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# Caged agonist of P2Y<sub>1</sub> and P2Y<sub>12</sub> receptors for light-directed facilitation of platelet aggregation

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## ABSTRACT

We have prepared a caged form (MRS2703) of a potent dual agonist of the P2Y<sub>1</sub> and P2Y<sub>12</sub> nucleotide receptors, 2-MeSADP, by blocking the β-phosphate group with a 1-(3,4-dimethoxyphenyl)eth-1-yl phosphoester. Although MRS2703 is itself inactive at human P2Y<sub>1</sub> and P2Y<sub>12</sub> receptors expressed heterologously in 1321N1 astrocytoma cells or in washed human platelets, this derivative readily regenerates the parent agonist upon mild irradiation with long-wave UV light (360 nm). The functional effect of the regenerated agonist was demonstrated by a rise in intracellular calcium mediated by either P2Y<sub>1</sub> or P2Y<sub>12</sub> receptors in transfected cells. Washed human platelets exposed to a solution of MRS2703 were induced to aggregate upon UV irradiation. At 1.0 μM MRS2703, full aggregation was achieved within 1 min of irradiation. Thus, this caged nucleotide promises to be a useful probe for potent P2Y receptor activation with light-directed spatial and temporal control.

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## 1. Introduction

Caged molecules, in which light is able to rapidly and site-specifically generate a reactive or biologically active chemical species, have been designed based on the photocleavage of the 2-nitrobenzyl protecting group. The 2-nitrobenzyl group was originally introduced as an orthogonal photoremovable protecting group for use in peptide synthesis by Patchornik et al. [1]. Since then various chemically improved modifications of the photolabile 2-nitrobenzyl group, such as the 1-(3,4-dimethoxyphenyl)eth-1-yl group, have been utilized [2]. The

term ‘caged’ was first coined by Kaplan et al. [19,20], and caged-ATP was the first to be introduced to study organized biological systems. A caged-ATP derivative (P3-1-(2-nitrophenylethyladenosine 5'-triphosphate)) has been used to probe the fast kinetics of muscle contraction, etc. [9]. This principle of photocleavage has also been effectively applied to selective deprotection for synthesis in a spatially addressable fashion on a surface array [3], masking of intracellular and extracellular signaling molecules for biological studies [4–6], and the light-reversible rapid activation of proteins [7,8]. In the area of platelet biology, an oligonucleotide aptamer consisting

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Abbreviations: FBS, fetal bovine serum; MRS2500, (1'R,2'S,4'S,5'S)-4-(2-iodo-6-methylamino-purin-9-yl)-1-[(phosphato)-methyl]-2-(phosphato)-bicyclo[3.1.0]hexane; MRS2703, P<sup>1</sup>-2-(methylthio)adenosyl-P<sup>2</sup>-(RS)-1-(4,5-dimethoxy-2-nitrophenyl)ethyl pyrophosphate, triethylammonium salt; 2-MeSADP, 2-methylthioadenosine 5'-diphosphate; DMEM, Dulbecco's modified Eagle's medium.

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of a ssDNA 15mer was caged, by preparation of a thymidine 4-O-(2-nitrophenylethyl) derivative, to provide a UV-light activatable derivative [10]. This derivative when unmasked with light bound to and inactivated R-thrombin, which is a key player in the blood-clotting cascade. Recently, coumarin-based photosensitive protecting groups have been applied to cage biologically active molecules [11].

The P2Y<sub>1</sub> and P2Y<sub>12</sub> receptors are G-protein-coupled receptors of the rhodopsin family present on the surface of platelets that respond to extracellular ADP to induce aggregation [12–14]. This activation is an essential step in the process of platelet aggregation *in vivo*. In *in vitro* studies of platelet function, the activation of both receptors by ADP is required for complete aggregation. Thus, application of a selective antagonist of either the P2Y<sub>1</sub> or the P2Y<sub>12</sub> receptor inhibits aggregation. A potent and selective P2Y<sub>1</sub> receptor nucleotide antagonist MRS2500, in which the ribose moiety is conformationally constrained to “fit” the preferred geometry of the binding site, inhibited both aggregation and the initial shape change in platelets, which are characteristic of the effects of ADP. MRS2500 was also shown to act as a P2Y<sub>1</sub> receptor antagonist *in vivo* to impede systemic thromboembolism and localized arterial thrombosis [14,15].

We previously applied the 2-nitrobenzyl group in the form of a nucleoside 5'-ether to prepare a caged agonist of the A<sub>1</sub> adenosine receptor [16]. In the present study we have synthesized and characterized pharmacologically a 2-nitrobenzyl-type derivative of the potent proaggregatory receptor probe, 2-methylthio-ADP (2-MeSADP), which was shown to activate both P2Y<sub>1</sub> and P2Y<sub>12</sub> receptors on the surface of platelets. 2-MeSADP is approximately three orders of magnitude more potent than ADP as an agonist of these two P2Y receptor subtypes and displays nanomolar-binding affinity at each. Structure-activity studies have been extensively reviewed [12]. This caged nucleotide allows spatial and temporal control over the proaggregatory signal, as demonstrated in *in vitro* models.

## 2. Materials and methods

### 2.1. Materials

The 1321N1 astrocytoma cells stably transfected with human P2Y<sub>1</sub> and P2Y<sub>12</sub> receptors were generously provided by Prof. T.K. Harden (University of North Carolina, Chapel Hill, NC). Dulbecco's modified Eagle's medium (DMEM) and fetal bovine serum (FBS) were purchased from Life Technologies (Rockville, MD). Plastic collagen-coated cellware was purchased from Becton Dickinson (Bedford, MA). Calcium Mobilization Assay Kit was purchased from Molecular Devices (Sunnyvale, CA).

<sup>1</sup>H NMR spectra were obtained with a Varian Gemini 300 spectrometer using D<sub>2</sub>O as a solvent. The chemical shifts are expressed as relative ppm from HOD (4.80 ppm). <sup>31</sup>P NMR spectra were recorded at room temperature by the use of Varian XL 300 spectrometer (121.42 MHz); orthophosphoric acid (85%) was used as an external standard. Purity of compounds was checked using a Hewlett-Packard 1100 HPLC equipped with a Zorbax Eclipse 5- $\mu$ m XDB-C18 analytical column (250 mm  $\times$  4.6 mm; Agilent Technologies Inc., Palo

Alto, CA). System A: linear gradient solvent system: 5 mM TBAP (tetrabutylammonium dihydrogenphosphate)-CH<sub>3</sub>CN from 80:20 to 40:60 in 20 min; the flow rate was 1 ml/min. Peaks were detected by UV absorption with a diode array detector. High-resolution mass measurements were performed on Micromass/Waters LCT Premier Electrospray Time of Flight (TOF) mass spectrometer coupled with a Waters HPLC system. Purification of the caged nucleotide was carried out by HPLC using a Luna 5  $\mu$ m RP-C18(2) semipreparative column (250 mm  $\times$  4.6 mm; Phenomenex, Torrance, CA). System B: 10 mM TEAA (triethylammonium acetate)-CH<sub>3</sub>CN from 90:10 to 60:40 in 30 min; the flow rate was 2 ml/min.

1-(4,5-Dimethoxy-2-nitrophenyl)-diazaoethane Generation Kit was purchased from Invitrogen Molecular Probes (Eugene, OR). 2-MeSADP (1) and other reagents and solvents were purchased from Sigma-Aldrich (St. Louis, MO).

### 2.2. Chemical synthesis

#### 2.2.1. P<sup>1</sup>-2-(methylthio)adenosyl-P<sup>2</sup>-(RS)-1-(4,5-dimethoxy-2-nitrophenyl)ethyl pyrophosphate, triethylammonium salt (4)

To prepare the reactive diazoethane (3) a solution of 4,5-dimethoxy-2-nitroacetophenone hydrazone (2, 25 mg, 0.10 mmol) in DMF (1 ml) was treated with MnO<sub>2</sub> (100 mg, 1.15 mmol). After stirring for 30 min at room temperature, the reaction mixture was filtered through Celite<sup>®</sup> diatomaceous earth (100 mg) contained in a Pasteur pipette to obtain a red solution that contains the 1-(4,5-dimethoxy-2-nitrophenyl)-diazaoethane (3). This solution was used in the next step without further purification. To a solution of 2-MeSADP (1, 5 mg, 0.008 mmol) in H<sub>2</sub>O (1 ml) was added the red solution that contains the 1-(4,5-dimethoxy-2-nitrophenyl)-diazaoethane (3). To avoid light the reaction flasks are covered with aluminum foil. The reaction mixture was stirred at room temperature for 24 h. The aqueous phase was treated with CH<sub>2</sub>Cl<sub>2</sub>. After removal of the solvent, the residue was purified by semipreparative HPLC as described above to obtain the mixture of diastereoisomers 4ab (4.8 mg, 66%) as a white solid. <sup>1</sup>H NMR (D<sub>2</sub>O)  $\delta$  8.05 (s, 1H), 7.97 (s, 1H), 7.44 (s, 1H), 7.39 (s, 1H), 7.32 (s, 1H), 7.19 (s, 1H), 6.04 (m, 2H), 5.90 (d, *J* = 4.8 Hz, 1H), 5.82 (d, *J* = 4.8 Hz, 1H), 4.50 (m, 1H), 4.41 (m, 1H), 4.33 (m, 1H), 4.30 (m, 1H), 4.21 (m, 1H), 4.13 (m, 3H), 3.99 (m, 2H), 3.94 (s, 1H), 3.91 (s, 1H), 3.71 (s, 1H), 3.68 (s, 1H), 2.56 (s, 1H), 2.54 (s, 1H), 1.50 (d, *J* = 6.3 Hz, 6H); <sup>31</sup>P NMR (D<sub>2</sub>O)  $\delta$  -11.17 (d, *J* = 20.8 Hz), -11.33 (d, *J* = 18.3 Hz), -11.82 (d, *J* = 20.8 Hz), -12.18 (d, *J* = 18.9 Hz); HRMS *m/z* found 681.0742 (*M* - H<sup>+</sup>)<sup>-</sup>. C<sub>21</sub>H<sub>27</sub>N<sub>6</sub>O<sub>14</sub>P<sub>2</sub>S requires 681.0781; HPLC (system A) 15.2 min.

HPLC analysis using System A was carried out on a solution of MRS2703 (50  $\mu$ M in PBS) before and after irradiation (as described in Section 2.4) for 2 min. Following this irradiation all of the MRS2703, having a retention time (r.t.) of 15.2 min, had disappeared and the major peak observed was a new peak with the identical r.t. as the parent 2-MeSADP (12.9 min).

### 2.3. Cell culture

Human 1321N1 astrocytoma cells stably transfected with the human P2Y<sub>1</sub> or P2Y<sub>12</sub> receptors were grown at 37 °C in a humidified incubator with 5% CO<sub>2</sub>/95% air in DMEM/F-12 medium (1:1) supplemented with 10% FBS, 100 units/ml

penicillin, 100 mg/ml streptomycin, and 2 mM L-glutamine. The cells were passaged using trypsinization every 4-5 days.

#### 2.4. Calcium mobilization assay

Cells were grown overnight in 100  $\mu$ l of medium in 96-well flat-bottom plates at 37 °C at 5% CO<sub>2</sub> or until they reached ~60-80% confluency. The calcium assay kit (Molecular Devices, Sunnyvale, CA, USA) was used as directed with no washing of cells and with probenecid added to the loading dye at a final concentration of 2.5 mM to increase dye retention. Cells were loaded with 50  $\mu$ l dye with probenecid in each well and incubated for 45 min at room temperature. The compound plate was prepared with dilutions of various compounds in Hank's buffer at pH 7.2. For irradiation of the caged agonist, a solution of MRS2703 in PBS was exposed to UV light under various conditions of wavelengths and for a range of times. A UV light (Spectroline model, UV-400; Spectronics Co., Westbury, USA) was outfitted with a 360 nm filter (Edmund Industrial Optics, Barrington, NJ, USA), and a PBS solution of MRS2703 (20  $\mu$ M) in a glass cuvette was irradiated at a distance of 1.0 cm. Room lighting was dimmed during the experiments using MRS2703 under non-irradiated conditions. Samples were run in duplicate with a Flexstation I (Molecular Devices) at room temperature. Cell fluorescence (excitation = 485 nm; emission = 525 nm) was monitored following exposure to a compound. Increases in intracellular calcium are reported as the maximum fluorescence value after exposure minus the basal fluorescence value before exposure.

#### 2.5. Preparation of human washed platelets

Human washed platelets were prepared as previously described [22]. Briefly, fresh blood obtained from healthy donors was centrifuged at 175  $\times$  g for 15 min at 37 °C, platelet rich plasma was removed, and centrifuged at 1570  $\times$  g for 15 min at 37 °C. The platelet pellet was washed twice in Tyrode's buffer (137 mM NaCl, 2 mM KCl, 12 mM NaHCO<sub>3</sub>, 0.3 mM NaH<sub>2</sub>PO<sub>4</sub>, 1 mM MgCl<sub>2</sub>, 2 mM CaCl<sub>2</sub>, 5.5 mM glucose, 5 mM Hepes, pH 7.3) containing 0.35% human serum albumin,

and finally resuspended at a density of 3  $\times$  10<sup>5</sup> platelets/ $\mu$ l in the same buffer in the presence of 0.02 U/ml of the ADP scavenger apyrase (adenosine 5'-triphosphate diphosphohydrolase, EC 3.6.1.5), a concentration sufficient to prevent desensitization of platelet ADP receptors during storage. Platelets were kept at 37 °C throughout all experiments.

#### 2.6. Platelet aggregation studies

Aggregation was measured at 37 °C by a turbidimetric method in a dual-channel Payton aggregometer (Payton Associates, Scarborough, Ontario, Canada). A solution of MRS2703 in PBS was exposed to UV light (12 W light source, with a peak UV wavelength of 254 nm) for various time periods and immediately added to a 450  $\mu$ l aliquot of platelet suspension stirred at 1100 rpm, in the presence of human fibrinogen (0.8 mg/ml), in a final volume of 500  $\mu$ l.

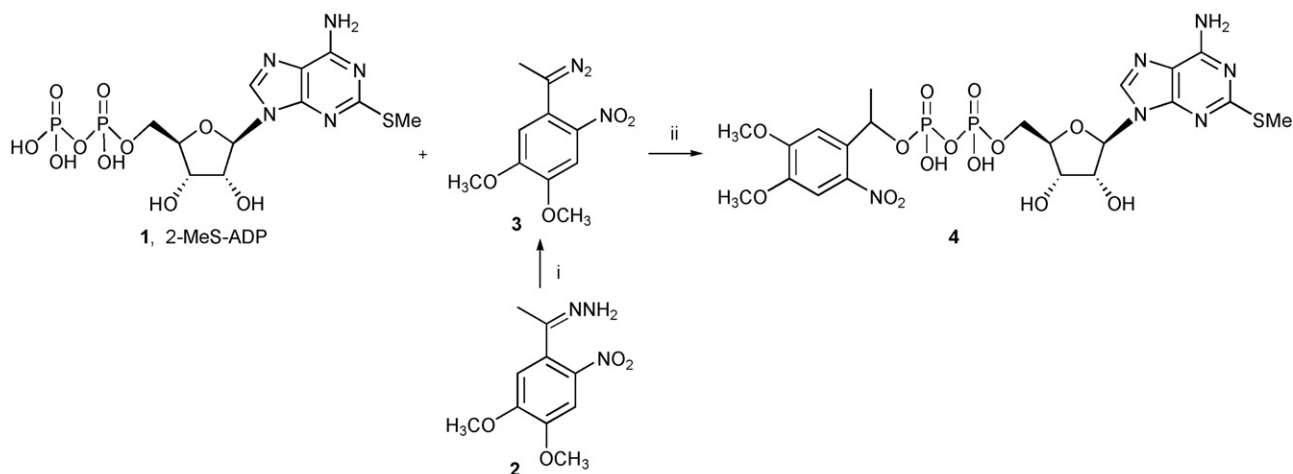
#### 2.7. Statistical analysis

Pharmacological parameters were analyzed with the GraphPAD Prism software (version 4.0, GraphPAD Prism, San Diego, CA). Data were expressed as mean  $\pm$  standard error. Statistical significance was calculated using the Student's t-test. *P* values less than 0.05 (*P* < 0.05) were considered to be statistically significant.

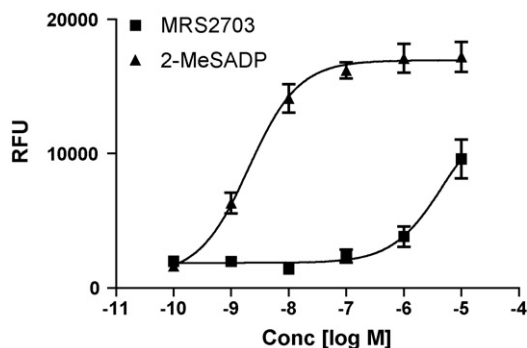
### 3. Results

#### 3.1. Design of reagent and chemical synthesis

The caged nucleotide MRS2703 (4) was prepared from 2-MeSADP (1) and the *in situ* generated phenyl diazoethane derivative (3) as shown in Fig. 1 [2]. The presence of the veratryl group is intended to allow unmasking by light of longer wavelength than is required for simple 2-nitrobenzyl derivatives. The presence of the ethyl moiety reduces side reactions as a result of the generation of photoproducts, which in this case would be an acetylaryl compound that is less reactive



**Fig. 1** – Structure and preparation of caged nucleotide MRS2703 4. The caged nucleotide could be reconverted to the starting material, agonist 2-MeSADP 1 upon irradiation. Reagents and conditions: (i) MnO<sub>2</sub>, DMF, r.t. (ii) H<sub>2</sub>O, r.t.

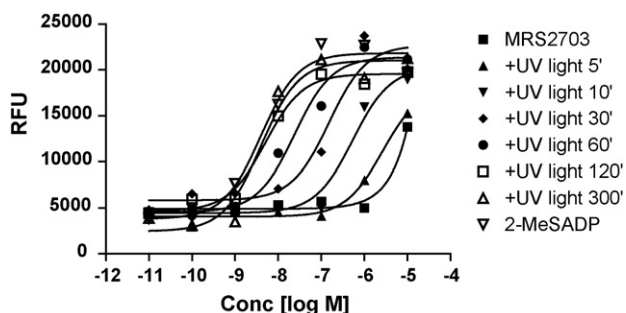


**Fig. 2** – The ability of 2-MeSADP, and the caged 2-MeSADP (MRS2703) to activate the human P2Y<sub>1</sub> receptor stably expressed in 1321N1 astrocytoma cells. Cells were loaded with 50  $\mu$ l dye with 2.5 mM probenecid in each well and incubated for 45 min at room temperature. The agonists were tested without exposure to UV light. 2-MeSADP induced a concentration-dependent rise in  $[Ca^{2+}]_i$  levels, with an EC<sub>50</sub> value of  $2.03 \pm 0.35$  nM ( $n = 3$ ), similar to previously reported values [18].

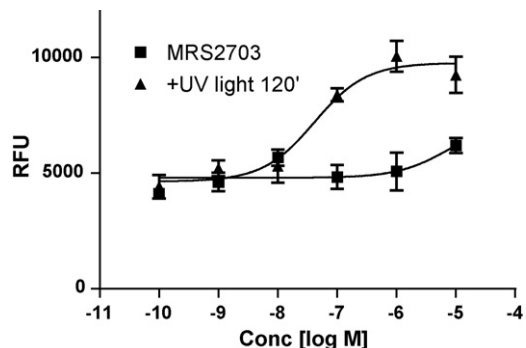
than the corresponding benzaldehyde. HPLC analysis indicated a nearly complete conversion of a dilute solution of MRS2703 to the parent 2-MeSADP during a 2 min irradiation.

### 3.2. Characterization in astrocytoma cells

The photoregeneration of the potent P2Y receptor agonist 2-MeSADP was first tested using the recombinant human P2Y<sub>1</sub> receptor expressed in 1321N1 astrocytoma cells (Fig. 2). In a



**Fig. 3** – Concentration–response curves for the increase in  $[Ca^{2+}]_i$  induced by the agonist mixtures, upon UV-light irradiation, in hP2Y<sub>1</sub>-1321N1 cells. Cells were loaded with 50  $\mu$ l dye with 2.5 mM probenecid in each well and incubated for 45 min at room temperature. The compound plate was prepared with dilutions of various compounds in Hank's buffer at pH 7.2. Samples were run with a Flexstation I (Molecular Devices), and cell fluorescence (excitation = 485 nm; emission = 525 nm) was monitored following exposure to the agonist. Increases in intracellular calcium are reported as the maximum fluorescence value after exposure minus the basal fluorescence value before exposure. The data shown are from an experiment performed in duplicate representative of two to four separate experiments of similar results performed in duplicate.



**Fig. 4** – The ability of MRS2703 and the un-caged MRS2703 (upon 120 s irradiation) to activate the human P2Y<sub>12</sub> receptor stably expressed in 1321N1 astrocytoma cells. Cells were loaded with 50  $\mu$ l dye with 2.5 mM probenecid in each well and incubated for 45 min at room temperature. Activation of the P2Y<sub>12</sub> receptor by either 2-MeSADP or the irradiated MRS2703 induced a concentration-dependent increase in  $[Ca^{2+}]_i$  that was not observed in control 1321N1 astrocytoma cells. The methods used were described in Section 2. Results are from three separate experiments performed in duplicate.

control experiment these cells were exposed to 2-MeSADP, which induced a concentration-dependent rise in  $[Ca^{2+}]_i$  levels, with an EC<sub>50</sub> value of  $2.03 \pm 0.35$  nM ( $n = 3$ ), similar to previously reported values [18]. MRS2703, without exposure to UV light, was weak in the stimulation of calcium mobilization, with an EC<sub>50</sub> value estimated to be  $\geq 10,000$  nM (Fig. 2).

For irradiation of the caged agonist, a dilute solution of MRS2703 (20  $\mu$ M) in PBS in a quartz cuvette was exposed to 360 nm UV light (from a portable light source equipped with a filter) for a range of times. The production of 2-MeSADP upon irradiation was determined using a bioassay consisting of stimulation of calcium mobilization in astrocytoma cells stably expressing the human P2Y<sub>1</sub> receptor. At 10  $\mu$ M MRS2703, a partial (~50% of maximal) stimulation of calcium mobilization was noted. The potency of the solution gradually increased over a period of 5 min, such that the final activity reached the same potency and maximal effect as 2-MeSADP (Fig. 3). This indicated that most of the MRS2703 was effectively un-caged upon irradiation to provide the biologically active P2Y<sub>1</sub> receptor agonist, 2-MeSADP. As a control, the activity of 2-MeSADP before and after 5 min irradiation under the same condition was not changed. The EC<sub>50</sub> values of the MRS2703 after exposure to UV light for 0, 5, 10, 30, 60, 120 and 300 s were demonstrated to be  $\geq 10,000$ ,  $3420 \pm 586$ ,  $633 \pm 128$ ,  $122 \pm 36$ ,  $22 \pm 7$ ,  $3.9 \pm 1.4$  and  $2.8 \pm 0.7$  nM ( $n = 3$ ), respectively. As a control, the EC<sub>50</sub> value of 2-MeSADP after 300 s exposure to UV light was shown to be  $2.6 \pm 0.8$  nM, which is similar to that of the non-irradiated. The results suggest that the UV-light exposure only induces the uncaging not the breakdown of 2-MeSADP.

2-MeSADP is an agonist of P2Y<sub>1</sub> and P2Y<sub>12</sub> receptors. Thus, we next tested if the caged 2-MeSADP, MRS2703, was able to activate P2Y<sub>12</sub> receptors, either before or following irradiation. The activity at the P2Y<sub>12</sub> receptor was tested using a bioassay consisting of stimulation of calcium mobilization in stably

transfected 1321N1 astrocytoma cells. Although in a previous study the 1321N1 cells were cotransfected with a chimeric G protein,  $G_{\alpha q/i}$ , to confer a capacity of the Gi-linked  $P2Y_{12}$  receptor to directly activate phospholipase C [26], in the current study only the endogenous G proteins were present. The coupling of the  $P2Y_{12}$  receptor to a calcium response in the 1321N1 cells was likely as reported previously [17] through G-protein  $\beta, \gamma$  subunits. Control 1321N1 cells did not display a rise in  $[Ca^{2+}]_i$  levels in response to 2-MeSADP, but in those cells expressing the  $P2Y_{12}$  receptor 2-MeSADP induced a concentration-dependent rise with an  $EC_{50}$  value of  $23 \pm 6$  nM. Fig. 4 shows that MRS2703, at concentrations up to  $10 \mu M$ , did not induce a rise in  $[Ca^{2+}]_i$  levels. However, upon 120 s irradiation, the un-caged nucleotide preparation showed an  $EC_{50}$  of  $41.4 \pm 12.8$  nM ( $n = 3$ ).

### 3.3. Characterization in human platelets

The photoregeneration of 2-MeSADP was then studied in washed human platelets (Fig. 5).

MRS2703 ( $1 \mu M$ ), without exposure to UV light, did not induce aggregation or shape change of human washed platelets (Fig. 5A). In contrast, exposure of the compound ( $1$  or  $0.05 \mu M$ ) to UV light for 5 s or longer resulted in platelet aggregation (Fig. 5A and B). At the highest concentration of MRS2703 tested ( $1 \mu M$ ), full aggregation was achieved within 30 s of irradiation. The extent of aggregation was comparable to aggregation induced by 2-MeSADP at a concentration of  $1 \mu M$ . The ability to induce platelet aggregation was slower at a lower concentration of MRS2703 ( $0.05 \mu M$ ) (Fig. 5B).

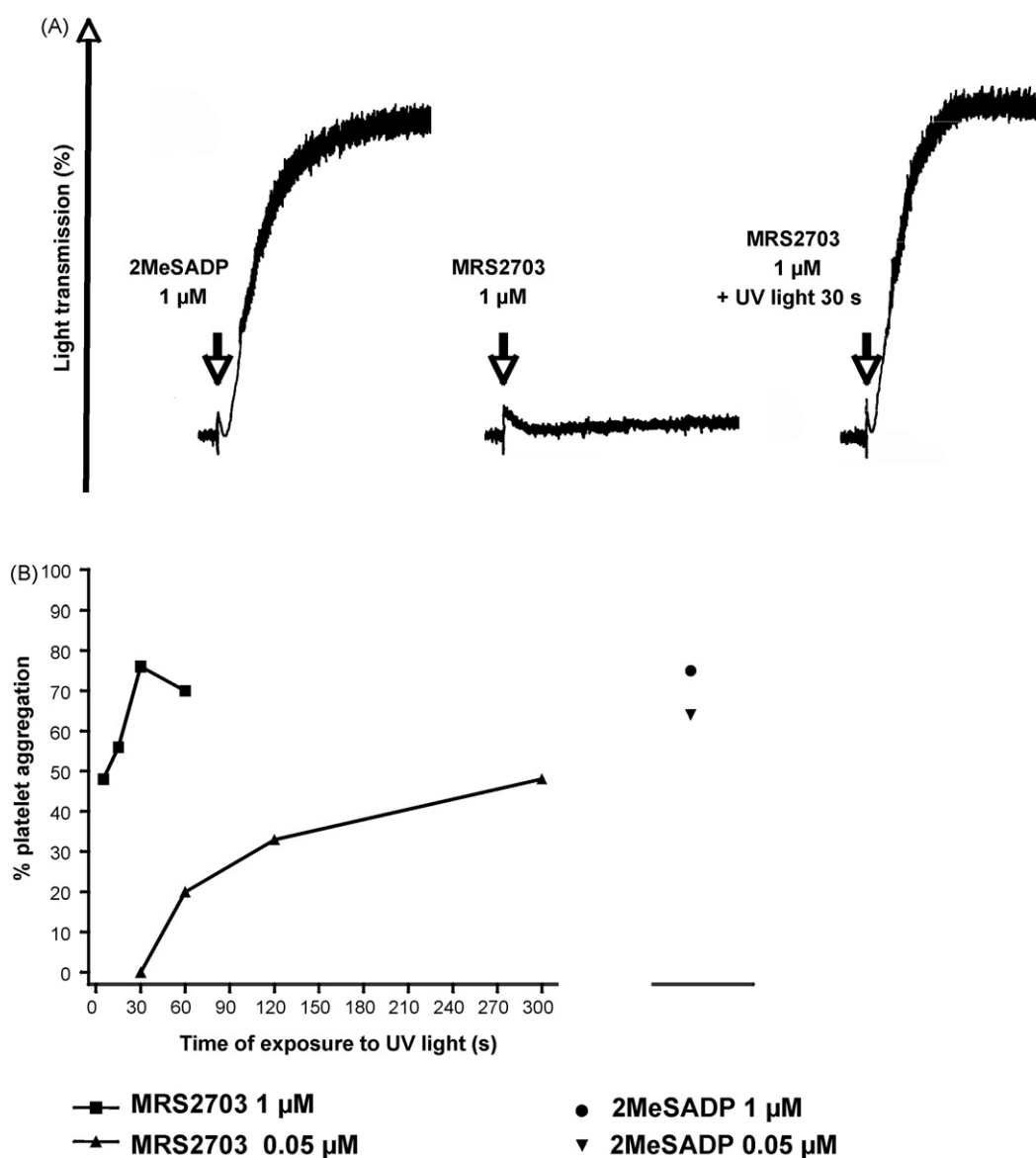


Fig. 5 – Aggregation of human washed platelets induced by MRS2703 following UV irradiation. (A) Representative washed human platelet aggregation measurements. (B) Effect of time exposure of MRS2703 ( $0.05$  or  $1 \mu M$ ) to UV light on the extent of platelet aggregation. The methods used were described in Section 2.

#### 4. Discussion

In the present study, we have synthesized and pharmacologically characterized the nucleotide derivative MRS2703. The photosensitive 1-(3,4-dimethoxyphenyl)eth-1-yl protecting group was placed at the  $\beta$ -phosphate of 2-MeSADP. This 2-nitrobenzyl-type group selected for "caging" the nucleotide agonist derivative is additionally substituted with 4,5-dimethoxy groups and an  $\alpha$ -methyl moiety for light absorption at longer wavelengths and less reactive photo-side products, respectively [2,6]. Prior to irradiation, MRS2703 is inactive at both P2Y<sub>1</sub> and P2Y<sub>12</sub> receptors. However, upon UV-light exposure, it potently activated both P2Y<sub>1</sub> and P2Y<sub>12</sub> receptors stably expressed in 1321N1 astrocytoma cells, and induced platelet aggregation *in vitro*. Thus, it is suggested that the caged agonist for P2Y<sub>1</sub> and P2Y<sub>12</sub> receptors should be useful in controlling cell signaling. The inactivity of non-irradiated MRS2703 at P2Y<sub>1</sub> and P2Y<sub>12</sub> receptors is consistent with the hypothesis that blocking the  $\beta$ -phosphate as a terminal ester precludes high-affinity binding to these two receptor subtypes. We have not evaluated the light-directed activation of the P2Y<sub>13</sub> receptor by MRS2703. However, such activation is expected because the parent nucleotide and photoproduct, 2-MeSADP, is a potent agonist of the P2Y<sub>13</sub> receptor [1,26].

Although caged-ADP and caged-ATP have been available previously, they are not selective agonists for the P2Y<sub>1</sub> and P2Y<sub>12</sub> receptors. Thus, potentially non-specific effects could result using these nonselective agonists. Compared with the non-caged 2-MeSADP, MRS2703 could be metabolically more stable, because it is predicted that caged 2-MeSADP as a  $\beta$ -phosphate ester would be resistant to certain phosphatases by analogy to dinucleotides [21]. Thus, caged 2-MeSADP may be used as a relatively stable source of 2-MeSADP to be used in mechanistic studies.

The caged molecules, such as MRS2703, have the potential to achieve precise temporal control of signaling in mechanistic studies. Investigation of the mechanisms of action of the biological systems, such as cells, requires special techniques to introduce effector molecules rapidly with known concentration and location. Time-resolved measurements on such systems may be impossible if the diffusion of target molecules to the cells is slow or if complications resulted from degradation of the molecules or desensitization of the biological response. One approach to this problem is to introduce the molecule rapidly and uniformly by pulse photolysis of precursor compounds that are biologically inactive. Thus, MRS2703 should be useful for the mechanistic studies of P2Y<sub>1</sub> and P2Y<sub>12</sub> receptor-related biological functions, such as platelet aggregation.

In a similar manner other nucleotides that act as selective P2Y receptor ligands could be caged for rapid photoregeneration. For example, regioselective conversion of one of the monophosphate groups of the selective P2Y<sub>1</sub> antagonist MRS2500, an (N)-methanocarba 3',5'-bisphosphate derivative, to a phosphodiester would be expected to greatly diminish its biological activity. Thus, use of an *o*-nitrobenzyl-type phosphodiester would allow that monophosphate moiety to be regenerated upon irradiation. Alternately, the P2Y<sub>1</sub> selective agonist MRS2365, an (N)-methanocarba derivative of 2-MeSADP [23,26], could be derivatized in a manner analogous

to MRS2703 for the selective light-directed activation of P2Y<sub>1</sub> receptors. Furthermore, caged P2Y receptor ligands, such as MRS2703 and caged MRS2500, may have potential for hemodynamic control in the clinic, in a manner that is more spatially selective than with use of anti-thrombotic drugs. The effects of the MRS2703 must now be evaluated in *in vivo* models. Perhaps this probe will be found useful in electrophysiological studies of the brain in which selected regions can be irradiated, by analogy to caged excitatory amino acid derivatives [24]. Methods for the rapid and noninvasive liberation of transmitters using irradiation have been shown to allow the manipulation of biochemical signals in the brain with submicron spatial resolution [24]. The effects of P2Y<sub>1</sub> receptors, P2Y<sub>12</sub> receptors, and other P2 receptors in cerebral ischemia and in the central nervous system in general are being studied [25]. Using a more sophisticated laser irradiation device, we would expect the *in situ* photocleavage of MRS2703 to occur on a much faster time scale than noted in this study.

In summary, the newly synthesized nucleotide derivative MRS2703 acts as a caged agonist for both P2Y<sub>1</sub> and P2Y<sub>12</sub> receptors, which can be rapidly liberated in biologically active form upon brief irradiation with UV light. Thus, MRS2703 and similar derivatives of other P2Y receptor ligands should be useful for precise control of signaling temporally and spatially in future mechanistic studies.

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