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Preparation of a set of 4,5-dihydro-3*H*-pyrrolo[3,2-*d*]pyrimidin-4-ones as potential Hsp90 ligands

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ABSTRACT

A synthetic route for the preparation of 4,5-dihydro-3*H*-pyrrolo[3,2-*d*]pyrimidin-4-ones characterized by a decorated benzyl moiety at different positions of the five-membered ring has been developed, and some compounds have been tested as Hsp90 ligands. One of them displayed IC_{50} = 50 μM representing an interesting starting point for further investigations.

The 90 kDa heat shock protein (Hsp90) is emerging as an important target for cancer chemotherapy because of its role in chaperoning proteins involved in signal transduction pathways that control tumor cell proliferation.[1–4] Among those disclosed being able to inhibit Hsp90, the purine (PU) class of inhibitors (Fig. 1)[5–9] represents the most investigated one, exhibiting biological activities in the nanomolar range and high levels of selectivity for tumor vs normal cell Hsp90.

Based on these observations and taking advantage of our experience in the synthesis of deazaguanine derivatives,[10] we planned to develop a synthetic route to access 4,5-dihydro-3*H*-pyrrolo[3,2-*d*]pyrimidin-4-ones of general structure **1**, characterized by the presence of a substituted benzyl moiety at different positions of the pyrrole ring, with the aim of exploring the structure–activity relationships for this class of compounds (Fig. 1).

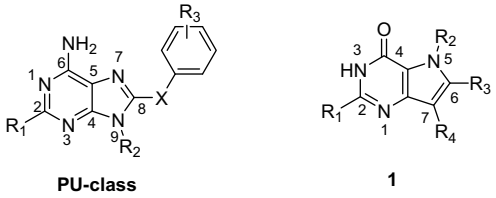
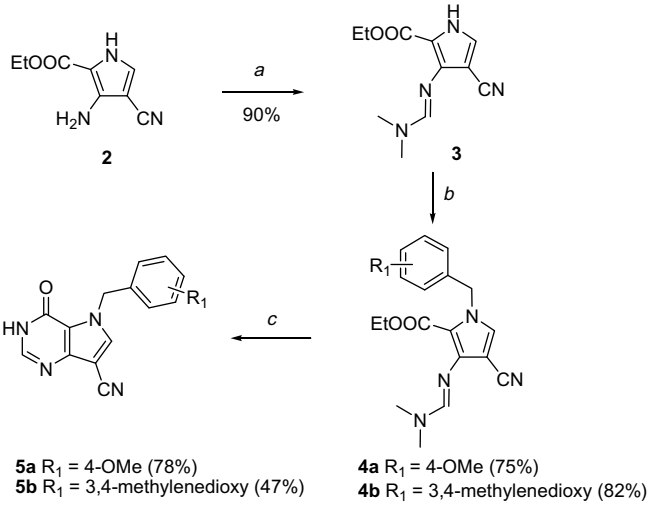


Figure 1. Purine and deazapurine Hsp90 ligands.

Compounds **1a**–**c** were prepared starting from ethyl 3-amino-4-cyano-1*H*-pyrrole-2-carboxylate (**2**) (Scheme 1), easily accessible by condensation of ethoxymethylenemalononitrile and diethyl aminomalonate. Reaction of **2** with DMF dimethyl acetal gave the protected derivative **3**, which was alkylated with the appropriate benzyl halide to give the corresponding benzyl derivatives **4a**,**b**. MW-aided cyclization of **4a**,**b** in the presence of ammonium



Scheme 1. Synthesis of compounds **5a**,**b**. Reagents and conditions: (a) $(MeO)_2CHNMe_2$, DCM, rt, 3 h; (b) benzyl halide, TBACl, NaI, 20% NaOH, DCM, rt, 1 h; (c) NH_4OH, EtOH, THF, MW, 100 °C, 20 min, sealed tube.

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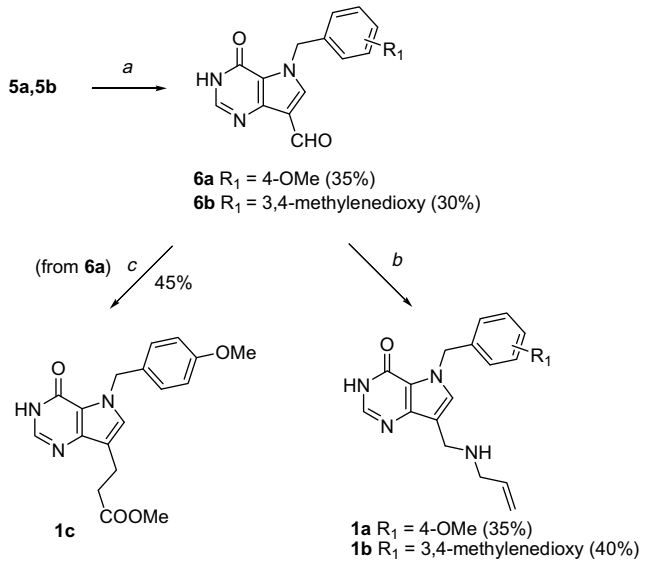


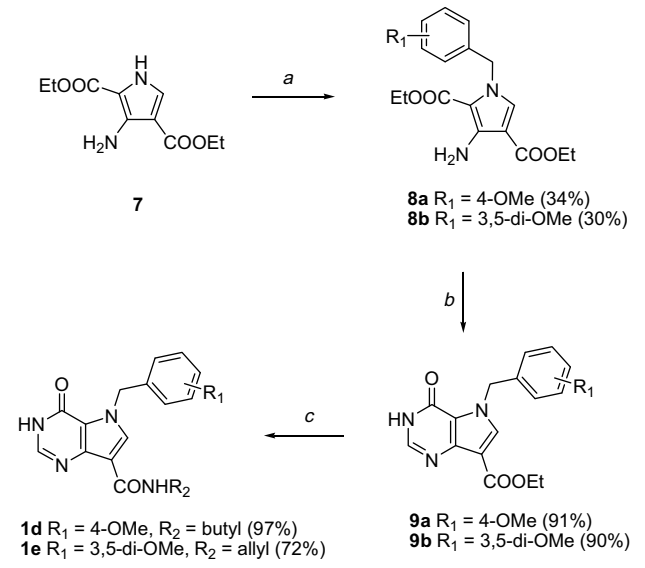
Scheme 2. Synthesis of compounds **1a**–**c**. Reagents and conditions: (a) $LiAlH_4$, THF, rt, 2 h; (b) allylamine, $Na(AcO)_3BH$, AcOH, DCE, rt, 48 h; (c) $Ph_3P{=}CHCOOMe$, MeOH, reflux, 20 h then H_2, Pd/C, EtOAc, rt, 4 h.

hydroxide afforded the N5-benzylated pyrrolo[3,2-*d*]pyrimidines **5a**,**b** functionalized at C7.

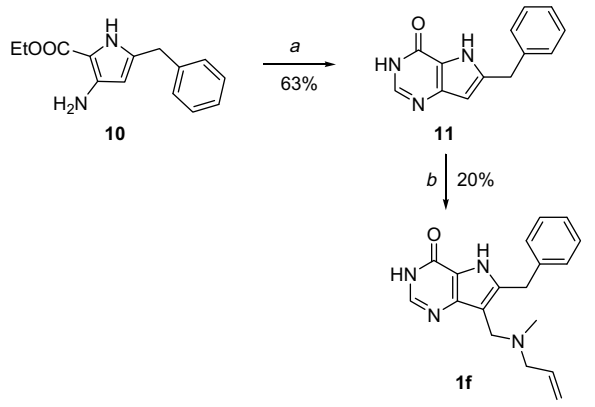
Elaboration of the CN group of **5a**,**b** (Scheme 2) to afford aldehydes **6a**,**b** failed under different experimental conditions using selective reducing agents, such as DIBAL, but was surprisingly accomplished by means of the strong reductant $LiAlH_4$. Subsequent reductive amination of the aldehydes **6a**,**b** gave the final compounds **1a**,**b** in an acceptable overall yield. Compound **1c** was obtained, starting from **6a**, through a Wittig olefination using methyl triphenylphosphoranylideneacetate followed by catalytic hydrogenation of the double bond.

A similar approach was followed for the synthesis of compounds **1d**,**e** which were prepared starting from diethyl 3-amino-1*H*-2,4-pyrroledicarboxylate (**7**) (Scheme 3).

Compound **7** was initially alkylated to give the corresponding benzyl derivatives **8a**,**b** which, in turn, were cyclized in the



Scheme 3. Synthesis of compounds **1d**,**e**. Reagents and conditions: (a) benzyl halide, TBACl, NaI, 20% NaOH, DCM, rt, 1 h; (b) $NH{=}CHNH_2{\cdot}AcOH$, EtOH or 2-methoxyethanol, reflux, 48 h; (c) R_2NH_2, $Ti(OiPr)_4$, reflux, 24 h.

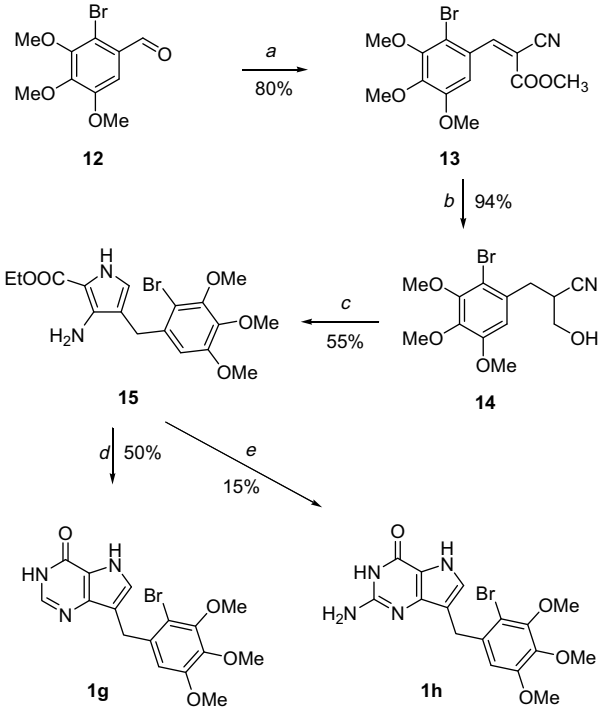


Scheme 4. Synthesis of compound **1f**. Reagents and conditions: (a) $NH{=}CHNH_2$ AcOH, EtOH, reflux, 5 h; (b) *N*-methylallylamine, CH_2O, CH_3COOH, H_2O, 95 °C, 24 h.

presence of formamidine acetate to afford the key intermediates **9a**,**b**. Treatment of **9a**,**b** with the appropriate amine in the presence of $Ti(OiPr)_4$ gave directly the final amides **1d**,**e**.

Compound **1f** was obtained starting from ethyl 3-amino-5-benzyl-1*H*-pyrrole-2-carboxylate (**10**)[11] by cyclization with formamidine acetate and subsequent Mannich reaction with formaldehyde and *N*-methylallylamine of the deazapurine derivative **11** (Scheme 4).

Finally, compounds **1g**,**h** were prepared according to Scheme 5. Aldehyde **12** was condensed with methyl cyanoacetate to give the intermediate **13**. Simultaneous reduction of the double bond and the ester function by means of $NaBH_4$ afforded the alcohol **14**, which was transformed into the pyrrole derivative **15** by oxidation to the corresponding aldehyde, reaction with diethyl aminomalonate, and subsequent cyclization in the presence of NaOMe.



Scheme 5. Synthesis of compounds **1g**,**h**. Reagents and conditions: (a) $CNCH_2COOCH_3$, piperidine, toluene, reflux, 1 h, (b) $NaBH_4$, EtOH, rt, 1 h; (c) (i) $(COCl)_2$, DMSO, DIPEA, DCM, −78 °C, 2 h; (ii) $NH_2CH(COOEt)_2{\cdot}HCl$, CH_3COONa, H_2O, EtOH, rt, 20 h; (iii) NaOMe, MeOH, rt, 3 h; (d) $NH{=}CHNH_2{\cdot}AcOH$, 2-methoxyethanol, reflux, 1 h; (e) 50% NH_2CN, 50% NaOH, rt, 16 h.

Condensation of **15** with formamidine acetate or cyanamide gave the target compounds **1g** and **1h**, respectively.

All the newly synthesized compounds were submitted to a *r*Hsp90 competitive binding assay in order to evaluate their ability to bind Hsp90.[12] Compounds **1a**,**b** and **1d**–**f** showed IC_{50} > 10,000 μM and thus were considered inactive. Among the compounds of the same series only **1c** elicited some activity (IC_{50} = 364 μM), while **1a** and **1d**, although bearing the same 4-OMe benzyl group as **1c**, but differing for the nature and length of the C7 chain, proved to be completely inactive. Interestingly, the activity was restored with compound **1h**[13] endowed with IC_{50} = 50 μM.

In summary, we have described the synthesis of new 4,5-dihydro-3*H*-pyrrolo[3,2-*d*]pyrimidin-4-ones substituted at different positions of the pentatomic ring with benzyl groups.

Some of the new compounds proved to be able to mimic members of the PU class in binding to Hsp90 protein. The synthetic approach described herein will be exploited for the preparation of several families of potential Hsp90 ligands.

Acknowledgments

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11. *Synthesis of compound* **10**. Acetic acid (21 μL, 0.37 mmol), diethyl aminomalonate hydrochloride (184 mg, 0.84 mmol) and sodium acetate (71 mg, 0.87 mmol) were added to a stirred solution of 3-oxo-4-phenylbutyronitrile (200 mg, 1.3 mmol) in dry EtOH (1 mL), and the mixture was stirred for 2 days, after which it was concentrated under vacuum. The residue was partitioned between DCM and water, and the organic phase was dried over Na_2SO_4. Removal of the solvent afforded a syrup that was dissolved in an ethanolic solution of EtONa (0.87 mmol, 1.8 mL); after 3 days of stirring, acetic acid (52 μL) was added and EtOH was removed under vacuum. The residue was dissolved in DCM and washed with $NaHCO_3$. The organic phase was evaporated to dryness. The crude product was purified by flash chromatography (eluent: EtOAc/PE, 2/3) to give **10** as a yellow solid (50% yield); mp 85–86 °C (EtOAc); ^{1}H NMR (200 MHz, $CDCl_3$): δ 7.31–7.15 (m, 5H), 5.48 (s, 1H), 4.24 (q, J = 7.1 Hz, 2H), 3.82 (s, 2H), 1.26 (t, J = 7.1 Hz, 3H); MS m/z 245 $(M+H)^+$; IR (Nujol): ν 3448, 2337, 1673 cm^{-1}. Anal. Calcd for $C_{14}H_{16}N_2O_2$: C, 68.83; H, 6.60; N, 11.47. Found: C, 68.98; H, 6.58; N, 11.46.
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13. *Analytical data for compound* **1h**: white solid; mp 289–290 °C; ^{1}H NMR (200 MHz, Me_2SO-d_6): δ 11.20 (br s, 1H), 10.32 (br s, 1H), 6.86 (s, 1H), 6.82 (s, 1H), 5.79 (s, 2H), 3.81 (s, 2H), 3.74 (s, 3H), 3.71 (s, 3H), 3.66 (s, 3H); MS m/z 411 $(M+H)^+$; IR (Nujol): ν 3423, 3134, 2752, 1671, 1629 cm^{-1}. Anal. Calcd for $C_{16}H_{17}BrN_4O_4$: C, 46.96; H, 4.19; N, 13.69. Found: C, 46.75; H, 4.27; N, 13.91.