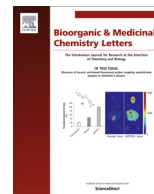




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## Synthesis and preliminary evaluation of 5,7-dimethyl-2-aryl-3H-pyrrolizin-3-ones as angiogenesis inhibitors

Nicholas S. Kirk<sup>a</sup>, Anna Bezos<sup>b</sup>, Anthony C. Willis<sup>c</sup>, Pichit Sudta<sup>d,e</sup>, Sunit Suksamrarn<sup>e</sup>, Christopher R. Parish<sup>b</sup>, Marie Ranson<sup>f</sup>, Michael J. Kelso<sup>a,\*</sup>

<sup>a</sup> Illawarra Health and Medical Research Institute, School of Chemistry, University of Wollongong, NSW 2522, Australia

<sup>b</sup> Cancer and Vascular Biology Group, Department of Cancer Biology and Therapeutics, John Curtin School of Medical Research, Australian National University, Canberra ACT 2601, Australia

<sup>c</sup> Single Crystal X-ray Diffraction Unit, Research School of Chemistry, Australian National University, Canberra ACT 2601, Australia

<sup>d</sup> Division of Chemistry, Phetchaburi Rajabhat University, Phetchaburi 76000, Thailand

<sup>e</sup> Department of Chemistry, Srinakharinwirot University, Bangkok 10110, Thailand

<sup>f</sup> Illawarra Health and Medical Research Institute, School of Biological Sciences, University of Wollongong, NSW 2522, Australia

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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-3H-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-1H-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-3H-pyrrolizin-3-ones represent a new class of angiogenesis inhibitors.

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Sunitinib **1** (Sutent®, Pfizer, Fig. 1)<sup>1</sup> is an indolin-2-one-based angiogenesis inhibitor approved for the treatment of vascularised renal cell carcinomas,<sup>2</sup> gastrointestinal stromal tumours<sup>3</sup> and pancreatic neuroendocrine tumours.<sup>4</sup> 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)  $\alpha$  and  $\beta$ , stem cell factor receptor (Kit), Fms-like tyrosine kinase 3 (FLT-3) and colony-stimulating factor-1 receptor (CSF-1R).<sup>5</sup> 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.<sup>6</sup> 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-1H-pyrrol-2-yl-2-arylacrylate **3** variants of semaxanib **2** inhibit angiogenesis in a rat aortic ring model.<sup>7</sup> 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-3H-pyrrolizin-3-ones **4** (Fig. 1).

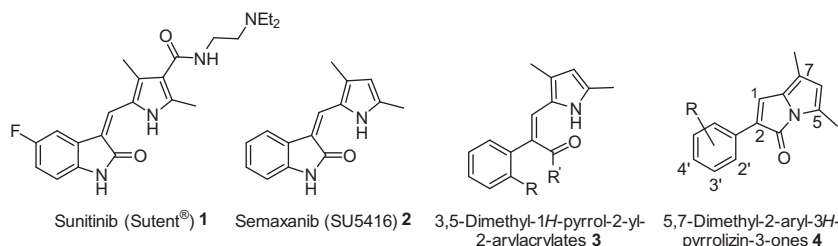
**Abbreviations:** HBTU, 2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate; CSF-1R, colony-stimulating factor-1 receptor; DIPEA, *N,N*-diisopropylethylamine; FLT-3, Fms-like tyrosine kinase 3; FOV, field of view; HUVEC, human umbilical vein endothelial cell; PDGFR, platelet-derived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

\* Corresponding author. Tel.: +61 2 4221 5085; fax: +61 2 4221 4287.

E-mail address: [mkelso@uow.edu.au](mailto:mkelso@uow.edu.au) (M.J. Kelso).

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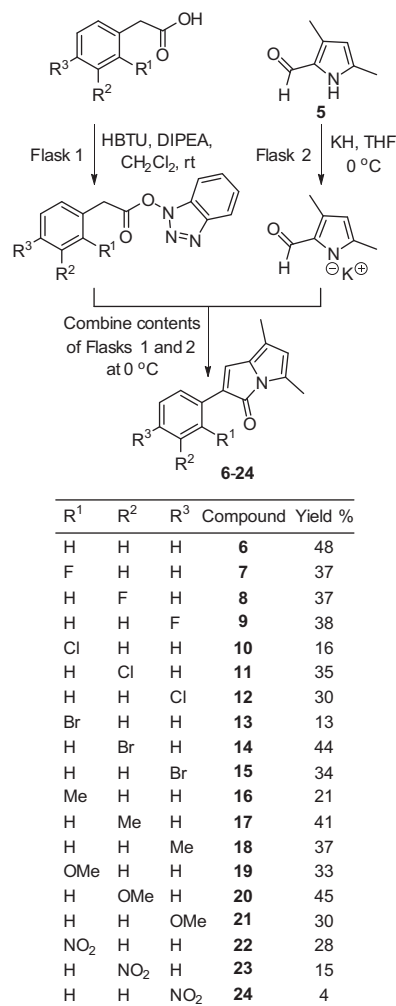
**Figure 1.** Angiogenesis inhibitors related to sunitinib **1**.

We hypothesised that 5,7-dimethyl-2-aryl-3H-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 3H-pyrrolizin-3-ones have appeared in the literature but little remains known about their chemistry and biological activity.<sup>8–13</sup> Complex structures incorporating a 3H-pyrrolizin-3-one-type core have been reported, including tricyclic pyrrolo-indolones, pyrrolo-isindolones, tetracyclic isindolo-indolones and other polycyclic heterocycles.<sup>14,15</sup> A number of these are marine natural products or their derivatives.<sup>16</sup>

Divergent access to 5,7-dimethyl-2-aryl-3H-pyrrolizin-3-ones appeared achievable in one-pot from 3,5-dimethyl-1H-pyrrole-2-carbaldehyde **5** and the many commercially available substituted phenylacetic acids. We had previously shown that the K<sup>+</sup> salt of **5** (generated using KH in THF) is smoothly N-acylated by methyl chloroformate,<sup>7</sup> 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<sup>+</sup> 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-3H-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-3H-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-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU) and 2.0 equiv of *N,N*-diisopropylethylamine (DIPEA) were stirred in CH<sub>2</sub>Cl<sub>2</sub> at room temperature with 1.0 equiv of phenylacetic acid to form the activated hydroxybenzotriazol-yl 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<sup>+</sup> 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).

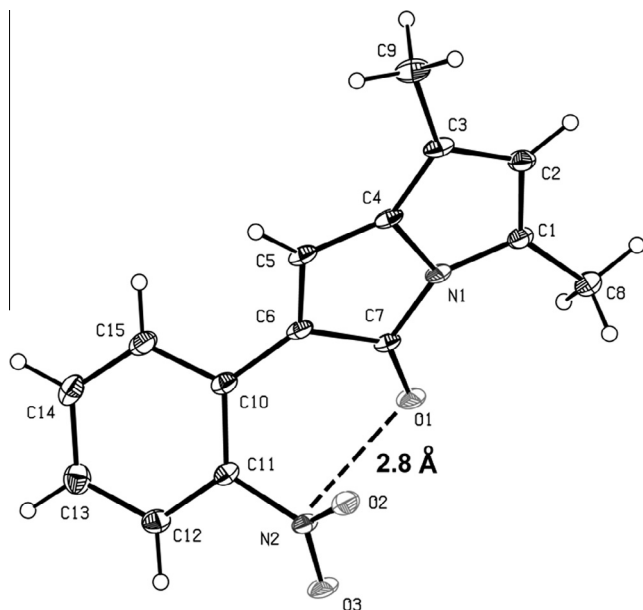
Employing the method with a systematic series of mono-substituted phenylacetic acid derivatives carrying F, Cl, Br, Me, OCH<sub>3</sub> and NO<sub>2</sub> groups at each of the 2′-, 3′- and 4′-positions similarly provided analogues **7–24** (Scheme 1). While the yields were low to moderate (4–48%) the simple procedure allowed rapid access to useable quantities of pure analogues for the SAR study. <sup>1</sup>H and <sup>13</sup>C NMR data for all compounds were unambiguous and consistent with 2-aryl-3H-pyrrolizin-3-ones. A single crystal X-ray structure obtained for 2′-NO<sub>2</sub> derivative **22** further confirmed its structure



**Scheme 1.** Divergent one-pot synthesis of 5,7-dimethyl-2-aryl-3H-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 3H-pyrrolizin-3-one ring system and positioned the electron-deficient NO<sub>2</sub> nitrogen in close proximity to the electronegative carbonyl oxygen (2.8 Å), suggesting a favourable interaction between these two atoms.

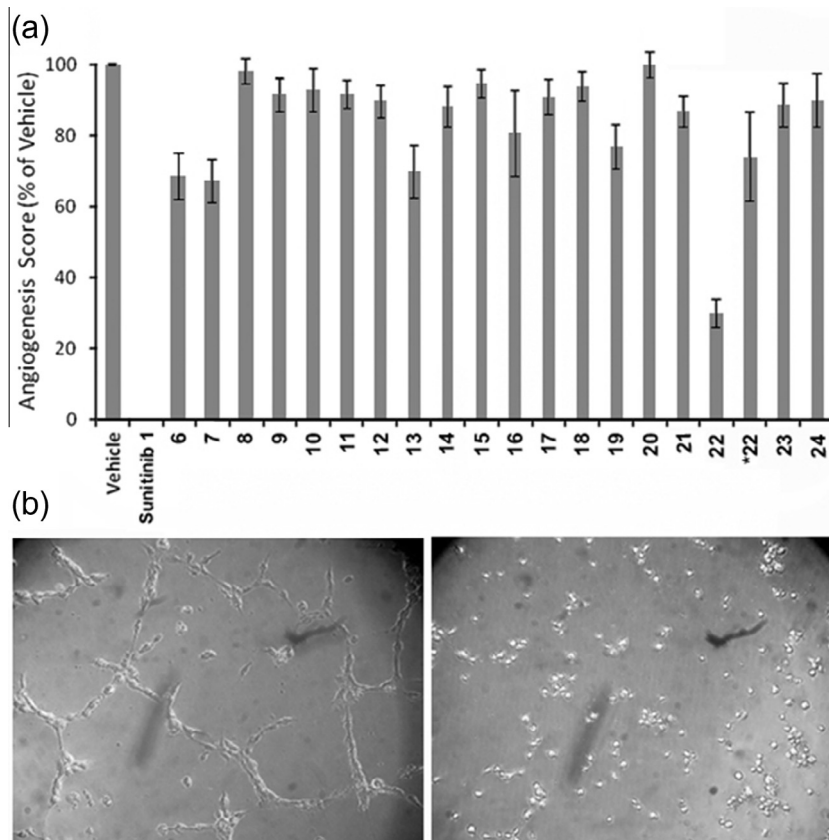
Compounds **6–24** were screened for in vitro angiogenesis inhibitory activity at 10 μM using the human umbilical vein endothelial cell (HUVEC)-based endothelial tube formation assay.<sup>17</sup> Matrix-cultured HUVECs differentiate in response to growth factors, becoming elongated and motile and able 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.<sup>18</sup> Quantification of angiogenesis and the inhibitory effects



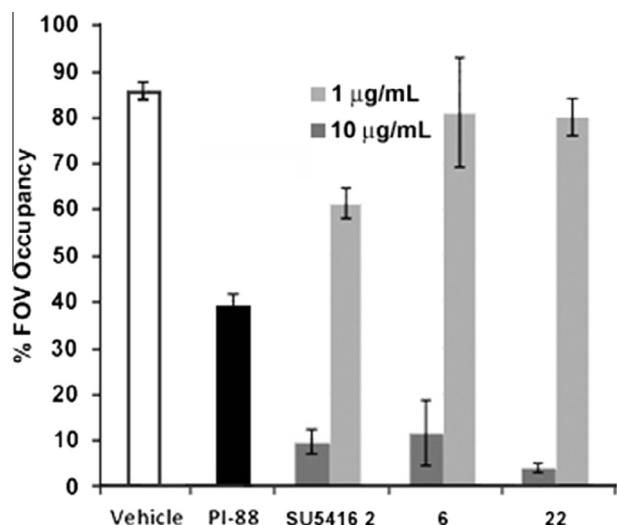
**Figure 2.** Single crystal X-ray structure (ORTEP) of **22**. Anisotropic displacement ellipsoids represent 30% probability levels. Hydrogen atoms are drawn as circles with small radii. Crystallographic data are provided in the [Supporting information](#). Atomic coordinates, bond lengths and angles and displacement parameters have been deposited at the Cambridge Crystallographic Data Centre (CCDC accession number 1439414).

of compounds uses the method of Aranda.<sup>17</sup> Briefly, images are captured and the cells tallied and categorised as ellipsoid (undifferentiated), sprouting (differentiating) or connected to other cells ([Supporting information, Fig. S1](#)). Total numbers of closed polyhedra are also counted, along with 'complex meshes' or polyhedral containing walls more than 2 cells thick. Input of the data into an equation ([Supporting information, Eq. S1](#)) generates an angiogenesis score, which is then expressed as a percentage relative to the angiogenesis score of the vehicle control (100% angiogenesis). Reductions in scores from 100% in the presence of added compounds correspond to angiogenesis inhibition.

[Figure 3a](#) shows that 2'-NO<sub>2</sub> analogue **22** was the most potent inhibitor in the series, reducing angiogenesis to 74.0% ± 12.6 of vehicle at 1 μM and 29.7% ± 4.1 at 10 μM. Sunitinib **1** completely inhibited angiogenesis at 10 μM. The 3'-NO<sub>2</sub> **23** and 4'-NO<sub>2</sub> **24** analogues showed almost no activity. Indeed all 3' (i.e., **8**, **11**, **14**, **17**, **20**) and 4' (i.e., **9**, **12**, **15**, **18**)-substituted compounds failed to show notable activity, confirming that these positions do not tolerate substitution. The unsubstituted derivative **6** showed modest activity (68.4% ± 6.6 of vehicle) and similar activities were observed with the 2'-fluoro **7**, 2'-bromo **13**, 2'-methyl **16** and 2'-methoxy **19** analogues. 2'-Chloro derivative **10** showed no activity. Compound **22** was shown to be 4-fold less toxic than sunitinib **1** towards related SVEC4-10 murine endothelial cells ([Supporting information, Fig. S2](#)), consistent with general cytotoxicity not being responsible for the observed anti-angiogenic effects. We conclude that while the 3'- and 4'-positions are intolerant of substitution, 2'-substituents are tolerated to some extent and in some cases (e.g., NO<sub>2</sub> group) they can increase activity. This is in agreement



**Figure 3.** (a) Inhibition of angiogenesis by 5,7-dimethyl-2-aryl-3H-pyrrolizin-3-ones **6–24** in HUVEC endothelial tube formation assay. Angiogenesis scores represent percentages (±SEM) relative to the angiogenesis score of the vehicle (DMSO) control (100% angiogenesis). All compounds were present at 10 μM. An additional 1 μM data point is shown for the most potent inhibitor **22**. (b) Representative images showing greater angiogenesis inhibition in the presence of 2'-NO<sub>2</sub> analogue **22** (right) relative to 2'-fluoro **7** (left, both compounds present at 10 μM).



**Figure 4.** Inhibition of angiogenesis by **6** and **22** in rat aortic ring assay. Data for positive controls PI-88 (100 µg/mL) and SU5416 **2** and negative control (DMSO vehicle, no compound) are shown for comparison. Data represent the mean % FOV occupancy ( $\pm$ SEM) generated from at least six replicate cultures of each compound.

with structure–activity conclusions from our previous work showing that *ortho*-nitro groups contribute favourably to anti-angiogenesis activity in the 3,5-dimethyl-1*H*-pyrrol-2-yl-2-arylacrylate series (Fig. 1, compound **3** R = NO<sub>2</sub>).<sup>7</sup>

Compounds **6** and **22** were examined next in a rat aortic ring assay to confirm their anti-angiogenic properties in a more physiologically relevant context. In this model, 1 mm rings sectioned from female rat aortas are suspended in a fibrin gel matrix and exposed to growth media. The aortic sections sprout new vessels over 5 days, which are scored manually as the % field of view (FOV) occupied (by vessels) under a light microscope (40X).<sup>7,19</sup> Inhibitors of angiogenesis produce a quantifiable reduction in % FOV occupancy (see [Supporting information](#) for further details). The vehicle control (DMSO, no compound) showed 86% FOV occupancy under the assay conditions (Fig. 4). Two positive control compounds PI-88 and SU5416 **2** were included in the study. PI-88 is a highly sulfated oligosaccharide-based angiogenesis inhibitor undergoing Phase III clinical trials as a treatment following cancer resection in patients with hepatitis virus-related hepatocellular carcinoma.<sup>20</sup> At 100 µg/mL PI-88 reduced % FOV occupancy to 39%. SU5416 **2** (synthesised in-house using the literature method)<sup>21</sup> reduced % FOV occupancy to 61% at 1 µg/mL and 10% at 10 µg/mL. Compound **22** produced no reduction in % FOV occupancy at 1 µg/mL but showed a marked effect at 10 µg/mL (4% FOV occupancy). Compound **6**, which showed lower potency than **22** in the endothelial tube formation assay, showed reductions in % FOV occupancy only slightly less than **22** at 10 µg/mL (Fig. 4).

In summary, a divergent one-pot synthesis of 5,7-dimethyl-2-aryl-3*H*-pyrrolizin-3-ones was developed using commercially available mono-substituted phenylacetic acids and pyrrole aldehyde **5**. Usable quantities of all analogues for the SAR study were obtained but scope remains for improving the yields. Nineteen analogues were synthesised and screened for anti-angiogenesis activity in an endothelial tube formation assay. The 2'-NO<sub>2</sub> analogue **22** was identified as the most potent inhibitor and the unsubstituted parent compound **6** and 2'-fluoro **7**, 2'-bromo **13**, 2'-methyl **16** and 2'-methoxy **19** derivatives showed activity. In contrast, all 3'- and 4'-substituted analogues were inactive. Com-

pounds **6** and **22** were advanced to a rat aortic ring angiogenesis assay to corroborate their anti-angiogenic properties in a more physiologically relevant context. Both compounds showed robust inhibitory effects at 10 µg/mL, confirming 5,7-dimethyl-2-aryl-3*H*-pyrrolizin-3-ones as a new class of angiogenesis inhibitors. Further structure–activity studies aimed at identifying more potent inhibitors are warranted. Key targets to pursue would include sunitinib-like analogues, where the basic *N,N*-diethylethylenediamine side chain amide of sunitinib is appended at the 6-position of 5,7-dimethyl-2-aryl-3*H*-pyrrolizin-3-ones. Profiling the class for activity against a panel of RTKs should provide insights into the mechanism of action of these compounds.

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## Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.bmcl.2016.02.033>.

## References and notes

- O'Farrell, A.-M.; Abrams, T. J.; Yuen, H. A.; Ngai, T. J.; Louie, S. G.; Yee, K. W. H.; Wong, L. M.; Hong, W.; Lee, L. B.; Town, A.; Smolich, B. D.; Manning, W. C.; Murray, L. J.; Heinrich, M. C.; Cherrington, J. M. *Blood* **2003**, *101*, 3597.
- Wood, L. *Expert Opin. Pharmacother.* **2012**, *13*, 1323.
- Koshenkov, V. P.; Rodgers, S. E. *Curr. Opin. Oncol.* **2012**, *24*, 414.
- Zhou, C.; Zhang, J.; Zheng, Y.; Zhu, Z. *Int. J. Cancer* **2012**, *131*, 1013.
- Roskoski, R., Jr. *Biochem. Biophys. Res. Commun.* **2007**, *356*, 323.
- Prakash, C. R.; Raja, S. *Mini-Rev. Med. Chem.* **2012**, *12*, 98.
- Sudta, P.; Kirk, N.; Bezos, A.; Gurlica, A.; Mitchell, R.; Weber, T.; Willis, A. C.; Prabpai, S.; Kongsaree, P.; Parish, C. R.; Suksamrarn, S.; Kelso, M. J. *Aust. J. Chem.* **2013**, *66*, 864.
- Despinoy, X. L. M.; McNab, H. *Org. Biomol. Chem.* **2009**, *7*, 4502.
- Pinho, T. M. V. D.; Soares, M. I. L.; Paixa, A. J. *Org. Chem.* **2005**, *70*, 6629.
- McNab, H.; Thornley, C. J. *Chem. Soc. Chem. Commun.* **1993**, 1570.
- Clark, B. A. J.; Despinoy, X. L. M.; McNab, H.; Sommerville, C. C.; Stevenson, E. J. *Chem. Soc., Perkin Trans. 1* **1999**, 2049.
- McNab, H.; Thornley, C. J. *Chem. Soc., Perkin Trans. 1* **2000**, 3584.
- McNab, H.; Montgomery, J.; Parsons, S.; Tredgett, D. G. *Org. Biomol. Chem.* **2010**, *8*, 4383.
- Morriello, G. J.; DeVita, R. J.; Mills, S. G.; Young, J. R.; Lin, P.; Doss, G.; Chicchi, G. G.; DeMartino, J.; Kurtz, M. M.; Tsao, K.-L. C.; Carlson, E.; Townson, K.; Wheeldon, A.; Boyce, S.; Collinson, Neil.; Rupniak, N.; Moore, S. *Bioorg. Med. Chem.* **2008**, *16*, 2156.
- Morriello, G. J.; Mills, S. G.; Johnson, T.; Reibarkh, M.; Chicchi, G.; DeMartino, J.; Kurtz, M.; Davies, P.; Tsao, K.-L. C.; Zheng, S.; Tong, X.; Carlson, E.; Townson, K.; Tattersall, F. D.; Wheeldon, A.; Boyce, S.; Collinson, N.; Rupniak, N.; Moore, S.; DeVita, R. J. *Bioorg. Med. Chem. Lett.* **2007**, *2010*, 20.
- Park, Y. C.; Gunasekera, S. P.; Lopez, J. V.; McCarthy, P. J.; Wright, A. E. *J. Nat. Prod.* **2006**, *69*, 580.
- Aranda, E.; Owen, G. I. *Biol. Res.* **2009**, *42*, 377.
- Invitrogen. Endothelial Tube Formation Assay (In Vitro Angiogenesis). [http://tools.lifetechnologies.com/content/sfs/manuals/Endothelial\\_Cells\\_Tube\\_Formation.pdf](http://tools.lifetechnologies.com/content/sfs/manuals/Endothelial_Cells_Tube_Formation.pdf).
- Brown, K. J.; Maynes, S. F.; Bezos, A.; Maguire, D. J.; Ford, M. D.; Parish, C. R. *Lab. Invest.* **1996**, *75*, 539.
- Clinicaltrials.gov at <http://clinicaltrials.gov/show/NCT01402908>.
- Sun, L.; Tran, N.; Tang, F.; App, H.; Hirth, P.; McMahon, G.; Tang, C. J. *Med. Chem.* **1998**, *41*, 2588.