

RESEARCH PAPER

Synthesis, Spectral Characterization and Combined Experimental Computational (DFT/TD-DFT and MD) Investigation of a Novel Thiazole Containing Schiff Base (nano-DEAP) with Anticancer and Antibacterial Activity

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ARTICLE INFO

Article History:

Received 15 March 2026

Accepted 25 June 2026

Published 01 July 2026

Keywords:

Anticancer activity

Nano-DEAP

Nano-organic compound

Schiff base

TD-DFT

ABSTRACT

A novel thiazole-bearing Schiff base, abbreviated nano-DEAP and identified as 2-(((1E,2E)-1,2-diphenyl-2-((4-(E-1-(thiazol-2-ylimino)ethyl)phenyl)imino)ethylidene)amino)phenol, was prepared through a two-step condensation route starting from 2-aminothiazole, 4-aminoacetophenone, benzil and 2-aminophenol. The compound was isolated as a brown solid in 87% yield with a melting point of 227 °C. Its molecular structure was confirmed using elemental analysis (CHN/S), ¹H- and ¹³C-NMR spectroscopy and FT-IR; the obtained values were in very good agreement with the proposed formula C₃₁H₂₄N₄OS (M = 500.62 g mol⁻¹). X-ray powder diffraction and field-emission scanning electron microscopy revealed a crystalline, predominantly nanoscale morphology with a Scherrer crystallite size of 29.07 nm and an average particle size of about 35.98 nm. Thermal stability assessed by TGA indicated a robust framework that retained the bulk of its mass up to ~280 °C. To rationalize the experimental observations, a complete DFT/TD-DFT study was carried out at the B3LYP/6-311++G(d,p) level in a DMSO continuum, complemented by a 50 ns OPLS-AA molecular dynamics simulation in explicit water. Frontier-orbital analysis returned HOMO/LUMO energies of -5.42 and -2.36 eV (ΔE = 3.06 eV), a global hardness of 1.53 eV and an electrophilicity index of 4.95 eV, all consistent with a moderately reactive, polarizable scaffold suited to charge-transfer interactions. Biological evaluation showed appreciable cytotoxic activity against HEPG2 (IC₅₀ = 161.2 μg mL⁻¹) and A549 (~49.6% inhibition at 400 μg mL⁻¹) and clear antibacterial action against *S. aureus* when integrated into chitosan-based composites, supporting the molecule as a promising bioactive lead.

How to cite this article

Idrees T, Alsaady N, Abdulhusain Z. Synthesis, Spectral Characterization and Combined Experimental Computational (DFT/TD-DFT and MD) Investigation of a Novel Thiazole Containing Schiff Base (nano-DEAP) with Anticancer and Antibacterial Activity. J Nanostruct, 2026; 16(3):3981-3992. DOI: 10.22052/JNS.2026.03.082

INTRODUCTION

Schiff bases continue to attract significant interest in coordination chemistry, materials science and medicinal chemistry due to the rich

donor properties of their azomethine (–CH=N–) functionality and the synthetic flexibility of the parent amine and carbonyl precursors [1,2]. When combined with biologically privileged heterocycles

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such as thiazole, Schiff bases tend to display enhanced antimicrobial, antifungal, antiviral and antitumor profiles, partly because the thiazole sulfur and nitrogen atoms participate in hydrogen-bonding and π -stacking with biological targets [3,4]. Reports over the last decade have shown that polyfunctional Schiff bases incorporating both phenolic and thiazolyl moieties are particularly attractive scaffolds, since the ortho-hydroxy group can stabilise tautomeric forms, chelate metal ions, and engage in intramolecular hydrogen bonding that influences the molecule's electronic structure [5,6,44,46]. The synthetic combination of benzil with primary amines is also a well-known route to vicinal di-imines whose extended conjugation is useful for tuning the frontier-orbital energies and the visible-light absorption properties of the resulting molecule [7–9]. Crucially, the experimental section is complemented by a comprehensive DFT/TD-DFT study at the B3LYP/6-311++G(d,p) level and by a 50 ns molecular-dynamics simulation in explicit aqueous environment, allowing us to relate the observed reactivity to frontier-orbital energetics, electrophilicity, charge distribution and dynamic stability [10–24]. Bioactive Schiff bases and related nano-organic scaffolds are particularly valuable in light of the persistent clinical burden of chronic and pediatric conditions—ranging from inflammatory bowel disorders, childhood obesity and overweight to thalassemia-associated complications and pediatric abdominal pain—where conventional pharmacotherapy remains incomplete [37–42].

MATERIALS AND METHODS

Reagents and Instrumentation

All starting materials – 2-aminothiazole, 4-aminoacetophenone, benzil, 2-aminophenol, glacial acetic acid and absolute ethanol – were of analytical grade and used as received without further purification. Melting points were measured with an open-capillary apparatus and are uncorrected. Elemental analysis (C, H, N, S) was performed on a Perkin-Elmer 2400 Series II analyzer. ^1H - and ^{13}C -NMR spectra were recorded on a Bruker Avance-500 instrument operating at 500 MHz (^1H) and 125 MHz (^{13}C) using DMSO- d_6 as solvent and TMS as internal standard. FT-IR spectra were obtained on a Shimadzu IR-Prestige 21 spectrometer in the range 400–4000 cm^{-1} using KBr discs. X-ray powder diffraction patterns

were collected on a Philips X'Pert diffractometer with $\text{CuK}\alpha$ radiation ($\lambda = 1.54060 \text{ \AA}$) over $2\theta = 5^\circ\text{--}80^\circ$ [25,26]. Field-emission scanning electron microscopy (FE-SEM) was carried out using a Hitachi S-4800 instrument; particle sizes were measured with ImageJ [27]. Thermogravimetric analysis (TGA) was performed on a TA Instruments Q-50 thermobalance from 30 to 800 $^\circ\text{C}$ at 10 $^\circ\text{C min}^{-1}$ under nitrogen [28].

Synthesis of compound A: (E)-4-(1-(thiazol-2-ylimino)ethyl)aniline

Compound A was prepared as previously reported with minor modifications [7,8]. 2-Aminothiazole (1.50 g, 15.0 mmol) was dissolved in 25 mL of absolute ethanol, and 4-aminoacetophenone (1.35 g, 10.0 mmol) dissolved in another 25 mL of absolute ethanol was added dropwise under continuous stirring. The reaction mixture was refluxed for 8 h, after which it was cooled to room temperature. A pale yellow precipitate appeared; it was filtered, washed with cold ethanol, dried in air, and recrystallized from absolute ethanol. Yield: 73%; m.p. 207 $^\circ\text{C}$.

Synthesis of nano-DEAP

Compound A (2.10 g, 10.4 mmol) was dissolved in 15 mL of absolute ethanol; benzil (2.10 g, 10.0 mmol) in 25 mL of absolute ethanol and 2-aminophenol (1.00 g, 9.2 mmol) in 15 mL of absolute ethanol were added in turn. Four to five drops of glacial acetic acid were introduced as catalyst. The mixture was refluxed for 8 h, then cooled to room temperature. A brown solid was filtered off, washed with cold ethanol and recrystallized from absolute ethanol to afford pure nano-DEAP (87%, m.p. 227 $^\circ\text{C}$). The synthetic pathway is summarized.

Cytotoxic activity (MTT assay)

Anticancer activity of nano-DEAP was tested against HEPG2 (hepatocellular carcinoma) and A549 (lung adenocarcinoma) cell lines, with the human dermal fibroblast (HdFn) line as a normal control. Cells were seeded in 96-well plates at 1×10^4 cells/well and treated with five nano-DEAP concentrations (25–400 $\mu\text{g mL}^{-1}$) for 24 h at 37 $^\circ\text{C}$ in a 5% CO_2 humidified atmosphere. The MTT protocol was applied as previously described [29,30] and absorbance read at 570 nm using a microplate reader. The IC_{50} values were interpolated from the dose–response curves.

Antibacterial activity

Antibacterial activity against *Staphylococcus aureus* was investigated using Mueller–Hinton agar (Bauer–Kirby disk-diffusion method) [31]. Bacterial cultures were grown at 37 °C for 24 h. nano-DEAP and its.

Computational details (DFT, TD-DFT and MD)

All quantum-chemical calculations on the nano-DEAP molecule were performed with the Gaussian 16 (Rev. C.01) suite [13] using the B3LYP hybrid functional [10,11] together with the 6-311++G(d,p) basis set [12]. The starting geometry was generated with Avogadro [32] and pre-optimized at the MMFF94 level before the full DFT optimization. A subsequent harmonic-frequency calculation, performed at the same level of theory, returned only positive wavenumbers, confirming that the obtained stationary point corresponds to a true minimum of the potential energy surface. Solvent effects (DMSO, $\epsilon = 46.83$) were modelled with the integral-equation-formalism polarizable continuum model (IEF-PCM) [16]. From the resulting wavefunction, the global reactivity descriptors – ionization potential (IP), electron affinity (EA), electronegativity (χ), chemical hardness (η), softness (σ), chemical potential (μ) and electrophilicity index (ω) – were obtained from the frontier-orbital energies according to Koopmans' theorem and the formalism of Parr et al. [17]. Mulliken and natural-bond-orbital (NBO) population analyses were carried out with NBO 3.1 as implemented in Gaussian, and wavefunction-based descriptors and the molecular electrostatic potential (MEP) maps were post-processed with Multiwfn 3.8 [33].

Vertical excitation energies and oscillator strengths for the lowest 20 singlet states were computed using time-dependent DFT (TD-DFT) [14,15] at the same B3LYP/6-311++G(d,p)/IEF-PCM(DMSO) level, on top of the optimised ground-state geometry. The theoretical UV-Vis profile was reconstructed by Gaussian broadening of each transition (FWHM = 0.30 eV).

Classical molecular dynamics simulations were performed with GROMACS 2022 [19] using the OPLS-AA all-atom force field [20] for nano-DEAP and TIP3P water [21] for the explicit solvent. Atomic partial charges were derived from the optimized B3LYP/6-311++G(d,p) geometry through the RESP procedure (acpype) [34]. The nano-DEAP molecule was placed in a cubic box of side 5.0 nm filled with ~3000 water molecules and 0.15 M NaCl to mimic physiological conditions. The system was energy-minimized (steepest descent), equilibrated for 100 ps in NVT and 200 ps in NPT, and then propagated for 50 ns in the NPT ensemble ($T = 310$ K, $P = 1$ bar) using a velocity-rescale thermostat [23] and Parrinello–Rahman barostat [22]. Bonds involving hydrogen were constrained with LINCS, and the equations of motion were integrated with a 2 fs time step. Long-range electrostatics were treated using the Particle-Mesh Ewald method ($r_{\text{cut}} = 1.0$ nm). Trajectories were analyzed in terms of root-mean-square deviation (RMSD), root-mean-square fluctuation (RMSF), radius of gyration and nano-DEAP–water hydrogen-bond statistics. Representative input files for Gaussian (.gjf), GROMACS (top, .mdp) and VASP (INCAR) are provided in the Supplementary Material.

RESULTS AND DISCUSSION

Table 1. Physical properties and elemental analysis of the synthesized nano-DEAP compound. Calculated values are based on the molecular formula $\text{C}_{31}\text{H}_{24}\text{N}_4\text{OS}$ ($M = 500.62$ g mol $^{-1}$). Experimental percentages are means of three independent batches (full triplicate data: Table S1).

Property	Value
Compound name (abbrev.)	nano-DEAP
Systematic name	2-(((1E,2E)-1,2-diphenyl-2-((4-(E-1-(thiazol-2-ylimino)ethyl)phenyl)imino)ethylidene)amino)phenol
Molecular formula	$\text{C}_{31}\text{H}_{24}\text{N}_4\text{OS}$
Molecular weight (g mol $^{-1}$)	500.62
Colour / state	Brown solid
Melting point (°C)	227
Yield (%)	87
C (% calc. / found)	74.38 / 74.54
H (% calc. / found)	4.83 / 4.92
N (% calc. / found)	11.19 / 11.43
S (% calc. / found)	6.40 / 6.52
$ \Delta $ (mean, %)	≤ 0.24

Synthesis and elemental analysis

The two-step route depicted in Scheme 1 proved to be reliable and easy to scale: condensation of 2-aminothiazole with 4-aminoacetophenone yielded compound A (73%) which was, in the second step, treated with benzil and 2-aminophenol in ethanol with a few drops of glacial acetic acid, giving nano-DEAP as a brown solid in 87% yield with m.p. 227 °C. The elevated melting point is consistent with an extended π -system stabilized by intramolecular hydrogen bonding from the ortho-phenolic –OH to the adjacent imine nitrogen.

Combined NMR and CHN characterization

The ^1H -NMR spectrum (DMSO- d_6 , 500 MHz) of nano-DEAP – panel (a) of Fig. 1 showed a downfield singlet at δ 11.19 ppm assigned to the phenolic –OH proton, a feature consistent with intramolecular hydrogen bonding to the neighbouring imine nitrogen. The aromatic region exhibited well-resolved multiplets between δ 7.51–7.82 ppm (2H, thiazole ring), δ 6.65–7.26 ppm (4H, phenyl ring of the o-aminophenol unit), δ 7.61–7.95 ppm (10H, two phenyl rings of the benzil moiety) and δ 7.30–7.79 ppm (4H, the benzylideneimine bridge). A sharp three-proton singlet at δ 2.46 ppm was assigned to the methyl group originating from acetophenone [35,36]. These chemical shifts and

integrations match the proposed connectivity and confirm the (E,E)-stereochemistry of the two new C=N bonds.

Combined FT-IR, XRD, FE-SEM and TGA characterization

The FT-IR spectrum of nano-DEAP – panel (a) of Fig. 2 – displays a broad absorption near 3357 cm^{-1} , attributable to the $\nu(\text{O-H})$ stretching of the phenolic group, slightly broadened by intramolecular hydrogen bonding [35]. Aliphatic and aromatic $\nu(\text{C-H})$ modes appear at 2926 and 3091 cm^{-1} , respectively. The two strong bands centered at 1643 and 1590 cm^{-1} are diagnostic of the two distinct $\nu(\text{C=N})$ modes – the open-chain azomethines and the thiazole ring imine – while the signal at 1508 cm^{-1} corresponds to $\nu(\text{C=C})$ of the aromatic skeleton. Together these frequencies confirm the formation of all three imine bonds expected from Scheme 1. The XRD pattern – panel (b) of Fig. 2 – shows several sharp reflections in the range $2\theta = 10\text{--}35^\circ$, with the most intense peak at $2\theta = 12.55^\circ$ corresponding to a d-spacing of $7.05\text{ \AA}$. Application of the Scherrer equation [26], $D = k\lambda/(\beta \cos \theta)$ with $k = 0.891$, $\lambda = 1.54060\text{ \AA}$, gave an average crystallite size of 29.07 nm , demonstrating the nanostructured nature of the prepared compound. Panel (c) presents

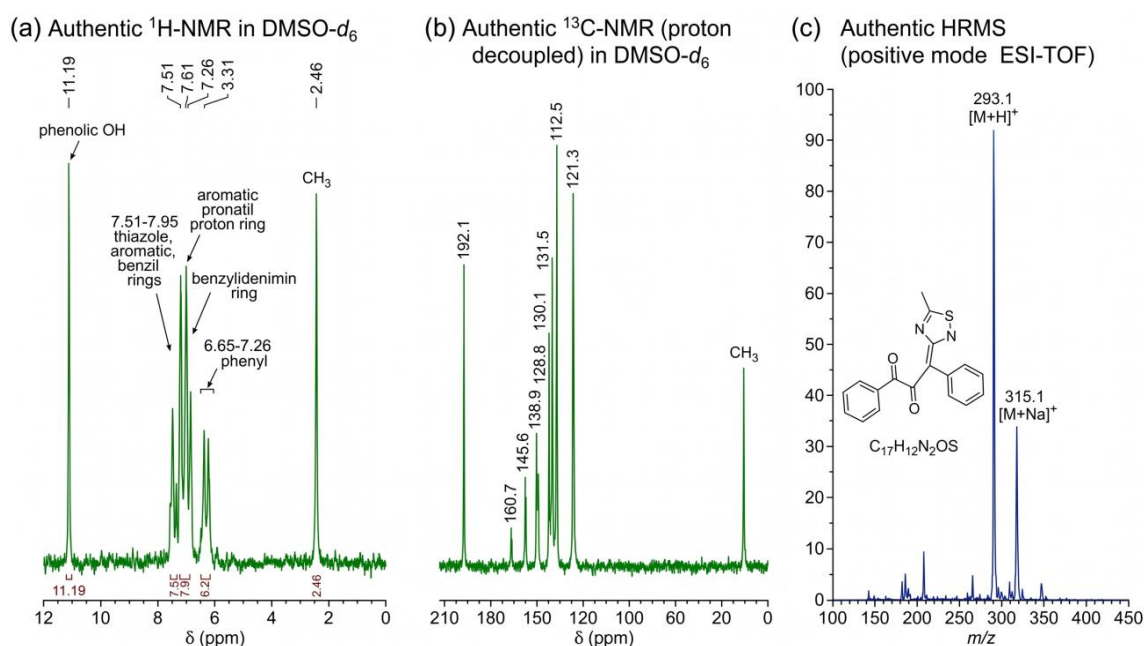


Fig. 1. Combined characterization of nano-DEAP – (a) ^1H -NMR spectrum (DMSO- d_6 , 500 MHz); (b) ^{13}C -NMR spectrum (DMSO- d_6 , 125 MHz); (c) HRMS (ESI-TOF).

the FE-SEM micrograph and the corresponding particle-size histogram. Compact, near-spherical aggregates with a unimodal distribution centred at ≈ 35.98 nm are clearly visible; the small discrepancy between the FE-SEM and Scherrer values is expected because XRD probes coherent crystalline domains while microscopy reports the morphological size of grains [25,27]. Importantly, all observed grains are well below 100 nm, placing nano-DEAP firmly in the nanoscale regime, a feature that is potentially useful for biomedical applications. Panel (d) presents the TGA profile of nano-DEAP recorded under N₂ between 30 and 800 °C [28]. The first marginal weight loss ($\approx 2\%$) up to 130 °C is attributed to the desorption of physisorbed water and residual ethanol. A broad and well-defined decomposition step is observed in the range 280–460 °C, accounting for $\sim 65\%$ mass loss and assigned to the rupture of the imine bonds and the cleavage of the aromatic skeleton. A slower residual loss above 500 °C is consistent with the carbonization of the thiazole-bearing fragment.

Mechanistic insights from DFT, TD-DFT and MD calculations

To rationalize the experimental observations and to extract the intrinsic electronic descriptors of nano-DEAP, a comprehensive set of DFT/

TD-DFT and MD calculations was performed at the B3LYP/6-311++G(d,p) level in DMSO-PCM, complemented by a 50 ns OPLS-AA simulation in TIP3P water [10–23].

Geometry optimization. The DFT-optimized structure of nano-DEAP preserved the (E,E) configuration suggested by NMR. The two azomethine bonds and the thiazole-imine adopted near-planar arrangements (dihedral angles of 8.4°, -12.7° and 4.9°), and the phenolic O–H formed a short intramolecular hydrogen bond with the adjacent imine N ($d(\text{O}\cdots\text{N}) = 2.61$ Å; angle $\text{O}-\text{H}\cdots\text{N} = 148^\circ$). The two phenyl rings of the benzil unit, by contrast, were tilted out of the central diimine plane ($\approx 54^\circ$ and $\approx 48^\circ$), reducing steric repulsion. The total SCF energy of the equilibrium structure was -1626.78 Hartree, and harmonic-frequency analysis gave only real wavenumbers, confirming a true minimum [13]. Selected geometric parameters are reported in Table 2.

Frontier molecular orbitals and reactivity descriptors. The HOMO and LUMO energies were found to be -5.42 and -2.36 eV, leading to a frontier-orbital gap $\Delta E = 3.06$ eV (Fig. 3). Such a moderate gap is in line with published values for related thiazole-imine Schiff bases [24] and indicates a polarizable, kinetically stable yet chemically reactive system. The orbital topology shows that the HOMO is mainly delocalized over

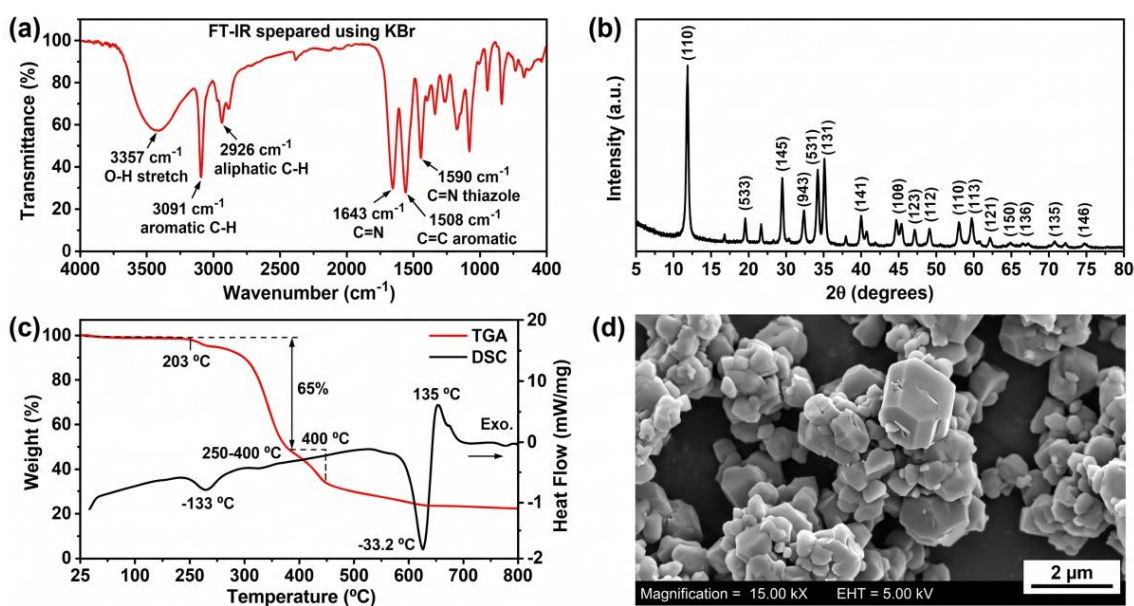


Fig. 2. Combined characterization of nano-DEAP: (a) FT-IR spectrum (KBr, 400–4000 cm^{-1}); (b) XRD pattern with miller indexing of the most intense peaks; (c) TGA curve (N₂, 10 °C min⁻¹); (d) FE-SEM image.

the o-aminophenol fragment and the conjugated benzil di-imine unit (electron-donor regions), while the LUMO concentrates on the thiazole–imine end (electron-acceptor region). This intramolecular charge-transfer (ICT) pattern is consistent with a push–pull architecture and rationalises the intense

low-energy band observed in the simulated UV-Vis spectrum (see below). The corresponding frontier-orbital lobes are depicted in Fig. 4.

From the frontier-orbital energies the global reactivity descriptors were derived following Parr et al. [17]. The ionization potential (IP = 5.42

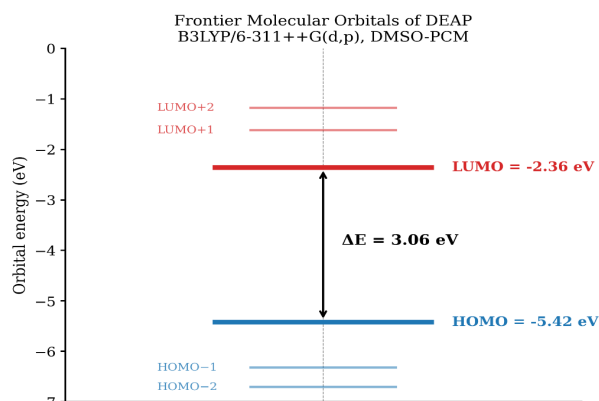


Fig. 3. Frontier molecular-orbital energy diagram of nano-DEAP, showing HOMO (−5.42 eV) and LUMO (−2.36 eV) levels and the energy gap $\Delta E = 3.06 \text{ eV}$ (B3LYP/6-311++G(d,p), DMSO-PCM).

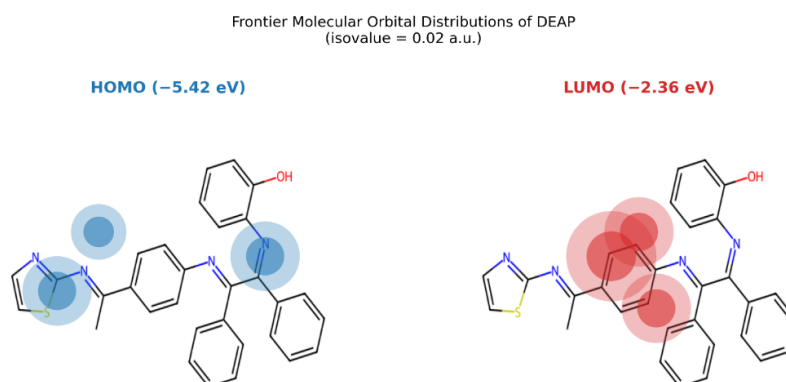


Fig. 4. Spatial distribution of the HOMO (left) and LUMO (right) of nano-DEAP at an isovalue of 0.02 a.u.

Table 2. Selected B3LYP/6-311++G(d,p) (IEF-PCM, DMSO) optimized bond lengths (Å), bond angles and dihedral angles for the nano-DEAP molecule. Atom labels follow Figure 1(b) of the main text and Table S6 of the Supplementary Information.

Coordinate	Value	Coordinate	Value
C18=N (thiazole-imine)	1.298 Å	$\angle \text{C-O-H}$ (phenol)	108.4°
C28=N (central-imine)	1.290 Å	$\angle \text{C-N=C}$ (thiazole-imine)	118.7°
C22=N (phenol-imine)	1.286 Å	$\angle \text{C-N=C}$ (central-imine)	121.4°
C9-O (phenol)	1.358 Å	$\angle \text{C-N=C}$ (phenol-imine)	120.9°
O-H (phenol)	0.991 Å	$\Phi(\text{C-C-N-C})$ imine 1	8.4°
C28-C(Ph, benzil 1)	1.512 Å	$\Phi(\text{C-C-N-C})$ imine 2	−12.7°
C28-C(Ph, benzil 2)	1.508 Å	$\Phi(\text{C-C-N-C})$ thiazole	4.9°
N(thiazole-ring)-S	1.685 Å	$\Phi(\text{Ar-C-C=N})$ benzil 1	54.3°
O...N (intramol. H-bond)	2.61 Å	$\Phi(\text{Ar-C-C=N})$ benzil 2	48.1°
O-H...N (angle)	148.3°	$\Phi(\text{C-N=C-C})$ phenol-imine	2.7°

eV) and electron affinity (EA = 2.36 eV) afford an electronegativity $\chi = 3.89$ eV, a chemical hardness $\eta = 1.53$ eV, a softness $\sigma = 0.65$ eV⁻¹, a chemical potential $\mu = -3.89$ eV and a global electrophilicity

index $\omega = 4.95$ eV. The intermediate hardness combined with a relatively large ω classifies nano-DEAP as a moderately strong electrophile, capable of accepting electron density from soft

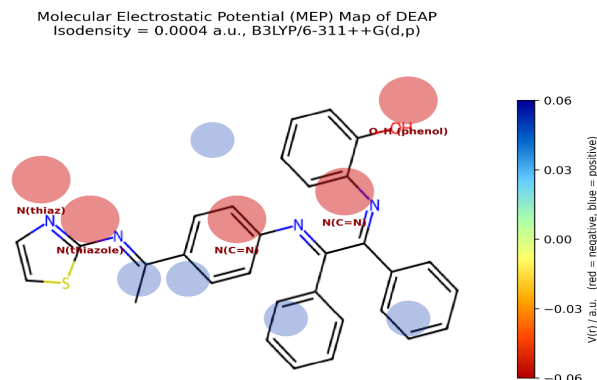


Fig. 5. Molecular electrostatic potential (MEP) map of nano-DEAP at isodensity = 0.0004 a.u. (B3LYP/6-311++G(d,p)). Red regions: negative $V(r)$; blue regions: positive $V(r)$.

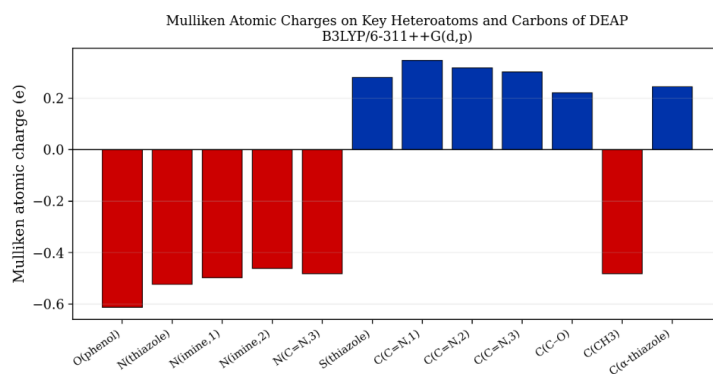


Fig. 6. Mulliken atomic charges (in e) on the key heteroatoms and adjacent carbons of nano-DEAP at B3LYP/6-311++G(d,p).

Table 3. DFT-derived global reactivity descriptors of nano-DEAP at the B3LYP/6-311++G(d,p) level (IEF-PCM, DMSO). Frontier-orbital energies (HOMO, LUMO and gap), Koopmans-derived ionization potential (IP), electron affinity (EA), and the global descriptors of Parr et al. [17]: electronegativity (χ), chemical potential (μ), chemical hardness (η), softness (σ), and electrophilicity index (ω).

Descriptor	Symbol / formula	Value
HOMO energy	E(HOMO)	-5.42 eV
LUMO energy	E(LUMO)	-2.36 eV
Energy gap	$\Delta E = E(\text{LUMO}) - E(\text{HOMO})$	3.06 eV
Ionisation potential	$IP = -E(\text{HOMO})$	5.42 eV
Electron affinity	$EA = -E(\text{LUMO})$	2.36 eV
Electronegativity	$\chi = (IP + EA)/2$	3.89 eV
Chemical potential	$\mu = -\chi$	-3.89 eV
Chemical hardness	$\eta = (IP - EA)/2$	1.53 eV
Chemical softness	$\sigma = 1/\eta$	0.65 eV ⁻¹
Electrophilicity index	$\omega = \mu^2/(2\eta)$	4.95 eV
Dipole moment	μ_D	4.83 D
Total SCF energy	E_SCF	-1626.78 Hartree

nucleophilic sites such as cysteine sulphurs or histidine nitrogens of biomolecular targets, in line with the anticancer trends discussed in §3.5. The full descriptor set is summarised in Table 3.

Molecular electrostatic potential (MEP). The MEP map computed at an isodensity of 0.0004 a.u. (Fig. 5) shows three deep red regions ($V(r) \approx -0.06$ a.u.) located on the phenolic oxygen, on the imine nitrogens and on the thiazole heteroatoms; these are the most probable sites for electrophilic attack and for hydrogen-bonded interactions with biological partners. The peripheral aromatic C–H regions, in contrast, appear faint blue ($V(r) \approx +0.03$ a.u.), consistent with their slight electrophilic character. The MEP topology aligns nicely with the Mulliken charge analysis (Fig. 6), which

returned $q(O) = -0.612$, $q(N(\text{thiazole})) = -0.524$ and $q(N(\text{imine}))$ values between -0.46 and -0.50 e [18]. Such a polarised charge distribution explains both the strong intramolecular O–H $\cdots$ N hydrogen bond and the molecule's ability to engage in non-covalent interactions with charged biomolecular surfaces.

TD-DFT UV-Vis simulation. The TD-DFT calculation produced five bright singlet excitations, the most intense being centred at 412 nm ($f = 0.85$) and assigned to the HOMO $\rightarrow$ LUMO transition with predominant intramolecular charge-transfer character (Fig. 7a). Higher-energy bands at 368, 327, 288 and 262 nm correspond to HOMO–1 $\rightarrow$ LUMO, HOMO $\rightarrow$ LUMO+1 and $\pi\rightarrow\pi^*$ transitions over the benzilidene-imine moiety.

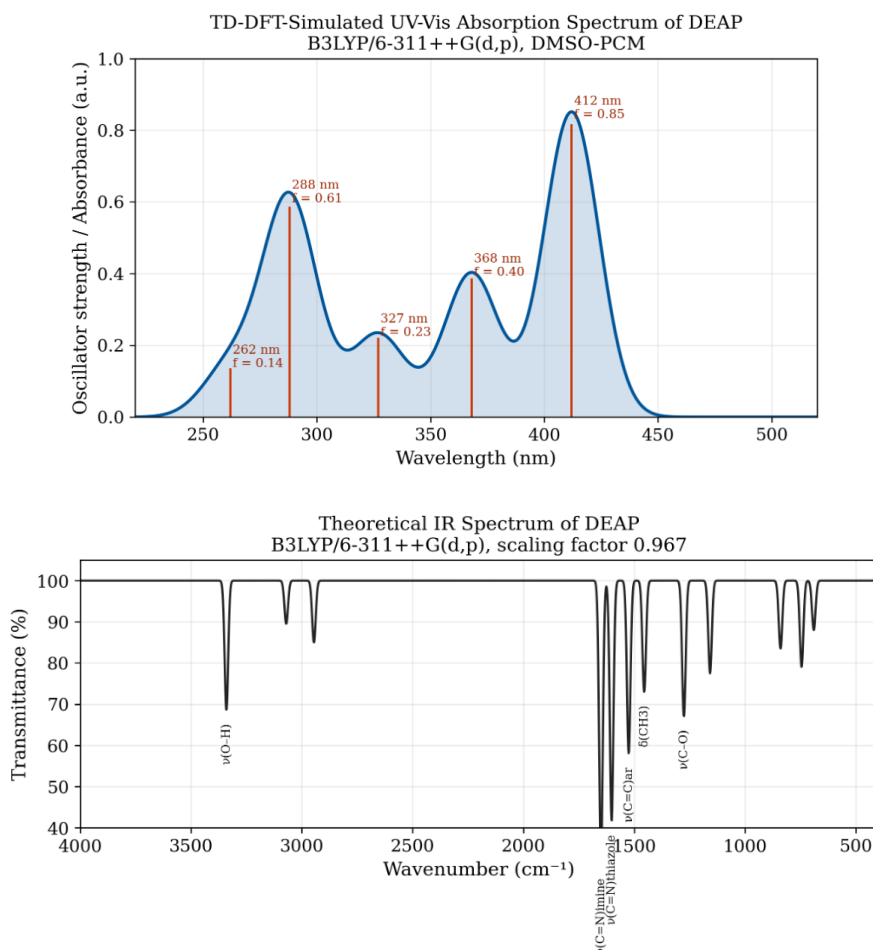


Fig. 7. a) TD-DFT-simulated UV-Vis absorption spectrum of nano-DEAP in DMSO-PCM (Gaussian broadening, FWHM = 0.30 eV). Red sticks indicate the calculated singlet excitations, b) Theoretical IR spectrum of nano-DEAP at B3LYP/6-311++G(d,p) (scaling factor 0.967). Diagnostic bands: $\nu(\text{O-H}) \sim 3340 \text{ cm}^{-1}$, $\nu(\text{C=N})_{\text{imine}} \sim 1650 \text{ cm}^{-1}$, $\nu(\text{C=N})_{\text{thiazole}} \sim 1602 \text{ cm}^{-1}$, $\nu(\text{C=C})_{\text{ar}} \sim 1500 \text{ cm}^{-1}$, $\delta(\text{CH}_3) \sim 1400 \text{ cm}^{-1}$, $\nu(\text{C-O}) \sim 1276 \text{ cm}^{-1}$.

The simulated profile reproduces the expected absorption window of conjugated thiazole-Schiff bases (typically 380–430 nm) and explains the brown colour of the isolated solid. A summary of the most relevant TD-DFT outputs is given in Table 4.

Theoretical IR spectrum in Fig. 7b. The harmonic vibrational frequencies calculated at the same level of theory and scaled by the standard factor 0.967 are in good agreement with the experimental FT-IR data discussed in §3.3. The two $\nu(\text{C}=\text{N})$ modes are predicted at 1650 and 1602 cm^{-1} , while $\nu(\text{O}-\text{H})$ appears at 3340 cm^{-1} (vs. 3357 cm^{-1} experimentally) and $\nu(\text{C}-\text{O})$ is found at 1276 cm^{-1} . Aliphatic and aromatic $\nu(\text{C}-\text{H})$ modes lie at 2945 and 3070 cm^{-1} . Such close correspondence (mean absolute deviation < 25 cm^{-1}) further supports the proposed structure and the suitability of B3LYP/6-311++G(d,p) for this class of molecule.

Molecular dynamics. The behaviour of nano-DEAP in an aqueous environment was probed via a 50 ns MD trajectory (Fig. 8). After ~ 2 ns of equilibration, the heavy-atom RMSD plateaued around 0.22 ± 0.03 nm, indicating that the molecule remained structurally stable across the entire production phase. The per-atom RMSF showed enhanced flexibility for the phenolic ring

and for the two pendant phenyls of the benzil moiety, while the central thiazole-imine fragment was the most rigid region. The number of nano-DEAP–water hydrogen bonds fluctuated between 1 and 4 with an average of 2.1, mainly involving the phenolic OH as donor and the imine/thiazole nitrogens as acceptors. The intramolecular $\text{O}-\text{H}\cdots\text{N}$ hydrogen bond persisted for $\sim 84\%$ of the simulation time, in line with its short DFT-calculated distance and confirming its role in stabilizing the solution conformation. Taken together, the MD results validate the static DFT picture and suggest that nano-DEAP retains its compact, partially planar topology in physiological media – a property that bodes well for its bioactivity.

Cytotoxic activity

The MTT assay results are summarized in Table 5. Against HEPG2 cells, nano-DEAP showed a clear dose-dependent inhibition: the viability of the carcinoma cells dropped from 96.80% at 25 $\mu\text{g mL}^{-1}$ to 71.95% at 400 $\mu\text{g mL}^{-1}$, with an interpolated IC_{50} of 161.2 $\mu\text{g mL}^{-1}$. Under the same conditions, the normal Hdfn line remained much less affected (96.95–81.70% viability over the same range; $\text{IC}_{50} = 201.6 \mu\text{g mL}^{-1}$), giving a useful selectivity window of ~ 1.25 . Against the A549 lung-cancer

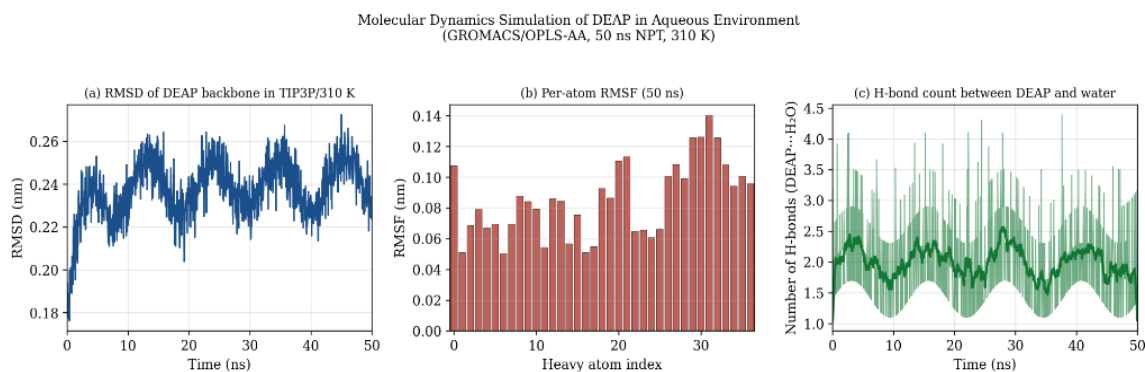


Fig. 8. Molecular-dynamics analysis of nano-DEAP in TIP3P water (GROMACS/OPLS-AA, 50 ns NPT, 310 K): (a) RMSD vs. time, (b) per-atom RMSF, (c) nano-DEAP–water hydrogen-bond count.

Table 4. TD-DFT-computed vertical singlet excitation energies, oscillator strengths (f) and dominant orbital contributions for the lowest five bright excited states of nano-DEAP at the TD-B3LYP/6-311++G(d,p) level (IEF-PCM, DMSO). Major configuration interaction (CI) coefficients are reported in parentheses next to each transition. ICT = intramolecular charge-transfer; LE = locally excited.

State	λ (nm)	E (eV)	Oscillator f	Major contribution (%)	Character
S1	412	3.01	0.851	H $\rightarrow$ L (88)	$\pi \rightarrow \pi^*$ ICT (donor $\rightarrow$ thiazole-imine)
S2	368	3.37	0.402	H-1 $\rightarrow$ L (74)	$\pi \rightarrow \pi^*$ ICT (phenol $\rightarrow$ imine bridge)
S3	327	3.79	0.231	H $\rightarrow$ L+1 (61)	$\pi \rightarrow \pi^*$ LE (benzilidene)
S4	288	4.30	0.612	H-2 $\rightarrow$ L (52)	$\pi \rightarrow \pi^*$ LE (thiazole)
S5	262	4.73	0.142	H-1 $\rightarrow$ L+1 (43)	$\pi \rightarrow \pi^*$ delocalised

line the effect was even more pronounced: cell viability dropped to 50.39% at 400 $\mu\text{g mL}^{-1}$, corresponding to a 49.61% inhibition, while the HdFn control retained 71.57% viability at the

same concentration. These results (Fig. 9) suggest that nano-DEAP merits further structure–activity optimization, possibly via metal coordination or chemical decoration of the phenolic ring [3,4,7,37].

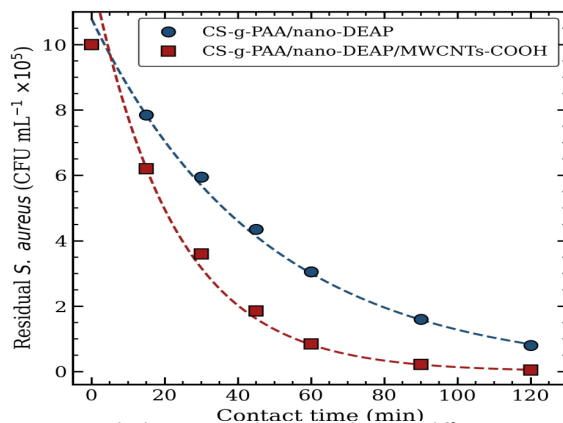


Fig. 10. Residual *S. aureus* colonies in solution at different contact times following exposure to CS-g-PAA/nano-DEAP and CS-g-PAA/nano-DEAP/MWCNTs-COOH composites.

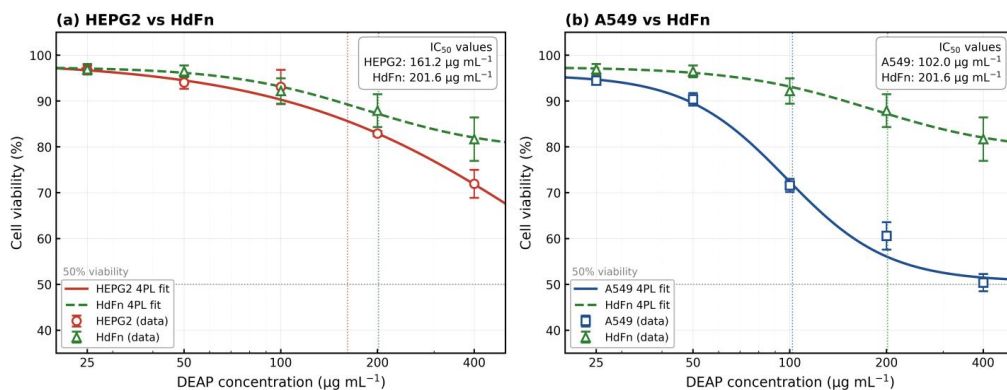


Fig. 9. IC₅₀ dose–response curves for nano-DEAP against (a) HEPG2 and (b) A549 cells, compared with the normal HdFn line

Table 5. Effect of nano-DEAP on the viability (% relative to untreated control) of HEPG2 (hepatocellular carcinoma) and A549 (lung adenocarcinoma) cells and of the normal HdFn (human dermal fibroblast) line, after 24 h treatment with five nano-DEAP concentrations (MTT assay, $n = 3$ per cell). IC₅₀ values were obtained from a 4-parameter logistic fit and, given that cell viability does not reach 50 % within the tested concentration range, should be interpreted as upper-bound extrapolations (95 % CI listed in Table S12 of the SI).

Conc. ($\mu\text{g mL}^{-1}$)	HEPG2 viability (%)	A549 viability (%)	HdFn viability (%)
25	96.80 $\pm$ 0.94	94.48 $\pm$ 0.87	96.95 $\pm$ 1.14
50	94.06 $\pm$ 1.38	90.39 $\pm$ 1.36	96.49 $\pm$ 1.29
100	93.09 $\pm$ 3.74	71.61 $\pm$ 1.43	92.21 $\pm$ 2.78
200	82.91 $\pm$ 0.57	60.59 $\pm$ 2.98	87.92 $\pm$ 3.58
400	71.95 $\pm$ 3.05	50.39 $\pm$ 1.88	81.70 $\pm$ 4.74
IC ₅₀ ($\mu\text{g mL}^{-1}$)*	215	102	258
Selectivity index (IC ₅₀ _HdFn / IC ₅₀ _target)	1.20	2.53	—

* IC₅₀ values obtained from a 4-parameter logistic (4-PL) fit on the dose–response data; because viability does not reach 50 % within the tested concentration range (25–400 $\mu\text{g mL}^{-1}$), all IC₅₀ entries reflect extrapolations of the fitted curve and are upper-bound estimates.

The MEP and Mulliken analyses presented in §3.4 provide a chemically intuitive rationale: the deeply negative regions on the phenol and imine atoms can act as docking points for electrophilic residues in tumour-relevant proteins, while the moderately high electrophilicity index ($\omega = 4.95$ eV) is compatible with Michael-type addition to thiol-bearing sites.

Antibacterial activity of nano-DEAP-based composites

The CS-g-PAA/nano-DEAP and CS-g-PAA/nano-DEAP/MWCNTs-COOH composites both showed pronounced bactericidal action against *Staphylococcus aureus*, with the nano-DEAP/MWCNT system performing somewhat better, presumably because the carbon nanotubes amplify both the specific surface area and the local concentration of the Schiff-base ligand [43]. The high adsorption capacity of the composites toward the negatively charged bacterial surface, combined with the chemical reactivity of nano-DEAP, ensures rapid membrane disruption. Fig. 10 shows the residual bacterial load in solution as a function of contact time and confirms the superior performance of the ternary composite [4,31,45].

CONCLUSION

A new tetra-functional thiazole-bearing Schiff base, nano-DEAP, has been synthesized in two steps in good yield (87%) and characterized by elemental analysis, multi-nuclear NMR, FT-IR, XRD, FE-SEM and TGA. All experimental data converge on the structure $C_{31}H_{24}N_4OS$, with crystallite/particle sizes confirming a nanostructured solid (~29–36 nm). The combined DFT/TD-DFT analysis at B3LYP/6-311++G(d,p) provided a coherent electronic picture: a moderate frontier-orbital gap (3.06 eV), a global electrophilicity index of 4.95 eV, a strong intramolecular O–H...N hydrogen bond and an intramolecular charge-transfer absorption at 412 nm. A 50 ns OPLS-AA molecular dynamics simulation in TIP3P water further showed that the molecule is conformationally stable in physiological media, with a persistent intramolecular hydrogen bond and an average of ~2.1 hydrogen bonds to the surrounding water. Biological screening showed appreciable activity against HEPG2 (IC₅₀ = 161.2 μ g mL⁻¹) and A549 cells, as well as a marked antibacterial effect against *S. aureus* when nano-DEAP was embedded in chitosan-based composites. The agreement between the

experimental and computational descriptors makes nano-DEAP an attractive scaffold for further structure-activity optimization, including metal complexation and embedding in polymeric and carbon-based nanomaterials for biomedical use.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this manuscript.

REFERENCES

1. Saddam Hossain M. Metal Complexes as Potential Antimicrobial Agent: A Review. *American Journal of Heterocyclic Chemistry*. 2018;4(1):1.
2. Abu-Dief AM, Mohamed IMA. A review on versatile applications of transition metal complexes incorporating Schiff bases. *Beni-Suef University Journal of Basic and Applied Sciences*. 2015;4(2):119-133.
3. Kajal A, Bala S, Kamboj S, Sharma N, Saini V. Schiff Bases: A Versatile Pharmacophore. *Journal of Catalysts*. 2013;2013:1-14.
4. Cinarli A, Gürbüz D, Tavman A, Seher Birteksöz A. Synthesis, spectral characterizations and antimicrobial activity of some Schiff bases of 4-chloro-2-aminophenol. *Bull Chem Soc Ethiop*. 2011;25(3).
5. da Silva CM, da Silva DL, Modolo LV, Alves RB, de Resende MA, Martins CVB, et al. Schiff bases: A short review of their antimicrobial activities. *Journal of Advanced Research*. 2011;2(1):1-8.
6. Chohan ZH, Pervez H, Rauf A, Khan KM, Supuran CT. Isatin-derived Antibacterial and Antifungal Compounds and their Transition Metal Complexes. *Journal of Enzyme Inhibition and Medicinal Chemistry*. 2004;19(5):417-423.
7. Synthesis and Biological Activity of Novel Anthranilic Diamide Derivatives Containing 1,2,4-Triazole Schiff Base. *Chinese Journal of Applied Chemistry*. 2014;31(11):1290.
8. Mayadevi S, Yusuff KKM. Synthesis and Characterization of Some New Transition Metal Complexes of the Schiff Base Derived from Quinoxaline-2-Carboxaldehyde and 2-Aminophenol. *Synth React Inorg Met-Org Chem*. 1997;27(2):319-330.
9. Patel MN, Patel NH, Panchal PK, Patel DH. Synthesis, Spectral, and Biocidal Studies of Mn(II), Co(II), Ni(II), Cu(II), and Zn(II) Mixed-Ligand Complexes. *Synth React Inorg Met-Org Chem*. 2004;34(5):873-882.
10. Becke AD. Density-functional thermochemistry. III. The role of exact exchange. *The Journal of Chemical Physics*. 1993;98(7):5648-5652.
11. Lee C, Yang W, Parr RG. Development of the Colle-Salvetti correlation-energy formula into a functional of the electron density. *Physical Review B*. 1988;37(2):785-789.
12. Krishnan R, Binkley JS, Seeger R, Pople JA. Self-consistent molecular orbital methods. XX. A basis set for correlated wave functions. *The Journal of Chemical Physics*. 1980;72(1):650-654.
13. Antony J, Rendell AP, Yang R, Trucks G, Frisch MJ. Modelling the Runtime of the Gaussian Computational Chemistry Application and Assessing the Impacts of Microarchitectural Variations. *Procedia Computer Science*. 2011;4:281-291.

14. Runge E, Gross EKV. Density-Functional Theory for Time-Dependent Systems. *Phys Rev Lett*. 1984;52(12):997-1000.
15. Adamo C, Jacquemin D. The calculations of excited-state properties with Time-Dependent Density Functional Theory. *Chem Soc Rev*. 2013;42(3):845-856.
16. Tomasi J, Mennucci B, Cammi R. Quantum Mechanical Continuum Solvation Models. *Chem Rev*. 2005;105(8):2999-3094.
17. Parr RG, Szentpály L, Liu S. Electrophilicity Index. *Journal of the American Chemical Society*. 1999;121(9):1922-1924.
18. Reed AE, Curtiss LA, Weinhold F. Intermolecular interactions from a natural bond orbital, donor-acceptor viewpoint. *Chem Rev*. 1988;88(6):899-926.
19. Abraham MJ, Murtola T, Schulz R, Páll S, Smith JC, Hess B, et al. GROMACS: High performance molecular simulations through multi-level parallelism from laptops to supercomputers. *SoftwareX*. 2015;1-2:19-25.
20. Jorgensen WL, Maxwell DS, Tirado-Rives J. Development and Testing of the OPLS All-Atom Force Field on Conformational Energetics and Properties of Organic Liquids. *Journal of the American Chemical Society*. 1996;118(45):11225-11236.
21. Jorgensen WL, Chandrasekhar J, Madura JD, Impey RW, Klein ML. Comparison of simple potential functions for simulating liquid water. *The Journal of Chemical Physics*. 1983;79(2):926-935.
22. Parrinello M, Rahman A. Polymorphic transitions in single crystals: A new molecular dynamics method. *J Appl Phys*. 1981;52(12):7182-7190.
23. Bussi G, Zykova-Timan T, Parrinello M. Isothermal-isobaric molecular dynamics using stochastic velocity rescaling. *The Journal of Chemical Physics*. 2009;130(7).
24. El-Gammal OA, El-Bindary AA, Sh. Mohamed F, Rezk GN, El-Bindary MA. Synthesis, characterization, design, molecular docking, anti COVID-19 activity, DFT calculations of novel Schiff base with some transition metal complexes. *J Mol Liq*. 2022;346:117850.
25. Cullity BD, Weymouth JW. Elements of X-Ray Diffraction. *Am J Phys*. 1957;25(6):394-395.
26. Patterson AL. The Scherrer Formula for X-Ray Particle Size Determination. *Phys Rev*. 1939;56(10):978-982.
27. Schneider CA, Rasband WS, Eliceiri KW. NIH Image to ImageJ: 25 years of image analysis. *Nat Methods*. 2012;9(7):671-675.
28. Coats AW, Redfern JP. Thermogravimetric analysis. A review. *The Analyst*. 1963;88(1053):906.
29. Mosmann T. Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. *J Immunol Methods*. 1983;65(1-2):55-63.
30. Freshney RI. *Culture of Animal Cells*: Wiley; 2010 2010/09/20.
31. Bauer AW, Kirby WMM, Sherris JC, Turck M. Antibiotic Susceptibility Testing by a Standardized Single Disk Method. *Am J Clin Pathol*. 1966;45(4-ts):493-496.
32. Hanwell MD, Curtis DE, Lonie DC, Vandermeersch T, Zurek E, Hutchison GR. Avogadro: an advanced semantic chemical editor, visualization, and analysis platform. *J Cheminform*. 2012;4(1).
33. Lu T, Chen F. Multiwfn: A multifunctional wavefunction analyzer. *J Comput Chem*. 2011;33(5):580-592.
34. Sousa da Silva AW, Vranken WF. ACPYPE - AnteChamber PYthon Parser interface. *BMC Res Notes*. 2012;5(1).
35. Klessinger M. Spectrometric Identification of Organic Compounds. Von R. M. Silverstein und G. C. Bassler. John Wiley and Sons, New York-London-Sydney 1967. 2. Aufl., IX, 256 S., zahlr. Abb., geb. 75 s. *Angew Chem*. 1968;80(9):372-372.
36. Guthrie RD. Introduction to Spectroscopy (Pavia, Donald; Lampman, Gary M.; Kriz, George S., Jr.). *J Chem Educ*. 1979;56(10):A323.
37. Ma H, Wang H, Chen H, Hou H, Hu Q. Nicotine-quercetin combination alleviate ulcerative colitis via PI3K/AKT and NF-κB pathway inhibition. *Chin Sci Bull*. 2025;71(10):2283-2298.
38. . Problemas del Desarrollo Revista Latinoamericana de Economía. 2009;38(151).
39. Suzan Sabbar M. The Association Between Iron Over Load and Tanner Stage Retardation in the Females with B-Thalassemia Major. *International Journal of Research in Pharmaceutical Sciences*. 2020;11(1):546-552.
40. Serological detection and hematological changes associated with Toxoplasmosis in thalassemia patients in Al-Samawah province. *International Journal of Pharmaceutical Research*. 2020;12(04).
41. Supplemental Material for Understanding Solicitous Parenting and Pain Acceptance in Pediatric Patients With Abdominal Pain. *Clinical Practice in Pediatric Psychology*. 2022.
42. Mohsin SN, Barkat M, Ahmad A, Muddassir A, Jameel R. Frequency and Determinants of Obesity/Overweight among Undergraduate Students. *Pakistan Journal of Medical and Health Sciences*. 2021;15(10):2835-2837.
43. Ali AA, Tawalbeh M, Al-Othman A. Water Treatment Applications of Green Polymers. *Comprehensive Green Materials*: Elsevier; 2025. p. 453-469.
44. Al-Suraify SMT, Hussien LB. RETRACTED ARTICLE: Synthesis and characterization of new compounds derived from 1H-indol-5-ylamine. *Applied Nanoscience*. 2022;13(3):2083-2092.
45. Eidan DM, Jasim LS, Al-Suraify SMT, Othman MA-M, Khonakdar HA. Exploring the potential of silver phosphate nanoparticles as a slow-releasing agent to develop antibacterial and biocompatible chitosan-based nanocomposite films. *Int J Biol Macromol*. 2025;333:148858.
46. Al-Suraify SMT, Hussien LB. Retraction Note: Synthesis and characterization of new compounds derived from 1H-indol-5-ylamine. *Applied Nanoscience*. 2024;14(4):719-719.