Synthesis, Characterization, and Biological Evaluation of Novel 3-(4-Chlorophenyl)-2-(substituted)quinazolin-4(3*H*)-one Derivatives as Multi-target Anti-inflammatory Agents

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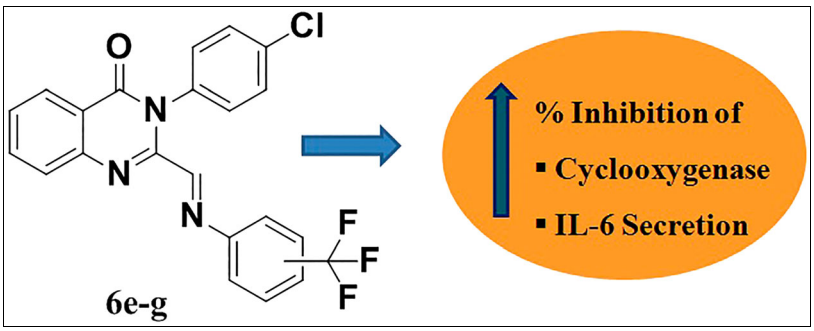


A novel class of 3-(4-chlorophenyl)-2-(substituted)quinazolin-4(3*H*)-one derivatives were synthesized, and the structure of synthesized compounds was characterized by IR, ^{1}H NMR, and mass spectroscopy. The newly synthesized compounds (**4a–g** and **6a–g**) were tested for their *in vitro* cyclooxygenase (COX) inhibition activity. The compounds have inhibitory profile against both COX-1 and COX-2, and some of the compounds are found to be selective against COX-2. The compound **6g** showed distinct inhibitory activity on COXs. The synthesized compounds were evaluated for their potential anti-inflammatory activity as inhibitors of the proinflammatory cytokines IL-6. Compounds **4d–g** showed the highest level of inhibition among all the tested compounds. Thus, our data suggested that these compounds may represent a new class of potent anti-inflammatory agents.

INTRODUCTION

Heterocyclic moieties play an important role in synthetic organic chemistry due to their extensive applications in pharmaceutical and agrochemical fields [1]. Among them, quinazolinone is a class of fused heterocyclic that has been consistently rewarded as a promising molecule because of its broad spectrum of pharmaceutical activities like antihistaminic, anti-inflammatory, antibacterial, antidiabetic, anticancer, antifungal, anthelmintics, and antiviral activities [2–4].

Nonsteroidal anti-inflammatory drugs (NSAIDs) are widely used for the treatment of inflammatory disorders. They help to manage the chronic pain, inflammation, and swelling tied to rheumatoid arthritis. But their long-term benefits are offset by an increased risk of gastric or duodenal perforation and bleeding [5]. The pharmacological effects of NSAIDs are due to inhibition of a membrane enzyme called cyclooxygenase (COX) that is involved in the prostaglandin (PG) biosynthesis. COX metabolizes arachidonic acid to PGH_2; this is the first step in prostanoid production and is the rate-limiting step in PG production. COX activity originates from two distinct and independently regulated isozymes, COX-1 and COX-2. The COX-1 is found primarily in a variety of tissues and appears to be important to the maintenance of physiological functions such as gastric protection and vascular homeostasis. Alternatively, the inductible COX-2 is selectively activated by proinflammatory stimuli and facilitates the release of PG involves in the inflammatory process [6]. Current NSAIDs inhibit both enzymes to varying degrees. The two COX isoforms (COX-1 and COX-2) have different functions, and the inhibition of these isoforms results in different therapeutic effects and side effects. The inhibition of COX-2 has generally been considered the basis for the anti-inflammatory effects of NSAIDs. However, traditional NSAIDs, which selectively inhibit COX-2 but these leads to gastrointestinal bleeding [7].

Among the highly marketed COX-2 inhibitors that comprise the pyrazole nucleus, celecoxib is the one that is treated as a safe anti-inflammatory agent. It is considered as a typical model of the diaryl heterocyclic template that is known to selectively inhibit the COX-2 enzyme. But due to serious adverse effects, the use of pyrazole derivatives is

limited. This limitation has led to the investigation of new N-heterocyclic derivatives with that more potent activity and less toxicity. With this background and in continuation on design and synthesis of biologically important N-heterocyclic compounds [8–12], we report here the synthesis of a quinazolinone derivatives as potential anti-inflammatory agents.

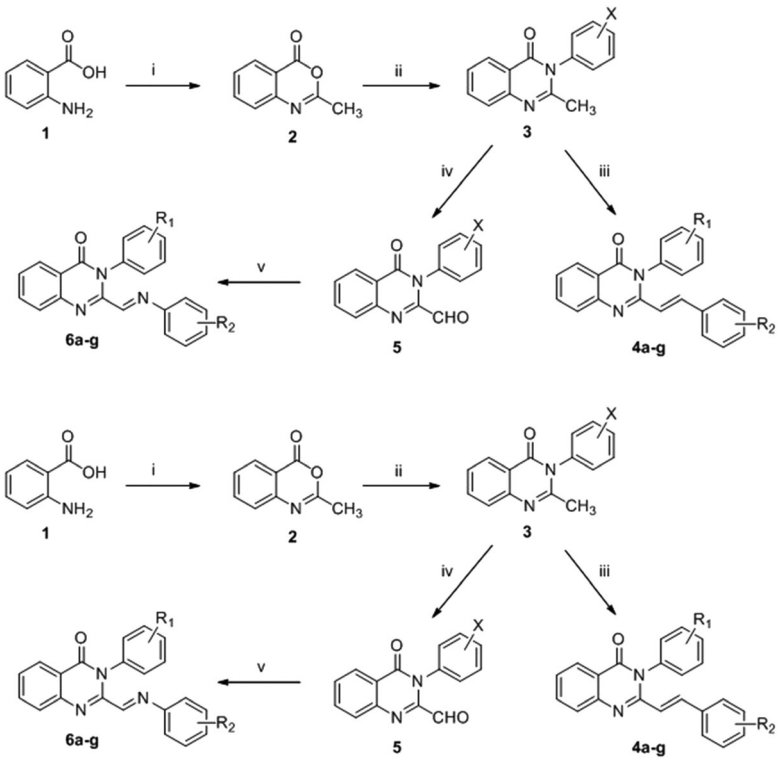
RESULTS AND DISCUSSION

Chemistry. The synthetic route to compound name is depicted in Scheme 1. The synthesis begins with the condensation of anthranilic acid (**1**) reacted with acetic anhydride yielded in compound **2**. The compound **2** further reacted with 4-(trifluoromethyl)aniline in acetic acid to yield 3-(4-chlorophenyl)-2-methylquinazolin-4(3*H*)-one (**3**) [13]. The resulting compound **3** was refluxed with different aldehydes, in the presence of acetic acid, which afforded the corresponding compounds **4a**–**g**. On the other hand, oxidation of methyl group in compound **3** to aldehydic function was successfully achieved with a SeO_2 in dioxane [14]. In this step, smooth conversion of compound **3** to compound **5** was achieved in 84% yield. The furnished product **5** was then allowed to react with fluoro-substituted anilines to afford the 3-(4-chlorophenyl)-2-((substituted) imino)methyl)quinazolin-4(3*H*)-one derivatives (**6a**–**g**) in excellent yields (81–91%). Thin-layer chromatography (TLC) was run throughout the reaction to optimize the reaction for purity and completion. Compounds **4a**–**g** and **6a**–**g** gave correct values in elemental analysis. The structure of all the target compounds was ascertained on the basis of various spectroscopic techniques.

BIOLOGICAL EVALUATION

***In vitro* inhibition of cyclooxygenase.** The primary aim of this study was to generate a library of 3-(4-chlorophenyl)- 2-(substituted)quinazolin-4(3*H*)-one compounds (**4a**–**g** and **6a**–**g**) with different scaffold decorations, to investigate their influence on the COX-1 and COX-2, and to identify preliminary structure–activity relationships (SARs). The synthesized compounds (**4a**–**g** and **6a**–**g**) are evaluated for their ability to inhibit COX-1 and COX-2 using an ovine COX-1/COX-2 assay kit. The efficacies of the test compounds were determined as the concentration causing 50% enzyme inhibition (IC_{50}, μM),

Scheme 1. General method for the synthesis of compounds 4a–g and 6a–g. Reagent and condition: (i) acetic anhydride, reflux, 2 h; (ii) substituted phenylamine, acetic acid, sodium acetate, reflux; (iii) substituted aldehydes (a–g), acetic acid, reflux, 8 h; (iv) dioxane, SeO2, reflux, 8 h; (v) substituted phenyl amine (a–g), EtOH, 8 h.



were calculated from the concentration–inhibition response curve (triplet determinations), and are represented in Table 1 as mean ± SD. The selectivity index was defined as IC_{50} (COX-1)/IC_{50} (COX-2). In the assay system, the IC_{50} values of celecoxib on COX-1 and COX-2 were determined to be >50 and 0.28 μM, respectively, indicating that celecoxib is a selective COX-2 inhibitor (selectivity index >178.57). As far as COX-1 inhibitory properties are concerned, all the tested compounds showed no inhibition of COX-1 up to 50 μM. Compound **6g** was the most potent inhibitor in this series with the COX-2 inhibiting activity (IC_{50} 0.28 μM) followed by **6e** (1.2 μM) and **6f** (1.6 μM) (Figure 1).

The different mono-substituents in the phenyl ring B is shown in Table 1. The compounds (**4a–g** and **6a–g**) include variations at three residue positions with fluorine and difluoromethane groups. It turned out that aromatic substitution was a determinant of the COX-2 inhibitory potency and selectivity; hence, variations in the position of fluorine and trifluoromethane groups on phenyl ring

Table 1

In vitro COX-1 and COX-2 enzyme inhibitory activities of synthesized compounds (**4a–g** and **6a–g**).

		IC_{50} (μM)[a]		
Compound	R	COX-1	COX-2	SI[b]
4a	H	>50	>50	1.00
4b	2-F	>50	14.5	3.44
4c	3-F	>50	16.8	2.97
4d	4-F	>50	12.3	4.06
4e	2-CF_3	>50	3.1	16.12
4f	3-CF_3	>50	3.7	13.51
4g	4- CF_3	>50	1.9	26.31
6a	H	>50	40.1	1.24
6b	2-F	>50	3.2	15.62
6c	3-F	>50	3.4	14.70
6d	4-F	>50	2.5	20.00
6e	2-CF_3	>50	1.2	83.33
6f	3-CF_3	>50	1.6	62.50
6g	4- CF_3	>50	0.26	192.30
Diclofenac	—	0.22	3.1	0.07
Celecoxib	—	>50	0.28	>178.57

COX, cyclooxygenase; SI, selectivity index.
[a]The determination was performed in duplicate for two independent experiments.
[b]SI = IC_{50} (COX-1)/IC_{50} (COX-2).

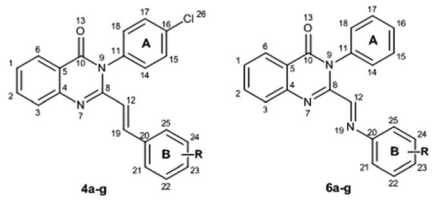


Figure 1. Structure of compounds **4a–g** and **6a–g**.

substantially increase the activity as compared with **4a** and **6a**. We observed that mono-substituents preferably at the 4-position of the phenyl ring B had the greatest influence on COX-2 selectivity. Within aromatic substitution, increasing the bulkiness at the para-position of aromatic ring resulted in enhanced COX-2 selectivity (**4g**). Therefore, it can be stated that steric effect contributes significantly toward the COX inhibition.

In order to extend SAR and design more efficacious and selective COX inhibitors with less cardiotoxicity, new 3-(4-chlorophenyl)-2-(substituted)quinazolin-4(3*H*)-one derivatives (**6a–g**) have been developed based on the replacement of the central —C═C— linkage with —C═N— double bond. The replacement of central —C═C— linkage of (**4a–g**) with —C═N— double bond to the phenyl ring of compounds **6a–g** caused an increase in potency toward COX-2 but had almost no impact on COX-1 activity. These results suggested that compounds (**6a–g**) having —C═N— linkage can lead to selective COX-2 inhibition.

On the other hand, the effects of a fluorine atom as a substituent on physical and chemical properties, as lipophilicity, electronic properties, hydrogen bonding, and metabolic stability are well known [15,16]. The difference in electronegativity between carbon and fluorine generates a large dipole moment in this bond that, when combined with the electrostatic distribution of a specific molecule, may contribute to the molecule's ability to establish intermolecular interactions. Hence, compared with the compounds **4b–g**, compounds **6b–g** caused an increase in potency. Cryer and Feldman [17] reported that COX enzymes are used to measure the anti-inflammatory effects. Because COX-2 enzyme is reported to be elevated in cells where inflammation has occurred, the inhibition of this enzyme by any compound could be regarded as one of the indices to judge the anti-inflammatory activity of that compound. We report here that these inhibitions were partly due to the inhibition at protein expression levels because COX-2–protein expression was significantly reduced by the treatment with quinazolinone derivatives. Thus, our data suggest that some of these novel compounds tested could act as new synthetic COX-2 inhibitors.

Enzyme-linked immunosorbent assay for IL-6 inhibition. As IL-6 was one of the crucial cytokines among the proinflammatory mediators, their blockers have been recognized as effective means for the treatment of inflammatory disease [18]. All the synthesized compounds were evaluated for anti-inflammatory activity against IL-6 at different doses (1, 10, and 20 μM). Inhibitory activity results are summarized in Table 2. A majority of tested compounds exhibited significant anti-inflammatory activity in inhibition of IL-6 expression *in vitro*. Meanwhile, with the higher concentration (20 μM), four of the studied compounds (**6d–g**) inhibited

Table 2

Inhibition of IL-6 secretion for newly synthesized compounds (**4a–g** and **6a–g**).

Compound	% inhibition of IL-6 secretion at different concentrations[a]		
	1 μM	10 μM	20 μM
4a	n/d	n/d	0.4 ± 0.1
4b	n/d	0.5 ± 0.3	20.7 ± 0.6
4c	n/d	n/d	8.0 ± 1.3.
4d	n/d	1.3 ± 0.5	21.9 ± 0.8
4e	n/d	3.7 ± 1.4	65 ± 0.9
4f	n/d	1.8 ± 1.7	39 ± 2.8
4g	n/d	7.2 ± 0.6	68 ± 0.9
6a	n/d	n/d	0.5 ± 0.6
6b	n/d	n/d	0.9 ± 0.1
6c	n/d	n/d	3.1 ± 0.2
6d	4.1 ± 1.1	86.4 ± 1.6	100 ± 0.0
6e	10.7 ± 2.7	96.3 ± 0.7	100 ± 0.0
6f	8.3 ± 0.9	91.1 ± 3.5	100 ± 0.0
6g	36.4 ± 2.5	99.1 ± 0.1	100 ± 0.0
DMS	n/d	84 ± 0.6	100 ± 0.0

DMS, dexamethasone; n/d, not determined.
[a]The means + standard deviations of triplicate assays were calculated; statistically different from control at $P < 0.01$.

IL-6 production by 100%, and the remaining compounds had an inhibitory effect lesser than 70%.

From the aforementioned data, a preliminary SAR for the compounds could be summarized. SAR studies indicated that the electronic nature of the substituent group in aromatic ring led to a significant variation in inhibition of IL-6. Considering the anti-inflammatory activity of the synthesized pyrazolones unsubstituted at the 4-position, compounds **6e**–g bearing a trifluoromethyl moiety in the 2/3/4-position showed superior activity than their analogs (**6a–d**). A similar rule was observed in thiazolidinones with a trifluoromethyl moiety in the 2/3/4-position of **4a–d**. Regarding the trifluoromethyl moiety itself, the strongly electron-withdrawing groups on the aromatic ring in heterocyclic groups seemed to be favorable to the anti-inflammatory activity than electron-donating ones.

CONCLUSION

In this work, we have synthesized new series of 3-(4-chlorophenyl)- 2-(substituted)quinazolin-4(3H)-one derivatives and screened for COX-1, COX-2, and IL-6 inhibition activity. The compounds **6d–g** showed distinct inhibition effect on COXs and serum production of IL-6. It was detected that both trifluoromethyl moiety at para-position on the substituted phenyl ring were highly efficient COX-2 and IL-6 inhibitors compared with other position (ortho and meta) and substituents (fluoro) incorporating the same scaffold. Aforementioned findings inferred **6g** as a potential COX-2 and IL-6 inhibitor and thus render it as a lead molecule for further development of new anti-inflammatory agents.

EXPERIMENTAL

Materials. All the chemicals and solvents were of AR grade. Solvents were used as supplied by commercial sources without any further purification. Elemental analysis (C, H, and N) was determined using a Carlo-Erba 1160 elemental analyzer. IR spectra were recorded on a JASCO FTIR-8400 spectrophotometer using Nujol mulls. The ^{1}H NMR spectra were recorded on a Varian AC 400 spectrometer instrument in $CDCl_3$ using TMS as the internal standard. Low-resolution ESI–MS spectra were obtained on a Varian 1200L model mass spectrometer (solvent: CH_3OH). Melting points were determined with a Buchi 530 melting point apparatus in open capillaries and are uncorrected. Compound purity was checked by TLC on precoated silica gel plates (Merck, Kieselgel 60 F254, layer thickness 0.25 mm).

Synthesis. ***Preparation of 2-methyl-4H-benzo[d][1,3] oxazin-4-one (2).*** Compound **1** (0.01 M, 1.37 g) was refluxed in acetic anhydride (0.01 M, 0.96 mL) for 2 h. The solvent was evaporated under reduced pressure, and recrystallization of the residue from acetonitrile gave the product **2**. Yield: (1.86 g, 84%), mp 82°C. *Anal*. Calcd for $C_9H_7NO_2$: C, 67.07; H, 4.38; N, 8.69. Found: C, 67.01; H, 4.25; N, 8.79. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.98 (s, 3H, —CH3), 7.57–8.01 (m, 4H, Ar—H). IR (nujol, cm^{-1}): 3049–2835 (Ar C—H), 1689 (C═O). MS, *m/z*: 162 (M + 1).

Preparation of 3-(4-chlorophenyl)-2-methylquinazolin-4(3H)-one (3). Equimolar quantities (0.01 M, 1.61 g) of compound **2** and the 4-chloroaniline (0.01 M, 0.9 mL) in acetic acid (5 mL) were refluxed for 5 h in the presence of sodium acetate. The resulting reaction mixture was cooled to room temperature and poured onto crushed ice. The separated out solid was filtered, washed thoroughly with cold distilled water, vacuum dried, and recrystallized from ethanol to obtain the product **3**. Yield: (2.18 g, 89%), mp 169°C. *Anal*. Calcd for $C_{15}H_{11}ClN_2O$: C, 66.55; H, 4.10; N, 10.35. Found: C, 66.38; H, 4.01; N, 10.41. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.01 (s, 3H, —CH3), 7.47–8.03 (m, 8H, Ar—H). IR (nujol, cm^{-1}): 3053–2841 (Ar C—H), 1685 (C═O). MS, *m/z*: 271 (M + 1).

General procedure for the synthesis of 4a–g. The compound **3** (1 mM, 0.27 g) was refluxed with substituted aldehydes (1 mM) in 10 mL of acetic acid for 8 h. After completion of the reaction (TLC), the mixture was extracted with ether, and the combined organic portions were dried (anhydrous Na_2SO_4). The solvent was evaporated under reduced pressure to give crude product, which was next purified by column chromatography on silica employing

chloroform : methanol (9:1) as eluted to obtain the pure product to give the product **4a**–**g**.

3-(4-Chlorophenyl)-2-styrylquinazolin-4(3H)-one (4a). Yield: (87%), mp 186°C. *Anal*. Calcd for $C_{22}H_{15}ClN_2O$: C, 73.64; H, 4.21; N, 7.81. Found: C, 73.51; H, 4.13; N, 7.98. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 6.35 (d, 1H, —CH), 7.15–8.03 (m, 12H, Ar—H), 8.42 (d, 1H, —CH). IR (nujol, cm^{-1}): 3053–2837 (Ar C—H), 1683 (C═O). MS, *m/z*: 359 (M + 1).

3-(4-Chlorophenyl)-2-(2-fluorostyryl)quinazolin-4(3H)-one (4b). Yield: (79%), mp 192°C. *Anal*. Calcd for $C_{22}H_{14}ClFN_2O$: C, 70.12; H, 3.74; N, 7.43. Found: C, 70.03; H, 3.65; N, 7.58. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 6.31 (s, 1H, —CH), 7.12–8.03 (m, 12H, Ar—H), 8.42 (d, 1H, —CH). IR (nujol, cm^{-1}): 3052–2832 (Ar C—H), 1682 (C═O). MS, *m/z*: 377 (M + 1).

3-(4-Chlorophenyl)-2-(3-fluorostyryl)quinazolin-4(3H)-one (4c). Yield: (82%), mp 190°C. Anal. Calcd for $C_{22}H_{14}ClFN_2O$: C, 70.12; H, 3.74; N, 7.43. Found: C, 70.05; H, 3.61; N, 7.61. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 6.33 (d, 1H, —CH), 7.35–8.04 (m, 12H, Ar—H), 8.52 (d, 1H, —CH). IR (nujol, cm^{-1}): 3055–2832 (Ar C—H), 1680 (C═O). MS, *m/z*: 377 (M + 1).

3-(4-Chlorophenyl)-2-(4-fluorostyryl)quinazolin-4(3H)-one (4d). Yield: (83%), mp 193°C. *Anal*. Calcd for $C_{22}H_{14}ClFN_2O$: C, 70.12; H, 3.74; N, 7.43. Found: C, 70.01; H, 3.63; N, 7.55. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 6.33 (d, 1H, —CH), 7.17–8.05 (m, 12H, Ar—H), 8.53 (d, 1H, —CH). IR (nujol, cm^{-1}): 3053–2832 (Ar C—H), 1682 (C═O). MS, *m/z*: 377 (M + 1).

3-(4-Chlorophenyl)-2-(2-(trifluoromethyl)styryl)quinazolin-4(3H)-one (4e). Yield: (80%), mp 199°C. *Anal*. Calcd for $C_{23}H_{14}ClF_3N_2O$: C, 64.72; H, 3.31; N, 6.56. Found: C, 64.61; H, 3.29; N, 6.65. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 6.32 (d, 1H, —CH), 7.13–8.04 (m, 12H, Ar—H), 8.41 (d, 1H, —CH). IR (nujol, cm^{-1}): 3052–2830 (Ar C—H), 1682 (C═O). MS, *m/z*: 427 (M + 1).

3-(4-Chlorophenyl)-2-(3-(trifluoromethyl)styryl)quinazolin-4(3H)-one (4f). Yield: (81%), mp 197°C. *Anal*. Calcd for $C_{23}H_{14}ClF_3N_2O$: C, 64.72; H, 3.31; N, 6.56. Found: C, 64.60; H, 3.21; N, 6.73. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 6.41 (d, 1H, —CH), 7.19–8.04 (m, 12H, Ar—H), 8.49 (d, 1H, —CH). IR (nujol, cm^{-1}): 3051–2830 (Ar C—H), 1682 (C═O). MS, *m/z*: 427 (M + 1).

3-(4-Chlorophenyl)-2-(4-(trifluoromethyl)styryl)quinazolin-4(3H)-one (4g). Yield: (83%), mp 200°C. *Anal*. Calcd for $C_{23}H_{14}ClF_3N_2O$: C, 64.72; H, 3.31; N, 6.56. Found: C, 64.63; H, 3.24; N, 6.69. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 6.33 (d, 1H, —CH), 7.11–8.06 (m, 12H, Ar—H), 8.43 (d, 1H, —CH). IR (nujol, cm^{-1}): 3053–2832 (Ar C—H), 1680 (C═O). MS, *m/z*: 427 (M + 1).

Preparation of 3-(4-chlorophenyl)-4-oxo-3,4-dihydroquinazoline-2-carbaldehyde (5). Compound **3** (0.01 mol, 2.85 g) was dissolved in hot dioxane (50 mL), and then powdered selenium dioxide (0.02 mol, 1.2 g) was added portion-wise while stirring. After complete addition, the reaction mixture was refluxed with stirring for 8 h. Then the reaction mixture was filtered off and quenched in ice-cold water. The solid product obtained was filtered and crystallized from benzene to give **5**. Yield: (2.83 g, 74%), mp 264°C. *Anal*. Calcd for $C_{15}H_9ClN_2O_2$: C, 63.28; H, 3.19; N, 9.84. Found: C, 63.16; H, 3.11; N, 9.87. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.41–8.01 (m, 8H, Ar—H), 9.43 (s, 1H, —CHO). IR (nujol, cm^{-1}): 3051–2827 (Ar C—H), 1678 (C═O). MS, *m/z*: 285 (M + 1).

General procedure for the synthesis of 6a–g. To a solution of compound **5** (0.001 mol, 0.285 g) in EtOH was added substituted phenyl amine (0.001 mol) and a few drops of acetic acid was added. The resulting mixture was refluxed 8 h. EtOH was removed under reduced pressure, and the product was recrystallized from absolute ethanol and gave the desired compounds **6a**–**g**.

3-(4-Chlorophenyl)-2-((phenylimino)methyl)quinazolin-4(3H)-one (6a). Yield: (90%), mp 206°C. *Anal*. Calcd for $C_{21}H_{14}ClN_3O$: C, 70.10; H, 3.92; N, 11.68. Found: C, 70.01; H, 3.83; N, 11.79. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.01–8.03 (m, 13H, Ar—H), 8.49 (s, 1H, —CH). IR (nujol, cm^{-1}): 3053–2835 (Ar C—H), 1680 (C═O). MS, *m/z*: 360 (M + 1).

3-(4-Chlorophenyl)-2-(((2-fluorophenyl)imino)methyl) quinazolin-4(3H)-one (6b). Yield: (86%), mp 211°C. *Anal*. Calcd for $C_{21}H_{13}ClFN_3O$: C, 66.76; H, 3.47; N, 11.12. Found: C, 66.61; H, 3.35; N, 11.29. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.01–8.01 (m, 12H, Ar—H), 8.52 (s, 1H, —CH). IR (nujol, cm^{-1}): 3053–2832 (Ar C—H), 1680 (C═O). MS, *m/z*: 378 (M + 1).

3-(4-Chlorophenyl)-2-(((3-fluorophenyl)imino)methyl) quinazolin-4(3H)-one (6c). Yield: (82%), mp 210°C. *Anal*. Calcd for $C_{21}H_{13}ClFN_3O$: C, 66.76; H, 3.47; N, 11.12. Found: C, 66.67; H, 3.41; N, 11.29. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.05–8.03 (m, 12H, Ar—H), 8.50 (s, 1H, —CH). IR (nujol, cm^{-1}): 3052–2837 (Ar C—H), 1683 (C═O). MS, *m/z*: 378 (M + 1).

3-(4-Chlorophenyl)-2-(((4-fluorophenyl)imino)methyl) quinazolin-4(3H)-one (6d). Yield: (91%), mp 214°C. *Anal*. Calcd for $C_{21}H_{13}ClFN_3O$: C, 66.76; H, 3.47; N, 11.12. Found: C, 66.63; H, 3.39; N, 11.25. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.08–7.93 (m, 12H, Ar—H), 8.50 (s, 1H, —CH). IR (nujol, cm^{-1}): 3051–2834 (Ar C—H), 1682 (C═O). MS, *m/z*: 378 (M + 1).

3-(4-Chlorophenyl)-2-(((3-(trifluoromethyl)phenyl)imino) methyl)quinazolin-4(3H)-one (6e). Yield: (83%), mp 218°C. *Anal*. Calcd for $C_{22}H_{13}ClF_3N_3O$: C, 61.77; H, 3.06; N, 9.82. Found: C, 61.63; H, 2.97; N, 9.91. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.01–8.01 (m, 12H, Ar—H), 8.51 (s, 1H, —CH). IR (nujol, cm^{-1}): 3055–2832 (Ar C—H), 1682 (C═O). MS, *m/z*: 428 (M + 1).

3-(4-Chlorophenyl)-2-(((3-(trifluoromethyl)phenyl)imino) methyl)quinazolin-4(3H)-one (6f). Yield: (81%), mp 216°C. *Anal*. Calcd for $C_{22}H_{13}ClF_3N_3O$: C, 61.77; H,

3.06; N, 9.82. Found: C, 61.65; H, 3.01; N, 9.93. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.03–8.01 (m, 12H, Ar—H), 8.52 (s, 1H, —CH). IR (nujol, cm^{-1}): 3052–2837 (Ar C—H), 1683 (C═O). MS, *m/z*: 428 (M + 1).

3-(4-Chlorophenyl)-2-((((4-(trifluoromethyl)phenyl)imino) methyl)quinazolin-4(3H)-one (6g). Yield: (86%), mp 220°C. *Anal*. Calcd for $C_{22}H_{13}ClF_3N_3O$: C, 61.77; H, 3.06; N, 9.82. Found: C, 61.71; H, 3.02; N, 9.97. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.19–8.03 (m, 12H, Ar—H), 8.62 (s, 1H, —CH). IR (nujol, cm^{-1}): 3051–2833 (Ar C—H), 1682 (C═O). MS, *m/z*: 428 (M + 1).

Cyclooxygenase inhibition assay. The colorimetric COX (ovine) inhibitor screening assay kit (kit catalog number 760111, Cayman Chemicals) was utilized to examine the ability of the test compounds and the reference drugs to inhibit the COX-1/COX-2 [19]. The tested compounds were dissolved in dimethyl sulfoxide. The tested compounds were added to both COX-1 and COX-2 with the substrates and buffers to perform the COX reaction according to kit instructions. The mixtures of these solutions were incubated for a period of 10 min at 37°C; after that, the COX reaction was initiated by adding the arachidonic acid solution. PGF2α, produced from PGH_2 by reduction with stannous chloride was measured by enzyme immunoassay. The product of this enzymatic reaction produces a distinct yellow color that absorbs at 410 nm. The intensity of this color, determined spectrophotometrically, is proportional to the amount of PG tracer bound to the well, which is inversely proportional to the amount of free PG present in the well during the incubation. Therefore, the more the inhibition of COX by any of the tested compounds, the less PG produced, and the more absorbance or color developed. The IC_{50} of inhibition of COX-1 and COX-2 was calculated by the comparison of the sample treated incubations to control incubations. Celecoxib was used as reference standard in the study.

Assay for IL-6 inhibition. Proinflammatory cytokine production by lipopolysaccharide in U937 human monocytes was measured according to the method described by Hwang's method [20]. U937 cells were maintained in RPMI-1640 supplemented with 10% fetal bovine serum and 100 mg/mL of penicillin G/streptomycin containing 10% fetal bovine serum at 37°C in a 5% CO_2 atmosphere. The cell viability was measured by the MTT staining assay cells (5×104 cells/cm^2) and was were seeded to the 96-well culture plate. After 24 h, the test compounds in 0.5% dimethyl sulfoxide were added to each well separately, and the plate was incubated at 37°C for 4 h. Finally, lipopolysaccharide was added, at a final concentration of l.0 mg/L in each well. Plates were further incubated at 37°C for 8 h in 5% CO_2. After incubation, supernatants were harvested and assayed for IL-6 by ELISA (Thermo Fischer Scientific) and were used to quantify IL-6 concentrations according to the manufacturer's protocols. Two independent experiments were performed in triplicate, and a representative set of data (means ± SDs) was used to determine the activity of each compound.

Statistical analysis. The results of all the activity were expressed as mean ± SEM. Results were statistically analyzed by using analysis of variance (two-way classification analysis). A probability value of less than 0.05 was considered to be statistically significant.

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SUPPORTING INFORMATION

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