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Suzuki Cross-coupling Approaches to the Synthesis of Bioactive 3-Substituted and 5-Substituted-4-methoxy-6-methyl-2-pyrones

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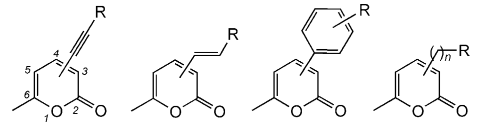
Abstract—Suzuki cross-coupling has been used to access a wide range of 3- and 5-substituted 2-pyrones, which show remarkable inhibitory activity against bacteria, yeasts and fungi. 3-Octenyl and 5-octenyl 2-pyrones inhibit human ovarium carcinoma (A2780) and human chronic myelogenous leukaemia (K562) cell lines at the micromolar level.

The 2-pyrone moiety is found in a large number of naturally-occurring biologically active compounds.[1] Simple changes in the substitution pattern on the 2-pyrone ring often leads to incredible diverse biological activity. For example, 4-hydroxy-2-pyrone moieties have been shown to be key components in non-peptidic HIV protease inhibitors,[2] whereas 4-alkenyl/ alkynyl/ aryl/ alkyl substituted-6-methyl-2-pyrones (**1–4**, Fig. 1), recently reported by us, demonstrate remarkable antimicrobial activities, human ovarium carcinoma (A2780) and human chronic myelogenous leukaemia (K562) inhibitory properties.[3] From a synthetic standpoint the pyrone ring system can be very useful, finding application as a cyclobutane precursor,[4] a diene component in Diels–Alder reactions,[5] and as a precursor to other heterocyclic systems.[6] Until very recently the construction of substituted 2-pyrones was fraught with difficulties, often requiring a multi-step synthesis via an acyclic precursor. Organometallic couplings (Suzuki, Sonogashira and Stille) onto the pyrone ring system have gone some way to overcoming these limitations, enjoying recent successes from the groups of Pleixats,[7] Cho[8] and ourselves.[9] Our continued drive towards identifying new biologically active substituted 2-pyrones, in particular 3- and 5-substituted-4-methoxy-6-methyl-2-pyrones, prompted our investigations on the Suzuki cross-couplings of 3-halo- and 5-halo-4-methoxy-6-methyl-2-pyrones. Herein we report synthetic details and some quite outstanding antimicrobial activities for 3- and 5-substituted-4-methoxy-6-methyl-2-pyrones.

Several Suzuki cross-couplings of 3-iodo- and 5-bromo-4-methoxy-6-methyl-2-pyrone with arylboronic acids have recently been reported.[7] However, we were somewhat surprised that 3-bromo-4-methoxy-6-methyl-2-pyrone could *not* be coupled under seemingly standard conditions. Moreover the reaction times presented were incredibly prolonged (19 h to 6 days!) using [$Pd_2dba_3{\cdot}dba$] (dba = dibenzylidene acetone) in refluxing toluene or $Pd(PPh_3)_4$ in refluxing dimethoxyethane. We decided to investigate the Suzuki cross-couplings of 3-bromo- and 5-bromo-4-methoxy-6-methyl-2-pyrone using our optimised conditions[9] for the synthesis of 3-alkenyl and 5-alkenyl-6-methyl-2-pyrones.

Suzuki Cross-Couplings

3-Bromo-4-methoxy-6-methyl-2-pyrone **5** was synthesised in two steps from commercially available triacetic acid lactone in 55% overall yield (Fig. 2).[10]



1 **2** **3** **4**

Figure 1. Bioactive 4-substituted-6-methyl-2-pyrones **1–4**.

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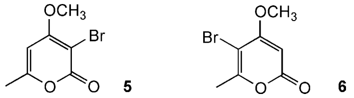
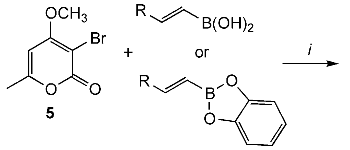


Figure 2. Substrates **5** and **6** for Suzuki cross-coupling.

Table 1. Coupling of various arylboronic acids with **5**[a]



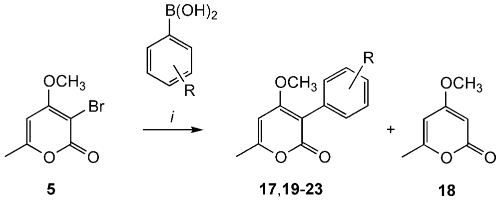
Entry	Pyrone (R)	Yield from boronic acid (%)[b]	Yield from benzodioxaboroles (%)[b]
1	$CH_3(CH_2)_2$, **7**	42	32
2	$CH_3(CH_2)_3$, **8**	64	30
3	$CH_3(CH_2)_4$, **9**	42	30
4	$CH_3(CH_2)_5$, **10**	43	34
5	Ph, **11**	29	23

[a] Conditions: (i), $Pd(OAc)_2$ (6 mol %), PPh_3 (18 mol %), alkenylboronic acid, Na_2CO_3, benzene, Δ, 6 h.
[b] Isolated yields after flash chromatography (%).

Bromination of commercially available dehydroacetic acid at 0 °C, followed by deacetylation with 90% sulphuric acid[11] and then methylation provides 5-bromo-methoxy-6-methyl-2-pyrone **6** starting material in 46% yield.

We have previously established for 4-bromo-6-methyl-2-pyrone that NaOEt and NaOH are far too strong for the 2-pyrone unit to withstand due to the ease of basic hydrolysis.[9] Indeed, rapid pyrone decomposition for **5** is observed in min. A mandatory base is Na_2CO_3 (2M), although K_2CO_3 can be used albeit less effectively. Using Na_2CO_3 and $Pd(OAc)_2$ (6 mol %) with PPh_3 (ligand for Pd) as the catalytic system in benzene/ethanol at reflux gave good reproducible results.[12] Thus compound **5** (Table 1) was coupled with alkenylboronic acids/alkenylbenzodioxaboroles in reaction times of less than 6 h! Substantially less time than originally reported for the coupling of arylboronic acids.[7]

Table 3. Coupling of various arylboronic acids with **5**[a]



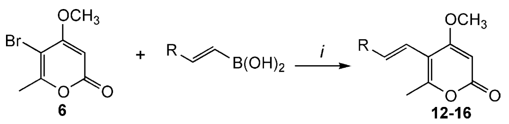
Entry	Pyrone (R)	Yield[b]
1	H-, **17**	79 (3)
2	p-CH_3-, **19**	73 (3)
3	p-Cl-, **20**	69 (3)
4	p-CH_3O-, **21**	65 (28)
5	m-NO_2-, **22**	38 (9)
6	p-CHO-, **23**	49 (3)

[a] Conditions: (i) $Pd(OAc)_2$ (6 mol %), PPh_3 (18 mol %), arylboronic acid, Na_2CO_3, benzene, Δ, 6 h.
[b] Isolated yields after flash chromatography (%). Yield of reduced product **18** in brackets (%).

Higher reaction yields were obtained from reactions where boronic acids were employed. This is advantageous, as the boronic acids are not air sensitive, unlike the benzodioxaboroles, which is presumably a contributing factor to the lower yields for the latter. For this reason, only the alkenylboronic acids were cross-coupled with **6** (Table 2). The yields demonstrate that it is easier to couple groups onto the 3-position as opposed to the 5-position.

With a view to optimizing the yield, it was predicted that alteration of the ligand would be of great benefit. Recent work in Pd-catalysed coupling reactions reveals that the steric (cone angle, θ) and electronic (σ and π donor/acceptor strengths) properties of the ligand for palladium play a significant role in the yields of cross-coupling reactions employing aryl and alkenyl halides/psuedo halides. Little is known about the effect of different ligands on the Pd-catalysed cross-coupling reactions of halogenated pyrone substrates.[9] Two electron-rich and sterically demanding ligands were chosen for evaluation in the Suzuki cross-coupling of **6**, namely PCy_3 and $P(t\text{-}Bu)_3$.[13] The yields of the cross-coupled products **12–16** increased substantially for each alkenyl

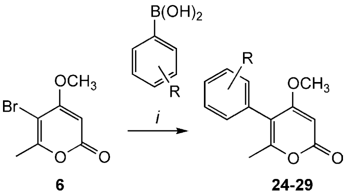
Table 2. Coupling of various arylboronic acids with **6**



Entry	Pyrone (R)	Isolated yields		
		$Pd(OAc)_2/PPh_3$[a]	$Pd(dba)_2/PCy_3$[b]	$Pd(dba)_2/P(t\text{-}Bu)_3$[b]
1	$CH_3(CH_2)_2$, **12**	34	64	54
2	$CH_3(CH_2)_3$, **13**	38	58	62
3	$CH_3(CH_2)_4$, **14**	42	67	65
4	$CH_3(CH_2)_5$, **15**	43	56	69
5	Ph, **16**	53	73	82

[a] Conditions: (i) $Pd(OAc)_2$ (6 mol %), L (18 mol %), alkenylboronic acid, Na_2CO_3, benzene, Δ, 6 h. Isolated yields after flash chromatography (%).
[b] Conditions: (i) $Pd_2dba_3{\cdot}dba$ (2.5 mol %), L (PCy_3 or P^tBu_3) (5 mol %), alkenylboronic acid, Na_2CO_3, benzene, Δ, 6 h.

Table 4. Coupling of various arylboronic acids with **6**[a]



Entry	Pyrone (R)	Yield[b]
1	H-, **24**	76
2	*p*-CH_3-, **25**	75
3	*p*-Cl-, **26**	77
4	*p*-CH_3O-, **27**	71
5	*m*-NO_2-, **28**	7
6	*p*-CHO-, **29**	9

[a] Conditions: (i) $Pd(OAc)_2$ (6 mol %), PPh_3 (18 mol %), arylboronic acid, Na_2CO_3, benzene, Δ, 6 h.
[b] Isolated yields after flash chromatography (%).

boronic acid. It has been suggested by Fu and co-workers[13b] that a palladium monophosphine $[Pd(PR_3)]$ might be the active catalyst. The steric bulk and the electron-richness (σ-donor) of the phosphine ligands are proposed as being important factors for increased reactivity. Compound **5** reacts with $Pd_2dba_3{\cdot}CHCl_3$ and $P(t\text{-}Bu)_3$ to give either a T-shaped or square planar (*O*-methoxy coordination) complex. ^{31}P NMR (202 MHz) shows a singlet at δ 61.47, indicating the presence of one phosphine ligand coordinated to Pd.

The aforementioned results extend on the trends observed in the cross-coupling reaction of aryl boronic acids and substrates containing sp^2 carbon–halide bonds. Moreover, they demonstrate that **5** and **6** can be used as benchmark substrates for testing new catalyst systems.

Initially we investigated both $P(t\text{-}Bu)_3$ and PPh_3 as ligands for Suzuki cross-coupling of **5** and **6** with aryl boronic acids. We believed that an improvement on the conditions previously reported could be made (2.5 mol % $Pd_2dba_3{\cdot}dba$ or 5 mol % $Pd(PPh_3)_4$, K_2CO_3, toluene or DME, rflx.).[7] For phenylboronic acid, we found that coupling to **5** proceeded equally well using $P(t\text{-}Bu)_3$ and PPh_3, providing **17** in 79 and 81% yields, respectively. We also encountered the formation of an unusual dehalogenated product **18** in minor quantities (3 and 5% for each ligand, respectively). Dehalogenated side-products have been recently observed in Suzuki cross-couplings of 4-bromopyrrole-2-carboxylates.[14] Given the small difference in yields observed for each ligand, we focused on the employment of Ph_3P for coupling arylboronic acids to both **5** and **6**, as it is relatively air and moisture stable and more easily handled than $t\text{-}Bu_3P$ (Tables 3 and 4). The results clearly demonstrate that a wide variety of arylboronic acids can be coupled to **5** (entries 2–6, Table 3). Surprisingly, **18** was formed in 28% yield when *p*-methoxyphenyl boronic acid was employed, at the expense of the coupled product **21** (entry 4, Table 3). The less activated arylboronic acids coupled less well, although modest yields for **22** and **23** were still obtained (entries 5 and 6, Table 3), providing a range of candidates for biological testing.

For substrate **6**, the coupled products **24**–**27** could be obtained in useful yields (entries 1–4, Table 4).

Table 5. Antimicrobial activities of 3-, 4- and 5-substituted-6-methyl-2-pyrones[a]

Compd	*Bacillus subtilis*[b]	*Escherichia coli*[b]	*Staphylococcus aureus*[b]	*Candida albicans*[c]	*Saccharomyces cerevisiae*[c]	*Schizosaccharomyces pombe*[c]
3-pent, **7**	18	21	0	0	0	13 (15)
3-hex, **8**	19	20	21	0	0	0
3-hept, **9**	20	0	0	0	0	0
3-oct, **10**	18	0	0	0	0	0
3-pheth, **11**	0	0	0	0	0	0
4-pent, **30**	16	19	20	16	0	28
4-hex, **31**	18	18	0	24	0	40
4-hept, **32**	18	18	17	21	0	34
4-oct, **33**	18	20	18	20	0	29
4-pheth, **34**	18	17	0	0	0	18
5-pent, **12**	16	17	0	11	10	20
5-hex, **13**	17	18	15	18	15	31
5-hept, **14**	19	20	16	12	12	26
5-oct, **15**	16	14	16	18	0	22
5-pheth, **16**	6	6	0	0	0	0
H-, **17**	6	0	0	0	0	0
p-CH_3-, **19**	16	20	0	0	0	0
p-Cl-, **20**	17	18	0	0	30	0
p-CH_3O-, **21**	0	0	0	0	0	0
m-NO_2-, **22**	6	0	0	0	0	0
p-CHO-, **23**	6	6	0	0	0	0
H-, **24**	6	6	0	0	0	0
p-CH_3-, **25**	8	6	0	0	0	20
p-CH_3O-, **27**	14	5	13	11	0	18

[a] Each cellulose disk contained 200 μg of the test compound. NT = not tested. Radii/mm of the inhibition zone. EtOH control (no inhibition).
[b] After 96 h incubation.
[c] After 48 h incubation. Control inhibitors, including squalestatin S1, as for ref 3 and 15a.

Table 6. Antimicrobial activities of 3-, 4- and 5-substituted-6-methyl-2-pyrones[a]

Compd	*Aspergillus niger*[b]	*Fusarium oxysporum*[b,c]	*Pythium ultimum*[b,d]	*Pythium mammillatum*[b]	*Rhizoctonia solani*[b,e]	*Botrytis cinerea*[b,f]
3-pent, **7**	93 (>95)	8 (48)	67 (80)	43 (100)	44 (100)	56 (100)
3-hex, **8**	77 (91)	8 (48)	37 (57)	35 (100)	43 (100)	51 (100)
3-hept, **9**	90 (95)	11 (56)	61 (75)	42 (100)	47 (100)	53 (100)
3-oct, **10**	89 (95)	11 (60)	100 (100)	25 (100)	53 (100)	60 (100)
3-pheth, **11**	>95 (100)	11 (60)	100 (100)	NT	92 (100)	58 (100)
4-pent, **30**	>95 (100)	5 (45)	100 (100)	NT	49 (100)	47 (100)
4-hex, **31**	92 (95)	2 (25)	96 (100)	30 (100)	36 (100)	44 (100)
4-hept, **32**	89 (94)	2 (24)	95 (100)	31 (93)	38 (100)	45 (100)
4-oct, **33**	89 (94)	5 (30)	100 (100)	NT	44 (100)	42 (100)
4-pheth, **34**	100 (100)	NT	100 (100)	NT	47 (100)	59 (100)
5-pent, **12**	82 (90)	8 (35)	100 (100)	43 (100)	50 (100)	39 (100)
5-hex, **13**	68 (>95)	7 (35)	100 (100)	34 (92)	41 (93.4)	33 (100)
5-hept, **14**	82 (95)	7 (24)	100 (100)	24 (94)	33 (100)	12 (100)
5-oct, **15**	90 (93)	10 (46)	100 (100)	22 (90)	40 (100)	29 (94)
5-pheth, **16**	71 (90)	12 (51)	NT	NT	67 (100)	47 (100)
H-, **17**	>95 (100)	7.4 (60)	100 (100)	NT	75 (100)	63 (100)
p-CH$_3$-, **19**	>95 (100)	11 (60)	100 (100)	NT	55 (100)	58 (100)
p-Cl-, **20**	>95 (95)	8 (60)	100 (100)	NT	51 (100)	58 (100)
p-CH$_3$O-, **21**	70 (82)	7 (60)	100 (100)	NT	81 (100)	62 (100)
m-NO$_2$-, **22**	100 (100)	12 (60)	100 (100)	NT	77 (100)	63 (100)
p-CHO-, **23**	83 (>95)	11 (60)	100 (100)	NT	79 (100)	65 (100)
H-, **24**	71 (>90)	12 (51)	NT	NT	67 (100)	47 (100)
p-CH$_3$-, **25**	>90 (>95)	10 (54)	NT	NT	61 (100)	49 (100)
p-CH$_3$O-, **27**	85 (90)	11 (61)	NT	NT	69 (100)	53 (100)

[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 center of the plate containing the organism.[18] Bioassays were performed using either plate or well methods.[19]

[b] Numbers represent percentage growth of the organism after 72 h incubation (NT = not tested). Numbers in brackets represent % growth after 7 days incubation.

[c] EtOH control (15% growth after 72 h, 65% after 7 days).

[d] EtOH control (100% growth after 72 h).

[e] EtOH control (>95% after 72 h).

[f] EtOH control (65% growth after 72 h, 100% after 7 days).

However, poor yields were obtained for **28** and **29** (entries 5 and 6, Table 4). Dehalogenation did not occur in reactions of **6**, suggesting that the 3-position is more susceptible to reduction, but also more reactive to cross-coupling. Similar differences in reactivity have been observed for 3,5-dibromo-2-pyrone, which allows for regioselective cross-coupling at the 3-position.[8]

Biological Activity

The 3-alkenyl (**7**–**11**) and 5-alkenyl (**12**–**16**) pyrones were screened for promising antimicrobial activity against bacteria, fungi and yeasts using reported assays.[15] For comparison, our previously reported 4-alkenyl pyrones (**30**–**34**) are given.[16] The screening results of the 3-aryl (**17**, **19**–**23**) and 5-aryl pyrones (**24**–**29**) are included. All anti-microbial activities are shown in Tables 5 and 6. These results clearly illustrate that a plethora of analogues show inhibitory activity. Notable examples, with potent inhibition, are given here. Analogue **20** inhibits *S. cerevisiae* (the only compound in the aryl class that is active). Analogue **31** shows excellent inhibitory activity against *S. pombe*, comparable to that of squalestatin S1! an extremely potent inhibitor of this organism.[15b] Inhibitory activity towards *P. ultimum* is seen for **8**, which retains potent inhibitory activity at 20 μg (39% inhibition), although activity is lost at 2 μg. An exceptional result is seen for **14** (5-heptenyl analogue) against *B. cinerea*. The 5-substituted analogues as a class show inhibitory activity against this organism.

The growth inhibitory activities of selected 2-pyrones were determined in A2780 human ovarium carcinoma and K562 human chronic myelogenous leukaemia cell lines using an in vitro cell culture system (MTT assay).[17] The 3-octenyl (**10**) and 5-octenyl derivatives **(15)** demonstrated promising activity against both cell lines (IC$_{50}$ (A2780) 30.3 μM; (K562) 30.1 μM; and (A2780) 26.9 μM; (K562) 33.3 μM, respectively.

In conclusion, we have synthesised a range of 3-alkenyl/aryl- and 5-alkenyl/aryl-6-methyl-2-pyrones using Suzuki cross-coupling. These studies represent the first *comprehensive* investigations into such coupling processes for 3-bromo- and 5-bromo-4-methoxy-6-methyl-2-pyrones and important reaction conditions are given, which improve substantially on those previously reported.[7] The 3-octenyl and 5-octenyl-4-methoxy-6-methyl-2-pyrones (**10** and **15**) have been identified as promising new cytotoxic agents against both A2780 and K562 cell lines. A significant study into the antimicrobial activities of the 2-pyrone system has been conducted. The results highlight remarkable inhibitory activity against a range of bacteria, yeasts and fungi. Substituted pyrones are clearly bioactive against a range of species and in the near future we hope to exploit their application. The complete results of these studies will be reported in due course.

Acknowledgements

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12. Representative procedure: $Pd(OAc)_2$ (6 mol %), PPh_3 (18 mol %) and **2** (1 mmol) in benzene (2 mL) and aq Na_2CO_3 (1 mL, 2M) 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, then allowed to cool to room temperature. The mixture was extracted into hexane (2×10 mL) and the combined extracts washed with saturated aq NaCl (2×10 mL), dried ($MgSO_4$) and concentrated in vacuo to give the crude product, which was purified by flash chromatography to give pale oils or solids.

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16. See ref 3 for synthetic details of the 4-alkenyl-6-methyl-2-pyrone ring systems.

17. See ref 3 for full details of the bioassay used to determine the IC_{50} values against A2780 and K562 cell lines.

18. For complete assay procedures for the 2-pyrones, see: Marrison, L. R. PhD Thesis, 'The synthesis and antimicrobial activities of 2*H*-pyran-2-ones', (Chapter 5), Manchester Metropolitan University (UK), **1998**; Dickinson, J. M. *PhD Thesis*, 'The chemistry of fungal biocontrol agents', University of Sussex (UK), **1988**. These assays will be reported in full in due course.

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