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Biological evaluation and molecular docking studies of new curcuminoid derivatives: Synthesis and characterization

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ABSTRACT

In the present study, three series of dimethylamino curcuminoids viz. 4-phenylaminomethyl curcumin (**3a–d**), arylidene curcumin (**3e**) and pyrazole curcumin (**3f–i**) derivatives have been synthesized and studied for their in vitro anti-inflammatory, antioxidant and antibacterial activities. Synthesized dimethylamino curcuminoid derivatives namely **3d**, **3e**, **3h** and **3i** have shown potent anti-inflammatory properties than parent curcumin. Molecular docking interactions of dimethylamino curcuminoids derivatives against cyclooxygenase enzymes (COX-1 and COX-2) were studied.

Curcumin is a naturally occurring phenolic compound and it is a major constituent of turmeric. It is used as a food based product and a traditional medicine to treat some skin diseases.[1] Turmeric is well known for its wound healing properties[2] and it is isolated from the root of rhizome *Curcuma longa* Linn.[3] Earlier Letters have revealed that the curcumin and curcumin related compounds possess multiple pharmaceutical applications such as anti-cancer,[4] antiangiogenesis,[5] antimutagenic,[6] antiproliferative,[7] antibacterial[8] and antifungal properties.[9] Generation of free radical initiates many diseases in biological system such as cardiovascular disease[10] and cancer.[11] Turmeric and its constituents show beneficial effects in the treatment of these diseases. Mainly curcumin is known for its remarkable antioxidant properties due to the presence of phenolic and enolic hydroxyl groups which leads to the delocalization of the electrons.[12] Further curcumin has been investigated for curing disease such as alzheimer disease[13] and arthritis.[14] The clinical trials have reported that ingestion of significant doses of curcumin (12 g/day) had no side effect.[15]

Even though the curcumin has several applications, which have the little of drawbacks like poor aqueous solubility, stability and also metabolically unstable.[16] Thus the researchers were focused to develop the new curcumin derivatives with enhanced biological activity and pharmacological property. Many structural modifications were reported in literature including curcumin amino acid conjugates,[17] hyaluronic acid–curcumin conjugate[18] curcumin PEG conjugates[19] and curcumin β diglucoside.[20] The variation of active methylene groups/replacing β-di-ketone bridge enhanced the biological activity than the natural curcumin.[21–24] Similarly, the dimethylaminocurcumin derivatives have shown enhanced biological activity.[25,26] In addition the dimethylamino curcumin can easily converted into a water soluble derivatives as hydrochloride salt.

Another way the replacement of beta diketo unit in curcumin by pyrazole moiety enhances the biological activity.[27] Based on the above outcome, it is decided to synthesize the new chemical entities of dimethylamino curcumin derivatives (**3a–i**) with different substituents. The synthesized compounds can easily converted into water soluble derivative like quaternary ammonium chloride. The synthesized compounds are characterized by ^{1}H NMR, ^{13}C NMR, Mass and IR spectroscopy and studied their biological evaluations such as antibacterial, anti-inflammatory and antioxidant activities. Along with molecular docking studies were studied for the synthesized curcumin derivative.

Molecular docking is the in silico method which is used to develop the homology model for the new drug candidate. This field will reduce the number of synthetic compound in drug discovery research. The synthesized dimethylamino curcuminoids compounds showed a very good anti-inflammatory activity. Thus, we continued to dock the ligand with enzyme like cyclooxygenase. This is one of the enzymes responsible to cause inflammation. The pharmacological inhibition of cyclooxygenase can provide relief from the symptoms of inflammation and pain. Here, the synthesized compounds were docked with COX-1 and COX-2 enzymes with

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the use molecular docking tools and the docking results are briefly explained.

The synthesis of dimethylaminocurcumin (Fig. 1) was carried out by aldol condensation. Boric anhydride acetylacetone complex was first prepared by the treatment of these two reagents at room temperature for 30 min which avoiding Knoevenagel condensation in C-3 position of 2,4-pentanedione. The boron complex then reacted with 4-dimethylaminobenzaldehyde in the presence of *n*-butylamine and tri-*n*-butyl borate to afford the products.[28] Whereas the 4-phenylaminomethyl curcumin compounds (**3a–d**) were prepared by the treatment of compound **2** with Formaldehyde and aromatic amine (aniline, 2-methoxy aniline, 3-trifluoro methyl aniline) at room temperature. For the preparation of arylidene curcumin (**3e**), 3-(4-hydroxy-3-methoxybenzylidene)pentane-2,4-dione was synthesized by the Knoevanagel condensation of vanillin with 2,4-pentanedione in the presence of piperidine and catalytic amount of acetic acid at room temperature.[29] Then 3-(4-hydroxy-3-methoxybenzylidene)pentane-2,4-dione was reacted with 4-dimethylaminobenzaldehyde in the presence of *n*-butylamine and tri-*n*-butyl borate produced the compound **3e**. The compound **3e** was prepared following the same procedure by used for the preparation of compound **2**.[28] Pyrazolyl curcumin compounds (**3f–i**) were synthesized by the irradiation of microwave for 2–5 min in Glacial acetic acid and various hydrazine hydrate derivatives.[30]

The synthesized compounds were characterized by ^{1}H NMR, ^{13}C NMR, mass and IR spectroscopies. The appearance of singlet for 12 proton around 3.02 ppm in ^{1}H NMR indicates the presence of *N*,*N*-dimethyl unit. Similarly ^{13}C NMR also confirms the presence of *N*, *N*-dimethyl unit, which is appeared around 40 ppm in all the synthesized compounds. The remaining characteristic peaks were appeared with appropriate chemical shift values. In addition the IR spectrum also gives the evidence for the presence of some functional groups such as carbonyl and dimethylamine. (The experimental characterizations were attached in Supporting information.) Further, all the synthesized compounds have been investigated for the biological activity in comparison with the parent curcumin and the standard.

These synthesized compounds (**3a–i**) were evaluated for their antioxidant activity by DPPH radical scavenging method[31] in comparison with curcumin and ascorbic acid. The free radical scavenging activity of each compound was tested in various concentrations measured by the change of absorbance and the reduction of DPPH radical spectrophotometric method. The IC_{50} values of parent curcumin and ascorbic acid were found to be 52.51 and 65.53 μg/mL, respectively. The IC_{50} values were found to be low for compounds **3b** and **3h** which are comparable with that of parent curcumin and ascorbic acid. But for the compounds **3e**, **3f**, **3g** and **3i**, the scavenging activity is moderate compared to the pyrazolyl curcumin. Other derivatives **3a**, **3c** and **3d** bearing aniline, 3-trifluoro methyl aniline and 2-methoxyaniline units displayed low scavenging activities. The IC_{50} values of all the synthesized compounds were listed in Table 1.

The synthesized dimethylaminocurcuminoid derivatives (**3a–i**) were carried out the antioxidant activity by the H_2O_2 scavenging method.[31] Especially fluoro substituted 4-phenylaminomethyl curcumin **3b** and arylidene curcumin **3e** showed potent H_2O_2 scavenging activity when compared to curcumin and ascorbic acid. Other derivatives **3a**, **3d** and **3h** having aniline, 2-methoxyaniline and pyrazole curcumin exhibited antioxidant activity nearer to curcumin activity. The compounds **2**, **3c**, **3f**, **3g** and **3i** have exhibited moderate H_2O_2 scavenging activity. The IC_{50} values of H_2O_2 scavenging activity of all the synthesized compounds were mentioned in Table 1.

The synthesized compounds were tested for their in vitro anti-inflammatory activity by protein denaturation technique using bovine serum albumin assay followed by the literature.[32] The half maximal inhibitory concentration (IC_{50}) values are represented in Table 2 using diclofenac sodium drug and parent curcumin to compare these activities. Among 4-phenylaminomethyl curcumin (**3a–d**), the compound **3d** with 2-methoxy aniline skeleton has shown more anti-inflammatory property than curcumin. But other compounds have low potency in this series. However pyrozolyl curcumin derivatives **3h** and **3i** have shown potent activity than the parent molecule as well as standard diclofenac sodium. But the other pyrazole derivatives **3g** and **3f** have shown lesser activity. The arylidene curcumin **3e** also exhibited potent anti-inflammatory activity than parent curcumin.

The study was designed to determine the zone of inhibition for dimethylamino curcuminoid derivatives following in the literature.[33] Antibacterial activity (well diffusion method) was evaluated

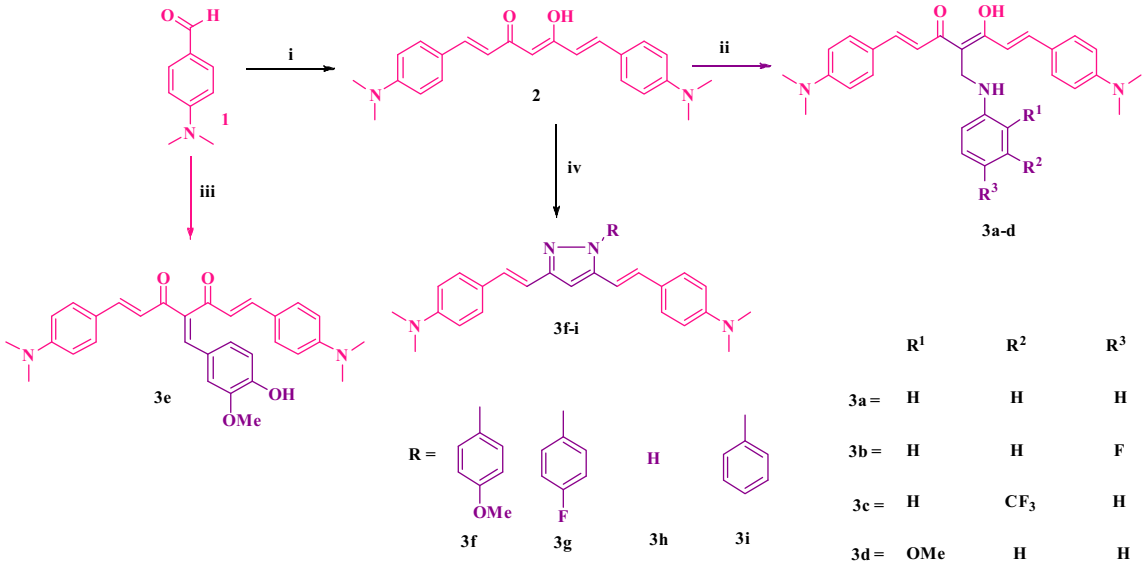

Figure 1. Synthesis of dimethylamino curcuminoid derivatives (**3a–i**). Reagents and conditions: (i) 2,4-pentanedione, B_2O_3, tri-*n*-butyl borate, *n*-butyl amine, ethyl acetate,0.4 N HCl, rt, 6 h; (ii) HCHO, amines, DCM, 4 h, rt; (iii) 3-(4-hydroxy-3-methoxybenzylidene)pentane-2,4-dione, B_2O_3, tri-*n*-butyl borate, *n*-butyl amine, ethyl acetate, 0.4 N HCl, rt, 6 h; (iv) hydrazine derivative,CH_3COOH, MW, 3 min.

Table 1
Antioxidant activity of synthesized compounds (**3a–i**)

S. no.	Compound	DPPH (IC_{50}) μg/mL	H_2O_2 (IC_{50}) μg/mL
1	**2**	78.52	74.56
2	**3a**	79.06	52.13
3	**3b**	48.16	46.90
4	**3c**	81.69	74.60
5	**3d**	76.28	53.94
6	**3e**	75.37	44.95
7	**3f**	88.05	79.59
8	**3g**	74.38	89.05
9	**3h**	46.05	50.78
10	**3i**	74.03	72.42
11	Curcumin	52.51	52.24
12	Vitamin C	65.53	68.82

Table 2
Anti-inflammatory activity of synthesized compounds (**3a–i**)

S. no.	Compound	IC_{50} (μg/ml)
1	**2**	180.38
2	**3a**	808.36
3	**3b**	226.71
4	**3c**	186.05
5	**3d**	81.36
6	**3e**	106.16
7	**3f**	751.12
8	**3g**	216.32
9	**3h**	51.79
10	**3i**	29.44
11	Curcumin	129.85
12	Diclofenac sodium	52.95

for all these derivatives of dimethylamino curcuminoid compounds (**3a–i**) against Gram-positive *Staphylococcus aureus* (ATCC 25923) and Gram-negative such as *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853), *Klebsiella pneumoniae* (ATCC 13883) bacteria by the zone of inhibition method. The growth inhibition was tested for these derivatives along with standard curcumin and antibacterial agent ciprofloxacin at different concentration 10 μg, 20 μg, 30 μg, 40 μg, and 50 μg, respectively. Each test has been evaluated twice and the zone of inhibition was mentioned in (Fig. 2). In the case of *Escherichia coli*, the compounds **3b**, **3e**, **3g** and **3i** have shown moderate to good activity, when compared to standard curcumin at 50 μg concentration. All the derivatives have shown equal activity compared to parent curcumin at 10 μg concentration except **3c**. With *Pseudomonas aeruginosa*, curcumin showed 11 mm inhibition at 50 μg whereas **3b** compound has shown 12 mm inhibition and the compounds **3e**, **3h** have shown moderate activity.

Similarly the growth inhibition was tested against *Staphylococcus aureus*. Significantly, **3i** has displayed a higher inhibition than curcumin and **3a**, **3d** and **3g** have shown moderate activity when compared to the standard curcumin at 50 μg concentration. Similarly at 10 μg concentration, **3a** and **3i** showed activity equal to the standard curcumin. **3b**, **3c**, **3h** and **3f** exhibited zero activity against *Staphylococcus aureus*. Further *Klebsiella pneumonia*, **3b**, **3g** and **3h** compound showed equal activity to curcumin at 50 μg concentration.

These series of dimethylamino curcuminoids derivatives were evaluated for cyclooxygenase inhibitors studies (COX-1 and COX-2) by using molecular docking method. The active site of 1PGG and 4COX is considered to be constituted of the amino acid residues ARG120, SER530, TYR385 and GLU524 following the reported literature.[34] As shown in Table 3, out of the 10 compounds, in the 4-phenylaminomethyl curcumin derivatives (**3a–d**), the compound **3b** and pyrazole curcumin **3i** have shown very good docking result comparable to other derivatives. The compound **3b** formed two hydrogen bond interaction with Leu531 and Val116 through the active site of COX-1 and bonding distance between NH of phenyl ring and OH of Leu531was found to be 2.15 Å (H. . .O); NH of phenyl ring and OH of Val 116 was found 2.28 Å (H. . .O). The two phenyl ring of dimethylamino curcumin was surrounded by the amino

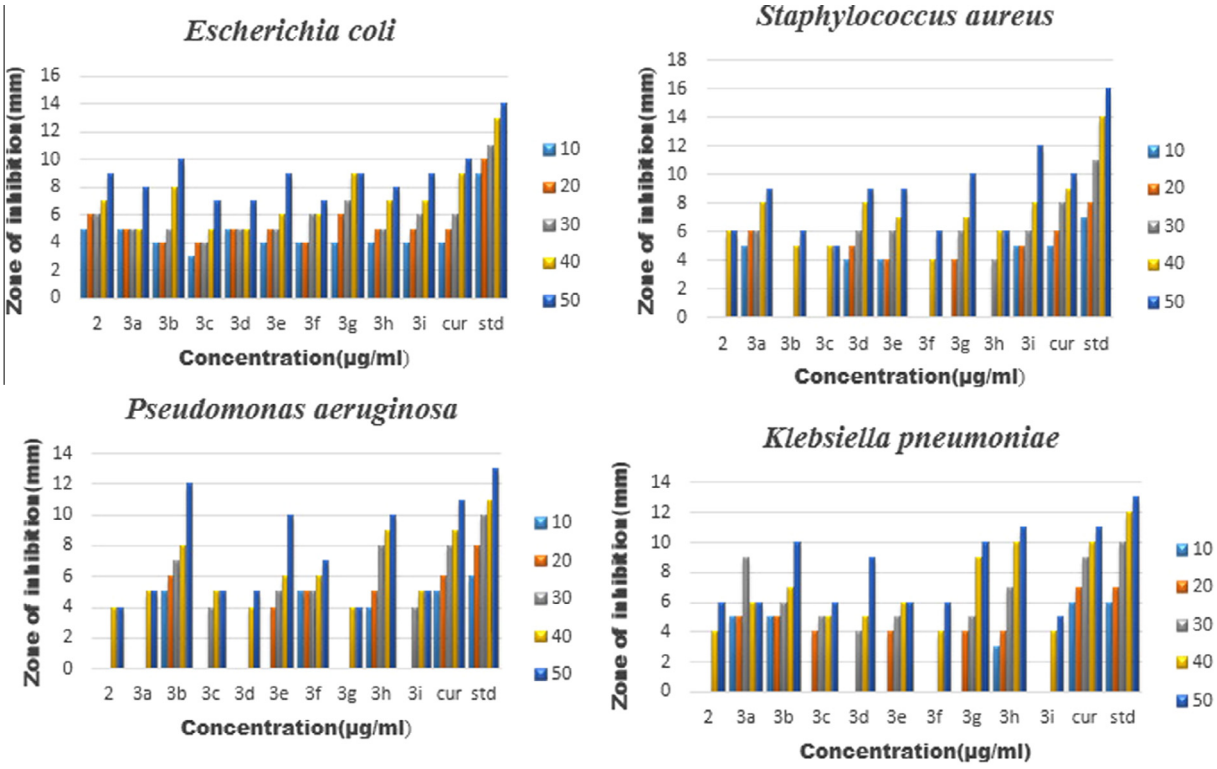

Figure 2. Anti-bacterial activity of dimethylamino curcuminoids derivatives (**3a–i**) (zone of inhibition in mm) against different bacterial strains.

Table 3
Molecular docking interaction of dimethylamino curcuminoids derivatives (**3a–i**) against cyclooxygenase inhibitors (COX-1)

Compound	Docking score (kcal/mol)	Number of hydrogen bonds	Interacting residues of 1PGG
2	−6.19	—	—
3a	−7.32	1	Ser530
3b	−10.01	2	Leu531, Val116
3c	−6.74	—	—
3d	−6.87	2	Gly214, Asp450
3e	1.03	2	Asn375, His90
3f	−8.96	—	—
3g	−8.58	—	—
3h	−6.27	1	Asn375
3i	−9.71	1	Tyr355

Table 4
Molecular docking interaction of dimethylamino curcuminoids derivatives (**3a–i**) against cyclooxygenase inhibitors (COX-2)

Compound	Docking score (kcal/mol)	Number of hydrogen bonds	Interacting residues of 4COX
2	−6.89	—	—
3a	−8.29	3	Tyr355, Arg120, Ser471
3b	−7.54	2	Tyr115, Tyr122
3c	−7.1	—	—
3d	−7.43	4	Tyr122, Tyr115, Ser471, Lys83
3e	−7.84	5	Tyr122, Ser471, Glu524, Arg120, Thr62
3f	−8.03	1	Pro84
3g	−8.16	—	—
3h	−7.36	3	Arg120, Glu524, Ser471
3i	−9.25	2	Pro84, Lys83

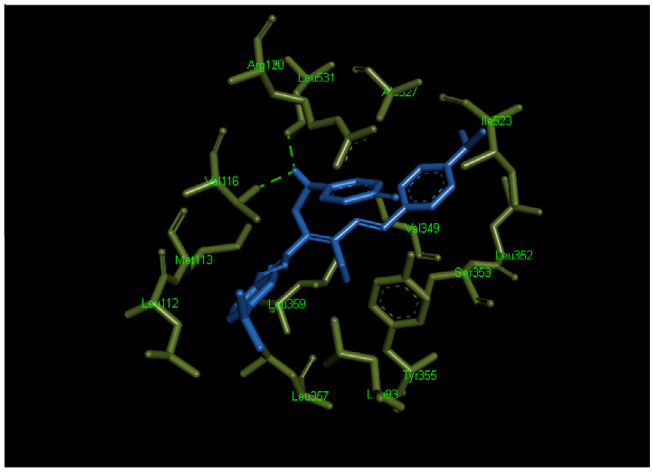

Figure 3. Binding of **3b** compound into the active site of COX-1.

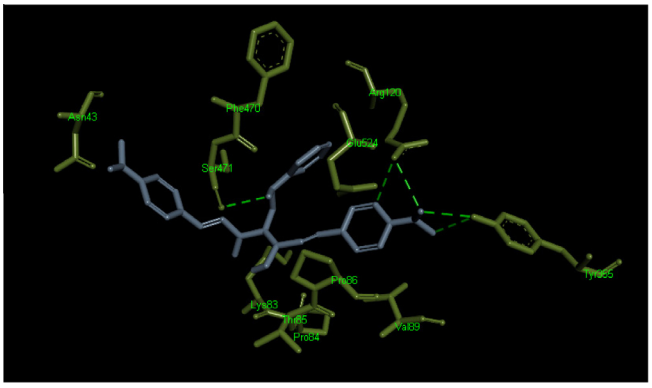

Figure 5. Binding of **3a** compound into the active site of COX-2.

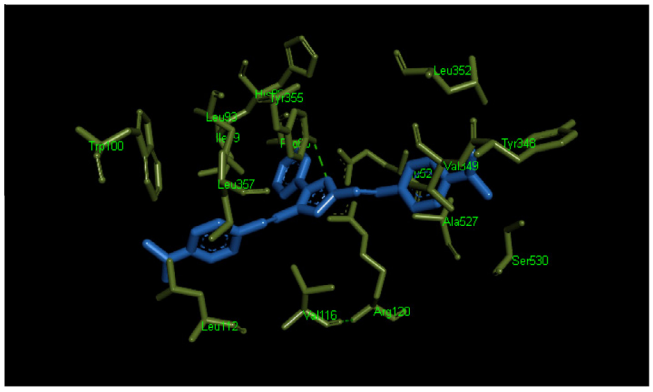

Figure 4. Binding of **3i** compound into the active site of COX-1.

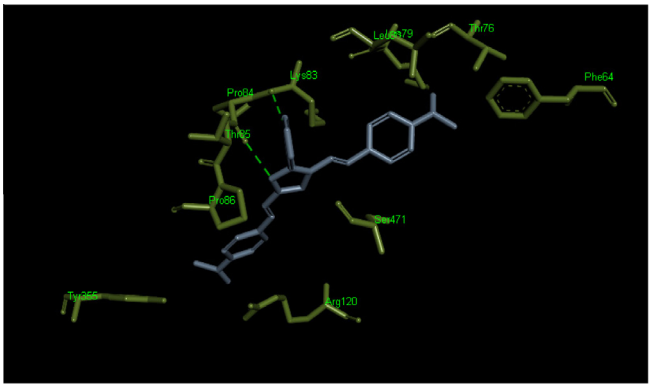

Figure 6. Binding of **3i** compound into the active site of COX-2.

acid residues such as Phe518, Leu352, Val349, Lle523, Lle89, Leu93, Leu112 and Leu357. The heptanoid part was surrounded by residues Leu359, Tyr355 and Met113. Another phenyl ring was surrounded by Ala527, Arg120, Leu117 and Tyr355 (Fig. 3).

Further the compound **3i**, *N*-substituted phenyl pyrazole was involved in the hydrogen bonding interaction with Tyr 355 (2.42 Å N...H) (Fig. 4). The pyrazole substituted phenyl ring was surrounded by residues Glu524, Arg120 and Lle89, respectively. Both dimethylamino substituted phenyl rings was surrounded by the amino acid residues like Val349, Ala527, Tyr348, Leu352, Leu112, Leu93, Leu357 and Trp100.

After docking simulations with 1PGG (COX-1) of these compounds were carried out with 4COX (COX-2) it was mentioned in Table 4. The compound **3a** and **3i** was found to better dock into the active site of COX-2 in least binding energy of −8.29 and −9.25 kcal/mol than other derivatives. Whereas, compound **3a** formed three hydrogen bond with Tyr355, Arg120 and Ser471. The bond distance between alkyl groups of dimethylamino group and hydroxyl of phenyl ring of Tyr355 is 2.90 Å (OH...C) and 3.22 Å (OH...C) respectively. One of the dimethylamino phenyl ring make the bonding with NH_2 of Arg120. It was found as 3.39 Å (N...H–C) and 3.31 Å (N...H–C). *N*-Substituted phenyl ring

of **3a** and OH of Ser471 also make the bonding which is found as 3.27 Å were observed (Fig. 5).

Similarly two hydrogen bond interactions were observed in **3i** compound. The pyrazole N was involved in hydrogen bonding interaction with Pro84 (2.91 Å N..H). Moreover favorable hydrophobic interactions were formed with the surrounded amino acids such as Pro86, Thr85 (Fig. 6).

In conclusion, the series of dimethylaminocurcumin were synthesized and proved as biologically active compounds. Replacing the substitution of beta diketone by pyrazole/phenylaminomethyl curcumin/arylidene curcumin groups are enhances the biological properties of **3b**, **3d**, **3e**, **3h** and **3i** compounds than parent. In addition, the antibacterial activities of these derivatives are good to moderate activity. Molecular docking studies also proves the compounds has very good cyclooxygenase inhibition.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2016.02.066.

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