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2-Pyrones possessing antimicrobial and cytotoxic activities

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Abstract—The 2-pyrone sub-unit is found in a number of natural products possessing broad spectrum biological activity. Such compounds are validated as being capable of binding to specific protein domains and able to exert a remarkable range of biological effects. In an effort to identify synthetic 2-pyrones with interesting biological effects, herein we report the synthesis and biological evaluation of 4-substituted-6-methyl-2-pyrones. Synthetic routes to 4-alkyl/alkenyl/aryl/alkynyl-6-methyl-2-pyrones have been developed utilising Sonogashira, Suzuki and Negishi cross-coupling starting from readily available 4-bromo-6-methyl-2-pyrone. Specific conditions for each organometallic protocol were required for successful cross-coupling. In particular, a triethylamine/acetonitrile—base/solvent mixture was crucial to Sonogashira alkynylation of 4-bromo-6-methyl-2-pyrone, whereas thallium carbonate was a mandatory base for the Suzuki cross-coupling of trialkylboranes. The 2-pyrones demonstrate potent inhibitory activity against *Bacillus subtilis*, *Escherichia coli*, *Staphylococcus aureus*, *Schizosaccharomyces pombe* and *Botrytis cinerea*. The growth inhibitory activities of selected 2-pyrones were determined in A2780 human ovarian carcinoma and K562 human chronic myelogenous leukaemia cell lines using an in vitro cell culture system (MTT assay). These studies demonstrate that 4-phenylethynyl-, 4-tetrahydropyranylpropargyl ether- and 4-ethynyl-6-methyl-2-pyrones have excellent potential as a new class of anticancer agents.

1. Introduction

The 2-pyrone ring **1** (2*H*-pyran-2-one), a six-membered cyclic unsaturated ester (Fig. 1),[1] is biodiverse and found in bacterial, microbial, plant, insect and animal systems.[2] The complexity of the 2-pyrones vary from simple substituted derivatives, such as triacetic acid lactone **2** and tetraacetic acid lactone **3**, to more complex systems, such as citreoviridin **4**, the bufadienolides **5**, the aureothins **6** and fusapyrone[3] **7**. 2-Pyrones demonstrate a whole spectrum of bioactivity and have been shown to be antifungal, antibiotic, cytotoxic, neurotoxic and phytotoxic.[4] Citreoviridin **4** was the principle toxin found responsible for cardiac beriberi in East Asia in the early part of the 20th century.[5] Fusapyrone **7** is a recent example of a 2-pyrone with considerable antifungal activity, low phytotoxicity and mycotoxicity. Bioactivity is not just associated with the more complex natural products. Indeed, some of the simplest 2-pyrones show remarkable biological effects. 6-Pentyl-2-pyrone **8** for example, isolated as a fungal product from several natural sources including *Trichoderma viride*,[6] demonstrates antimicrobial activity against *Rhizoctonia cerealis*, *Gaeumannomyces graminis* and *Botrytis cinerea*. Similarly, other 6-substituted-2-pyrones have been shown to display phytotoxic insecticidal and cytotoxic properties. The phomenins **9** and **10** (A and B) are two polypropionate 2-pyrones isolated from the phytopathogenic fungus *Phoma tracheiphila*, which have a phytotoxicity of ca. 100 μg/mL.[7] Radicinin **11** has been shown to inhibit the germination of seeds of *Lepidum sativum* (cress) at concentrations of 50 μM.[8] At lower concentrations, considerable damage to root development has been noted. This compound demonstrates inhibitory activity towards the growth of some Gram-positive bacteria, such as *Staphylococcus aureus* and *Clostridium* species, but in general its antifungal properties are negligible. The steroidal 2-pyrones poaefusarin **12** and sporofusarin **13** prevent the complete germination of pea, beans and barley seeds. These 2-pyrones also show acute mammalian toxicity including skin inflammation,

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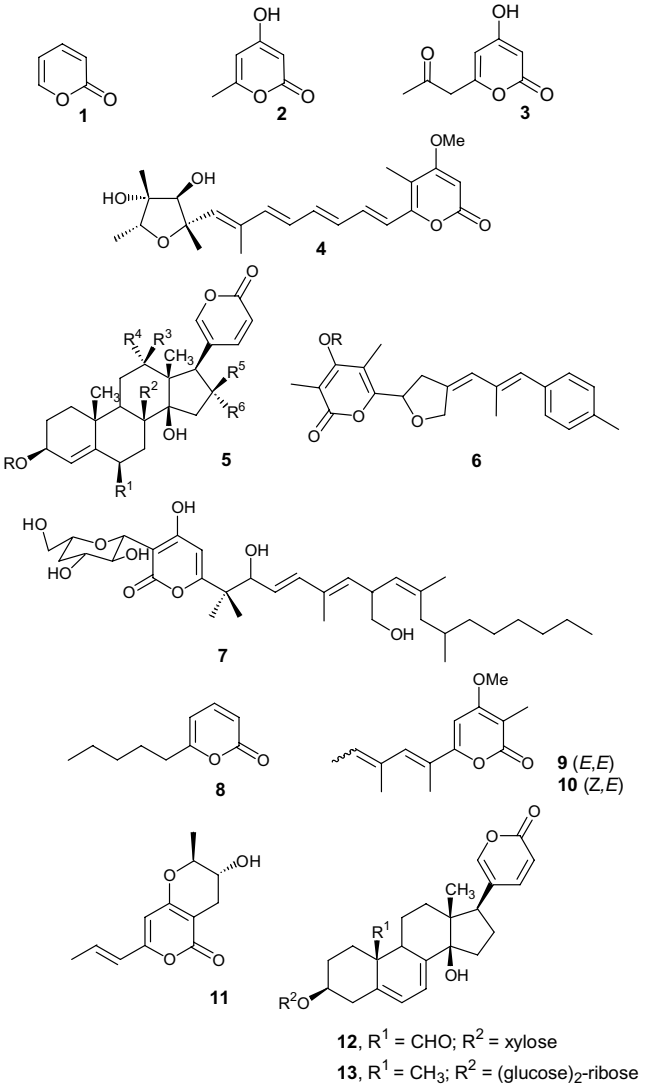


Figure 1. Natural products containing the 2-pyrone sub-unit.

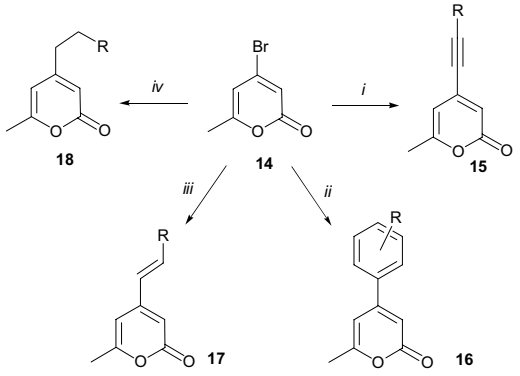
haemorrhagic diathesis and necrosis of the alimentary tract and exhaustion of the bone marrow.

The chemistry of 2-pyrone derivatives is no less fascinating. It finds a wide variety of synthetic applications as a diene component in Diels–Alder reactions,[9] and as a precursor to other heterocyclic systems, such as 2-pyridinones.[10] The aromatic nature of the 2-pyrone ring system is exhibited by its selective substitution patterns in electrophilic reactions, for example, nitration, sulfonation and halogenation, which all occur selectively at the 3- or 5-positions. In contrast, Michael addition reactions proceed selectively at the 4- and 6-positions. However, such methods can be cumbersome and do have limitations. Despite the synthetic utility of 2-pyrones, the synthesis and construction of substituted derivatives are fraught with difficulties, often requiring multi-step syntheses via acyclic precursors. These reactions usually lead to ring opening and rearrangements and the 2-pyrone ring is difficult to regenerate. Direct substitutions onto the 2-pyrone ring, in particular organometallic couplings (Suzuki, Sonogashira, Stille and amination reactions), offer a versatile approach to the synthesis of a plethora of 2-pyrone analogues. The groups of Cho,[11] Moreno-mañas and Pleixats,[12] Rossi[13] and ourselves[14] have recently reported the uses of halogenated 2-pyrones as substrates for organometallic cross-coupling reactions. Furthermore, Meinwald and co-workers have coupled a halogenated 2-pyrone to an alkylzinc reagent, which was a key step in the synthesis of the cockroach sex pheromone, supellapyrone (5-(2′R,4′R-dimethylheptanyl)-3-methyl-2-pyrone).[15] These combined efforts are beginning to overcome the limitations previously associated with the chemistry of the 2-pyrone ring system.[16]

Very recently Waldmann proposed that key to the efficient discovery of new ligands and inhibitors is to identify compound classes already validated as being capable of binding to specific protein domains.[17] 2-Pyrone natural products clearly for fill this description and thus to take advantage of the wide spectrum of biological effects associated with natural 2-pyrones and to utilise the organometallic methodology recently developed for the synthesis of various substituted 2-pyrone derivatives (alkyl, alkenyl, aryl and alkynyl) we have engaged in a programme of research directed towards the identification of simple 2-pyrones with interesting biological effects. We herein report synthetic details and biological results (antimicrobial and anticancer) of 4-substituted-6-methyl-2-pyrones.

2. Chemistry

We chose 4-bromo-6-methyl-2-pyrone **14** for a range of Pd-catalysed reactions (Scheme 1). It was predicted that the 4-carbon centre should be very reactive towards oxidative addition to Pd(0) species as it is an electron deficient carbon within the 2-pyrone ring and therefore an *activated* position. It should be appreciated that the methyl group at the 6-position would be slightly deactivating (Fig. 2).



Scheme 1. (i) Pd-catalysed C–C bond couplings of **14**: (i), Pd/C, Ph_3P, CuI, Et_3N, CH_3CN, Δ; (ii), $Pd(OAc)_2$, $PPh_3RCH=CHM$ (*E*), Na_2CO_3, benzene, Δ; (iii), $Pd(OAc)_2$, Ph_3P, arylboronic acid, Na_2CO_3, benzene, Δ; (iv), BH_3·THF, alkene, 0 °C then $Pd(dppf)Cl_2$, Tl_2CO_3, THF/H_2O, 25 °C.

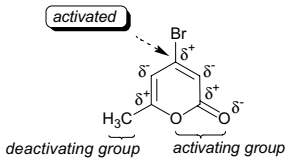
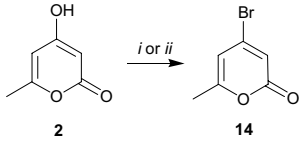


Figure 2. Latent polarities in 2-pyrone **14**.

An efficient synthesis for **14** was achieved by bromination of commercially available **2** using phosphorus tribromide in DMF at 70 °C (Vilsmeier Haack type reaction) to provide **14** in 79% yield.[18] Alternatively, treatment of **2** with tetrabutylammonium bromide and phosphorus pentoxide in toluene at 100 °C provided **14** in 72% yield.[19] The latter method in our hands was lower yielding than that reported (95%), but was more amenable to large-scale synthesis (100 g scale) mechanical stirring is required (Scheme 2).



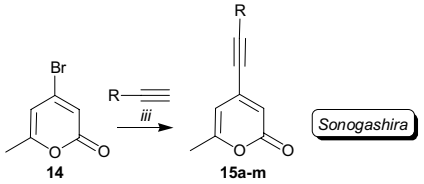
Scheme 2. (i), **2** (1 equiv), PBr_3 (0.33 equiv), DMF–Et_2O, reflux with mechanical stirring, N_2, 16 h; (ii), **2** (1 equiv), *n*-Bu_4NBr (1.1 equiv), P_2O_5 (2.4 equiv), toluene, 100 °C, 1 h.

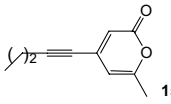
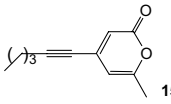
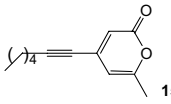
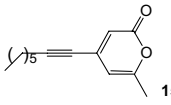
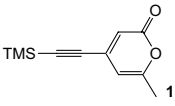
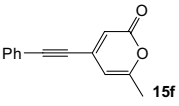
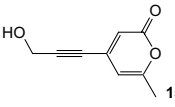
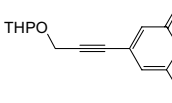
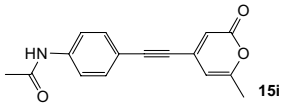
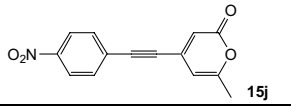
3. Sonogashira alkynylation reactions

Over the last 30 years, the Pd-catalysed alkynylation reaction has emerged as one of the more general and reliable methods for the synthesis of disubstituted alkynes.[20] The Sonogashira reaction, which involves the reaction of a terminal alkyne with an organohalide in the presence of base, Pd catalyst and Cu co-catalyst, is generally one of the most useful procedures. Although there are number of other metals that may be employed for Pd-catalysed alkynylation, in particular Mg, Sn and Zn that are arguably better for a number of substrates, we felt that the Sonogashira protocol would suffice. The Sonogashira alkynylations of **14** provided a series of 4-alkynyl-6-methyl-2-pyrones (**15a**–**m**) (Scheme 2).

The optimum catalyst[14] was found to be a 10% Pd/C (20 wt%) and Ph_3P (25 wt%) combination; in the presence of CuI (4 mol%) in dry acetonitrile/dry triethylamine (1.5:2.5, v/v) at reflux. These conditions could be used with great success (Table 1).[21]

Table 1. Sonogashira alkynylation of **14**[a]

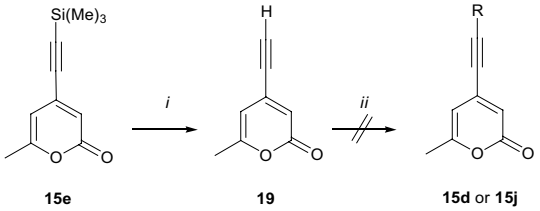


Entry	Coupled product	Yield[b] (%)
1	 **15a**	72
2	 **15b**	77
3	 **15c**	79
4	 **15d**	81
5	 **15e**	82
6	 **15f**	74
7	 **15g**	5
8	 **15h**	81
9	 **15i**	95
10	 **15j**	95

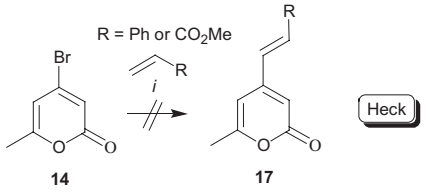
[a] All coupling reactions were conducted at reflux with dry Et_3N and dry CH_3CN (2.5:1.5, v/v), CuI (4 mol%), 10% Pd/C (20 wt%), PPh_3 (25 wt%), under N_2 or argon for 3 h.
[b] Isolated yields after flash chromatography.

At the onset of this project, we identified 4-ethynyl-6-methyl-2-pyrone **19** as a versatile intermediate that could facilitate coupling to a variety of alkenyl and aryl halides under Sonogashira alkynylation conditions (Scheme 3).

We realised that the most efficient route to **19** was going to be via deprotection of 4-trimethylsilylethynyl-6-methyl-2-pyrone **15e**. However, it turned out that this reaction was not straightforward by standard silyl cleavage using methanolic KOH and tetrabutylammonium fluoride (TBAF, 1 M in THF). The reaction at room temperature surprisingly resulted in spontaneous decomposition. Lowering the reaction temperature to −78 °C allowed effective deprotection to occur in 2 h, providing **19** in 80% yield with no observable decomposition. We believe that it is the high reactivity of this compound with basic solvents, such as THF, diethyl



Scheme 3. (i), TBAF (1 M), THF, −78 °C; (ii), [Pd] Cat., CuI, Et_3N, CH_3CN.



Scheme 4. (i), $Pd(OAc)_2$ (5 mol%), PPh_3 (15 mol%), Et_3N or *n*-Bu_3N, DMF, 80–100 °C.

ether and ethanol at temperatures of >−30 °C, which promotes rapid decomposition. Compound **19** is stable in weakly acidic solvents such as chloroform, dichloromethane and hexane at room temperature. For this reason, it is important to note that the isolation of the product must be done using nonpolar, neutral solvents. We undertook a series of Sonogashira alkynylation reactions with **19**, although frustratingly we have so far been unable to couple this pyrone with aryl halides under a variety of standard conditions (Et_3N as base and either CH_3CN, THF or DMF at reflux) using $Pd(OAc)_2/PPh_3$ or $Pd(PPh_3)_4$ precatalysts/catalysts. This is presumably due to the aforementioned reasons, where **19** decomposes under the Sonogashira conditions. It should be noted that we are unable to recover **19** from these reactions (alkyne dimer predominates). Very recently it has been reported that it is possible to take substituted trimethylsilyl protected alkynes as substrates for the Sonogashira reaction. This reaction requires caesium carbonate as a base and serves to deprotect the trimethylsilyl derivative 'in situ'. However, we have yet been unable to obtain useful yields of the cross-coupled products from the Sonogashira reactions of **15e** with aryl bromides (conditions: $Pd(PPh_3)_2Cl_2$ (5 mol%), CuI (4 mol%), 1,4-dioxane, reflux, $CsCO_3$, 3 h).

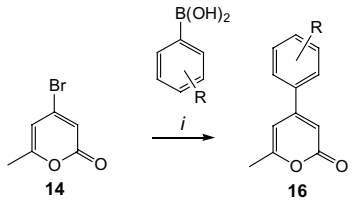
4. Heck, Negishi and Suzuki alkenylation reactions

The direct cross-coupling of alkenes with aryl and alkenyl halides, the Heck reaction, is the most widely used and well known cross-coupling procedure.[22] It is generally effected by Pd(0) catalysts, which are conveniently prepared in situ from a precursor catalyst, such as palladium(II)acetate ($Pd(OAc)_2$)/triphenylphosphine combination. Following general and modified Heck coupling procedures, a number of reactions were conducted with **14** (as the electrophilic component) and styrene or methyl acrylate in the presence of triethylamine or tributylamine as the base, in *N*,*N*-dimethylformamide (DMF) at 80–100 °C, however as of yet we been unable to isolate the coupled product (Scheme 4).

In order to achieve coupling with alkenes, attention was diverted to activated metallated alkenes. There are various procedures in the literature for the synthesis of alkenyl metals, and many of these have been used in the cross-coupling reactions with aryl and alkenyl halides. Metals reported to date include aluminium,[23,24] boron,[25] magnesium,[26] silicon,[27] tin,[28] zinc[29] and zirconium.[30,31] Depending on the metal employed, the alkenyl metal reagent is formed either by selective addition of a metal hydride across a triple bond, by treatment of a vinyl halide directly with the metal or by a metal–metal exchange reaction. Some of these metal reagents have immediate disadvantages when considering the reactivity of the 2-pyrone ring. Organomagnesium reagents would tend to attack the pyrone ring at the 2-, 4- or the 6-positions and cause ring-opening. Aluminium hydrides are reactive toward many types of oxygen functionalities, as are the corresponding organoaluminates, and this was unappealing to us. We decided to turn our attention to organoboranes, which have been shown to be extremely versatile nucleophilic reagents.[32]

Suzuki coupling with arylboronic acids: The Suzuki coupling of **14** with several arylboronic acids proceeded well using Pd(0) based catalysts, such as tetrakistriphenylphosphinepalladium(0) $(Ph_3P)_4Pd$ or $Pd(OAc)_2$/triphenylphosphine (Pd:PPh_3, 1:3). The latter precatalyst proved more versatile, in that $Pd(PPh_3)_2$—*the active catalyst*—was formed in situ, whereas $Pd(PPh_3)_4$ had to prepared fresh and used within a month stored at −30 °C in the dark under an atmosphere of argon (commercial sources of $Pd(PPh_3)_4$ were unreliable in our reactions!). A wide range of bases are used in Suzuki couplings, in particular NaOEt and NaOH are commonly employed. These bases were too strong for the 2-pyrone unit to withstand due to ease of hydrolysis. We found that use of Na_2CO_3 was crucial to high reproducible yields of **16**. In Table 2, the coupling of several commercially boronic acids can be found. The electron-donating arylboronic acids provided the coupled products in generally good yields (entries 2 and 4, Table 2). Changing the ligand to tri-2-furylphosphine ($P(2\text{-furyl})_3$, electron withdrawing ligand) improved the yields. Although little problems were seen with the chloro-substituent (entry 3, Table 2), changing to the more electron withdrawing nitro and formyl substituents had a dramatic effect on the yields (entries 5 and 6, Table 2). For the latter, the $P(2\text{-furyl})_3$ ligand again improved the yield to 43%. The reasons for the higher yields may be attributed to a key phosphine dissociation event during the transmetallation step of the catalytic cycle. We presume that it is the lower nucleophilicity of these arylboronic acids, which contributes to the poor yields of the cross-coupled products. Buchwald and co-workers have developed an efficient ligand (catalyst system) for cross-coupling arylboronic acids with aryl halides, which is particularly useful for less reactive substrates.[33] The conditions: $Pd(OAc)_2$ (1 mol%)/di-*tert*-butylphosphino)biphenyl (2.0 mol%) ($P(t\text{-Bu})_2$Biph), potassium fluoride (3 equiv), THF;

Table 2. Cross-coupling of various arylboronic acids with **4**[a]



Entry	Pyrone (R)	Yield (%)		
		PPh_3	P(2-furyl)$_2$[b]	P(*t*-Bu)$_2$Biph[c]
1	H–, **16a**	56	81	92
2	*p*-CH_3–, **16b**	52	76	85
3	*p*-Cl–, **16c**	53	53	72
4	*p*-CH_3O–, **16d**	62	86	84
5	*m*-NO_2–, **16e**	0	—	—
6	*p*-CHO–, **16f**	20	43	56

[a] Conditions: (i), $Pd(OAc)_2$ (6 mol%), PPh_3 (18 mol%), arylboronic acid (1.1 equiv), Na_2CO_3, benzene/ethanol, Δ, 6 h.
[b] Using P(2-furyl)$_3$ (18 mol%) as the ligand in place of PPh_3, yields in parenthesis.
[c] Using the Buchwald catalytic system; $Pd(OAc)_2$ (1 mol%), 2-di-*tert*-butylphosphino)biphenyl (2 mol%), arylboronic acid (1.1 equiv), KF (3 equiv), THF, 25 °C, 6–16 h.

allow a variety of aryl bromides and aryl chlorides to be coupled to arylboronic acids at room temperature. In all of the arylboronic acids tested, improved yields were observed for Suzuki cross-coupling of **14**. Indeed, the procedure could be run at room temperature. Generally the reactions required stirring overnight, that is with the exceptions of **16b** and **16d** (entries 2 and 4, Table 2), which were complete within 6 h.

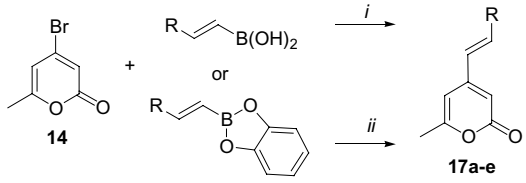
Suzuki coupling with E-alkenyl-1,3,2-benzodioxaboroles and E-alkenyl boronic acids: We decided to explore the uses of both *E*-alkenyl-1,3,2-benzodioxaboroles and *E*-alkenyl boronic acids. The synthesis of the *E*-alkenyl-1,3,2-benzodioxaboroles was accomplished by *syn* addition of catechol borane (1.0 M solution in THF) to a number of terminal acetylenes at 70 °C for 1 h.[34] The pure products were distilled under high vacuum and then stored under nitrogen (*air and moisture sensitive*). The initial investigations into coupling *E*-alkenyl-1,3,2-benzodioxaboroles with **14** under Pd(0) catalysis gave the coupled products in ~30–40% yields (Table 3). However, we felt these reactions were cumbersome and the air-sensitive benzodioxaboroles made them unattractive. The corresponding boronic acids were expected to be less sensitive. Their synthesis was achieved via hydrolysis of the *E*-alkenyl-1,3,2-benzodioxaboroles, which occurred rapidly within 1 h at room temperature.

The cross-coupling of **14** to the *E*-alkenyl boronic acids was facile and yields of the 4-alkenyl-6-methyl-2-pyrones improved dramatically (Table 3). As for the arylboronic acids, use of Na_2CO_3 proved a mandatory base.

Negishi coupling of E-alkenyl zirconocenes: Zirconocene chloride hydride (Cp_2ZrHCl) is a commercially available white solid, which adds to terminal alkynes regioselectively and stereospecifically to provide *E*-alkenyl zirconocenes.[35,36] Cross-coupling of *E*-alkenyl zirconocenes with β-bromo-α,β-unsaturated esters in the presence of catalytic amounts of $PdCl_2(PPh_3)_2$ (5 mol%)/*i*-Bu_2AlH (10 mol%) has been shown to be an effective protocol for cross-coupling (*i*-Bu_2AlH serves to reduce Pd(II) to Pd(0)).[37] We found that compound **14** and (*E*)-hex-1-enyl zirconocene chloride coupled to give the product **17b** in 72% yield. We initially had problems extending the protocol to other alkynes. The air and light sensitivity of Cp_2ZrHCl caused some problems. By conducting the reactions in the dark and using freshly prepared Cp_2ZrHCl, the yields from the cross-coupling were increased (Table 4). It should be noted that we have only detected the *E*-isomer in these reactions (by NMR and HRGC).

Suzuki cross-coupling with trialkylboranes: hydroboration-Suzuki coupling: To the best of our knowledge there are no reports, outside our recent work, on the transfer of organoboranes, containing alkyl groups, to 2-pyrones. The synthesis of 4-alkyl-6-methyl-2-pyrones (**18**) was initially envisaged as being easily accessible from alkylboronic acids and alkylbenzodioxaboroles.

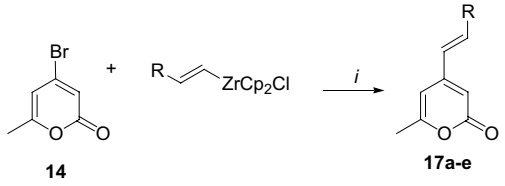
Table 3. Suzuki cross-coupling of alkenyl boronic acids with **14**[a]



Entry	Pyrone (R)	Yield[b] (%)
1	$CH_3(CH_2)_2$–, **17a**	58 (35)
2	$CH_3(CH_2)_3$–, **17b**	66 (32)
3	$CH_3(CH_2)_4$–, **17c**	60 (41)
4	$CH_3(CH_2)_5$–, **17d**	56 (32)
5	Ph–, **17e**	27 (31)

[a] Conditions: (i), $Pd(OAc)_2$ (6 mol%), PPh_3 (18 mol%), alkenyl boronic acid, Na_2CO_3, benzene/ethanol, reflux, 6 h; (ii), as for (i), but benzene only.
[b] Numbers in brackets correspond to the yields from the alkenyl benzodioxaboroles.

Table 4. Negishi cross-coupling of alkenyl zirconocenes with **14**[a]



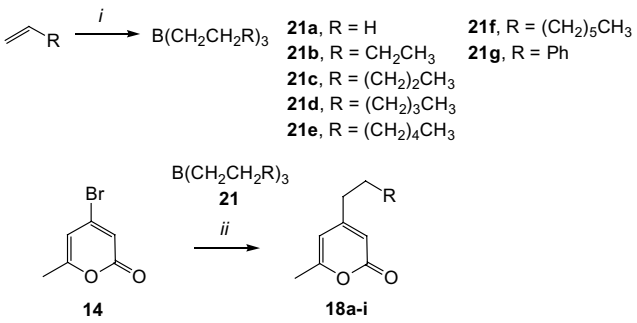
Entry	Alkyne (R)	Yield (%)
1	$CH_3(CH_2)_2$–, **17a**	67
2	$CH_3(CH_2)_3$–, **17b**	72
3	$CH_3(CH_2)_4$–, **17c**	76
4	$CH_3(CH_2)_5$–, **17d**	53
5	Ph–, **17e**	74

[a] Conditions: (i), $Pd(PPh_3)_2Cl_2$ (5 mol%), *i*-Bu_2AlH (1 M, 10 mol%), alkenyl zirconocene (1.1 equiv), THF, reflux, 5–6 h.

Attempts to couple either have so far proved unsuccessful. A variety of conditions ([Pd] catalyst, THF, 1,4-dioxane, Na_2CO_3 or K_2CO_3 at rt to reflux) were tried. Attention focused on an alternative method, again using boranes but utilising the readily accessible trialkylboranes. Alkylboranes are prepared quantitatively by hydroboration of alkenes, proceeding through a *cis*-Markovnikov addition from the less hindered side of the alkene bond.[38] Alkylboranes are usually quite inert towards many types of functional groups, and we felt that these nucleophilic coupling partners would be ideal for the coupling reactions of brominated 2-pyrones. A number of trialkylboranes were coupled to **14** using some important conditions. We chose to employ [1,1′-bis(diphenylphosphino)ferrocene] dichloropalladium(II)·dichloromethane [Pd(dppf)Cl_2·CH_2Cl_2] as the precatalyst. The dppf ligand thwarts β-hydride elimination of the alkyl-[Pd] intermediates, reduction of the halide (hydrodehalogenation) and isomerisation of the alkyl groups and essentially facilitates the efficient transfer of the alkyl group to the 2-pyrone ($Pd(PPh_3)_4$ produced complex mixtures of products).[39] We found that thallium carbonate (Tl_2CO_3) was a mandatory base in a THF–H_2O (6:1, v/v) solvent mixture, which allows the reactions to be conducted effectively at 25 °C (Table 5). Several trialkylboranes (**21a**–**g**) were cross-coupled (**18a**–**g**) and the isolated yields were good. The addition of base specifically increases the reactivity (nucleophilicity) of the organic group on boron, promoting the transmetallation step between the trialkylborane and the 16-electron (dppf)$RPd^{(II)}X$ species (Table 5). Alteration of the base to K_2CO_3 or Na_2CO_3 resulted in lower yields and the reactions had to be performed at reflux. The large thallium cation is expected to be weakly coordinated, increasing the nucleophilicity of the resultant $[R_3BOH]^-$ species.

Alteration of the base to Cs_2CO_3 provides similar yields of the cross-coupled product, thus providing some credibility for this argument. Recent work on the reaction of alkylboronic acids with aryl halides and aryl triflates by Molander and Yun has demonstrated that Tl_2CO_3 can be replaced by Cs_2CO_3 or K_2CO_3 in THF/H_2O (10:1, v/v) at reflux using [Pd(dppf)Cl_2·CH_2Cl_2] as the catalyst precursor, to provide high yields of the coupled products. However, as with our initial studies we have yet to obtain reproducible yields for the cross-coupled products using this method. Therefore at the present time the most efficient way to introduce the alkyl groups to **14** is via hydroboration of the terminal alkene, followed by Suzuki cross-coupling (Table 5).

Table 5. Suzuki cross-coupling of trialkylboranes with **14**[a]



Entry	Pyrone (R)	Yield (%)
1	H, **18a**	56
2	CH_3CH_2–, **18b**[b]	60
3	$CH_3(CH_2)_2$–, **18c**[c]	50
4	$CH_3(CH_2)_3$–, **18d**	46
5	$CH_3(CH_2)_4$–, **18e**	54
6	$CH_3(CH_2)_5$–, **18f**	41
7	Ph–, **18g**	59

[a] Conditions: (i) BH_3·THF, alkene, 0 °C, 1 h; (ii) Pd(dppf)Cl_2 (5 mol%), Tl_2CO_3, THF/H_2O, 25 °C.
[b] Et_3B (1 M in THF) was employed.
[c] *n*-Bu_3B (1M in THF) was employed.

Table 6. In vitro IC_{50} cytotoxicity results against A2780 and K562 cell lines[a]

R	A2780 (μM)	K562 (μM)
Pentynyl, **15a**	4.8	17.8
Hexynyl, **15b**	7.0	15.5
Heptynyl, **15c**	2.9	11.1
Octynyl, **15d**	3.4	20.3
Phenylethynyl, **15f**	1.8	4.0
$THPOCH_2$, **15h**	2.1	4.0
Ph, **16a**	>50	>50
p-CH_3–, **16b**	>50	>50
p-Cl–, **16c**	>50	>50
p-CH_3O–, **16d**	>50	>50
p-CHO–, **16f**	19.2	26.0
Hexenyl, **17b**	>50	>50
Heptenyl, **17c**	>50	>50
Phenylethenyl, **17e**	>50	>50
Ethyl, **18a**	>50	>50
Butyl, **18b**	>50	>50
Pentyl, **18c**	>50	>50
Hexyl, **18d**	>50	>50
Heptyl, **18e**	41.8	28.7
2-Phenylethyl, **18g**	>50	>50
Ethynyl, **19**	3.1	2.0

[a] The IC_{50} values represent an average of three experiments.

5. Biological results

With a large number of 4-subsituted-6-methyl-2-pyrones synthesised we embarked on thorough systematic biological screening. We were encouraged by reports on the antitumour activities of a variety of pyrone analogues.[40] A number of our compounds were evaluated for growth inhibitory activities against A2780 human ovarian carcinoma and K562 human chronic myelogenous leukaemia cell lines using an in vitro cell culture system (MTT assay) (Table 6). The assay itself is based on the reduction of 3-(4′,5′-dimethylthiazol-2′-yl)-2,5-diphenyltetrazolium bromide (MTT, yellow colour) by mitochondrial dehydrogenases of metabolically active cells to a purple-blue formazan. The IC_{50} concentration for each derivative was calculated with reference to a standard curve (control cells), which represents the concentration that results in a 50% decrease in cell growth after 5 days incubation. It should be noted that comparable

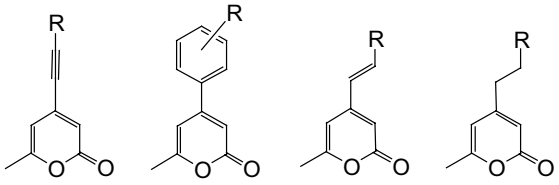
potent inhibitors against A2780 and K562 cell lines have been reported[41] (within the same laboratory).[41a]

The antiproliferative activity of the 4-substituted-6-methyl-2-pyrones is distributed in both A2780 and K562 cell lines. It is possible to discern some quite prominent structure–activity relationships for the compounds. Just over a third of the compounds screened demonstrate prominent activity.

The 4-alkynyl derivatives (**15a**–**d**, **15f**, **15h** and **19**) are the most potent compounds in the series, with phenylethynyl derivative **15f** possessing an IC_{50} of 1.8 and 4.0 μM against A2780 and K562 cell lines, respectively. An interesting trend is observed for the alkynyl series against the both cell lines, particularly K562. Increasing the alkynyl chain from pentynyl through to a heptynyl chain (**15a**–**c**) sees an improvement in activity against the K562 cell line, peaking at **15c**. By extending the alkynyl chain by one-carbon to **15d**, we see a fall off in activity. A slightly different trend for the A2780 cell line is observed, although the activity again peaks with **15c**. A somewhat striking result is observed by comparing the activities of **15d** for both cell lines (IC_{50} = (A2780) 3.4 μM and (K562) 20.3 μM). Such differences are observed with alkynyl derivatives **15a**–**d** for both cell lines to more or a lesser degree. Two further potent analogues, in addition to **15f**, are **15h** and **19** with an IC_{50} (A2780) of 2.1 and 3.1 μM and IC_{50} (K562) of 4.0 and 2.0 μM, respectively.

The antimicrobial effects of the 2-pyrones were evaluated for against selected bacteria, yeasts and fungi (Tables 7 and 8).

Table 7. Antimicrobial activities of 4-substituted-6-methyl-2-pyrones[a]



15 **16** **17** **18**

Compound (R, No.)	*Bacillus subtilis*[b]	*Escherichia coli*[b]	*S. aureus*[b]	*C. albicans*[c]	*S. cerevisiae*[c]	*Schizosaccharomyces pombe*[c]
$CH_3(CH_2)_2$–, **15a**	18 (20.5)	22 (25.0)	21 (23.9)	11 (12.5)[d]	0	29 (32.9)[e]
$CH_3(CH_2)_3$–, **15b**	17 (19.3)	20 (22.7)	0	20 (22.7)[e]	0	45 (51.1)[f]
$CH_3(CH_2)_4$–, **15c**	19 (21.6)	19 (21.6)	21 (23.9)	22 (25.0)[e]	0	50 (56.8)[f]
$CH_3(CH_2)_5$–, **15d**	17 (19.3)	17 (19.3)	18 (20.5)	0	0	48 (54.5)[f]
Ph–, **15f**	19 (21.6)	20 (22.7)	0	0	0	37 (42.0)[f]
$THPOCH_2$–, **15h**	20 (22.7)	17 (19.3)	30 (34.1)	13 (14.8)[d]	0	18 (20.5)[d]
p-NHAc-Ph–, **15i**	6 (6.8)	0	16 (18.2)	0	0	0
p-NO_2-Ph–, **15j**	6 (6.8)	0	0	0	0	9 (10.2)
H–, **16a**	18 (20.5)	20 (22.7)	18 (20.5)	0	0	0
p-CH_3–, **16b**	19 (21.6)	18 (20.4)	0	0	0	0
p-Cl–, **16c**	18 (20.5)	20 (22.7)	20 (22.7)	0	0	0
p-CH_3O–, **16d**	17 (19.3)	20 (22.7)	0	0	0	20 (22.7)[e]
p-CHO–, **16f**	18 (20.5)	15 (17.0)	18 (20.5)	0	0	14 (15.9)[d]
$CH_3(CH_2)_2$–, **17a**	16 (18.2)	19 (21.6)	20 (22.7)	16 (18.2)[d]	0	28 (31.8)[e]
$CH_3(CH_2)_3$–, **17b**	18 (20.5)	18 (20.5)	0	24 (27.3)[e]	0	40 (45.5)[f]
$CH_3(CH_2)_4$–, **17c**	18 (20.5)	18 (20.5)	17 (19.3)	21 (23.9)[e]	0	34 (38.6)[e]
$CH_3(CH_2)_5$–, **17d**	18 (20.5)	20 (22.7)	18 (20.5)	20 (22.7)[e]	0	29 (32.9)[e]
Ph–, **17e**	18 (20.5)	17 (19.3)	0	0	0	18 (20.5)[d]
H, **18a**	0	21 (23.8)	0	0	0	0
CH_3CH_2–, **18b**	20 (22.7)	22 (25.0)	0	0	0	0
$CH_3(CH_2)_2$–, **18c**	23 (26.1)	22 (25.0)	0	0	0	22 (25.0)[e]
$CH_3(CH_2)_3$–, **18d**	21 (23.9)	25 (28.4)	0	11 (12.5)[d]	0	26 (29.5)[e]
$CH_3(CH_2)_4$–, **18e**	16 (18.2)	17 (19.3)	14 (15.9)	16 (18.2)[d]	5 (5.7)	30 (34.1)[f]
$CH_3(CH_2)_5$–, **18f**	19 (21.6)	22 (25.0)	18 (20.5)	20 (22.7)[e]	0	30 (34.1)[f]
Ph–, **18g**	18 (20.5)	20 (22.7)	0	0	0	10 (11.4)
H–, **19**	17 (19.3)	16 (18.2)	30 (34.1)	36 (40.9)[f]	40 (45.4)[f]	40 (45.5)[f]
Zaragozic acid A 'Control'	—	—	—	27 (30.7)	27 (30.7)	37 (42.0)

NT = not tested; radii/mm (percent) of inhibition. The values given averages of at least two separate experiments.

[a] Each cellulose disk contained 200 μg of the test compound.

[b] After 4 d incubation.

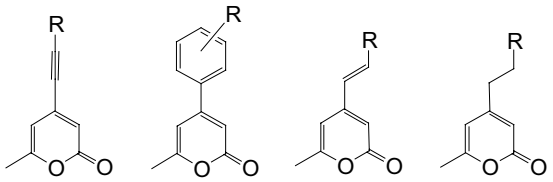
[c] After 2 d incubation.

[d] Represents modest inhibitory activity.

[e] Good inhibitory activity.

[f] Excellent inhibitory activity with respect to the control sample.

Table 8. Antimicrobial activities of 4-substituted-6-methyl-2-pyrones[a]



15 **16** **17** **18**

Compound (R, No.)	*Aspergillus niger*[b]	*Fusarium oxysporum*[b,c]	*Pythium ultimum*[b,d]	*Rhizoctonia solani*[b,e]	*B. cinered*[b,f]
Ph–, **15b**	88 (>95)	6 (51)[g]	100 (100)	56 (100)	56 (100)
$CH_3(CH_2)_2$–, **15a**	>95 (100)	2 (26)[h]	100 (100)	28 (78)[h]	39 (100)[i]
$CH_3(CH_2)_3$–, **15b**	92 (>95)	2 (32)[h]	100 (100)	26 (84)[h]	31 (100)[g]
$CH_3(CH_2)_4$–, **15c**	89 (100)	3 (32)[g]	100 (100)	23 (84)[h]	39 (100)[i]
$CH_3(CH_2)_5$–, **15d**	90 (>95)	6 (35)[g]	100 (100)	41 (100)[i]	38 (100)[i]
Ph–, **15f**	88 (>95)	6 (51)[g]	100 (100)	56 (100)	56 (100)
$THPOCH_2$–, **15h**	89 (>95)	5 (47)[g]	89 (100)	20 (72)[h]	40 (100)[i]
p-NHAc-Ph–, **15i**	>95 (100)	11 (53)	100 (100)	55 (100)	58 (100)
p-NO_2-Ph–, **15j**	87 (91)	6 (56)[g]	100 (100)	71 (100)	56 (100)
H–, **16a**	91 (>95)	2 (30)[h]	100 (100)	42 (100)[i]	55 (100)
p-CH_3–, **16b**	>95 (100)	6 (47)[g]	100 (100)	39 (100)[g]	58 (100)
p-Cl–, **16c**	82 (91)	8 (51)[i]	100 (100)	51 (100)	63 (100)
p-CH_3O–, **16d**	NT	14 (100)	100 (100)	57 (100)	56 (100)
p-CHO–, **16f**	79 (93)	8 (56)[i]	100 (100)	59 (100)	63 (100)
$CH_3(CH_2)_2$–, **17a**	>95 (100)	5 (45)[g]	100 (100)	49 (100)	47 (100)
$CH_3(CH_2)_3$–, **17b**	92 (>95)	2 (25)[h]	96 (100)	35 (100)[g]	44 (100)
$CH_3(CH_2)_4$–, **17c**	89 (94)	2 (24)[h]	95 (100)	38 (100)[g]	45 (100)
$CH_3(CH_2)_5$–, **17d**	89 (94)	5 (30)[g]	100 (100)	44 (100)[i]	42 (100)[i]
Ph–, **17e**	NT	NT	100 (100)	47 (100)	59 (100)
CH_3CH_2–, **18b**	>95 (100)	11 (60)	100 (100)	47 (100)	63 (100)
$CH_3(CH_2)_2$–, **18c**	80 (90)	5 (39)[g]	100 (100)	56 (100)	47 (100)
$CH_3(CH_2)_3$–, **18d**	87 (94)	5 (47)[g]	100 (100)	52 (100)	51 (100)
$CH_3(CH_2)_4$–, **18e**	79 (92)	5 (51)[g]	100 (100)	51 (100)	53 (100)
$CH_3(CH_2)_5$–, **18f**	80 (80)	5 (48)[g]	100 (100)	51 (100)	47 (100)
Ph–, **18g**	93 (93)	11 (60)	100 (100)	51 (100)	50 (100)
H–, **19**	59 (71)	2 (35)[h]	100 (100)	17 (93)[h]	30 (100)[g]

[a] Each test plate was inoculated by pippetting 20 μL of a 10 mg/mL solution of the pyrone in absolute EtOH, 2 cm offset from the centre of the plate containing the organism.[43] The values given averages of at least two separate experiments.

[b] Numbers represent percentage growth of the organism after 3 d incubation (NT = not tested). Numbers in brackets represent % growth after 7 d incubation.

[c] EtOH control (15% growth after 3 d, 65% after 7 d).

[d] EtOH control (100% growth after 3 d).

[e] EtOH control (61% growth after 3 d, 100% after 7 d).

[f] EtOH control (65% growth after 3 d, 100% after 7 d).

[g] Good inhibitory activity.

[h] Excellent inhibitory activity. Bioassays were performed using either plate or well methods.[44]

[i] Represents modest inhibitory activity.

The vast majority of the 2-pyrones screened against *B. subtilis* demonstrated some inhibitory activity. The most potent analogue was pentyl derivative **18c**. Compounds **18b**, **18d** and **15h** share similar inhibitory activity. As a class, the alkyl derivatives (**18**) show changes in activity according to changing alkyl chain length.

Inhibitory activity against *E. coli* was observed for a large number of the 2-pyrones. The alkyl class again showed potent inhibitory properties. Here the hexyl derivative **18d** was the most potent. Similar inhibitory activity is observed for the alkenyl class (**17**). The alkynyl derivatives (**15**) as a class showed promising inhibition, particularly the pentynyl and hexynyl derivatives (**15a** and **15b**, respectively). The phenylethynyl derivative **15f** showed good inhibition, however the *para*-acetamide and para-nitro derivatives **15i** and **15j**, respectively, were poor inhibitors of both *E. coli* or *B. subtilis*.

S. aureus proved a harder organism to inhibit, although selected analogues did demonstrate some inhibitory activity. Analogues containing the tetrahydropyranyl-ether ethynyl and the ethynyl substitutents, **15h** and **19**, respectively, stand out as the most potent analogues. Note that no inhibitory activity was observed for **15f**, nor **15b** or **15j**.

Twelve of the twenty six 2-pyrones tested for inhibitory activity against *Candida albicans* demonstrated promising results. It is particularly clear that a longer alkyl chain is beneficial for potency in the alkyl class of

2-pyrones (**18**). Inhibitory activity increases in order hexyl **18d**, heptyl **18e**, octyl **18f**. this is not however the trend for the alkenyl class (**17**). The most potent analogue of this class is the hexyl derivative **17b**. Inhibitory activity falls off, either by loss or gain of a 'CH_2'. The aryl class (**16**) showed no activity against this organism. Several alkynyl derivatives (**15a**–**c**, **h**) did demonstrate inhibitory activity, not least the ethynyl derivative **19**, which is as potent as zaragozic acid A (ZA-A, control—a potent *squalene synthase* inhibitor) against the organism.[42]

The 2-pyrones on the whole did not show any inhibitory activity against *S. cerevisiae*. This was of great surprise to us, given that a number of analogues inhibited *C. albicans* and also *S. pombe*. The exception was **19**, which again shared similar activity to ZA-A. Clearly the free alkyne is important. *S. pombe* was inhibited by twenty of the 2-pyrones tested. The aryl substituted 2-pyrones (**16**) as a class were poor inhibitors, except for the *para*-methoxy and *para*-formyl derivatives **16d** and **16f**, respectively, which demonstrated modest inhibition. The alkyl, alkenyl and alkynyl classes all demonstrated some quite remarkable inhibitory activities. In particular, a number of analogues (**15b**–**d**) belonging to the alkynyl class demonstrated inhibitory activity greater than ZA-A. The potency of **19** was apparent once again, although slightly less potent than **15d**–**f**. It is worth noting that growth inhibition was retained after 5 days (Fig. 3, picture A).

A. niger was appreciably resistant to the majority of compounds tested. However, a few 2-pyrones did exhibit some antifungal properties. The alkyl class of 2-pyrones (**18**) were active after 3 days, but inhibitory activity disappeared after 7 days. This implies that these compounds are bacteristatic and only hinder the growth rather than stopping it completely. The most active compound by against *A. niger* was **19**, which after 7 days still remained considerably active, preventing any further growth of the organism at the point of inoculation. The extent of inhibition of growth of *A. niger* in the presence of **19** can be seen in Figure 3 (pictures B and C).

It was clear that the 2-pyrones retarded the growth of *F. oxysporum*, but the limited growth after 3 days makes it difficult to identify useful inhibition data. After 7 days, a number of analogues were retarding growth. In particular, analogues **15ab**, **16a**, **17bc** and **19** showed promising inhibitory activity compared to the control over the same time.

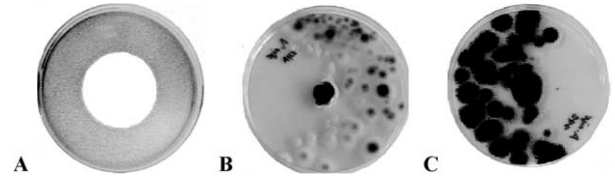


Figure 3. A, *S. Pombe* with **19** after 5 days. **B**, *A. niger* with **19** after 3 days. **C**, *A. niger* with **19** after 7 days.

P. ultimum was not sensitive to any of the 2-pyrones screened.

The vast majority of the 2-pyrones demonstrated growth retardation against *R. solani* away from the point of inoculation, although by far the most active class were the alkynyl derivatives (**15**). Indeed, it was only these compounds that remained sufficiently active after 7 days. In particular, analogues **15a**–**c**, **15h** and **19** demonstrated excellent inhibition.

A significant amount of growth retardation was observed for the 2-pyrones against *B. cinerea*, although unfortunately in all cases, the inhibition disappeared after 7 days. The alkynyl class (**15**) once again were the most potent analogues. The hexynyl and ethynyl derivatives, **15b** and **19**, were the most active, showing over a 50% reduction in growth as compared to the control.

The specific biological process being inhibited by the 4-substituted-6-methyl-2-pyrones remains unidentified. Recent work by Deck and co-workers has demonstrated that 6-chloro-2-pyrones inhibit yeast cholesterol esterase from *Candida cylindracea* (also called *C. rugose* CRL3).[45] Given the broad spectrum biological activity associated with the compounds described herein, we believe that it is the 2-pyrone unit, which is responsible for inhibitory activity. Whether it is cholesterol esterase or a related enzyme that is the target and hence the mode of action of these compounds is currently being investigated.

In summary, we have synthesised an array of 4-substituted-6-methyl-2-pyrone derivatives, utilising Pd-catalysed C–C bond forming processes (Negishi, Sonogashira and Suzuki methodologies). These methods facilitate access to substituted 2-pyrones, which would be otherwise difficult to achieve using traditional synthetic methods. The biological results demonstrate an abundance of biological activity associated with this class of simple *synthetic* 2-pyrones. The study highlights the importance of testing compounds that are biologically validated as being capable of exerting a biological effect in a range of organisms. We hope researchers within this field will be encouraged to screen other simple 2-pyrones in a similar manner. Our future endeavours are geared towards identifying the mode of action of these compounds.

6. Experimental

6.1. General methods

Terminal alkynes were purchased from Aldrich or Lancaster and redistilled or recrystallised. THF was dried over sodium-benzophenone ketyl (distilled prior to use). Dry CH_2Cl_2, Et_3N and CH_3CN were distilled over calcium hydride. All reactions were conducted under an inert atmosphere of Ar or N_2 on a Schlenk line. $Pd(PPh_3)_4$ was prepared prior to use from $Pd(OAc)_2$ and PPh_3 (1:5) in diethyl ether, stirring at room temperature (1 h, in the dark!), then filtered through a sinter glass funnel (under N_2) to give $Pd(PPh_3)_4$ as a yellow solid.

$Pd(OAc)_2$ was purchased from Aldrich or Strem. $PdCl_2$ was provided by Johnson Matthey as a loan. $(PPh_3)_2PdCl_2$ was prepared from $PdCl_2$ in refluxing DMSO and PPh_3 (2 equiv) using a known procedure.[46] 10% Pd/C was purchased from Lancaster. Compound **3a** was synthesised according to the literature procedure(s).[18,47] Melting points were recorded on an electrothermal IA9000 Digital Melting Point Apparatus and are uncorrected. TLC analysis was performed on Merck 5554 aluminium backed silica gel plates and compounds visualised by ultraviolet light (254 nm), phosphomolybdic acid solution (5% in EtOH), or 1% ninhydrin in EtOH. The relative proportion of solvents in mixed chromatography solvents refers to the volume/volume ratio. Infrared spectra were recorded on a ATI Mattson Genesis FT-IR. Mass spectrometry was carried out using a Fisons Analytical (VG) Autospec instrument. High resolution masses are within 5 ppm of theoretical values. ^1H NMR spectra were recorded at 270 MHz using a JEOL EX270 spectrometer or at 400 MHz using a JEOL ECX400 spectrometer; ^{13}C NMR spectra at 67.9 or 100.5 MHz. Chemical shifts are reported in parts per million (δ) downfield from an internal tetramethylsilane reference. Coupling constants (J values) are reported in hertz (Hz), and spin multiplicities are indicated by the following symbols: s (singlet), d (doublet), t (triplet), q (quartet), qn (quintet), sx (sextet), m (multiplet), br (broad).

The characterisation data for compounds **15a**–**k** can be found elsewhere.[21]

6.1.1. 4-Bromo-6-methyl-2-pyrone 14. Phosphorous tribromide (32.48 g, 120 mmol) in dry diethyl ether (88 mL) was added to mechanically stirred *N*,*N*-dimethylformamide (130 mL) at 0 °C over a period of 1 h. A thick white precipitate was formed. 4-Hydroxy-6-methyl-2-pyrone (5.04 g, 40 mmol) was dissolved in DMF (88 mL) and added to the precipitate. The resulting solution was stirred at 60 °C for 20 h. The mixture was cooled and water (200 mL) was added. The solution was then extracted with diethyl ether (6 × 300 mL) and the ethereal extracts back-washed with water (3 × 200 mL). Concentration of the combined organic layers in vacuo yielded an orange solid, which could be purified by filtering through a pad of silica with hexane/ether (1:1, v/v), or by vacuum sublimation at 70 °C/1 mmHg gave the known *title compound* as a cream solid (5.93 g, 78.5%). Mp 87–89 °C.[18] δ_H (270 MHz, $CDCl_3$) 6.44 (s, 1H), 6.18 (s, 1H), 2.23 (s, 3H). δ_C (68 MHz, $CDCl_3$) 162.0, 160.3, 140.9, 114.5, 108.2, 19.5. ν_{max} (neat, cm^{-1}) 3083, 1725, 1619, 1544, 1305, 1209, 1130, 1027, 868, 805. LREI m/z 188/190 [M^+], 173/175 [M^+-CH_3], 160/162 (100, [M^+−C≡O]), 151, 129, 117/119, 109, 97, 81, 66, 57. LRCI m/z 189/191 [M^++1], 206/208 (100), 160/162, 128, 111. HRCI: m/z exact mass calculated for $C_6H_6O_2{}^{79}Br$ 188.9551; Found 188.9551.

6.1.2. Representative procedure for the Sonogashira reaction. To a flame-dried flask under an atmosphere of N_2 or Ar was added **3a** (1 mmol), 10% Pd/C (20 mol %, 2 mol % based on Pd), PPh_3 (2.5 mol %) and CuI (4 mol %). Dry Et_3N (2.5 mL) and dry CH_3CN (1.5 mL) were added via cannula and the mixture was magnetically stirred and heated to reflux for 3 h. The mixture was cooled to room temperature and filtered through Celite, washed with CH_2Cl_2 and the filtrate concentrated in vacuo to give an oil. The products were isolated by either direct recrystallisation or by extraction into hot hexane, followed by chromatography on silica gel using petroleum ether 40–60 °C/EtOAc, 9/1, v/v) to provide the products as crystalline solids or viscous oils.

6.1.3. Synthesis of (*E*)-alkenyl-1,3,2-benzodioxaboroles. *Representative procedure*: To the alkyne (50 mmol) in a flame-dried flask (under vacuum) under a nitrogen atmosphere, was added catechol borane (50 mmol) via a syringe and the mixture heated to 70 °C for 1 h. The products were obtained by high vacuum distillation to yield the (*E*)-alkenyl-1,3,2-benzodioxaboroles as colourless oils, which were stored under argon. The characterisation data for these compounds is in accordance to that reported.[48] (*E*)-Pentenyl-1,3,2-benzodioxaborole, bp 63–66 °C/0.3 mmHg. Lit. 67 °C/0.4 mmHg; (*E*)-Hexenyl-1,3,2-benzodioxaborole, bp 81–85 °C/0.3 mmHg. Lit. 82 °C/0.3 mmHg; (*E*)-Heptenyl-1,3,2-benzodioxaborole, bp 100–104 °C/0.3 mmHg; (*E*)-Octenyl-1,3,2-benzodioxaborole, bp 120–125 °C/0.3 mmHg; (*E*)-Styryl-1,3,2-benzodioxaborole, as a white solid, mp 77–78 °C. Lit. 78–78.5 °C.

6.1.4. Hydrolysis of (*E*)-alkenyl-1,3,2-benzodioxaboroles to (*E*)-alkenyl boronic acids. *Representative procedure*: Water (100 mmol) was added to the neat (*E*)-alkenyl-1,3,2-benzodioxaboroles (50 mmol) and shaken at room temperature for 1 h. The resultant precipitate was filtered and recrystallised from hot water to yield the pure (*E*)-alkenyl boronic acids as white solids. In the case of (*E*)-phenylethenyl-1,3,2-benzodioxaborole, hydrolysis was carried out at 70 °C.

6.1.5. (*E*)-Pentene boronic acid. Mp 89–92 °C. Lit. 81 °C.[49] 87% yield. δ_H (270 MHz, CD_3OD) 6.41–6.57 (m, 1H), 5.45 (dd, 1H, J = 17.59, 51.10), 4.77 (s, 2H), 2.10 (m, 2H), 1.42 (m, 2H), 0.90 (td, 3H, J1.46, 7.32). δ_C (68 MHz, CD_3OD) 153.7, 152.1, 38.8, 22.8, 14.0. δ_B (CD_3OD) 30.0. ν_{max} (KBr, cm^{-1}) 3251, 2932, 2422, 1642, 1358, 1142, 994.

6.1.6. (*E*)-Hexene boronic acid. Mp 90–93 °C.[50] 85% yield. δ_H (270 MHz, CD_3OD) 6.40–6.57 (m, 1H), 5.68 (dd, 1H, J = 17.59, 55.14), 4.85 (s, 2H), 2.07–2.19 (m, 2H), 1.26–1.45 (m, 4H), 0.91 (t, 3H, J = 7.33). δ_C (68 MHz, CD_3OD) 153.9, 152.3, 36.7, 32.0, 23.3, 14.3. δ_B (CD_3OD) 30.0. ν_{max} (KBr, cm^{-1}) 3225, 2928, 2408, 1641, 1357, 1151, 995.

6.1.7. (*E*)-Heptene boronic acid. Mp 89–94 °C. Lit. 88–90 °C.[51] 90% yield. δ_H (270 MHz, CD_3OD) 6.41–6.55 (m, 1H), 5.45 (dd, 1H, J = 17.59, 54.22), 4.87 (s, 2H), 2.06–

2.17 (m, 2H), 1.23–1.50 (m, 6H), 0.89 (t, 3H, $J = 6.96$). δ_C (68 MHz, CD_3OD) 154.0, 152.4, 36.7, 32.5, 29.4, 23.5, 14.4. δ_B (CD_3OD) 30.0. ν_{max} (KBr, cm^{-1}) 3236, 2925, 2412, 1640, 1342, 1112, 995.

6.1.8. (*E*)-Octene boronic acid. Mp 77–83 °C.[52] 82% yield. δ_H (270 MHz, CD_3OD) 6.41–6.55 (m, 1H), 5.45 (dd, 1H, $J = 17.59$, 53.13), 4.86 (s, 2H), 2.07–2.17 (m, 2H), 1.20–1.50 (m, 8H), 0.89 (t, 3H, $J = 6.97$). δ_C (68 MHz, CD_3OD) 153.9, 152.3, 36.8, 32.9, 30.0, 29.7, 23.7, 14.5. δ_B (CD_3OD) 30.0. ν_{max} (KBr, cm^{-1}) 3270, 2925, 2432, 1637, 1350, 1234, 995.

6.1.9. (*E*)-Phenylethenyl boronic acid. Mp 162–164 °C. Lit. 165 °C.[53] 91% yield. δ_H (270 MHz, CD_3OD) 7.19–7.52 (m, 6H), 6.23 (dd, 1H, $J = 19.26$, 54.1), 4.82 (s, 2H). δ_C (68 MHz, CD_3OD) 149.5, 148.1, 119.8, 119.6, 118.0, 117.9. δ_B (CD_3OD) 25.9. ν_{max} (KBr, cm^{-1}) 3349, 3003, 2558, 2478, 1622, 1353, 989, 744, 691.

6.2. General procedure for the Suzuki coupling of 4-bromo-6-methyl-2-pyrone with arylboronic acids using $Pd(OAc)_2$ and L ($L = PPh_3$ or P(2-furyl)$_3$)

Representative procedure: $Pd(OAc)_2$ (6 mol %), PPh_3 (18 mol %), and 4-bromo-6-methyl-2-pyrone (1 equiv 1 mmol scale) were stirred in benzene (2 mL) and aq Na_2CO_3 (1 mL, 2 M), under a nitrogen atmosphere for 0.5 h. To this solution was added a solution of the boronic acid (1.1 equiv) in ethanol (1 mL). The solution was then refluxed for 6 h, allowed to cool to room temperature and the excess boronic acids oxidised for 1 h at room temperature by addition of 30% hydrogen peroxide (0.1 mL). Extraction into hexane or ether (2×10 mL), followed by washing with saturated aqueous NaCl (2×10 mL), dried ($MgSO_4$) and the concentrated in vacuo to afford the crude product. Purification by chromatography on silica gel (ether or ethyl acetate/hexanes mixtures) gave the products as solids.

6.3. General procedure for the Suzuki coupling of aryl halides using KF as base

$Pd(OAc)_2$ (2.2 mg, 0.01 mmol, 1.0 mol %), (di-*tert*-butyl)biphenyl (6.0 mg, 0.020 mmol, 2.0 mol %), the arylboronic acid (1.5 mmol), the aryl halide (1.0 mmol), and potassium fluoride (174 mg, 3.0 mmol). The flask was evacuated and backfilled with nitrogen. THF (5 mL) was added and the reaction mixture stirred at room temperature until the starting material was consumed, as judged by TLC. The mixture was diluted with ether (30 mL) and poured into a separatory funnel, washed with aqueous NaOH (1 M, 20 mL), and the aqueous layer extracted with ether (20 mL). The combined organic layers were washed with brine (20 mL), dried ($MgSO_4$), filtered and concentrated in vacuo. Purification by chromatography on silica gel (ether or ethyl acetate/hexanes mixtures) gave the products as solids.

6.3.1. 4-Phenyl-6-methyl-2-pyrone 16a. Mp 89–91 °C. δ_H (270 MHz, $CDCl_3$) 7.47–7.58 (m, 5H), 6.35 (s, 1H), 6.32 (s, 1H), 2.32 (s, 3H). δ_C (68 MHz, $CDCl_3$) 163.4, 162.1, 155.5, 135.9, 130.5, 129.1, 126.6, 108.2, 103.4, 20.1. ν_{max} (KBr, cm^{-1}) 3084, 1740, 1712, 1633, 1544, 1449, 1328, 1140, 890, 833, 770, 701. LREI m/z 186 [M^+], 158 [$M^+-C{\equiv}O$], 129, 115 (100), 102, 89, 77, 63, 51, 43. LRCI m/z 187 [MH^+]. HRCI m/z exact mass calculated for $C_{12}H_{11}O_2$ 187.0759; Found 187.0757.

6.3.2. 4-(*p*-Methylphenyl)-6-methyl-2-pyrone 16b. Mp 117–118 °C. δ_H (270 MHz, $CDCl_3$) 7.47 (d, 2H, $J = 8.06$), 7.27 (d, 2H, $J = 7.70$), 6.33 (s, 1H), 6.31 (s, 1H), 2.41 (s, 3H), 2.31 (s, 3H). δ_C (68 MHz, $(CD_3)_2CO$) 164.1, 164.0, 156.6, 142.8, 134.6, 128.5, 108.5, 104.4, 22.3, 21.0. ν_{max} (KBr, cm^{-1}) 2919, 1727, 1707, 1635, 1328, 1138, 1025, 838, 817. LREI m/z 200 [M^+], 172 (100, [$M^+-C{\equiv}O$]), 143, 129, 115, 91, 77, 63. LRCI m/z 201 [MH^+] (100). HRCI m/z exact mass calculated for $C_{13}H_{13}O_2$ 201.0915; Found 201.0914.

6.3.3. 4-(*p*-Chlorophenyl)-6-methyl-2-pyrone 16c. Mp 122–123 °C. δ_H (270 MHz, $CDCl_3$) 7.40–7.50 (m, 4H), 6.30 (s, 1H), 6.24 (s, 1H), 2.30 (s, 3H). δ_C (68 MHz, $CDCl_3$) 162.9, 162.3, 154.0, 136.7, 134.0, 129.2, 127.8, 108.0, 102.9, 19.9. ν_{max} (KBr, cm^{-1}) 1712, 1639, 1546, 1410, 1327, 1089, 838, 817. LREI m/z 222/220 [M^+], 194/192 [$M^+-C{\equiv}O$], 151/149, 129, 114, 69, 43. LRCI m/z 221 [MH^+] (100). HRCI m/z exact mass calculated for $C_{12}H_{10}O_2Cl$ 221.0369; Found 221.0371.

6.3.4. 4-(*p*-Methoxyphenyl)-6-methyl-2-pyrone 16d. Mp 110–113 °C. δ_H (270 MHz, $CDCl_3$) 7.54 (d, 2H, $J = 9.2$), 6.98 (d, 2H, $J = 9.2$), 6.30 (br s, 2H), 3.86 (s, 3H), 2.31 (s, 3H). ν_{max} (KBr, cm^{-1}) 3100, 2929, 1703, 1634, 1604, 1545, 1612, 1293, 1246, 1182, 1031, 830. LREI m/z 216 [M^+], 188 (100, [$M^+-C{\equiv}O$]), 173, 159, 145, 131, 102, 91, 69. LRCI m/z 217 [MH^+] (100). HRCI m/z exact mass calculated for $C_{13}H_{13}O_3$ 217.0864; Found 217.0864.

6.3.5. 4-(*p*-Formylphenyl)-6-methyl-2-pyrone 16f. Mp 128–130 °C. δ_H (270 MHz, $CDCl_3$) 10.09 (s, 1H), 7.99 (d, 2H, $J = 8.06$), 7.73 (d, 2H, $J = 8.06$), 6.41 (s, 1H), 6.31 (s, 1H), 2.35 (s, 3H). δ_C (68 MHz, $CDCl_3$) 191.2, 162.8, 154.1, 141.6, 137.5, 130.3, 127.4, 109.6, 103.1, 20.2. ν_{max} (KBr, cm^{-1}) 3070, 2854, 1744, 1703, 1639, 1550, 1329, 1200, 891, 912. LREI m/z 214 [M^+], 199 [M^+-CH_3], 186 [$M^+-C{\equiv}O$], 157, 143, 129, 115, 97, 83, 69, 57, 43. LRCI m/z 215 [MH^+] (100). HRCI m/z exact mass calculated for $C_{13}H_{11}O_3$ 215.0708; Found 215.0707.

6.4. Suzuki cross-coupling of 4-bromo-6-methyl-2-pyrone with (*E*)-alkenyl-1,3,2-benzodioxaboroles

Representative procedure: The palladium catalyst (6 mol %) and 4-bromo-6-methyl-2-pyrone (1 equiv, 1 mmol scale) were stirred under a nitrogen atmosphere for 0.5 h. The (*E*)-alk-1-enyl-1,3,2-benzodioxaborole

(1.1 equiv) in benzene (1.1 mL, 1 M) were added, followed by an aqueous solution of sodium carbonate (1 mL, 2 M). The mixture was refluxed for 2 h, and the residual organoborane was oxidised by addition of NaOH (0.4 mL, 3 M) and 30% hydrogen peroxide (0.4 mL). The product was extracted with hexane or ether (2×10 mL), washed with water (4×10 mL) and saturated aqueous NaCl, dried ($MgSO_4$), concentrated in vacuo and purified by chromatography on silica gel (ether/hexane mixtures) to give the products as pale yellow oils.

6.5. Suzuki cross-coupling of 4-bromo-6-methyl-2-pyrone with (*E*)-alkenyl boronic acids

Representative procedure: $Pd(OAc)_2$ (6 mol%), PPh_3 (18 mol%) and 4-bromo-6-methyl-2-pyrone (1 mmol) in benzene (2 mL) and aq Na_2CO_3 (1 mL, 2 M) were magnetically stirred under N_2 for 0.5 h. To this was added a solution of the boronic acid (1.1 mmol) in EtOH (1 mL) and the solution was refluxed for 6 h, and then allowed to cool to room temperature. The excess boronic acids were oxidised with 30% H_2O_2 (0.1 mL) for 1 h. The mixture was extracted into hexane (2×10 mL) and the combined extracts washed with saturated aq NaCl (2×10 mL), dried ($MgSO_4$), concentrated in vacuo and purified by chromatography on silica gel (ether/hexane mixtures) to give the products as pale yellow oils.

6.5.1. (*E*)-4-Pentenyl-6-methyl-2-pyrone 17a. δ_H (270 MHz, $CDCl_3$) 6.45 (dt, 1H, $J = 6.96, 15.75$), 6.14 (d, 1H, $J = 15.75$), 6.15 (s, 1H), 5.91 (s, 1H), 2.17–2.27 (m, 5H), 1.44–1.57 (sx, 2H, $J = 7.69$), 0.95 (t, 3H, $J = 7.33$). δ_C (68 MHz, $CDCl_3$) 163.5, 160.9, 152.0, 140.3, 126.6, 107.9, 100.7, 34.9, 21.6, 19.7, 13.4. ν_{max} (neat, cm^{-1}) 2959, 1727, 1630, 1547, 1324, 956, 851. LREI m/z 178 [M^+], 163 [M^+-CH_3], 150 [$M^+-C{\equiv}O$], 135 (100, [M^+-CH_3 and $C{\equiv}O$]), 121, 107, 91, 77, 65, 51, 43. Anal. Calcd for $C_{11}H_{14}O_2$: C, 74.13; H, 7.92. Found: C, 73.78; H, 7.81.

6.5.2. (*E*)-4-Hexenyl-6-methyl-2-pyrone 17b. δ_H (270 MHz, $CDCl_3$) 6.28–6.42 (dt, 1H, $J = 6.76, 15.94$), 6.03 (d, 1H, $J = 15.94$), 6.04 (s, 1H), 5.80 (s, 1H), 2.15 (t, 2H, $J = 7.02$), 1.18–1.41 (m, 4H), 0.81 (t, 3H, $J = 7.02$). δ_C (68 MHz, $CDCl_3$) 163.3, 160.8, 151.9, 140.4, 126.4, 107.7, 100.6, 32.4, 30.4, 21.9, 19.6, 13.5. ν_{max} (neat, cm^{-1}) 2958, 2870, 1730, 1651, 1548, 1324, 1140, 856. Anal. Calcd for $C_{12}H_{16}O_2$: C, 74.97; H, 8.39. Found: C, 74.68; H, 8.21.

6.5.3. (*E*)-4-Heptenyl-6-methyl-2-pyrone 17c. δ_H (270 MHz, $CDCl_3$) 6.30–6.43 (dt, 1H, $J = 6.96, 15.76$), 6.06 (d, 1H, $J = 15.76$), 6.07 (s, 1H), 5.83 (s, 1H), 2.16 (s, 3H), 2.14 (t, 2H, $J = 7.33$), 1.17–1.45 (m, 6H), 0.82 (t, 3H, $J = 6.42$). δ_C (68 MHz, $CDCl_3$) 163.7, 161.0, 152.1, 140.6, 126.6, 108.1, 100.9, 33.0, 31.3, 28.2, 22.3, 19.9, 13.9. ν_{max} (neat, cm^{-1}) 2956, 2926, 1729, 1653, 1548, 1325, 1140, 856. LREI m/z 206 [M^+], 191 [M^+-CH_3], 176, 162, 148, 135 (100), 121, 105, 91, 77, 65, 55. Anal. Calcd for $C_{13}H_{18}O_2$: C, 75.69; H, 8.80. Found: C, 75.82; H, 9.12.

6.5.4. (*E*)-4-Octenyl-6-methyl-2-pyrone 17d. δ_H (270 MHz, $CDCl_3$) 6.43 (dt, 1H, $J = 6.96, 15.75$), 6.13 (d, 1H, $J = 15.02$), 6.11 (s, 1H), 5.92 (s, 1H), 2.10–2.28 (m, 5H), 1.19–1.49 (m, 8H), 0.89 (t, 3H, $J = 6.96$). δ_C (68 MHz, $CDCl_3$) 163.7, 161.1, 152.1, 140.7, 126.7, 108.1, 100.9, 33.1, 31.6, 28.8, 28.6, 22.5, 19.9, 14.0. ν_{max} (neat, cm^{-1}) 2956, 2928, 1729, 1651, 1548, 1323, 1156, 854. LREI m/z 220 [M^+], 205 [M^+-CH_3], 192 [$M^+-C{\equiv}O$], 177, 163, 149, 135 (100), 121, 108, 96, 77, 69, 55. Anal. Calcd for $C_{14}H_{20}O_2$: C, 76.33; H, 9.15. Found: C, 75.94; H, 8.94.

6.5.5. (*E*)-4-Phenylethenyl-6-methyl-2-pyrone E-17e. Mp 123–125 °C. δ_H (270 MHz, $CDCl_3$) 7.29–7.46 (m, 5H), 7.11 (d, 1H, $J = 16.48$), 6.72 (d, 1H, $J = 16.48$), 6.20 (s, 1H), 6.00 (s, 1H), 2.19 (s, 3H). δ_C (68 MHz, $CDCl_3$) 163.5, 161.4, 151.7, 136.6, 135.4, 129.6, 128.9, 127.5, 124.5, 109.4, 100.7, 20.0. ν_{max} (KBr, cm^{-1}) 3086, 1703, 1621, 1539, 1449, 1329, 1140, 961, 822, 755, 693. LREI m/z 212 [M^+], 197 [M^+-CH_3], 184 [M^+-CO], 170, 155, 141 (100), 128, 115, 102, 91, 77, 57, 43. Anal. Calcd for $C_{14}H_{12}O_2$: C, 79.23; H, 5.70. Found: C, 78.95; H, 6.03.

6.5.6. (*Z*)-4-Phenylethenyl-6-methyl-2-pyrone Z-17e. 4-Phenylethynyl-6-methyl-2-pyrone (50 mg), Pd/C (5 mg) and quinoline (1 mg) were stirred under a hydrogen atmosphere in dry benzene (1 mL) for 1 h. The solution was diluted with ether (10 mL), filtered and washed with sodium carbonate (2 M, 10 mL). The ether layer was dried ($MgSO_4$), concentrated in vacuo and purified by chromatography on silica gel (ethyl acetate/hexane = 1:4, v/v) to provide the *title compound* as a colourless oil (44 mg, 87.2%). δ_H (270 MHz, $CDCl_3$) 7.19 (m, 5H), 6.81 (d, 1H, $J = 12.5$), 6.20 (d, 1H, $J = 12.5$), 5.98 (s, 1H), 5.65 (s, 1H), 2.02 (s, 3H). δ_C (68 MHz, $CDCl_3$) 161.8, 137.1, 132.0, 130.1, 128.9, 128.1, 125.8, 114.6, 111.8, 105.1, 103.9, 19.7. ν_{max} (neat, cm^{-1}) 3061, 2960, 2924, 1726, 1642, 1543, 1408, 1261, 1025. LREI m/z 212 [M^+], 197 [M^+-CH_3], 184 [$M^+-C{\equiv}O$], 170, 155, 141 (100), 128, 115, 102, 91, 77, 57, 43. Anal. Calcd for $C_{14}H_{12}O_2$: C, 79.23; H, 5.70. Found: C, 79.15; H, 5.84.

6.6. Synthesis of the trialkylboranes

The alkene (60 mmol) was placed under a nitrogen atmosphere at 0 °C. A solution of borane in THF (20 mmol, 1 M) was added and the solution stirred for 1 h. No attempt was made to isolate the trialkylboranes.

6.7. Suzuki cross-coupling of 4-bromo-6-methyl-2-pyrone and trialkylboranes

Representative procedure: 4-Bromo-6-methyl-2-pyrone (1 mmol), $PdCl_2$ (dppf) (6 mol%) and Tl_2CO_3 (3 equiv)

were dissolved in the appropriate solvent (5 mL) under an argon atmosphere. The trialkylborane in THF (1.1 mmol, 1 M) was added and the solution stirred for 16 h at the appropriate temperature. NaOH (0.5 mL, 3 M) and H_2O_2 (0.5 mL, 30%) were added and the mixture further stirred for 1 h to facilitate hydrolysis of the remaining boranes. The mixture was then extracted with hexane (2×20 mL), the hexane back-washed with water (4×10 mL), dried ($MgSO_4$) and concentrated in vacuo to yield the crude product. The products were purified by chromatography on silica gel (hexane/ether mixtures).

6.7.1. 4-Ethyl-6-methyl-2-pyrone 18a. δ_H (270 MHz, $CDCl_3$) 5.94 (s, 1H), 5.91 (s, 1H), 2.42 (q, 2H, $J = 7.33$), 2.23 (s, 3H), 1.18 (t, 3H, $J = 7.33$). δ_C (68 MHz, $CDCl_3$) 163.5, 161.6, 161.2, 108.7, 105.3, 28.2, 19.7, 12.1. ν_{max} (neat, cm^{-1}) 3070, 2972, 1729, 1644, 1562, 1450, 1332, 1228, 1151, 1071, 915, 849. LREI m/z 138 [M^+], 123 [M^+-CH_3], 110 [$M^+-C{\equiv}O$], 95, 67. LRCI m/z 139 [MH^+] (100). HRCI m/z exact mass calculated for $C_8H_{11}O_2$ 139.0759; Found 139.0759.

6.7.2. 4-Butyl-6-methyl-2-pyrone 18b. δ_H (270 MHz, $CDCl_3$) 5.85 (s, 1H), 5.80 (s, 1H), 2.30 (t, 2H, $J = 7.33$), 2.16 (s, 3H), 1.54 (m, 4H), 1.37 (t, 3H, $J = 7.70$). δ_C (68 MHz, $CDCl_3$) 163.0, 161.0, 160.3, 109.1, 105.3, 34.5, 29.9, 21.8, 19.4, 13.4. ν_{max} (neat, cm^{-1}) 3070, 2953, 1731, 1645, 1562, 1449, 1326, 1228, 1150, 1029, 948, 849. LREI m/z 166 [M^+], 151 [M^+-CH_3], 137 [$M^+-C_2H_5$], 124, 109, 96, 81, 69, 53. LRCI m/z 167 [MH^+] (100). HRCI m/z exact mass calculated for $C_{10}H_{15}O_2$ 167.1072; Found 167.1070.

6.7.3. 4-Pentyl-6-methyl-2-pyrone 18c. δ_H (270 MHz, $CDCl_3$) 5.93 (s, 1H), 5.87 (s, 1H), 2.35 (t, 2H, $J = 7.32$), 2.22 (s, 3H), 1.56 (qn, 2H, $J = 7.33$), 1.27–1.34 (m, 4H), 0.90 (t, 3H, $J = 6.96$). ν_{max} (neat, cm^{-1}) 3070, 2956, 1731, 1644,1562, 1449, 1327, 1228, 1149, 1029, 853. LREI m/z 180 [M^+], 165 [M^+-CH_3], 148, 137, 124, 109, 96 (100), 84, 67. LRCI m/z 181 [MH^+] (100). HRCI m/z exact mass calculated for $C_{11}H_{17}O_2$ 181.1228; Found 181.1230.

6.7.4. 4-Hexyl-6-methyl-2-pyrone 18d. δ_H (270 MHz, $CDCl_3$) 5.93 (s, 1H), 5.89 (s, 1H), 2.36 (t, 2H, $J = 6.97$), 2.23 (s, 3H), 1.2–1.7 (m, 8H), 0.89 (t, 3H, $J = 6.96$). δ_C (68 MHz, $CDCl_3$) 162.9, 160.9, 160.2, 109.1, 105.2, 34.8, 31.1, 28.3, 27.7, 22.1, 19.3, 13.6. ν_{max} (neat, cm^{-1}) 2955, 1738, 1640, 1562, 1460, 1338, 1228, 1149, 1030, 847. LRCI m/z 195 [MH^+], 124, 96. HRCI m/z exact mass calculated for $C_{12}H_{19}O_2$ 195.1385; Found 195.1382.

6.7.5. 4-Heptyl-6-methyl-2-pyrone 18e. δ_H (270 MHz, $CDCl_3$) 5.93 (s, 1H), 5.88 (s, 1H), 2.36 (t, 2H, $J = 7.32$), 2.22 (s, 3H), 1.20–1.65 (m, 10H), 0.88 (t, 3H, $J = 6.97$). δ_C (68 MHz, $CDCl_3$) 163.2, 161.1, 160.4, 109.3, 105.4, 35.0, 31.4, 28.8, 28.7, 27.9, 22.4, 19.5, 13.8. ν_{max} (neat, cm^{-1}) 2955, 2928, 1731, 1646, 1562, 1460, 1030, 850. LRCI m/z 209 [MH^+] (100). HRCI m/z exact mass calculated for $C_{13}H_{21}O_2$ 209.1541; Found 209.1540.

6.7.6. 4-Octyl-6-methyl-2-pyrone 18f. δ_H (270 MHz, $CDCl_3$) 5.93 (s, 1H), 5.86 (s, 1H), 2.35 (t, 2H, $J = 7.33$), 2.22 (s, 3H), 1.17–1.59 (m, 12H), 0.88 (t, 3H, $J = 6.96$). ν_{max} (neat, cm^{-1}) 2927, 2857, 1738, 1710, 1563, 1464, 1327, 1229, 1029, 958, 849. LREI m/z 222 [M^+], 205, 180, 164, 138, 124, 112, 97, 84, 70. LRCI m/z 223 [MH^+], 124, 96. HRCI m/z exact mass calculated for $C_{14}H_{23}O_2$ 223.1698; Found 223.1696.

6.7.7. 4-Phenylethyl-6-methyl-2-pyrone 18g. δ_H (270 MHz, $CDCl_3$) 7.13–7.30 (m, 5H), 5.89 (s, 1H), 5.85 (s, 1H), 2.84 (t, 2H, $J = 6.96$), 2.64 (t, 2H, $J = 6.96$), 2.19 (s, 3H). ν_{max} (neat, cm^{-1}) 3063, 3027, 2923, 1728, 1644, 1563, 1453, 1328, 1029, 848, 751, 701. LREI m/z 214 [M^+], 186 [$M^+-C{\equiv}O$], 171, 143, 124, 107, 95, 91 (100), 77, 65, 51, 43. LRCI m/z 187 [MH^+] (100), 158 [M–CO], 115. HRCI m/z mass calculated for $C_{14}H_{15}O_2$ 215.1072; Found 215.1073.

6.7.8. 4-Ethynyl-6-methyl-2-pyrone 19. 4-(Trimethylsilyl)ethynyl-6-methyl-2-pyrone (0.206 g, 1 mmol) was dissolved in dry THF (1 mL) and cooled under nitrogen to −78 °C. A 1 M solution of TBAF (2 equiv) in THF was added and the mixture stirred at −78 °C for 2 h. At the end of the reaction, water (10 mL) was added and the solution immediately extracted with hexane (2×25 mL). The hexane was concentrated in vacuo at <25 °C to afford the product as a white solid. Mp 67–68 °C (decomp.). δ_H (270 MHz, $CDCl_3$) 6.20 (s, 1H), 5.92 (s, 1H), 3.47 (s, 1H), 2.16 (s, 3H). δ_C (68 MHz, $CDCl_3$) 162.3, 161.7, 137.7, 116.0, 105.1, 86.1, 79.3, 19.8. ν_{max} (KBr, cm^{-1}) 3261, 2955, 2114, 1717, 1632, 1535, 1316, 1034, 849. LREI m/z 134 [M^+], 119 [M^+-CH_3], 106 (100, [$M^+-C{\equiv}O$]), 78, 69, 63, 50. Anal. Calcd for $C_8H_6O_2$: C, 71.64; H, 4.51. Found: C, 71.60; H, 4.46.

7. Biology

7.1. The standard assay for cytotoxicity

The assay used a standard 96 well format (Bibby Sterilin). Between 400 and 1000 cells were plated into each well, in a total volume of 50 μL. The cells were allowed to recover for 24 h before 150 μL of medium containing the drug was added to each well. The control wells just received 150 μL of medium alone. Each drug dose was represented by three wells. The plates were then incubated at 37 °C in a 5% CO_2 atmosphere for 5 days. After incubation, 50 μL of MTT solution (3 mg/mL) was added to each well and the plate returned to the incubator for 3 h. After this time, the medium and excess MTT was aspirated from each well and the formazan crystals were solubilised in 200 μL of DMSO. The plate was then read using a multiscan microplate reader (Titretech,

Labsystems UK Ltd.) at 540 nm with subtraction at 620 nm to allow for turbidity. The resultant output was processed and the percentage growth inhibition for each dose calculated, each experiment was performed in duplicate. Mean and standard deviations for each dose were calculated from duplicate experiments. Based on a standard assay.[54]

7.2. Antimicrobial assay

The antimicrobial assays were performed as previously described.[55] An antibiotic 'ring', which contained several known potent antibacterial agents (Novobiocin, Penicillin G, Streptomycin, Tetracyline, Chloroamphenicol, Erythromycin, Fusidic acid and Methicillin), was used as the control in the bacterial assays (individual discs contain 1 unit). Zones of inhibition (~5–8 mm) were observed for these compounds. Squalestatin S1 (Zaragozic acid A), an extremely potent yeast inhibitor, was used as a control in the yeast assay (200 μg/disk). The zones of inhibition observed against *C. albicans*, *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe* were 27, 27 and 37 mm, respectively.

References and notes

1. Staunton, J. In *Comprehensive Organic Chemistry*; Burton, D. H., Ollis, W. D., Eds.; Pergamon: New York, 1979; p 629.
2. Moreno-Manas, M.; Pleixats, R. *Naturally Occurring Oxygen Ring Systems*; Academic: New York, 1992; p 21.
3. Altomare, C.; Perrone, G.; Zonno, M. C.; Evidente, A.; Pengue, R.; Fanti, F.; Polonelli, L. *J. Nat. Prod.* **2000**, *63*, 1131.
4. Dickinson, J. M. *Nat. Prod. Rep.* **1993**, *10*, 71.
5. Uen, Y. In *Mycotoxins*; Purchase, I. F. H., Ed.; Elsevier: New York, 1974; p 283.
6. Spencer, H. C.; Rowe, K.; McCollister, D. D. *J. Pharmacol. Exp. Ther.* **1950**, *99*, 57.
7. Tringali, C.; Parisi, A.; Piatelli, M.; Di San Lio, G. *Nat. Prod. Lett.* **1993**, *3*, 101.
8. Hansen, O. R. *Acta Chem. Scand.* **1954**, *8*, 1332.
9. (a) Bu'Lock, J. D.; Smith, H. E. *J. Chem. Soc.* **1960**, *1*, 502; (b) Effenberger, F.; Ziegler, T. *Chem. Ber.* **1987**, *120*, 1339; (c) Tam, T. F.; Coles, P. *Synthesis* **1988**, 383; (d) Moody, C. J.; Rahimtoola, K. F. *J. Chem. Soc., Perkin Trans. 1* **1990**, 673; (e) Moody, C. J.; Rahimtoola, K. F. *J. Chem. Soc., Perkin Trans. 1* **1990**, 681.
10. (a) Afridi, A. S.; Katritzky, A. R.; Ramsden, C. A. *J. Chem. Soc., Perkin Trans. 1* **1977**, 1428; (b) Kiang, A. K.; Tang, S. F.; Wang, W. S. *J. Chem. Soc.* **1971**, *1*, 2721.
11. (a) Lee, J.-H.; Kim, W.-S.; Lee, Y. Y.; Cho, C.-G. *Tetrahedron Lett.* **2002**, *43*, 5779; (b) Lee, J.-H.; Park, J.-S.; Cho, C.-G. *Org. Lett.* **2002**, *4*, 1171.
12. Cerezo, S.; Moreno-mañas, M.; Pleixats, R. *Tetrahedron* **1998**, *54*, 7813.
13. Bellina, F.; Biagetti, M.; Carpita, A.; Rossi, R. *Tetrahedron Lett.* **2001**, *42*, 2859.
14. (a) Marrison, L. R.; Dickinson, J. M.; Fairlamb, I. J. S. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 3509; (b) Marrison, L. R.; Dickinson, J. M.; Ahmed, R.; Fairlamb, I. J. S. *Tetrahedron Lett.* **2002**, *43*, 8853; (c) Marrison, L. R.; Dickinson, J. M.; Ahmed, R.; Fairlamb, I. J. S. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 2667.
15. (a) Shi, X.; Leal, W. S.; Liu, Z.; Schrader, E.; Meinwald, J. *Tetrahedron Lett.* **1995**, *36*, 71; (b) Leal, W. S.; Shi, X.; Liang, D.; Schal, C.; Meinwald, J. *Proc. Natl. Acad. Sci. U.S.A.* **1995**, *92*, 1033.
16. (a) Danieli, B.; Lesma, G.; Martinelli, M.; Passarella, D.; Peretto, I.; Silvani, A. *Tetrahedron* **1998**, *54*, 14081; (b) Liu, Z.; Meinwald, J. *J. Org. Chem.* **1996**, *61*, 6693.
17. Waldmann, H. *Bioorg. Med. Chem.* **2003**, *11*, 3045.
18. Cervera, M.; Moreno-mañas, M.; Pleixats, R. *Tetrahedron* **1990**, *46*, 7885.
19. Kato, Y.; Okada, S.; Tomimoto, K.; Mase, T. *Tetrahedron Lett.* **2001**, *42*, 4849.
20. Negishi, E.; Anastasia, L. *Chem. Rev.* **2003**, *103*, 1979.
21. Fairlamb, I. J. S.; Lu, F.-J.; Schmidt, J.-P. *Synthesis* **2003**, 2564.
22. Beletskaya, I. P.; Cheprakov, A. V. *Chem. Rev.* **2000**, *100*, 3009.
23. Takia, K.; Sato, M.; Oshima, A.; Nozaki, H. *Bull. Chem. Soc. Jpn.* **1984**, *57*, 108.
24. Baba, S.; Negishi, E. *J. Am. Chem. Soc.* **1976**, *98*, 6729.
25. Miyaura, N.; Suzuki, A. *Chem. Commun.* **1979**, 866.
26. Negishi, E.; Valente, L. F.; Kobayashi, M. *J. Am. Chem. Soc.* **1980**, *102*, 3298.
27. Hatanaka, Y.; Ebina, Y.; Hiyama, T. *J. Am. Chem. Soc.* **1991**, *113*, 7075, and references cited therein.
28. Stille, J. K. *Angew. Chem., Int. Ed. Engl.* **1986**, *25*, 508.
29. Negishi, E.; Takahashi, T.; Baba, S.; Van Horn, D. E.; Okukado, N. *J. Am. Chem. Soc.* **1987**, *109*, 2393.
30. Negishi, E.; Van Horn, D. E. *J. Am. Chem. Soc.* **1978**, *100*, 2252.
31. Negishi, E.; Okukado, N.; King, A.; Spiegel, B. I.; Van Horn, D. E. *J. Am. Chem. Soc.* **1978**, *100*, 2254.
32. (a) Miyaura, N. *J. Organomet. Chem.* **2002**, *653*, 54; (b) Suzuki, A. *Acc. Chem. Res.* **1982**, *15*, 178; (c) Suzuki, A. *Pure Appl. Chem.* **1985**, *57*, 1749; (d) Miyaura, N.; Suzuki, A. *Chem. Rev.* **1995**, *95*, 2457; (e) Stanforth, S. P. *Tetrahedron* **1998**, *54*, 263.
33. (a) Wolfe, J. P.; Singer, R. A.; Yang, B. H.; Buchwald, S. L. *J. Am. Chem. Soc.* **1999**, *121*, 9550; (b) Wolfe, J. P.; Buchwald, S. L. *Angew. Chem., Int. Ed.* **1999**, *38*, 2413.
34. Brown, H. C.; Gupta, S. K. *J. Am. Chem. Soc.* **1975**, *97*, 5249.
35. Wailes, P. C.; Weigold, H.; Bell, A. P. *J. Organomet. Chem.* **1967**, *8*, 239.
36. Schwartz, J. *J. Organomet. Chem. Libr.* **1976**, *1*, 461.
37. Okukado, N.; Van Horn, D. E.; Klima, W. L.; Negishi, E. *Tetrahedron Lett.* **1978**, *19*, 1027.
38. Brown, H. C. *Organic Synthesis via Boranes*; New York: Wiley, 1975.
39. Castle, P. L.; Widdowson, D. A. *Tetrahedron Lett.* **1986**, *27*, 6013.
40. (a) Perchellet, E. M.; Ladesich, J. B.; Chen, Y.; Sin, H. S.; Hua, D. H.; Kraft, S. L.; Perchellet, J. P. *Anticancer Drugs* **1998**, 565; (b) Perchellet, J. P.; Newell, S. W.; Ladesich, J. B.; Perchellet, E. M.; Chen, Y.; Hua, D. H.; Kraft, S. L.; Basaraba, R. J.; Omura, S.; Tomoda, H. *Anticancer Res.* **1997**, *1*, 2427.
41. A variety of *E*-chalcones have been reported with potent inhibitory activity against the K562 cell line, see: Ducki, S.; Forrest, R.; Hadfield, J. A.; Kendall, A.; Lawrence, N. J.; McGown, A. T.; Rennison, D. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 1051, For references relating to inhibitory activity against the A2780 cell line, see: (a) McClusky, A.; Bowyer, M. C.; Collins, E.; Sim, A. T. R.; Sakoff, J. A.; Baldwin, M. L. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 1687; (b) Kokotos, G.; Constantinou-Kokotou, V.; Padrón, J. M.; Peters, G. *J. Bioorg. Med. Chem. Lett.* **2001**, *11*, 861.
42. Dufresne, C.; Wilson, K. E.; Zink, D.; Smith, J.; Bergstrom, J. D.; Kurtz, M.; Rew, D.; Nallin, M.; Jenkins,

R.; Bartizal, K.; Trainor, C.; Bills, G.; Meinz, M.; Huang, L.; Onishi, J.; Milligan, J.; Mojena, M.; Pelaez, F. *Tetrahedron* **1992**, *48*, 10221–10226.
43. For complete assay procedures for the 2-pyrones see: Marrison, L. R., Ph.D. Thesis, The synthesis and antimicrobial activities of 2H-pyran-2-ones, (Chapter 5), Manchester Metropolitan University (UK), 1998; Dickinson, J. M., Ph.D. Thesis, The chemistry of fungal biocontrol agents, University of Sussex (UK), 1988.
44. *CRC Handbook of Microbiology*; Laskin, A. I., Lechevailer, H. A., Eds.; *Organismic Microbiology*; CRC: Ohio, 1973; Vol. I.
45. (a) Hatch, M. S.; Brown, W. M.; Deck, J. A.; Hunsaker, L. A.; Deck, L. M.; Vander Jagt, D. L. *Biochim. Biophys. Acta* **2002**, *1596*, 381; (b) Deck, L. M.; Baca, M. L.; Salas, S. L.; Hunsaker, L. A.; Vander Jagt, D. L. *J. Med. Chem.* **1999**, *42*, 4250.
46. Mann, F. G.; Purdie, D. *J. Chem. Soc.* **1935**, 1549.
47. Kato, Y.; Okada, S.; Tomimoto, K.; Mase, T. *Tetrahedron Lett.* **2001**, *42*, 4849.
48. Brown, H. C.; Gupta, S. K. *J. Am. Chem. Soc.* **1972**, *94*, 4370.
49. Matteson, D. S. *Synthesis* **1975**, 147.
50. Dieck, H. A.; Heck, R. F. *J. Org. Chem.* **1975**, *40*, 1083.
51. Matteson, D. S.; Jesthi, P. K. *J. Organomet. Chem.* **1976**, *10*, 25.
52. Hamaoka, T.; Brown, H. C. *J. Org. Chem.* **1975**, *40*, 1189.
53. Matteson, D. S. *J. Am. Chem. Soc.* **1975**, *97*, 5608.
54. (a) Mossmann, T. *J. Immunol. Meth.* **1983**, *63*, 55; (b) Edmondson, J. M.; Armstrong, L. S.; Martinez, A. O. *J. Tissue Culture Meth.* **1988**, *11*, 15.
55. Fairlamb, I. J. S.; Dickinson, J. M.; Higson, S.; Grieveson, L.; O'Connor, R.; Marin, V. *Bioorg. Med. Chem.* **2002**, *10*, 2641, Each disk contained 20 μL of a 10 mg/mL solution of the test compound in DMSO, ethanol or H_2O to give a final concentration of 200 μg/disk.