Influence of the diversified structural variations at the imine functionality of 4-bromophenylacetic acid derived hydrazones on alkaline phosphatase inhibition: Synthesis and molecular modelling studies

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Supporting Information

X-ray crystallographic analysis

The X-ray diffraction measurements were carried out for the synthesized compounds **4i** and **4q**. The crystal and instrumental parameters used in the unit cell determination, the data collection, and structure refinement parameters are presented in the Table S1 with selected bond lengths and angles in Table S2.

Table S1 Crystal data and structure refinement for **4i** and **4q**

Structural parameters	**Compound 4i**	**Compound 4q**
Empirical Formula	$C_{18}H_{17}BrN_2O_2$	$C_{21}H_{17}BrN_2O$
Formula weight (g/mol)	373.24	393.27
Temperature (K)	100(2)	100(2)
Wavelength (Å)	1.5418	1.5418
Crystal system	Monoclinic	Monoclinic
Space group	P 2_1/n	P c
Unit cell dimensions (Å)		
	a = 14.6957(3), α = 90°	a = 20.4423(11), α = 90°
	b = 4.8449(11), β = 102.2190(2)°	b = 5.5758(3), β = 98.6210(7)°
	c = 23.1542(5), γ = 90°	c = 7.6350(7), γ = 90°
Volume (Å^3)	1611.23(6)	860.43(10)
Z	4	2
D_{calc} (gcm^{-3})	1.539	1.518
μ (mm^{-1})	3.571	3.335
F (000)	760	400
Crystal size (mm^3)	0.20 × 0.03 × 0.03	0.20 × 0.18 × 0.06
Theta range for data collection (°)	3.277 to 76.730	4.375 to 76.12
Reflections collected	7820	7517
Independent reflections	3335 [R_{int} = 0.0662]	3007 [R_{int} = 0.0657]
Reflections observed	2903	2814
Min. and max. transmission	1.00000 and 0.51682	1.00000 and 0.51682
Data/restraints/parameters	3335 / 37 / 211	3007 / 8 / 229
Goodness-of-fit on F^2	1.039	1.130
Final R indices [I >2σ(I)]	R_1 = 0.0531, wR_2 = 0.1342	R_1 = 0.0858, wR_2 = 0.2443
R indices (all data)	R_1 = 0.0616, wR_2 = 0.1408	R_1 = 0.0914, wR_2 = 0.2495
Absolute structure parameter	-	0.01(4)
Largest diff. peak and hole (e Å^{-3})	1.164 and -1.149	3.267 and -0.995
CCDC reference number	1401865	1401866

Table S2 Selected bond lengths, (Å), and angles, (°), for **4i** and **4q**

	Compound 4i	**Compound 4q**
Bond length (Å)		
O2-C2	1.369(4)	-
O2-C21	1.414(4)	-
C21 C22	1.494(4)	-
C22-C23	1.317(5)	-
C1-C1'	-	1.48(2)
C4-C7	-	1.447(19)
C1-C7	1.465(4)	-
C7-N1	1.281(4)	1.281(9)
N1-N2	1.377(3)	1.332(17)
N2-C8	1.348(4)	1.385(19)
C8-O1	1.238(3)	1.23(2)
C8-C9	1.514(4)	1.50(2)
C9-C11	1.512(4)	1.50(3)
C14-Br14	1.908(3)	1.882(15)
Bond angles (°)		
C2-C1-C1'	-	119.8(13)
C2'-C1'-C1	-	122.3(13)
C23-C22-C21	126.9(3)	-
C22-C21-O2	109.1(2)	-
C1-C7-N1	120.9(2)	-
C4-C7-N1	-	
C7-N1-N2	113.7(2)	118.5(13)
N1-N2-C8	122.3(2)	119.5(13)
N2-C8-O1	120.0(2)	120.9(14)
C8-C9-O1	121.7(2)	123.5(15)
N2-C8-C9	118.3(2)	

Structural description

In the molecular structure of the **4i**, the (allyloxy)benzylidene)acetohydrazide segment of the molecule is close to planar with an root-mean-square (rms) deviation of 0.0315 Å from the best fit meanplane through all 15 non-hydrogen atoms. This plane is inclined at 64.83(6)° to the bromophenyl ring plane. Bond lengths in the C7—N1—N2—C8—O8 section of the molecule, Table S2, suggest a considerable degree of delocalization that would contribute to this planarity. In **4q**, the allyloxybenzene substituent is replaced by a biphenyl. This somewhat destroys the planarity of the biphenyl-4-ylmethylene-acetohydrazide segment of the molecule despite the biphenyl itself being reasonably planar, rms deviation 0.0346 Å for the 12 non-hydrogen atoms and the two phenyl rings inclined to one another at an angle of 3.7(6)°. The biphenyl plane is inclined to that through the C7—N1—N2—C8—O8 section of the molecule at 25(1)° while this plane subtends an angle of 52.0(7)° to the plane of the bromophenyl substituent. The delocalization in the central section of the molecule noted for **4i** clearly extends to **4q**, Table S2. Structures of compounds with an (*E*)-*N*'-benzylidene-2-phenylacetohydrazide framework are scarce, with only 4 unique examples in the Cambridge Structural Database.[1] These vary only in the substituents on the benzene rings of the benzylidene and/or the phenylacetohydrazide units,[2-5] and none closely resemble the two molecules reported here.

In the crystal structure of **4i**, inversion dimers form through classical N—H…O hydrogen bonds, Table S3, supported by C—H…O contacts and these dimers are stacked along the *b* axis in a three-dimensional network (Fig. S1). For **4q** dimers again form through N—H…O hydrogen bonds, Table S3, supported in this instance by C—H…O and C—H…Br contacts. Additional C—H…O contacts generate a three dimensional network of molecules stacked along the *c* axis direction (Fig. S2).

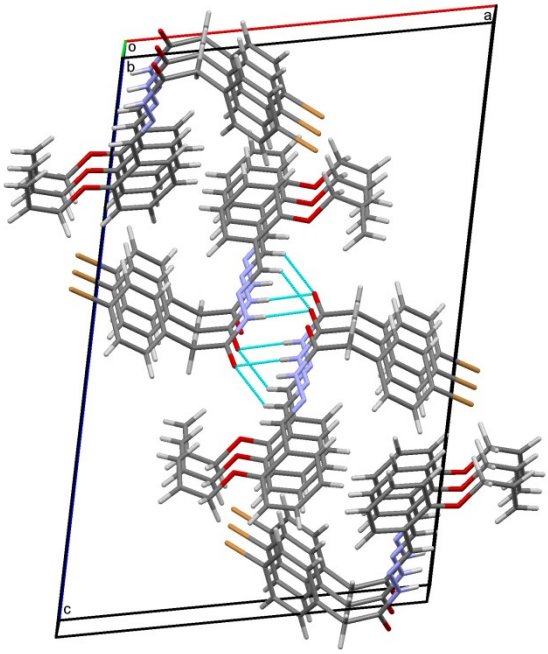

Fig. S1 Three-dimensional network of **4i** along *b* axis.

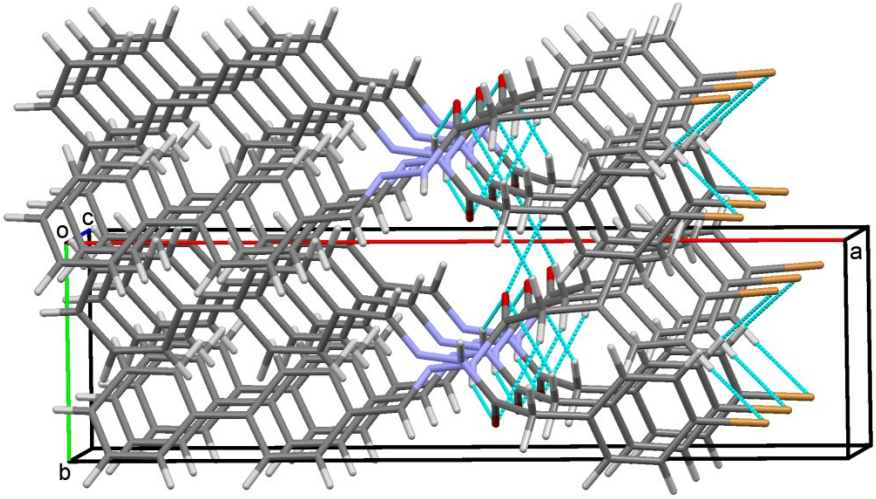

Fig. S2 Three-dimensional network of **4q** along *c* axis.

Table S3 Hydrogen bond geometry for **4i** and **4q** [Å, and °]

Compound	D-H...A	d(D-H)	d(H...A)	d(D...A)	<(DHA)
4i	N2—H2N…O8^{Ii}	0.783(19)	2.09(2)	2.874(3)	176(4)
	C7—H7…O8^{Ii}	0.95	2.68	3.464(3)	141
4q	C9--H9B…O1^{Iii}	0.99	2.48	3.46(2)	170
	N2—H2A…O1^{IIii}	0.9(2)	2.1(2)	2.951(18)	160(15)
	C9—H9A…O1^{IIii}	0.99	2.55	3.45(2)	151
	C13--H13…Br1^{IIii}	0.95	3.00	3.836(14)	147

Symmetry codes: (Ii) $-x+1, -y+1, -z+1$; (IIi) $x, -y, z-1/2$; (IIii) $x, -y+1, z-1/2$.

X-ray structure determination

The X-ray measurements for **4i** and **4q** were carried out on an Agilent Supernova Dual diffractometer. CuKα radiation (λ = 1.54184 Å) was used for both collections, which were controlled by CrysAlisPro[6] with data collected at 100(2)K. Data were corrected for Lorentz and polarization effects using and multi-scan absorption corrections were applied also using CrysAlisPro. Both structures were solved by direct methods (SHELXS-97),[7] and refined using full-matrix least-squares procedures (SHELXL-2014/7)[8] and Titan2000.[9] All non-hydrogen atoms were refined anisotropically and hydrogen atoms bound to carbon were placed in the calculated positions, and their thermal parameters were refined isotropically with Ueq(H) = 1.2U_{eq}(C). The hydrogen atoms on N2 in both molecules were located in difference Fourier syntheses and their coordinates refined with U_{eq}(H) = 1.2U_{eq}(N). All molecular plots and packing diagrams were drawn using Mercury,[10] and additional metrical data were calculated using PLATON.[11] Tables were prepared using WINGX.[12] Details of the X-ray measurements and crystal data for both compounds are given in Table S1. Data for compound **4q** were not of the

best quality. However, the data used here were obtained from the best of several samples that were tried. As a result the residuals for this structure are somewhat high which is clearly reflected in the uncertainties in the metrical data. Very high residual density in the vicinity of the Br14 atom in this structure may be the result of unresolved disorder but a suitable disorder model could not be established.

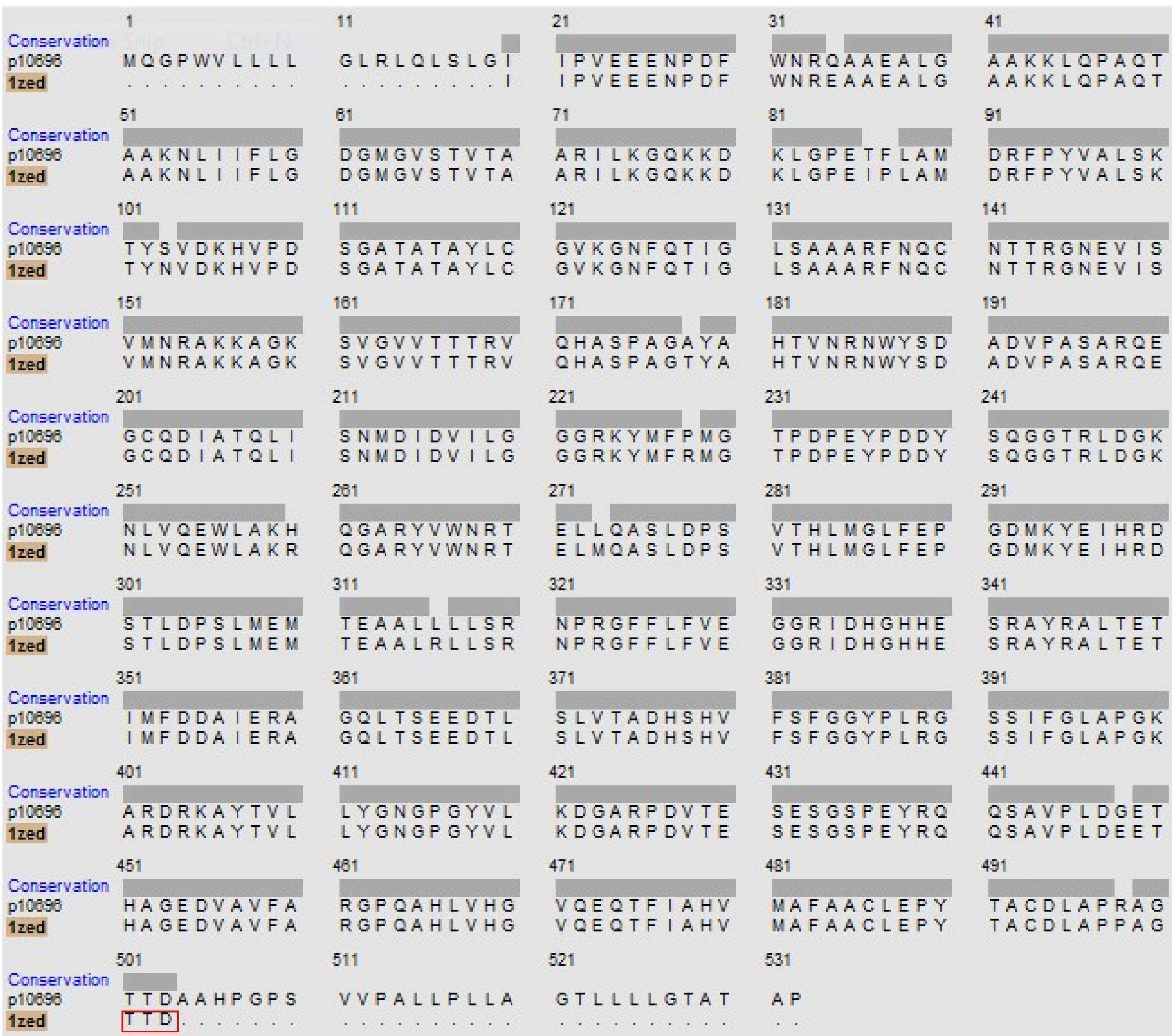

Fig. S3 Sequence Alignment of target human derived germ cells alkaline phosphatase (h-GCAP) with template human derived placental alkaline phosphatase (PDB ID 1ZED). Where, the gray bars show the conservation of amino acids.

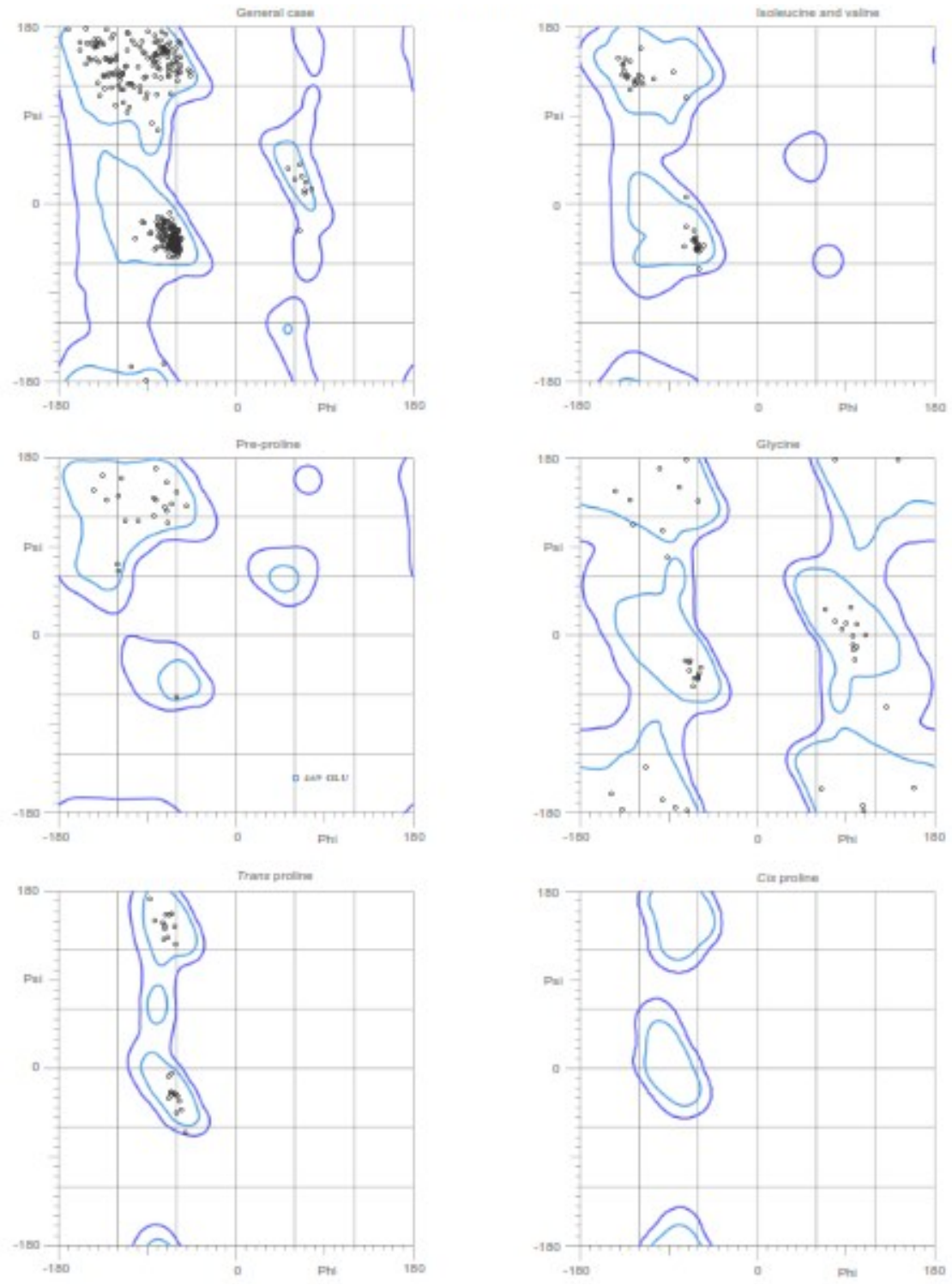

Fig. S4 Ramachandran Plot of h-GCAP, 97.7% (471/482) of all residues lies in favored (98%) regions. 99.8% (481/482) of all residues lies in allowed (>99.8%) regions. Amino acid Glu469 is the only outlier.

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