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In silico study of CYP450 inhibitor activity of (*E*)-1-(3-((4-chlorophenyl) diazenyl)-4-hydroxyphenyl)ethanone

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Molecular docking and single crystal study of azo dye (*E*)-1-(3-((4-chlorophenyl)diazenyl)-4-hydroxyphenyl)ethanone (**3**) is reported here. It has been synthesized by diazotization of 4-chloroaniline followed by coupling with 4-hydroxyacetophenone. Molecule is almost planar with acetyl as well as azo groups only slightly deviating from the plane of C3–C8 phenyl ring as evident by C1–C2–C3–C8 and N2–N1–C7–C6 torsion angles of –3.6(3)° and –3.9(3)°, respectively. Torsion angle N1–N2–C9–C14 between azo group and chlorophenyl ring is somewhat larger being –13.1(3)° leading to torsion angle between phenyl C3–C8 and chlorophenyl C9–C14 ring of 17.26(11)°. Intramolecular O2–H2···N2 hydrogen-bonding is observed here. Pillars along *b*-axis are formed due to π···π stacking interactions of parallel molecules in head-to-head fashion with centroid-to-centroid distance of 3.8829(14) Å and ring slippage of 1.416 Å. Title compound shows good binding affinity towards six enzymes of CYP450 family. Both nitrogens of the azo bond show significant involvement in bonding interactions with proteins in case of all the six enzymes.

Keywords: Azo dye, Molecular docking, CY450 inhibitor, X-ray diffraction, π···π stacking interactions

Azo dyes can be prepared straightforward in high purity and in very good yield by coupling a diazonium compound with coupling component in a mixture of water with simple alkanol[1].Azo-coupling of halogen-substituted benzenediazonium salt with hydroxyl-acetophenone is one of the most important reaction. It leads to formation of azo-compound with three functional groups i.e. –X, –OH, –$COCH_3$. It acts as a precursor for diazinyl chalcones[2], pyrazolines[3,4], ethers by coupling hydroxyl group with aryl halide[5] and metal complexes[6]. Azo dyes are studied for their anticancer activity against breast and liver cancer cell lines[7,8]. Report of antitubercular activity of azo dye is also known[9]. 90% of all metabolic reactions shows that carcinogen metabolism have involvement of six CYP isoforms (CYP1A2, CYP2C8, 2C9, 2C19, 2D6, and 3A4)[10-12]. Malignancy related to breast, liver, lung or stomach cells can be doubted if one or more of these six cytochromes are overexpressed[13-16]. Present study reports the interaction of synthesized azo dye with six CYP isoforms using molecular docking method.

Experimental Details

Materials and method

The starting materials 4-chloroaniline, 4-hydroxy-acetophenone, sodium nitrite and solvent were purchased from commercial sources and were used without further purification. Melting point was determined in open capillary using electro thermal melting point apparatus and is uncorrected. Progress of reactions was monitored by TLC. Infrared (IR) spectra (4000–600 cm^{-1}) of the samples were recorded using a Perkin–Elmer Spectrum 100, equipped with a Specac Golden Gate Diamond ATR as a solid sample support. ^{1}H NMR spectra were recorded with a Bruker Avance III 500 NMR spectrometer with TMS as internal reference. MS spectra were recorded with an Agilent 6624 Accurate Mass TOF LC/MS instrument (ESI ionization). Molecular docking was performed using Pyrx platform and Autodock Vina. The mol2 files of all compounds were prepared using Avogadro. The UCSF Chimera 1.15 and Discovery Studio visualizer software were used to study the molecular interactions with proteins. 3D structure of 3a was drawn using software package Avogadro, an open-source molecular builder and visualization tool, version 1.2.0. http://avogadro.cc/[17]. Structure file was used for docking in mol2 format after geometry optimization. Six cytochrome 450 enzymes namely CYP1A2, CYP2C9, CYP2C19, CYP2C8, CYP2D6 & CYP3A4 were used as target protein structures. The .pdb files of these enzymes were sourced from the website

rscb.org[18]. The .pdb files downloaded from the website and prepared for docking by removing the ligand from complex (if any) and prepared for docking using UCSF Chimera[19]. Solvent molecules and co-crystalized ligand, if any was removed in the process. Docking was performed using Pyrxsoftware[20]. Interactions between selected ligand and enzyme were calculated and analysed in UCSF Chimera[19] and BIOVIA Discovery Studio Visualizer [21].

Single-crystal X-ray diffraction (XRD) data were collected on an Agilent Technologies SuperNova Dual diffractometer with an Atlas detector using monochromated Mo-Kα radiation (λ = 0.71073 Å) at room temperature. The data were processed using CrysAlis Pro [22]. Structure was solved by direct methods and refined on F^2 using full-matrix least-squares procedures using SHELX2014[23]. All non-hydrogen atoms were refined anisotropically. Hydrogen atoms were readily located in a difference Fourier maps and were subsequently treated as riding atoms in geometrically idealized positions, with C–H = 0.93 (aromatic) or 0.96 Å (methyl), O–H = 0.82 Å and with U_{iso}(H) = kU_{eq}(C,O), where k = 1.5 for OH and methyl groups, which were permitted to rotate but not to tilt, and 1.2 for all other H atoms. CCDC 1899453 contains the supplementary crystallographic data for this paper. These data are provided free of charge by The Cambridge Crystallographic Data Centre. Crystallographic data is listed in Table 1.

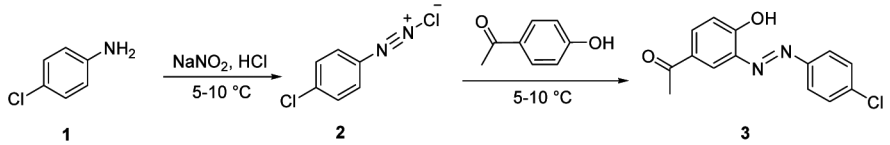
Molecular modeling and docking approach were implemented to compare the binding efficiency of erlotinib, gemcitabine as standard inhibitors along with synthesized azo dye with six enzymes from CYP450 family. Comparison of binding energies and pharmacophoric interactions showed the interaction of synthesized compound to these enzymes compared to the standard drugs. Looking at the acceptable docking values and pharmcophoric interactions, compound was synthesized and analyzed spectroscopically. Single crystal XRD study of the compound was also carried out to study the bonding interaction in solid state.

Synthesis of (*E*)-1-(3-((4-chlorophenyl)diazenyl)-4-hydroxyphenyl)ethenone (3): 4-chloro aniline (1.90 g, 15 mmol) was dissolved in dilute HCl (1.5 mL in 15 mL water) and cooled in an ice bath to 0–5°C. The reaction mixture was added to the solution of sodium nitrite (1.043 g, 15 mmol in 15 mL of water) and at about 0–5°C with continuous stirring to give yellow coloured solution[24]. To this solution, ethanolic solution of 4-hydroxyacetophenone [2.04 g,15 mmol in 10 mL 80% ethanolic solution (80 part of ethanol and 20 part of water)] was added in an ice bath with continuous stirring for 30 min to get orange yellow precipitate. The pH of reaction mixture was maintained between 5 and 6 using sodium acetate solution. The reaction mixture was kept overnight at about 15°C for the completion of the reaction. The precipitate obtained was washed with cold water, dried and recrystallized using ethanol (Scheme 1). Crystals suitable for XRD analysis were obtained by slow evaporation using mixture of ethanol and chloroform (1:1 v/v) at room temperature in dark chamber[25].

Table 1 — Crystallographic data for **3**

Compound code	**3**
CCDC number	1899453
Molecular formula	$C_{14}H_{11}ClN_2O_2$
Molecular weight	274.70
Crystal System	Monoclinic
Space group	*C*2/*c*
a (Å)	24.4962(16)
b (Å)	3.8829(3)
c (Å)	26.6781(13)
α (°)	90
β (°)	95.167(5)
γ (°)	90
Z	8
V (Å^3)	2527.2(3)
D_{calc} (g cm^{-3})	1.444
μ (mm^{-1})	0.301
F(000)	1136
Reflections collected	6003
Independent reflections	2873
R_{int}	0.0229
Parameters	174
R_1, wR_2 [$I > 2\sigma(I)$][a]	0.0479, 0.1104
R_1, wR_2 (all data)[a]	0.0727, 0.1226
GOF[b]	1.025
$\Delta\rho_{min}$, $\Delta\rho_{max}$ (e Å^{-3})	0.240, –0.211

[a] $R = \sum||F_o| - |F_c||/\sum|F_o|$; $wR_2 = \{\sum[w(F_o^2 - F_c^2)^2]/\sum[w(F_o^2)^2]\}^{1/2}$. [b] $S = \{\sum[(F_o^2 - F_c^2)^2]/(n/p)\}^{1/2}$ where n is the number of reflections and p is the total number of parameters refined



1 2 3

Scheme 1 — Synthesis of (*E*)-1-(3-((4-chlorophenyl)diazenyl)-4-hydroxyphenyl)ethenone (**3**)

Spectral data of compound 3: Orange yellow crystalline solid, Yield 83%, Melting point 145°C, (ESI+) *m/z*: 275.0581 (MH^+), HRMS: calcd for $C_{14}H_{12}ClN_2O_2$: 275.0582, found: 275.0581.^{1}H NMR (500 MHz, DMSO-d_6, ppm):2.57(s,3H,CH_3);7.20(d, 2H, Ar-H); 7.67 (d, 2H, Ar-H); 8.02–8.07(m,3H,Ar-H); 8.24 (d,1H,Ar-H); 11.49 (s, 1H,Ar-OH), IR: (υ_{max}/cm^{-1}): 2918 (OH), 1672 (C=O), 1562 (N=N), 1421 (C=C).

Result and Discussion

Compound **3** (*E*)-1-(3-((4-chlorophenyl)diazenyl)-4-hydroxyphenyl)ethanone was synthesized by diazotization of 4-chloroaniline followed by coupling with 4-hydroxyacetophenone in aqueous ethanolic solution. The compound was obtained as orange yellow solid with good yield. The IR spectra shows characteristic (Ar-OH) absorption band at 2918 cm^{-1}, strong stretching vibration in the region 1672 cm^{-1} attributed to υ (-C=O), υ (N=N) absorption band found in the region 1562 cm^{-1}, bands at 1419 cm^{-1} and 1482 cm^{-1} may be attributed to phenyl ring C=C stretching vibrations[26]. A strong band due to OH deformation vibrations is observed at 1266 cm^{-1} (Ref 27). Band at 1355 cm^{-1} is observed due to C-O-H deformation [28](Fig. 1).The ^{1}H NMR spectrum of compound **3** in DMSO exhibits characteristic singlet at 11.49 ppm attributed to phenolic OH proton, a doublet at 7.20 ppm showing 1H represent proton at C5, a doublet at 6.67 ppm showing 2H represent protons at C10 and C14, a doublet at 8.24 ppm showing 1H represent proton at C8 whereas a multiplate between 8.02-8.07 ppm showing 3H represent three protons at C4, C11 and C13, respectively, a singlet at 2.57 ppm showing three protons attributed to the methyl group.(Figs 2, 3, 4) The mass spectra and XRD diffraction study confirms the structure of compound **3.**

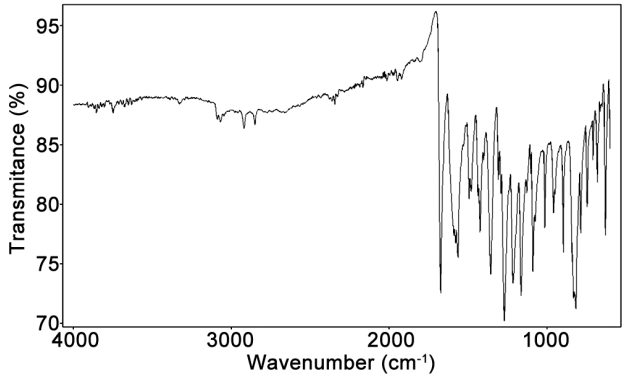


Fig. 1 — FTIR spectrum of compound **3**

The single-crystal study shows that molecule **3** is almost planar with acetyl as well as azo groups only slightly deviating from the plane of C3–C8 phenyl ring as evident by C1–C2–C3–C8 and N2–N1–C7–C6 torsion angles of –3.6(3)° and –3.9(3)°, respectively. Torsion angle N1–N2–C9–C14 between azo group and chlorophenyl ring is somewhat larger being –13.1(3)° leading to torsion angle between phenyl C3–C8 and chlorophenyl C9–C14 ring of 17.26(11)° (Fig. 4**).**

Beside the intramolecular O2–H2···N2 hydrogen-bonding there is present no other significant hydrogen-bonding (Table 2, Fig. 5). Pillars along *b*-axis are formed due to π···π stacking interactions of parallel molecules in head-to-head fashion with centroid-to-centroid distance of 3.8829(14) Å and ring slippage of 1.416 Å (Fig. 6).

Highest docking values obtained from the molecular docking study of compound **3** with proteins

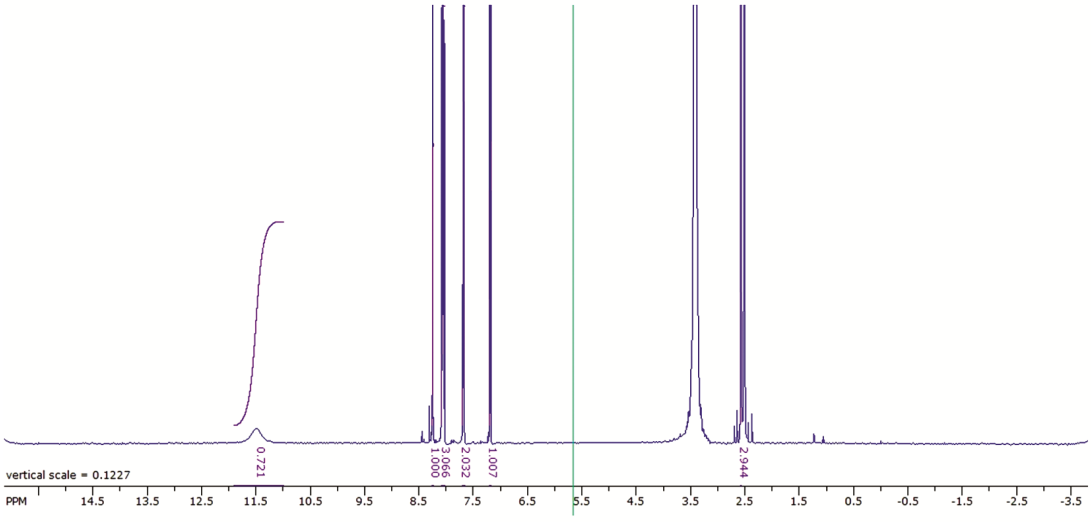


Fig. 2 — ^{1}H NMR spectrum of compound **3**

Table 2 — Hydrogen bond geometry of **3**

D–H···A	D–H (Å)	H···A (Å)	D···A (Å)	D–H···A (°)	Symmetry code
O2–H2···N2	0.82	1.88	2.573(2)	141.8	*x*, *y*, *z*

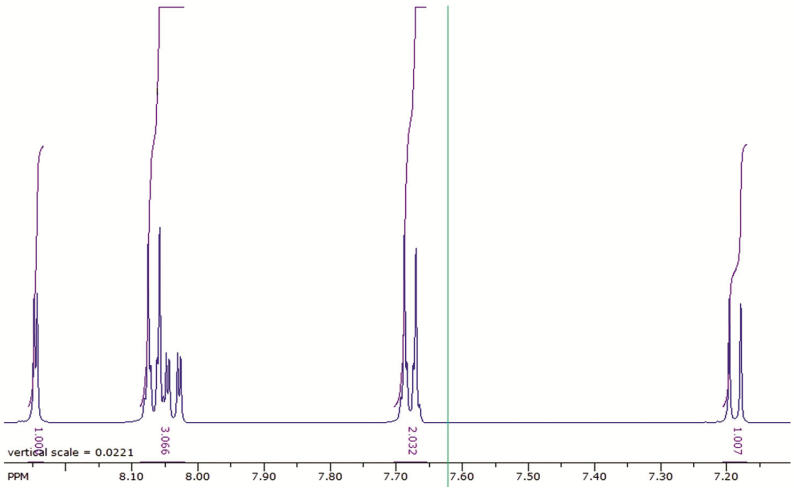


Fig. 3 — ^{1}H NMR spectrum of **3** showing protonic peaks in aromatic region

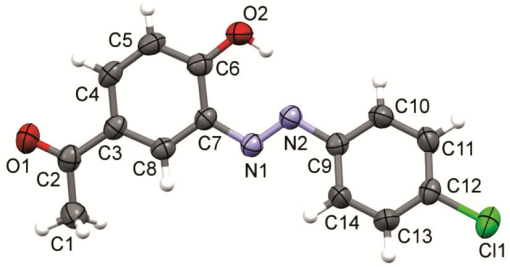


Fig. 4 —Molecular structures and atom numbering scheme for compound **3**; Probability ellipsoids are drawn at the 50% level

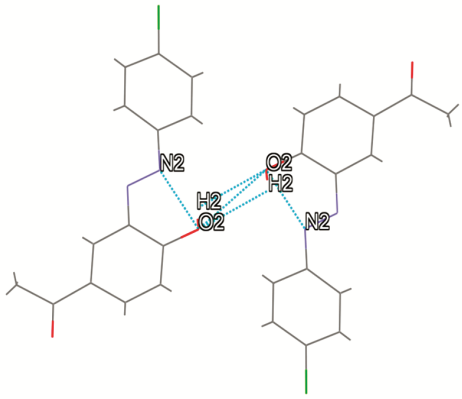


Fig. 5 — O2–H2···N2 Hydrogen bonding in compound **3**

from CYP450 family are shown in Table 3. Compound **3** shows highest docking score with CYP2D6 and CYP2C9 as compared to erlotinib as standard and it shows higher docking score than gemcitabine in case of all six enzymes. Compound **3** show hydrogen bonding interactions with Lys420 and Glu400 of CYP2C9 through nitrogen atom of the azo bond. Another nitrogen atom of the azo bond shows similar interaction with Phe419. Compound **3** also shows π----π interaction with Asp349 (A), Lys421(A), Lys420(A), Glu400(A), Lys423(A) and Lys421(B). Chloro group forms hydrogen bonding interaction with Tyr424(A). Fig. 7 show two-dimensional and three-dimensional view of the interaction of **3** with amino acids of CYP2C9.

Compound **3** show interaction with Glu216 and Phe120 through both the nitrogens of azo bond of CYP2D6. One of the nitrogen bond show hydrogen bond interactions with Phe120 with CYP2D6. It shows π----π interaction with Phe120 of CYP2D6. -OH and -C=O groups form hydrogen bond interaction with CYP2D6 through Glu216 and Ser304. Chloro group show acceptor type hydrogen bonding with Val370, Phe483, Val374 and Hem601 of CYP2D6. Fig. 8 show two-dimensional and three-dimensional view of the interaction of 3 with amino acids of CYP2D6.

Compound **3** also show significant interactions with CYP3A4. Both nitrogens from azo bond show interaction with Glu374. Nitrogen shows hydrogen bond interaction with CYP3A4 through Arg372. Compound **3** shows π----π stacked type of interaction with Ala370 and π----σ interaction with Phe57 and π----alkyl interaction with Arg372. It also shows interaction with Phe57 through chloro group. Fig. 9 show two-dimensional and three-dimensional view of the interaction of **3** with amino acids of CYP3A4.

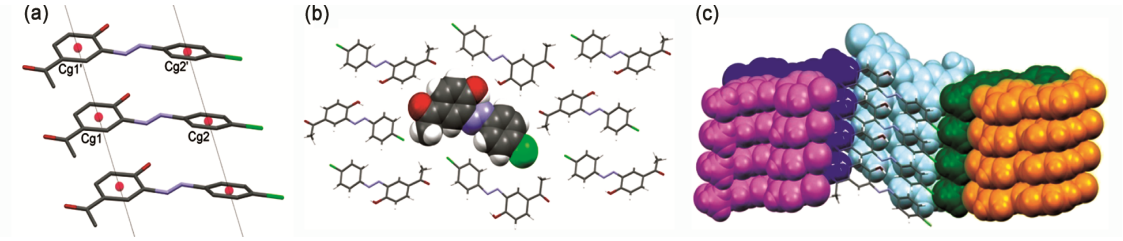


Fig. 6 — (a) π···π stacking interactions along *b*-axis. Hydrogen atoms have been omitted for clarity. (b) Packing of molecules along *ac*-plane. (c) Pacing along *b*-axis (color code: arbitrary colors)

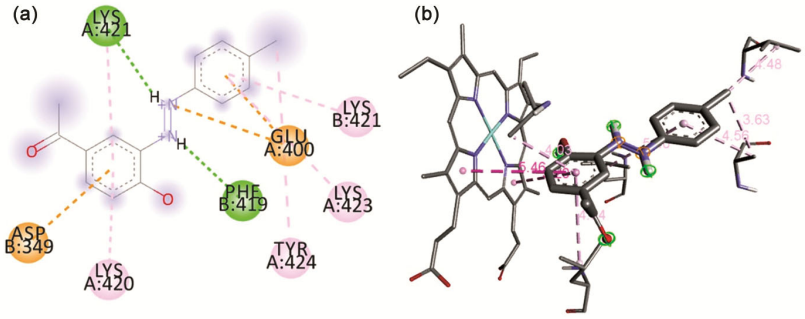


Fig. 7 — Molecular docking interactions of **3** with CYP2C9 **(a)** 2-Dimensional representation of various interactions of ligand molecule with amino acids and (b) 3-Dimensional view of the interaction

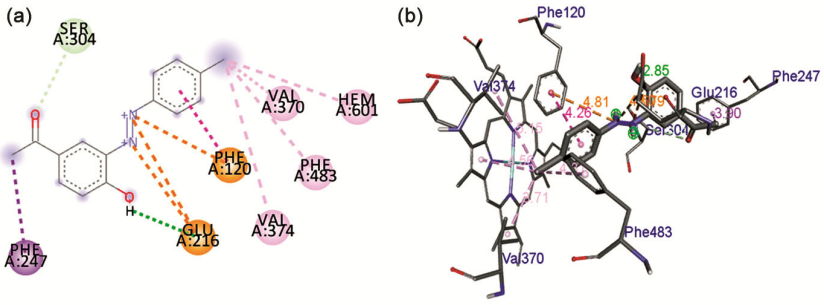


Fig. 8 — Molecular docking interactions of **3** with CYP2D6 (a) 2-Dimensional representation of various interactions of ligand molecule with amino acids and (b) 3-Dimensional view of the interaction

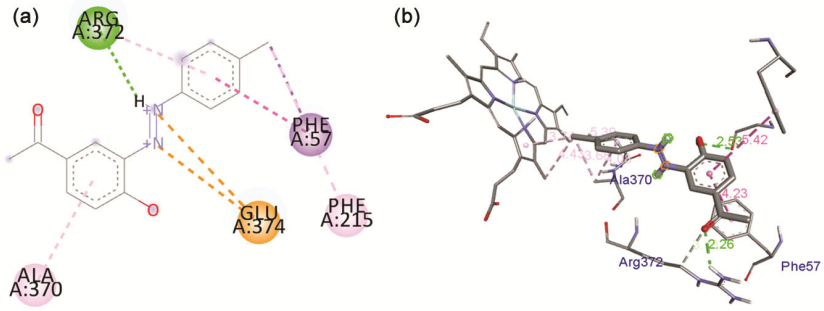


Fig. 9 — Molecular docking interactions of **3** with CYP3A4 (a) 2-Dimensional representation of various interactions of ligand molecule with amino acids and (b) 3-Dimensional view of the interaction

Table 3 — Docking score (kcal/mol) of 3 and standard reference compounds with CYP450 enzymes

Protein Compound	CYP1A2	CYP2C19	CYP2C8	CYP2C9	CYP2D6	CYP3A4
3	-6.5	-7.2	-7.2	-7.6	-8.6	-7.5
Erlotinib	-6.8	-7.4	-8.1	-7.4	-7.5	-7.8
Gemcitabine	-6.2	-6.8	-6.2	-7.1	-7.0	-7.2

Conclusion

The azodye **3** was synthesized in mild condition with high purity and good yield. The molecule is formed with three functional groups –Cl, –OH, and –$COCH_3$.Its single-crystal study shows its planar nature with acetyl as well as azo group but slight deviation from plane of C3-C8 phenyl ring. Crystal system found to be monoclinic. It shows Intra molecular O2–H2···N2 hydrogen-bonding as well as π···π stacking interactions between parallel molecules in head-to-head fashion with centroid-to-centroid distance of 3.8829(14) Å. Structural and molecule docking study of **3** shows that the both the nitrogen of azo group of the compound are actively involved in hydrogen bonding interaction with majority of the proteins from CYP450 family which are responsible for over expression and malignancy in some cancer tissues. Synthesis of compounds which can fit well in the cavity of these proteins and give good hydrogen bonding as well as π interaction with hem group can lead to finding new drug like molecules with better activity. Any small advancement in this direction can help the field of drug discovery in this important domain.

Supplementary Information

Supplementary information is available in the website http://nopr.niscpr.res.in/handle/123456789/58776.

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