J. Chem. Sci. Vol. 129, No. 8, August 2017, pp. 1247–1256.
DOI 10.1007/s12039-017-1320-4

REGULAR ARTICLE

Phthalocyanine and azaphthalocyanines containing eugenol: synthesis, DNA interaction and comparison of lipase inhibition properties

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MS received 6 December 2016; revised 27 January 2017; accepted 20 May 2017

Abstract. Novel eugenol-substituted zinc(II) azaphthalocyanines (ZnAzaPcs) were synthesised and their lipase inhibition and DNA binding properties compared with phthalocyanines (Pcs) containing eugenol. This is the first study on lipase inhibition and DNA binding of Pcs and AzaPcs containing a pharmacophore group, such as eugenol. The novel ZnAzaPcs were characterised using a combination of FT-IR, ^{1}H NMR, ^{13}C NMR, UV–Vis, MS and elemental analysis. The crystal structures of two pyrazine compounds were also determined by the single crystal diffraction technique. This study showed that two phthalocyanines compounds (**3a** and **4a**) could be potential lipase inhibitor agents due to greater hydrophobicity than other azaphtalocyanines. Compound **4a** displayed lowest IC_{50} value. Non-intercalative binding to DNA was identified only for compound **2a**.

Keywords. Azaphthalocyanine; eugenoe; lipase inhibition; phthalocyanine; DNA interaction.

1. Introduction

Azaphthalocyanines (AzaPcs) are aza-analogs of phthalocyanines (Pcs), in which some of the C atoms in the Pc macrocycle are replaced with N atoms.[1] These macrocycles have attracted considerable attention due to their promising photosensitive,[2] fluorescent,[3] non-linear optical[4] and oxidative properties.[5] Eight additional N atoms on the periphery of AzaPcs increase the polarities of these macrocycles, and substituents may be used to modify solubility, UV–Vis absorption, i.e., shifts of Q-bands, and acid-base properties.[6] Substituted AzaPcs are generally more soluble than the corresponding Pcs, and therefore they may have more technological applications.[7]

Two synthetic procedures were performed to prepare a unique type of substituted AzaPc: (i) direct cyclotetramerisation of the corresponding substituted 2,3-dicyanopyrazines; (ii) further modification of the formed substituted AzaPc macrocycle.[8] Procedure (i) is the most common method used.[9]

Eugenol is a phytochemical obtained from sources such as *Syzygium aromaticum* and *Ocimum sanctum*, and has wide applications.[10] Eugenol shows antidepressant-like,[11] antibacterial[12] and antioxidant[13] activities. Eugenol substituted Pcs were synthesised and some properties investigated by some of our group members.[14] Further, single crystals of phthalonitrile compounds were published[15] beforehand. However, synthesis and characterisation of tetra- and octa-eugenol-substituted AzaPcs have not been reported prior to the present study.

DNA binding studies are important in the development of new anticancer agents. Metal complexes may alter the DNA duplication and avoiding the growth of the tumor cells.[16] In the pursuit of tools, phthalocyanines that bind to DNA have been extensively studied.[17]

Obesity is widely recognised as a major health issue that is caused by an imbalance between energy intake and expenditure. Obesity often provokes more serious diseases, such as type II diabetes, hypertension, arteriosclerosis and hyperlipidemia. Pancreatic lipase has a key role in fat digestion. Moreover, drugs such as orlistat (tetrahydrolipstatin), which inhibit pancreatic

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Electronic supplementary material: The online version of this article (doi:10.1007/s12039-017-1320-4) contains supplementary material, which is available to authorized users.

lipase are used as therapeutic agents to treat obesity.[18] Orlistat is an anti-obesity agent acting locally in the gastrointestinal tract by reversibly inhibiting pancreatic and gastric lipases. Pancreatic and gastric lipases are involved in the disruption of long chain triglycerides. Controlling obesity with orlistat is limited because of its adverse metabolic effects (borborygmi, intestinal flatulence and abdominal cramps) and poor patient compliance.[19] Therefore, more successful therapeutics should be investigated. Studies on enzyme inhibition of Pcs have been scarcely reported, due to the macrocyclic and bulky structure of Pcs. However, some Pc compounds were previously reported as xanthine oxidase inhibitory by our group.[20] The Pcs and AzaPcs containing pharmacophore group, like eugenol, are expected to display effective porcine pancreatic lipase inhibitory properties. Because, Pcs and AzaPcs containing apolar group like eugenol can bind to enzyme active site and inhibit enzyme.

The purposes of this research are, (i) to synthesise and characterise eugenol-substituted AzaPc, and (ii) to compare the spectroscopic and porcine pancreatic lipase (PPL) inhibitory properties of the novel AzaPc with Pc containing eugenol in the literature.[14a,b]

2. Experimental

2.1 *Materials*

5-Chloropyrazine-2,3-dicarbonitrile and 5,6-dichloropyrazine-2,3-dicarbonitrile were prepared according to published procedures.[21] The Pcs containing eugenol moieties (**3a** and **4a**) were synthesised according to literature method.[14a,b] FT-IR spectra were recorded on a Perkin-Elmer Spectrum 100 Infrared Spectrometer. Sources of other chemicals: Eugenol (Merck Chemical Company (Darmstadt, Germany)), Orlistat (Xenical, Hoffman, La Roche,Segrate, Italy), p-Nitrophenol palmitate (pNPP) (Sigma, UK), porcine pancreatic lipase (PPL) (Applichem, Germany). Spectroscopic analyses were accomplished by UV–Vis spectrophotometer (Perkin-Elmer), ^{1}H NMR and ^{13}C NMR (Agilent 400 FT-NMR), and Elemental analyses at RTEÜ Research Centre (Rize, TURKEY); Mass analyses (Agilent LC/MS-TOF) at GRÜMLAB Research Centre (Giresun, TURKEY).

2.2 *5-(4-Allyl-2-methoxyphenoxy)pyrazine-2,3-dicarbonitrile **(1)***

5-Chloropyrazine-2,3-dicarbonitrile (0.3 g, 1.83 mmol) and eugenol (0.3 g, 1.83 mmol) were stirred in 15 mL tetrahydrofuran (THF) at 65°C. Triethylamine (0.25 mL, 1.8 mmol) was then added and the mixture stirred at 65°C for 24 h, then it was evaporated to dryness. The residue was dissolved in ethanol and then precipitated by addition of water. The light yellow precipitate was collected by filtration. The product was purified by crystallisation from dry amyl alcohol.

The yield of white crystals was 87% (0.46 g). Melting point (M.p.): 96–97°C. $C_{16}H_{12}N_4O_2$: Anal. Found: C, 65.74; H, 4.15; N, 19.15% Calc. C, 65.75; H, 4.14; N, 19.17%. IR, υ_{max}(cm^{-1}): 3059 (CH, aromatic), 2969, 2943, 2843 (CH, aliphatic), 2239 (C ≡ N), 1637 (C=C, aliphatic), 1606, 1557, 1534, 1503 (C=C, aromatic), 1279, 1263 (O-CH_3) 1190, 1034, 833, 751. ^{1}H NMR (δ ppm in Dmso-d_6): 9.006 (s, 1H, Ar-H), 7.179–7.159 (d, J = 8, 1H, Ar-H) 7.052–7.048 (d, J = 1.6, 1H, Ar-H), 6.866–6.846 (dd, J = 2, J = 2, 1H, Ar–H), 6.052–5.950 (m, 1H, CH), 5.158–5.063 (m, 2H, =CH_2), 3.703 (s, 3H, OCH_3), 3.415–3.398 (d, J = 6.8, 4H, -CH_2). ^{13}C NMR (100 MHz, DMSO-d_6): 39.77, 56.30, 114.00 and 114.02 (CN), 114.60, 116.72, 121.19, 122.47, 127.05, 131.28, 137.65, 138.28, 140.33, 140.84, 150.70, 159.74.

2.3 *5,6-Bis(4-allyl-2-methoxyphenoxy)pyrazine-2,3-dicarbonitrile **(2)***

5,6-Dichloropyrazine-2,3-dicarbonitrile (1 g, 5 mmol) and eugenol (1.64 g, 10 mmol) were stirred in 25 mL THF at 65°C. Triethylamine (1.5 mL, 10.6 mmol) was then added and the mixture stirred at 65°C for 24 h, then it was evaporated to dryness. The remaining residue was dissolved in ethanol and then precipitated by addition of water. The light yellow precipitate was collected by filtration. The crude product was purified by crystallisation from dry amyl alcohol.

The yield of light yellow crystals was 83% (1.89 g). Melting point (M.p.): 109–110°C. $C_{26}H_{22}N_4O_4$: Anal. Found: C, 68.61; H, 4.84; N, 12.34%. Calc. C, 68.71; H, 4.88; N, 12.33%. IR υ_{max}(cm^{-1}): 3087, 3059 (CH, aromatic), 2984, 2944, 2845 (CH, aliphatic), 2234 (C ≡ N), 1639 (C=C, aliphatic), 1605, 1545, 1501 (C=C, aromatic), 1356, 1272, 1221 (O-CH_3), 1115, 1031, 904, 814, 738. ^{1}H NMR (δ ppm in Dmso-d_6): 7.240–7.220 (d, J = 8 Hz, 2H, Ar-H) 7.076–7.071 (d, J = 2, 2H, Ar-H), 6.882–6.862 (dd, J = 2, J = 1.6, 2H, Ar–H), 6.065–5.964 (m, 2H, =CH), 5.172–5.067 (m, 4H, =CH_2), 3.753 (s, 6H, OCH_3), 3.428–3.411 (d, J = 6.8, 4H, -CH_2). ^{13}C NMR (100 MHz, DMSO-d_6): 39.76, 56.39, 114.05, 114.10 (CN), 116.75, 121.17, 122.56, 124.03, 137.64, 138.37, 140.37, 150.63, 151.21

2.4 *General procedure of zinc(II)azaphthalocyanines (**1a** and **2a**)*

Synthesised pyrazine dinitrile derivatives **1** and **2** (2 mmol) with zinc acetate (0.5 mmol) were mixed and heated at 250°C for 20 min, respectively. After cooling, the blue product was washed with common solvents (water, ethanol, methanol, ether). The obtained blue-green products were purified using column chromatography ($CHCl_3$-EtOH, 10:1, silica gel).

2.4a *Zinc(II)azaphthalocyanine **1a***: Yield 78% (0.48 g). Melting point (M.p.)>300°C. $C_{64}H_{48}N_{16}O_8Zn$: Anal. Found: C, 62.33; H, 3.98; N, 18.15%. Calc. C, 62.26; H, 3.92; N, 18.15%; IR, υ_{max} (cm^{-1}): 3067, 3003 (CH, aromatic),

Table 1. Crystal data and structure refinement parameters for compounds **1** and **2**.

Crystal data	**1**	**2**	Crystal data	**1**	**2**
Empirical formula	$C_{16}H_{12}N_4O_2$	$C_{26}H_{22}N_4O_4$	V (Å^3)	1512.57 (13)	4848.0 (8)
Formula weight	292.30	454.48	Z	4	8
Crystal system	Monoclinic	Monoclinic	D_c(g cm^{-3})	1.284	1.245
Space group	$P2_1/n$	C2/c	μ (mm^{-1})	0.09	0.09
a(Å)	9.8466 (5)	33.876 (3)	θ range (°)	3.2-28.1	2.2-28.0
b(Å)	8.3460 (4)	8.6637 (4)	Measured refls.	36735	12392
c(Å)	18.4843 (10)	29.924 (3)	Independent refls.	3761	4656
α (°)	90.00	90.00	R_{int}	0.040	0.041
β (°)	95.286 (2)	146.495 (3)	S	1.12	0.91
γ (°)	90.00	90.00	R1/wR2	0.060/0.171	0.075/0.241
			Δ ρ_{max} /Δ ρ_{min} (eÅ^{-3})	0.18/-0.23	0.33/-0.20

2972, 2936, 2833 (CH, aliphatic), 1637 (C=C, aliphatic), 1603, 1532, 1505 (C=C, aromatic), 1341, 1322, (O-CH_3), 1292, 1195, 1149, 1120, 1031, 987, 746. ^{1}H NMR (δ ppm in Dmso-d_6)9.269 (s, 4H, Ar-H), 7.682–6.978 (m, 12H, Ar-H), 6.249–6.148 (m, 4H, CH), 5.332–5.223 (m, 8H, =CH_2), 3.780 (s, 12H, OCH_3), 3.625–3.570 (d, 8H, -CH_2). MASS (m/z): 1234.30 [M]$^+$. UV–Vis (DMSO): λ_{max}/nm: 355, 576, 635.

2.4b *Zinc(II)azaphthalocyanine **2a***: Yield 75% (0.7 g). Melting point (M.p.)>300°C. $C_{104}H_{88}N_{16}O_{16}Zn$: Anal. Found: C, 66.39; H, 4.74; N, 11.94%. Calc. C, 66.33; H, 4.71; N, 11.90%. IR, υ_{max}(cm^{-1}): 3071, 3007 (CH, aromatic), 2968, 2935, 2837 (CH, aliphatic), 1635 (C=C, aliphatic), 1602, 1538, 1502 (C=C, aromatic), 1395, 1268, 1216 (O-CH_3), 1117, 922, 745. ^{1}H NMR (δ ppm in Dmso-d_6): δ 7.553–7.533 (d, J = 8, 4H, Ar-H) 7.125–7.121 (d, J = 1.6, 4H, Ar-H), 7.036–7.016 (dd, J = 1.6, J = 2, 4H, Ar–H), 6.241–6.140 (m, 8H, CH), 5.352–5.241 (m, 16H, =CH_2), 3.738 (s, 24H, OCH_3), 3.641–3.625 (d, J = 6.4, 16H, -CH_2). MASS (m/z): 1882.94 [M]$^+$. UV–vis (DMSO): λ_{max}/nm: 365, 574, 630.

2.5 *X-ray diffraction analysis*

Single crystals of compounds **1** and **2** were picked for data collection, which was performed on a Stoe IPDS diffractometer equipped with a graphite-monochromatic Mo-K_α radiation at 296 K. The structure of compounds **1** and **2** was solved using SHELXS-97[22] and refined by full-matrix least-squares methods on F^2, using SHELXL-97,[22] from within the WINGX[23] suite of software. The H atoms of C atoms were located from different maps and then treated as riding atoms with C-H distances of 0.93–0.97 Å. Molecular diagrams of compounds **1** and **2** were created using MERCURY.[24] Geometric calculations were performed with PLATON.[25] The crystallographic data and other parameters for compounds **1** and **2** are summarized in Table 1.

2.6 *DNA-binding assay*

All phthalocyanines and azaphthalocyanines, except compound **2a,** formed aggregate in reaction buffer (pH 7.4 in a 10 mM Tris HCl buffer containing 50 mM NaCl, % 5 DMSO), so the electronic spectral titration experiment was performed only for compound **2a**. CT-DNA concentration per nucleotide phosphate was calculated by absorption spectroscopy at 260 nm using a DNA extinction coefficient value of 6600 M^{-1} cm^{-1}.[26] Absorption spectra were recorded in the region of 250–900 nm by fixing the concentration of compound **2a** (20 × 10^{-6} mol L^{-1}), while increasing the concentration of CT-DNA (0 to 20 × 10^{-6} mol L^{-1}) step by step. An equal quantity of CT-DNA was added to both the reference and complex solutions to eliminate the absorbance of DNA itself. The binding constants (K_b) was determined for compound **2a** using eq. 1.[27]

$$[DNA]/(\varepsilon_a - \varepsilon_f) = [DNA]/(\varepsilon_b - \varepsilon_f) + 1/K_b(\varepsilon_b - \varepsilon_f) \quad (1)$$

Here, ε_a, ε_f and ε_b correspond to the apparent absorption coefficient (A_{obsd}/[DNA]), the extinction coefficient of the free compound and the extinction coefficient of the compound when fully bound to DNA, respectively. In the plots of [DNA]/(ε_a - ε_f) versus [DNA], K_b is given by the ratio of slope to the intercept

2.7 *Lipase inhibition assay*

The ability of the test compounds to inhibit lipase was evaluated against porcine pancreatic lipase (15μg/cm^3) using the method described previously[28] with slight modifications. p-Nitrophenol palmitate (pNPP) was used as substrate which was hydrolyzed by lipase to p-nitrophenol (pNP). In brief, 970 μL of 0.05 M sodium phosphate buffer (pH 8.0, 5% DMSO), containing sodium cholate (2.00 mg/mL) and gum arabic (1.00 mg/mL) prewarmed at 37°C was mixed with 5 μL of Pc compounds at different concetrations and 20μL of porcine pancreatic lipase. This mixture was incubated 5 minute at 37°C. Then, 20μL of pNPP (0.01 mM in acetonitrile) was added to the mixture. After 15 min of incubation

at 37°C, the absorbance was measured at 410 nm (UV-1600, Shimadzu Co., Japan) against an enzyme-free control. Orlistat was used as positive control. In order to calculate IC_{50} values, different concentrations of Pc compounds and standards were performed at the same reaction conditions. The half-maximal inhibitory concentration (IC_{50}) was calculated from the absorbance data.

3. Results and Discussion

3.1 *Synthesis and characterisation*

5-Chloropyrazine-2,3-dicarbonitrile and 5,6-dichloro pyrazine-2,3-dicarbonitrile were prepared according to literature procedures.[21] The synthetic route of the novel metalloazaphthalocyanines (M: Zn) is shown in Scheme 1.

The novel pyrazine dinitrile derivatives **1** and **2** were prepared by treatment of 5-chloropyrazine-2,3-dicarbonitrile and 5,6-dichloropyrazine-2,3-dicarbonitr ile with eugenol, respectively. Afterwards, zinc(II) AzaPcs (ZnAzaPcs) were synthesised by heating pyrazine dinitrile compounds **1** and **2** with $Zn(CH_3COO)_2$ salt for 20 min. Characterisation of the compounds has been completed by FT-IR, X-ray diffraction analysis, UV–Vis spectroscopy, ^{1}H NMR, ^{13}C NMR, elemental analysis and MS. Elemental analysis results of the compounds **1, 2, 1a** and **2a** showed good overlap with the calculated values.

In the FT-IR spectrum, formation of compound **1** was indicated by the appearance of a sharp CN band at 2239 cm^{-1}. In the ^{1}H NMR spectrum of compound **1,** aromatic peaks appeared at 9.006 ppm as a singlet, at 7.179–7.159 and 7.052–7.048 ppm as two doublets and at 6.866–6.846 as a doublet-doublet, allyl (-CH=CH_2) protons at 6.052–5.950 and 5.158–5.063 ppm as two multiplets and at 3.415–3.398 ppm as a doublet and methoxy protons at 3.703 ppm as a singlet. The ^{13}C NMR spectrum of compound **1** indicated the existence of nitrile C atoms at 114.00 and 114.02 ppm.

FT-IR spectrum of the AzaPc **1a** signified the cyclotetramerisation of compound **1** with the disappearance of the CN peak at 2239 cm^{-1}. In the ^{1}H NMR spectrum, formation of compound **1a** was indicated by aromatic peaks appearing at 7.682–6.978 ppm as a multiplet and 9.269 ppm as a singlet, allyl (-CH=CH_2) protons at 6.249–6.148 and 5.332–5.223 ppm as two multiplets and at 3.625-3.570 ppm as a doublet and methoxy protons at 3.780 ppm as a singlet.

The formation of compound **2** was indicated by the appearance of a sharp CN band at 2234 cm^{-1} in the FT-IR spectrum. In the ^{1}H NMR spectrum, formation of compound **2** was indicated by the appearance of aromatic peaks at 7.240–7.220 and 7.076–7.071 ppm as two doublets and at 6.882–6.862 as a doublet-doublet, allyl (-CH=CH_2) protons at 6.065–5.964 and 5.172–5.067 ppm as two multiplets and at 3.428–3.411 ppm as a doublet and methoxy (-OCH_3) protons at 3.753 ppm as a singlet. The ^{13}C NMR spectrum of compound **2** showed the presence of nitrile C atoms at 114.10 ppm.

FT-IR spectrum of the AzaPc **2a** signified cyclotetramerisation of the compound **2** derivative with the disappearance of the CN peak at 2234 cm^{-1}. In the ^{1}H NMR spectrum of compound **2a**, structure was indicated by the appearance of aromatic peaks at 7.553–7.533 and 7.125–7.121 ppm as two doublets and at 7.036–7.016 as a doublet-doublet, allyl (-CH=CH_2) protons at 6.241–6.140 and 5.352–5.241 ppm as two multiplets and at 3.641–3.625 ppm as a doublet and methoxy protons at 3.738 ppm as a singlet.

The symmetric compound **2** affords only one macrocyclic isomer, namely compound **2a**; but compound **1** is nonsymmetrical and consequently, four constitutional isomers of compound **1a** may be formed. The NMR spectra are in accordance with this, since the H signals of compound **2a** are sharp and have well defined coupling constants, whereas the corresponding signals of compound **1a** are broad with unresolved splittings.

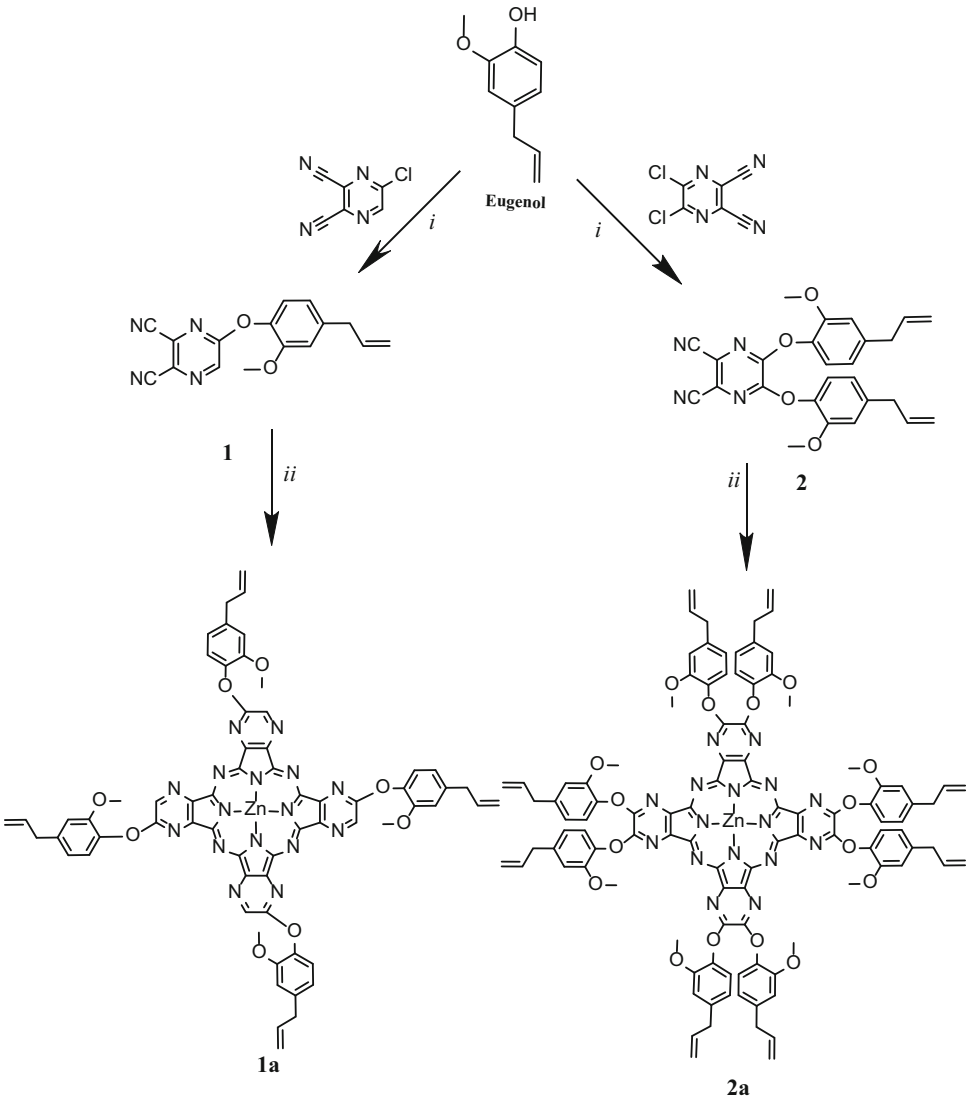
High resolution MS spectra (ESI-TOF) of compounds **1a** and **2a** provided definitive proof of their structures. Ionisation took place in DMSO solution. Molecular ion peaks of compounds **1a** and **2a** were detected. MS spectrum measurements confirmed unambiguously the molecular mass of compounds **1a** (m/z = 1234.30 M^+), and **2a** (m/z = 1882.94 M^+).

The synthesised AzaPcs (**1a** and **2a**) showed two strong absorption regions, one in the UV region between 355 and 365 nm (B band) and the other in the visible region between 635 and 630 nm (Q band) in DMSO, respectively. The UV–Vis spectra of the synthesised AzaPcs are shown in Figure 1.

UV–Vis spectra of eugenol-substituted ZnPcs (**3a** and **4a**) and ZnAzaPcs (**1a** and **2a**) show Q-bands at 680 and 630 nm, respectively. The blue shift of 50 nm for ZnAzaPc compared to ZnPc is ascribed to the additional N atoms in the AzaPc macrocycle.[29]

3.2 *Crystallographic analysis of compounds **1** and **2***

The molecular structure of compound **1** with atom labelling is indicated in Figure 2. The nitriles are equivalent and typical of N ≡ C triple bonds. The N ≡ C bond distances are 1.135(3) Å and 1.138(3) Å. The dihedral angle between pyrazine and phenyl rings is 74.76(5)°.



Scheme 1. Synthesis route of compounds **1, 2** and azaphthalocyanines **1a** and **2a:** (i) Triethylamine/THF, 65°C, 24 h. (ii) Metal salt, 250°C, 20 min.

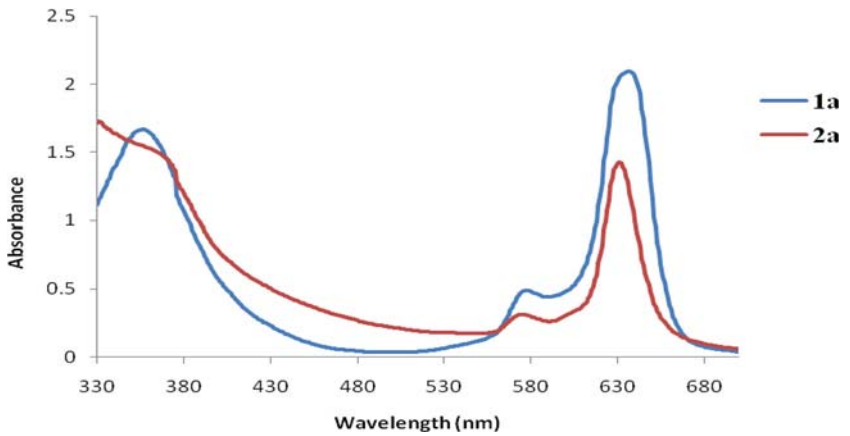


Figure 1. UV–Vis spectra of azaphthalocyanines **1a** and **2a** in DMSO (Concn.: 5×10^{-5} M, Optical path length: 10 mm).

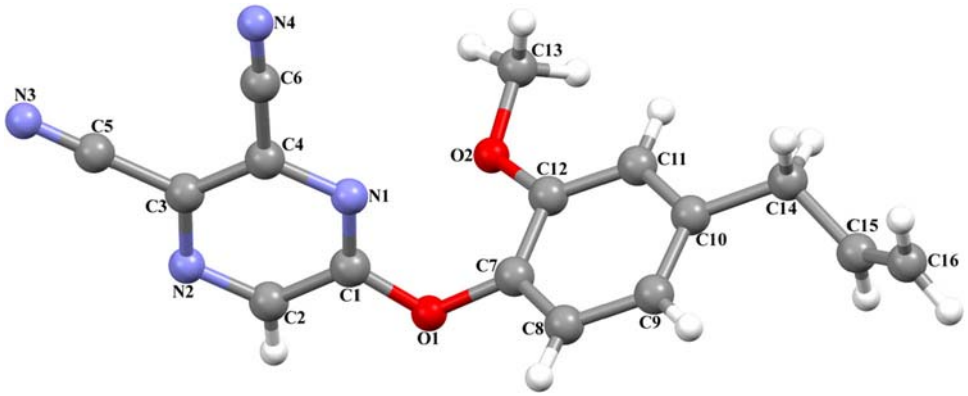


Figure 2. The structure of compound **1** with the atom numbering scheme.

Table 2. $\pi \cdots \pi$ interaction distances (Å) of compounds **1** and **2**.

Cg(I)	Cg(J)	Cg-Cg	Perpendicular distance
1			
Cg(1)	Cg(2)^{i}	3.555	3.379
2			
Cg(1)	Cg(1)^{i}	3.875 (4)	3.695
Cg(2)	Cg(2)^{ii}	3.903 (2)	3.604

Symmetry codes: (i) 3/2-x, 1/2+y, 3/2-z; Cg(1)=N1/C1/C2/N2/C3/C4; Cg(2)=C7-C12 for **1**; (i) 1-x, -y, 1-z; (ii) 1/2-x, 1/2-y, 1-z; Cg(1)=C1-C6; Cg(2)=C14-C19 for compound **2**.

The pyrazine ring plane is approximately planar, with maximum deviation from the least-squares plane being 0.0040(13) Å for atom C1. The molecules in the crystal of compound **1** are connected by $\pi \cdots \pi$ interactions. An intermolecular $\pi \cdots \pi$ contact occurs between pyrazine and phenyl rings of neighbouring molecules (Figure 3). The distance between the ring centroids is 3.379 Å. Details of this interaction are presented in Table 2.

The molecular structure of **2** with atom labelling is shown in Figure 4. The N ≡ C bond distances are 1.127(5) Å and 1.127(13) Å. The C-N bond distances range from 1.298(5) to 1.354(5) Å. The pyrazine ring forms dihedral angles of 75.71(16) and 88.57(23)° with the two phenyl rings. The dihedral angle of the phenyl rings is 20.41(42)°. The pyrazine ring plane is approximately planar, with maximum deviation from the least-squares plane being 0.0226(23) Å for atom C8. The molecules in the crystal of compound **2** are linked into sheets by a combination of C-H $\cdots \pi$ and $\pi \cdots \pi$ interactions. Atom C7 in the molecule (at x, y, z) acts as a H-bond donor to the C1 ∼ C6 phenyl ring in the molecule (at 1-x, 1-y, 1-z), forming a centrosymmetric $R_2^2(10)$ ring centred at 1/2,1/2,1/2. Compound **2** also contains two $\pi \cdots \pi$ interactions. An intermolecular $\pi \cdots \pi$ contact occurs between the two symmetry-related phenyl rings of neighbouring molecules (Figure 5). The distances between the ring centroids are 3.604 and 3.695 Å. Details of these interactions are presented in Table 2.

3.3 *DNA-binding assay analysis*

The absorption spectrum of the compound **2a** in the presence and absence of CT-DNA was assayed to determine the binding mode of Pc with DNA. A molecule can bind to DNA *via* intercalation, electrostatic or groove binding. In general, DNA intercalative binder agents result in large shifts in wavelength because of π-stacking interactions between aromatic chromophore groups of DNA and binder while DNA stacking agents or groove binders create small shifts in absorbance wavelengths in the spectra.[30] The absorption spectra span the range of 250 to 900 nm. The absorption spectrum of compound **2a** (Figure 6) showed small hypochromic shift by increasing the concentration of CT-DNA. No red shift was observed. The small hypochromic shift endorses non-intercalative binding mode between DNA and compound **2a**. The apparent binding constant (K_b) values of compound **2a** was calculated as 9.2×10^4 M^{-1}. The small hypochromic shift and calculated value of K_b endorse non-intercalative binding mode between DNA and compound **2a**.

3.4 *Lipase inhibition assay analysis*

All the compounds were evaluated for their lipase activity. The lipase inhibition activity of the studied

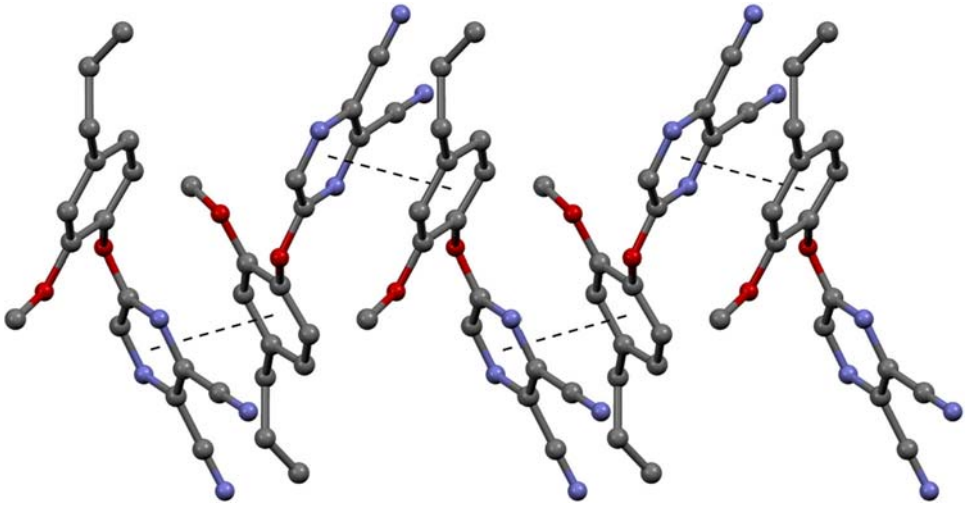


Figure 3. Showing the formation of a chain along [010] generated by $\pi \cdots \pi$ interactions as part of crystal structure of compound **1**.

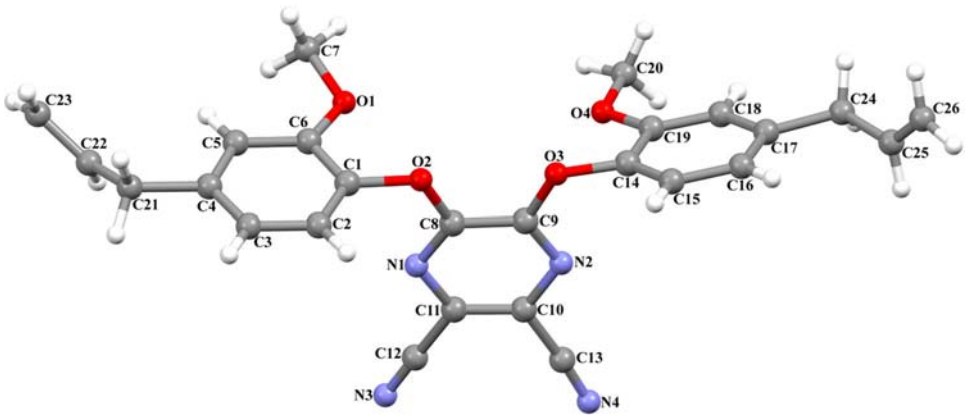


Figure 4. The structure of compound **2** with the atom numbering scheme.

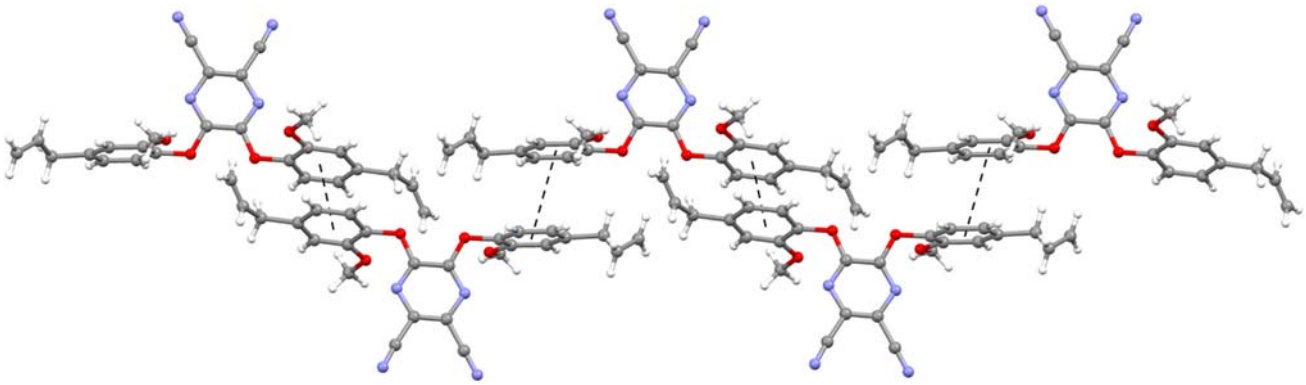


Figure 5. Showing the formation of a chain along [110] generated by $\pi \cdots \pi$ interactions as part of crystal structure of compound **2**.

compounds was estimated using a spectrophotometric test, with *p*NPP as the substrate. The lipase inhibition was not observed for all starting compounds (**1**–**4**). The compounds **1a, 2a**, **3a**, and **4a** displayed potent PPL inhibition in a dose-dependent manner, with IC_{50} values of 8.01 ± 0.52, 3.20 ± 0.03, 2.15 ± 0.21 and

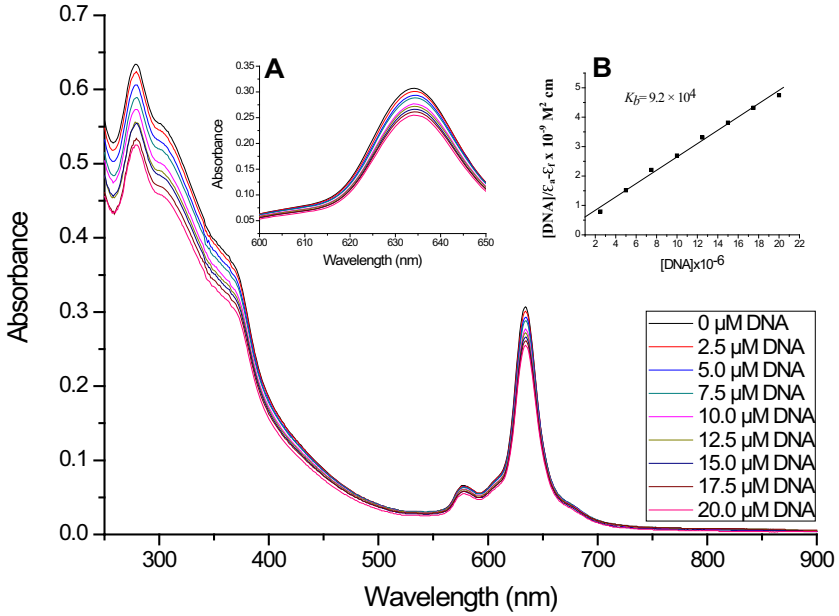


Figure 6. Absorption spectra (top) for the titration of compound **2a** by increasing concentrations of CT-DNA (pH 7.4 in a 10 mM Tris HCl buffer containing 50 mM NaCl, 5% DMSO). Inset: (A) Magnified plot of titration of compound **2a** with as indicated above. (B) The linear fit using eq. 1.

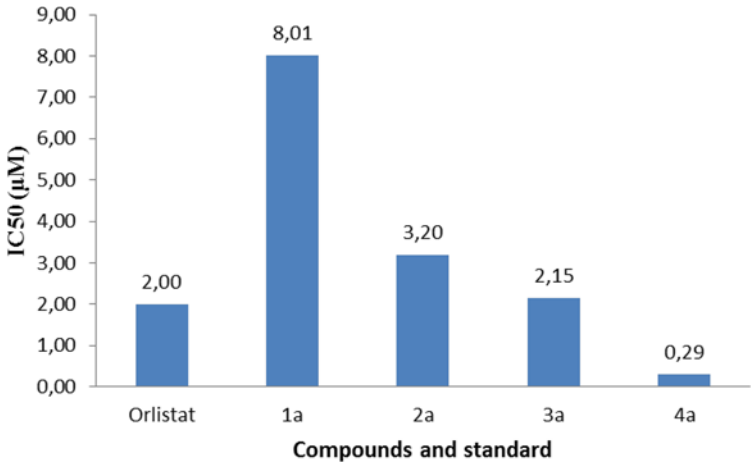


Figure 7. IC_{50} values of compounds (**1a, 2a, 3a, 4a**) and orlistat.

0.29 ± 0.03 µM, respectively (Figure 7). The IC_{50} for orlistat was 2.00 µM. IC_{50} is the concentration of tested compound required for 50% inhibition of the PPL activity. In particular, compound **4a** inhibited the PPL to a greater extent than the standard inhibitor, orlistat. The active site of the lipase is covered with an amphipathic lid, which creates a hydrophobic environment around the active residue.[31] The opening of this flap extends the hydrophobic surface around the active site. The hydrophobic surface surrounding the enzyme facilitates penetration of substrate molecules into the active site. This hydrophobic region binds them to the water–triglyceride interface, where the triglyceride can partition into the active site.[32] The inhibition observed may be due to non-covalent interactions between Pc molecules with the amphipathic lid or hydrophobic surface of lipase. This interpretation is supported by the hydrophobicity order of the Pc molecules: **1a**<**2a**<**3a**<**4a**. It is important and valuable that phthalocyanine molecules have better inhibitory properties than the starting compounds on lipase enzyme.

4. Conclusions

The synthesis and characterisation of peripheral tetra- and octa-eugenol-substituted novel ZnAzaPcs were accomplished. Spectroscopic and porcine pancreatic lipase inhibitor properties of the novel AzaPcs (**1a** and **2a**) were compared to the previously reported properties for eugenol-substituted Pc compounds **3a** and **4a**. Pc compounds (**3a** and **4a**) inhibited lipase to a greater extent than the AzaPcs (**1a** and **2a**) compounds. When compared to orlistat, compound **4a** exhibited potent PPL inhibitory activity in a dose-dependent manner.

Supplementary Information (SI)

Crystallographic data for the structural analysis have been deposited with the Cambridge Crystallographic Data Centre, CCDC No. 1058245 for compound **1**, and 1024371 for compound **2**. Copies of this information may be obtained free of charge from the Director, CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: +44-1223-336033; e-mail: deposit@ccdc.cam.ac.uk or www: http://www.ccdc.cam.ac.uk). All additional information, IR spectra (Figures S1, S4, S7, S10), 1H NMR, 13C MNR spectra (Figures S2, S3, S5, S6, S8 and S11), MS (Figures S9 and S12), synthesis route of compounds **3, 4** and phthalocyanines **3a** and **4a** (Figure S13) are given in the Supplementary Information, which is available at www.ias.ac.in/chemsci.

Acknowledgements

This study was supported by Recep Tayyip Erdogan University under Project No: 2014.102.02.04.

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