these studies small molecular weight ligands were prepared and enzyme structure active site was retrieved from the Protein Data Bank. The structures were prepared in Chem Draw 3D and were saved as MDL Mol File. The structure of alkaline phosphatase was retrieved from Protein Data Bank as cav1B8 J alkvan_SVA.pdb file (Holtz *et al.*, 1999; Murphy *et al.*, 1997; Kim and Wyckoff, 1991). After loading the prepared ligands and the binding site Docking was started at Standard Docking Accuracy Settings.

Results and discussion

The synthesis of bischalcones was achieved through base catalyzed Claisen–Schmidt reaction (Scheme 1) between cyclohexanone and *p*-substituted aryl aldehydes. The synthesis of compounds was confirmed with the help of their physical data, IR, and ^{1}H NMR spectra.

Bishalcones showed a characteristic IR absorption peak at *v* 1,690–1,650 cm^{-1} indicating the presence of a conjugated carbonyl group (>C=O) as well as an olfenic C=C band in the region 1,400–1,605 cm^{-1}. In ^{1}H NMR the characteristic peak of 2H near δ7.50–δ7.99 was observed.

Table 1 and Fig. 1 show the effect of bischalcones on the activity of acid phosphatase, it can be observed that bischalcones showed an activating effect on the enzyme. It can be the inference that bischalcones can not be used as inhibitors for the conditions related to elevated acid phosphatase levels, and therefore such moieties are of no use in the treatment of diseases where elevated acid phosphatase



Here, Ar' = *p*-F-C_6H_4, *p*-Cl-C_6H_4, *p*-Br-C_6H_4, *p*-CH_3-C_6H_4, *p*-OCH_3-C_6H_4, *p*-NO_2-C_6H_4, C_6H_5-CH=CH, *p*-$N(CH_3)_2$-C_6H_4, H

Scheme 1 Synthesis of bischalcones

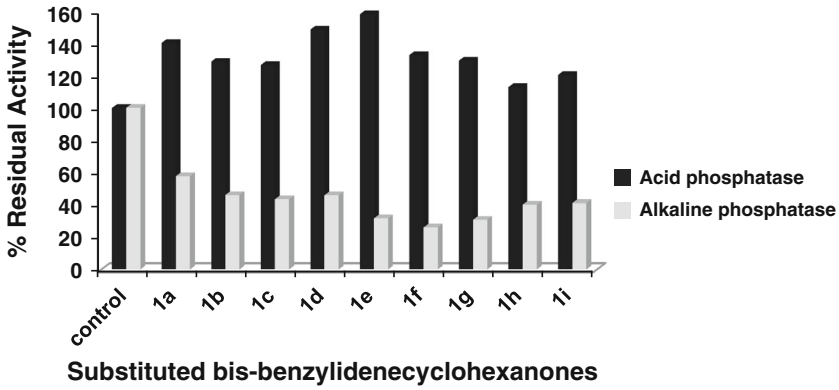


Fig. 1 Effect of substituted bis-benzylidenecyclohexanones (**1a–1i**) on Acid and alkaline phosphatase activity

level is observed but at the same time, the compounds can be beneficial at low cellular acid phosphatase levels which have been reported in prostate cancer cells (Veeramani *et al.*, 2005).

From the results, it can also be observed that bischalcones exerted an inhibitory effect on alkaline phosphatase (Table 1; Fig. 1). The inhibition has been found between ~40 and 75 % in different bischalcones.

Table 2 represents the data of in vitro inhibition and docking studies of bischalcones on alkaline phosphatase activities. This gives 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 alkaline phosphatase available through RCSB Protein Data Bank (PDB entry cav1B8 J alkvan_SVA.pdb) (Holtz *et al.*, 1999; Murphy *et al.*, 1997; Kim and Wyckoff, 1991). The decrease in total energy of bischalcone–alkaline phosphatase complex (in the range of approximately −75.5084 to −100.845) has been found to be less than the levamisole–enzyme complex (approximately −59.6946), a well reported inhibitor of alkaline phosphatase (Van Belle, 1976). It is also used as an anthelmentic agent. It has been reported by Kelleher *et al.* (1996) that the inhibitors of alkaline phosphatase can be successfully used in the treatment of neurological disorders, where the inhibitors were administered to reduce degeneration of cells and reduces peripheral neuropathy. The compound and its derivatives have been successfully used in the treatment of Alzheimer's disease. The present study can lead to the discovery of novel class of alkaline phosphatase inhibitors.

The binding of levamisole with the binding site of the enzyme shows a slight overlap with the *p*-nitrophenyl-phosphate binding with the enzyme active site (Fig. 2).

The lowest energy has been shown in case of 2,6-bis(4′-nitro benzylidene)cyclohexanone and out of this total energy stabilization −78.0976 is due to van der Waal interactions and −24.3751 is the contribution of H-bonds. The *p*-nitrophenyl group of 1f and substrate are perfectly aligned.

The results clearly indicate a higher binding affinity of designed compounds over the existing inhibitor of enzyme

Table 2 Docking and in vitro inhibition studies of alkaline phosphatase in presence of bischalcones

S. no.	Name of ligand	Total energy	VDW	H bond	K_i value (mM)	Type of inhibition
1.	2,6-Bis(4′-fluoro benzylidene)cyclohexanone (**1a**)	−76.5245	−71.3748	−5.14966	1.09	Competitive
2.	2,6-Bis(4′-chloro benzylidene)cyclohexanone (**1b**)	–82.5661	–77.591	–4.97508	0.670	Competitive
3.	2,6-Bis(4′-bromo benzylidene)cyclohexanone (**1c**)	–81.4958	–75.1658	–6.33004	0.565	Competitive
4.	2,6-Bis(4′-methyl benzylidene)cyclohexanone (**1d**)	–75.5744	–69.9312	–5.64319	0.845	Non-competitive
5.	2,6-Bis(4′-methoxy benzylidene)cyclohexanone (**1e**)	–88.0326	–76.3711	–11.6615	0.389	Competitive
6.	2,6-Bis(4′-nitro benzylidene)cyclohexanone (**1f**)	–100.845	–78.0976	–24.3751	0.250	Competitive
7.	2,6-Bis(3′-phenylallylidene)cyclohexanone (**1g**)	–75.5084	–68.2076	–7.30077	0.441	Non-competitive
8.	2,6-Bis(4′-(dimethyl amino)benzylidene)cyclohexanone (**1h**)	–93.9001	–84.0486	–9.85157	0.470	Competitive
9.	2,6-Di-benzylidene-cyclo-hexanone (**1i**)	–75.0789	–67.1408	–7.93812	0.470	Competitive
10.	Levamisole	–59.6946	–49.5207	–10.1739	4	Competitive
11.	*p*-Nitrophenylphosphate	–114.225	–48.2566	–58.6114	–	–

The results presented are one of the docking studies carried out using iGemdock at standard docking accuracy settings. The K_i values and the type of inhibition were determined at varying substrate concentrations 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

but at the same time it is less than the substrate. The affinity with the substrate *p*-nitrophenylphosphate has been shown to be the highest and the total energy stabilization is −114.225, of which −48.2566 is the contribution of van der Waal interactions and -58.6114 is the stabilization resulting due to H-bonds.

It was found that in vitro studies correlated well with the results of the docking studies. The 2,6-bis(4′-nitro benzylidene)cyclohexanone was found to most inhibitory to alkaline phosphatase showing an inhibition of ∼26 % at 1 mM concentration (Table 1; Fig. 1) and the compound resulted in the highest binding energy –100.845 into the active site of the enzyme (Table 2). In most of the bischalcones docking results showing more stabilization were found to be more inhibitory and those showing less stabilization were less inhibitory. The effect of synthesized compounds was further studied on alkaline phosphatase at varying concentration of substrates in presence and absence of compounds to determine the K_i value and to establish the type of inhibition (Nelson and Cox, 2008). It was found that most of the compounds inhibited the enzyme in a competitive manner except that of 2,6-bis(4′-methylbenzylidene) cyclohexanone and 2,6-bis(3′-phenyl-allylidene)cyclohexanone, where the inhibition has been found to be of non-competitive type. In a more detailed study (Figs. 3, 4, 5) of the docking results it can be observed that all the bischalcones except these two interact at the binding site of *p*-nitrophenylphosphate. From Figs. 4 and 5 it is clear that two compounds, i.e., **1d** and **1g** are showing interactions at a site other than the binding of *p*-nitrophenyl phosphate.

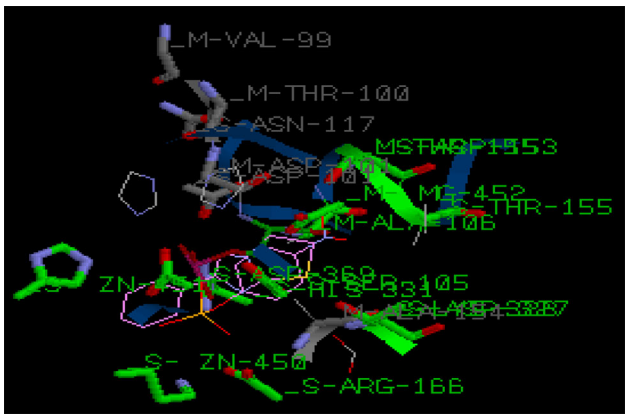


Fig. 2 Binding of levamisole and *p*-nitrophenyl phosphate into the binding site of alkaline phosphatase

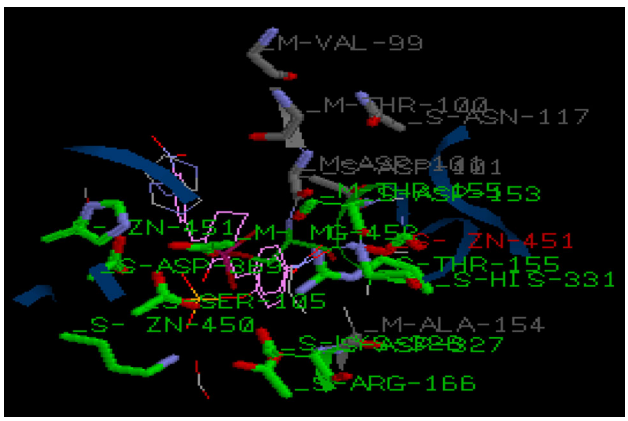


Fig. 3 Binding of 2,6-bis(4′-nitro benzylidene)cyclohexanone **1f**, into the binding site of alkaline phosphatase

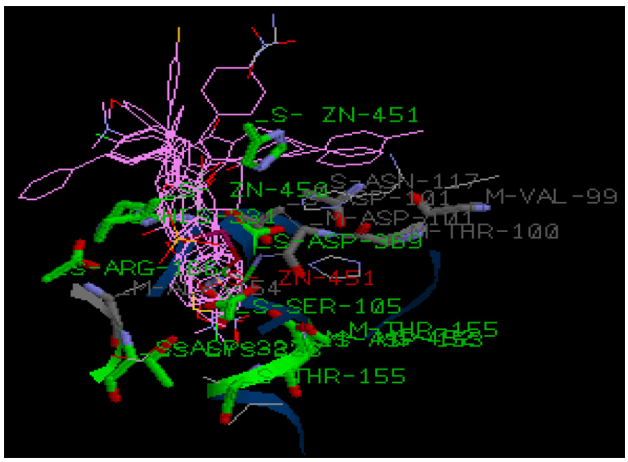


Fig. 4 Binding of bischalcones into the binding site of alkaline phosphatase. Two compounds **1d** and **1g** are showing the binding at a site other than the binding site

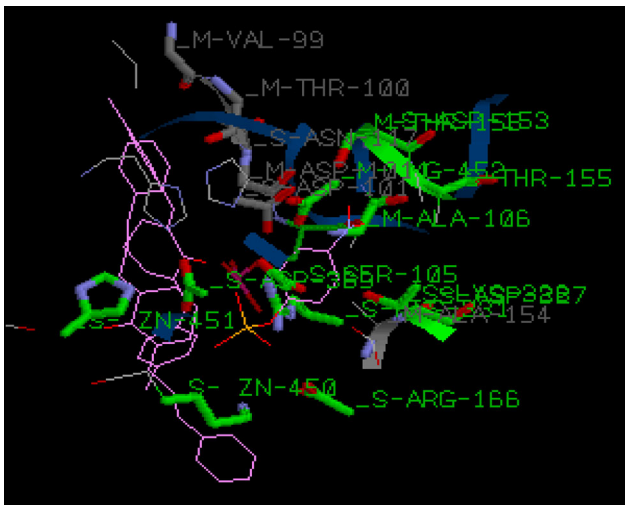


Fig. 5 Another view of binding of bischalcones into the binding site of alkaline phosphatase. Two compounds **1d** and **1g** are showing the binding at a site other than the binding site

Conclusion

The compounds under study have been synthesized successfully as characterized by the IR and ^{1}H NMR spectral data. From the enzymatic studies, it was concluded that the compounds show a differential effect on acid and alkaline phosphatases of liver. The acid phosphatase activity was enhanced in presence of these compounds, whereas the activity of alkaline phosphatase was inhibited. All the bischalcones showed a greater affinity with alkaline phosphatase than levamisole, an established inhibitor of alkaline phosphatase. 2,6-bis(4′-nitrobenzylidene)cyclohexanone was found to be most inhibitory to alkaline phosphatase The compounds inhibited the enzyme activity in a competitive manner except that of 2,6-bis(4′-methylbenzylidene)cyclohexanone and 2,6-bis(3′-phenylallylidene)cyclohexanone, where the inhibition has been found to be of non-competitive type.

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Conflict of interest The authors have declared no conflict of interest.

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