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Original article

Isoxazole analogs of curcuminoids with highly potent multidrug-resistant antimycobacterial activity

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ABSTRACT

Curcumin (**1**), demethoxycurcumin (**2**) and bisdemethoxycurcumin (**3**), the curcuminoid constituents of the medicinal plant *Curcuma longa* L., have been structurally modified to 55 analogs and antimycobacterial activity against *Mycobacterium tuberculosis* has been evaluated. Among the highly active curcuminoids, the isoxazole analogs are the most active group, with mono-*O*-methylcurcumin isoxazole (**53**) being the most active compound (MIC 0.09 μg/mL). It was 1131-fold more active than curcumin (**1**), the parent compound, and was approximately 18 and 2-fold more active than the standard drugs kanamycin and isoniazid, respectively. Compound **53** also exhibited high activity against the multidrug-resistant *M. tuberculosis* clinical isolates, with the MICs of 0.195–3.125 μg/mL. The structural requirements for a curcuminoid analog to exhibit antimycobacterial activity are the presence of an isoxazole ring and two unsaturated bonds on the heptyl chain. The presence of a suitable *para*-alkoxyl group on the aromatic ring which is attached in close proximity to the nitrogen function of the isoxazole ring and a free *para*-hydroxyl group on another aromatic ring enhances the biological activity.

1. Introduction

Tuberculosis is the leading cause of mortality among all infectious diseases worldwide and is responsible for over two million deaths annually [1]. The incident of the human immunodeficiency virus (HIV) pandemic and the increased prevalence of multidrug-resistant strains of *Mycobacterium tuberculosis* caused this disease to be more complicated [2,3]. The recent increase in the number of multidrug-resistant clinical isolates of *M. tuberculosis* has created an urgent need for the evolution of new antituberculosis therapeutics [4,5]. Naturally occurring compounds have demonstrated significant activity in the in vitro assays against *M. tuberculosis* [6]. The structural modification of natural products is one of the potential strategies for the development of new anti-TB drugs which are different from the drugs currently used. In the search for compounds with anti-TB property, a number of medicinal plants have been investigated by our group. Among the tested extracts, the curcuminoid fraction of *Curcuma longa* L. was shown to exhibit antimycobacterial activity. Curcuminoids are the major constituents of *C. longa* and some other *Curcuma* species. It has been known for some time that curcuminoids have been used as a natural food additive. The major curcuminoid isolated from *C. longa* is curcumin (**1**). The minor constituents include demethoxycurcumin (**2**) and bisdemethoxycurcumin (**3**). Curcuminoids exhibited many interesting biological activities [7], for example, antioxidant activity [8,9], anti-inflammatory activity [10,11], anticancer activity [8,12,13], anti-trypanosomal activity [14] and anti-HIV activity [15]. In the present work, the crude curcuminoids were separated into compounds **1**, **2** and **3** and the antimycobacterial activity of these compounds against the non-virulent *M. tuberculosis* H37Ra was 100, 50 and 25 μg/mL, respectively. These curcuminoids displayed much lower activity than the standard antitubercular drugs kanamycin, which exhibited MIC of 2.5 μg/mL, and especially isoniazid and rifampin, which showed MIC of 0.06 and 0.004 μg/mL, respectively. However, the well known medicinal use of curcuminoids, the relatively less toxicity of these food ingredients and the unlimited availability of the compounds from *C. longa* are of special interest. The unique skeleton of curcuminoids which is different from the current anti-TB drugs could also avoid any possible cross resistance of the curcuminoid-derived compounds with the current anti-TB drugs. This work deals with the structural modification of the

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curcuminoids **1**–**3** to analogs with exceptionally high activity against the multidrug-resistant (MDR) strains of *M. tuberculosis*.

2. Chemistry

2.1. Chemical modification of curcuminoids

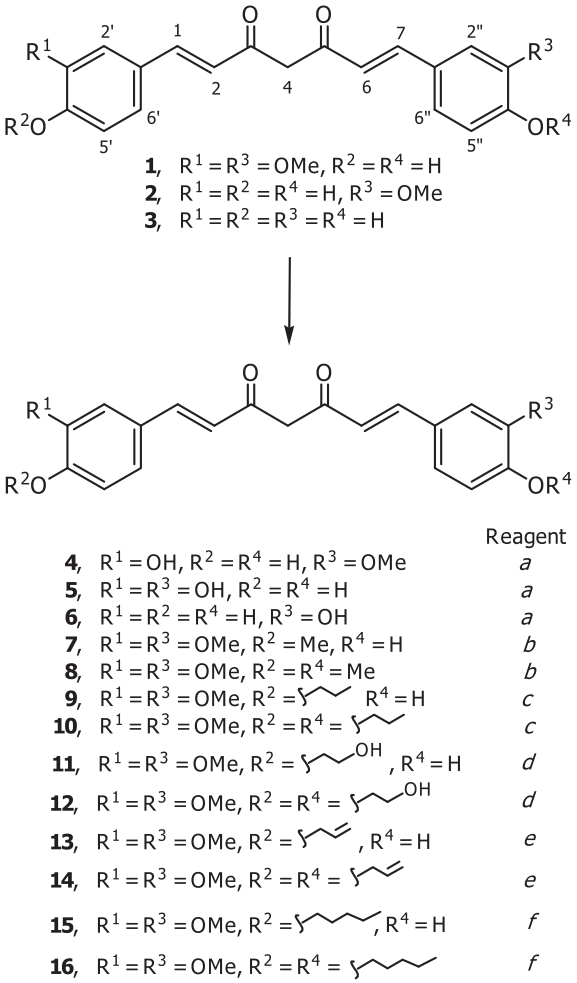
The natural curcuminoids **1**–**3** obtained from *C. longa* were subjected to chemical modifications for antimycobacterial evaluations.

2.1.1. Demethylated analogs

The parent curcuminoids **1** and **2** were demethylated as described previously [14,16] to yield the corresponding demethylated analogs **4**, **5** and **6** (Scheme 1).

2.1.2. Methyl ether and higher alkyl ether analogs

Alkylation of the curcuminoid **1** is outlined in Scheme 1. Methylation of **1** was achieved by modification of the literature procedure [14,17] to afford mono-*O*-methylcurcumin (**7**) and di-*O*-methylcurcumin (**8**) in 39 and 47%, respectively. The spectroscopic (IR, ^{1}H NMR and mass spectra) data of compounds **7** and **8** were consistent with the literature values [17]. Compound **1** was subjected to *n*-propylation by modification of the reported procedure to give mono-*O*-*n*-propylcurcumin (**9**) and di-*O*-*n*-propylcurcumin (**10**) in 25 and 50% yield, respectively. The spectroscopic data of **9** (see Section 5) were in agreement with the structure. The spectroscopic data of **10** were identical to those of reported values [14]. Mono-*O*-(2-hydroxyethyl)curcumin (**11**), di-*O*-(2-hydroxyethyl) curcumin (**12**), mono-*O*-allylcurcumin (**13**) and di-*O*-allylcurcumin (**14**) were prepared by appropriate alkylation of curcumin (**1**) using the literature procedures [14]. *n*-Pentylation of compound **1** was achieved by reacting **1** with *n*-propyl iodide in acetone in the presence of anhydrous potassium carbonate to afford mono-*O*-*n*-pentylcurcumin (**15**) and di-*O*-*n*-pentylcurcumin (**16**) in 34 and 40% yields, respectively. The spectroscopic data of **15** and **16** (see Section 5) were consistent with the structures.



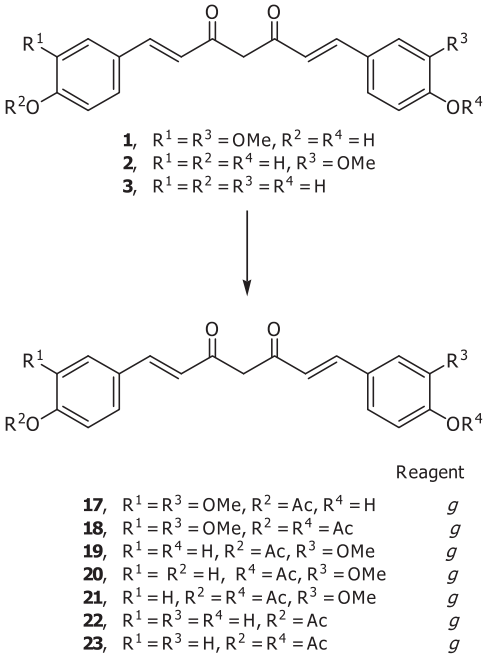
Scheme 1. Demethylation and alkylation of curcuminoids **1**–**3**. Reagents and conditions: (*a*) BBr_3, CH_2Cl_2, 0 °C, then ambient temp.; (*b*) MeI, K_2CO_3, acetone, reflux; (*c*) *n*-propyl iodide, K_2CO_3, acetone, reflux; (*d*) 2-bromoethanol, K_2CO_3, acetone, reflux; (*e*) allyl bromide, K_2CO_3, acetone, reflux; (*f*) *n*-pentyl iodide, K_2CO_3, acetone, reflux.

2.1.3. Acetate analogs

In order to have both the mono and diacetate derivatives for biological evaluation, the curcuminoids **1**–**3** were subjected to partial acetylation as shown in Scheme 2. Reaction of **1** with acetic anhydride in pyridine–$CHCl_3$ afforded the monoacetate **17** and diacetate **18** in 25 and 70% yields. The spectroscopic data of **17** (Section 5) were in agreement with the structure, and those of **18** were consistent with the literature values [18]. Compounds **2** and **3** were similarly subjected to partial acetylation to give the corresponding mono and diacetate analogs **19**, **20**, **21**, **22** and **23**. The spectroscopic data of these compounds (Section 5) were consistent with the structures and were in agreement with the reported data [14,18,19]. The isomeric monoacetates **19** and **20** were distinguished by ^{1}H NMR spectral data. Thus, the presence of acetate group at the 4′-position was evident from the downfield shift of the two aromatic protons, H-3′ and H-5′, at δ 7.11. This was different from the ^{1}H NMR spectrum of compound **20** that the H-3′ and 5′ signals appeared at δ 6.81, and H-5″ at δ 7.02.

2.1.4. Dihydro, tetrahydro, hexahydro and octahydro analogs

Dihydrocurcumin (**24**) was prepared in 30% yield by zinc–acetic acid reduction of the parent curcuminoid **1**. The structure of the product **24** was confirmed by ^{1}H NMR spectral data. The differences



Scheme 2. Acetylation of curcuminoids **1**–**3**. Reagents and condition: (*g*) Ac_2O, pyridine-$CHCl_3$ (1:1), ambient temp.

from the starting curcuminoid **1** being the absence of two olefinic protons of H-6 and H-7 at ca. δ 6.47 and 7.57, and the presence of two methylene signals at δ 2.64 and 2.87. Tetrahydrocurcumin (**25**), hexahydrocurcumin (**26**) and octahydrocurcumin (**27**) were prepared by catalytic hydrogenation of **1** according to the literature procedure [20,21] (Scheme 3).

2.1.5. Unsaturated and saturated mono-keto analogs

The enone **28**, the dienones **29** and **30**, the trienone **31** and the unconjugated ketone **32** were prepared by the literature methods [14,22,23] (Scheme 4).

2.1.6. Pyrazole analogs

The pyrazole analogues of the curcuminoids **1**–**3** were prepared as outlined in Scheme 5. Using the literature procedure [24] with a slight modification, curcumin pyrazole (**33**) was obtained in 71% yield by treatment of **1** with hydrazine hydrate in AcOH at 50 °C. Reaction of **2** and **3** with the same reagent afforded the pyrazole analogs **34** and **35** in 77% and 41% yields, respectively. The spectroscopic data of these compounds were consistent with the structures and were in agreement with the reported data [20,24]. The *N*-phenyl substituted pyrazole **36** was similarly prepared in 81% yield by reaction of compound **1** with phenylhydrazine hydrate. The spectroscopic data (Section 5) were consistent with the structure and were in agreement with the reported data [24].

2.1.7. Isoxazole analogs

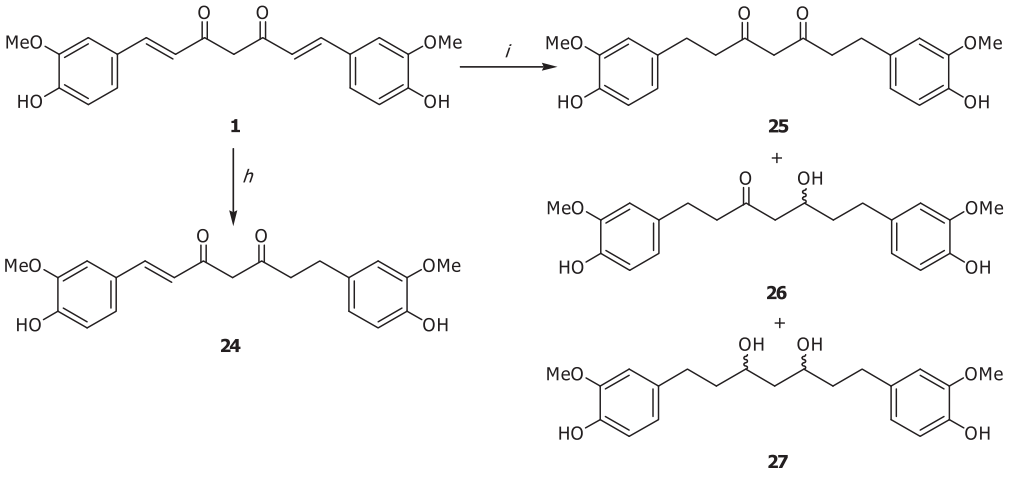
The isoxazole analog **37** was prepared in 70% yield by treatment of **1** with hydroxylamine hydrochloride in pyridine (Scheme 5). The spectroscopic data of **37** (see Section 5) were consistent with the structure and were in agreement with those reported previously [24]. Treatment of **2** with the same reagent afforded a 1:1 mixture (65%) of two isomeric isoxazoles **38a** and **38b**, which could not be separated by column chromatography (Scheme 5). However, separation of the mixture was achieved by normal phase HPLC (see Section 5) to give pure **38a** and **38b**. The structures of these isoxazoles were distinguished by ^{1}H and ^{13}C NMR analysis. Data supporting this interpretation were provided by 2D NMR experiments in which the HMBC correlations between H-2′ and C-3, C-2‴, C-6‴; H-2″ and C-5, C-2⁗, C-6⁗ were observed. The isoxazole analog **39** was similarly prepared in 68% yield from the curcuminoid **3**. The spectroscopic data (Section 5) were consistent with the structure.

2.1.8. Isoxazole analogs of dihydro and tetrahydrocurcumins

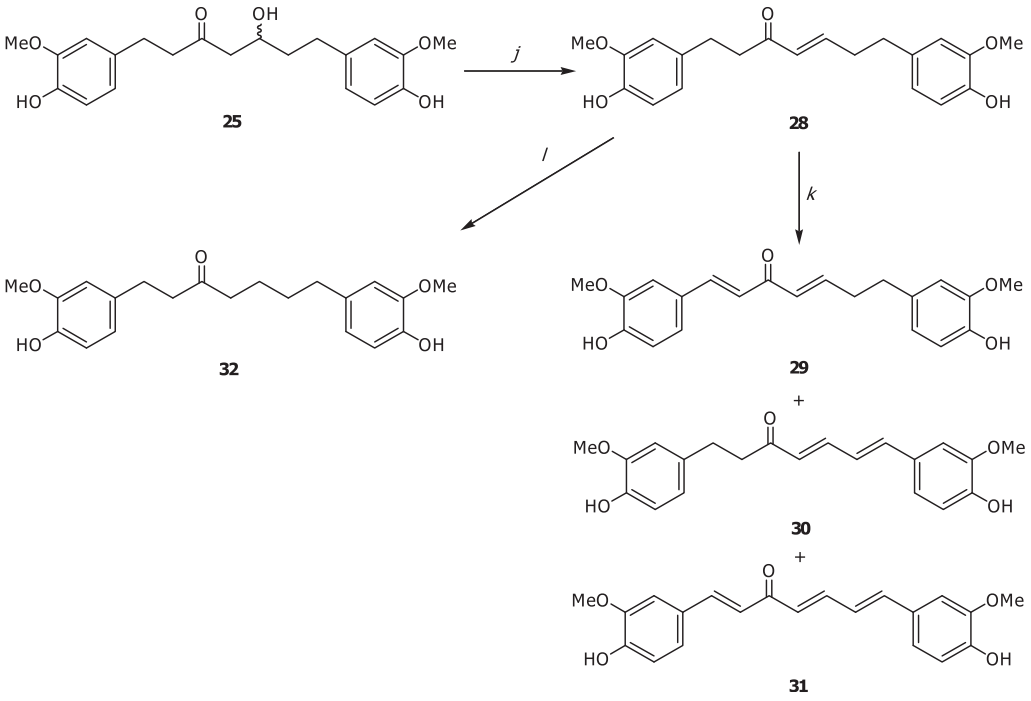
The isoxazole analog **40** was prepared in 63% yield by treatment of dihydrocurcumin (**24**) with hydroxylamine hydrochloride in pyridine (Scheme 5). The structure of the product was established as **40**, not the isomeric structure **41**, by the spectroscopic evidence. Thus, the ^{1}H NMR spectrum showed a four-proton broad singlet signal of methylene group at δ 2.92 (H-1″ and H-2″), and two olefinic protons at δ 6.73 (H-1′) and 7.19 (H-2′). The evidence was supported by the 2D NMR experiments in which the HMBC correlations between H-2′ and C-3, C-2‴, C-6‴; H-2″ and C-5, C-2⁗, C-6⁗ were observed. The isoxazole analog **42** was similarly prepared in 77% yield from tetrahydrocurcumin (**25**) (Scheme 5). Thus, treatment of **25** in pyridine with hydroxylamine hydrochloride to give the intermediate monoxime, which was subsequently treated with *p*-toluenesulfonic acid to yield **42** in 54% overall yield from **25**. The spectroscopic data of **42** were different from those of compound **40** by the absence of olefinic signal at δ 6.73 (H-1′) and 7.19 (H-2′) and the presence of additional two methylene signals at δ 2.87–2.96 (m, 8H). The mass spectral data were also consistent with its structure.

2.1.9. Alkyl ethers and methyl ethers of isoxazole analogs

Antimycobacterial evaluation of the foregoing curcuminoid **1** and its mono-*O*-*n*-pentyl ether analog **15** indicated that introduction of an *n*-pentyl group resulted in 8-fold increase in activity (Table 1). In order to see whether the *n*-pentyl group would enhance the activity in the case of the isoxazole analog **37**, the mono-*O*-*n*-pentyl ether analog **43** and the isomeric isoxazole analog **44** (1:1 mixture), were prepared in 24%, together with the di-*O*-*n*-pentyl ether analog **45** in 62% (Scheme 6). The **43**/**44** mixture was separated by HPLC (see detail in Section 5). The structures of the isomeric pentyl ether analogs **43** and **44** were distinguished by ^{1}H and ^{13}C NMR data (see Section 5). The present of the pentyl group was evident from the signals of the methyl group at δ 0.91 (t, J = 6.8 Hz, H-5⁗′), two methylene groups at δ 1.40 (m, H-3⁗′ and H-4⁗′), one methylene group at δ 1.84 (m, H-2⁗′), and another one methylene group at δ 4.02 (t, J = 6.4 Hz, H-1⁗′). The ^{13}C NMR data of C-5⁗′, C-2⁗′, C-3⁗′, C-4⁗′ and C-1⁗′ appeared at δ 13.9, 22.4, 28.0, 28.8 and 69.0, respectively. The evident was supported by 2D NMR experiments in which the HMBC correlations between H-2′ and C-3, C-2‴, C-6‴; H-2″ and C-5, C-2⁗, C-6⁗; H-1⁗′ and C-3⁗′, C-3‴ were observed. Although compound **43** was very active, this compound encountered solubility problem during the dilution of sample solution for biological evaluation. The



Scheme 3. Reduction of curcumin (**1**). Reagents and condition: (*h*) Zinc powder, AcOH, ambient temp.; (*i*) H_2/Pd–C, EtOH.



Scheme 4. Dehydration of hexahydrocurcumin (**25**), and dehydrogenation and catalytic hydrogenation of **28**. Reagents and conditions: (*j*) *p*-TsOH, C_6H_6, reflux; (*k*) DDQ, THF; (*l*) H_2/Pd–C, EtOH.

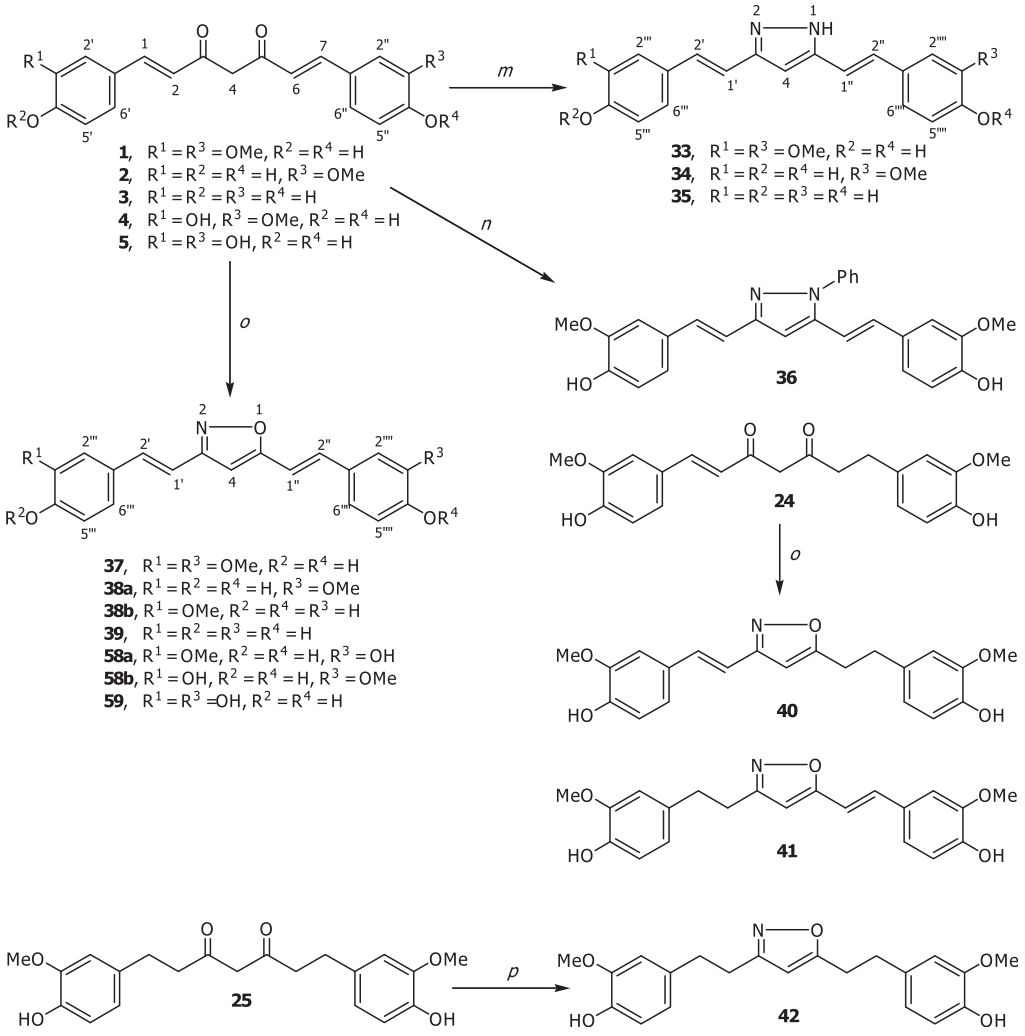
relatively more soluble and lower alkyl ether analogs, the mono-*O*-(3,3-dimethylallyl) ether analogs **46a**/**46b** (2:1 mixture) and the di-*O*-(3,3-dimethylallyl) ether analogs **47**, the mono-*O*-ethyl ether analogs **48a**/**48b** (5:2 mixture) and the di-*O*-ethyl ether analogs **49**, and the acetate analogs **50**, **51** and **52**, were prepared (Scheme 6). The structures of the isomeric mono-*O*-(3,3-dimethylallyl) ether analogs **46a**/**46b**, the mono-*O*-ethyl ether analogs **48a**/**48b**, and the monoacetates **50** and **51** were distinguished by ^{1}H and ^{13}C NMR spectral data in similar manner to those of **43** and **44**. After the assay results were obtained, the methyl ether analogs **53**, **54**, **55**, **56a**/**56b** (1:1 mixture) and **57** were also prepared (Scheme 6). The structures of the isomeric methyl ether analogs **54** and **55**, and **56a** and **56b** were distinguished by ^{1}H and ^{13}C NMR data in similar manner to those of **43** and **44** (see Section 5). The mono-demethylated analogs **58a**/**58b** (1:2 mixture) and the di-demethylated analog **59** were also prepared (Scheme 5) and structural characterization was achieved by similar spectroscopic spectral analysis.

3. Results and discussion

The antimycobacterial activity of the parent curcuminoids **1**–**3** against the non-virulent *M. tuberculosis* H37Ra was 100, 50 and 25 μg/mL, respectively (Table 1). The first type of analogs selected for antimycobacterial evaluation was the demethylated analogs. However, the mono-*O*-demethylated analog **4** and the di-*O*-demethylated analog **5** were only as active as, or even less active than, the parent compound **1** (see Table 1). The demethylated analog **6** was much less active than its parent compound **2**. The results have indicated that increase in polarity of the curcuminoids caused decrease in activity. We therefore moved to analogs which were more lipophilic than their respective parent compounds. The-mono-*O*-methyl analogs **7** was 4-fold more active than the parent compound **1**. However, further methylation to the di-*O*-methyl analog **8** resulted in decrease in activity (MIC 100 μg/mL) when compared with the mono-*O*-methyl analog **7** (MIC 50 μg/mL). Since it seemed that increase in lipophilicity resulted in increase in activity, we therefore synthesized the higher alkyl ether analogs of the parent curcuminoid **1** starting with the mono-*O*-*n*-propyl ether **9**. As expected, the antimycobacterial activity of **9** was higher than the parent compound **1**; it was approximately 4-fold more active than compound **1**. However, the di-*O*-*n*-propyl analog **10** was very weakly active (MIC 200 μg/mL). The relatively nonpolar nature of the alkyl group is required for high activity of the analogs. This was evident from the relatively low activity of the mono-*O*-(2-hydroxyethyl) analog (**11**) (MIC 200 μg/mL), which was more polar than the *n*-propyl ether analog **9**. The di-*O*-(2-hydroxyethyl) analog (**12**) also showed low activity. The activity of the ether analogs was sensitive to the nature of the alkyl group, as seen from the relatively lower activity of the mono-*O*-allyl ether **13** and the di-*O*-allyl ether **14**, the unsaturated ether analog of **9** and **10**, than the analogs **9** and **10**, respectively. To see whether saturated higher alkyl ether analogs contributed to high activity of the curcuminoid, the mono and di-*O*-*n*-pentyl ether analogs **15** and **16** were synthesized and assessed for antimycobacterial activity and it was found that compounds **15** and **16** (MIC 12.5 and 100 μg/mL) were more active than the corresponding *n*-propyl ether analogs **9** and **10** (MIC 25 and 200 μg/mL), respectively. At this point, it is concluded that in going from the mono alkyl analogs to dialkyl analogs, a sharp decrease in activity was observed.

We next evaluated the potency of the acetate analogs of curcuminoids. The results have indicated that the monoacetates **17**, **19**, **20** and **22** exhibited comparable activity to that of their respective parent compounds **1**, **2**, and **3**. As expected, the corresponding diacetate analogs **18** and **21** showed lower activity than the monoacetate analogs **17**, **19** and **20**. The exception was for the analog **23**, which showed comparable activity to that of the parent compound **3**.

The chemical modification of the curcuminoids **1**–**3** to the above corresponding ether and acetate derivatives did not seem to give promising analogs with high antimycobacterial activity. We therefore chose to modify the skeleton of the curcuminoids. The reduced analogs of curcuminoid **1**, viz. **24**, **25**, **26** and **27**, were



Scheme 5. Preparation of pyrazole and isoxazole analogs. Reagents and conditions: (*m*) NH_2NH_2, hydrate, AcOH; (*n*) phenylhydrazine hydrate, AcOH; (*o*) $NH_2OH.HCl$, pyridine, 50 °C; (*p*) (1) $NH_2OH.HCl$, pyridine, ambient temp., (2) *p*-TsOH, C_6H_6, 60 °C.

prepared and assessed for antimycobacterial activity. The assay results indicated that, except for the tetrahydro analog **25** that was approximately 2-fold more active than the parent compound **1**, the activity of the rest were approximately the same as (in the case of the hexahydro analog **26**) or less active (in the case of the dihydro analog **24** and the octahydro analog **27**) than the parent compound **1**.

We then explored the biological activity of the mono-keto analogs of the parent compound **1**. The enone **28**, the dienones **29** and **30**, the trienone **31** and the unconjugated ketone **32** were assessed for antimycobacterial activity. Except for the ketone **32** which exhibited comparable activity to that of the parent curcuminoid **1**, the rest showed 4-fold (compounds **28** and **30**) and 8-fold (compounds **29** and **31**) more active than the parent compound **1**.

Although the above conjugated mono-keto analogs exhibited much improved antimycobacterial activity than that of the parent compound **1**, it was still less active than the standard drug kanamycin. We therefore changed our strategy to analogs with more rigid heptyl chain. In order to keep the heptyl chain less flexible, a cyclic structure was introduced into the chain. The five-membered cyclic dinitrogen analog, the pyrazole analog, was first chosen and the analogs **33**, **34** and **35** were prepared from the curcuminoids **1**, **2** and **3**, respectively. The antimycobacterial activity of these cyclic analogs, however, was not promising; the MICs of these compounds were 200, 100, and 25 μg/mL, respectively. The N-substituted analog **36** was also prepared, but it was only as active as compound **35**.

We then turned our attention to the five-membered cyclic N–O analogs, the isoxazole analogs. Curcumin isoxazole (**37**) exhibited interesting biological activities, for example, anti-inflammatory activity [25] and anticancer activity [26,27]. Potent antimycobacterial compounds with the isoxazole functionality in the molecules have also been reported [28–30]. In the present work, the isoxazole analogs **37**, **38a**/**38b** (1:1 mixture), and **39** were prepared from the parent compounds **1**, **2**, and **3**, respectively, and subjected to antimycobacterial evaluation. The results indicated that the isoxazole analog **37** was highly active; its MIC was 1.56 μg/mL, which was about 64-fold more active than the parent curcuminoid **1**. The isoxazole analogs **38a**/**38b** and **39** were less active; their MICs were both 12.5 μg/mL. It was worth noting that the 3,4-dioxygenated groups on both aromatic rings are required for an isoxazole analog to exhibit high antimycobacterial activity. The isoxazole **37** was thus selected as the lead compound.

In order to see the influence of unsaturation at the alkyl chain, the corresponding dihydro analog **40** and the tetrahydro analog **42** were prepared and their MICs were determined. The gradual decrease in activity in going from the fully unsaturated analog **37** (MIC 1.56 μg/mL) to the dihydro analog **40** (MIC 6.25 μg/mL) and

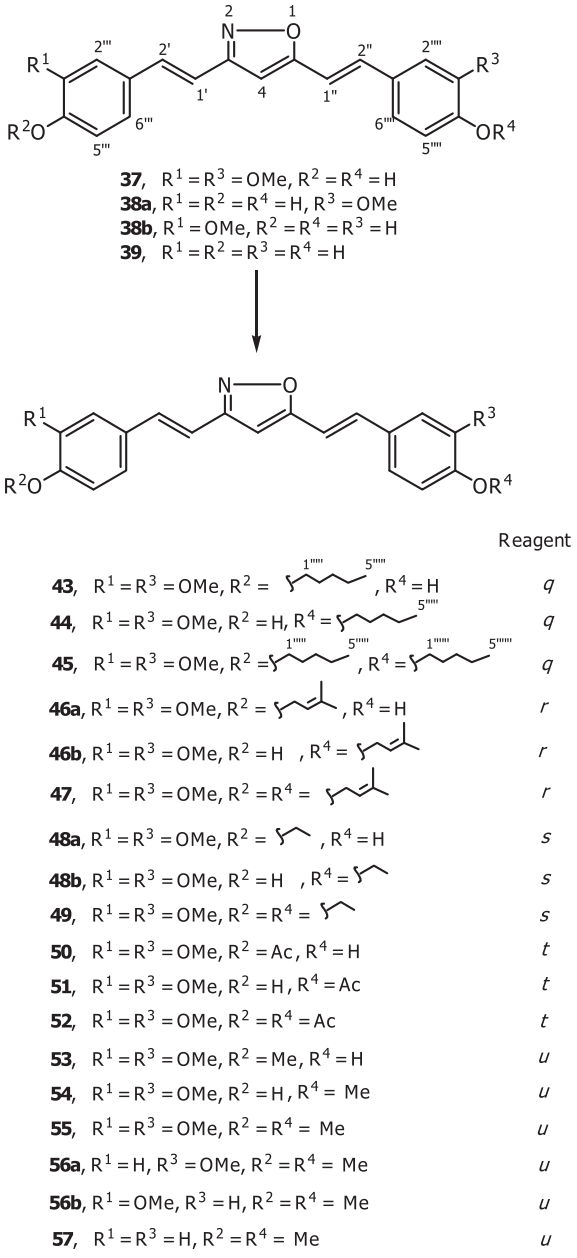
Table 1
The in vitro activity of the curcuminoid analogs against *M. tuberculosis* H37Ra strain.

Compound	MIC (μg/mL)	Compound	MIC (μg/mL)
1	100	**32**	100
2	50	**33**	200
3	25	**34**	100
4	200	**35**	25
5	100	**36**	25
6	200	**37**	1.56
7	50	**38a/38b** (1:1)	12.5
8	100	**39**	12.5
9	25	**40**	6.25
10	200	**41**	ND[b]
11	200	**42**	100
12	200	**43**	0.39
13	100	**44**	6.25
14	Inactive[a]	**45**	Inactive[a]
15	12.5	**46a/46b** (2:1)	1.56
16	100	**47**	ND[b]
17	100	**48a/48b** (5:2)	12.5
18	200	**49**	ND[b]
19	50	**50**	1.56
20	50	**51**	1.56
21	100	**52**	1.56
22	25	**53**	0.09
23	25	**54**	0.78
24	200	**55**	0.39
25	50	**56a/56b** (1:1)	100
26	100	**57**	Inactive[a]
27	Inactive[a]	**58**	6.25
28	25	**59**	200
29	12.5	Kanamycin	2.5
30	25	Isoniazid	0.06
31	12.5	Rifampin	0.004

[a] Inactive at MIC > 200 μg/mL.
[b] ND, not determined.

finally to the tetrahydro analog **42** (MIC 100 μg/mL) has led to a conclusion that full conjugated system of the C_7 chain is required for high antimycobacterial activity of the isoxazole analogs.

Since the mono-*O*-*n*-pentyl analog **15** was 8-fold more active than the parent compound **1**, the lead compound **37** was transformed to the corresponding mono-*O*-*n*-pentyl analogs **43** and its isomeric analog **44**, and the di-*O*-*n*-pentyl ether analog **45**. It was found that the analog **43** was highly active (MIC 0.39 μg/mL) which was about 256-fold more active than the parent compound **1**. However, its isomeric analog **4** was less active than the analog **43** (MIC of compound **44** was 6.25 μg/mL). As expected, compound **45** was inactive to the test. The low activity of **45** was possibly, at least partly, due to its low solubility. Since compound **43** (and **44**) has some solubility problem upon dilution with water, which was due to the lipophilic nature of the *n*-pentyl group, we then further explored other alkyl group that exhibited comparable activity to that of compound **43**, but with higher solubility than that of compound **43**. A number of relatively less polar alkyl ether analogs, including the mono-*O*-(2,2-dimethylallyl) ether analogs **46a**/**46b** (2:1 mixture) and the mono-*O*-ethyl ether analogs **48a**/**48b**, and the acetate analogs **50**, **51** and **52**, were prepared and assessed for anti-mycobacterial activity. However, none of them was as active as the analog **43** (see Table 1). The di-*O*-(3,3-dimethylallyl) ether analogs **47** and the di-*O*-ethyl ether analogs **49** have not been subjected to biological evaluation. We eventually discovered that the mono-*O*-methyl analog **53** exhibited the highest activity; its the MIC was 0.09 μg/mL or 0.24 μM, that is 1131-fold more active than the parent curcuminoid **1** (MIC 100 μg/mL or 271.45 μM). Comparison of the MIC of the isoxazole **53** with those of the standard drugs, it was evident that compound **53** was approximately 18 and 2-fold more active than kanamycin sulfate (MIC 2.50 μg/mL or 4.29 μM) and isoniazid (MIC 0.06 μg/mL or 0.44 μM), respectively. Its isomeric analog **54** was less active, the MIC of which is 0.78 μg/mL. Comparison of the activity of



Scheme 6. Alkylation and acetylation of isoxazole analogs **37**, **38a**, **38b** and **39**. Reagents and conditions: (*q*) *n*-pentyl iodide, K_2CO_3, acetone, reflux; (*r*) 3,3-dimethylallyl bromide, K_2CO_3, acetone, reflux; (*s*) diethyl sulfate, K_2CO_3, acetone, reflux; (*t*) Ac_2O, pyridine; (*u*) MeI, K_2CO_3, acetone, reflux.

compound **53** with that of compound **54** and the activity of compound **43** with that of **44** have led to a conclusion that one of the structural requirements for a mono-*O*-alkylated analog of isoxazole to exhibit high antimycobacterial activity is that the alkoxyl group is located at the aromatic ring which is closer to the nitrogen function than the oxygen function of the isoxazole ring. It should be noted that, despite it being a di-*O*-methyl ether analog which was much less active than the mono-*O*-methyl ether analog, the isoxazole analog **52** still exhibited high activity, with MIC of 0.39 μg/mL, though it was less active than its mono-*O*-methyl ether analog. In contrast, the activity of **56a**/**56b** and **57**, the fully methylated analogs of the parent compounds **2** and **3**, was very low (see Table 1). The findings have pointed out that the number of oxygenation on the aromatic rings of curcuminoids, the number and nature of the substituents on the oxygen function of the aromatic rings are important for high

Table 2
The in vitro activity of the isoxazole analogs **43**, **44**, **53** and **54** against multidrug-resistant *M. tuberculosis* compared with H37Ra strain.

Entry	Code	Isolate resistant profile[a]	MIC (μg/mL)			
			43	**44**	**53**	**54**
1	H37Ra	Pan sensitive	0.39	6.25	0.09	0.78
2	M3	INH, RIF, SM	0.39	1.56	0.195	0.78
3	M4	INH, RIF, EMB, SM	1.56	1.56	1.56	6.25
4	M5	INH, RIF, SM	1.56	1.56	3.125	12.5
5	M6	INH, RIF	0.78	3.125	0.39	1.56
6	M8	INH, RIF, EMB, SM	3.125	6.25	3.125	12.5
7	M11	INH, RIF	3.125	6.25	3.125	12.5
8	M16	INH, RIF, EMB	1.25	1.56	1.56	12.5
9	M21	INH, RIF, EMB, SM	3.125	6.25	3.125	12.5
10	M22	INH, RIF	0.78	6.25	0.39	1.56
11	M27	INH, RIF	0.39	1.56	0.195	0.78
12	M46	INH, RIF, EMB, SM, OFX, CIP	1.56	1.56	1.56	6.25
13	M48	INH, RIF, EMB, SM, OFX, CIP	3.125	3.125	1.56	12.5
14	M53	INH, RIF, EMB, SM, OFX, CIP	3.125	3.125	3.125	12.5

[a] INH, isoniazid; RIF, rifampin; EMB, ethambutol; SM, streptomycin; OFX, ofloxacin; CIP, ciprofloxacin.

antimycobacterial activity. It should also be noted that, like the curcuminoid analogs, demethylation of the isoxazole analogs resulted in decrease in activity. This was exemplified by the low activity of the isoxazoles **58a**/**58b** and **59** (MICs 6.25 and 200 μg/mL, respectively).

The two most potent analogs, **43** and **53**, together with their isomers, **44** and **54**, were assessed for various multidrug-resistant (MDR) clinical isolates *M. tuberculosis* (Table 2, entries 2–14). For the isoniazid (INH)- and rifampin (RIF)-resistant isolates (entries 5, 7, 10 and 11), the most active analog **53** were still very active (MICs 0.195–3.125 μg/mL) whereas the analog **43** were also very active (MICs 0.39–3.125 μg/mL). For the INH-, RIF-, and streptomycin (SM)-resistant isolates (entries 2 and 4), the analog **53** also exhibited comparable activity against those of the INH- and RIF-resistant isolates (MICs 0.195 and 3.125 μg/mL) whereas compound **43** showed similar activity (MICs 0.39 and 1.56 μg/mL). For the INH-, RIF-, and ethambutol (EMB)-resistant isolates (entry 8), and INH-, RIF-, EMB- and SM-resistant isolates (entries 3, 6 and 9), both the analogs **43** and **53** showed MICs in the range 1.56–3.125 μg/mL. More interestingly, the isolates that also resistant to the second-line drugs ofloxacin (OFX) and ciprofloxacin (CIP) (entries 12–14) were also sensitive to compounds **43** and **53** (MICs 1.56–3.125 μg/mL). As expected, compound **44** was less active than its isomer **43** (MICs 1.56–6.25 μg/mL). Compound **54** exhibited varying activity against different MDR isolates (MICs 0.78–12.5 μg/mL). The isoxazole analog **53** is, therefore, the potent structure lead for the development of MDR antitubercular agents.

4. Conclusion

Structural modifications of the natural curcuminoids, curcumin (**1**), demethoxycurcumin (**2**) and bisdemethoxycurcumin (**3**), to 55 analogs have been undertaken and antimycobacterial activity against *M. tuberculosis* has been evaluated. The isoxazole analogs are the most active class of analogs, with mono-*O*-methylcurcumin isoxazole (**53**) being the most active compound (MIC 0.09 μg/mL). It was 1131-fold more active than the parent compound **1**. Compound **53** also exhibited high activity against the multidrug-resistant *M. tuberculosis* (MDR-TB) strains, with the MICs of 0.195–3.125 μg/mL. The structural requirements for a curcuminoid analog to exhibit antimycobacterial activity are the presence of an isoxazole ring and two unsaturated bonds on the heptyl chain. The presence of a suitable *para*-alkoxyl group on the aromatic ring which is attached in close proximity to the nitrogen function of the isoxazole ring and a free *para*-hydroxyl group on another aromatic ring enhances the biological activity.

5. Experimental

5.1. General

Melting points were determined on an Electrothermal melting point apparatus and are uncorrected. IR spectra were recorded in KBr on a Perkin–Elmer FT-IR Spectrum BX spectrophotometer. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AVANCE 400 spectrometer operating at 400 and 100 MHz, respectively. Electron impact (EI) and electrospray (ES) mass spectra were obtained using a Finnigan Polaris Q and a Finnigan LC-Q mass spectrometer. High resolution mass spectra were obtained using a Bruker micrOTOF mass spectrometer. Column chromatography and TLC were carried out using Merck silica gel 60 (<0.063 mm) and precoated silica gel 60 F_{254} plates, respectively. Spots on TLC were detected under UV light (254 nm) and by spraying with anisaldehyde–H_2SO_4 reagent followed by heating.

5.2. Chemical modifications of curcuminoids

The three natural curcuminoids, curcumin (**1**), demethoxycurcumin (**2**) and bisdemethoxycurcumin (**3**), were obtained as described previously [14].

5.2.1. Demethylation of compounds *1* and *2*

Compound **1** was demethylated in the same manner described previously [14,16] to yield mono-*O*-demethylcurcumin (**4**) (42%) and di-*O*-demethylcurcumin (**5**) (33%). Compound **2** was also subjected to demethylation in similar manner to that of compound **1** to give *O*-demethyldemethoxycurcumin (**6**) (64%). The spectroscopic (IR, ^{1}H NMR and mass spectra) data of these analogs were consistent with the reported values [14,16].

5.2.2. Methylation of curcumin (*1*)

Compound **1** was subjected to methylation by modification of the literature procedure [14,17]. Thus, compound **1** (100 mg, 0.27 mmol) was dissolved in dry acetone (3 mL) and anhydrous K_2CO_3 (100 mg) and MeI (0.8 mL) were added. The reaction mixture was refluxed for 3 h; water was added and the mixture was extracted with CH_2Cl_2 (100 mL × 2). The combined organic phase was washed with H_2O, dried over anhydrous Na_2SO_4 and the solvent was removed under vacuum. The products were separated by column chromatography using CH_2Cl_2 as eluent to yield curcumin mono-*O*-methyl ether (**7**) (40 mg, 39%) and curcumin di-*O*-methyl ether (**8**) (50 mg, 47%). The spectroscopic (IR, ^{1}H NMR, and mass spectra) data were consistent with the reported values [17].

5.2.3. Propylation of curcumin (*1*)

Compound **1** (120 mg, 0.32 mmol) was dissolved in dry acetone (2 mL) and anhydrous K_2CO_3 (40 mg) and *n*-propyl iodide (0.6 mL) were added. The reaction mixture was reflux for 5 h, water was added and the mixture was extracted with CH_2Cl_2. The combined organic phase was washed with H_2O, dried over anhydrous Na_2SO_4 and the solvent was removed under vacuum. The crude products were isolated and purified by column chromatography using CH_2Cl_2 to yield mono-*O*-*n*-propylcurcumin (**9**) (34 mg, 25%) and di-*O*-*n*-propylcurcumin (**10**) (72 mg, 50%). The spectroscopic data of compound **10** were consistent with the reported values [14].

*5.2.3.1. Mono-O-n-propylcurcumin (**9**).* IR ν_{max}: 3398, 2965, 2935, 1618, 1563, 1508, 1258, 1133, 1030, 968, 840 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.03 (t, J = 7.3 Hz, 3H, H-3‴), 1.86 (sextet, J = 7.1 Hz, 2H, H-2‴), 3.89 and 3.93 (each s, 2 × 3H, 2 × OMe), 4.00 (t, J = 6.8 Hz, 2H, H-1‴), 5.78 (s, 1H, H-4), 5.83 (br s, 1H, OH), 6.45 and 6.46 (each d, J = 15.7 Hz, 2 × 1H, H-2 and H-6), 6.85 and 6.91

(each d, $J = 8.2$ Hz, 2 × 1H, H-5′ and H-5″), 7.03 and 7.06 (each br d, $J = 1.4$ Hz, 2 × 1H, H-2′ and H-2″), 7.15 (br d, $J = 8.2$ Hz, 2 × 1H, H-6′ and H-6″), 7.57 and 7.58 (each d, $J = 15.7$ Hz, 2 × 1H, H-1 and H-7); ESMS (+ve): *m*/*z* (% rel. abund.) 411 $[M + H]^+$ (100).

*5.2.4. 2-Hydroxyethylation and allylation of curcumin (**1**)*

Compound **1** was subjected to 2-hydroxyethylation as described previously [14] to afford mono-*O*-(2-hydroxyethyl)curcumin (**11**) and di-*O*-(2-hydroxyethyl)curcumin (**12**) in 48 and 35%. Compound **1** was similarly subjected to allylation [14] to afford mono-*O*-allylcurcumin (**13**) and di-*O*-allylcurcumin (**14**) in 33 and 46%, respectively. The spectroscopic data were consistent with the reported values [14].

*5.2.5. Pentylation of curcumin (**1**)*

Compound **1** was subjected to pentylation in similar manner to that of methylation of compound **1**, but using *n*-pentyl iodide in place of methyl iodide to give mono-*O*-*n*-pentylcurcumin (**15**) and di-*O*-*n*-pentylcurcumin (**16**) in 34 and 40% yields, respectively.

*5.2.5.1. Mono-O-n-pentylcurcumin (**15**).* IR ν_{max}: 3420, 2934, 2869, 1625, 1582, 1511, 1465, 1424, 1262, 1136, 1032, 967, 847, 811 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 0.91 (t, $J = 7.1$ Hz, 3H, H-5‴), 1.40 (m, 2 × 2H, H-3‴ and H-4‴), 1.84 (br q, 2H, H-2‴), 3.89 and 3.93 (each s, 2 × 3H, 3′-OMe and 3″-OMe), 4.03 (t, $J = 6.8$ Hz, 2H, H-1‴), 5.79 (s, 1H, H-4), 5.84 (br s, 1H, 4″-OH), 6.46 and 6.47 (each d, $J = 15.8$ Hz, 2 × 1H, H-2 and H-6), 6.85 (d, $J = 8.2$, 1H, H-5′), 6.92 (d, $J = 8.2$ Hz, 1H, H-5″), 7.03 and 7.06 (each br s, 2 × 1H, H-2′ and H-2″), 7.10 (br d, $J = 8.2$ Hz, 2 × 1H, H-6′ and H-6″), 7.57 and 7.58 (d, $J = 15.8$ Hz, 2 × 1H, H-1 and H-7); EIMS: *m*/*z* (% rel. abund.) 438 $[M]^+$ (10), 420 (100), 350 (29), 349 (22), 191 (36), 190 (51), 177 (38).

*5.2.5.2. Di-O-n-pentylcurcumin (**16**).* IR ν_{max}: 2958, 2940, 2857, 1625, 1581, 1511, 1466, 1420, 1337, 1256, 1227, 1133, 1026, 969, 845, 793 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 0.91 (t, $J = 7.0$ Hz, 2 × 3H, H-5‴ and H-5⁗), 1.40 (m, 4 × 2H, H-3‴, H-4‴, H-3⁗ and H-4⁗), 1.84 (br q, 2 × 2H, H-2‴ and H-2⁗), 3.89 (s, 2 × 3H, 3′-OMe and 3″-OMe), 4.03 (t, $J = 6.8$ Hz, 2 × 2H, H-1‴ and H-1⁗), 5.79 (s, 1H, H-4), 6.46 (d, $J = 15.8$ Hz, 2 × 1H, H-2 and H-6), 6.85 (d, $J = 8.2$ Hz, 2 × 1H, H-5′ and H-5″), 7.05 (br s, 2 × 1H, H-2′ and H-2″), 7.09 (br d, $J = 8.2$ Hz, 2 × 1H, H-6′ and H-6″), 7.58 (d, $J = 15.8$ Hz, 2 × 1H, H-1 and H-7); EIMS: *m*/*z* (% rel. abund.) 508 $[M]^+$ (5), 420 (16), 344 (100), 234 (77), 220 (25), 177 (17).

*5.2.6. Acetylation of curcuminoids **1**–**3***

Ac_2O (0.5 mL) was added to a solution of compound **1** (100 mg, 0.27 mmol) in pyridine–$CHCl_3$ (1:1, 2 ml) and the reaction mixture was stirred at ambient temperature for 2 h. After the usual work up, the mixture was extracted with CH_2Cl_2 and the organic phase was washed with H_2O, dried over anhydrous Na_2SO_4 and the solvent was evaporated to dryness. The crude product was purified by column chromatography eluting with CH_2Cl_2 to afford mono-*O*-acetylcurcumin (**17**) (28 mg, 25%) and di-*O*-acetylcurcumin (**18**) (85 mg, 70%) as yellow amorphous solid; m.p. 163–165 °C (lit. [18] 160 °C). The spectroscopic (IR, ^1H NMR and mass spectra) data of compound **18** were consistent with the reported values [18].

*5.2.6.1. Mono-O-acetylcurcumin (**17**).* Orange foam: ^1H NMR (400 MHz, $CDCl_3$) δ 2.30 (s, 3H, OAc), 3.86 and 3.93 (each s, 2 × 3H, 2 × OMe), 5.81 and 5.85 (each s, 2 × 1H, H-4 and OH), 6.44 (d, $J = 15.8$ Hz, 1H, H-6), 6.35 (d, $J = 15.8$ Hz, 1H, H-2), 6.92 (d, $J =$ ca 8.0 Hz, H-5″), 7.03 (br s, 2H, H-2′ and H-2″), 7.11 (br d, $J =$ ca 8.0 Hz, 1H, H-5′), 7.59 (br d, $J = 15.8$ Hz, 2H, H-1 and H-7); EIMS *m*/*z* (% rel. abund.) 410 $[M]^+$ (17), 368 (40), 350 (100), 191 (33), 190 (57), 177 (24).

Compound **2** was subjected to acetylation in the same manner to that of compound **1** to give Mono-*O*′-acetyldemethoxycurcumin (**19**), mono-*O*″-acetyldemethoxycurcumin (**20**) and di-*O*-acetyldemethoxycurcumin (**21**) in 28%, 21% and 33% yield, respectively. The spectroscopic data of compound **21** were consistent with the reported values [14].

*5.2.6.2. Mono-O′-acetyldemethoxycurcumin (**19**).* Orange amorphous solid; m.p. 127–129 °C; IR ν_{max}: 3757, 3758, 1762, 1750, 1718, 1685, 1654, 1627, 1560, 1542, 1508, 1458, 1429, 1207, 1137, 968, 838 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 2.29 (s, 3H, OAc), 3.92 (s, 3H, OMe), 5.80 (s, 1H, H-4) and 5.85 (br s, 1H, OH), 6.47 (d, $J = 15.7$ Hz, 1H, H-6), 6.54 (d, $J = 15.7$, 1H, H-2), 6.91 (d, $J = 8.2$ Hz, 1H, H-5″), 7.03 (br s, 1H, H-2″), 7.11 (d, $J = 8.5$ Hz, 2H, H-3′ and H-5′), 7.11 (obscured signal, 1H, H-6″), 7.55 (d, $J = 8.5$ Hz, 2H, H-2′ and H-6′), 7.59 (d, $J = 15.7$ Hz, 1H, H-7), 7.61 (d, $J = 15.8$ Hz, 1H, H-1); ESMS (+ve) *m*/*z* (% rel. abund.) 403 $[M + Na]^+$ (29), 381 $[M + H]^+$ (100).

*5.2.6.3. Mono-O″-acetyldemethoxycurcumin (**20**).* Yellow amorphous solid; m.p. 166–168 °C; IR ν_{max}: 3448, 1757, 1732, 1718, 1701, 1654, 1627, 1602, 1576, 1559, 1508, 1458, 1599, 1199, 1169, 1145, 1032, 961, 831 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$ + 2 drops of CD_3OD) δ 2.30 (s, 3H, OAc), 3.85 (s, 3H, OMe), 5.78 (s, 1H, H-4), 6.45 and 6.51 (each d, $J = 15.8$ Hz, 2H, H-2 and H-6), 6.81 (d, $J = 8.5$ Hz, 2H, H-3′ and H-5′), 7.02 (d, $J = 8.1$ Hz, 1H, H-5″), 7.09 (br s, 1H, H-2″), 7.12 (dd, $J = 8.1$, 1.4 Hz, 1H, H-6″), 7.42 (d, $J = 8.5$ Hz, 2H, H-2′ and H-6′), 7.55 and 7.59 (each d, $J = 15.8$ Hz, 2H, H-1 and H-7); ESMS (+ve) *m*/*z* (% rel. abund.), 403 $[M + Na]^+$ (27), 381 $[M + H]^+$ (100).

Compound **3** was subjected to acetylation in the same manner to that of compound **1** to give mono-*O*-acetylbisdemethoxycurcumin (**22**) and di-*O*-acetylbisdemethoxycurcumin (**23**) in 25% and 60% yield, respectively. The spectroscopic (IR, H NMR and mass spectra) data of compound **23** were consistent with the reported values [19].

*5.2.6.4. Mono-O-acetylbisdemethoxycurcumin (**22**).* Yellow powder, m.p. 178–180 °C; IR ν_{max}: 3872, 3448, 1735, 1718, 1685, 1654, 1637, 1604, 1508, 1458, 1374, 1235, 1169, 972, 833 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 2.30 (s, 3H, OAc), 5.78 (s, 1H, H-4), 6.48 and 6.54 (each d, $J = 15.8$ Hz, 2 × 1H, H-2 and H-6), 6.83 (d, $J = 8.3$ Hz, 2H, H-3″ and H-5″), 7.11 (d, $J = 8.3$ Hz, 2H, H-3′ and H-5′), 7.45 (d, $J = 8.3$ Hz, 2H, H-2″ and H-6″), 7.54 (d, $J = 8.3$ Hz, 2H, H-2′ and H-6′), 7.60 (d, $J = 15.8$ Hz, 2H, H-1 and H-7); ESMS (+ve) *m*/*z* (% rel. abund.) 723 $[2M + Na]^+$ (83), 373 $[M + Na]^+$ (8), 351 $[M + H]^+$ (56).

*5.2.7. Reduction of curcumin (**1**) with zinc–acetic acid*

To a solution of curcumin (**1**) (100 mg, 0.27 mmol) in acetic acid (3 ml) was added Zn dust (20 mg) and the mixture was stirred at ambient temperature for 4 h. The reaction was worked up with H_2O and the solution was extracted with EtOAc. The organic phase was washed with H_2O, dried over anhydrous Na_2SO_4 and the solvent was evaporated to dryness. The crude products were purified by column chromatography using *n*-hexane–EtOAc (3:2) to give dihydrocurcumin (**24**) (30 mg, 30%).

*5.2.7.1. Dihydrocurcumin (**24**).* ^1H NMR (400 MHz, $CDCl_3$ + 2 drops of CD_3OD) δ 2.64 (t, $J = 7.7$ Hz, 2H, H-7), 2.87 (t, $J = 7.7$ Hz, 2H, H-6), 3.83 and 3.90 (each s, 2 × 3H, 2 × OMe), 5.56 (s, 1H, H-4), 6.27 (d, $J = 15.7$ Hz, 1H, H-2), 6.66 (partially obscured signal, 1H, H-5″), 6.68 (s, 1H, H-2″), 6.79 (d, $J = 7.8$ Hz, 1H, H-6″), 6.88 (d, $J = 8.2$ Hz, 1H, H-5′), 6.98 (d, $J = 1.4$ Hz, 1H, H-2′), 7.04 (dd, $J = 8.2$, 1.4 Hz, 1H, H-6′), 7.48 (d, $J = 15.7$ Hz, 1H, H-1); EIMS *m*/*z* (% rel. abund.): 370 $[M]^+$ (24), 352 (97), 305 (47), 177 (100), 150 (46), 137 (90), 135 (36).

*5.2.8. Catalytic hydrogenation of curcumin (**1**)*

Tetrahydrocurcumin (**25**), hexahydrocurcumin (**26**) and octahydrocurcumin (**27**) were prepared by catalytic hydrogenation of curcumin (**1**) according to the literature procedure [20,21]. Their

identities were confirmed by ^{1}H NMR spectroscopic data comparison with those of the reported values [20,21].

5.2.9. Synthesis of unsaturated and saturated mono-keto analogs

The enone **28** was prepared by dehydration of hexahydrocurcumin (**26**) by the literature method [14]. The dienones **29** and **30**, and the trienone **31** were prepared by DDQ oxidation of the enone **28** using the literature procedure [14]. Catalytic hydrogenation of the enone **28** using palladium on charcoal as a catalyst furnished the saturated ketone **32**. The spectroscopic data of the synthesized compounds were consistent with the reported values [14,21].

*5.2.10. Synthesis of pyrazole analogs of the curcuminoids **1**, **2** and **3***

Curcumin (**1**) (5 g, 13.6 mmol) was dissolved in AcOH (150 ml) and hydrazine hydrate (1.5 mL, 30.9 mmol) was added. The reaction mixture was stirred at 50 °C for 24 h; water (100 mL) was then added and the mixture was extracted with EtOAc (150 mL × 3). The combined organic phase was washed with H_2O, dried over anhydrous Na_2SO_4. The solvent was evaporated and the residue was purified by column chromatography using CH_2Cl_2–MeOH (50:2) to yield compound **33** (3.5 g, 71%).

*5.2.10.1. Curcumin pyrazole (**33**).* Yellow crystals (from CH_2Cl_2–MeOH); m.p. 223–224 °C (lit. [20] 215 °C): ^{1}H NMR data were in agreement with those reported previously [24]; ESMS (−ve) *m*/*z* (% rel. abund.) 363 $[M - H]^-$ (100).

By using the same procedure for the preparation of compound **33**, compound **2** was converted to the corresponding pyrazole analog **34** in 77%. ^{1}H NMR data were in agreement with those reported previously [20].

By using the same procedure for the preparation of compound **33**, compound **3** was converted to the corresponding pyrazole **35** as white solid, m.p. >250 °C (lit. [20] 272–273 °C) in 41%. ^{1}H NMR data were in agreement with those reported previously [20].

*5.2.11. Synthesis of phenylpyrazole analog of curcumin (**1**)*

Compound **1** was converted to the corresponding phenylpyrazole **36** in 81% by the same procedure for the preparation of compound **33**, but using phenylhydrazine in place of hydrazine hydrate. ^{1}H NMR data were in agreement with those reported previously [24].

*5.2.12. Synthesis of isoxazole analog of the curcuminoids **1**, **2** and **3***

A solution of curcumin (**1**) (100 mg, 0.27 mmol) in pyridine (3 mL) was treated with $NH_2OH{\cdot}HCl$ (100 mg) and the mixture was stirred at 50 °C for 6 h. The reaction was worked up with H_2O and the solution was extracted with EtOAc. The organic phase was washed with H_2O, dried over anhydrous Na_2SO_4 and the solvent was evaporated to dryness. The crude product was purified by column chromatography using CH_2Cl_2–MeOH (100:1.5) to give compound **37** (70 mg, 70%).

*5.2.12.1. Curcumin isoxazole (**37**).* Needles (from CH_2Cl_2–*n*-hexane); m.p. 177–178 °C (lit. [24] m.p. 162 °C); IR ν_{max}: 3447, 1646, 1606, 1575, 1513, 1433, 1369, 1277, 808, 734 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.93 (s, 2 × 3H, 2 × OMe), 6.41 (s, 1H, H-4), 6.79 (d, J = 16.3 Hz, 1H, H-1′), 6.89 (br d, J = 8.0 Hz, 1H, H-5‴), 6.90 (br d, J = 8.0 Hz, 1H, H-5⁗), 6.94 (d, J = 16.7 Hz, 1H, H-1″), 7.01 (overlapping signals, H-2‴, H-6⁗), 7.04 (m, 1H, H-6‴), 7.06 (br s, 1H, H-2⁗), 7.08 (d, J = 16.7 Hz, 1H, H-2″), 7.26 (d, J = 16.3 Hz, 1H, H-2′); ESMS (+ve) *m*/*z* (% rel. abund.) 366 $[M + H]^+$ (100).

A 1:1 mixture of **38a** and **38b** was prepared from **2** in 65% yield by the method employed for compound **37**. The **38a**/**38b** mixture was further purified by normal phase HPLC (column: Luna Silica (2) 100 A, 5 μm, 4.6 × 250 mm, mobile phase: CH_2Cl_2–MeOH (99:1), flow rate: 1 mL/min, detector: 254 nm) to yield **38a** (t_R 13.25 min) and **38b** (t_R 14.39 min).

*5.2.12.2. Demethoxycurcumin isoxazole isomer1 (**38a**).* White solid; m.p. 198–200 °C; IR ν_{max}: 3406, 1645, 1606, 1590, 1513, 1433, 1277, 1171, 1033, 960, 820 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$ + 5 drops of CD_3OD) δ 3.91 (s, 3H, OMe), 6.38 (br s, 1H, H-4), 6.76 (d, J = 16.4 Hz, 1H, H-1′), 6.81 (br d, J = 8.4 Hz, 2H, H-3‴ and H-5‴), 6.87 (d, J = 8.1 Hz, 1H, H-5⁗), 6.92 (d, J = 16.4 Hz, 1H, H-1″), 6.98 (br d, J = 8.1 Hz, 1H, H-6⁗), 7.05 (br s, 1H, H-2⁗), 7.07 (d, J = 16.4 Hz, 1H, H-2″), 7.25 (obscured signal, 1H, H-2′), 7.31 (br d, J = 8.4 Hz, 2H, H-2‴ and H-6‴); ESMS (−ve) *m*/*z* (% rel. abund.) 334 $[M - H]^-$ (100).

*5.2.12.3. Demethoxycurcumin isoxazole isomer 2 (**38b**).* White solid; m.p. 194–195 °C; IR ν_{max}: 3406, 1645, 1606, 1590, 1513, 1433, 1277, 1171, 1033, 960, 820 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$ + 5 drops of CD_3OD) δ 3.90 (s, 3H, OMe), 6.37 (br s, 1H, H-4), 6.76 (partial obscured signal, 1H, H-1′), 6.79 (br d, J = 8.4 Hz, 2H, H-3⁗ and H-5⁗), 6.86–6.89 (m, 2H, H-1′ and H-5‴), 6.98 (br s, 1H, H-2‴), 7.01 (br d, J = 8.1 Hz, 1H, H-6‴), 7.06 (d, J = 16.4 Hz, 1H, H-2″), 7.22 (obscured signal, 1H, H-2′), 7.25 (obscured signal, 1H, H-2′), 7.31 (br d, J = 8.4 Hz, 2H, H-2‴ and H-6‴); ^{13}C NMR (100 MHz, $CDCl_3$ + 5 drops of CD_3OD) δ 55.9 (3‴-OMe), 97.5 (C-4), 108.9 (C-2‴), 110.7 (C-1′), 113.1 (C-1″), 114.8 (C-5‴), 115.7 (C-3⁗, C-5⁗), 121.4 (C-6‴), 128.6 (C-2⁗, C-6⁗), 134.8 (C-2′), 135.6 (C-2″), 146.9 (C-3‴, C-4‴), 157.4 (C-4⁗), 162.3 (C-5), 168.4 (C-3); ESMS (−ve): *m*/*z* (% rel. abund.) 334 $[M - H]^-$ (100).

Compound **39** was prepared in 68% yield by the method employed for compound **37**.

*5.2.12.4. Bisdemethoxycurcumin isoxazole (**39**).* Needles (from CH_2Cl_2–MeOH); m.p. >250 °C; IR ν_{max}: 3341, 1637, 1604, 1514, 1450, 1282, 1254, 970, 835 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$ + 12 drops of CD_3OD) δ 6.35 (s, 1H, H-4), 6.72 (d, J = 16.3 Hz, 1H, H-1′), 6.77 (br d, J = 8.4 Hz, 4H, H-3‴, H-5‴, H-3⁗, H-5⁗), 6.85 (d, J = 16.4 Hz, 1H, H-1″), 7.04 (d, J = 16.4 Hz, 1H, H-2″), 7.21 (d, J = 16.3 Hz, 1H, H-2′), 7.33 (d, J = 8.4 Hz, 4H, H-2‴, H-6‴, H-2⁗, H-6⁗); ESMS (+ve): *m*/*z* (% rel. abund.) 633 $[2M + Na]^+$ (100); HR-TOFMS (ES^+): *m*/*z* 306.1130 $[M + H]^+$; calcd for $C_{19}H_{15}NO_3$ + H, 306.1130.

*5.2.13. Synthesis of isoxazole analog of dihydrocurcumin (**24**) and tetrahydrocurcumin (**25**)*

Starting from dihydrocurcumin (**24**), the isoxazole analog **40** was prepared in 63% yield by the same method employed for the preparation of compound **37**.

*5.2.13.1. Dihydrocurcumin isoxazole (**40**).* White solid; m.p. 168–170 °C; ^{1}H NMR ($CDCl_3$) δ 2.92 (br s, 4H, H-1″ and H-2″), 3.84 and 3.92 (each s, 6H, 2 × OMe), 5.94 (br s, 1H, H-4), 6.68 (br s, 1H, H-2⁗), 6.70 (obscured signal, H-6⁗), 6.73 (d, J = 16.3 Hz, 1H, H-1′), 6.84 (d, J = 8.5 Hz, 1H, H-5⁗), 6.89 (d, J = 8.1 Hz, 1H, H-5‴), 6.98 (br s, 1H, H-2‴), 7.01 (d, J = 8.1 Hz, 1H, H-6‴), 7.19 (d, J = 16.3 Hz, 1H, H-2′); ^{13}C NMR (100 MHz, $CDCl_3$) δ 28.2 (C-6), 34.2 (C-7), 55.9 (3‴-OMe and 3⁗-OMe), 100.7 (C-4), 108.6 (C-2‴), 110.9 (C-2⁗), 111.0 (C-2), 114.3 (C-5⁗), 114.7 (C-5‴), 121.0 (C-6⁗), 121.4 (C-6‴), 128.2 (C-1‴), 132.6 (C-1⁗), 134.6 (C-1), 144.0 (C-4⁗), 146.4 (C-3⁗), 146.8 (C-4‴, C-3‴), 163.6 (C-5), 168.4 (C-3); ESMS (+ve): *m*/*z* (% rel. abund.) 368 $[M + H]^+$ (100); HR-TOFMS (ES^+): *m*/*z* 368.1505 $[M + H]^+$; calcd for $C_{21}H_{21}NO_5$ + H, 368.1498.

The isoxazole analog **42** was prepared as followed. A solution of **25** (116 mg, 0.31 mmol) in pyridine (3 mL) was treated with $NH_2OH{\cdot}HCl$ (60 mg) and the mixture was stirred at ambient temperature for 1 h. The reaction was worked up with H_2O and the solution was extracted with EtOAc. The organic phase was washed with H_2O, dried over anhydrous Na_2SO_4 and the solvent was

evaporated to dryness. The crude product was purified by column chromatography using CH_2Cl_2–MeOH (100:1.5) to give the monoxime of **25** (85 mg, 70%), which was dissolved in benzene (2 mL) and *p*-toluenesulfonic acid monohydrate (50 mg) was added. The reaction mixture was stirred at 60 °C for 1.5 h; water was added and the mixture was extracted with EtOAc. The combined organic phase was washed with water and dried over anhydrous Na_2SO_4. The solvent was evaporated and the residue was chromatographed using CH_2Cl_2–MeOH (10:0.1) to yield **42** (62 mg, 77%).

*5.2.13.2. Tetrahydrocurcumin isoxazole (**42**).* White amorphous solid. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 2.87–2.91 (m, 6H), 2.96 (m, 2H) (H-1′, H-2′, H-1″, H-2″), 3.82 and 3.83 (each s, 2 × 3H, 2 × OMe), 5.50 and 5.51 (each s, 2 × 1H, 2 × OH), 5.67(s, 1H, H-4), 6.61–6.67 (m, 4H, H-2‴, H-6‴, H-2⁗, H-6⁗), 6.80 and 6.81 (each d, J = 7.8 Hz, H-5‴ and H-5⁗); ESMS (+ve): m/z (% rel. abund.) 370 $[M + H]^+$ (100).

*5.2.14. Pentylation of the isoxazole **37***

The isoxazole **37** was subjected to *n*-pentylation in similar manner to that of methylation of compound **1**, but using *n*-pentyl iodide in place of methyl iodide, to afford **43** and **44** (1:1 mixture) and **45** in 24 and 62%, respectively. The **43**/**44** mixture was separated by normal phase HPLC (column: Luna Silica (2) 100A, 5 μm, 4.6 × 250 mm, mobile phase: *n*-hexane–$CHCl_3$ (1:1), flow rate: 1.2 mL/min, detector: 254 nm) to yield **43** (t_R 21.42 min) and **44** (t_R 19.02 min).

*5.2.14.1. Compound **43**.* White solid; m.p. 116–117 °C; IR ν_{max}: 3376, 2937, 1644, 1597, 1509, 1467, 1428, 1279, 1225, 1163, 1140, 1032, 962, 807, 734 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.91 (t, J = 6.8 Hz, 3H, H-5⁗′), 1.40 (m, 4H, H-3⁗′ and H-4⁗′), 1.84 (m, 2H, H-2⁗′), 3.90 and 3.93 (each s, 2 × 3H, 2 × OMe), 4.02 (t, J = 6.4 Hz, 2H, H-1⁗′), 6.40 (s, 1H, H-4), 6.80 (d, J = 16.3 Hz, 1H, H-1′), 6.85 (d, J = 8.0 Hz, 1H, H-5‴), 6.90 (d, J = 8.0 Hz, 1H, H-5⁗), 6.95 (d, J = 16.5 Hz, 1H, H-1″), 6.99–7.09 (m, 5H, H-2″, H-2‴, H-2⁗, H-6‴, H-6⁗), 7.27 (d, J = 16.3 Hz, 1H, H-2′); ^{13}C NMR (100 MHz, $CDCl_3$) δ 13.9 (C-5⁗′), 22.4 (C-2⁗′), 28.0 (C-3⁗′), 28.8 (C-4⁗′), 55.9 (3‴-OMe), 56.0 (3⁗-OMe), 69.0 (C-1⁗′), 97.6 (C-4), 108.2 (C-2‴), 109.6 (C-2⁗), 110.0 (C-1′), 111.2 (C-5‴), 113.8 (C-1″), 114.6 (C-5⁗), 121.0 (C-6‴), 121.6 (C-6⁗), 128.4 (C-1⁗), 128.5 (C-1‴), 134.8 (C-2′), 135.5 (C-2″), 146.6 (C-4⁗), 146.8 (C-3⁗), 149.6 (C-3‴, C-4‴), 162.1 (C-5), 168.5 (C-3); ESMS (−ve): m/z (% rel. abund.) 434 $[M - H]^-$ (100); HR-TOFMS (APCI$^+$): m/z 436.2121 $[M + H]^+$; calcd for $C_{26}H_{29}NO_5$ + H, 436.2118.

*5.2.14.2. Compound **44**.* White solid; m.p. 115–116 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.91 (t, J = 6.8 Hz, 3H, H-5⁗′), 1.40 (m, 4H, H-3⁗′ and H-4⁗′), 1.84 (m, 2H, H-2⁗′), 3.90 and 3.93 (each s, 2 × 3H, 2 × OMe), 4.02 (t, J = 6.4 Hz, 2H, H-1⁗′), 6.40 (s, 1H, H-4), 6.79 (d, J = 16.3 Hz, 1H, H-1′), 6.85 (d, J = 8.2 Hz, 1H, H-5‴), 6.91 (d, J = 8.0 Hz, 1H, H-5⁗), 6.97 (d, J = 16.4 Hz, 1H, H-1″), 7.01–7.08 (m, 5H, H-2″, H-2‴, H-2⁗, H-6‴, H-6⁗), 7.26 (d, J = 16.3 Hz, 1H, H-2′); ESMS (−ve): m/z (% rel. abund.) 434 $[M - H]^-$ (100); HR-TOFMS (APCI$^+$): m/z 436.2125 $[M + H]^+$; calcd for $C_{26}H_{29}NO_5$+H, 436.2118.

*5.2.14.3. Compound **45**.* Aggregated needles (from *n*-hexane–CH_2Cl_2); m.p. 130–131 °C; IR ν_{max}: 3446, 2952, 2869, 1634, 1596, 1581, 1558, 1514, 1467, 1393, 1325, 1268, 1241, 1138, 1050 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.89 (t, J = 6.9 Hz, 6H, H-5⁗′ and H-5⁗′′), 1.37 (m, 8H, H-3⁗′, H-3⁗′′, H-4⁗′, H-4⁗′′), 1.82 (m, 4H, H-2⁗′ and H-2⁗′′), 3.87 and 3.88 (each s, 2 × 3H, 2 × OMe), 4.60 (br t, J = 5.4 Hz, 4H, H-1⁗′ and H-1⁗′′), 6.38 (s, 1H, H-4), 6.78 (d, J = 16.4 Hz, 1H, H-1′), 6.82 and 6.83 (each d, J = 8.2 Hz, 2H, H-5‴ and H-5⁗), 6.95 (d, J = 16.4 Hz, 1H, H-1″), 6.99–7.06 (m, 5H, H-2″, H-2‴, H-2⁗, H-6‴, H-6⁗), 7.25 (d, J = 16.3 Hz, 1H, H-2′); ESMS (+ve): m/z (% rel. abund.) 506 $[M + H]^+$ (100).

*5.2.15. 3,3-Dimethylallylation of the isoxazole **37***

The isoxazole **37** was subjected to 3,3-dimethylallylation in similar manner to that of methylation of compound **1**, but using 3,3-dimethylallyl bromide in place of methyl iodide, to afford a mixture of **46a** and **46b** and **47** in 25 and 73% yields, respectively.

*5.2.15.1. Compounds **46a**/**46b** (2:1 mixture).* IR ν_{max}: 3427, 2934, 1646, 1559, 1511, 1430, 1269, 1137, 962, 810 cm^{-1}; ***46a***: ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.73 and 1.76 (each s, 2 × 3H, 2 × Me), 3.91 and 3.93 (each s, 2 × 3H, 2 × OMe), 4.60 (br d, J = 6.1 Hz, 2H, H-1⁗′), 5.50 (m, 1H, H-2⁗′), 5.74 (s, 1H, OH), 6.40 (s, 1H, H-4), 6.80 (d, J = 16.3 Hz, 1H, H-1′), 6.86 (d, J = 8.0 Hz, 1H, H-5‴), 6.90 (d, J = 8.0 Hz, 1H, H-5⁗), 6.95 (d, J = 16.4 Hz, 1H, H-1″), 7.00–7.11 (m, 5H, H-2″, H-2‴, H-2⁗, H-6‴, H-6⁗), 7.27 (d, J = 16.3 Hz, 1H, H-2′); ESMS (+ve): m/z (% rel. abund.) 434 $[M + H]^+$ (100); ***46b***: ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.73 and 1.76 (each s, 6H, 2 × Me), 3.91 and 3.93 (each s, 6H, 2 × OMe), 4.60 (br d, J = 6.1 Hz, 2H, H-1⁗′), 5.50 (m, 1H, H-2⁗′), 5.77 (s, 1H, OH), 6.40 (s, 1H, H-4), 6.79 (d, J = 16.4 Hz, 1H, H-1′), 6.85 (br d, J = 8.0 Hz, 1H, H-5⁗), 6.91 (br d, J = 8.0 Hz, 1H, H-5‴), 6.98 (d, J = 16.4 Hz, 1H, H-1″), 7.00–7.07 (m, 5H, H-2″, H-2‴, H-2⁗, H-6‴, H-6⁗), 7.26 (d, J = 16.3 Hz, 1H, H-2′); ***46a***/***46b***: ESMS (+ve): m/z (% rel. abund.) 434 $[M + H]^+$ (100).

*5.2.15.2. Compound **47**.* White solid; m.p. 167–186 °C; IR ν_{max}: 2928, 1645, 1581, 1512, 1432, 1312, 1136, 1028, 989, 818 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.73 and 1.76 (each s, 4 × 3H, 4 × Me), 3.90 and 3.91 (each s, 2 × 3H, 2 × OMe), 4.60 (br d, J = 6.1 Hz, 4H, H-1⁗′ and H-1⁗′′), 5.50 (m, 2H, H-2⁗′ and H-2⁗′′), 6.40 (s, 1H, H-4), 6.81 (d, J = 16.3 Hz, 1H, H-1′), 6.86 (br d, J = 8.3 Hz, 2H, H-5‴ and H-5⁗), 6.97 (d, J = 16.4 Hz, 1H, H-1″), 7.01–7.11 (m, 5H, H-2″, H-2‴, H-2⁗, H-6‴, H-6⁗), 7.27 (d, J = 16.3 Hz, 1H, H-2′); ESMS (−ve) m/z (% rel. abund.) 500 $[M - H]^-$ (100); HR-TOFMS (ES$^-$): m/z 500.2436 $[M - H]^-$; calcd for $C_{31}H_{35}NO_5$–H, 500.2437.

*5.2.16. Ethylation of the isoxazole **37***

The isoxazole **37** was subjected to ethylation in similar manner to that of methylation of compound **1**, but using diethyl sulfate in place of methyl iodide, to afford a 5:2 mixture of **48a** and **48b** in 23% and **49** in 60%.

*5.2.16.1. Compound **48a**/**48b** (5:2 mixture).* IR ν_{max}: 3420, 2934, 1644, 1598, 1509, 1430, 1267, 1138, 1032, 961, 808 cm^{-1}; ***48a***: ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.46 (t, J = 6.9 Hz, 3H, H-2⁗′), 3.92 and 3.93 (each s, 2 × 3H, 2 × OMe), 4.12 (m, 2H, H-1⁗′), 5.75 (s, 1H, OH), 6.40 (s, 1H, H-4), 6.80 (d, J = 16.3 Hz, 1H, H-1′), 6.86 (d, J = 8.0 Hz, 1H, H-5‴), 6.90 (d, J = 8.0 Hz, 1H, H-5⁗), 6.95 (d, J = 16.4 Hz, 1H, H-1″), 7.00–7.11 (m, 5H, H-2″, H-2‴, H-2⁗, H-6‴, H-6⁗), 7.27 (d, J = 16.3 Hz, 1H, H-2′); ***48b***: ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.46 (t, J = 6.9 Hz, 3H, H-2⁗′), 3.91 and 3.94 (each s, 2 × 3H, 2 × OMe), 4.12 (m, 2H, H-1⁗′), 5.78 (s, 1H, OH), 6.40 (s, 1H, H-4), 6.79 (d, J = 16.4 Hz, 1H, H-1′), 6.85 (br d, J = 8.0 Hz, 1H, H-5⁗), 6.91 (br d, J = 8.0 Hz, 1H, H-5‴), 6.98 (d, J = 16.4 Hz, 1H, H-1″), 7.00–7.11 (m, 5H, H-2″, H-2‴, H-2⁗, H-6‴, H-6⁗), 7.26 (d, J = 16.3 Hz, 1H, H-2′); ***48a***/***48b***: ESMS (+ve) m/z (% rel. abund.) 434 $[M + H]^+$ (100).

*5.2.16.2. Compound **49**.* White plates (from CH_2Cl_2–*n*-hexane); m.p. 175 °C; IR ν_{max}: 2976, 1633, 1581, 1558, 1514, 1473, 1264, 1239, 1136, 1026, 967, 800 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.46 (t, J = 6.9 Hz, 6H, H-2⁗′ and H-2⁗′′), 3.91 and 3.92 (each s, 2 × 3H, 2 × OMe), 4.12 (m, 4H, H-1⁗′ and H-1⁗′′), 6.40 (s, 1H, H-4), 6.81 (d, J = 16.3 Hz, 1H, H-1′), 6.85 and 6.86 (each d, J = 8.0 Hz, 2H, H-5‴ and H-5⁗), 6.97 (d, J = 16.4 Hz, 1H, H-1″), 7.01–7.11 (m, 5H, H-2″, H-2‴, H-2⁗, H-6‴, H-6⁗), 7.27 (d, J = 16.3 Hz, 1H, H-2′); ESMS (+ve): m/z (% rel. abund.) 422 $[M + H]^+$ (100); HR-TOFMS (ES$^+$): m/z 422.1974 $[M + H]^+$; calcd for $C_{25}H_{27}NO_5$ + H, 422.1967.

*5.2.17. Acetylation of the isoxazole **37***

Ac_2O (0.05 mL) was added to a solution of compound **37** (90 mg, 0.25 mmol) in pyridine (2 mL) and the reaction mixture was stirred at ambient temperature for 1 h. The crude product which were obtained by the usual work up followed by solvent extraction were subjected to column chromatography eluting with CH_2Cl_2:MeOH (100:0.8) to afford a mixture of **50** and **51** (31 mg, 30%), and **52** (62 mg, 55%). The **50**/**51** mixture was subjected to normal phase HPLC separation (column: Luna Silica (2) 100A, 5 μm, 4.6 × 250 mm, mobile phase: *n*-hexane–$CHCl_3$ (30:50), flow rate: 0.8 mL/min, detector: 254 nm) to yield **47** (t_R 30.99 min) and **48** (t_R 33.62 min).

*5.2.17.1. Compound **50**.* White solid; m.p. 167–168 °C; IR ν_{max}: 3421, 1759, 1647, 1602, 1561, 1513, 1429, 1371, 1276, 1220, 1121, 1031, 962, 828, 739 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 2.30 (s, 3H, OAc), 3.86 and 3.94 (each s, 2 × 3H, 2 × OMe), 6.41 (s, 1H, H-4), 6.79 (d, *J* = 16.3 Hz, 1H, H-1′), 6.91 (d, *J* = 8.1 Hz, 1H, H-5‴″), 7.00–7.14 (m, 7H, H-1″, H-2″, H-2‴, H-2‴″, H-5‴, H-6‴, H-6‴″), 7.27 (d, *J* = 16.3 Hz, 1H, H-2′); ^{13}C NMR (100 MHz, $CDCl_3$) δ 20.6 (COMe), 55.9 (3‴-OMe), 56.0 (3‴″-OMe), 97.6 (C-4), 108.8 (C-2‴), 110.2 (C-2‴″), 110.8 (C-1′), 114.8 (C-5‴″), 116.5 (C-1″), 120.0 (C-6‴″), 121.5 (C-6‴), 121.6 (C-6‴″), 123.1 (C-5‴), 128.1 (C-1‴ and C-1‴″), 134.9 (C-2″), 135.0 (C-2′), 146.8 (C-4‴″), 147.0 (C-3‴″), 151.1 (C-3‴), 152.2 (C-4‴), 161.8 (C-5), 168.7 (C-3), 168.8 (COMe); ESMS (–ve): *m*/*z* (% rel. abund.) 406 $[M - H]^-$ (100); HR-TOFMS ($APCI^+$): *m*/*z* 408.1449 $[M + H]^+$; calcd for $C_{23}H_{21}NO_6$ + H, 408.1442.

*5.2.17.2. Compound **51**.* White solid; m.p. 159 °C; ^1H NMR (400 MHz, $CDCl_3$) δ 2.30 (s, 3H, OAc), 3.87 and 3.92 (each s, 2 × 3H, 2 × OMe), 6.44 (s, 1H, H-4), 6.87 (d, *J* = 16.3 Hz, 1H, H-1′), 6.90 (d, *J* = 8.1 Hz, 1H, H-5‴″), 6.95 (d, *J* = 16.4 Hz, 1H, H-1″), 6.99–7.10 (m, 6H, H-2″, H-2‴, H-2‴″, H-5‴, H-6‴, H-6‴″), 7.29 (d, *J* = 16.3 Hz, 1H, H-2′); ESMS (–ve): *m*/*z* (% rel. abund.) 406 $[M - H]^-$ (100); HR-TOFMS ($APCI^+$): *m*/*z* 408.1444 $[M + H]^+$; calcd for $C_{23}H_{21}NO_6$ + H, 408.1442.

*5.2.17.3. Compound **52**.* White solid; m.p. 188–189 °C; IR ν_{max}: 3015, 2924, 1753, 1638, 1601, 1566, 1514, 1470, 1396, 1207, 1225, 1203, 971, 832, 740, 656 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 2.31 (s, 2 × 3H, 2 × OAc), 3.87 (s, 2 × 3H, 2 × OMe), 6.47 (br s, 1H, H-4), 6.89 (d, *J* = 16.3 Hz, 1H, H-1′), 7.02–7.15 (m, 8H, H-1″, H-2″, H-2‴, H-2‴″, H-5‴, H-5‴″, H-6‴, H-6‴″), 7.31 (d, *J* = 16.3 Hz, 1H, H-2′); ^{13}C NMR (100 MHz, $CDCl_3$) δ 20.5 (COMe), 55.8 (3‴-OMe, 3‴″-OMe), 98.5 (C-4), 110.1, 110.6 (C-2‴, C-2‴″), 119.8, 120.0 (C-6‴, C-6‴″), 123.0, 123.1 (C-5‴, C-5‴″), 134.2 (C-2′), 134.4, 134.7 (C-1‴, C-1‴″), 135.0 (C-2″), 140.2, 140.4 (C-4‴, C-4‴″), 151.3 (C-3‴, C-3‴″), 161.7 (C-5), 168.0 (C-3), 168.8 (2 × COMe); ESMS (+ve): *m*/*z* (% rel. abund.) 450 $[M + H]^+$ (100); HR-TOFMS (ES^+): *m*/*z* 450.1566 $[M + H]^+$; calcd for $C_{25}H_{23}NO_7$ + H, 450.1553.

*5.2.18. Methylation of the isoxazole **37***

Compound **37** (1.5 g, 4.1 mmol) was dissolved in dry acetone (45 mL) and anhydrous K_2CO_3 (1.1 g) and MeI (4.5 mL) was added. The reaction mixture was reflux for 5 h. Water was added and the mixture extracted with CH_2Cl_2. The combined organic phase was washed with H_2O, dried over anhydrous Na_2SO_4 and the solvent was removed under vacuum. The crude products were separated and purified by column chromatography using CH_2Cl_2–MeOH (100:1) to yield a 1:1 mixture of isomeric mono-methylated analogs **53** and **54** (590 mg, 38%) and the di-methylated analog **55** (805 mg, 50%). A mixture of **53** and **54** was separated by normal phase HPLC (column: Luna Silica (2) 100A, 5 μm, 4.6 × 250 mm, mobile phase: *n*-hexane–EtOAc (72:28), flow rate: 1 mL/min, detector: 254 nm) to yield pure **53** (t_R 31.38 min) and **54** (t_R 32.01 min).

*5.2.18.1. Compound **53**.* Colorless foam; IR ν_{max}: 3413, 2935, 2836, 1645, 1598, 1513, 1431, 1268, 1159, 1139, 1025, 961, 809, 736, 607 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 3.90 and 3.93 (each s, 2 × 3H and 1 × 3H, 3 × OMe), 6.40 (s, 1H, H-4), 6.81 (d, *J* = 16.3 Hz, 1H, H-1′), 6.86 (br d, *J* = 8.3 Hz, 1H, H-5‴), 6.90 (br d, *J* = 8.3 Hz, 1H, H-5‴″), 6.94 (d, *J* = 16.4 Hz, 1H, H-1″), 7.00 (br d, *J* = 8.3 Hz, 1H, H-6‴″), 7.04–7.08 (m, 4H, H-2″, H-2‴, H-2‴″, H-6‴), 7.28 (d, *J* = 16.3 Hz, 1H, H-2′); ^{13}C NMR (100 MHz, $CDCl_3$) δ 55.9 (3‴-OMe and 3‴″-OMe), 56.0 (4‴-OMe), 97.7 (C-4), 108.2 (C-2‴″), 109.1 (C-2‴), 111.1 (C-1′), 111.2 (C-5‴), 113.8 (C-1″), 114.6 (C-5‴″), 121.1 (C-6‴), 121.6 (C-6‴″), 128.5 (C-1‴″), 128.6 (C-1‴), 134.7 (C-2′), 135.6 (C-2″), 146.7 (C-4‴″), 146.8 (C-3‴″), 149.3 (C-3‴), 150.2 (C-4‴), 162.1 (C-5), 168.4 (C-3); ESMS (+ve): *m*/*z* (% rel. abund.) 380 $[M + H]^+$ (100); HR-TOFMS ($APCI^+$): *m*/*z* 380.1497 $[M + H]^+$; calcd for $C_{22}H_{21}NO_5$ + H, 380.1492.

*5.2.18.2. Compound **54**.* Colorless foam; ^1H NMR (400 MHz, $CDCl_3$) δ 3.89, 3.92 and 3.94 (each s, 3 × 3H, 3 × OMe), 6.40 (s, 1H, H-4), 6.79 (d, *J* = 16.4 Hz, 1H, H-1′), 6.85 (br d, *J* = 8.0 Hz, 1H, H-5‴″), 6.91 (br d, *J* = 8.0 Hz, 1H, H-5‴), 6.98 (d, *J* = 16.4 Hz, 1H, H-1″), 7.04 (br s, 1H, H-2‴), 7.06–7.09 (m, 4H, H-2″, H-2‴″, H-6‴, H-6‴″), 7.26 (d, *J* = 16.3 Hz, 1H, H-2′); ^{13}C NMR (100 MHz, $CDCl_3$) δ 55.9 (3‴-OMe and 3‴″-OMe), 56.0 (4‴-OMe), 97.6 (C-4), 108.8 (C-2‴), 108.9 (C-2‴″), 110.9 (C-1′), 111.2 (C-5‴″), 114.2 (C-1″), 114.7 (C-5‴), 120.8 (C-6‴″), 121.5 (C-6‴), 128.2 (C-1‴), 129.0 (C-1‴″), 134.9 (C-2′), 135.4 (C-2″), 146.8 (C-4‴), 146.9 (C-3‴), 149.2 (C-3‴″), 150.0 (C-4‴″), 162.1 (C-5), 168.5 (C-3); ESMS (+ve): *m*/*z* (% rel. abund.) 380 $[M + H]^+$ (100); HR-TOFMS ($APCI^+$): *m*/*z* 380.1501 $[M + H]^+$; calcd for $C_{22}H_{21}NO_5$ + H, 380.1492.

*5.2.18.3. Compound **55**.* White powder; m.p. 159–160 °C; IR ν_{max}: 3449, 2999, 2936, 2838, 1646, 1601, 1561, 1513, 1428, 1266, 1224, 1140, 1026, 966, 807, 767, 736 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 3.89 and 3.91 (each s, 2 × 3H and 2 × 3H, 4 × OMe), 6.41 (s, 1H, H-4), 6.81 (d, *J* = 16.3 Hz, 1H, H-1′), 6.84 (br d, *J* = 8.2 Hz, 1H, H-5‴), 6.86 (br d, *J* = 8.2 Hz, 1H, H-5‴″), 6.98 (d, *J* = 16.4 Hz, 1H, H-1″), 7.03–7.10 (m, 5H, H-2″, H-2‴, H-2‴″, H-6‴, H-6‴″), 7.27 (d, *J* = 16.3 Hz, 1H, H-2′); ^{13}C NMR (100 MHz, $CDCl_3$) δ 55.8 and 55.9 (3‴-OMe, 3‴″-OMe, 4‴-OMe, 4‴″-OMe), 97.6 (C-4), 108.7, 109.0 (C-2‴, C-2‴″), 111.0 (C-1′), 111.1 (C-5‴, C-5‴″), 114.0 (C-1″), 120.8, 121.0 (C-6‴, C-6‴″), 128.5, 128.8 (C-1‴, C-1‴″), 134.6 (C-2′), 135.3 (C-2″), 149.1, 149.8, 150.1 (C-3‴, C-3‴″, C-4‴, C-4‴″), 162.0 (C-5), 168.3 (C-3); ESMS (+ve): *m*/*z* (% rel. abund.) 394 $[M + H]^+$ (100); HR-TOFMS ($APCI^+$): *m*/*z* 394.1650 $[M + H]^+$; calcd for $C_{22}H_{23}NO_5$ + H, 394.1649.

*5.2.19. Methylation of a mixture of the isoxazole mixture **38a**/**38b***

The 1:1 mixture of the isoxazoles **38a**/**38b** was subjected to methylation in similar manner to that of compound **37** to give a 1:1 mixture of **56a** and **56b** in 80%.

*5.2.19.1. Compounds **56a**/**56b** (1:1 mixture).* IR ν_{max}: 2962, 2838, 1644, 1603, 1577, 1509, 1430, 1307, 1263, 1139, 1025, 961, 821 cm^{-1}; ***56a***: ^1H NMR (400 MHz, $CDCl_3$) δ 3.82, 3.89 and 3.92 (each s, 3 × 3H, 3 × OMe), 6.41 (s, 1H, H-4), 6.83 (d, *J* = 16.4 Hz, 1H, H-1′), 6.86 (d, *J* = 8.2 Hz, 1H, H-5‴″), 6.89 (br d, *J* = 8.4 Hz, 2H, H-3‴ and H-5‴), 6.98 (d, *J* = 16.4 Hz, 1H, H-1″), 7.03 (br s, 1H, H-2‴″), 7.06–7.12 (m, 2H, H-2″ and H-6‴″), 7.28 (d, *J* = 16.4 Hz, 1H, H-2′), 7.45 (br d, *J* = 8.4 Hz, 2H, H-2‴ and H-6‴); ***56b***: ^1H NMR (400 MHz, $CDCl_3$) δ 3.82, 3.89 and 3.92 (each s, 3 × 3H, 3 × OMe), 6.40 (s, 1H, H-4), 6.83 (d, *J* = 16.4 Hz, 1H, H-1′), 6.85 (d, *J* = 8.2 Hz, 1H, H-5‴), 6.89 (br d, *J* = 8.4 Hz, 2H, H-3‴″ and H-5‴″), 6.97 (d, *J* = 16.4 Hz, 1H, H-1″), 7.03 (br s, 1H, H-2‴), 7.06–7.12 (m, 2H, H-2″ and H-6‴), 7.27 (d, *J* = 16.4 Hz, 1H, H-2′), 7.45 (br d, *J* = 8.4 Hz, 2H, H-2‴″ and H-6‴″); ***56a***/***56b***: ESMS (+ve) *m*/*z* (% rel. abund.) 749 $[2M + Na]^+$ (100).

*5.2.20. Methylation of the isoxazole **39***

The isoxazole **39** was subjected to methylation in similar manner to that of compound **37** to give the corresponding dimethyl ether analog **57** in 78%.

*5.2.20.1. Compound **57***. Aggregated needles (from CH_2Cl_2–*n*-hexane), m.p. 180–181 °C; IR ν_{max}: 2934, 1646, 1604, 1578, 1559, 1510, 1307, 1248, 1174, 1092, 969, 819 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 3.82 (s, 2 × 3H, 2 × OMe), 6.39 (s, 1H, H-4), 6.85 (d, J = 16.4 Hz, 1H, H-1′), 6.90 (br d, J = 8.4 Hz, 4H, H-3‴, H-5‴, H-3⁗, H-5⁗), 6.97 (d, J = 16.4 Hz, 1H, H-1″), 7.10 (d, J = 16.4 Hz, 1H, H-2″), 7.28 (d, J = 16.4 Hz, 1H, H-2′), 7.45 (d, J = 8.4 Hz, 4H, H-2‴, H-6‴, H-2⁗, H-6⁗); ESMS (+ve): *m*/*z* (% rel. abund.) 334 $[M + H]^+$ (100); HR-TOFMS (ES^+): *m*/*z* 334.1442 $[M + H]^+$; calcd for $C_{21}H_{19}NO_3$ + H, 334.1443.

*5.2.21. Synthesis of isoxazole analogs of mono-O-demethylcurcumin (**4**)*

A 1:2 mixture of the isoxazole analogs **58a** and **58b** was prepared from **4** in 60% yield by the same method employed for the preparation of compound **37** from compound **1**.

*5.2.21.1. Compounds **58a**/**58b** (1:2 mixture).* IR ν_{max}: 3453, 3134, 1645, 1601, 1561, 1509, 1434, 1376, 1277, 1118, 1023, 962, 816 cm^{-1}; ***58a***: ^1H NMR (400 MHz, $CDCl_3$ + 6 drops of CD_3OD) δ 3.88 (s, 3H, OMe), 6.37 (s, 1H, H-4), 6.74 (d, J = 16.3 Hz, 1H, H-1′), 6.81 and 6.84 (each d, J = 7.9 Hz, 1H, H-5⁗ and H-5‴), 6.87 (br d, J = 8.1 Hz, 1H, H-6⁗), 6.92 (d, J = 16.3 Hz, 1H, H-1″), 6.96–7.06 (m, 4H, H-2″, H-2‴, H-2⁗, H-6‴), 7.17 (d, J = 16.3 Hz, 1H, H-2′); ***58b***: ^1H NMR (400 MHz, $CDCl_3$ + 6 drops of CD_3OD) δ 3.88 (s, 3H, OMe), 6.36 (s, 1H, H-4), 6.70 (d, J = 16.3 Hz, 1H, H-1′), 6.78 and 6.83 (each d, J = 8.1 Hz, 1H, H-5⁗ and H-5‴), 6.87 (br d, J = 8.1 Hz, 1H, H-6‴), 6.92 (d, J = 16.3 Hz, 1H, H-1″), 6.96–7.06 (m, 4H, H-2″, H-2‴, H-2⁗, H-6⁗), 7.16 (d, J = 16.3 Hz, 1H, H-2′); ***58a***/***58b***: ESMS (−ve): *m*/*z* (% rel. abund.) 350 $[M - H]^-$ (100).

*5.2.22. Synthesis of isoxazole analog of di-O-demethylcurcumin (**5**)*

The isoxazole analog **59** was prepared in 67% yield by the same method employed for the preparation of compound **37** from compound **1**.

*5.2.22.1. Compound **59***. Pale white solid; m.p. >250 °C; IR ν_{max}: 3367, 1647, 1603, 1565, 1520, 1440, 1288, 1113, 958, 808 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$ + 6 drops of CD_3OD) δ 6.35 (s, 1H, H-4), 6.69 (d, J = 16.3 Hz, 1H, H-1′), 6.71–6.87 and 6.96–7.06 (each m, 8H, H-1″, H-2″, H-2‴, H-2⁗, H-5‴, H-5⁗, H-6‴, H-6⁗), 7.15 (d, J = 16.3 Hz, 1H, H-2′); ESMS (−ve): *m*/*z* (% rel. abund.) 336 $[M - H]^-$ (100); HR-TOFMS (ES^-): *m*/*z* 306.1130 $[M - H]^-$; calcd for $C_{19}H_{15}NO_5$–H, 306.1130.

5.3. Biological activities

5.3.1. Mycobacterial strains

The reference strain *M. tuberculosis* H37Ra and the clinical isolates of MDR-TB were obtained from Ramathibodi Hospital, Mahidol University, Bangkok, Thailand.

5.3.2. Antimycobacterial assay

Antimycobacterial activities were determined by microplate Alamar blue assay (MABA) [31]. Briefly, the compounds were dissolved in dimethyl sulfoxide (Sigma) and subsequently diluted twofold in 100 μL of Middlebrook 7H9GC in clear flatbottom, 96-well microplates. A mycobacterial suspension was prepared in 0.04% Tween 80 and diluted with sterile distilled water to a turbidity of the McFarland no. 1. The suspension was then diluted 1:50 with 7H9GC, and 100 μL was added to the wells. After incubated at 37 °C for 7 days, 12.5 μL of 20% Tween 80 and 20 μL of Alamar blue (SeroTec Ltd., Oxford, UK) were added to all wells. Growth of the organisms was determined after reincubation at 37 °C for 16–24 h by visual determination of a color change from blue to pink. The MIC was defined as the lowest concentration which prevented the color change. NIH, RIF and kanamycin (Sigma) were included as controls.

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