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Synthesis and biological activity of novel 4-nicotinoyl-1,7-di(pyridine-3-yl)-3,5-diaryl heptane-1,7-dione under Claisen-Schmidt condensation

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ABSTRACT

The focus of the research is on the unexpected products that resulted from the condensation reaction between 3-acetyl pyridine **(1)** and various heterocyclic aldehydes **2(a-h)** under the conditions of the Claisen-Schmidt reaction. Chalcone derivatives were produced in good yield **3(a-h)** by various heterocyclic aldehydes in the presence of aqueous alkaline bases.

The latest synthesis of chalcones with N, O, S heterocycles has shown their potential biological value in the post-decade period. The chalcone compounds formed, which are immediately converted into unexpected 4-nicotinoyl-1,7-di(pyridine-3-yl)-3,5-diaryl heptane-1,7-dione in the same reaction. The melting point, elemental analysis, MS, FTIR, ^{1}H NMR, and ^{13}C NMR spectroscopic data were used to confirm the structure of produced compounds. A large number of heterocyclic chalcones have better activity than the standard ones.

The antibacterial activity of the produced compounds against various bacterial and fungal species was also assessed *in vitro*. Studies on docking and anticancer were also conducted. Of the compounds that were synthesised, **3g** and **3h** showed the highest potency due to the presence of a halogen atom.

The synthesised compounds were assessed for their *in vitro* antibacterial and antifungal potential towards several pathogenic bacterial and fungal strains by the agar well diffusion method. All these compounds showed moderate to good antimicrobial activity. The most active antimicrobial compounds, **3g** and **3h,** were further evaluated for their antioxidant potency by 2,2-Diphenyl-1-picrylhydrazyl (DPPH) and 2,2′-azinobis(3-ethylbenzothiazolin-6-sulfonic acid) ($ABTS^{\cdot+}$) cation radical decolorisation assays. Anti-inflammatory efficacy of these compounds was tested by lipoxygenase (LOX) inhibition assay. These derivatives were also assessed for their cytotoxicity towards human lung cancer cells by MTT assay. The compounds **3g** and **3h** have shown good antioxidative, anti-inflammatory, and anti-cancer activity.

The molecular docking analysis was performed using the compound **3d** with *S. aureus* enterotoxin, *P. aeruginosa* bacterioferritin B, anticancer protein human BCL-2 isoform, and lipoxygenase to ascertain the probable binding model.

1. Introduction

Chalcones are bioactive compounds derived from both natural and synthetic sources. The scientific community is well aware of their physicochemical characteristics, reactivity, and biological activities. Chalcone is the name for a straightforward chemical framework that is present in a variety of naturally occurring chemicals, mostly found in plants. The name "chalcone" originates from the Greek word Chalcos, which means bronze. The yellow and orange (bronze-like) hues of the vegetable tissues that contain these chemicals are the cause of this relationship. Stanislaw Kostanecki and Josef Tambor, the first scientists to synthesise these naturally occurring chemicals with distinctive colours, were the ones who originally mentioned this phrase. Nevertheless, chalcones have been used for therapeutic purposes for a very long time. These substances were employed in the treatment of a variety of illnesses through the usage of plants and herbs [1]. Currently, chalcones (1, 3-diarylprop-2-en-1 ones) (Fig. 1), encompassing both synthetic and natural variants, as well as derivatives of the chalcone structure, have been associated with a diverse array of biological activities, including anti-inflammatory [2], antioxidant [3], anticancer [4], antidiabetic [5],

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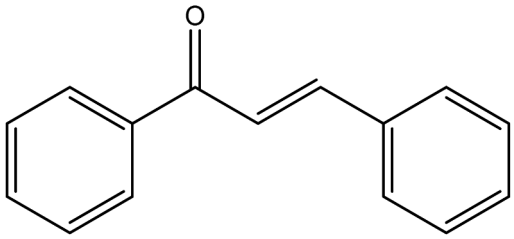


Fig. 1. (E)-1,3-diphenylprop-2-en-1-one.

antimicrobial [6], anti-HIV [7,8], antibacterial [9], antifungal [10], DNA binding [11], enzyme inhibition [12], and antitumor [13] properties.

The simplicity of synthesising chalcone derivatives has resulted in the creation of many chemicals.They are widely distributed in fruits, vegetables, teas, and other plants. They are a basic chemical scaffold composed of numerous naturally occurring chemicals [14]. Because chalcones and their derivatives have a double bond in conjugation with a carbonyl group, they exhibit strong biological activities that are crucial and useful in the design of drugs. The Claisen-Schmidt condensation method is an efficient approach for synthesising chalcones.

Chalcones and their derivatives are excellent physiologically active substances.Chalcones have pharmacological characteristics that have substantial therapeutic applications because of α, β-unsaturation. Strong interaction between molecules results from the transfer of charge carriers via donor and acceptor groups affixed to two aromatic rings at distinct positions in chalcones [15,16]. The preparation and derivatisation of chalcones, a significant class of privileged structures and building blocks used for the synthesis of new biologically active compounds with a wide range of possible therapeutic applications, is carried out using the most recent environmentally friendly and sustainable synthetic methods [17]. The biological activity of chalcones is due to the presence of the keto-ethylene group, the type of substituents in the aromatic rings, and the heterocycles. Heteroatoms, which include heterocyclic blocks, offer a compelling chance to find novel compounds that can be used to cure cancer. A scientific study reveals that over 85 % of all biologically active molecules either contain or are heterocycles, with nitrogen heterocycles frequently serving as the structural foundation of these complex compounds [18]. This underscores the essential function of heterocycles in innovative drug discovery and design.Many synthetic medications work well as chemotherapeutics. Pyridines are a type of nitrogenous heterocycles that can be synthesised through several pathways, leading to the production of new compounds with anticancer and antitumor properties [19].

Pyridine and its heterocyclic derivatives are the most extensively applied scaffolds for drug design and synthesis in organic compounds, and they play important roles in the organic chemistry field. In fact, molecules containing pyridine scaffolds have drawn a lot of attention from researchers in a variety of domains. Their distinct heteroaromatic functional significance in organic chemistry, ease of conversion into various functional derivatives, significant impact on pharmacological activity, and use as pharmacophores in medicinal chemistry are the key reasons for this. Numerous broad-spectrum medicines and agricultural chemical products were discovered as a result of these qualities [20]. Recent papers highlighting the efficacy of pyridine derivatives and the antioxidant capabilities of chalcones motivate us to develop a new category of chalcones incorporating a pyridine moiety [21]. Since chloroquine also has a pyridine moiety in its chemical structure, the mechanism of action of pyridine and chloroquine are comparable. Methoxy chalcone and a pyridine structure together have been the subject of little research attempts to develop new antimalarial drugs [22]. Nowadays, nitrogen, oxygen, and sulphur-containing heterocyclic compounds have been widely studied due to their interesting applications as bioactive molecules [23]. Neurotoxin 3-acetylpyridine, an antimetabolite of nicotinamide, specifically damages calbindin-expressing neurones in the inferior olive, which sends axons, termed climbing fibres, to form synapses on Purkinje cells. Destruction of the nerve fibres innervating the cerebellum by 3-acetylpyridine can induce cerebellar ataxias and provoke inflammatory reactivity present in animal brains [24].

Attempting the synthesis of novel heterocyclic chalcones containing the thiophene moiety is therefore a logical step towards developing potential new drugs to counter drug resistance, the antibacterial cefoxitin and antifungal tioconazole possessing the thiophene moiety [25]. Furan derivatives are an imperative class of heterocyclic compounds that have important biological properties. Furan is rapidly and extensively absorbed from the intestine and the lung. It can pass through biological membranes and enter various organs. Compounds comprising the furan are biologically active and are existent in a number of pharmaceutical products [26].

All the halogenated benzaldehydes can undergo Claisen-Schmidt condensation reactions in the same manner. Although different halogenated benzaldehydes do have different rates of reaction,in which brominated benzaldehyde reacts more readily than others due to the larger size and less negative inductive effect of bromine. It is important to design a structural activity relationship and low-cost, less toxic, and powerful chalcone-based halogens, in which fluorine has become an important tool in drug discovery, since the inclusion of a fluorine atom or fluorinated group into drugs or drug leads allows simultaneous modulations of lipophilic, electronic, and steric parameters, all of which can critically influence both the pharmacokinetics and pharmacodynamic properties of drugs [27].

Claisen-Schmidt simultaneously reported the condensation process for the first time in 1881. It is the formation of an α,β-unsaturated aldehyde or ketone with high chemoselectivity by condensation of an aromatic aldehyde with an aromatic ketone or an aromatic heterocyclic ketone in the presence of a base or an acid. Nevertheless, this process is frequently tainted with a few small byproduct reactions, such as dimerisation, bis-condensation, and the aromatic aldehyde Cannizzaro or Tischenko reactions. Consequently, we have also prepared chalcone, flavanone, 1, 3-diarylpropane derivatives, and a novel family of macrocycles in a single step using the Claisen-Schmidt condensation [28].

Chalcones, characterised by an α,β-unsaturated carbonyl structure including two aromatic rings, are classified as open-chain flavonoids, in which a third carbon that serves as a Michael acceptor group facilitates the effective attachment of nucleophiles to diverse biological targets [29]. The easiest way to describe the broad scope of the Michael reaction is as a “conjugate addition” or “1,4 addition” to an α,β-unsaturated carbonyl compound [30]. The Michael addition is a crucial reaction for organic synthesis because it creates a carbon-carbon bond. Although the conversion of a carbon-carbon double bond into a carbon-carbon single bond serves as the driving force, the equilibrium is also influenced by the specifics of the structure and reaction conditions [31].

Researchers synthesized the symmetrically halogenated bischalcones (SHBCs) and examined their Michael addition reaction with reduced glutathione (GSH) to determine whether the compounds cytotoxic effects were associated with their GSH reactivity. They performed the Michael addition of GSH to the SHBCs in methanol solutions. They synthesised molecule possesses two polarised carbon-carbon double bonds, each of which can theoretically undergo successive interactions with the thiol group of GSH. In the synthesised compounds, thiol reactivity may influence the molecular mechanism of cytotoxicity [32].

The biological activity, encompassing the cytotoxic effects of chalcones and their derivatives, arises from non-covalent interactions between these chemicals and cellular macromolecules (proteins, DNA). As DNA serves as a crucial biological receptor, numerous chemicals exert their tumour cytotoxic effects by binding to DNA, there by affecting the cell cycle, altering replication, limiting cell development, obstructing division, and ultimately leading to opoptosis or cell death. Research demonstrated that the most potent chemicals against tumour cells are

Mannich derivatives of acetophenones and structurally analogous α,β-unsaturated ketones, which exert their cytotoxic effects via the alkylation of cellular thiols, including glutathione and cysteine [33,34].

An enantioselective intramolecular oxa-Michael conversion of alcohols to tethered, low electrophilicity Michael acceptors, catalysed by a bifunctional imminophosphorane, is presented. The recently established catalytic enantioselective method was executed on a multigram scale, and several Michael adducts were then derivatised into a range of valuable building blocks, facilitating the synthesis of enantioenriched physiologically active compounds and natural products [35].

Chiral carbocation intermediates can be effectively intercepted by several nucleophiles, including alcohols, water, and thiols, exhibiting strong stereoselectivity. The principal S_N1 mechanism occurs through a tertiary amine-mediated proton transfer that enables facial selectivity in the reaction with carbocation [36].

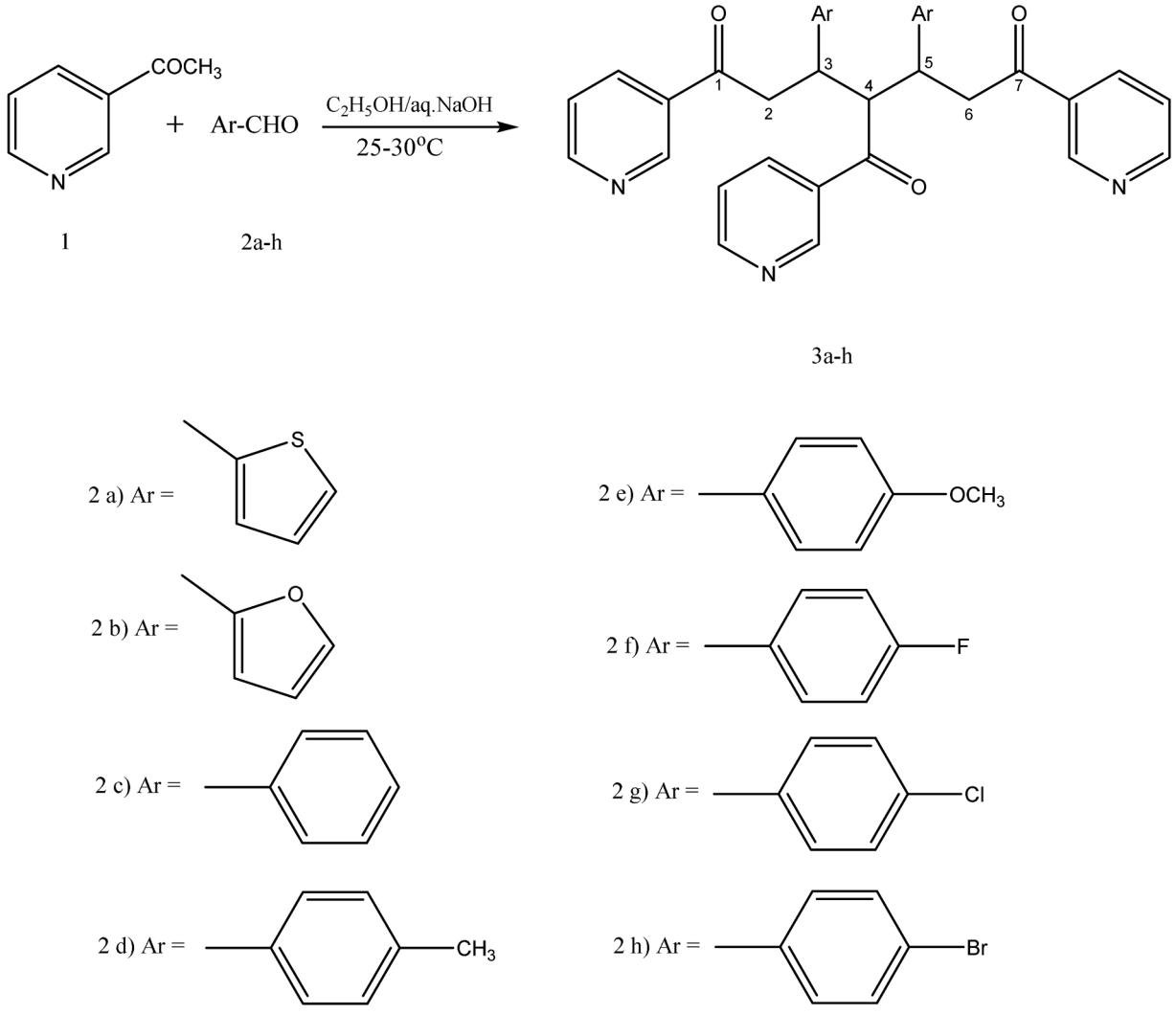
We are driven to participate in this domain due to the extensive pharmacological applications associated with chalcone-derived heterocycles. As we proceed with our work on the synthesis of pyridine in basic conditions, we are also screening a series of unexpected novel 1,7-di (pyridine-3-yl)heptane-1,7-dione derivatives in moderate to excellent yields via Claisen-Schmidt condensation and itself continued to Michael addition in a single step of pyridine ketone with heterocyclic aldehydes and substituted benzaldehyde. The synthesised compounds were assessed *in vitro* for their antibacterial, antifungal, and anti-inflammatory activities, as well as for docking studies and anticancer evaluations concerning lung and breast cancers.

In consideration of the aforementioned aspects, this study aims to expand our synthesis to encompass novel pyridine chalcones and assess their antimicrobial, antioxidant, anti-inflammatory, anticancer, and docking studies.

2. Material and methods

2.1. Chemistry

The synthesis of chalcones and their heterocyclic chalcone derivatives, the chemicals were bought from TCL (Japan) and Merck (Germany). The melting points of the synthesised compounds were measured using the 'Stuart-SMP10 melting point instrument' in open-glass capillaries and are uncorrected. Using KBr pellets, IR absorption spectra were collected between 4000 and 400 cm^{-1} on a 'Perkin-Elmer Spectrum 100 FTIR spectrometer'. On a Bruker-NMR 400 MHz spectrophotometer, ^1H and ^{13}C NMR spectra were obtained using $CDCl_3$ solvent.$CDCl_3$ solvent had a solvent residual peak of 7.6, which can serve as a reference ppm. The chemical shifts measured by ^1H NMR and ^{13}C NMR were expressed as parts per million (ppm) downfield from TMS (Me_4Si). The following are the names of the splitting patterns: s for singlet, d for doublet, t for triplet, q for quartet, and m for multiplet. Mass spectra were recorded using the Lynx SCN781 spectrometer in TOF mode. The progress and purity of the compounds were checked by thin-



Scheme 1. Synthesis of novel heterocyclic chalcones **(3a-h)**.

layer chromatography (TLC) on Merck silica gel 60 F_{254} precoated sheets, in an ethyl acetate and benzene mixture of the ratio 0.5:7 v/v, and spots were developed using iodine vapours and ultraviolet light as a visualising agent. The synthetic scheme for the preparation of heterocyclic chalcone derivatives is shown in Scheme 1.

- Mechanism

The unexpected products are formed *in situ*. Chalcones are not isolated. But reaction occurs through chalcones.

- **Step 1: Formation of chalcone.**
- **Step 2: Formation of Novel 4-nicotinoyl-1,7-di(pyridine-3-yl)-3,5-diaryl heptane-1,7-dione.**

2.1.1. 4-nicotinoyl-1,7-di(pyridin-3-yl)-3,5-di(thiophen-2-yl)heptane-1,7-dione (3a)

3-Acetyl pyridine **(1)** (0.0041 mol, 0.5g) and thiophene-2-carboxaldehyde **(2a)** (0.0041 mol, 0.46g) were stirred in water (15 ml) and ethanol (10 ml) in the presence of sodium hydroxide (0.0041 mol) at 25–30°C for eight hours. The reaction mixture was kept overnight in an ice bath. The precipitated solid product was filtered, washed with ice-cold water and recrystallised from ethanol (Fig. 2).

Light brown solid; yield: 87 %; m.p.: 198–200°C; m.f.: $C_{31}H_{25}N_3O_3S_2$; IR (KBr, ν_{max}, cm^{-1}): 1681 (*C = O*), 1582 (*C = N*), 1083 (C-N), 696 (C-S-C); ^{1}H NMR (400 MHz, $CDCl_3$) in δ ppm: 2.44 (t, 1H, -CH-, *J* = 7.0 Hz), 4.14 (q, 2H, -CH-, *J* = 7.1 Hz), 4.57 (d, 4H, -CH_2CO, *J* = 7.1 Hz), 6.78–7.28 (m, 6H, Th-H, *J* = 6.8 Hz), 8.34–8.83 (m, 12H, Py-H, *J* = 7.6 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) in δ ppm: 205.1 (1C, *C = O*), 201.7 (2C, *C = O*), 152.7 (3C, *C = N*), 149.1 (3C, C-N), 144.2 (2C), 134.9 (3C), 132.5 (3C), 126.6 (2C), 125.7 (2C), 124.6 (2C), 123.9 (3C), 57.7 (1C), 46.7 (2C), 38.8 (2C); MS *m/z*: 552.12 (*M* + 1).

2.1.2. 3,5-di(furan-2-yl)-4-nicotinoyl-1,7-di(pyridin-3-yl)heptane-1,7-dione (3b)

3-Acetyl pyridine **(1)** (0.0041 mol, 0.5g) and 2-furaldehyde **(2b)** (0.0041 mol, 0.39g) were stirred in water (15 ml) and ethanol (12 ml) in the presence of sodium hydroxide (0.0041 mol) at 25–30°C for twelve hours. The reaction mixture was kept overnight in an ice bath. The precipitated solid product was filtered, washed with ice-cold water and recrystallised from ethanol (Fig. 3).

Brown solid; yield: 80 %; m.p.: 108–110°C; m.f.: $C_{31}H_{25}N_3O_5$; IR (KBr, ν_{max}, cm^{-1}): 1680 (*C = O*), 1584 (*C = N*), 1069 (C-N), 1234 (C-O-C); ^{1}H NMR (400 MHz, $CDCl_3$) in δ ppm: 2.26 (t, 1H, -CH-, *J* = 7.0 Hz), 4.21 (q, 2H, -CH-, *J* = 7.1 Hz), 4.49 (d, 4H, -CH_2CO, *J* = 7.1 Hz), 6.03–7.28 (m, 6H, Fu-H, *J* = 7.3 Hz), 7.84–8.80 (m, 12H, Py-H, *J* = 7.6 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) in δ ppm: 205.3(1C, *C = O*), 201.7 (2C, *C = O*), 154.0 (2C), 150.5 (3C, *C = N*), 149.1 (3C, C-N), 141.5 (2C), 134.9 (3C), 132.4 (3C), 123.3 (3C), 110.2 (2C), 106.6 (2C), 53.8 (1C), 43.7 (2C), 36.3 (2C); MS *m/z*: 520.16 (*M* + 1).

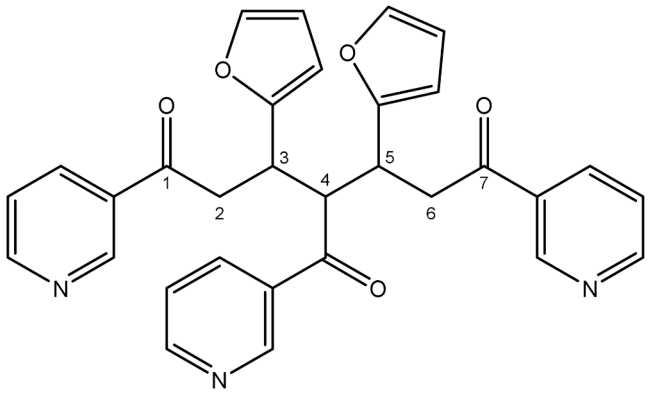


Fig. 3. Structure of chalcone derivative (**3b**).

2.1.3. 4-nicotinoyl-3,5-diphenyl-1,7-di(pyridin-3-yl)heptane-1,7-dione (3c)

3-Acetyl pyridine **(1)** (0.0041 mol, 0.5g) and benzaldehyde **(2c)** (0.0041 mol, 0.44g) were stirred in water (15 ml) and ethanol (12 ml) in the presence of sodium hydroxide (0.0041 mol) at 25–30°C for six hours. The reaction mixture was kept overnight in an ice bath. The precipitated solid product was filtered, washed with ice-cold water and recrystallised from ethanol (Fig. 4).

Pale yellow solid; yield: 86 %; m.p.: 158–160°C; m.f.: $C_{35}H_{29}N_3O_3$; IR (KBr, ν_{max}, cm^{-1}): 3333 (Ar-H), 1673 (*C = O*), 1584 (*C = N*), 1076 (C-N); ^{1}H NMR (400 MHz, $CDCl_3$) in δ ppm: 4.17 (t, 1H, -CH-, *J* = 7.0 Hz), 4.31 (q, 2H, -CH-, *J* = 7.1 Hz), 5.06 (d, 4H, -CH_2CO, *J* = 7.1 Hz), 7.19–7.28 (m, 10H, Ar-H, *J* = 7.6 Hz), 7.45–9.04 (m, 12H, Py-H, *J* = 7.6 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) in δ ppm: 206.0 (1C, *C = O*), 205.1 (2C, *C = O*), 152.8 (3C, *C = N*), 149.3 (3C, C-N), 141.5 (2C), 135.0 (3C), 132.8 (3C), 129.0 (4C), 128.9 (4C), 123.2 (2C), 123.0 (3C), 52.7 (1C), 50.7 (2C), 46.4 (2C); MS *m/z*: 540.19 (*M* + 1).

2.1.4. 4-nicotinoyl-1,7-di(pyridin-3-yl)-3,5-di-p-tolylheptane-1,7-dione (3d)

3-Acetyl pyridine **(1)** (0.0041 mol, 0.5g) and 4-methyl benzaldehyde **(2d)** (0.0041 mol, 0.49g) were stirred in water (15 ml) and ethanol (12 ml) in the presence of sodium hydroxide (0.0041 mol) at 25–30°C for twelve hours. The reaction mixture was kept overnight in an ice bath. The precipitated solid product was filtered, washed with ice-cold water and recrystallised from ethanol (Fig. 5).

Pale yellow solid; yield: 79 %; m.p.: 132–134°C; m.f.: $C_{37}H_{33}N_3O_3$; IR (KBr, ν_{max}, cm^{-1}): 3510 (Ar-H), 1662 (*C = O*), 1582 (*C = N*), 1076 (C-N); ^{1}H NMR (400 MHz, $CDCl_3$) in δ ppm: 2.41 (s, 6H, -CH_3), 4.31 (t, 1H,

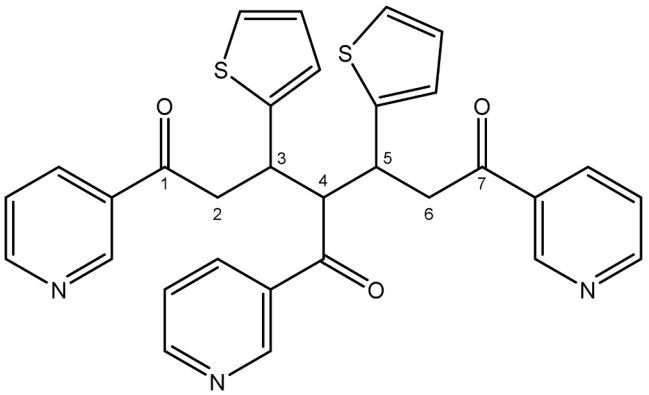


Fig. 2. Structure of chalcone derivative (**3a**).

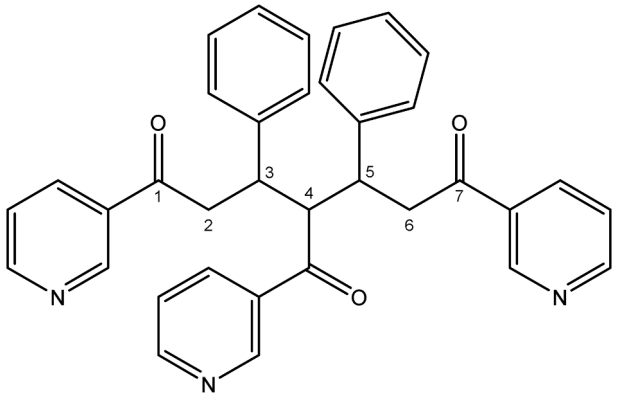


Fig. 4. Structure of chalcone derivative (**3c**).

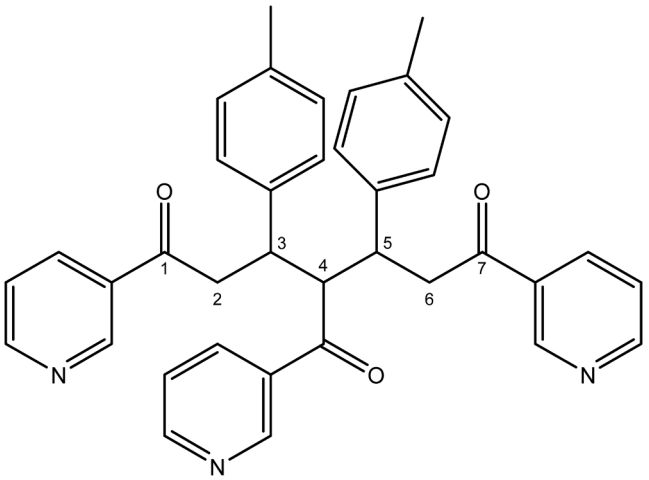


Fig. 5. Structure of chalcone derivative (**3d**).

-CH-, $J = 7.0$ Hz), 5.03 (q, 2H, -CH-, $J = 7.1$ Hz), 5.64 (d, 4H, -CH_2CO, $J = 7.1$ Hz), 7.05–7.07 (m, 8H, Ar-H, $J = 7.6$ Hz), 8.28–9.05 (m, 12H, Py-H, $J = 7.6$ Hz); ^{13}C NMR (100 MHz, $CDCl_3$) in δ ppm: 206.5 (1C, *C* = *O*), 205.7 (2C, *C* = *O*), 152.2 (3C, *C* = *N*), 149.4 (3C, C-N), 141.8 (2C), 136.6 (3C), 135.3 (2C), 132.8 (3C), 128.7 (4C), 128.3 (4C), 123.1 (3C), 53.3 (1C), 46.9 (2C), 41.4 (2C), 20.8 (2C, CH_3); MS *m/z*: 568.23 (*M* + 1).

2.1.5. 3,5-bis(4-methoxyphenyl)-4-nicotinoyl-1,7-di(pyridin-3-yl)heptane-1,7-dione(3e)

3-Acetyl pyridine **(1)** (0.0041 mol, 0.5g) and 4-methoxy benzaldehyde **(2e)** (0.0041 mol, 0.56g) were stirred in water (15 ml) and ethanol (12 ml) in the presence of sodium hydroxide (0.0041 mol) at 25–30°C for twelve hours. The reaction mixture was kept overnight in an ice bath. The precipitated solid product was filtered, washed with ice-cold water and recrystallised from ethanol (Fig. 6).

Pale yellow solid; yield: 75 %; m.p.: 153–155°C; m.f.: $C_{37}H_{33}N_3O_5$; IR (KBr, ν_{max}, cm^{-1}): 3499 (Ar-H), 1660 (*C* = *O*), 1585 (*C* = *N*), 1075 (C-N); ^{1}H NMR (400 MHz, $CDCl_3$) in δ ppm: 3.49 (t, 1H, -CH-, $J = 7.0$ Hz), 3.61 (q, 2H, -CH-, $J = 7.1$ Hz), 3.84 (d, 4H, -CH_2CO, $J = 7.1$ Hz), 4.30 (s, 6H, –OCH_3), 6.96–7.40 (m, 8H, Ar-H, $J = 7.6$ Hz), 7.69–9.04 (m, 12H, Py-H, $J = 7.6$ Hz); ^{13}C NMR (100 MHz, $CDCl_3$) in δ ppm: 206.5 (1C, *C* = *O*), 205.9 (2C, *C* = *O*), 158.4 (2C), 152.3 (3C, *C* = *N*), 149.6 (3C, C-N), 135.8 (3C), 134.8 (2C), 132.8 (3C), 129.5 (4C), 123.1 (3C), 114.5 (4C), 55.4 (2C, –OCH_3), 55.1 (1C), 46.4 (2C), 40.9 (2C); MS *m/z*: 600.22 (*M* + 1).

2.1.6. 3,5-bis(4-fluorophenyl)-4-nicotinoyl-1,7-di(pyridin-3-yl)heptane-1,7-dione(3f)

3-Acetyl pyridine **(1)** (0.0041 mol, 0.5g) and 4-fluoro benzaldehyde **(2f)** (0.0041 mol, 0.51g) were stirred in water (15 ml) and ethanol (12 ml) in the presence of sodium hydroxide (0.0041 mol) at 25–30°C for eight hours. The reaction mixture was kept overnight in an ice bath. The precipitated solid product was filtered, washed with ice-cold water and recrystallised from ethanol (Fig. 7).

Pale yellow solid; yield: 85 %; m.p.: 143–145°C; m.f.: $C_{35}H_{27}F_2N_3O_3$; IR (KBr, ν_{max}, cm^{-1}): 3334 (Ar-H), 1673 (*C* = *O*), 1584 (*C* = *N*), 1077 (C-N), 973 (Ar-F); ^{1}H NMR (400 MHz, $CDCl_3$) in δ ppm: 4.17 (t, 1H, -CH-, $J = 7.0$ Hz), 4.21 (q, 2H, -CH-, $J = 7.1$ Hz), 4.67 (d, 4H, -CH_2CO, $J = 7.1$ Hz), 7.18–7.32 (m, 8H, Ar-H, $J = 8.0$ Hz), 7.56–9.02 (m, 12H, Py-H, $J = 7.6$ Hz); ^{13}C NMR (100 MHz, $CDCl_3$) in δ ppm: 205.6 (1C, *C* = *O*), 204.8 (2C, *C* = *O*), 153.7 (2C, Ar-F), 152.9 (3C, *C* = *N*), 149.4 (3C, C-N), 135.4 (2C), 134.5 (3C), 129.7 (3C), 129.1(4C), 128.7 (3C), 123.0 (4C), 56.4 (1C), 45.5 (2C), 42.6 (2C); MS *m/z*: 576.17 (*M* + 1).

2.1.7. 3,5-bis(4-chlorophenyl)-4-nicotinoyl-1,7-di(pyridin-3-yl)heptane-1,7-dione(3g)

3-Acetyl pyridine **(1)** (0.0041 mol, 0.5g) and 4-chloro benzaldehyde **(2g)** (0.0041 mol, 0.57g) were stirred in water (15 ml) and ethanol (12 ml) in the presence of sodium hydroxide (0.0041 mol) at 25–30°C for four hours. The reaction mixture was kept overnight in an ice bath. The precipitated solid product was filtered, washed with ice-cold water and recrystallised from ethanol (Fig. 8).

Pale yellow solid; yield: 89 %; m.p.: 198–200°C; m.f.: $C_{35}H_{27}Cl_2N_3O_3$; IR (KBr, ν_{max}, cm^{-1}): 3172 (Ar-H), 1674 (*C* = *O*), 1582 (*C* = *N*), 1090 (C-N), 699 (Ar-Cl); ^{1}H NMR (400 MHz, $CDCl_3$) in δ ppm: 3.52 (t, 1H, -CH-, $J = 7.0$ Hz), 3.64 (q, 2H, -CH-, $J = 7.1$ Hz), 4.31 (d, 4H, -CH_2CO, $J = 7.1$ Hz), 7.11–7.28 (m, 8H, Ar-H, $J = 8.0$ Hz), 7.44–9.05 (m, 12H, Py-H, $J = 7.6$ Hz); ^{13}C NMR (100 MHz, $CDCl_3$) in δ ppm: 206.6 (1C, *C* = *O*), 205.9 (2C, *C* = *O*), 152.4 (3C, *C* = *N*), 149.6 (3C, C-N), 141.8 (2C), 135.0 (3C), 132.8 (3C), 130.5 (2C, Ar-Cl), 129.6 (4C), 128.5 (4C), 123.6 (3C), 55.4 (1C), 46.5 (2C), 40.9 (2C); MS *m/z*: 608.11 (*M* + 1).

2.1.8. 3,5-bis(4-bromophenyl)-4-nicotinoyl-1,7-di(pyridin-3-yl)heptane-1,7-dione(3h)

3-Acetyl pyridine **(1)** (0.0041 mol, 0.5g) and 4-bromo benzaldehyde **(2h)** (0.0041 mol, 0.75g) were stirred in water (15 ml) and ethanol (12 ml) in the presence of sodium hydroxide (0.0041 mol) at 25–30°C for four hours. The reaction mixture was kept overnight in an ice bath. The precipitated solid product was filtered, washed with ice-cold water and recrystallised from ethanol (Fig. 9).

Pale yellow solid; yield: 93 %; m.p.: 170–172°C; m.f.:

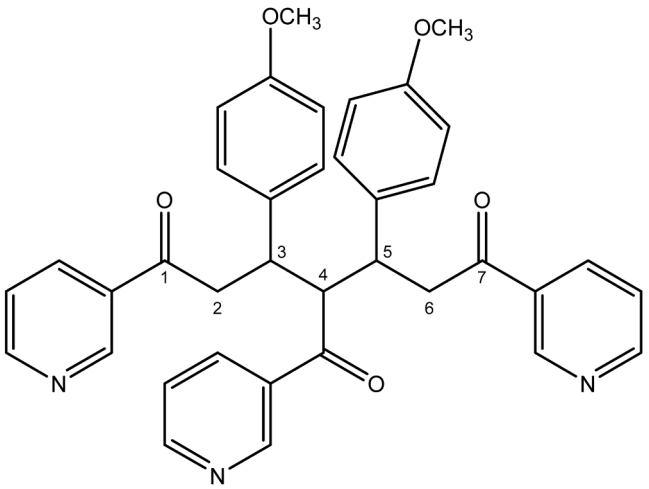


Fig. 6. Structure of chalcone derivative (**3e**).

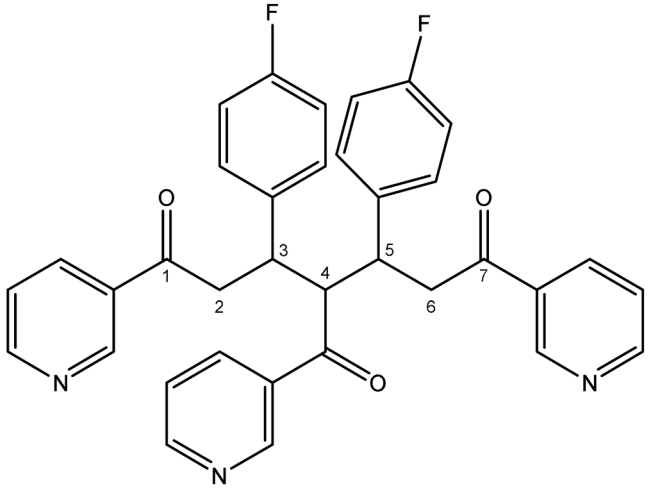


Fig. 7. Structure of chalcone derivative (**3f**).

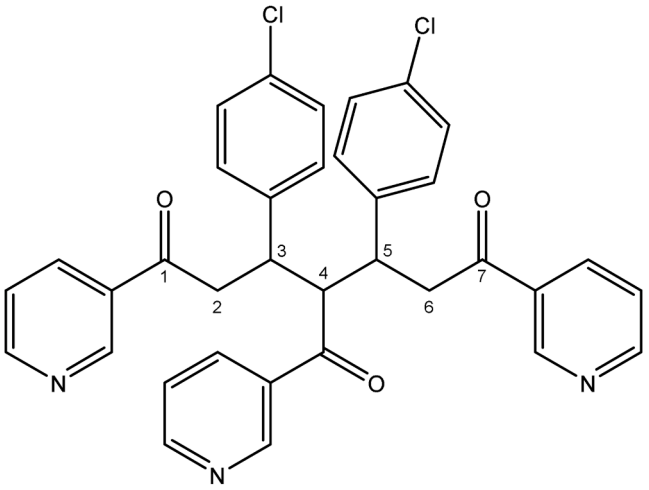


Fig. 8. Structure of chalcone derivative (**3g**).

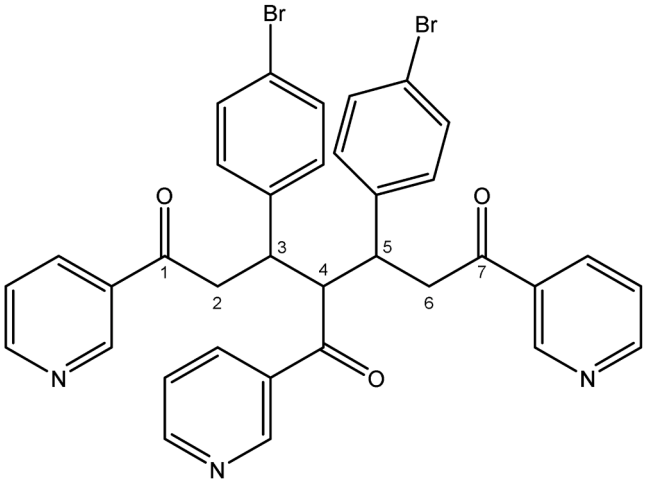


Fig. 9. Structure of chalcone derivative (**3h**).

$C_{35}H_{27}Br_2N_3O_3$; IR (KBr, ν_{max}, cm^{-1}): 3332 (Ar-H), 1667 (*C = O*), 1584 (*C = N*), 1072 (C-N), 650 (Ar-Br); ^{1}H NMR (400 MHz, $CDCl_3$) in δ ppm: 4.23 (t, 1H, -CH-, *J* = 7.0 Hz), 4.34 (q, 2H, -CH-, *J* = 7.1 Hz), 5.65 (d, 4H, $-CH_2CO$, *J* = 7.1 Hz), 7.26–8.38 (m, 8H, Ar-H, *J* = 8.0 Hz), 8.25–9.06 (m, 12H, Py-H, *J* = 7.6 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) in δ ppm: 206.2 (1C, *C = O*), 205.4 (2C, *C = O*), 152.3 (3C, *C = N*), 149.4 (3C, C-N), 141.8 (2C), 135.1 (3C), 132.97 (4C), 132.93 (3C), 128.7 (4C), 123.1 (3C), 122.7 (2C, C-Br), 53.0 (1C), 47.3 (2C), 41.7 (2C); MS *m/z*: 696.00 (*M* + 1).

2.2. Antimicrobial activity

In vitro antimicrobial activity of the compounds 3(a, b, c, d, e, f, g, h) was performed by the agar well diffusion method. The compounds were prepared in three different concentrations (5 mg/mL, 2.5 mg/mL, and 1.25 mg/mL) in 20 % DMSO.

Antibacterial activity was performed against *Escherichia coli, S. aureus, and P. aeruginosa.* The bacterial subcultures of 18–24 hrs old were plated on Mueller-Hinton agar medium. Three wells of 6 mm diameter were created by using a sterile borer. The test compounds at three different concentrations were introduced to wells. The plates were incubated at 37 °C for 24 hrs. The zone of inhibition was measured. The diameter of the zone of inhibition was compared with those produced by standard antibiotics, streptomycin, ciprofloxacin, and chloramphenicol. 20 % DMSO was used as a negative control.

Antifungal efficacy of the compounds 3 (a, b, c, d, e, f, g, h) was investigated against *C. albicans, A. brasiliensis,* and *A. flavous.* The spore suspension from the fungal suspension was swabbed on Sabouraud Dextrose agar. Three wells were created by using a sterile cork borer and loaded with test compounds at two different concentrations (5 mg/mL and 2.5 mg/mL). The plates were incubated at 27 °C for 2–3 days. The zone of inhibition was measured. Standard antibiotic fluconazole (Diflucan) was used as a positive control, and DMSO was used as a negative control [37–39].

2.3. Antioxidant activity

Antioxidant activity was determined by using DPPH (DPPH·) and ABTS radical cation scavenging assays.

2.3.1. DPPH radical scavenging activity

The DPPH (2,2-Diphenyl-1-picrylhydrazyl) radical scavenging activity for synthetic compounds was performed by combining 50 μL of sample solutions (25–500 μg/mL) with 300 μL of an ethanolic solution comprising 0.5 mmol/L DPPH (2,2-Diphenyl-1-picryhydrazyl). The tubes were placed in a dark atmosphere and kept at a temperature of 37 °C for a duration of 5 min [41]. The absorbance was determined relative to blank at a wavelength of 517 nm. The radical scavenging activity of the compounds **3g** and **3h** is expressed as a percentage of inhibition determined using the Formula.1. Ascorbic acid was used as a standard.

$$\% \text{ Inhibition } = \frac{Absorbance\ of\ control - Absorbance\ of\ test}{Absorbance\ of\ control} X\ 100 \qquad (1)$$

2.3.2. ABTS radical cation decolorization assay

In order to perform the ABTS radical scavenging activity, the $ABTS^+$ radical was produced by combining an aqueous solution of 2,2′-azinobis (3-ethylbenzothiazolin-6-sulfonic acid) (ABTS) (7 mmol/L) with 2.45 mmol/L potassium persulfate in a 1:1 ratio.

The reaction mixture was incubated at room temperature for 16–20 h. We utilise methanol as a solvent to create the solution of $ABTS^+$with an absorbance of 0.7 at a wavelength of 734 nm. Following a 30 min incubation period different amounts of synthesized **3g** and **3h** (ranging from 10–500 μg/mL) were added to 3.99 mL of $ABTS^+$solution. The absorbance at 734 nm was then measured. The outcomes were quantified using the % scavenging of the compounds **3g** and **3h** and were expressed as percentages in Formula.2. Ascorbic acid was used as a standard [40,41].

$$\% \text{ Inhibition} = \frac{Absorbance\ of\ control - Absorbance\ of\ test}{Absorbance\ of\ control} X\ 100 \qquad (2)$$

2.4. Anti-inflammatory activity

Evaluation of *invitro,* anti-inflammatory activity of compounds **3g** and **3h** based on detecting the hydroperoxide created in the lipoxygenation process using a pure LOX. The anti-inflammatory properties of the compounds **3g** and **3h** were assessed by using soybean lipoxygenase. Lipoxygenase solution was prepared by using a 100 mmol/L sodium phosphate buffer of pH 8.0 [41]. 20 μL of lipoxygenase solution (15U/mL) was mixed with 10 μL of synthesized compounds and standard with different concentrations and made up the volume to 190 μL in each well of flat bottom 96 well plate with 160 μL of 100 m mol/L of sodium phosphate buffer. Sodium phosphate buffer alone was used as a blank, and lipoxygenase with buffer was considered as a control for the study. Celecoxib with various concentrations was used as a standard control for the study. The plate was incubated for 10 min at 25°C. This was followed by the addition of 10 μL substrate mixture (linoleic acid), and absorbance was measured at 234 nm.

The percentage inhibition for compounds **3g** and **3h** can be calculated by using the Formula.3.

$$\% \text{ Inhibition} = \frac{Absorbance\ of\ control - Absorbance\ of\ test}{Absorbance\ of\ control} X\ 100 \quad (3)$$

A graph was drawn to establish the relationship between the inhibitor conc. and the % inhibition in order to get the IC_{50} value, which corresponds to the conc. at which 50 % inhibition occurs [41–43].

2.5. Anticancer activity

2.5.1. Cell viability assay using MTT assay

The cell viability assay was conducted in accordance with [44].The proliferation rates of the human lung cancer cell line (A549 cell line) at various concentrations of compounds **3g** and **3h** (12.5, 25, 50, 100, 200, and 400 μg/mL) were determined by 3-(4,5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide) (MTT) assay. MTT is reduced by mitochondrial lactate dehydrogenase to the water-insoluble pink compound formazan, which upon dissolution into appropriate solvent exhibits a purple colour. Depending on the viability of the cells, colour intensity is produced.

The cells were maintained in Dulbecco's Modified Eagle Medium (DMEM) media with an F12 mixture supplemented with 10 % Foetal bovine serum (FBS) along with 1 % antibiotic-antimycotic solution in the atmosphere of 5 % CO_2 and 18–20 % O_2 at 37 °C in the CO_2 incubator. 200 μL of the cells (10,000 cells per well) were seeded into 96-well plates and incubated at 37 °C, 5 % CO_2, for 24 h. After 24 h of incubation, the cells were treated with 100 μL of 12.5, 25, 50, 100, 200, and 400 μg/mL of compounds **3 g** and **3h** Plates were incubated at 37 °C, 5 % CO_2,for 24 h. After incubation, the morphology of the cells was examined under a microscope. 20 μL of MTT solution was added to each well. Plates were further incubated for 3 h, and the medium was removed. Formazan crystals were dissolved in 100 μL of DMSO. Absorbance was measured at 570 nm, and 630 nm was used as a reference wavelength. % of the cell viability was calculated by using the Formula.4.

$$\% \text{ of Cell viability} = \frac{Absorbance\ of\ the\ treated\ cells}{Absorbance\ of\ the\ untreated\ cells}\ X\ 100 \quad (4)$$

The IC_{50} value was determined by using the linear regression equation, *i.e.,* y=mx+c. Here $y = 50$, and m and c values were derived from the viability graph [44–46].

2.6. Docking studies

Molecular docking studies were carried out for the screening of antibacterial, anti-inflammatory, and anticancer proteins against the compound **3d**

Crystal structures of *Staphylococcus aureus* enterotoxin C-2 (PDB ID 1STE), *Pseudomonas aeruginosa* Bacterioferritin B (PDB ID: 3IS7), lipoxygenase (PDB ID: 2IUJ), and anti-cancer protein human BCL-2, isoform 1 (PDB ID: 1G5M) were retrieved from the PDB database from www.rcsb.org. These proteins were used as receptor molecules.

The compound was drawn with proper two-dimensional orientation. The target proteins and ligands were optimised for docking studies. The energy was minimised using Marvin sketch software. The energy-minimised ligand was then used to carry out docking simulations. All the water molecules from proteins were removed using Discovery Studio software. The grid box is set for docking simulations so that it surrounds the region of interest in the macromolecule by using the graphical user interface program MGL tool. The grid box volume was set to 30 Å for the x, y, and z dimensions, respectively, and the grid centre was set for x, y, and z, respectively, which covered all the amino acids in the considered active pocket. The interaction between protein and ligand was predicted by Autodock 4.2. During docking, a maximum of 15 conformations were considered for the ligand. The discovery studio was used to deduce 2D and 3D representations of the interaction between ligand and protein [47,48].

3. Results and discussion

3.1. Chemistry

The objective under Claisen-Schmidt condensation was set from 3-acetylpyridine and aryl/heteroaryl aldehyde that react in an ethanol-water mixture in the presence of sodium hydroxide base. This resulted in the acquisition of not only the target chalcones but also unanticipated products **(3a-h)** in **75–93 %** yield that were determined by IR, ^{1}H NMR, and ^{13}C NMR to be uncommon Michael addition products.

The characteristic peaks of heterocyclic chalcone derivatives, shown by the absorption bands at 1660–1681 cm^{-1}, 1582–1585 cm^{-1}, and 1069–1090 cm^{-1} in the FT-IR spectra of the heterocyclic chalcone derivatives **(3a-h),** are caused by the $C = O$, $C = N$, and C-N functional groups, respectively. The C-S-C group in thiophene **(3a)** and the C-O-C group in furan **(3b)** are responsible for the significant peaks at 696 cm^{-1} and 1234 cm^{-1}, respectively. Ar-F **(3f)**, Ar-Cl **(3g),** and Ar-Br **(3h)** correspond to the sharp peak that emerged in the range of 973 cm^{-1}, 699 cm^{-1}, and 650 cm^{-1} (Figures in spectra supplemental file).

^{1}H NMR spectra provided additional confirmation of the heterocyclic chalcone product structures. This signals assignment is determined by its intensity pattern and chemical shift. The doublet at 3.84–5.65 ppm (J = 7.1 Hz) in the ^{1}H NMR spectra of **(3a-h)** indicates that the product's acetyl group contains two methylene protons. At 3.61 −5.05 ppm (J = 7.1 Hz), a quartet was found, suggesting that there are two -CH- groups in a molecule, showing the same chemical environment by being attached to the same type of aryl group in a derivative. A triplet within the range of δ 2.26–4.17 ppm was observed (J = 7.0 Hz) as a result of the product's single-CH- group. The pyridine ring's typical chemical shift values were revealed in the series of ^{1}H NMR spectra. Twelve hydrogen atoms from three rings were found to be in a multiplet at δ 7.44–9.06 ppm (J = 7.6 Hz). Similarly, two thiophene rings cause the signal multiplet for six protons at δ 6.78–7.28 (J = 6.8 Hz) **(3a)**. Due to two furan rings, the signal multiplet occurred for six protons at δ 6.03–7.28 ppm **(3b).** At δ 7.19 ppm to 7.28 ppm, the signals appeared as multiplets for ten aromatic protons from two benzene rings **(3c),** and at δ 6.96 ppm to 8.38 ppm for eight aromatic protons **(3d-h),** they showed as multiplets. For the derivative **(3d)**, the six protons of the two methyl groups displayed a singlet at δ 2.41 ppm. Comparably, two methoxy groups with six protons each displayed a singlet at δ 4.30 ppm **(3e).**

All of the substances ^{13}C NMR spectra structural features and the spectral signals correlate well. You can find this data under the experimental section. The ^{13}C NMR spectra showed that one nicotinoyl $C = O$ group had a large resonance at approximately δ 201–205 ppm, while two 3-acetyl pyridine moiety-linked $C = O$ groups **(3a-b)** had a major resonance at approximately δ 201.7 ppm. Similar signals were seen for one nicotinyl $C = O$ group at about δ 206 ppm and two groups linked by a 3-acetyl pyridine moiety that contained carbonyl carbon at about δ 205 ppm **(3c-h)**. Signals at about δ 152 ppm ($C = N$), 149 ppm (C-N), 136–134, 132, and 123 ppm were found for five carbon atoms in each of the three pyridine rings in the **(3a-h)** series. Due to carbon, which is linked to two chalcone groups and one nicotinoyl group, the signal ranges from δ 52.7 ppm to 57.7 ppm. Next, the δ values for the α-carbon of each chalcone -CH_2- group ranged from 43.7 to 50.7 ppm, while the δ values for the β-carbon of each chalcone -CH- group ranged from 36.3 to 46.4. A range of signals detected at δ 144.2, 126.6, 125.7, and 124.6 ppm were unclearly attributed to the carbons in each thiophene ring **(3a).** A range of signals observed at 154.0, 141.5, 110.2, and 106.6 ppm were inconclusively attributed to the carbon atoms in each furan ring **(3b).**The aromatic carbons in the **(3e)** and **(3f)** series showed signals at around δ 114.5–115.9 ppm for two carbons, 129.5 ppm for an additional two carbons, and 158 ppm (C-OCH_3)–160 ppm (C-F) for a single carbon in each aromatic ring, respectively. The aromatic ring carbons are linked to the β-position of chalcone, shown by the signals at 134 ppm **(3e)** and 139 ppm **(3f)**, respectively.

The signals attributed to two carbon atoms at approximately 128

ppm and another two carbon atoms at 129 ppm were found in each aromatic ring in the **3(c,d, g, and h)** series. The signal for ring carbon, which is connected to the β-carbon of the chalcone ring, emerged at about 141 ppm. Remaining carbon atoms for derivatives: 122.7 (C-Br) in **(3h)**, 135.3 ppm (C-CH_3) in **(3d),** 130.5 (C-Cl) in **(3g)**, and 123.2 (Ar-CH) in **(3c)**. Methoxy carbon **(3e)** and methyl carbon **(3d)** were indicated by the signals δ 55.4 ppm and δ 20.8 ppm, respectively.

Finally, characteristic peaks were observed in the mass spectra of heterocyclic chalcone derivatives. The compound **(3a-h)** showed $\boldsymbol{M}^+$ ion peaks corresponding to molecular mass at *m/z* **(*M* + 1)** of **552.12, 520.16, 540.19, 568.23, 600.22, 576.17, 608.11,** and **696.00,** respectively.

The ^{1}H NMR, ^{13}C NMR, mass, and IR spectra of the chalcone derivative 3a are as shown in Fig. 10 (the ^{1}H NMR, ^{13}C NMR, mass, and IR spectra of the chalcone derivatives 3b-3h can be found in the Supplementary Material_Figure).

3.2. Antimicrobial activity

3.2.1. Antibacterial activity

All the synthesized compounds have shown antibacterial activity against pathogens *E. coli, S. aureus, and P. aeruginosa.* Antibacterial activity of all the compounds is greater than that of streptomycin for *S. aureus.* All the compounds exhibit antibacterial efficacy in a concentration-dependent manner.

Among the synthesized compounds, **3h** and **3g** have shown good antibacterial activity against *E. coli.,* which is followed by **3e** with mean zone of inhibition values of 16.21±0.02 mm,16.05±0.05 mm, and 15.05 ±0.05 mm, respectively. All other compounds have the same antibacterial efficacy with mean zone of inhibition values around 13.05±0.05 mm.

Compound **3b** has shown good antibacterial efficacy against *S. aureus*, with a mean zone of inhibition value of 16.05±0.05 mm, which is followed by **3f** with a mean zone of inhibition value of 15.05 ±0.05 mm. All other compounds have nearly the same zone of inhibition values around 14.07±008 mm.

Compounds **3g** and **3b** demonstrated the highest antibacterial efficacy against *P. aeruginosa*, with mean zone of inhibition values of 15.05 ±0.06 mm. and 15.21±0.25 respectively.

We can conclude that, among the synthesized compounds **3g, 3h**, and **3b** have demonstrated good antibacterial efficacy.

The compounds **3g** and **3h** possess electron-withdrawing chloro and bromo groups, respectively, and exhibit the highest antibacterial efficacy against *E. coli.* Chalcones containing nitro, chloro, and bromo groups, which are electron-withdrawing groups, have more effective antibacterial activity [46].The compounds **3g** and **3h**, which have electron-withdrawing chloro and bromo groups, have shown excellent antibacterial activity. This information is further supported by [49], which further demonstrated that halo chalcones (F, Cl, Br) have shown significant antibacterial activity against *B. subtilis.* significant antibacterial action due to the electron-withdrawing chloro group was observed [50].

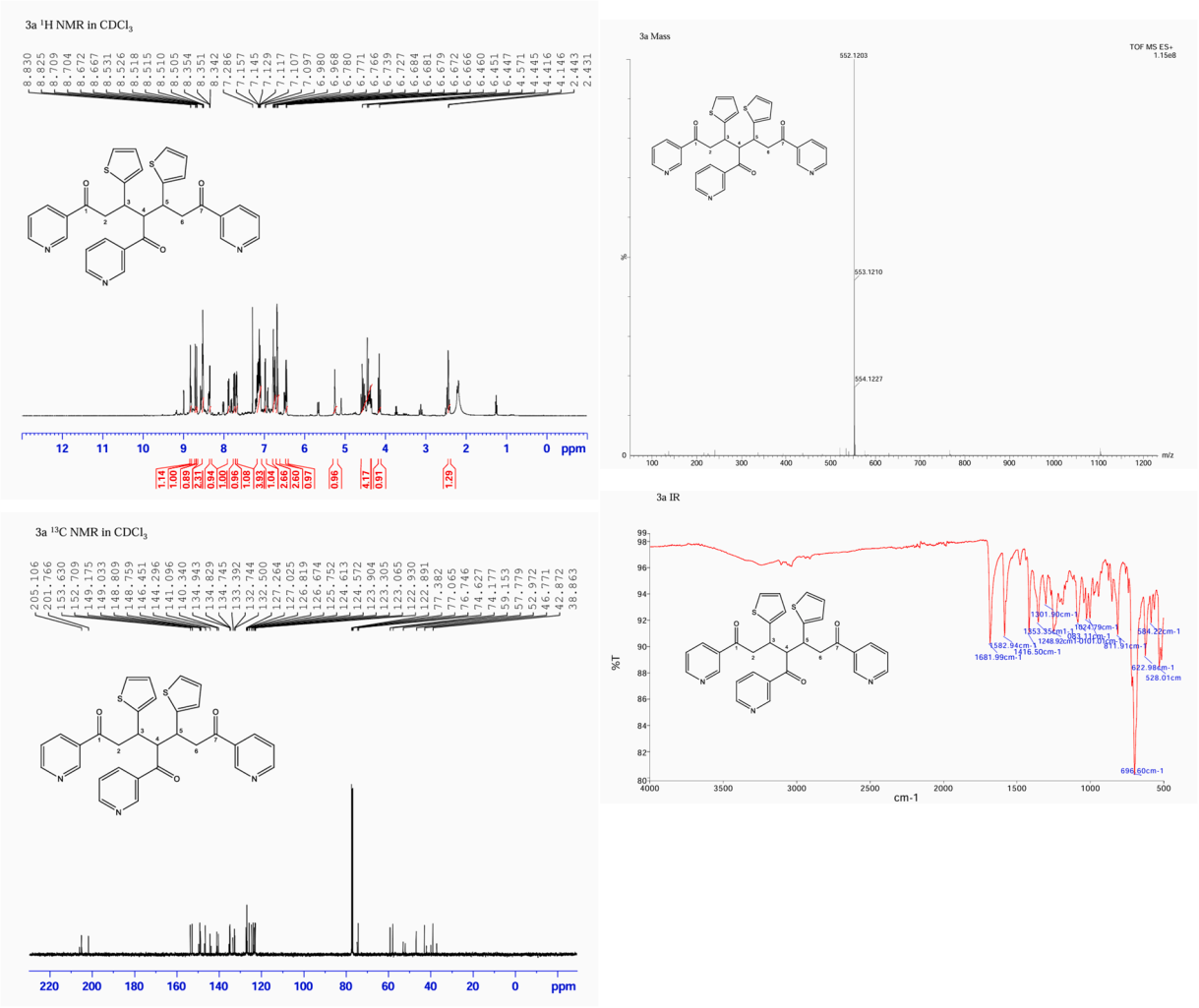


Fig. 10. ^{1}H NMR,^{13}C NMR, Mass and IR spectra of the chalcone derivative **3a**.

Compound **3b** contains a furan ring. Similar antibacterial activity by furan ring-containing chalcones against *E. coli* at the concentration of 128 μg/mL and also against *S. aureus* at the concentration of 64 μg/mL was observed [51] (Figs. 11-13.

3.2.2. Antifungal activity

All the compounds have shown good antifungal activity against *Candida albicans, Aspergillus brasiliensis, and Aspergillus flavus.*

Among the synthesized compounds, greater antifungal activity is exhibited by **3b** and **3e** towards *Candida albicans* with a mean value of zone of inhibition of 14.02±0.03 mm and 15.05±0.05 mm, respectively. All other compounds have nearly the same antifungal efficacy, with a mean inhibition zone around 11.05±0.05 mm.

All the compounds except **3c** and **3h** have nearly the same antifungal activity towards *A. brasiliensis*. Compounds **3c** and **3h** have produced the mean zone of inhibition value of 25.05±0.05 mm and 22.05±0.05 mm, respectively.

Among all the compounds, **3b, 3c**, and **3h** have shown good antifungal efficacy against *Aspergillus flavus*. Their mean zones of inhibition are 27.05±0.05 mm, 25.15±0.05 mm, and 24.05±0.05 mm, mm, respectively, for compounds **3h, 3c**, and **3b**

We can conclude that compounds **3b, 3c, 3e**, and **3h** have good antifungal efficacy.

Compound **3c** has demonstrated exceptional antifungal efficacy against both *Aspergillus* species utilised in the investigation. The antifungal activity may be ascribed to its diminutive size. Among the synthesised compounds, **3c** is the smallest. The phenyl ring in **3c** facilitates enhanced electron delocalisation and hydrophobicity, thereby improving the molecule's interaction with the fungal cell membrane and increasing its permeability, which effectively disrupts the structure and function of the fungal cell [52]. The methoxy group in compound **3e** functions as an electron donor by resonance. This may have augmented the antifungal efficacy of this drug. This is additionally corroborated by [53], which noted that methoxy-substituted compounds exhibit significant antifungal activity against *C. albicans*. The notable antifungal activity is attributed to the presence of the methoxy group detected [54]. The bromo group, including **3h**, has demonstrated significant antifungal effectiveness against *A. flavus.* This is additionally corroborated by the research of [55], which noted that bromo derivatives of chalcones have significant antifungal activity (Figs. 14-16).

3.3. Antioxidant activity

3.3.1. DPPH assay

Antioxidative properties of the compounds were evaluated based on their capacity to scavenge DPPH radicals. The DPPH radical scavenging ability of the compounds **3g** and **3h** was determined through the changes in absorbance of DPPH at 517 nm. Both the compounds effectively scavenged the DPPH radical in a concentration-dependent manner. The antioxidative activity of the compound **3g** > **3h** with IC_{50} values of 49.12 μg/mL and 66.13 μg/mL, respectively. The IC_{50} value of ascorbic acid is 25.33 μg/mL. Outcomes are the mean standard deviation of three separate experiments. Similar results were observed with chloro-substituted chalcones [56]. Chloro substituted compounds 6e, 6j and 6n have shown good DPPH radical scavenging activity with IC_{50} values of 63.92, 27.71 and 35.02 respectively [50] (Fig. 17).

3.3.2. ABTS scavenging activity

Compounds **3g** and **3h** exhibited dose-dependent ABTS radical scavenging (100–500 μg/mL), demonstrating remarkable radical scavenging activity. The percentage inhibition of ABTS scavenging was recorded. The compounds **3g** and **3h** have IC_{50} values of 74.64 μg/mL and 68.74 μg/mL, respectively, while ascorbic acid had an IC_{50} value of 12.44 μg/mL. All values are the standard deviation of the mean of three independent experiments. Halo substituted compounds have demonstrated good antioxidant activity. This is further supported by [57] who observed that the strong ABTS radical scavenging activity of the compounds is due to the presence of electron withdrawing chloro substituted group on their bis chlorine molecules (Fig. 18).

3.4. Anti-inflammatory activity

The detection and measurement of hydroperoxides generated in the lipoxygenation reaction using purified Lipoxygenase (LOX) enzyme are the basis of the LOX inhibitory assay. The compounds **3g** and **3h** were evaluated for their LOX inhibitory activity in dose dependent manner and IC_{50} value was determined. The results have shown that both the compounds have good anti-inflammatory activity, which is slightly less than that of standard Celecoxib. The IC_{50} values of **3g** and **3h** were 267.9 μg/mL and 55.83 μg/mL, respectively, whereas IC_{50} value of standard Celecoxib was 20.08 μg/mL. The compounds **3g** and **3h** having chloro and bromo-substituted groups, respectively, are good in anti-inflammation action. Compound **3h** exhibited more effective inhibition of LOX than **3g** This was further supported by [58], who have shown the electron-withdrawing group-bearing compounds have better anti-inflammatory action. Although both chlorinated and brominated chalcones have demonstrated good anti-inflammatory activity, generally brominated chalcones tend to exhibit stronger anti-inflammatory activity [59] (Fig. 19).

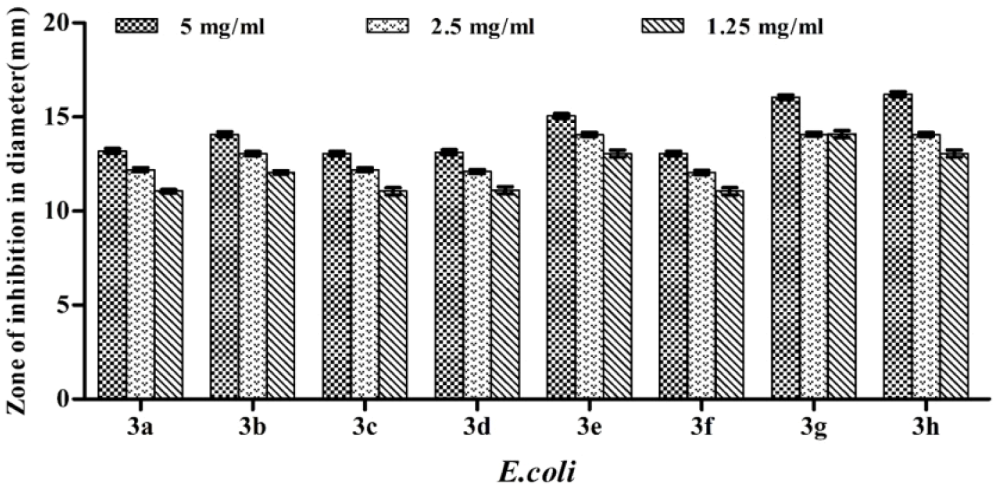


Fig. 11. Antibacterial activity of the compounds **3(a-h)** towards *E.coli.*

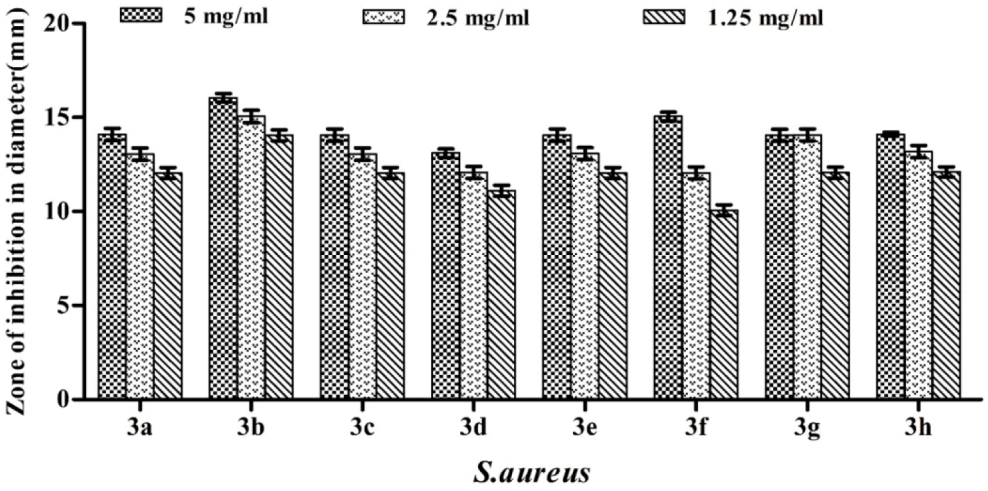


Fig. 12. Antibacterial activity of the compounds **3(a-h)** towards *S.aureus*.

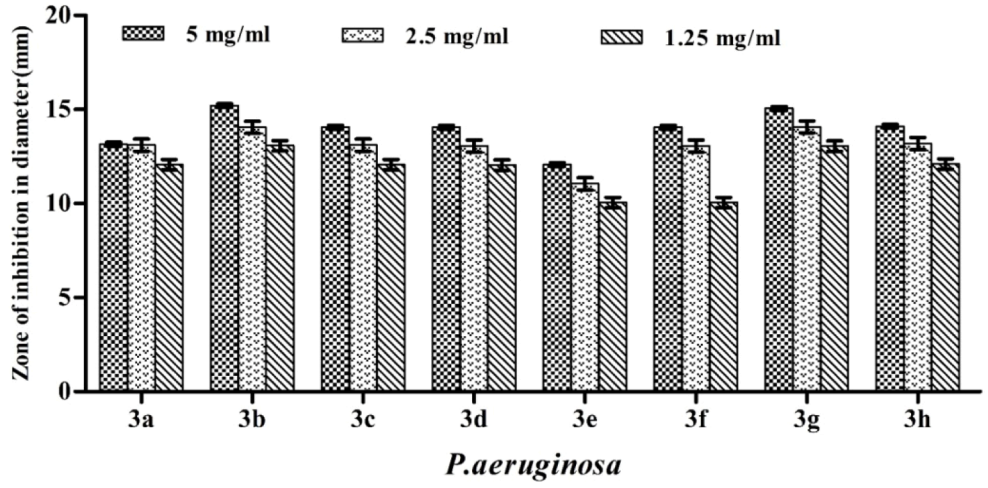


Fig. 13. Antibacterial activity of the compounds **3(a-h)** towards *P.aeruginosa.*

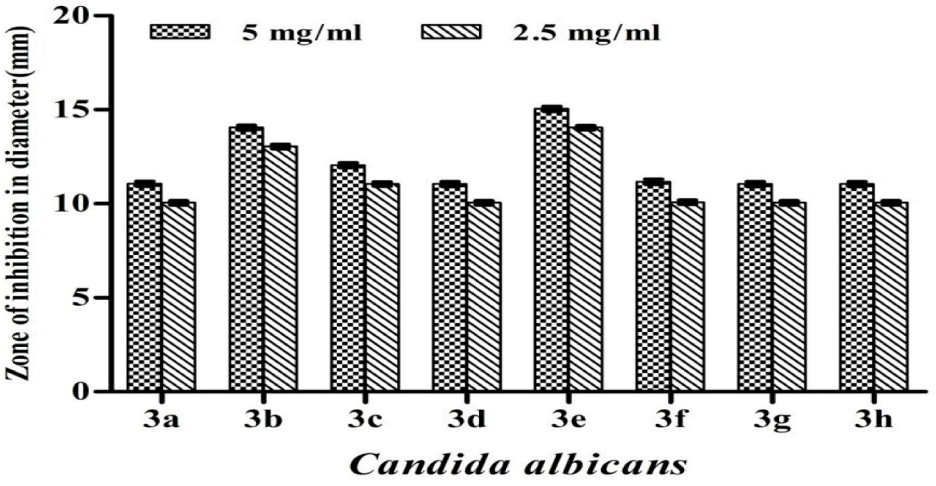


Fig. 14. Antifungal activity of the compounds **3(a-h)** towards *C.albicans.*

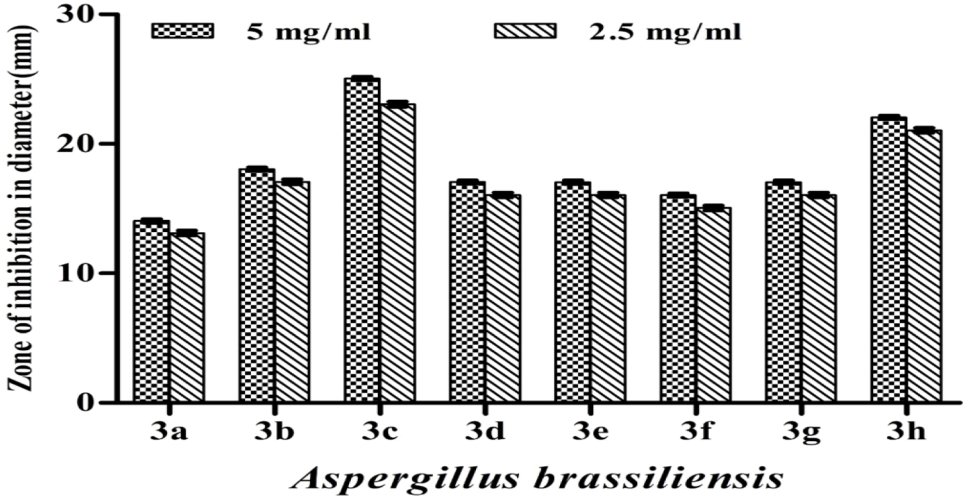


Fig. 15. Antifungal activity of the compounds **3(a-h)** towards *A.brasiliensis.*

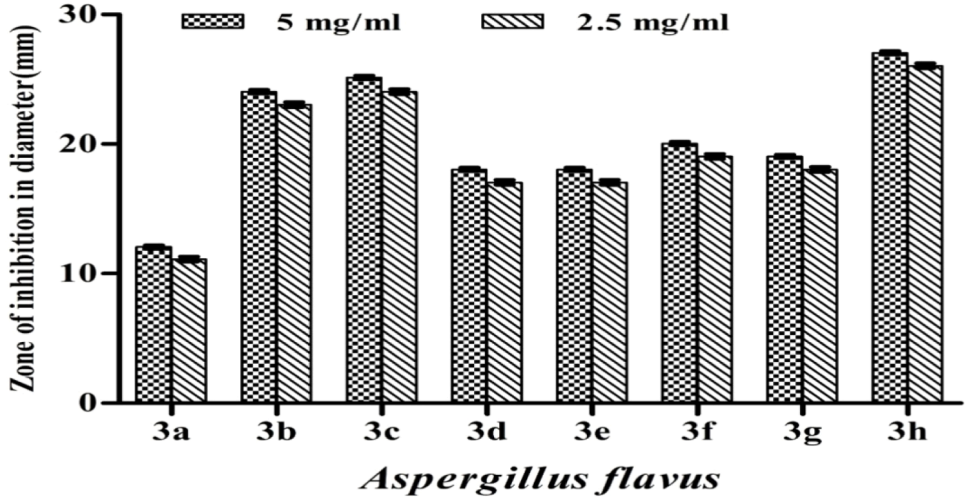


Fig. 16. Antifungal activity of the compounds **3(a-h)** towards *A.flavus.*

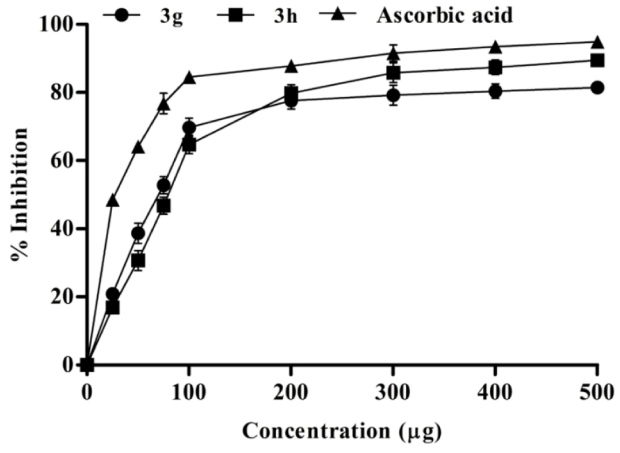


Fig. 17. Antioxidative action of compounds **3g** and **3h** with DPPH.

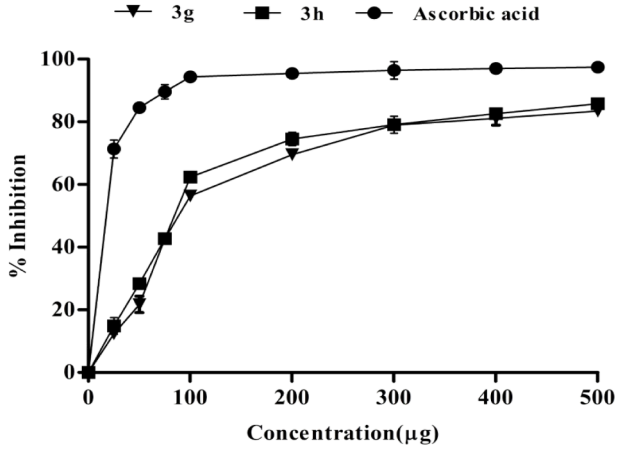


Fig. 18. ABTS cation radical scavenging activity of compounds **3g** and **3h**.

3.5. Anticancer activity

3.5.1. Cell viability assay using MTT assay

Cytotoxicity of the compounds, **3g** and **3h**, was tested on A549 cell lines and compared to that of the standard anticancer drug Erlotinib. Cancer cells treated with compounds **3g** and **3h** showed dose-dependent cytotoxicity with an increase in cell cytotoxicity as the concentrations of **3g** and **3h** increased. In this cell line, the percentage of cell viability

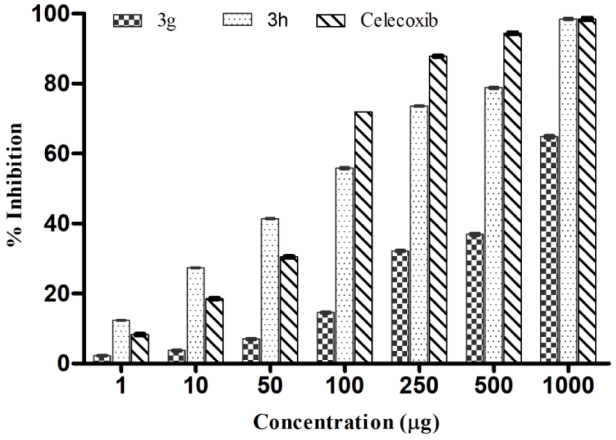


Fig. 19. Anti-inflammatory activity of the compounds **3g** and **3h**.

based on the IC_{50} value was observed to be 69.77 % at 100 µg/mL for **3g** and 52.73 % at 100 µg/mL for **3h** Cytotoxicity was observed at concentrations ranging from 12.5 µg/mL to 400 µg/mL for both the compounds. The series of halo-substituted chalcones synthesized exhibited anticancer activity [60]. The cytotoxic activity was observed both *in vitro* and *in vivo* in gastric cancer cells by bromo substituted chalcones [61].

The chloro- and bromo-derived chalcones exhibited the highest antiproliferative activity in a dose-dependent manner with an IC_{50} value below 1.0 and 1.57 µg/mL for AGS and HL-60 cells, respectively [62].

Halogenated chalcones show strong anticancer activity. Halogen-bond interactions usually follow donor-acceptor complex formation between donor halogen and acceptor atom. Their excellent ability to reduce the cancer cell viability, lack of toxicity towards erythrocytes, and favourable physicochemical and pharmacokinetic properties [63]. Even though bromine is less electronegative than chlorine, it is more resonance stabilized, and brominated derivatives have shown good anti proliferative action against HCT 116 cells [64] (Fig. 20).

3.6. Docking studies

Docking analysis of the compound **3d** was performed to identify the antibacterial, anti-inflammatory, and anticancer target proteins in an attempt to justify all these activities of the synthesised compound **3d** (Table 1).

The docking result of the antibacterial target protein *Staphylococcus aureus* enterotoxin C-2 (PDB ID: 1STE) revealed that the compound has a significant binding mode with a docking score of −7.4 kcal/mol. The compound formed three hydrogen bonds with Tyr 32, Asn 60, and Tyr 26. Further interaction was stabilised by alkyl bonds and van der Waals interactions.

The docking result of the antibacterial target protein *Pseudomonas aeruginosa* Bacterioferritin B (PDB ID: 3IS7) showed that the compound has interacted with the target protein with a good docking score of −6.6 kcal/mol. The compound formed H bonds with Asn 70 and Asp 73. Further interaction is stabilised by van der Waals forces and alkyl bonds.

The docking result of the anti-inflammatory target lipoxygenase (PB ID: 2IUJ) demonstrated that the synthesised compound has a good docking mode with a docking score of −8.8 kcal/mol. The interaction is established through four H bonds formed with Gln 277, Asn 562, Asp 205, and Arg 557. It is further stabilised by a large number of van der Waals forces.

The docking analysis of the anticancer target protein Human BCL-2, Isoform 1 (PDB ID: 1G5M), establishes that the interaction is good with a docking score of −7.5 kcal/mol. The hydrogen bond is formed with Tyr 18. Interaction is further stabilised by a large number of van der Waals forces and electrostatic interaction with Glu 152.

All these results suggest that the synthesised molecule has shown antibacterial, anti-inflammatory, and anticancer activity (Figs. 21-28).

4. Conclusion

Substituted 1-(pyridine-3-yl) prop-2-en-1-one chalcone is formed when 3-acetyl pyridine **(1)** condenses with heterocyclic aldehyde and substituted benzaldehyde **(2a-h)**. This compound cannot be eliminated under the circumstances of the Claisen-Schmidt reaction. As a result of Michael's addition reaction of chalcone with 3-acetyl pyridine, it has now been demonstrated that chalcone, in the first stage, produces the molecule of 1,7-di(pyridine-3-yl) heptane-1,7-dione chalcone derivatives in the second stage.

The novel products are formed in a single step using stoichiometric amounts of 3-acetyl pyridine and aromatic aldehydes, including heterocyclic aldehydes. The chalcones are not isolated in the reaction. The chalcones formed immediately undergo Michael addition to give novel products. Pyridine chalcone derivative synthesis proved incredibly easy, quick, and environmentally benign. Less money was spent on the synthesis chemical, which produced a favourable outcome. The goal of synthesis was to create a low-cost, effective medication design without any negative effects.

In the present study, innovative substituted pyridine heterocyclic chalcones(**3a-h**) were successfully synthesized. These compounds

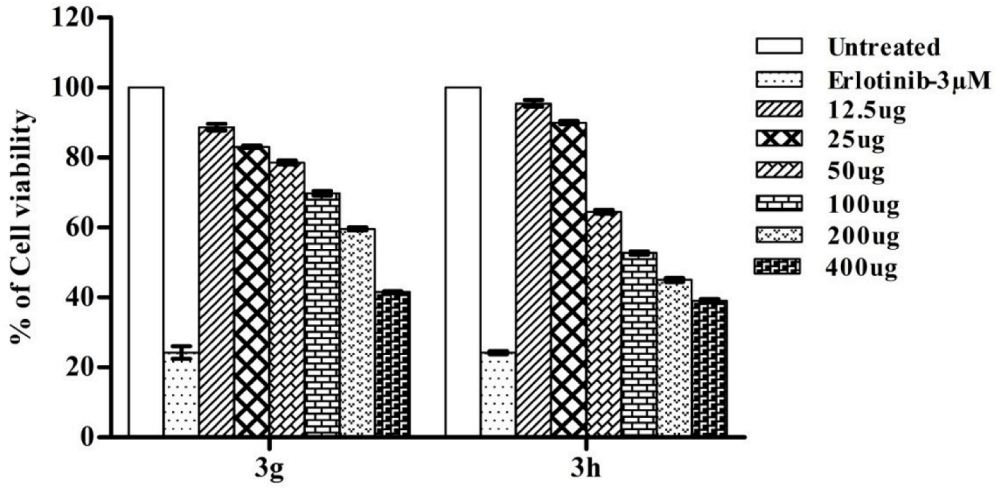
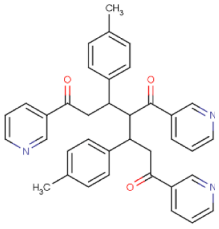


Fig. 20. Comparative % of cell viability of **3g** and **3h** treated cells at different concentrations.

Table 1
Docking of the ligand**3d** with *Staphylococcus aureus* enterotoxin C-2 (PDB ID 1STE), *Pseudomonas aeruginosa* Bacterioferritin B (PDB ID: 3IS7), lipoxygenase (PDB ID: 2IUJ), and anti-cancer protein human BCL-2, isoform 1 (PDB ID: 1G5M).

Receptor	Ligand (compound 3d)	Binding energy (kcal/J)	No.of hydrogen bonds	Amino acid interaction with ligand
Staphylococcus aureus (PDB ID 1STE),		−7.5	3H	Tyr A: 32 Asn A:60 Tyr A: 26
Pseudomonas aeruginosa (PDB ID: 3IS7)		−6.6	2H	Asn H: 70 Asp H: 73
LOX (PDB ID: 2IUJ)		−8.8	4H	Gln A: 277 Asn A: 562 Asp A:205 Arg A:557
Apoptosis (PDB ID: 1G5M)		−7.5	1H	Tyr A: 18

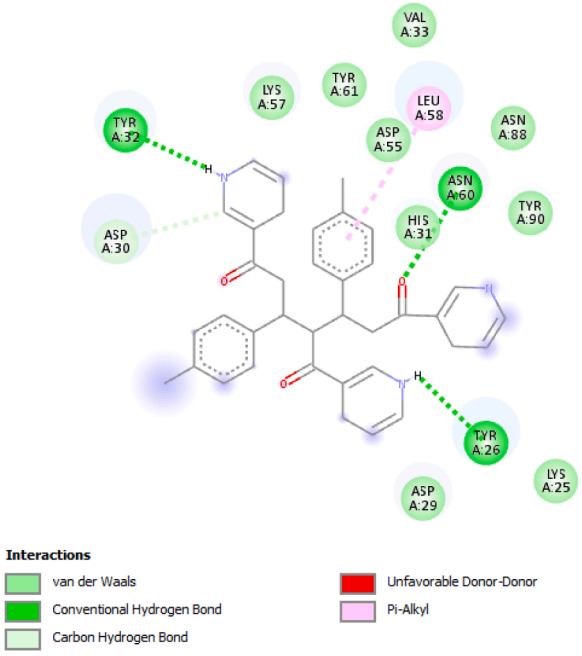


Fig. 21. 2D structure of *Staphylococcus aureus* enterotoxin C-2(PDB ID:1STE).

Fig. 22. 3D structure of *Staphylococcus aureus* enterotoxin C-2(PDB ID:1STE).

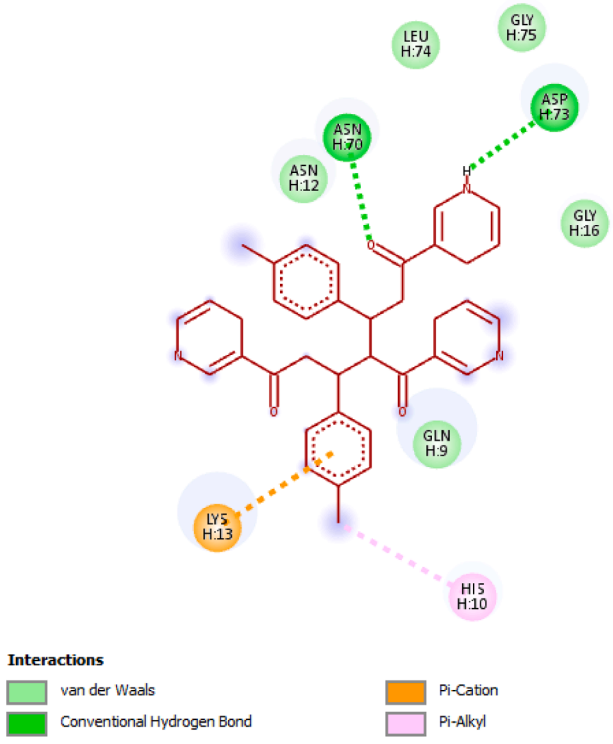


Fig. 23. 2D structure of *Pseudomonas aeruginosa* Bacterioferritin B (PDB ID: 3IS7).

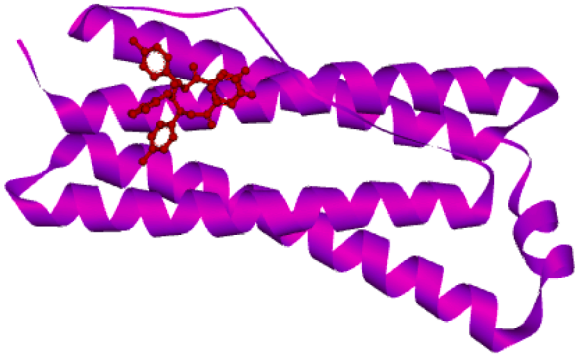


Fig. 24. 3D structure of *Pseudomonas aeruginosa* Bacterioferritin B (PDB ID: 3IS7).

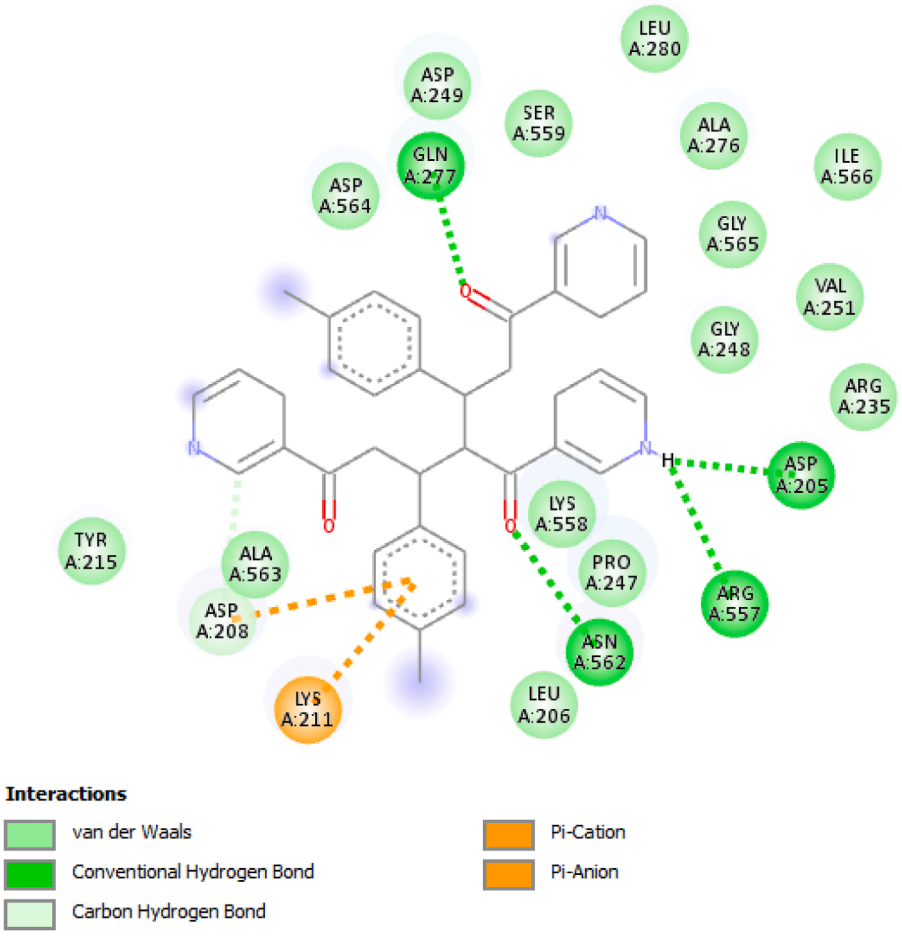


Fig. 25. 2D structure of Lipoxygenase (PB ID: 2IUJ).

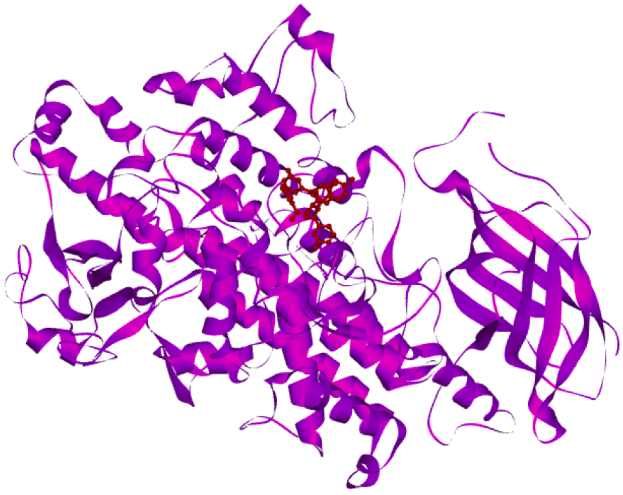


Fig. 26. 3D structure of Lipoxygenase (PB ID: 2IUJ).

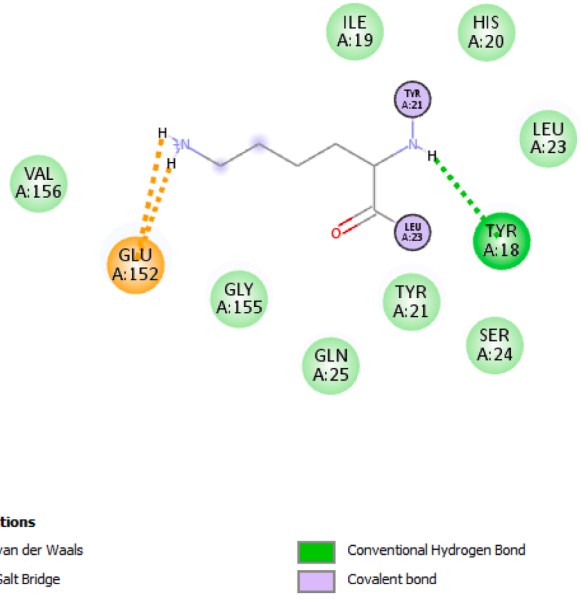


Fig. 27. 2D structure of anti-cancer protein Human BCL-2, Isoform 1 (PDB ID: 1G5M).

underwent rigorous evaluation for their *in vitro* antimicrobial, antioxidant, anti-inflammatory, and anticancer properties and were subjected to molecular docking studies with potent receptors demonstrating these activities. The findings indicated that the synthesised compound **3g** exhibited the highest antibacterial, antioxidant, anti-inflammatory, and cytotoxic activities. Additionally, molecular docking studies of the compound **3d** suggested that the potential interaction sites for the antibacterial activity are *S. aureus* enterotoxin C-2 and *P. aeruginosa* bacterioferritin B, for anti-inflammatory activity are lipoxygenase, and for anticancer activity are human BCL-2 isoform 1. The pharmacokinetic profile of these compounds indicates that these compounds can be

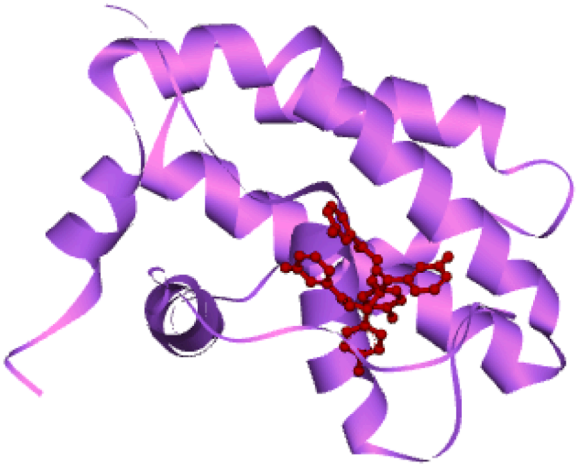


Fig. 28. 3D structure of anti-cancer protein Human BCL-2, Isoform 1 (PDB ID: 1G5M).

further used to explore their beneficial effect at the molecular level.

There was a lot of biological activity in all the compounds **(3a–h)**, but compounds **3g** and **3h** were more effective because they had a chloro group and a bromo group added to them, respectively. Chloro and bromo substituents are electron-withdrawing groups.

CRediT authorship contribution statement

Geetha Doreswamy: Writing – review & editing, Writing – original draft, Resources, Methodology, Formal analysis, Conceptualization. **BasavarajuYeriyur Basavaiah:** Writing – review & editing, Supervision, Methodology, Conceptualization. **Roopa Kallappa:** Resources, Methodology.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.molstruc.2025.142979.

Data availability

No data was used for the research described in the article.

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