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Efficient synthesis of anacardic acid analogues and their antibacterial activities

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ABSTRACT

Anacardic acid derivatives exhibit a broad range of biological activities. In this report, an efficient method for the synthesis of anacardic acid derivatives was explored, and a small set of salicylic acid variants synthesised retaining a constant hydrophobic element (a naphthyl tail). The naphthyl side chain was introduced via Wittig reaction and the aldehyde installed using directed *ortho*-metalation reaction of the substituted *o*-anisic acids. The failure of *ortho*-metalation using unprotected carboxylic acid group compelled us to use directed *ortho*-metalation in which a tertiary amide was used as a strong *ortho*-directing group. In the initial route, tertiary amide cleavage during final step was challenging, but cleaving the tertiary amide before Wittig reaction was beneficial. The Wittig reaction with protected carboxylic group (methyl ester) resulted in side-products whereas using sodium salt resulted in higher yields. The novel compounds were screened for antibacterial activity and cytotoxicity. Although substitution on the salicylic head group enhanced antibacterial activities they also enhanced cytotoxicity.

The advent of bacterial resistance due to global gene transfer (metallo-β-lactamase NDM-1),[1] and excessive use of antibiotics is life threatening posing a loss of therapeutic options.[2,3] Hence, there is an urgent need to develop new antibacterial agents to combat bacterial resistance. Anacardic acid and its derivatives were first isolated from the cashew *Anacardium occidentale* (Anacardiaceae)[4] and exhibited a broad range of biological activities such as antioxidant, anti-inflammatory, anti-cancer and antibacterial, including activity against MRSA.[5] During the course of our work[6] on the discovery of new antibiotics, we have synthesised a number of anacardic acid analogues and were interested to further explore their potential as antimicrobial agents. Naturally occurring anacardic acid is comprised of a salicylic acid group substituted with alkyl chains of 15–17 carbon length. Previous antibacterial studies based on this simple scaffold,[7,8] revealed a number of structure–activity relationships (SAR) (Figs. 1 and 2); (a) the salicylic acid moiety is important for antibacterial activity and protection leads to inactive compounds,[7] (b) modifying the carboxylic acid group to benzyl amine and its derivatives does not improve the activity,[9] (c) the hydrophobic 'tail' makes a significant contribution to the antimicrobial activity where an aliphatic alkyl chain or *cyclo*-octane were the most active and substituted phenolic groups were inactive, (d) protection of phenolic hydroxyl (ethyl or isopropyl ether) and benzamide derivatives of salicylic group are cytotoxic.[10] It is assumed that antibacterial activity of these molecules might be due to disorder in the fluid bilayer of the membrane or surfactant properties and non-specific activity. In this report, we describe an efficient method for the synthesis of anacardic acid derivatives and explore their antibacterial and cytotoxic activities. Our analogues modify the salicylic acid core structure by modulating the electron density of the aromatic ring and acidity of the protons while retaining a consistent 2-carbon linker to a naphthyl group.

A small set of salicylic acid variants were synthesised while retaining a constant hydrophobic naphthyl tail and short two carbon linker. The naphthyl side chain was introduced *via* Wittig reaction and aldehyde was installed using *ortho*-metalation of the substituted *o*-anisic acids (Scheme 1).

The initial synthetic route started from 2-methoxybenzoic acid (**1a**) that was subjected to an *ortho*-metalation reaction[11,12] with *s*-BuLi and in situ electrophilic substitution by formyl group (using

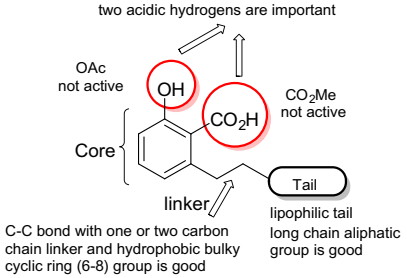

Figure 1. Preliminary SAR of anacardic acid analogues.

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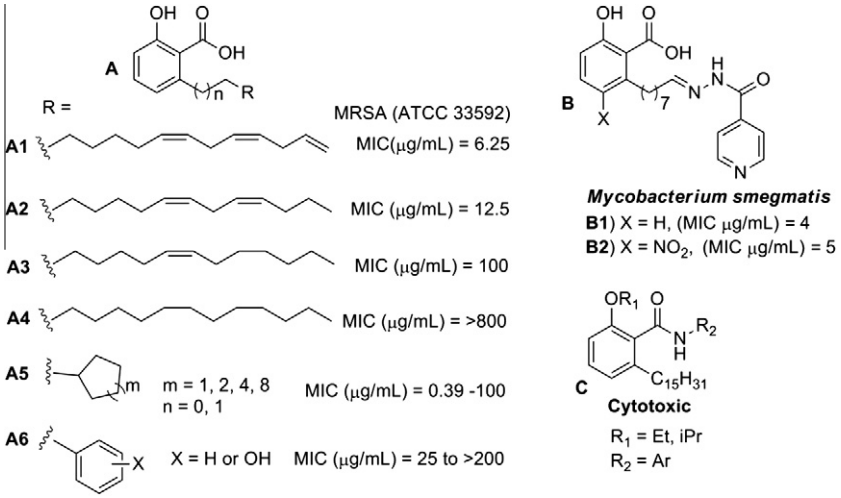
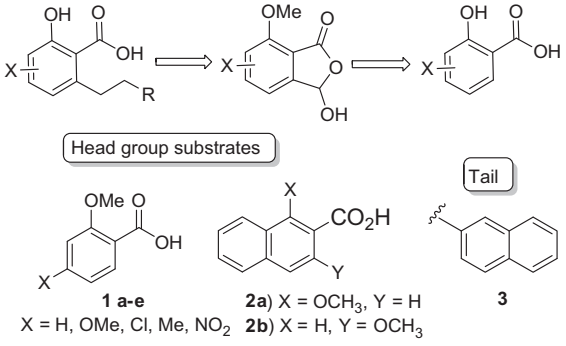


Figure 2. Known anacardic acid analogues.



Scheme 1. Retrosynthetic analysis.

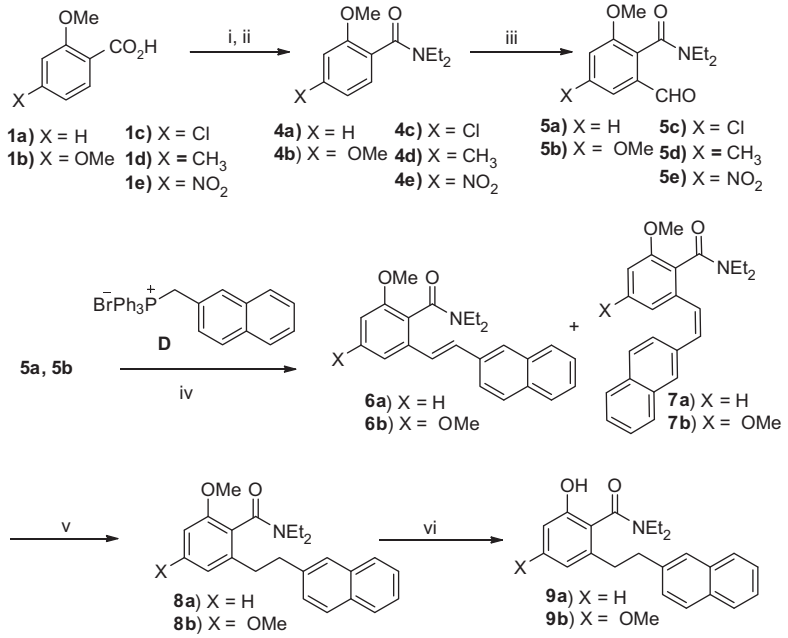
DMF as a source). The formation of the *ortho* substituted product was not observed. A similar reaction with 4-methoxy or 4-chloro substituted 2-methoxybenzoic acids (**1b**, **1c**) also failed to give the required product and instead resulted in a complex mixture. Challenges encountered in performing the *ortho*-metalation in the presence of an unprotected carboxylic acid group compelled us to use directed *ortho*-metalation (DoM)[13] where a tertiary amide was used as a strong *ortho*-directing group.[14] Thus, 2-methoxybenzoic acid (**1a**), on reaction with thionyl chloride at reflux, gave the acid chloride, which on treating with diethyl amine resulted in the formation of diethyl benzamide **4a**.[15] The compound **4a** was subjected to *ortho*-metalation using *s*-BuLi followed by in situ formylation to give the aldehyde **5a** in quantitative yield (Scheme 2).[16]

The aldehyde **5a** on Wittig reaction[17] with naphthyl ylide using *t*-BuOK in THF resulted in the formation of a mixture of *cis*/*trans* olefins **6a**, **7a.** The olefins were separated by column chromatography and characterised by LCMS, ^{1}H and ^{13}C NMR.[18] The double bond was reduced using 10% Pd/C under H_2 atmosphere to give the compound **8a**. The methyl ether of the compound **8a** was deprotected using 48% aq HBr solution at reflux temperature to give the compound **9a**. Similar reactions were performed with 2,4-dimethoxybenzoic acid (**1b**) to obtain the compound **9b**. The deprotection of diethylamide group was not achieved when conducted using aq HBr, alternative attempts to cleave diethylamide were also unsuccessful. Hence, we decided to cleave the diethyl amide group before conducting the Wittig reaction. The compound **5a** on treating with AcOH/HCl[19] at reflux temperature for 12 h gave the acid **10a**, which was in equilibrium with its corresponding lactol. The compound **10a** on reaction with naphthyl ylide using *t*-BuOK resulted in a complex mixture and failed to give the olefin.[20] Hence, the lactol was methylated using the known procedure by DBU and MeI,[21] to give methyl ester **11a** in 95% yield (Scheme 3). The compound **11a** on subjecting to the Wittig olefination reaction also failed to give the olefin resulting in the isolation of side products. The formation of by-products was explained based on the formation of methylated lactol and may be the formation of alkyne by ring opening with strong base such as *t*-BuOK.[20]

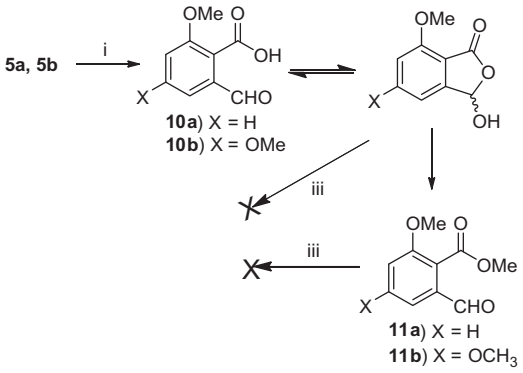
Alternatively, it was evident from the earlier report[20] that olefination reaction of the lactol using sodium salt would result in the formation of olefin in much higher yields than methyl ester. Hence, the olefination reaction of lactol **10a** was performed by the use of NaH at 50 °C, which resulted in the formation of olefin **12a** in quantitative yields (see Supplementary data). The olefin **12a** on deprotection with BBr_3 gave compound **13a**, which on reduction with Pd/C gave the compound **14a** (Scheme 4). Once the synthetic route was established we pursued the other 4-substituted 2-methoxybenzoic acid derivatives (viz. 4-Cl, 4-OMe, 4-CH_3, and 4-NO_2)[22] by, performing similar reactions that resulted in the formation analogues of **14a** (see Table S1 in Supplementary data). The *ortho*-metalation reaction 4-nitro-2-methoxybenzoic acid derivative (**4e**) failed to give the substituted aldehyde (**5e**) due to the strong deactivating nature of nitro group.

Once we accomplished the synthesis of 4-substituted analogues, we focused on sterically bulky groups. Thus, 1-methoxy and 3-methoxy naphthoic acids **2a**, **2b** were converted to tertiary amides **15a**, **15b**, respectively (Scheme 5). The compound **15a** on subjecting to directed *ortho*-metalation reaction gave the required aldehyde **16a** whereas the 3-methoxy derivative **15b** failed to give the *ortho*-substituted product. The aldehyde **16a** on deprotection with HCl/AcOH resulted in the formation of decomposition products. Therefore, the synthetic strategy failed with the bulky naphthyl derivatives. In continuing efforts to explore the new modification, we also explored other head group such as phenolic head group by performing similar reactions as explored for the previous compounds (Supplementary data).

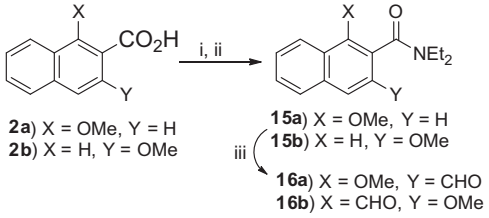
The compounds synthesized were tested for antibacterial activity and cytotoxicity using standard assay procedures. The antibacterial MIC were evaluated against sensitive and resistant Gram-positive strains (*Staphylococcus aureus-ATCC25923*, *MRSA-ATCC43300*,, *S. aureus-NRS 19*) and Gram-negative organisms (*Escherichia coli*, *Klebsiella pneumonia*, *Pseudomonas aeruginosae*)



Scheme 2. Reagents and conditions: (i) $SOCl_2$, reflux, 3 h; (ii) Et_2NH, CH_2Cl_2, 0 °C–rt, 12 h; (ii) *s*-BuLi/TMEDA, −78 °C, DMF; (iv) *t*-BuOK, THF, 0 °C–rt, 12 h; (v) Pd/C (10%) H_2 atm, rt, 1 h; (vi) 48% aq HBr, reflux, 5 h.

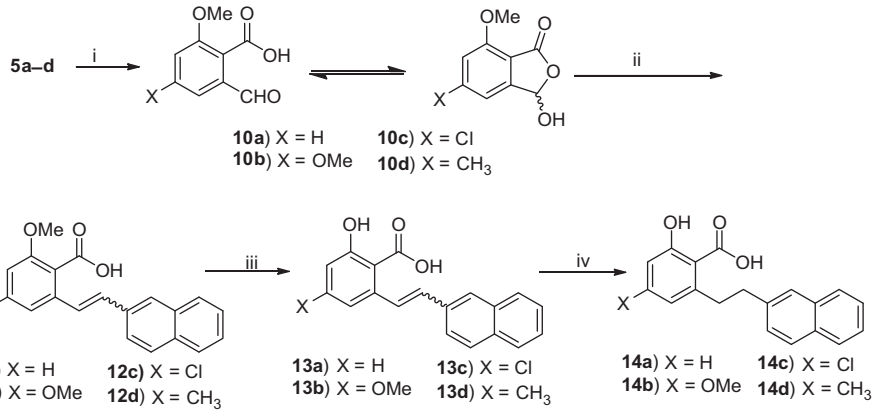


Scheme 3. Reagents and conditions: (i) AcOH/HCl, reflux, 12 h; (ii) DBU-MeI, MeCN, rt; (iii) D, *t*-BuOK, THF, 0 °C–rt, 12 h.



Scheme 5. Reagents and conditions: (i) $SOCl_2$, reflux, 3 h; (ii) Et_2NH, CH_2Cl_2, 0 °C–rt, 12 h; (iii) *s*-BuLi/TMEDA, −78 °C, DMF.

using CLSI standards[23] by twofold serial broth micro dilution. Of the compounds tested for antibacterial MIC, no compounds showed activity against G-negative bacteria and the compounds with tertiary amide did not show any activity against G-positive bacteria (Supplementary data). The compounds with free phenolic and carboxylic groups were active against Gram-positive bacteria. Among the compounds containing carbon–carbon double bond with free carboxylic acid, methyl ether compound **12c** showed 64 μg/mL against MRSA. The compounds containing carbon–carbon double bond linker with free carboxylic acid and phenolic group showed 16–64 μg/mL activities against Gram-positive



Scheme 4. Reagents and conditions: (i) AcOH/HCl, reflux, 12 h; (ii) D, NaH, THF, 50 °C, 2 h; (iii) BBr_3, CH_2Cl_2; (iv) Pd/C (10%), H_2 atm., rt, 1 h.

Table 1
Biological assay results

	Compounds											
	Vancomycin	**12a**	**13a**	**14a**	**12b**	**13b**	**14b**	**12c**	**13c**	**14c**	**12d**	**13d**	**14d**
G-positive (MIC μg/mL)													
S. aureus[a]	1	>128	64	>128	>128	>128	64	>128	16	16	>128	16	32
MRSA[b]	2	>128	64	>128	>128	128	64	64	16	16/32	>128	16	64
S. aureus[c]	1	>128	64	>128	>128	128	128	128	16	128	>128	32	128
Cytotoxicity (CC_{50} μM)													
HEK293	Nd	>100	97.0	>100	>100	65	65	93	38	35	119.2	52	40
HepG2	Nd	>100	71	>100	>100	79	71	104	25	35	117	30	38

[a] *ATCC25923*.
[b] *ATCC43300*.
[c] *NRS 19* (GISA, glycopeptide-intermediate *S. aureus*).

bacteria. The compounds containing carbon–carbon single bond linker with free carboxylic acid and phenolic group showed 16–128 μg/mL activities against Gram-positive bacteria. The cytotoxicity of the compounds was evaluated against HepG2 and HEK293 cell lines and is detailed in the Supplementary data. Compared to *o*-anisic acid derivatives the substituted *o*-anisic acid derivatives gave no significant improvement in antimicrobial activities but were found to be more cytotoxic (Table 1).

In summary we have developed an efficient method for the synthesis of anacardic acid derivatives. The failure of *ortho*-metalation in the presence of an unprotected carboxylic acid group compelled us to use directed *ortho*-metalation where a tertiary amide was used as a strong *ortho*-directing group. In the initial route using tertiary amide cleavage during the final step was challenging and cleaving the tertiary amide before Wittig reaction was beneficial. The Wittig reaction with protected carboxylic group (methyl ester) resulted in side-products and using sodium salt resulted in higher yields. The new compounds synthesized were screened for antibacterial activity and cytotoxicity. Although substitution on the salicylic head group improved antibacterial activities the compounds were cytotoxic and were not pursued as antibacterial leads.

Acknowledgements

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Supplementary data

Supplementary data (experimental details for the synthesis of new compounds, analytical data including NMR spectra and details of biological assays) associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2013.01.074.

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