COMMUNICATION

Expedient method for the solid-phase synthesis of some 4-substituted-4,5-dihydropyridazin-3(2*H*)-ones

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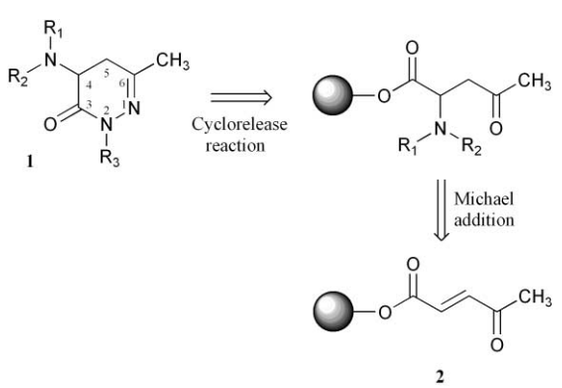
The solid-phase synthesis of some 4-amino-4,5-dihydropyridazin-3(2*H*)-ones has been realised using regiospecific Michael addition and subsequent cyclorelease reactions.

The development of solid-phase combinatorial and multiple parallel synthesis methods has stimulated considerable interest in the discovery and optimisation of new leads in the pharmaceutical industry, since most of the recent publications in this field are focused on the synthesis of small, drug-like molecules.[1] Specifically, the access to heterocyclic compounds by solid-phase synthesis[2,3] has emerged as a powerful tool, since small, substituted heterocycles offer a high degree of structural diversity and have proven to be exceptionally useful as therapeutic agents. As a result, a number of pharmaceutically useful heterocyclic compounds have recently been prepared by solid-phase methodology, which proved to be a convenient method for the generation of combinatorial libraries.[4,5]

Great attention has been paid to the 6-substituted-4,5-dihydropyridazin-3-ones, due to their potential biological activities.[6–9] However little has been reported on the synthesis and pharmacological screening of 4-substituted compounds.[10,11]

As part of our efforts towards the solid-phase synthesis and biological evaluation of diverse heterocycles, we have previously described a cyclorelease approach for the construction of the pyridazinone nucleus on Wang resin.[12] In this paper, we report the development of an efficient method for the parallel synthesis of 4-amino-4,5-dihydropyridazin-3(2*H*)-one derivatives **1** and more specifically the use of a polymer-supported Michael adduct **2** to introduce diversity at the C-4 position of the final ring (Scheme 1).



Scheme 1

The synthetic route is characterised by three essential steps (Scheme 2): (1) synthesis of the starting Michael adduct in the solution phase and coupling to the resin, (2) addition of diverse amines and (3) cyclisation–cleavage (condensation with hydrazines). Furthermore, such an approach would have the advantages of solution-phase chemistry, allowing a large amount of precursor **5** as well as solid-phase synthesis for generating diversity.

Our initial efforts were directed towards the preparation of the building block **2**.

This first step could not be accomplished starting from polymer-supported levulinic acid since bromination was revealed to be inappropriate when applied to the solid phase. Thus, various conditions investigated for this reaction always gave a mixture of mono-, di- and tribromolevulinic derivatives.

Consequently the desired α,β-unsaturated acid **5** was prepared in the solution phase from levulinic acid[13] **3** by bromination providing the corresponding 3-bromolevulinic acid **4** in good yield. Without any purification, **4** was converted to 4-oxopent-2-enoic acid **5** by treatment with sodium acetate in glacial acetic acid. Compound **5** was obtained after recrystallisation from chloroform in moderate yield (40%).

Our solid-phase route began with anchoring **5** on Wang resin in useful conditions (DIC and DMAP)†. The reaction could easily be monitored by FT-IR spectroscopy of the resin **2**, which showed a strong appearance of the characteristic C=O stretches of γ-ketoester at 1730 cm^{-1} and disappearance of the ν_{OH} vibration of Wang resin at 3400 cm^{-1}.

The first element of diversity was introduced by Michael addition with a variety of primary or secondary amines to **2** in DMSO for 1 hour under ambient conditions. The complete addition was checked by ^1H NMR analyses of the products released from the resins **6**–**9** upon treatment with a 10% solution of trifluoroacetic acid in dichloromethane for 2 hours at room temperature. The Michael addition on a solid phase is particularly suitable for primary amines since an excess could be used, thus reducing the formation of bis-addition side products.[14,15]

In the final step, which was an intramolecular cyclisation with concomitant release of the final products under acid-free conditions, a second element of diversity was incorporated. Addition of an excess of hydrazine[12,16] (10 equiv., 1 h, 70 °C) in THF–EtOH (1 : 1) to the resins **6**–**9** afforded the corresponding hydrazone intermediates (*E*/*Z* hydrazone isomers in equilibrium at room temperature) which cyclise according to reported solution-phase procedures[6,7] (Scheme 2). After removal of excess hydrazine by evaporation under reduced pressure (in the case of volatile hydrazines), the desired pyridazinones **10**–**16** were obtained in good yields (Table 1)†. Furthermore, the purity of the crude products was evaluated at 90% after inspection of the ^1H NMR spectra. In the cases of other hydrazines, flash chromatography would be necessary.

HRMS and collected NMR data are in agreement with the proposed structures.

A ^{13}C NMR study was performed on product **14** to ensure the regiospecificity of the Michael addition. The spectrum showed a typical triplet of quartets signal ($^1J_{CH}$ = 29.6 Hz, $^3J_{CH}$ = 3.2 Hz, C-5) at 30 ppm which collapsed to a triplet during a gated decoupling experiment at δ = 2.07 ppm (6-CH_3). The loss of the $^3J_{CH}$ coupling constant from the resonance of C-5 confirmed the proposed structure with the amino group at the C-4 position and not at C-5.

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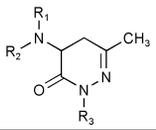
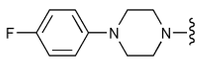
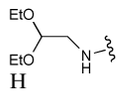
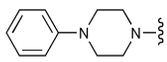
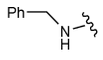
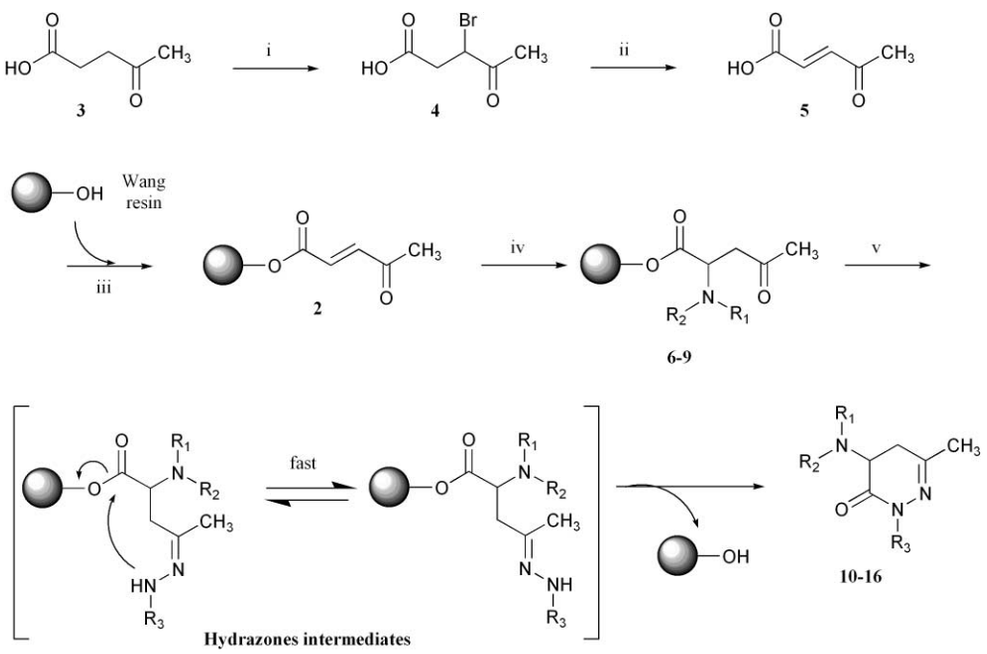
Table 1 Synthesis of compounds **10**–**16**



Product	**10**	**11**	**12**	**13**	**14**	**15**	**16**
							
R_3	H	CH_3	H	CH_3	H	CH_3	CH_3
Yield[a]	73	66	80	70	72	75	70

[a] Isolated overall yield (based upon loading of Wang resin, 0.65 mmol g^{-1}).



Scheme 2 *Reagents and conditions:* (i) Bromine, conc. HCl, −15 °C ⟶ r.t., 5 h; (ii) sodium acetate, glacial acetic acid, r.t. then 100 °C, 45 min; (iii) Wang resin, DIC, DMAP, anhydrous DMF, r.t.; (iv) R_1R_2NH, DMSO, r.t., 1 h; (v) R_3NHNH_2, THF–EtOH (1 : 1), 70 °C, 1 h.

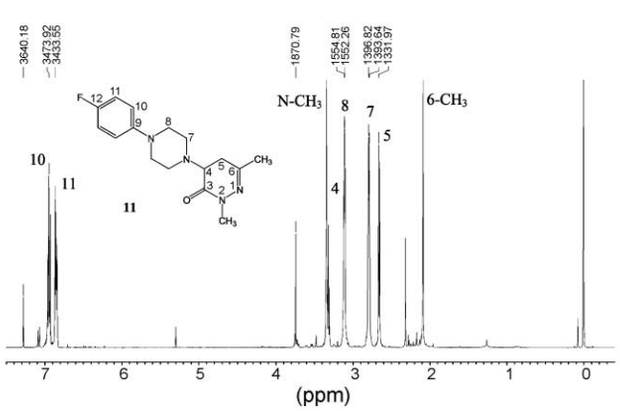
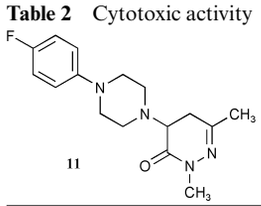


Fig. 1 ^{1}H NMR spectrum of crude product **11** (500 MHz, $CDCl_3$).

Fig. 1 represents the ^{1}H NMR spectrum in $CDCl_3$ of the crude pyridazinone **11**, whose analytical data are given in notes†. The yield and purity levels are representative of the other derivatives **10**–**16** of the series.

Using procedures reported in the literature,[10] we screened diverse pyridazinones previously synthesized,[12] including compound **11**, on murine tumor lines (3LL and L1210 cells) to evaluate their cytotoxic effect‡. Among the investigated derivatives we only report in Table 2 the cytotoxicity of **11** and two 4-unsubstituted analogs (**17** and **18**).

Table 2 Cytotoxic activity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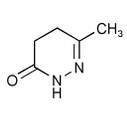
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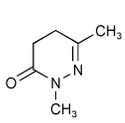
18

	CC_{50}[a]/μM		
Murine tumor lines	**11**	**17**	**18**
3LL	46	> 800	> 800
L1210	39	> 800	> 800

[a] CC_{50}: 50% Cytotoxic Concentration.

From the inspection of these first results, compound **11** with a substituent at the C-4 position on the final ring showed greater cytotoxicity on the two tumor lines.

In summary, we have shown that 4-amino-4,5-dihydropyridazin-3(2*H*)-one derivatives can be produced in good yields and purities from a resin-bound Michael adduct. Diversity is brought in by using various amines (step 2) and hydrazines (step 3) in the ring construction. In order to confirm their presumed efficacy as cytotoxic agents, the successful application of this strategy for the preparation of a large library of 4-amino derivatives implementing the protocol with a robotic system is currently in progress.

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Notes and references

† Typical experimental procedure: A solution of 4-oxopent-2-enoic acid **5** (1.30 mmol) in anhydrous DMF (3 ml) was added to a suspension of Wang resin (1 g) (Sigma-Aldrich, loading of 0.65 mmol g^{-1}) in anhydrous DMF (8 ml) in the presence of a catalytic amount of dimethylaminopyridine (DMAP, 0.1%) and diisopropylcarbodiimide (DIC, 1.30 mmol). After agitating for 18 hours at room temperature, resin **2** was washed successively with DMF (2 × 5 ml), THF (2 × 5 ml), THF–H_2O (1 : 1, 10 ml), H_2O (10 ml), THF (2 × 5 ml), CH_2Cl_2 (2 × 5 ml) and then dried under reduced pressure at 40 °C for 2 hours. FT-IR (ν cm^{-1}): 1730 (CO ester), 1700 (CO ketone). The resin **2** (500 mg, 0.32 mmol) was next shaken with *p*-fluorophenylpiperazine (3.25 mmol) in DMSO (6 ml) for 1 hour at room temperature. Resin **6** was washed successively with DMSO (2 × 5 ml), THF (2 × 5 ml), CH_2Cl_2 (2 × 5 ml) and then dried under reduced pressure at 40 °C for 2 hours. To the resin **7** (500 mg, 0.31 mmol) in THF (4 ml) was added a solution of methylhydrazine (3.25 mmol) in ethanol (4 ml). The resulting mixture was stirred for 1 hour at 70 °C. The polymer was removed by filtration and washed with CH_2Cl_2 (3 × 3 ml). The combined filtrates were concentrated to dryness to give the crude product **11** (89 mg), which was purified on silica gel eluting with CH_2Cl_2–MeOH (90 : 10) as a white solid (65 mg, yield: 66%), mp 122–123 °C. ^{1}H NMR δ (500 MHz; $CDCl_3$) 2.09 (3H, s, 6-CH_3), 2.65 (2H, d, 5-H), 2.79 (4H, t, 7-H), 3.10 (4H, t, 8-H), 3.31 (1H, t, 4-H), 3.34 (3H, s, N-CH_3), 6.86 (2H, m, 11-H), 6.94 (2H, t, 10-H). ^{13}C NMR δ (125 MHz; $CDCl_3$) 23.6 (6-CH_3), 30.4 (C_5), 36.2 (N-CH_3), 49.4 (C_7), 50.5 (C_8), 58.6 (C_4), 115.5 (C_{11}, d, $^2J_{CF}$ = 21.8 Hz), 118.0 (C_{10}, d, $^3J_{CF}$ = 7.3 Hz), 147.9 (C_9), 152.6 (C_6), 157.1 (C_{12}, d, $^1J_{CF}$ = 238.6 Hz), 163.4 (C_3). HRMS [$M^{+\bullet}$] calcd. for $C_{16}H_{21}N_4OF$: *m*/*z* 304.16994. Found: 304.1703.

‡ Tumor cells were incubated with products (25–800 μM) at 37 °C in a humidified atmosphere of 5% CO_2 in air for 48 hours. MTT[17] (1 mg ml^{-1}) in phosphate-buffered saline was added to each well and incubated for a further 4 hours. After centrifugation, the blue formazan crystals produced by viable cells were dissolved in DMSO to allow assay using a microplate spectrophotometer (Multiscan Life-Science) at 540 nm (test) and 690 nm (background). Cytotoxicity was calculated in comparison with control cell growth and the CC_{50} was calculated using the Biolise software.

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