Synthesis and biological evaluation of (E)-cinnamic acid, (E)-2-styrylthiazole and (E)-2-[2-(naphthalen-1-yl)vinyl]thiazole derivatives

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Abstract
Cinnamyl- and thiazole-based compounds have been shown to exhibit diverse medicinal properties and a series of twelve (E)-2-styrylthiazole and (E)-2-[2-(naphthalen-1-yl)vinyl]thiazole derivatives, which are conjugates of both systems and which satisfy the “Lipinski rule of 5”, have been synthesised and subjected to in vitro biological screening. While insignificant inhibition (60-98% viability at 10 µM) of HeLa (cervical cancer) cells was noted, all five of the (E)-2-[2-(naphthalen-1-yl)vinyl]thiazole derivatives proved remarkably active against SH-SY5Y (neuroblastoma) cells with IC⁵₀ values ranging from 2.09 to 8.64 µM. Two of the seven (E)-2-styrylthiazoles were found to be moderately active (with IC⁵₀ values of 10.8 and 11.7 mM), whereas the remaining five analogues exhibit significant proliferation of SH-SY5Y cells (with IC⁵₀ values of 180-1000 mM). The results warrant further studies on the effects of styrylthiazoles on the differentiation and extension of SH-SY5Y cells in order to assess their activity in neurological degenerative diseases.

Keywords: Synthesis, cinnamic acids, styrylthiazoles, 2-[2-(naphthalen-1-yl)vinyl]thiazoles, biological activity
Introduction

The naturally occurring cinnamic acid derivatives, \( p \)-coumaric acid 1, caffeic acid 2 and ferulic acid 3, are small phenolic compounds found in fruits, vegetables and flowers (Figure 1)\(^1\) or, as their esters, in essential oils, resins and balsams.\(^2\) Cinnamic acid, an important intermediate in the biochemical shikimic and phenylpropanoic acid pathways,\(^3\) belongs to the class of plant hormones (viz., auxins) which regulate cell growth and differentiation.\(^4\) Cinnamic acid analogues act as precursors of many commercially important synthetic cinnamic esters\(^5\) and as reactants in the preparation of chalcones and stilbenes.\(^6\) Cinnamic acid derivatives have also been reported to possess antidiabetic,\(^7\) hepato-protective,\(^8\) antioxidant,\(^9\) antimicrobial,\(^10\) anti-tuberculosis\(^11\) and anti-cancer properties.\(^12\) On the other hand, compounds containing the thiazole nucleus have also been reported to exhibit various biological activities, the specificity of action often being dictated by the attached functionalities.\(^13\) Thiazole-containing heterocyclic peptides, such as nosiheptide, GE2270 A and nocathiacin,\(^14\)-\(^17\) isolated from marine organisms, exhibit potent biological profiles — observations which support the inclusion of the thiazole nucleus in the design of lead compounds in the development of new active pharmaceutical ingredients (APIs).\(^18\) Attention has thus been given to the development of novel compounds which contain both the styryl and thiazole moieties and, in this communication, we report the preparation and biological screening of a series of styrylthiazoles 4, \((E)\)-2-[(naphthalen-1-yl)vinyl]thiazoles and their cinnamic acid precursors.

\[ \text{Figure 1. Structures of cinnamic acid derivatives: } p \text{-coumaric acid 1, caffeic acid 2 and ferulic acid 3 and the proposed styrylthiazoles 4.} \]

Results and Discussion

Access to the cinnamic acids 7a-k and the targeted \((E)\)-styrylthiazoles 4a-g and the \((E)\)-2-[(naphthalen-1-yl)vinyl]thiazoles 4h-l, all of which satisfy the “Lipinski rule of 5”,\(^19\) is outlined in Scheme 1. Various synthetic methods have been used for the preparation of cinnamic acid and its derivatives,\(^6\)^\(^20\),\(^21\) including compound 7k, as reported by Master \textit{et al.}\(^16\) whose approach has been adopted in the current study. Thus, the commercially available aldehydes 6a-k were reacted with malonic acid in pyridine, in the presence of a catalytic quantity of piperidine, at 90 °C for 1 hour (Scheme 1). [The lower temperature (90 °C) gave yields comparable with those obtained at 120-130 °C.]\(^16\) This procedure permitted the diastereoselective synthesis of the desired \((E)\)-cinnamic acid analogues 7a-f in good yields (68-98%). Confirmation of the \(E\)-configuration is provided by
the large $^1$H NMR vicinal coupling constant ($ca.$ 16 Hz) between the vinylic protons which typically resonate at $ca.$ 7.0 and 9.0 ppm. All of the cinnamic acids 7a-k were subjected to biological screening, while cinnamic acids 7a and 7k served as precursors for the synthesis of the styrylthiazoles 4a-g and the (E)-2-[2-(naphthalen-1-yl)vinyl]thiazoles 4h-l.

The synthesis of the (E)-2-styrylthiazoles 4a-g involved three steps, viz., i) conversion of (E)-cinnamic acid 7a to cinnamamide 8a; ii) thionation to obtain the thioamide 9a; and iii) condensation with $\alpha$-halo carbonyl derivatives to give the corresponding thiazoles 4a-f (Scheme 1). Following the method developed by Pozdnev et al.,$^{22,23}$ (E)-cinnamic acid 7a was reacted with di-tert-butyl dicarbonate [(Boc)$_2$O], ammonium hydrogen carbonate and pyridine in tetrahydrofuran to afford cinnamamide 8a in good yield (78.5%). Thionation was achieved by stirring cinnamamide 8a with Lawesson’s reagent in dry THF at ambient temperature for 8 h,$^{24}$ the progress of the reaction being monitored by thin layer chromatography (TLC). Work-up and column chromatography gave (E)-3-phenylprop-2-enethioamide 9a in 52% yield, with retention of the (E)-configuration about the double bond being confirmed by the large $^1$H NMR vinylic coupling constant ($J = 15.6$ Hz) and conversion of the carbonyl group to thiocarbonyl by the significant downfield shift ($\Delta\delta = 32$ ppm) of the thiocarbonyl (C=S) signal to $\delta$ 198.1 ppm. Application of the conventional Hantzsch method,$^{13}$ involving reaction of (E)-3-phenylprop-2-enethioamide 9a with each of the $\alpha$-halo carbonyl compounds 10a-f ($X=Br$ or Cl) afforded the (E)-2-styrylthiazoles 4a-f. Hydrolysis of the styrylthiazole-5-carboxylate ester 4f afforded the corresponding acid 4g as a white solid (65%, method 1).

Similar methods were used to obtain the (E)-2-[2-(naphthalen-1-yl)vinyl]thiazoles 4h-l, starting from (E)-3-(naphthalen-1-yl)-2-propenoic acid 7k and proceeding via the two intermediates, (E)-3-(naphthalen-1-yl)-2-propenamide 8k (98%) and (E)-3-(naphthalen-1-yl)prop-2-enethioamide 9k (77%).

The synthetic derivatives 7a-k and 4a-l were screened for anti-malarial (Plasmodium falciparum), anti-tuberculosis (Mycobacterium tuberculosis) and anti-bacterial (Pseudomonas aeruginosa) activity as well as for cytotoxicity, in terms of their capacity to inhibit HeLa and SH-SY5Y cells. All of these compounds {with the exception of the (E)-2-[2-(naphthalen-1-yl)vinyl]thiazoles (4h-l), and the para-chlorophenyl (4b) and para-bromophenyl (4c) (E)-styrylthiazoles which have predicted Log P values of 5.83-6.99 (i.e. slightly > 5)} satisfy the requirements of the “Lipinski rule of five” for in vivo transport and the capacity to traverse biological membranes.$^{19}$
Scheme 1. Synthesis of (E)-cinnamic acid derivatives, (E)-styrlythiazoles and (E)-2-[2-(naphthalen-1-yl)vinyl]thiazoles: Reagents and conditions: (i) Piperidine, pyridine, 90 °C, 1 h; (ii) (Boc)$_2$O, THF, pyridine, NH$_4$HCO$_3$, rt, 6 h; (iii) Lawesson’s reagent, THF, rt, 8 h; (iv) EtOH, 70 °C, 1 h; v) KOH, MeOH-H$_2$O.

Preliminary in vitro cytotoxic screening of the synthesised compounds 7a-k and 4a-l was conducted using HeLa cells, while further cytotoxicity studies were conducted on the human neuroblastoma SH-SY5Y cells using an xCELLigence Real-Time Cell Analyzer (RTCA), the output of which is illustrated in Figure 2. The real-time monitoring permits label-free analysis of
cell viability providing insight into the mode of action of the test compounds.\textsuperscript{28,29} From the HeLa cell inhibition data (Table 1) it was apparent that with the exception of the \(p\)-chlorophenyl- (7c) and 2-thiophenyl- (7g) cinnamic acid analogues, which exhibited 35-40\% inhibition at 10 \(\mu\)M, the remaining cinnamic acid analogues exhibited low levels of inhibition (\(\leq 20\%\) at 10 \(\mu\)M). The styrylthiazoles 4a-g and the \((E)\)-2-[2-(naphthalen-1-yl)vinyl]thiazoles 4h-l showed 2-40\% inhibition of HeLa cells at 10 \(\mu\)M, but remarkably variable activity against SH-SY5Y cells (Table 1, Figure 2). Thus, while the RTCA data revealed that of all the \((E)\)-2-[2-(naphthalen-1-yl)vinyl]thiazoles 4h-l inhibited the SH-SY5Y cells remarkably with IC\textsubscript{50} values in the range of 2.09 to 8.64 \(\mu\)M. Compounds 4a and 4b inhibited the SH-SY5Y cells moderately with IC\textsubscript{50} values of ca. 11 mM, the remaining compounds exhibited IC\textsubscript{50} values ranging from 180 to 1000 mM, with the carboxy analogue 4g exhibiting the lowest toxicity on SH-SY5Y cells with a predicted IC\textsubscript{50} value > 1000 mM. The proliferative effects may be due to the extended \(\pi\)-delocalisation in the styryl- and naphthalenylthiazole scaffolds — a structural feature of all-trans-retinoic acid which has been reported to: i) activate survival signalling in SH-SY5Y cells; ii) promote cell survival; and iii) reduce cell susceptibility to neurotoxins.\textsuperscript{30,31} Further studies are required to compare the effects of compounds 4a-l and all-trans-retinoic acid on the morphology and differentiation of SH-SY5Y cells,\textsuperscript{32} and explore their potential activity against neurodegenerative diseases (e.g., Parkinson’s and Alzheimer’s diseases).

Figure 2: RTCA dose-response curves for compound 4l (1-100 \(\mu\)M) on SH-SY5Y cells Cell.

Certain substituted cinnamic acids have been shown to exhibit anti-malarial potential,\textsuperscript{3,33} and compounds 7a-k, the styrylthazole derivatives 4a-g and the \((E)\)-2-[2-(naphthalen-1-yl)vinyl]thiazoles 4h-l were subjected to \textit{in vitro} whole cell \textit{PfLDH-based (Plasmodium falciparum parasite lactate dehydrogenase)} bioassay,\textsuperscript{34} the results of which are summarised in Table 1. Gravina \textit{et al.}\textsuperscript{35} found that, while \(\alpha\)-cyano- and \(\alpha\)-fluorocinnamate exhibited promising anti-malarial activity, these compounds were unfortunately toxic to human cells — a pattern
mirrored in the cytotoxicity levels observed for the cinnamic acid analogues 7a-k. The PfLDH inhibition levels exhibited by the cinnamic acid derivatives 7b-k at a concentration of 20 µM lie in the range 10-30% (chloroquine exhibits 98% inhibition at 2 nM), the 2-furanyl 7h and 2-pyrrolyl 7h derivatives being the least active and the para-fluorophenyl derivative 7f the most active. The (E)-2-styrylthiazoles 4a-g and the (E)-2-[(naphthalen-1-yl)vinyl]thiazoles 4h-l also typically exhibited low inhibition levels against PfLDH (< 20% inhibition at 20 µM).

Cinnamic acid analogues only appear to feature in anti-tuberculosis agents when present as components of more complex scaffolds\(^3\) and, consequently, the in vitro assays for activity against *M. tuberculosis* H\(^{37}\)Rv were limited to the (E)-2-styrylthiazoles 4a-g and (E)-2-[2-(naphthalen-1-yl)vinyl]thiazoles 4h-l. While exhibiting some activity, the MIC90 and MIC99 values for these compounds were found to exceed 20 µM. Thiazole derivatives have been reported to exhibit antimicrobial activity\(^3\) and compounds 4a-l were also subjected to the antibacterial disc diffusion susceptibility assay against *P. aeruginosa* at concentrations of 10–2000 µM. The para-bromophenyl styrylthiazole derivative 4c and (E)-4-(4-fluorophenyl)-2-[2-(naphthalen-1-yl)vinyl]thiazole 4k exhibited low-level zones of inhibition of ca. 7 and 9 mm at 1000 and 2000 µM, respectively (cf- ampicillin, 24.7 mm at 0.0715 µM and streptomycin, 20 mm at 0.0172 µM).

Table 1. Bioassay data showing: the effects of the cinnamic acid analogues 7a-k, the styrylthiazoles 4a-g and the (E)-2-[2-(naphthalen-1-yl)vinyl]thiazoles 4h-l on the viability of HeLa cells; IC\(_{50}\) values for the inhibition of SH-SY5Y cells by compounds 4a-l; the effects of compounds 7a-k and 4a-l on the viability of PfLDH at 20 µM; and the effects of compounds 4a-g and 4h-l against *Mycobacterium tuberculosis* H\(^{37}\)Rv

<table>
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<th>Compd.</th>
<th>R</th>
<th>% HeLa viability at 10 µM</th>
<th>SH-SY5Y IC(_{50}) (µM)</th>
<th>% PfLDH viability at 20 µM</th>
<th>MIC90 (µM)</th>
<th>MIC99 (µM)</th>
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Table 1 (continued)

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<th>Compd.</th>
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<th>% HeLa viability at 10 µM</th>
<th>SH-SY5Y IC₅₀ (µM)</th>
<th>% P/LDH viability at 20 µM</th>
<th>MIC₉₀ (µM)</th>
<th>MIC₉₉ (µM)</th>
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Control compounds: aUntreated HeLa cells: 100% viability; bUntreated SH-SY5Y cells: 100% viability; cChloroquine: 4% viability at 2 nM; dRifampicin: 0.0015µM (MIC₉₀) & 0.00167 µM (MIC₉₉).

Conclusions

Various cinnamic acid analogues 7a-k have been prepared and used as precursors for the synthesis of 2-styrylthiazole derivatives 4a-g and (E)-2-[(naphthalen-1-yl)vinyl]thiazoles 4h-l. None of these compounds exhibited significant inhibition of HeLa cells nor significant antimalarial, antituberculosis or antibacterial activity. However, the (E)-2-[2-[(naphthalen-1-yl)vinyl]thiazoles 4h-l exhibited remarkable activities against SH-SY5Y cells (with IC₅₀ values ranging from 2.09 to 8.64 µM), while the 2-styrylthiazole derivatives 4a-g showed moderate activities against SH-SY5Y cells ranging from inhibition in two cases (with IC₅₀ values of 10.8 and 11.7 mM) to proliferation, with IC₅₀ values ranging from 180 to > 1000 mM. The results indicate that studies are warranted on the effects of styrylthiazoles on the differentiation and extension of SH-SY5Y cells in order to assess their therapeutic potential in the treatment of neurological degenerative diseases.
Experimental Section

All reagents were obtained from Sigma-Aldrich (South Africa) and used without further purification. Tetrahydrofuran (THF) and methylene chloride were stored over 4 Å molecular sieves. Reaction progress and purity of the compounds were checked by thin layer chromatography (TLC) on pre-coated silica gel G60 F\textsubscript{254} plates (Merck®), and viewed under UV light (Syngiene LF-206.LS lamp, South Africa) at 254 and 365 nm. Melting points were recorded, uncorrected, using Reichert(R) slide warmer hot plate microscopy. Nuclear magnetic resonance (NMR) spectra were recorded on Bruker Avance™ II 600 MHz, Bruker Avance™ III HD 400 MHz and Bruker Fourier™ 300 MHz spectrometers. The NMR chemical shifts are reported in ppm downfield from tetramethylsilane (TMS), and the coupling constants are given in Herz (Hz). The NMR analyses were carried out in deuterated solvents, such as DMSO-\textit{d}_6, CDCl\textit{3}, acetone-\textit{d}_6 and methanol-\textit{d}_4, and the spectra calibrated using solvent signals [δ\textsubscript{H}: 7.26 ppm for residual CHCl\textit{3}, 2.50 ppm for residual DMSO, 2.05 ppm for residual acetone and 3.31 ppm for residual MeOH; δ\textsubscript{C}: 77.2 ppm (CDCl\textit{3}), 39.5 ppm (DMSO-\textit{d}_6), 29.8 ppm (acetone-\textit{d}_6) and 49.0 ppm (MeOH-\textit{d}_4)]. Infrared (IR) spectra were obtained using a Perkin Elmer Spectrum 400 Frontier / FT-IR spectrometer; compounds were analysed neat. High resolution mass spectra (HRMS) were recorded on a Waters API Q-TOF Ultima spectrometer (University of Stellenbosch, Stellenbosch, South Africa).

Compounds 7a-k, 37, 8a,\textsuperscript{16,37} 8b,\textsuperscript{38} 9a,\textsuperscript{16,24} 9b,\textsuperscript{39} 4a,\textsuperscript{39-41} 4c-g,\textsuperscript{42} 4h\textsuperscript{39} and 4k\textsuperscript{43,44} are known. The preparation and characterisation of the known compound 4a and the new compounds are summarised below. NMR data for new compounds and bioassay procedures are provided in the supplementary data.

Formation of the \((E)\)-styrylthiazoles 4a-g and the \((E)\)-2-[2-(naphthalen-1-yl)vinyl]thiazoles 4h-l.

\textbf{General procedure, exemplified by the preparation of 4-phenyl-2-styrylthiazole (4a).} A mixture of \((E)\)-3-phenylprop-2-enethioamide (9) (0.082 g, 0.5 mmol) and 2-bromoacetophenone (0.099 g, 0.5 mmol) in ethanol (1.5 mL) was stirred at 70 °C for 1 h. The solvent was removed \textit{in vacuo} and the crude product was extracted with EtOAc. The resulting solution was washed successively with satd. aq. NaHCO\textsubscript{3} and water, and dried (anh. Na\textsubscript{2}SO\textsubscript{4}), and the solvent was removed \textit{in vacuo}. The crude product was purified using column chromatography on silica gel G\textsubscript{60}, eluting with hexane-EtOAc (3:2) to give, as a yellowish, fluffy solid, 4-phenyl-2-styrylthiazole (4a) (0.109 g, 82.6%), mp 130-132 °C (Lit.\textsuperscript{39-41} 131.0-131.5 °C) [HRMS: m/z calculated for C\textsubscript{17}H\textsubscript{14}NS (MH\textsuperscript{+}) 264.0847. Found M+1, 264.0841]; δ\textsubscript{H}/ppm (400 MHz; DMSO-\textit{d}_6) 8.07 (1H, s, thiazolyl-H), 8.00 (2H, d, J = 7.6 Hz, ArH), 7.72 (2H, d, J = 7.3 Hz, ArH), 7.55 (2H, s, HC=CH ), 7.50–7.40 (4H, overlapping m, ArH) and 7.39–7.33 (2H, overlapping m, ArH); δ\textsubscript{C}/ppm (100 MHz; DMSO-\textit{d}_6) 165.8, 154.8, 135.3, 133.9, 133.7, 128.7, 128.6, 128.5, 127.9, 127.0, 125.9, 121.1 and 113.9 (ArC and HC=CH).

\((E)\)-4-(4-Chlorophenyl)-2-styrylthiazole (4b) as a yellow solid (0.140 g, 94%), mp 150-153 °C [HRMS: m/z calculated for C\textsubscript{17}H\textsubscript{13}NS\textsuperscript{35}Cl (MH\textsuperscript{+}) 298.0457. Found M+1, 298.0448]; δ\textsubscript{H}/ppm (400 MHz; DMSO-\textit{d}_6) 8.07 (1H, s, thiazolyl-H), 8.00 (2H, d, J = 7.6 Hz, ArH), 7.72 (2H, d, J = 7.3 Hz, ArH), 7.55 (2H, s, HC=CH ), 7.50–7.40 (4H, overlapping m, ArH) and 7.39–7.33 (2H, overlapping m, ArH); δ\textsubscript{C}/ppm (100 MHz; DMSO-\textit{d}_6) 165.8, 154.8, 135.3, 133.9, 133.7, 128.7, 128.6, 128.5, 127.9, 127.0, 125.9, 121.1 and 113.9 (ArC and HC=CH).
MHZ; DMSO-d6) 8.15 (1H, s, thiazolyl-H), 8.04 (2H, d, J = 7.6 Hz, ArH), 7.73 (2H, d, J = 7.3 Hz, ArH), 7.56 (2H, s, HC=CH), 7.53 (2H, d, J = 8.3 Hz, ArH), 7.42 (2H, t, J = 7.3 Hz, ArH) and 7.37 (1H, t, J = 7.1 Hz, ArH); δc/ppm (100 MHz; DMSO-d6) 166.2, 153.7, 135.4, 134.3, 132.8, 132.6, 129.0, 128.8, 128.7, 127.8, 127.3, 121.2 and 114.8 (ArC and HC=CH).

(E)-4-(4-Bromophenyl)-2-styrylthiazole (4c) as a fluffly, yellow solid (0.103 g, 60.1%), mp 169-170 ºC [HRMS: m/z calculated for C17H13NS79Br (MH+), 341.9952. Found M+1, 341.9945]; δH/ppm (400 MHz; DMSO-d6) 8.15 (1H, s, thiazolyl-H), 7.96 (2H, d, J = 8.0 Hz, ArH), 7.73 (2H, d, J = 7.0 Hz, ArH), 7.66 (2H, d, J = 8.0 Hz, ArH), 7.55 (2H, s, HC=CH), 7.42 (2H, dd, J = 6.6 Hz and J = 7.1 Hz, ArH), 7.37 (1H, t, J = 7.2 Hz, ArH); δc/ppm (100 MHz; DMSO-d6) 166.8, 154.2, 153.9, 134.8, 133.7, 132.2, 129.5, 129.4, 128.6, 127.8, 121.8, 121.7 and 115.4 (ArC and HC=CH).

(E)-4-(4-Fluorophenyl)-2-styrylthiazole (4d) as a yellow solid (0.091 g, 64.9%), mp 126-128 ºC [HRMS: m/z calculated for C17H13NSF (MH+), 282.0753. Found M+1, 282.0742]; δH/ppm (400 MHz; DMSO-d6) 8.06 (1H, s, thiazolyl-H), 8.04 (2H, m, ArH), 7.73 (2H, d, J = 7.1 Hz, ArH), 7.55 (2H, s, HC=CH), 7.42 (2H, t, J* = 6 Hz, ArH), 7.37 (1H, m, ArH) and 7.30 (2H, t, J* = 10 Hz, ArH); δc/ppm (100 MHz; DMSO-d6) 166.5, 165.5 (1JFC= 246 Hz), 155.3, 136.0, 134.6, 131.0 (1JFC= 3.6 Hz), 129.5, 129.4, 128.7 (1JFC= 9.8 Hz), 127.8, 121.8, 116.1 (1JFC= 21.9 Hz) and 114.4 (ArC, HC=CH and thiazolyl-C).

* Overlapping doublets (JH,HH and JF,HH).

(E)-4-(4-Chlorophenyl)-2-[2-(1-naphthalenyl)vinyl]thiazole (4i) as a yellow solid, mp 118-120 ºC; [HRMS: m/z calculated for C21H15NS5Cl (MH+), 348.0614. Found M+1, 348.0606]; v/cm-1 1560 (C=C) and 3143 (C=CH, ArH); δH/ppm (400 MHz; CDCl3) 8.30 (1H, d, J = 16.1 Hz, CH=CH2), 8.26 (1H, d, J = 8.4 Hz, ArH), 7.93-7.86 (4H, m, ArH), 7.83 (1H, d, J = 7.3 Hz, ArH), 7.62-7.57 (1H, m, ArH), 7.56-7.50 (2H, m, ArH), 7.44-7.39 (4H, overlapping m, 2 x ArH, CH=CH2 and thiazolyl-H); δc/ppm (100 MHz; CDCl3) 167.2, 155.3, 134.2, 133.9, 133.2, 133.0, 131.8, 131.5, 129.5, 129.1, 128.9, 127.9, 126.7, 126.3, 125.8, 124.4, 124.1, 123.7 and 112.7 (ArC, HC=CH and thiazolyl-C).

(E)-4-(4-Bromophenyl)-2-[2-(1-naphthalenyl)vinyl]thiazole (4j) as a yellow solid, mp 120-122 ºC; [HRMS: m/z calculated for C21H15NS79Br (MH+), 392.0109. Found M+1, 392.0090]; v/cm-1 1513 (C=C) and 3028 (C=CH, ArH); δH/ppm (400 MHz; CDCl3) 8.30 (1H, d, J = 15.9 Hz, CH=CH2), 8.26 (1H, d, J = 8.3 Hz, ArH), 7.90-7.84 (4H, overlapping m, ArH), 7.83 (1H, d, J = 7.3 Hz, ArH) 7.62-7.49 (5H, overlapping m, ArH), 7.45 (1H, s, thiazolyl-H) and 7.41 (1H, d, J = 15.9 Hz, CH=CH2); δc/ppm (100 MHz; CDCl3) 167.0, 155.2, 133.8, 133.3, 131.9, 131.9, 131.7, 131.3, 129.4, 128.8, 128.0, 126.6, 126.1, 125.7, 124.2, 124.0, 123.5, 122.3 and 112.7 (ArC, HC=CH and thiazolyl-C).

(E)-2-[2-(1-Naphthalenyl)vinyl]-4-(4-nitrophenyl)thiazole (4l) as a bright yellow solid, mp 148-149 ºC; [HRMS: m/z calculated for C21H15N2O2S (MH+), 359.0854. Found M+1, 359.0839]; v/cm-1 1598 (C=C) and 3101 (C=CH, ArH); δH/ppm (400 MHz; DMSO-d6) 8.52 (1H, s, thiazolyl-H), 8.41 (1H, d, J = 15.8 Hz, CH=CH2), 8.39-8.32 (5H, overlapping m, ArH), 8.05 (1H, d, J = 7.3 Hz, ArH), 8.02-7.98 (2H, overlapping m, ArH), 7.68-7.64 (2H, overlapping m, ArH) and 7.63-7.58 (2H, overlapping m, ArH, CH=CH2); δc/ppm (100 MHz; DMSO-d6) 166.8, 152.8, 146.8,
139.9, 133.4, 132.3, 131.1, 130.7, 129.5, 128.7, 127.1, 126.9, 126.3, 125.9, 124.4, 124.3, 123.5, 123.4 and 118.8 (ArC, HC=CH and thiazolyl-C).

**Hydrolysis of ethyl (E)-2-styrylthiazole-4-carboxylate (4f).**

This was achieved using two different methods.

**Method 1.**\(^{45}\) A solution of KOH (0.093 g, 0.5 mmol) in MeOH-H\(_2\)O [(2:1), 300 µL] was added to ethyl (E)-2-styrylthiazole-4-carboxylate (4f) (0.088 g, 0.25 mmol) in MeOH (250 µL). The resulting solution was stirred at room temperature for 2 h, and the reaction was monitored by TLC [hexane-EtOAc (1:1)]. Addition of HCl (20%; 0.5 mL) to the reaction mixture precipitated (E)-2-styrylthiazole-4-carboxylic acid (4g) (0.038 g, 65.4%) as a yellow solid, mp 178-180 ºC (this compound has been cited in the literature\(^ {44}\) without a mp) [HRMS: m/z calculated for C\(_{12}\)H\(_{10}\)NO\(_2\)S (MH\(^+\)) 232.0422. Found M\(^+\), 232.0431]; ν/cm\(^{-1}\) 1730 (C=O) and 2598-3140 (COOH); δ\(_{H}\)/ppm (400 MHz; DMSO-d\(_6\)) 8.39 (1H, s, thiazolyl-H), 7.71 (2H, \(J = 8.2\) Hz, ArH), 7.53 (2H, apparent d, \(\Delta\nu = 17.6\) Hz, CH=CH), 7.42 (2H, t, \(J = 7.2\) Hz, ArH) and 7.36 (1H, m, ArH); δ\(_{C}\)/ppm (100 MHz; DMSO-d\(_6\)) 166.4 (C=O), 162.0, 147.8, 135.2, 128.9, 128.1, 128.1, 128.0, 127.4 and 121.0 (ArC and HC=CH).

**Method 2.**\(^ {43}\) The procedure for the synthesis of compound 4a was employed, using (E)-3-phenylprop-2-enethioamide 9 (0.163 g, 1 mmol) and bromopyruvic acid (0.167 g, 1 mmol) to obtain the desired product 4g as a yellow solid (0.175 g, 75.5%).

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