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Curcuminoid-derived 3,5-bis(styryl)isoxazoles - Mechanochemical synthesis and antioxidant activity

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Abstract. Mechanochemical synthesis of curcuminoid-derived 3,5-bis(styryl)isoxazoles and their antioxidant activities are reported.

Keywords. Curcuminoids; 3,5-bis(styryl)isoxazoles; antioxidant

1. Introduction

Curcumin, the active ingredient of turmeric, is a diarylheptanoid natural product that is endowed with much bioactivity;[1–3] yet it has limited applications as a drug due to its low bioavailability.[4] Structural modification of curcumin has been explored much as a strategy to circumvent this inadequacy. Among such structurally modified curcumin derivatives, the curcumin-derived pyrazoles and isoxazoles have been investigated extensively.[5–13] With our long standing interest in the synthesis and bioactivity studies on curcumin, its analogs and derivatives,[14–18] we have also investigated the structural modification approach.[12,19] The natural curcumin is a mixture of three compounds, namely 1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione (**1**) (curcumin I), its demethoxy (curcumin II) and bisdemethoxy (curcumin III) derivatives. Recently, we have reported the rapid, mechanochemical synthesis and antioxidant assay of 3,5-bis(4-hydroxy-3-methoxystyryl) pyrazole (**2a**), its 1-aryl derivatives (**2b**) and 3,5-bis(4-hydroxy-3-methoxystyryl)isoxazole (**2c**) prepared from curcumin I (**1**).[19] In continuation, we have now extended the above method for the synthesis of 3,5-bis(styryl) isoxazoles (**4a-g)** from other, differently substituted curcuminoids (**3a-g**), with a view to understand how the substituents on the aryl ring would affect the antioxidant activity, in the light of the continued interest on the antioxidant activity of curcumin, its derivatives and analogs.[20–22] The thermal methods that have been reported earlier for the synthesis of curcumin-derived isoxazoles take, in general, 6-40 h for completion.[6–9,13]

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2. Experimental

2.1 *General*

Reagents and solvents used were of analytical grade. Thin Layer Chromatography (TLC) and Preparative Thin Layer Chromatography (PTLC) were performed on glass plates (20 × 75 mm and 20 × 20 cm, respectively) coated with TLC grade silica gel-G (E. Merck, India). The spots or bands were visualized directly or under UV light. The C, H, N analyses were carried out on Vario EL Elementar analyzer. ^{1}H and ^{13}C NMR were recorded on Bruker Topspin and Bruker AV, 500 MHz (500 MHz for ^{1}H and 125 MHz for ^{13}C NMR), Bruker Avance III, 400 MHz (400 MHz for ^{1}H and 100 MHz for ^{13}C NMR) at room temperature. Chemical shifts are given in ppm relative to TMS as an internal standard. HRMS were recorded on JEOL JMS 600H mass spectrometer. The absorbance values were recorded on a Jasco V-550 spectrophotometer. The curcuminoids (**3a-g**) were obtained from suitably substituted benzaldehydes by adopting the method reported earlier by us and their identities were established by spectral characterization and comparison with samples available with us.[12,14,23]

2.2 *General procedure for the synthesis of 3,5-bis (styryl)isoxazoles (**4a-g**)*

A mixture of curcuminoid **3a-g** (0.1 mmol), hydroxylamine hydrochloride (0.1 mmol) and glacial acetic acid (0.1 mL) were vigorously ground in an agate mortar for 1–2 mins. The crude product, obtained in a powder form, was then purified by PTLC ($CHCl_3$: EtOAc, 3:2) to afford the 3,5-bis(styryl)isoxazoles (**4a-g**).

2.2a *3,5-Bis(4-hydroxystyryl)isoxazole (**4a**)*: Yield 87%; M.p. 198°C; ^{1}H NMR (500 MHz, Acetone-d_6): δ 5.62 (s, 1H, 4-H), 5.94 (s, 2H, ArOH), 6.64 (d, 2H, 2,6-H, J = 16.0 Hz), 6.86 (d, 2H, ArH, J = 8.0 Hz), 7.14–7.15 (dd, 4H, ArH), 7.37 (s, 2H, ArH), 7.52 (d, 2H, 1,7-H, J = 16.0 Hz); ^{13}C NMR (100 MHz, DMSO-d_6): δ 168.6, 162.1, 148.1, 147.9, 147.8, 136.3, 134.7, 127.3, 126.9, 121.4, 121.2, 115.6, 115.5, 112.6, 110.4, 110.3, 110.1, 97.8; HRMS (ESI): Calcd for $C_{19}H_{15}NO_3$, (M+H): 306.11302; Found: 306.11308; Anal. Calcd. (%) For $C_{19}H_{15}NO_3$: C, 74.74; H, 4.95; N, 4.59; Found (%): C, 74.53; H, 5.17; N, 4.69.

2.2b *3,5-Bis(3-hydroxy-4-methoxystyryl)isoxazole (**4b**)*: Yield 85%; M.p. 168°C; ^{1}H NMR (500 MHz, Acetone-d_6): δ 3.82 (s, 6H, OCH_3), 5.60 (s, 1H, 4-H), 5.92 (s, 2H, ArOH), 6.64 (d, 2H, 2,6-H, J = 16.0 Hz), 6.86 (d, 2H, ArH, J = 8.0 Hz), 7.14-7.16 (dd, 2H, ArH), 7.36 (s, 2H, ArH), 7.52 (d, 2H, 1,7-H, J = 16.0 Hz); ^{13}C NMR (100 MHz, DMSO-d_6): δ 168.3, 162.1, 148.1, 147.9, 147.9, 147.8, 136.3, 134.7, 127.3, 126.9, 121.6, 121.2, 115.6, 115.5, 112.6, 110.4, 110.3, 110.1, 97.8, 55.7, 55.6 HRMS (ESI): Calcd. for $C_{21}H_{19}NO_5$, (M+Na): 388.11609; Found: 388.11608; Anal. Calcd. (%) For $C_{21}H_{19}NO_5$: C, 69.03; H, 5.24; N, 3.83; Found (%): C, 68.98; H, 5.28; N, 3.96.

2.2c *3,5-Bis(2-hydroxystyryl)isoxazole (**4c**)*: Yield 84%; M.p. 152°C; ^{1}H NMR (500 MHz, Acetone-d_6): δ 5.64 (s, 1H, 4-H), 5.97 (s, 2H, ArOH), 6.64 (d, 2H, 2,6-H, J = 16.0 Hz), 6.85 (d, 2H, ArH, J = 8.0 Hz), 7.14-7.15 (dd, 4H, ArH), 7.37 (s, 2H, ArH), 7.52 (d, 2H, 1,7-H, J = 16.0 Hz); ^{13}C NMR (100 MHz, DMSO-d_6): δ 168.9, 162.1, 148.3, 147.9, 147.8, 147.6, 136.1, 134.7, 127.6, 126.9, 121.4, 121.2, 115.5, 115.3, 112.8, 110.4, 110.3, 110.1, 97.6; HRMS (ESI): Calcd for $C_{19}H_{15}NO_3$, (M+H): 306.11302; Found: 306.11306; Anal. Calcd. (%) For $C_{19}H_{15}NO_3$: C, 74.74; H, 4.95; N, 4.59; Found (%): C, 74.51; H, 5.12; N, 4.66.

2.2d *3,5-Bis(4-methoxystyryl)isoxazole (**4d**)*: Yield 85%; M.p. 143°C; ^{1}H NMR (500 MHz, Acetone-d_6): δ 3.84 (s, 6H, OCH_3), 5.64 (s, 1H, 4-H), 6.64 (d, 2H, 2,6-H, J = 16.0 Hz), 6.86 (d, 2H, ArH, J = 8.0 Hz), 7.14-7.15 (dd, 4H, ArH), 7.36 (s, 2H, ArH), 7.52 (d, 2H, 1,7-H, J = 16.0 Hz); ^{13}C NMR (100 MHz, DMSO-d_6): δ 168.7, 162. 9, 148.1, 147.9, 147.8, 136.3, 134.3, 127.3, 126.1, 121.6, 121.2, 115.6, 115.4, 112.6, 110.5, 110.3, 110.1, 55.71, 55.67; HRMS (ESI): Calcd for $C_{21}H_{19}NO_3$, (M+Na): 356.12626; Found: 356.12628; Anal. Calcd. (%) For $C_{21}H_{19}NO_3$: C, 75.66; H, 5.74; N, 4.20; Found (%): C, 75.52; H, 5.79; N, 4.06.

2.2e *3,5-Bis(3,4-dimethoxystyryl)isoxazole (**4e**)*: Yield 82%; M.p. 16°C; ^{1}H NMR (500 MHz, Acetone-d_6): δ 3.84 (s, 12H, OCH_3), 5.64 (s, 1H, 4-H), 6.64 (d, 2H, 2,6-H, J = 16.0 Hz), 6.86 (d, 2H, ArH, J = 8.0 Hz), 7.14-7.15 (dd, 2H, ArH), 7.36 (s, 2H, ArH), 7.52 (d, 2H, 1,7-H, J = 16.0 Hz); ^{13}C NMR (100 MHz, DMSO-d_6): δ 168.9, 162.1, 148.3, 147.9, 147.1, 136.7, 134.3, 127.3, 126.8, 121.5, 121.2, 115.6, 115.4, 112.6, 110.6, 110.3, 110.1, 55.7, 55.6HRMS (ESI): Calcd for $C_{23}H_{23}NO_5$, (M+Na): 416.14739; Found: 416.14736; Anal. Calcd. (%) For $C_{23}H_{23}NO_5$: C, 70.21; H, 5.89; N, 3.56; Found (%): C, 70.06; H, 6.13; N, 3.83.

2.2f *3,5-Bis(3,4-methylenedioxystyryl)isoxazole (**4f**)*: Yield 82%; M.p. 152°C; ^{1}H NMR (500 MHz, Acetone-d_6): δ 5.55 (s, 1H, 4-H), 5.99 (s, 4H, CH_2), 6.59 (d, 2H, 2,6-H, J = 16.0 Hz), 6.90 (s, 2H, ArH), 7.11 (m, 4H, ArH), 7.52 (d, 2H, 1,7-H, J = 15.5 Hz); ^{13}C NMR (125 MHz, Acetone-d_6): δ 150.1, 148.6, 141.8, 128.1, 123.6, 122.3, 116.2, 111.1, 106.5, 92.6, 91.5; HRMS (ESI): Calcd for $C_{21}H_{15}NO_5$, (M+H): 362.10285; Found: 362.10286; Anal. Calcd. (%) For $C_{21}H_{15}NO_5$: C, 69.80; H, 4.18; N, 3.88; Found (%): C, 69.98; H, 4.39; N, 3.52.

2.2g *3,5-Bis(styryl)isoxazole (**4g**)*: Yield 83%; M.p. 128°C; ^{1}H NMR (500 MHz, Acetone-d_6): δ 5.54 (s, 1H, 4-H), 6.59 (d, 2H, 2,6-H, J = 16.0 Hz), 6.90 (s, 2H, ArH), 7.11 (m, 8H, ArH), 7.52 (d, 2H, 1,7-H, J = 15.5 Hz); ^{13}C NMR (125 MHz, Acetone-d_6): δ 141.1, 133.0, 128.0, 123.4, 122.3, 116.1, 111.6, 108.1; HRMS (ESI): Calcd for $C_{19}H_{15}NO$, (M+Na): 296.10513; Found: 296.10511; Anal. Calcd. (%) For $C_{19}H_{15}NO$: C, 83.49; H, 5.53; N, 5.12; Found (%): C, 83.67; H, 5.75; N, 5.23.

2.3 *Antioxidant capacity assays*

2.3a *DPPH assay*: Methanolic solutions of 3,5-bis (styryl)isoxazoles (**4a**-**g**), at six different concentrations (0.05, 0.1, 0.25, 0.5, 0.75 and 1 mM), were prepared. These test solutions (0.04 mL) containing bis(styryl)isoxazoles (**4a**-**g**) were added to DPPH solution in methanol (10^{-5} M; 2.8 mL). The absorbance was monitored at 517 nm, after the reaction mixture was allowed to stand for 30 min. From the absorbance values monitored, the percentage inhibition was calculated and the result is expressed in terms of EC_{50}. The percentage of inhibition of DPPH by the antioxidant at a particular time was calculated as % inhibition of DPPH = $[(Ab_c - Ab_s)/Ab_c] \times 100$, where Ab_c is the absorbance of the control and Ab_s is the absorbance of the sample.

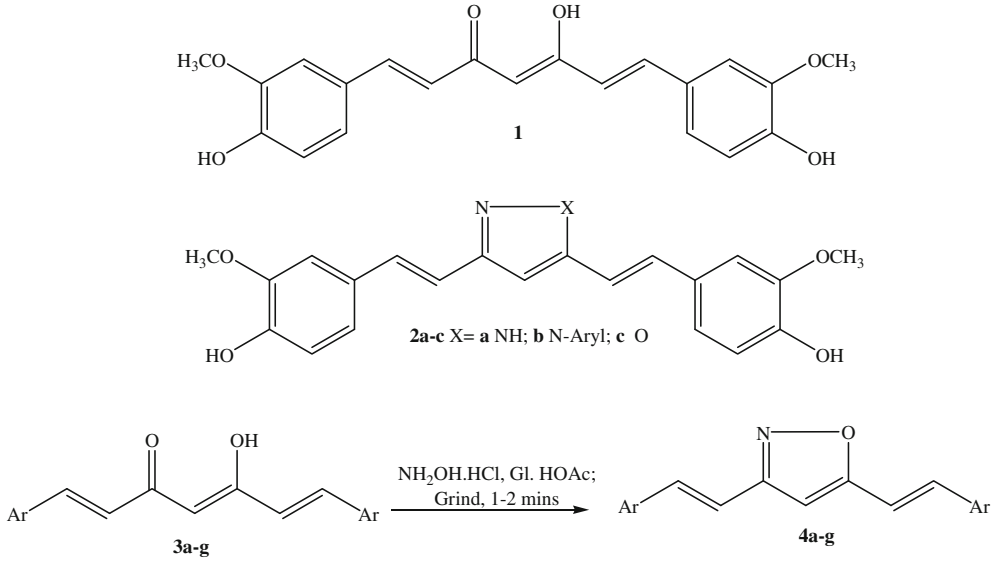
2.3b *Ferric ion reducing antioxidant power (FRAP) assay*: Freshly prepared FRAP reagent was used for the analysis. It was obtained by mixing acetate buffer (300 mM each of acetic acid and sodium acetate), tripyridyltriazine (10 mM in 40 mM aqueous hydrochloric acid solution) and $FeCl_3.6H_2O$ (20 mM) aqueous solutions in the ratio 10:1:1. Methanolic solution of $FeSO_4.6H_2O$ was prepared at different concentrations (0.05, 0.1, 0.25, 0.5, 0.75, 1.0, 1.5 and 2.0 mM). The solutions of bis(styryl)isoxazoles (**4a-g**) were also prepared in methanol (0.5 mM) and after adding these (0.04 mL) to the FRAP reagent (3 mL), the reaction mixtures were incubated at 37°C for 30 min. The absorbance was measured at 596 nm and the results were expressed as micro molar Fe^{2+} equivalents.

2.3c *β-Carotene bleaching assay*: β-Carotene (0.2 mg), linoleic acid (0.02 mL) and Tween 20 (0.18 mL) were mixed with chloroform (0.5 mL) and the solvent was removed by evaporation. The residue obtained was mixed with distilled water (50 mL) and the resulting emulsion (4 mL aliquots each) was added to 3,5-bis(styryl)isoxazoles (**4a-g**) in methanol (0.2 mL, 1 mmol). Absorbance was measured at 470 nm, using a mixture of the emulsion (4 mL) and methanol (0.2 mL) as the control. The absorbance was again recorded after incubation for 1 h at 50°C in the dark. The antioxidant activity (AA) was evaluated in terms of bleaching of the β-carotene using the formula $AA = [1 - (A_o - A_t)/(A_o^o - A_t^o)] \times 100$, where A_o and A_o^o are the absorbance values measured at zero time of the incubation for test sample and control, respectively. A_t and A_t^o are the absorbance values of the sample and control after incubation time.

3. Results and Discussion

3.1 *Synthesis*

The precursor curcuminoids (**3a-g**) were obtained by microwave assisted synthetic protocol[23] by the modification of a conventional thermal method developed earlier by our group.[14] Sui *et al.*, seemingly in the first report on the synthesis of an isoxazole derivative (**2c**) of a curcuminoid, had reacted curcumin I (**1**) with hydroxylamine hydrochloride in ethanol under reflux for 16 h.[13] The methods subsequently reported for this reaction could be seen to generally take 6–40 h for the completion under conventional heating.[5–9] In continuation to our work on mechanochemical synthesis of curcumin derivatives,[19] we have now reacted curcuminoids (**3a-g**) with hydroxylamine hydrochloride in presence of catalytic amount of acetic acid. The room temperature mechanical grinding in an agate mortar for about 1–2 minutes rapidly afforded the 3,5-bis(styryl)isoxazoles (**4a-g**) in powder form. We observed that the compounds obtained in better purity and yield than reported earlier, as judged by TLC. The formation and identity of these isoxazoles were confirmed by physical and spectral methods. It appears that the initially formed oxime derivatives proceeded to cyclize affording the isoxazole ring (scheme 1). Among the 3,5-bis(styryl)isoxazoles



Scheme 1. Structures of curcumin I (**1**) and its 3,5-bis(styryl)azole derivatives.

(**4a-g**) now synthesized, five examples (**4b**, **4c**, **4d**, **4f** and **4g**) appear to be hitherto unreported.

3.2 *Antioxidant Studies*

The antioxidant (AO) activities of the 3,5-bis(styryl) isoxazoles (**4a-g**) were evaluated by 2,2-diphenylpicrylhydrazyl (DPPH), ferric ion reducing antioxidant power (FRAP) and β-carotene assays. The AO activity by the DPPH assay is expressed in terms of EC_{50} values, calculated by plotting the percentage inhibition of DPPH against various test concentrations (figure 1, table 1). We have earlier shown that the isoxazole derivative of curcumin I (**2c**) exhibits better AO capacity than the parent curcumin I (**1**) in DPPH assay under similar experimental conditions;[19] the corresponding EC_{50} values for the percentage inhibition of DPPH being 8 ± 0.011 μmol and 40 ± 0.06 μmol, respectively for (**2c**) and (**1**). A comparison of this data with those obtained now for the 3,5-bis(styryl)isoxazoles (**4a-g**) shows that the isoxazoles (**4a-g**) are less active than the isoxazole derivative of curcumin I (**2c**). However, two among these, **4a** and **4b**, are more active than curcumin I (**1**). Thus, the presence of a hydroxy group juxtaposed with a methoxy group on the terminal aryl rings enhances the AO activity of curcuminoid derived isoxazoles.

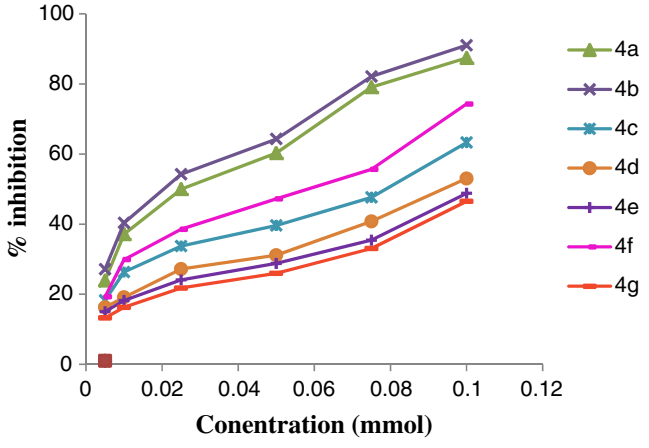


Figure 1. Percentage inhibition of DPPH (10^{-2} mM) at various concentrations of 3,5-bis(styryl)isoxazoles (**4a-g**).

Table 2. The $FeSO_4$ equivalent of 3,5-bis(styryl)isoxazoles (**4a-g**) in the presence of FRAP reagent and the Antioxidant Activity (AA) % of **4a-g** in β–carotene assay.

Entry	$FeSO_4$ equivalents (μmol)	AA %
4a	1182	87.59
4b	1195	90.91
4c	997	76.99
4d	883	69.52
4e	592	65.31
4f	1036	80.23
4g	475	61.12

The results of FRAP assay are expressed as micromolar Fe^{2+} equivalents (table 2); a higher value of ferrous sulphate equivalent being indicative of a better AO capacity. Our earlier sttudies[19] have shown that under identical assay conditions, the curcumin I derived isoxazole (**2c**) and curcumin I (**1**) showed Fe^{2+} equivalent of 1304 μmol and 1164 μmol, respectively. The present results based on FRAP assay is now seen to follow a similar pattern as seen in the DPPH assay, wherein the presence of a hydroxy group along with a methoxy group on the terminal aryl rings is found to be beneficial. A similar conclusion could now be drawn based on the β-carotene bleaching assay of the 3,5-bis(styryl)isoxazoles (**4a-g**) (table 2) and in the light of our earlier study.[19]

4. Conclusions

We report the synthesis of curcuminoid-derived 3,5-bis(styryl)isoxazoles (**4a-g)** by mechanochemical grinding of the respective curcuminoid and hydroxylamine hydrochloride catalyzed by gl. acetic acid. The anti-oxidant studies of **4a-g** revealed that hydroxy and methoxy groups present in the terminal aryl moieties of 3,5-bis(styryl)isoxazoles improve their anti-oxidant activity.

Table 1. Percentage inhibition of DPPH (10^{-2} mmol) by 3,5-bis(styryl)isoxazoles (**4a-g**).

	Percentage inhibition by 3,5-bis(styryl)pyrazoles (**4a-g**) at various concentrations (mM)						
Entry	0.005	0.01	0.025	0.05	0.075	0.1	EC_{50} (μmol)
4a	23.91 ± 0.21	37.15 ± 0.06	49.98 ± 0.31	60.29 ± 0.18	79.08 ± 0.12	87.42 ± 0.16	28 ± 0.19
4b	27.12 ± 0.18	40.36 ± 0.11	54.25 ± 0.22	64.29 ± 0.37	82.13 ± 0.19	91.05 ± 0.16	20 ± 0.11
4c	18.28 ± 0.23	26.32 ± 0.09	33.74 ± 0.16	39.62 ± 0.22	47.64 ± 0.07	63.29 ± 0.34	80 ± 0.48
4d	16.27 ± 0.16	19.11 ± 0.23	27.18 ± 0.17	31.13 ± 0.25	40.77 ± 0.06	53.02 ± 0.14	96 ± 0.52
4e	15.03 ± 0.29	18.20 ± 0.18	24.09 ± 0.08	28.78 ± 0.24	35.43 ± 0.17	48.78 ± 0.09	102 ± 0.49
4f	19.26 ± 0.33	29.88 ± 0.17	38.56 ± 0.24	47.23 ± 0.29	55.64 ± 0.07	74.28 ± 0.13	60 ± 0.31
4g	13.26 ± 0.11	16.27 ± 0.21	21.78 ± 0.09	25.98 ± 0.12	33.06 ± 0.19	46.52 ± 0.17	108 ± 0.22

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References

1. Esatbeyoglu T, Huebbe P, Ernst I M, Chin D, Wagner A E and Rimbach G 2012 *Angew. Chem. Int. Ed. Engl.* **51** 5308
2. Gupta S C, Prasad Y, Kim J H, Patchva S, Webb L J, Priyadarsini I K and Aggarwal B B 2011 *Nat. Prod. Rep.* **28** 1937
3. Teiten M-H, Eifes S, Dicato M and Diederich M 2010 *Toxins* **2** 128
4. Anand P, Kunnumakkara A B, Newman R A and Aggarwal B B 2007 *Mol. Pharm.* **4** 807
5. Narlawar R, Pickhardt M, Leuchtenberger S, Baumann K, Krause S, Dyrks T, Weggen S, Mandelkow E and Schmidt B 2008 *Chem. Med. Chem.* **3** 165
6. Mishra S, Karmodiya K, Surolia N and Surolia A 2008 *Bioorg. Med. Chem.* **16** 2894
7. Changtam C, Hongmanee P and Suksamrarn A 2010 *Eur. J. Med. Chem.* **45** 4446
8. Amolins M W, Peterson L B and Blagg B S J 2009 *Bioorg. Med. Chem.* **17** 360
9. Das J, Pany S, Panchal S, Majhi A and Rahman G M 2011 *Bioorg. Med. Chem.* **19** 6196
10. Liu Y, Dargusch R, Maher P and Schubert D 2008 *J. Neurochem.* **105** 1336
11. Maher P, Akaishi T, Schubert D and Abe K 2010 *Neurobiol. Aging* **31** 706
12. Mayadevi M, Sherin D R, Keerthi V S, Rajasekharan K N and Omkumar R V 2012 *Bioorg. Med. Chem.* **20** 6040
13. Sui Z, Salto R, Li J, Craik C and Montellano P R O 1993 *Biorg. Med. Chem.* **1** 415
14. Babu K V D and Rajasekharan K N 1994 *Org. Prep. Procedures Intl.* **26** 674
15. Ruby A J, Kuttan G, Babu K V D, Rajasekharan K N and Kuttan R 1995 *Cancer Lett.* **94** 79
16. Devasena T, Rajasekharan K N and Menon V P 2002 *Pharmcol. Res.* **46** 39
17. Kiran M S, Sameerkumar V B, Viji R I, Sherin G T, Rajasekharan K N and Sudhakaran P R 2008 *J. Cell Physiol.* **215** 251
18. Manju M, Sherin G T, Rajasekharan K N and Oommen O V 2009 *Fish Physiol. Biochem.* **35** 413
19. Sherin D R and Rajasekharan K N 2015 *Arch. Pharm.* **348** 1
20. Ak T and Gulcin I 2008 *Chemico-Biol. Inter.* **174** 27
21. Chakraborti S, Dhar G, Dwivedi V, Das A, Poddar A, Chakraborti G, Basu G, Chakrabarti P, Surolia A and Bhattacharyya B 2013 *Biochemistry* **52** 7449
22. Puneeth H R and Chandrashekariah S A 2015 *Int. J. Pharm. Pharm. Sci.* **7** 244
23. Sherin D R, Thomas S G and Rajasekharan K N 2015 *Het. Commun.* **21** 381