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SCIENCE DIRECT
Bioorganic & Medicinal Chemistry Letters
Bioorganic & Medicinal Chemistry Letters 16 (2006) 3998–4001

Discovery of protein–protein binding disruptors using multi-component condensations small molecules

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Received 28 March 2006; revised 21 April 2006; accepted 4 May 2006
Available online 9 June 2006

Abstract—A series of small molecule compounds interfering with the binding process of VEGF and NRP1 has been identified and further optimized. Full synthetic details as well as SAR are reported which demonstrate that expeditious MCC-based syntheses may lead to valuable molecules addressing challenging targets such as protein–protein interactions. Preliminary functional assay data confirm that these compounds may be further developed toward drug candidates.

Vascular endothelial growth factor (VEGF) has been described as a cytokine triggering one of the major pathways controlling angiogenesis. This growth factor, which is expressed as two major isoforms, VEGF165 and VEGF121, binds several tyrosine kinase receptors like VEGFR2/KDR or FLT1. Neuropilin-1 (NRP1), a non-tyrosine kinase receptor, has been recently identified as a VEGF165 co-receptor. Coexpression of NRP1 and VEGFR2 in endothelial cells (EC) promotes VEGF165 binding to VEGFR2 leading to EC migration and proliferation, compared with EC expressing VEGFR2 alone.[1] NRP1 has also been associated with neuronal guidance[2] and epithelial cell migration. In a prior study,[3] NRP1 has been validated as a target of interest while addressing prostate cancer, showing that this co-receptor is involved in migration and proliferation of tumor cell lines. Prevention of VEGF–NRP1 hetero-dimerization by a small molecule appeared therefore to us as both a promising and a challenging task of relevance in the pursuit of novel prostate cancer treatments.[4]

Several examples of protein–protein binding disruption by small molecules have already been reported proving that this approach constitutes a viable therapeutic strategy. Nutlins and 1,4-benzodiazepine-2,5-diones for example have been proposed to inhibit the p53–HDM2 interaction which has also proved to be of pharmaceutical relevance while developing new cancer cures.[5] In a similar approach, the Bcl-XL hetero-dimer has also been reported as a target for small molecule disruptors.[6,7]

Multi-component condensations (MCCs) are among the most useful chemical reactions known in the context of drug discovery. Because they combine two important attributes of synthetic efficiency, convergence and atom economy, they were naturally recognized and used in combinatorial chemistry.[8] One aspect of MCCs that have been overlooked in the past is their capacity to create structurally complex molecules in a single chemical step.[9] This property is of obvious interest in the context of protein–protein interactions, where structurally elaborate natural products have a demonstrated track-record.

In this report are described for the first time to our knowledge the use of MCCs for the discovery of small molecule antagonists of a protein–protein interaction between VEGF and NRP1, and the identification of potent and functional antagonists.

To assess the potential of organics to disrupt the interaction between VEGF and NRP1, an original ELISA-based binding assay was developed. This assay is using a luminescent mode of detection supported by a VEGF specific antibody–enzyme (HRP) conjugate and recombinant NRP1.[10] The screening was performed with a

Keywords: VEGF-NRP1; Protein–protein interaction; Multi-component condensations; 3-Amino-1-cyano-indolizines.
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doi:10.1016/j.bmcl.2006.05.014

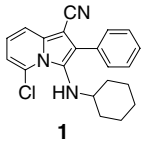

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Figure 1. 5-Chloro-3-cyclohexylamino-2-phenyl-indolizine-1-carbonitrile.

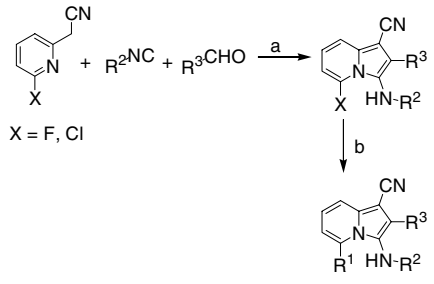
collection of approximately 20,000 small molecules selected for their structural relevance and diversity: 11,800 compounds purchased from dedicated suppliers and 8160 compounds prepared in-house using 22 different, often proprietary, MCCs.

While none of the commercially available compounds inhibited significantly the VEGF–NRP1 interaction, several compounds clustered on three different MCC-based chemotypes showed some activity. Among those chemotypes, 3-amino-1-cyano-indolizines were the most potent antagonists. A prototypical indolizine, 5-chloro-3-cyclohexylamino-2-phenyl-indolizine-1-carbonitrile (**1**) (Fig. 1), showed an IC_{50} of 29 μM in our ELISA assay.

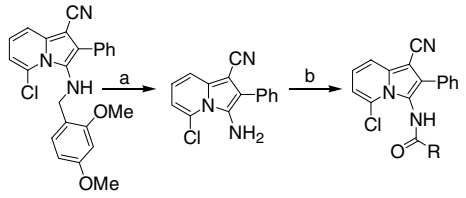
3-Amino-1-cyano-indolizines were easily prepared using a recent three-component condensation discovered in our laboratory (Scheme 1, step a).[11] Isocyanides underwent condensation with aldehydes and cyano-2-methylene-pyridines, in the presence of a catalytic amount of base, to give 3-amino-1-cyano-indolizines in fair to good yields. The mechanism is thought to proceed through the cheletropic addition of the isocyanide moiety to the Knoevenagel-type hetero-diene intermediate.[12]

A great benefit of MCC is that each substituent is addressable in the course of the reaction, greatly facilitating the lead optimization phase. In the case of 3-amino-1-cyano-indolizines, the C-5 position of the indolizine ring could also be varied easily by conducting a subsequent S_NAr substitution of the fluoro group on the compound **2** (Scheme 1, step b).

As shown in Scheme 2, removal of the 2,4-dimethoxybenzyl group in **11** with TFA afforded primary amine **9**, followed by N-acylation with benzoyl chloride and acetyl chloride, giving the N-acylated compounds **12** and **13**, respectively.



Scheme 1. Reagent and conditions: (a) 10% DBU, *n*-butanol, rt then 100 °C, 16 h; (b) methyl thioglycolate for **3**, mercaptoacetic acid for **4**, methyl mercaptan for **5**, TEA, DMF, 70 °C, 16 h; 3-dimethylamino-1-propanol for **6**, NaH, DMF, 70 °C, 16 h; morpholine for **7**, unsymdimethylethylenediamine for **8**, DMF, 70 °C, 16 h.

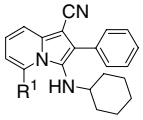


Scheme 2. Reagent and conditions: (a) TFA, DCM, 1 h; (b) BzCl for **12**, AcCl for **13**, TEA, DCM, 16 h.

An optimization program was therefore designed from **1** in which the three substitutable positions, R^1 to R^3, were sequentially and orthogonally varied. Despite several variations at the R^1 position, no improvement over the chloro group was found and this substituent was therefore considered to be optimum and conserved for the next round of optimization (Table 1). Whereas chloro derivatives seem to be associated with the strongest inhibitory activities, it should be noted that significant activities remain by changing it for other substituents, ruling out consequently that activity is mediated by the alkylation of chloro-indolizine through a S_NAr mechanism.

R^2 optimization was conducted by running MCCs using an extended set of isocyanides of either commercial origin or custom-made according to published procedures (Table 2).[13] Great variations among different substituents were observed for this position. While aromatic moieties seemed to be better tolerated, a two-carbon aliphatic linker was preferred to maintain the higher inhibitory activities (compounds **23**–**25**). However, acylation of the amino function did not show any biological activity (compounds **12** and **13**).

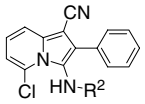
Table 1. Yield and inhibitory activity of 3-amino-1-cyano-indolizines—variations at the R^1 position



Compound	R^1	Yield %	ELISA[c] IC_{50} (μM)
1	Cl	68[a]	29
2	F	65[a]	na
3	SCH_2CO_2Me	98[b]	na
4	SCH_2CO_2H	95[b]	na
5	SMe	49[b]	na
6	$O(CH_2)_3NMe_2$	17[b]	79
7	1-Morpholinyl	61[b]	49
8	$NH(CH_2)_2NMe_2$	48[b]	42

[a] Isolated yield based on cyano-2-methylene-pyridine.
[b] Scheme 1, step b.
[c] na, not active.

Table 2. Yield and inhibitory activity of 3-amino-1-cyano-indolizines—variations at the R^2 position



Compound	R^2	Yield[a] %	ELISA[d] IC_{50} (μM)
9	H	74[b]	na
10	$(CH_2)_2NEt_2$	7	na
11	2,4-Dimethoxybenzyl	73	na
12	Benzoyl	9[c]	na
13	Acetyl	32[c]	na
14	3-Aminophenyl	11	80
15	$(CH_2)_3NMe_2$	11	62
16	2,6-Dimethylphenyl	7	60
17	4-Dimethylaminophenyl	18	47
18	$(CH_2)_2NH_2$	17	43
19	$CH_2CONHCH_2$(2-furyl)	17	19
20	3,4-Dichlorobenzyl	11	13
21	$(CH_2)_2OMe$	18	9
22	$(CH_2)_2N$(Me)Benzyl	10	4
23	2-(4-Chlorophenyl)ethyl	41	4
24	2-(2-Chlorophenyl)ethyl	36	3
25	2-Phenylethyl	11	2

[a] Isolated yield based on cyano-2-methylene-pyridine.
[b] Scheme 2, step a.
[c] Scheme 2, step b.
[d] na, not active.

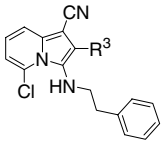
R^3 optimization was addressed by using a variety of commercial aldehydes (compounds **26**–**53**, Table 3). It appeared that aromatic moieties, presence were related to activities, while higher ranking compounds are characterized by substituted phenyl rings. Nature and position of the aromatic subtituents proved to have little influence on potency (compounds **39**–**43**).

From the hit identified in the primary screening campaign (compound **1**), we were able to rapidly improve potency by an order of magnitude to reach low micromolar inhibitory activity within a four months timeframe. To further validate the potential relevance of cyano-indolizine for cancer treatment, several compounds from the 3-amino-1-cyano-indolizines series were selected and evaluated in an in vitro assay for measuring their inhibitory potency against migration of PC3 prostate cancer cell lines[14] (see Table 4).

Compounds **1**, **19**, **24**, **25**, and **53** inhibited migration of PC3 cell lines in the range from 8 to 72 μM, while maintaining a correlation between the biochemical and functional efficacy.[15]

In conclusion, the use of MCCs allowed us to conduct expeditious lead optimization process even in the challenging domain of protein–protein interaction disruption historically addressed by more elaborate peptidomimetics and natural compounds.

Table 3. Yield and inhibitory activity of 3-amino-1-cyano-indolizines - variations at the R^3 position



Compound	R^3	Yield[a] (%)	ELISA[b] IC_{50}, (μM)
26	Cyclohexyl	76	na
27	4-Quinolinyl	38	na
28	4-Piperidinyl	98	95
29	2-(5-Methylthiazoyl)	7	22
30	4-*tert*-Butylphenyl	3	14
31	$(CH_2)_2OH$	44	13
32	4-Trifluoromethoxyphenyl	54	13
33	4-$(Me_2N(CH_2)_3O)$-Phenyl	8	10
34	2-Thiophenyl	2	10
35	2-Toyl	8	9
36	4-Pyridyl	12	6
37	3-Thiophenyl	2	6
38	3,5-Dimethylphenyl	62	6
39	4-Fluorophenyl	10	5
40	4-Toyl	6	5
41	4-Methoxyphenyl	5	5
42	3-Pyridyl	45	4
43	4-Fluoronaphthyl	10	4
44	3-Chlorophenyl	13	3
45	4-Methylthiophenyl	11	3
46	2-Methoxyphenyl	15	3
47	2-Chlorophenyl	13	3
48	2-Fluorophenyl	24	3
49	2-Phenylethyl	21	2
50	2-Pyridyl	46	2
51	3-Methoxyphenyl	12	2
52	4-Chlorophenyl	6	2
53	3-Fluorophenyl	10	2

[a] Isolated yield base on cyano-2-methylene-pyridine.
[b] na, not active.

Table 4. Migration assay on PC3 cells with compounds **1**, **19**, **24**, **25**, and **53**

Compound	ELISA IC_{50} (μM)	Migration[a] IC_{50} (μM)
1	29	72
19	19	54
24	3	23
25	2	21
53	2	8

[a] All experiments were repeated atleast twice.

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