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# Selective COX-2 inhibitors. Part 1: Synthesis and biological evaluation of phenylazobenzenesulfonamides

Wei-Jern Tsai,[a] Young-Ji Shiao,[a] Shwu-Jiuan Lin,[b,*] Wen-Fei Chiou,[a] Lie-Chwen Lin,[a] Tsang-Hsiung Yang,[b] Che-Ming Teng,[c] Tain-Shung Wu[a] and Li-Ming Yang[a,b,*]

[a]*Division of Medicinal Chemistry, National Research Institute of Chinese Medicine, 155-1, Li-Nong Street, Sec. 2, Taipei 112, Taiwan*
[b]*Department of Medicinal Chemistry, College of Pharmacy, Taipei Medical University, 250 Wu-Hsing Street, Taipei 110, Taiwan*
[c]*Pharmacological Institute, College of Medicine, National Taiwan University, Taipei, Taiwan*

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**Abstract**—A series of phenylazobenzenesulfonamide derivatives were designed and synthesized for the evaluation as selective cyclooxygenase-2 (COX-2) inhibitors in a cellular assay using human whole blood (HWB) and an enzymatic assay using purified ovine enzymes. Extensive structure–activity relationships (SAR) were studied within this series, and several of selective COX-2 inhibitors have been identified. Among them, compound **8**, 4-(4-amino-2-methylsulfanyl-phenylazo)benzenesulfonamide, showed a potent inhibitory activity to the cyclooxygenase enzymes ($IC_{50}$'s for COX-1: 23.28 μM; COX-2: 2.04 μM), being active but less COX-2 selective than celecoxib.


Nonsteroidal anti-inflammatory drugs (NSAIDs) are widely used for the treatment of pain, fever, and inflammation. Conventional NSAIDs act as nonselective inhibitors of cyclooxygenase (COX) enzymes, which catalyze the formation of prostaglandins (PGs) from arachidonic acid. There are at least two mammalian COX 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, such as gastrointestinal integrity and renal function. Traditional NSAIDs such as aspirin, indomethacin, and diclofenac are nonselective inhibitors and are associated with gastric ulceration, bleeding, and renal function suppression.[3] Generally, prostacyclin and thromboxane normally balance each other's opposing effects. Introducing the selective COX-2 inhibitor may bias the balance dangerously toward thromboxane, raising blood pressure, possibly hardening arteries and certainly promoting heart-attack and stroke-causing clots in some patients.[4] This could explain the cardiotoxicity caused by rofecoxib (Vioxx®),[5] and subsequently valdecoxib (Bextra®),[6] which were recently withdrawn from the market.[7] Thus, there is an urgent need to search for new selective COX-2 inhibitors with a mild therapeutic effect on COX-1, which could theoretically reduce cardiovascular side effects due to their antiplatelet and anti-thrombotic activities, and improve the safety profiles.[8]

The majority of selective COX-2 inhibitors belong to a class of tricyclic compounds possessing 1,2-diaryl substitution on a central heterocyclic, or carbocyclic ring system.[9] Extensive structure–activity relationship (SAR) studies for the diarylheterocycle compounds have shown that a $SO_2NH_2$, or a $SO_2Me$ substituent at the *para*-position of one of the phenyl rings often provides optimum selective COX-2 inhibitory potency (Fig. 1).[10] 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

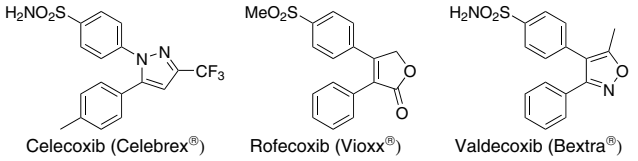


**Figure 1.** Selective tricyclic COX-2 inhibitors.

*Keywords*: Anti-inflammatory; Selective COX-2 inhibitors; Phenylazobenzenesulfonamide; Resveratrol; Isosterism; SAR.
* Corresponding authors. Tel.: +886 2 2820 1999x8551; fax: +886 2 2826 4276; e-mail addresses: lmyang@nricm.edu.tw; shwu-lin@tmu.edu.tw

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found mainly in the skin of grapes and red wine. It has been demonstrated to have antioxidant, anti-inflammatory, cardioprotective (the so-called 'French paradox'),[11] and cancer chemopreventive properties.[12] It has also been shown to exhibit moderate selective COX-1 inhibitory activity.[13]

As an alternative to convert a COX-1 selective compound into a COX-2 selective inhibitor, we have designed and modified the basic skeleton of *trans*-stilbene by the concept of isosterism,[14] which lacks a traditional central heterocyclic or carbocyclic ring template such as celecoxib (Celebrex®).[15] We have previously reported that the 4-(4-dimethylamino-phenylazo)benzenesulfonamide (**1**) demonstrates a high potency against the amyloid-β (Aβ) aggregation, which is responsible for neuro-inflammation in Alzheimer's disease.[16] However, whether these phenylazobenzenesulfonamide derivatives possess a selective COX-2 inhibitory activity has not yet been investigated. In this paper, we describe the synthesis of these compounds and evaluate their inhibitory potency against COX-1/COX-2 in vitro.

Structural modifications of the phenylazobenzenesulfonamide derivatives include (a) replacing the *para*-hydroxyl group of resveratrol by the *para*-sulfonamide moiety, and (b) the bioisosteric replacement of the central ethylenic linkage with an azo *N*,*N*-double bond. Additionally, we modified the various substituents on the phenylazo ring B in order to obtain compounds with the high COX-2 selectivity and potency that we were seeking. These strategies are illustrated in Figure 2.

The target phenylazobenzenesulfonamides (**1**–**12**) were synthesized in a manner similar to that described in our previous publication.[16] The procedures for preparation of these compounds are summarized in Schemes 1–3. Although the synthesis of these derivatives has already been reported,[17] neither their selective COXs inhibitory activity nor the possible chemical conversion has been described or characterized previously. The synthesis involved initial diazotization of sulfanilamide with sodium nitrite in hydrochloride solution to afford the diazonium salt **13**, followed by coupling with a molar equivalent of the appropriate aromatic compounds **14a**–**g**, in sodium acetate or sodium hydroxide solution at 0 °C. The reactions were accompanied by a color change from pale yellow to deep brown or reddish orange and gave the corresponding phenylazobenzenesulfonamide derivatives **1**–**4** and **8**–**10** (Scheme 1). The phenol **2** was treated with diazomethane to give anisole derivative **6** in 94% yield. Similarly, catechol **3** was converted to the monomethylated **5** in 50% yield

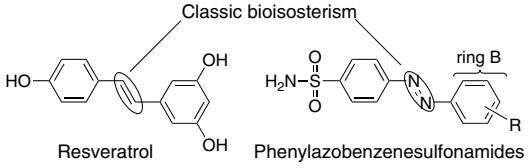
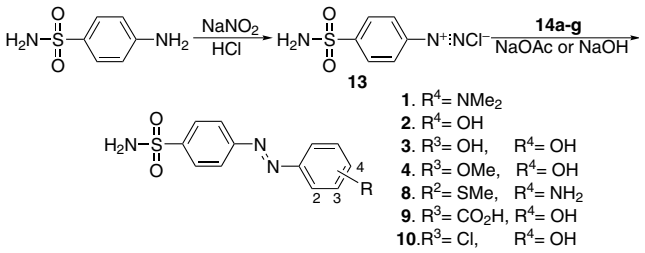
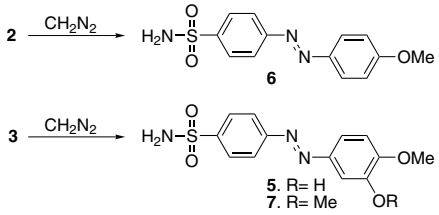


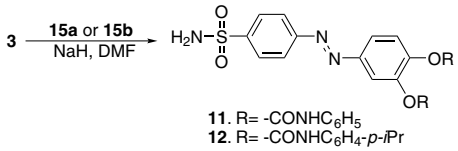

**Figure 2.** Design concept of phenylazobenzenesulfonamides.




**Scheme 1.** Synthesis of phenylazobenzenesulfonamide derivatives **1**–**4** and **8**–**10**. Reagents: **14a**, *N*,*N*-dimethylaniline; **14b**, phenol; **14c**, catechaol; **14d**, guaiacole; **14e**, 3-(methylthio)aniline; **14f**, salicyclic acid; **14g**, 2-chlorophenol.




**Scheme 2.** Synthesis of phenylazobenzenesulfonamide derivatives **5**–**7**.




**Scheme 3.** Synthesis of phenylazobenzenesulfonamide derivatives **11**–**12**. Reagents: **15a**, phenyl isocyanate; **15b**, 4-isopropylphenyl isocyante.

and, with a large amount of diazomethane, to veratrole derivative **7** in almost a quantitative yield (Scheme 2). The carbamate derivatives were readily prepared from the catechol **3** with 2.2 molar excess of phenyl isocyanate or 4-isopropylphenyl isocyanate in the presence of sodium hydride in DMF at 0 °C to give the desired carbamates **11** and **12**, respectively, in yields ranging from 70% to 75% (Scheme 3). 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.

SAR studies of the phenylazobenzenesulfonamides for the selective COX-2 inhibitors. We have evaluated the selectivity of COXs inhibitory potency in a cellular assay using human whole blood (HWB) and in an enzymatic assay using purified ovine COX enzymes.[18,19] The $IC_{50}$ micromolar values for COXs were determined for selected/promising compounds. Indomethacin, resveratrol, and celecoxib were evaluated as reference compounds for the in vitro assay. The COX-1 activity was measured as the $TXB_2$ production after stimulation of platelet aggregation by calcium ionophore A-23,187. The COX-2 activity was measured as the $PGE_2$ levels produced by leukocytes after stimulation by LPS.[20]

**Table 1.** Inhibitory activity of phenylazobenzenesulfonamides using human whole blood assays

| Compound | R | HWB $IC_{50}$[a] (μM) | | Selectivity index |
|---|---|---|---|---|
| | | COX-1 | COX-2 | COX-1/COX-2 |
| **1** | 4-$N(CH_3)_2$ | >100 | na | ns |
| **2** | 4-OH | >100 | na | ns |
| **3** | 3,4-$(OH)_2$ | 59.96 | 12.42 | 4.83 |
| **4** | 3-$OCH_3$, 4-OH | >100 | 16.85 | 7.91 |
| **5** | 3-OH, 4-$OCH_3$ | 66.41 | na | ns |
| **6** | 4-$OCH_3$ | >100 | 14.63 | 9.48 |
| **7** | 3,4-$(OCH_3)_2$ | 61.73 | 4.28 | 14.42 |
| **8** | 2-$SCH_3$, 4-$NH_2$ | 23.28 | 2.04 | 11.40 |
| **9** | 3-$CO_2H$, 4-OH | 31.57 | 8.89 | 3.55 |
| **10** | 3-Cl, 4-OH | 33.32 | 11.34 | 2.94 |
| **11** | 3,4-$(OCONHC_6H_5)_2$ | 35.07 | 11.07 | 3.17 |
| **12** | 3,4-$(OCONHC_6H_4$–*p*-*i*-$C_3H_7)_2$ | 26.26 | 12.37 | 2.12 |
| Indomethacin | | 0.005 ± 0.001 | 0.036 ± 0.004 | 0.13 |
| Resveratrol | | 2.84 ± 1.90 | 33.15 ± 3.50 | 0.09 |
| Celecoxib | | 20.03 ± 1.58 | 0.33 ± 0.10 | 60.25 |

na, no activity; ns, nonselective.

[a] Values are means ± SEM from three independent experiments using COXs 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.

**Table 2.** In vitro COXs enzyme inhibition data for compounds **7**–**10**

| Compound | $IC_{50}$[a] (μM) | | Selectivity index |
|---|---|---|---|
| | COX-1 | COX-2 | COX-1/COX-2 |
| **7** | >100 | 84.5 | ns |
| **8** | 34.09 | 18.0 | 1.89 |
| **9** | 29.25 | 23.6 | 1.24 |
| **10** | 40.53 | 39.7 | 1.02 |
| Indomethacin | 0.004 ± 0.001 | 0.34 ± 0.07 | 0.01 |
| Resveratrol | 0.38 ± 0.17 | 3.49 ± 1.44 | 0.11 |
| Celecoxib | 26.61 ± 8.49 | 0.44 ± 0.06 | 60.48 |

ns, nonselective.

[a] Values are means ± SEM from three independent experiments using an ovine COXs assay kit (Catalog No. 560101, Cayman Chemicals Inc., Ann Arbor, MI, USA). Since SEM values never exceeded 15% of the media, they have been omitted.

The selectivity index (SI, COX-1 $IC_{50}$/ COX-2 $IC_{50}$) was calculated. The results of inhibition are summarized in Tables 1 and 2.

Compound **1** showed a remarkable inhibitory activity against amyloid-β (Aβ) aggregation.[16] Surprisingly, this compound completely lost the COX-2 inhibitory activity in the HWB assay.[19,20] Compounds with various substituents on the phenylazo ring B were examined to determine whether the substituents affect the COX-2 activity and selectivity, as outlined in Table 1. The introduction of a 4-hydroxy, **2**, or a 3-hydroxy-4-methoxy, **5**, group showed to be devoid of COX-2 inhibitory activity. The presence of a 4-hydroxy-3-methoxy, **4**, or a 4-methoxy, **6**, group led to a complete loss of both COX-2 and COX-1 inhibitory activities.

The SAR studies demonstrated that the parent compound, 3,4-dihydroxyl (**3**), exhibited moderate COX-2 inhibitory potency. However, replacement of the 3-hydroxy moiety in **3** with a 3-chloro, **10**, retained some COX-2 potency, but less active than that of a 3-carboxy, **9**, group. Also, these compounds enhanced COX-1 inhibitory activity. Interestingly, introduction of the 3,4-dimethoxy moiety, **7** (COX-2 $IC_{50}$ = 4.28 μM), provided excellent activity and selectivity than the parent compound **3** (COX-2 $IC_{50}$ = 12.42 μM).

Replacement of the 3,4-dihydroxy moiety of **3** by a 3,4-di-*N*-phenylcarbamate, **11**, and a 3,4-di-*N*-(4-isopropylphenyl)carbamate, **12**, group was investigated for selectivity and potency. As shown in Table 1, the selective COX-2 inhibitory activities were decreased gradually in proportion to the size of the substituents. But interestingly, the COX-1 activities were enhanced as the substituents became bulkier. Consequently, compound **7** was found to be a preferential COX-2 inhibitor (SI value of 14.42). Indeed, compound **8** displayed a greater COX-2 inhibitory profile when compared to phenylazobenzenesulfonamide in this series (COX-2 $IC_{50}$ = 2.04 ± 1.57 μM), although it appeared to be less selective against COX-2 (SI of 11.40 and 60.25 for **8** and celecoxib, respectively).

From these results, we further evaluated more potent derivatives, **7**–**10**, as selective COX-2 inhibitors in purified ovine enzymes assays.[21] As described in Table 2, all of the substituents mentioned above lost or showed diminished COX-2 selectivity. However, compound **8** showed a remarkable increase in COX-2 selectivity ($IC_{50}$ = 18.0 μM; SI = 1.89). The reasons for these discrepancies are at present unknown.

In conclusion, we have identified a series of novel phenylazobenzenesulfonamides that are potent but moderately selective COX-2 inhibitors with an appropriate balance between COX-1 and COX-2 inhibition. These results confirm that the dihydroxy compound **3** was a less potent COX-2 selective inhibitor than its corresponding dimethoxy compound **7**. However, replacement of the dimethoxy moiety in **7** with carbamate analogues yielded **11** and **12**, which showed diminished COX-2 inhibitory activity. Among the SAR examined, compound **8** was

proved to be the most potent COX-2 inhibitor ($IC_{50}$'s for COX-1: 23.28 μM; COX-2: 2.04 μM) in this series, but moderately selective than celecoxib (Tables 1 and 2). Further modification of **8** for more potent and selective COX-2 inhibitors with improved safety profile and less cardiovascular risk is in progress.

## Acknowledgments

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