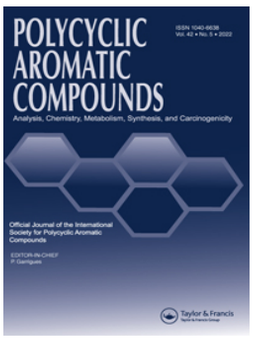


Polycyclic Aromatic Compounds

ISSN: (Print) (Online) Journal homepage: https://www.tandfonline.com/loi/gpol20

Design, Synthesis and Biological Evaluation of Novel Furan & Thiophene Containing Pyrazolyl Pyrazolines as Antimalarial Agents

Hemantkumar N. Akolkar, Sujata G. Dengale, Keshav K. Deshmukh, Bhausaheb K. Karale, Nirmala R. Darekar, Vijay M. Khedkar & Mubarak H. Shaikh

To cite this article: Hemantkumar N. Akolkar, Sujata G. Dengale, Keshav K. Deshmukh, Bhausaheb K. Karale, Nirmala R. Darekar, Vijay M. Khedkar & Mubarak H. Shaikh (2022) Design, Synthesis and Biological Evaluation of Novel Furan & Thiophene Containing Pyrazolyl Pyrazolines as Antimalarial Agents, Polycyclic Aromatic Compounds, 42:5, 1959-1971, DOI: 10.1080/10406638.2020.1821231

To link to this article: https://doi.org/10.1080/10406638.2020.1821231

POLYCYCLIC AROMATIC COMPOUNDS
2022, VOL. 42, NO. 5, 1959–1971
https://doi.org/10.1080/10406638.2020.1821231

Taylor & Francis
Taylor & Francis Group

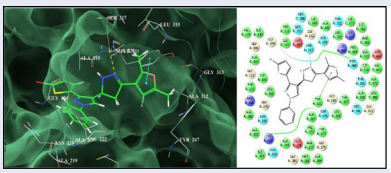
Design, Synthesis and Biological Evaluation of Novel Furan & Thiophene Containing Pyrazolyl Pyrazolines as Antimalarial Agents

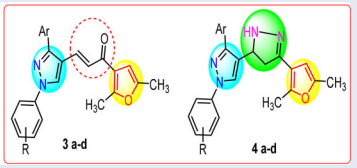
Hemantkumar N. Akolkar[a], Sujata G. Dengale[b], Keshav K. Deshmukh[b], Bhausaheb K. Karale[a], Nirmala R. Darekar[a], Vijay M. Khedkar[c], and Mubarak H. Shaikh[a]

[a]P.G. and Research, Department of Chemistry, Radhabai Kale Mahila Mahavidyalaya, Ahmednagar, India; [b]P.G. and Research, Department of Chemistry, Sangamner Nagarpalika Arts, D. J. Malpani Commerce, B.N. Sarada Science College, Sangamner, India; [c]Department of Pharmaceutical Chemistry, School of Pharmacy, Vishwakarma University, Pune, India

ABSTRACT

In search for novel compounds targeting Malaria, based on the *in silico* molecular docking binding affinity data, the novel furans containing pyrazolyl chalcones (**3a-d**) and pyrazoline derivatives (**4a-d**) were synthesized. The formation of the synthesized compound were confirmed by spectral analysis like IR, ^{1}H NMR, ^{13}C NMR and mass spectrometry. Compounds with thiophene and pyrazoline ring **4b** (0.47 μM), **4c** (0.47 μM) and **4d** (0.21 μM) exhibited excellent anti-malarial activity against *Plasmodium falciparum* compared with standard antimalarial drug Quinine (0.83 μM). To check the selectivity furthermore, compounds were tested for antimicrobial activity and none of the synthesized compound exhibited significant potency compared with the standard antibacterial drug Chloramphenicol and antifungal drug Nystatin respectively. So, it can be resolved that the produced compounds show selectively toward antimalarial activity and have the potential to be explored further.





ARTICLE HISTORY
Received 7 August 2020
Accepted 5 September 2020

KEYWORDS
Antimalarial; antimicrobial; chalcones; pfENR inhibitor; pyrazole-pyrazolines; thiophene

Introduction

Life-threatening disease Malaria is caused by *Plasmodium* parasites that are spread to people through the bites of infected female Anopheles mosquitoes. Out of five *Plasmodium* Parasites *Plasmodium falciparum* produces high levels of blood-stage parasites that sequester in critical organs in all age groups.[1] As per the World Health Organization report in 2018, in sub Saharan Africa 11 million pregnant women were infected with malaria and 872 000 children were born with a low birth weight. Around 24 million children estimated to be infected with the *P. falciparum* parasite in the region; out of these, 1.8 million had severe anemia and 12 million had

CONTACT Hemantkumar N. Akolkar hemantakolkar@gmail.com P.G. and Research, Department of Chemistry, Radhabai Kale Mahila Mahavidyalaya, Ahmednagar, Maharashtra 414001, India.

Supplemental data for this article is available online at https://doi.org/10.1080/10406638.2020.1821231.

moderate anemia.[2] Mortality and morbidity caused by malaria are continually increasing. This subject is the consequence of the ever-increasing development of parasite resistance to drugs and also increased mosquito resistance to insecticides which is one of the most critical complications in controlling malaria over recent years.[3]

P. falciparum enoyl-acyl carrier protein (ACP) reductase (ENR) is an enzyme in type II fatty acid synthesis (FAS II) pathway which catalyzes the NADH-dependent reduction of trans-2-enoyl-ACP to acyl-ACP and plays important role in completion of the fatty acid elongation cycles. Due to its role in the parasite's fatty acid pathway, *Pf*ENR has been known as one of the most promising antimalarial targets for structure-based drug design.[4–6] Triclosan, a broadly used antibiotic, is effective inhibitor of *Pf*ENR enzyme activity. Several efforts have been taken in the recent past in the direction of the identification of new antimalarials using pharmacophore modeling, molecular docking and MD simulations.[7–12]

Pyrazole is a well-known class of nitrogen containing heterocyclic compounds and play important role in agricultural and medicinal field. Pyrazole and its derivatives are known to possess antibacterial,[13] antipyretic,[14] fungistatic,[15] anticonvulsant,[16] antitubercular,[17] antipyretic,[18] insecticides,[19] and anti-inflammatory[20] activities. Pyrazoline containing compounds are recognized to possess various pharmacological activities like antimalarial,[21,22] anticancer,[23] anti-inflammatory,[24] analgesic,[24] antitumor,[25] antimicrobial[26] and antidepressant activities.[27] Furan containing compounds possess lipoxygenase inhibitor,[28] urotensin-II receptor antagonists,[29] fungicidal,[30] epidermal growth factor receptor inhibitors and anticancer[31] etc. activities. Chalcone is a natural pigment found in plant and is an important intermediate for the synthesis of flavonoids. Varieties of biological activities are associated with chalcones and their derivatives such as antiplasmodial,[32] nematicide,[33] antiallergenic,[34] antimalarial,[35] anti-HIV,[36] anti-cancer,[37] anti-inflammatory[38] and anti-tuberculosis.[39]

So, considering the biological importance of pyrazoles, furan and chalcone, herein we report the design of a small library of furan containing pyrazolyl pyrazoline derivatives by molecular hybridization approach targeting *Pf*ENR using the *in silico* molecular docking technique. The promising results obtained from this *in silico* study served the basis for the synthesis of these molecules followed by evaluation of their antimalarial potential.

Molecular docking technique plays significant role in lead identification/optimization and in the mechanistic study by predicting the binding affinity and the thermodynamic interactions leading the binding of a ligand to its biological receptor. Thus, with the objective to identify novel leads targeting the crucial antimalarial target *Plasmodium falciparum enoyl-ACP reductase* (*Pf*ENR or FabI) (pdb code: 1NHG), molecular docking was carried out using the GLIDE (Grid-based LIgand Docking with Energetics) program of the Schrodinger Molecular modeling package.[40–42] A small library of 8 molecules comprising furan containing pyrazolyl pyrazoline derivatives (**3a-3d**, **4a-4d**) was docked against *Pf*ENR. The ensuing docking conformation revealed that these molecules changed a binding mode which is corresponding with the active site of pfENR and were found to be involved in a series of bonded and non-bonded interactions with the residues lining the active site. Their docking scores varied from −6.979 to −8.222 with an average docking score of −7.563 signifying a potent binding affinity to *Pf*ENR. In order to get a quantitative insight into the most significantly interacting residues and their associated thermodynamic interactions, a detailed per-residue interaction analysis was carried out (Table S1, Supporting Information). This analysis showed that the furan containing pyrazolyl chalcones (**3a-d**) (Figure 1) were deeply embedded into the active site of *Pf*ENR engaging in a sequence of favorable *van der Waals* interactions observed with Ile:C369, Phe:C368, IleA323, Ala:A320, Ala:A319, Arg:A318, Ser:A317, Leu:A315, Pro:A314, Gly:A313, Ala:A312, Lys:A285, Met:A281, Tyr:A277, Tyr:A267, Thr:A266, Leu:A265, Gly:A112, Tyr:A111, Gly:A110 and Asp:A107 residues through the 1,3-substituted-1*H*-pyrazol-4-yl scaffold while the 1-(2,5-Dimethylfuran-3-yl) prop-2-en-1-one

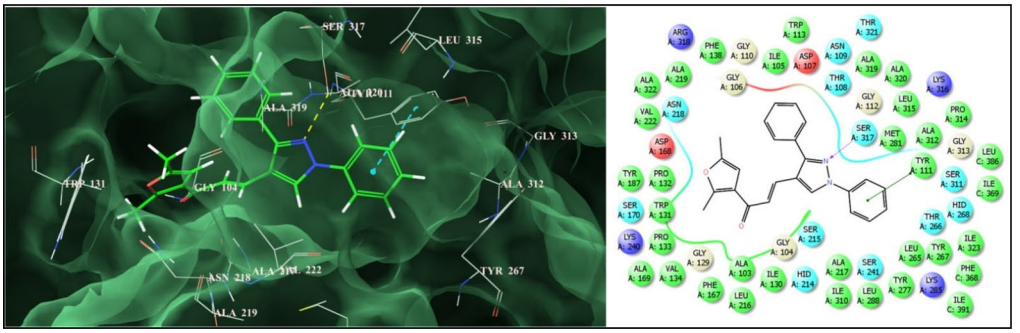


Figure 1. Binding mode of **3a** into the active site of *Plasmodium falciparum enoyl-ACP reductase* (on right side: pink lines represent the hydrogen bond while green lines signify π-π stacking interactions).

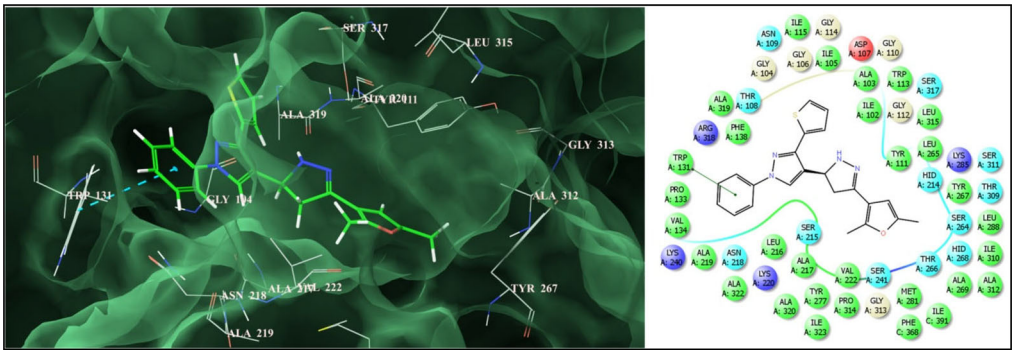
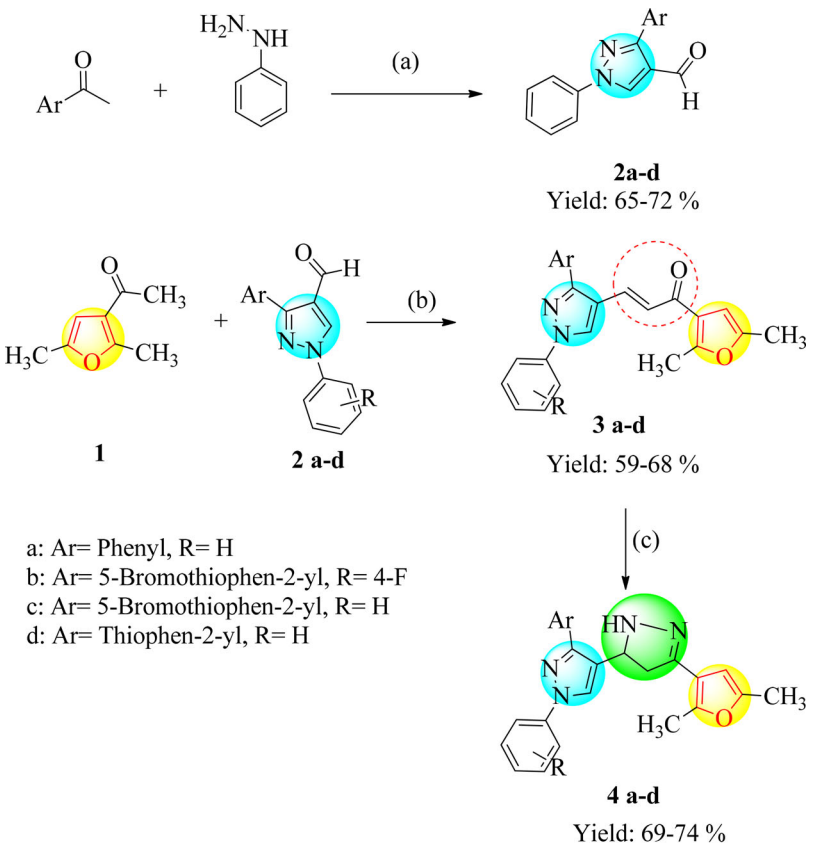


Figure 2. Binding mode of **4d** into the active site of *Plasmodium falciparum enoyl-ACP reductase* (on right side: pink lines represent the hydrogen bond while green lines signify π-π stacking interactions).

component of the molecules was seen to be involved in similar interactions with Asn:A218, Ala:A217, Leu:A216, Ser:A215, Trp:A131, Gly:A106, Ile:A105, Gly:A104 residues of the active site.

Furthermore the enhanced binding affinity of these molecule is also attributed to significant electrostatic interactions observed with Arg:A318, Ser:A317, Lys:A285, Asp:A236, Asn:A218, Ala:A217, Ser:A215, Tyr:A111, Gly:A110, Asp:A107, Gly:A104 residues lining the active site. On the other hand, the furan containing pyrazoline derivatives (**4a-d**) (Figure 2) were also seen to be stabilized into the active of *Pf*ENR through a network of significant *van der Waals* interactions observed with (2,5-dimethylfuran-3-yl)-1*H*-pyrazolyl scaffold *via* Ile:C369, Phe:C368, Ala:A320, Ser:A317, Leu:A315, Pro:A314, Gly:A313, Ala:A312, Lys:A285, Tyr:A267, Thr:A266, Leu:A265, Gly:A112, Tyr:A111, Gly:A110, Gly:A106 and Ile:A105 while other half of the molecule i.e., 2-thiophenyl-1-phenyl-1*H*-pyrazole showed similar type of interactions with IleA323, Ala:A319, Arg:A318, Met:A281, Tyr:A277, Val:A222, Ala:A219, Asn:A218, Ala:A217, Leu:A216, Ser:A215, Trp:A131, Ile:A130, Trp:A113, Asp:A107, Gly:A104 residues.

Further the enhanced binding affinity of the molecules is also attributed to favorable electrostatic interactions observed with Arg:A318, Ser:A317, Glu:A289, Lys:A285, Asp:A236, Asn:A218, Ala:A217, Ser:A215, Tyr:A111, Gly:A110, Asp:A107 and Gly:A104. While these non-bonded interactions (*van der Waals* and electrostatic) were observed to be the major driving force for the mechanical interlocking of these novel furan containing pyrazolyl pyrazoline derivatives into the active site *Pf*ENR, the enhanced binding affinity of these molecules is also contributed by very prominent hydrogen bonding interaction observed for **3a** (Ser:A317(2.708 Å)), **4a** (Ser:A317(2.783 Å)), **4b** (Ser:A317(2.462 Å)) and **4c** (SerA317(2.462 Å)). Furthermore these



Reagents and conditions: (a): i) EtOH, reflux, 2 hr ii) $DMF/POCl_3$, 0-10° C; (b) 10 % aq. KOH, EtOH, RT, 14hr; (c) $NH_2NH_2.H_2O$, EtOH, AcOH, 6hr

Scheme 1. Synthesis of pyrazolyl chalcones (**3a-d**) and pyrazolyl pyrazolines (**4a-d**).

molecules were also engaged in a very close π-π stacking interactions: **3a**: Tyr: A111(2.669 Å), **3b**: Tyr:A267(2.529 Å), **3c**: Tyr:A267(2.541 Å), **3d**: Tyr:A267(2.335 Å), **4a**: Tyr:A111(2.602 Å), **4b**: Trp:A131(2.073 Å), **4c**: TyrA:111(2.073 Å) and **4d**: TrpA131(2.538 Å) (Figures S1–S6, Supporting Information).

This type of bonded interactions i.e., hydrogen bonding and π-π stacking are known to serve as an "anchor" to guide the alignment of a molecule into the 3D space of enzyme's active site and facilitate the non-bonded interactions (*Van der Waals* and electrostatic) as well. Overall, the in-silico binding affinity data suggested that these furans containing pyrazolyl pyrazoline derivatives (**3a-d**, **4a-d**) could be developed as novel scaffold to arrive at compounds with high selectivity and potency *Plasmodium falciparum*.

Results and discussion

Chemistry

The novel series of furan containing pyrazolyl chalcones (**3a-d**) and pyrazoline derivatives (**4a-d**) were synthesized from commercially available starting materials (Scheme 1). Initially, pyrazole aldehyde **2a-d** was formed by the condensation between substituted acetophenone and phenyl

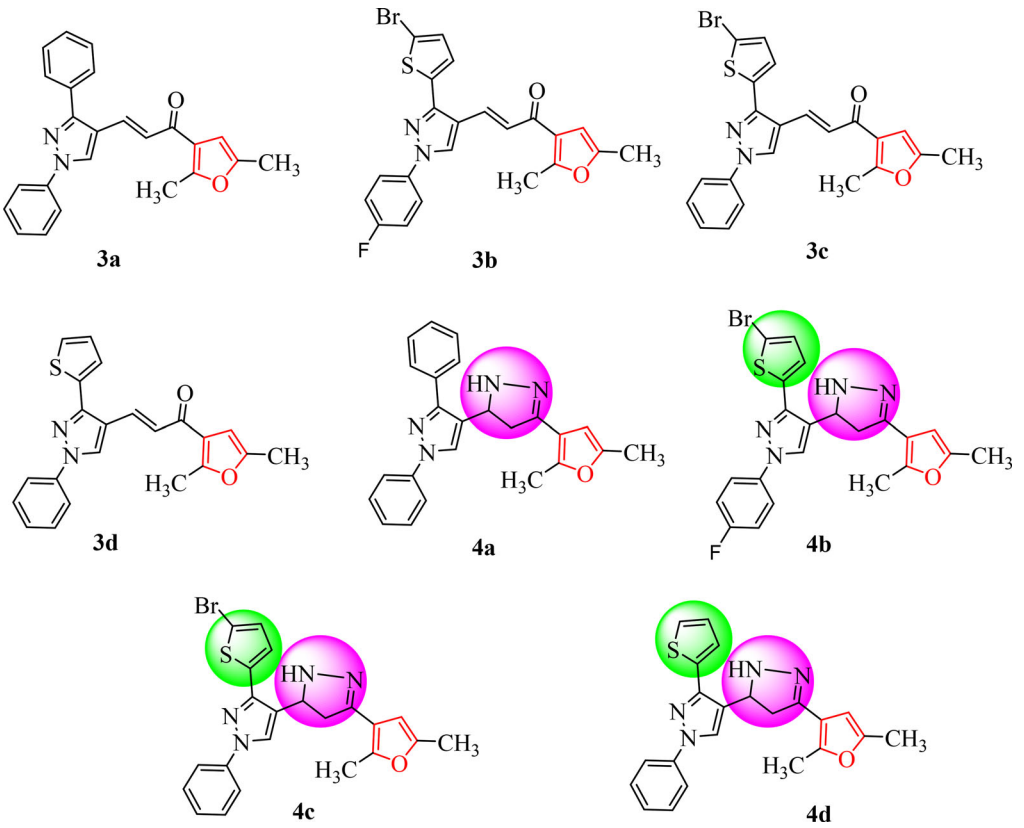


Figure 3. The newly synthesized compounds structure **3a-d** & **4a-d**.

hydrazine followed by Vilsmeier-Haack formylation reaction (Scheme 1). Then furan containing pyrazolyl chalcones **3a-d** were synthesized by base-catalyzed Claisen-Schmidt condensation of 1-(2,5-dimethylfuran-3-yl)ethanone **1** and substituted pyrazole aldehyde **2a-d**.[43] Finally, the furan containing pyrazolyl chalcones **3a-d** and hydrazine hydrate in ethanol solvent using catalytic amount of acetic acid at reflux condition for 6 hr afforded the corresponding pyrazolyl pyrazolines (**4a-d**) in quantitative isolated yield (69–74%) (Scheme 1).

The newly synthesized compounds structures were shown in Figure 3. The newly synthesized compound's structures were confirmed by IR, ^{1}H NMR, ^{13}C NMR, mass spectral data. For compound **3a**, in IR spectrum the stretching band for C = O was detected at 1657 cm^{-1}. In the ^{1}H NMR spectrum of compound **3a**, the proton of pyrazole and furan ring resonate as a singlet at δ 9.31 and δ 6.60 ppm respectively. Also, singlet for two $-CH_3$ were observed at δ 2.27 and δ 2.50 ppm. The ^{13}C NMR spectrum of compound **3a** showed signal at δ 184.41 ppm due to C = O and δ 12.89 and δ 13.93 ppm is due to two $-CH_3$. Mass spectrum confirms the formation of compound **3a** showed m/z = 369 $(M+H)^{+}$.

Secondly, in the IR spectrum of compound **4a**, –N–H stretching band observed at 3252 cm^{-1}. The 1H NMR spectrum of compound **4a**, the CH_2 protons of the pyrazoline ring resonated as a pair of doublets of doublets at δ 2.88 ppm and 3.35 ppm. The CH proton appeared as triplet at δ 4.87 ppm due to vicinal coupling with two protons of the methylene group. In the ^{13}C NMR spectra of the compound **4a** carbons of the pyrazoline ring were observed at δ 41.97 ppm and 54.67 ppm. All the other aromatic and aliphatic protons and carbons were observed at expected regions. Mass spectrum confirms the formation of compound **4a** showed m/z = 383 $(M+H)^{+}$.

Table 1. Antimalarial (μM), Antibacterial (MIC in μg/mL) & Antifungal (MIC in μg/mL) activity.

Cpd	Antimalarial activity Plasmodium falciparum	Antibacterial activity				Antifungal activity			Molecular Docking Score
		EC	PA	SA	SP	CA	AN	AC	
3a	1.46	200	200	250	250	500	500	500	−7.814
3b	3.93	100	250	250	200	1000	500	500	−7.032
3c	2.16	62.5	200	125	250	500	>1000	>1000	−7.192
3d	3.07	100	100	200	200	1000	500	500	−7.118
4a	6.31	125	100	100	100	500	500	500	−6.979
4b	0.47	100	200	100	100	250	500	500	−8.157
4c	0.47	125	125	200	200	1000	>1000	>1000	−8.222
4d	0.21	200	100	125	100	500	500	500	−7.988
Chloroquine	0.06	–	–	–	–	–	–	–	–
Quinine	0.83	–	–	–	–	–	–	–	–
CP	–	50	50	50	50	–	–	–	–
NS	–	–	–	–	–	100	100	100	–

Cpd: Compound; EC: Escherichia coli; PA: Pseudomonas aeruginosa; SA: Staphylococcus aureus; SP: Streptococcus pyogenes; CA: Candida albicans; AN: Aspergillus niger; AC: Aspergillus clavatus; CP: Chloramphenicol; NS: Nystatin.

Biological evaluation

In vitro antimalarial screening

All the synthesized novel compounds were tested for antimalarial activities. The *in vitro* antimalarial assay was carried out according to the micro assay protocol of Rieckmann and coworkers with minor modifications.[44–47] The results were recorded as the minimum inhibitory concentrations (μM MIC) chloroquine and quinine were used as the reference drug (Table 1).

Herein, we have synthesized four chalcone and pyrazoline derivatives respectively. Structure activity relationship (SAR) plays very important role while displaying the antimalarial activity. All the synthesized chalcone derivatives (**3a-d**) exhibited less potency compared to the standard drug. But pyrazoline derivatives exhibited excellent antimalarial activity compared to the standard drug. In compound **4a**, thiophene ring was absent and pyrazoline ring is present, so, the compound **4a** exhibited less potency compared to the standard drug. Now, in compound **4b**, bromo substituted thiophene and pyrazoline rings are present along with the fluorine at the para position on benzene ring. Interestingly, this compound **4b** (0.47 μM), exhibited excellent activity compared to the standard drug quinine (0.83 μM). Again, in compound **4c**, bromo substituted thiophene and pyrazoline rings are present but no fluorine at the para position of benzene ring. Though fluorine is absent on benzene ring in compound **4c** (0.47 μM), it exhibited same potency as that of compound **4b** compared to the standard drug quinine (0.83 μM). Finally, in compound **4d**, there were no substitution on the thiophene and benzene ring. In compound **4d** plane thiophene, plane benzene ring and pyrazoline ring constructed in a single molecular framework. Compound **4d** (0.21 μM), exhibited four-fold more antimalarial activity compared to the standard drug quinine (0.83 μM). From SAR, we can conclude that for the antimalarial activity thiophene, pyrazoline and benzene ring were very important in a single molecular framework.

Antimicrobial activities

Further, all the novel synthesized compounds were also screened for antimicrobial activities against the bacterial strains *Escherichia coli* (MTCC 443), *Staphylococcus aureus* (MTCC 96), *Pseudomonas aeruginosa* (MTCC 1688), *Streptococcus pyogenes* (MTCC 442) and fungal strains *Aspergillus clavatus* (MTCC 1323), *Candida albicans* (MTCC 227) and *Aspergillus niger* (MTCC 282). The minimum inhibitory concentration (MIC) was determined by the broth dilution method. Chloramphenicol and Nystatin were used as reference drugs for antibacterial and antifungal activity, respectively. The results of antibacterial and antifungal activity were given in Table 1.

The results given in Table 1 indicated that none of the synthesized compound exhibited significant potency toward the standard antibacterial drug Chloramphenicol and antifungal drug Nystatin. Hence, from above result we can conclude that the synthesized compounds show selectively antimalarial activity and negligible antimicrobial activity.

Conclusion

In conclusion, Considering the importance of enoyl-ACP reductase (*Pf*ENR) in *Plasmodium*, a small library of 8 molecules comprising furan containing pyrazolyl pyrazoline derivatives (**3a-d**, **4a-d**) was designed and docked against *Pf*ENR. Based on the *in silico* binding affinity data, synthesis was carried out for these novel furans containing pyrazolyl pyrazoline derivatives (**3a-d**, **4a-d**) and was evaluated for activity against *Plasmodium falciparum*. The synthesized compounds shown selectively antimalarial activity with minimal antimicrobial activity. Compounds (**3a-d**) exhibited less antimalarial activity compared to the standard drug. From the series of compounds (**4a-d**), compound **4b** (0.47 μM), **4c** (0.47 μM) and **4d** (0.21 μM) exhibited more antimalarial activity compared to the standard drug quinine (0.83 μM). Compound 4d shows four-fold more activity compared to the standard drug quinine. From the SAR, we have distinguished areas of the pyrazolyl chalcones and pyrazolyl pyrazolines framework where variations can be made to expand the pharmacokinetic profile as well as features required to improve inhibitor effectiveness. This innovative molecular scaffold presents breakthrough for optimization to develop effective *Pf*ENR inhibitors.

Experimental

General

All the reagents, solvents and chemicals were taken from commercial sources found to be and used as such without purification. The physical constant like melting points were measured on a DBK melting point apparatus and are uncorrected. IR spectra were recorded on Shimadzu IR Affinity 1S (ATR) FTIR spectrophotometer. ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectra were recorded on Bruker Advance II 400 spectrophotometer using TMS as an internal standard and DMSO-d6 as solvent and chemical shifts were expressed as δ ppm units. Mass spectra were obtained on Waters, Q-TOF micro mass (ESI-MS) mass spectrometer.

General procedure for the synthesis of pyrazolyl chalcones (3a-d)

A mixture of 1-(2,5-dimethylfuran-3-yl)ethanone 1 (0.05 mol), substituted pyrazole aldehyde **2** (0.05 mol) and 10% aqueous potassium hydroxide (10 mL) in ethanol (50 mL) was stirred at room temperature for 14 h. The progress of the reaction was monitored by TLC. After completion of the reaction, the reaction mixture was transferred into crushed ice and neutralized by dil. HCl. The precipitation observed, filtered it, washed with water and dried. The crystallization of product carried out in ethanol.

(E)-1-(2,5-Dimethylfuran-3-yl)-3-(1,3-diphenyl-1H-pyrazol-4-yl)prop-2-en-1-one (3a)

Yield: 61%, yellow solid; mp: 80–82 °C; IR (νmax, cm^{-1}): 2921 (=C–H), 2855 (C–H), 1657 (C = O), 1454 (C = N); ^{1}H-NMR (400 MHz, DMSO-d6, δ, ppm): 9.31 (s, 1H, Pyrazole-H), 7.93 (d, 2H, J = 7.9 Hz), 7.38–7.68 (m, 10H, Ar–H), 6.60 (s, 1H, Furan-H), 2.53 (s, 3H, –CH_3), 2.27 (s, 3H, –CH_3); ^{13}C NMR (100 MHz, DMSO-d6, δ, ppm): 184.4 (C = O), 159.9, 152.8, 149.7, 138.9, 132.2, 132.0, 129.6, 128.8, 128.5, 128.6, 128.4, 127.1, 123.8, 122.1, 118.6, 117.6, 105.9, 13.9 (CH_3), 12.9 (CH_3); MS(ESI-MS): m/z 369.11 (M + H).$^{+}$

(E)-3-(3-(5-Bromothiophen-2-yl)-1-(4-fluorophenyl)-1H-pyrazol-4-yl)-1-(2,5-dimethylfuran -3-yl) prop-2-en-1-one (3b)

Yield: 59%, yellow solid, mp: 112–114 °C; IR (νmax, cm^{-1}): 2923 (=C–H), 2856 (C–H), 1656 (C = O), 1455 (C = N); ^{1}H-NMR (400 MHz, DMSO-d6, δ, ppm): 9.25 (s, 1H, Pyrazole-H), 7.90 (dd, 2H, J = 4.7 & 9.0 Hz, Ar–H), 7.64 (d, 1H, J = 15.4 Hz, olefinic-H), 7.39–7.45 (m, 3H, Ar–H), 7.34 (d, 1H, J = 3.8 Hz, Ar–H), 7.25 (d, 1H, J = 3.8 Hz, Ar–H), 6.61 (S, 1H, Furan-H), 2.55 (s, 3H, $-CH_3$), 2.28 (s, 3H, $-CH_3$); ^{13}C NMR (100 MHz, DMSO-d6, δ, ppm): 184.2, 162.0, 159.6, 157.1, 149.7, 145.7, 135.4, 135.1, 131.4, 130.8, 128.9, 127.3, 124.6, 122.0, 120.7, 120.6, 117.3, 116.6, 116.3, 112.5, 105.9, 13.9, 12.9; MS (ESI-MS): m/z 472.89 $(M + H)^{+}$.

(E)-3-(3-(5-Bromothiophen-2-yl)-1-phenyl-1H-pyrazol-4-yl)-1-(2,5-dimethylfuran-3-yl)prop -2-en-1-one (3c)

Yield: 68%, yellow solid, mp120–114 °C; IR (νmax, cm^{-1}): 2921 (=C–H), 2855 (C–H), 1699 (C = O), 1454 (C = N); ^{1}H-NMR (400 MHz, DMSO-d6, δ, ppm): 9.14 (s,1H, Pyrazole-H), 7.87 (d, 2H, J = 7.8 Hz, Ar–H), 7.70 (d, 1H, J = 15 Hz, olefinic-H), 7.52 (t, 2H, J = 8 Hz, Ar–H), 7.36–7.40 (m, 2H, Ar–H), 7.20 (s, 2H, Ar–H), 6.55 (s, 1H, Furan-H), 2.57 (s, 3H, $-CH_3$), 2.29 (s, 3H, $-CH_3$); ^{13}C NMR (100 MHz, DMSO-d6, δ, ppm): 184.3, 157.1, 149.7, 145.7, 138.6, 135.5, 131.4, 130.9, 129.7, 128.8, 127.3, 127.3, 124.6, 122.0, 118.6, 117.4, 112.5, 105.9, 13.9, 12.9; MS(ESI-MS): m/z 454.57 $(M + H)^{+}$.

(E)-1-(2,5-Dimethylfuran-3-yl)-3-(1-phenyl-3-(thiophen-2-yl)-1H-pyrazol-4-yl)prop-2-en-1-one (3d)

Yield: 62%, yellow solid, mp 124–126 °C; IR (νmax, cm^{-1}): 2921 (=C–H), 2715 (C–H), 1652 (C = O),1456 (C = N); ^{1}H-NMR (400 MHz, DMSO-d6, δ, ppm): 8.56 (s, 1H, Pyrazole-H), 7.91 (d, 2H, J = 7.8 Hz, Ar–H), 7.76 (d, 1H, J = 15.4 Hz, olefinic-H), 7.60 (d, 1H, J = 5.1 Hz, Ar–H), 7.54 (t, 2H, J = 8.2 Hz, Ar–H), 7.35–7.44 (m, 3H, Ar–H), 7.21 (dd, 1H, J = 5.0 & 3.7 Hz, Ar–H), 6.59 (s, 1H, Furan-H), 2.57 (s, 3H, $-CH_3$), 2.29 (s, 3H, $-CH_3$); ^{13}C NMR (100 MHz, DMSO-d6, δ, ppm): 184.4, 157.0, 149.7, 146.8, 138.7, 133.5, 131.5, 129.7, 128.7, 128.1, 127.3, 127.2, 126.8, 124.3, 122.1, 118.6, 117.4, 105.9, 13.9, 12.9; MS(ESI-MS): m/z 375.10 $(M + H)^{+}$.

General procedure for synthesis of pyrazolyl-pyrazoline (4a-d)

A mixture of chalcone **3a-d** (0.001 mol) and hydrazine hydrate (0.004 mol) in solvent ethanol (10 ml) was refluxed in presence of catalytic amount of glacial acetic acid for 6 h. The progress of the reaction was monitored by TLC. After completion of the reaction, the reaction mixture was transferred into crushed ice. The precipitation observed, filtered it, washed with water and dried. The crystallization of product carried out in ethanol to get pure pyrazolines.

4-(4,5-Dihydro-3-(2,5-dimethylfuran-3-yl)-1H-pyrazol-5-yl)-1,3-diphenyl-1H-pyrazole (4a)

Yield: 74%, white solid, mp 102–104 °C; IR (νmax, cm^{-1}): 3306 (N–H), 3049 (Ar–H), 1592 (C = N); ^{1}H-NMR (400 MHz, DMSO-d6, δ, ppm): 8.56 (s, 1H, pyrazole-H), 7.90 (d, 2H, J = 7.8 Hz, Ar–H), 7.76 (d, 2H, J = 8.3 Hz, Ar–H), 7.47–7.52 (m, 4H, Ar–H), 7.41 (t, 1H, J = 7.3 Hz, Ar–H), 7.31 (t, 1H, J = 7.4 Hz, Ar–H), 7.20 (s, 1H, N–H), 6.19 (s, 1H, furan-H), 4.87 (t, 1H, J = 10.7 Hz, pyrazoline-H), 3.34 (dd, 1H, J = 10.5 & 15.6 Hz, pyrazoline-H), 2.88(dd, 1H, J = 11.1 & 16.1 Hz, pyrazoline-H), 2.38 (s, 3H, CH_3), 2.20 (s, 3H, CH_3); ^{13}C NMR (100 MHz, DMSO-d6, δ, ppm): 150.4, 149.3, 147.6, 145.1, 139.5, 132.9, 129.5, 128.6, 127.9, 127.2, 126.2, 123.2, 118.1, 115.2, 105.9, 54.7, 41.9, 13.3, 12.9; MS (ESI-MS): m/z 383.04 $(M + H)^{+}$.

3-(5-Bromothiophen-2-yl)-1-(4-fluorophenyl)-4-(4,5-dihydro-3-(2,5-dimethylfuran-3-yl)-1H-pyrazol-5-yl)-1H-pyrazole (4b)

Yield: 69%, white solid, mp 98–100 °C; IR (νmax, cm^{-1}):3310 (N–H), 3046 (Ar–H), 1594 (C = N); ^{1}H NMR (400 MHz, DMSO-d6, δ, ppm): 8.54 (s, 1H, pyrazole-H), 7.88 (m, 2H, Ar–H), 7.35 (t, 2H, J = 8.7 Hz, Ar–H), 7.28 (dd, 2H, J = 3.8 Hz, Ar–H), 7.21 (s, 1H, N–H), 6.20 (s, 1H, furan-H), 4.93 (t, 1H, J = 10.68 Hz, pyrazoline-H), 3.37 (dd, 1H, J = 10.7 & 16.5 Hz, pyrazoline-H), 2.86 (dd, 1H, J = 10.9 & 16.1 Hz, pyrazoline-H), 2.38 (s, 3H, CH_3), 2.20 (s, 3H, CH_3); ^{13}C NMR (100 MHz, DMSO-d6, δ, ppm): 161.6, 159.1, 149.3, 147.7, 145.3, 144.1, 136.9, 135.6, 131.2, 128.0, 126.6, 122.6, 120.2, 120.2, 116.4, 116.2, 115.1, 111.5, 105.9, 54.3, 41.1, 13.3, 12.9; MS (ESI-MS): m/z 486.93 $(M + H).^+$

3-(5-Bromothiophen-2-yl)-4-(4,5-dihydro-3-(2,5-dimethylfuran-3-yl)-1H-pyrazol-5-yl)-1-phenyl-1H-pyrazole (4c)

Yield: 72%, white solid, mp 122–124 °C; IR (ν max, cm^{-1}): 3303 (N–H), 3096 (Ar–H), 1593 (C = N), ^{1}H NMR (400 MHz, DMSO-d6, δ, ppm): 8.55 (s, 1H, pyrazole-H), 7.84 (d, 2H, J = 7.9 Hz, Ar–H), 7.51 (t, 2H, J = 7.6 Hz, Ar–H), 7.22–7.34 (m, 4H, Ar–H), 6.20 (s, 1H, furan-H), 4.94 (t, 1H, J = 10.6 Hz, pyrazoline-H), 3.38 (m, 1H, pyrazoline-H), 2.88 (dd, 1H, J = 12.1 & 16.1 Hz, pyrazoline-H), 2.39 (s, 3H, CH_3), 2.20 (s, 3H, CH_3); ^{13}C NMR (100 MHz, DMSO-d6, δ, ppm): 149.3, 147.7, 145.2, 144.0, 139.0, 137.0, 131.2, 129.6, 127.8, 126.6, 126.5, 122.5, 118.0, 115.1, 111.4, 105.9, 54.4, 41.1, 13.3, 12.9; MS (ESI-MS): m/z 468.95 $(M + H).^+$

4-(4,5-Dihydro-3-(2,5-dimethylfuran-3-yl)-1H-pyrazol-5-yl)-1-phenyl-3-(thiophen-2-yl)-1H-pyrazole (4d)

Yield: 70%, white solid, mp 96–98 °C; IR (ν max, cm^{-1}): 3336 (N–H), 3067 (Ar–H), 1501 (C = N); ^{1}H NMR (400 MHz, DMSO-d6, δ, ppm): 8.53 (s, 1H, pyrazole-H), 7.86 (d, 1H, J = 8 Hz, Ar–H), 7.58 (d, 1H, J = 4.9 Hz, Ar–H), 7.47–7.52 (m, 3H, Ar–H), 7.31 (t, 1H, J = 7.3 Hz, Ar–H), 7.15–7.20 (m, 2H, Ar–H), 6.21 (s, 1H, furan-H), 4.98 (t, 1H, J = 10.5 Hz, pyrazoline-H), 3.42 (m, 1H, pyrazoline-H), 2.89 (dd, 1H, J = 10.7 & 16.1 Hz, pyrazoline-H), 2.39 (s, 3H, CH_3), 2.20 (s, 3H, CH_3); ^{13}C NMR (100 MHz, DMSO-d6, δ, ppm): 149.3, 147.7, 145.1, 144.9, 139.2, 135.0, 129.6, 127.9, 127.4, 126.3, 126.0, 125.8, 122.6, 118.1, 115.1, 105.9, 54.5, 41.3, 13.3, 12.9; MS (ESI-MS): m/z 389.03 $(M + H).^+$

Experimental protocol for biological activity

Antimalarial assay

The antimalarial activity of the synthesized compounds was carried out in the Microcare laboratory & TRC, Surat, Gujarat. According to the micro assay protocol of Rieckmann and coworkers the *in vitro* antimalarial assay was carried out in 96 well microtiter plates. To maintain *P. falciparum* strain culture in medium Roswell Park Memorial Institute (RPMI) 1640 supplemented with 25 mM (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid) (HEPES), 1% D-glucose, 0.23% sodium bicarbonate and 10% heat inactivated human serum. To obtain only the ring stage parasitized cells, 5% D-sorbitol treatment required to synchronized the asynchronous parasites of *P. falciparum*. To determine the percent parasitaemia (rings) and uniformly maintained with 50% RBCs (O^+) an initial ring stage parasitaemia of 0.8 to 1.5% at 3% hematocrit in a total volume of 200 µl of medium RPMI-1640 was carried out for the assay. A stock solution of 5 mg/ml of each of the test samples was prepared in DMSO and subsequent dilutions were prepared with culture medium. To the test wells to obtain final concentrations (at five-fold dilutions) ranging between 0.4 µg/ml to 100 µ g/ml in duplicate well containing parasitized cell preparation the diluted samples in 20 µl volume were added. In a candle jar, the culture plates were incubated at 37 °C. Thin

blood smears from each well were prepared and stained with Jaswant Singh-Bhattacharji (JSB) stain after 36 to 40 h incubation. To record maturation of ring stage parasites into trophozoites and schizonts in presence of different concentrations of the test agents the slides were microscopically observed. The minimum inhibitory concentrations (MIC) was recorded as the test concentration which inhibited the complete maturation into schizonts. Chloroquine was used as the reference drug.

After incubation for 38 hours, and percent maturation inhibition with respect to control group, the mean number of rings, trophozoites and schizonts recorded per 100 parasites from duplicate wells.

Molecular docking

The crystal structure of *Plasmodium Falciparum Enoyl-Acyl-Carrier-Protein Reductase* (*Pf*ENR or FabI) in complex with its inhibitor Triclosan was retrieved from the protein data bank (PDB) (pdb code: 1NHG) and refined using the protein preparation wizard. It involves eliminating all crystallographically observed water (as no conserved interaction is reported with co-crystallized water molecules), addition of missing side chain/hydrogen atoms. Considering the appropriate ionization states for the acidic as well as basic amino acid residues, the appropriate charge and protonation state were assigned to the protein structure corresponding to pH 7.0 followed by thorough minimization, using OPLS-2005 force-field, of the obtained structure to relieve the steric clashes due to addition of hydrogen atoms. The 3D structures of the furan containing pyrazolyl chalcones (**3a-d**) were sketched using the build panel in Maestro and were optimized using the Ligand Preparation module followed by energy minimization using OPLS-2005 force-field until their average root mean square deviation (RMSD) reached 0.001 Å. The active site of *Pf*ENR was defined using receptor grid generation panel to include residues within a 10 Å radius around the co-crystallized ligand. Using this setup, flexible docking was carried using the extra precision (XP) Glide scoring function to gauze the binding affinities of these molecules and to identify binding mode within the target. The obtained results as docking poses were visualized and analyzed quantitatively for the thermodynamic elements of interactions with the residues lining the active site of the enzyme using the Maestro's Pose Viewer utility.

Acknowledgements

Authors are thankful to Microcare laboratory and TRC, Surat, Gujarat for providing antimicrobial and antimalarial activities and Director, SAIF, Panjab University, Chandigarh for providing spectral data; Schrodinger Inc. for providing the software to perform the *in silico* study.

Disclosure statement

No potential conflict of interest was reported by the author(s).

Funding

Authors are thankful to Department of Science and Technology, New Delhi for providing financial assistance for research facilities under DST-FIST programme.

References

1. E. A. Ashley, A. Pyae Phyo, and C. J. Woodrow, "Malaria," *The Lancet* 391, no. 10130 (2018): 1608–21.
2. World Health Organization. "World Malaria Report 2019" (World Health Organization, Geneva, 2019). Licence: CC BY-NC-SA 3.0 IGO.

3. J. Talapko, I. Skrlec, T. Alebic, M. Jukic, and A. Vcev, "Malaria: The Past and the Present," *Microorganisms* 7 (2019): 179.
4. R. J. Heath, and C. O. Rock, "Enoyl-Acyl Carrier Protein Reductase (fabI) Plays a Determinant Role in Completing Cycles of Fatty Acid Elongation in Escherichia coli," *The Journal of Biological Chemistry* 270, no. 44 (1995): 26538–42.
5. R. F. Waller, S. A. Ralph, M. B. Reed, V. Su, J. D. Douglas, D. E. Minnikin, A. F. Cowman, G. S. Besra, and G. I. McFadden, "A Type II Pathway for Fatty Acid Biosynthesis Presents Drug Targets in Plasmodium falciparum," *Antimicrobial Agents and Chemotherapy* 47, no. 1 (2003): 297–301.
6. G. Nicola, C. A. Smith, E. Lucumi, M. R. Kuo, L. Karagyozov, D. A. Fidock, J. C. Sacchettini, and R. Abagyan, "Discovery of Novel Inhibitors Targeting Enoyl-Acyl Carrier Protein Reductase in Plasmodium falciparum by Structure-Based Virtual Screening," *Biochemical and Biophysical Research Communications* 358, no. 3 (2007): 686–91.
7. N. Surolia, and A. Surolia, "Triclosan Offers Protection against Blood Stages of Malaria by Inhibiting enoyl-ACP Reductase of Plasmodium falciparum," *Nature Medicine* 7, no. 2 (2001): 167–73.
8. S. Sharma, T. N. C. Ramya, A. Surolia, and N. Surolia, "Triclosan as a Systemic Antibacterial Agent in a Mouse Model of Acute Bacterial Challenge," *Antimicrobial Agents and Chemotherapy* 47, no. 12 (2003): 3859–66.
9. R. P. Samal, V. M. Khedkar, R. R. S. Pissurlenkar, A. G. Bwalya, D. Tasdemir, R. A. Joshi, P. R. Rajamohanan, V. G. Puranik, and E. C. Coutinho, "Design, Synthesis, Structural Characterization by IR, (1) H, (13) C, (15) N, 2D-NMR, X-Ray Diffraction and Evaluation of a New Class of Phenylaminoacetic Acid Benzylidene Hydrazines as pfENR Inhibitors," *Chemical Biology & Drug Design* 81, no. 6 (2013): 715–29.
10. M. Chhibber, G. Kumar, P. Parasuraman, T. N. C. Ramya, N. Surolia, and A. Surolia, "Novel Diphenyl Ethers: Design, Docking Studies, Synthesis and Inhibition of Enoyl ACP Reductase of Plasmodium falciparum and Escherichia coli,"*Bioorganic & Medicinal Chemistry* 14, no. 23 (2006): 8086–98.
11. V. A. Morde, M. S. Shaikh, R. R. S. Pissurlenkar, and E. C. Coutinho, "Molecular Modeling Studies, Synthesis, and Biological Evaluation of Plasmodium falciparum Enoyl-Acyl Carrier Protein Reductase (PfENR) Inhibitors,"*Molecular Diversity* 13, no. 4 (2009): 501–17.
12. A. Manhas, A. Patel, M. Y. Lone, P. K. Jha, and P. C. Jha, "Identification of PfENR Inhibitors: A Hybrid Structure-Based Approach in Conjunction with Molecular Dynamics Simulations," *Journal of Cellular Biochemistry* 119, no. 10 (2018): 8490–500.
13. M. A. Berghot, and E. B. Moawad, "Convergent Synthesis and Antibacterial Activity of Pyrazole and Pyrazoline Derivatives of Diazepam," *European Journal of Pharmaceutical Sciences: Official Journal of the European Federation for Pharmaceutical Sciences* 20, no. 2 (2003): 173–9.
14. J. N. Dominguez, J. E. Charris, M. Caparelli, and F. Riggione, "Synthesis and Antimalarial Activity of Substituted Pyrazole Derivatives," *Arzneimittel-Forschung* 52, no. 6 (2002): 482–8.
15. R. Sridhar, P. T. Perumal, S. Etti, G. Shanmugam, M. N. Ponnuswamy, V. R. Prabavathy, and N. Mathivanan, "Design, Synthesis and anti-Microbial Activity of 1*H*-Pyrazole Carboxylates," *Bioorganic & Medicinal Chemistry Letters* 14, no. 24 (2004): 6035–40.
16. Shivapura Viveka, Dinesha Dinesha, Prasanna Shama, Shivalingegowda Naveen, Neratur Krishnappagowda Lokanath, and Gundibasappa Karikannar Nagaraja, "Design, Synthesis, Anticonvulsant and Analgesic Studies of New Pyrazole Analogues: A Knoevenagel Reaction Approach,"*RSC Advances* 5no. 115 (2015): 94786–95.
17. Z. Xu, C. Gao, Q. C. Ren, X. F. Song, L. S. Feng, and Z. S. Lv, "Recent Advances of Pyrazole-Containing Derivatives as Anti-Tubercular Agents," *European Journal of Medicinal Chemistry* 139, (2017): 429–40.
18. R. S. Fabiane, T. S. Vanessa, R. Viviane, P. B. Lysandro, R. O. Marli, G. B. Helio, Z. Nilo, A. P. M. Marcos, and F. M. Carlos, "Hypothermic and Antipyretic Effects of 3-Methyl- and 3-Phenyl-5-Hydroxy-5-Trichloromethyl-4,5-Dihydro-1*H*-Pyrazole-1-Carboxyamides in Mice," *European Journal of Pharmacology* 451 (2002): 141–7.
19. A. Ana, R. C. Jose, P. C. Fernando, D. O. Agel, J. G. Maria, H. Antonio, L. Fernando, and M. Andres, "Efficient Tautomerization Hydrazone-Azomethine Imine under Microwave Irradiation. Synthesis of [4,3] and [5,3]Bipyrazoles," *Tetrahedron* 54 (1998): 13167–80.
20. K. O. Mohammed, and Y. M. Nissan, "Synthesis, Molecular Docking, and Biological Evaluation of Some Novel Hydrazones and Pyrazole Derivatives as Anti-Inflammatory Agents," *Chemical Biology & Drug Design* 84, no. 4 (2014): 473–88.
21. B. Insuasty, A. Montoya, D. Becerra, J. Quiroga, R. Abonia, S. Robledo, I. D. Velez, Y. Upegui, M. Nogueras, and J. Cobo, "Synthesis of Novel Analogs of 2-pyrazoline Obtained from [(7-Chloroquinolin-4-yl)Amino]Chalcones and Hydrazine as Potential Antitumor and Antimalarial Agents," *European Journal of Medicinal Chemistry* 67 (2013): 252–62.
22. G. Kumar, O. Tanwar, J. Kumar, M. Akhter, S. Sharma, C. R. Pillai, M. M. Alam, and M. S. Zama, "Pyrazole-Pyrazoline as Promising Novel Antimalarial Agents: A Mechanistic Study," *European Journal of Medicinal Chemistry* 149 (2018): 139–47.

23. R. Alam, A. Alam, and A. K. Panda Rahisuddin, "Design, Synthesis and Cytotoxicity Evaluation of Pyrazolyl Pyrazoline and Pyrazolyl Aminopyrimidine Derivatives as Potential Anticancer Agents," *Medicinal Chemistry Research* 27 (2018): 560–70.
24. S. Viveka, D. P. Shama, G. K. Nagaraja, S. Ballav, and S. Kerkar, "Design and Synthesis of Some New Pyrazolyl-Pyrazolines as Potential Anti-Inflammatory, Analgesic and Antibacterial Agents," *European Journal of Medicinal Chemistry* 101 (2015): 442–51.
25. H. Khanam, A. Mashrai, A. Sherwani, M. Owais, and N. Siddiqui, "Synthesis and Anti-Tumor Evaluation of B-Ring Substituted Steroidal Pyrazoline Derivatives," *Steroids* 78, (2013): 1263–72.
26. S. K. Sahu, M. Banerjee, A. Samantray, C. Behera, and M. A. Azam, "Synthesis, Analgesic, anti-Inflammatory and Antimicrobial Activities of Some Novel Pyrazoline Derivatives," *Tropical Journal of Pharmaceutical Research* 7, no. 2 (2008): 961–8.
27. M. Johnson, B. Younglove, L. Lee, R. LeBlanc, H. Holt, Jr, P. Hills, H. Mackay, T. Brown, S. L. Mooberry, and M. Lee, "Design, Synthesis, and Biological Testing of Pyrazoline Derivatives of combretastatin-A4," *Bioorganic & Medicinal Chemistry Letters* 17, no. 21 (2007): 5897–901.
28. J. Vinayagam, R. L. Gajbhiye, L. Mandal, M. Arumugam, A. Achari, and P. Jaisankar, "Substituted Furans as Potent Lipoxygenase Inhibitors: Synthesis, in Vitro and Molecular Docking Studies," *Bioorganic Chemistry* 71 (2017): 97–101.
29. C. J. Lim, N. H. Kim, H. J. Park, B. H. Lee, K. S. Oh, and K. Y. Yi, "Synthesis and SAR of 5-Aryl-Furan-2-Carboxamide Derivatives as Potent urotensin-II Receptor Antagonists," *Bioorganic & Medicinal Chemistry Letters* 29, no. 4 (2019): 577–80.
30. B. Wang, Y. Shi, Y. Zhan, L. Zhang, Y. Zhang, L. Wang, X. Zhang, Y. Li, Z. Li, and B. Li, "Synthesis and Biological Activity of Novel Furan/Thiophene and Piperazine-Containing (Bis)1,2,4-Triazole Mannich Bases," *Chinese Journal of Chemistry* 33, no. 10 (2015): 1124–34.
31. Z. Lan, D. Xinshan, W. Jiaofeng, M. Guangpeng, L. Congchong, C. Guzhou, Z. Qingchun, and H. Chun, "Design, Synthesis and Biological Activities of N-(Furan-2-Ylmethyl)-1H-Indole-3-Carboxamide Derivatives as Epidemal Growth Factor Receptor Inhibitors and Anticancer Agents," *Chemical Research in Chinese Universities* 33, no. 3 (2017): 365–72.
32. M. L. Go, M. Liu, P. Wilairat, P. J. Rosenthal, K. J. Saliba, and K. Kirk, "Antiplasmodial Chalcones Inhibit Sorbitol-Induced Hemolysis of Plasmodium Falciparum-Infected Erythrocytes," *Antimicrobial Agents and Chemotherapy* 48, no. 9 (2004): 3241–5.
33. J. A. Gonzalez, and A. Estevez-Braun, "Effect of (*E*)-Chalcone on Potato-Cyst Nematodes Globodera Pallida and G. rostochiensis," *Journal of Agricultural and Food Chemistry* 46 (1998): 1163–5.
34. M. Yoshimura, A. Sano, J. L. Kamei, and A. Obata, "Identification and Quantification of Metabolites of Orally Administered Naringenin Chalcone in Rats," *Journal of Agricultural and Food Chemistry* 57, no. 14 (2009): 6432–7.
35. M. Chen, T. G. Theander, S. B. Christensen, L. Hviid, L. Zhai, and A. Kharazmi, "Licochalcone A, a New Antimalarial Agent, Inhibits in Vitro Growth of the Human Malaria Parasite Plasmodium falciparum and Protects Mice from P. yoelii Infection," *Antimicrobial Agents and Chemotherapy* 38, no. 7 (1994): 1470–5.
36. L. Mishra, R. Sinha, H. Itokawa, K. F. Bastow, Y. Tachibana, Y. Nakanishi, N. Kilgore, and K. H. Lee, "Anti-HIV and Cytotoxic Activities of Ru(II)/Ru(III) Polypyridyl Complexes Containing 2,6-(2'-Benzimidazolyl)-Pyridine/Chalcone as Co-Ligand," *Bioorganic & Medicinal Chemistry* 9, no. 7 (2001): 1667–71.
37. C. Jin, Y. J. Liang, H. He, and L. Fu, "Synthesis and Antitumor Activity of Novel Chalcone Derivatives," *Biomedicine & Pharmacotherapy = Biomedecine & Pharmacotherapie* 67, no. 3 (2013): 215–7.
38. F. Herencia, M. L. Ferrándiz, A. Ubeda, J. N. Domínguez, J. E. Charris, G. M. Lobo, and M. J. Alcaraz, "Synthesis and anti-Inflammatory Activity of Chalcone Derivatives," *Bioorganic & Medicinal Chemistry Letters* 8, no. 10 (1998): 1169–74.
39. Y. Qian, G. Y. Ma, Y. Yang, K. Cheng, Q. Z. Zheng, W. J. Mao, L. Shi, J. Zhao, and H. L. Zhu, "Synthesis, Molecular Modeling and Biological Evaluation of Dithiocarbamates as Novel Antitubulin Agents," *Bioorganic & Medicinal Chemistry* 18, no. 12 (2010): 4310–6.
40. R. A. Friesner, J. L. Banks, R. B. Murphy, T. A. Halgren, J. J. Klicic, D. T. Mainz, M. P. Repasky, E. H. Knoll, M. Shelley, J. K. Perry, et al. "Glide: A New Approach for Rapid, Accurate Docking and Scoring. 1. Method and Assessment of Docking accuracy," *Journal of Medicinal Chemistry* 47, no. 7 (2004): 1739–49.
41. R. A. Friesner, R. B. Murphy, M. P. Repasky, L. L. Frye, J. R. Greenwood, T. A. Halgren, P. C. Sanschagrin, and D. T. Mainz, "Extra Precision Glide: Docking and Scoring Incorporating a Model of Hydrophobic Enclosure for Protein-Ligand Complexes," *Journal of Medicinal Chemistry* 49, no. 21 (2006): 6177–96.
42. T. A. Halgren, R. B. Murphy, R. A. Friesner, H. S. Beard, L. L. Frye, W. T. Pollard, and J. L. Banks, "Glide: A New Approach for Rapid, Accurate Docking and Scoring. 2. Enrichment Factors in Database Screening," *Journal of Medicinal Chemistry* 47, no. 7 (2004): 1750–9.

43. S. J. Takate, A. D. Shinde, B. K. Karale, H. Akolkar, L. Nawale, D. Sarkar, and P. C. Mhaske, "Thiazolyl-Pyrazole Derivatives as Potential Antimycobacterial Agents," *Bioorganic & Medicinal Chemistry Letters* 29, no. 10 (2019): 1199–202.
44. K. H. Rieckmann, G. H. Campbell, L. J. Sax, and J. E. Ema, "Drug Sensitivity of plasmodium falciparum. An In-Vitro Micro Technique," The *Lancet* 311, no. 8054 (1978): 22–3.
45. R. Panjarathinam, *Text Book of Medical Parasitology*, 2nd ed. (Chennai: Orient Longman Pvt. Ltd., 2007), 329–331.
46. C. Lambros, and J. P. Vanderberg, "Synchronization of Plasmodium falciparum Erythrocytic Stages in Culture," *The Journal of Parasitology* 65, no. 3 (1979): 418–20.
47. J. S. B. Singh, "Stain; a Review," *Indian Journal of Malariology* 10 (1956): 117–29.