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Synthesis and evaluation of electron-rich curcumin analogues

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ABSTRACT

The natural product curcumin has long been recognized for its medicinal properties and is utilized for the treatment of many diseases. However, it remains unknown whether this activity is based on its presumably promiscuous scaffold, or if it results from the Michael acceptor properties of the α,β-unsaturated 1,3-diketone moiety central to its structure. To probe this issue, electron-rich pyrazole and isoxazole analogues were prepared and evaluated against two breast cancer cell lines, which resulted in the identification of several compounds that exhibit low micromolar to mid nanomolar anti-proliferative activity. A conjugate addition study was also performed to compare the relative electrophilicity of the diketone, pyrazole and isoxazole analogues.

1. Introduction

Turmeric has been utilized for centuries in Eastern medicine as a topical treatment for wounds, inflammation, and tumors.[1] The active component was identified as curcumin (**1**, Fig. 1), and its isolation from the rhizome of *Curcuma longa L. (Zingiberaceae)* over two centuries ago eventually allowed for structural identification and testing against various diseases.[2] Properties exhibited by curcumin include anti-inflammatory, anti-oxidant, anti-viral, cutaneous wound healing, hypocholesterolemic effects in diabetic patients, anti-angiogenic, and stimulatory response to stress-induced biological activity.[3,4] Curcumin has demonstrated preventative activity against Aβ aggregation in Alzheimer's models[5], and several studies have shown that curcumin manifests anti-proliferative activity against various cancers, including leukemia, colon, liver, breast, and prostate cancers.[4,6–8] However, the truly unique feature of this molecule is its lack of toxicity. Large quantities of curcumin can be consumed without toxicity, suggesting this molecule may serve as a valuable scaffold for therapeutic development. Three phase I clinical trials have demonstrated tolerances as high as 12 g per day.[9,10] These distinctive properties make curcumin a valuable lead compound for drug development, and it remains the focus of several clinical trials.[4,11]

The ability of curcumin to exhibit such versatility can be attested to the large number of biological targets that are affected in response to administration of this compound. Anti-inflammatory activity has been ascribed to result from curcumin's ability

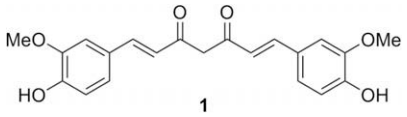

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Figure 1. Curcumin.

to hinder pathways responsible for inflammatory cascades through down regulation of the nuclear transcription factor-κB (NF-κB), activator protein-1 (AP-1), and by decreasing levels of prostaglandin E_2 synthase (PGE_2).[3,12] Curcumin manifests anti-oxidant activity by scavenging superoxides and hydrogen peroxide, and by decreasing levels of nitric oxide synthase activity through its electron rich, aromatic appendages composed of masked catechols.[3,13] Its anti-viral activity is most notably recognized by its ability to inhibit type I Human Immunodeficiency Virus (HIV), and its wound healing properties result from the increased biosynthesis of TGF-β1.[3] Curcumin also promotes apoptosis in cancer cells by suppressing cyclin D and endothelial growth factor receptor (EGFR) function, while simultaneously decreasing levels of phosphokinase B (AKT), c-myc, and phospho-AKT (pAKT).[13]

In total, there are 97 previously reported biological targets for curcumin, ranging from transcription factors to various enzymes and receptors.[4] However, it is not known whether these activities are due to covalent modification of targets through its Michael acceptor properties or general promiscuity resulting from the moderately simple scaffold. It has been postulated that conversion of the α,β-unsaturated 1,3-diketone moiety into an electron-rich ring system would lessen the potential for nucleophilic addition.[14] Therefore, it was proposed that modification of the 1,3-diketone into a comparatively electron-rich isoxazole moiety that contains

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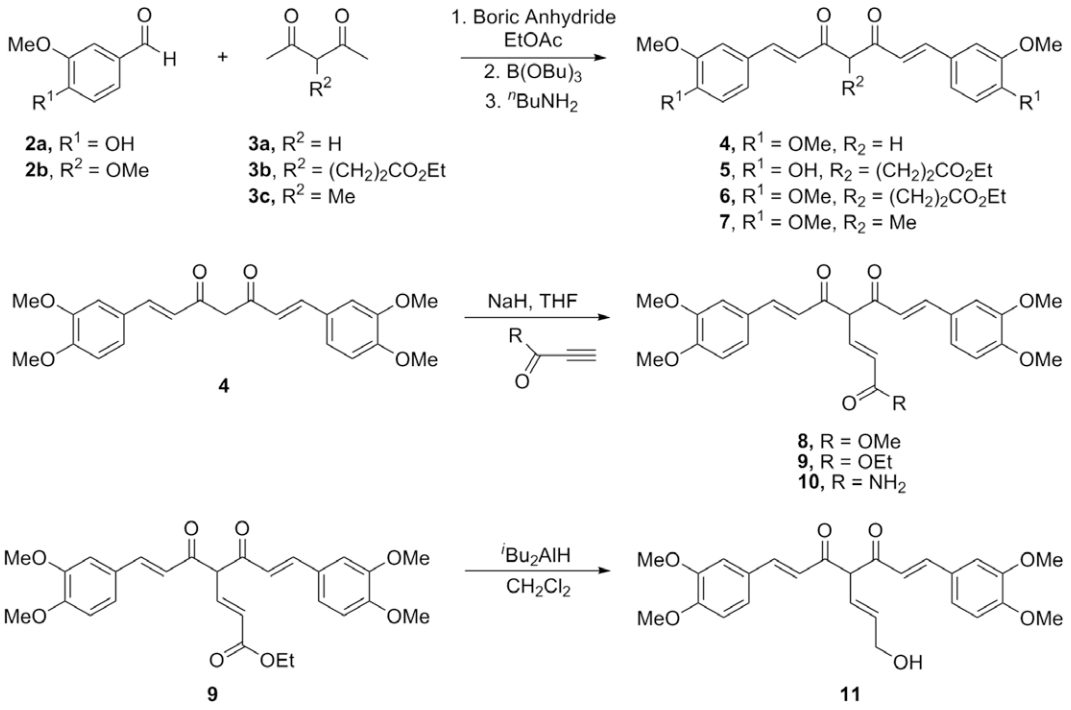


Scheme 1. Synthesis of 1,3-diketone angalogues.

two adjacent hydrogen bond acceptors in similar proximity to the original ketones would accomplish this objective. Furthermore, since the 1,3-diketone can tautomerize into the corresponding enol[15], it was proposed that a pyrazole aromatic nucleus would represent an electron-rich mimetic of this hydrogen-bonding network and also serve to suppress nucleophilic addition to the adjacent, conjugated olefins. Identification of previously reported curcumin analogues that exhibit increased anti-proliferative,[16,17] anti-malarial,[18] anti-oxidant, and anti-inflammatory activity,[19] as well as improved anti-neurodegenerative properties[20,21] provided the basis for the heteroaromatic species reported in this article.

2. Results and discussion

2.1. Synthesis of 1,3-diketone analogues

Chemistry developed by Lin et al.[16,17] was utilized and expanded upon to prepare 1,3-diketone analogues for the purpose of determining structure–activity relationships (Scheme 1). A complex was formed between boric anhydride and 2,4-pentanedione, facilitating a 1,5-bis-aldol condensation with 3,4-dimethoxybenzaldehyde to afford dimethylcurcumin, **4**. Similar methodology was used to derive compounds **5**, **6**, and **7** from their respective 3,4-disubstituted benzaldehydes and 3-substituted pentanediones. Compounds **8**, **9**, and **10** were obtained from dimethylcurcumin (**4**) via Michael addition with methyl propiolate, ethyl propiolate, and propiolamide, respectively. Reduction of the α,β-unsaturated ethyl ester **9** with diisobutyl aluminum hydride at −78 °C produced the allylic alcohol **11**, in good yield.

2.2. Synthesis of pyrazole and isoxazole analogues

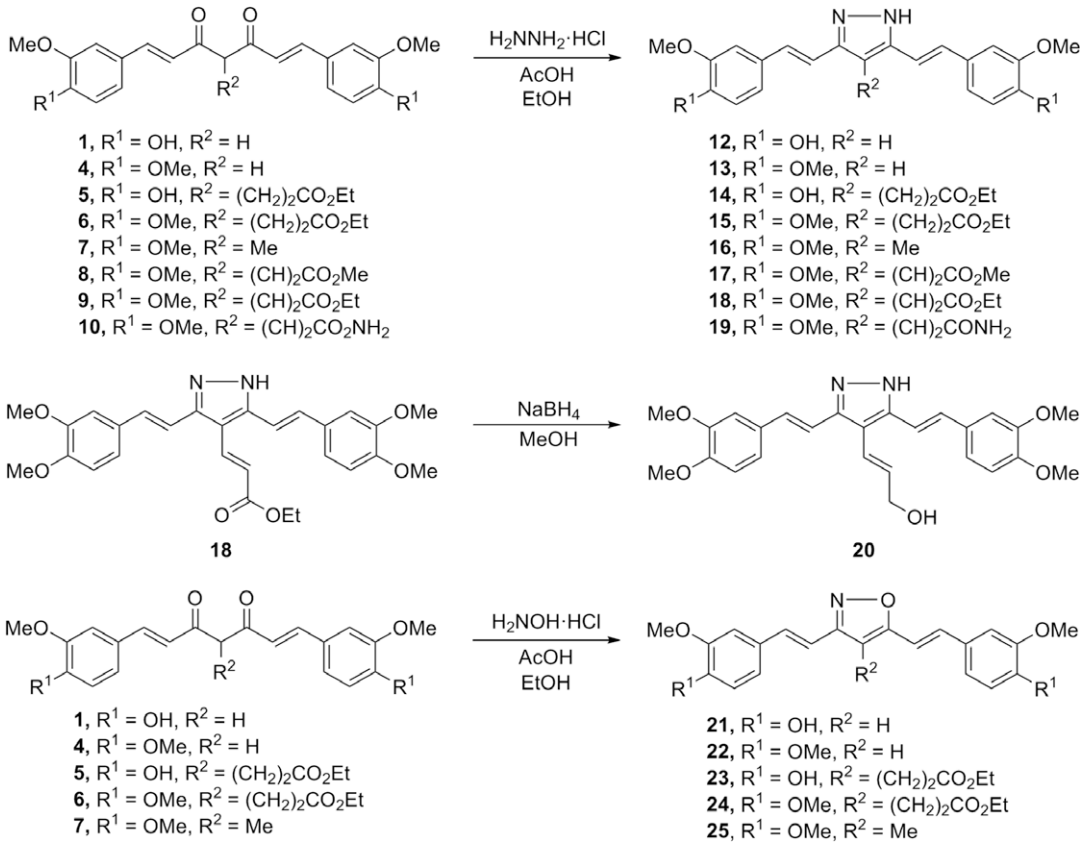
The completed diketone species were used to prepare pyrazole analogues through an acid-catalyzed condensation with hydrazine hydrochloride, while stirring at reflux for forty hours. The isoxazole analogues were prepared in similar fashion enlisting hydroxylamine hydrochloride (Scheme 2). After discovering the instability of allylic alcohol **11** to these cyclization conditions, an alternative pathway was utilized in which the α,β-unsaturated ethyl ester analogue (**9**) was first cyclized, and subsequently reduced with sodium borohydride to give the cyclized allylic alcohol, **20**.

2.3. Electrophilicity of curcumin and analogues

To compare the electrophilicity of curcumin to the electron-rich pyrazole and isoxazole analogues, 1,4-conjugate addition of benzyl mercaptan to the benzylic olefins was monitored over time by ^{1}H NMR. After incubating an equimolar mixture of curcumin and benzyl mercaptan for 1 h, new aliphatic protons began to appear at δ2.96 and δ4.17 (Fig. 2), indicative of conjugate addition. Additionally, the appearance of a multiplet at δ3.57 was attributed to the α-methylene group adjacent to sulfur in the conjugated product. Furthermore, two singlets appeared at δ3.76 and δ3.83 corresponding to the disulfide product of benzyl mercaptan and the desymmetrization of the curcumin scaffold, respectively.

Quantitative addition to the olefin was not observed due to competing disulfide formation, however, integration of the emerging aliphatic protons with respect to the aromatic methoxy protons (Table 1) indicated 53% addition after 24 h. Additionally, mass spectrometry data provided evidence that addition of benzyl mercaptan to curcumin resulted in both mono- and di-conjugate-addition products, as masses were observed for both products. In contrast, when the related isoxazole and pyrazole analogues were incubated with benzyl mercaptan for 48 h, no change in the ^{1}H NMR was observed (see Supporting Information), indicating that these species were not as susceptible to nucleophilic attack by benzyl mercaptan.

This observation can be explained by the electron rich and heteraromatic nature of the pyrazole and isoxazole rings as compared to the diketone species.[22] The benzylic olefin is less susceptible to Michael addition due to decreased electrophilicity and because the mechanism by which such a transformation occurs involves a non-aromatic intermediate. The electron-rich and heteroaromatic properties of the pyrazole and isoxazole rings suggest these compounds



Scheme 2. Synthesis of pyrazole and isoxazole analogues.

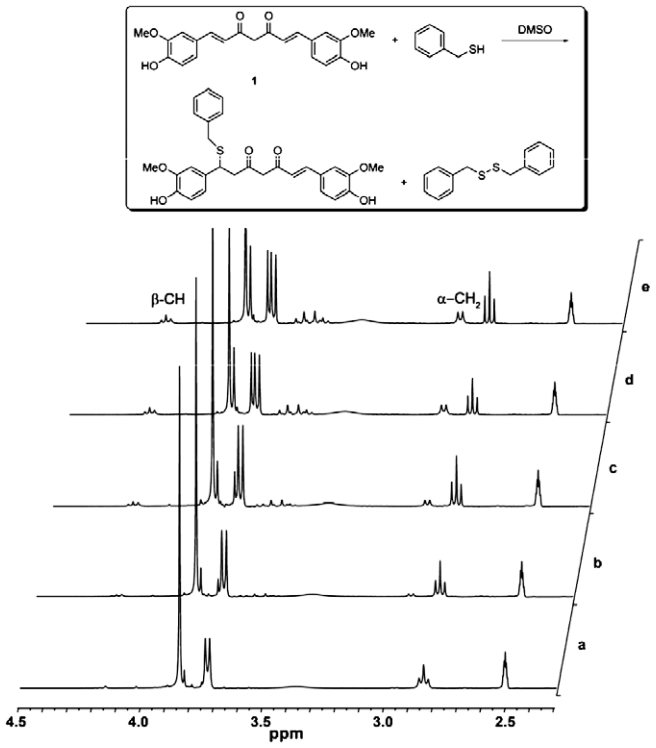


Figure 2. [1]H NMR spectra for the addition of benzyl mercaptan to curcumin (a) 10 min (b) 1 h (c) 5 h (d) 14 h (e) 24 h after addition.

are less electrophilic and therefore, less promiscuous than curcumin as a "general" alkylating agent within the cell.

Table 1
Relative integration[a] of protons resulting from 1,4-conjugate addition to curcumin.

Elapsed time	δ 2.96 (d, J = 7.7, α-C**H**$_2$)	δ 4.16 (t, J = 7.8, β-C**H**)
10 min	0.08	0.03
1 h	0.22	0.12
5 h	0.37	0.21
14 h	0.91	0.56
24 h	1.06	0.62

[a] Integrations relative to aromatic OCH_3 substituents (6H).

2.4. Growth inhibitory activity by curcumin and analogues

The data shown in Table 2 represent the growth inhibitory activity of curcumin and its analogues against MCF-7 (ER+) and SKBr-3 (ER-, HER2 overexpressing) breast cancer cell lines. The results indicate that compounds **4**, **7**, **8**, **9**, and **11** represent the most potent analogues, manifesting submicromolar to low micromolar IC_{50} values against both cell lines. Upon analysis of the anti-proliferation data for these analogues, it is evident that in most cases, IC_{50} values for the parent dione are better than the pyrazole and isoxazole analogues. This can be attributed to the promiscuity of the α,β-unsaturated ketone, as well as conformational differences between the 6-membered hydrogen-bonding network created by the enol form of the diketone species and the rigid, more conformationally constrained 5-membered ring derivatives. Additionally, the pyrazole analogues exhibited lower IC_{50} values than the corresponding isoxazole analogues, highlighting the potential significance of a hydrogen bond donor on the pyrazole ring and its related nature to the corresponding enol form of the diketone species. Another interesting observation is the difference in IC_{50} values between analogues with 3,4-dimethoxy substitution on each of the

Table 2
Anti-proliferative activities of curcumin and analogues[a].

Compound	MCF-7 (IC_{50}, μM)[b]	SKBR3 (IC_{50}, μM)
1	5.58 ± 0.97	2.11 ± 0.09
4	1.07 ± 0.05	0.955 ± 0.145
5	1.96 ± 0.35	4.38 ± 1.18
6	>100	>100
7	1.09 ± 0.03	1.02 ± 0.05
8	0.512 ± 0.004	0.564 ± 0.020
9	1.28 ± 0.14	1.96 ± 0.65
10	5.35 ± 0.28	9.83 ± 1.12
11	0.796 ± 0.067	1.24 ± 0.58
12	4.19 ± 1.59	0.258 ± 0.034
13	12.7 ± 0.3	1.58 ± 0.46
14	6.01 ± 0.39	5.60 ± 1.24
15	10.1 ± 0.7	11.7 ± 0.5
16	8.16 ± 3.73	>100
17	49.5 ± 2.9	>100
18	11.4 ± 0.5	5.99 ± 0.61
19	39.9 ± 2.6	52.6 ± 7.8
20	>100	>100
21	1.76 ± 0.39	4.00 ± 0.13
22	59.5 ± 6.5	>100
23	13.2 ± 1.6	13.9 ± 1.9
24	>100	>100
25	55.7 ± 5.7	42.5 ± 12.2

[a] Values represent mean ± standard error for at least two separate experiments performed in triplicate.
[b] IC_{50} is defined as the concentration of compound necessary to inhibit cellular growth by 50%.

aromatic rings and the analogues with the 3-methoxy-4-hydroxy substitution, which is similar to the natural product. The diketone species with a 3,4-dimethoxy substitution generally manifested lower IC_{50} values than the analogues that contain phenols. In contrast, the pyrazole and isoxazole derivatives exhibited the opposite trend, as the phenolic analogues exhibited lower IC_{50} values than analogues with dimethoxy substitution. This can be attributed to the electronic effect induced by the pyrazole and isoxazole rings, which impacts the hydrogen bond capability and pK_a of the phenols as compared to the diketo species.[23] Finally, the difference in IC_{50} values between the diketo species with substitutions at the α position indicates that an unsaturated and oxygen-containing moiety generally lowers the IC_{50} value, as observed with compounds **8** and **11**. Compound **6**, containing 3,4-dimethoxy substitution and a saturated R^2 substituent (Scheme 2) displayed no anti-proliferative activity at the concentrations tested. Further SAR is required to elucidate the origin of this effect as no other compounds in this library shared this group of functionalities. It should be noted that similar compounds bearing the unsaturated amide substituent also manifested decreased activity, indicating that nitrogen-bearing substituents may be detrimental to their anti-proliferative activity. In summary, it was observed that the most active analogues were those that contained a 3,4-dimethoxy aromatic substitution and unsaturated, oxygen-containing substituents flanking the α position, which exhibited low to sub micromolar IC_{50} values.

3. Conclusion

We have completed a library of curcumin analogues in an effort to elucidate structure–activity relationships, as well as to determine the relevance of curcumin's Michael acceptor properties and its ability to act as an anti-cancer agent. Anti-proliferative data from two breast cancer cell lines were obtained, which led to identification of several compounds that exhibit increased growth inhibitory activity versus the natural product. Furthermore, it was determined that the Michael acceptor properties were not critical to retention of inhibitory activity for curcumin and analogues. In fact, both the isoxazole and pyrazole analogues displayed only slightly lower activities than the related diketone species, with the pyrazole species manifesting slightly better activity than the related isoxazole. These studies highlighted the importance of the hydrogen-bonding network demonstrated by curcumin in its enol form, which corresponds favorably to pyrazoles H-bond donor/acceptor moieties. Curcumin analogues with diminished electrophilicity that maintain anti-proliferative activity are exciting and potentially viable targets for future drug development.

4. Experimental

4.1. (1*E*,16*E*)-1,7-Bis(3,4-dimethoxyphenyl)hepta-1,6-diene-3,5-dione (4)

2,4-Pentanedione (0.32 mL, 3.0 mmol) and boric anhydride (0.15 g, 2.1 mmol) were dissolved in EtOAc (3.0 mL). The solution was stirred for 30 min at 40 °C before 3,4-dimethoxybenzaldehyde (1.0 g, 6.0 mmol) and tributyl borate (1.64 mL, 6.0 mmol) were added and stirred for 30 min at 40 °C. *n*-Butylamine (0.45 mL, 4.5 mmol) was dissolved in EtOAc (3.0 mL), and added dropwise over 15 min. The reaction mixture stirred for 24 h at 40 °C, at which point it was quenched by the addition of 4 N HCl (10 mL). The mixture was stirred at 60 °C for 30 min, and then the aqueous layer extracted with EtOAc (3× 50 mL). The combined organic layers were washed with saturated aqueous $NaHCO_3$ and saturated aqueous NaCl, dried (Na_2SO_4), filtered, and concentrated. The residue was purified by flash chromatography (SiO_2, 2:3 EtOAc in hexanes, then 3:2 EtOAc in hexanes) to afford **4** (438.1 mg, 37%) as a yellow amorphous solid: ^{1}H NMR ($CDCl_3$, 500 MHz) δ 3.86 (d, *J* = 6.20 Hz, 12H), 5.76 (s, 1 H), 6.43 (d, *J* = 15.8 Hz, 2H), 6.81 (d, *J* = 8.35 Hz, 2H), 7.01 (d, *J* = 1.85 Hz, 2H), 7.08 (dd, *J* = 1.90 Hz, 8.30 Hz, 2H), 7.54 (d, *J* = 15.8 Hz, 2H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 55.9 (2C), 56.0 (2C), 77.3, 101.4 (2C), 109.6 (2C), 111.1 (2C), 122.0, 122.7, 128.0 (2C), 140.4 (2C), 149.2 (2C), 151.0 (2C), 183.3 (2C); IR (film) ν_{max} 3001, 2959, 2934, 2912, 2837, 1624, 1582, 1556, 1512, 1462, 1454, 1441, 1421, 1339, 1300, 1263, 1232, 1198, 1159, 1136, 1022, 966 cm^{-1}; HRMS (ES^+) *m*/*z*: [M+H] calcd for $C_{23}H_{24}O_6$ 397.1651; found 397.1640.

4.2. (*E*)-Ethyl 7-(4-hydroxy-3-methoxyphenyl)-4-((*E*)-3-(4-hydroxy-3-methoxyphenyl)acryloyl)-5-oxohept-6-enoate (5)

Compound **5** was prepared following the same procedure used for the preparation of **4**. The residue was purified by flash chromatography (SiO_2, 5:3:2Hexanes:EtOAc:CH_2Cl_2) to afford **5** (490.0 mg, 32%) as an orange amorphous solid: ^{1}H NMR ($CDCl_3$, 500 MHz) δ 1.18 (t, *J* = 9.5 Hz, 3H), 2.48 (t, *J* = 8 Hz, 2H), 2.87 (t, *J* = 9.5 Hz, 2H), 3.90 (s, 6H), 4.06 (q, *J* = 7.15 Hz, 2H), 5.82 (s, 2H), 6.87 (s, 1 H), 6.88–6.89 (m, 2H), 6.92 (s, 1 H), 7.02 (d, *J* = 2Hz, 2H), 7.10 (d, *J* = 8.25 Hz, 2H), 7.64 (d, *J* = 15.3 Hz, 2H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 14.2, 21.7, 36.6, 56.0 (2C), 60.7, 109.5, 110.1 (2C), 114.9 (2C), 117.8 (2C), 122.9 (2C), 128.0 (2C), 142.2 (2C), 146.8 (2C), 147.9 (2C), 173.0, 182.9 (2C); IR (film) ν_{max} 3400, 3074, 2961, 2926, 2852, 2400, 1722, 1663, 1618, 1587, 1514, 1454, 1429, 1393, 1377, 1275, 1207, 1186, 1161, 1124, 1101, 1032, 976, 945 cm^{-1}; HRMS (ES^+) *m*/*z*: [M+Na] calcd for $C_{26}H_{28}O_8$ 491.1682; found 491.1677.

4.3. (*E*)-Ethyl-7-(3,4-dimethoxyphenyl)-4-((*E*)-3-(3,4-dimethoxyphenyl)acryloyl)-5-oxohept-6-enoate (6)

Compound **6** was prepared following the same procedure used for the preparation of **4**. The residue was purified by flash chroma-

tography (SiO_2, 5:3:2Hexanes:EtOAc:CH_2Cl_2) to afford **6** (351.1 mg, 23%) as a yellow amorphous solid: ^{1}H NMR ($CDCl_3$, 500 MHz) As a mixture of tautomers: δ 1.26 (t, J = 7.2 Hz, 3H), 1.26 (t, J = 7.2Hz, 3H), 2.36 (t, J = 6.9 Hz, 2H), 2.40 (t, J = 6.9, 2H), 2.57 (t, J = 7.4 Hz, 2H), 2.97 (t, J = 7.3 Hz, 2H), 3.93 (12H), 3.97 (12H), 4.15 (q, J = 7.1 Hz, 2H), 4.15 (q, J = 7.1 Hz, 2H), 4.26 (t, J = 7.1 Hz, 1H), 6.73 (d, J = 15.9 Hz, 2H), 6.87 (d, J = 8.3 Hz, 2H), 6.90 (d, J = 8.3 Hz, 2H), 7.01 (d, J = 15.3 Hz, 2H), 7.07 (d, J = 1.8 Hz, 2H), 7.14 (d, J = 1.7 Hz, 2H), 7.16 (dd, J_1 = 8.4 Hz, J_2 = 1.8 Hz, 2H), 7.20 (dd, J_1 = 8.4 Hz, J_2 = 1.6 Hz, 2H), 7.67 (d, J = 15.9 Hz, 2H), 7.74 (d, J = 15.3 Hz, 2H); ^{13}C NMR ($CDCl_3$, 125 MHz) As a mixture of tautomers δ 14.1, 14.2, 23.3, 29.7, 31.6, 36.6, 56.0 (4C), 60.6 (2C), 63.5, 108.9, 109.6, 109.9, 110.2, 110.4, 111.0 (2C), 111.1 (2C), 118.0, 121.7 (2C), 122.7 (2C), 123.9, 126.9 (2C), 127.1 (3C), 128.4 (2C), 142.1, 145.0, 149.2 (4C), 151.1 (2C), 151.8 (2C), 172.9 (2C), 182.9, 190.9, 194.9 (2C); IR (film) ν_{max} 3061, 2959, 2930, 2853, 2617, 2357, 2029, 1732, 1681, 1666, 1639, 1593, 1574, 1556, 1514, 1504, 1454, 1444, 1421, 1377, 1339, 1308, 1263, 1231, 1186, 1161, 1138, 1109, 1022, 984, 945 cm^{-1}; HRMS (ES$^+$) m/z: [M+Na] calcd for $C_{28}H_{32}O_8$ 519.1995; found 519.1944.

4.4. (1*E*,6*E*)-1,7-Bis(3,4-dimethoxyphenyl)-4-methylhepta-1,6-diene-3,5-dione (7)

Compound **7** was prepared following the same procedure used for the preparation of **4**. The residue was purified by flash chromatography (SiO_2,1:1Hexanes:EtOAc) to afford **7** (573.7 mg, 44%) as a dark yellow amorphous solid: ^{1}H NMR ($CDCl_3$, 400 MHz) As a mixture of tautomers: δ 1.51(d, J = 6.9 Hz, 3H), 2.19(s, 3H), 3.92–3.96(24H), 6.72(d, J = 15.9 Hz, 2H), 6.89(d, J = 8.4 Hz, 2H), 6.99(d, J = 15.3 Hz, 2H), 7.06(d, J = 1.68, 2H), 7.10(s, 2H), 7.16(dd, J_1 = 8.5, J_2 = 1.9, 2H), 7.19(d, J = 8.2, 2H), 7.43(s, 1H), 7.48(dd, J_1 = 8.1, J_2 = 2.2, 1 H), 7.65(d, J = 16.0 Hz, 2H), 7.70(d, J = 15.4, 2H); ^{13}C NMR ($CDCl_3$, 125 MHz) As a mixture of tautomers δ 12.1 29.7, 55.9 (4C), 56.0 (4C), 59.0, 105.7, 109.9, 110.0, 110.1, 110.4, 111.0, 111.0, 111.1, 111.2, 116.6, 118.7 (2C), 121.4 (2C), 122.5, 123.7, 124.4, 127.1 (2C), 128.5, 141.3, 144.7, 146.2 (2C), 149.2 (2C), 149.2 (2C), 151.0 (2C), 151.7, 152.0, 182.5, 191.0, 196.1, 196.8; IR (film) ν_{max} 3412, 3059, 2999, 2959, 2928, 2853, 2839, 2613, 2598, 2536, 2359, 2035, 1713, 1668, 1591, 1514, 1464, 1454, 1443, 1421, 1377, 1340, 1308, 1265, 1238, 1198, 1161, 1140, 1107, 1045, 1024, 982, 947, 924 cm^{-1}; HRMS (ES$^+$) m/z: [M+H] calcd for $C_{24}H_{26}O_6$ 411.1808; found 411.1800.

4.5. (2*E*,6*E*)-Methyl-7-(3,4-dimethoxyphenyl)-4-((*E*)-(3,4-dimethoxyphenyl)acryloyl)-5-oxohepta-2,6-dienoate (8)

Compound **4** (500.0 mg, 1.26 mmol) was dissolved in THF (6.0 mL) and stirred at rt before NaH (45.4 mg, 60% wt in mineral oil, 1.17 mmol) was added. The solution was stirred for 30 min at rt. In a separate flask, methyl propiolate (0.17 mL, 1.95 mmol) was dissolved in THF (9.0 mL) and stirred at room temperature. Contents from the first flask were slowly added via cannula to the methyl propiolate solution over 10 min, and subsequently stirred at rt for 24 h. The reaction was quenched by addition of 5% sulfuric acid (25 mL) and the aqueous layer extracted with EtOAc (3× 50 mL). The combined organic layers were washed with saturated aqueous $NaHCO_3$ and saturated aqueous NaCl, dried (Na_2SO_4), filtered, and concentrated. The residue was purified by flash chromatography (SiO_2, 2:3 EtOAc in hexanes, then 3:2 EtOAc in hexanes) to afford **8** (291.0 mg, 53%) as an orange amorphous solid: ^{1}H NMR ($CDCl_3$, 500 MHz) δ 3.85 (s, 3H), 3.96 (d, J = 1.65, 12H), 6.00 (d, J = 11.7 Hz, 1H), 6.92 (d, J = 11.7 Hz, 2H), 7.00 (d, J = 11.6 Hz, 2H), 7.09 (s, 2H), 7.22 (d, J = 6.15 Hz, 2H), 7.79 (d, J = 11.6 Hz, 2H), 7.93 (d, J = 11.7 Hz, 1H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 51.8, 56.02 (2C), 56.04 (2C), 109.9, 110.0 (2C), 110.5 (2C), 111.2 (2C), 118.8, 121.9, 123.0, 128.0 (2C), 139.3 (2C), 143.0, 149.3 (2C), 151.5 (2C), 167.3, 183.8 (2C); IR (film) ν_{max} 2999, 2949, 2935, 2910, 2837, 2604, 2359, 1715, 1614, 1597, 1580, 1508, 1437, 1420, 1342, 1308, 1261, 1225, 1190, 1161, 1138, 1022, 1001, 982, 964, 932 cm^{-1}; HRMS (ES$^+$) m/z: [M + Na] calcd for $C_{27}H_{28}O_8$ 503.1682; found 503.1667.

4.6. (2*E*,6*E*)-Ethyl-7-(3,4-dimethoxyphenyl)-4-((*E*)-3-(3,4-dimethoxyphenyl)acryloyl)-5-oxohepta-2,6-dienoate (9)

Compound **9** was prepared following the same procedure used for the preparation of **8**. The residue was purified by flash chromatography (SiO_2, 1:199 MeOH in CH_2Cl_2, then 1:66 MeOH in CH_2Cl_2) to afford **9** (636.0 mg, 94.4%) as a red amorphous solid: ^{1}H NMR ($CDCl_3$, 500 MHz) δ 1.35 (t, J = 7.16 Hz, 3H), 3.95 (s, 12H), 4.30 (q, J = 7.12Hz, 2H), 5.98 (d, J = 15.64 Hz, 1 H), 6.91 (d, J = 8.34 Hz, 2H), 7.00 (d, J 15.42Hz, 2H), 7.09 (d, J = 1.84 Hz, 2H), 7.21 (dd, J = 1.84, 8.34 Hz, 2H), 7.78 (d, J = 15.42 Hz, 2H), 7.91 (d, J = 15.64 Hz, 1 H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 14.6, 56.17 (2C), 56.24 (2C), 60.8, 110.2 (3C), 110.5 (2C), 111.4 (2C), 119.2, 122.7, 123.3, 128.3 (2C), 139.2 (2C), 143.1, 149.5 (2C), 151.6 (2C), 167.1, 183.9 (2C); IR (film) ν_{max} 3061, 2978, 2961, 2935, 2907, 2872, 2837, 2598, 2033, 1715, 1614, 1595, 1580, 1512, 1464, 1443, 1421, 1367, 1308, 1265, 1161, 1140, 1097, 1024, 976 cm^{-1}; HRMS (ES$^+$) m/z: [M+Na] calcd for $C_{28}H_{30}O_8$ 517.1838; found 517.1826.

4.7. (2*E*,6*E*)-7-(3,4-Dimethoxyphenyl)-4-((*E*)-3-(3,4-dimethoxyphenyl)acryloyl)-5-oxohepta-2,6-dienamide (10)

Concentrated aqueous ammonia (6.5 mL) was cooled in a dry ice-isopropyl alcohol bath. Methyl propiolate (2.0 mL, 22.4 mmol) was added dropwise, and after 10 min, the organic layer was extracted with EtOAc (3× 5 mL), dried over Na_2SO_4, and concentrated, affording crystalline propiolamide (quantitative). Compound **4** (600.0 mg, 1.52 mmol) was dissolved in THF (10.0 mL) and stirred at rt before NaH (54.52 mg, 60% wt in mineral oil, 1.36 mmol) was added. The solution was stirred for 30 min at rt. In a separate flask, propiolamide (209.3 mg, 3.03 mmol) was dissolved in THF (6.0 mL) and stirred at rt. Contents from the first flask were slowly added via cannula to the propiolamide solution over 10 min, and subsequently stirred at rt for 24 h. The reaction was quenched by addition of 5% sulfuric acid (25 mL) and the aqueous layer extracted with EtOAc (3× 50 mL). The combined organic layers were washed with saturated aqueous $NaHCO_3$ and saturated aqueous NaCl, dried (Na_2SO_4), filtered, and concentrated. The residue was recrystallized from EtOAC/hexanes to afford **10** (557.7 mg, 88%) as an orange amorphous solid: ^{1}H NMR ($CDCl_3$, 500 MHz) δ 3.94 (d, J = 1.9 Hz, 12H), 5.45 (s, -N**H$_2$**), 5.95 (d, J = 15.2Hz, 1H), 6.90 (d, J = 8.4 Hz, 2H), 6.98 (d, J = 15.4 Hz, 2H), 7.07 (d, J = 1.9 Hz, 2H), 7.21 (dd, J = 1.9, 8.4 Hz, 2H), 7.76 (d, J = 15.4 Hz, 2H), 7.90 (d, J = 15.2 Hz, 1H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 55.9 (2C), 56.0 (2C), 110.1 (3C), 110.3 (2C), 111.1 (2C), 118.8, 122.9, 124.1, 128.0 (2C), 137.2 (2C), 142.6, 149.2 (2C), 151.3 (2C), 166.9, 183.4 (2C); IR (film) ν_{max} 3412, 3323, 3292, 3215, 2999, 2943, 2837, 1663, 1614, 1597, 1582, 1510, 1466, 1441, 1421, 1385, 1342, 1308, 1263, 1225, 1161, 1140, 1111, 1020, 1001, 978, 966 cm^{-1}; HRMS (ES$^-$) m/z: [M−H] calcd for $C_{26}H_{27}NO_7$ 464.1709; found 464.1731.

4.8. (1*E*,6*E*)-1,7-Bis(3,4-dimethoxyphenyl)-4-((*E*)-3-hydroxyprop-1-enyl)hepta-1,6-diene-3,5-dione (11)

Compound **9** (500.0 mg, 1.0 mmol) was dissolved in CH_2Cl_2 (10.0 mL) and cooled to −78 °C before dibal-H (3.0 mL, 1 M in CH_2Cl_2, 3.0 mmol) was added drop wise. After 30 min, the solution was warmed to rt and stirred for an additional 1.5 h. The solution was quenched using saturated aqueous sodium potassium tartrate

(Rochelle's salt) and the aqueous layer extracted with CH_2Cl_2 (3× 50 mL). The combined organic layers were washed with saturated aqueous NaCl, dried (Na_2SO_4), and concentrated. The residue was purified by flash chromatography (SiO_2, 1:5:9:10 MeOH:CH_2Cl_2:EtOAc:hexanes) to afford **11** (301.3 mg, 66%) as a red amorphous solid: ^{1}H NMR ($CDCl_3$, 500 MHz) δ 3.92 (d, J = 3.9 Hz, 12H), 4.40 (d, J = 4.6 Hz, 2H), 5.88 (dt, J = 5.7, 15.6 Hz, 1H), 6.58 (dt, J = 1.4, 15.6 Hz, 1H), 6.87 (d, J = 8.3 Hz, 2H), 6.97 (d, J = 15.6 Hz, 2H), 7.05 (d, J = 1.8 Hz, 2H), 7.16 (dd, J = 1.8, 8.4 Hz, 2H), 7.67 (d, J = 15.6 Hz, 2H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 55.94, 55.98, 56.0 (2C), 63.6, 110.4, 111.0 (2C), 111.1 (2C), 111.5 (2C), 119.7, 122.5, 124.2, 128.4 (2C), 136.5 (2C), 141.2, 149.2 (2C), 151.0 (2C), 182.5 (2C); IR (film) ν_{max} 3458, 3443, 2997, 2926, 2874, 2853, 2835, 1620, 1595, 1580, 1556, 1510, 1464, 1454, 1435, 1421, 1337, 1306, 1261, 1192, 1159, 1138, 1094, 1022, 959, 930 cm^{-1}; HRMS (ES^-) m/z: [M−H] calcd for $C_{26}H_{28}O_7$ 451.1757; found 451.1766.

4.9. 4,4′-(1*E*,1′*E*)-2,2′-(1-*H*-Pyrazole-3,5-diyl)bis(ethene-2,1-diyl)bis(2-methoxyphenol) (12)

Compound **1** (300.0 mg, 0.81 mmol) was dissolved in absolute EtOH (5.0 mL). Hydrazine hydrochloride (66.9 mg, 0.984 mmol) was added followed by a catalytic amount of glacial acetic acid (1.0 mL). The solution stirred at reflux for 40 h. The crude reaction mixture was concentrated in vacuo and redissolved in EtOAc (200 mL). The organic layer was washed with saturated aqueous $NaHCO_3$ and saturated NaCl, dried (Na_2SO_4), filtered, and concentrated in vacuo. The residue was purified by column chromatography (SiO_2, 5:3:2 hexanes:EtOAc:CH_2Cl_2) to afford compound **12** (262.0 mg, 87%) as a dark orange amorphous solid: ^{1}H NMR ($CDCl_3$ w/MeOD, 500 MHz) δ 3.79 (s, 6H), 6.43 (s, 1H), 6.72 (s, 2H), 6.73 (s, 1H), 6.75 (s, 1H), 6.85 (dd, J_1 = 8.2 Hz, J_2 = 1.8 Hz, 2H), 6.90(d, J = 8.3 Hz, 2H), 6.92(d, J = 6.4 Hz, 2H); ^{13}C NMR ($CDCl_3$ w/MeOD, 125 MHz) δ 55.4 (2C), 98.8, 108.6 (2C), 114.8 (2C), 115.3 (2C), 120.1 (2C), 127.6 (2C), 128.8 (2C), 130.5 (2C), 146.1 (2C), 147.3 (2C); IR (film) ν_{max} 3383, 2359, 2341, 2332, 1593, 1558, 1512, 1456, 1429, 1277, 1259, 1231, 1211, 1155, 1122 cm^{-1}; HRMS(ES^+) m/z: [M+H] calcd for $C_{21}H_{20}N_2O_4$ 365.1505; found 365.1484.

4.10. 3,5-Bis(3,4-dimethoxystyryl)-1-*H*-pyrazole (13)

Compound **13** was prepared following the same procedure used for the preparation of **12**. The residue was purified by flash chromatography (SiO_2, 2:3:5 (CH_2Cl_2, EtOAc, hexanes)) to afford **13** (54.0 mg, 90%) as a yellow-orange amorphous solid: ^{1}H NMR ($CDCl_3$, 500 MHz) δ 3.85 (d, J = 15.5 Hz, 12H), 6.62 (s, 1H), 6.74 (d, J = 8.10 Hz, 2H), 6.95 (m, 6H), 7.05 (d, J = 16.5 Hz, 2H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 55.6 (2C), 55.8 (2C), 99.4, 99.9 (2C), 108.5 (2C), 111.0 (2C), 115.8 (2C), 119.9 (2C), 129.6 (2C), 130.6 (2C), 149.0 (2C), 149.1 (2C); IR (film) ν_{max} 3331, 3177, 3126, 3078, 3065, 2999, 2959, 2934, 2910, 2835, 1599, 1583, 1558, 1514, 1464, 1441, 1420, 1331, 1311, 1265, 1196, 1157, 1138, 1103, 1024, 960 cm^{-1}; HRMS (ES^+) m/z: [M+H] calcd for $C_{23}H_{24}N_2O_4$ 393.1814; found 393.1801.

4.11. Ethyl 3-(3,5-bis(4-hydroxy-3-methoxystyryl)-1-*H*-pyrazol-4-yl)propanoate (14)

Compound **14** was prepared following the same procedure used for the preparation of **12**. The residue was purified by flash chromatography (SiO_2, 5:3:2 Hexanes:EtOAc:CH_2Cl_2) to afford **14** (63.5 mg, 81%) as a yellow-orange amorphous solid: ^{1}H NMR ($CDCl_3$, 500 MHz) δ 1.23 (t, J = 7.1 Hz, 3H), 2.57 (t, J = 7.8 Hz, 2H), 3.01 (t, J = 7.8 Hz, 2H), 3.83 (s, 6H), 4.12 (q, J = 7.2 Hz, 2H), 6.82 (d, J = 16.6 Hz, 2H), 6.85 (d, J = 8.3 Hz, 2H), 6.89 (d, J = 1.5 Hz, 2H), 6.93 (dd, J_1 = 8.2 Hz, J_2 = 1.6 Hz, 2H), 7.06 (d, J = 16.5 Hz, 2H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 14.2, 18.8, 35.5, 55.8 (2C), 60.6, 108.4 (2C), 113.9, 114.6 (2C), 115.3 (2C), 120.5 (2C), 129.4 (2C), 130.1 (2C), 144.0 (2C), 145.9 (2C), 146.8 (2C), 173.0; IR (film) ν_{max} 3323, 3041, 3034, 2957, 2924, 2851, 2320, 1724, 1661, 1593, 1556, 1514, 1464, 1429, 1375, 1277, 1238, 1205, 1159, 1124, 1099, 1068, 1034, 962 cm^{-1}; HRMS (ES^+) m/z: [M+H] calcd for $C_{26}H_{28}N_2O_6$ 465.2026; found 465.2005.

4.12. Ethyl 3-(3,5-bis(3,4-dimethoxystyryl)-1-*H*-pyrazol-4-yl)propanoate (15)

Compound **15** was prepared following the same procedure used for the preparation of **12**. The residue was purified by flash chromatography (SiO_2, 5:3:2 Hexanes:EtOAc:CH_2Cl_2) to afford **15** (43.0 mg,73%) as a yellow–orange amorphous solid: ^{1}H NMR ($CDCl_3$, 500 MHz) δ 1.23 (t, J = 7.1 Hz, 3H), 2.58 (t, J = 7.7 Hz, 2H), 3.03 (t, J = 7.8 Hz, 2H), 3.87 (12H), 4.12 (q, J = 4.1 Hz, 2H), 6.77 (m, 2H), 6.85 (m, 2H), 6.93 (d, J = 8.25 Hz, 2H), 6.97 (s, 2H), 7.07 (m, 2H); ^{13}C NMR ($CDCl_3$, 125 MHz) 14.2, 18.8, 35.6, 55.9 (4C), 60.6, 108.2 (2C), 111.1, 114.3 (2C), 115.4 (2C), 120.1 (2C), 129.3 (2C), 129.7 (2C), 129.9 (2C), 149.1 (2C), 149.1 (2C), 173.0; IR (film) ν_{max} 3333, 3173, 3078, 3030, 2957, 2924, 2851, 1728, 1666, 1601, 1583, 1556, 1514, 1464, 1443, 1420, 1373, 1335, 1265, 1234, 1159, 1138, 1095, 1068, 1026, 962 cm^{-1}; HRMS (ES^+) m/z: [M+H] calcd for $C_{28}H_{32}N_2O_6$ 493.2339; found 493.2321.

4.13. 3,5-Bis(3,4-dimethoxystyryl)-4-methyl-1-*H*-pyrazole (16)

Compound **16** was prepared following the same procedure used for the preparation of **12**. The residue was purified by flash chromatography (SiO_2, 2:3:5 (CH_2Cl_2, EtOAc, hexanes)) to afford **16** (60.8 mg, 58%) as a yellow–orange amorphous solid: ^{1}H NMR ($CDCl_3$, 500 MHz) δ 2.22 (s, 3H), 3.82 (s, 6H), 3.82 (s, 6H), 6.73 (d, 2H, J = 8.8 Hz), 6.81 (d, 2H, J = 16.5 Hz), 6.92 (m, 4H), 6.97 (d, 2H, J = 16.5 Hz); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 9.1, 55.8 (2C), 55.9 (2C), 108.6, 108.7 (2C), 111.1 (2C), 112.2 (2C), 115.1 (2C), 119.9 (2C), 129.5 (2C), 130.1 (2C), 149.1 (2C), 149.1 (2C); IR (film) ν_{max} 3416, 3240, 3229, 3111, 2999, 2957, 2932, 2835, 2359, 1636, 1601, 1514, 1464, 1420, 1333, 1265, 1238, 1159, 1138, 1024, 960 cm^{-1}; HRMS (ES^+) m/z: [M+H] calcd for $C_{24}H_{26}N_2O_4$ 407.1971; found 407.1954.

4.14. (*E*)-Methyl 3-(3,5-bis(3,4-dimethoxystyryl)-1-*H*-pyrazol-4-yl)acrylate (17)

Compound **17** was prepared following the same procedure used in the preparation of compound **12**. The residue was purified by flash chromatography (SiO_2, 1:1 Hexanes:EtOAc) to afford **17** (43.3 mg,15%) as a yellow–orange amorphous solid: ^{1}H NMR ($CDCl_3$, 500 MHz) δ 3.76 (s, 3H), 3.83 (s, 6H), 3.84 (s, 6H), 6.15 (d, J = 16.0 Hz, 1H), 6.75 (d, J = 8.1 Hz, 2H), 6.86 (d, J = 16.3 Hz, 2H), 6.93 (d, J = 2.5 Hz, 2H), 6.95 (d, J = 1.7 Hz, 2H), 7.09 (d, J = 16.3, 2H), 7.81 (d, J = 16.0 Hz, 2H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 51.7, 56.0 (4C), 109.0 (2C), 111.1, 113.0 (2C), 113.5 (2C), 117.8 (2C), 120.6 (2C), 129.3 (2C), 132.6 (2C), 135.2 (2C), 149.2 (2C), 149.7 (2C), 167.9; IR (film) ν_{max}3317, 2923, 2849, 2837, 1701, 1624, 1601, 1582, 1514, 1464, 1437, 1420, 1310, 1265, 1198, 1159, 1138, 1024, 960, 933 cm^{-1}; HRMS (ES^+) m/z: [M+H] calcd for $C_{27}H_{28}N_2O_6$ 477.2026; found 477.1993.

4.15. (*E*)-Ethyl-3-(3,5-bis(3,4-dimethoxystyryl)-1-*H*-pyrazol-4-yl)acrylate (18)

Compound **18** was prepared following the same procedure used for the preparation of **12**. The residue was purified by flash chro-

matography (SiO_2, 1:7.5:15:26.5 MeOH:CH_2Cl_2:EtOAc:hexanes) to afford **18** (37.5 mg, 76%) as a yellow–orange amorphous solid: ^{1}H NMR ($CDCl_3$, 500 MHz) δ 1.35 (t, J = 7.15 Hz, 3H), 3.93 (d, J = 8.30 Hz, 12H), 4.29 (q, J = 7.15 Hz, 2H), 6.23 (d, J = 16.0 Hz, 1H), 6.86 (d, J = 8.20 Hz, 2H), 6.96 (d, J = 16.3 Hz, 2H), 7.04 (d, J = 1.80 Hz, 2H), 7.06 (dd, J = 1.80, 8.25 Hz, 2H), 7.17 (d, J = 16.3 Hz, 2H), 7.89 (d, J = 16.0 Hz, 2H); ^{13}C NMR ($CDCl_3$, 125 MHz) 14.4, 55.90 (2C), 55.92 (2C), 60.5, 108.9 (2C), 111.1 (2C), 113.0, 113.6, 118.3 (2C), 120.5 (2C), 129.2 (2C), 132.4 (2C), 134.9 (2C), 149.06, 149.11 (2C), 149.6 (2C), 167.5; IR (film) ν_{max} 3321, 2995, 2957, 2935, 2835, 1701, 1684, 1624, 1601, 1583, 1558, 1514, 1464, 1443, 1420, 1394, 1367, 1308, 1263, 1240, 1159, 1140, 1094, 1026, 962 cm^{-1}; HRMS (ES$^+$) m/z: [M+H] calcd for $C_{28}H_{30}N_2O_6$ 491.2182; found 491.2168.

4.16. (*E*)-3-(3,5-Bis(3,4-dimethoxystyryl)-1-*H*-pyrazol-4-yl)acrylamide (19)

Compound **19** was prepared following the same procedure used for the preparation of **12**. The residue was purified by flash chromatography (SiO_2, 1:99 MeOH in CH_2Cl_2) to afford **19** (41.7 mg, 84%) as a yellow-orange amorphous solid: ^{1}H NMR ($CDCl_3$, 500 MHz) δ 3.92 (s, 12H), 5.62 (s, -N**H$_2$**), 6.34 (d, J = 15.6 Hz, 1H), 6.87 (d, J = 8.2 Hz, 2H), 6.92 (d, J = 16.4 Hz, 2H), 7.03 (d, J = 16.4 Hz, 2H), 7.04 (d, J = 1.7 Hz, 2H), 7.10 (dd, J = 1.7, 8.2 Hz, 2H), 7.60 (d, J = 15.6 Hz, 1H); ^{13}C NMR ($CDCl_3$, 125 Hz) δ 55.88 (2C), 55.98 (2C), 105.4 (2C), 109.6 (2C), 111.0 (2C), 111.1(2C), 111.2, 117.1 (2C), 120.1, 122.3 (2C), 127.4 (2C), 142.5 (2C), 149.1 (2C), 150.8, 168.0; IR (film) ν_{max}3339, 3325, 3198, 3186, 2999, 2957, 2935, 2912, 2835, 1717, 1668, 1634, 1595, 1514, 1464, 1441, 1420, 1394, 1337, 1308, 1265, 1186, 1159, 1140, 1024, 978, 968; HRMS (ES$^+$) m/z: [M+H] calcd for $C_{26}H_{27}N_3O_5$ 462.2029; found 462.2010.

4.17. (*E*)-3-(3,5-Bis(3,4-dimethoxystyryl)-1-*H*-pyrazol-4-yl)prop-2-en-1-ol (20)

Compound **18** (80.0 mg, 0.16 mmol) was dissolved in anhydrous MeOH (5.0 mL) and stirred at rt. $NaBH_4$ (61.7 mg, 1.6 mmol) was added and the solution stirred at reflux. After 1 h, additional $NaBH_4$ (61.7 mg, 1.6 mmol) was added, and the solution stirred at reflux for 15.5 h. The reaction was quenched by the addition of H_2O, resulting in the formation of a white solid. The precipitate was dissolved by the addition of 10% HCl (10 mL) and the aqueous layer was extracted with CH_2Cl_2 (3× 20 mL). The combined organic layers were washed with saturated aqueous $NaHCO_3$ and saturated aqueous NaCl, dried (Na_2SO_4), filtered, and concentrated. The residue was purified by flash chromatography (SiO_2, 1:7.5:15:26.5 MeOH:CH_2Cl_2:EtOAc:hexanes) to afford **20** (41.0 mg, 83%) as a yellow-orange amorphous solid: ^{1}H NMR ($CDCl_3$, 500 MHz) δ 3.84 (s, 2H), 3.87 (d, J = 9.7 Hz, 12H), 6.21 (d, J = 16.0 Hz, 1H), 6.76 (d, J = 8.45 Hz, 2H), 6.89 (d, J = 16.3 Hz, 2H), 6.92 (m, 4H), 7.16 (d, J = 16.3 Hz, 2H), 7.87 (d, J = 16.0 Hz, 1H); ^{13}C NMR ($CDCl_3$, 125 Hz) δ 51.7, 55.84 (2C), 55.87 (2C), 108.8 (2C), 111.0 (2C), 112.9, 113.3, 117.6 (2C), 120.6 (2C), 129.1 (2C), 132.6 (2C), 135.2 (2C), 149.0, 149.6 (2C), 167.9 (2C); IR (film) ν_{max} 3315, 3153, 3059, 2999, 2949, 2935, 2835, 1713, 1626, 1601, 1583, 1514, 1464, 1441, 1421, 1312, 1265, 1198, 1161, 1140, 1097, 1026, 960, 933 cm^{-1}; HRMS (ES$^+$) m/z: [M+H] calcd for $C_{26}H_{28}N_2O_5$ 449.2076; found 449.2096.

4.18. 4,4′-(1*E*,1′*E*)-2,2′-(Isoxazole-3,5-diyl)bis(ethene-2,1-diyl)bis(2-methoxyphenol) (21)

Compound **1** (250 mg, 0.69 mmol) was dissolved in absolute EtOH (5.0 mL). Hydroxylamine hydrochloride (56.6 mg, 0.83 mmol) was added followed by a catalytic amount of glacial acetic acid (750 μL). The solution stirred at reflux for 40 h. The crude reaction mixture was concentrated in vacuo and redissolved in EtOAc (200 mL). The organic layer was washed with saturated aqueous $NaHCO_3$ and saturated NaCl, dried (Na_2SO_4), filtered, and concentrated in vacuo. The residue was purified by column chromatography (SiO_2, 5:3:2 hexanes:EtOAc:CH_2Cl_2) to afford **21** (218.0 mg, 89%) as a dark orange amorphous solid: ^{1}H NMR (*d*-DMSO, 500 MHz) δ 3.89 (s, 6H), 6.85 (d, J = 8.1 Hz, 1H), 6.87 (d, J = 8.1 Hz, 1H), 6.92 (s, 1H), 7.10 (dd, J_1 = 8.3 Hz, J_2 = 1.9 Hz, 1H), 7.13 (d, J = 12.4 Hz, 1H), 7.13 (dd, J_1 = 8.3 Hz, J_2 = 1.8 Hz, 1 H), 7.16 (d, J = 12.3 Hz, 1 H), 7.33–7.37 (m, 4H), 9.45 (s, 1H), 9.51 (s, 1H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 55.6, 55.6, 97.8, 109.9, 110.2, 110.3, 112.6, 115.5, 115.5, 121.3, 121.7, 126.9, 127.3, 134.7, 136.4, 147.8, 147.9, 147.9, 148.1, 162.2, 168.3; IR (film) ν_{max} 3402, 2961, 2924, 2851, 2359, 2341, 2332, 1645, 1605, 1593, 1562, 1514, 1464, 1433, 1375, 1279, 1231, 1207, 1188, 1159, 1122, 1032, 960 cm^{-1}; HRMS (ES$^+$) m/z: [M+H] calcd for $C_{21}H_{19}NO_5$ 366.1342; found 366.1348.

4.19. 3,5-Bis(3,4-dimethoxystyryl)isoxazole (22)

Compound **22** was prepared following the same procedure used for the preparation of **21**. The residue was purified by flash chromatography (SiO_2, 2:3:5 (CH_2Cl_2, EtOAc, hexanes)) to afford **22** (54.0 mg, 90%) as a yellow-orange amorphous solid: ^{1}H NMR ($CDCl_3$, 500 MHz) δ 3.85 (d, J = 2.55 Hz, 6H), 3.88 (d, J = 4.20 Hz, 6H), 6.37 (s, 1 H), 6.77 (d, J = 16.35, 1H), 6.81 (dd, J = 3.75, 8.20 Hz, 2H), 6.94 (d, J = 16.4 Hz, 1H), 7.03 (m, 5H), 7.23 (d, J = 16.4 Hz, 1H); ^{13}C NMR ($CDCl_3$, 125 Hz) δ 55.89, 55.93, 55.98, 56.0, 97.8, 108.6, 108.9, 111.0, 111.1, 114.2 (2C), 120.9, 121.2, 128.6, 128.9, 134.7, 135.4, 149.2 (2C), 149.9, 150.1, 162.2, 168.5; IR (film) ν_{max} 2995, 2953, 2932, 2916, 2835, 1643, 1599, 1583, 1512, 1504, 1462, 1427, 1416, 1265, 1225, 1159, 1140, 1024, 964 cm^{-1}; HRMS (ES$^+$) m/z: [M+H] calcd for $C_{23}H_{23}NO_5$ 394.1655; found 394.1649.

4.20. Ethyl 3-(3,5-bis(4-hydroxy-3-methoxystyryl)isoxazol-4-yl)propanoate (23)

Compound **23** was prepared following the same procedure used for the preparation of **21**. The residue was purified by flash chromatography (SiO_2, 2:3:5 (CH_2Cl_2, EtOAc, hexanes)) to afford **23** (60.7 mg, 76%) as a yellow amorphous solid: ^{1}H NMR ($CDCl_3$, 500 MHz) δ 1.23 (t, J = 7.2 Hz, 3H), 2.60 (t, J = 7.6 Hz, 2H), 2.97 (t, J = 7.6 Hz, 2H), 3.96 (s, 6H), 4.13 (q, J = 7.2 Hz, 2H), 5.90 (bs, 2H), 6.79 (d, J = 16.5 Hz, 1H), 6.80 (d, J = 16.3 Hz, 1H), 6.94 (d, J = 8.1 Hz, 1H), 6.94 (d, J = 8.2 Hz, 1H), 7.05 (d, J = 1.8 Hz, 1H), 7.06 (d, J = 1.7 Hz, 1H), 7.08 (dd, J_1 = 8.3 Hz, J_2 = 1.8 Hz, 1H), 7.11 (dd, J_1 = 8.3 Hz, J_2 = 1.8 Hz, 1H), 7.31 (d, J = 16.3 Hz, 1H), 7.37 (d, J = 16.5 Hz, 1H) ^{13}C NMR ($CDCl_3$, 125 MHz) δ 14.2, 17.9, 34.6, 56.0 (2C), 60.9, 108.7, 108.9, 109.4, 112.1, 112.3, 114.7, 114.8 (2C), 121.3, 128.5, 128.8, 133.9, 135.2, 146.7, 146.8, 146.8 (2C), 160.0, 164.7, 172.5; IR (film) ν_{max} 3499, 3416, 3049, 2962, 2935, 2872, 2853, 2758, 2748, 2644, 2590, 2548, 2311, 2060, 1728, 1634, 1593, 1514, 1450, 1439, 1373, 1275, 1236, 1207, 1184, 1159, 1122, 1095, 1063, 1032, 964, 939 cm^{-1}, HRMS (ES$^+$) m/z: [M+H] calcd for $C_{26}H_{27}NO_7$ 466.1866; found 466.1860.

4.21. Ethyl 3-(3,5-bis(3,4-dimethoxystyryl)isoxazol-4-yl)propanoate (24)

Compound **24** was prepared following the same procedure used for the preparation of **21**. The residue was purified by flash chromatography (SiO_2, 2:3:5 (CH_2Cl_2, EtOAc, hexanes)) to afford **24** (64.4 mg, 61%) as a dark yellow amorphous solid: ^{1}H NMR ($CDCl_3$,

500 MHz) δ 1.15 (t, J = 7.2 Hz, 3H), 2.52 (t, 7.5 Hz, 2H), 2.91 (t, J = 7.5 Hz, 2H), 3.86 (m, 12H), 4.05 (q, J = 7.15 Hz, 2H), 6.73 (d, J = 4.15 Hz, 1H), 6.75 (d, J = 4.0 Hz, 1H), 6.79–6.82 (m, 2H), 7.01–7.06 (m, 4H), 7.21 (d, J = 19.0 Hz, 1H), 7.31 (d, J = 16 Hz, 1H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 14.2, 17.9, 34.6, 56.0, 60.8, 109.0, 109.1, 109.7, 111.1, 111.2, 112.4, 112.5, 120.9, 121.0, 129.0, 133.8, 135.1, 149.2 (2C), 149.9, 150.0, 160.0, 164.7, 172.5; IR (film) ν_{max} 3541, 3435, 3053, 2959, 2930, 2853, 2839, 2598, 2033, 1728, 1674, 1634, 1591, 1514, 1454, 1443, 1421, 1375, 1339, 1263, 1236, 1159, 1140, 1094, 1063, 1024, 966 cm^{-1}; HRMS (ES$^+$) m/z: [M+H] calcd for $C_{28}H_{31}NO_7$ 494.2179; found 494.2163.

4.22. 3,5-Bis(3,4-dimethoxystyryl)-4-methylisoxazole (25)

Compound **25** was prepared following the same procedure used for the preparation of **21**. The residue was purified by flash chromatography (SiO_2, 2:3:5 (CH_2Cl_2, EtOAc, hexanes)) to afford **25** (59.2 mg, 59%) as a yellow–orange amorphous solid: ^{1}H NMR ($CDCl_3$, 500 MHz) 2.18 (s, 3H), 3.85 (s, 3H), 3.85 (s, 3H), 3.88 (s, 3H), 3.89 (s, 3H), 6.70 (d, J = 16.3 Hz, 1H), 6.79–6.83 (3H), 6.99–7.05 (4H), 7.22 (d, J = 16.4 Hz, 1H), 7.26 (d, J = 16.7 Hz, 1H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 29.7, 55.9 (2C), 56.0 (2C), 108.8, 109.0, 109.2, 110.0, 111.1, 111.2, 113.5, 120.8, 129.1, 129.3, 133.2, 134.9, 149.2 (2C), 149.8, 149.9, 160.5, 164.2; IR (film) ν_{max} 3541, 3445, 3194, 3051, 2999, 2959, 2930, 2853, 2837, 2600, 2361, 2322, 2280, 2033, 1682, 1645, 1634, 1593, 1514, 1506, 1454, 1441, 1421, 1387, 1339, 1312, 1263, 1236, 1196, 1159, 1138, 1092, 1024, 964 cm^{-1}; HRMS (ES$^+$) m/z: [M+H] calcd for $C_{24}H_{25}NO_5$ 408.1811; found 408.1797.

4.23. Anti-proliferation assays

MCF-7 and SKBr3 cells were maintained in a 1:1 mixture of Advanced DMEM/F12 (Gibco) supplemented with non-essential amino acids, L-glutamine (2 mM), streptomycin (500 μg/mL), penicillin (100 units/mL), and 10% FBS. Cells were grown to confluence in a humidified atmosphere (37 °C, 5% CO_2), seeded (2000/well, 100 μL) in 96-well plates, and allowed to attach overnight. Compound or geldanamycin at varying concentrations in DMSO (1% DMSO final concentration) was added, and cells were returned to the incubator for 72 h. At 72 h, the number of viable cells was determined using an MTS/PMS cell proliferation kit (Promega) per the manufacturer's instructions. Cells incubated in 1% DMSO were used as 100% proliferation, and values were adjusted accordingly. IC_{50} values were calculated from separate experiments performed in triplicate using GraphPad Prism.

4.24. Benzyl mercaptan addition studies

In a 1 dram vial, 15.0 mg curcumin (**4**) (0.041 mmol) was dissolved in 0.60 mL d-DMSO. Benzyl mercaptan (4.7 μL, 0.041 mmol) was then added, marking time zero. The contents of the vial were transferred to an NMR tube and ^{1}H NMR spectra were recorded at set intervals (10 min, 1 h, 5 h, 14 h, and 24 h) to monitor the progress of the reaction. The presence of the benzyl mercaptan-curcumin conjugate was confirmed by HRMS (ES+) m/z: single addition product: [M+H] calcd for $C_{28}H_{28}O_6S$ 493.1685; found 493.1664; double addition product: [M+Na] 639.1851; found 639.1835. ^{1}H NMR (d_6-DMSO, 400 MHz) As a mixture of curcumin, benzyl mercaptan and conjugated product: 2.84 (t, J = 7.7, 1 H), 2.96 (d, J = 7.7 Hz, 2H), 3.58 (m, 2H), 3.73 (d, J = 8 Hz, 2H), 3.81 (s, 3H), 3.85 (s, 3H), 3.85 (s, 6H), 4.17 (t, 7.8, 1H), 5.78 (s, 1H), 6.07 (s, 1H), 6.59 (d, J = 15.9 Hz, 1H), 6.74 (d, J = 11.2 Hz, 2H), 6.77 (d, J = 15.8 Hz, 2H), 6.83 (d, J = 8.2 Hz, 2H), 7.11 (dd, J_1 = 1.9, J_2 = 8.3, 2H), 7.16 (dd, J_1 = 1.9 Hz, J_2 = 8.3 Hz, 2H), 7.23 (m, 3H), 7.30 (d, J = 8 Hz, 2H), 7.32 (m, 5H), 7.46 (d, J = 15.8 Hz, 1H), 7.56 (d, J = 16 Hz, 2H), 8.97 (s, 1H) 9.68 (s, 2H) 10.07 (s, 1H); ^{13}C NMR (d_6-DMSO, 500 MHz) δ 34.8, 34.8, 34.8, 43.1, 43.1, 44.0, 48.8, 55.5, 55.6, 79.1, 101.2 101.3, 111.1, 111.5, 111.6, 115.1, 115.6, 119.4, 120.0, 120.1, 120.1, 126.8, 128.3, 128.8, 131.6, 131.7, 131.9, 138.1, 140.5, 145.6, 145.6, 145.7, 147.4, 147.5, 149.3, 156.3, 156.4, 177.5, 190.7, 196.7, 202.1; IR (film) ν_{max} 3028, 2959, 2930, 2916, 1601, 1574, 1556, 1514, 1494, 1452, 1429, 1367, 1360, 1269, 1236, 1209, 1177, 1153, 1121, 1070, 1032, 916 cm^{-1}. Similar experiments were carried out with the pyrazole and isoxazole derivatives of curcumin, compounds **12** and **21**, respectively.

Supporting information

Characterization for all compounds and spectra for the benzyl mercaptan addition studies. This material is available via the Internet at http://www.sciencedirect.com.

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References and notes

1. Sharma, R. A.; Gescher, A. J.; Steward, W. P. *Eur. J. Cancer* **2005**, *41*, 1955.
2. Aggarwal, B. B.; Sundaram, C.; Malani, N.; Ichikawa, H. *Adv. Exp. Med. Biol.* **2007**, *595*, 1.
3. Joe, B.; Vijaykumar, M.; Lokesh, B. R. *Crit. Rev. Food Sci. Nutr.* **2004**, *44*, 97.
4. Goel, A.; Kunnumakkara, A. B.; Aggarwal, B. B. *Biochem. Pharmacol.* **2008**, *75*, 787.
5. Park, S-Y.; Kim, D. S. H. L. *J. Nat. Prod.* **2002**, *65*, 1227.
6. Wahlstrom, B.; Blennow, G. *ActaPharmacol. Toxicol.* **1978**, *43*, 83.
7. Ammon, H. P. T.; Wahl, M. A. *Planta Med.* **1991**, *57*, 1.
8. Rashmi, R.; Kumar, T. R.; Santhosh, K. D. *FEBS Lett.* **2003**, *538*, 19.
9. Cheng, A.-L.; Hsu, C.-H.; Lin, J.-K.; Hsu, M.-M.; Ho, Y.-F.; Shen, T.-S.; Ko, J.-Y.; Lin, J.-T.; Lin, B.-R.; Wu, M.-S.; Yu, H.-S.; Jee, S.-H.; Chen, G.-S.; Chen, T.-M.; Chen, C.-A.; Lai, M.-K.; Pu, Y.-S.; Pan, M.-H.; Wang, Y.-J.; Tsai, C.-C.; Hsieh, C.-Y. *Anticancer Res.* **2001**, *21*, 2895.
10. Shoba, G.; Joy, D.; Joseph, T.; Majeed, M.; Rajendran, R.; Srinivas, P. S. *Planta Med.* **1998**, *64*, 353.
11. Strimpakos, A. S.; Sharma, R. A. *Antioxid. Redox Signal.* **2008**, *10*, 511.
12. Kao, E.-S.; Wang, C.-J.; Lin, W.-L.; Yin, Y.-F.; Wang, C.-P.; Tseng, T. H. *J. Agric. Food Chem.* **2005**, *53*, 430.
13. Chan, M. M.; Huang, H. I.; Fenton, M. R. *Biochem. Pharmacol.* **1998**, *55*, 1955.
14. Simoni, D.; Rizzi, M.; Rondanin, R.; Baruchello, R.; Marchetti, P.; Invidiata, F. P.; Labbozzetta, M.; Poma, P.; Carina, V.; Notarbartolo, M.; Alaimo, A.; D'Alessandro, N. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 845.
15. Payton, F.; Sandusky, P.; Alworth, A. L. *J. Nat. Prod.* **2007**, *70*, 143.
16. Lin, L.; Shi, Q.; Su, C-Y.; Shih, C. C-Y.; Lee, K-H. *Bioorg. Med. Chem.* **2006**, *14*, 2527.
17. Lin, L.; Shi, Q.; Nyarko, A. K.; Bastow, K. F.; Wu, C.-C.; Su, C.-Y.; Shih, C. C.-Y.; Lee, K.-H. *J. Med. Chem.* **2006**, *49*, 3963.
18. Mishra, S.; Karmodiya, K.; Surolia, N.; Surolia, A. *Bioorg. Med. Chem.* **2008**, *16*, 2894.
19. Selvam, C.; Jachak, S. M.; Thilagavathi, R.; Chakraborti, A. K. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 1793.
20. Narlawar, R.; Pickhardt, M.; Leuchtenberger, S.; Baumann, K.; Krause, S.; Dyrks, T.; Weggen, S.; Mandelkow, E.; Schmidt, B. *ChemMedChem* **2008**, *3*, 165.
21. Narlawar, R.; Baumann, K.; Schubenel, R.; Schmidt, B. *Neurodegen. Dis.* **2007**, *4*, 88.
22. Bird, C. W. *Tetrahedron* **1992**, *48*, 335.
23. Ohtsu, H.; Xiao, Z.; Ishida, J.; Nagai, M.; Wang, H.-K.; Itokawa, H.; Su, C.-Y.; Shih, C.; Chiang, T.; Chang, E.; Lee, Y.; Tsai, M.; Chang, C.; Lee, K.-H. *J. Med. Chem.* **2002**, *46*, 5037.