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SYNTHESIS AND BIOLOGICAL EVALUATION OF 2,3-DIARYLTHIOPHENES AS SELECTIVE COX-2 INHIBITORS. PART II: REPLACING THE HETEROCYCLE

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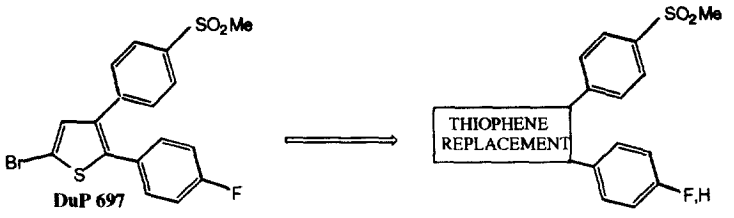
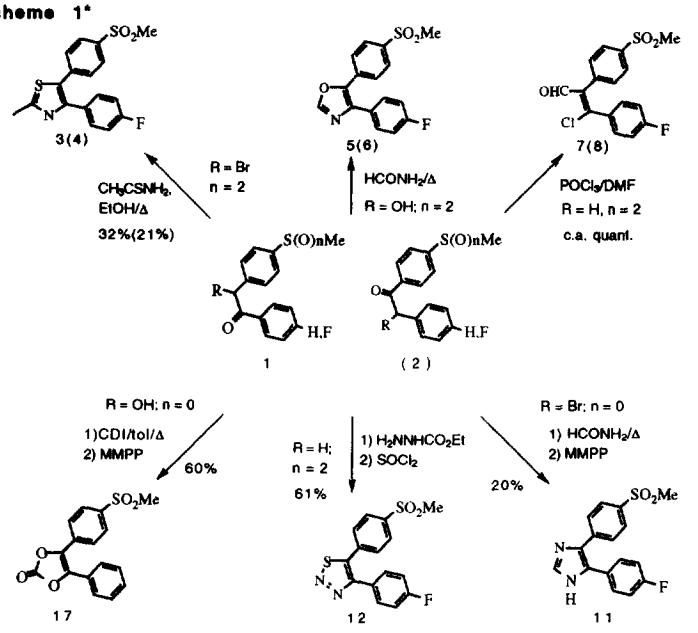
Abstract. The thiophene ring of DuP 697 was replaced by a variety of heterocycles and the products were tested for their ability to inhibit human Cox-2 and Cox-1, the isozymes of cyclooxygenase.

A highly selective inhibitor of the newly discovered cyclooxygenase (Cox) isozyme,[1] Cox-2, that would be bioavailable and metabolically stable, is a strongly pursued objective. This is because such an inhibitor has the potential to produce a new generation of nonsteroidal antiinflammatory drugs (NSAIDs) with improved therapeutic properties and fewer side effects such as gastropathy.[2] This potential was explored in the "tricyclic" class of NSAIDs, best represented by DuP 697,[3] a series that exhibited excellent potency for the inhibition of Cox-2 and moderate selectivity over Cox-1.[4] In an earlier letter,[5] we established that the structure-activity relationship (SAR) for the methylsulfonylphenyl ring of this series is particularly tight. Only the relatively small and polar primary sulfonamide group is tolerated at this position without appreciable loss of potency on Cox-2. Unfortunately, despite the promises of improved bioavailability with such a derivative, the concomitant loss of selectivity required that we investigate the SAR in other areas of the molecule. Removal of the bromo substituent at the C5 position of the thiophene ring yielded a potent and more selective Cox-2 inhibitor. In fact, this compound is inactive in our Cox-1 assay at concentrations up to 100 μM.[5] Furthermore, the 2-bromothiophene moiety of DuP 697 has been reported to be metabolized to the 2-methylsulfonyl derivative, a long lasting metabolite.[6] Consequently, a simple, preferably unsubstituted ring to replace the thiophene nucleus was sought. In this letter, our SAR investigation focuses on ring replacements for the thiophene template.[7]



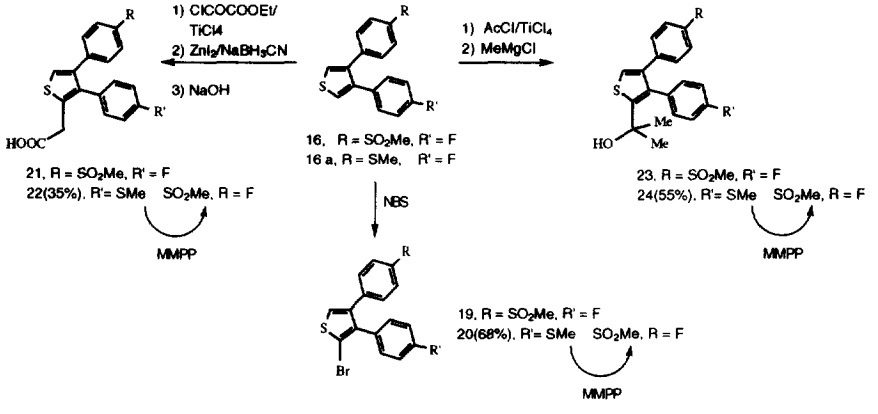
Synthesis: All the compounds were prepared following classical procedures that are well described in heterocyclic chemistry literature[8] and were generally derived from the appropriately substituted diarylethanone intermediate, **1** or **2** (R'' = H, OH or Br)[9a,5] (scheme 1). When appropriate, typical yields were indicated. The isothiazoles, **9** and **10** (Table 1), were prepared in 54% and 75% yield from the reaction of the corresponding β-chlorovinylaldehydes **7** and **8**[9b] with ammonium thiocyanate in refluxing acetone. Triazole **13** was prepared by heating 4-(phenylethynyl)thioanisole with $TMSiN_3$ at 150 °C in a sealed tube[9c] (53 %) followed by oxidation of the resulting cyclized product to the sulfone derivative **13** with magnesium monoperoxyphthalate (MMPP). The isomeric tetrazoles, **14** and **15**, were obtained by refluxing in acetonitrile a mixture of 4-benzoylthioanisole, sodium azide and titanium tetrachloride according to the known procedure,[9d] followed by oxidation with MMPP and separation of the isomers by chromatography. The 3,4-diarylthiophene, **16**, was prepared (71%) via the intramolecular reductive coupling of the corresponding diketo sulfide to sulfide **16a** using the procedure ($Zn/TiCl_4$) described by Nakayama,[9e] followed by oxidation with MMPP. The 2,3-norbornenyl derivative **18** was produced (65%) by heating to reflux with NBS in the presence of AIBN and light the 2,3-diarylnorbornanyl intermediate which had been obtained in the palladium catalyzed cross-coupling of norbonylene, 4-fluorobenzeneboronic acid and 4-(methylsulfonyl)bromobenzene according to the procedure of Kosugi et al.[9f] Derivatives **19**, **21** and **23** of the thiophene **16** (scheme 2) were prepared by conducting the appropriate aromatic electrophilic substitution[8] on **16** providing, in each case, the desired compound as the major isomer which was isolated by simple chromatography. Alternatively, derivatives **20**, **22** and **24** were obtained from conducting the same transformations on the methylsulfide-analog **16a**, followed by oxidation to the sulfone using MMPP. The compounds were tested in vitro for their ability to inhibit the Cox-2 and Cox-1 enzymes. The results are presented in Table 1.

Scheme 1*



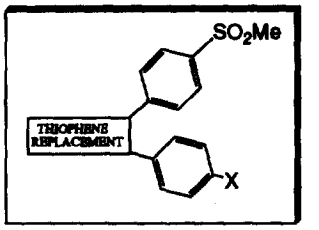
* Compounds in parenthesis refer to regioisomers derived from ketone 2 (see text).

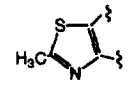
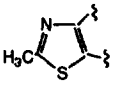
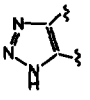
Scheme 2

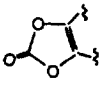
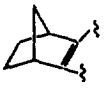
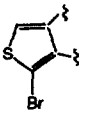
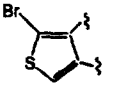
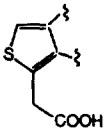


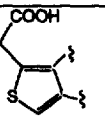
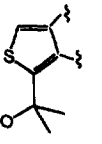
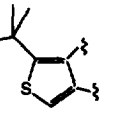
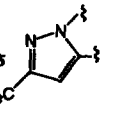
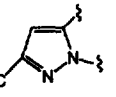
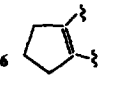
Discussion: The isothiazoles **9**, **10** and the 1,2,3-thiadiazole **12** showed only moderate Cox-2 activity (IC_{50} = 1.4-11.3 μM) and were essentially inactive against Cox-1. In addition, other 5-membered heterocycles that contained 2 or more nitrogen atoms in the ring, such as the imidazole **11**, the triazole **13**, the tetrazoles **14** and **15**, did not show significant activity in our assays. Although these compounds should have similar spatial disposition of the rings, it appears that the active site of the isozymes do not tolerate a polar replacement for the thiophene moiety. The carbonate **17** was also inactive at concentration up to 30 μM. This unambiguously demonstrates that not all 5-membered ring templates will be tolerated and that the SAR is also

Table 1. IC_{50} values (μM) against human Cox-2 and Cox-1 enzymes.
Microsomes from baculovirus-infected Sf9 cells expressing either recombinant human Cox-2 or Cox-1 were used as enzyme source. Microsomal membrane containing COX-1 or COX-2 enzymes were used at protein concentration of 25 and 4 μg/mL respectively and were preincubated for 15min with the inhibitor followed by a 15 min incubation with arachidonic acid at1 μM. PGE_2 was measured by EIA.[5]



Compound, X	Cox-1 Cox-2	RATIO
3, F 	>100 0.31	>320
4, F 	>100 0.31	>320
5, F 	>100 >30	-
6, F 	>100 >30	-
9, F 	>100 2.1	>47
10, F 	>100 5.1	>19
11, F 	>100 >30	-
12, F 	>100 6.5	>15
13, H 	>50 >30	-
14, 3,4-diF 	>100 >30	-

Compound, X	Cox-1 Cox-2	RATIO
15, 3,4-diF 	>50 >30	-
16, F 	>100 0.47	>210
17, H 	>50 >30	-
18, F 	>50 30	>1.6
19, F 	1.2 0.03	40
20, F 	>50 >30	-
21, F 	>50 >10	-

Compound, X	Cox-1 Cox-2	RATIO
22, F 	>50 >10	-
23, F 	>50 5.9	>8.5
24, F 	>50 >30	-
25, F SC-58125 REF 11A 	>50 0.2	>250
26, F  REF 11B	>1.1 1.2	>.92
27, F SC 57666  REF 11C	>50 0.16	>310
DuP-697	0.6 0.007	85

very tight in this region of the tricyclic series. The present study shows that unsubstituted oxazoles, such as **5** or **6**, are virtually inactive against both enzymes. However, there is a report indicating that 2-aryloxazoles are potent Cox-2 inhibitors.[10] This suggests the importance of a properly chosen substituent in this region of the tricyclic series and will be the subject of a study to be publish soon. Larger rings such as the norbornenyl derivative **18**, were also evaluated. This compound was essentially inactive (IC_{50} > 30 μM) against both enzymes in our assay conditions. In cases where the thiophene ring could be replaced by another ring resulting in a compound showing cyclooxygenase inhibition activity (i.e. IC_{50} < 1 μM), the compounds were selective Cox-2 inhibitors (i.e. Cox-2/Cox-1 >200). This is the case for the isomeric thiazoles **3** and **4** and the 3,4-diarylthiophene **16**. They are at least 200-fold more potent for the inhibition of Cox-2 and have IC_{50}s < 0.47 μM. A number of these analogs were evaluated for their oral absorption and for their efficacy in vivo. In rats, most compounds were found to have poor bioavailability and pharmacokinetics. This is the case for **3**, **4**, **16**, and **19**. However, **9** and **12** were more promising and the data obtained are listed in Table 2. Although these two compounds are not the most potent among the analogs presented, they show good in vivo activity, particularly for compound **12** which has excellent bioavailability and sustained plasma levels as opposed to **9** (low absorption, fast clearance). This demonstrates that good oral bioavailability compensates for a slight drop in their in vitro potency. In addition, **12** was found to be clean in the ^{51}Cr excretion model of ulcerogenicity (100 mpk, bid, 4 days) which indicates a higher level of GI tolerance than recognized standards such as indomethacin[12] and validates the hypothesis supporting the study.

Table 2. Plasma concentration(PC) in ug/mL at 2 hrs in rats at 20 mpk(1% methocel), ED_{50} values (mg/kg) in the rat paw model and effect on gastrointestinal integrity(GI) in rats in the ^{51}Cr assay.[12]

Compound	PC	ED_{50}	GI
Indomethacin	n/a	2.0	+
DuP-697	0.7	1.3	+
9	2.2	10.0	n/a
12	12.8	0.7	-

n/a : not available
+ : causes lesions; - no lesion observed.

In conclusion, this study has identified a number of useful heterocycles that can be used to replace the thiophene ring of DuP 697. The thiadiazole **12** is an orally potent and selective Cox-2 inhibitor with a remarkable in vivo potency with an ED_{50} of 0.7 mg/kg in the rat paw edema model and free of GI toxicity.

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