Structural studies on bioactive compounds. 1 Heck reactions on 4-methylenepyrazolo[5,1-*c*][1,2,4]triazines

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1-(3-Methyl-4-methylenepyrazolo[5,1-*c*][1,2,4]triazin-6(4*H*-yl)ethanone **9a** and its 7-methyl- **9b** and 7-phenyl-derivative **9c** undergo Heck coupling to afford (*Z*)-1-{4-benzylidene-3-methylpyrazolo[5,1-*c*][1,2,4]triazin-6(4*H*)-yl}ethanones **10a–c** and substituted benzylidene analogues **10d–m** as the major geometrical isomers in low yields. The most potent agent in a human tumour screen *in vitro* was **10b** (mean GI_{50} value 4.9 μM in a panel of 60 human cancer cell lines), with evidence of selective action against colon KM12 (GI_{50} 0.02 μM) and breast MCF-7 tumour cell lines (GI_{50}1.35 μM).

Keywords: Heck reactions, 4-methylenepyrazolo[5,1-*c*][1,2,4]triazines, human cancer screening, 60 cell in *vitro tumour* screen

In the 1960s it was sufficient to justify the antitumor screening of new bicyclic heterocycles on the scientifically fragile rationale that they were "structural analogues" of purines.[2] In the intervening decades, the drug discovery process has become more challenging and the tumor models used to select molecules for closer evaluation have passed through several evolutionary phases – sequentially, the *in vivo* mouse sarcoma 180 and P388/L1210 leukaemia systems, selected human xenograft models and finally, notably, the National Cancer Institute *in vitro* human 60 cell panel.[3] Irrespective of the provenance of new lead agents, whether they be identified by chemistry-driven approaches[4] or by molecular target-guided molecule hunts,[5,6] interrogation of the information-rich qualities of the 60 cell panel can be instrumental in corroborating a predicted mechanism or, possibly more valuably, devining *de novo* the molecular mechanism underlying an unique phenotypic response through COMPARE analysis.[7]

Derivatives of the bicyclic pyrazolo[5,1-*c*][1,2,4]triazine ring system **1**, including unusual 6-acyl modifications with an *exo*-cyclic methylene group at C-4 **2**, were originally reported in the early 1960s (Fig. 1). These compounds were referred to as pyrazolo[3,2-*c*]-*as*-triazines in earlier papers.[8,9] Some compounds had *in vivo* antitumor activity against the mouse sarcoma 180 and a transplanted methylcholanthrene-induced rat sarcoma model.[10] We were keen to revisit this series because of the opportunity to exploit (the then unknown) Heck palladium(0)-mediated chemistry[11-14] in an attempt to synthesise modifications at the methylene group (Fig. 1) and to subject selected products to a 60-cell *in vitro* evaluation.

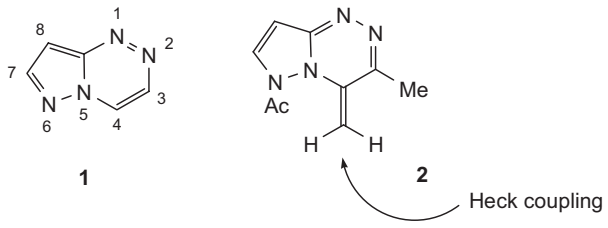
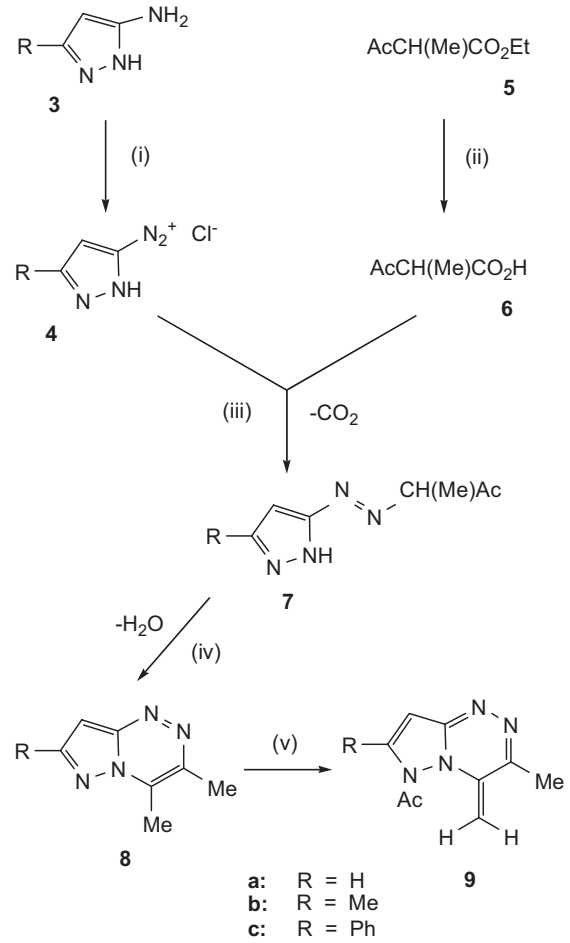


Fig. 1 The pyrazolo[5,1-*c*][1,2,4]triazine ring and numbering system **(1)** and 6-acyl **(2)** derivatives with antitumour activity.

Chemistry

Following the synthetic route applied previously, 3-aminopyrazole **3a** was diazotised in 5 M-hydrochloric acid to furnish the diazonium chloride **4a** which was coupled at pH 7–8 with the β-keto acid **6**, derived by alkaline hydrolysis of ethyl methylacetoacetate **5**. The unstable pyrazolylazoalkane **7a** underwent slow spontaneous, but unpredictable, decarboxylation and cyclisation to the 3,4-dimethylpyrazolotriazine **8a**.[8] We now show that cyclisation can be accelerated by boiling **7a** in ethanol containing catalytic *p*-TsOH. Similarly, the 7-methyl- **8b** and 7-phenyl-pyrazolotriazine **8c** were formed in 30 and 38% overall yields, respectively, from 3-amino-5-methylpyrazole **3b** and 3-amino-5-phenylpyrazole **3c**. The corresponding 6-acetyl-derivatives



Scheme 1 *Reagents and conditions*: (i) $NaNO_2$ in 2 M HCl, 0°C; (ii) KOH, H_2O, 25°C; (iii) AcONa, H_2O, 0°C; (iv) *p*-TsOH (cat.), EtOH, reflux; (v) Ac_2O, reflux.

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9a–c were derived from the dimethylpyrazolotriazines with refluxing acetic anhydride (Scheme 1). The presence of *exo*-cyclic methylene groups in **9a–c** was confirmed by the appearance (^{1}H NMR spectroscopy) of two coupled doublets for the olefinic protons.

Classical Heck reactions involve the palladium(0)-catalysed coupling of alkenes with aryl or vinyl iodides, bromides or triflates in the presence of base and typically proceed in high yields.[15-17] Our desire to generate new carbon–carbon bonds between the sterically-shielded *exo*-methylene residue of substrates **9a–c** and aryl groups represents an extreme challenge and difficulties became apparent when the coupling between pyrazolotriazine **9a** and bromobenzene was investigated under a range of typical Heck conditions. Initially DMF was employed as solvent (100°C) in the attempted coupling of **9a** with bromobenzene in the presence of $Pd(OAc)_2$ with a range of organic bases at different stoichiometries; reaction outcomes are recorded in Table 1.

No coupled products were obtained in the absence of a phosphine ligand (Table 1; entries 1 to 3). A coupled product was detected (TLC) employing K_2CO_3 as base and PPh_3 (0.3 equiv.) as ligand (entry 4) but application of palladacycles[18] as catalysts in the presence of Cs_2CO_3 or Et_3N failed to secure any coupled product (entries 5–7); replacement of bromobenzene with iodobenzene in the presence of $Pd(OAc)_2$ was similarly unsuccessful (entry 8). The first modest success was achieved with the latter catalyst and a combination of Et_3N (4 equiv.) and PPh_3 (0.5 equiv.) which afforded an isolable benzylidenepyrazolotriazine in 19% yield following chromatographic purification. This compound was subsequently shown to have the *Z*-configuration **10a** (see later) at the olefinic bond (entry 9). Surprisingly, use of Cs_2CO_3 and iodobenzene for a short reaction time (1.5 h) led to the isolation of a mixture of **10a** and the diphenylmethylene pyrazolotriazine **11a** in a combined yield of 32% (entry 10). Presumably, the initial product **10a** can undergo a second Heck reaction because, when reaction time under these conditions was extended to 24 h, only the doubly-coupled product was isolated (22%) (entry 11) (Scheme 2). Use of dimethylacetamide (DMAC) as solvent met with only partial success depending on the base used: NaOAc led to no reaction taking place with bromobenzene (entries 12 and 13); similarly K_3PO_4 and iodobenzene gave no coupling (entry 14); employment of CsF led to decomposition of the substrate and no identifiable coupling products (entry 15); and K_3PO_4 and Cs_2CO_3, for short reaction times, gave mixtures of **10a** and **11a** (entries 16 and 17, respectively). Overall Cs_2CO_3 as base gave the highest yields but usually the doubly-coupled pyrazolotriazine **11a** was the major product. The influence of a substituent at C-7 in the pyrazole ring on yields from Heck reactions between **9b,c** and bromobenzene was explored using 'standardised' conditions: $Pd(OAc)_2/PPh_3$ as catalyst/ligand, Et_3N as base and DMF as solvent at 100°C. The yields of coupled products **10b** and **c** were a disappointing 11 and 37%, respectively.

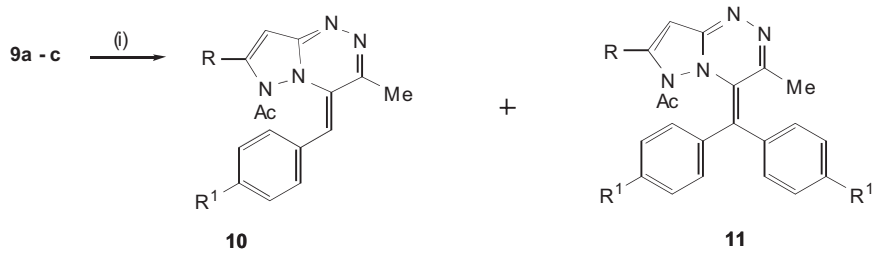
Purified monocoupled benzylidenepyrazolotriazine **10a** showed a ^{1}H NMR spectroscopy peak for the methine proton at δ 6.47 and for analogues **10b–m** this peak was within the range δ 6.31–6.47. However, NMR spectra of crude mixtures showed additional minor resonances in the δ 6.5–7.0 range implying that mixtures of geometric isomers were formed from which only the major product was chromatographically purified and suitably characterised. A full spectroscopic assignment of purified **10a** was completed employing 1D ^{1}H and ^{13}C experiments as well as 2D heteronuclear single quantum coherence (^{13}C HSQC) to resolve direct coupling between ^{1}H and ^{13}C and longer range heteronuclear multi-bond coherence (^{13}C HMBC) experiments to couple isolated spin systems and resolve quaternary centres. This gave unambiguous assignment for all the non-phenyl ^{1}H and ^{13}C resonances for compound **10a** (see Experimental). Interatom distances were assessed by use of the double pulse-field gradient spin echo (DPFGSE) experiment to measure transfer NOEs.

Expected NOEs were observed linking the protons at C-7 and C-8 of **10a**. Irradiation of the methine proton at δ 6.47 shows enhancement at both the C-4 methyl group (7.4%) as well as the adjacent phenyl proton (3.4%); irradiation of C-4 methyl shows enhancement at the methine proton only. In contrast, no NOEs were observed on irradiation of the *N*-acyl methyl proton signal. These observations confirm that **10a** adopts the *Z*-configuration, implying that there is considerable distortion from coplanarity at the olefinic bond to accommodate both aryl and acyl substituents on the same sectors of the molecule. Molecular modelling studies were carried out to investigate potentially available geometries. Starting structures were built from planar arrangements of the favoured Z-isomers in which the rotation of the amide bond and phenyl group were varied; geometries were optimised using a semi-empirical approach followed by geometry optimisation at the Hartree-fock level using the 6–31* basis

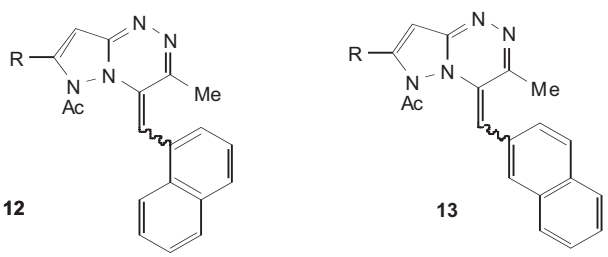
Table 1 Heck reactions on 1-{3-methyl-4-methylenepyrazolo[5,1-*c*][1,2,4]triazin-6(4*H*)-yl}ethanone **9a**

Entry no.	Base	Molar equivalents of base	Solvent	Conditions[a]	Time/h	Outcome
1	Et_3N	2.5	DMF	A	24	No reaction
2	Na_2CO_3	2.5	DMF	A	24	No reaction
3	K_2CO_3	2.5	DMF	A	24	No reaction
4	K_2CO_3	2.5	DMF	B	24	Coupling[b]
5	Cs_2CO_3	2.0	DMF	C	4	Decomp
6	Et_3N	10.0	DMF	C	4	No reaction
7	Et_3N	10.0	DMF	C	24	No reaction
8	Et_3N	10.0	DMF	D	24	No reaction
9	Et_3N	4.0	DMF	E	24	**10a** (19%)
10	Cs_2CO_3	2.0	DMF	D	1.5	**10a + 11a** (32%)
11	Cs_2CO_3	2.5	DMF	B	24	**11a** (22%)
12	NaOAc	1.1	DMAC	B	24	No reaction
13	NaOAc	1.1	DMAC	C	24	No reaction
14	K_3PO_4	2.0	DMAC	D	1.5	No reaction
15	CSF	1.2	DMAC	B	24	Decomp
16	K_3PO_4	1.0	DMAC	B	1.5	**10a + 11a** (27%)
17	Cs_2CO_3	2.0	DMAC	B	1.5	**10a + 11a** (32%)

[a]Conditions: A, $Pd(OAc)_2$ (0.1 equiv), C_6H_5Br (2.0 equiv.), base, solvent at 100°C; B, As A plus PPh_3 (0.3 equiv.); C, As A with palladacycles (0.5 equiv. as catalyst); D, As A with Pd $(OAc)_2$ (0.05 equiv.) and C_6H_5I (2.0 equiv.); E, As A plus PPh_3 (0.5 equiv.).
[b]Detected by TLC and presence of absorptions (^{1}H NMR spectroscopy) in the δ 6.3–6.5 range.



	R	R^1			R	R^1
a:	H	H		**a:**	H	H
b:	Me	H		**b:**	H	Me
c:	Ph	H		**c:**	H	F
d:	H	OMe				
e:	Me	OMe				
f:	Ph	OMe				
g:	H	Me				
h:	H	F				
i:	Me	Me				
j:	Me	Et				
k:	Me	F				
l:	Me	CO_2Et				
m:	Ph	Et				



a: R = H
b: R = Me
c: R = Ph

Scheme 2 *Reagents and conditions*: (i) $Pd(OAc)_2$, Et_3N, PPh_3 in DMF, 100°C; (ii) 2N H_2SO_4, 100°C, then 2N NaOH

set. Distortions of the bond by ~5 degrees between C-4 and the bridgehead N-5 perturbs the planarity of the bicyclic core and minimises steric interactions between the phenyl and amide groups; these interactions are further reduced by rotation of the phenyl group from the plane of the bicyclic heterocycle, an arrangement consistent with the NOE data (Fig. 2a). A greater displacement from planarity is required in a putative *E*-isomer of **10a** to avoid steric clashes between the phenyl group and the methyl fragment at C-3 (Fig. 2b), although these interactions still have to be accommodated in the doubly-coupled compounds **11a–c**. The above structural considerations clearly have a major bearing on the low yields from these Heck reactions.

The exact orientation of the amide function in **10a** is less certain. The lack of NOE data suggests that it is in a relatively isolated position. However, it is not possible to rule out an unobserved weak interaction to the phenyl group. *Ab initio* modelling suggests that the amide is not coplanar with the distorted pyrazole ring.

The further scope of the Heck coupling was then investigated. Because yields were very low often it was not possible to isolate pure mono- **10** and di-coupled **11** products

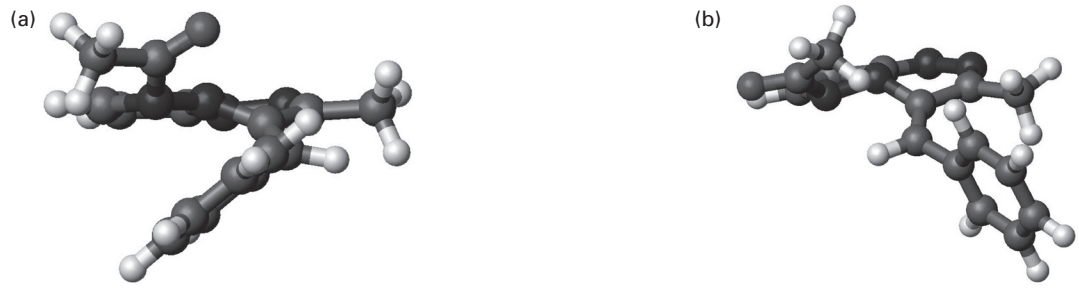


Fig. 2 (a) Minimised steric interactions between the phenyl and amide groups give rise to the *Z*-isomer of **10a** an arrangement consistent with the NOE data. (b) A putative *E*-isomer of **10a**.

by chromatographic separation and reaction mixtures were generally monitored by TLC, ^{1}H NMR spectroscopy and HRMS. When 4-bromoanisole was used as aryl halide in the coupling of **9a** in the presence of $Pd(OAc)_2/Et_3N/PPh_3$/DMF at 100°C, the expected benzylidenepyrazolotriazine **10d** (12%) was accompanied by the previously synthesised desmethoxy analogue **10a** (24%). This type of aryl-aryl exchange reaction has been reported previously in the presence of Pd(0) catalysts.[19] Whereas the 7-methylpyrazolotriazine **9b** with 4-bromoanisole and $Pd(OAc)_2$ with Et_3N/PPh_3 in DMAC at 100°C gave only a 3% isolated yield of the Heck product **10e**, identified by ^{1}H NMR spectroscopy, the corresponding 7-phenylpyrazolotriazine **9c** gave a mixture of the required 4-methoxybenzylidene derivative **10f** (9%) and the desmethoxy product **10c** (38%), which was the highest overall conversion into Heck products seen in this work. In a few other cases, it was possible to detect mixtures of coupled products by HRMS analysis. Thus, with Cs_2CO_3 as base, compound **9a** and 4-methylbromobenzene afforded an inseparable mixture of **10g** and the doubly-coupled product **11b** (combined yield 24%). Appropriate molecular ions for both components ($C_{16}H_{16}N_4O$ and $C_{23}H_{22}N_4O$) were identified in the HRMS spectrum of the mixture, as well as expected fragment $M^+ - 42$ (CH_2 =C=O) ions from both components; similarly, with 4-fluorobromobenzene an unseparated mixture of **10h** ($C_{15}H_{13}FN_4O$) and **11c** ($C_{21}H_{16}F_2N_4O$), with HRMS showing respective M^+-42 fragment ions, was formed (25%). In other cases Heck coupling of pyrazolotriazines **9b** and **9c** with a range of 4-substituted bromobenzenes gave substituted benzylidene pyrazolotriazines **10i–m** in isolated yields < 10%. Couplings between **9a,b** and 1- and 2-bromonaphthalenes gave crude products which were identified, tentatively (HRMS), as mixtures of (*Z*) and (*E*) geometric isomers **12a,b** and **13a,b**, respectively: their ^{1}H NMR spectra showed multiple peaks in the δ 6.6–6.8 region assigned to the methine protons. Physical characteristics of pyrazolotriazines **10a–f** and **11a** are recorded in the Experimental Section and for compounds **10i–m** in Table 2.

(*Z*)-1-{4-Benzylidene-3-methylpyrazolo[5,1-*c*][1,2,4]triazin-6(4*H*)-yl}ethanone **10a** underwent *N*-dealkylation in hot 1M sulfuric acid to yield the 4-benzylpyrazolo[5,1-*c*][1,2,4]triazine **14** (Scheme 2). The ^{1}H NMR spectrum showed a methyl signal at δ 2.82 and a benzylic methylene signal at δ 4.60. This compound was unstable on addition of base showing the emergence of multiple methyl signals, presumably attributable to a facile ring-opening of the 1,2,4-triazine ring promoted by nucleophilic attack at C-4, a feature of the chemistry of this bicyclic system.[8,9]

Biology

Six compounds were tested in the National Cancer Institute (USA) 60-cell *in vitro* tumour screen.[7] The pyrazolotriazines **8a,b** and the 6-acetyl-derivatives **9a,b** were essentially inactive showing mean GI_{50} values ~ 100 μM. The benzylidene derivatives **10a,b** were more potent and the most active agent was compound **10b** (mean GI_{50} 4.9 μM), with evidence of selective action against the colon KM12 (GI_{50} 0.02 μM) and breast MCF7 tumour cell lines (GI_{50} 1.35 μM).

Experimental

General methods

Melting points were measured on a Galenkamp apparatus and are uncorrected. IR spectra were recorded on a Mattson 2020 Galaxy series FT-IR spectrometer and UV spectra on a Pharmacia Biotech Ultraspec 2000 UV/visible spectrophotometer. Mass spectra were recorded on either a Micromass Platform spectrometer, an AEI MS-902 (nominal mass), or a VG Micromass 7070E or Finigan MAT900XLT

Table 2 Physical properties of pyrazolotriazines **10i–10m**

Compound	M.p./°C	Formula	Found (M + 1)	Required (M)	^{1}H NMR (δ values)
10i	74–76	$C_{17}H_{18}N_4O$	295.145	294.1481	2.23 (3H, s, CH_3), 2.37 (3H, s, CH_3), 2.40 (3H, s, CH_3), 2.53 (3H, s, CH_3), 6.34 (1H, s, = CH), 6.60 (1H, s, H-8), 7.13–7.18 (2H, m, ArH), 7.42 (2H, m, J^* = 8.0, ArH)
10j	72–74	$C_{18}H_{20}N_4O$	309.146	308.1637	1.25 (3H, t, J = 7.6, CH_2CH_3), 2.24 (3H, s, CH_3), 2.40 (3H, s, CH_3), 2.53 (3H, s, CH_3), 2.67 (2H, t, J = 7.6, CH_2CH_3), 6.34 (1H, s, = CH), 6.61 (1H, s, H-8), 7.14 (2H, m, J^* = 8.2, ArH), 7.45 (2H, m, J^* = 8.2, ArH)
10k	120–121	$C_{16}H_{15}FN_4O$	299.126	298.125	2.23 (3H, s, CH_3), 2.41 (3H, s, CH_3), 2.54 (3H, s, CH_3), 6.31 (1H, s, = CH), 6.97 (1H, s, H-8), 7.02 (2H, m, ArH), 7.49–7.64 (2H, m, ArH)
10l	104–105	$C_{19}H_{20}N_4O_3$	353.1432	352.1535	1.41 (3H, t, J = 7.12, CH_2CH_3), 2.22 (3H, s, CH_3), 2.44 (3H, s, CH_3), 2.55 (3H, s, CH_3), 4.39 (2H, q, J = 7.12, CH_2CH_3), 6.35 (1H, s, =CH), 6.64 (1H, s, H-8), 7.54 (2H, m, J^* = 8.4, ArH), 7.97 (2H, m, J^* = 8.4, ArH)
10m	86–88	$C_{23}H_{22}N_4O$	371.1865	370.1794	1.23 (3H, t, J = 7.8, CH_2CH_3), 2.37 (3H, s, CH_3), 2.49 (3H, s, CH_3), 2.64 (2H, q, J = 7.8, CH_2CH_3), 6.39 (1H, s, =CH), 7.09 (1H, s, H-8), 7.10–7.61 (9H, m, ArH)

spectrometer (accurate mass). Compounds were confirmed as pure (single spot TLC) prior to submission for HRMS. Merck silica gel 60 (40–60 μM) was used for column chromatography. Routine NMR spectra were recorded in $CDCl_3$ on a Bruker ARX 250 instrument; coupling constants in Hz (For AA'XX' systems $J^* = J_{23} + J_{25}$). NMR spectroscopy experiments on compound **10a** were carried out on a Bruker Avance 400; NOE experiments were recorded using the DPFGSE method. On-resonance inversion was achieved by the use of a Gaussian shaped pulse that was optimised to invert only a narrow bandwidth of the peak of interest. The effects of spin diffusion were also considered by repeating the experiment at a number of different mixing times between 0.6 and 1.3 s. Molecular modelling was performed using the Gaussian 98 package. Starting structures were initially geometry-optimised using the semi-empirical AM1 method with the 6–31* basis set. The geometry was then further refined using the Hartree–Fock level with a 6–31* basis set.

General method for the synthesis of 3,4-dimethylpyrazolo[5,1-c][1,2,4]triazines **8**

Ethyl methylacetoacetate (34.7 g) was hydrolysed in a solution of aqueous potassium hydroxide (15.9 g) in water (120 ml) for 24 h at 0°C, acidified to pH 6 with 10N-hydrochloric acid and added at 0°C to a solution of 3-aminopyrazole **3a** (20.0 g) which had been diazotised in 5 M-hydrochloric acid (160 ml) with aqueous sodium nitrite (18.8 g) in water (40 ml). Portionwise addition of sodium acetate trihydrate (120 g) caused effervescence and formation of a gum, which slowly solidified at 0°C to give **7** (79%); ^{1}H NMR δ 2.00 (3H, s, CH_3), 2.48 (3H, s, CH_3), 6.31 (1H, d, J = 2.3), 7.51 (1H, d, J = 2.3), 8.20 (1H, brs, NH), 9.58 (1H, brs, NH). Crude **7** (31.6 g) was refluxed in EtOH (200 ml) containing *p*-TsOH (0.32 g) for 3 h. Evaporation of solvent under reduced afforded an oil which was partitioned between $CHCl_3$ and brine. Evaporation of the organic layer, followed by chromatographic fractionation silica-gel chromatography gave a solid which was crystallised from AcOEt-hexane to yield 3,4-dimethylpyrazolo[5,1-*c*][1,2,4]triazine **8a** (5.55 g, 86%) as a yellow solid, m.p. 101–103°C (Lit.,[8] m.p. 105–106°C); ^{1}H NMR δ 2.87 (3H, s, CH_3), 2.90 (3H, s, CH_3), 7.20 (1H, d, J = 2.56, H-8), 8.22 (1H, d, J = 2.51, H-7). Similarly prepared were the following dimethylpyrazolotriazines:

3,4,7-Trimethylpyrazolo[5,1-c][1,2,4]triazine (**8b**): From **3b** (46%) as an orange solid, m.p. 125–127°C; ^{1}H NMR δ 2.59 (3H, d, J = 0.40, CH_3), 2.80 (3H, s, CH_3), 2.83 (3H, s, CH_3), 6.91 (1H, d, J = 0.40, H-8); HRMS (ES) *m/z* 163.1063 (M + 1). $C_8H_{10}N_4$ requires 162.0905.

3,4-Dimethyl-7-phenylpyrazolo[5,1-c][1,2,4]triazine (**8c**): From **3c** (38%), m.p. 177–179°C; ^{1}H NMR δ 2.87(3H, s, CH_3), 2.88 (3H, s, CH_3), 7.41 (1H, s, H-8), 7.43–7.47 (1H, m, ArH), 7.48–7.53 (2H, m, ArH), 8.05–8.08 (2H, m, ArH); HRMS (ES) *m/z* 225.1132 (M + 1). $C_{13}H_{12}N_4$ requires 224.1062.

General method for the synthesis of 4-methylenepyrazolo[5,1-c][1,2,4-triazines (**9**): 3,4-Dimethylpyrazolo[5,1-*c*][1,2,4]triazine **6a** (1.86 g) was refluxed for 1 h in acetic anhydride (20 ml). After cooling, the reaction mixture was poured into water to afford pale yellow crystals (from light petroleum) of 1-{3-methyl-4-methylenepyrazolo[5,1-*c*][1,2,4]triazin-6(4*H*)-yl}ethanone **9a** (1.32 g, 55.4%), m.p. 98–100°C (Lit.,[8] m.p. 97–98°C); ^{1}H NMR δ 2.46 (3H, s, CH_3), 2.66 (3H, s, CH_3), 5.12 (1H, d, J = 1.48, =CH), 5.88 (1H, d, J = 1.46, =CH), 6.98 (1H, d, J = 1.84, H-8), 7.81 (1H, d, J = 1.84, H-7); HRMS (ES) *m/z* 191.0878 (M + 1). $C_9H_{10}N_4O$ requires 190.0855. Similarly prepared were the following 6-substituted pyrazolotriazines:

1-{3,7-Dimethyl-4-methylenepyrazolo[5,1-c][1,2,4]triazin-6(4H)-yl}ethanone (**9b**): From **8b** (87%), as yellow crystals, m.p. 193–195°C; ^{1}H NMR δ 2.28 (3H, s, CH_3), 2.37 (3H, s, CH_3), 2.52 (3H, s, CH_3), 4.92 (1H, d, J = 1.5, =CH), 5.65 (1H, d, J = 1.5, =CH), 6.67 (1H, s, H-8); HRMS (ES) *m/z* 205.1053 (M + 1). $C_{10}H_{12}N_4O$ requires 204.1012

1-{3-Methyl-4-methylene-7-phenylpyrazolo[5,1-c][1,2,4]triazin-6(4H)-yl}ethanone (**9c**):From **8c** (64%), m.p. 150–151°C; ^{1}H NMR δ 2.28 (3H, s, CH_3), 2.49 (3H, s, CH_3), 4.95 (1H, d, J = 1.6, =CH), 5.79 (1H, d, J = 1.6, =CH), 7.13 (1H, s, H-8), 7.34–7.38 (3H, m, ArH), 7.85–7.87 (2H, m, ArH); HRMS (ES) *m/z* 267.121 (M + 1). $C_{15}H_{14}N_4O$ requires 266.1168.

General method for the Heck synthesis of 4-benzylidenepyrazolo[5,1-c]- [1,2,4]triazines (**10**)

Into an ACE pressure tube (Aldrich) was placed **9a** (0.2 g), bromobenzene (1.66 g, 10 mol. equiv.), triethylamine (0.43 g, 4 mol. equiv.), $Pd(OAc)_2$ (23.6 mg, 0.1 mol. equiv.) and triphenylphosphine (0.14 g, 0.5 mol. equiv.) and DMF (10 ml). The tube was sealed under an atmosphere of nitrogen and heated at 100°C for 24 h. The cooled reaction mixture was shaken with EtOAc (10 ml) and saturated aqueous $NaHCO_3$ (10 ml). The organic layer was washed with water and brine and solvent evaporated to give a residue which was fractionated on a silica gel column. The product, *(Z)-1-{4-benzylidene-3-methylpyrazolo[5,1-c][1,2,4]triazin-6(4H)-yl}ethanone* **10a** (19%) formed pale yellow crystals, m.p. 128–130°C; ^{1}H NMR δ 2.44 (3H, s, CH_3), 2.65 (3H, s, $COCH_3$), 6.47 (1H, s, =CH), 6.82 (1H, d, J = 1.95, H-8), 7.29–7.37 (3H, m, ArH), 7.44–7.47 (2H, m, ArH), 7.59 (1H, d, J = 1.94, H-7); ^{13}C NMR δ 169.9 (C=O), 144.0 (C-4), 141.0 (C-7), 133.5 (C-8a), 123.6 (C-3), 113.0 =CH), 95.3 (C-8), 21.5 ($COCH_3$), 19.8 (CCH_3); HRMS (ES) *m/z* 267.1221 (M + 1). $C_{15}H_{14}N_4O$ requires 266.1168. Similarly prepared (method for **10a**) were the following benzylidene-pyrazolotriazines:

(Z)-1-{4-Benzylidene-3,7-dimethylpyrazolo[5,1-c][1,2,4]triazin-6(4H)-yl}ethanone (**10b**): From **9b** (11%) as pale yellow crystals, m.p. 90–92°C; ^{1}H NMR δ 2.22 (3H, s, CH_3), 2.42 (3H, s, CH_3), 2.54 (3H, s, CH_3), 6.37 (1H, s, =CH), 6.61 (1H, s, H-8), 7.26–7.28 (3H, m, ArH), 7.50 (2H, d, J = 6.21 Hz, ArH); HRMS (ES) *m/z* 281.1374 (M + 1). $C_{16}H_{16}N_4O$ requires 280.1324.

(Z)-1-{4-Benzylidene-3-methyl-7-phenylpyrazolo[5,1-c][1,2,4]triazin-6-(4H)-yl}ethanone (**10c**): From **9c** (37%), m.p. 168–169°C; ^{1}H NMR δ 2.38 (3H, s, CH_3), 2.50 (3H, s, CH_3), 6.41 (1H, s, =CH), 7.10 (1H, s, H-8), 7.21–7.31 (6H, m, ArH), 7.48–7.50 (2H, m, ArH), 7.58–7.60 (2H, m, ArH); HRMS (ES) *m/z* 343.1015 (M + 1). $C_{21}H_{18}N_4O$ requires 342.1482. The difference here is greater then in other cases but the totality of the evidence supports the assigned structure.

(Z)-1-{4-(4-Methoxybenzylidene)-3-methylpyrazolo[5,1-c][1,2,4]triazin-6(4H)-yl}-ethanone (**10d**): From **9a** and 4-bromoanisole (12%), m.p. 129–131°C; ^{1}H NMR δ 2.42 (3H, s, CH_3), 2.54 (3H, s, CH_3), 3.84 (3H, s, CH_3), 6.42 (1H, s, =CH), 6.80 (1H, d, J = 1.95, H-8), 6.86 (2H, m, J^* = 8.58), 7.45 (2H, m, J^* = 8.58), 7.60 (1H, d, J = 1.95, H-7); HRMS (ES) *m/z* 297.1312 (M + 1). $C_{16}H_{16}N_4O_2$ requires 296.1273).

(Z)-1-{4-(4-Methoxybenzylidene)-3,7-dimethylpyrazolo[5,1-c][1,2,4]triazin-6-(4H)-yl}ethanone (**10e**): From **9b** and 4-bromoanisole (3%); ^{1}H NMR δ 1.93 (3H, s, CH_3), 2.25 (3H, s, CH_3), 2.52 (3H, s, CH_3), 3.84 (3H, s, CH_3), 6.32 (1H, s, =CH), 6.60 (1H, s, H-8), 6.86 (2H, m, J^* = 12.0, ArH), 7.51 (2H, m, J^* = 12.0, ArH).

(Z)-1-{4-(4-Methoxybenzylidene)-3-methyl-7-phenylpyrazolo[5,1-c][1,2,4]triazin-6(4H)-yl}ethanone (**10f**): From **9c** and 4-bromoanisole (9%), m.p. 148–150°C; ^{1}H NMR δ 2.35 (3H, s, CH_3), 2.48 (3H, s, CH_3), 3.80 (3H, s, CH_3), 6.34 (1H, s, =CH), 6.82 (2H, d, J = 8.84), 7.10 (1H, s, H-8), 7.21–7.30 (3H, m, ArH), 7.51 (2H, d, J = 8.68, ArH), 7.66 (2H, m, J^* = *9.74*, ArH); HRMS (ES) *m/z* 373.1575 (M + 1). $C_{22}H_{20}N_4O_2$ requires 372.1586). Chromatographic fractionation of the products also afforded **10c** (38%).

1-{4-(Diphenylmethylene)-3-methylpyrazolo[5,1-c][1,2,4]triazin-6(4H)-yl}ethanone (**11a**): When the reaction leading to **10a** (above) was conducted in DMF at 100°C, employing Cs_2CO_3 as base (2.5 mol. equiv.) and triphenylphosphine (0.3 mol. equiv.), this (diphenylmethylene)pyrazolotriazine **11a** was isolated (22%), m.p.146–148°C; ^{1}HNMR δ 1.72(3H,s,CH_3),2.52(3H,s,CH_3),6.68(1H, d J = 1.95, H-8), 6.95–6.99 (2H, m,), 7.19–7.22 (3H, m, ArH), 7.28–7.30 (2H, m, ArH), 7.36–7.39 (4H, m, ArH); HRMS (ES) *m/z* 343.155 (M + 1). $C_{21}H_{18}N_4O$ requires 342.1481.

4-Benzyl-3-methylpyrazolo[5,1-c][1,2,4]triazine (**14**): Pyrazolotriazine **10a** (10 mg) was boiled in 1 M H_2SO_4 (1 ml) for 4 h. The cooled mixture was basified (to pH 8) with 2 M NaOH/ice and product was extracted with DCM. Evaporation of the dried (Na_2SO_4) organic layer, followed by preparative TLC (10% MeOH in DCM), afforded the benzylpyrazolotriazine (3 mg, 36%), ^{1}H NMR δ 2.82 (3H, CH_3), 4.60 (2H, CH_2), 7.21 (1H, d, J = 2.5, H-8), 7.26–7.34 (5H, m, ArH), 8.21 (1H, d, J = 2.5, H-7); HRMS (ES) *m/z* 225.1123 (M + 1). $C_{13}H_{12}N_4$ requires 224.1062

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