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Synthesis, characterization and catalytic optimization of Schiff bases containing 8-methyl-quinazolinones as potent anti-inflammatory and antimicrobial scaffolds

Naimish P Ramani[a], Ishita J Modasiya[b] & Bonny Y Patel*[a]

[a] School of Science, Department of Chemistry, R K University, Rajkot 360 020, Gujarat, India

[b] Department of Microbiology, School of Science, R K University, Rajkot 360 020, Gujarat, India

E-mail: bonny.y.patel@gmail.com

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A set of twelve new quinazoline arylidene/heteroarylidene motifs have been synthesized by forming Schiff bases from the 3-amino-8-methyl-2-(*p*-tolyl)quinazolin-4(3*H*)-one intermediate. These compounds have been analyzed and tested for their anti-inflammatory and antimicrobial potential. The *in vitro* results for anti-inflammatory activity show that compound 6g (2-Br) exhibits the most potent activity with an inhibition of 96.89±0.83 against the standard medication. Compound 6d (4-OH) has the second-highest anti-inflammatory inhibition of 92.64±0.38. In terms of antimicrobial activity, compound 6d shows the highest inhibitory impact ranging from 12.5 to 50 µg/mL against specific bacterial and fungal pathogens. Compound 6g also demonstrates good action against *E. coli*, *Klebsiella sp.*, *B. megaterium*, *S. aureus*, and *A. niger* species. Compounds 6e, 6j and 6k have strong antibacterial activity but only modest antifungal efficacy in antimicrobial activity tests. According to the SAR study, compounds 6d, 6e and 6k that have EDGs such as hydroxyl at the *ortho*, *meta* and *para* positions have both potent antimicrobial and anti-inflammatory activities. Moreover, compounds 6c, 6g, and 6j which have EWGs (–Cl, –Br, and –NO_2) in the *ortho* and *para* positions exhibit better anti-inflammatory activity as compared to the *meta*-derivative 6b. Further evaluation of compounds 6d and 6g with standard antibiotics is crucial for treating harmful microorganisms.

Keywords: Quinazolinone, Schiff base, Catalytic optimization, Anti-inflammatory, Antimicrobial

There exist three distinct forms of quinazolinone, referred to as isomers, that arise from different substitution patterns of the carbonyl group. These isomers are precisely known as 2(1*H*)-quinazolinones, 4(3*H*)-quinazolinones, and 2,4(1*H*,3*H*)-quinazoline-dione. The most widely utilized isomer among these three is the 4(3*H*)-quinazolinones. This isomer is extensively used as intermediates or can be found as natural products in various suggested biosynthetic pathways (Fig. 1)[1,2].

Quinazolinones are a group of organic compounds that exhibit diverse biological activities, including anticancer, anticonvulsant, anti-inflammatory, antitubercular, and antibacterial properties[3]. Due to their versatile medicinal potential, quinazolinone and similar fused ring heterocycles have been considered as one of the most important categories[4-7]. Additionally, the quinazolinone heterocycle is also present in several natural anti-inflammatory alkaloids, such as Tryptanthrin and Rutaecarpine[8,9]. Nonsteroidal anti-inflammatory drugs (NSAIDs) work by inhibiting COX, an enzyme that produces prostaglandins. COX-1 is present in the kidneys and stomach, while COX-2 is induced in response to inflammation and produces prostaglandins that cause pain and support inflammation[10,11]. Several selective COX-2 inhibitors that belong to different structural classes, such as vicinal diaryl heterocycles, diaryl carbocycles, and heteroaryl-ethers, have been developed as pharmacophores to treat inflammation[12]. NSAIDs have adverse effects, including gastrointestinal injury, bleeding, intolerance, and renal toxicity[13,14]. Some selective COX-2 inhibitors are approved by the FDA as safe anti-inflammatory medications, including celecoxib, rofecoxib, valdecoxib, and parecoxib[15].

Furthermore, reported for their versatile pharmacological activities, quinazolinones and their derivatives are particularly effective in treating microbial infections[16,17]. Various structural classes of

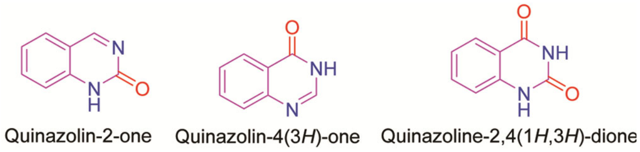


Fig. 1 — Three isomeric forms of quinazolinone

quinazolinone include a variety of FDA-approved medications. Febrifugine is a medicine that contains quinazolinone and is used to treat malaria. Methaqualone is a sedative, Albuconazole is an antifungal medication, and Balaglitazone is used to lower blood sugar levels. Quinethazone belongs to the class of anti-hypertensive drugs, while Halofuginone is an anti-protozoal medication. Proquazone is a well-known nonsteroidal anti-inflammatory drug (Fig. 2)[18,19]. Schiff bases possess important biological properties such as antibacterial, antifungal, anti-inflammatory, antiviral, and anticancer effects mainly due to the azomethine group (-CH=N-)[20,21]. In the current study, we aimed to develop novel compounds that contain quinazolinone and Schiff bases template, which have limited citations as bicyclic central scaffolds as COX-2 inhibitors. These compounds are known to be among the most active classes of substances with potent anti-inflammatory and antimicrobial properties[22,23]. Our research is dedicated to improving our procedures for catalytic optimization, increasing yields, and exploring novel approaches to medication development, as previously studied[24-34].

Experimental Section

The chemical utilized was of commercial standard procured from Sigma-Aldrich. The Bruker ultra-shield-400 spectrometer was used to record the ^1H NMR (400 MHz) and ^{13}C NMR (125 MHz) spectra. Tetramethylsilane (TMS) was used as an internal standard, and DMSO-d_6 was used as a solvent. Coupling constants (J) were designated in hertz (Hz), and abbreviations such as s (singlet), d (doublet), t (triplet), and m (multiplet) were used for indicating multiplicity. Using KBr, all of the compounds' infrared spectra (ν, cm^{-1}) were captured on a Shimadzu FTIR 8400S spectrophotometer. Mass spectra were acquired on using the Shimadzu GC-2010+ ultra-spectrometer. An ECS 4010 Elemental Combustion System (Costech Instruments, Pioltello) was used to perform elemental analyses, and the results were within the acceptable range (±0.40) of the computed values. Melting point measurements were recorded without correction after being verified with an electro thermal melting point device (model 9100, Electrothermal Engineering Ltd.). The completeness of the reaction and the purity of the compounds were tested using a mixture of toluene and ethyl acetate (in

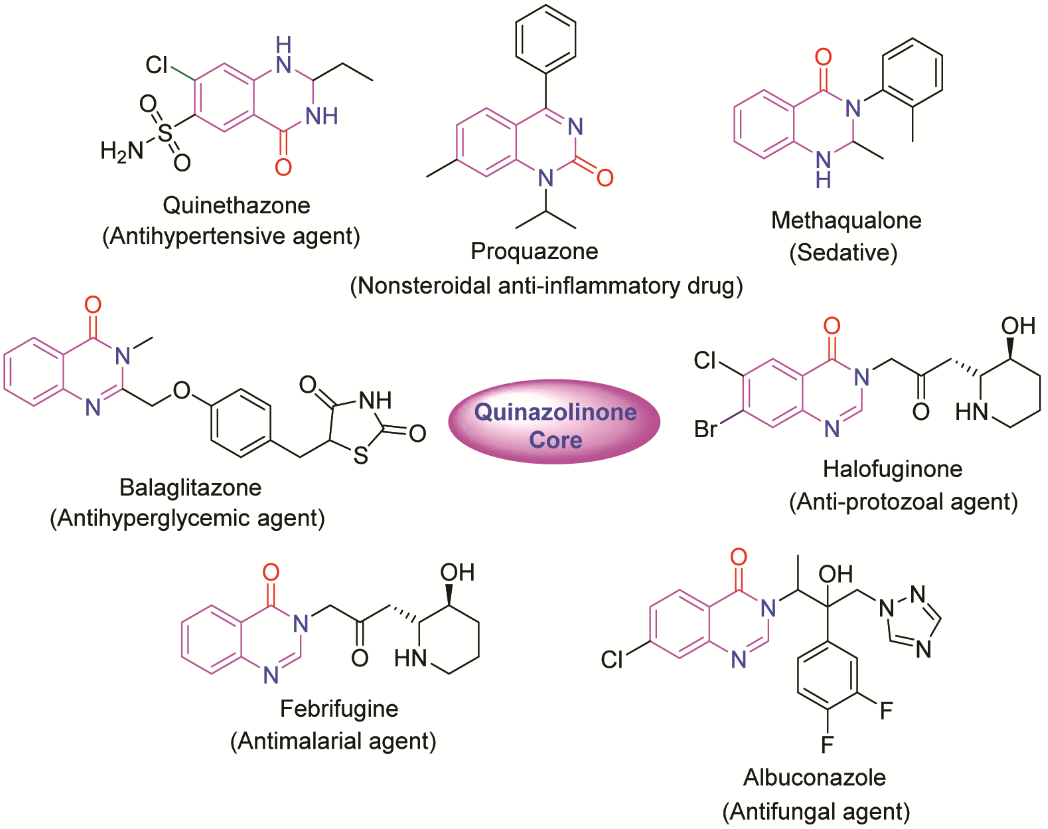
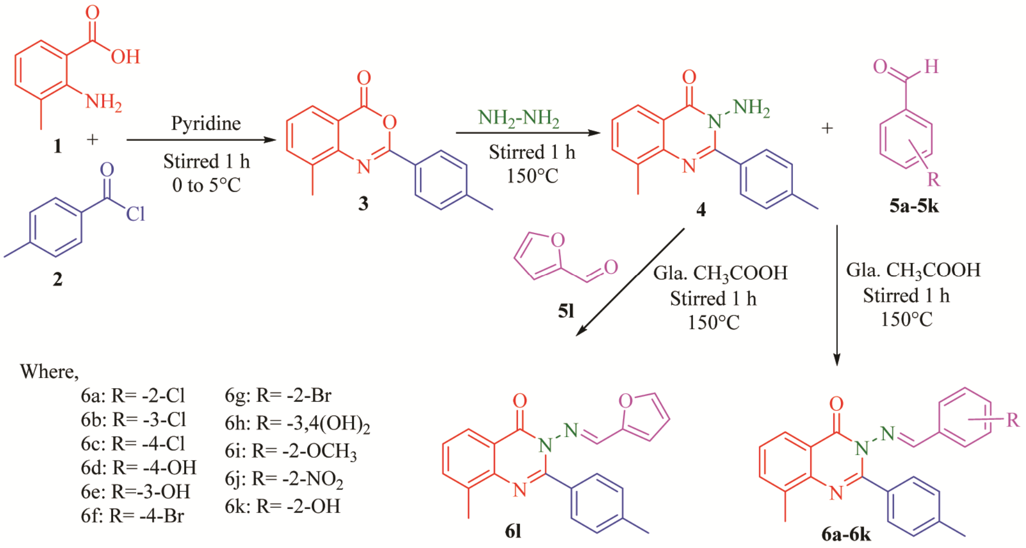


Fig. 2 — Quinazolinone-based medicine



Scheme 1 — Synthetic route for the preparation of compounds **6a-l**

a 5:5 ratio) and a mixture of ethyl acetate and *n*-hexane (in a 3:7 ratio) as mobile phases. This test was conducted on aluminum-coated TLC plates 60 F245 (manufactured by E. Merck) and the results were observed under ultraviolet (UV) light.

Synthesis of 8-methyl-2-(*p*-tolyl)-4*H*-benzo[*d*][1,3] oxazin-4-one, 3

2-amino-3-methylbenzoic acid (1) (0.1 mol) was dissolved in 10 mL of pyridine by stirring. Progressively add 0.2 mol of con. 4-methaylbenzoyl chloride (2) to the mixture at 0° to 5°C and maintain the mixture's temperature for 2 h. The reaction progress was monitored by aluminum-coated TLC using ethyl acetate: toluene (5:5) solvent system. Stir the reaction mixture for a further 1 h at RT after the completion of reaction. Add ice-cooled process water (30 mL) while stirring. The separated product was filtered, washed with cooled water and purification was performed with IPA slurry on the product. The solid was then vacuum dried at 30°C to 35°C to get the desired compound 3.

Synthesis of 3-amino-8-methyl-2-(*p*-tolyl) quinazolin-4(3*H*)-one, 4

The compound 3 (0.1 mol) and hydrazine hydrate (0.5 mol, 99%) were mixed in RBF. Stir the mixture for an hour at 150°C, and the reaction progress was monitored by TLC using ethyl acetate: toluene (5:5) solvent system. Stir the mixture and allow it to cool before adding ice-cold distilled water (10 mL) and methanol (2-3 mL). Then, rinse the reaction material with a solution made of methanol and water (2:8) after filtering it. The solid was then vacuum dried at 30°C to 35°C to get the targeted compound **4**. Yield 95%. m.p.250-252°C. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 8.01 (d, 1H, *J* = 8.0 Hz, Ar-*H*), 7.77 (d, 2H, *J* = 8.0 Hz, Ar-*H*), 7.69 (d, 1H, *J* = 7.1 Hz, Ar-*H*), 7.42 (t, 1H, *J* = 7.6 Hz, Ar-*H*), 7.29 (d, 2H, *J* = 7.7 Hz, Ar-*H*), 5.68 (s, 2H, N*H*$_2$), 2.54 (s, 3H, Ar-C*H*$_3$), 2.39 (s, 3H, Ar-C*H*$_3$); ^{13}C NMR (DMSO-d_6, 125 MHz): δ 161.38, 154.41, 145.19, 139.23, 135.49, 134.45, 132.26, 129.83, 127.93, 126.20, 123.69, 119.82, 20.97, 17.04. Anal. Calcd for $C_{16}H_{15}N_3O$: C, 72.43; H, 5.70; N, 15.84. Found: C, 72.47; H, 5.68; N, 15.82%.

Catalytic optimization of reaction

According to literature studies, dehydrative cyclization has been completed over the years using a variety of catalysts to get a greater yield while decreasing reaction time. In order to improve the yield, we conducted a series of reactions using methanol and ethanol as solvents and hydrazine hydrate as a catalyst under different catalytic conditions and temperatures to produce compound 4 (Scheme 1). As a result, we discovered that neat hydrazine hydrate at 150°C, without any solvent or additional catalyst, was the most effective condition for obtaining the highest yield in the shortest amount of time (Table 1).

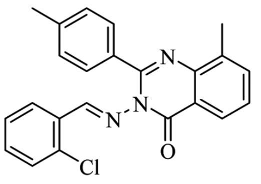
General preparation of 3-[(substituted-benzylidene)amino]-8-methyl-2-(*p*-tolyl)quinazolin-4(3*H*)-one, 6a-l

A solution of 3-amino-8-methyl-2-(*p*-tolyl) quinazolin-4(3*H*)-one **4** (0.1 mol) was prepared in

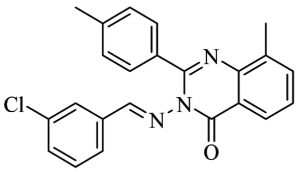
Table 1 — Optimization processes of compound **4**

S. No.	Catalyst	Solvent	Temperature (°C)	Time (h)	Yield (%)
1	NH_2NH_2 with AcOH	Methanol	65-70	6	52
2	NH_2NH_2 with Pyridine	Methanol	65-70	6	10
3	NH_2NH_2 with Piperidine	Methanol	65-70	6	–
4	NH_2NH_2 with AcOH	Ethanol	65-70	6	42
5	NH_2NH_2 with Pyridine	Ethanol	65-70	6	25
6	NH_2NH_2 with Piperidine	Ethanol	65-70	6	–
7	NH_2NH_2	Ethanol	65-70	6	60
8	NH_2NH_2	–	100	1	80
9	NH_2NH_2	–	150	1	95

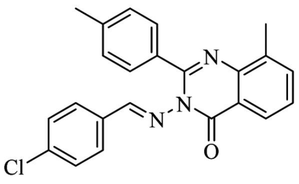
Table 2 — Structures of synthesized compounds **6a-l**



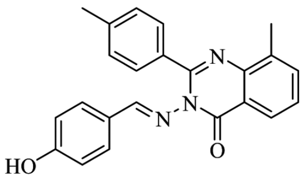
6a



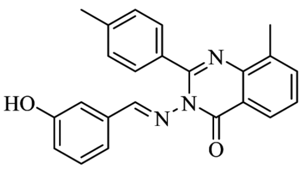
6b



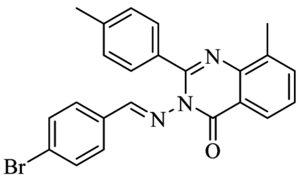
6c



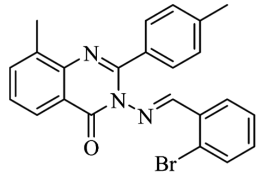
6d



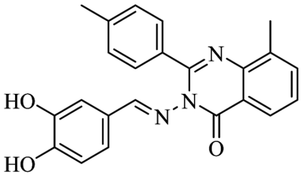
6e



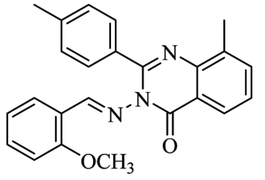
6f



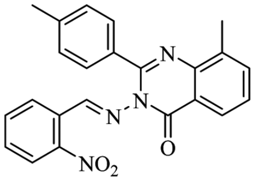
6g



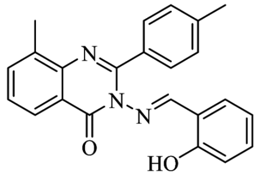
6h



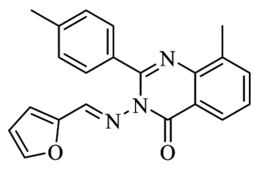
6i



6j



6k



6l

glacial acetic acid (2-3 mL). While continuously stirring the solution between 25°C and 35°C, gradually add a mixture of glacial acetic acid and benzaldehyde derivatives until the solution turns translucent. The mixture was stirred and refluxed at 150°C for 1 h, and the reaction progress was monitored by TLC using ethyl acetate: *n*-hexane (2:8) solvent system. After chilling, the reaction mixture was poured into a mixture of methanol (1-2 mL) and ice-cold process water (10 mL). The solid material thus obtained was wash with methanol and water solution (1:9), vacuum dry the solid at 30°C to 35°C to afford the desired final compounds 6a-l (Scheme 1, Table 2).

3-[(2-Chlorobenzylidene)amino]-8-methyl-2-(*p*-tolyl)quinazolin-4(3*H*)-one, 6a: Yield 89%. m.p.251-253°C. IR (KBr): 3020 (Aromatic C-H), 2911 (C-H), 1685 (C=O), 1555 (C=N), 1453 (C=C), 1370 cm^{-1} (C-

N); ^{1}H NMR (DMSO-d_6, 400 MHz): δ 8.74 (s, 1H, N=C*H*), 7.92 (d, 1H, *J* = 8.1 Hz, Ar - *H*), 7.75 (m, 3H, *J* = 4.4 Hz, Ar-*H*), 7.35 (t, 3H, *J* = 8.1 Hz, Ar-*H*), 7.24 (t, 1H, *J* = 7.4 Hz, Ar-*H*), 7.20 (t, 1H, *J* = 7.8 Hz, Ar-*H*), 7.18 (d, 2H, *J* = 8.2 Hz, Ar-*H*), 2.53 (s, 3H, Ar-CH_3), 2.33 (s, 3H, Ar-CH_3); ^{13}C NMR (DMSO-d_6, 125 MHz): δ 165.3, 154.9, 152.8, 143.7, 139.4, 134.6, 133.0, 132.4, 131.8, 131.2, 131.0, 130.1, 129.5, 128.3, 127.5, 126.1, 126.0, 124.9, 121.5, 21.5, 19.1; MS: *m/z* 387 [M]$^+$. Anal. Calcd for $C_{23}H_{18}N_3OCl$: C, 71.22; H, 4.68; N, 10.83. Found: C, 71.18; H, 4.65; N, 10.81%.

3-[(3-Chlorobenzylidene)amino]-8-methyl-2-(*p*-tolyl)quinazolin-4(3*H*)-one, 6b: Yield : 90%. m.p.224-226°C. IR (KBr): 3016 (Aromatic C-H), 2916 (C-H), 1681 (C=O), 1558 (C=N), 1458 (C=C), 1373 cm^{-1} (C-N); ^{1}H NMR (DMSO-d_6, 400 MHz): δ 9.09 (s, 1H, N=C*H*), 8.06 (d, 1H, *J* = 8.0 Hz, Ar - *H*), 7.74 (m, 3H, *J* = 4.0 Hz, Ar-*H*), 7.65 (m, 3H, *J* = 8.0 Hz, Ar-*H*), 7.55 (t, 1H, *J* = 7.8 Hz, Ar-*H*), 7.48 (t, 1H, *J* = 7.6 Hz, Ar-*H*), 7.27 (d, 2H, *J* = 8.0 Hz, Ar-*H*), 2.58 (s, 3H, Ar-CH_3), 2.35 (s, 3H, Ar-CH_3); ^{13}C NMR (DMSO-d_6, 125 MHz): δ 167.5, 157.9, 151.8, 144.7, 139.7, 135.7, 135.0, 134.4, 133.8, 132.2, 131.9, 131.1, 129.9, 128.2, 127.8, 127.1, 126.6, 124.5, 120.7, 20.9, 17.0; MS: *m/z* 387 [M]$^+$. Anal. Calcd for $C_{23}H_{18}N_3OCl$: C, 71.22; H, 4.68; N, 10.83. Found: C, 71.20; H, 4.66; N, 10.80%.

3-[(4-Chlorobenzylidene)amino]-8-methyl-2-(*p*-tolyl)quinazolin-4(3*H*)-one, 6c: Yield 79%. m.p.210-212°C. IR (KBr): 3021 (Aromatic C-H), 2920 (C-H), 1683 (C=O), 1555 (C=N), 1450 (C=C), 1370 cm^{-1} (C-N); ^{1}H NMR (DMSO-d_6, 400 MHz): δ 8.43 (s, 1H, N=C*H*), 7.88 (d, 1H, *J* = 8.2 Hz, Ar - *H*), 7.56 (t, 3H, *J* = 4.5 Hz, Ar-*H*), 7.46 (t, 3H, *J* = 8.2 Hz, Ar-*H*), 7.39 (m, 1H, *J* = 7.5 Hz, Ar-*H*), 7.34 (t, 1H, *J* = 7.6 Hz, Ar-*H*), 7.18 (d, 2H, *J* = 8.2 Hz, Ar-*H*), 2.49 (s, 3H, Ar-CH_3), 2.39 (s, 3H, Ar-CH_3); ^{13}C NMR (DMSO-d_6, 125 MHz): δ 165.5, 154.9, 152.8, 149.7, 135.7, 134.7, 134.0, 133.4, 132.8, 132.2, 130.9, 130.1, 128.9, 128.4, 127.5, 127.1, 126.3, 125.5, 121.7, 21.9, 18.3; MS: *m/z* 387 [M]$^+$. Anal. Calcd for $C_{23}H_{18}N_3OCl$: C, 71.22; H, 4.68; N, 10.83. Found: C, 71.21; H, 4.67; N, 10.85%.

3-[(4-Hydroxybenzylidene)amino]-8-methyl-2-(*p*-tolyl)quinazolin-4(3*H*)-one, 6d: Yield 80%. m.p.284-286°C. IR (KBr): 3302 (O-H), 3024 (Aromatic C-H), 2924 (C-H), 1658 (C=O), 1589 (C=N), 1450 (C=C), 1327 cm^{-1} (C-N); ^{1}H NMR (DMSO-d_6, 400 MHz): δ 10.33 (s, 1H, O*H*), 8.77 (s, 1H, N=C*H*), 8.04 (m, 1H, *J* = 8.0 Hz, Ar-*H*), 7.73 (d, 1H, *J* = 7.1 Hz, Ar-*H*), 7.62 (t, 4H, *J* = 8.8 Hz, Ar-*H*), 7.46 (t, 1H, *J* = 7.7 Hz, Ar-*H*), 7.25 (d, 2H, *J* = 8.0 Hz, Ar-*H*), 6.87 (d, 2H, *J* = 8.8 Hz, Ar-*H*), 2.58 (s, 3H, Ar-CH_3), 2.34 (s, 3H, Ar-CH_3); ^{13}C NMR (DMSO-d_6, 125 MHz): δ 169.5, 161.6, 158.1, 151.8, 144.8, 139.5, 135.6, 134.6, 132.0, 130.8, 129.9, 128.2, 126.4, 124.3, 123.2, 120.8, 115.9, 20.9, 17.0; MS: *m/z* 369 [M]$^+$. Anal. Calcd for $C_{23}H_{19}N_3O_2$: C, 74.78; H, 5.18; N, 11.37. Found: C, 74.75; H, 5.15; N, 11.30%.

3-[(3-Hydroxybenzylidene)amino]-8-methyl-2-(*p*-tolyl)quinazolin-4(3*H*)-one, 6e: Yield 83%. m.p.270-272°C. IR (KBr): 3310 (O-H), 3029 (Aromatic C-H), 2928(C-H), 1653 (C=O), 1585 (C=N), 1451 (C=C), 1324 cm^{-1} (C-N); ^{1}H NMR (DMSO-d_6, 400 MHz): δ 10.29 (s, 1H, O*H*), 8.75 (s, 1H, N=C*H*), 8.28 (t, 1H, *J* = 8.2 Hz, Ar-*H*), 7.69 (d, 1H, *J* = 7.0 Hz, Ar-*H*), 7.34 (m, 4H, *J* = 8.0 Hz, Ar-*H*), 7.20 (m, 1H, *J* = 7.7 Hz, Ar-*H*), 7.12 (d, 2H, *J* = 8.3 Hz, Ar-*H*), 6.79 (d, 2H, *J* = 8.0 Hz, Ar-*H*), 2.59 (s, 3H, Ar-CH_3), 2.35 (s, 3H, Ar-CH_3); ^{13}C NMR (DMSO-d_6, 125 MHz): δ 168.5, 162.9, 156.1, 153.8, 145.5, 140.5, 134.6, 134.3, 132.5, 130.4, 129.8, 128.6, 125.4, 123.9, 123.5, 120.5, 116.7, 20.7, 18.5; MS: *m/z* 369 [M]$^+$. Anal. Calcd for $C_{23}H_{19}N_3O_2$: C, 74.78; H, 5.18; N, 11.37. Found: C, 74.73; H, 5.17; N, 11.35%.

3-[(4-Bromobenzylidene)amino]-8-methyl-2-(*p*-tolyl)quinazolin-4(3*H*)-one, 6f: Yield 88%. m.p.246-248°C. IR (KBr): 3016 (Aromatic C-H), 2915 (C-H), 1685 (C=O), 1554 (C=N), 1457 (C=C), 1377 cm^{-1} (C-N); ^{1}H NMR (DMSO-d_6, 400 MHz): δ 8.45 (s, 1H, N=C*H*), 7.96 (d, 1H, *J* = 8.0 Hz, Ar - *H*), 7.65 (m, 3H, *J* = 4.0 Hz, Ar-*H*), 7.47 (m, 3H, *J* = 8.0 Hz, Ar-*H*), 7.45 (t, 1H, *J* = 7.8 Hz, Ar-*H*), 7.35 (t, 1H, *J* = 7.6 Hz, Ar-*H*), 7.25 (d, 2H, *J* = 8.0 Hz, Ar-*H*), 2.58 (s, 3H, Ar-CH_3), 2.35 (s, 3H, Ar-CH_3); ^{13}C NMR (DMSO-d_6, 125 MHz): δ 162.5, 152.9, 150.8, 145.3, 134.9, 134.7, 133.9, 133.5, 133.3, 132.2, 131.8, 131.5, 128.9, 128.5, 127.7, 127.3, 125.4, 125.3, 121.8, 20.5, 18.0; MS: *m/z* 431 [M]$^+$. Anal. Calcd for $C_{23}H_{18}N_3OBr$: C, 63.90; H, 4.20; N, 9.72. Found: C, 63.95; H, 4.15; N, 9.75%.

3-[(2-Bromobenzylidene)amino]-8-methyl-2-(*p*-tolyl)quinazolin-4(3*H*)-one, 6g: Yield 90%. m.p.232-234°C. IR (KBr): 3021 (Aromatic C-H), 2915 (C-H), 1682 (C=O), 1557 (C=N), 1455 (C=C), 1373 cm^{-1} (C-N); ^{1}H NMR (DMSO-d_6, 400 MHz): δ 8.72 (s, 1H, N=C*H*), 7.97 (d, 1H, *J* = 8.0 Hz, Ar - *H*), 7.75 (t, 3H, *J* = 4.2 Hz, Ar-*H*), 7.47 (m, 3H, *J* = 8.2 Hz, Ar-*H*), 7.35 (t, 1H, *J* = 7.8 Hz, Ar-*H*), 7.23 (t, 1H, *J* = 7.5 Hz, Ar-*H*), 7.25 (m, 2H, *J* = 8.1 Hz, Ar-*H*), 2.58 (s, 3H, Ar-C*H*$_3$), 2.35 (s, 3H, Ar-C*H*$_3$); ^{13}C NMR (DMSO-*d*$_6$, 125 MHz): δ 160.5, 154.9, 150.4, 149.4, 139.4, 137.6, 135.7, 134.3, 133.4, 133.2, 132.9, 131.7, 130.9, 129.2, 128.8, 126.1, 125.6, 124.6, 120.8, 20.4, 17.0; MS: *m/z* 431 [M]$^+$. Anal. Calcd for $C_{23}H_{18}N_3OBr$: C, 63.90; H, 4.20; N, 9.72. Found: C, 63.93; H, 4.22; N, 9.77%.

3-[(3,4-Dihydroxybenzylidene)amino]-8-methyl-2-(*p*-tolyl)quinazolin-4(3*H*)-one, 6h: Yield 82%. m.p.288-290°C. IR (KBr): 3310 (O-H), 3029 (Aromatic C-H), 2925 (C-H), 1658 (C=O), 1587 (C=N), 1452 (C=C), 1327 cm^{-1} (C-N); ^{1}H NMR (DMSO-*d*$_6$, 400 MHz): δ 10.45 (s, 1H, O*H*), 10.40 (s, 1H, O*H*), 8.77 (s, 1H, N=C*H*), 8.31 (m, 1H, *J* = 8.0 Hz, Ar-*H*), 7.67 (m, 4H, *J* = 8.8 Hz, Ar-*H*), 7.35 (d, 1H, *J* = 7.7 Hz, Ar-*H*), 7.25 (d, 2H, *J* = 8.0 Hz, Ar-*H*), 6.87 (m, 2H, *J* = 8.8 Hz, Ar-*H*), 2.58 (s, 3H, Ar-C*H*$_3$), 2.34 (s, 3H, Ar-C*H*$_3$); ^{13}C NMR (DMSO-*d*$_6$, 125 MHz): δ 169.5, 161.6, 158.1, 151.8, 144.8, 139.5, 135.6, 134.6, 132.0, 130.8, 129.9, 128.2, 126.4, 124.3, 123.2, 120.8, 115.9, 20.9, 17.0; MS: *m/z* 385 [M]$^+$. Anal. Calcd for $C_{23}H_{19}N_3O_3$: C, 71.68; H, 4.97; N, 10.90. Found: C, 71.65; H, 4.95; N, 10.88%.

3-[(2-Methoxybenzylidene)amino]-8-methyl-2-(*p*-tolyl)quinazolin-4(3*H*)-one, 6i: Yield 70%. m.p 276-278°C. IR (KBr): 3302 (O-H), 3024 (Aromatic C-H), 2924 (C-H), 1658 (C=O), 1589 (C=N), 1450 (C=C), 1327 (C-N), 1035 cm^{-1} (C-O); ^{1}H NMR (DMSO-*d*$_6$, 400 MHz): δ 8.55 (s, 1H, N=C*H*), 7.96 (m, 1H, *J* = 8.2 Hz, Ar-*H*), 7.76 (d, 1H, *J* = 7.1 Hz, Ar-*H*), 7.52 (m, 3H, *J* = 8.7 Hz, Ar-*H*), 7.34 (t, 2H, *J* = 7.5 Hz, Ar-*H*), 7.20 (d, 2H, *J* = 8.0 Hz, Ar-*H*), 6.95 (d, 2H, *J* = 8.4 Hz, Ar-*H*), 3.58 (s, 3H, OC*H*$_3$), 2.58 (s, 3H, Ar-C*H*$_3$), 2.34 (s, 3H, Ar-C*H*$_3$); ^{13}C NMR (DMSO-*d*$_6$, 125 MHz): δ 162.4, 160.6, 157.1, 155.8, 144.8, 139.4, 135.4, 134.2, 132.0, 130.7, 129.3, 128.2, 126.4, 124.4, 123.3, 120.5, 115.4, 20.9, 17.0; MS: *m/z* 383 [M]$^+$. Anal. Calcd for $C_{24}H_{21}N_3O_2$: C, 75.18; H, 5.52; N, 10.96. Found: C, 75.20; H, 5.53; N, 10.92%.

8-Methyl-3-[(2-nitrobenzylidene)amino]-2-(*p*-tolyl)quinazolin-4(3*H*)-one, 6j: Yield 75%. m.p.264-266°C. IR (KBr): 3024 (Aromatic C-H), 2924 (C-H), 1689 (C=O), 1527 (C=N), 1458 (C=C), 1334 cm^{-1} (C-N); ^{1}H NMR (DMSO-*d*$_6$, 400 MHz): δ 9.45 (s, 1H, N=C*H*), 8.18 (m, 1H, *J* = 4.0 Hz, Ar - *H*), 8.07 (d, 1H, *J* = 7.1 Hz, Ar-*H*), 7.83 (m, 3H, *J* = 4.0 Hz, Ar-*H*), 7.75 (d, 1H, *J* = 7.1 Hz, Ar-*H*), 7.64 (d, 2H, *J* = 8.2 Hz, Ar-*H*), 7.48 (t, 1H, *J* = 7.7 Hz, Ar-*H*), 7.27 (d, 2H, *J* = 8.2 Hz, Ar-*H*), 2.58 (s, 3H, Ar-C*H*$_3$), 2.35 (s, 3H, Ar-C*H*$_3$); ^{13}C NMR (DMSO-*d*$_6$, 125 MHz): δ 165.5, 157.9, 151.8, 144.6, 139.8, 135.1, 134.2, 132.9, 132.0, 129.8, 128.9, 128.3, 127.0, 126.7, 124.9, 124.6, 20.9, 17.0; MS: *m/z* 398 [M]$^+$. Anal. Calcd for $C_{23}H_{18}N_4O_3$: C, 69.34; H, 4.55; N, 14.06. Found: C, 68.92; H, 4.52; N, 14.20%.

3-[(2-Hydroxybenzylidene)amino]-8-methyl-2-(*p*-tolyl)quinazolin-4(3*H*)-one, 6k: Yield 80%. m.p.292-294°C. IR (KBr): 3310 (O-H), 3029 (Aromatic C-H), 2928 (C-H), 1653 (C=O), 1585 (C=N), 1451 (C=C), 1324 cm^{-1} (C-N); ^{1}H NMR (DMSO-*d*$_6$, 400 MHz): δ 10.29 (s, 1H, O*H*), 8.75 (s, 1H, N=C*H*), 8.28 (m, 1H, *J* = 8.2 Hz, Ar-*H*), 7.69 (d, 1H, *J* = 7.0 Hz, Ar-*H*), 7.34 (m, 3H, *J* = 8.0 Hz, Ar-*H*), 7.20 (t, 1H, *J* = 7.7 Hz, Ar-*H*), 7.12 (m, 3H, *J* = 8.3 Hz, Ar-*H*), 6.79 (d, 2H, *J* = 8.0 Hz, Ar-*H*), 2.59 (s, 3H, Ar-C*H*$_3$), 2.35 (s, 3H, Ar-C*H*$_3$); ^{13}C NMR (DMSO-*d*$_6$, 125 MHz): δ 168.5, 162.9, 156.1, 153.8, 145.5, 140.5, 134.6, 134.3, 132.5, 130.4, 129.8, 128.6, 125.4, 123.9, 123.5, 120.5, 116.7, 20.7, 18.5; MS: *m/z* 369 [M]$^+$. Anal. Calcd for $C_{23}H_{19}N_3O_2$: C, 74.78; H, 5.18; N, 11.37. Found: C, 74.72; H, 5.12; N, 11.31%.

3-[(Furan-2-ylmethylene)amino]-8-methyl-2-(*p*-tolyl)quinazolin-4(3*H*)-one, 6l: Yield 79%. m.p.308-310°C. IR (KBr): 3063 (Aromatic C-H), 2924 (C-H), 1674 (C=O), 1597 (C=N), 1465 (C=C), 1327 cm^{-1} (C-N); ^{1}H NMR (DMSO-*d*$_6$, 400 MHz): δ 8.85 (s, 1H, N=CH), 8.04 (d, 1H, *J* = 7.4 Hz, Ar - *H*), 8.01 (d, 1H, *J* = 1.4 Hz, Ar-*H*), 7.74 (d, 1H, *J* = 7.1 Hz, Ar-*H*), 7.61 (d, 2H, *J* = 8.2 Hz, Ar-*H*), 7.47 (t, 1H, *J* = 7.6 Hz, Ar-*H*), 7.26 (m, 3H, *J* = 8.0 Hz, Ar-*H*), 6.75 (q, 1H, *J* = 1.7 Hz, Ar-*H*), 2.58 (s, 3H, Ar-C*H*$_3$), 2.36 (s, 3H, Ar-C*H*$_3$); ^{13}C NMR (DMSO-*d*$_6$, 125 MHz): δ 158.0, 157.9, 151.8, 147.9, 147.4, 139.6, 135.7, 134.8, 132.1, 129.9, 128.2, 126.5, 124.4, 120.7, 120.0, 112.8, 20.9, 17.0; MS: *m/z* 343 [M]$^+$. Anal. Calcd for $C_{21}H_{17}N_3O_2$: C, 73.45; H, 4.99; N, 12.24. Found: C, 73.50; H, 4.95; N, 12.26%.

***In-vitro* anti-inflammatory assay**

This study investigated the anti-inflammatory activity of synthesized compounds using an albumin denaturation assay based on the method of Mizushima and Kobayashi, with minor modifications[35]. For the preparation of samples, both the standard drug (Diclofenac sodium) and test compounds were first minimally dissolved in dimethyl formamide (DMF) before being diluted with 0.2 M phosphate buffer solution (PBS - *p*H 7.4). The final DMF content in every solution was less than 2.5%. The test solution (100 µg/mL) was mixed with 450 µL of 5% bovine serum albumin that was previously prepared in PBS. For 15 min, this reaction mixture was incubated at 37ºC in an incubator. After that, the temperature was raised to 60°C for 10 min to denaturize the samples. After cooling to RT, the mixture was diluted with 2.5 mL of PBS. The resulting turbidity, a measure of protein aggregation, was then quantified using a UV spectrophotometer (Shimadzu-UV-1900) at 660 nm. The percentage inhibition of protein denaturation was calculated using the following formula: % inhibition = [(Abs control – Abs test) / Abs control] × 100. As a control, double-distilled water was used; neither drug nor test solution was added. Each experiment was performed in triplicates.

In-vitro antimicrobial screening assay

The minimum inhibitory concentrations (MICs) of the most active synthesized compounds were assessed using the serial broth dilution method. The MIC is the lowest concentration of a sample that prevents the visible growth of a microorganism after a specific incubation period. We tested molecules **6a** to **6l** for their antimicrobial activity against Gram-negative cultures (*E. coli, Klebsiella)*, Gram-positive cultures *(B. megaterium, S. aureus)*, and antifungal activity against fungal cultures *A. niger*, and *F. solani*. Dimethyl sulfoxide (DMSO) was used to prepare stock solutions (2 mg/mL) of each test compound for MIC assays. To test a wider range of doses, serial dilutions were prepared for different concentrations of these test compounds ranging from 100 to 2000 µg/mL. Test tubes containing culture medium like LB broth for bacterial cultures and sabouraud dextrose broth supplemented with different concentrations of the test compounds were prepared. Subsequently, each tube was inoculated with a distinct bacterial strain, ensuring a consistent initial concentration of 10^8 cells/mL. After 24 to 48 h of incubation at 37°C (antibacterial) and 30°C (antifungal), the tubes were examined to determine if the tested organisms were growing or absent. This study utilized Chloramphenicol, Ciprofloxacin, and Nystatin as standard drugs to assess the activity of the test compounds. After a primary screening of test compounds, activated test compounds were serially diluted (50, 25, 12.5 and 10 µg/mL) and inoculated in suitable media and growth was observed after 24 or 48 h. The experiment was performed in three individual sets.

Results and Discussion

Chemistry

The synthetic route for the preparation of targeted compounds **6a-l** is depicted as Scheme 1. A reaction was carried out using 4-methylbenzoyl chloride **2** and 2-amino-3-methylbenzoic acid **1** in the presence of pyridine, resulting in the production of 8-methyl-2-(*p*-tolyl)-4*H*-benzo[*d*][1,3]oxazin-4-one **3** through dehydrative cyclization. Subsequently, compound **3** was heated to 150°C with hydrazine hydrate (99%) to produce 3-amino-8-methyl-2-(*p*-tolyl)quinazolin-4(3*H*)-one **4**. Further, compound **4** was effectively combined in glacial acetic acid with various substituted aromatic aldehydes to obtain the corresponding quinazolinone arylidene/ heteroarylidene derivatives **6a-l**. The reaction mechanism may have proceeded as per the Fig. 3. Possibly, intermediate **3** was produced by the reaction of 2-amino-3-methylbenzoic acid 4-methaylbenzoyl chloride at the initial stage with the removal of the water molecule. Further, Intermediate **3** react with hydrazine hydride by the simple condensation reaction to form intermediate **4**. Intermediate **4** reacted with various aldehyde derivatives to afford the final products **6a-l**.

In vitro Anti-inflammatory evaluation

Protein denaturation describes the process where external forces like heat, harsh chemicals, or even certain compounds disrupt the bonds and interactions that hold a protein's structure together. This disruption unfolds the secondary structures and tertiary structures of protein[36,37]. The new compounds were tested at 100 µg/mL to assess their protein denaturation-inhibiting potential and compared with standard drug diclofenac sodium. The numerical results were presented in Table 3 and their bar graph represented in Fig. 4. According to the data, all the tested compounds showed more than 72% anti-inflammatory inhibition. Among these compounds, compound **6g** was considered the most potent anti-inflammatory inhibitor compared to the standard drug, diclofenac sodium. Compound **6g** demonstrated the highest anti-inflammatory inhibition, with a percentage of 96.9±0.83%. Compound **6d** also exhibited good inhibition in anti-inflammatory study

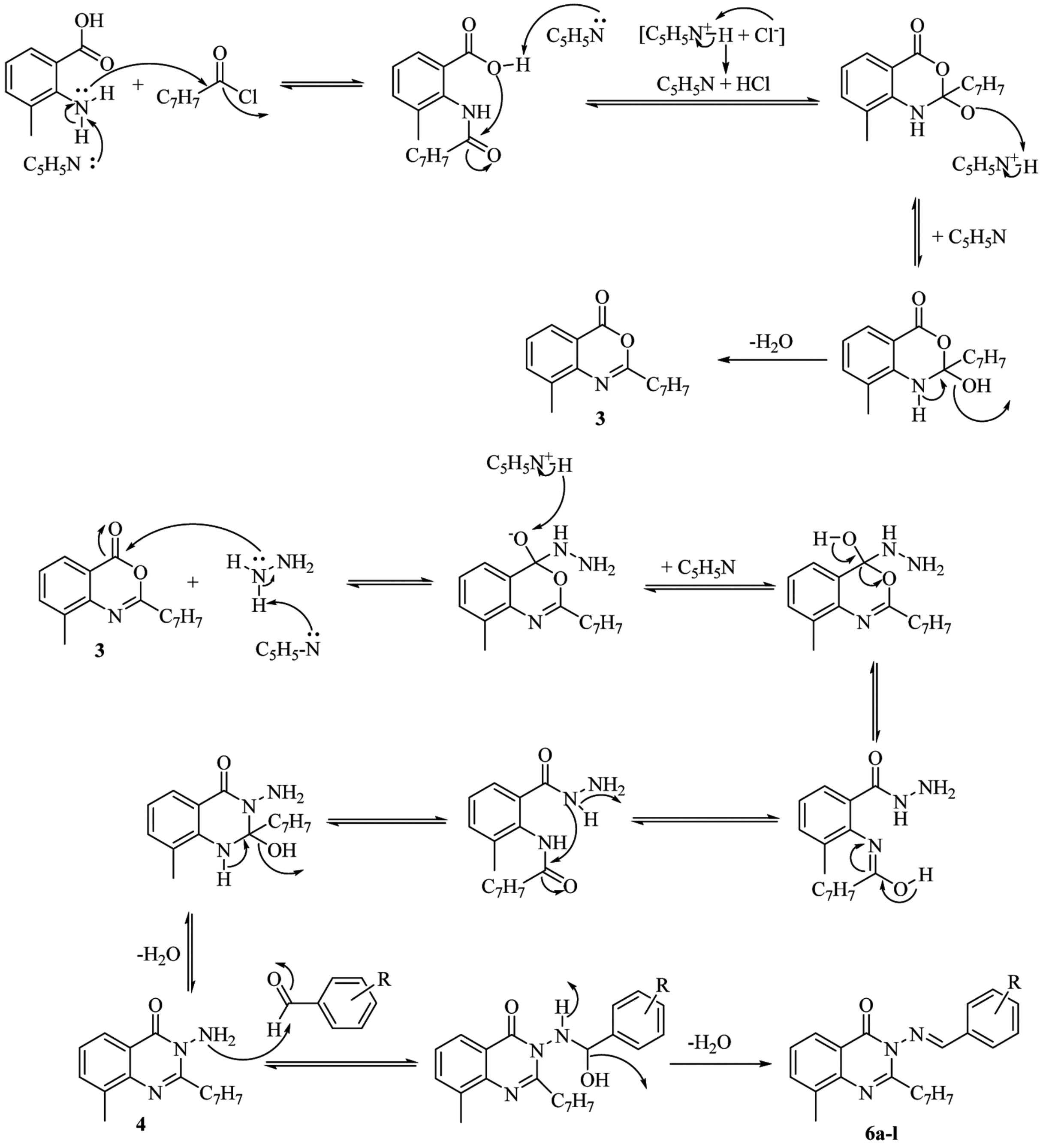


Fig. 3 — Possible reaction mechanism pathway

with 92.64 ±0.38%. Additionally, compounds **6c**, **6e**, **6j** and **6k** showed moderate inhibition in the activity study, with percentage inhibition ranging from 85.14% to 86.71%. The results indicate that all compounds have the ability to inhibit protein denaturation and protect against inflammation. The data provided in the figure suggests that each chemical differs significantly (*p<0.0001, **p<0.001, ***p<0.05) from the reference medication.

Antimicrobial evaluation

The antimicrobial potential of all synthesized compounds was evaluated by determining their minimum inhibitory concentrations (MICs) against various gram-positive, gram-negative bacteria, and fungi. The results presented in Table 4 and Fig. 5 indicate that all compounds showed a diverse range of MIC. The compounds **6d**, **6e**, **6g**, **6j** and **6k** displayed potent antibacterial activity (range: 12.5 – 100 µg/mL)

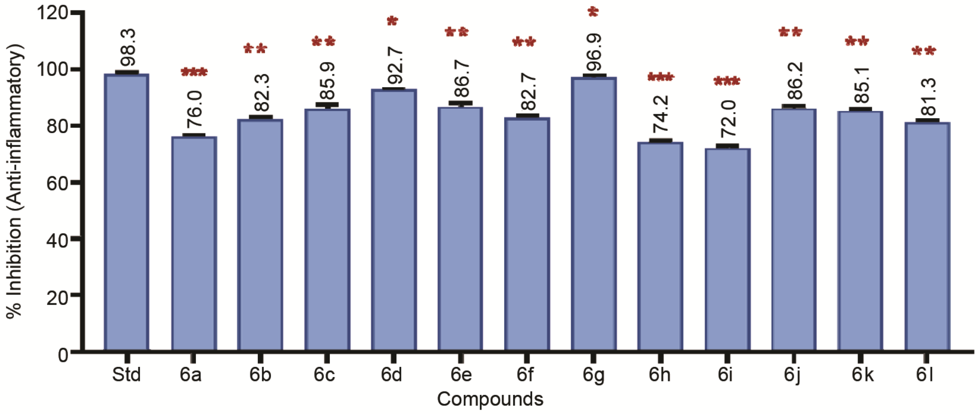


Fig. 4 — *In vitro* anti-inflammatory data represents the mean ± standard deviation of the mean (n = 3). Values represent the Mean ± SD *p<0.0001, **p<0.001, and ***p<0.05: Significantly different from control (One-way ANOVA followed by Tukey test by Graph Pad Prism version 8). Std- Standard drug. The same superscripts are non-significant to each other.

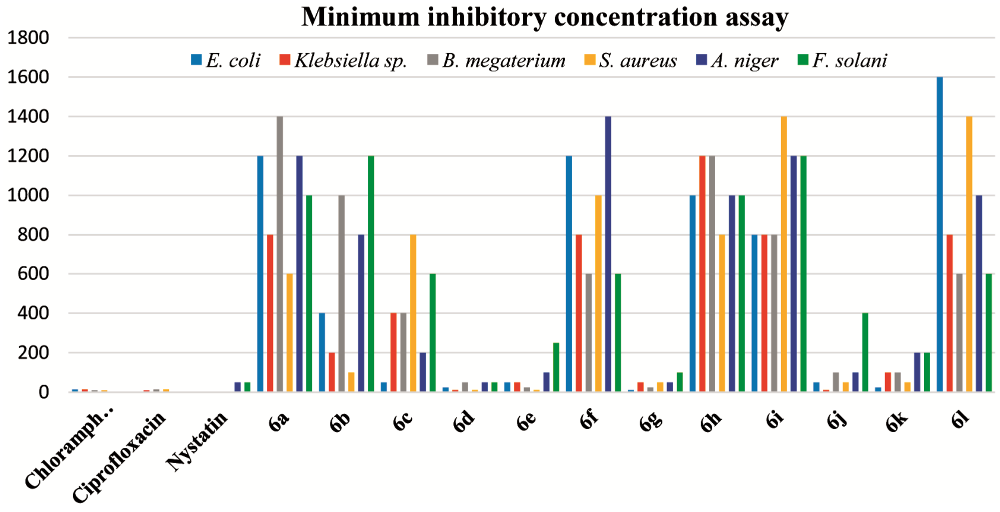


Fig. 5 — Minimum inhibitory concentration data of antimicrobial study

Table 3 — *In-vitro* anti-inflammatory of compounds **6a-l**

Compd	% Inhibition (Anti-inflammatory)
6a	76.04 ±0.56
6b	82.24 ±0.67
6c	85.93 ±1.62
6d	92.64 ±0.38
6e	86.71 ±1.36
6f	82.67 ±0.68
6g	96.89 ±0.83
6h	74.20 ±0.43
6i	72.04 ±0.95
6j	86.15 ±0.74
6k	85.14 ±0.76
6l	81.25 ±0.68
Std	98.25 ±0.52

Data represents the mean ± standard deviation of the mean (n = 3). Values represent the Mean ± SD *p<0.0001, **p<0.001, and ***p<0.05: Significantly different from control. (One-way ANOVA followed by Tukey test by Graph Pad Prism version 8). Std-Standard drug. Same superscript is non-significant to each other.

compared to their good to moderate antifungal efficacy (range: 50 – 400 µg/mL). Among them, compound **6d** demonstrated the best antimicrobial activity, effectively inhibiting the growth of all bacterial and fungal strains. On the other hand, compound **6g** showed good activity against *E. coli*, *Klebsiella sp.*, *B. megaterium*, *S. aureus*, and *A. niger*, and moderate activity against *F. solani*[38].

SAR Study

A study was conducted to investigate the connection between the structure of a compound and its antimicrobial and anti-inflammatory properties. The research discovered that the only factors that affect the behavior of the produced compounds are the various substitutions (-R) attached to the aromatic ring. The study found that compounds containing electron-donating group (EDG) (-OH) at the *ortho*, *meta*, and

Table 4 — Antimicrobial screening data of compounds **6a-l**

Compd	Minimum inhibitory concentration (µg/mL)					
	Bacterial strains				Fungal strains	
	E. coli	*Klebsiella sp.*	*B. megaterium*	*S. aureus*	*A. niger*	*F. solani*
6a	1200±1.1	800±1.6	1400±0.85	600±2.7	1200±0.55	1000±1.0
6b	400±2.5	200±0.55	1000±0.65	100±1.35	800±1.5	1200±0.5
6c	50±1.0	400±0.5	400±2.5	800±3.2	200±0.85	600±0.55
6d	25±0.65	12.5±0.55	50±0.55	12.5±2.5	50±0.85	50±0.55
6e	50 ±1.0	50±2.0	25±2.5	12.5±3.0	100±2.0	250±1.0
6f	1200±1.5	800±0.7	600±3.0	1000±0.05	1400±0.10	600±0.15
6g	12.5±2.7	50±1.0	25±2.2	50±2.5	50±2.0	100±0.10
6h	1000±0.05	1200±1.5	1200±0.9	800±1.5	1000±1.0	1000±2.0
6i	800±1.8	800±0.5	800±0.5	1400±2.5	1200±2.0	1200±3.0
6j	50±0.8	12.5±0.45	100±3.0	50±2.3	100±1.2	400±0.05
6k	25±2.7	100±1.5	100±0.6	50±1.2	200±0.5	200±0.8
6l	1600±1.0	800±0.55	600±3.0	1400±2.5	1000±1.5	600±3.5
Chloramphenicol	15±0.6	15±0.09	10±0.8	10±0.15	–	–
Ciprofloxacin	5±0.2	10±1.0	15±0.25	15±1.2	–	–
Nystatin	–	–	–-	–	50±0.75	50±1.5

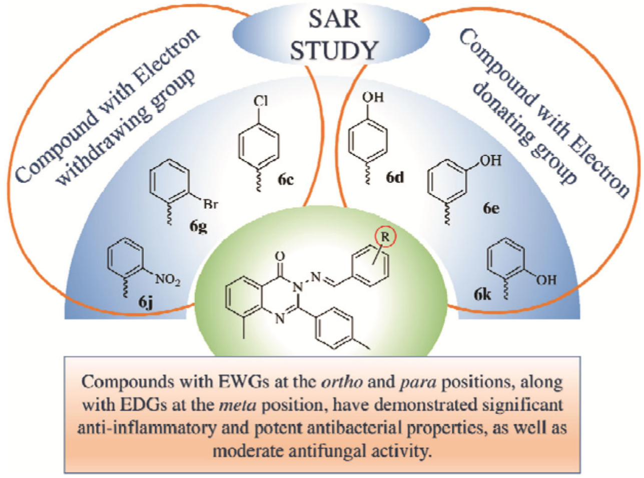


Fig. 6 — SAR study concept of synthesized compounds

para positions, such as compounds **6d**, **6e** and **6k** exhibited potent systemic antimicrobial and anti-inflammatory activities. Meanwhile, compounds **6c**, **6g** and **6j**, which have electron-withdrawing groups (EWGs) in the *ortho* and *para* positions (-Cl, -Br, and -NO_2), displayed greater anti-inflammatory activity in comparison to the *meta*-substituted molecule **6b**. It has been observed that compounds **6g** and **6j**, which have electron-withdrawing groups (EWGs) in the *ortho* position (-Br and -NO_2), exhibit better antimicrobial activity when compared to compounds **6b**, **6c** and **6f** which have EWGs in the *meta* and *para* positions (Fig. 6). This suggests that the location of EWGs and EDGs has a significant impact on the therapeutic efficacy of antimicrobials and anti-inflammatory potency. Additionally, aromatic aldehyde-containing compounds have been found to be more active than heterocyclic aldehydes **6l**.

Conclusion

New quinazolinone Schiff base molecules were synthesized from 3-amino-8-methyl-2-(*p*-tolyl)quinazolin-4(3*H*)-one intermediates that were obtained by catalytic optimized methods. Anti-inflammatory activity results indicated that compound **6g**, which contains halogen, exhibited the highest potential with an inhibition of 96.89±0.83 and compound 6d showed the second-highest inhibition of 92.64±0.38 against diclofenac sodium. In antimicrobial activity, compound **6d** demonstrated the highest inhibitory impact ranging from 12.5 to 50 µg/mL against selected bacterial and fungal pathogens, while **6g** also demonstrated good action against all bacterial strains and *A. niger* species. Moreover, compounds **6e**, **6j**, and **6k** had excellent antibacterial activity but only modest antifungal ability in their antimicrobial activity study. According to the study on structure-activity relationship (SAR), compounds **6d**, **6e**, and **6k** that have electron-donating groups (EDGs) such as hydroxyl at all positions have both potent antimicrobial and anti-inflammatory activities. Compounds **6c** (-Cl), **6g** (-Br), and **6j** (-NO_2) in the *ortho* and *para* positions exhibit excellent to good anti-inflammatory activity as compared to the *meta*-derivative **6b** (-Cl). Additionally, compounds **6g** and **6j** demonstrate good antimicrobial activity when compared to other EWGs derivatives of the *meta* and *para* positions. Therefore, further evaluation of compounds **6d** and **6g** in combination with standard antibiotics is a vital to increase their synergistic outcome and to utilize them for the treatments of harmful microorganisms in the future.

Supplementary Information

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

Conflict of Interests

The authors have declared that there is no conflict of interests.

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