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Synthesis and preliminary evaluation of 5,7-dimethyl-2-aryl-3*H*-pyrrolizin-3-ones as angiogenesis inhibitors

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ABSTRACT

Sunitinib (Sutent®) is a receptor tyrosine kinase (RTK) and angiogenesis inhibitor approved for the treatment of renal cell carcinomas, gastrointestinal stromal tumours and pancreatic neuroendocrine tumours. A key structural motif retained throughout medicinal chemistry efforts during sunitinib's development was the indoline-2-one group. In the search for new anti-angiogenic scaffolds, we previously reported that non-indoline-2-one-based derivatives of semaxanib (SU5416, a structurally simpler sunitinib predecessor that underwent Phase III trials) are active as angiogenesis inhibitors, indicating that the group is not essential for activity. This Letter describes the synthesis and structure–activity relationships of another class of non-indoline-2-one angiogenesis inhibitors related to sunitinib/semaxanib; the 5,7-dimethyl-2-aryl-3*H*-pyrrolizin-3-ones. A focussed library of 19 analogues was prepared using a simple novel process, wherein commercially available substituted arylacetic acids activated with an amide coupling reagent (HBTU) were reacted with the potassium salt of 3,5-dimethyl-1*H*-pyrrole-2-carbaldehyde in one-pot. Screening of the library using a cell-based endothelial tube formation assay identified 6 compounds with anti-angiogenesis activity. Two of the compounds were advanced to the more physiologically relevant rat aortic ring assay, where they showed similar inhibitory effects to semaxanib at 10 μg/mL, confirming that 5,7-dimethyl-2-aryl-3*H*-pyrrolizin-3-ones represent a new class of angiogenesis inhibitors.

Sunitinib **1** (Sutent®, Pfizer, Fig. 1)[1] is an indolin-2-one-based angiogenesis inhibitor approved for the treatment of vascularised renal cell carcinomas,[2] gastrointestinal stromal tumours[3] and pancreatic neuroendocrine tumours.[4] Its mechanism of action involves inhibition of at least eight different receptor tyrosine kinases (RTKs), including vascular endothelial growth factor receptors 1–3 (VEGFR1–VEGFR3), platelet-derived growth factor receptors (PDGFRs) α and β, stem cell factor receptor (Kit), Fms-like tyrosine kinase 3 (FLT-3) and colony-stimulating factor-1 receptor (CSF-1R).[5] The indolin-2-one (oxindole) portion was considered crucial for activity of sunitinib **1** and was retained throughout development. Indeed, the structurally simpler indolin-2-one predecessor semaxanib (SU5416) **2** underwent a Phase III clinical trial for advanced colorectal cancer.[6] In recent work, we showed that in spite of its perceived importance the indolin-2-one moiety is not essential for anti-angiogenic activity in this class, demonstrating that ring-opened 3,5-dimethyl-1*H*-pyrrol-2-yl-2-arylacrylate **3** variants of semaxanib **2** inhibit angiogenesis in a rat aortic ring model.[7] We now report the discovery of a second class of non-indolin-2-one-based angiogenesis inhibitors related to sunitinib **1**/semaxanib **2**; the 5,7-dimethyl-2-aryl-3*H*-pyrrolizin-3-ones **4** (Fig. 1).

Abbreviations: HBTU, 2-(1*H*-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate; CSF-1R, colony-stimulating factor-1 receptor; DIPEA, *N*,*N*-diisopropylethylamine; FLT-3, Fms-like tyrosine kinase 3; FOV, field of view; HUVEC, human umbilical vein endothelial cell; PDGFR, platelet-derived growth factor receptor; RTK, receptor tyrosine kinase; THF, tetrahydrofuran; VEGFR, vascular endothelial growth factor receptor.

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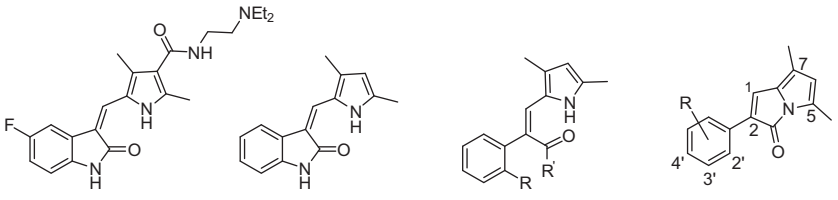


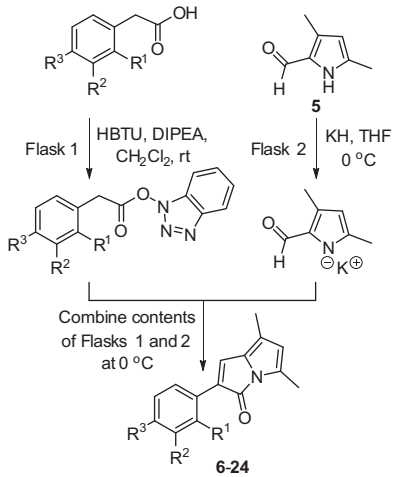
Figure 1. Angiogenesis inhibitors related to sunitinib **1**.

We hypothesised that 5,7-dimethyl-2-aryl-3*H*-pyrrolizin-3-ones might possess anti-angiogenic properties due to their structural similarity to sunitinib **1**, SU5416 **2** and acrylates **3**. The hypothesis was tested by developing new chemistry to create a small library of analogues carrying systematic variations at the 2-aryl ring and investigating their structure–activity relationships (SAR). Sporadic reports of 3*H*-pyrrolizin-3-ones have appeared in the literature but little remains known about their chemistry and biological activity.[8–13] Complex structures incorporating a 3*H*-pyrrolizin-3-one-type core have been reported, including tricyclic pyrrolo-indolones, pyrrolo-isoindolones, tetracyclic isoindolo-indolones and other polycyclic heterocycles.[14,15] A number of these are marine natural products or their derivatives.[16]

Divergent access to 5,7-dimethyl-2-aryl-3*H*-pyrrolizin-3-ones appeared achievable in one-pot from 3,5-dimethyl-1*H*-pyrrole-2-carbaldehyde **5** and the many commercially available substituted phenylacetic acids. We had previously shown that the K^+ salt of **5** (generated using KH in THF) is smoothly *N*-acylated by methyl chloroformate,[7] leading us to consider that the same salt might also undergo N-acylation reactions with phenylacetic acids pre-activated as acid halides or with amide coupling reagents. A simple, divergent synthesis was envisaged wherein activated phenylacetic acids generated in one flask are combined with the K^+ salt of **5**, which had been freshly prepared in a separate flask. Mixing of the flasks would initiate pyrrole N-acylation/amide formation and ensuing benzylic proton abstraction under the basic reaction conditions (excess KH) would trigger an intramolecular Knoevenagel condensation to deliver 5,7-dimethyl-2-aryl-3*H*-pyrrolizin-3-ones.

Preliminary synthetic efforts surveying a variety of amide coupling reagents, procedures and rates and orders of addition in model reactions with phenylacetic acid and pyrrole aldehyde **5** yielded 5,7-dimethyl-2-phenyl-3*H*-pyrrolizin-3-one **6** as the major product in many instances, as observed by TLC analysis. The highest yield of **6** (48%, Scheme 1) was obtained when 1.1 mol equiv of amide coupling reagent 2-(1*H*-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU) and 2.0 equiv of *N*,*N*-diisopropylethylamine (DIPEA) were stirred in CH_2Cl_2 at room temperature with 1.0 equiv of phenylacetic acid to form the activated hydroxybenxzotriazoyl ester in one flask (Flask 1) and 2.0 mol equiv of KH was stirred in THF at 0 °C with 1.0 equiv of aldehyde **5** in a separate flask (Flask 2) to generate the K^+ pyrrolate salt. Pre-cooling Flask 1 to 0 °C and pouring its contents into Flask 2 at 0 °C in one portion completed the procedure (Scheme 1).

Employing the method with a systematic series of mono-substituted phenylacetic acid derivatives carrying F, Cl, Br, Me, OCH_3 and NO_2 groups at each of the 2′-, 3′- and 4′-positions similarly provided analogues **7**–**24** (Scheme 1). While the yields were low to moderate (4–48%) the simple procedure allowed rapid access to useable quantities of pure analogues for the SAR study. ^1H and ^{13}C NMR data for all compounds were unambiguous and consistent with 2-aryl-3*H*-pyrrolizin-3-ones. A single crystal X-ray structure obtained for 2′-NO_2 derivative **22** further confirmed its structure



R^1	R^2	R^3	Compound	Yield %
H	H	H	**6**	48
F	H	H	**7**	37
H	F	H	**8**	37
H	H	F	**9**	38
Cl	H	H	**10**	16
H	Cl	H	**11**	35
H	H	Cl	**12**	30
Br	H	H	**13**	13
H	Br	H	**14**	44
H	H	Br	**15**	34
Me	H	H	**16**	21
H	Me	H	**17**	41
H	H	Me	**18**	37
OMe	H	H	**19**	33
H	OMe	H	**20**	45
H	H	OMe	**21**	30
NO_2	H	H	**22**	28
H	NO_2	H	**23**	15
H	H	NO_2	**24**	4

Scheme 1. Divergent one-pot synthesis of 5,7-dimethyl-2-aryl-3*H*-pyrrolizin-3-ones **6**–**24**.

(Fig. 2). The X-ray structure revealed that the 2′-nitrophenyl ring was tilted 35.5° relative to the plane of the 3*H*-pyrrolizin-3-one ring system and positioned the electron-deficient NO_2 nitrogen in close proximity to the electronegative carbonyl oxygen (2.8 Å), suggesting a favourable interaction between these two atoms.

Compounds **6**–**24** were screened for in vitro angiogenesis inhibitory activity at 10 μM using the human umbilical vein endothelial cell (HUVEC)-based endothelial tube formation assay.[17] Matrix-cultured HUVECs differentiate in response to growth factors, becoming elongated and motile and able to self-organise into capillary-like structures over a 2–12 h period. This process is disrupted in a dose-dependent manner by small molecule inhibitors of angiogenesis.[18] Quantification of angiogenesis and the inhibitory effects