SYNTHESIS, MOLECULAR DOCKING STUDIES AND EVALUATION OF PHARMACOLOGICAL ACTIVITIES OF *N*'-BENZYLIDENE-2-([SUBSTITUTED-PHENYL](3-(5-(4- NITROPHENYL)THIOPHENE-2-yl) OXIRAN-2-yl) METHOXY) ACETOHYDRAZIDE

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ABSTRACT

A series of novel *N*'-benzylidene-2-([substituted-phenyl](3-(5-(4-nitrophenyl)thiophen-2-yl) oxiran-2-yl)methoxy)acetohydrazide have been prepared through a multistep synthesis. The key featuresof this reaction are the incorporation of four heterocyclic rings in the structure of target productsusing commonly available and inexpensive catalysts, high yields (up to 60-87%), and simple reaction conditions.Final salt products were obtained by self-capturing a proton by the nitrogen of the thiophene moiety. The easy work-up and mild reaction conditions are notable features of this protocol. Theantibacterial, and anti-inflammatory activities of the synthetic products were examined in comparison with standard drugs such as Rifampicin and fluconazole for antimicrobial and diclofenac sodium for anti-inflammatory. Most of the compounds showed good activities in comparison to the references. The docking study revealed that the target compounds exhibited strong interactions with the protein, surpassing those of the standard drug rifampicin, with binding affinities ranging from -9.6 to -8.1 kcal/mol.

KEYWORDS: Propanones, Hydrazide, Pharmacological, Anti-inflammatory, Anti-bacterial.

1.INTRODUCTION

Inflammation, as a defense mechanism in higher organisms, plays a pivotal role in maintaining health by detecting and addressing harmful stimuli such as damaged cells, irritants,and pathogens[1]. This essential immune response is critical for healing infections, injuries, and tissue damage. The immune system initiates a variety of physical responses that result in inflammation, which can manifest in both acute and chronic forms[2]. Conditions like acute bronchitis, infected ingrown toenails, sore throats from colds or flu, skin cuts or scratches, intense exercise, acute appendicitis, dermatitis, tonsillitis, bacterial meningitis, sinusitis, and physical injuries are all known to trigger acute inflammation[3].[4].

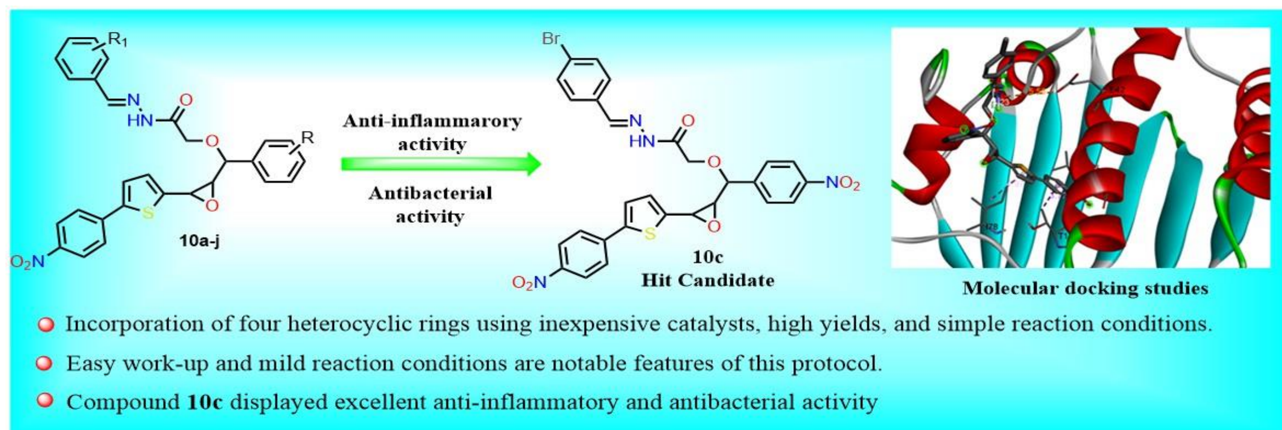
Chronic inflammation is associated with a range of diseases, including asthma, hepatitis, ulcerative colitis, Crohn's disease, periodontitis, rheumatoid arthritis, tuberculosis, and chronic peptic ulcers[5]To manage the pain and discomfort caused by inflammation, various treatments are commonly employed, including NSAIDs, corticosteroids, and herbal supplements[6]. The search for safe and effective treatments for these conditions is ongoing. This underscores the need for continued research and innovation in the field of inflammatory disease management[7].

The rise in immune-compromised patients in recent years is closely linked to the rise in fungalinfections[8,9].The majority of mycoses that are found are categorized as superficial or systemic. While *Aspergillus* sp., and *Candida* spp., are the principal culprits behind systemic mycoses, dermato phytes, a group of fungi that includes species from the *Microsporum* and *Trichophyton* genera, are also responsible for superficial infections[10,11]. Even though various antifungal medications are available to treat fungal infections, issues withtoxicity, resistance, and efficacy profiles severely restrict their use[12,13].

Chalcones wide range of actions as anti-inflammatory[14], antifungal[15], antibacterial[16], antioxidant [17], antimalarial[18], and anticancer [19]drugs have been thoroughly investigated. One of our earlier investigations showed that the inhibition of the manufacture of fungal cell-wall polymers like (1,3)β-D-glucansynthase is linked to the mode of action of several chalcones against fungal infections. From amedicinal perspective, chalcones can inhibit glutathione-*S*-transferases (GSTs), which are enzymes linked to drug resistance[20]. This may lead to the development of new drugs forthe treatment of infections brought on by fungi. Furthermore, earlier research has shown that theyeast genotype, strain cell density, and chalcone substitution pattern all affect the antifungal efficacyof the compound. On the other hand, hydrazones are

known to possess several biological properties such as anti-inflammatory[21], antimicrobial[22], anticancer[23], antioxidant[24], antibacterial[25], antiviral[26], anticholinesterase[27], etc.

Graphical abstract:



2. MATERIAL AND METHODS

2.1 Chemistry

2.1.1 General

Solvents (analytical grade)and reagents (Laboratory grade) from Merck Millipore (Billerica, MA, USA) or Sigma-Aldrich(St.Louis, MO, USA)were used without purification. Thin layer chromatography (TLC)was performed on pre-coated silica gel60GF254(Merck) platesmeasuring 0.2 mm in diameter. Melting points were measured using SMP3 melting point equipment (Stuart, Staffordshire, UK). An ATR-equipped Nicolet 6700 from Thermo Scientific (Waltham, MA, USA) was used to obtain FT-IRspectra.The ^{1}H- and ^{13}C-NMR spectra were recorded on a DPX400 spectrometer (Bruker, Billerica, MA, USA) operating at 400 and 100 MHz respectively, using $CDCl_3$ as the solvent and TMS as the internal standard. The spectrometer used was GCMS-QP2010.

2.1.2. Generalprocedureforthesynthesisof1,3-diaryl-2-propene-1-ones (3)

To solution of 3 g of sodium hydroxide (1.5 mol) in 30 mL of water and 18 mL of ethyl alcohol is taken in a 500 mL round-bottomed flask. In the flask provided with a mechanical stirrer, 7 mL of aceto phenone (**2**,1 mol) was added. The reaction mixture cooled to 0-5^{0}C in to which 6mL of pure aryl aldehyde (**1**,1 mol) was added. Stirred the reaction mixture for 3 h at 20-25^{0}C

temperature, the reaction was monitored by the TLC (Ethyl acetate :Hexane, 7:3) technique upto completion. After the reaction was completed, pH6-7was adjusted by using diluted hydrochloric acid. The reaction mixture was washed with ether, ether layer was separated. Theorganic layer was dried with anhydrous Na_2SO_4 and the solvent using a vacuum[28]. The isolated crude chalcone (**3**) was purified by silica column chromatography technique with methanol and dichloromethane with good yield (96%).

^{1}HNMR:δ6.55(1H,d,*J*=9.5Hz),6.79(2H,d,*J*=8.9Hz),7.03(1H,d,*J*=8.6Hz),7.42-7.56(4H,7.49 **x**`(d,*J*=8.9,Hz),7.50(d,*J*=8.7Hz),7.63-7.80(3H,Ar-H),7.69(d,*J*=8.6Hz),7.74(d,*J*=8.7 Hz),8.03(1H, d, *J* = 9.5 Hz).

IR (KBr, cm^{-1}): 1600 (-C=O- stretching), 1620 (C=C stretching), Mass(m/z): 380.

2.1.3.General procedure for the synthesis of *para*-substituted phenyl)(3-(5-(4-nitrophenyl) thiophen-2-yl)oxiran-2-yl)methanone (4)

To the reaction mixture (**3**,1 mol), a solution of 30% H_2O_2 (2 mol) was added dropwise at ^{0}C and a small amount of sodium hydroxide (6 mL) was added in the presence of alcohol medium, stirred for 1.5 h to complete the epoxidation step. Progress of the reaction mixture was monitored by TLC by using a mobile phase (Ethyl acetate : Hexane, 8:2). The reaction was completed for 3 h. Vacuum filtration progressed, and the solid product (**4**) was recovered. The highest yield of 43% was obtained in ethanol when a 2.5molar equivalent of H_2O_2 was used[29].

^{1}HNMR:δ4.20-4.32(2H,-CH_2),4.26(d,*J*=8.1Hz),4.26(d,*J*=8.1Hz),6.83-7.16(4H,6.89(d,*J*=8.9 Hz), 6.99(d,*J* =8.5Hz),7.10(d,*J* =8.5Hz),7.40(2H,d, *J* =8.9Hz),7.54(2H,d,*J*=8.7 Hz),7.94(2H,d, *J* = 8.7,1.9,0.4Hz).

IR(KBr, cm^{-1}): 1610 (-C=O-stretching), 1650 (C=Cstretching), 2980 (-C=N-stretching), Mass (m/z): 396.

2.1.4.General procedure for the synthesis of (*para*-substitutedphenyl)(3-(5-(4-nitrophenyl) thiophen-2-yl)oxiran-2-yl)methanol (5)

To a 25 mL round-bottom flask, *para*-substituted phenyl)(3-(5-(4-nitrophenyl)thiophen-2-yl) oxiran-2-yl)methanone(**4**,1mmol) and methanol(20mL) were added, followed by $NaBH_4$ (38mg,1mmol),and the solution was stirred for three min. The reaction was quenched with1M HCl until a pH of 6 was reached (~1.5mL) and evaporated in a vacuum to dryness. The crude product (**5**) was extracted with ethyl acetate (3x20 mL). The combined organic layers were washed with brine, dried over anhydrous sodium sulfate, filtered, and evaporated in a vacuum to

dryness to obtain yield 72%[30].

^{1}HNMR:δ3.65(1H,d, J=8.1 Hz),3.91(1H,d,J=8.1Hz),5.00(1H,d,J=5.0Hz),6.89(2H,d,J=0.5Hz), 6.99-7.16(2H,7.05(d,J=8.5Hz),7.10(d,J=8.5Hz),7.22-7.47(4H,7.28(d,J=8.3,Hz),7.40(d,J =8.9Hz) 7.56(2H,d,J =8.3,Hz).

2.1.5.General procedure for the synthesis of methyl-2-([substituted phenyl](3-(5-(4-nitrophenyl) thiophen-2-yl) oxiran-2-yl) methoxy) acetate (6)

To the reaction mixture, a solution of (*para*-substituted phenyl)(3-(5-(4-nitrophenyl)thiophen-2-yl)oxiran-2-yl)methanol(**5**,**0.0**1mol) which was dissolved in DMF was refluxed in the presence of methyl bromo acetate(0.01 mol) for 5 h, Progress of the reaction mixture was monitored by TLC by using mobile phase (Ethyl acetate: Hexane, 7:3). Reaction was completed in 5 h. The reaction mixture was allowed to cool and poured into the ice mixture. The precipitate obtained was filtered by using vacuum filtration and dried. Recrystallization was done by using ethanol to get **6** to get 69% of the yield[31].

^{1}HNMR:δ3.63-3.77(4H,3.70(d, J=8.1,Hz),3.72(s),3.88(1H,d,J =8.1Hz),4.11-4.21(2H,4.16(s), 4.16(s),5.04(1H,d,J=5.3Hz),6.89(2H,d,J=8.9,Hz),6.99-7.16(2H,Ar-H),7.05(d,J=8.5Hz), 7.10(d,J= 8.5Hz),7.40(d,J=8.9 Hz),7.49(d, J= 8.3Hz),7.56 (d,J= 8.3Hz).

^{13}CNMR:δ52.2,57.1,58.8,65.5,73.9,114.3,124.0,127.0,127.2,128.5,128.6,128.7,128.8,131.6,133. 7,134.3,134.6,148.4,151.1,168.5.

2.1.6. General procedure for the synthesis of 2-([substituted phenyl](3-(5-(4-nitrophenyl) thiophen-2-yl)oxiran-2-yl) methoxy) acetic acid (7)

To the reaction mixture, methyl-2-([substituted phenyl](3-(5-(4-nitrophenyl)thiophen-2-yl)oxiran-2-yl)methoxy)acetate (**6**)was dissolved in water and it was refluxed for 2h by using sodium hydroxide solution(2 mL). After the completion of the reaction as monitored by TLC (Ethyl acetate: Hexane, 7:3), the reaction mixture was allowed to cool and poured into an ice mixture. The precipitate obtained was filtered by using vacuum filtration and dried to get **7** to get 75% of the yield[32]

^{1}H NMR (400 MHz, $CDCl_3$): δ 12.80 (s, 1H), 7.57–7.52 (m, 4H), 7.54 –7.47 (m, 3H), 7.43-7.36 (m, 3H), 7.01 (d, J = 8.5 Hz, 1H), 5.03 (d, J = 5.3 Hz, 1H), 4.22 (s, 2H), 3.81 (d, J = 7.7 Hz, 1H), 3.54 (dd, J = 7.7, 5.3 Hz, 1H).

^{13}C NMR (100 MHz, $CDCl_3$): δ 170.5, 147.6, 146.5, 138.1, 135.6, 133.7, 128.6, 128.10, 128.0, 127.3, 124.2, 123.8, 117.0, 111.6, 67.6, 59.1, 51.8.

2.1.7.General procedure for the synthesis of ethyl 2-([substituted-phenyl](3-(5-(4-nitrophenyl)thiophen-2-yl)oxiran-2-yl)methoxy)acetate (8)

Intermediate 2-([substituted phenyl](3-(5-(4-nitrophenyl)thiophen-2-yl)oxiran-2-yl)methoxy) acetic acid (**7**)(0.01mol) in 20mL of absolute alcohol and 0.5mL dil sulfuric acid was refluxed for 12h. The excess alcohol was removed by distillation. The contents were cooled and poured into ice. The product obtained was filtered through a sintered glass crucible and dried to get **8** obtained yield of 69%[33].

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.59–7.51 (m, 4H), 7.50–7.47 (m, 2H), 7.43–7.36 (m, 3H), 7.39 (d, J = 8.5 Hz, 1H), 7.01 (d, J = 8.5 Hz, 1H), 5.02 (d, J = 5.4 Hz, 1H), 3.98 (s, 2H), 3.81 (d, J = 7.7 Hz, 1H), 3.53 (dd, J = 7.7, 5.4 Hz, 1H), 2.49 (q, J = 7.4 Hz, 2H), 1.02 (t, J = 7.4 Hz, 3H).

^{13}C NMR (100 MHz, $CDCl_3$): δ 170.5, 147.6, 146.5, 138.1, 135.6, 133.7, 128.6, 128.1, 128.0, 127.3, 124.2, 123.8, 117.0, 111.6, 67.6, 59.1, 51.8.

2.1.8. General procedure for the synthesis of 2-([substituted -phenyl](3-(5-(4-nitrophenyl) thiophen-2-yl)oxiran-2-yl)methoxy)acetohydrazide (9)

Ethyl2-([susbtituted-phenyl](3-(5-(4-nitrophenyl)thiophen-2-yl)oxiran-2-yl)methoxy)acetate(**8**, 0.01 mol) in 20 mL of absolute alcohol and 0.5 mL hydrazine hydrate was refluxed for 8 h.The contents were cooled to room temperature and poured into ice. The product (**9**) obtained was filtered through a sintered glass crucible and dried to get a yield of 76%[34].

^{1}HNMR:δ3.70(1H,d, J=8.1 Hz),3.88(1H,d,J=8.1Hz),4.20-4.30(2H),4.25(s, 2H),5.04(1H, d, J=5.3Hz),6.89(2H,d,J =8.9 Hz),6.99-7.16(2H,7.05(d,J=8.5Hz),7.10(d,J=8.5Hz),7.34-7.63 (6H, Ar-H), 7.40(d,J=8.9 Hz),7.49(d,J=8.3Hz),7.56(d,J = 8.3,Hz).

^{13}CNMR:δ57.1,58.8,65.5,73.9,114.3,124.0,127.0,127.2,128.5,128.6,128.7,128.8,131.6,133.7,134.3,134.6,148.4,151.1,169.8.

2.1.9.General procedure for the synthesis of *N*'-benzylidene-2-([substituted-phenyl](3-(5-(4-nitrophenyl) thiophen-2-yl)oxiran-2-yl)methoxy) aceto hydrazide (10a-j)

Final intermediate *N*'-benzylidene-2-([substituted-phenyl](3-(5-(4-nitrophenyl)thiophen-2-yl) oxiran-2-yl)methoxy)acetohydrazide (0.01mol) in 20mL of absolute alcohol and aceticacid(5 mL) in the presence of substituted aldehyde(0.01mol)was refluxed for 3h.The contents were cooled to room temperature and poured into ice. The product obtained was filtered through a sintered glass crucible and dried to get final products **10a-j**[24].

Compound 10a

^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.08 (s, 1H), 7.68–7.65 (m, 2H), 7.59–7.55 (m, 2H), 7.55–7.52 (m, 2H), 7.52 (d, J = 1.6 Hz, 1H), 7.51–7.47 (m, 2H), 7.44–7.40 (m, 4H), 7.39 (d, J = 8.6 Hz, 1H), 7.01 (d, J = 8.5 Hz, 1H), 5.03 (d, J = 5.4 Hz, 1H), 4.14 (s, 2H), 3.81 (d, J = 7.7 Hz, 1H), 3.54 (dd, J = 7.7, 5.4 Hz, 1H).

^{13}CNMR:δ57.1,58.8,65.5,73.9,114.3,117.7,124.0,127.0,127.2,128.5,128.6,128.7,128.8,129.4,131.6,133.6, 133.7,133.8,133.9,134.3,134.6,139.5,146.5,148.4, 151.1,169.8.

Compound 10b

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.08 (s, 1H), 7.68–7.65 (m, 2H), 7.55–7.52 (m, 2H), 7.42 (dd, J = 7.9, 1.9 Hz, 3H), 7.40 (dt, J = 3.9, 1.9 Hz, 3H), 7.37 (d, J = 2.2 Hz, 2H), 7.34–7.30 (m, 2H), 7.01 (d, J = 8.5 Hz, 1H), 5.03 (d, J = 5.4 Hz, 1H), 4.14 (s, 2H), 3.81 (d, J = 7.7 Hz, 1H), 3.53 (dd, J = 7.7, 5.4 Hz, 1H).

^{13}CNMR:δ57.1,58.8, 65.5, 73.9, 114.3, 117.7, 122.3, 124.0,127.0,127.3,128.6,129.4,131.6,131.7, 133.8,134.3,134.6,139.5,146.5,148.4,151.1, 169.8.

Compound 10c

^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.09 (s, 1H), 7.60 (d, J = 8.6 Hz, 2H), 7.53 (d, J = 8.6 Hz, 4H), 7.42 (d, J = 1.6 Hz, 1H), 7.40 (t, J = 1.8 Hz, 1H), 7.37 (d, J = 3.5 Hz, 2H), 7.37 (s, 1H), 7.35 (s, 1H), 7.13–7.09 (m, 2H), 7.01 (d, J = 8.5 Hz, 1H), 4.99 (d, J = 5.4 Hz, 1H), 4.14 (s, 2H), 3.81 (d, J = 7.7 Hz, 1H), 3.66-3.63 (m, 1H).

^{13}C NMR:δ57.1, 58.8,65.5,73.9,114.3, 117.7, 122.3, 124.0,127.0,127.5,127.6,128.6,131.5,131.6, 131.7,133.8,134.3,134.6,139.5,146.5,148.4,151.1,169.8.

Compound 10d

^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.09 (s, 1H), 7.53 (d, J = 8.6 Hz, 4H), 7.49 (t, J = 1.5 Hz, 1H), 7.44–7.42 (m, 1H), 7.41–7.39 (m, 2H), 7.38 (d, J = 5.0 Hz, 1H), 7.27–7.23 (m, 1H), 7.12–7.05 (m, 3H), 7.07 (t, J = 7.8, Hz, 1H), 7.01 (d, J = 8.5 Hz, 1H), 5.01 (d, J = 5.4 Hz, 1H), 4.10 (s, 2H), 3.81 (d, J = 7.7 Hz, 1H), 3.53 (dd, J = 7.7, 5.4 Hz, 1H).

^{13}CNMR:δ57.1,58.8,65.5,73.9,114.3,122.3,124.0,127.0,127.2,127.5,128.5,128.6,128.7,128.8,131.5, 131.6, 131.7,133.7,133.8, 134.3, 134.3,134.3,134.6,146.5, 148.4,151.1,169.8.

Compound 10e

^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.09 (s, 1H), 7.53 (d, J = 8.6 Hz, 4H), 7.42 (d, J = 1.6 Hz, 1H), 7.40 (dd, J = 7.1, 5.0 Hz, 2H), 7.39 (d, J = 8.6 Hz, 1H), 7.30 (d, J = 8.3 Hz, 2H), 7.17 (d, J = 8.3

Hz, 2H), 7.12–7.08 (m, 2H), 7.01 (d, *J* = 8.5 Hz, 1H), 5.03 (d, *J* = 5.4 Hz, 1H), 4.10 (s, 2H), 3.81 (d, *J* = 7.7 Hz, 1H), 3.53 (dd, *J* = 7.7, 5.4 Hz, 1H), 2.27 (s, 3H).

^{13}CNMR:*δ*21.3,57.1,58.8,65.5,73.9,114.3,122.3,124.0,126.4,127.0,127.5,128.6,129.1,131.6,131.7,133.8,134.3,134.6, 141.5,146.5,148.4, 151.1,169.8.

Compound 10f

^{1}HNMR:*δ*3.65(1H,d, *J*=8.1Hz),3.88(1H,d,*J*=8.1Hz),4.20(s, 2H),5.02(1H, d, *J*=5.3Hz),6.89(d, *J* =8.9Hz),7.00(d,*J*=8.0 Hz),7.05(d,*J*=8.5Hz),7.09(d,*J*=8.6 Hz),7.10(d,*J*=8.5Hz),7.30(t, *J*=8.0 Hz),7.37(d,*J* =8.0 Hz),7.40(d, *J*=8.9 Hz),7.51 (d, *J* =8.6Hz),7.60(d,*J* =1.5 Hz),7.85(1H,s).

^{13}CNMR:*δ*57.1,58.8,65.5,73.9,114.3,118.4,122.3,124.0,126.5,127.0,127.5,128.6,130.1,131.0,131.5, 131.6,131.7,133.8,134.3,134.6,146.5,148.4,151.1,169.8.

Compound 10g

^{1}HNMR:*δ*3.58(1H,d,*J*=8.1Hz),3.88(1H,d,*J*=8.1Hz),4.20(s,2H),4.88(1H, d,*J*=5.5Hz),6.89(d,*J*=8.9 Hz),6.90 (d, *J*=8.2 Hz),7.03 (d,*J*= 8.2 Hz),7.05 (d,*J*= 8.5 Hz), 7.10 (d,*J*=8.5Hz),7.13 (d, *J*= 1.8 Hz),7.17 (d,*J*= 8.3 Hz),7.17(d,*J*= 8.2 Hz)),7.40 (d, *J* =8.9 Hz),7.42(d,*J* =8.3Hz),7.83(1H,s).

^{13}C NMR:*δ*57.1,58.8, 65.5,73.9, 113.6,114.2, 114.3,115.7,124.0,126.5,127.0, 128.6, 129.0, 130.1,131.6,133.8,134.3,134.6,146.5, 146.6, 146.6,146.6,148.4,151.1, 157.4,169.8.

Compound 10h

^{1}H NMR (400 MHz, $CDCl_3$) *δ*: 8.11 (s, 1H), 7.58–7.52 (m, 4H), 7.46–7.41 (m, 2H), 7.42 (d, *J* = 1.6 Hz, 1H), 7.40 (dd, *J* = 6.7, 5.0 Hz, 2H), 7.33–7.31 (m, 1H), 7.13–7.09 (m, 2H), 7.06 (dd, *J* = 8.0, 2.5, 1.8 Hz, 1H), 7.01 (d, *J* = 8.5 Hz, 1H), 6.91 (t, *J* = 7.9, 1.7 Hz, 1H), 4.96 (d, *J* = 5.4 Hz, 1H), 4.15 (s, 2H), 3.81 (d, *J* = 7.7 Hz, 1H), 3.52 (dd, *J* = 7.7, 5.4 Hz, 1H).

^{13}C NMR:*δ*57.1,58.8,65.5,73.9, 113.6, 114.2, 114.3,124.0,126.5, 127.0,128.5, 128.6,128.7, 128.8,129.0,129.2,131.6,133.7,133.8,134.3,134.6,146.5,146.6,148.4,151.1, 169.8.

IR (KBr, cm^{-1}): 1600 (-C=N- stretching), 1680(-C=C- stretching), 3100 (-NH- stretching).

Mass(m/z): 593.

Compound 10i

^{1}H NMR:*δ* 3.67 (1H,d, *J*=8.1 Hz),3.88 (1H,d,*J*=8.1Hz),4.20(s, 2H),5.03 (1H, d,*J*=5.3Hz),6.89(2H,d,*J*=8.9 Hz),7.05(d,*J*=8.5Hz),7.10(d, *J*=8.5 Hz),7.10(d,*J*= 8.5 Hz),7.33(d, *J*=8.3 Hz),7.38 (d, *J*= 8.3 Hz),7.40(d, *J*= 8.9 Hz),7.55(2H,d,*J*= 8.5 Hz),7.86(1H,s).

^{13}CNMR:*δ*57.1,58.8,65.5,73.9,114.3,122.3,124.0,127.0,127.3,128.5,128.6,128.7,128.8,129.2,131.5, 131.6, 131.7,133.7,133.8, 134.3,134.3,134.6,146.5, 148.4,151.1,169.8.

Compound 10j

^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.11 (s, 1H), 7.57 (d, J = 1.6 Hz, 1H), 7.55 (q, J = 1.5 Hz, 2H), 7.52 (d, J = 1.6 Hz, 1H), 7.49 (t, J = 1.5 Hz, 1H), 7.42 (dd, J = 4.6, 3.1 Hz, 2H), 7.41–7.40 (m, 2H), 7.38 (d, J = 5.0 Hz, 1H), 7.25 (t, J = 8.1, 1.6 Hz, 1H), 7.13–7.10 (m, 2H), 7.07 (t, J = 7.8, 1.5 Hz, 1H), 7.01 (d, J = 8.5 Hz, 1H), 5.01 (d, J = 5.4 Hz, 1H), 4.10 (s, 2H), 3.81 (d, J = 7.7 Hz, 1H), 3.53 (dd, J = 7.7, 5.4 Hz, 1H).

^{13}CNMR:δ57.1,58.8,65.5,73.9,114.3,124.0,126.5,127.0,127.5,128.5,128.6,128.7,128.8,129.2,130.4,131.6,133.7,133.8,134.3, 134.6, 146.5,148.4,151.1,169.8.

2.2 Biology

Pharmacological Activity

2.2.1. Anti-inflammatoryActivity

The denaturation of bovine serum albumin technique was employed to assess the anti-inflammatory activity of the target compounds, according to the methods described in the literature[35,36]. The pH of the combination, which contained the test chemical and a 1% aqueous solution of bovine albumin fraction, was increased to 7.4 in the test sample. In addition, test samples were heated to 51 °C for 20 minutes after incubating at 37 °C for 20 minutes. After cooling to room temperature, the sample's turbidity was measured at 660 nm with a UV-visible spectrophotometer. The typical medicine used in the investigation, diclofenac sodium, was administered in triplicate.

The title compounds' anti-inflammatory efficacy was calculated using the following formula based on the percentage of inhibition of albumin denaturation:

$$\%\ \text{Inhibition} = \frac{[\text{Control absorbance - Sample absorbance}]}{\text{Control absorbance}} \times 100$$

2.2.2. Antimicrobial activity

All the compounds were tested against four different microorganisms: *Escherichia coli*, and *Candida albicans.* The agar well-diffusion method was applied for the determination of the inhibition zone and minimum inhibitory concentration (MIC). Briefly, broth culture(0.75mL) containing *ca.*106 colon-forming units(CFU)per mL of the test strain was added to nutrient agar medium(75mL) at 45 °C, mixed well, and then poured into a 15 cm sterile metallic petriplate.

The medium was allowed to solidify, and 8mm wells were dug with a sterile metallic borer, then a DMSO solution of the test sample (1 mL)at 1mg/mL was added to the respective wells. DMSO served as negative control, and the standard antimicrobial drugs rifampicin (5µg/disc) and ampicillin(10µg/disc) were used as positive controls. Triplicate plates for each microorganism strains were prepared and were incubated aerobically at37°C for 24h.The activity was determined by measuring the diameter of the zone showing complete inhibition(mm), there by, the zones were precisely measured with the aid of a vernier caliper(precision0.1mm). The growth inhibition was calculated concerning the positive control.

2.3. Molecular docking studies

2.3.1. Protein preparation

The crystal structures of *E. coli* 24kDa domain in complex with clorobiocin (PDB:1KZN) were downloaded from the protein database (www.rcsb.org)[37]. Before protein preparation, the inhibitors, other ligands, and water molecules were deleted from the protein to obtain clean protein. Polar hydrogen atoms with Gasteiger-Huckel charges were added to the protein before docking[38]. The protein (PDB ID: 3LN1) grid box's center was set to 62, 66, and 38, while the number of points in the x, y, and z dimensions was set to 37.738, -26.122, and -6.412 Å, respectively.

2.3.2. Ligand preparation

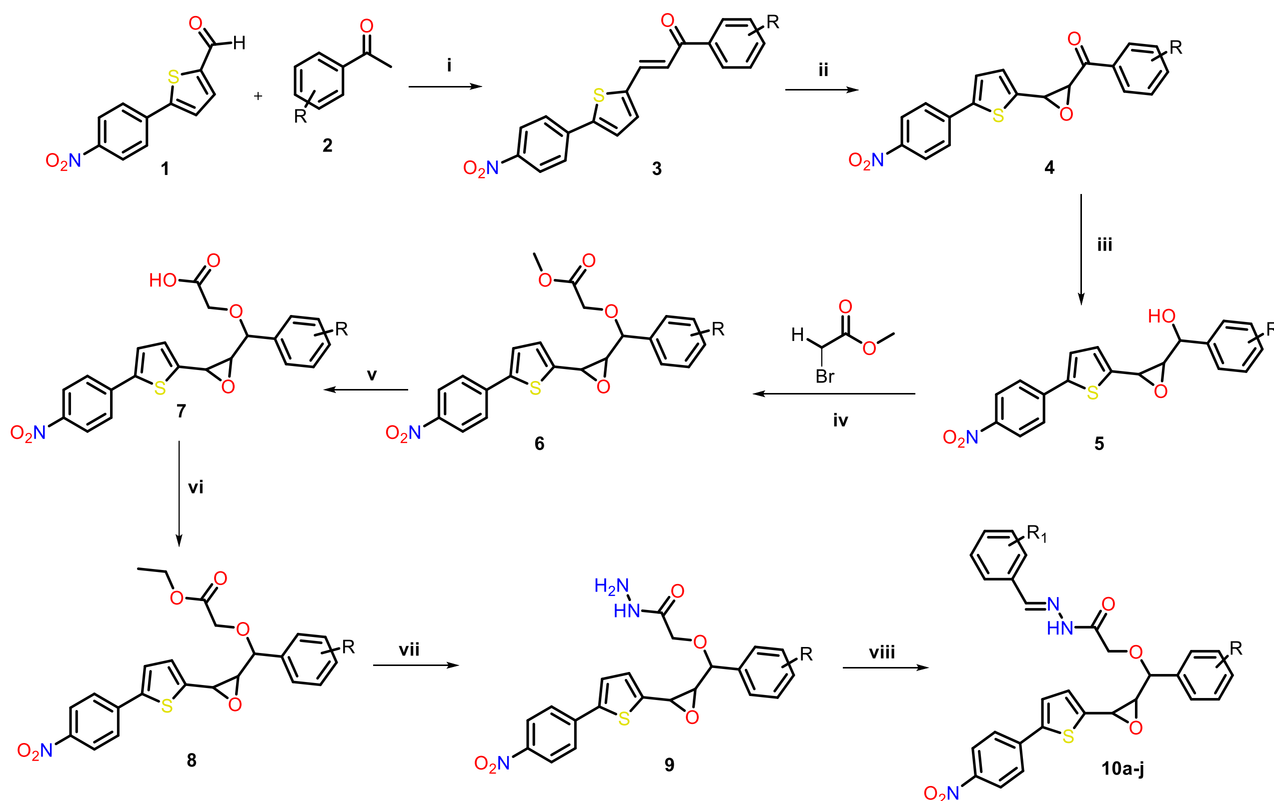
The Marvin Sketch tool was used to determine the 2D orientations of the synthesized molecules, which were then converted into the most energy-efficient 3D structures *via* a minimization method. Furthermore, the claims made by Gasteiger Rotatable bonds and nonpolar hydrogen atoms were constructed using AutoDock 4.2. When docking each compound with Auto Dock Vina. The docking result is displayed by Discovery Studio software[39].

3. RESULTS AND DISCUSSION

3.1 Chemistry

The synthetic route for the preparation of hydrazones coupled with thiophenyl ring was given in Scheme 1.The synthesis began with the Claisen-Schmidt condensation of compounds **1** and **2** in a mixture of ethanol and water using NaOH, resulting in the formation of chalcone**3**. This intermediate was then treated with 30% H_2O_2 at 0 °C in the presence of NaOH and ethanol to

yield the oxirane derivative **4**. The carbonyl group in compound **4** was reduced to the alcoholic group using $NaBH_4$ in methanol, producing compound **5**. Subsequently, compound **5** was refluxed with methyl-2-bromobutanoate in DMF to obtain the methyl ester derivative **6**. This derivative was converted to ethyl ester derivative **8** through a two-step process: first, treatment with NaOH to form the acid derivative **7**, followed by esterification with ethanol in the presence of H_2SO_4. The ethyl ester derivative **8** was then reacted with hydrazine hydrate in ethanol to produce the hydrazine derivative **9**, which was further treated with substituted benzaldehyde derivatives to yield the final compound **10a-j**. The physical parameters of the hydrazones are tabulated in **Table.1**.



Scheme 1: Schematic representation for *N'*-benzylidene-2-([substituted-phenyl](3-(5-(4-nitro phenyl) thiophen-2-yl)oxiran-2-yl)methoxy)acetohydrazide (**10a-j**)

Reagents and conditions: i) NaOH, EtOH+H_2O, 0-5^{0}C, 3 h, stir, RT; ii) 30% H_2O_2, 0^{0}C, NaOH, EtOH, stir, 3 h, RT; iii) MeOH, $NaBH_4$, 3 min, stir, RT; iv) DMF, reflux, 5 h; v) H_2O, reflux, 2 h, 30%NaOH; vi) EtOH, dil. H_2SO_4, reflux, 12 h; vii) EtOH, $N_2H_4.H_2O$, reflux, 8 h; viii) EtOH, acetic acid, reflux, 3 h.

Table 1: Characterization data of *N*'-benzylidene-2-([substituted-phenyl](3-(5-(4-nitro phenyl)thiophen-2-yl)oxiran-2-yl)methoxy)acetohydrazide 10a-j

Comp. No.	R	R^1	Melting Point (℃)	Molecular Weight	%Yield
10a	*p*-Cl	*p*-NO2	198	593	87
10b	*p*-Br	*p*-NO2	196	637	73
10c	*p*-NO2	*p*-Br	203	637	80
10d	*m*-Cl	*p*-Br	198	626	78
10e	*p*-CH3	*p*-Br	199	606	60
10f	*m*-Br	*p*-Br	196	670	65
10g	*m*-OH	*p*-Cl	195	564	55
10h	***m*-NO2**	*p*-Cl	**194**	**593**	**87**
10i	*p*-Br	*p*-Cl	193	626	65
10j	*m*-Cl	*p*-Cl	192	582	60

3.2 Pharmacological Activity

Anti-inflammatory Activity

Diclofenac sodium was considered as a standard medication to assess the *in vitro* anti-inflammatory effectiveness of all the synthesized hydrazone derivatives through the inhibition of protein denaturation (bovine albumin). The findings of the determination of the IC_{50} values are summarized in **Table 2**. Based on the findings, it was evident that several synthetic compounds (**compounds 10a-j**) had superior activity.

Table 2: Summarizes the IC_{50} values and anti-inflammatory ratios of hydrazones

Entry	IC_{50} hydrazone release (µM)
10a	35.41 ± 2.04
10b	**13.04 ± 2.47**
10c	**7.92 ± 1.07**
10d	10.67 ± 1.22
10e	20.88 ± 3.21
10f	9.90 ± 2.18
10g	57.45 ± 2.34
10h	60.41 ± 8.34
10i	91.88 ± 2.64
10j	9.88 ± 2.21
Diclofenac sodium	5.01 ± 1.32

3.3. Antimicrobial Activity

All our compounds, *i.e.*, hydrazones**10(a-j)** were tested for antimicrobial activity using rifampicin(5µg/disc) and Fluconazole (10 µg/disc) as standard drugs.MBC were determined and results are listed in**Table 3** below.

Table 3: Antimicrobial activities of all the synthesized compounds

Compound	Zoneof inhibition(mm)		Minimum inhibition concentration (MIC)g/mL	
	E. coli	*C.albicans*	*E. coli*	*C.albicans*
10a	-	20	-	250
10b	-	15	-	-
10c	17	20	100	50
10d	14	20	120	500
10e	12	15	-	-
10f	19	22	63	31
10g	18	25	125	31
10h	22	26	50	50
10i	18	20	63	125
10j	17	17	-	-
Rifampicin	32	-	-	-
Fluconazole	30	-	-	-
DMSO	-	14	-	-

(-) Indicates no activity

The results of antimicrobial activities of our synthesized compounds are shown in **Table 3** as ZOI (in mm) and MIC (mg/mL). These results are listed in below**Table 4**.

Table 4.Determination of minimum bactericidal and fungicidal concentration (μg/mL) of chloro-derivatives.

Concentrations μg/Ml	1000	500	250	125	63	31	1000	500	250	125	63	31
Microorganism Growth	***E. coli***						***C.albicans***					
10c	-	-	-	+	+	+	-	-	-	-	+	+
10f	-	-	-	+	+	+	-	-	+	+	+	+
10i	-	-	+	+	+	+	-	-	-	-	+	+

3.4. Molecular docking studies

To determine the potential binding mode of the active compounds against the target protein *E. coli* 24kDa domain in complex with clorobiocin, docking studies were conducted. The molecular docking analysis showed promising binding energies for all the compounds, which ranged from to -9.6 to -8.1 kcal/mol, as detailed in **Table 5**. The most active compound **10c** displayed the highest binding affinity with the protein showing three hydrogen bonding interactions. All the interactions were raised from oxygen atoms of the nitro groups with ILE64 (2.60 Å), GLY63 (2.31 Å), and LYS89 (2.10 Å) amino acid residues.

Similarly, another active compound **10f** forms two hydrogen bonding interactions at the active site (**Figure 2**). The most active compounds exhibited similar hydrogen bonding interactions with the target protein compared to the standard drug rifampicin displayed three hydrogen bonding interactions with LYS89 (2.26 Å), SER94 (2.19 Å), and ASP92 (1.83 Å) amino acid residues.

Table 5. The binding affinity of the synthesized compounds.

Code	Binding affinity (kcal/mol)
10a	-8.9
10b	-8.7
10c	-9.6
10d	-9.0
10e	-8.9
10f	-8.1
10g	-9.4
10h	-8.5
10i	-9.0
10j	-9.3

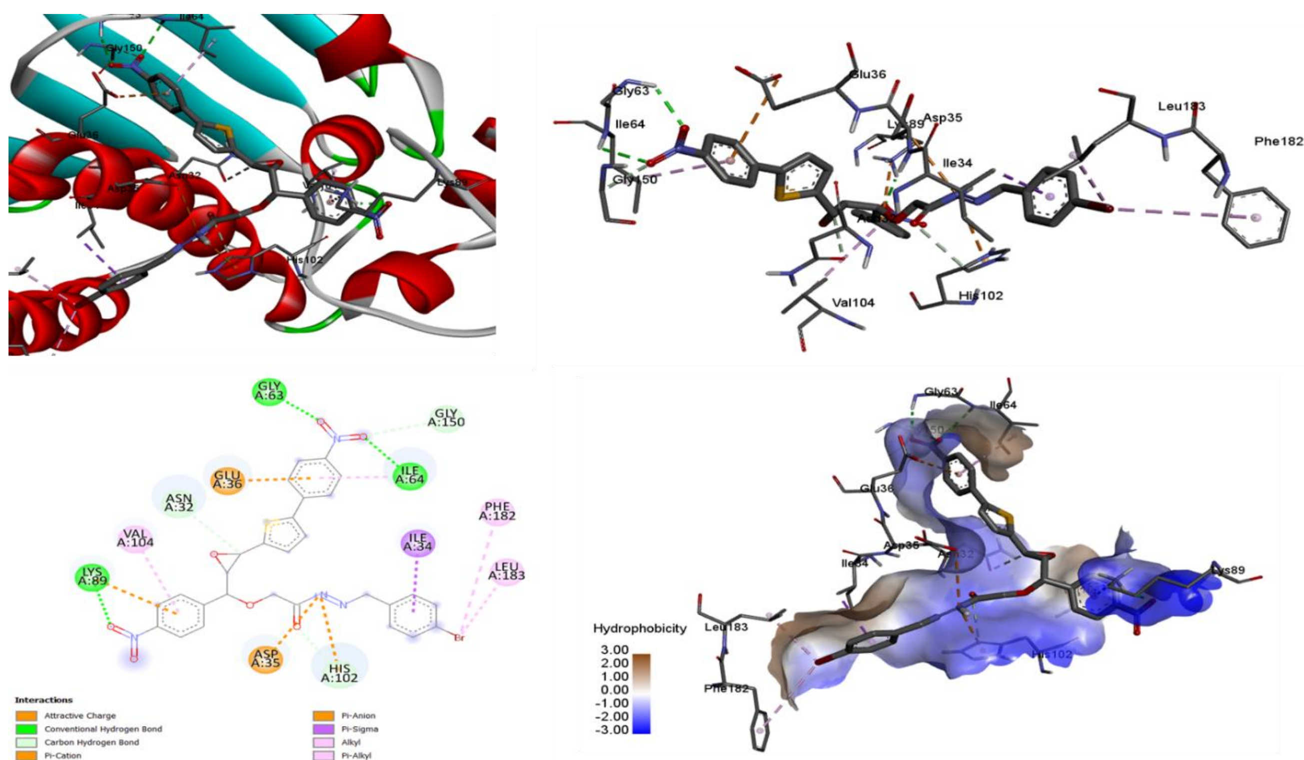


Figure 1: The crystal structure of *E. coli* 24kDa domain (PDB:1KZN) with the active compound **10c.**

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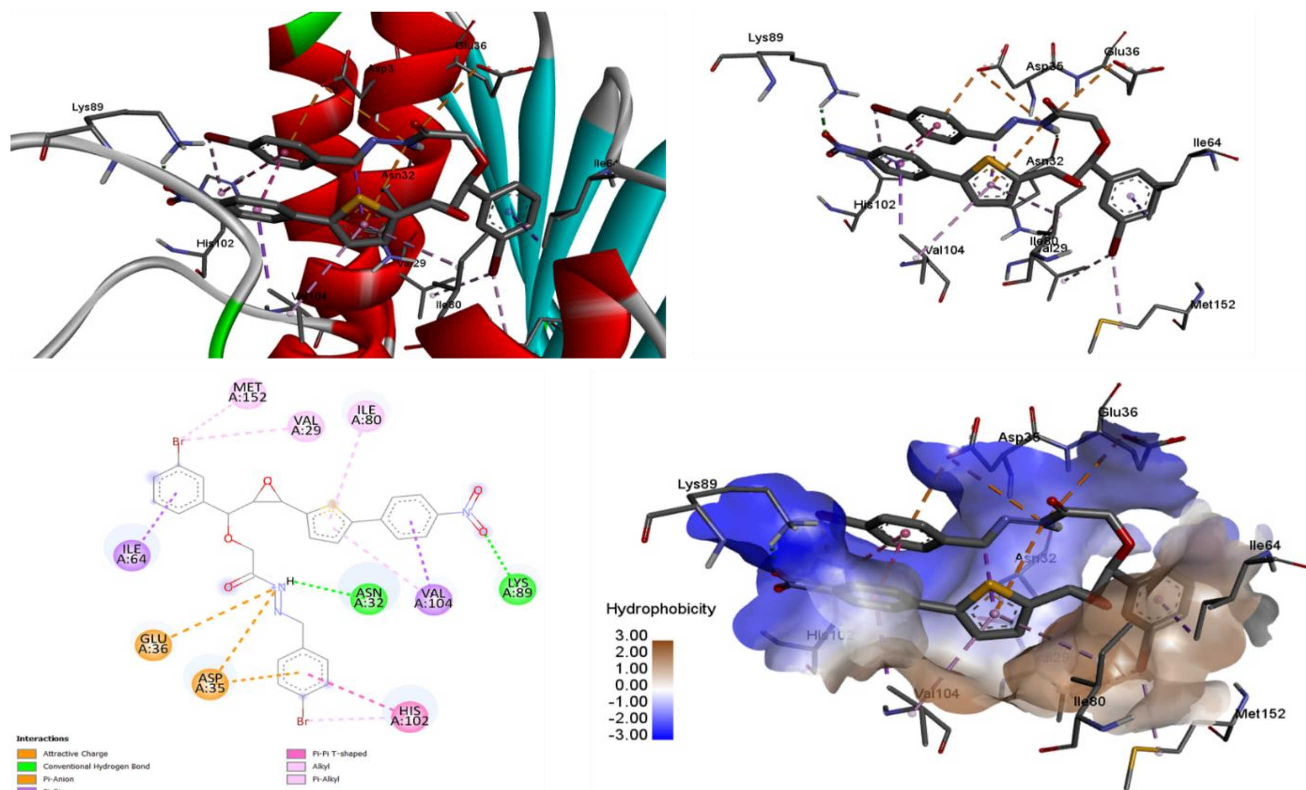

Figure 2: The crystal structure of *E.coli* 24kDa domain(PDB:1KZN) with the active compound **10f.**

CONCLUSION

This study reports on the production of thiophenyl hydrazones. A simple synthetic route has been used with a short reaction duration with excellent yields and without tedious purification techniques. The manufactured hydrazone molecules were assessed for anti-inflammatory and antimicrobial studies. Compounds **10b** and **10c** exhibit good anti-inflammatory properties. In the series (**10a-j**), compounds **10c**, **10f**,and **10i** showed excellent antimicrobial properties. The docking studies reveal that the most active compounds, particularly **10c** and **10f**, demonstrated strong binding affinities with the *E. coli* 24kDa domain, showing multiple hydrogen bonds and other non-covalent interactions, comparable to the standard drug rifampicin. These interactions highlight the potential of these compounds as promising therapeutic agents, warranting further optimization and development.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES:

[1] A.U. Ahmed, An overview of inflammation: mechanism and consequences, Front.Biol 6(4) (**2011**) 274-281.

[2] C. Nordqvist, Everything you need to know about inflammation, Med News Today [Internet]. Medilexicon (**2017**).

[3] P.A. Ward, Acute and chronic inflammation, Fundament Inflam 3 (**2010**) 1-16.

[4] E.O. Iwalewa, L. McGaw, V. Naidoo, J. Eloff, Inflammation: the foundation of diseases and disorders. A review of phytomedicines of South African origin used to treat pain and inflammatory conditions, Afric J of Biotech 6(25) (**2007**).

[5] R. Pahwa, A. Goyal, I. Jialal, Chronic Inflammation.[Updated 2022 Aug 8], StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing (**2022**).

[6] J.C. Maroon, J.W. Bost, A. Maroon, Natural anti-inflammatory agents for pain relief, Surg Neurol Intl 1 (**2010**).

[7] H. ur Rashid, Y. Xu, N. Ahmad, Y. Muhammad, L. Wang, Promising anti-inflammatory effects of chalcones via inhibition of cyclooxygenase, prostaglandin E2, inducible NO synthase and nuclear factor κb activities, Bioorg Chem 87 (**2019**) 335-365.

[8] F.C. Odds, Antifungal agents: their diversity and increasing sophistication, Mycologist 17(2) (**2003**) 51-55.

[9] D.W. Denning, M.J. Bromley, How to bolster the antifungal pipeline, Sci. 347(6229) (**2015)** 1414-1416.

[10] B. Havlickova, V.A. Czaika, M. Friedrich, Epidemiological trends in skin mycoses worldwide, Mycoses 51 (**2008**) 2-15.

[11] I. Weitzman, R.C. Summerbell, The dermatophytes, Clin Microbiol Rev 8(2) (**1995**) 240-259.

[12] M.C. Fisher, N.J. Hawkins, D. Sanglard, S.J. Gurr, Worldwide emergence of resistance to antifungal drugs challenges human health and food security, Sci 360(6390) (**2018**) 739-742.

[13] J. Bastert, M. Schaller, H. Korting, E. Evans, Current and future approaches to antimycotic treatment in the era of resistant fungi and immunocompromised hosts, Intl J of Antimicrob Agents 17(2) (**2001**) 81-91.

[14] V. Kamat, B.C. Yallur, B. Poojary, V.B. Patil, S.P. Nayak, P.M. Krishna, S.D. Joshi, Synthesis, molecular docking, antibacterial, and anti-inflammatory activities of benzimidazole-containing tricyclic systems, J. Chin Chem Soc 68(6) (**2021**) 1055-1066.

[15] P. Mao, T. Deng, J. Tian, Y. Liu, H. Xin, Y. An, Y. Hu, Y. Qin, W. Xue, Design, synthesis, and antifungal activities of chalcone derivatives containing piperidine and sulfonamide moiety, J Saudi Chem Soc. 28(1) (**2024**) 101791.

[16] V. Kamat, S.P. Nayak, G. Adiga, A.C. Rajeena, S.U. Kanekar, P. Rekha, Design, facile synthesis and pharmacological evaluation of hybrid 1, 3, 4-thiadiazole linked chalcone confined via sulphur bridge, Heterocyc Lett 8(3) (**2018**) 671-678.

[17] M.S. Ahmad, F.E. Hawaiz, Novel chalcone-based crown ethers: synthesis, characterization, antioxidant activity, biological evaluations, and wastewater remediation, RSC Adv 14(4) (**2024**) 2369-2379.

[18] S. Sinha, A. Kaur, R. Sehgal, Synthesis and characterization of silver nanoparticle conjugated-chalcones and their evaluation for antimalarial, cytotoxicity and haemolytic potential at in vitro level, Disc. Appl. Sci. 6(6) (**2024**) 273.

[19] M.A. El-Atawy, D.H. Hanna, A.H. Bashal, H.A. Ahmed, E.M. Alshammari, E.A. Hamed, A.R. Aljohani, A.Z. Omar, Synthesis, Characterization, Antioxidant, and Anticancer Activity against Colon Cancer Cells of Some Cinnamaldehyde-Based Chalcone Derivatives, Biomol 14(2) (**2024**) 216.

[20] K. Fatemeh, Studies on glutathione conjugation reactions of some cyclic chalcone analogs to seek reactivity-biological activity relationships, Int J Mol Sci. 10;24(10):8557(**2023**).

[21] V. Kamat, K. Venuprasad, A. Shadakshari, R.S. Bhat, A. D'souza, S. Chapi, A. Kumar, P.V. Kuthe, M. Sankaranarayanan, K.N. Venugopala, Synthesis, anti-inflammatory, antibacterial, and antioxidant evaluation of novel pyrazole-linked hydrazone derivatives, J. Mol. Str. 1312 (**2024**) 138634.

[22] V. Kamat, R. Santosh, B. Poojary, S.P. Nayak, B.K. Kumar, M. Sankaranarayanan, Faheem,

S. Khanapure, D.A. Barretto, S.K. Vootla, Pyridine-and thiazole-based hydrazides with promising anti-inflammatory and antimicrobial activities along with their in silico studies, ACS omega 5(39) (**2020**) 25228-25239.

[23] S.S. Çakmak, Ö. Erdoğan, F. Başoğlu, U. Çoruh, Ö. Çevik, S. Karakuş, Exploring etofenamate hydrazide-hydrazone/copper (II) complexes: Synthesis, anticancer activity, carbonic anhydrase IX inhibition and docking studies, J.Mol.Str. 1312 (**2024**) 138555.

[24] Y.I. Touahria, N. Chafai, O. Moumeni, A. Boublia, M. Mehri, Y. Benguerba, Synthesis, characterization, and comprehensive computational analysis of aromatic hydrazone compounds: Unveiling quantum parameters, evaluating antioxidant activity, and investigating molecular docking interactions, J. Mol. Liq. 403 (**2024**) 124897.

[25] V. Kamat, R. Santosh, S.P. Nayak, (E)-N′-(4-Fluorobenzylidene)-5-methyl-2-(pyridin-3-yl) thiazole-4-carbohydrazide, Molbank (**2019**)(2) M1058.

[26] D. Geetha, C. Sharath, N. Shivakumar, B. Lakshminarayana, K. Chandini, K. Balakrishna, Novel series of hydrazones carrying pyrazole and triazole moiety: Synthesis, structural elucidation, quantum computational studies and antiviral activity against SARS-Cov-2, J.Mol.Str. (**2024**) 139016.

[27] A. Tabbiche, A. Bouchama, K. Fadli, B. Ahmad, N. Kumar, C. Chiter, M. Yahiaoui, F. Zaidi, K. Boudjemaa, N. Dege, Development of new benzil-hydrazone derivatives as anticholinesterase inhibitors: synthesis, X-ray analysis, DFT study and in vitro/in silico evaluation, J. Biomol Str Dyn (**2024**) 1-16.

[28] V. Kamat, N. Bhavya, B. Poojary, V.B. Patil, G. Ramesh, M. Mahendra, Emphasized DFT, DNA binding, and electrochemical studies of hybrid 1, 3, 4-thiadiazole-linked chalcone confined via a sulfur bridge, J.Chem Sci136(2) (**2024**) 1-11.

[29] E.C.C. Esquivel, V.C. Rufino, M.H.T. Nogueira, A.C.C. Souza, J.R.P. Júnior, M.S. Valle, Synthesis and characterization of 1, 3, 5-triarylpyrazol-4-ols and 3, 5-diarylisoxazol-4-ols from chalcones and theoretical studies of the stability of pyrazol-4-ol toward acid dehydration, J.Mol.Str 1204 (**2020**) 127536.

[30] P. Zhang, E.A. Terefenko, J. Bray, D. Deecher, A. Fensome, J. Harrison, C. Kim, E. Koury, L. Mark, C.C. McComas, 1-or 3-(3-Amino-2-hydroxy-1-phenyl propyl)-1, 3-dihydro-2 H-benzimidazol-2-ones: Potent, Selective, and Orally Efficacious Norepinephrine Reuptake Inhibitors, J.Med Chem 52(18) (**2009**) 5703-5711.

[31] E.M. Flefel, W. Tantawy, A. Fayed, H.H. Sayed, M. Khedr, Synthesis, Molecular Docking and Biological Evaluation of Some Novel Heterocyclic Derivatives.Curr Pharm Des. 29(1) (**2023**) :48-59.

[32] V. Theodorou, K. Skobridis, A.G. Tzakos, V. Ragoussis, A simple method for the alkaline hydrolysis of esters, Tetrahed Lett 48(46) (**2007**) 8230-8233.

[33] M. Rakse, C. Karthikeyan, N.S. Narayana Moorthy, R.K. Agrawal, Design, Synthesis and Biological Evaluation of 3-(2-(benzo [d] thiazol-2-ylthio) acetamido) benzoic Acid Derivatives as Inhibitors of Protein Tyrosine Phosphatase 1B, Lett Drug Des Disc 18(1) (**2021**) 46-56.

[34] M. Rani, S. Jayanthi, S. Kabilan, R. Ramachandran, Synthesis, Spectral, Crystal structure, Hirshfeld surface, Computational analysis, and Antimicrobial studies of Ethyl-(E)-4-(2-(2-arylidenehydrazinyl)-2-oxoethyl) piperazine-1-carboxylates, J.Mol Str.1252 (**2022**) 132082.

[35] Y. Mizushima, M. Kobayashi, Interaction of anti-inflammatory drugs with serum proteins, especially with some biologically active proteins, J. Pharm Pharmacol 20(3) (**1968**) 169-173.

[36] S. Sakat, A.R. Juvekar, M.N. Gambhire, In vitro antioxidant and anti-inflammatory activity of methanol extract of Oxalis corniculata Linn, Int J Pharm Pharm Sci 2(1) (**2010**) 146-155.

[37] D. Lafitte, V. Lamour, P.O. Tsvetkov, A.A. Makarov, M. Klich, P. Deprez, D. Moras, C. Briand, R. Gilli, DNA gyrase interaction with coumarin-based inhibitors: the role of the hydroxybenzoate isopentenyl moiety and the 5 ‘-methyl group of the noviose, Biochem 41(23) (**2002**) 7217-7223.

[38] V. Babagond, K. Katagi, M. Akki, D.S. Reddy, H. Shanavaz, A. Jaggal, S. DK, Design and Synthesis of Potent Anticancer Agents Using Coumarin-Quinazolinone Based Scaffolds: Exploration of ADME Properties and Molecular Docking Studies, ChemSelect 9(17) (**2024**) e202400435.

[39] O. Trott, A.J. Olson, AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading, J.Comput Chem 31(2) (**2010**) 455-461.