Research Article

In vitro antimalarial evaluation and molecular docking analysis of Mannich base curcumin analogues against the artemisinin target PfATP6

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Abstract

Objective: The aim was to design twenty Mannich bases of curcumin based on their structural analogy to curcumin, dock them against the *Plasmodium falciparum* ATP6 (PfATP6) gene and based on their binding energies with the protein, select, synthesize and evaluate them for their *in vitro* antimalarial activity. **Methods and Results:** Compounds were designed and docked onto the active site of PfATP6 to determine their binding affinities with the protein. The selected compounds were synthesized using the Mannich reaction and their antimalarial activity was evaluated *in vitro* against chloroquine-sensitive 3D7 strain and mutant RKL2 strain of *P. falciparum*. The compounds CDM3 (-3.11 Kcal/mol, 4.11 µg/ml against 3D7 and 10.89 µg/ml against RKL2 strains) and CDM1 (-4.11 Kcal/mol, 4.09 µg/ml against 3D7 and 8.2 µg/ml against RKL2 strains) showed the best binding energies and antimalarial activity with IC_{50} values comparable to that of curcumin (-4.95 Kcal/mol, 2.13 µg/ml, 5.59 µg/ml) but less than that of chloroquine (0.7 µg/ml against 3D7 and 1.4 µg/ml against RKL2 strains). **Conclusion:** Mannich base curcumin derivatives showed substantial antimalarial activity compared to curcumin and showed proper binding energies with the PfATP6 protein through hydrophobic binding interactions. The major binding point was found to be with the residue Lys1213 which could be the reason for the antimalarial activity of the compounds and the site could be exploited for further antimalarial drug targeting. The study provided a detailed insight into the necessary structural modifications to be made in the curcumin molecule to create more potent drug candidates for rationale-based antimalarial drug design and development.

***Keywords*:** Malaria, Mannich base, Curcumin, PfATP6, Molecular docking

Introduction

Despite years of intense research, malaria remains a deadly infectious disease worldwide. According to World Health Organization (WHO), Malaria is the fourth most widespread infectious disease after tuberculosis, HIV and pneumonia (Gupta and Guin, 2010). The development of resistance by malaria parasite to the first line and second line antimalarial drugs, has contributed to the persistence of this infectious disease and hence there is an urgent need of new drugs with more safety and efficacy to overcome the burden. Currently artemisinin-based combination treatments are the mainstay of treatment for *P. falciparum* malaria globally, but emergence of artemisinin resistance in Southeast Asia poses a serious threat to the global control of *P. falciparum* malaria (WHO, 2016). Therefore, need of identification of an antimalarial drug that is easy to produce/isolate, is inexpensive, and demonstrates little toxicity across a diverse population is very much important for global malaria control programs and eradication of this deadly infectious disease. Drug discovery for malaria is a time consuming and expensive process, but continuous evolution of the drug discovery methods and high-quality lead generation process is likely to deliver potential compounds with better therapeutic activity. Numerous reports are available

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mentioning the traditional use of turmeric in the treatment of malaria in many countries including India (Aggarwal et al., 2006). Curcumin 1, 7-bis-(4-hydroxy-3-methoxyphenyl)-1, 6-heptadiene-3, 5-dione (diferuloyl methane), a hydrophobic polyphenol is one of the important phytoconstituent derived from the rhizome part of the plant *Curcuma longa*. It gained importance due to wide range of therapeutic properties and was also found to be safe in high doses in various animal and human models. It is also a promising lead compound for antimalarial drug development and its antimalarial activity has been established both in *in vitro* and *in vivo* studies (Reddy et al., 2015; Chakrabarti et al., 2013). A detailed structural elucidation of the curcumin molecule had earlier revealed that the phenolic groups in the curcumin nucleus play a crucial role in its anti-malarial activity. Replacement or removal of the phenolic group leads to a loss of activity which suggests that two unsubstituted phenolic groups are necessary for curcumin's antimalarial activity (Mishra et al., 2008). The antiparasitic action of curcumin has been hypothesized to be due to its binding to a hydrophobic pocket in the transmembrane region of the PfATP6 (modelled) protein in the parasite and thereby interfering with its calcium transport (Naik et al., 2011). *P. falciparum* Ca(2+)-ATPase (*Pf*ATP6) the parasite orthologue of mammalian Sarcoplasmic–Endoplasmic Reticulum Ca2+–ATPase (SERCA), has been confirmed to be the molecular target of the most potent animalarial artemisinins (Jung et al., 2005). Through docking simulation studies, it was found that curcumin effectively inhibited PfATP6 through hydrophobic interactions and hydrogen bonds, leading to its antimalarial action (Ji and Shen, 2009). According to recent researches, derivatives and analogues of curcumin have been proclaimed to have improved efficacy against *P. falciparum* cultures than the parent molecule (Awsthi et al., 2009; Dohutia et al., 2017). Mannich bases are a structurally heterogeneous class of chemical compounds that are generated from various substrates through the introduction of an aminomethyl function by means of the mannich reaction and have several important biological activities including antimalarial activity (March, 2005; Raynes et al., 1999). In our study for the first time we have synthesized curcumin analogues for antimalarial activity targeting the PfATP6 protein. Most of the compounds were designed keeping the pharmacologically active hydroxy and methoxy groups which are present in curcumin apart from a few compounds which contained halogens. The compounds were designed keeping in mind the feasibility of the reaction to proceed. The work is likely to help in developing potential leads for the development of newer drugs/ hybrid molecules against malaria.

Materials and methods

The Mannich base series of curcumin analogues were designed based on the Mannich reaction involving formaldehyde with that of the active hydrogen (-CH_2-) present in the seven membered carbon chain of curcumin in presence of a primary/secondary amine. Most of the compounds were designed keeping the hydroxy and methoxy groups which are present in curcumin. The ligands were prepared using the 'Prepare ligand' protocol of DS 4.5 which eliminates corresponding structures, standardizes the charges of common groups, calculates the ions and ionization of the ligand's functional groups, generates isomers and tautomers, carries out 2D-3D conversion, verifies and optimizes the structures, and other tasks established by user-defined parameters. Energy minimizations of all the ligands were done by applying Chemistry at Harvard Macromolecular Mechanics (CHARMM) force field. All the designed compounds were subjected to molecular analysis prior to docking studies using the online Molinspiration Chemoinformatics software (http://www.molinspiration.com/cgi-bin/properties). Important molecular properties such as molecular weight, logP values, polar surface area of the molecules (Ertl et al., 2000) number of hydrogen bond donors/acceptors and number of rotatable bonds all of which contribute to the Lipinsky Rules have been estimated through Molinspiration (Lipinsky, 2009). PfATP6 was used as receptor molecule for the docking study to probe the binding free energy between the ligand library and receptor using AutoDock 4.2 (Morris et al., 2009). Autodock Tools (ADT) was used to optimize the receptor and ligand molecules. Addition of polar hydrogen's, Kollman charges and AD4 type of atoms was carried out for the preparation of the receptor molecule, while Gasteiger charges were combined to the ligands and maximum numbers of active torsions were given. AutoGrid4 was used to prepare a grid map of interaction energies around the residues Leu263, Phe264, Gln267, Ile977, Ile981, Ala985, Asn1039, Leu1040, Ile1041 and Asn1042 with a grid box of 90 X 90 X 90 Å3 centred on X, Y, Z = 52.27, 16.45, 11.48 with a grid spacing of 0.375Å. The residues in the current study have been used with reference to earlier reports of their interactions with artemisinin and curcumin (Garah et al., 2009; Shandilya et al., 2013). Molecular docking was performed using Lamarckian Genetic Algorithm (LGA), keeping the receptor molecule rigid throughout the docking simulation and rest of the docking parameters was set to default values. Ten different poses were generated for each ligand and scored using AutoDock 4.2 scoring functions and were ranked according to their docked energy. AutoDock Tools, PyMOL (Delano, 2002; Lerner and Carlson, 2008) and Discovery studio 4.5 client (BIOVIA Discovery Studio Client 4.5) were used for post-docking analysis.

(1E,6E)-4-[(diethylamino)methyl]-1,7-bis(4-hydroxy-3-methoxyphenyl)hepta-1,6-diene-3,5-dione (CDM-6)

Unimolar amounts of curcumin and N,N-diethylamine were taken in separate beakers and dissolved in 1:1 ratio of 95% ethanol and tetrahydrofuran. A few drops of concentrated HCl was added to both the solutions. The contents were then mixed together while adding formaldehyde drop wise and constantly stirring in an ice bath. The mixture was then refluxed in an ice bath for 10-18 hrs. The progress of the reaction was checked by thin layer chromatography (TLC).

TLC: Toluene/Methanol (7:2). Rf = 0.61, yield: 59%, mp: 128-131°C. IR (cm-1): 3394, 2956, 2825, 1678. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.0548 (s, 1H), 3.6033-3.7232 (m, 3H), 2.033-2.2232 (t, 2H), 1.4990-1.5193 (m, 6H), 2.6093-2.7291 (t, 2H), 9.7291 (s, 1H), 7.0149-7.7291 (m, 6H). ^{13}C NMR (CDCl3, 400 MHz): δ 192.10, 152.60, 151.89, 147.61, 138.22, 134.89, 132.85, 130.60, 129.00, 128.67, 128.55, 128.50, 122.10, 67.22, 61.57, 59.10, 55.61, 22.67. MS (EI, m/z): 453(M-60+).

(1E,6E)-4-[(dimethylamino)methyl]-1,7-bis(4-hydroxy-3-methoxyphenyl)hepta-1,6-diene-3,5-dione (CDM-3)

Unimolar amounts of curcumin and N,N-dimethylamine were taken in separate beakers and dissolved in 1:1 ratio of 95% ethanol and tetrahydrofuran. A few drops of concentrated HCl was added to both the solutions. The contents were then mixed together while adding formaldehyde drop wise and constantly stirring in an ice bath. The mixture was then refluxed in an ice bath for 10-18 hrs. The progress of the reaction was checked by TLC.

TLC: Toluene/Methanol (6:1). Rf = 0.78, yield: 51%, mp: 191-195°C. IR (cm-1): 3329, 2939, 2836, 1609, 1271. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.0548 (s, 1H), 3.6093-3.7291 (m, 3H), 3.2033-3.2232 (m, 2H), 2.2232 (m, 3H), 2.2532 (m, 3H), 2.4990 (m, 2H), 2.5193 (m, 2H). ^{13}C NMR (CDCl3, 400 MHz): δ 198.22, 152.20, 151.00, 147.61, 132.85, 130.60, 129.00, 128.67, 128.55, 123.50, 122.10, 68.22, 61.50, 59.10, 54.89. MS (EI, m/z): 425(M-60+).

(1E,6E)-1,7-bis(4-hydroxy-3-methoxyphenyl)-4-(piperidin-1-ylmethyl)hepta-1,6-diene-3,5-dione (CDM-2)

Unimolar amounts of curcumin and piperidine were taken in separate beakers and dissolved in 1:1 ratio of 95% ethanol and tetrahydrofuran. A few drops of concentrated HCl was added to both the solutions. The contents were then mixed together while adding formaldehyde drop wise and constantly stirring in an ice bath. The mixture was then refluxed in an ice bath for 10-18 hrs. The progress of the reaction was checked by TLC.

TLC: Toluene/Methanol (7:3). Rf = 0.78, yield: 52%. m.p. 185-187 °C; IR (cm^{-1}): 3629, 2930, 2850, 1250, 1579. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 9.71 (s, H), 7.0149-7.7291 (m, 6H), 3.7532 (t, 4H), 3.2033-3.2532 (d, 2H), 2.2149-2.5532 (m, 4H) 1.0548-1.4990 (m, 4H). ^{13}C NMR (400 MHz) δ: 191.61, 147.61, 145.10, 144.90, 138.22, 134.89, 132.85, 130.60, 129.00, 128.67, 128.55, 128.50, 122.10, 67.22, 61.56, 59.10, 55.61, 24.89, 22.62. MS (EI, m/z): 465.32 (M-60+).

(1E,6E)-1,7-bis(4-hydroxy-3-methoxyphenyl)-4-[(4-oxopiperidin-1-yl)methyl]hepta-1,6-diene-3,5-dione (CDM-14)

Unimolar amounts of curcumin and piperidinone were taken in separate beakers and dissolved in 1:1 ratio of 95% ethanol and tetrahydrofuran. A few drops of concentrated HCl was added to both the solutions. The contents were then mixed together while adding formaldehyde drop wise and constantly stirring in an ice bath. The mixture was then refluxed in an ice bath for 10-18 hrs. The progress of the reaction was checked by TLC.

TLC: Dichloromethane/Methanol (5:2). Rf = 0.55, yield: 57%. m.p. 191-193 °C; IR (cm^{-1}): 3629, 2930, 2850, 1250, 1579. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 9.7291 (s, H), 7.0149-7.7291 (m, 6H), 3.7532 (t, 4H), 3.2033-3.2532 (d, 2H), 2.2149-2.5532 (m, 4H) 1.0548-1.4990 (m, 4H). ^{13}C NMR (400 MHz) δ: 191.61, 147.61, 145.10, 144.90, 138.22, 134.89, 132.85, 130.60, 129.00, 128.67, 128.55, 128.50, 122.10, 67.22, 61.56, 59.10, 55.61, 24.89, 22.62. MS (EI, m/z): 465.32 (M-60+).

(1E,6E)-1,7-bis(4-hydroxy-3-methoxyphenyl)-4-(thiomorpholinomethyl)hepta-1,6-diene-3,5-dione (CDM-1)

Unimolar amounts of curcumin and thiomorpholine were taken in separate beakers and dissolved in 1:1 ratio of 95% ethanol and tetrahydrofuran. A few drops of concentrated HCl was added to both the solutions. The contents were then mixed together while adding formaldehyde drop wise and constantly stirring in an ice bath. The mixture was then refluxed in an ice bath for 10-18 hrs. The progress of the reaction was checked by TLC.

TLC: Dichloromethane/Methanol (5:1). Rf = 0.53, yield: 61%. m.p. 177-182 °C; IR (cm^{-1}): 3381, 2935, 2821, 1633, 1247. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 9.7291 (s, H), 7.0149-7.7291 (m, 6H), 3.7532 (t, 4H), 3.2033-3.2532 (d, 2H), 2.2149-2.5532 (m, 4H) 1.0548-1.4990 (m, 4H). ^{13}C NMR (400 MHz) δ: 190.55, 144.90, 128.50-138.22, 122.10, 68.90, 61.50, 58.10, 53.89, 24.89. MS (EI, m/z): 483.1872 (M-60+).

***In vitro* antimalarial evaluation**

The *in vitro* antimalarial screening was carried out following the microassay methods of Reickmann and Desjardins with minor modifications (Rieckmann et al.,

1978; Desjardins et al., 1979). We used two different dosages 5 µg/ml and 50 µg/ml for preliminary screening of the synthesized products and the ones with good antimalarial activity were further tested for determination of IC_{50} values. The compounds were dissolved in 1:200 dimethyl sulfoxide (DMSO) to get a stock solution of 5 mg/ml concentration. The further required dilutions of the stock solutions were prepared with incomplete media (without serum). To a 96-well flat bottom microtiter plate required amount of the test compounds of the secondary standard curcumin and different test dosages were charged per well in triplicates and accordingly the required volume of synchronized parasites in 3% hematocrit containing 1% parasitemia was added to get the final test dose. Chloroquine (Sigma chemicals) as a primary standard and curcumin (Sigma chemicals) as a secondary standard was used for this test to validate the integrity of the assay. The negative control wells inoculated with 20 µl of CRPMI (Complete Roswell Park Memorial Institute) to which 180 µl of 3% hematocrit with 1% parasitaemia were added while another set of negative control wells received 20 µl of incomplete culture medium and 0.5 % DMSO. The plates were incubated for 40hrs in a water jacketed incubator at 37ºC and 5% CO_2 environment, after which thin blood smears were prepared from each well, fixed with methanol, stained with 3% Giemsa and observed under microscope. The level of parasitemia in terms of % dead rings and trophozoites was determined by counting a total of 100 asexual parasites (both live and dead) microscopically.

Results and discussion

Molecular docking studies

From table 1 we observed that the Mannich base drivatives CDM-15 and CDM-16 violated two of the five Lipinsky rules. In both the cases it was observed that they have a logP value over five which is likely to affect their partition across membranes and a molecular weight above five hundred daltons. As a result these compounds were not processed further. The rest of the compounds fulfilled all the criteria of Lipinsky rules as no violations were observed and were further processed for their docking studies. The docking results of Mannich based derivatives of curcumin indicated that the compounds with aliphatic secondary amines showed better binding energies than their aromatic counterparts. The derivatives containing dimethylamine (CDM-3) and diethylamine (CDM-6) showed a much better affinity to the binding site of the PfATP6 protein than aromatic substituents. Dimethylamine and diethylamine containing analogues showed proper fitting to the binding pocket of PfATP6 and had a root mean square deviation (RMSD) value of **-3.11** and **-3.23** Kcal/mol respectively. Of the aryl substituents, piperdine, piperdinone and thiomorpholine containing derivatives showed the best binding affinity (**-3.09, -3.29, -4.11** Kcal/mol respectively). However cyclic groups with substituents showed less binding affinity and more unfavourable bumps, possibly due to steric hindrance caused by the bulkiness of the

Table 1. Molecular properties of the designed Mannich condensates

Compound code	miLogP	TPSA (Å)	MW (g/mol)	nON (H bond acceptor)	nOHNH (H-bond donor)	Nrotb (no. of rotatable bonds)	Violations
CDM-6	3.18	96.30	453.54	7	2	12	0
CDM-3	2.43	96.30	425.48	7	2	10	0
CDM-2	3.34	96.30	465.55	7	2	10	0
CDM-14	2.00	113.38	479.53	8	2	10	0
CDM-1	2.82	96.30	483.59	7	2	10	0
CDM-4	1.73	108.33	466.53	8	3	10	0
CDM-5	2.28	105.54	467.52	8	2	10	0
CDM-8	3.58	96.30	479.57	7	2	10	0
CDM-9	2.70	99.54	494.59	8	2	11	0
CDM-11	4.19	96.30	481.59	7	2	14	0
CDM-10	3.67	96.30	479.57	7	2	10	0
CDM-12	2.74	108.33	494.59	8	3	10	0
CDM-13	1.46	116.53	481.55	8	3	10	0
CDM-15	**5.31**	96.30	**509.64**	7	2	16	2
CDM-16	**8.85**	96.30	**621.86**	7	2	24	2
CDM-17	2.16	113.38	465.50	8	2	10	0
CDM-18	2.71	105.09	439.51	7	3	12	0
CDM-19	2.61	105.54	481.55	8	2	10	0
CDM-7	2.64	105.54	481.55	8	2	10	0
CDM-20	3.67	96.30	479.57	7	2	10	0

*The bold characters indicate compounds which were unsuitable for further processing.

structure. From *in silico* studies, it was seen that aliphatic secondary amines with shorter and unbranched carbon chains showed better binding energies and any increase in the number of carbon chains decreased the activity of the compounds. Similarly in the case of cyclic secondary amines, compounds with one or no substituents showed better binding affinity as evidenced by the RMSD scores of piperidine, 4-piperidinone and thiomorpholine and morpholine. Substituents containing piperazine did not show good binding affinities.

Chemistry

Mannich bases of curcumin were synthesized with curcumin, formaldehyde and secondary amines as per the practicality of the reaction and with slight modifications to the method of Liu and his co-workers (Figure 1) (Zhichang et al., 2014).

(1E,6E)-1,7-bis(4-hydroxy-3-methoxyphenyl)-4-(thiomorpholinomethyl)hepta-1,6-diene-3,5-dione (CDM-1)

The IR spectra of the compound CDM1 shows a broad peak at 3381 cm^{-1} indicating the presence of an OH group. Due to tertiary nature of the Nitrogen added via the mannich reaction, there is no IR absorbance due to absence of N-H stretching. The peaks at 2935 and 2821 cm^{-1} signified the formation of a new alkyl chain as the peaks were absent from the standard curcumin spectra. The peak at 1633 showed the presence of the ketone groups of curcumin. The peak at 1247 cm^{-1} showed the presence of a methoxy group. The ^{1}H-NMR spectra of CDM1 was carried out using $CDCl_3$ as a solvent, the peak of which can be seen as a singlet at 7.0548 ppm. The peak at 3.7532 ppm indicates the presence of a methoxy group. The deshielded peak at 9.7291 ppm indicates the presence of a hydroxyl hydrogen. The peaks from 1.0548-1.4990, 2.2149-2.5532, 3.2033-3.2532 ppm depict the hydrogen atoms present in the methylene linkage of the thiomorpholine substituent. The other signals and peaks of ^{13}C NMR were in complete agreement with the assigned structures. The fragmentation pattern of CDM-1 at 100% (483) showed that the molecular weight of the synthesized compound corroborated with that of the theoretical value indicating proper formation of the compound.

(1E,6E)-1,7-bis(4-hydroxy-3-methoxyphenyl)-4-(piperidin-1-ylmethyl)hepta-1,6-diene-3,5-dione (CDM-2)

For the compound CDM2, the IR transmittance peak at 3629 cm^{-1} shows the trademark stretching of the OH group, while the peaks at 2930 and 2850 cm^{-1} hint at the formation of a new alkyl linkage. The peak at 1250 cm^{-1} signifies the methoxy group while the peak at 1579 cm^{-1} is likely to represent the ketone groups present in curcumin. The ^{1}H-NMR spectra of CDM2 was carried out using $CDCl_3$ as a solvent, the peak of which can be seen as a singlet at 7.0548 ppm. The peak at 3.7232 ppm indicates the presence of a methoxy group. The peaks at 1.4990-1.5193, 2.2033-2.2232 and 2.6893-2.7921 ppm indicate the hydrogen atoms present in the substituent piperidine ring. The lone singlet peak at 9.7291 ppm shows the presence of the phenolic hydrogen. The other signals and peaks of ^{13}C NMR were in complete agreement with the assigned structures. The fragmentation pattern of CDM-2 at 100% (465) showed that the molecular weight of the synthesized compound corroborated with that of the theoretical value indicating proper formation of the compound.

(1E,6E)-4-[(dimethylamino)methyl]-1,7-bis(4-hydroxy-3-methoxyphenyl)hepta-1,6-diene-3,5-dione (CDM-3)

The IR spectra of the compound CDM3 shows a broad peak at 3329 cm^{-1}, hinting at the presence of a hydroxyl group. The peaks at 2939 and 2836 cm^{-1} signify the formation of a new alkyl chain. The peak at 1609 cm^{-1} shows the presence of ketone while the peak at 1271 cm^{-1} denotes the presence of a methoxy group. The ^{1}H-NMR spectra of CDM3 was carried out using $CDCl_3$ as a solvent, the peak of which can be seen as a singlet at 7.0548 ppm. The peak at 3.6093-3.7291 ppm shows the presence of a methoxy group. The peak at 3.2033-

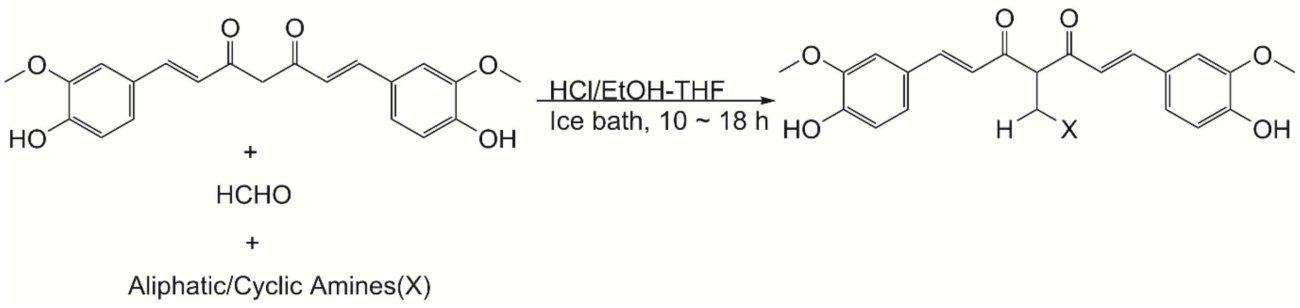

Figure 1. Synthesis of Mannich based derivatives of curcumin. Reaction Conditions: Curcumin, Formaldehyde, X= Aliphatic/Cyclic secondary amines, [X=N,N-diethylamine(CDM-6), X= N,N-dimethylamine(CDM-3), X= piperidine(CDM-2), X= piperidinone (CDM-14), X= thiomorpholine(CDM-1). Conc. HCl, 1:1 ratio of 95% ethanol and THF, refluxed in an ice bath for 10-18 hrs

3.2232 ppm indicates the methylene bridge between the parent compound and the substituent. The strong triplet at 2.2232 and 2.2532 ppm confers to the methyl groups present in the dimethyalmine group. The peaks at 2.4990 and 2.5193 ppm are indicative of the methylene linkage of the dimethylamine group. The other signals and peaks of ^{13}C NMR were in complete agreement with the assigned structures. The fragmentation pattern of CDM-3 at 100% (425) showed that the molecular weight of the synthesized compound corroborated with that of the theoretical value indicating proper formation of the compound.

(1E,6E)-4-[(diethylamino)methyl]-1,7-bis(4-hydroxy-3-methoxyphenyl)hepta-1,6-diene-3,5-dione (CDM-6)

In CDM6, the O-H stretching peak is observed at 3394 cm^{-1}. The formation of a new alkyl chain is observed through the peaks at 2956 and 2825 cm^{-1} respectively. The ketone peak is observed at 1678 cm^{-1} while the methoxy group is depicted through the peak at 1278 cm^{-1}. The ^{1}H-NMR spectra of CDM6 was carried out using $CDCl_3$ as a solvent, the peak of which can be seen as a singlet at 7.0548 ppm. The peak at 3.6033-3.7232 ppm shows the presence of a methoxy group. The peak at 2.033-2.2232 ppm indicates the methylene bridge between the parent compound and the substituent. The triplet at 1.4990 and 1.5193 ppm confers to the methyl groups present in the diethyalmine group. The peaks at 2.6093 and 2.7291 ppm are indicative of the methylene linkage of the dimethylamine group. The hydroxyl group of the curcumin molecule is depicted by the de-shielded peak at 9.7291 ppm. The peaks 7.0149-7.7291 ppm corresponds to that of the hydrogen atoms in the phenyl rings. The other signals and peaks of ^{13}C NMR were in complete agreement with the assigned structures. The fragmentation pattern of CDM-6 at 100% (453) showed that the molecular weight of the synthesized compound corroborated with that of the theoretical value indicating proper formation of the compound.

(1E,6E)-1,7-bis(4-hydroxy-3-methoxyphenyl)-4-[(4-oxopiperidin-1-yl)methyl]hepta-1,6-diene-3,5-dione (CDM-14)

For the compound CDM14, the broad peak at 3379 cm^{-1} depicts O-H stretching while the peaks at 2927, 2864 and 2831 cm^{-1} indicate the formation of a new alkyl chain. The peaks at 1707 and 1243 cm^{-1} correspond to ketone and methoxy groups respectively. The ^{1}H-NMR spectra of CDM6 was carried out using $CDCl_3$ as a solvent, the peak of which can be seen as a singlet at 7.0548 ppm. The triplet at 3.7532 ppm shows the presence of a methoxy group. The peak at 3.2033-3.2532 ppm indicates the hydrogen atoms present in link between the parent compound and the substituent. The peaks at 2.2149-2.3548 ppm are indicative of the methylene linkage of the diethylamine group. The hydroxyl group of the curcumin molecule is depicted by the de-shielded peak at 9.7290 ppm. The peaks at 1.0548-1.4990 ppm corroborate with the hydrogen atoms present in the piperidinone ring. The peaks 7.0148-7.7290 ppm correspond to that of the hydrogen atoms in the phenyl rings. The signals and peaks of ^{13}C NMR were in complete agreement with the assigned structures. The fragmentation pattern of CDM-14 at 100% (465) showed that the molecular weight of the synthesized compound corroborated with that of the theoretical value indicating proper formation of the compound.

Table 2. Binding energies, substituents and IC_{50} values of Mannich base derivatives of curcumin

Compound code	Substituents	Binding energy	3D7			RKL-2		
		(Kcal/mol)	IC_{50} (µg/ml)	*$R^{2=}$	#Y=	IC_{50} (µg/ml)	*$R^{2=}$	#Y=
CDM1	Thiomorpholine	-4.11	4.09	0.809	3.691 X +32.5	8.2	0.851	0.977X + 38.96
CDM2	Piperadine	-3.09	5.13	0.945	0.828X + 25.42	12.3	0.957	0.606X + 23
CDM3	n-Dimethylamine	-3.11	4.11	0.860	0.507X + 31.66	11.89	0.908	0.650X + 23.62
CDM4	Piperazine	+7.14	19.11	0.886	7.182X +37.33	36.40	0.843	1.116X + 41.74
CDM5	Morpholine	+1.79	9.9	0.924	13.17X + 28.37	19.3	0.935	15.3X + 3.5
CDM6	n-Diethylamine	-3.23	4.81	0.876	1.885X + 14.82	12.13	0.900	0.68X + 25.79
CDM7	n-Diisopropylamine	+15.66	49.1	0.844	1.901X + 25.91	90.5	0.865	1.293X + 21.73
CDM8	4-methylpiperazine	+1.90	10.1	0.900	0.561X + 26.79	22.1	0.865	1.293X + 21.73
CDM9	4-ethylpiperazine	+33.36	Higher than 100ug/ml			Higher than 100ug/ml		
CDM10	2-methylpiperadine	+14.26	40.26	0.860	0.507X + 31.66	86.33	0.935	15.3X + 3.5
CDM11	n-Dipropylamine	-2.11	5.62	0.886	7.182X +37.33	11.91	0.900	0.68X + 25.79
CDM12	2,5-dimethylpiperazine	+4.19	17.7	0.924	13.17X + 28.37	37.6	0.865	1.293X + 21.73
CDM13	4-hydroxypiperidine	+2.47	15.3	0.876	1.885X + 14.82	33.1	0.865	1.293X + 21.73
CDM14	4-piperidinone	-3.29	4.66	0.891	2.11X + 16.13	8.9	0.869	1.91X + 17.16
Curcumin	-	-4.95	2.13	0.903	13.11X + 26.33	5.59	0.893	14.79X +4.1
Chloroquine	-	-	-	-	0.7	-	-	1.44

*R^{2}= Chi-square value; #Y= mx + c

In vitro antimalarial evaluation

The compounds which showed a minimal antimalarial activity of 20% (at 5µg/ml) to 80% (at 50 µg/ml) of inhibition were selected for determination of their IC_{50} values. Multiple doses were taken based on their parasite inhibiting properties as determined microscopically. The compounds and the parasite concentrations were adjusted in the same way as that of the screening method. Percentage reductions were used to plot percentage inhibition of growth as a function of drug concentrations. IC_{50} values were determined by log-concentration–response Non-Lin! (https://nees.org/resources/nonlin) and probit analysis (Chi, 1997). The results are provided in table 2.

Among the aliphatic substituents, the product containing dimethylamine substituent (CDM3) showed the best binding energy followed by CDM6 which had a diethylamine

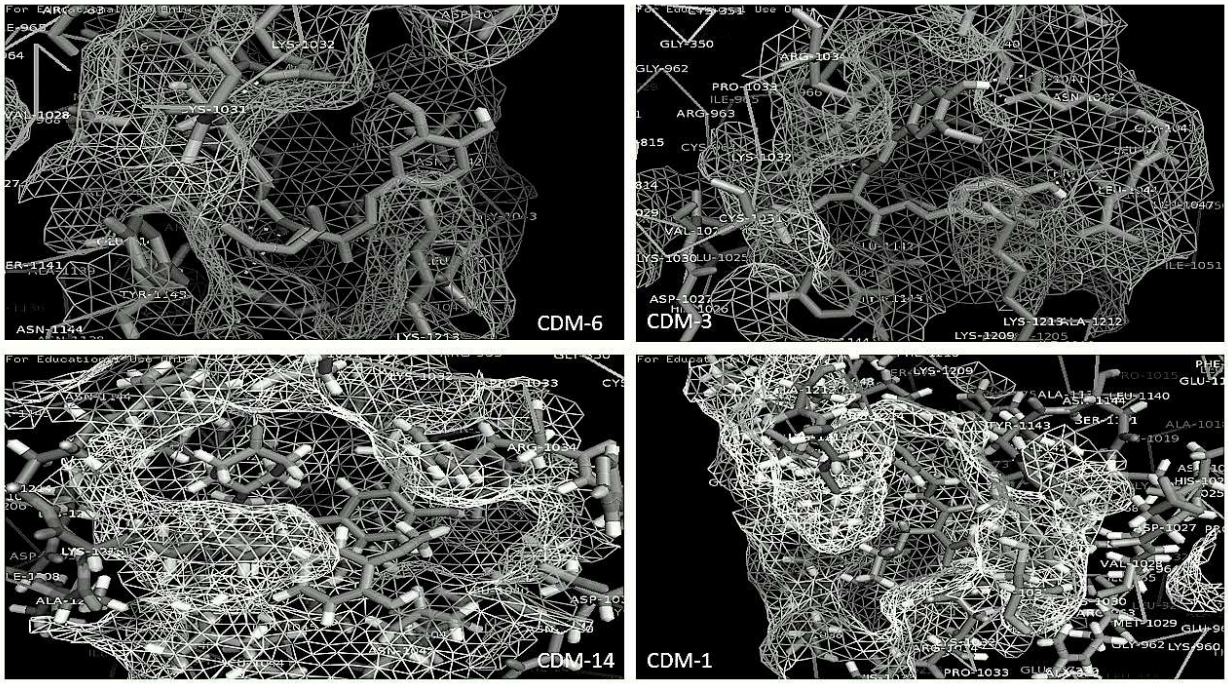

Figure 2. Docked orientation of the Mannich base derivatives showing the fitting of compounds CDM-6, CDM-3, CDM-14 and CDM-1 into the binding pockets of PfATP6 protein

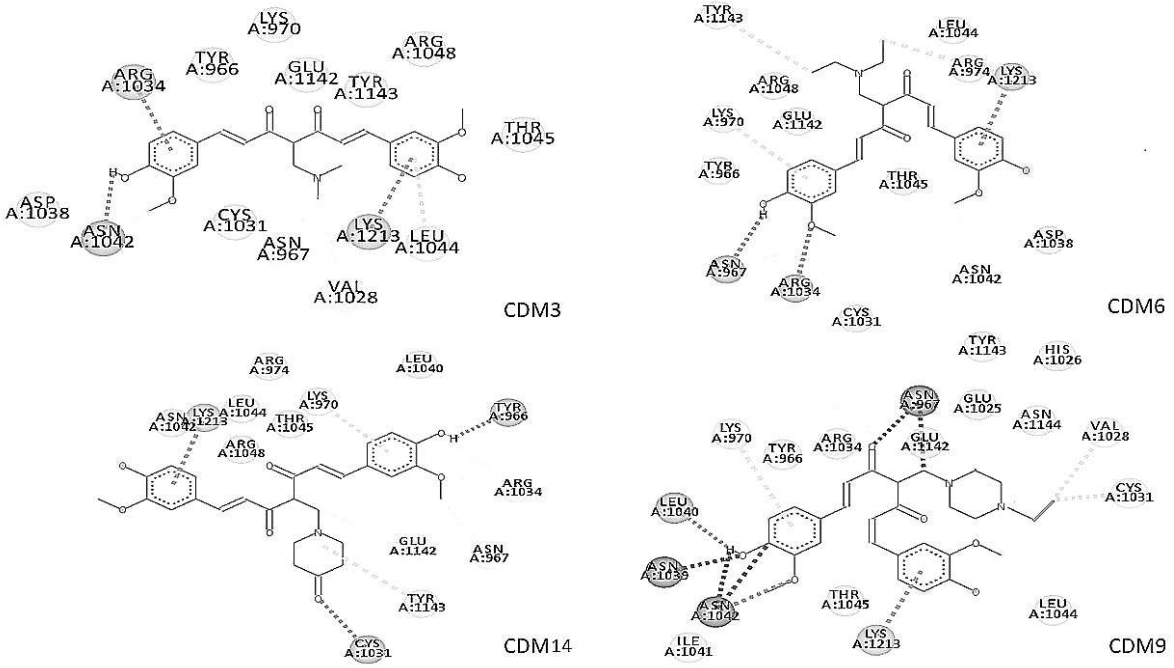

Figure 3. Interactions of the aliphatic secondary amines (CDM3, CDM6) and cyclic secondary amines (CDM14, CDM9) with the PfATP6 protein. All the four compounds show hydrophobic interaction with the Lys1213 residue

substituent. Among the cyclic groups, piperidinone (CDM8) and thiomorpholine (CDM11) substituents showed good binding affinities. Elongation and branching of the carbon chain decreases the affinity of compounds as evidenced by the high binding energies of the compounds CDM1 and CDM7. Cyclic compounds containing substituents showed high binding energies. This could be attributed to steric hindrance as evidenced by the presence of unfavorable bumps in CDM9 which essentially contains an ethyl piperazine group. Both CDM3 and CDM6 showed conventional bonding with Asn1042, Asn967 and Arg1034 respectively and were found to fit in the PfATP6 groove (Figure 2). However, the interaction via π-σ bonds with the residue Lys1213 is found to be common for all the compounds (Figure 3). Therefore the antimalarial activity of the Mannich base derivatives could be attributed to the interaction with Lys1213 residue of PfATP6. It was evident from the docking analysis as well as antimalarial evaluation that the title molecules disclosed admirable scoring and significant bioactivity. It was also confirmed that hydrophobic interaction was the only major force playing a role between the ligand–receptor interactions.

Conclusion

In our study we analysed the different binding affinities of the designed Mannich base structural analogues of curcumin and studied the various interactions of the compounds with the protein PfATP6. The selected compounds were synthesized and evaluated for their antimalarial activity. From the *in silico* studies we found that the common point of interaction of the compounds which showed the best antimalarial studies was that with the amino acid Lys1213. Newer drug candidates could be designed to target this specific residue. IC_{50} values of the compounds were found to be satisfactory in comparison to that of the standard curcumin but less than that of chloroquine. It was further observed that substituents containing a secondary amine group showed far better results than primary or tertiary groups, a fact which would help researchers in designing more potent molecules. The study provided a detailed insight into the necessary structural modifications to be made in the curcumin molecule to create more effective hybrid drug candidates for rationale-based antimalarial drug design and development, which is the need of the hour due to emergence of rapid resistance to the standard antimalarial drugs.

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Conflict of Interest

Authors declare no conflict of interest.

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