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Synthesis, Antioxidant and Docking Studies of Some Unsymmetrical Curcuminoids

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Abstract

The synthesis of unsymmetrical curcuminoids has garnered significant interest due to their promising bioactive properties, including anti-inflammatory, antioxidant, and anticancer activities. This study explores novel synthetic pathway for the preparation of unsymmetrical curcuminoids, focusing on the variation of substitution patterns on the aromatic rings. Key factors such as reaction conditions, yields, and the influence of substituents on the stability and reactivity of the curcuminoids are examined. The resulting compounds are characterized using techniques such as NMR spectroscopy, mass spectrometry, and their biological activities are evaluated through preliminary *in vitro* assays. Molecular docking studies were also carried out to examine *in silico* tyrosinase inhibitory activity. This research provides valuable insights into the design and synthesis of curcuminoid derivatives with potential therapeutic applications and lays the foundation for further development of curcuminoid-based drug candidates

Keywords: Unsymmetrical curuminoids, synthesis, *invitro* antioxidant study, DPPH, docking.

Introduction

Curcumin present in turmeric (*Curcuma Longa*) possesses a myriad of therapeutic activities ranging from anti-inflammatory, anti-oxidant, anti-hepatotoxic, anti-microbial, angiogenic, anti-depressant, chemo preventive, anti-fertility, neuroprotective, HIV-I and HIV-II protease inhibitor and many more[1,2]. Hence turmeric is treated as a holistic gift of nature[3]. Its synthetic modification and laboratory synthesis also gained considerable attention. Several methods are reported for the synthesis of symmetrical C-7 curcuminoids[4-8].

After long term research in the field of synthesis of curcuminoids, we paid our focus to the synthesis of curcumin analogs that bear non-identical aryl rings at the termini of the heptadienedione carbon chain. A simple retro-synthetic analysis

suggested that the synthesis of cinnamoylacetones and their subsequent condensation, as their boron complex, with another araldehyde, bearing a dissimilar aryl group, would permit an access to unsymmetrical curcumin analogs. Several reports are available citing the synthesis of unsymmetrical curcuminoids. Yongfu et al in 2013 reported the synthesis of several unsymmetrical curcuminods with pentadiene chain and screened for their biological activity[9]. The synthesis of C5-unsymmetrical curcuminoids were also synthesized and screened for their anticancer activity[10]. Reports were available about the synthesis of monocarbonyl unsymmetrical curcuminoids. In 2022 synthesized several unsymmetrical curcuminoids, employing Pabon's method. They were successful in synthesizing the halogenated, nitro and heterocyclic derivatives, but in a low yield[11]. The synthesis of C7-unsymmetrical curcuminoids with considerable yield in a comparatively lesser time is the need of the time, owing to its variable biological activity. In 1994, a new synthetic route for the synthesis of curcumoinoids was reported utilizing tetrahydroquinoline-aectic acid as the catalyst[6]. This reduced the reaction time considerably. We decided to adapt the method for the synthesis of unsymmetrical curcuminoids, but in two steps. We reasoned that the use of a large excess of the acetylacetone-boron complex in its reaction with a minimal amount of one aryl aldehyde would be a feasible approach for the synthesis of cinnamoyl acetone. After isolation of the cinnamoylacetone so formed, a subsequent condensation with a different aryl aldehyde could follow, yielding the desired product. Applying this reaction strategy, five cinnamoylacetones were successfully synthesized as the precursors of novel unsymmetrical curcuminoids.

MATERIALS AND METHODS: The IR spectra were recorded in Bruker WM-400 (400 MHz, FTIR). The 1H NMR spectra were recorded on a Jeol EX 90 (90 MHz) and Bruker DRX-300 (300 MHz, 500 MHz FT NMR spectrometers. FAB MS spectra were recorded on Jeol SX- 102 (FAB) spectrometer and EI mass spectra were recorded on a Jeol D-300 or SX-102 mass spectrometer. ^{13}C NMR spectra were recorded on a Jeol GSX (100 MHz) spectrometer. C, H, N analysis was done at STIC, CUSAT, Kochi. The spectra were recorded at CDRI, Lucknow. TLC analysis was done on glass plate coated with silica gel (E Merck, India) prepared from aqueous slurry and activated prior to use. All chemicals used were of AR grade (E Merck, India)

Synthesis of cinnamoylacetones

Synthesis of 6-(4-hydroxy-3-methoxyphenyl)-hex-5-ene-2,4-dione (1a)

Vanillin (1 mM, 0.154 g) was mixed with boric acid and acetyl acetone (3 mM, 0.3 ml) in 2 ml DMF. Then THQ (0.02 ml) in acetic acid (0.06 ml) was added and heated for 4 hours. When 20 % acetic acid was used for final work up, the product got dissolved to some extent in acetic acid and the extraction with ethyl acetate was difficult. Thus we decided to use aqueous hydrochloric acid and subsequent extraction with ethyl acetate yielded the product. The product was then purified by dry column chromatography. The reaction mixture was then added to 30 ml 1N HCl and then extracted with ethyl acetate. The organic layer was separated and dried. The product was then purified using dry column chromatography using mixture of petroleum ether and chloroform in the ratio 2:1.The yield obtained was 54% (m.p. – 148-150^{o}C)

Anal. Calcd. for $C_{13}H_{14}O_4$: C, (66.7); H, (6.02). Found C, (65.9); H, (6.82)
^{1}H NMR: δ 2.153 (3H,s), 3.94 (3H, s), 5.6,5.799 (1H, s), 6.29 (H, d, J=16 Hz), 6.91(1H,d, J= 8.5 Hz), 7.00 (Ar-H,1H,s), 7.07-7.09 (Ar-H, 2,5H,m), 7.52 (Ar-H, 6H,d, J=16 Hz)
IR (KBr): 3247, 1600, 1513, 1288, 1201, 1125, 966, 839, 764, 571, 470, 428, 405
FAB MS (NBA): 177.29, 216.31, 235.39$[M+1]^+$

Synthesis of 6-(4-Hydroxyphenyl)-hex-5-ene-2,4-dione (1b)
Boric acid was mixed with 4-hydroxy benzaldehyde (1mM, 0.122 g) and acetyl acetone(3mM, 0.3 ml)was added in 2 ml DMF. Then THQ (0.02 ml) in acetic acid(0.06 ml) was added and heated for 4 hours. The reaction mixture was then added to 30 ml 1N HCl and then extracted with ethyl acetate. The organic layer was separated and dried. The product was then purified using dry column chromatography using mixture of petroleum ether and DCM in the ratio 1:2. The yield obtained was 50%. m.p. - 92-95^{o}C.
Anal. Calcd. for $C_{12}H_{12}O_3$: C, (70.6); H, (6.0). Found C, (71.0); H, (5.6)
^{1}H NMR: 5.6 (1H, s), 6.3 (1H, d, J=16 Hz), 6.9 (1H, d, J= 8 Hz), 7.42 (Ar- H, 2,6-H, d, J= 15 Hz), 7.52(Ar-H, 3,5-H, d, J=15 Hz)
IR (KBr): 3491, 2875, 1596, 1381, 1212, 1131, 1011, 989, 875, 760, 730, 700 cm^{-1}
FAB MS (NBA): 123.22, 161.27, 189.31, 204.35(M^+), 205.36(M+1)

Synthesis of 6-(4-Methoxyphenyl)-hex-5-ene-2,4-dione (1c)
Anisaldehyde (1 mM, 0.136 ml) was mixed with boric acid and acetyl acetone (3mM, 0.3 ml) in 2 ml DMF. Then THQ (0.02 ml) in acetic acid (0.06 ml) was added and heated for 4 hours. The reaction mixture was then added to 30 ml 1N HCl and then extracted with ethyl acetate. The organic layer was separated and dried. The product was then purified using dry column chromatography using petroleum ether. The yield obtained was 66%. m.p.- 103-105^{o}C
Anal. Calcd. for $C_{13}H_{14}O_3$**:** C, (71.54); H, (6.47). Found C, (71.6); H, (6.37)
IR (KBR): 1632, 1508, 1361, 1256, 1109, 974, 825, 727, 522, 460, 434, 412
^{1}H NMR: δ 2.15(3H,s), 3.94(3H,s), 5.598(1H,s), 6.32(1H,d), 6.89(1H,d), 7.46(Ar-H, 3,5H,d), 7.5 (Ar-H, 2,6H,d)
FAB MS (NBA): 121.22, 133.25, 161.27, 175.31, 200.31, 219.36 $[M+1]^+$

Synthesis of 6-(3,4-dimethoxyphenyl)-hex-5-ene-2,4-dione (1d)
Veratraldehyde (1 mM, 0.167g) was mixed with boric acid and acetyl acetone (3mM, 0.3 ml) in 2 ml DMF. Then THQ (0.02 ml) in acetic acid (0.06 ml) was added and heated for 4 hours. The reaction mixture was then added to 30 ml 1N HCl and then extracted with ethyl acetate. The organic layer was separated and dried. The product was then purified using dry column chromatography using petroleum ether. The yield obtained was 48%. m.p. - 84-86^{o}C
Anal. Calcd. for $C_{14}H_{16}O_4$: C, (67.7); H, (6.5). Found C, (67.9); H, (6.29)
IR (KBr): 3430, 2842, 1651, 1514, 1425, 1353, 1235, 1159, 955, 910, 808, 675, 603, 533, 481, 415

^{1}H NMR: δ 2.15 (3H, s), 3.923 (3H, s), 5.617 (1H, s), 6.32 (1H, d, J=16 Hz), 6.9 (1H, d, J= 6 Hz), 7.09-7.11 (Ar-H, 2,5H, m), 7.53 (Ar-H, 6H, d, J=16 Hz)
FAB MS (NBA): 191.32, 205.33, 233.35, 248.41, 249.43$[M+1]^+$

Synthesis of 6-(3,4-methylenedioxophenyl)-hex-5-ene-2,4-dione (1e)

Piperonaldehyde (1 mM, 0.150 g) was mixed with boric acid and acetyl acetone (3mM, 0.3 ml) in 2 ml DMF. Then THQ (0.02 ml) in acetic acid (0.06 ml) was added and heated for 4 hours. The reaction mixture was then added to 30 ml 1N HCl and then extracted with ethyl acetate. The organic layer was separated and dried. The product was then purified using dry column chromatography using petroleum ether. The yield obtained was 50% . m.p.- 90-92ºC
Anal. Calcd. for $C_{15}H_{17}O_3$: C, (73.5); H, (7.0). Found C, (73,8); H, (6.8)
IR (KBr): 3434, 2787, 1586, 1439, 1301, 1166, 1043, 938, 813, 724, 602, 529, 435, 419 cm^{-1}
^{1}H NMR: δ 2.15 (3H, s), 5.590 (2H, s), 6.006 (2H, s), 6.27 (1H, d, J=15.5 Hz), 6.81 (1H, d, J=8 Hz), 6.982 – 7.02 (Ar-H, 2,6-H, m), 7.49 (Ar-H, 5H, d, J=16 Hz)
FAB MS (NBA): 200.42, 215.12, 246.3, 247.8 (M^+)

Synthesis of curcuminoids from cinnamoyl acetones

Synthesis of 1-(4-hydroxy-3-methoxyphenyl)-7-(4-hydroxyphenyl) hepta-1,6-diene-3,5-dione (2a)[curcumin II]

Cinnamoylacetone (**1a**) (1mM, 0.254g) was mixed with boric acid (1mM, 0.061g) in 2ml N,N-DMF. Then 4-hydroxybenzaldehyde (1mM, 0.122 mg) was added and THQ (0.02 ml) in acetic acid (0.06 ml) was added and heated for 4 hours. The reaction mixture was then added to 20% acetic acid under vigorous stirring. The separated product was then filtered and dried. The product was purified by dry column chromatography using chloroform. The yield obtained was 42 %. The m.p is 183-185ºC
Anal. Calcd. for $C_{20}H_{18}O_5$: C, (71.0); H, (5.36). Found C, (71.5); H, (4.82)
IR (KBr):
^{1}H NMR: δ 3.9 (3H,s),5.79 (1H, s), 6.488 (2H, 2-H, d, J=15 Hz), 6.865(2H, 6-H, d, J=7 Hz), 7.58-7.62(2H,1,7-H,m), 7.058(Ar-H, 1H, s, 2'-H), 7.47(Ar-H, 1H, d, J=7 Hz 6'-H), 7.128(Ar-H, 2H, d, J= 8 Hz, 2,6-H), 6.93-6.97(Ar-H, 2H,m,3,5,5'-H,)
FABMS(NBA): 176.38, 203.45, 239.54, 323.61, 338.85(M+1)

Synthesis of 1-(4-hydroxy-3-methoxyphenyl)-7-(4-methoxyphenyl) hepta-1,6-diene-3,5-dione (2b)

Cinnamoylacetone (**1a**) (1mM, 0.254g) was mixed with boric acid (1Mm, 0.061g) in 2ml N,N-DMF. Then 4-methoxybenzaldehyde (1mM, **0.234** mg) was added and THQ (0.02 ml) in acetic acid (0.06 ml) was added and heated for 4 hours. The reaction mixture was then added to 20% acetic acid under vigorous stirring. The separated product was then filtered and dried. The product was purified by dry column chromatography using a 3:1 mixture of petroleum ether and DCM. The yield obtained was 52%. The m.p.is 132-133ºC
Anal. Calcd. for $C_{21}H_{20}O_5$: C, (71.6); H, (5.7). Found C, (71.2); H, (6.18)

IR (KBr): 3170, 1666, 1598, 1515, 1451, 1285, 1159, 1666, 1598, 1515, 1451, 1285, 1159,788, 640, 534, 506, 450, 422
^{1}H NMR: δ 3.93(6H,s), 5.794(2H,s), 6.49 (2H, dd, 2,6-H), 7.5-7.65(5H, m, 1,7-H, 3'-H,5,5'-H), 7.06-7.14(Ar-H, 4H, m, 2,2'-H,5,5'-H)
FAB MS (NBA): 176.25, 203.31, 239.42, 257.39, 338.58, 353.35(M+1)

Synthesis of 1-(4-hydroxyphenyl)-7-(4-methoxyphenyl) hepta-1,6-diene-3,5-dione (2c)

Cinnamoyl acetone (**1c**) (1mM, 0.254g) was mixed with boric acid (1mM, 0.061g) in 2ml DMF. Then 4-hydroxybenzaldehyde (1mM, 0.122 mg) was added and THQ (0.02 ml) in acetic acid (0.06 ml) was added and heated for 4 hours. The reaction mixture was then added to 20% acetic acid under vigorous stirring. The separated product was then filtered and dried. The product was purified by dry column chromatography using a 1:1 mixture of petroleum ether and DCM. The yield obtained as 42 %. m.p.- 88-90^{o}C
Anal. Calcd. for $C_{20}H_{18}O_4$: C, (74.5); H, (5.6). Found C, (74.62); H, (4.63)
IR (KBr) : 3430, 1600, 1451, 1217, 830, 603, 483, 466, 438, 430, 410, 401 cm^{-1}
^{1}H NMR: δ 3.85(3H,s), 5.79(2H,s), 6.49(2H, dd, 2,6-H), 7.62(2H, dd, 1,7-H), 7.41-7.52(Ar-H, 4H, m, 2,2'-H,6,6'-H), 6.785-6.905(Ar-H, 4H, m, 3,3'-H, 5,5'-H)
FAB MS (NBA): 176, 203.45, 239.42, 316, 323 (M^+)

Synthesis of 1-(4-hydroxyphenyl)-7-(3,4-dimethoxyphenyl) hepta-1,6-diene-3,5-dione (2d)

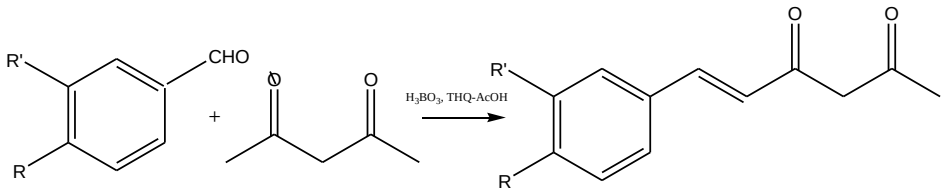
Cinnamoylacetone (**1d**) (1mM, 0.254g) was mixed with boric acid (1mM, 0.061g) in 2ml N,N-DMF. Then 4-hydroxybenzaldehyde (1mM, 0.122 mg) was added and THQ (0.02 ml) in acetic acid (0.06 ml) was added and heated for 4 hours. The reaction mixture was then added to 20% acetic acid under vigorous stirring. The separated product was then filtered and dried. The product was purified by dry column chromatography using a 1:6 mixture of petroleum ether and DCM. The yield obtained was 46%. m.p -115-117^{o}C
Anal. Calcd. for $C_{21}H_{20}O_5$: C, (71.6); H, (5.72). Found C, (71.5); H, (5.82)
IR (KBr): 3168, 1612, 1458, 1301, 1175, 790, 716, 640, 623, 545, 500, 475, 478, 466, 434
^{1}H NMR: 3.92 (6H, s), 5.82 (2H, s), 6.67 (2H, 2,6- H, m), 6.61 (2H, 1,7-H, d, J=15 Hz), 7.24 (4H, 2,6-H, 2',5'-H, Ar-H, m), 7.54 (3H, 3,5, 6'-H, Ar-H, m)
FAB MS (NBA): 176.25, 197.33, 242.39, 273.31, 329.4, 353.3(M+1)

Results and Discussion

Acetylacetone in DMF was treated with boric acid and vanillin in the molar ratio 1:3:3 and the reaction mixture was treated with 1,2,3,4-tetrahydroquinoline-acetic acid as a catalyst and heated for four hours. The reaction mixture was then added to dilute hydrochloric acid and the aqueous mixture was extracted with ethyl acetate. Work-up and further processing, including column chromatography, afforded a product, the high resolution FAB mass spectrum of which showed a [M+1] peak at 235.39. In its ^{1}H NMR spectrum, a singlet at δ 2.15 could be assigned to methyl hydrogens of a –CO-CH_3 group. The peaks at δ 5.6 and 5.79 were due to the keto hydrogens of the 1,3-enol unit.

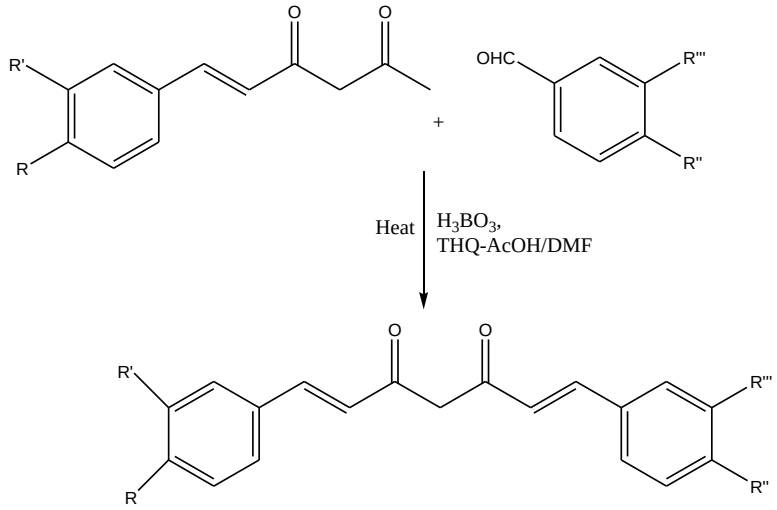
The two doublets at δ 6.29 and 6.91could be accounted by the two hydrogens of the 1-hexene chain. A doublet at δ 7.51 could be assigned to the aryl ring hydrogen ortho to the -C=C- bond. A multiplet at δ 7.07-7.09 was attributed to the 3,6-aryl ring hydrogens. Based on the above data, the product now obtained was formulated as (4-hydroxy-3-methoxycinnamoyl) acetone **1a**, which was obtained in 54% yield.

By repeating similar reaction with four other aryl aldehydes, we have now successfully synthesized four additional cinnamoylacetones, required as the precursors for the synthesis of unsymmetrical curcuminoids. The reaction in a generalized manner is schematically shown below.



R'= OCH_3, R= OH (**1a**)
R'= H, R= OH (**1b**)
R'= H, R = OCH_3 (**1c**)
R' = OCH_3, R = OCH_3 (**1d**)
R'=R= -O-CH_2-O- (**1e**)

We next attempted to use the cinnamoylacetones obtained as described above as the starting material for the synthesis of curcumin analogs bearing non-identical aryl rings at the termini of the heptadiene carbon chain of curcuminoids. Thus, the cinnamoylacetones at a 1 mM scale was mixed with equimolar amounts of boric acid and an aryl araldehyde in DMF. The Knoevenagel condensation catalyst THQ in acetic acid was added and the whole mixture was heated on a water bath for 4 hours. The reaction mixture was then added to 20 % aqueous acetic acid under rapid stirring to obtain a powdery product. This crude products thus obtained were then purified by dry column chromatography.



R = OH, R' = -OCH_3, R"= OH, R''' =H **(2a)**
R = OH, R' =OCH_3, R"= OCH_3, R''' =H (**2b**)
R = OH, R' =H, R"= OCH_3, R''' = H (**2c**)
R = OH, R' =H, R"=OCH_3, R''' = OCH_3(**2d**)

Antioxidant activity study

Curcumin is a well-known natural antioxidant (AO), and numerous studies have demonstrated its effectiveness in this role. A comparative scientific study of curcumin components was appeared in the literature and found that curcumin III exhibited potent AO properties[12]. Cousins et al the antioxidant capacity of methanolic extracts from fresh and dried turmeric rhizomes[13].It is also reported on the AO capabilities of curcumin components using *in vitro* models, such as phosphomolybdenum and linoleic acid peroxidation methods[14]. The $NO^{\cdot}$ radical scavenging activity of manganese complexes of curcumin was evaluated and found them to have much lower IC50 values than curcumin and standard antioxidants[15]. Curcumin's AO activity through inhibition of controlled styrene oxidation was also determined, concluding that non-phenolic curcuminoids lacked AO activity, reinforcing the idea that the phenolic groups, not the keto-enol CO–CH_2-CO group, are essential for AO activity[16]. Several ring-substituted symmetric analogs of curcumin were synthesized by some workers and studied their AO properties using three models: inhibition of lipid peroxide, DPPH (diphenylpicrylhydrazyl) scavenging, and ABTS (2,2'-azinobis-3-ethylbenzthiazoline-6-sulphonate) assays[17]. Their study showed that phenolic analogs were more active than non-phenolic ones, with the activity increasing when the phenolic group was sterically hindered by ortho-methyl groups. Sun et al. studied the AO mechanism of curcumin using density functional theory (DFT), proving that its AO mechanism involves hydrogen atom abstraction from the phenolic group[18]. The bond dissociation energy (BDE) were calculated and concluded that phenolic curcumin analogs are more potent antioxidants than those without –OH groups[19]. The AO behavior of curcumin and demethoxycurcumin was determined through various assays[20], reaffirming the importance of phenolic –OH groups for both AO activity and free radical kinetics. The

AO activity of curcumin and its hydrogenated derivatives (THC, HHC, and OHC) was compared, finding that the hydrogenated derivatives exhibited much higher biological activity than curcumin[21]. And concluded that hydrogenation of conjugated double bonds in the central carbon chain enhanced AO activity significantly. A series of curcumin analogs with varying carbon spacers between the two aryl rings were synthesized and measured their AO activity using TRAP and FRAP assays[22]. Although non-phenolic analogs also exhibited some AO activity, phenolic analogs were generally more effective.

Further investigation into the AO properties of curcumin was reported by Alk and Gulcin, who studied its inhibition of lipid peroxidation, Fe^{3+} ion reduction, DPPH radical scavenging, $ABTS^{\bullet+}$ radical scavenging, superoxide anion radical scavenging, hydrogen peroxide scavenging, and Fe^{2+} ion chelation[23]. One of the earliest studies on the AO activity of curcuminoids found that phenolic groups are crucial for their AO properties. Sharma observed that Curcumin I and its bisdemethyl analog, bis-(3,4-dihydroxycinnamoyl) methane, were potent inhibitors of in vitro lipid peroxidation in rat brain homogenates[24]. Disagreement remains regarding the molecular mechanism of curcuminoid AO activity, with some studies suggesting that the phenolic groups are crucial, while others propose that the central methylene hydrogen plays an important role [25]. It has been suggested that both the central methylene hydrogen and the phenolic hydrogen may be involved in the AO mechanism[26]. Some studies concluded that the phenolic group is essential for free radical scavenging activity, with the methoxy group further enhancing this activity[27-28].

Based on the light of above literature review, here is the comparison the AO activity of the newly synthesized unsymmetrical curcuminoids by DPPH and FRAP assays. The results were compared with the standard compound. We attempt to provide a structure activity relation for the unsymmetrical compounds by examining the influence of OH group substituted at varying position of the curcuminoid moiety.

Methanol was commonly used as the solvent in the reactions of AO with DPPH[29]. The increased reactivity of DPPH in alcohols has been postulated to be due to the formation of hydrogen bond between the nitrogen of DPPH and alcohol, thereby decreasing the radical delocalization and thus increasing its reactivity. After several trial experiments, the concentration of DPPH was set at 10^{-5} M, and the test compounds were taken in four different concentrations such as 0.01 mM, 0.025 mM, 0.05mM, 0.075 mM and 0.1 mM in methanol. The solutions of synthetic AO, BHA was also prepared in the above concentrations. DPPH (2.8 ml) was pipetted out into 46 test tubes. The eight test compounds (0.04 ml) in five different concentrations was pipetted out into 40 test tubes. Simultaneously, 0.05 ml of BHA in five different concentrations was pipetted out into 5 test tubes. Methanol (0.04 ml) was pipetted out into the last test tube, which served as the blank and all the mixtures were incubated for 30 minutes at room temperature. Subsequently, absorbance was measured at 517 nm in each case.

Table 1 Percentage Inhibition of DPPH by the unsymmetrical curcuminoids

Concentration(mM)	2a	2b	2c	2d	BHA
0.01	37	26	24	24	31
0.025	60	36	30	39	46
0.05	74	44	42	46	65
0.075	83	55	50	51	84
0.1	96	74	69	67	93

Table 2. EC_{50} values for the inhibition of DPPH (10^{-5}M) by Unsymmetrical curcuminoids

Compound	EC50(μM)
2a	18
2b	64.5
2c	76
2d	73
BHA	30

The results were substantiated by doing FRAP assay also. All the test compounds were prepared at a concentration of 500 μM and $FeSO_4.7H_2O$ at a concentration range of 100 μM to 4000 μM was used for the purpose of calibration. The mixtures of test compounds with the FRAP reagent were incubated for 20 minutes at 37ºC and the absorbance was read at 593 nm. As the AO activity of the compounds increases, the color of the mixture and hence the absorbance decreases. Better AO molecules would possess a higher FRAP assay value. The result is expressed in terms of micromolar $FeSO_4$ equivalents. The experiment was repeated in duplicate. The results obtained were in agreement with that obtained by the DPPH assay. The result seems to indicate that the FRAP values are generally better if the compound possesses an ability to co-ordinate metal through a phenolic group which bears an ortho methoxy group.

Table 3. FeSO4 equivalent values of unsymmetrical curcuminoids by FRAP assay

Compound	$FeSO_4$ (μM)
2a	1350
2b	710
2c	610
2d	650

Molecular Docking Studies of Curcumin derivatives in Bacterial tyrosine kinase Protein

Recently, docking and scoring softwares are widely used to hasten the drug design and product development in pharmaceutical industry[30-32]. A preliminary understanding on bioactivity was obtained by subjecting the novel compounds synthesized to *in silico* analysis using docking algorithm, LibDock, a commercially available docking programme; and a module of Discovery Studio v4.0. The maximum binding interactions were observed using Discovery Studio Visualizer v4.0 and the scores corresponding to maximum binding affinity were calculated.

Yongfu et al in 2013 synthesized several unsymmetric curcuminoids with pentadiene chain and carried out *in vitro* and *in silico* studies to check the activities of them as tyrosinase inhibitors[9]. They proved that the one with phenolic group in the fourth position of benzene ring of the curcuminoid is much better tyrosinase inhibitor than the other analogs. Here, we selected the four synthesized unsymmetrical curcuminoids with –OH group in the fourth position for the molecular docking study

The crystal structure of mutated Bacterial tyrosine kinase protein was downloaded from Research Collaboratory Structural Bioinformatics (RCSB) protein data bank having the PDB ID 2WJE. The prepared protein was energy minimized and saved as 2WJE.pdb . The ligands were designed using Marvinsketch 5.3.0. The ligand preparation was done using Discovery studio 2022. The minimized receptor Bacterial tyrosine kinase and ligand was docked with Libdock, a relatively fast algorithm that conducts 'Hotspots' matching with ligand conformation. The binding affinity of the four ligands with the protein was compared with that of the standard drug Metronidazole

Preparation of the Protein (Scaffold protein-2WJE)

The X-ray structure of protein containing water molecules and hetero atoms were refined using Accelrys Discovery studio 2022 and the protein crystal structure was energy optimized after energy minimization. The protein was then saved as 2WJE.pdb and subjected to docking studies[33]

Preparation of Ligands:

The structure of the ligands **2a**, **2b**, **2c**, **2d** and the standard drug Metronidazole(write the names of compounds) were prepared using Marvinsketch 5.3.0 and saved as sdf file.

Docking Methodology

The software for molecular docking used in this study was Discovery studio 2022 (DS 2022, Accerlrys Inc. SanDiego, CA). Discovery Studio 4.0 software refers to a computational platform designed for molecular modeling and simulation tasks in the field of life sciences. The docking between the ligand and protein was evaluated by using Libdock docking program. Libdock is a high-throughput algorithm for docking ligands into an active binding site on the receptor, which is also a site feature docking algorithm[34]. Prior to docking, the targets and ligands were preprocessed for optimizing and minimizing the structure and generating its conformers respectively[35]. The 4 ligands and the standard drug were docked with the binding site of Bacterial tyrosine kinase. Ligand conformations were aligned to the receptor interaction sites and the best poses were reported in the end of the docking simulations. Each pose was evaluated

according to the Libdock score. The scores obtained from docking studies are given in **Table 4**. The 3D and 2D Docking images are given in **Fig.1-5.**

Table 4 : Libdock score obtained from the docking study of unsymmetrical curcuminoids

Ligand	**Type of Interaction with Amino acid residue**				**Score**
	van der Waals	**Hydrogen Bonding**	**Pi-alkyl**	**Pi-cation**	
2a	LEUA:168,LYS:181,PROA:170,LYSA:169,GLUA:189,TYRA:221,GLNA:185	VALA:217	LEUA:188,LEUA:216,VALA:167,	LYSA:220	93.5318
2b	LYSA:169,PROA:170,LEUA:168,LYSA:181,HISA:166,VALA217,TRYA:221,LEUA:188,ALAA:184	GLNA:185	LEUA:216	LYSA:220,GLUA:189	81.1854
2c	GLUA:189,PROA:170,LYSA:169,VALA:167,TYRA:221,VALA:216	GLNA:185	LEUA:216 LEUA:188	LYSA:220	83.6059
2d	TYRA:221	LEUA:168 LYSA:169		PROA:170 LEUA:216	97.1186
METRONIDAZOLE (Std.Drug)	LEUA:216, TYRA:221,LEUA:188,VALA:167,ALAA:184,HISA:166,LEUA:168,LYSA:182, LYSA:169	GLNA:145		PROA:170 LYSA:181	74.207

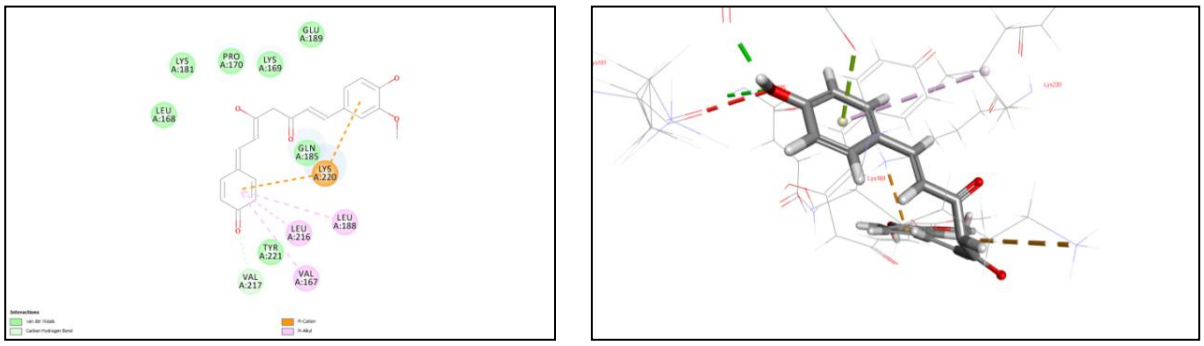


Fig.1 2D and 3D image of docking of 2a with Protein (2WJE)

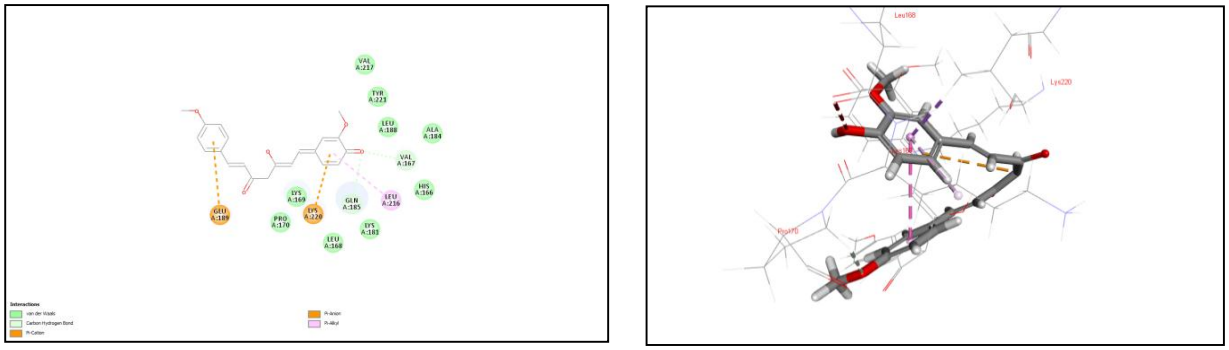


Fig.2 2D and 3D image of docking of 2b with Protein(2WJE)

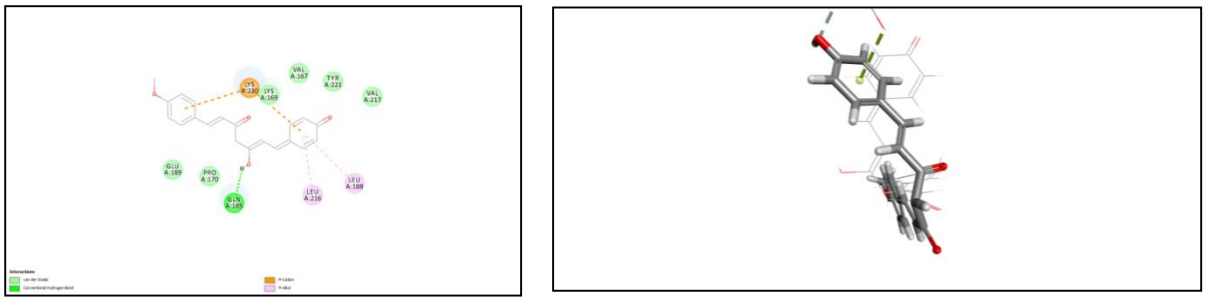


Fig.3 2D and 3D image of docking of 2c with Protein(2WJE)

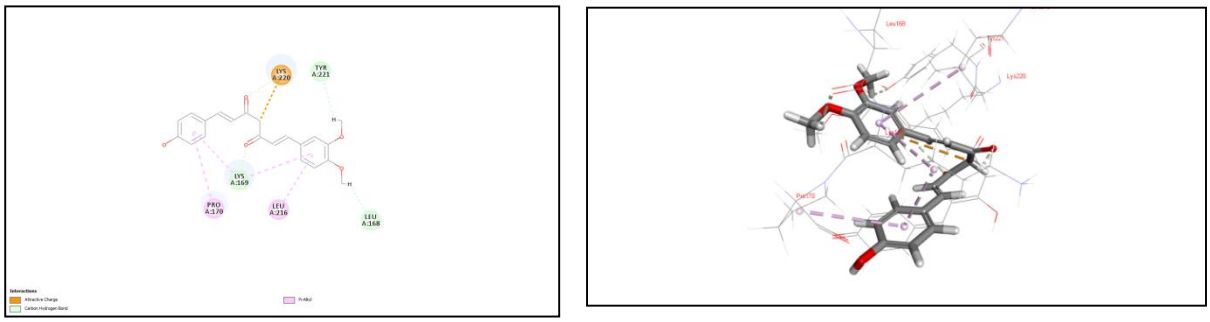


Fig.4 2D and 3D image of docking of 2d with Protein(2WJE)

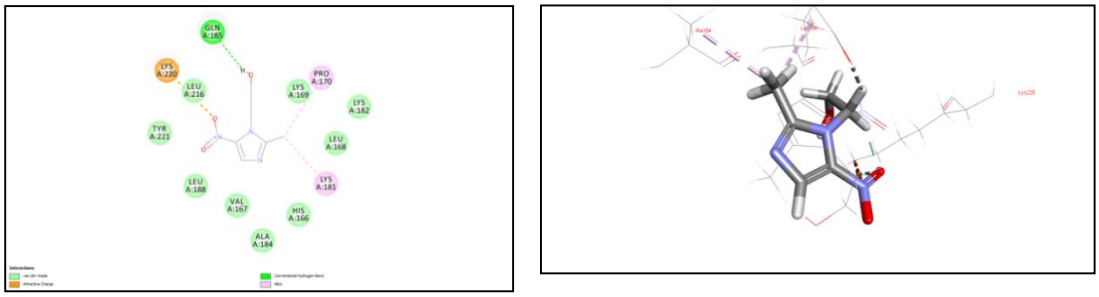


Fig.5 2D and 3D image of docking of Std. Drug with Protein(2WJE)

Table 4. represents the Libdock scores of the various ligands. The docking was performed using the software Discovery studio 2022. The five ligands were successfully docked into the predicted binding cavity of protein Bacterial tyrosine kinase using Libdock module. Metronidazole was taken as the standard drug. Multiple conformations were generated for each compound. All the ligands, **2a, 2b, 2c** and **2d** and were found to have good Libdock scores when compared with that of the standard Metronidazole . The Libdock ranking follows the order **2d> 2a > 2c >2b** >Metronidazole (Standard Drug).The docking with the protein binding site showed four different kinds of interactions like van der Waals, pi-alkyl, pi-cation and hydrogen bonding among them the predominant interaction is through van der Waals force .

Conclusion

Four novel unsymmetrical curcuminoids are prepared in lesser time and comparatively appreciable yield. They were characterized using various the spectroscopic techniques to confirm their structures, and they were screened for *in vitro* antioxidant study using DPPH assay and FRAP assay. From the antioxidant activity it was observed that compound 2a showed higher activity than the standard BHA, with order of antioxidant activity as 2a>BHA>2b>2d>2c. The phenolic –OH group at fourth position of the phenyl ring proved to be beneficial for the increased activity. Also, the presence of methoxy group in ortho position to –OH group increased the antioxidant activity, as it was proved earlier in the literature review. This presents a new promising step into the drug design chemistry

According to the Libdock algorithm, higher the docking score, higher the strength and vice versa. Hence it can be inferred that all the ligands showed good docking score when compared to that of the standard drug antibacterial drug, Metrimidazole. Among the four ligands, 1-(4-hydroxy phenyl)-7-(3,4-dimethoxy phenyl)-hepta-1,6-diene-3,5-dione gave the best score with greater binding affinity towards the protein, bacterial tyrosine kinase. From the *in silico* studies it was proved that all the derivatives are having greater inhibition towards the tyrosine kinase protein than the standard drug, indicating all are novel and promising drug candidates for further drug discovery. The findings are consistent with earlier studies, which indicated that the presence of a hydroxyl group at the fourth position of a ring enhances tyrosinase inhibitory activity.

Conflict of Interest

The author declares no conflict of interest with the publication of research work

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