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Selective COX-2 inhibitors. Part 2: Synthesis and biological evaluation of 4-benzylideneamino- and 4-phenyliminomethyl-benzenesulfonamides☆

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Abstract—Two series of 4-benzylideneamino- and 4-phenyliminomethyl-benzenesulfonamide derivatives were designed and synthesized for the evaluation as selective cyclooxygenase-2 (COX-2) inhibitors in a cellular assay using human whole blood (HWB). Extensive structure–activity relationships (SAR) were studied within these series. Several compounds were found to be novel and selective COX-2 inhibitors. Among them, the most potent and selective was 4-(3-carboxy-4-hydroxy-benzylideneamino)benzenesulfonamide (**20**, **LA2135**), (IC_{50}'s for COX-1: 85.13 μM; COX-2: 0.74 μM; SI: 114.5), being more active COX-2 selective than celecoxib.

1. Introduction

Nonsteroidal anti-inflammatory drugs (NSAIDs) are widely used for the treatment of pain, fever, and inflammation. Traditional NSAIDs act as nonselective inhibitors of cyclooxygenase (COX) enzymes, which catalyze the formation of prostaglandins (PGs) from arachidonic acid. COX exists in at least two isoforms.[1,2] COX-2 is induced in response to proinflammatory conditions, while COX-1 is constitutive and responsible for the maintenance of physiological homeostasis. This discovery led to the theory that inhibition of COX-1 causes the side effects of NSAIDs such as gastric ulceration, bleeding, and renal function suppression, whereas inhibition of COX-2 accounts for their therapeutic effects.[3] Normally, prostacyclin (PGI_2) and thromboxane (TxA_2) balance each other's opposing effects. Introducing the selective COX-2 inhibitor may disrupt the balance dangerously toward thromboxane, which can increase the risk of cardiovascular event, coronary artery diseases, heart attack, and stroke in some patients.[4] This could explain the cardiotoxicity caused by rofecoxib (Vioxx®) and valdecoxib (Bextra®), which were recently withdrawn from the market.[5] Thus, there is an urgent need to search for new selective COX-2 inhibitors with a mild therapeutic effect on COX-1 inhibition, which could theoretically reduce the cardiovascular side effects due to their antiplatelet and anti-thrombotic activities, and improve the safety profiles.[6]

The majority of selective COX-2 inhibitors belong to a class of tricyclic sulfone/sulfonamide compounds possessing 1,2-diaryl substitution on a central heterocyclic or carbocyclic ring system.[7,8] (Fig. 1). Recently, a number of naturally occurring *trans*-stilbenoids have been reported as inhibitors of COX. For example, resveratrol (3,4′,5-trihydroxy-*trans*-stilbene) is a phytoalexin present mainly in the skin of grapes and red wine. It has broad spectrum pharmacological activities (antioxidant, neuroprotective, anti-inflammatory, cardioprotective, cancer chemopreventive, etc.)[9,10] and has been shown to exhibit moderate selective COX-1 inhibitory activity.[11,12]

As an alternative to convert a COX-1 selective compound into a COX-2 selective inhibitor, we have de-

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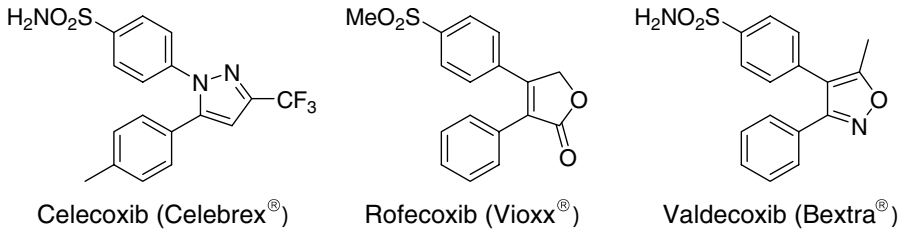


Figure 1. Selective tricyclic COX-2 inhibitors.

signed and modified the basic skeleton of *trans*-stilbene based on the concept of isosterism,[13] which lacks a traditional central heterocyclic or carbocyclic ring template such as celecoxib (Celebrex®).[14] We previously reported that the 4-(4-amino-2-methylsulfanyl-phenylazo)benzenesulfonamide (**38**) demonstrates a moderate COX-2 selectivity.[15] However, it is still unclear if the central double bond linkage within the scaffold optimizes their activity. In order to extend SAR and design more efficacious and selective COX-2 inhibitors with less cardiotoxicity, new benzenesulfonamides have been developed based on the bioisosteric replacement of the central –N=N– linkage with –N=C– or –C=N– double bond. These include the 4-benzylideneamino- and 4-phenyliminomethyl-benzenesulfonamide derivatives (**6**–**26** and **27**–**36**, respectively). We further investigated various substituents on the phenyl B-ring segment in order to obtain compounds with high COX-2 selectivity and potency that we were seeking. These strategies are illustrated in Figure 2.

2. Synthesis

4-Benzylideneaminobenzenesulfonamide derivatives were synthesized by condensation of sulfanilamide with the appropriately substituted benzaldehyde according to the standard procedures[16] (Scheme 1). The usual work-up was followed by recrystallization from ethanol to give the corresponding Schiff bases (**6**–**16** and **21**–**26**, respectively). However, attempts to use this general approach for the coupling of sulfanilamide with 3-hydroxybenzaldehyde, 3,4-dihydroxybenzaldehyde, vanillin, or 5-formylsalicylic acid, respectively, to give the desired compounds (**17**–**20**) failed. Thus, these benzaldehydes were protected with benzyl bromide followed

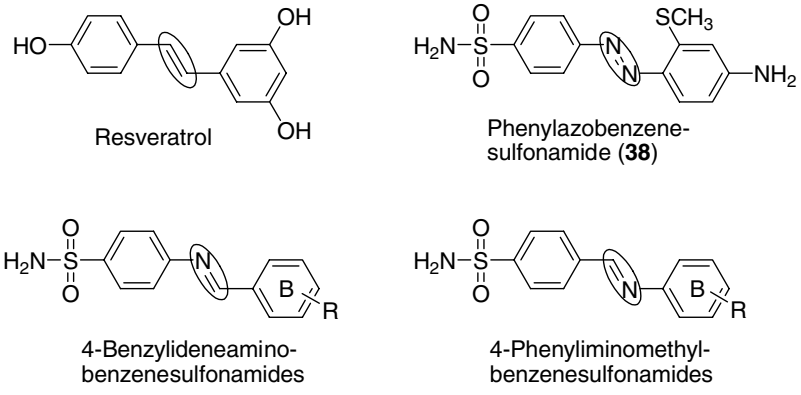
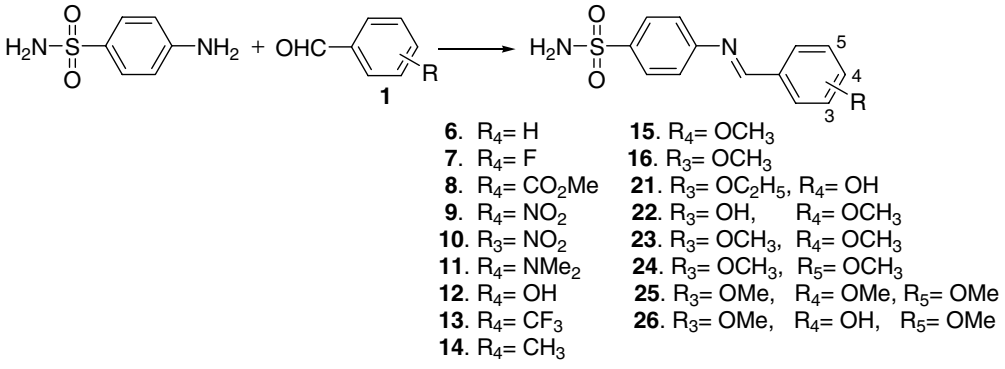
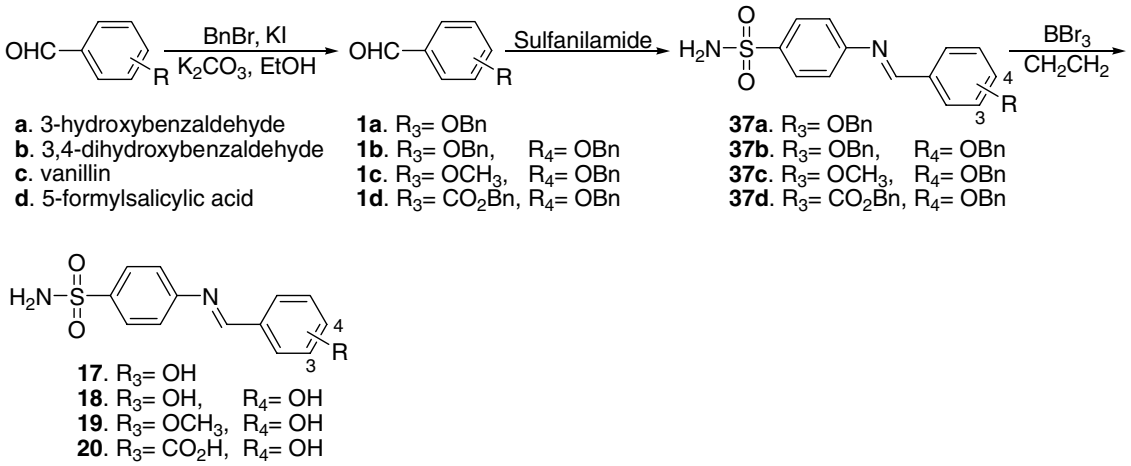


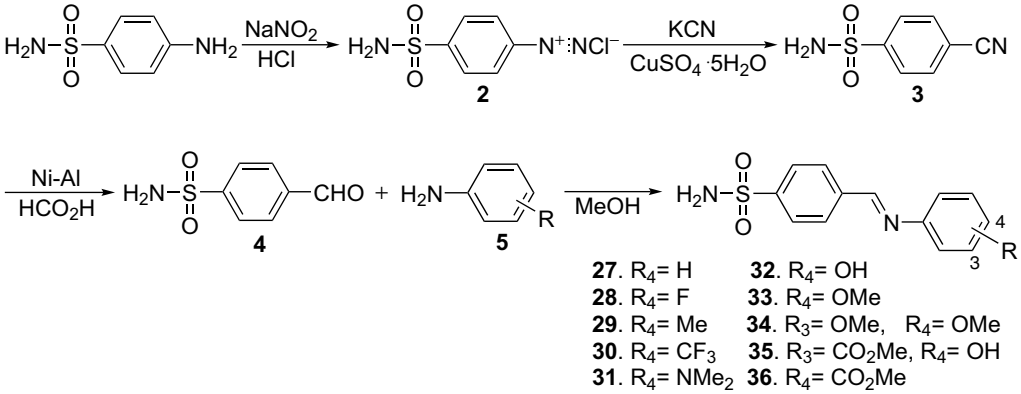
Figure 2. Classic bioisosterism.



Scheme 1. Synthesis of 4-benzylideneaminobenzenesulfonamides **6**–**16** and **21**–**26**.



Scheme 2. Synthesis of 4-benzylideneaminobenezenesulfonamides **17**–**20**.



Scheme 3. Synthesis of 4-phenyliminomethylbenzenesulfonamides **27**–**36**.

by coupling with sulfanilamide to give the protected intermediates (**37a**–**d**). Subsequent acidic cleavage of the benzyl protecting group afforded the target compounds (**17**–**20**) (Scheme 2).

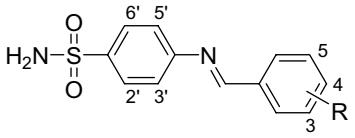
4-Phenyliminomethylbenzenesulfonamide derivatives (**27**–**36**) were synthesized according to synthetic procedures described previously to prepare the key intermediate of 4-formylbenzenesulfonamide **4**[17] as shown in Scheme 3. Following the coupling of aldehyde **4** with the appropriate aniline in methanol yielded the corresponding imines (**27**–**36**). The proposed structures were characterized by detailed ^{1}H, ^{13}C NMR (HMQC, HMBC, COSY), high resolution electron impact (HR-EIMS) analyses, and analytical thin-layer chromatography.

3. Results and discussion

The extensive SAR studies of the 4-benzylideneamino- and 4-phenyliminomethyl-benzenesulfonamides as the selective COX-2 inhibitors have been investigated. The IC_{50} μM values for COX isozymes activity and the selectivity index (SI) calculation of COX-1/COX-2 IC_{50} ratios for different COX inhibitory activities allowed us to evaluate the selectivity of COX inhibitory potency by the human whole blood (HWB) assay.[18] The SAR exploration is summarized in Tables 1 and 2. Resveratrol, NS398, and celecoxib were evaluated as reference compounds in the in vitro assay. COX-1 activity was measured as TxB_2 production after stimulation of platelet aggregation by calcium ionophore A-23,187. COX-2 activity was measured as PGE_2 levels produced by leukocytes after stimulation by LPS.[19]

The SAR of different monosubstituents in the phenyl ring B is shown in Table 1. Different monosubstituents preferably at the 3- or 4-position of the phenyl ring B had the greatest influence on COX-2 selectivity. These studies demonstrated that the 4-hydroxy derivative **12** as the parent compound exhibited moderate COX-2 selectivity (COX-2 IC_{50} = 3.42 μM; SI = 43.4). Compared to compound **12**, replacement of the 4-hydroxy moiety **12** with an electron withdrawing group such as 4-fluoro (**7**), 4-methoxycarbonyl (**8**), or 4-nitro (**9**) substituents increased COX-2 inhibitory potency and selectivity. However, compound **10** with a 3-nitro substituent resulted in loss of COX-2 inhibitory potency. On the other hand, the 4-*N*,*N*-dimethylamino substituent **11** exhibited potent and selective inhibition of COX-2 (COX-2 IC_{50} = 3.36 μM; SI = 58.2). Among these com-

Table 1. Inhibitory activity of 4-benzylideneaminobenzenesulfonamides using human whole blood assay



Compound	R	HWB IC_{50}* (μM)		Selectivity index
		COX-1	COX-2	COX-1/COX-2
6	4-H	108.34	2.87	37.8
7	4-F	159.07	2.22	71.5
8	4-CO_2Me	183.50	2.73	67.3
9	4-NO_2	141.25	3.00	47.1
10	3-NO_2	105.63	6.75	15.6
11	4-NMe_2	195.74	3.36	58.2
12	4-OH	148.20	3.42	43.4
13	4-CF_3	182.19	4.60	39.6
14	4-CH_3	190.47	4.94	38.5
15	4-OCH_3	313.83	9.88	31.8
16	3-OCH_3	383.36	5.45	70.4
17	3-OH	38.20	2.78	13.7
18	3,4-$(OH)_2$	23.15	2.85	8.1
19	3-OCH_3, 4-OH	78.20	2.95	26.5
20 (LA2135)	3-CO_2H, 4-OH	85.13	0.74	114.5
21	3-OC_2H_5, 4-OH	47.91	3.69	13.0
22	3-OH, 4-OCH_3	43.80	3.50	12.5
23	3,4-$(OCH_3)_2$	110.27	3.09	35.6
24	3,5-$(OCH_3)_2$	109.69	3.40	32.3
25	3,4,5-$(OCH_3)_3$	167.09	2.93	57.0
26	3,5-$(OCH_3)_2$, 4-OH	155.95	2.71	57.5
Resveratrol		4.12 ± 1.76	34.61 ± 1.57	0.1
NS398		35.68 ± 5.80	0.61 ± 0.12	59.0
Celecoxib		23.47 ± 3.45	0.30 ± 0.06	78.0

* Values are the means ± SEM from three independent experiments using COX assay kits (Catalog Nos.: 519031 and 514010, Cayman Chemicals Inc., Ann Arbor, MI, USA). Since SEM values never exceeded 15% of the media, they have been omitted.

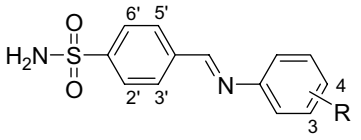
pounds (**7**–**12**) with monosubstituted at the *para*-position, the COX-2 inhibitory selectivity order was 4-F > 4-CO_2Me > 4-NMe_2 > 4-NO_2 > 4-OH > unselective 3-NO_2. In contrast, introduction of a 4-trifluoromethyl (**13**), 4-methyl (**14**), 4-methoxy (**15**), or the unsubstituted analogue **6** exhibited slightly less selectivity than compound **12** as shown in Table 1. Interestingly, substitution by a 3-methoxy (**16**) led to a drop in potency but was quite selective for COX-2 (SI = 70.4). Thus, we investigated the hydroxy derivatives (**12**, **17**, and **18**), except for the 4-hydroxy substituent **12**, exhibiting a marked loss of COX-2 inhibitory selectivity. These results suggest that compounds **17**, **18**, and **22** with an electron donating hydroxyl substituent effect at the 3-position may display a dramatic loss of COX-2 inhibitory selectivity.

Other disubstitution at the 3- and 4-positions of the phenyl ring B, except for compound **20**, did not further enhance COX-2 selectivity compared to the monosubstitution. The 4-hydroxy-3-methoxy substitution **19** showed a potent and moderate COX-2 selectivity (SI = 26.5). Moreover, the substitution of the 3-ethoxy-4-hydroxy (**21**) or 3-hydroxy-4-methoxy (**22**) reduced potency and COX-2 selectivity more significantly. However, the corresponding 4-(3-carboxy-4-hydroxy-benzylideneamino)benzenesulfonamide (**20**, **LA2135**) resulted in a dramatic increase in potency (IC_{50} = 0.74 μM) with an excellent selective COX-2 inhibitory activity (SI = 114.5) comparable to the selectivity of the reference compounds resveratrol (SI = 0.1) and celecoxib (SI = 78). One explanation for this dramatic substituent effect is the electron withdrawing properties of the 3-carboxylic acid moiety attached adjacent to the electron donating group, thus amplifying the conjugative effects of the 4-hydroxy group. The counterbalancing effect of these two groups results in the phenyl ring B having an optimal potency to achieve the desired COX-2 selectivity.

In general, trisubstitution on the phenyl ring B further increased the potency and selectivity of the COX-2 inhibitors. The 3,4,5-trimethoxy derivative **25** and 4-hydroxy-3,5-dimethoxy substituent **26** were more selective against COX-2 (SI values of 57.0 and 57.7, respectively) than the corresponding disubstituted analogues 3,4-dimethoxy substituent **23** and 3,5-dimethoxy substituent **24** (SI values of 35.6 and 32.3, respectively). Again, the more electron donating methoxy substituents on the phenyl ring B made positive contributions to the COX-2 inhibitory selectivity.

To extend the exploration for the different central double bond linkage, the bridge –N=C– was replaced by

Table 2. Inhibitory activity of 4-phenyliminomethylbenzenesulfonamides using human whole blood assay



Compound	R	HWB IC_{50}* (μM)		Selectivity index
		COX-1	COX-2	COX-1/COX-2
27	4-H	63.59	3.11	20.5
28	4-F	80.20	4.38	18.3
29	4-Me	63.22	4.62	13.7
30	4-CF_3	43.89	6.54	6.7
31	4-NMe_2	64.42	1.95	33.0
32	4-OH	51.83	5.09	10.2
33	4-OMe	60.74	4.14	14.7
34	3,4-$(OMe)_2$	31.27	4.28	7.3
35	3-CO_2Me, 4-OH	23.99	3.13	7.7
36	4-CO_2Me	56.73	3.72	15.3
Resveratrol		4.12 ± 1.76	34.61 ± 1.57	0.1
NS398		35.68 ± 5.80	0.61 ± 0.12	59.0
Celecoxib		23.47 ± 3.45	0.30 ± 0.06	78.0

* Values are the means ± SEM from three independent experiments using COX assay kits (Catalog Nos.: 519031 and 514010, Cayman Chemicals Inc., Ann Arbor, MI, USA). Since SEM values never exceeded 15% of the media, they have been omitted.

isosteric –C═N– bond, and the 4-phenyliminomethylbenzenesulfonamide series were synthesized and evaluated for their ability to inhibit COX isozymes in vitro. Bioassay exploration results are shown in Table 2. These series of compounds showed a poor COX-2 inhibitory activity with the lower selectivity indices than the exhibiting of 4-benzylideneaminobenzenesulfonamides, but **31** with a 4-*N,N*-dimethylamino moiety exhibited a moderate COX-2 inhibitory selectivity in this series with an SI value of 33.0.

4. Conclusions

In summary, an isosteric modification and optimization of a central double bond of *trans*-stilbene resveratrol, a cyclooxygenase inhibitor with a moderate activity against COX-1, can yield benzenesulfonamides with altered COX-2 inhibitory potency and selectivity. Based on these results, it is clear that the novel 4-benzylideneaminobenzenesulfonamides (the central –N═C– series) are more potent and selective for COX-2 inhibitory activity than the 4-phenyliminomethylbenzenesulfonamides (the central –C═N– series). Several compounds (**7**, **20**, **25**, and **26**) exhibited good COX-2 inhibitory potency and selectivity. Among the SAR studies, compound **20** (**LA2135**) was proved to be the most potent and selective COX-2 inhibitor (COX-1 IC_{50} = 85.13 μM; COX-2 IC_{50} = 0.74 μM; SI = 114.5) exhibiting the comparable selectivity as the reference compounds celecoxib, NS398, and resveratrol in this series. Mechanisms of the COX-2 inhibitory selectivity remain to be elucidated, but compound **20** (**LA2135**) can be considered as a lead compound in the design of a new class of potent drugs. Further optimization based on **20** will be described in the near future.

5. Experimental

5.1. General

All commercial chemicals were used as obtained without further purification. Melting points were determined with a Büchi capillary melting point apparatus and were uncorrected. NMR spectra were recorded on Varian Unity Inova-500 spectrometer. The samples were dissolved in DMSO-d_6 and measured in 5 mm NMR tubes. Chemical shifts values (δ) are expressed in ppm referred to TMS and coupling constants (J) in hertz. The EIMS spectra were measured with direct insertion probe on a Finnigan GCQ spectrometer at 30 eV. HR-EIMS spectral data were recorded on a Finnigan MAT 95S mass spectrometer. Thin-layer chromatography was performed on Merck Kieselgel 60 F_{254} precoated aluminum silica gel sheets. Flash column chromatography was carried out on Merck Kieselgel 60 (230–400 mesh).

5.2. General procedure for the synthesis of 4-benzylideneaminobenzenesulfonamides

A mixture of sulfanilamide and the appropriate benzaldehyde (1.15 equiv) was stirred in oil bath for 5 min at 150 °C and then cooled to room temperature. The crude crystalline obtained was filtered and purified by recrystallization from ethanol to afford the corresponding Schiff bases. Physical and spectral data for **6**–**16** and **21**–**26** are listed below.

5.2.1. 4-Benzylideneamino-benzenesulfonamide (6). Compound **6** was synthesized using benzaldehyde (yield 78%); mp 188–190 °C; ^{1}H NMR: δ 7.34 (s, –NH_2), 7.38 (d, J = 8.5 Hz, H-3′, H-5′), 7.52–7.57 (m, H-3,4,5), 7.84 (d, J = 8.5 Hz, H-2′, H-6′), 7.95 (d, J = 8.0 Hz, H-2, H-6), 8.63 (s, –NCH). ^{13}C NMR: δ

121.3 (C-3′, C-5′), 127.0 (C-2′, C-6′), 128.9 (C-3, C-5), 129.0 (C-2, C-6), 132.1 (C-4), 135.6 (C-1), 141.2 (C-1′), 154.4 (C-4′), 162.8 (–N*C*H). HR-MS *m*/*z* calcd for $C_{13}H_{12}N_2O_2S$ 260.0619, found 260.0622.

5.2.2. 4-(4-Fluoro-benzylideneamino)-benzenesulfonamide (7). Compound **7** was synthesized using 4-fluorobenzaldehyde (yield 80%); mp 145–147 °C; ^{1}H NMR: δ 7.34 (s, –NH_2), 7.37 (t, J = 9.0 Hz, H-3, H-5), 7.38 (d, J = 8.5 Hz, H-3′, H-5′), 7.84 (d, J = 8.5 Hz, H-2′, H-6′), 8.01 (dd, J = 6.0, 9.0 Hz, H-2, H-6), 8.63 (s, –NCH). ^{13}C NMR: δ 116.1 (d, J = 21.9 Hz, C-3, C-5), 121.3 (C-3′, C-5′), 127.0 (C-2′, C-6′), 131.5 (d, J = 9.0 Hz, C-2, C-6), 132.4 (d, J = 2.9 Hz, C-1), 141.2 (C-1′), 154.3 (C-4′), 161.6 (–N*C*H), 164.4 (d, J = 248.9 Hz, C-4). HR-MS *m*/*z* calcd for $C_{13}H_{11}FN_2O_2S$ 278.0525, found 278.0525.

5.2.3. 4-(4-Methoxycarbonyl-benzylideneamino)-benzenesulfonamide (8). Compound **8** was synthesized using methyl 4-formylbenzoate (yield 84%); mp 202–204 °C; ^{1}H NMR: δ 3.87 (s, –OCH_3), 7.36 (s, –NH_2), 7.43 (d, J = 8.5 Hz, H-3′, H-5′), 7.86 (d, J = 8.5 Hz, H-2′, H-6′), 8.08 (d, J = 8.5 Hz, H-2, H-6), 8.11 (d, J = 8.5 Hz, H-3, H-5), 8.74 (s, –NCH). ^{13}C NMR: δ 52.4 (–O*C*H$_3$), 121.4 (C-3′, C-5′), 127.0 (C-2′, C-6′), 129.1 (C-2, C-6), 129.7 (C-3, C-5), 132.1 (C-4), 139.5 (C-1), 141.6 (C-1′), 153.9 (C-4′), 162.0 (–N*C*H), 165.8 (C═O). HR-MS *m*/*z* calcd for $C_{15}H_{14}N_2O_4S$ 318.0674, found 318.0674.

5.2.4. 4-(4-Nitro-benzylideneamino)-benzenesulfonamide (9). Compound **9** was synthesized using 4-nitrobenzaldehyde (yield 30%); mp 185–188 °C; ^{1}H NMR: δ 7.38 (s, –NH_2), 7.47 (d, J = 8.5 Hz, H-3′, H-5′), 7.88 (d, J = 8.5 Hz, H-2′, H-6′), 8.21 (d, J = 8.5 Hz, H-2, H-6), 8.38 (d, J = 8.5 Hz, H-3, H-5), 8.82 (s, –NCH). ^{13}C NMR: δ 121.5 (C-3′, C-5′), 124.1 (C-3, C-5), 127.0 (C-2′, C-6′), 130.0 (C-2, C-6), 141.0 (C-1), 142.0 (C-1′), 149.2 (C-4), 153.5 (C-4′), 161.2 (–N*C*H). HR-MS *m*/*z* calcd for $C_{13}H_{11}N_3O_4S$ 305.0470, found 305.0471.

5.2.5. 4-(3-Nitro-benzylideneamino)-benzenesulfonamide (10). Compound **10** was synthesized using 3-nitrobenzaldehyde (yield 23%); mp 169–171 °C; ^{1}H NMR: δ 7.37 (s, –NH_2), 7.46 (d, J = 8.5 Hz, H-3′, H-5′), 7.84 (t, J = 8.0 Hz, H-5), 7.87 (d, J = 8.5 Hz, H-2′, H-6′), 8.38 (d, J = 8.0 Hz, H-6), 8.41 (m, H-4), 8.75 (d, J = 1.7 Hz, H-2), 8.83 (s, –NCH). ^{13}C NMR: δ 121.5 (C-3′, C-5′), 123.1 (C-2), 126.2 (C-4), 127.0 (C-2′, C-6′), 130.7 (C-5), 134.8 (C-6), 137.1 (C-1), 141.8 (C-1′), 148.2 (C-3), 153.5 (C-4′), 161.1 (–N*C*H). HR-MS *m*/*z* calcd for $C_{13}H_{11}N_3O_4S$ 305.0470, found 305.0473.

5.2.6. 4-(4-Dimethylamino-benzylideneamino)-benzenesulfonamide (11). Compound **11** was synthesized using 4-(dimethylamino)benzaldehyde (yield 65%); mp 212–214 °C; ^{1}H NMR: δ 3.01 (s, –$N(CH_3)_2$), 6.79 (d, J = 8.5 Hz, H-3, H-5), 7.28 (s, –NH_2), 7.30 (d, J = 8.5 Hz, H-3′, H-5′), 7.75 (d, J = 8.5 Hz, H-2, H-6), 7.80 (d, J = 8.5 Hz, H-2′, H-6′), 8.41 (s, –NCH). ^{13}C NMR: δ 39.8 (–N(*C*H$_3$)$_2$), 111.4 (C-3, C-5), 121.1 (C-3′, C-5′), 123.3 (C-1), 126.9 (C-2′, C-6′), 130.7 (C-2, C-6), 140.1 (C-1′), 152.7 (C-4), 155.3 (C-4′), 161.7 (–N*C*H). HR-MS *m*/*z* calcd for $C_{15}H_{17}N_3O_2S$ 303.1042, found 303.1041.

5.2.7. 4-(4-Hydroxy-benzylideneamino)-benzenesulfonamide (12). Compound **12** was synthesized using 4-hydroxybenzaldehyde (yield 40%); mp 208–210 °C; ^{1}H NMR: δ 6.89 (d, J = 8.5 Hz, H-3, H-5), 7.31 (s, –NH_2), 7.32 (d, J = 8.5 Hz, H-3′, H-5′), 7.79 (d, J = 8.5 Hz, H-2, H-6), 7.81 (d, J = 8.5 Hz, H-2′, H-6′), 8.47 (s, –NCH). ^{13}C NMR: δ 115.8 (C-3, C-5), 121.1 (C-3′, C-5′), 126.9 (C-2′, C-6′), 127.1 (C-1), 131.1 (C-2, C-6), 140.6 (C-1′), 154.9 (C-4′), 161.1 (C-4), 161.9 (–N*C*H). HR-MS *m*/*z* calcd for $C_{13}H_{12}N_2O_3S$ 276.0569, found 276.0571.

5.2.8. 4-(4-Trifluoromethyl-benzylideneamino)-benzenesulfonamide (13). Compound **13** was synthesized using 4-(trifluoromethyl)benzaldehyde (yield 67%); mp 188–191 °C; ^{1}H NMR: δ 7.37 (s, –NH_2), 7.44 (d, J = 8.5 Hz, H-3′, H-5′), 7.87 (d, J = 8.5 Hz, H-2′, H-6′), 7.91 (d, J = 8.5 Hz, H-3, H-5), 8.16 (d, J = 8.5 Hz, H-2, H-6), 8.76 (s, –NCH). ^{13}C NMR: δ 121.4 (C-3′, C-5′), 124.0 (q, J = 270.8 Hz, *C*F$_3$), 125.9 (d, J = 4.0 Hz, C-3, C-5), 127.0 (C-2′, C-6′), 129.6 (C-2, C-6), 131.4 (q, J = 31.3 Hz, C-4), 139.2 (C-1), 141.7 (C-1′), 153.8 (C-4′), 161.7 (–N*C*H). HR-MS *m*/*z* calcd for $C_{14}H_{11}F_3N_2O_2S$ 328.0493, found 328.0494.

5.2.9. 4-(4-Methyl-benzylideneamino)-benzenesulfonamide (14). Compound **14** was synthesized using *p*-tolualdehyde (yield 55%); mp 198–200 °C; ^{1}H NMR: δ 2.38 (s, –CH_3), 7.33 (s, –NH_2), 7.35 (d, J = 8.0 Hz, H-3, H-5), 7.37 (d, J = 8.5 Hz, H-3′, H-5′), 7.83 (d, J = 8.5 Hz, H-2′, H-6′), 7.84 (d, J = 8.0 Hz, H-2, H-6), 8.58 (s, –NCH). ^{13}C NMR: δ 21.3 (–*C*H$_3$), 121.3 (C-3′, C-5′), 127.0 (C-2′, C-6′), 129.1 (C-2, C-6), 129.6 (C-3, C-5), 133.1 (C-1), 141.0 (C-1′), 142.3 (C-4), 154.6 (C-4′), 162.6 (–N*C*H). HR-MS *m*/*z* calcd for $C_{14}H_{14}N_2O_2S$ 274.0776, found 274.0777.

5.2.10. 4-(4-Methoxy-benzylideneamino)-benzenesulfonamide (15). Compound **15** was synthesized using *p*-anisaldehyde (yield 70%); mp 195–197 °C; ^{1}H NMR: δ 3.83 (s, –OCH_3), 7.08 (d, J = 8.5 Hz, H-3, H-5), 7.32 (s, –NH_2), 7.34 (d, J = 8.5 Hz, H-3′, H-5′), 7.82 (d, J = 8.5 Hz, H-2′, H-6′), 7.90 (d, J = 8.5 Hz, H-2, H-6), 8.54 (s, –NCH). ^{13}C NMR: δ 55.5 (–O*C*H$_3$), 114.4 (C-3, C-5), 121.2 (C-3′, C-5′), 127.0 (C-2′, C-6′), 128.6 (C-1), 130.9 (C-2, C-6), 140.8 (C-1′), 154.8 (C-4′), 161.9 (–N*C*H), 162.4 (C-4). HR-MS *m*/*z* calcd for $C_{14}H_{14}N_2O_3S$ 290.0725, found 290.0728.

5.2.11. 4-(3-Methoxy-benzylideneamino)-benzenesulfonamide (16). Compound **16** was synthesized using *m*-anisaldehyde (yield 32%); mp 145–148 °C; ^{1}H NMR: δ 3.82 (s, –OCH_3), 7.13–7.15 (m, H-4), 7.35 (s, –NH_2), 7.38 (d, J = 8.5 Hz, H-3′, H-5′), 7.45 (t, J = 8.0 Hz, H-5), 7.51 (d, J = 1.5 Hz, H-2), 7.53 (m, H-6), 7.84 (d, J = 8.5 Hz, H-2′, H-6′), 8.60 (s, –NCH). ^{13}C NMR: δ 55.3 (–O*C*H$_3$), 112.8 (C-2), 118.3 (C-4), 121.3 (C-3′, C-5′), 122.0 (C-6), 127.0 (C-2′, C-6′), 130.1 (C-5), 137.1 (C-1), 141.2 (C-1′), 154.3 (C-4′), 159.6 (C-3), 162.7 (–N*C*H). HR-

MS *m/z* calcd for $C_{14}H_{14}N_2O_3S$ 290.0725, found 290.0725.

5.2.12. 4-(3-Ethoxy-4-hydroxy-benzylideneamino)-benzenesulfonamide (21). Compound **21** was synthesized using 3-ethoxy-4-hydroxybenzaldehyde (yield 15%); mp 173–175 °C; ^{1}H NMR: δ 1.36 (t, J = 7.0 Hz, –CH_3), 4.08 (q, J = 7.0 Hz, –OCH_2), 6.91 (d, J = 8.0 Hz, H-5), 7.31 (s, –NH_2), 7.32 (d, J = 8.5 Hz, H-3′, H-5′), 7.35 (dd, J = 1.5, 8.0 Hz, H-6), 7.50 (d, J = 1.5 Hz, H-2), 7.81 (d, J = 8.5 Hz, H-2′, H-6′), 8.44 (s, –NCH), 9.78 (br, –OH). ^{13}C NMR: δ 14.7 (–CH_3), 63.9 (–OCH_2), 111.9 (C-2), 115.5 (C-5), 121.2 (C-3′, C-5′), 124.5 (C-6); 126.9 (C-2′, C-6′), 127.5 (C-1), 140.6 (C-1′), 147.2 (C-3), 151.0 (C-4), 154.9 (C-4′), 162.1 (–N*C*H). HR-MS *m/z* calcd for $C_{15}H_{16}N_2O_4S$ 320.0830, found 320.0831.

5.2.13. 4-(3-Hydroxy-4-methoxy-benzylideneamino)-benzenesulfonamide (22). Compound **22** was synthesized using 3-hydroxy-4-methoxybenzaldehyde (yield 40%); mp 215–218 °C; ^{1}H NMR: δ 3.83 (s, –OCH_3), 7.05 (d, J = 8.5 Hz, H-5), 7.31 (s, –NH_2), 7.32 (d, J = 8.5 Hz, H-3′, H-5′), 7.34 (dd, J = 2.0, 8.5 Hz, H-6), 7.42 (d, J = 2.0 Hz, H-2), 7.81 (d, J = 8.5 Hz, H-2′, H-6′), 8.44 (s, –NCH), 9.39 (br, –OH). ^{13}C NMR: δ 55.7 (–O*C*H$_3$), 111.7 (C-5), 113.7 (C-2), 121.2 (C-3′, C-5′), 122.9 (C-6), 126.9 (C-2′, C-6′), 128.8 (C-1), 140.7 (C-1′), 146.8 (C-3), 151.4 (C-4), 154.7 (C-4′), 162.1 (–N*C*H). HR-MS *m/z* calcd for $C_{14}H_{14}N_2O_4S$ 306.0674, found 306.0668.

5.2.14. 4-(3,4-Dimethoxy-benzylideneamino)-benzenesulfonamide (23). Compound **23** was synthesized using veratraldehyde (yield 46%); mp 187–189 °C; ^{1}H NMR: δ 3.83 (s, –OCH_3), 3.84 (s, –OCH_3), 7.11 (d, J = 8.5 Hz, H-5), 7.32 (s, –NH_2), 7.35 (d, J = 8.5 Hz, H-3′, H-5′), 7.47 (dd, J = 2.5, 8.5 Hz, H-6), 7.55 (d, J = 2.5 Hz, H-2), 7.82 (d, J = 8.5 Hz, H-2′, H-6′), 8.52 (s, –NCH). ^{13}C NMR: δ 55.5, 55.7 (3- & 5-O*C*H$_3$), 109.5 (C-2), 111.3 (C-5), 121.2 (C-3′, C-5′), 124.7 (C-6), 126.9 (C-2′, C-6′), 128.6 (C-1), 140.8 (C-1′), 149.1 (C-3), 152.3 (C-4), 154.7 (C-4′), 162.1 (–N*C*H). HR-MS *m/z* calcd for $C_{15}H_{16}N_2O_4S$ 320.0831, found 320.0832.

5.2.15. 4-(3,5-Dimethoxy-benzylideneamino)-benzenesulfonamide (24). Compound **24** was synthesized using 3,5-dimethoxybenzaldehyde (yield 58%); mp 197–199 °C; ^{1}H NMR: δ 3.80 (s, –$OCH_3 \times 2$), 6.69 (t, J = 2.5 Hz, H-4), 7.12 (d, J = 2.5 Hz, H-2, H-6), 7.35 (s, –NH_2), 7.37 (d, J = 8.5 Hz, H-3′, H-5′), 7.84 (d, J = 8.5 Hz, H-2′, H-6′), 8.55 (s, –NCH). ^{13}C NMR: δ 55.5 (3- & 5-O*C*H$_3$), 104.3 (C-4), 106.6 (C-2, C-6), 121.3 (C-3′, C-5′), 127.0 (C-2′, C-6′), 137.6 (C-1), 141.3 (C-1′), 154.2 (C-4′), 160.7 (C-3, C-5), 162.7 (–N*C*H). HR-MS *m/z* calcd for $C_{15}H_{16}N_2O_4S$ 320.0830, found 320.0831.

5.2.16. 4-(3,4,5-Trimethoxy-benzylideneamino)-benzenesulfonamide (25). Compound **25** was synthesized using 3,4,5-trimethoxybenzaldehyde (yield 85%); mp 227–230 °C; ^{1}H NMR: δ 3.74 (s, –OCH_3), 3.85 (s, –$OCH_3 \times 2$), 7.29 (s, –NH_2), 7.34 (s, H-2, H-6), 7.36 (d, J = 8.5 Hz, H-3′, H-5′), 7.84 (d, J = 8.5 Hz, H-2′, H-6′), 8.54 (s, –NCH). ^{13}C NMR: δ 56.0 (3- & 5-O*C*H$_3$), 60.2 (4-O*C*H$_3$), 106.2 (C-2, C-6), 121.2 (C-3′, C-5′), 127.0 (C-2′, C-6′), 131.1 (C-1), 140.8 (C-4), 141.1 (C-1′), 153.2 (C-3, C-5), 154.4 (C-4′), 162.3 (–N*C*H). HR-MS *m/z* calcd for $C_{16}H_{18}N_2O_5S$ 350.0936, found 350.0935.

5.2.17. 4-(4-Hydroxy-3,5-dimethoxy-benzylideneamino)-benzenesulfonamide (26). Compound **26** was synthesized using syringaldehyde (yield 52%); mp 228–231 °C; ^{1}H NMR: δ 3.83 (s, –$OCH_3 \times 2$), 7.25 (s, –NH_2), 7.32 (s, H-2, H-6), 7.33 (d, J = 8.5 Hz, H-3′, H-5′), 7.82 (d, J = 8.5 Hz, H-2′, H-6′), 8.46 (s, –NCH), 9.22 (br, –OH). ^{13}C NMR: δ 56.0 (3- & 5-O*C*H$_3$), 106.6 (C-2, C-6), 121.1 (C-3′, C-5′), 126.1 (C-1), 127.0 (C-2′, C-6′), 139.7 (C-4), 140.6 (C-1′), 148.1 (C-3, C-5), 154.8 (C-4′), 162.3 (–N*C*H). HR-MS *m/z* calcd for $C_{15}H_{16}N_2O_5S$ 336.0779, found 336.0778.

5.3. General procedure for the reaction of sulfanilamide with benzyl-protected benzaldehydes

The hydroxy groups of benzaldehyde (3-hydroxybenzaldehyde, 3,4-dihydroxybenzaldehyde, vanillin, or 5-formylsalicylic acid, respectively) were protected with benzyl bromide to give benzyl ether or ester derivatives **1a**–**d**, followed by coupling with sulfanilamide to give the protected intermediates (**37a**–**d**). Subsequent BBr_3/CH_2Cl_2 cleavage of the protecting group afforded the target compounds. Physical and spectral data for **17**–**20** are listed below.

5.3.1. 4-(3-Hydroxy-benzylideneamino)-benzenesulfonamide (17). Compound **17** was synthesized using the benzyl-protected 3-hydroxybenzaldehyde (yield 47%); mp 206–208 °C; ^{1}H NMR: δ 6.80 (d, J = 8.5 Hz, H-3′, H-5′), 7.08–7.11 (m, H-4), 7.13 (s, –NH_2), 7.23 (d, J = 1.5 Hz, H-2), 7.34 (m, H-6), 7.40 (t, J = 8.0 Hz, H-5), 7.54 (d, J = 8.5 Hz, H-2′, H-6′), 9.89 (s, –NCH). ^{13}C NMR: δ 114.7 (C-2), 115.2 (C-3′, C-5′), 121.2 (C-6), 121.9 (C-4), 127.5 (C-2′, C-6′), 130.4 (C-5), 133.2 (C-1′), 137.7 (C-1), 148.1 (C-4′), 158.1 (C-3), 193.3 (–N*C*H). HR-MS *m/z* calcd for $C_{13}H_{12}N_2O_3S$ 276.0568, found 276.0567.

5.3.2. 4-(3,4-Dihydroxy-benzylideneamino)-benzenesulfonamide (18). Compound **18** was synthesized using the benzyl-protected 3,4-dihydroxybenzaldehyde (yield 88%); mp 242–244 °C; ^{1}H NMR: δ 6.80 (d, J = 8.5 Hz, H-3′, H-5′), 6.89 (d, J = 8.0 Hz, H-5), 7.22 (d, J = 2.0 Hz, H-2), 7.47 (dd, J = 2.0, 8.0 Hz, H-6), 7.55 (d, J = 8.5 Hz, H-2′, H-6′), 9.68 (s, –NCH). ^{13}C NMR: δ 114.5 (C-2), 115.2 (C-3′, C-5′), 115.6 (C-5), 124.6 (C-6), 127.5 (C-2′, C-6′), 128.9 (C-1), 146.0 (C-1′), 149.1 (C-3), 148.0 (C-4′), 152.2 (C-4), 191.2 (–N*C*H). HR-MS *m/z* calcd for $C_{13}H_{12}N_2O_4S$ 292.0518, found 292.0518.

5.3.3. 4-(4-Hydroxy-3-methoxy-benzylideneamino)-benzenesulfonamide (19). Compound **19** was synthesized using the benzyl-protected vanillin (yield 84%); mp 212–214 °C; ^{1}H NMR: δ 3.82 (s, –OCH_3), 6.80 (d,

J = 8.5 Hz, H-3′, H-5′), 6.96 (d, J = 8.0 Hz, H-5), 7.37 (d, J = 2.0 Hz, H-2), 7.40 (dd, J = 2.0, 8.0 Hz, H-6), 7.55 (d, J = 8.5 Hz, H-2′, H-6′), 9.75 (s, –NCH). ^{13}C NMR: δ 55.6 (–O*C*H$_3$), 110.8 (C-2), 115.2 (C-3′, C-5′), 115.5 (C-5), 126.1 (C-6), 127.4 (C-2′, C-6′), 128.7 (C-1), 133.3 (C-1′), 148.0 (C-4′), 148.2 (C-3), 153.1 (C-4), 191.1 (–N*C*H). HR-MS *m*/*z* calcd for $C_{14}H_{14}N_2O_4S$ 306.0674, found 306.0674.

5.3.4. 4-(3-Carboxy-4-hydroxy-benzylideneamino)-benzenesulfonamide (20). Compound **20** was synthesized using the benzyl-protected 5-formylsalicylic acid (yield 73%); mp 213–215 °C; ^{1}H NMR: δ 6.88 (d, J = 8.5 Hz, H-3′, H-5′), 7.13 (d, J = 8.5 Hz, H-5), 7.58 (d, J = 8.5 Hz, H-2′, H-6′), 8.45 (dd, J = 2.0, 8.5 Hz, H-6), 8.34 (d, J = 2.0 Hz, H-2), 9.87 (s, –NCH). ^{13}C NMR: δ 113.8 (C-3), 116.1 (C-3′, C-5′), 118.3 (C-5), 127.4 (C-2′, C-6′), 128.4 (C-1), 133.9 (C-2), 134.3 (C-1′), 135.2 (C-6), 146.8 (C-4′), 165.7 (C-4), 171.1 (C═O), 191.1 (–N*C*H). HR-MS *m*/*z* calcd for $C_{14}H_{12}N_2O_5S$ 320.0467, found 320.0467.

5.4. 4-Cyanobenzenesulfonamide (3)

A cold solution of $NaNO_2$ (11.7 g, 0.17 mol) was added to a suspension of sulfanilamide (25 g, 0.15 mol) in aqueous HCl (2.3 N, 200 mL) at 0 °C with stirring for 10 min. The diazotized solution was poured with vigorous stirring into a suspension of KCN (41.6 g, 0.64 mol) and $CuSO_4{\cdot}5H_2O$ (38.5 g, 0.15 mol) in 200 mL of water. The mixture was stirred at 0 °C for 30 min and was then heated to 80 °C for 30 min and then chilled. The brown solid precipitate was collected, dried, and successively extracted with 5% ethanol in benzene. The combined organic extracts were concentrated and recrystallized from water to afford benzonitrile **3** (17.39 g, 66%); mp 155–157 °C; ^{1}H NMR: δ 7.63 (s, –NH$_2$), 7.97 (d, J = 8.5 Hz, H-2′, H-6′), 8.05 (d, J = 8.5 Hz, H-3′, H-5′). ^{13}C NMR: δ 114.3 (C-4′), 117.9 (–*C*N), 126.5 (C-2′, C-6′), 133.3 (C-3′, C-5′), 148.0 (C-1′).

5.5. 4-Formylbenzenesulfonamide (4)

To a solution of 4-cyanobenzenesulfonamide **3** (5.85 g, 32.15 mmol) in formic acid (75%, 84 mL) was added Raney nickel (6.2 g). This stirred mixture was refluxed for 1 h and then filtered through Celite. The combined filtrate was concentrated to afford benzaldehyde **4** (5.1 g, 86%); mp 105–107 °C; ^{1}H NMR: δ 7.55 (s, –NH$_2$), 8.02 (d, J = 8.5 Hz, H-2′, H-6′), 8.08 (d, J = 8.5 Hz, H-3′, H-5′), 10.1 (–CHO). ^{13}C NMR: δ 126.3 (C-2′, C-6′), 130.0 (C-3′, C-5′), 138.0 (C-4′), 148.7 (C-1′), 192.4 (C═O).

5.6. General procedure for the synthesis of 4-phenyliminomethylbenzenesulfonamides

A mixture of 4-formylbenzenesulfonamide **4** and the appropriate aniline (1.05 equiv) in methanol (2 mL) was stirred in oil bath for 5 min at 150 °C, and then allowed to cool to room temperature. The crude product was purified by recrystallization from isopropanol and ether to give the corresponding imines. Physical and spectral data for **27**–**36** are listed below.

5.6.1. 4-Phenyliminomethyl-benzenesulfonamide (27). Compound **27** was synthesized using aniline (yield 43%); mp 201–203 °C; ^{1}H NMR: δ 7.27–7.32 (m, H-2,4,6), 7.44 (t, J = 8.0 Hz, H-3, H-5), 7.49 (s, –NH$_2$), 7.95 (d, J = 8.0 Hz, H-2′, H-6′), 8.10 (d, J = 8.0 Hz, H-3′, H-5′), 8.71 (s, –NCH). ^{13}C NMR: δ 121.1 (C-2, C-6), 126.1 (C-2′, C-6′), 126.5 (C-4), 129.0 (C-3′, C-5′), 129.3 (C-3, C-5), 138.7 (C-4′), 146.1 (C-1′), 150.1 (C-1), 159.4 (–N*C*H). HR-MS *m*/*z* calcd for $C_{13}H_{12}N_2O_2S$ 260.0619, found 260.0621.

5.6.2. 4-(4-Fluoro-phenyliminomethyl)-benzenesulfonamide (28). Compound **28** was synthesized using 4-fluoroaniline (yield 72%); mp 179–181 °C; ^{1}H NMR: δ 7.25–7.29 (m, H-3, H-5), 7.38–7.40 (m, H-2, H-6), 7.46 (s, –NH$_2$), 7.94 (d, J = 8.5 Hz, H-2′, H-6′), 8.09 (d, J = 8.5 Hz, H-3′, H-5′), 8.73 (s, –NCH). ^{13}C NMR: δ 116.0 (d, J = 22.4 Hz, C-3, C-5), 123.1 (d, J = 8.0 Hz, C-2, C-6), 126.1 (C-2′, C-6′), 129.0 (C-3′, C-5′), 138.6 (C-4′), 146.2 (C-1′), 147.1 (C-1), 159.4 (–N*C*H), 160.8 (d, J = 242.0 Hz, C-4). HR-MS *m*/*z* calcd for $C_{13}H_{11}FN_2O_2S$ 278.0525, found 278.0525.

5.6.3. 4-(4-Methyl-phenyliminomethyl)-benzenesulfonamide (29). Compound **29** was synthesized using *p*-toluidine (yield 66%); mp 206–208 °C; ^{1}H NMR: δ 2.33 (s, –CH$_3$), 7.24 (s, H-2, H-3, H-5, H-6), 7.47 (s, –NH$_2$), 7.93 (d, J = 8.5 Hz, H-2′, H-6′), 8.09 (d, J = 8.5 Hz, H-3′, H-5′), 8.72 (s, –NCH). ^{13}C NMR: δ 20.6 (-*C*H$_3$), 121.1 (C-2, C-6), 126.1 (C-2′, C-6′), 128.9 (C-3′, C-5′), 129.7 (C-3, C-5), 136.1 (C-4), 138.9 (C-4′), 146.0 (C-1′), 148.2 (C-1), 158.3 (–N*C*H). HR-MS *m*/*z* calcd for $C_{14}H_{14}N_2O_2S$ 274.0776, found 274.0775.

5.6.4. 4-(4-Trifluoromethyl-phenyliminomethyl)-benzenesulfonamide (30). Compound **30** was synthesized using 4-(trifluoromethyl)aniline (yield 63%); mp 165–167 °C; ^{1}H NMR: δ 7.46 (d, J = 8.5 Hz, H-2, H-6), 7.51 (s, –NH$_2$), 7.79 (d, J = 8.5 Hz, H-3, H-5), 7.97 (d, J = 8.5 Hz, H-2′, H-6′), 8.13 (d, J = 8.5 Hz, H-3′, H-5′), 8.74 (s, –NCH). ^{13}C NMR: δ 121.7 (C-2, C-6), 125.4 (q, J = 270.0 Hz, *C*F$_3$), 126.2 (C-2′, C-6′), 126.4 (d, J = 3.3 Hz, C-3, C-5), 131.4 (q, J = 31.3 Hz, C-4), 129.4 (C-3′, C-5′), 138.2 (C-4′), 146.6 (C-1′), 154.5 (C-1), 162.0 (–N*C*H). HR-MS *m*/*z* calcd for $C_{14}H_{11}F_3N_2O_2S$ 328.0493, found 328.0494.

5.6.5. 4-(4-Dimethylamino-phenyliminomethyl)-benzenesulfonamide (31). Compound **31** was synthesized using 4-(dimethylamino)aniline (yield 59%); mp 246–248 °C; ^{1}H NMR: δ 2.94 (s, –N(CH$_3$)$_2$), 6.76 (d, J = 8.5 Hz, H-3, H-5), 7.33 (d, J = 8.5 Hz, H-2, H-6), 7.43 (s, –NH$_2$), 7.90 (d, J = 8.5 Hz, H-2′, H-6′), 8.04 (d, J = 8.5 Hz, H-3′, H-5′), 8.73 (s, –NCH). ^{13}C NMR: δ 40.1 (–N(*C*H$_3$)$_2$), 112.4 (C-3, C-5), 122.7 (C-2, C-6), 126.0 (C-2′, C-6′), 128.2 (C-3′, C-5′), 138.9 (C-1), 139.6 (C-4′), 145.1 (C-1′), 149.8 (C-4), 153.2 (–N*C*H). HR-MS *m*/*z* calcd for $C_{15}H_{17}N_3O_2S$ 303.1042, found 303.1043.

5.6.6. 4-(4-Hydroxy-phenyliminomethyl)-benzenesulfonamide (32). Compound **32** was synthesized using 4-aminophenol (yield 75%); mp 242–244 °C; ^{1}H NMR: δ 6.81 (d, J = 8.5 Hz, H-3, H-5), 7.26 (d, J = 8.5 Hz, H-2, H-6), 7.45 (s, –NH_2), 7.91 (d, J = 8.0 Hz, H-2′, H-6′), 8.05 (d, J = 8.0 Hz, H-3′, H-5′), 8.70 (s, –NCH). ^{13}C NMR: δ 115.7 (C-3, C-5), 122.8 (C-2, C-6), 126.1 (C-2′, C-6′), 128.5 (C-3′, C-5′), 139.2 (C-4′), 141.9 (C-1), 145.5 (C-1′), 155.5 (–N*C*H), 156.8 (C-4). HR-MS *m*/*z* calcd for $C_{13}H_{12}N_2O_3S$ 276.0569, found 276.0570.

5.6.7. 4-(4-Methoxy-phenyliminomethyl)-benzenesulfonamide (33). Compound **33** was synthesized using *p*-anisidine (yield 76%); mp 207–209 °C; ^{1}H NMR: δ 3.78 (s, –OCH_3), 7.00 (d, J = 8.5 Hz, H-3, H-5), 7.36 (d, J = 8.5 Hz, H-2, H-6), 7.46 (s, –NH_2), 7.93 (d, J = 8.5 Hz, H-2′, H-6′), 8.07 (d, J = 8.5 Hz, H-3′, H-5′), 8.74 (s, –NCH). ^{13}C NMR: δ 55.3 (–O*C*H_3), 114.5 (C-3, C-5), 122.7 (C-2, C-6), 126.1 (C-2′, C-6′), 128.7 (C-3′, C-5′), 139.1 (C-4′), 143.4 (C-1), 145.7 (C-1′), 156.8 (–N*C*H), 158.4 (C-4). HR-MS *m*/*z* calcd for $C_{14}H_{14}N_2O_3S$ 290.0725, found 290.0725.

5.6.8. 4-(3,4-Dimethoxy-phenyliminomethyl)-benzenesulfonamide (34). Compound **34** was synthesized using 3,4-dimethoxyaniline (yield 76%); mp 221–223 °C; ^{1}H NMR: δ 3.78 (s, 4-OCH_3), 3.81 (s, 3-OCH_3), 6.95 (dd, J = 2.5, 8.5 Hz, H-6), 7.00 (d, J = 8.5 Hz, H-5), 7.06 (d, J = 2.5 Hz, H-2), 7.47 (s, –NH_2), 7.93 (d, J = 8.0 Hz, H-2′, H-6′), 8.07 (d, J = 8.0 Hz, H-3′, H-5′), 8.77 (s, –NCH). ^{13}C NMR: δ 55.5 (4-O*C*H_3), 55.7 (3-O*C*H_3), 105.3 (C-2), 111.9 (C-5), 114.0 (C-6), 126.1 (C-2′, C-6′), 128.7 (C-3′, C-5′), 139.1 (C-4′), 143.6 (C-1), 145.7 (C-1′), 148.1 (C-4), 149.2 (C-3), 156.8 (–N*C*H). HR-MS *m*/*z* calcd for $C_{15}H_{16}N_2O_4S$ 320.0831, found 320.0832.

5.6.9. 4-(4-Hydroxy-3-methoxycarbonyl-phenyliminomethyl)-benzenesulfonamide (35). Compound **35** was synthesized using methyl 5-aminosalicylate (yield 64%); mp 183–185 °C; ^{1}H NMR: δ 3.91 (s, –OCH_3), 7.07 (d, J = 8.5 Hz, H-5), 7.47 (s, –NH_2), 7.61 (dd, J = 2.5, 8.5 Hz, H-6), 7.76 (d, J = 2.5 Hz, H-2), 7.93 (d, J = 8.5 Hz, H-2′, H-6′), 8.09 (d, J = 8.5 Hz, H-3′, H-5′), 8.78 (s, –NCH). ^{13}C NMR: δ 52.5 (–O*C*H_3), 113.6 (C-3), 118.3 (C-5), 122.5 (C-2), 126.1 (C-2′, C-6′), 128.8 (C-3′, C-5′), 128.9 (C-6), 138.8 (C-4′), 142.1 (C-1), 145.9 (C-1′), 158.0 (–N*C*H), 158.7 (C-4), 168.7 (C=O). HR-MS *m*/*z* calcd for $C_{15}H_{14}N_2O_5S$ 334.0467, found 334.0468.

5.6.10. 4-(4-Methoxycarbonyl-phenyliminomethyl)-benzenesulfonamide (36). Compound **36** was synthesized using methyl 4-aminobenzoate (yield 65%); mp 226–228 °C; ^{1}H NMR: δ 3.86 (s, –OCH_3), 7.39 (d, J = 8.0 Hz, H-2, H-6), 7.51 (s, –NH_2), 7.96 (d, J = 8.0 Hz, H-2′, H-6′), 8.02 (d, J = 8.0 Hz, H-3, H-5), 8.13 (d, J = 8.0 Hz, H-3′, H-5′), 8.74 (s, –NCH). ^{13}C NMR: δ 52.1 (–O*C*H_3), 121.3 (C-2, C-6), 126.1 (C-2′, C-6′), 127.2 (C-4), 129.4 (C-3′, C-5′), 130.5 (C-3, C-5), 138.3 (C-4′), 146.6 (C-1′), 155.2 (C-1), 161.5 (–N*C*H), 165.9 (C=O). HR-MS *m*/*z* calcd for $C_{15}H_{14}N_2O_4S$ 318.0674, found 318.0673.

5.7. Cyclooxygenase inhibition assays in human whole blood (HWB)

The assays for COX isozymes activity in human whole blood were performed according to Patrignani et al.[18,19] In blood, thromboxane B_2 (TxB_2) production from platelets is a substitute measure of COX-1 activity, while PGE_2 production is a substitute measure of COX-2 activity. Blood samples were divided in two parts for parallel experiments to test COX-1 and COX-2 inhibition. Test compounds were dissolved and diluted in DMSO, and 2 μL of each dilution of the test compound was added into each well in duplicate. Then, 500 μL blood aliquots were dispensed into each well and incubated at 37 °C for 15 min with shaking. To induce COX-2, lipopolysaccharide (LPS, 2 μL of 5 mg/mL) was added to each well, except the basal control 15 min after the addition of the test compounds. To stimulate COX-1, the calcium ionophore A23187 (2 μL of 5 mM stock in DMSO) was added to each well, except the basal control, 15 min after the addition of the test compounds. At 1 h after A23187 addition or 8 h after LPS addition, the blood samples were transferred and centrifuged at 1000 ×g for 3 min. The supernatants were collected and stored at −80 °C until ready for analysis. After reconstitution with EIA buffer and appropriate dilution, the samples were assayed for TxB_2 (COX-1) and PGE_2 (COX-2) using EIA kits supplied by Cayman Chemical Co. (Ann Arbor, MI, Cat. Nos.: 519031 and 514010). The IC_{50} of the tested compounds was calculated from the concentration–inhibition response curves.

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