Cite this: *Med. Chem. Commun.*,
2019, 10, 310

Identification of the first enantiopure Rac1–Tiam1 protein–protein interaction inhibitor and its optimized synthesis *via* phosphine free remote group directed hydroarylation†

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Received 24th September 2018,
Accepted 21st December 2018

DOI: 10.1039/c8md00477c

rsc.li/medchemcomm

A phosphine free hydroarylation reaction applied to norbornene derivatives is described for the first time and was exploited for the regioselective gram scale synthesis of **AR-148**, a known Rac1–Tiam1 PPI inhibitor. Umpolung conversion of the nitro group into free amine allowed the regiocontrol of the key arylation step *via* a long range effect. The effect of **AR-148** in comparison with its enantiomers on Rac1 activation of has been evaluated and (–)**AR-148** has been identified as the first enantiomerically pure inhibitor of Rac1–Tiam1 PPI.

Introduction

The small GTPase protein Rac1 has gained attention for its role in different pathologies, among which are cancer and cardiovascular diseases.¹ Rac1 regulates events such as smooth muscle cell (SMC) migration² and proliferation,³ and leukocyte-endothelial cell interaction.⁴ Rac1 activity depends on the equilibrium between the inactive GDP-bound and the active GTP-bound forms. This cycling is regulated by guanine nucleotide exchange factors (GEFs) which act as activators, and GTPase activating proteins (GAPs) and GDP dissociation inhibitors (GDIs) which act as negative regulators. The T-cell lymphoma invasion and metastasis 1 (Tiam1) protein, a specific GEF for Rac1, is crucial for cell–cell adhesion and cell migration. Small molecules interfering with the Rac1–Tiam1 protein–protein interaction (PPI) were identified and reported by us^{5,6} and by others^{7–11} (Fig. 1a). Afterwards we reported the identification of 2-amino-3-(phenylsulfanyl)norbornane-2-carboxylate as a privileged scaffold for the *de novo* design and synthesis of a structurally original family of Rac1 inhibitors¹² (Fig. 1b). A G-LISA assay on SMCs demonstrated that among

all the compounds tested, **1** (named **AR-148**) selectively and potently inhibits Rac1 without interfering with RhoA. A Boyden chamber chemotaxis assay and cell movement video microscopy analyses have shown how **1** (**AR-148**) affects cell migration in response to the chemotactic agent platelet derived growth factor BB (PDGF-BB).¹²

In our previous work we designed and performed a divergent synthetic strategy from which a small library of aryl 2-amino-3-(phenylsulfanyl)norbornane-2-carboxylate was obtained.

Fig. 1 Previous work: a) first generation of Rac–Tiam1 inhibitors; b) target compound **AR-148** obtained in racemic form; c) **AR-148** synthesis drawbacks: –compound **4**, precursor of **AR-148**, is the minor reaction product, –**2*** used as the racemate of the two enantiomers.

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† Electronic supplementary information (ESI) available. See DOI: 10.1039/c8md00477c

The main difference between AR-148 and the other known Rac1-Tiam1 PPI inhibitors (Fig. 1a and b) consists in the presence of a three dimensional core heavily functionalized which position the aromatic groups involved in π - π interactions or π -cation interactions in very precise way (Fig. 2).

In modern drug discovery, compounds with three dimensional structures that escape the “flatland” of multiple arenes, become more central because of higher levels of insaturation and the presence of multiple stereocenters. The resulting structural complexity seems to provide better receptor ligand interaction with consequent improved potency, selectivity and decreased off-target effects.¹³

Focusing our attention on the efficient preparation of the most active compound AR-148, we identified two main drawbacks.

Firstly, the hydroarylation reaction was performed in a classical way in the presence of Ph_3P and an excess of iodoarene (3 equiv.) which gave the C-6 arylated isomer, precursor of AR-148, as the minor isomer (3 : 4 = 70 : 30)¹⁴ (Fig. 1c). Secondly, compound AR-148 was obtained in racemic form. Nowadays, defining the activity of both enantiomers is extremely important because of the great impact on reducing unexpected toxicity in further drug development. Moreover, once the eutomer is defined, its precise pharmacophore model could deeply improve the rational design of new derivatives. As a consequence, the preparation of the two enantiomers in pure form is crucial for evaluating their activity independently. In this work, we report an efficient protecting group free, gram scale selective synthesis of compound AR-148, the obtainment, *via* chromatographic separation, of both enantiomers and their pharmacological evaluation.

Results and discussion

Chemistry

To optimize the synthesis of AR-148 in an eco-friendly way, we reasoned that a phosphine-free hydroarylation protocol could be of outstanding interest. Many phosphine ligands are expensive, toxic, unrecoverable and often water- and air-sensitive. In large-scale applications on industrial and semi-industrial scales, the phosphines might be a serious eco-

nomic burden. Therefore we considered performing the reaction in the absence of phosphine.¹⁵ To our knowledge, phosphine-free Heck hydroarylation has never been realized on olefins and the only examples reported are limited to alkyne substrates.^{16,17} So, we planned to perform the hydroarylation using the same conditions described before, but in the absence of the phosphine ligand ($\text{Pd}(\text{OAc})_2/\text{TEA}/\text{HCOOH}$ in CH_3CN). The progressive addition to racemate **2** of 3-iodoaniline (1 equiv. $\times$ 3 every 4 hours) at a lower temperature (50 °C instead of 82 °C) gave the same mixture of regioisomers **3** and **4** in higher yield (80% instead of 75%) but with the same ratio (Scheme 1).

The theoretical work done by M. B. Hall on the mechanism of ligand-free Heck type arylation^{18–20} and the works published by Cacchi on phosphine-free Heck hydroarylation on alkyne,^{16,17} shed light on the different possible scenarios regarding the mechanism of our reaction. Unfortunately, the presence of a slight excess of an organic base like TEA, the use of a coordinative solvent such as CH_3CN , the requirement of formic acid and the substrate itself, containing the norbornene core (ligand in the Catellani reaction²¹), make it very difficult to prove which catalytic cycle is really operating or if there are more than one operating at the same time. It is worthy to report that different solvents have been screened in the model reaction between simple norbornene **SI5** and iodobenzene **SI6** (DMF, DMSO, CH_3CN , DCM, MeOH, THF, DCE, Et_2O) and have always led, even if with variable yield, to the formation of the desired product **SI7** (Fig. S3†). The good conversion obtained in DCE underlined how the reaction works, even in the absence of a coordinative solvent, is in accordance with the formation of the dianionic species isolated by Hartwig and co-workers for the ligand-free Heck arylation.¹⁹ Once a fairly satisfactory result was obtained which demonstrated the feasibility of a phosphine free hydroarylation protocol, we turned our attention to the selectivity of the Heck hydroarylation which is in fact the divergent point in the synthesis of **4**, a precursor of the desired compound AR-148. In order to modulate the regioselectivity in favour of **4** instead of **3**, several attempts were performed by using different reaction conditions but without satisfactory results^{22–24} (Table S1†).

Our previous studies^{14,25} disclosed that the regioselectivity is mainly driven by a long range effect exerted by the nitro group on C-2, (Path a, Fig. 3).^{26,27} We thus decided to exploit

Fig. 2 AR-148 molecular interaction surface of Rac1-AR-148.¹²

Scheme 1 Phosphine free methodology. Reagents and conditions: (a) $\text{Pd}(\text{OAc})_2$ 5%, TEA 3.5 equiv., HCOOH 3.0 equiv., CH_3CN , 50 °C, (80%) *used as racemate of the 2 enantiomers.

Fig. 3 Umpolung conversion of nitro- to amino group to modulate the long range effect on the hydroarylation reaction, (*data for regioselectivity in NBoc protected norbornene have been previously reported^{12,14}).

the umpolung conversion of the nitro- to the amine group, reasoning that an opposite long range effect should induce an inverse charge distribution of the double bond and consequently an opposite regiochemistry could be obtained (Path b, Fig. 3).

By treating compound **2** with $\text{Zn}/\text{H}_3\text{PO}_4$ in THF from 0 °C to r.t., the amine precursor **5** has been prepared in very good yield (97%) (Scheme 2).

After a brief optimization (Table S2†) to identify the adequate reaction conditions, compounds **7** and **8** have been obtained (yield 88%, 82% for the grams scale) by reacting **5** under the phosphine-free protocol previously optimized ($\text{Pd}(\text{OAc})_2/\text{TEA}/\text{HCOOH}$ in CH_3CN).

As expected thanks to an opposite long range effect, an inverse C5/C6 ratio was found to belong to the regioisomer **8**, precursor of **AR-148**, the main product of the reaction (7 : 8 = 30 : 70).

Coordination of palladium by the free amine at the *endo* position of **5** cannot be excluded but is a non-productive pathway for the Heck hydroarylation reaction, in fact no *endo*-product has been detected on the norbornene core^{28–30} (see ESI† page S7 for NMR characterization and assignment of *exo*-substituted compounds **7** and **8**).

The use of the phosphine free protocol has been fundamental to obtain the desired compound as a single reaction product and to improve the atom economy of the process. Indeed, we hypothesized that the undesired Buchwald–Hartwig reaction of *m*-iodoaniline on itself or on the norbornene aliphatic amine of **5** does not work in absence of phosphine while the Heck arylation is not affected. Polymers of the *m*-iodoaniline and polyarylated products were in fact always detected by mass analysis of the crude using phosphine catalysed reaction conditions (Table S2†). Such undesired derivatives are completely suppressed using the phosphine free protocol and the amount of *m*-iodoaniline **6** is reduced from 3.0 to 1.1 equivalents.

Buchwald–Hartwig amination has been tested directly on the inseparable mixture of **7** and **8**. Using $\text{Pd}(\text{tetrakis})$, K_3PO_4 and 1.4 equiv. 4-iodonitrobenzene in MeCN, compounds **9** and **10** were obtained in an almost quantitative yield (90%). K_3PO_4 is required instead of Cs_2CO_3 to avoid the arylation of the aminoester functionality on the norbornene core. The compounds **9** and **10** have been separated by flash chromatography and their structures were confirmed by NMR analysis (structural elucidation, ESI†). By performing the reduction of the nitro group of **10** with Zn/HCl 1 M in MeOH at 0 °C, compound ($\pm$)**AR-148** was obtained in a very efficient way.

The whole protocol was scaled up and 1 gram of compound ($\pm$)**AR-148** was obtained without the use of any protective group, with a higher atom economy, higher overall yield and in a regioselective way avoiding the use of phosphine in the Heck reaction (Scheme 2).

Scheme 2 Reagents and conditions: a) Zn , H_3PO_4 1 M, THF, 0 °C–rt, 2 gram scale; b) phosphine free methodology *m*-iodoaniline 1.1 equiv., $\text{Pd}(\text{OAc})_2/\text{TEA}$, HCOOH , CH_3CN , 45 °C, 1 gram scale; c) 4-iodonitrobenzene 1.1 equiv., $\text{Pd}(\text{tetrakis})$, K_2PO_4 , CH_3CN , 70 °C, 1 gram scale; d) Zn , HCl 1 M, MeOH, 0 °C, 1 h, 1 gram scale; *used as the racemate of the 2 enantiomers.

Fig. 4 Dose-dependent effect of **AR148** and its enantiomers on Rac1-GTP levels. SMCs were seeded at a density of 2×10^5 per 35 mm Petri dish and incubated with DMEM supplemented with 10% FCS. After 24 h, the compounds were added to the cultured medium at final concentrations of 1, 2.5, 5 and 10 μM , and after 4 h Rac1 GTP levels were evaluated by G-LISA assays.

Finally, we faced the problem related to the availability of the two enantiomers of **AR-148** for the pharmacological tests. We focused on the enantioselective synthesis of norbornene scaffold **2** obtained through the Diels–Alder cycloaddition reaction.¹² We tried to perform the reaction by using chiral catalysts, but although we observed enhanced selectivity, very low yields always affected our results. The synthesis of chiral dienophiles (β -sulfanyl nitroacrylate esters of (–)-menthol and (–)-8-Ph-menthol) was very troublesome and the yields were not suitable as a first step of the synthesis. Therefore, we considered the exploitation of a removable chiral auxiliary to obtain the separation of the two corresponding diastereoisomers of **AR-148**. By reacting the free amine of **AR-148** with (–)-menthylchloroformate the two corresponding monomenthylcarbamate diastereoisomers were obtained and easily separated by HPLC, but unfortunately further hydrolysis of the chiral auxiliary always led to unreacted compounds or tarry materials.

Finally, through the use of chiral HPLC, we separated the two enantiomers of **AR-148** with unsatisfactory results. However, excellent results were obtained in the chiral HPLC resolution of racemate ($\pm$)-**10** (see the ESI[†]), (Scheme 2). The two enantiomers (–)-**10** and (+)-**10** were obtained with high enantiomeric purity (purity: >99% ee: >99).

After reduction of the single enantiomers (Zn/HCl in MeOH at 0 °C), the corresponding amines (–)-**AR-148** and (+)-**AR-148** were obtained. NMR analyses were performed confirming the structure and the purity of the final compounds.

Pharmacology

The effect of **AR-148** and its enantiomers on Rac1 activation was investigated in human cultured smooth muscle cells (SMCs). Interestingly, the (–)-**AR-148** enantiomer re-

duced the intracellular levels of Rac1-GTP by $63.9 \pm 15.0\%$ at 10 μM , while (+)-**AR-148** was inactive. Accordingly, the racemate 10 μM **AR-148** only partially inhibited the Rac1 activity ($-17.2 \pm 4.1\%$). These data clearly demonstrated an enantioselective effect of (–)-**AR-148** on Rac1 (Fig. 4, Experimental ESI[†]).

Conclusions

In summary, we reported a gram scale, protecting group-free synthesis of compound **AR-148**, a known potent inhibitor of the Rac1–Tiam1 protein–protein interaction. The synthetic strategy applied includes the first example of a phosphine free Heck hydroarylation reaction on an olefin, which represents an improvement also in terms of eco-friendliness and atom economy of the process. We were able to exploit the remote directing group effect of the free amine for the selective obtainment of the desired regioisomer of the arylation reaction. Despite the impossibility of an enantiopure synthesis, we obtained the two enantiomers of **AR-148**, via HPLC separation of the precursors, and we demonstrated for the first time the enantioselectivity in the inhibition of Rac1–Tiam1 PPI by (–)-**AR-148**. Characterization of the absolute stereochemistry of (–)-**AR-148** and study of cocrystallization with the Rac1 protein are proceeding in order to define the pharmacophoric model for further drug design.

Conflicts of interest

The authors confirm that this article has no conflict of interest.

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