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Original article

Synthesis and evaluation of antimicrobial activity of 4*H*-pyrimido[2,1-*b*] benzothiazole, pyrazole and benzylidene derivatives of curcumin

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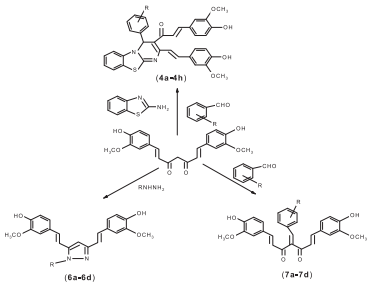
HIGHLIGHTS

- One-pot syntheses of novel curcumin derivatives have good biological activities.
- First example of three component reaction of curcumin using pyridine as catalyst.
- First time that we have isolated and characterized the reaction intermediates.
- Easy synthesis of pyrazoles and benzylidenes of curcumin which possesses good antimicrobial activity.

GRAPHICAL ABSTRACT



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ABSTRACT

A novel, one-pot, simple, efficient procedure for 4*H*-pyrimido[2,1-*b*]benzothiazole (**4a**–**h**), pyrazole (**6a**–**d**) and benzylidene (**7a**–**d**) derivatives of curcumin under solvent and solvent free conditions in microwave with good yield is have been synthesized. The synthesized compounds were evaluated for their antibacterial activity against gram-positive and gram-negative bacteria viz. *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Escherichia coli*, *Bacillus cereus* and *Providencia rettgeri* and antifungal activity against fungi viz *Aspergillus niger*, *Aspergillus fumigates*, *Aspergillus flavus*. Detailed mechanistic study shows reaction proceeds through Knoevenagel type intermediate **3a** which has been suggested as key intermediate for reaction (Fig. 3).

1. Introduction

Multi-component reactions (MCRs) are powerful tool and have attracted much attention of synthetic organic chemists because of the building of complex molecules with diverse range of complexity which can easily be achieved from readily available starting material [1–3]. The synthesis of heterocycles using MCRs is

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a domain of classic carbonyl condensation chemistry. Pyrimidines have attracted considerable interest because of their wide range of biological activities. Some of which have anti-viral, anti-tumour, anti-inflammatory, antihypertensive activities [4–6], calcium channel modulators [7] and antimicrobial agents [8–10]. Pharmacologically, pyrazoles and its derivatives represent one of the most important class of heterocyclic compounds and have attracted considerable attention from both synthetic and medicinal chemists due to their biological activities covering a wide ranges of application of pyrazole moiety as potential antimicrobial [11], anti-viral [12], anticancer [13], herbicide [14]. Some of its derivatives have been reported to exhibit significant cyclooxygenase (COX-2) inhibitors [15], molluscicidal activity [16], antiglaucoma activity [17], anti-inflammatory [18], antioxidant [19] and anti-HCV activity [20].

Curcumin possess antimicrobial [21], antioxidant [22], anti-HIV protease activity [23], and cancer preventive properties [24]. Extensive research has revealed that this polyphenol can both prevent and treat cancer [25]. Phenolic O–H and methylene hydrogen of curcumin revealed antioxidant activity by free radical reactions [26]. Elias et al. [27] have reported the dihydropyridones synthesis by microwave assisted reaction of curcumin with either primary amines or the amine acetate in the presence of montmorillonite K-10 as a catalyst but with poor yield. Various substituted thiazoles have been synthesized and examined for their antifungal and antibacterial activities [28]. Objective of the present MCR is to use biologically important component viz. curcumin, 2-amino benzothiazole, thiosemicarbazide, semicarbazide, isoniazide to convert it into other potential antimicrobial agents. Our target molecules have shown good antibacterial and antifungal activity. We have developed the pyrazoles of curcumin using acetic acid as potential active antimicrobial agents. The synthesis of 4*H*-pyrimido [2,1-*b*]benzothiazole and benzylidene derivatives of curcumin was tried with acetic acid which resulted in 55% yield. The use of pyridine (base) as catalyst led to formation of 67% yield of 4*H*-pyrimido[2,1-*b*]benzothiazole. Pyridine has not been reported as catalyst, hence this paper also report this new catalyst for multi-component reactions. The intermediate of this reaction has been isolated and characterized for the first time, hence mechanism could be established.

2. Chemistry

In this study we have developed a simple and efficient synthetic method for novel derivatives of curcumin. 4*H*-Pyrimido[2,1-*b*] benzothiazole (**4a**–**h**) derivatives of curcumin has been synthesized by three component reaction of curcumin, substituted aromatic aldehydes and 2-amino benzothiazole using pyridine under solvent and solvent free conditions (Scheme 1). Benzylidene derivatives of curcumin (**7a**–**d**) have also been synthesized under optimized reaction conditions using curcumin and substituted aromatic aldehydes (Scheme 3). Our synthetic method for pyrazole derivatives of curcumin (**6a**–**d**) has been developed in acetic acid (Scheme 4).

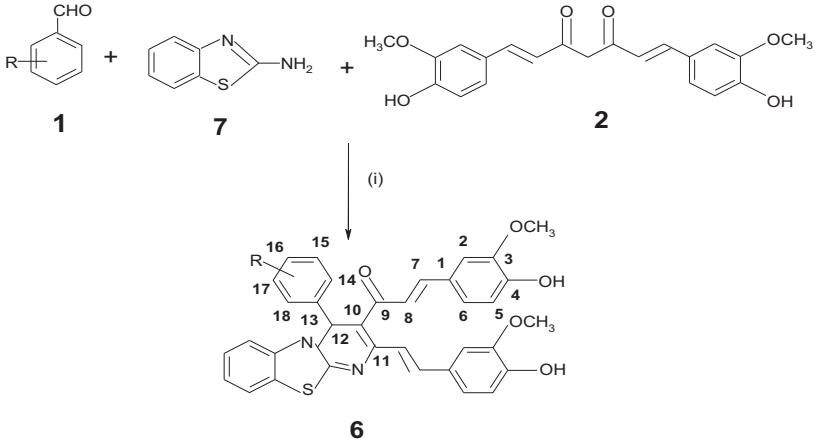
3. Pharmacology

3.1. To determine MIC by the micro dilution broth susceptibility test

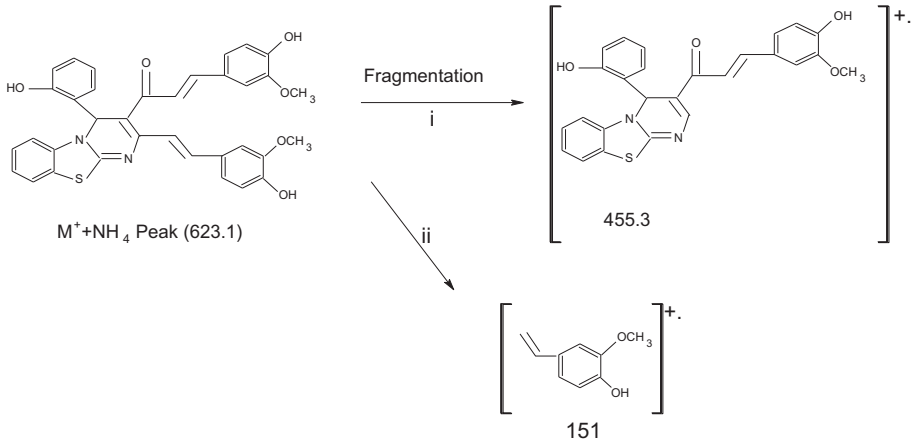
Different concentrations 20, 10, 5, 2.5, 1.25 and 0.625 μM/mL of the compound (**4a**–**h**, **6a**–**d** and **7a**–**d**) were prepared in sterile microwell to determine minimum inhibitory concentration (MIC). Nutrient broth was adjusted to pH 7.0 used for the determination of MIC. The inoculum of the test microorganisms was prepared by using 16 hold cultures adjusted by reference to the 0.5 McFarland standards (1.5×10^8 CFU/mL). Brain heart infusion broth was prepared and 150 μL of it was taken in each well and 10 μL of each compound was added in broth with different concentrations, then 10 μL of bacterial culture broth was added. The plate was shaken to uniformly mix the inoculum with the broth. Optical density was taken by photo spectrometer (μQuant, Biotek Ltd. USA) and incubated for 24 h at 37 °C. Appearance of any turbidity shows that the compound is not able to inhibit the growth of the bacteria, while no turbidity indicates the inhibition of microorganism by the sample (Table 1).

3.2. In-vitro antifungal test [29]

For antifungal testing the pyrimidine derivatives of curcumin, curcumin and fluconazole (standard drug) solution was prepared in acetone at initial concentration (120 μM/mL) and serially diluted to make effective concentration of 20, 10, 5.0, 2.5, 1.25, 0.625 and 0.315 μM/mL. Isolated fungal species *A. niger*, *A. fumigates* and *A. flavus* were selected. Subourad dextrose agar was prepared and 150 μL of it was taken in each well and 10 μL of each compound was added in broth with different concentrations, then 10 μL of fungi culture broth was added. The plate was shaken to uniformly mix the inoculum with the broth and note the optical density. The well incubated for 72 h at 28 °C. Appearance of any turbidity shows that the compound is not able to inhibit the growth of the bacteria,



Scheme 1. Conditions (i) refluxing in methanol at 60–65 °C using pyridine as catalyst.



Scheme 2. Mass fragmentation patterns of product **4b**.

while no turbidity indicates the inhibition of microorganism by the sample. To ensure that solvent had no effect on fungal growth a control test was performed with test medium supplemented with acetone at the same dilutions as used in the experiment. The results incorporated in Table 2.

To determine zone of inhibition, sterilized filter discs were dipped in these solutions and subsequently dried to remove acetone. Subourad dextrose agar was prepared and allowed to solidify. One of these discs was kept free from antifungal drug (fluconazole) and served as growth control. Fungi were selected viz *A. niger*, *A. fumigates*, *A. flavus* and 1 mL of each fungi culture were added in the Subourad dextrose agar plates and spread with the help of sterile spreader. The filter paper discs soaked in above mentioned dilutions of curcumin, fluconazole and curcumin derivatives were placed aseptically over the inoculated plates using sterile forceps. The plates were incubated at 28 °C for 72 h, in upright position. The zone of inhibition was measured using scale (Table 2).

4. Result and discussion

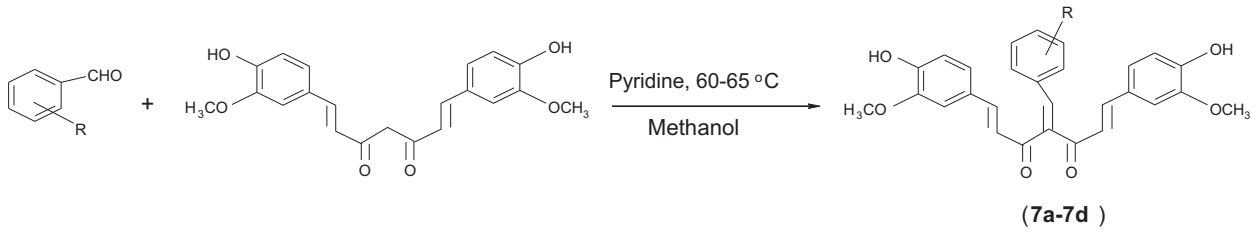
4.1. Chemistry

Reaction of curcumin, 2-amino benzothiazole and benzaldehyde at 60–65 °C in the absence of pyridine as a catalysts fail to give product. This shows that pyridine is needed to get the product. In order to evaluate the appropriate catalyst loading, a model reaction using curcumin (5 mmol), 2-amino benzothiazole (5 mmol) and substituted aromatic aldehydes (5 mmol) was carried out using 0.25 mL, 0.5 mL, 0.75 mL and 1 mL of pyridine at 60–65 °C with methanol as solvent (Fig. 1). It was found that 1 mL of pyridine resulted in the product formation. Higher quantity of pyridine (1.5 mL and 2 mL) neither increases the yield nor improves the conversion time. So, 1 mL of pyridine was found to be the optimal quantity.

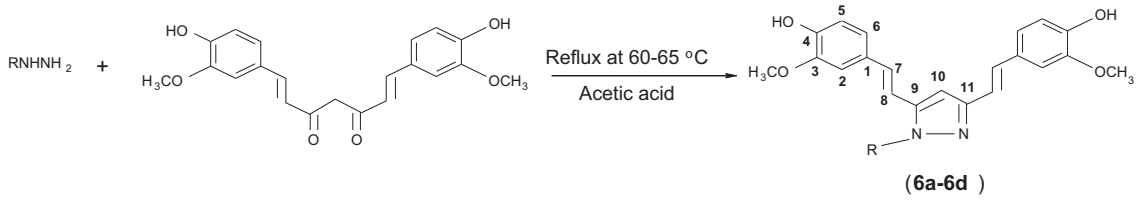
The multi-component reaction of curcumin (5 mmol), 2-amino benzothiazole (5 mmol) and substituted aromatic aldehydes (5 mmol) dissolved in methanol using pyridine (1 mL), resulted product formation within 18–20 h with yield ranges from 66 to 76% (Scheme 1). The structure of 4*H*-pyrimido[2,1-*b*]benzothiazole derivatives of curcumin, was confirmed by IR, NMR, Mass spectral analysis. The ^{1}H NMR spectra of the product **4a**–**h** were characterized by singlet around 7.26 δ due to asymmetric C–H hydrogen and the multiplet at 7.1–7.61 δ with unsymmetrical pattern for aromatic hydrogens of benzothiazole and benzene ring of aldehyde, which is not shown by curcumin. Mass spectra of product **4b** shows the $[M + NH_4]^+$ molecular ion peak at 623.1 and other fragments peak at 455.3, 151 for corresponding fragments which are shown in Scheme 2. This supports the probable structure of product **4b**. A variety of electron donating and electron withdrawing groups on aromatic aldehydes have been studied (4a-4h) (Table 3).

Optimized reaction conditions in hand lead us to synthesize the benzylidene derivatives of curcumin using curcumin (5 mmol) and substituted aromatic aldehydes (5 mmol) in the presence of pyridine in methanol at 60–65 °C (Scheme 3). Methodology involves for synthesis of pyrazole derivative, curcumin (5 mmol), and hydrazines (5 mmol) were refluxed in acetic acid at 60–65 °C (Scheme 4). Synthesized benzylidene and pyrazole derivatives have been characterized by ^{1}H NMR, ^{13}C NMR and mass spectral study.

The reaction in solvent took longer time (6–20 h), hence there is need to find methods to reduce reaction time. Therefore the reaction was carried out in microwave under solvent free condition. The reaction was completed in 8–10 min with good yield (Table 3). Thus synthesis under microwave is very simple, giving high yield (69–89%) in shorter duration and greatly reducing the environmental pollution by eliminating volatile organic solvent. Various substituted aromatic aldehydes containing electron withdrawing and electron donating substituents at ortho, meta or para positions show equal ease towards the product formation in high yields.



Scheme 3. Synthesis of benzylidene derivatives of curcumin.



Scheme 4. Preparation of pyrazoles of curcumin.

There was no significant effect of electron donating and electron withdrawing substituents.

4.2. Mechanism

The mechanistic pathway of three component reaction using aldehyde, dicarbonyl and 2-amino benzothiazole has not been reported so far. In a previous report, Kappe proposed that Lewis acid or protic acid-mediated Biginelli reaction proceeded via the formation of an iminium ion (formed by acid-catalyzed condensation of aldehyde with urea) as a key intermediate rather than carbenium ion intermediate (derived from the acid-catalyzed aldol reaction of aldehyde and ethyl acetoacetate) [30]. Literature survey revealed that till now the intermediate of these three component condensation reaction has not been isolated and characterized. Shen et al. [31] have suggested that mechanism of Biginelli reaction proceeded with urea and thiourea in different ways when t-BuOK was used. But they failed to isolate and characterize the intermediate. They have synthesized compound **6** using literature procedure (currently, there is no report on the successful isolation of intermediate **6**). Further, they have synthesized the intermediates **9** (derived from the acid-mediated condensation of 4-chloro benzaldehyde and 2-phenyl acetophenone) intermediates **7** and **8** under acidic conditions by the well known method documented in literature [32,33] (Fig. 2), and further reacts with third component which gives the target product. Shen et al. have synthesized the intermediate **8** in toluene solvent using acid-mediated conditions which gave target product but author have reported that target product was formed with low yield (15%) in toluene; hence there is minimum possibility of this type of intermediate in reaction because this reaction does not proceed well in toluene. Intermediate **9** proposed by Shen et al. also was prepared in benzene but author have reported that reaction was well tolerated in protic solvent, so there is also minimum possibility to follow the mechanistic pathway by this type of intermediate.

The mechanism of three component reaction has been studied in the present paper (Scheme 5). For this, three set of reactions of two components each were carried out. The intermediate formed was reacted with third component and the product formed was analyzed by mp, IR, NMR and mass spectral studies. It was found that only one set of reaction gave the product i.e. the reaction proceeds in two steps: condensation of benzaldehyde **1** and curcumin **2** according to Knoevenagel type reaction. Then 2-amino benzothiazole **3** is reacted with compound **3a** through Michael addition (**3a** characterized by IR, NMR and mass spectra) to afford pyrimidine derivative of curcumin having 4*H*-pyrimido[2,1-*b*]benzothiazoles ring system in structure **4**.

The mass fragmentation of intermediate **3a** (prepared by reacting of benzaldehyde with curcumin) shows [M + H] ion peak at 457.7. NMR spectra of intermediate **3a** shows the singlet at 10.03 δ (=CH–Ar), and the multiplet between 7.32 and 7.90 unsymmetrical pattern due to hydrogen of benzene ring of benzaldehyde, which is not shown by the NMR spectra of the curcumin and multiplet between 6.67 and 7.15 due to 6 hydrogen of two aromatic

Table 1
Minimum inhibitory concentration (MIC_{95} in μM/mL)[a] of curcumin derivatives against bacteria strain.

Compounds	Bacteria species					
	S. aureus (ATCC 11632)	*S. typhi* (ATCC 15499)	*P. aeruginosa* (ATCC 23564)	*E. coli* (ATCC 35218)	*Bacillus cereus* (MTCC 7350)	*Providencia rettgeri* (DRDE strain)
4a	20	20	10	—	5	20
4b	20	—	20	—	5	—
4c	—	—	20	10	5	—
4d	—	20	10	5	2.5	10
4e	—	10	5	10	2.5	10
4f	1.25	20	10	5	2.5	—
4g	20	—	—	10	5	20
4h	—	—	20	20	5	10
6a	—	—	5	2.5	—	2.5
6b	1.25	2.5	2.5	—	0.625	1.25
6c	1.25	5	1.25	2.5	—	—
6d	1.25	2.5	—	—	1.25	—
7a	1.25	2.5	—	1.25	0.625	0.625
7b	5	2.5	1.25	—	0.625	—
7c	1.25	5	1.25	2.5	1.25	1.25
7d	2.5	5	2.5	—	1.25	2.5
Acetone	—	—	—	—	—	—
Curcumin	20	20	—	20	10	20
Ciprofloxacin	1.25	2.5	1.25	1.25	0.625	1.25
Inoculum control	Growth in all concentration	Growth in all concentration	Growth in all concentration	Growth in all concentration	Growth in all concentration	Growth in all concentration
Broth control	No growth	No growth	No growth	No growth	No growth	No growth

(—) Resistant.
[a] MIC (μM) = Minimum inhibitory concentration.

Table 2
Antifungal activity (zone of inhibition and MIC_{95} in μM/mL) of curcumin derivatives and their cytotoxicity.

Compounds	Fungal strains						Cytotoxicity activity IC_{50} (in μM/mL) (L123 cell)
	Zone of inhibition (in mm)			MIC_{95} (in μM/mL)			
	A. niger	*A. fumigates*	*A. flavus*	*A. niger*	*A. fumigates*	*A. flavus*	
4a	—	—	—	—	—	—	>100
4b	—	—	—	—	—	—	50
4c	—	—	—	—	—	—	>100
4d	10	15	—	10	20	—	>100
4e	—	—	11	—	—	12	ND
4f	—	12	—	—	5	—	ND
4g	—	—	—	—	—	—	ND
4h	—	—	—	—	—	—	>100
6a	15	20	—	5	1.25	—	ND
6b	22	25	20	2.5	2.5	1.25	ND
6c	18	—	22	2.5	—	2.5	>100
6d	17	—	20	2.5	—	1.25	>100
7a	—	15	—	—	2.5	—	ND
7b	15	20	—	5	1.25	—	>100
7c	17	20	—	2.5	2.5	—	50
7d	—	—	20	—	—	1.25	>100
Acetone	No activity	No activity	No activity	No activity	No activity	No activity	
Curcumin	9	12	—	20	20	—	
Fluconazole	18	22	25	2.5	1.25	1.25	
Broth control	No growth	No growth	No growth	No growth	No growth	No growth	

(—) Resistant.
ND = Not determined.

ring of curcumin. IR spectra of intermediate **3a** show peaks between 2864 and 2916 cm^{-1} due to C—H_{str} of benzene ring, peak around 3736 cm^{-1} due to stretching of hydroxyl group, peak around 1600 cm^{-1} can be assigned to carbonyl group. Mass fragments, IR and NMR spectra of **3a** support its structure. This is first time that we have isolated the intermediate **3a** which has been characterized and confirmed by comparing the data published in literature [34], hence it can be suggested that it may be the key intermediate for mechanistic study (Fig. 3). We have successfully isolated the intermediate **5a** (prepared by reacting benzaldehyde and 2-amino benzothiazole). The mass fragmentation of intermediate **5a** show $[M - H]^+$ ion peak at 387. ^1H NMR spectra of intermediate shows the triplet at 6.99 (—CH), doublet at 7.19 due to two similar hydrogen of —NH and multiplet are observed between 7.37 and 8.02 due to hydrogen of aromatic rings. This intermediate fail to produce the target product, hence it involvement as intermediate can be ruled out.

4.3. Antimicrobial activity

4.3.1. Dilution

The antibacterial activity of curcumin derivatives was compared with curcumin **2** based on micro dilution broth susceptibility test method. The stock solution of curcumin derivatives (**4a**–**h**, **6a**–**d**, **7a**–**d**) and curcumin **2** were prepared in acetone. The stock solution of each of these derivatives was serially diluted (20, 10, 5, 2.5, 1.25, 0.625, 0.312 μM/mL) and added to Brain Heart Infusion broth, after which a standardized bacterial suspension was added. The lowest concentration of curcumin derivatives in μM/mL that prevented in-vitro growth of microorganism has been represented as MIC_{95} (minimum inhibitory concentration) shown in Table 1.

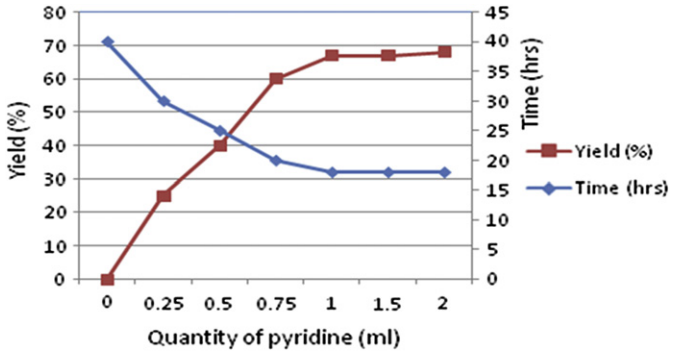


Fig. 1. Optimization of catalyst loading. Three component reaction of curcumin, benzaldehyde and 2-amino benzothiazole proceeds in methanol in the presence of pyridine as catalyst at 60–65 °C.

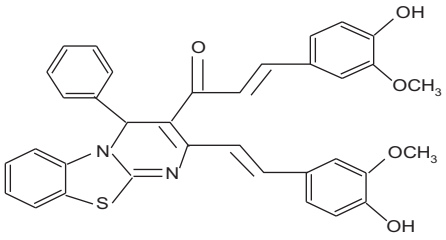
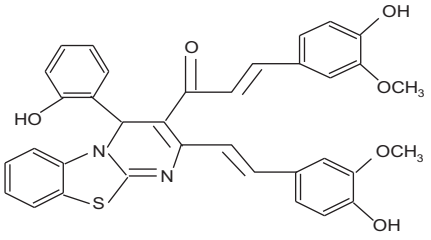
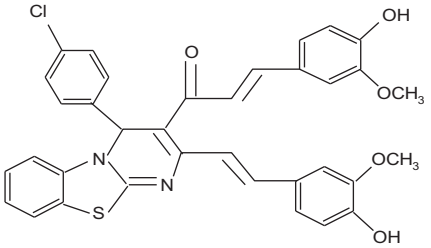
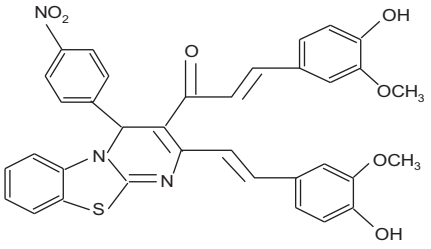
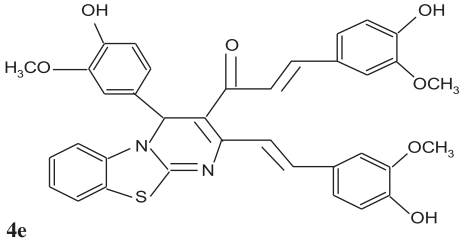
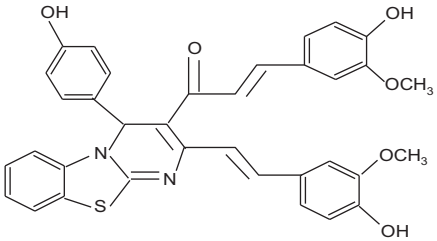
4.3.2. Antibacterial activity (interpretation)

Susceptibility test in-vitro was done on bacteria *Staphylococcus aureus* (ATCC 11632), *Pseudomonas aeruginosa* (ATCC 15499), *Salmonella typhi* (ATCC 23564), *Escherichia coli* (ATCC 35218), *Bacillus cereus* (MTCC 7350) and *Providencia rettgeri*. Each test was performed in triplicate and the MICs reported represent the best of at least two repetitions.

4.3.3. Structure activity relationships

We have investigated curcumin derivatives in order to access their potential effect against different bacterial strains. The effects of various substituents in the phenyl group attached and substituted hydrazines in order to find out their structure activity relationship. As evident from Table 1, substitution at para position as nitro and hydroxyl group (derivatives **4d**–**f**) demonstrated good activity on microorganism. Particularly the derivatives **4f** was active on all species of bacteria tested and the MIC_{95} value was as low as 1.25 μM/mL for *S. aureus* followed by 2.5 μM/mL for *B. cereus*. Electron releasing group at para and meta positions particularly as hydroxy and methoxy substituents shows good activity against *P. aeruginosa* and *B. cereus* with 5 and 2.5 μM/mL respectively. SAR study showed that introduction of hydroxyl group at para position only (compound **4f**) showed equipotent activity as ciprofloxacin against *S. aureus*. Electron withdrawing group as nitro group at the para position of C-4 aryl ring exhibited good activity against *P. aeruginosa*, *E. coli* and *B. cereus*. SAR also showed that any other substitution at the meta and para position of the C-4 aryl ring retarded the efficiency of the resulting compounds as can be seen in the derivative **4h**, **4g**, **4c** and **4b**. Overall structure activity relationship results show that all curcumin derivatives effectively work as antibacterial agents

Table 3
One-pot synthesis of 4*H*-pyrimido[2,1-*b*]benzothiazole, pyrazoles and benzylidene derivatives of curcumin.

Entry	R	Product	Time (h)/yield (%)[a,b]	Time (min)/yield (%)[a,c]	M.P. (°C)
1	H	 **4a**	18/67	8/69	97–98
2	2-OH	 **4b**	18/69	8/73	155–156
3	4-Cl	 **4c**	20/66	8/77	112–113
4	4-NO_2	 **4d**	20/71	10/73	85–86
5	4-OH–3-OCH_3	 **4e**	18/74	10/79	80–81
6	4-OH	 **4f**	20/75	8/71	101–102

(*continued on next page*)

Table 3 (*continued*)

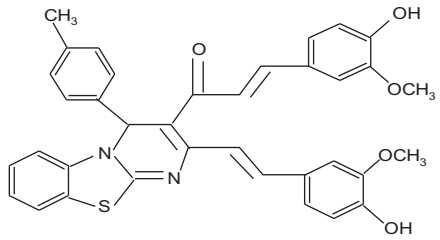
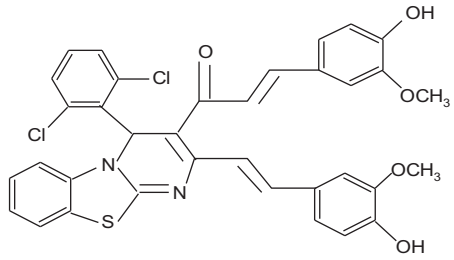
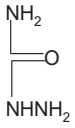
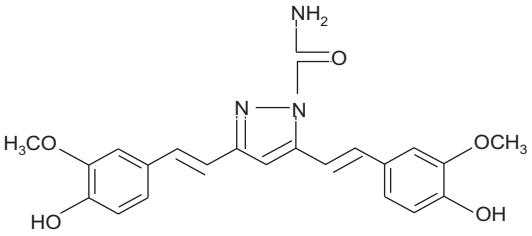
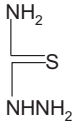
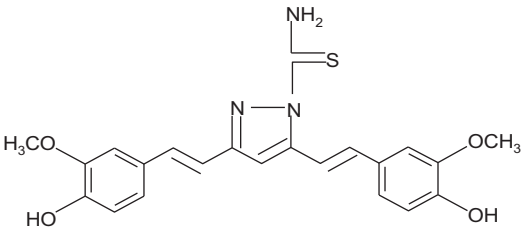
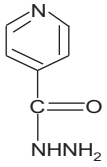
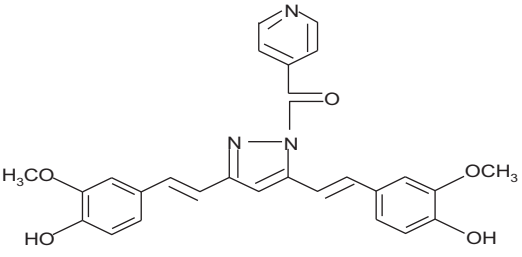
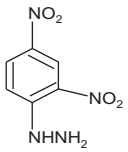
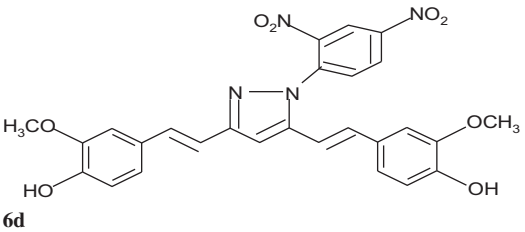
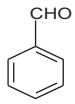
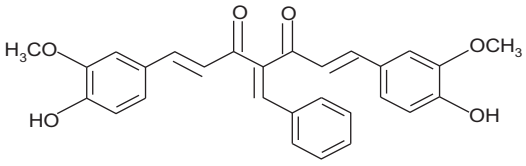
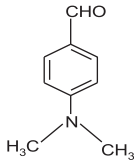
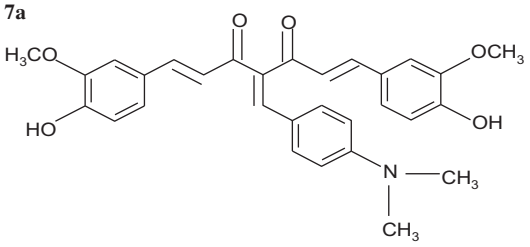
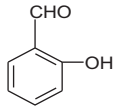
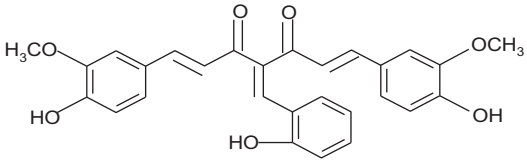
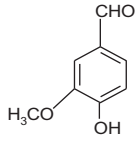
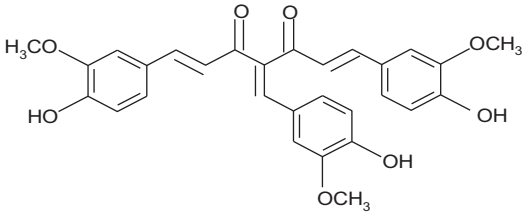
Entry	R	Product	Time (h)/yield (%)[a,b]	Time (min)/yield (%)[a,c]	M.P. (°C)
7	4-CH_3	 **4g**	20/76	8/70	108–109
8	2,6-Cl_2	 **4h**	20/72	8/71	90–92
9		 **6a**	6/85	10/89	204–206
10		 **6b**	7/82	10/83	100–101
11		 **6c**	7/78	10/80	105–106
12		 **6d**	6/86	10/88	118–119

Table 3 (*continued*)

Entry	R	Product	Time (h)/yield (%)[a,b]	Time (min)/yield (%)[a,c]	M.P. (°C)
13		 **7a**	12/72	8/76	119–120
14		 **7b**	13/76	8/77	104–106
15		 **7c**	12/78	8/78	92–94
16		 **7d**	14/76	10/78	98–100

[a] Isolated yield.
[b] Reaction carried out in solvent.
[c] Reaction carried out under solvent free conditions in microwave.

against *B. cereus* (Fig. 4 and Table 1). The data also revealed that the activity of all compounds follow the order **4f** > **4e** > **4d** > **4a**>**4h** > **4g** > **4c** > **4b**.

The structural activity data suggests the derivatives **6a** having amide moiety at hydrazine scaffold don't show significant activity but replacement of oxygen atom from sulphur atom showed immense antibacterial activity as **6b** was effective against all species except *E. coli*. It may be due to difference between atomic size of sulphur and oxygen atoms. SAR study also revealed that **6c** has isoniazide moiety shows equipotent antibacterial activity as ciprofloxacin against *S. aureus* (1.25 μM/mL) and *P. aeruginosa* (1.25 μM/mL). Electron withdrawing substituent at ortho and para positions as 2,4-dinitro phenyl group (derivatives **6d**) have shown excellent activity against *S. aureus* and *B. cereus*. SAR study indicates that presence of pyrazole moiety is additive towards the antibacterial activity. Furthermore, SAR exhibited electron releasing substituents at ortho para positions (benzylidene derivatives **7b** and **7c**) showed immense activity against *S. aureus* and *P. aeruginosa*. Literature survey however suggests that pyrimidine, benzylidene and pyrazole derivatives of curcumin have not been evaluated so far for their antibacterial activity.

4.4. Antifungal activity (interpretation)

Literature survey however suggests that synthesized curcumin derivatives so far have not been evaluated for their antifungal

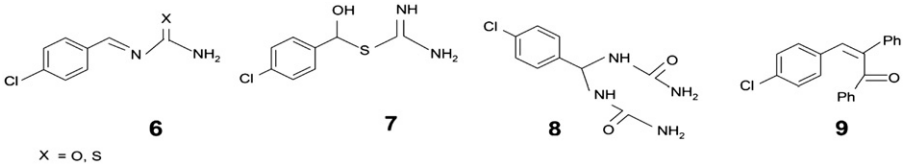
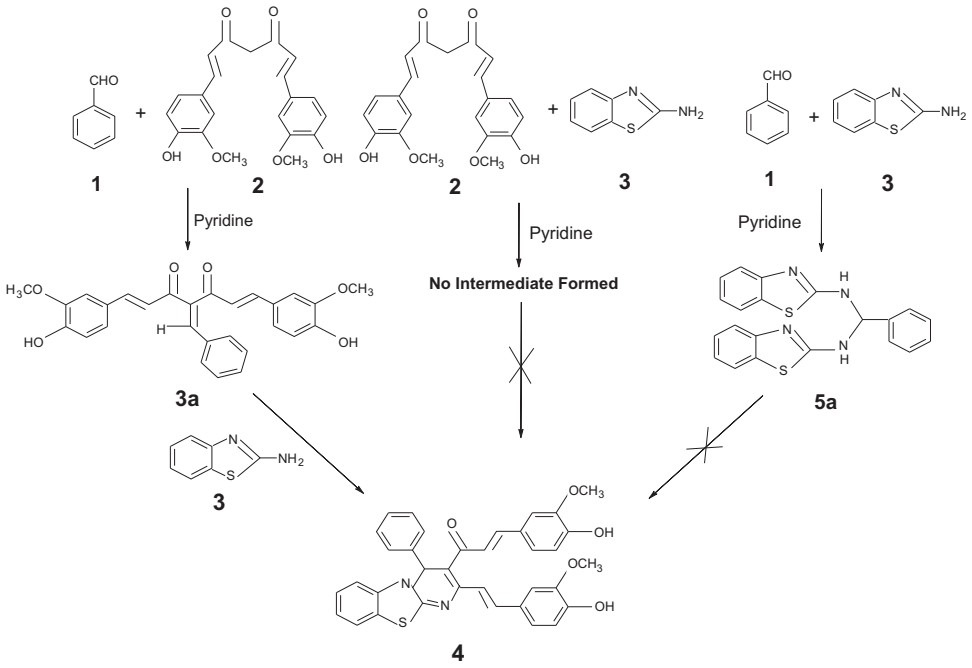


Fig. 2. Proposed intermediate for mechanistic study. Suggested by Shen et al.



Scheme 5. Proposed mechanism for synthesis of 4*H*-pyrimido[2,1-*b*]benzothiazole derivatives of curcumin **4**.

activity. We have now evaluated the antifungal activity of curcumin derivatives against isolated fungi viz. *A. niger*, *A. fumigates*, *A. flavus*. A broad spectrum of antifungal activity of derivatives **4d** (*p*-nitro), **4e** (4-hydroxy-3-methoxy) and **4f** (4-hydroxy) were obtained against tested fungal strain. The antifungal activity of curcumin derivatives was compared with the standard antifungal drugs fluconazole. Regarding the SAR study from the analysis data, it is suggested that *p*-substitution by electron withdrawing (**4d**) exhibited significant activity against *A. niger* and *A. fumigates.* Electron releasing substituents at para positions (**4e** and **4f**) also revealed good spectrum against derivatives show the antifungal activity against *A. fumigates* and *A. flavus* respectively. SAR study confirmed that excellent activity was demonstrated by pyrazole derivative **6b** against *A. niger*, *A. fumigates* and *A. flavus* with MIC 2.5, 2.5 and 1.25 μM/mL which is quite comparable with standard drug fluconazole (MIC 2.5, 1.25 and 1.25 μM/mL) tested under similar conditions. It is interesting that electron releasing substituents at para and ortho positions (benzylidene derivatives **7b** and **7c**) was effective against *A. niger* and *A. fumigates*, but decreased the in-vitro activity by introducing the electron releasing group at meta position (compound **7d**). Analysis of result showed that substitution at para position by electron releasing group increased the antifungal activity. It opens a new era for exploring suitably designed curcumin derivatives as potential antibacterial/antifungal drugs.

Overall structure activity relationship showed that a minor alteration in the molecular configuration of investigated compounds may have a pronounced effect on antibacterial and antifungal screening. However, this approach of combining different active molecules present in nature, resulting in enhancement of their bioavailability, is certainly encouraging. These derivatives of curcumin are more hydrophilic than the parent molecule. The hydrophilic nature of pyrimidines, pyrazoles and benzylidenes may also help in their active transport across the cellular membrane.

4.5. Cytotoxicity against L123 (human lung cells)

Cytotoxicity was performed by MTT assay method [35,36]. A 96 well flat bottom tissue culture plate was seeded with 2 × 103 cells in 0.1 mL of MEM medium supplemented with 10% FBS and allowed to attach for 24 h. After 24 h of incubation, cells were treated with test compounds to get a concentration of 5, 10, 20, 50 and 100 μM/mL incubated for 48 h. The cells in the control group received only the medium containing the 0.2% DMSO. Each treatment was performed in duplication. After the treatment, drug containing media was removed and washed with 200 μL of PBS. To each well of the 96 well plate, 100 μL of MTT reagent (stock: 1 mg/mL in serum free medium) was added and incubated for 4 h at 37 °C. After 4 h of incubation the plate was inverted on tissue paper to remove the MTT reagent. To solubilize formazan crystals in the wells, 100 μL of 100% DMSO was added to each well. The optical density was measured by microtiter plate reader at 590 nm. Compound concentration (μM) required to reduce the viability of mock-infected cells by 50% as determined by MTT method which summarized in Table 2. Results of MTT assay mentioned on the cell

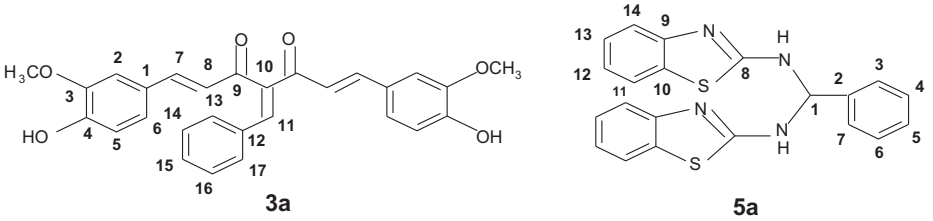


Fig. 3. Key intermediate for mechanistic study. Suggested by present study.

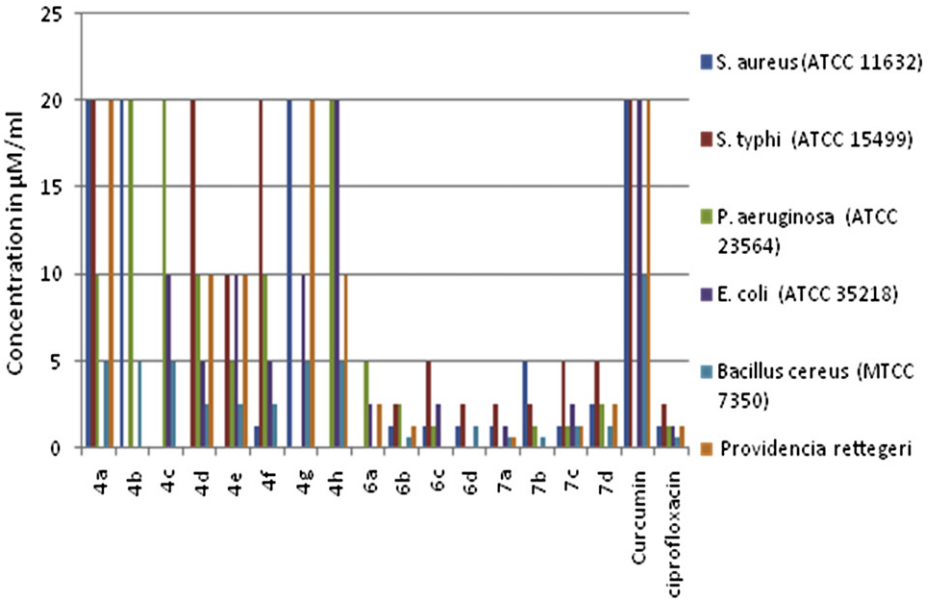


Fig. 4. Comparable chart for MIC_{95} of curcumin and its derivatives against tested bacteria strain.

viability based on ability to metabolize the compounds. The results show that derivatives **4b** and **7c** are comparatively more cytotoxic than **4a**, **4c**, **4g**, **6c**, **4d**, **7d** and **7d** as these derivatives showed IC_{50} values more than 100 μM/mL. It means para chloro (**4c**) and ortho hydroxyl (**7c**) substituted derivatives showed the higher cytotoxicity that is doses related.

5. Conclusion

It can be concluded that reaction of substituted aromatic aldehydes, curcumin and 2-amino benzothiazole in presence of catalyst under solvent free conditions in microwave produces novel derivatives of curcumin. A rapid and environmentally benign method for synthesizing 4*H*-pyrimido[2,1-*b*]benzothiazole, pyrazole and benzylidene derivatives of curcumin has been developed, the short reaction times and ease of work-up make the method advantageous. During the present work we have concluded that the synthesized curcumin derivatives are more effective than curcumin itself against many common strains of bacteria and fungal. Structure activity relationship studies revealed that para substituted derivatives play important role in antimicrobial activity. The present work suggests that there is need to develop nonantibiotic drugs which may overcome antibiotic resistance. Such highly bioactive drugs preferably herbal preparations with least toxicity. A little structure variation can cause immense difference in the activity of the drug. This approach can open new vistas in the chemotherapy of the infective disease. The field is further open for pharmacokinetics and clinic studies to establish these molecules as drugs in the market. In addition, Knoevenagel type intermediate **3a** is suggested as key intermediate for this reaction.

6. Experimental protocols

6.1. General

The ^{1}H NMR spectra were measured by BRUKER AVANCE II 400 NMR spectrometer with tetramethylsilane as an internal standard at 20–25 °C; data for ^{1}H NMR are reported as follow: chemical shift (ppm), integration, multiplicity (s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet and br, broad), coupling constant (Hz). IR spectra were recorded by SHIMADZU; IR spectrometer of sample dispersed in KBr pellet or nujol and is reported in terms of frequency of absorption (cm^{-1}). MS 1927 microwave starter kit was used for microwave reactions. Reaction was carried out under microwave conditions at 300 W in open to air conditions. The output power can be controlled by controller button. E-Merck precoated TLC plates, RANKEM silica gel G for preparative thin-layer chromatography were used. Melting points were determined in open capillaries and was uncorrected. Curcumin, hydrazines, ciprofloxacin and substituted aromatic aldehydes were purchased from Hi media Laboratory Ltd., Mumbai, India. 2-Amino benzothiazole was purchased from Sigma Aldrich. Brain heart infusion™, Mueller–Hinton agar, Subourad dextrose agar and Subourad dextrose broth were purchased from OXOID LTD., Basingstoke, Hampshire, England. 96 Microwell with lid was purchased from IWAKI Brand SCITECH DIV. Asahi Techno Glass, Japan.

6.2. Typical procedure for preparation of 4H-pyrimido[2,1-b] [1,3] benzothiazole curcumin (*4a–h*)

A mixture of curcumin (5 mmol), substituted aromatic aldehydes (5 mmol) and 2-amino benzothiazole (5 mmol) was refluxed at 60–65 °C in presence of pyridine (1 mL) as catalysts in methanol (10 mL). The reaction was monitored by TLC. After completion the reaction, mixture was then poured in cold water and filtered. The residue was purified via column chromatography on silica gel using EtOAc/MeOH (3:1). The pyrimidine derivatives were collected as yellow crystals.

6.3. Microwave synthesis of 4H-pyrimido[2,1-b] [1,3]benzothiazole curcumin (*4a–h*)

A mixture of curcumin (5 mmol), substituted aromatic aldehydes (5 mmol) and 2-amino benzothiazole (5 mmol) was irradiated in microwave under solvent free conditions using pyridine (1 mL) as catalyst. After completion of the reaction, the contents

were transferred in water with the help of methanol and filtered. The residue was purified via column chromatography on silica gel using EtOAc/MeOH (3:1). The pyrimidine derivatives of curcumin were collected as yellow crystals.

6.4. Synthesis of pyrazoles of curcumin (**6a–d**)

A mixture of curcumin (5 mmol) and hydrazines (5 mmol) was refluxed at 65–70 °C in presence of acetic acid as solvent. The reaction was monitored by TLC. After completion the reaction mixture was then poured in cold water and the acetic acid was neutralized using sodium carbonate till then effervescences came from the solution. The solid mass was filtered and recrystallized by ethyl acetate. The pyrazole derivatives of curcumin were collected as yellow crystals (Scheme 4).

6.5. General procedure for synthesis of Knoevenagel condensate of curcumin (**7a–d**)

A mixture of curcumin (5 mmol) and substituted aromatic aldehydes (benzaldehyde, 4-dimethylamino benzaldehyde, 2-hydroxy benzaldehyde and 4-hydroxy-3-methoxy benzaldehyde) (5 mmol) was refluxed at 60–65 °C in methanol using pyridine. After completion the reaction (judged by TLC analysis), the reaction mixture was cooled at room temperature and poured in cold water. The solid mass was filtered. It was dissolved in ethanol and filtered again. The residue was purified via column chromatography on silica gel using EtOAc/MeOH (3:1). The solid was recrystallized by ethyl acetate to give the product (Scheme 3).

6.6. Synthesis of intermediate **3a**

A mixture of curcumin (5 mmol) and benzaldehyde (5 mmol) was refluxed at 60–65 °C for 15 h in presence of pyridine (1 mL) as catalysts in methanol (10 mL). The reaction was monitored by TLC. After completion the reaction, mixture was cooled to room temperature and poured in cold water and filtered. The residue was purified via column chromatography on silica gel using EtOAc/MeOH (3:1). The solid was recrystallized by ethyl acetate. Intermediate **3a** was collected as yellow crystals.

6.7. Synthesis of intermediate **5a**

A mixture of benzaldehyde (5 mmol) and 2-amino benzothiazole (5 mmol) was refluxed at 60–65 °C in presence of pyridine (1 mL) as catalysts in methanol (10 mL) for 5 h. The reaction was monitored by TLC. After completion the reaction, mixture was then poured in cold water and filtered. The residue was purified via column chromatography on silica gel using Hexane/MeOH (1:1). The solid was recrystallized by ethyl alcohol. Intermediate **5a** was collected as yellow crystal.

6.8. 4-Phenyl-4H-pyrimido[2,1-b] [1,3]benzothiazole curcumin (**4a**)

Yellow crystal, mp 97–98 °C, *Rf* = 0.52 (DCM/Toluene = 3:2); IR (KBr) (ν_{max}, cm^{-1}): 3348 (C–H_{str}), 2933 (C–H_{str}), 1625 (C=O_{str}), 1510 (C–H_{def}), 1267 (C–H_{str}), 1122 (C–S_{str}), 962–812 (C–H_{def}); ^{1}H NMR (400 MHz, $CDCl_3$): δ_H 3.93 (6H, s, OCH_3), 5.80 (1H, d, 6-H, *J* = 6.44 Hz), 6.49 (1H, d, 5-H, *J* = 5.76 Hz), 6.87 (1H, d, 8-H, *J* = 8.56 Hz), 7.05 (1H, d, 7-H, *J* = 1.8 Hz), 7.1–7.6 (15H, m, arom), 7.26 (1H, s, C–H); ^{13}C NMR (100 MHz, $CDCl_3$): 178.06 (C-9), 149.74 (C-1), 142.66 (C-3), 141.59 (C-4), 135.38 (C-7), 123.61 (C-5), 123.51 (C-6), 122.39 (C-11), 117.67 (C-7), 116.46 (C-8), 109.64 (C-2), 104.42 (C-10), 50.71 (OCH_3); ESI-MS: *m*/*z* calculated for $C_{35}H_{28}N_2O_5S$ 588.62, found $[M + NH_4]^+$ 607.2.

6.9. 4-(2-Hydroxy phenyl)-4H-pyrimido[2,1-b] [1,3]benzothiazole curcumin (**4b**)

Yellow crystal, mp 155–156 °C, *Rf* = 0.54 (DCM/Toluene = 3:2); IR (KBr) (ν_{max}, cm^{-1}): 3504 (OH_{str}), 2941 (C–H_{str}), 1627 (C=O_{str}), 1508 (C–H_{str}), 1280 (C–H_{str}) 1153 (C–S_{str}), 962–813 (C–H_{def}); ^{1}H NMR (400 MHz, $CDCl_3$): δ_H 3.93 (6H, s, OCH_3), 5.78 (1H, d, 6-H, *J* = 5.8 Hz), 6.49 (1H, d, 5-H, *J* = 5.76 Hz), 6.87 (1H, d, 8-H, *J* = 8.52 Hz), 7.05 (1H, d, 7-H, *J* = 3.04 Hz), 7.1–7.6 (14H, m, arom), 7.26 (1H, s, C–H), 8.83 (1H, s, OH); ^{13}C NMR (100 MHz, $CDCl_3$): 182.89 (C-9), 149.11 (C-1), 147.74 (C-3), 140.36 (C-4), 129.80 (C-7), 126.22 (C-5), 122.70 (C-11), 120.79 (C-6), 115.77 (C-7), 115.53 (C-8), 110.63 (C-2), 100.79 (C-10), 55.47 (OCH_3); ESI-MS: *m*/*z* calculated for $C_{35}H_{28}N_2O_6S$ 604.62, found $[M + NH_4]^+$ 623.1.

6.10. 4-(4-Chloro phenyl)-4H-pyrimido[2,1-b] [1,3]benzothiazole curcumin (**4c**)

Yellow crystal, mp 112–113 °C, *Rf* = 0.49 (DCM/Toluene = 3:2); IR (KBr) (ν_{max}, cm^{-1}): 3510 (OH_{str}), 3012 (C–H_{str}), 2922 (C–H_{str}), 1625 (C=O_{str}), 1500 (C–H_{def}), 1278 (C–H_{def}), 1155 (C–S_{str}), 960–812 (C–H_{def}); ^{1}H NMR (400 MHz, $CDCl_3$): δ_H 3.93 (6H, s, OCH_3), 5.78 (1H, d, 6-H, *J* = 6.76 Hz), 6.49 (1H, d, 5-H, *J* = 5.76 Hz), 6.94 (1H, d, 8-H, *J* = 8.12 Hz), 7.05 (1H, d, 7-H, *J* = 1.8 Hz), 7.1–7.6 (14H, m, arom), 7.26 (1H, s, C–H); ^{13}C NMR (100 MHz, $CDCl_3$): 182.86 (C-9), 149.10 (C-1), 147.73 (C-3), 140.35 (C-4), 140.09 (C-12), 129.77 (C-13), 129.39 (C-18 and C-14), 129.34 (C-17 and C-15), 126.21 (C-7), 125.61 (C-5), 122.63 (C-11), 120.77 (C-6), 120.44 (C-16), 115.76 (C-7), 115.51 (C-8), 110.59 (C-2), 100.78 (C-10), 55.46 (OCH_3); ESI-MS: *m*/*z* calculated for $C_{35}H_{27}N_2ClO_5S$ 623.15, found $[M + NH_4]^+$ 641.1.

6.11. 4-(4-Nitro phenyl)-4H-pyrimido[2,1-b] [1,3]benzothiazole curcumin (**4d**)

Yellow crystal, mp 112–113 °C, *Rf* = 0.49 (DCM/Toluene = 3:2); IR (KBr) (ν_{max}, cm^{-1}): 3007 (C–H_{str}), 2922 (C–H_{str}), 1625 (C=O_{str}), 1510 (C–H_{def}), 1267 (C–H_{def}), 1122 (C–S_{str}), 962–812 (C–H_{str}); ^{1}H NMR (400 MHz, $CDCl_3$): δ_H 3.94 (6H, s, OCH_3), 5.78 (1H, d, 6-H, *J* = 6.36 Hz), 6.49 (1H, d, 5-H, *J* = 5.76 Hz), 6.93 (1H, d, 8-H, *J* = 3.4 Hz), 7.04 (1H, d, 7-H, *J* = 1.76 Hz), 7.1–7.61 (14H, m, arom), 7.26 (1H, s, C–H); ^{13}C NMR (100 MHz, $CDCl_3$): 182.89 (C-9), 149.12 (C-1), 147.75 (C-3), 140.37 (C-4), 130.34 (C-13), 129.81 (C-18 and C-15), 126.21 (C-7), 123.90 (C-5), 123.46 (C-11), 122.90 (C-6), 120.79 (C-16), 115.77 (C-7), 115.52 (C-8), 110.66 (C-2), 100.78 (C-10), 55.47 (OCH_3); ESI-MS: *m*/*z* calculated for $C_{35}H_{27}N_3O_7S$ 633.7, found $[M + NH_4]^+$ 652.1.

6.12. 4-(4-Hydroxy-3-methoxy phenyl)-4H-pyrimido[2,1-b] [1,3] benzothiazole curcumin (**4e**)

Yellow crystal, mp 80–81 °C, *Rf* = 0.49 (DCM/Toluene = 3:2); IR (KBr) (ν_{max}, cm^{-1}): 3504 (OH_{str}), 1627 (C=O_{str}); ^{1}H NMR (400 MHz, $CDCl_3$): δ_H 3.13 (3H, s, OCH_3), 3.97 (6H, s, OCH_3), 5.80 (1H, d, 6-H, *J* = 5.6 Hz), 6.49 (1H, d, 5-H, *J* = 5.76 Hz), 6.88 (1H, d, 8-H, *J* = 8.52 Hz), 7.05 (1H, d, 7-H, *J* = 1.76 Hz), 6.92–7.62 (13H, m, arom) 7.26 (1H, s, C–H), 7.85 (1H, s, OH); ^{13}C NMR (100 MHz, $CDCl_3$): 182.91 (C-9), 149.15 (C-1), 147.77 (C-3), 140.38 (C-4), 126.21 (C-7), 125.08 (C-5), 122.76 (C-6), 120.82 (C-18 and C-14), 120.59 (C-16), 120.36 (C-17), 117.66 (C-16), 115.77 (C-7), 115.53 (C-8), 115.14 (C-14), 110.71 (C-2), 99.49 (C-10), 55.50 (OCH_3); ESI-MS: *m*/*z* calculated for $C_{36}H_{30}N_2O_7S$ 634.65, found $[M + NH_4]^+$ 652.2.

*6.13. 4-(4-Hydroxy phenyl)-4H-pyrimido[2,1-b] [1,3]benzothiazole curcumin (**4f**)*

Yellow crystal, mp 101–102 °C, *Rf* = 0.58 (DCM/Toluene = 3:2); IR (KBr) (ν, cm^{-1}): 3504 (OH_{str}), 1724 (C=O_{str}); ^{1}H NMR (400 MHz, $CDCl_3$): δ_H 3.92 (6H, s, OCH_3), 5.82 (1H, d, 6-H, *J* = 8.48 Hz), 6.48 (1H, d, 5-H, *J* = 5.76 Hz), 6.88 (1H, d, 8-H, *J* = 8.52 Hz), 7.05 (1H, d, 7-H, *J* = 1.76 Hz), 6.85–7.58 (14H, m, arom), 7.07 (1H, s, C–H), 9.73 (1H, s, OH); ^{13}C NMR (100 MHz, $CDCl_3$): 182.89 (C-9), 149.11 (C-1), 147.74 (C-3), 140.36 (C-4), 129.80 (C-16), 126.22 (C-7), 122.70 (C-5), 120.79 (C-18 and C-14), 115.77 (C-7), 115.53 (C-8), 110.63 (C-2), 100.79 (C-10), 55.47 (OCH_3); ESI-MS: *m*/*z* calculated for $C_{35}H_{28}N_2O_6S$ 604.62, found $[M + NH_4]^+$ 623.1.

*6.14. 4-(4-Methyl phenyl)-4H-pyrimido[2,1-b] [1,3]benzothiazole curcumin (**4g**)*

Yellow crystal, mp 108–109 °C, *Rf* = 0.59 (DCM/Toluene = 3:2); IR (KBr) (ν_{max}, cm^{-1}): 3292 (C–H_{str}), 3007 (C–H_{str}), 1720 (C=O_{str}); ^{1}H NMR (400 MHz, $CDCl_3$): δ_H 3.13 (3H, s, CH_3), 3.97 (6H, s, OCH_3), 5.80 (1H, d, 6-H, *J* = 5.6 Hz), 6.50 (2H, d, 5-H, *J* = 5.76 Hz), 6.88 (2H, d, 8-H, *J* = 8.96 Hz), 7.05 (1H, d, 7-H, *J* = 5.24 Hz), 6.92–7.85 (14H, m, arom); ^{13}C NMR (100 MHz, $CDCl_3$): 182.86 (C-9), 149.08 (C-1), 147.72 (C-3), 147.57 (C-13), 146.55 (C-11), 140.33 (C-4), 129.39 (C-16), 128.25 (C-12), 126.20 (C-7), 122.67 (C-5), 120.75 (C-6), 119.76 (C-17 and C-15), 115.75 (C-7), 115.50 (C-8), 115.37 (C-14 and C-18), 110.53 (C-2), 100.92 (C-10), 55.47 (OCH_3); ESI-MS: *m*/*z* calculated for $C_{36}H_{30}N_2O_5S$ 602.82, found $[M + NH_4]^+$ 623.1.

*6.15. 4-(2,6-Dichloro phenyl)-4H-pyrimido[2,1-b] [1,3] benzothiazole curcumin (**4h**)*

Yellow crystal, mp 90–92 °C, *Rf* = 0.54 (DCM/Toluene = 3:2); IR (KBr) (ν_{max}, cm^{-1}): 3078 (C–H_{str}), 1716 (C=O_{str}); ^{1}H NMR (400 MHz, $CDCl_3$): δ_H 3.90 (6H, s, OCH_3), 5.87 (1H, d, 6-H, *J* = 5.6 Hz), 6.53 (1H, d, 5-H, *J* = 5.88 Hz), 6.77–7.02 (7H, m, arom), 7.06–7.59 (6H, m, arom), 7.74 (1H, s, C–H), ^{13}C NMR (100 MHz, $CDCl_3$): 182.78 (C-9), 148.91 (C-1), 147.59 (C-3), 140.26 (C-4), 133.25 (C-11), 129.57 (C-12), 127.70 (C-15), 126.22 (C-17), 125.09 (C-16), 122.54 (C-5), 120.84 (C-6), 120.24 (C-13), 117.78 (C-7), 115.71 (C-8), 115.71 (C-18), 115.39 (C-14), 110.11 (C-2), 100.72 (C-10), 55.42 (OCH_3); ESI-MS: *m*/*z* calculated for $C_{35}H_{26}N_2O_5Cl_2S$ 657.67, found $[M + NH_4]^+$ 675.6.

*6.16. 4-Benzylidene curcumin (Intemediate **3a**)*

Yellow crystal, mp 120–122 °C, *Rf* = 0.54 (DCM/Toluene = 3:2); ^{1}H NMR (400 MHz, $CDCl_3$): δ_H 3.93 (6H, s, OCH_3), 5.80 (2H, s, C_2), 6.50 (2H, d, 5-H, *J* = 15.8 Hz), 6.67–7.15 (6H, m, arom), 7.32–7.90 (5H, m, arom), 10.03 (1H, s, =CH–Ar); ^{13}C NMR (100 MHz, $CDCl_3$): 182.81 (C-9), 149.04 (C-1), 147.68 (C-3), 145.96 (C-4), 140.30 (C-11), 139.88 (C-12), 134.13 (C-13), 129.83 (C-17), 128.69 (C-14 and C-16), 129.21 (C-15), 126.18 (C-5), 125.87 (C-6), 123.38 (C-7), 122.64 (C-8), 115.74 (C-2), 110.36 (C-10), 55.45 (OCH_3); ESI-MS: *m*/*z* calculated for $C_{28}H_{24}O_6$ 456.5, found $[M + H]^+$ 457.7.

*6.17. Intermediate **5a***

Off white crystal, mp 117–119 °C, *Rf* = 0.61 (Hexane/Methanol = 1:1); ^{1}H NMR (400 MHz, $CDCl_3$): δ_H 6.99 (t, 1H, –CH, *J* = 6.96 Hz), 7.19 (d, 2H, NH, *J* = 6.72 Hz), 7.37–8.02 (13H, m, Ar–H); ^{13}C NMR (100 MHz, $CDCl_3$): 166.72 (C-1), 152.51 (C-8), 132.41 (C-2), 130.86 (C-3), 128.15 (C-7), 125.25 (C-5), 120.77 (C-6), 120.52 (C-4), 117.69 (C-9), 78.97 (C-10), 78.64 (C-11 and C-14), 78.31 (C-12 and C-13); ESI-MS: *m*/*z* calculated for $C_{21}H_{16}N_4S_2$ 388.52, found $[M - H]^+$ 387.2.

*6.18. N-Amido pyrazole curcumin (**6a**)*

Yellow Powder, mp 204–206 °C, *Rf* = 0.70 (DCM/Toluene = 3:1); ^{1}H NMR (400 MHz, $CDCl_3$): δ_H 1.56 (2H, s, NH_2), 3.95 (6H, s, OCH_3), 5.80 (2H, s, 2-H), 6.49 (2H, d, 5-H, *J* = 15.76 Hz), 6.89–7.86 (8H, m, Ar–H); ^{13}C NMR (100 MHz, $CDCl_3$): 182.86 (C-9), 147.56 (C-1), 146.55 (C-3), 129.42 (C-4), 128.25 (C-5), 127.29 (C-6), 119.76 (C-7), 115.46 (C-8), 115.37 (C-11), 108.89 (C-2), 98.87 (C-10), 55.38 (OCH_3); ESI-MS: *m*/*z* calculated for $C_{22}H_{21}O_5N_3$ 407.41, found $[M + H]^+$ 408.3.

*6.19. N-Thiamido pyrazole curcumin (**6b**)*

Yellow crystal, mp 100–101 °C, *Rf* = 0.69 (DCM/Toluene = 3:1); ^{1}H NMR (400 MHz, $CDCl_3$): 2.04 (2H, s, NH_2), 3.95 (6H, s, OCH_3), 5.80 (2H, s, 2-H), 6.49 (2H, d, 5-H, *J* = 15.76 Hz), 6.91–7.61 (6H, m, Ar–H); ^{13}C NMR (100 MHz, $CDCl_3$): 149.32 (C-1), 147.65 (C-3), 146.65 (C-4), 129.44 (C-5), 128.25 (C-6), 127.38 (C-7), 119.84 (C-8), 115.45 (C-11), 109.08 (C-10), 55.42 (OCH_3); ESI-MS: *m*/*z* calculated for $C_{22}H_{21}O_4N_3S$ 423.12, found $[M + H]^+$ 424.2.

*6.20. N-Carbonyl pyridine pyrazole curcumin (**6c**)*

Yellow crystal, mp 105–106 °C, *Rf* = 0.68 (DCM/Toluene = 3:1); ^{1}H NMR (400 MHz, $CDCl_3$): 3.91 (6H, s, OCH_3), 5.88 (2H, s, 2-H), 6.56 (2H, d, 5-H, *J* = 15.76 Hz), 6.79–7.89 (10H, m, Ar–H); ^{13}C NMR (100 MHz, $CDCl_3$): 182.86 (C-9), 149.08 (C-1), 147.72 (C-3), 147.57 (C-4), 146.55 (C-pyridine ring), 140.33 (C-C-pyridine ring), 129.39 (C-5), 128.25 (C-6), 126.20 (C-pyridine ring), 122.67 (C-pyridine ring), 120.75 (C-7), 119.76 (C-8), 115.75 (C-11), 110.53 (C-2), 108.92 (C-10), 53.47 (OCH_3); ESI-MS: *m*/*z* calculated for $C_{27}H_{23}O_5N_3$ 469.22, found $[M + 2]^+$ 471.9.

*6.21. N-(2,4-Dinitro phenyl) pyrazole curcumin (**6d**)*

Yellow powder, mp 118–119 °C, *Rf* = 0.68 (DCM/Toluene = 3:1); ^{1}H NMR (400 MHz, $CDCl_3$): 3.89 (6H, s, OCH_3), 5.80 (2H, s, 2-H), 6.49 (2H, d, 5-H, *J* = 15.56 Hz), 6.86–7.53 (9H, m, Ar–H); ^{13}C NMR (100 MHz, $CDCl_3$): 149.11 (C-1), 147.73 (C-3), 147.03 (C-4), 129.50 (C-5), 122.71 (C-6), 120.97 (C-7), 120.43 (C-8), 115.51 (C-11), 110.60 (C-2), 109.10 (C-10), 55.48 (OCH_3); ESI-MS: *m*/*z* calculated for $C_{27}H_{22}O_8N_4$ 530.14, found $[M + H]^+$ 531.5.

*6.22. 4-(4-N-Dimethyl benzylidene) curcumin (**7b**)*

Yellow powder, mp 104–106 °C, *Rf* = 0.67 (DCM/Toluene = 3:2); ^{1}H NMR (400 MHz, $CDCl_3$): δ_H 3.13 (6H, s, $N(CH_3)_2$), 3.95 (6H, s, OCH_3), 5.80 (2H, s, 2-H), 6.50 (2H, d, 5-H, *J* = 15.8 Hz), 6.85–7.15 (6H, m, Ar–H), 7.45–7.85 (4H, m, Ar–H), 9.84 (1H, s, =CH–Ar); ^{13}C NMR (100 MHz, $CDCl_3$): 182.79 (C-9), 148.76 (C-1), 147.63 (C-3), 140.27 (C-11), 126.19 (C-12), 122.57 (C-17 and C-13), 120.69 (C-15), 115.92 (C-14 and C-16), 110.21 (C-2), 100.72 (C-10), 55.43 (OCH_3); ESI-MS: *m*/*z* calculated for $C_{30}H_{29}O_6N$ 499.58, found $[M + H]^+$ 500.2.

*6.23. 4-(2-Hydroxy benzylidene) curcumin (**7c**)*

Yellow crystal, mp 92–94 °C, *Rf* = 0.65 (DCM/Toluene = 3:2); ^{1}H NMR (400 MHz, $CDCl_3$): δ_H 2.55 (3H, s, CH_3), 3.89 (6H, s, OCH_3), 5.92 (2H, s, 2-H), 6.57 (2H, d, 5-H, *J* = 12.12 Hz), 6.72 (2H, d, 8-H, *J* = 8.0 Hz), 6.97–7.77 (12H, m, Ar–H); ^{13}C NMR (100 MHz, $CDCl_3$): 188.10 (C-9), 154.32 (C-1), 152.96 (C-3), 145.57 (C-11), 134.98 (C-12), 131.45 (C-13 and C-17), 127.91 (C-5), 126.00 (C-6), 121.00 (C-7), 120.74 (C-8), 115.77 (C-14 and C-16), 110.21 (C-2), 105.99 (C-10),

60.71 (OCH_3); ESI-MS: *m*/*z* calculated for $C_{29}H_{26}O_6$ 470.53, found $[M + 2]^+$ 472.3.

6.24. 4-(4-Hydroxy-3-methoxy benzylidene) curcumin (**7d**)

Yellow crystal, mp 98–100 °C, *Rf* = 0.64 (DCM/Toluene = 3:2); ^{1}H NMR (400 MHz, $CDCl_3$): δ_H 3.25 (3H, s, OCH_3), 3.93 (6H, s, OCH_3), 5.80 (2H, s, 2-H), 6.49 (2H, d, 5-H, *J* = 15.76 Hz), 6.87 (2H, d, 8-H, *J* = 8.52 Hz), 6.92–7.61 (11H, m, Ar–H), 9.84 (1H, s, OH); ^{13}C NMR (100 MHz, $CDCl_3$): 182.85 (C-9), 149.05 (C-1), 147.70 (C-3), 140.34 (C-11), 126.21 (C-12), 122.65 (C-7), 120.78 (C-8), 115.49 (C-14 and C-16), 110.49 (C-10), 55.45 (OCH_3); ESI-MS: *m*/*z* calculated for $C_{29}H_{26}O_8$ 502.53, found $[M + NH_4]^+$ 521.1.

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