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ARTICLE *in* CHEMICO-BIOLOGICAL INTERACTIONS · JANUARY 2015

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Identification of new inhibitors for human hematopoietic prostaglandin D_2 synthase among FDA-approved drugs and other compounds

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ARTICLE INFO

Article history:
Received 26 September 2014
Received in revised form 22 November 2014
Accepted 8 January 2015
Available online 17 January 2015

Keywords:
Prostaglandin D_2 synthase
FDA-approved drugs
HPGDS inhibitors
Glutathione transferase S1-1

ABSTRACT

Objective: Hematopoietic prostaglandin D_2 synthase (HPGDS) is a member of the Sigma class glutathione transferases (GSTs) catalyzing the isomerization of prostaglandin H_2 to prostaglandin D_2, a mediator of allergy and inflammation responses. Selective inhibitors of human HPGDS are expected to be of therapeutic importance in relieving symptoms related to allergy and asthma. Hence, a collection of diverse FDA-approved compounds was screened for potential novel applications as inhibitors of HPGDS.
Methods: The catalytic activity of purified HPGDS was used for inhibition studies in vitro.
Results: Our inhibition studies revealed 23 compounds as effective inhibitors of HPGDS with IC_{50} values in the low micromolar range. Erythrosine sodium, suramin, tannic acid and sanguinarine sulfate were characterized with IC_{50} values of 0.2, 0.3, 0.4, and 0.6 μM, respectively. Kinetic inhibition analysis showed that erythrosine sodium is a nonlinear competitive inhibitor of HPGDS, while suramin, tannic acid and sanguinarine sulfate are linear competitive inhibitors.
Conclusion: The results show that certain FDA-approved compounds may have pharmacological effects not previously realized that warrant further consideration in their clinical use.

1. Introduction

Prostaglandins (PGs) are bioactive lipid messengers involved in the regulation of various physiological and pathophysiological processes [1]. Biosynthesis of prostaglandins is a complex series of reactions, which is regulated by a variety of enzymes. The process initiates when membrane phospholipids release arachidonic acid (AA) by the action of phospholipases (PLAs). Subsequently, PG endoperoxide synthase known as cyclooxygenase (COX) catalyzes a reaction that converts AA to an unstable prostanoid intermediate PGH_2, which can be converted into various bioactive prostaglandin isomers PGD_2, PGE_2, $PGF_2\alpha$, and PGI_2 by specific enzymes [2,3].

Isomerization of PGH_2 to PGD_2 is performed by the actions of two genetically distinct types of prostaglandin D_2 synthase enzymes, lipocalin PGDS (LPGDS) and hematopoietic PGDS (HPGDS). The former is present in the central nervous system, testis, and the human heart and is also known as glutathione-independent PGDS [4]. The second is a glutathione-dependent PGDS, present in the spleen and the hematopoietic system (HPGDS), being widely distributed in antigen-presenting cells, Th2 lymphocytes, mast cells, and megakaryocytes [5–7]. PGD_2 is synthesized both in the central nervous system and in peripheral tissues, where it appears to mediate various allergic and inflammatory responses by activating two G-protein-coupled receptors, the DP1-receptor and CRTH2 (chemoattractant receptor-homologous molecule expressed on T helper type 2 (Th2) cells), also known as DP2-receptor. In the central nervous system, PGD_2 regulates sleep [8], pain [9] and other activities via the DP1-receptor [10]. In peripheral tissues, it is involved in mediating chemotaxis of eosinophils and basophils to the lung via the DP2-receptor [11] and is considered as a central player in promoting Th2-related allergic inflammation [12]. It has also been shown that overproduction of PGD_2 exacerbates asthmatic reactions in humans [4] and mice models [13]. Thus, HPGDS is considered as a promising therapeutic target for the design of anti-allergic and anti-inflammatory drugs.

HPGDS is the only vertebrate Sigma-class member of the GST superfamily and it requires glutathione (GSH) for isomerization of PGH_2 to PGD_2. HPGDS is a cytosolic homodimeric enzyme with a molecular weight of 23 kDa, and has a similar three-dimensional structure and active site topology as the other GSTs despite low sequence similarity [14]. The monomer can be divided into two domains, with the active site existing at the domain interface. The N-terminal domain contains the GSH binding site, while the C-terminal domain contributes to the PGH_2 binding site or hydrophobic substrate binding site. In addition to the isomerization

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http://dx.doi.org/10.1016/j.cbi.2015.01.014
0009-2797/

reaction, HPGDS catalyzes the conjugation reaction of GSH with many compounds bearing electrophilic functional groups. The reaction mechanism involves activation of GSH by forming and stabilizing a reactive thiolate anion. The hydroxyl group of Tyr8 in the N-terminal domain is involved in the process of stabilization of GSH thiolate anion. The 3-D structural studies of GSTs with GSH, substrate analogue, or with the product reveal that the hydroxyl group of tyrosine and the sulfur atom of the GSH lie within a distance of hydrogen bonding [15].

In the present study we have screened a collection of diverse 1040 FDA-approved compounds as inhibitors of HPGDS, and determined the IC_{50} values of the 23 most effective compounds. We further selected the most potent inhibitors suramin, sanguinarine sulfate, erythrosine sodium, and tannic acid for further kinetic studies, determined the type of inhibition and calculated the inhibition constant (K_i) values. In addition, HPGDS was characterized by determination of activities with a series of substrates used with other members of the GST superfamily.

2. Materials and methods

2.1. Materials

Unless stated otherwise, all the chemicals used for enzymatic activity measurements and kinetic studies were purchased from Sigma–Aldrich (Steinheim, Germany). The US Drug Collection, comprising a set of 1040 compounds was purchased from MicroSource Discovery Systems, Inc.

2.2. Plasmid DNA synthesis and transformation

The gene encoding His_6-tagged human HPGDS was custom synthesized by DNA 2.0, Inc., Menlo Park, CA, and was provided in pJexpress401 expression vector. The construct was introduced into electro-competent *Escherichia coli* XL-1 blue strain via electroporation. Briefly, 2 μl of DNA (20 pg/μl) and 45 μl of *E. coli* XL-1 blue cells were gently mixed and placed in a Gene Pulser® cuvette with 0.1 cm electrode gap (Bio-Rad). After an electric pulse of 4.5 s at a voltage of 1.25 kV, 960 μl of LB medium was added and gently mixed with repeated pipetting. Finally the cells containing DNA and LB medium were incubated at 37 °C in an incubator-shaker at 200 rpm for 45 min in a 10 ml tube. After incubation cells were spread on LB agar plates containing kanamycin and incubated for 16 h at 37 °C. Colonies on the agar plates verified the successful transformation.

2.3. Protein expression and purification

The recombinant HPGDS was expressed in *E. coli* XL-1 blue strain from the pJexpress401expression vector. The cells were grown in 2× YT medium containing 50 μg/ml kanamycin at 37 °C to an OD_{600} of 0.285 and induced with 0.1 mM isopropyl-β-D-thiogalactopyranoside (IPTG) as described by Kolm et al. [16]. After incubation for 16 h at 37 °C, the cells were harvested at 7000 rpm for 10 min at 4 °C (Avanti J-20 XP Beckman Coulter USA). The supernatant was discarded and the pellet containing bacteria was resuspended in 30 ml of ice-cold buffer A (20 mM sodium phosphate pH 7.4, containing 85 mM imidazole, 0.5 M NaCl, 10 mM β-mercaptoethanol, 0.02% NaN_3), and 0.2 mg/ml of lysozyme and one tablet of EDTA-free protease inhibitor (Roche Germany). After incubation for 30 min on ice bath, the cells were lysed by sonication (Vibra cell USA) and 5 × 20 s treatments were employed at an output control of 7.5 with an interval of 1 min on ice bath to avoid heating the sample. The resultant suspension was centrifuged at 15,000 rpm for 1 h at 4 °C. The cell pellet was discarded and the supernatant containing the enzyme was collected and loaded on pre-equilibrated Ni-IMAC (Amersham Biosciences, Uppsala Sweden). Unbound proteins were washed away with buffer A until base line absorbance at 280 nm was recorded by using Nanodrop. The HPGDS was eluted with buffer A containing 0.5 M imidazole at a flow rate of 2 ml/min. The eluted enzyme fractions were pooled and dialyzed overnight against 10 mM Tris HCl buffer pH 7.8, containing 0.2 mM DTT and 1 mM EDTA. The protein was concentrated and the concentration was measured by Bradford Standard Assay (Bio-Rad USA). The homogeneity and the purity of the enzyme was confirmed by SDS–PAGE analysis, using a 12.5% (w/v) polyacrylamide resolving gel [17], and small aliquots were saved at −80 °C.

2.4. Enzyme activity assay

The enzymatic activities of purified HPGDS with 1 mM 1-chloro-2,4-dinitrobenzene (CDNB), 1 mM 1-fluoro-2,4-dinitrobenzene (FDNB), and 0.4 mM isothiocyanates accompanying 1 mM GSH conjugation were measured spectrophotometrically in 0.1 M sodium phosphate buffer, pH 6.5 at 30 °C. The stock solutions for isothiocyanates were prepared in acetonitrile (2% final concentration in the assay), and for CDNB and FDNB in ethanol (5% final concentration in the assay). The molar extinction coefficients used for calculations were $\Delta\varepsilon_{340nm} = 9600\ M^{-1}cm^{-1}$ for CDNB and FDNB [18], $\Delta\varepsilon_{274nm} = 7450\ M^{-1}cm^{-1}$ for allyl isothiocyanate, $\Delta\varepsilon_{274nm} = 9250\ M^{-1}cm^{-1}$ for benzyl isothiocyanate, $\Delta\varepsilon_{274nm} = 8520\ M^{-1}cm^{-1}$ for cyclohexyl isothiocyanate, $\Delta\varepsilon_{274nm} = 8350\ M^{-1}cm^{-1}$ for propyl isothiocyanate and $\Delta\varepsilon_{274nm} = 8890\ M^{-1}cm^{-1}$ for phenethyl isothiocyanate [19], respectively.

2.5. Enzyme inhibition assays

The conjugation activity of GSH with CDNB by HPGDS was used as a biochemical assay for inhibition studies. Screening of the US drug library was carried out in triplicate by using 96 wells plate reader (Spectramax PLUS 384 Molecular Devices). Reactions were monitored at 340 nm for a time course of 1 min at 30 °C in 0.1 M sodium phosphate buffer pH 6.5. The concentrations of GSH and CDNB were kept constant at 1 mM in the final reaction volume of 300 μl. For screening of the whole library, a final drug concentration of 10 μM was used in the reaction mixture of 300 μl. The concentration of the enzyme used was 4.85 ng per 300 μl. All the drugs were available as stock solutions in 10 mM in DMSO, and the final concentration of DMSO in the 300 μl reaction volume was 3.3% v/v for the measurements of any compound. This DMSO concentration did not inhibit the enzyme activity. IC_{50} values of potent compounds were calculated by different concentration points where the solubility of the drug allowed and the background activities were corrected by same solvent concentrations. For the determination of K_i values of the most potent compounds, the concentration of CDNB was kept constant at 1 mM, while the concentrations of the second substrate GSH and the inhibitors were varied [20].

2.6. Data analysis

GraphPad Prism version 6.0 was used for the analysis of inhibition data. IC_{50} values of the selected compounds were calculated by plotting percentage remaining activities of the enzymes against $\log_{10}$ of at least 7 different concentrations of the inhibitor using nonlinear regression (one site competition). For the determination of K_i values and the type of inhibition, two methods were employed to see the effect of the inhibitor on both V_{max} and K_m values. This was primarily done by plotting the reciprocal of the rate of reactions against the different concentrations of inhibitor as a Dixon plot and secondarily by double reciprocal plots.

3. Results

3.1. Enzymatic activities of HPGDS with alternative substrates

The recombinantly expressed and purified HPGDS was functionally characterized with alternative electrophilic substrates comprising aryl halides and isothiocyanates (Table 1). Our results were essentially in agreement with previous investigations on the catalytic activities of HPGDS with aryl halides [21]. In addition our study included a range of isothiocyanates, featuring both aliphatic and aromatic substituents. The results revealed that the enzyme has lower specific activities with aliphatic isothiocyanates than with their aromatic analogs, indicating that the aromatic substituents promote high affinity for the active site of HPGDS.

Table 1
Specific activities of human HPGDS with alternative substrates. The results are the means of three replicate measurements ± S.E. The background reactions without enzyme were measured by using the same concentration of the solvent and subtracted from the rates in the presence of enzyme.

Substrate	Specific activity (μmol min^{-1} mg^{-1})
Aryl halides	
1-chloro-2,4-dinitrobenzene	4.0 ± 0.5
2,4-dinitro-1-fluorobenzene	28.7 ± 2.6
Organic isothiocyanates	
Allyl isothiocyanate	4.0 ± 0.2
Benzyl isothiocyanate	32.3 ± 1.2
Cyclohexyl isothiocyanate	14.2 ± 0.9
Propyl isothiocyanate	7.3 ± 0.5
Phenethyl isothiocyanate	57.6 ± 3.4

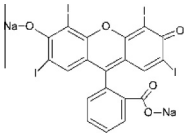
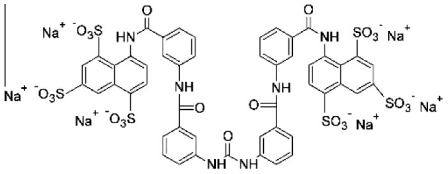
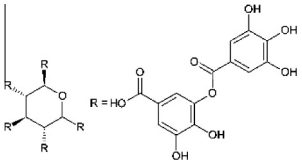
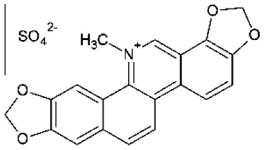
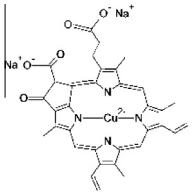
3.2. Inhibition of HPGDS by US drug library compounds

The catalytic activity of HPGDS with CDNB was used for screening the inhibitory effects of the compounds in the US drug library. The preliminary screening of the library was carried out at 10 μM inhibitor concentration. Out of this diverse drug library, 23 compounds giving more than 60% inhibition were selected as the best inhibitors of HPGDS and their IC_{50} values were determined by varying the concentration of the drug. A number of additional compounds were also inhibitory, but their potencies were lower than those in Table 2 and were therefore not further investigated. Fig. 1 shows a representative plot of percentage remaining activity of the enzyme against inhibitor concentration. The IC_{50} values of the selected best inhibitors of HPGDS and their corresponding chemical structures are summarized in Table 2. Erythrosine sodium, suramin, tannic acid and sanguinarine sulfate were the most potent inhibitors of HPGDS with IC_{50} values of 0.2, 0.3, 0.4, and 0.6 μM, respectively. Ethacrynic acid was found to be the least potent of the selected inhibitors with an IC_{50} value of 44 μM.

3.3. Kinetic analysis of HPGDS inhibition by the most potent inhibitors

On the basis of IC_{50} values, the compounds erythrosine sodium, suramin, sanguinarine sulfate and tannic acid were selected as the most potent inhibitors of HPGDS. The effect of the compounds was determined at different concentrations with respect to GSH as var-

Table 2
IC_{50} values of the most potent inhibitors of HPGDS. The fractional activities were measured at 340 nm using constant GSH and CDNB concentrations of 1 mM as a function of varying concentrations of the selected compounds in 0.1 M sodium phosphate buffer, pH 6.5 at 30 °C. The IC_{50} values were obtained by nonlinear regression analysis.

No	Compound	Structural formula	IC_{50} value (μM)
1	Erythrosine sodium		0.2
2	Suramin		0.3
3	Tannic acid		0.4
4	Sanguinarine sulfate		0.6
5	Chlorophyllide Cu complex Na salt		1.7

(*continued on next page*)

Table 2 (*continued*)

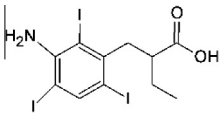
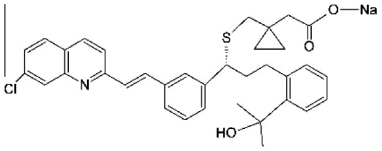
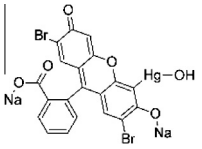
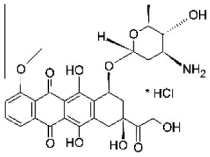
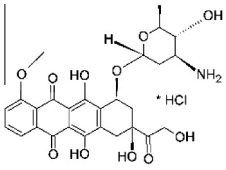
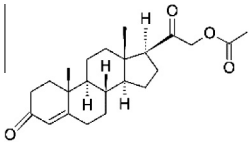
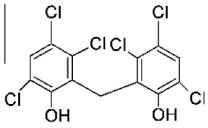
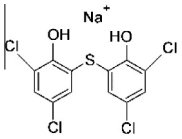
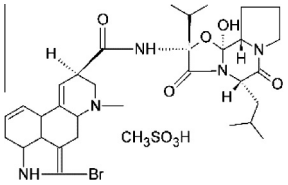
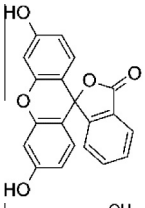
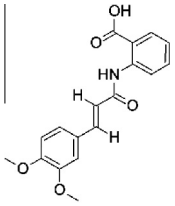
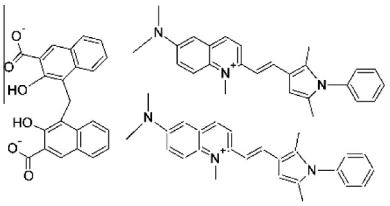
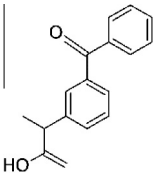
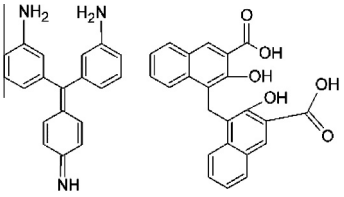
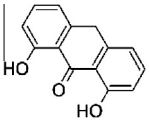
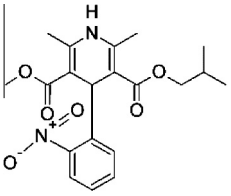
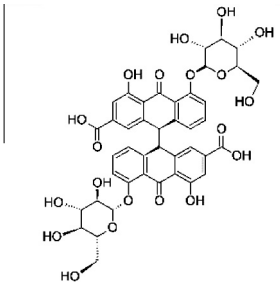
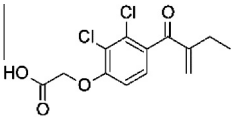
No	Compound	Structural formula	IC_{50} value (μM)
6	Iopanic acid		1.8
7	Montelukast sodium		2.0
8	Merbromin		3.3
9	Candesartan cilextil		5.5
10	Epirubicin hydrochloride		8.4
11	Desoxycorticosterone acetate		8.6
12	Hexachlorophene		8.9
13	Bithionate sodium		12.0
14	Bromocriptine mesylate		13.2
15	Fluorescein		13.4
16	Tranilast		13.7

Table 2 (*continued*)

No	Compound	Structural formula	IC_{50} value (μM)
17	Pyrvinium pamoate		17.2
18	Ketoprofen		20.3
19	Pararosaniline pamoate		20.8
20	Anthralin		23.7
21	Nisoldipine		29.0
22	Sennoside A		39.3
23	Ethacrynic acid		44

ied substrate. The type of inhibition was evaluated from Dixon and double reciprocal plots and the K_i values were determined by non-linear regression analysis (Table 3). The Dixon plot of the most potent inhibitor, erythrosine sodium showed nonlinear behavior with the increase in the concentration of the inhibitor (Fig. 2A), so a single K_i value for this compound could not be obtained due to the nonlinear effect. The Dixon and double reciprocal plots of erythrosine sodium (Fig. 2A and B) showed nonlinear competitive inhibition, while tannic acid (Fig. 3A and B), suramin (Fig. 4A and B), and sanguinarine sulfate (Fig. 5A) exhibited linear competitive inhibition with K_i values 0.1, 0.2, and 1.0 μM, respectively. In the case of sanguinarine sulfate the double reciprocal plot showed nonlinearities, but the inhibitory effect was clearly competitive as shown by the convergence at the 1/*V*-axis (Fig. 5B).

4. Discussion

PGD_2 is a major pro-inflammatory mediator of the allergic responses [6,10,22]. Its actions include vasodilation, attraction of inflammatory cells, platelet aggregation, sleep induction and smooth muscle relaxation [11,23,24]. It has also been shown that overproduction of PGD_2 exacerbates the allergic asthmatic responses in animal models [13,25,26]. Involvement of HPGDS in the biosynthesis of PGD_2 and its contribution to allergic rhinitis makes it an important therapeutic target for anti-allergic and anti-inflammatory drugs. In this perspective, the development and exploration of new compounds for synthase as well as receptor targets will provide new opportunities to prevent the bronchoconstrictor effects of PGD_2 and is supported by the clinical uses of

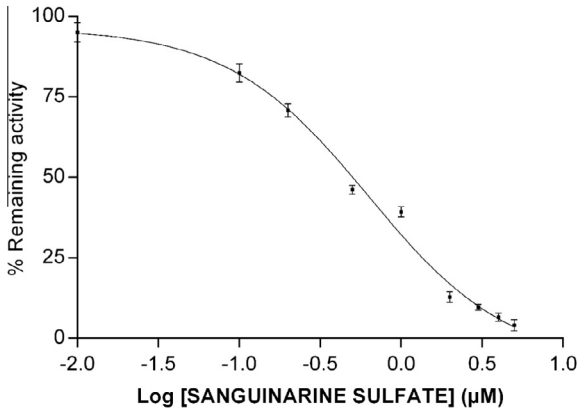


Fig. 1. Inhibition of HPGDS by sanguinarine sulfate using CDNB as the substrate. The IC_{50} value is the concentration of the inhibitor giving 50% inhibition of the enzyme activity in the standard assay system was calculated by plotting log concentration of the inhibitor against percent remaining activity of the enzyme.

Table 3
Characteristics of the selected most potent inhibitors of the HPGDS.

Compound	K_i value (μM)	Type of inhibition
Erythrosine sodium	N.D.	Nonlinear competitive
Tannic acid	0.1	Linear competitive
Suramin	0.2	Linear competitive
Sanguinarine sulfate	1.0	Linear competitive

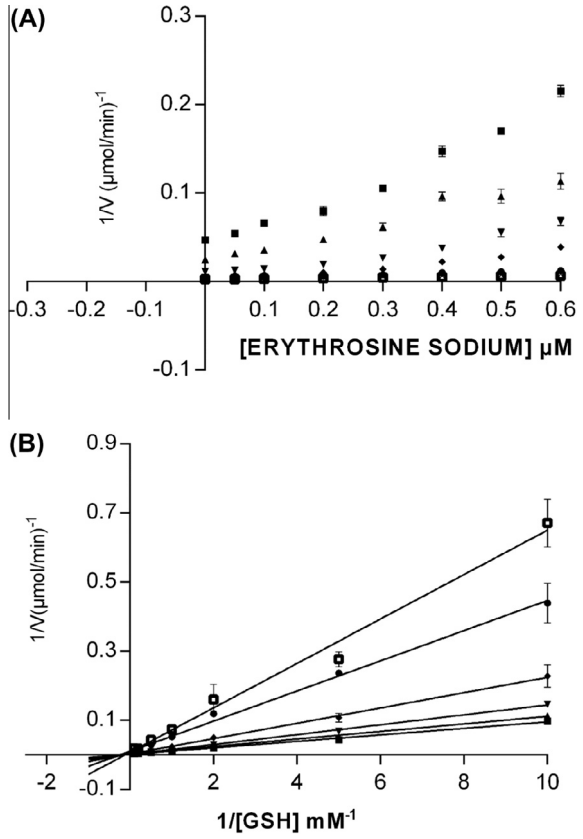


Fig. 2. (A) Dixon plot of reciprocal rates of reactions $(\mu mol/min)^{-1}$ as a function of the concentration of the inhibitor erythrosine sodium for HPGDS at different concentrations of GSH: (■) 0.1 mM, (▲) 0.2 mM, (▼) 0.5 mM, (♦) 1.0 mM, (●) 2.0 mM, (□) 5.0 mM, and (▲) 10 mM by keeping the second substrate (CDNB) constant at 1 mM. Each point in the graph represents the average of three replicate measurements with ±S.E. (B) Double reciprocal plot of inhibitor erythrosine sodium with HPGDS at varied concentrations of GSH. The reaction rates were measured in the absence (■) or presence of (▲) 0.05 μM (▼) 0.1 μM (♦) 0.2 μM (●) 0.4 μM (□) 0.5 μM of the inhibitor. Each point in the graph represents the average of three replicate measurements ±S.E.

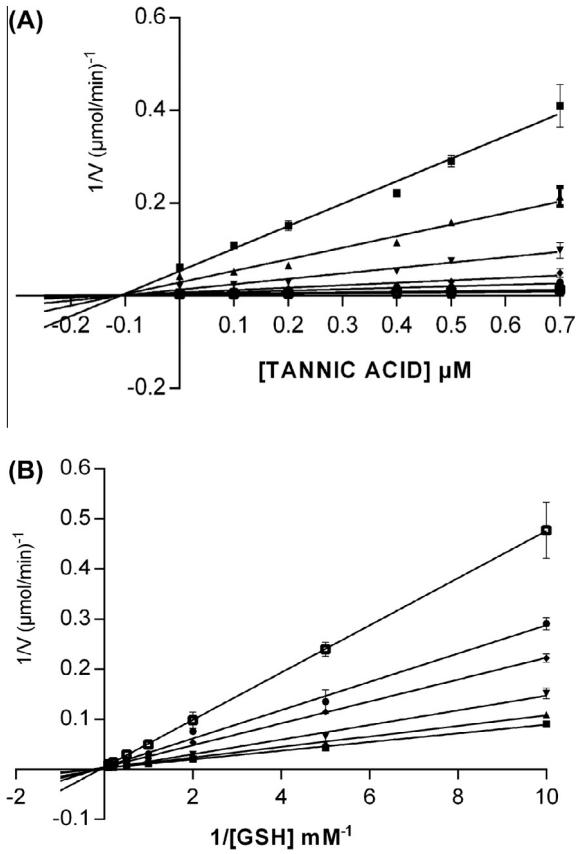
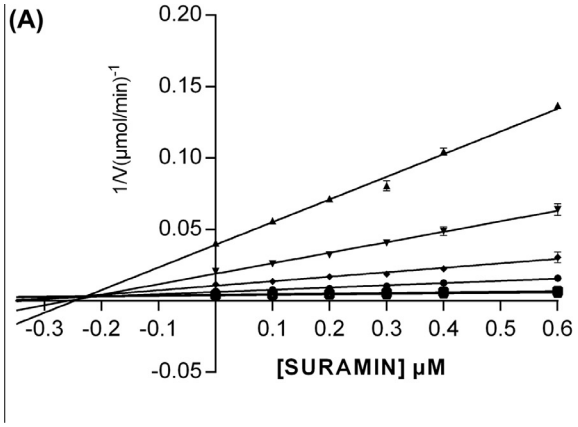


Fig. 3. (A) Dixon plot of reciprocal rates of reactions $(\mu mol/min)^{-1}$ as a function of the concentration of the inhibitor tannic acid for HPGDS at different concentrations of GSH: (■) 0.1 mM, (▲) 0.2 mM, (▼) 0.5 mM, (♦) 1.0 mM, (●) 2.0 mM, (□) 5.0 mM, and (▲) 10 mM by keeping the second substrate (CDNB) constant at 1 mM. Each point in the graph represents the average of three replicate measurements with ±S.E. (B) Double reciprocal plot of inhibitor tannic acid with HPGDS at varied concentrations of GSH. The reaction rates were measured with 1 mM (CDNB) in the absence (■) or presence of (▲) 0.1 μM, (▼) 0.2 μM, (♦) 0.4 μM, (●) 0.5 μM, and (□) 0.7 μM of the inhibitor. Each point in the graph represents the average of three replicate measurements ± S.E.

tranilast, an inhibitor of HPGDS [27] and ramatroban, a receptor antagonist [28] during allergic diseases. Recently, a very comprehensive review has been published, which disclosed a broad range of HPGDS inhibitors and described their potential therapeutic applications [29].

In the present study we have explored novel inhibitors for HPGDS from a wide range of commonly used compounds ranging from small drug-like molecules to very high molecular weight dyes already approved for their use in various therapeutic and other applications. The rationale behind the screening of this diverse collection of 1040 compounds was that such compounds could find their way to clinical use more rapidly as compared to other newly developed drug candidates, as the established compounds are already tested in terms of toxicity and other important pharmacokinetic parameters.

Among all the tested compounds, at least 23 compounds exhibited interesting inhibitory effects on HPGDS activity with IC_{50} values in low micromolar concentrations. Our results (Table 2) demonstrated erythrosine sodium as the most potent inhibitor of HPGDS with an IC_{50} value of 0.2 μM and ethacrynic acid, a well characterized inhibitor and a substrate for other GSTs [30,31] as the weakest inhibitor with an IC_{50} value of 44 μM. Further inhibition kinetic analysis of the four most potent inhibitors namely, erythrosine sodium, suramin, tannic acid and sanguinarine sulfate



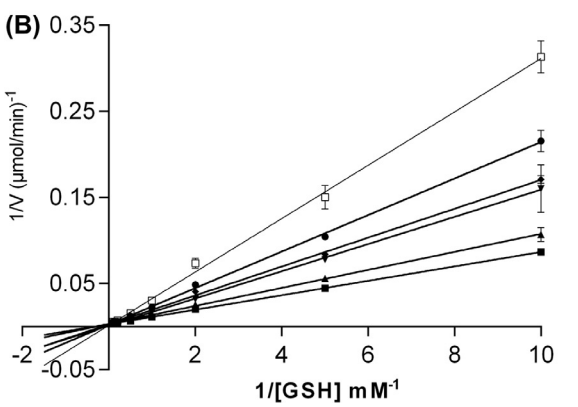
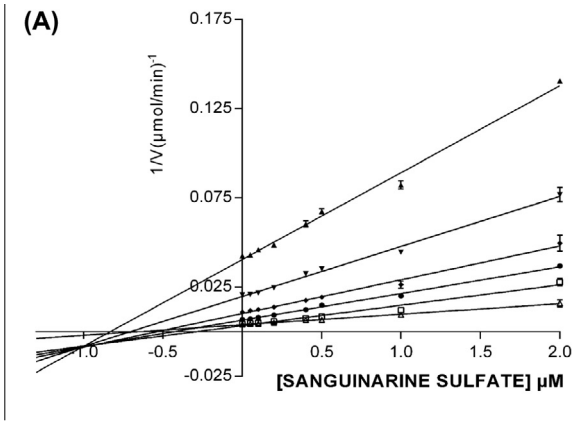


Fig. 4. (A) Dixon plot of reciprocal rates of reactions $(\mu mol/min)^{-1}$ as a function of the concentration of the inhibitor suramin for HPGDS at different concentrations of GSH: (▲) 0.2 mM, (▼) 0.5 mM, (♦) 1.0 mM, (●) 2.0 mM, (◘) 5.0 mM, and (▲) 10 mM by keeping the second substrate (CDNB) constant at 1 mM. Each point in the graph represents the average of three replicate measurements with ±S.E. (B) Double reciprocal plot of inhibitor suramin with HPGDS at varied concentrations of GSH. The reaction rates were measured with 1 mM (CDNB) in the absence (■) or presence of (▲) 0.1 µM, (▼) 0.2 µM, (♦) 0.3 µM, (●) 0.4 µM, and (□) 0.6 µM of the inhibitor. Each point in the graph represents the average of three replicate measurements ±S.E.



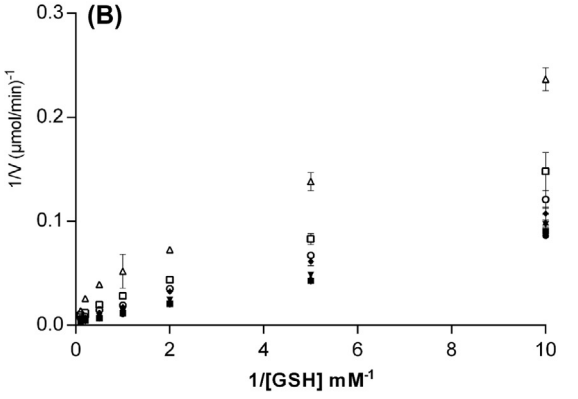


Fig. 5. (A) Dixon plot of reciprocal rates of reactions $(\mu mol/min)^{-1}$ as a function of the concentration of the inhibitor sanguinarine sulfate for HPGDS at different concentrations of GSH: (▲) 0.2 mM, (▼) 0.5 mM, (♦) 1.0 mM, (●) 2.0 mM, (◘) 5.0 mM, and (Δ) 10 mM by keeping the second substrate (CDNB) constant at 1 mM. Each point in the graph represents the average of three replicate measurements with ±S.E. (B) Double reciprocal plot of inhibitor sanguinarine sulfate with HPGDS at varied concentrations of GSH. The reaction rates were measured with 1 mM (CDNB) in the absence (●) or presence of (■) 0.05 µM, (▲) 0.1 µM, (▼) 0.2 µM, (♦) 0.4 µM, (○) 0.5 µM, (□) 1.0 µM and (Δ) 2.0 µM of the inhibitor. Each point in the graph represents the average of three replicate measurements ±S.E.

was done and the results are summarized in Table 3. The compounds tannic acid, suramin, and sanguinarine sulfate showed linearity in their Dixon plots (Figs. 3A, 4A, and 5A), hence their K_i values were determined. However, erythrosine sodium showed nonlinearity in the Dixon plot (Fig. 2A), so no K_i value could be found for this compound. Investigations based on the inhibition studies of various homodimeric and heterodimeric GSTs and their activity measurements with different substrates proposed that the soluble GSTs subunits are kinetically independent [32,33]. However, with some substrates and inhibitors cooperative behavior has been uncovered [34]. HPGDS is a cytosolic homodimeric enzyme, belonging to the Sigma class of GSTs and catalyzing the isomerization of cyclooxygenase product PGH_2 to PGD_2 (Fig. 6). A reasonable explanation for the nonlinear behavior of erythrosine sodium in Dixon plot could possibly be cooperativity between the two subunits of the enzyme. Our kinetic results revealed that erythrosine sodium is a nonlinear competitive inhibitor of HPGDS while suramin, tannic acid, and sanguinarine sulfate showed linear competitive inhibition.

Bioactivities of these novel HPGDS inhibitors enlighten their therapeutic potential, as these compounds are already being used in medical applications. Suramin a polysulfonated naphthylurea, having antiprotozoal, trypanocidal and antiviral effects and is used in the treatments of sleeping sickness caused by trypanosomes [35], onchocerciasis [36], and prostate cancer [25], with few adverse effects that include nausea, vomiting, urticarial rash that disappears in days without stopping treatment, and adrenal cortical damage. Tannic acid has been considered to have potential as an anti-cancer agent for cholangiocarcinoma. The oral administration of tannic acid in humans is regarded as safe and this drug is currently being used commercially as a food additive [37]. Erythrosine sodium is a color additive that is commonly used in food industry as well as in pharmaceuticals for coating of tablets. As a biological stain, erythrosine is used as a radiopaque medium and a dental plaque disclosing agent. A comparative toxicological profile of sodium fluorescein and erythrosine on animal models suggests toxicity of erythrosine in certain dose ranges [38]. Studies on sanguinarine have also disclosed its diverse biological activities such as antimicrobial activity [39], anti-plaque activity [40], and, furthermore, this drug was reported to have anti-inflammatory activities [41]. Our findings suggest that these compounds may have additional pharmacological effects by suppressing PGD_2 formation.

Additionally, in the list of our selected 23 most effective inhibitors, we have identified two compounds montelukast sodium and tranilast as inhibitors of HPGDS with IC_{50} values of 2.0 and 13.7 µM, respectively. Both of these drugs are already in the

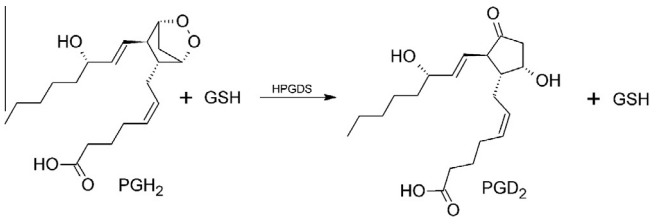


Fig. 6. HPGDS catalyzed isomerization of PGH_2 to PGD_2.

market for clinical use under trade names Singulair and Rizaben. Montelukast sodium is a leukotriene receptor antagonist (LTRA) used for the treatments of mild asthma and other seasonal allergies [42], while tranilast is an anti-allergic drug that inhibits fibroblast proliferation and production of interleukin-6, suppresses collagen formation and is prescribed for the symptoms of asthma and allergic rhinitis. Its role as a potential anti-proliferative drug is under investigation [43]. In contrast to tranilast, which has been reported as an inhibitor of HPGDS [27], herein for the first time, we report montelukast sodium as an inhibitor of HPGDS. These findings suggests that tranilast and montelukast sodium exert some of their therapeutic effects by preventing the production of PGD_2 via inhibiting HPGDS activity.

5. Conclusion

In conclusion, our screening results suggest that these potent and novel inhibitors of HPGDS have potential to be used in the treatment of conditions involved with inappropriate PGD_2 synthesis such as allergic asthma. However, we suggest that further characterization of these compounds should be done by using natural substrate PGH_2 in the inhibition assays. Additionally, we conclude that these drugs could possibly serve as lead compounds for the synthesis of new inhibitors for human HPGDS.

Conflict of Interest

The authors declare that there is no conflict of interest.

Transparency Document

The Transparency document associated with this article can be found in the online version.

Acknowledgments

This work was supported by grants from the Swedish Research Council and Swedish Cancer Society.

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