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Design, synthesis, and biological evaluation of 1,3,6,7-tetrahydroxyxanthone derivatives as phosphoglycerate mutase 1 inhibitors

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ABSTRACT

Phosphoglycerate mutase 1 (PGAM1) is a promising target for cancer treatment. Herein, we found that α-mangostin and γ-mangostin exhibited moderate PGAM1 inhibitory activities, with IC_{50} of 7.2 μM and 1.2 μM, respectively. Based on α-mangostin, a series of 1,3,6,7-tetrahydroxyxanthone derivatives were designed, synthesized and evaluated in vitro for PGAM1 inhibition. The significant structure–activity relationships (SAR) and a fresh binding mode of this kind of new compounds were also clearly described. This study provides valuable information for further optimization of PGAM1 inhibitors with 1,3,6,7-tetrahydroxyxanthone backbone or de novo design of novel inhibitor.

Introduction

Increased glucose metabolism is one of the distinguishing features between normal cells and highly proliferating cells like cancer, stem and immune cells.[1] German scientist Otto Warburg and his coworkers found that tumor tissues metabolize nearly ten-fold more glucose to lactate in a given time than normal tissues under aerobic conditions. Even in the non-hypoxia environment, tumor cells prefer the metabolic pathway of glycolysis to the more efficient oxidative phosphorylation for energy production.[2] This observation is called Otto Warburg effect.[3] Cancer cells tend to competently coordinate bioenergetics, anabolic biosynthesis, and balanced redox status to provide an optimum microenvironment for cancer cell proliferation and tumor growth.[4] Therefore, aerobic glycolysis has been recognized as a hallmark of cancer, and targeting the key enzyme involved in aerobic glycolysis may be a promising therapeutic approach for cancer treatment.

Phosphoglycerate mutase 1 (PGAM1), an important mutase in the glycolytic pathway that catalyzes the conversion of 3-phosphoglycerate (3-PG) into 2-phosphoglycerate (2-PG), plays an important role in coordinating glycolysis and anabolic activity to promote the proliferation of cancer cells.[5] In humans, PGAM1 is found to be overexpressed in various types of cancer, such as breast cancer,[6] hepatocellular carcinoma,[7] and colorectal cancer.[8] Jiang et al. had demonstrated that PGAM1 simultaneously affects anabolic and glycolysis reactions in cells by modulating the cellular levels of two key intermediates 3-PG and 2-PG. Elevated 3-PG is able to competitively inhibit 6-phosphogluconate (6-PGD), resulting in reduced oxidative pentose phosphate pathway (PPP) flux and ultimately inhibition of nucleotide synthesis.[9,10] Meanwhile, 2-PG is a positive regulator of 3-phosphoglycerate dehydrogenase (3-PHGDH) which participates in amino acid synthesis.[9] In conclusion, inhibition of PGAM1 is not only reducing the rate of glycolysis but also can affect the biosynthetic pathway of nucleotide and amino acid. Developing PGAM1 inhibitors is a promising direction toward potential cancer treatments.

To date, only a few types of PGAM1 inhibitors have been reported. The reported inhibitors can generally be divided into two types: (i) substrate competition inhibitor, such as MJE3, which was found to covalently label PGAM1 on Lys 100 by its unique spiroepoxide pharmacophore.[11] (ii) allosteric site inhibitors, such as PGMI-004A, which is obtained from the structure modification of Alizarin Red S (ARS) to improve permeability (Fig. 1).[10] Moreover, although (-)-Epigallocatechin-3-gallate (EGCG) was also identified as a non-substrate competitive inhibitor (Fig. 1),[12] it belongs to pan-assay interference compounds (PAINS) due to its promiscuous actions.[13]

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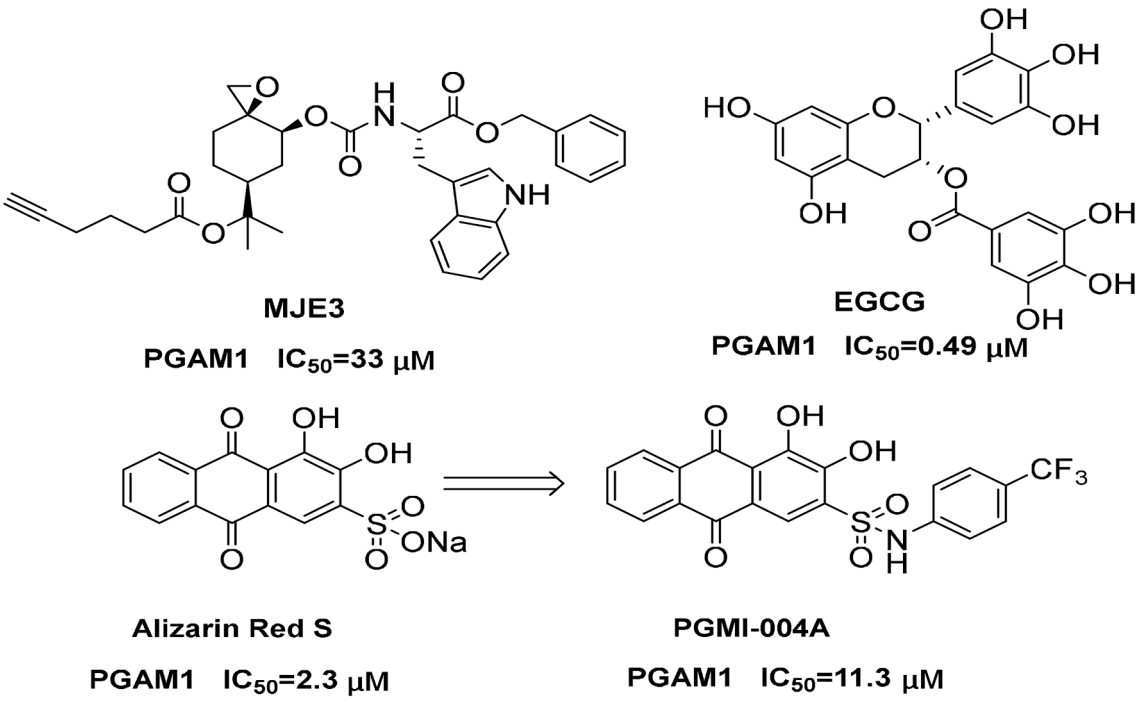


Fig. 1. The chemical structures of mentioned PGAM1 inhibitors.

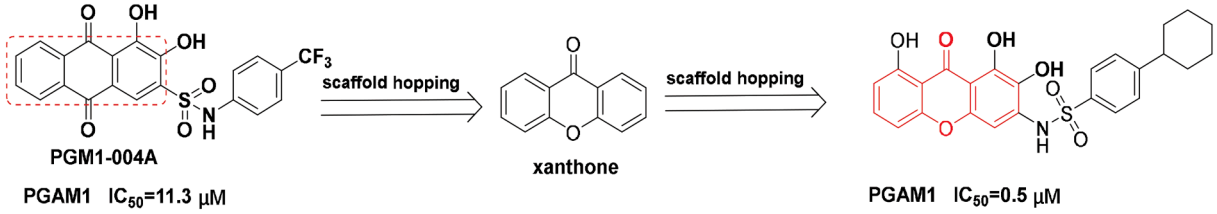


Fig. 2. xanthone as a novel scaffold.

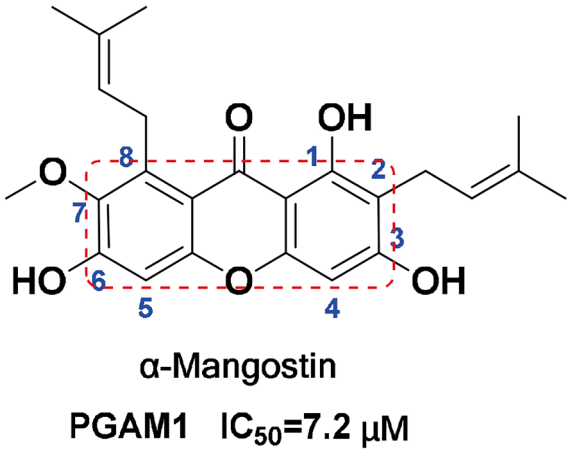


Fig. 3. The chemical structure ofα-mangostin.

PGMI-004A contains an anthraquinone core, which belongs to structural alerts in medicinal chemistry and may lead to potential side effects due to its high reactivity. Base on this, a scaffold hopping was conducted to replace the anthraquinone scaffold with xanthone scaffold to avoid quinone structure, and a series of xanthone derivatives were disclosed. Many of them showed higher potency against PGAM1 than PGMI-004A (Fig. 2).[14,15]

In the past few years, our research group has devoted to the related study of natural product α-mangostin extracted from the pericarp of mangosteen and exhibiting numerous pharmacological and biological activities such as antifungal, anticancer, antibacterial, antioxidant, antimalarial and anti-inflammatory.[16,17] In vitro screening assays α-mangostin (IC_{50} = 7.2 μM) exhibited better PGAM1 inhibitory activity than PGMI-004A (IC_{50} = 11.3 μM) (Fig. 3). We assumed that it may bind to the allosteric site due to its high similarity with reported allosteric inhibitor mentioned above. Herein, we described our efforts aimed at the discovery of novel potent PGAM1 inhibitors based on α-mangostin.

Initially, a series of substituents were introduced on the exposed hydroxyl groups at C3 and C6 of α-mangostin (**1a-f**), which led to a significant reduction of enzyme activities against PGAM1, with IC_{50} > 20 μM (Fig.4). The results suggested that the hydrogen bond donor of

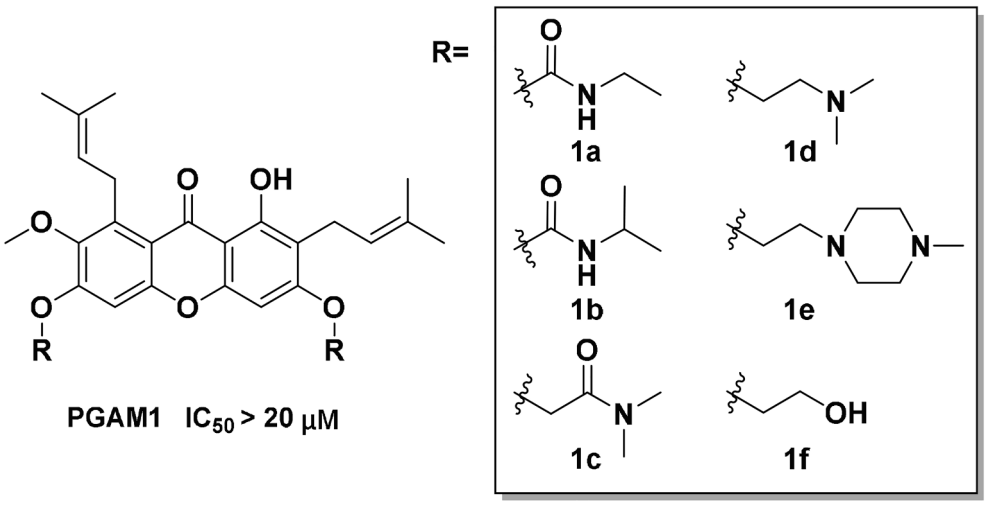
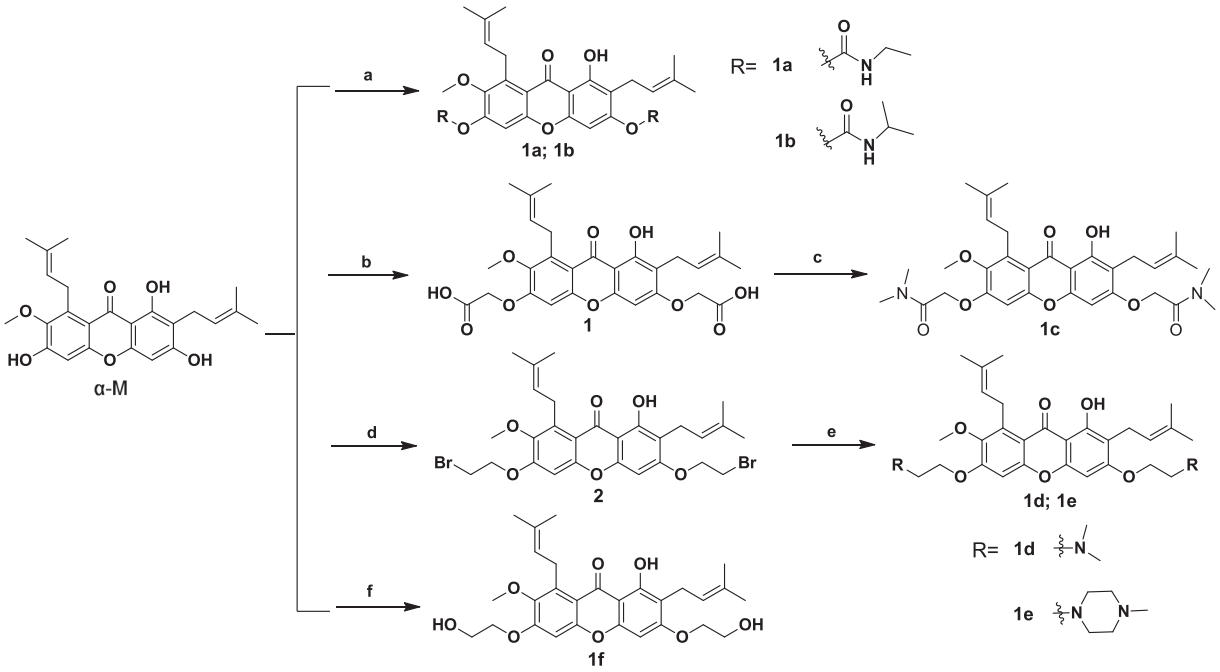


Fig. 4. The chemical structures of **1a-g**.



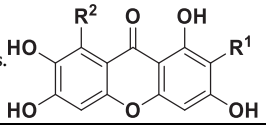
Scheme 1. Synthesis of compounds **1a-1f**. Reagent and condition:(a) Isocyanate, triethylamine, acetonitrile, reflux, 5 h; (b) (i) Ethyl chloroacetate, Potassium carbonate, acetone, reflux, 6h; (ii) NaOH ,H_2O, methanol, reflux, 4 h;(c) dimethylamine solution in water (40%), EDCI, HOBT, TEA, RT, 10 h; (d) 1,2-Dibromoethane, Potassium carbonate, acetone, reflux, 20 h; (e) Secondary amine, Potassium carbonate, acetone, reflux, 10 h; (f) 2-Bromoethanol, Potassium carbonate, acetone, reflux, 20 h.

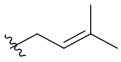
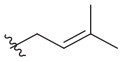
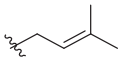
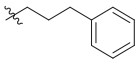
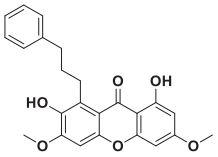
hydroxyls at C3 and C6 may play an important role in its activity. The synthetic pathways for the target compounds **1a-g** are provided in Scheme 1.

To further explore the structure–activity relationship (SAR), we exposed the last phenolic hydroxyl at C7 in α-mangostin to obtain **2a** (also called γ-mangstion). As shown in Table 1, compound **2a** showed higher inhibitory activity than α-mangostin with IC_{50} of 1.2 μM. Then, we retained the fully exposed hydroxyls and transformed the isopentenyl at C2 into hydrogen atoms (**2b**) to elucidate the role of isopentenyls for enzymatic inhibitory activity. Compound **2b** possessed a similar inhibitory effect against PGAM1 compared with **2a**, which suggested that the isopentenyl at the C2 is not necessary for enzyme

Table 1

The structures of **2a-f** and observed activities.



NO	R^1	R^2	IC_{50}(μM)[a]
2a (γ-M)[a]			1.9
2b	H		3.4
2c	H		20
2d	H		2.8
2e	H		1.5
2f			>20
PGMI-004A[b]			13.0

[a] γ-M: γ-mangostin.
[b] Active control.

inhibition. Afterwards, replacements of the isopentenyl at C8 with shortened (**2b**) and extended (**2c**) aliphatic chains and bulky phenyl substituents (**2e**) were conducted. Compound **2c** showed an obvious reduction of potency, but extended lipophilic chain (**2c**) and bulky phenyl group (**2e**) led to comparable enzyme inhibition with **2b**. Based on the results, we hypothesized that the lipophilic substituents in this position may extend into a hydrophobic pocket in the binding site. Entropy loss caused by long flexible chains may explain the similar activities of **2d** and **2e** with **2b**, although they owned hydrophobic substituents of different lengths and volumes.

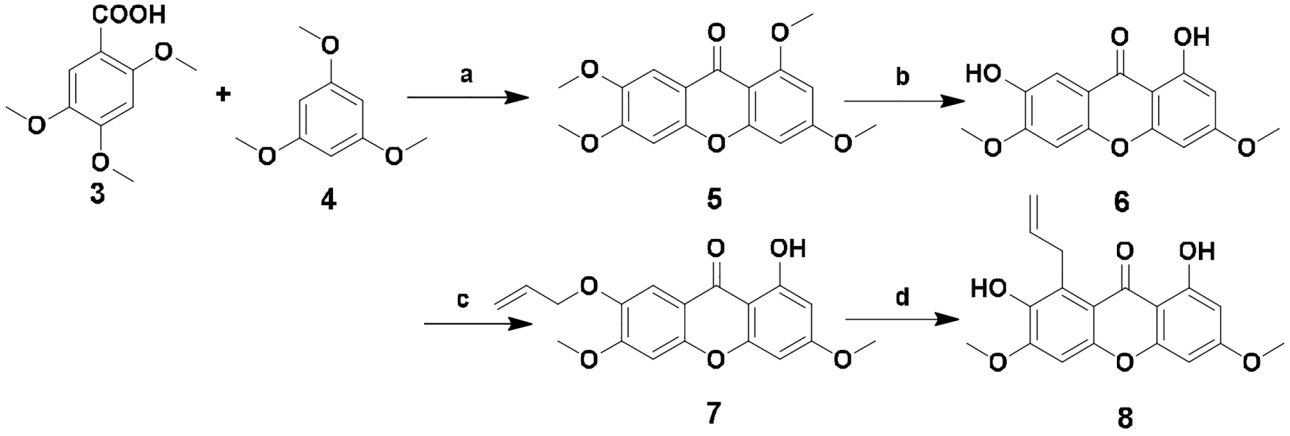
Moreover, the enzyme inhibitory activity of methylation precursor **(2f)** of compound **2e** was also determined, with a complete loss of potency ($IC_{50} > 20$ μM), suggesting that exposed hydroxyl groups at C3 and C6 were essential to activities (Table 1). The synthetic pathways for the target compounds **2a-e** are shown in Scheme 2 and Scheme 3.

In addition, we found that introducing lipophilic groups on the hydroxyl group at C1, without any substituent at C8, could also lead to good potencies (Table 2). The inhibitory activities increased with the extension of the hydrophobic alkane chain substituents on the hydroxyl (**3a-b**). Unfortunately, the introduction of a piperidine ring at the end of the alkane chain (**3c-d**) resulted in a significant drop in activities. By contrast, a relatively lipophilic indoline substituent (**3e**) could obtain a comparable potency with **3b**. This might be due to the decrease of hydrophobic interactions when putting relatively polar groups (tertiary amine **3c** or amide group **3f**) into a completely lipophilic cavity. Calculated LogP of the terminal structures in the substituents are also shown in Fig. 5 (by Chemdraw professional 17.0), which is consistent with our hypothesis. The synthetic pathways for the target compounds **3a-e** are shown in Scheme 4 and Scheme 5.

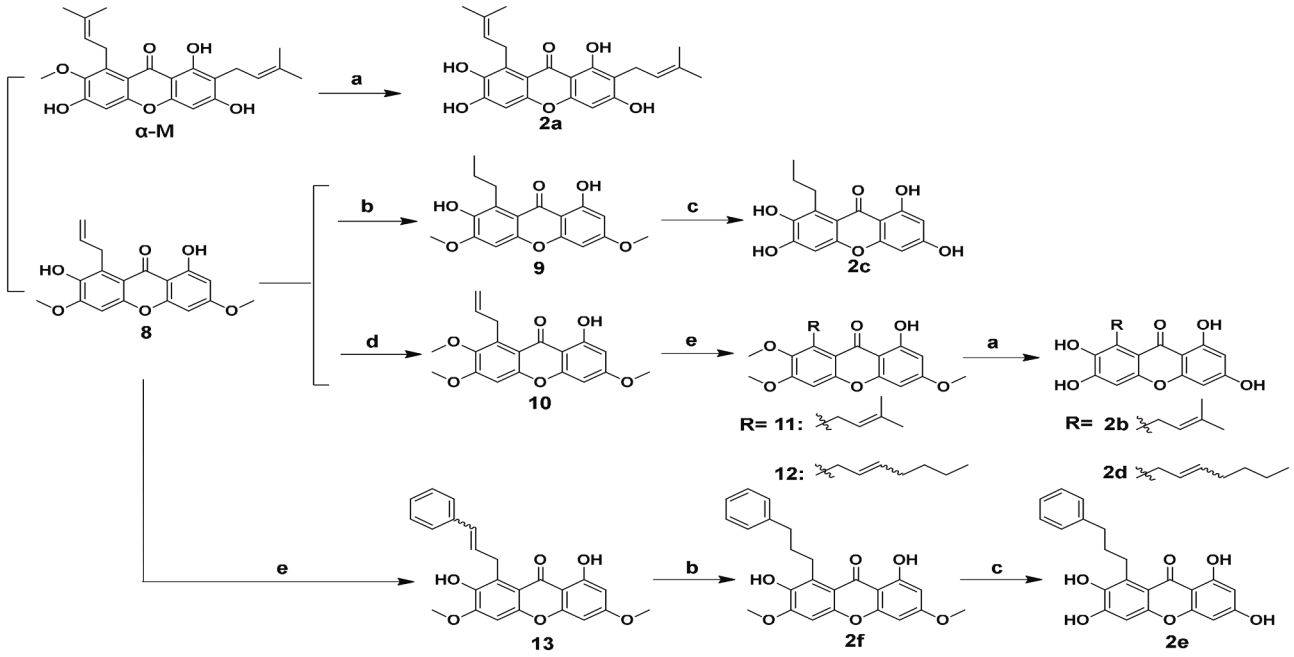
Cellular level experiments on the selected compounds with good PGAM1 inhibitory activity were also carried out (Table 3). *In vitro* cell anti-proliferation assays indicated that **2a** and **3b** exhibited good inhibitory activities against Human lung adenocarcinoma cell H1299 with EC_{50} of 2.2 μM and 3.4 μM, respectively.

To further understand the binding mode of this kind of inhibitors, we carried out docking studies of compounds **2b** and **3b** with PGAM1(**PDB code: 5Y2I**). A comparison with the co-crystal of PGMI-004A with PGAM1(**PDB code: 5Y2I**)[18] was also made (Fig.6).

The docking result of **2b** (Fig.6**, B**) revealed that the 1,3,6,7-tetrahydroxyxanthone scaffold bound into a shallow groove in the binding site, and the isopentenyl substituent, instead of the anthraquinone scaffold of PGMI-004A in the co-crystal (Fig.6**, A**),[18] extended into the primary hydrophobic pocket (constituted by Phe22, Leu95, Val112 and Trp115). Besides, the hydroxyls at C3 and C6 respectively formed hydrogen bonds with Asn 20, Glu 19 and Arg 10, which may illustrate the reason for the loss of potency when methylations were performed on hydroxyls in



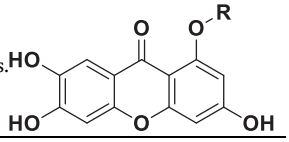
Scheme 2. Synthesis of key intermediate **8**. Reagent and condition: (a) (i) $SOCl_2$, CH_2Cl_2, reflux, 10 h; (ii) $AlCl_3$, CH_2Cl_2, 0 °C to reflux, 10 h; (b) Acetic acid, HBr, reflux, N_2, 3 h; (c) Allyl bromide, Potassium carbonate, acetone, reflux, 4 h; (d) N,N-Dimethylaniline, reflux, 3 h, N_2.

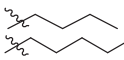
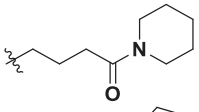
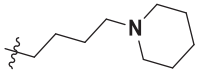
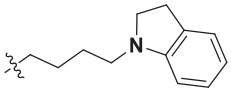


Scheme 3. Synthesis of compounds **2a-2e**. Reagent and condition: (a) NaH, DMF, ethyl mercaptan, reflux, N_2, 5 h; (b) Pd/C, ethanol, 40 °C, 10 h, H_2; (c) Acetic acid, HBr, reflux, N_2, 20 h; (d) Dimethyl sulfate, Potassium carbonate, acetone, reflux, 6 h; (e) Olefins, Grubbs II, CH_2Cl_2, 40 °C, 12 h, N_2.

Table 2

The structures of **3a-e** and observed activities.



NO	R	IC_{50}(μM)
3a		12.8
3b		2.8
3c		>20
3d		>20
3e		5.7
PGMI-004A[a]		13.0

[a] Active control.

these positions (**2f**). A π-π stacking interaction was also observed between Phe 22 and 1,3,6,7-tetrahydroxyxanthone scaffold (Fig. 6, **D**). The docking result of **3b** showed an analogous bind mode compared to **2b**, with a horizontally flipped 1,3,6,7-tetrahydroxyxanthone scaffold

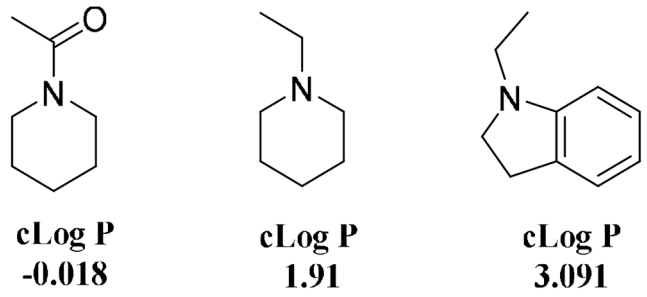
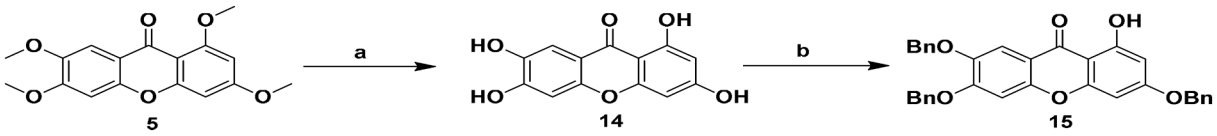


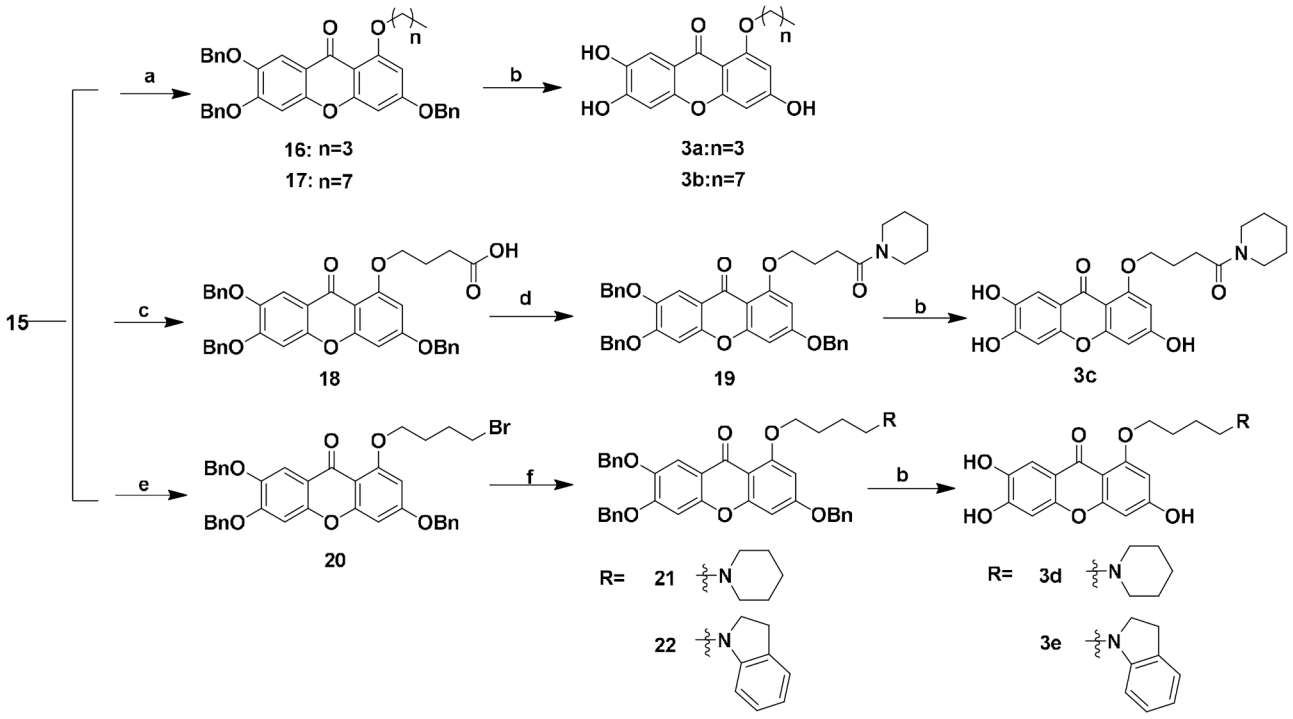
Fig. 5. cLogP of terminal structures in substituents of **3c-e**.

occupying the same groove (Fig. 6, **C**). The aliphatic chain on the C1 phenolic hydroxyl bent and then occupied the main hydrophobic cavity. The flipped binding mode may be due to the high symmetry of the scaffold and hydroxyl substitution, and interactions observed in the docking study of **3b** were similar to **2b**. Overall, the meaningful results suggested an apparently different binding mode compared to previously reported allosteric site inhibitors PGMI-004A.[10,18]

In summary, we first reported that α-Mangostin and γ-mangostin (**2a**) target PGAM1 in the glycolytic pathway. Subsequently, a series of tetrahydroxyxanthone derivatives were designed, synthesized and evaluated for their potential as novel PGAM1 inhibitors. In vitro cell growth inhibition assays indicated that compound **2a**, **3b** exhibited



Scheme 4. Synthesis of key intermediate **15**. Reagent and condition: (a) Acetic acid, HBr, N_2, reflux, 24 h; (b) Benzyl bromide, potassium carbonate, DMF, 40 °C, 4 h.



Scheme 5. Synthesis of compounds **3a-3e**. Reagent and condition:(a) Brominated alkanes, Caesium carbonate, acetonitrile, reflux, 5 h; (b) Pd/C, ethanol, 40 °C, 10 h, H_2; (c) (i) Ethyl 4-bromobutyrate, Caesium carbonate, acetonitrile, reflux, 5 h; (ii) NaOH, H_2O, methanol, reflux, 4 h; (d) Piperidine, EDCI, HOBT, TEA, RT, 10 h; (e) Dibromoalkane, Caesium carbonate, acetonitrile, reflux, 5 h; (f) amine substrates, Potassium carbonate, acetone, reflux, 6 h.

Table 3
Antiproliferative activities of selected compounds against H1299 cells.

Compound	EC_{50}(μM)[a]	compound	EC_{50}(μM)[a]
2a	2.2	**3a**	60
2b	47.7	**3b**	3.4
2d	13.5	**3e**	28.1
2e	41.6	**PGMI-004A**[a]	26.2

[a] Active control.

better inhibitory activities against Human lung adenocarcinoma cell H1299 with EC_{50} of 2.2 μM and 3.4 μM, respectively. The SAR studies suggested that exposed hydroxyls at C3 and C6 of 1,3,6,7-tetrahydroxyxanthone scaffold and suitable hydrophobic substituents at C1 or C8 are beneficial for the potency. Notably, the docking studies revealed a novel binding mode that hydroxyls at C3 and C6 formed hydrogen bond interaction with the protein and lipophilic substituents at C1 or C8 extended into the unique hydrophobic pocket in the allosteric site. This research provides valuable information and guideline for further exploration of novel PGAM1 inhibitors.

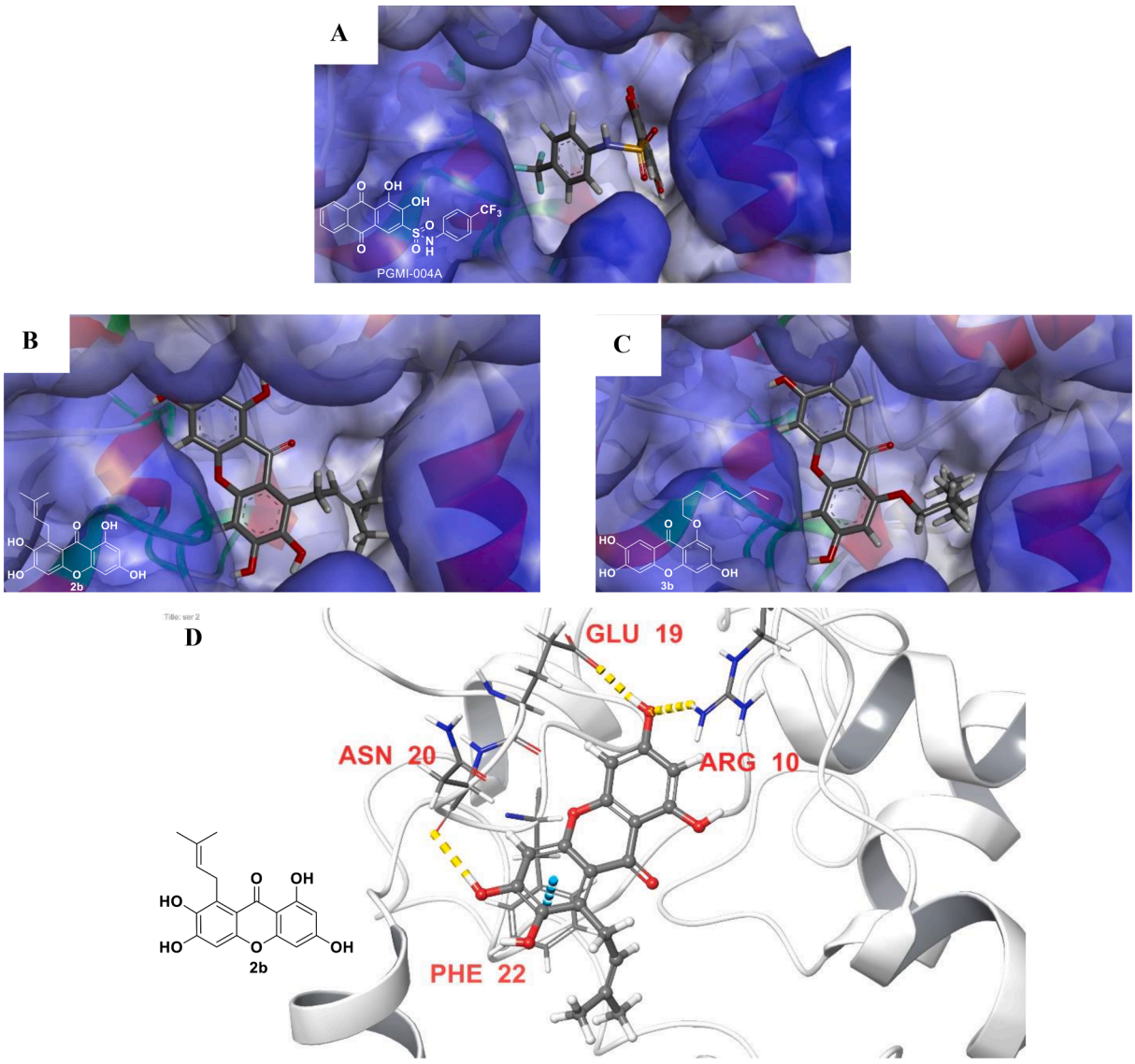


Fig. 6. The binding modes of PGAM1- inhibitors, with carbon atoms colored in gray, oxygen atoms in red, nitrogen atoms in blue and hydrogen atoms in off-white (A: the crystal structure of PGMI-004A-PGAM1 complex{PDB 5Y2I}; B: the docking result of **2b**; C: the docking result of **3b**; D: interactions observed in **2b**).

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bmcl.2021.127820.

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