Discovery and Preliminary SAR of 5-Arylidene-2,2-Dimethyl-1,3-Dioxane-4,6-Diones as Platelet Aggregation Inhibitors

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Abstract: We herein document the discovery of 5-arylidene-2,2-dimethyl-1,3-dioxane-4,6-diones as a novel family of platelet aggregation inhibitors. The preliminary optimization study enabled us to establish the most salient features of the structure-activity relationships in this series as well as to identify novel derivatives that are upto 60 times more potent than the hit structure **1** and slightly superior to the reference drug Milrinone.

Keywords: Antiplatelet agents, Meldrum´s acid, platelet aggregation inhibitors.

INTRODUCTION

The hemostatic system is designed to maintain fluid blood flow under physiological conditions and to react rapidly to form a clot at sites of vascular damage [1]. In this exquisitely regulated system platelets play key roles as sentinels of vascular integrity [2]. The interaction of platelets with collagen and/or thrombin initiates a complex biochemical sequence of signal transduction resulting in a functional cell response [3]. Although this mechanism is vital when tissue is damaged, inappropriate activation of platelets is an important pathogenic factor in widespread cardiovascular diseases, such as myocardial and cerebral circulatory disorders, venous thrombosis and arteriosclerosis. However, failure of platelets to become activated can lead to excessive bleeding.

Small molecules that are able to modulate platelet function represent a well-established approach to prevent the diseases mentioned above [4]. Inhibitors of platelet aggregation currently employed in therapy (Fig. **1**) are represented by three main drug categories, which include non-steroidal anti-inflammatory drugs (Aspirin)[5], adenosine diphosphate receptor antagonists (Clopidogrel or Ticlopidine) [6], and glycoprotein IIb/IIIa (GPIIb/IIIa) antagonists (Tirofiban) [7]. Despite the documented utility of these drugs, their efficacy and selectivity are not sufficiently high and there are reasons to believe that substantial improvements in antiplatelet therapy can be made. This issue is of primary importance since none of the currently marketed antithrombotic agents, or indeed most of the compounds in advanced clinical trials, are free of significant bleeding risk or meet the basic requirements expected for the ideal antiplatelet agent [8]. As a result, the discovery of novel chemotypes that exhibit antiplatelet activity is a goal of great interest, not only for their possible evolution into clinical candidates but also because such compounds could be employed as valuable pharmacological tools to provide comprehensive information about platelet function.

Meldrum´s acid (2,2-dimethyl-1,3-dioxane-4,6-dione) was first synthesized and misassigned by A. N. Meldrum in 1908 [9] and correctly elucidated in 1948 by Davidson and Bernhard [10]. This compound exhibits unique structural features that make it a tremendously attractive molecule from practical and theoretical points of view [11, 12]. While Meldrum´s acid and its derivatives have been extensively employed in the preparation of heterocyclic libraries that show manifold biological activities [13], the pharmacological properties associated with the 1,3-dioxane-4,6-dione core remain practically unexplored. Some recent reports on remarkable biological activities have highlighted the as yet untapped potential of such a chemotype in medicinal chemistry [14, 15]. Within the framework of a chemical genomics project aimed at the discovery of novel chemotypes that exhibit antiplatelet activity through original mechanisms of action, we recently started a high-throughput screening program of our in-house library (*ca*. 4000 compounds) in the search for new chemotypes with antiplatelet effect. Herein we document the discovery and some preliminary features of the structure-activity relationship of 5-arylidene-2,2-dimethyl-1,3-dioxane-4,6-diones as a novel family of structurally simple and potent antiplatelet agents.

Two hit compounds (Fig. **2**) were identified during a screening campaign performed on a library containing diverse 5-substituted Meldrum´s acid derivatives (e.g. 5-acyl, 5-alkyl, 5-alkylidene and 5-arylidene). While most of the 1,3-dioxane-4,6-dione congeners were inactive, 5-benzylidene derivative **1** and its azaanalog **2** (Fig. **2**) emerged as encouraging hit structures due to their structural

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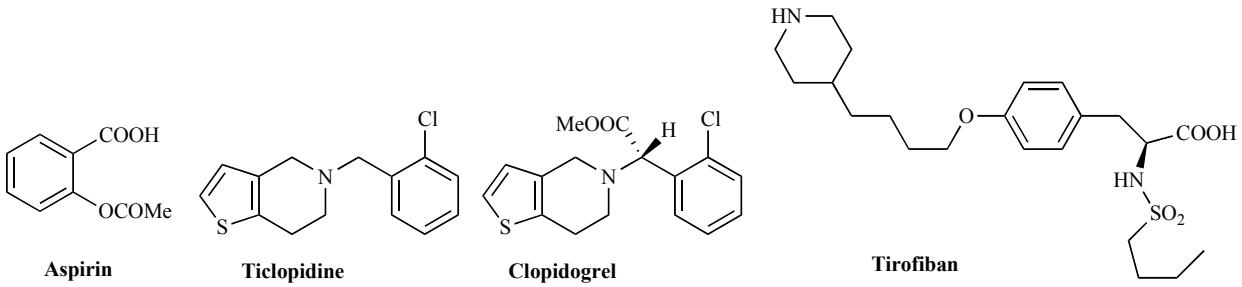


Aspirin **Ticlopidine** **Clopidogrel** **Tirofiban**

Fig. (1). Structures of representative antiplatelet agents in clinical use.

1

5-benzylidene-2,2-dimethyl-1,3-dioxane-4,6-dione

IC_{50} = 121.42 μM

2

2,2-dimethyl-5-(pyridin-2-ylmethylene)-1,3-dioxane-4,6-dione

IC_{50} = 82.12 μM

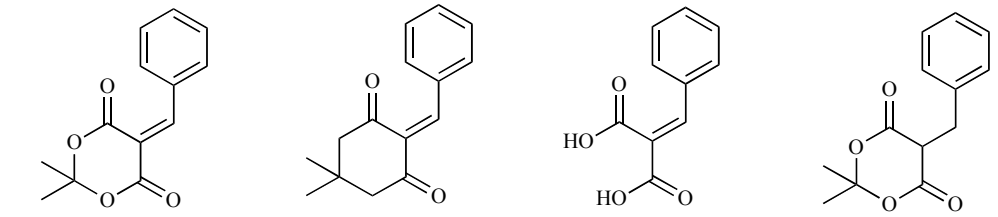
Fig. (2). Structure and antiplatelet activity of the Hit Compounds **1** and **2**.

simplicity and relatively potent platelet inhibitory activity (IC_{50} = 121.42 μM and 82.12 μM, respectively). As a preliminary step in assessing the SAR in this series we decided to evaluate the platelet inhibitory activity of three structural analogs of hit compound **1** [Fig. **3**, 2-benzylidene-5,5-dimethylcyclohexane-1,3-dione (**3**), 2-benzylidenemalonic acid (**4**), and 5-benzyl-2,2-dimethyl-1,3-dioxane-4,6-dione (**5**)]. All compounds proved to be inactive, a result that highlights the contribution of the heterocyclic scaffold and the exocyclic double bond to the documented biological activity.

In an effort to complete the SAR studies a focused library of 2,2-dimethyl-1,3-dioxane-4,6-diones (**8**) incorporating different arylidene, heteroarylidene, and alkylidene residues at position 5 of the heterocyclic scaffold was synthesized (Scheme **1**) by following a recently reported environmentally friendly procedure [16]. Target compounds were isolated by precipitation and purified by recrystallization (or chromatography). The analytical and spectral characterization enabled unequivocal assignments and the results are consistent with data reported previously.

The platelet aggregation inhibitory activity of the obtained library (**8a-t**) and two reference compounds (Sulfinpyrazone and Milrinone) was examined on washed human platelets by the turbidimetric method of Born employing thrombin as agonist [17]. Compounds were tested in DMSO solutions and reported IC_{50} values are the mean of at least three experiments (in duplicate) in which human blood from different individuals was used. Active compounds inhibited platelet aggregation in a dose-dependent manner. The results of these experiments are summarized in Table **1**.

Inspection of the biological data (Table **1**) shows that structural manipulation of hit structure **1** enabled the identification of novel derivatives that are generally more potent (2- to 60-fold) than **1** and the reference compound Sulfinpyrazone. Several of the derivatives described here



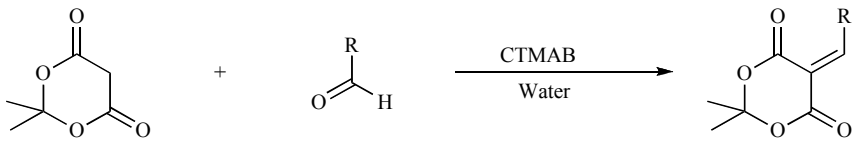
1
IC_{50} = 121.42 μM

3
Inactive

4
Inactive

5
Inactive

Fig. (3). Comparison of the antiplatelet activity of **1** and three analogs (**3**-**5**).

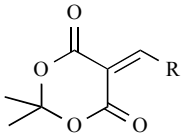


6 **7** **8a-t**

Scheme 1. Synthesis of the 5-alkylidene-2,2-dimethyl-1-3-dioxane-4,6-dione library.

have IC_{50} values in the low micromolar range (2-25 μM). In particular, compounds **8j** and **8m** (IC_{50} = 2.10 μM and 3.40 μM, respectively) exhibit antiplatelet activity that is slightly superior to that determined for Milrinone. Analysis of the data in Table **1** reveals the substantial contribution of the group(R) on the exocyclic double bond to the documented platelet inhibitory activity. As it can be observed, active compounds incorporate aryl (**8a-n**) or heteroaryl (**8o-q**) fragments, whereas derivatives containing alkyl residues (**8r-t**) were inactive. This result confirms the relevance of the (het)aryl group, which together with the 1,3-dioxane-4,6-dione and the exocyclic double bond can be considered part of the pharmacophore.

Table 1. Antiplatelet Activity of 5-Arylidene-2,2-dimethyl-1-3-dioxane-4,6-diones (8)



Compound	R	Yield (%)	Antiplatelet Activity IC_{50} (μM)[Ψ]
8a	Ph	82	121.42
8b	Ph-4-OMe	72	43.21
8c	Ph-4-OH	45	47.60
8d	Ph-4-Cl	64	21.58
8e	Ph-4-F	58	33.94
8f	Ph-2-OMe	68	57.83
8g	Ph-2-Cl	90	19.45
8h	Ph-2-NO_2	74	16.33
8i	Ph-2,4-OMe	76	39.27
8j	Ph-2,4-Cl	71	2.10
8k	Ph-2-Cl-5-OMe	77	73.25
8l	Ph-2,6-OMe	59	28.03
8m	Ph-2,6-Cl	56	3.40
8n	Ph-2-Cl-6-F	63	6.84
8o	2-Thiophene	71	17.36
8p	2-Furane	65	29.46
8q	2-Pyridine	76	82.12
8r	CH_2CH_3	37	*
8s	$CH(CH_3)_2$	43	*
8t	CH_2 CH_2CH_3	48	*
Sulfinpyrazone			509.49
Milrinone			4.70

[Ψ]The antiplatelet activities of the standards and the synthesized compounds were tested by the turbidimetric method in human washed platelets using thrombin as agonist. Methods for these assays have been published elsewhere [17]. * Inactive or IC_{50} >200μM. Results shown are means of at least three experiments (in duplicate). The highest mean standard error of IC_{50} values was 10%.

The screening of compounds that incorporate substituents at diverse positions of the phenyl residue (Table **1**, compounds **8b-n**) showed that such a modification provides derivatives with improved platelet aggregation inhibitory activity in comparison to the reference compound (**8a**, R = Ph IC_{50} = 121.42μM). Although favorable, the change in biological profile was clearly dependent on the nature of the introduced group and, more importantly, on the substitution pattern at the phenyl ring. A common structural feature of all compounds that showed potent antiplatelet activity (IC_{50}< 20 μM) was the presence of a substituent at position 2, a factor that is particularly important for derivatives that have a 2,4- or 2,6-substitution pattern (e.g., **8g**, **8h**, **8i**, **8m**, **8n**). The exceptions to this trend are those derivatives that contain methoxy groups (**8f**, **8i**, **8k** and **8l**). Although the limited number of compounds incorporating heterocyclic moieties precludes a detailed analysis of the structure-activity relationship, the superior antiplatelet effect observed for the 2-thiophenyl and 2-furyl derivatives suggests that a similar SAR trend could be operating in these series. It is important to highlight that compounds **8j** and **8m** exhibited outstanding antiplatelet activity (IC_{50} = 2.10 μM and 3.40 μM, respectively) -i.e. 2-times higher than that determined for the reference compound Milrinone under the same experimental conditions.

In summary, we have documented the discovery and structural optimization of a new family of potent antiplatelet agents. The preliminary exploration around the hit structure **1** generated new potent derivatives and also highlighted the most salient features of the SAR in this series. Further studies are in progress in our laboratories to identify novel derivatives exhibiting sub-micromolar activity derived from the chemotype documented here and to establish the mechanism of action of these promising derivatives.

EXPERIMENTAL

The compounds described here were obtained according to previously reported procedures. The synthesis, isolation and purification of all compounds were accomplished using equipment routinely available in laboratories for parallel synthesis. Commercially available starting materials and reagents were purchased and used without further purification from freshly opened containers. Isolation of precipitated/ triturated products was performed in a 12-channel vacuum manifold from Aldrich. The NMR spectra were recorded on a Bruker AM300 MHz (^{1}H) and 75 MHz (^{13}C) and XM500 spectrometers. The spectroscopic and analytical data, which are consistent with previously reported data, for representative compounds are presented in the experimental section. The platelet aggregation inhibitory activity of the obtained library was determined according to a previously described procedure.

5-Benzylidene-2,2-dimethyl-1,3-dioxane-4,6-dione (8a). Yield: 82%, mp 83-85°C (*i*-PrOH). IR (KBr): ν_{max}= 1765, 1730, 1580 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$, ppm) δ 8.36 (s, 1H), 7.18 - 7.16 (d, 2H, *J* = 8.2Hz), 7.05 (t, 2H, *J* = 8.2 Hz), 6.93 - 6.90 (m, 1H), 1.70 (s, 6H). ^{13}C NMR (75 MHz, $CDCl_3$, ppm) δ 162.9, 159.4, 157.7, 133.3, 131.5, 128.5, 114.7, 104.3, 27.4.

5-(4-Methoxybenzylidene)-2,2-dimethyl-1,3-dioxane-4, 6-dione (8b): Yield: 72%, mp 126-127°C (EtOH). IR (KBr) ν_{max}= 1747, 1574 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$, ppm) δ 8.39 (s, 1H), 8.25 (d, *J* = 8.7 Hz, 2H), 6.99 (d, *J* = 8.7 Hz, 2H), 3.90 (s, 3H), 1.75 (s, 6H); ^{13}C NMR (75 MHz, $CDCl_3$, ppm) δ 164.2, 160.4, 157.8, 137.6, 124.8, 114.4, 111.0, 104.1, 55.7, 27.5.

5-(4-Hydroxybenzylidene)-2,2-dimethyl-1,3-dioxane-4, 6-dione (8c): Yield: 45%, mp 200-201°C (EtOH), IR (KBr) ν_{max}= 1745, 1571 cm^{-1}. ^1H NMR (300 MHz, DMSO-d_6, ppm) δ 10.92 (s, 1H), 8.24 (s, 1H), 8.15 (d, *J* =8.6 Hz, 2H), 6.88 (d, *J* = 8.6 Hz, 2H), 1.70 (s, 6H). ^{13}C NMR (75 MHz, DMSO-d_6, ppm) δ 163.7, 156.9, 138.1, 123.0, 116.5, 109.8, 103.9, 26.8.

(4-Chlorobenzylidene)-2,2-dimethyl-1,3-dioxane-4,6-dione (8d): 64%, mp 157-159°C (EtOH). IR (KBr) ν_{max}= 1738, 1732, 1586 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$, ppm) δ 8. 38 (s, 1H), 8.05 (d, *J* = 8.1 Hz, 2H), 7.48 (d, *J* = 8.1 Hz, 2H), 1.80 (s, 6H). ^{13}C NMR (75 MHz, $CDCl_3$, ppm) δ 162.8, 159.5, 156.7, 134.3, 133.2, 129.8, 116.2, 104.7, 27.6.

5-(Furan-2-ylmethylene)-2,2-dimethyl-1,3-dioxane-4,6-dione(8p): Yield: 65%, mp 91-92°C (EtOH). IR (KBr) ν_{max}= 1743, 1585 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$, ppm) δ 8.45 (d, *J* = 3.9 Hz, 1H), 8.35 (s, 1H), 7.83 (d, *J* = 0.9 Hz, 1H), 6.74 (dd, *J* = 3.9 Hz, 0.9 Hz, 1H), 1.76 (s, 6H). ^{13}C NMR (75 MHz, $CDCl_3$, ppm) δ 163.4, 160.4, 150.6, 150.4, 141.4, 128.2, 115.3, 107.7, 104.7, 27.8.

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CONFLICT OF INTEREST

Declared none.

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