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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DOI: https://doi.org/10.24820/ark.5550190.p009.905

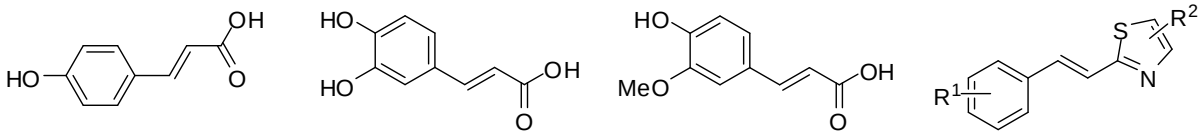
Abstract

Cinnamyl- and thiazole-based compounds have been shown to exhibit diverse medicinal properties and a series of twelve (*E*)-2-styrylthiazole and (*E*)-2-[(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-[naphthalen-1-yl)vinyl]thiazole derivatives proved remarkably active against SH-SY5Y (neuroblastoma) cells with IC_{50} values ranging from 2.09 to 8.64 µM. Two of the seven (*E*)-2-styrylthiazoles were found to be moderately active (with IC_{50} values of 10.8 and 11.7 mM), whereas the remaining five analogues exhibit significant proliferation of SH-SY5Y cells (with IC_{50} 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.



1 *p*-Coumaric acid **2** Caffeic acid **3** Ferulic acid **4** Styrythiazoles

Figure 1. Structures of cinnamic acid derivatives: *p*-coumaric acid **1**, caffeic acid **2** and ferulic acid **3** and the proposed styrylthiazoles **4**.

Results and Discussion

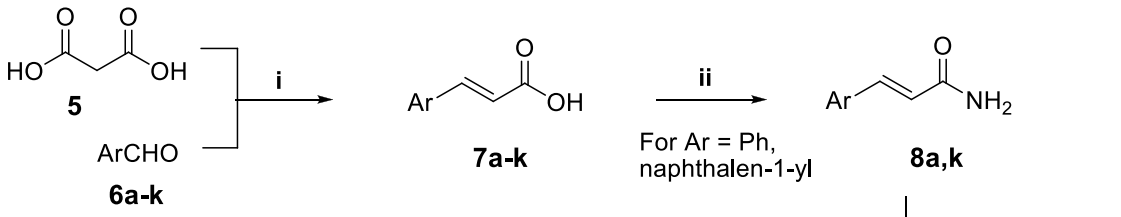
Access to the cinnamic acids **7a-k** and the targeted (*E*)-styrylthiazoles **4a-g** and the (*E*)-2-[2-(naphthalen-1-yl)viny]thiazoles **4h-l**, all of which satisfy the "Lipinski rule of 5",[19] is outlined in Scheme 1. Various synthetic methods have been used for the preparation of cinnamic acid and its derivatives,[6,20,21] including compound **7k**, as reported by Master *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 α-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 [$(Boc)_2O$], 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**[22-27] 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 δ 198.1 ppm. Application of the conventional Hantzsch method,[13] involving reaction of (*E*)-3-phenylprop-2-enethioamide **9a** with each of the α-halo carbonyl compounds **10a-f** (X=Br or Cl) afforded the (*E*)-2-styrylthiazoles **4a-f.** Hydrolysis of the styrythiazole-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*)-styrythiazoles 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]



	Ar	R	% Yield
7a	Phenyl	-	99
7b	*N,N*-Dimethylanilin-4-yl	-	88
7c	*p*-Chlorophenyl	-	94
7d	*p*-Bromophenyl	-	98
7e	*p*-Fluorophenyl	-	93
7f	Quinolin-2-yl	-	68
7g	Thiophen-2-yl	-	90
7h	Furan-2-yl	-	73
7i	Pyrrol-2-yl	-	90
7j	3,4-Methylenedioxyphenyl	-	73
7k	Naphthalen-1-yl	-	68
4a	Phenyl	Phenyl	83
4b	Phenyl	*p*-Chlorophenyl	94
4c	Phenyl	*p*-Bromophenyl	60
4d	Phenyl	*p*-Fluorophenyl	85
4e	Phenyl	*p*-Nitrophenyl	79
4f	Phenyl	Carbethoxy	77
4g	Phenyl	Carboxy	78
4h	Naphthalen-1-yl	Phenyl	69
4i	Naphthalen-1-yl	*p*-Chlorophenyl	58
4j	Naphthalen-1-yl	*p*-Bromophenyl	63
4k	Naphthalen-1-yl	*p*-Fluorophenyl	94
4l	Naphthalen-1-yl	*p*-Nitrophenyl	65

v

Scheme 1. Synthesis of (*E*)-cinnamic acid derivatives, (*E*)-styrylthiazoles and (*E*)-2-[2-(naphthalen-1-yl)vinyl]thiazoles: Reagents and conditions: (i) Piperidine, pyridine, 90 ºC, 1 h; (ii) $(Boc)_2O$, THF, pyridine, NH_4HCO_3, rt, 6 h; (iii) Lawesson's reagent, THF, rt, 8 h; (iv) EtOH, 70 ºC, 1 h; v) KOH, MeOH-H_2O.

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.[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 μM, the remaining cinnamic acid analogues exhibited low levels of inhibition (≤ 20% at 10 μM). 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 μM, 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_{50} values in the range of 2.09 to 8.64 μM. Compounds **4a** and **4b** inhibited the SH-SY5Y cells moderately with IC_{50} values of *ca.* 11 mM, the remaining compounds exhibited IC_{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_{50} value > 1000 mM. The proliferative effects may be due to the extended π-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.[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,[32] and explore their potential activity against neurodegenerative diseases (*e.g.*, Parkinson's and Alzheimer's diseases).

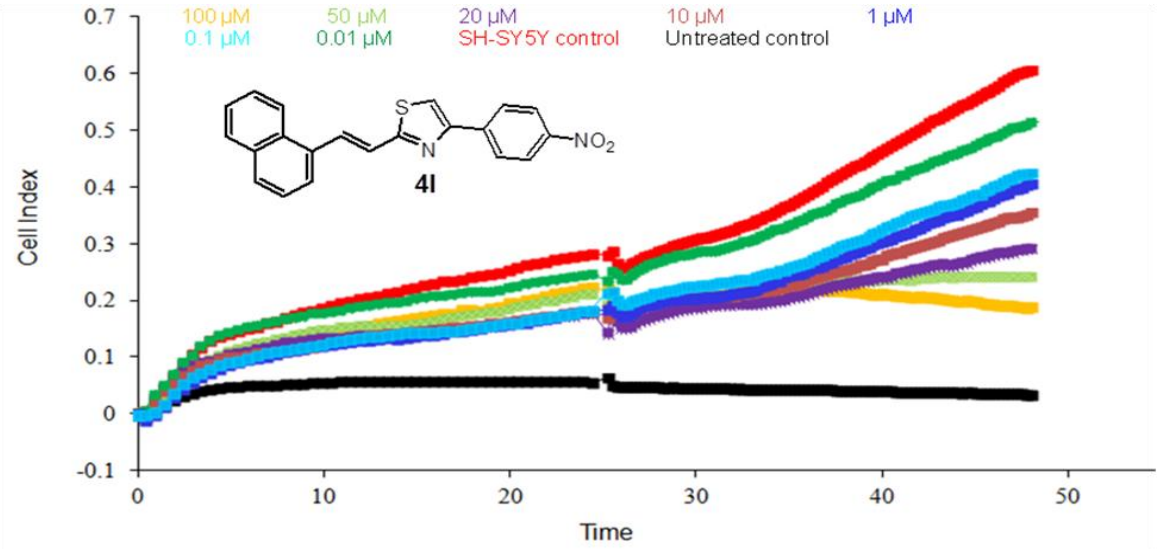


Figure 2: RTCA dose-response curves for compound **4l** (1-100 μM) on SH-SY5Y cells Cell.

Certain substituted cinnamic acids have been shown to exhibit anti-malarial potential,[3,33] and compounds **7a-k**, the styrylthazole derivatives **4a-g** and the (*E*)-2-[(naphthalen-1-yl)vinyl)]thiazoles **4h-l** were subjected to *in vitro* whole cell *Pf*LDH-based (*Plasmodium falciparum* parasite lactate dehydrogenase) bioassay,[34] the results of which are summarised in Table 1. Gravina *et al.*[35] found that, while α-cyano- and α-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 *Pf*LDH 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 **7g** 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 *Pf*LDH (< 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[36] 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*-bromophenylstyrythiazole 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 *Pf*LDH at 20 μM; and the effects of compounds **4a-g** and **4h-l** against *Mycobacterium tuberculosis* H_{37}Rv

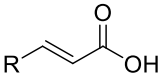
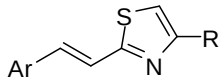
Compd.	R	% HeLa viability at 10 μM[a]	SH-SY5Y IC_{50} (μM)[b]	% *Pf*LDH viability at 20 μM[c]	MIC90 (μM)[d]	MIC99 (μM)[d]
						
7a	Phenyl	-	-	-	-	-
7b	*N*,*N*-dimethylanilin-4-yl	85	-	80	-	-
7c	*p*-Chlorophenyl	65	-	75	-	-
7d	*p*-Bromophenyl	90	-	75	-	-
7e	*p*-Fluorophenyl	85	-	70	-	-
7f	Quinolin-2-yl	90	-	75	-	-
7g	Thiophen-2-yl	60	-	90	-	-
7h	Furan-2-yl	80	-	90	-	-
7i	Pyrrol-2-yl	75	-	80	-	-
7j	3,4-Methylenedioxyphenyl	80	-	80	-	-
7k	Naphthalen-1-yl	85	-	80	-	-

Table 1 (continued)

Compd.	R	% HeLa viability at 10 µM[a]	SH-SY5Y IC_{50} (µM)[b]	% *Pf*LDH viability at 20 µM[c]	MIC90 (µM)[d]	MIC99 (µM)[d]
	 **4a-g**: Ar = Phenyl **4h-l**: Ar = Naphthalen-1-yl					
4a	Phenyl	60	$1.08x10^4$	95	> 20	> 20
4b	*p*-Chlorophenyl	62	$1.17 x10^4$	102	> 20	> 20
4c	*p*-Bromophenyl	60	$1.80 x10^5$	95	> 20	> 20
4d	*p*-Fluorophenyl	70	$5.73 x10^5$	92	> 20	> 20
4e	*p*-Nitrophenyl	90	$4.03 x10^5$	82	> 20	> 20
4f	Carbethoxy	82	$3.51 x10^5$	98	> 20	> 20
4g	Carboxy	98	$> 1x10^6$	90	> 20	> 20
4h	Phenyl	60	2.09	82	> 20	> 20
4i	*p*-Chlorophenyl	65	5.19	90	> 20	> 20
4j	*p*-Bromophenyl	70	8.64	95	> 20	> 20
4k	*p*-Fluorophenyl	75	4.87	105	> 20	> 20
4l	*p*-Nitrophenyl	80	2.23	112	> 20	> 20

Control compounds: [a]Untreated HeLa cells: 100% viability; [b]Untreated SH-SY5Y cells: 100% viability; [c]Chloroquine: 4% viability at 2 nM; [d]Rifampicin: 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, anti-tuberculosis or antibacterial activity. However, the (*E*)-2-[2-[(naphthalen-1-yl)vinyl]thiazoles **4h-l** exhibited remarkable activities against SH-SY5Y cells (with IC_{50} 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 IC_{50} values of 10.8 and 11.7 mM) to proliferation, with IC_{50} 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_{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-d_6, $CDCl_3$, acetone-d_6 and methanol-d_4, and the spectra calibrated using solvent signals [δ_H: 7.26 ppm for residual $CHCl_3$, 2.50 ppm for residual DMSO, 2.05 ppm for residual acetone and 3.31 ppm for residual MeOH; δ_C: 77.2 ppm ($CDCl_3$), 39.5 ppm (DMSO-d_6), 29.8 ppm (acetone-d_6) and 49.0 ppm (MeOH-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**,[16,37] **8b**,[38] **9a**,[16,24] **9b**,[39] **4a,**[39-41] **4e-g,**[42] **4h**[39] and **4k**[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.

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 ^{0}C for 1 h. The solvent was removed *in vacuo* and the crude product was extracted with EtOAc. The resulting solution was washed successively with satd. aq. $NaHCO_3$ and water, and dried (anhydr. Na_2SO_4), and the solvent was removed *in vacuo*. The crude product was purified using column chromatography on silica gel $G_{60,}$ 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 ºC (Lit.[39-41] 131.0-131.5 ºC) [HRMS: *m/z* calculated for $C_{17}H_{14}NS$ (MH^+) 264.0847. Found *M*+1, 264.0841]; δ_H/ppm (400 MHz; DMSO-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_C/ppm (100 MHz; DMSO-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 ºC [HRMS: *m/z* calculated for $C_{17}H_{13}NS^{35}Cl$ (MH^+), 298.0457. Found *M*+1, 298.0448]; δ_H/ppm (400

MHz; DMSO-d_6) 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); δ_C/ppm (100 MHz; DMSO-d_6) 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 fluffy, yellow solid (0.103 g, 60.1%), mp 169-170 ºC [HRMS: *m/z* calculated for $C_{17}H_{13}NS^{79}Br$ (MH^+), 341.9952. Found M+1, 341.9945]; δ_H/ppm (400 MHz; DMSO-d_6) 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); δ_C/ppm (100 MHz; DMSO-d_6) 166.8, 154.2, 135.9, 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 $C_{17}H_{13}NSF$ (MH^+), 282.0753. Found M+1, 282.0742]; δ_H/ppm (400 MHz; DMSO-d_6) 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); δ_C/ppm (100 MHz; DMSO-d_6) 166.5, 163.5 ($^1J_{F,C}$ = 246 Hz), 155.3, 136.0, 134.6, 131.0 ($^4J_{F,C}$ = 3.6 Hz), 129.5, 129.4, 128.7 ($^3J_{F,C}$ = 9.8 Hz), 127.8, 121.8, 116.1 ($^2J_{F,C}$ = 21.9 Hz) and 114.4 (ArC, HC=CH and thiazolyl-C).

* Overlapping doublets ($J_{H,H}$ and $J_{F,H}$).

(*E*)-4-(4-Chlorophenyl)-2-[2-(1-naphthalenyl)vinyl]thiazole *(4i)* as a yellow solid, mp 118-120 ºC ; [HRMS: *m/z* calculated for $C_{21}H_{15}NS^{35}Cl$ (MH^+), 348.0614. Found M+1, 348.0606]; ν/cm^{-1} 1560 (C=C) and 3143 (C=CH, ArH); δ_H/ppm (400 MHz; $CDCl_3$) 8.30 (1H, d, J = 16.1 Hz, CH=CH_a), 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=CH_b, and thiazolyl-H); δ_C/ppm (100 MHz; $CDCl_3$) 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 $C_{21}H_{15}NS^{79}Br$ (MH^+) 392.0109. Found M+1, 392.0090]; ν/cm^{-1} 1513 (C=C) and 3028 (C=CH, ArH); δ_H/ppm (400 MHz; $CDCl_3$) 8.30 (1H, d, J = 15.9 Hz, CH=CH_a), 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=CH_b); δ_C/ppm (100 MHz; $CDCl_3$) 167.0, 155.2, 133.8, 133.3, 133.1, 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 $C_{21}H_{15}N_2O_2S$ (MH^+) 359.0854. Found M+1, 359.0839]; ν/cm^{-1} 1598 (C=C) and 3101 (C=CH, ArH); δ_H/ppm (400 MHz; DMSO-d_6) 8.52 (1H, s, thiazolyl-H), 8.41 (1H, d, J = 15.8 Hz, CH=CH_a), 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 and CH=CH_b); δ_C/ppm (100 MHz; DMSO-d_6) 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_2O [(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 $C_{12}H_{10}NO_2S$ (MH^+) 232.0422. Found *M*+1, 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, Δν = 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%).

Acknowledgements

The authors thank Rhodes University for a bursary (E.O.O) and Rhodes University and the South African Medical Research Council (MRC) for generous financial support. This research project was supported by the South African Medical Research Council (MRC) with funds from National Treasury under its Economic Competitiveness and Support Package. The xCELLigence Real-Time Cell Analyzer SP Station was supported through the National Research Foundation (NRF) – Department of Science & Technology (DST) Research Infrastructure Support Programme (National Nanotechnology Equipment Programme) grant.

References

1. Hoskins, J. A. *J. Appl. Toxicol.* **1984**, *4*, 283. https://doi.org/10.1002/jat.2550040602
2. Kamínek, M.; Ludwig-Müller, J.; Vaňková, R.; Zažímalová, E. *J. Plant Growth Regul*. **2006**, *25*, 89. https://doi.org/10.1007/s00344-005-0120-0
3. Sharma, P. *Prateek. J. Chem. Pharm. Res*. **2011**, *3*, 403.
4. Maeda. H.; Dudareva, N. *Annu. Rev. Plant Bio*. **2012**, 73.

5. Lee, G. S.; Widjaja, A, Ju Y-H. *Biotechnol. Lett*. **2006**, *28*, 581–585. https://doi.org/10.1007/s10529-006-0019-2
6. Mobinikhaledi, A; Foroughifar, N; Jirandehi, H. F. *Synth. React. Inorganic, Met. Nano-Metal Chem.* **2008**, *38*, 428–430.
7. Joshi, B. P.; Sharma, A.; Sinha, A. K. *Tetrahedron* **2006**, *62*, 2590–2593. https://doi.org/10.1016/j.tet.2005.12.028
8. Simonyan, A. V. *Pharm. Chem. J*. **1999**, *33*, 158. https://doi.org/10.1007/BF02508456
9. Joshi, S. D.; Vagdevi, H. M; Vaidya, V. P; Gadaginamath, G. S. *Eur. J. Med. Chem*, **2008**, *43*, 1989. https://doi.org/10.1016/j.ejmech.2007.11.016
10. Haddow, A.; Haris, R. J. C.; Kon, G. A. R.; Roe, E. M. F. *Phil. Trans. Roy. Soc. (London)* **1948**, *241A*, 147. https://doi.org/10.1098/rsta.1948.0011
11. Alajarín, M.; Cabrera, J.; Pastor, A.; Sánchez-Andrada, P.; Bautista, D. *J. Org. Chem.* **2007**, *72*, 2097. https://doi.org/10.1021/jo062417e
12. Alajarín, M.; Cabrera, J.; Pastor, A.; Sánchez-Andrada, P.; Bautista, D. *J. Org. Chem.* **2008**, *73*, 963–973. https://doi.org/10.1021/jo7021668
13. Prakash, O.; Sharma, N.; Ranjan, P. *Synth. Commun.* **2013**, *43*, 582. https://doi.org/10.1080/00397911.2011.604815
14. Moody, C. J.; Bagley, M. C. *J. Chem. Soc., Perkin Trans. 1*, **1998**, 601. https://doi.org/10.1039/a704094f
15. Parmeggiani, A.; Krab, I. M.; Okamura, S.; Nielsen, R. C.; Nyborg, J.; Nissen, P. *Biochemistry* **2006**, *45*, 6846. https://doi.org/10.1021/bi0525122
16. Master, H. E.; Khan, S. I.; Poojari, K. A. *Bioorg. Med. Chem.* **2005**, *13*, 4891. https://doi.org/10.1016/j.bmc.2005.04.075
17. Sureshbabu, V. V.; Venkataramanarao, R.; Naik, S. A.; Chennakrishnareddy, G. *Tetrahedron Lett.* **2007**, *48*, 7038. https://doi.org/10.1016/j.tetlet.2007.07.129
18. Mandal, S.; Moudgil, M.; Mandal, S. K. *Eur. J. Pharmacol*. **2009**, *625*, 90. https://doi.org/10.1016/j.ejphar.2009.06.065
19. Lipinski, C. A.; Lombardo, F.; Dominy, B. W.; Feeney, P. J. *Adv. Drug Deliv. Rev.* **1997**, *23*, 3. https://doi.org/10.1016/S0169-409X(96)00423-1
20. Constantin, I. C.; Fulga, T.; Marioara, O. *Tetrahedron Lett*. **2003**, *44*, 3579. https://doi.org/10.1016/S0040-4039(03)00529-X
21. Wang, J. Wang, C. Li, H. Wang, Y. *Green Chem*. **2006**, *8*, 96.

22. Pozdnev, V. F. *Int. J. Pept. Protein Res.* **1994**, *44*, 36. https://doi.org/10.1111/j.1399-3011.1994.tb00402.x
23. Wang, S.; Hazelrigg, T. *Nature* **1994**, *369*, 400. https://doi.org/10.1038/369400a0
24. Jesberger, M.; Davis, T. P.; Barner, L. *Synthesis* **2003**, 1929.
25 Xia, Z.; Smith, C. D. *J. Org. Chem.* **2001**, *66*, 3459. https://doi.org/10.1021/jo005783l
26. Wang, Z.Y.; Zhang, C. *Macromolecules* **1992**, *25*, 5851. https://doi.org/10.1021/ma00047a043
27. Just-Baringo, X.; Bruno, P.; Albericio, F.; Álvarez, M. *Tetrahedron Lett.* **2015**, *52*, 5435. https://doi.org/10.1016/j.tetlet.2011.07.128
28. Goodyer, I. D.; Taraschi, T. F. *Exp. Parasitol.* **1997**, *86*, 158. https://doi.org/10.1006/expr.1997.4156
29. O'Brien, J.; Wilson, I.; Orton, T.; Pognan, F. *Eur. J. Biochem.* **2000**, *267*, 5421. https://doi.org/10.1046/j.1432-1327.2000.01606.x
30. Southwick, P. L.; Sapper, D. I. *J. Org. Chem.* **1954**, *19*, 1926. https://doi.org/10.1021/jo01377a009
31. Jämsä, A.; Hasslund, K.; Cowburn, R. F.; Bäckström, A, Vasänge M. *Biochem. Biophys. Res. Commun*. **2004**, *319*, 993. https://doi.org/10.1016/j.bbrc.2004.05.075
32. Ollinger, J.; Bailey, M. A.; Moraski, G. C.; Casey, A.; Florio, S.; Alling, T.; Miller, M. J.; Parish, T. *PLoS One* **2013**, *8*, e60531. https://doi.org/10.1371/journal.pone.0060531
33. Kanaani, J.; Ginsburg, H. *Antimicrob. Agents Chemother*. **1992**, *36*, 1102. https://doi.org/10.1128/AAC.36.5.1102
34. Yoshifuji, M.; An, D.-L.; Toyota, K.; Yasunami, M. *Tetrahedron Lett.* **1994**, *35*, 4379. https://doi.org/10.1016/S0040-4039(00)73361-2
35. Gravina, H. D.; Tafuri, N. F.; Silva Júnior, A.; Fietto, J. L. R.; Oliveira, T. T.; Diaz, M. A. N.; Almeida, M. R. *Res. Vet. Sci.* **2011**, *91*, e158. https://doi.org/10.1016/j.rvsc.2010.11.010
36. Narasimhan, B.; Belsare, D.; Pharande, D.; Mourya, V.; Dhake, A. *Eur. J. Med. Chem.* **2004**, *39*, 827. https://doi.org/10.1016/j.ejmech.2004.06.013
37. Jover, J.; Bosque, R.; Sales, J. *QSAR Comb. Sci.* **2008**, *27*, 1204. https://doi.org/10.1002/qsar.200810049
38. Baek, G. H.; Cho, S. J.; Jung, Y. S., Seong, C. M.; Lee, C. W.; Park, N. S. *Bull. Korean Chem. Soc*. **1999**, *20*, 232.
39. Giovanni, P.; Benedetto, T.; Scapini, G. *Helv. Chim. Acta* **1948**, *31*, 1142. https://doi.org/10.1002/hlca.19480310421
40. Yu, Y. B., Chen, H. L., Wang, L. Y., Chen, X. Z., Fu, B. *Molecules* **200**9, *14*, 4858.

https://doi.org/10.3390/molecules14124858
41. Ishiwata, Y.; Togo, H. *Synlett* **2008**, *17*, 2637
42. Glover, C.; Merritt, E. A.; Bagley, M. C. *Tetrahedron Lett.* **2007**, *48*, 7027. https://doi.org/10.1016/j.tetlet.2007.07.111
43. Karegoudar, P.; Karthikeyan M.S.; Prasad D.J.; Mahalinga, M.; Holla, B.S.; Kumari, N.S. *Eur. J. Med. Chem.* **2008**, *43*, 261. https://doi.org/10.1016/j.ejmech.2007.03.014
44. Guernon, J. M.; Wu, Y. J. *Tetrahedron Lett*. **2011**, *52*, 3633. https://doi.org/10.1016/j.tetlet.2011.05.028
45. Theodorou, V.; Skobridis, K.; Tzakos, A. G.; Ragoussis, V. *Tetrahedron Lett.* **2007**, *48*, 8230. https://doi.org/10.1016/j.tetlet.2007.09.074

