1-1-2019

Prototyping kinase inhibitor-cytotoxin anticancer mutual prodrugs activated by tumour hypoxia: A chemical proof of concept study

Geraud Sansom

University of Wollongong, gs254@uowmail.edu.au

Nicholas Kirk

University of Wollongong, nsk597@uowmail.edu.au

Christopher Guise

University of Auckland

Robert Anderson

University of Auckland

Jeff Smaill

University of Auckland

See next page for additional authors

Follow this and additional works at: <https://ro.uow.edu.au/ihmri>

Part of the [Medicine and Health Sciences Commons](#)

Recommended Citation

Sansom, Geraud; Kirk, Nicholas; Guise, Christopher; Anderson, Robert; Smaill, Jeff; Patterson, Adam; and Kelso, Michael J., "Prototyping kinase inhibitor-cytotoxin anticancer mutual prodrugs activated by tumour hypoxia: A chemical proof of concept study" (2019). *Illawarra Health and Medical Research Institute*. 1392.

<https://ro.uow.edu.au/ihmri/1392>

Prototyping kinase inhibitor-cytotoxin anticancer mutual prodrugs activated by tumour hypoxia: A chemical proof of concept study

Abstract

Amide- and ester-linked kinase inhibitor-cytotoxin conjugates were rationally designed and synthesised as prototype hypoxia-activated anticancer mutual prodrugs. Chemical reduction of an aryl nitro trigger moiety was shown to initiate a spontaneous cyclisation/fragmentation reaction that simultaneously released the kinase inhibitor semaxanib (SU5416) and the amine- or alcohol-linked cytotoxin from the prodrugs. Preliminary cell testing and reduction potential measurements support optimisation of the compounds towards tumour-selective mutual prodrugs.

Disciplines

Medicine and Health Sciences

Publication Details

Sansom, G. N., Kirk, N. S., Guise, C. P., Anderson, R. F., Smaill, J. B., Patterson, A. V. & Kelso, M. J. (2019). Prototyping kinase inhibitor-cytotoxin anticancer mutual prodrugs activated by tumour hypoxia: A chemical proof of concept study. *Bioorganic and Medicinal Chemistry Letters*, 29 (10), 1215-1219.

Authors

Geraud Sansom, Nicholas Kirk, Christopher Guise, Robert Anderson, Jeff Smaill, Adam Patterson, and Michael J. Kelso

Prototyping Kinase Inhibitor–Cytotoxin Anticancer Mutual Prodrugs Activated by Tumour Hypoxia: A Chemical Proof of Concept Study

Geraud N. Sansom^{a,b}, Nicholas S. Kirk^{a,b}, Christopher P. Guise^{c,d}, Robert F. Anderson^{c,d}, Jeff B.

Smaill^{c,d}, Adam V. Patterson^{c,d}, and Michael J. Kelso^{a,b,*}

^aMolecular Horizons and School of Chemistry & Molecular Bioscience, University of Wollongong, NSW 2522, Australia.

^bIllawarra Health & Medical Research Institute, Wollongong, NSW 2522, Australia.

^cAuckland Cancer Society Research Centre, Faculty of Medical and Health Sciences, The University of Auckland, Private Bag 92019, Auckland 1142, New Zealand.

^dThe Maurice Wilkins Centre for Molecular Biodiscovery, The University of Auckland, Private Bag 92019, Auckland 1142, New Zealand

ABSTRACT: Amide- and ester-linked kinase inhibitor–cytotoxin conjugates were rationally designed and synthesised as prototype hypoxia-activated anticancer mutual prodrugs. Chemical reduction of an aryl nitro trigger moiety was shown to initiate a spontaneous cyclisation/fragmentation reaction that simultaneously released the kinase inhibitor semaxanib (SU5416) and the amine- or alcohol-linked cytotoxin from the prodrugs. Preliminary cell testing and reduction potential measurements support optimisation of the compounds towards tumour-selective mutual prodrugs.

KEYWORDS: Hypoxia, mutual prodrug, anticancer, bioreductive activation, Sunitinib, Semaxinib, Floxuridine, 4-aminoaniline mustard

Cytotoxic drugs have been the mainstay of many anticancer treatment regimens but their effectiveness is limited by low tumour cell selectivity and, as a consequence, dose-limiting side effects. Tumour-activated prodrugs that selectively deliver cytotoxins to the immediate tumour microenvironment are sought as a means of improving the pharmacodynamics of cytotoxic drugs and reducing their toxicity towards non-cancerous tissues.^{1–4} Nitroaromatics have received intense study as prodrugs that can undergo reductive metabolism (bioactivation) to hydroxylamine or amine derivatives within the hypoxic environment of

solid tumours.⁵⁻⁸ The newly revealed amine nucleophiles can then serve to trigger reactions that release the active cytotoxin. In 1994, the Denny group reported 2-nitrophenyl acetamides as hypoxia-activated prodrug scaffolds,⁹ where spontaneous cyclisation of the nascent 2-aminophenyl acetamide expelled an activated 4-aminoaniline mustard cytotoxin and an indoline-2-one waste fragment. More recently, we reported the synthesis and anti-angiogenic properties of 2-nitrophenylacetate derivatives related to the drug sunitinib (Sutent®), an indoline-2-one-based multi-kinase inhibitor used in the treatment of highly vascularised renal cell carcinomas and gastrointestinal stromal tumours (Figure 1(a)).¹⁰

When considered in the context of Denny and colleagues' work,⁹ we noted that our 2-nitrophenylacetates could potentially serve as tumor-activated 'mutual' prodrug scaffolds,¹¹ wherein hypoxia-dependent bioactivation of the 2-nitrophenyl moiety and ensuing cyclisation of the 2-aniline might simultaneously evolve an active cytotoxin (formerly incorporated within the prodrug as an amide or ester) along with the kinase inhibitor semaxanib (SU5416) **3**, a clinically evaluated predecessor of sunitinib¹²⁻¹⁴ as a functionalised version of Denny's indolin-2-one waste fragment. Furthermore, we noted that a hydrogen bond was invariably present between the pyrrole-NH and carbonyl oxygen atoms in our 2-nitrophenylacetic amides and esters (Figure 1(a)), suggesting the scaffold may be pre-organised and activated for rapid indoline-2-one cyclisation following reduction to the aniline.¹⁰ Conceptually, mutual prodrugs of this type could mitigate side effects arising from either or both drugs while at the same time increasing tumor concentrations of the actives and improving pharmacodynamics.

Figure 1. (a) Anti-angiogenic 2-nitrophenylacetate derivatives related to sunitinib.¹⁰ (b) Prototype mutual prodrugs **1** and **2** and their proposed mechanism of bioreductive activation to selectively release the indoline-2-one kinase inhibitor semaxanib **3** and 4-aminoaniline mustard or floxuridine cytotoxins within hypoxic regions of tumors.

Two mutual prodrug prototypes were conceived to explore the concept. Prodrug **1** incorporated Denny's 4-aminoaniline mustard linked to the 2-nitrophenylacetate scaffold via an amide (Figure 1(b)). In this compound, the amide would serve to withdraw electron density from the mustard nitrogen, thereby reducing DNA reactivity of the prodrug. The 4-aminoaniline mustard released into hypoxic tumors following prodrug activation would be vastly more DNA-reactive and cytotoxic due to increased electron density on the mustard nitrogen driving formation of DNA-alkylating aziridinium ions.^{9,15} Prodrug **2** linked the 2-nitrophenylacetate scaffold via an ester to the primary alcohol of the anticancer drug floxuridine (Figure 1(b)). As a nucleoside antimetabolite requiring phosphorylation as part of its mechanism, the drug should have reduced activity while tethered to the prodrug. Within normoxic tissues, the nitro radical anion intermediate formed on each prodrug following one-electron reduction of the nitro group by human reductases is predicted to be back-scavenged by molecular oxygen, regenerating the prodrug. Within hypoxic tissues, further reduction of this intermediate to the amine 6-electron reduction product of each prodrug could occur, giving rise to hypoxia-selective triggering.¹⁶

The synthesis of **1** commenced with the coupling of 2-nitrophenylacetic acid and *p*-phenylenediamine using HBTU/diisopropylethylamine to give **4** in 80% yield (Scheme 1). *N,N*-dialkylation of **4** with 2-bromoethanol in the presence of K₂CO₃ afforded bis-alcohol **5** in 62% yield. Mono-alkylation was achieved within hours but prolonged heating at 50 °C was required to obtain an acceptable yield of **5**. Bis-alcohol protection of **5** using *tert*-butyldimethylsilyl chloride (TBDMSCl)/imidazole provided **6** in 86% yield. Reacting **6** with *N*-methylcarbamoyl pyrrole aldehyde **7** using our reported Knoevenagel conditions¹⁰ (K₂CO₃, 18-crown-6, THF, reflux) delivered the advanced precursor **8** in modest yield (21%) with no evidence of formation of the *E*-isomer in the ¹H NMR spectrum of the crude reaction mixture. Despite trialling many other pyrrole protecting groups, bases, solvents and reaction conditions, the yield of **8** could not be improved. Performing the Knoevenagel reaction with **7** and unprotected **5** gave none of the desired bis-alcohol. Deprotection of **8** using TBAF buffered with acetic acid in THF at 0 °C delivered the bis-alcohol **9** in 86% yield. Final conversion to the target mustard **1** was accomplished in 80% yield over 2 steps by first converting **9** to the bis-mesylate (MsCl, triethylamine) and then heating the freshly prepared crude with LiCl at 75 °C in DMF.

Scheme 1. Synthesis of mutual prodrug **1**.

The synthesis of **2** commenced with bis-TBS protection of the 1° and 2° alcohols of commercially available floxuridine (Scheme 2). The reaction proceeded well under standard conditions (TBDMS-Cl/imidazole) to give **10** in excellent yield (97%). Similarly high yields were previously reported for the bis-TBS protection of thymidine.¹⁷ Treatment of **10** with freshly distilled 2,4-dimethoxybenzoyl chloride, diisopropylethylamine and DMAP gave the *N*-protected derivative **11** in 87% yield. Selective silyl-deprotection of the less hindered 1° alcohol using aqueous trichloroacetic acid (4.2 M) in THF (1:4)¹⁸ delivered **12** in 50% yield. Steglich esterification¹⁹ of **12** with 2-nitrophenylacetic acid proceeded smoothly to give **13** (87%). Condensation of **13** with pyrrole aldehyde **7** using our Knoevenagel conditions¹⁰ produced a mixture of *cis* and *trans* isomers in modest yield ((*Z*)-**14** 10%; (*E*)-**14** 11%). Extensive efforts to improve the yield were unsuccessful. Removal of the 2,4-dimethoxy benzoyl protecting group from (*Z*)-**14** with ammonia in methanol gave the penultimate precursor **15** (75%) and final deprotection of the TBS group with TBAF/acetic acid delivered prodrug **2** (69%).²⁰ Attempts to access the two prodrugs by an alternative route involving direct ester/amide coupling of cytotoxins to a 2-nitrophenylacetic acid derivative containing the pre-installed pyrrole group (obtained from the allyl ester in Figure 1(a)) were not successful, but led to the discovery of novel bioactive 3-imino-2-(pyrrol-2-yl) isatogens²¹ and pyrrolizin-3-ones.²²

Scheme 2. Synthesis of mutual prodrug **2**.

Proof of concept reactions were performed with **1** and **2** to establish whether chemical reduction of the aryl nitro groups would trigger spontaneous cyclisation of the nascent anilines to generate semaxanib **3** and, by inference, the respective 4-aminoaniline mustard and floxuridine cytotoxins. Three standard room temperature aryl nitro reduction reactions were employed: $\text{Fe}/\text{CH}_3\text{COOH}$ in $\text{EtOH}/\text{H}_2\text{O}$,²³ $\text{FeCl}_3\cdot 6\text{H}_2\text{O}/\text{Zn}$ in $\text{DMF}/\text{H}_2\text{O}$ ²⁴ and $\text{NaBH}_4/\text{Pd-C}$ in $\text{MeOH}/\text{H}_2\text{O}$ ²⁵ (Table 1). Semaxanib **3** was isolated from all reactions within 0.5-3 h in yields ranging from 37–84%. *N*-hydroxy semaxanib **16**, formed by cyclisation of the intermediate *N*-hydroxylamine 4-electron reduction product, was also isolated from reactions in 11-51% yield. The combined yields of indoline-2-ones **3** and **16** were invariably high (80-94%) suggesting cyclisation within the scaffold is highly favoured. Downfield-shifting of the pyrrole-NH signals in the ^1H NMR spectrum of **1** (δ 12.04 ppm) and **2** (δ 11.66 ppm) (and all pyrrole-containing intermediates) confirmed both *cis* stereochemistry for the compounds and the presence of an intramolecular hydrogen bond between the pyrrole-NH and carbonyl oxygen atoms.¹⁰ This H-bond in **1** and **2** likely serves as a pseudo-acid catalyst that promotes spontaneous cyclisation.

Table 1. Proof of concept chemical reductions with mutual prodrugs **1** and **2**.

Prodrug	Reductant	Solvent	t (h)	Yield 3 (%)	Yield 16 (%)	Yield 3+16 (%)
1	Fe/CH ₃ COOH	EtOH/H ₂ O	1	80	14	94
	FeCl ₃ .6H ₂ O/Zn	DMF/H ₂ O	3	53	32	85
	NaBH ₄ /Pd-C	MeOH/H ₂ O	0.5	43	37	80
2	Fe/CH ₃ COOH	EtOH/H ₂ O	1	84	11	95
	FeCl ₃ .6H ₂ O/Zn	DMF/H ₂ O	3	40	51	91
	NaBH ₄ /Pd-C	MeOH/H ₂ O	0.5	37	45	82

Preliminary biological testing of **1** and **2** was carried out using MDA-MB-468 triple-negative breast cancer cells and SW620 colon cancer cells (Table 2). The prodrugs were assessed first for their degree of deactivation relative to parent cytotoxin control compounds under aerobic conditions. For prodrug **1**, melphalan was selected as the clinically relevant nitrogen mustard control²⁶ and for prodrug **2** the comparator was Floxuridine. The degree of prodrug deactivation was determined using the deactivation ratio (DR = Aerobic IC₅₀_{prodrug}/Aerobic IC₅₀_{control}). For prodrug **1**, 8.5-fold and 3.0-fold deactivation was observed in the two cell-lines, while 3.0-fold and 6.9-fold deactivation was seen with **2**. It is proposed that for **1**, the amide bond pulls electron density away from the mustard nitrogen leading to decreased DNA reactivity (and hence cytotoxicity) in the prodrug. In the case of **2**, Floxuridine is an antimetabolite and would be inactivated by the ester linkage to the bulky 2-nitrophenylacetate moiety. The deactivation observed with prodrug **2** relative to floxuridine suggests that the ester bond remained intact under the assay conditions. The ability of the prodrugs to undergo nitroaromatic bioreduction and cyclisation to release the respective cytotoxins was determined using the hypoxic cytotoxicity ratio (HCR = Aerobic IC₅₀/Anoxic IC₅₀). Under the assay conditions used here, however, neither prodrug showed evidence for activation under anoxia.

Table 2. Biological evaluation of mutual prodrugs.

Cell line	Drug	Aero IC50 (μ M)	Anox IC50 (μ M)	DR ^a	HCR ^b
MDA-MB-468	Melphalan	1.00 $\pm$ 0.11	0.81 $\pm$ 0.16	-	1.2-fold
	Floxuridine	27.42 $\pm$ 12.52	45.53 $\pm$ 6.10	-	<1-fold
	1	8.31 $\pm$ 0.95	7.38 $\pm$ 0.88	8.3-fold	1.1-fold
	2	83.88 $\pm$ 40.93	247.96 $\pm$ 80.56	3.1-fold	<1-fold
	Tirapazamine	105.2	1.304	-	81-fold
	TH-302	4.403	0.0062	-	710-fold
SW620	Melphalan	1.81 $\pm$ 0.25	1.22 $\pm$ 0.10	-	1.5-fold
	Floxuridine	0.96 $\pm$ 0.40	1.48 $\pm$ 0.53	-	<1-fold
	1	11.48 $\pm$ 6.57	12.67 $\pm$ 4.93	6.3-fold	<1-fold
	2	18.70 $\pm$ 14.81	13.96 $\pm$ 5.39	19.4-fold	1.3-fold
	Tirapazamine	91.03	1.2	-	76-fold
	TH-302	20.6	0.052	-	396-fold

Sensitivity of MDA-MB-468 and SW620 cells to hypoxia-activated mutual prodrugs **1** and **2** and their parent cytotoxins under aerobic (Aero) and anoxic (Anox) conditions. Antiproliferative IC50 values are shown (mean $\pm$ std dev, n = 3). The activity of two positive control hypoxia-activated cytotoxins Tirapazamine²⁷ and TH-302²⁸ are included for comparison (n = 1). ^aThe deactivation ratio (DR) indicates the degree of deactivation of the prodrug relative to the effector under aerobic conditions (i.e. DR = Aero prodrug IC50/Aero control cytotoxin IC50). ^bThe hypoxic cytotoxicity ratio (HCR) indicates whether cells are more sensitive to the prodrugs or effectors under anoxic conditions (HCR = aerobic IC50/anoxic IC50).

To examine why **1** and **2** did not show enhanced cytotoxicity under anoxia, one-electron reduction potentials ($E[1]$) for the aryl nitro groups in the prodrugs were measured using pulse radiolysis. This thermodynamic parameter characterises the electron affinity of nitroaromatics and hence their willingness to accept an electron from human oxidoreductases. Pulse radiolysis of **2** returned $E[1] = -509 \pm 8$ mV, but measurements for **1** were confounded by poor aqueous solubility. Measurements were instead obtained using the bis-alcohol precursor **9** as a proxy. It was rationalised that the remoteness of the alcohols from the

arylnitro group would limit their influence on the reduction potential. Pulse radiolysis of **9** returned $E[1] = -550 \pm 8$ mV. Thus, we conclude that the lack of bioreductive activation observed with the prodrugs under anoxia arises from the low nitroaromatic one-electron reduction potentials. It has been reported that nitroaromatic prodrugs show greatest hypoxia-selectivity when $E[1]$ falls within the range -330 to -450 mV.² With $E[1]$ for **1** and **2** falling below this, the prodrugs are unable to accept electrons from one-electron reductases at appreciable rates and therefore incapable of undergoing bioreduction in cells under anoxia.

In conclusion, we have rationally-designed and synthesized amide and ester-linked hypoxia-activated mutual prodrug prototypes and provided chemical proof of concept showing that reduction of the aryl nitro group triggers simultaneous release of two cancer drugs. Release of *N*-hydroxy semaxanib **16** was also noted but if generated *in vivo*, this compound could serve as an active kinase inhibitor, especially against VEGFR-2.²⁹ Reduction potentials in the prototypes are at this stage too low to confer efficient bioreductive activation. Appending electron withdrawing groups to the nitroaryl ring could increase the reduction potential towards the target minimum of -450 mV. Attaching electron withdrawing groups to the indoline-2-one of Semaxanib is known to produce only minor changes in kinase inhibition.³⁰ Additionally, Denny showed that electron withdrawing groups do not affect cyclisation rates of 2-aminophenyl acetamides formed by reduction of 2-nitrophenyl acetamide prodrugs.⁹ Based on this, a second generation of compounds containing electron withdrawing groups on the nitroaryl ring are the focus of efforts to optimise the mutual prodrugs for clinical use.

Appendix A. Supplementary data

Synthesis, compound characterisation and ¹H NMR/¹³C NMR spectra for compounds **1**, **2**, **3**, **4**, **5**, **6**, **8**, **9**, **10**, **11**, **12**, **13**, (*Z*)-**14**, (*E*)-**14**, **15**. Chemical reductions, pulse radiolysis experiments and in vitro cell experiments. The Supplementary Data is available free of charge on the ACS Publications website.

AUTHOR INFORMATION

Corresponding Author

*Email: mkelso@uow.edu.au

Funding Sources

Funded in part by a Health Research Council of New Zealand grant 17/255 to Jeff Smaill and Adam Patterson and Australian National Health and Medical Research Council (NHMRC) Project Grant (APP1100432) to Michael Kelso.

ACKNOWLEDGMENT

We thank the University of Wollongong (Wollongong, Australia) and University of Auckland (Auckland, NZ) for supporting this work.

REFERENCES

- (1) Bildstein, L.; Dubernet, C.; Couvreur, P., Prodrug-based intracellular delivery of anticancer agents. *Adv. Drug Del. Rev.* **2011**, *63*, 3-23.
- (2) Denny, W. A., Prodrug strategies in cancer therapy. *Eur. J. Med. Chem.* **2001**, *36* (7), 577-595.
- (3) Chari, R. V. J., Targeted delivery of chemotherapeutics: tumor-activated prodrug therapy. *Adv. Drug Del. Rev.* **1998**, *31*, 89-104.
- (4) Mahato, R.; Tai, W.; Cheng, K., Prodrugs for improving tumor targetability and efficiency. *Adv. Drug Del. Rev.* **2011**, *63*, 659-670.
- (5) Chen, Y.; Hu, L., Design of anticancer prodrugs for reductive activation. *Med. Res. Rev.* **2009**, *29* (1), 29-64.
- (6) Denny, W. A., Hypoxia-activated prodrugs in cancer therapy: progress to the clinic. *Future Oncol.* **2010**, *6* (3), 419-28.
- (7) Wilson, W. R.; Hay, M. P., Targeting hypoxia in cancer therapy. *Nat. Rev. Cancer* **2011**, *11* (6), 393-410.
- (8) Hunter, F. W.; Wouters, B. G.; Wilson, W. R., Hypoxia-activated prodrugs: paths forward in the era of personalised medicine. *Br. J. Cancer* **2016**, *114* (10), 1071-7.
- (9) Atwell, G. J.; Sykes, B. M.; O'Connor, C. J.; Denny, W. A., Relationships between structure and kinetics of cyclization of 2-aminoaryl amides: potential prodrugs of cyclization-activated aromatic mustards. *J. Med. Chem.* **1994**, *37* (3), 371-380.
- (10) 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., Synthesis, Structural Characterisation, and Preliminary Evaluation of Non-Indolin-2-one-based Angiogenesis Inhibitors Related to Sunitinib (Sutent®). *Aust. J. Chem.* **2013**, *66* (8), 864-873.
- (11) Tranoy-Opalinski, I.; Fernandes, A.; Thomas, M.; Gesson, J. P.; Papot, S., Design of Self-Immolative Linkers for Tumour-Activated Prodrug Therapy. *Anticancer Agents Med. Chem.* **2008**, *8* (6), 618-637.
- (12) Fiedler, W.; Mesters, R.; Tinnefeld, H.; Loges, S.; Staib, P.; Dührsen, U.; Flasshove, M.; Ottmann, O. G.; Jung, W.; Cavalli, F.; Kuse, R.; Thomalla, J.; Serve, H.; O'Farrell, A. M.; Jacobs, M.; Brega, N. M.; Scigalla, P.; Hossfeld, D. K.; Berdel, W. E., A phase 2 clinical study of SU5416 in patients with refractory acute myeloid leukemia. *Blood* **2003**, *102* (8), 2763-2767.

- (13) Fury, M. G.; Zahalsky, A.; Wong, R.; Venkatraman, E.; Lis, E.; Hann, L.; Aliff, T.; Gerald, W.; Fleisher, M.; Pfister, D. G., A Phase II study of SU5416 in patients with advanced or recurrent head and neck cancers. *Invest. New Drugs* **2007**, *25* (2), 165-72.
- (14) Zangari, M.; Anaissie, E.; Stopeck, A.; Morimoto, A.; Tan, N.; Lancet, J.; Cooper, M.; Hannah, A.; Garcia-Manero, G.; Faderl, S.; Kantarjian, H.; Cherrington, J.; Albitar, M.; Giles, F. J., Phase II study of SU5416, a small molecule vascular endothelial growth factor tyrosine kinase receptor inhibitor, in patients with refractory multiple myeloma. *Clin. Cancer Res.* **2004**, *10* (1 Pt 1), 88-95.
- (15) Palmer, B. D.; Wilson, W. R.; Pullen, S. M.; Denny, W. A., Hypoxia-selective antitumor agents. 3. Relationships between structure and cytotoxicity against cultured tumor cells for substituted N,N-bis(2-chloroethyl)anilines. *J. Med. Chem.* **1990**, *33* (1), 112-121.
- (16) Siim, B. G.; Denny, W. A.; Wilson, W. R., Nitro reduction as an electronic switch for bioreductive drug activation. *Oncol. Res.* **1997**, *9* (6-7), 357-69.
- (17) Madhuri, V.; Kumar, V. A., Design and synthesis of dephosphono DNA analogues containing 1,2,3-triazole linker and their UV-melting studies with DNA/RNA. *Nucleosides Nucleotides Nucl. Acids* **2012**, *31* (2), 97-111.
- (18) Zhu, X.-F.; Williams, H. J.; Ian Scott, A., Aqueous Trichloroacetic Acid: Another Useful Reagent for Highly Selective 5'-Desilylation of Multisilylated Nucleosides. *Synth. Commun.* **2003**, *33* (12), 2011.
- (19) Bernhard, N.; Wolfgang, S., Simple Method for the Esterification of Carboxylic Acids. *Angew. Chem. Int. Ed. Engl.* **1978**, *17* (7), 522-524.
- (20) Dreier, I.; Kumar, S.; Søndergaard, H.; Rasmussen, M. L.; Hansen, L. H.; List, N. H.; Kongsted, J.; Vester, B.; Nielsen, P., A Click Chemistry Approach to Pleuromutilin Derivatives, Part 2: Conjugates with Acyclic Nucleosides and Their Ribosomal Binding and Antibacterial Activity. *J. Med. Chem.* **2012**, *55* (5), 2067-2077.
- (21) Kirk, N. S.; Sansom, G. N.; Sudta, P.; Suksamrarn, S.; Willis, A. C.; Bremner, J. B.; Kelso, M. J., Unexpected synthesis of 3-imino-2-(pyrrol-2-yl) isatogen derivatives affords facile access to a 2-pyrrolyl isatogen. *Synth. Commun.* **2017**, *47* (1), 62-67.
- (22) Kirk, N. S.; Bezos, A.; Willis, A. C.; Sudta, P.; Suksamrarn, S.; Parish, C. R.; Ranson, M.; Kelso, M. J., Synthesis and preliminary evaluation of 5,7-dimethyl-2-aryl-3H-pyrrolizin-3-ones as angiogenesis inhibitors. *Bioorg. Med. Chem. Lett.* **2016**, *26* (7), 1813-1816.
- (23) Gamble, A. B.; Garner, J.; Gordon, C. P.; O'Conner, S. M. J.; Keller, P. A., Aryl Nitro Reduction with Iron Powder or Stannous Chloride under Ultrasonic Irradiation. *Synth. Commun.* **2007**, *37* (16), 2777-2786.

- (24) Desai, D. G.; Swami, S. S.; Hapase, S. B., "Rapid and Inexpensive Method for Reduction of Nitroarenes to Anilines". *Synth. Commun.* **1999**, 29 (6), 1033-1036.
- (25) Pogorelić, I.; Filipan-Litvić, M.; Merkaš, S.; Ljubić, G.; Capanec, I.; Litvić, M., Rapid, efficient and selective reduction of aromatic nitro compounds with sodium borohydride and Raney nickel. *J. Mol. Catal. A: Chem.* **2007**, 274 (1), 202-207.
- (26) Facon, T.; Mary, J. Y.; Hulin, C.; Benboubker, L.; Attal, M.; Pegourie, B.; Renaud, M.; Harousseau, J. L.; Guillermin, G.; Chaletteix, C.; Dib, M.; Voillat, L.; Maisonneuve, H.; Troncy, J.; Dorvaux, V.; Monconduit, M.; Martin, C.; Casassus, P.; Jaubert, J.; Jardel, H.; Doyen, C.; Kolb, B.; Anglaret, B.; Grosbois, B.; Yakoub-Agha, I.; Mathiot, C.; Avet-Loiseau, H., Melphalan and prednisone plus thalidomide versus melphalan and prednisone alone or reduced-intensity autologous stem cell transplantation in elderly patients with multiple myeloma (IFM 99-06): a randomised trial. *Lancet* **2007**, 370 (9594), 1209-18.
- (27) Zeman, E. M.; Brown, J. M.; Lemmon, M. J.; Hirst, V. K.; Lee, W. W., SR-4233: a new bioreductive agent with high selective toxicity for hypoxic mammalian cells. *Int. J. Radiat. Oncol. Biol. Phys.* **1986**, 12 (7), 1239-42.
- (28) Huang, Y.; Tian, Y.; Zhao, Y.; Xue, C.; Zhan, J.; Liu, L.; He, X.; Zhang, L., Efficacy of the hypoxia-activated prodrug evofosfamide (TH-302) in nasopharyngeal carcinoma in vitro and in vivo. *Cancer Commun.* **2018**, 38 (1), 15.
- (29) Bouérat, L.; Fensholdt, J.; Liang, X.; Havez, S.; Nielsen, S. F.; Hansen, J. R.; Bolvig, S.; Andersson, C., Indolin-2-ones with High in Vivo Efficacy in a Model for Multiple Sclerosis. *J. Med. Chem.* **2005**, 48 (17), 5412-5414.
- (30) Sun, L.; Tran, N.; Tang, F.; App, H.; Hirth, P.; McMahon, G.; Tang, C., Synthesis and Biological Evaluations of 3-Substituted Indolin-2-ones: A Novel Class of Tyrosine Kinase Inhibitors That Exhibit Selectivity toward Particular Receptor Tyrosine Kinases. *J. Med. Chem.* **1998**, 41 (14), 2588-2603.