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SHORT COMMUNICATION

## Bioactive compounds of *Crocus sativus* L. and their semi-synthetic derivatives as promising anti-*Helicobacter pylori*, anti-malarial and anti-leishmanial agents

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### Abstract

*Crocus sativus* L. is known in herbal medicine for the various pharmacological effects of its components, but no data are found in literature about its biological properties toward *Helicobacter pylori*, *Plasmodium* spp. and *Leishmania* spp. In this work, the potential anti-bacterial and anti-parasitic effects of crocin and safranal, two important bioactive components in *C. sativus*, were explored, and also some semi-synthetic derivatives of safranal were tested in order to establish which modifications in the chemical structure could improve the biological activity. According to our promising results, we virtually screened our compounds by means of molecular modeling studies against the main *H. pylori* enzymes in order to unravel their putative mechanism of action.

### Keywords

Anti-leishmanial agents, anti-malarial agents, *Crocus sativus* L., *Helicobacter pylori*, safranal

### History

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### Introduction

One of the most important microbial infections affecting the human stomach is due to *Helicobacter pylori*, a Gram-negative bacterium that inhabits the inner mucosa, causing not only gastritis but also gastroduodenal ulcers and, in the more severe stages, tumors. One of the reasons for the failure of *H. pylori* eradication in many countries is the increasing antibiotic resistance<sup>1</sup>. Among the new strategies for the treatment of *H. pylori* infection, it can be mentioned the inhibition of urease<sup>2,3</sup>, an enzyme that permits the survival of *H. pylori* in the harsh acidic environment of the stomach and its colonization of the gastric mucosa, by neutralization of the gastric acidity thanks to the ammonia production from host urea. Several natural and synthetic compounds such as sulforaphane, an isothiocyanate extracted from crucifers<sup>4</sup>, allyl isothiocyanates<sup>5</sup>, flavonoids and their corresponding reductive derivatives<sup>6</sup>, quercetin and its analogues<sup>7</sup> and some synthetic thiosemicarbazones<sup>3</sup> are effective urease inhibitors. Instead, caffeic acid phenethyl ester, one of the principal components of propolis, was found to be a competitive inhibitor of *H. pylori* peptide deformylase (HpPDF), an enzyme that catalyzes the removal of the formyl group from the N-terminus of nascent polypeptide chains, which is essential for

*H. pylori* survival<sup>8</sup>. Other targets for the treatment of *H. pylori* infection are as follows: (1) the type II dehydroquinase (DHQ2), the third enzyme of the shikimic acid pathway<sup>9</sup>; (2) glutamate racemase, which provides D-glutamate for the construction of N-acetylglucosamine-N-acetylmuramic acid peptidoglycan subunits<sup>10</sup>; (3) *H. pylori*  $\beta$ -hydroxyacyl-ACP (FabZ), an important enzyme involved in the bacterial type II fatty acid synthetic pathway<sup>11,12</sup>; (4) *H. pylori* phosphopantetheine adenylyltransferase (HpPPAT), which catalyzes the penultimate step in coenzyme A biosynthesis<sup>13</sup>; (5) the class II fructose-bisphosphate (FBP) aldolase, an enzyme involved in microbial glycolysis and gluconeogenesis<sup>14</sup>; and (6) NADPH oxidase<sup>15</sup>.

Among the parasitic infections, malaria has always attracted attention because of the increasing resistance of *Plasmodium falciparum* and *Plasmodium vivax* parasites to traditional drugs; therefore, nowadays research is focused on the development of drugs and vaccines with the aim of targeting not only the blood stage of infection but also the pre-erythrocytic and sexual stages<sup>16</sup>. Beside artemisinin and quinine (found in *Artemisia annua* and *Cinchona* spp., respectively), and their semi-synthetic derivatives that represent the most important drugs in current therapies, additional natural products content in Amazonian plants possess anti-plasmodial effects against *P. falciparum* and *P. vivax*<sup>17</sup>. It is noteworthy that crude plant extracts could have a better anti-plasmodial activity than the individual components at the same dose. This effect is due to the pharmacodynamic synergy that could be found between *Cinchona* alkaloids, and/or to the pharmacokinetic interactions such as those between natural

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products in *A. annua* tea, making artemisinin more rapidly absorbed than the single drug<sup>18</sup>.

Another disease caused by parasite infection is leishmaniasis, which is present especially in poor populations characterized by low healthcare conditions like some disadvantaged regions of South America, Africa and Asia. Leishmaniasis is caused by protozoan parasites whose transmission is through the bite of the phlebotomine sand fly: cutaneous leishmaniasis is mostly due to *Leishmania major* infection, while visceral leishmaniasis is due to *Leishmania donovani*, widespread in India and East Africa. Anti-leishmanial drugs used in current therapies, such as meglumine antimoniate, sodium stibogluconate, pentamidine, miltefosine and amphotericin B, cause several side effects and are too expensive for these populations. Hence, in the research of new naturally occurring anti-leishmanial drugs, we can mention chloroform extract of *Valeriana wallichii* roots, thanks to the caffeic acid bornyl ester<sup>19</sup> and natural product-inspired quinazolinone hybrids<sup>20</sup>. Disuccinyl betulin, diglutaryl dihydrobetulin and disuccinyl dihydrobetulin, derivatives of the natural triterpene betulin present in the *Betula* spp. cork layer, inhibit the relaxation activity of the enzyme IB type topoisomerase of *L. donovani*; this effect decreases the intracellular parasites number in macrophages<sup>21</sup>.

With the aim of finding new natural or semi-synthetic antimicrobial drugs, we focused our attention on *Crocus sativus* L. (also called saffron), that is a herbaceous perennial-cormous plant containing in the stigmas a great variety of bioactive compounds: the volatile oil safranal, a carotenoid derivative (crocin) and its di-glycosidic esters (crocins), picrocrocin and still other components such as anthocyanins, flavonoids, vitamins, amino acids and proteins. In fact, in addition to its use as food additive, saffron is also known in natural medicine for improving memory and learning abilities and for the treatment of sick, spasm, asthma, bronchitis, fever, colds and cardiovascular diseases; furthermore, it also possesses anticancer, antioxidant, hypolipemic and anticonvulsant activities<sup>22</sup>. Despite all these pharmacological effects, few data are available in literature, regarding exclusively the activity of its aqueous and methanolic extracts, about antimicrobial and anti-parasitic activities (*Bacillus subtilis* and *cereus*, *Staphylococcus aureus* and *Staphylococcus pyogenes*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Cladosporium herbarum*, *Brucella melitensis*, *Candida albicans*, *Cryptococcus neoformans*, *Trichophyton rubrum*, *Aspergillus fumigatus*, *Pyricularia oryza*, and *L. major*)<sup>23,24</sup>. Given our interest in this field<sup>25–28</sup>, we explored the potential anti-*H. pylori*, anti-malarial and anti-leishmanial effects of crocin (**1**) and safranal (**2**), which are two important products found in *C. sativus*, and tested some semi-synthetic derivatives of safranal (**3–9**, Scheme 1) in order to establish, with the support of computational studies, which modifications in the chemical structures could improve their biological activity to provide new leads for the development of promising antimicrobial and anti-parasitic agents. In addition, we obtained a direct correlation between specific biological activity and active principles of this spice.

## Materials and methods

### Chemistry

Commercial samples of crocin (crocin-1, crocetin di-gentiobiose ester) and safranal (>88%) were purchased from Sigma-Aldrich (Milan, Italy). Safranal was further purified by column chromatography on silica gel (230–400 mesh, G60 Merck, ethyl acetate:*n*-hexane 1:3). The other compounds (**3–9**) have been synthesized and characterized to ensure purity as reported in the literature<sup>29</sup>.

## Antimicrobial studies

### *H. pylori* culture

Nineteen clinical strains of *H. pylori*, isolated from patients with duodenal ulcer or gastritis, and reference strain ATCC 43504 were used for this study. The *H. pylori* strains were maintained at  $-80^{\circ}\text{C}$  in Wilkins–Chalgren broth with 10% (v/v) horse serum (Seromed) and 20% (v/v) glycerol (Merck) until required for the experiments. Before being used, the bacteria were subcultured twice on Columbia agar base (Difco Laboratories) supplemented with 10% horse serum and 0.25% Bacto yeast extract (Difco). Plates were incubated for 72 h at  $37^{\circ}\text{C}$  in an atmosphere of 10%  $\text{CO}_2$  in a gas incubator.

### Anti-*H. pylori* activity

MICs were determined by modified broth dilution method as previously described<sup>30</sup>. Briefly, an inoculum equivalent to 1 McFarland standard was prepared in Wilkins–Chalgren broth and diluted in MegaCell™ RPMI-1640 medium. Each well was inoculated with *H. pylori* at a final concentration of approximately  $5 \times 10^5$  CFU/well. The plates were incubated at  $37^{\circ}\text{C}$  under microaerophilic conditions (10%  $\text{CO}_2$  in a gas incubator) and examined after 72 h of incubation. In order to confirm MIC results, a comparative evaluation with agar dilution method<sup>31</sup> was also performed. The MICs obtained for each compounds by microdilution test were the same to those obtained by agar dilution (data not shown).

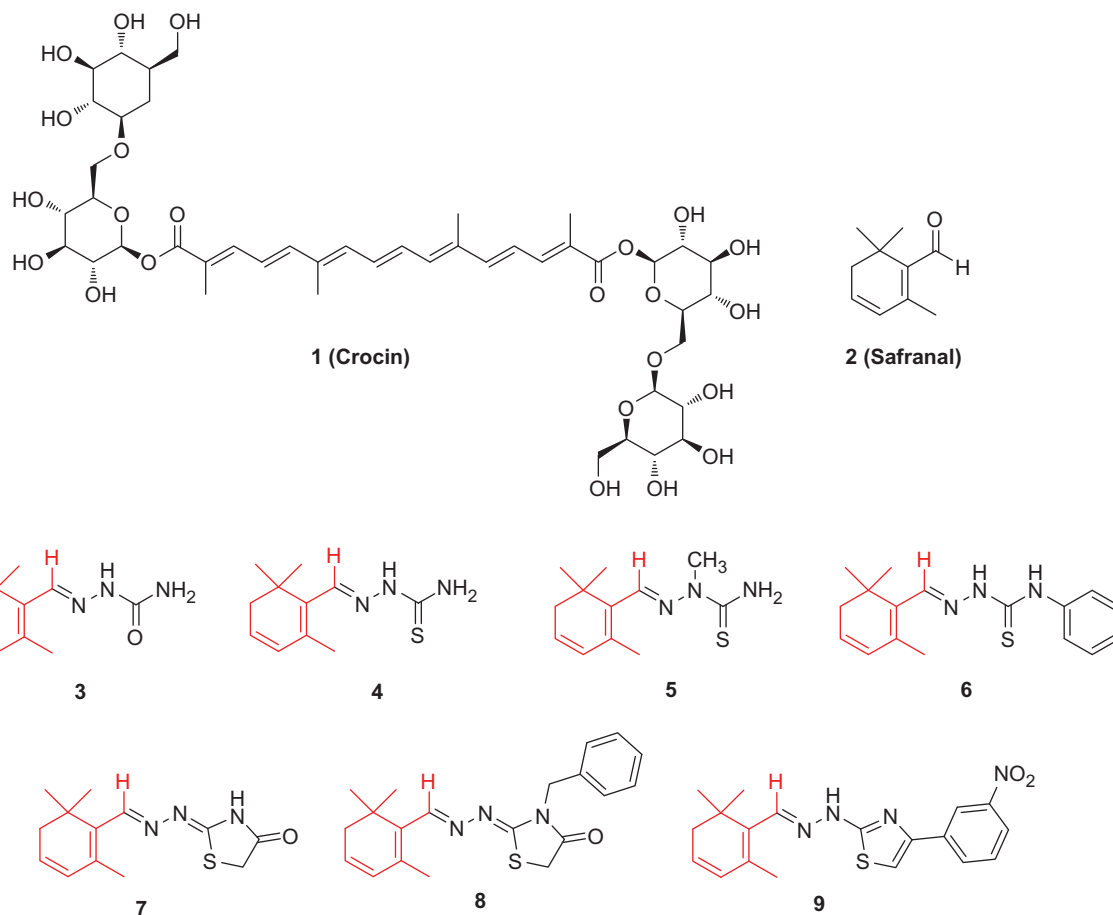
### *Plasmodium falciparum* cultures and drug susceptibility assay

*Plasmodium falciparum* cultures were carried out according to Trager and Jensen with slight modifications<sup>32</sup>. The CQ-susceptible strain D10 and the CQ-resistant strain W2 were maintained at 5% hematocrit (human type A-positive red blood cells) in RPMI-1640 (EuroClone, Celbio) medium with the addition of 1% AlbuMax (Invitrogen, Milan, Italy), 0.01% hypoxanthine, 20 mM HEPES and 2 mM glutamine. All the cultures were maintained at  $37^{\circ}\text{C}$  in a standard gas mixture consisting of 1%  $\text{O}_2$ , 5%  $\text{CO}_2$  and 94%  $\text{N}_2$ . Compounds were dissolved in either water or DMSO and then diluted with medium to achieve the required concentrations (final DMSO concentration <1%, which is non-toxic to the parasite). Drugs were placed in 96-well flat-bottomed microplates (COSTAR) and serial dilutions made. Asynchronous cultures with parasitemia of 1–1.5% and 1% final hematocrit were aliquoted into the plates and incubated for 72 h at  $37^{\circ}\text{C}$ . Parasite growth was determined spectrophotometrically ( $\text{OD}_{650}$ ) by measuring the activity of the parasite lactate dehydrogenase, according to a modified version of the method of Makler & Hinrichs in control and drug-treated cultures<sup>33</sup>. The anti-malarial activity is expressed as 50% inhibitory concentrations ( $\text{IC}_{50}$ ); each  $\text{IC}_{50}$  value is the mean and standard deviation of at least three separate experiments performed in duplicate.

### Evaluation of anti-leishmanial activity

Promastigote stage of *Leishmania infantum* strain MHOM/TN/80/IPT1 (kindly provided by Dr. M. Gramiccia and Dr. T. Di Muccio, ISS, Roma) and clinical isolate of *Leishmania tropica* (MHOM/IT/2012/ISS3130) were cultured in RPMI-1640 medium (EuroClone) supplemented with 15% heat-inactivated fetal calf serum (EuroClone), 20 mM HEPES and 2 mM *L*-glutamine at  $22^{\circ}\text{C}$ .

To estimate the  $\text{IC}_{50}$ , the 3-[4.5-dimethylthiazol-2-yl]-2.5-diphenyltetrazolium bromide (MTT) method<sup>34</sup> was used with modifications. Compounds were dissolved in DMSO and then diluted with medium to achieve the required concentrations.



Scheme 1. Structures of bioactive compounds (crocin **1** and safranal **2**) of *Crocus sativus* L. and semi-synthetic safranal derivatives **3–9**.

Drugs were placed in 96-well round-bottom microplates and seven serial dilutions were made. Amphotericin B was used as the reference anti-*Leishmania* drug. Parasites were diluted in complete medium to  $5 \times 10^6$  parasites/mL, and 100  $\mu$ L of the suspension was seeded into the plates, incubated at 22 °C for 72 h and then 20  $\mu$ L of MTT solution (5 mg/mL) was added into each well for three hours. The plates were then centrifuged, the supernatants discarded and the resulting pellets dissolved in 100  $\mu$ L of lysing buffer consisting of 20% (w/v) of a solution of SDS (Sigma), 40% of *N,N*-dimethylformamide (Merck) in H<sub>2</sub>O. The absorbance was measured spectrophotometrically at a test wavelength of 550 nm and a reference wavelength of 650 nm. The results are expressed as IC<sub>50</sub>, which is the dose of compound necessary to inhibit cell growth by 50%; each IC<sub>50</sub> value is the mean  $\pm$  standard deviation of at least three separate experiments performed in duplicate.

### Molecular modeling studies

Docking experiments were performed using the X-ray crystallographic structures of six new strategic targets for the treatment of *H. pylori* infection, downloaded from the Protein Data Bank site (PDB)<sup>35</sup> (Table S1). The treatment of X-ray enzyme structures was carried out by ‘‘Protein Preparation Wizard’’<sup>36</sup> function: non-protein residues were removed, hydrogen atoms were added and finally, energy minimization was performed until the root-mean squared deviation (RMSD) of all heavy atoms was within 0.3 Å X-ray PDB model. Both *E* and *Z* isomers of the 3D semisynthetic derivatives **4**, **5** and **9** were prepared, before the docking simulations, using the latest version of the software Maestro GUI 9.7 in the Schrodinger Suite 2014<sup>37</sup>. For each molecule,

several protonated and tautomeric forms were calculated by means of the ‘‘LigPrep’’ module<sup>38</sup> and used as starting points for the molecular recognition studies. Glide 6.2 SP<sup>39</sup> protocol was applied to dock the flexible 3D ligands within the active sites of every rigid target. The ‘‘Receptor Grid Generation’’ panel was used to define a cube of length 25 Å as binding region, centered on the former positions of the removed X-ray co-crystallized inhibitors, into each catalytic site. Low values of RMSD between experimental and docked structures, after re-docking procedure, have been shown the good ability of Glide protocol to accurately predict the ligand binding conformations (as shown in Table S1). Ligand-binding energies (dG Bind) in Kcal/mol were estimated by ‘‘Prime MM-GBSA’’ utility<sup>40</sup>. With regard to FabZ and HpPDF enzymes, the most stable conformations of docked compounds were submitted to a molecular dynamic (MD) simulation of 1.0 ns carried out by Desmond package<sup>41</sup> and were also analyzed using ‘‘Simulation Interactions Diagram’’ panel. All figures were generated by means of the Pymol 1.7.0.0 version molecular graphic system<sup>42</sup>.

### Results and discussion

Starting from the commercially available natural product safranal (**2**), purified by column chromatography, we previously synthesized semicarbazone (**3**), thiosemicarbazone (**4–6**), thiazolidinone (**7,8**) and hydrazothiazole (**9**) derivatives. For these products, the safranal nucleus was maintained to establish the impact of the chemical modifications, such as the increasing steric hindrance and the different substituents, on the tested biological activity.

Concerning the biological activity of compounds **1–9** against *H. pylori* strains (Table 1), safranal (**2**) showed an MIC<sub>50</sub> value of



32 µg/mL against some strains while its semi-synthetic derivatives **4**, **5** and **9** showed an effective anti-bacterial activity; and for some strains, they are markedly more active than the reference drugs, metronidazole (>32 µg/mL) and clarithromycin (≥32 µg/mL). In detail, the two thiosemicarbazonic derivatives (**4** and **5**) proved their activity in the range of 4–8 µg/mL (MIC<sub>50</sub> and MIC<sub>90</sub>, respectively), while the most effective compound is the hydrazothiazole **9**, which contains a phenyl moiety substituted with a nitro group at C3, with a biological activity ranging from 2 to 4 µg/mL (MIC<sub>50</sub> and MIC<sub>90</sub>, respectively), suggesting that the presence of the hydrazone moiety could be important for the explication of the anti-bacterial effect.

With the purpose to elucidate a putative mechanism of action for the active semi-synthetic derivatives of safranal (**4**, **5** and **9**), a virtual screening against some *H. pylori* targets was performed. Therefore, six enzyme crystal structures were considered for the docking simulations downloaded from the PDB<sup>35</sup>: DHQ2, FabZ, FBP aldolase, glutamate racemase, HpPDF and urease represent, in fact, new strategic targets for the treatment of the resistant *H. pylori* infection. Protein X-ray models were selected, for the structure-based virtual screening, considering low values of resolution and the presence of a co-crystallized inhibitor (Table S1).

No crystallographic models of HpPPAT are deposited in the PDB, for this reason, it has not been considered for the molecular recognition studies. For each selected target, both *E* and *Z* isomers of the 3D molecules were docked into the binding pocket by means of Glide 6.2 SP<sup>39</sup> protocol, and the binding energy values (dG Bind in Kcal/mol), for the best produced ligand-protein complexes, were estimated by Prime-MMGBSA utility<sup>40</sup> considering as final score the average of the two isomers values.

Table 1. Anti-bacterial activity (µg/mL) of compounds **1–9** and reference drugs against *Helicobacter pylori* strains.

Compounds	Range	MIC <sub>50</sub>	MIC <sub>90</sub>
1	32–0.03	32	>32
2	32–0.03	>32	>32
3	32–0.03	32	>32
4	32–0.03	4	8
5	32–0.03	4	8
6	32–0.03	>32	>32
7	32–0.03	16	32
8	32–0.03	16	32
9	32–0.03	2	4
Metronidazole	32–0.03	0.5	>32
Clarithromycin	32–0.06	<0.06	>32

The hydrazothiazole **9** showed the highest dG Bind value of –81.94 Kcal/mol for the FabZ enzyme, conversely the thiosemicarbazonic derivatives **4** and **5**, that displayed also a good accommodation into the binding pocket of this target, gave the best results for the HpPDF with the respective dG bind values –54.32 and –56.49 Kcal/mol (Table S2).

In order to investigate the stereochemical requirements on the basis of a possible inhibition mechanism, a MD of 1.0 ns were also performed *in silico*, by Desmond package<sup>41</sup>, to gain insight into the interactions of *E–Z* isomers with the FabZ and HpPDF amino acids residues lining the catalytic sites. With regard to the FabZ MD analysis, carried out by the “Simulation Interaction Diagram” panel, the last frames of the semisynthetic isomers revealed a possible target inhibition by a competitive mechanism of action. Moreover, for compound **9**, a further investigation suggested that the *E* isomer is localized inside the binding cavity with the nitrophenyl and the hydrazothiazole (tautomeric form) moieties sandwiched between the entrance residues TYR100 and PRO112' by  $\pi$ – $\pi$  interactions. The stabilization of both the **9** *E–Z* isomers is associated with several hydrophobic contacts formed by residues of the lipophilic tunnel entrance (Figure 1), by contrast the active compounds **4** and **5** establish only fewer H-bond contacts with HIS58 and GLU72, located near the FabZ active site, and no hydrophobic interactions with the key TYR100 residue (Figures S1 and S2)<sup>11</sup>. Taking into account the HpPDF MD analysis, all compounds showed the same binding mode within the catalytic site of the target. In agreement with the theoretical data reported in Table S2, the ligand–HpPDF complexes of the derivatives **4** and **5** are stabilized by a great number of hydrophobic/H-bond interactions involving the thiosemicarbazide moiety: in this case, ILE45, TYR92, TYR103, LEU131 and ALA135 residues, forming the lipophilic task, could play an important role for the ligand binding mode (Figures 2 and 3). However, the hydrazothiazole **9** might be a better HpPDF inhibitor than the derivatives **4** and **5**: its *E–Z* isomers make not only hydrophobic but also H-bond contacts by the hydrazonic moiety and one ionic interaction, for the *E* isomer, by the nitro group of the phenyl substituent (Figure S3).

On the basis of these virtual screening studies, we concluded that a probably enzymatic inhibition might be involved in the biological activity of safranal derivatives against the *H. pylori* strains, and the hydrazothiazole **9** might be considered as a new hit compound for future investigation and enzymatic assays.

With regard to the anti-malarial activity of these products (Table 2), crocin (**1**) displayed a biological effect against chloroquine-sensitive D10 strain (IC<sub>50</sub> = 18.93 µg/mL).

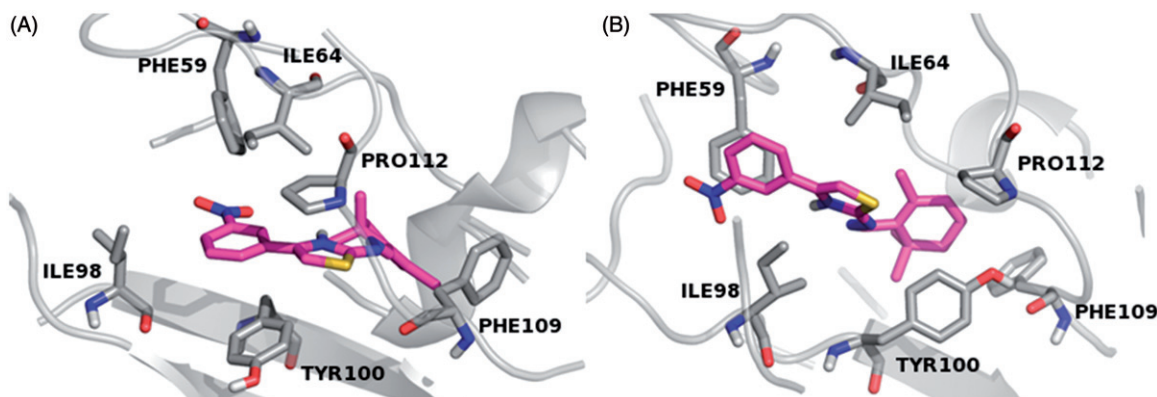


Figure 1. MD simulation last frame of both *E* (A) and *Z* (B) isomers of compound **9** and their hydrophobic contacts into the FabZ binding site. The *E–Z* isomers are rendered as carbon-pink sticks, the pocket amino acids are labeled as carbon-gray sticks and the target is described as gray cartoon. All non-carbon atoms are colored according to atom types. For the colored version of this Figure, please refer to the online article.

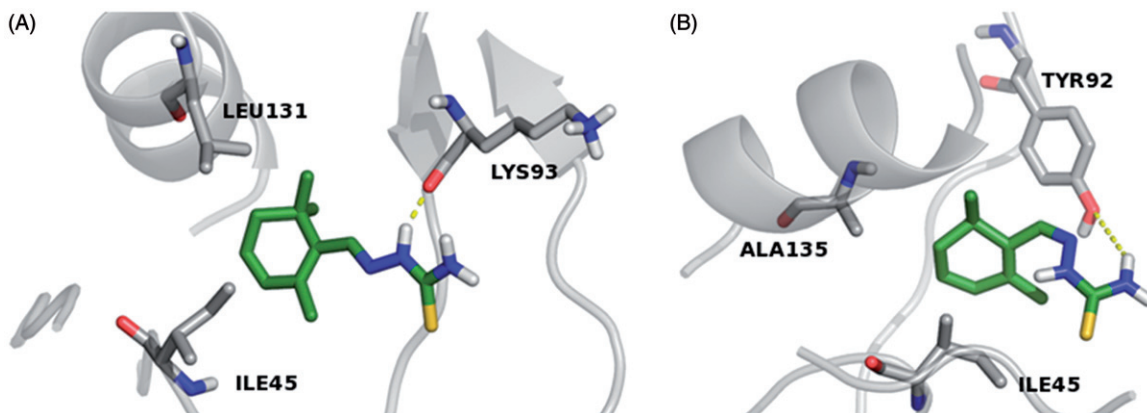


Figure 2. MD simulation last frame of both *E* (A) and *Z* (B) isomers of compound **4** and their H-bond/hydrophobic interactions within the HpPDF binding site. The *E*-*Z* isomers are rendered as carbon-green sticks, the pocket amino acids are labeled as carbon-gray sticks and the target is described as gray cartoon. All non-carbon atoms are colored according to atom types. For the colored version of this Figure, please refer to the online article.

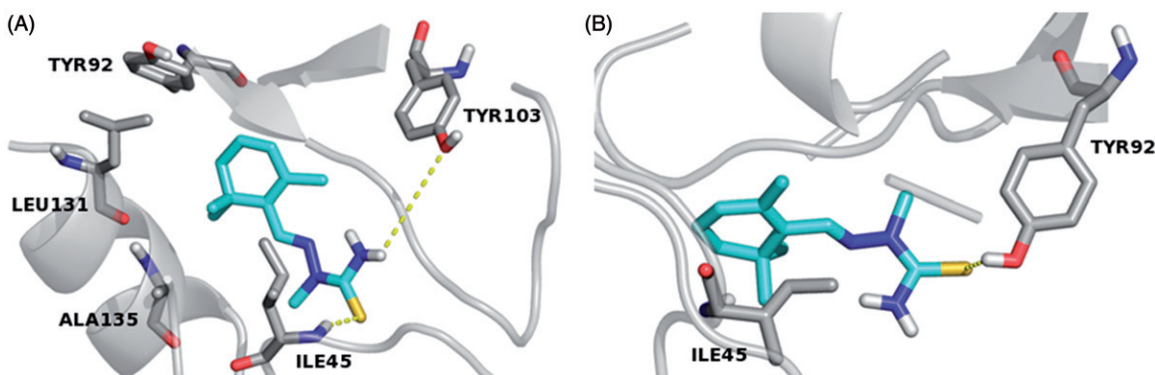


Figure 3. MD simulation last frame of both *E* (A) and *Z* (B) isomers of compound **5** and their H-bond/hydrophobic interactions within the HpPDF binding site. The *E*-*Z* isomers are rendered as carbon-cyan sticks, the pocket amino acids are labeled as carbon-gray sticks and the target is described as gray cartoon. All non-carbon atoms are colored according to atom types. For the colored version of this Figure, please refer to the online article.

Table 2. Anti-malarial activity ( $\mu\text{g/mL}$ ) against chloroquine-sensitive D10 strain and chloroquine-resistant W2 strain and anti-leishmanial activity ( $\mu\text{g/mL}$ ) of compounds **1–9** and reference drugs.

Compound	D10 IC <sub>50</sub>	W2 IC <sub>50</sub>	<i>L. infantum</i> (IPT1) IC <sub>50</sub>	<i>L. tropica</i> IC <sub>50</sub>
1	18.93 $\pm$ 1.24	>20	>20	>20
2	>20	>20	>20	>20
3	13.26 $\pm$ 1.49	10.28 $\pm$ 1.5	>20	>20
4	10.15 $\pm$ 1.54	13.07 $\pm$ 4.56	>20	11.51 $\pm$ 4.76
5	6.01 $\pm$ 0.69	6.75 $\pm$ 2.53	12.50 $\pm$ 3.93	14.69 $\pm$ 6.71
6	4.92 $\pm$ 1.88	>20	>20	>20
7	>20	>20	>20	15.64 $\pm$ 3.52
8	5.98 $\pm$ 0.48	7.22 $\pm$ 0.41	6.10 $\pm$ 1.39	1.98 $\pm$ 0.13
9	8.53 $\pm$ 1.64	7.22 $\pm$ 1.37	>20	>20
Chloroquine	0.011 $\pm$ 0.002	0.233 $\pm$ 0.07	Nd	Nd
Amphotericin B	nd	nd	0.11 $\pm$ 0.04	0.07 $\pm$ 0.02

Nd, not determined.

The (thio)semicarbazone derivatives **3**, **4** and **5** were active against both chloroquine-sensitive D10 strain and chloroquine-resistant W2 strains (IC<sub>50</sub> = 13.26 and 10.28  $\mu\text{g/mL}$ , 10.15 and 13.07  $\mu\text{g/mL}$ , 6.01 and 6.75  $\mu\text{g/mL}$ , respectively), while 4-phenylthiosemicarbazone **6** only inhibited D10 strain (IC<sub>50</sub> = 4.92  $\mu\text{g/mL}$ ). Unlike the thiazolidinone **7**, which was not active, the corresponding *N*-benzylated compound **8** showed an inhibitory effect of 5.98 and 7.22  $\mu\text{g/mL}$  against D10 and W2 strains, respectively. Furthermore, the hydrazothiazole **9** displayed its activity against both strains (IC<sub>50</sub> = 8.53  $\mu\text{g/mL}$  against D10 and 7.22  $\mu\text{g/mL}$  against W2). With regard to the anti-leishmanial

effect, thiosemicarbazone **4** displayed an IC<sub>50</sub> value of 11.51  $\mu\text{g/mL}$  toward *L. tropica*, whereas the *N*-methylated **5** was active not only against *L. tropica* (IC<sub>50</sub> = 14.69  $\mu\text{g/mL}$ ) but also against *L. infantum* (IC<sub>50</sub> = 12.50  $\mu\text{g/mL}$ ). Thiazolidinone **7** showed an IC<sub>50</sub> of 15.64  $\mu\text{g/mL}$  against *L. tropica* while its derivative **8**, a benzylated substituent at the lactamic nitrogen, was more active than the others toward *L. tropica* (IC<sub>50</sub> = 1.98  $\mu\text{g/mL}$ ) and also on *L. infantum* with an IC<sub>50</sub> of 6.10  $\mu\text{g/mL}$ . These encouraging results showed that safranal and its semi-synthetic derivatives could be further investigated to develop new anti-*H. pylori* and anti-parasitic agents.

## Conclusions

Many reports have explained the great variety of biological activities possessed by saffron and its natural components, but no data in literature are present with regard to the anti-bacterial activity against *H. pylori* and the anti-parasitic activity against *Plasmodium* and *Leishmania*. Starting from this point, we assessed the anti-*H. pylori*, anti-malarial and anti-leishmanial effects of two natural products found in *C. sativus* L., crocin (**1**) and safranal (**2**), and of the semi-synthetic safranal derivatives (**3–9**). The thiosemicarbazonic derivatives **4** and **5** and the (thiazol-2-yl)hydrazonic compound **9** exhibited the best anti-*H. pylori* activities and a possible mechanism of action, corroborated by molecular modeling studies, might be the inhibition of several targets as FabZ and HpPDF. With regard to the anti-parasitic effect, compounds **5**, **6**, **8** and **9** possessed moderate anti-malarial activity, whereas the *N*-benzylated thiazolidinone **8** has proven to be the best anti-leishmanial agent of the series. These biological studies, supported by molecular modeling studies, demonstrated that unsaturated nucleus of safranal could have the pharmacophoric prerequisites to provide new leads for the development of promising agents for the management of *H. pylori* and parasitic infections caused by *Plasmodium* and *Leishmania*.

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## Declaration of interest

The authors report no declaration of interest.

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**Supplementary material available online**

Supplementary Tables S1 and 2; Figures S1–3 and Supporting information.