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Development of Carprofen analogues with activity against *Mycobacterium tuberculosis*

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ABSTRACT

Carprofen, a veterinary non-steroidal anti-inflammatory drug, has demonstrated bactericidal activity against *Mycobacterium tuberculosis* and the closely related model organism *M. bovis* BCG. Herein, we present the SAR-driven optimisation of three series of carbazole-based carprofen analogues for increased antimycobacterial potency and selectivity over the human monocyte-derived THP-1 cell line. An efficient synthetic route was employed to assemble a range of carprofen analogues which were then evaluated in whole-cell phenotypic assays to establish their activity against well-studied model organisms for *M. tuberculosis*. The most promising compound was further profiled against *M. tuberculosis* H37Rv, confirming the identification of a potent antitubercular carbazole with significantly enhanced therapeutic potential.

1. Introduction

Tuberculosis (TB) remains one of the leading challenges to global health. The emergence of extensively drug-resistant (XDR) strains of *Mycobacterium tuberculosis* threatens decades of progress in the treatment of this disease. In their Global Tuberculosis Report 2024, the World Health Organization (WHO) stated that TB was the world's leading cause of death from a single infectious agent with an estimated 1.25 million deaths in 2023 and over 10 million new cases every year.[1] Of those who developed active TB in 2023, an estimated 440,000 have rifampicin-resistant or multidrug-resistant TB (RR- or MDR-TB). There is, therefore, an urgent need for the identification of new drug candidates to strengthen the antitubercular drug development pipeline.

In recent years, a range of synthetic antimicrobial carbazoles have been reported in the literature with potent activities against the fungal pathogen *Candida albicans*,[2,3] the Gram-positive ESKAPE pathogens: Enterococcus faecalis,[3,4] Methicillin-resistant *Staphylococcus aureus* (MRSA),[3,5,6] and the Gram-negative ESKAPE pathogens: *Escherichia coli*,[3,5] *Klebsiella pneumoniae*[5] and *Pseudomonas aeruginosa*.[5]

Several antitubercular carbazoles have also been reported. In 2005, Ma et al.[7] described the identification of a small group of six antitubercular carbazole alkaloids from the stem bark of *Micromelum hirsutum*. This work was built upon by Choi et al.[8] in 2006, and Börger et al.[9] in 2016, who identified potent synthetic antitubercular carbazoles with MIC values as low as 1.5 μM and SI values of >10. The presence of oxygenated functional groups in the 3-position of the carbazole scaffold appear frequently among the most potent and selective examples, as do C-2 hydroxyl groups. However, the metabolic liabilities associated with the aldehyde and phenol moieties prevalent among these compounds make them challenging candidates for development as antitubercular drug candidates.

In our previous work we have identified carprofen, a carbazole-based non-steroidal anti-inflammatory drug (NSAID), as a compound of interest due to its moderate potency (MIC = 146 μM (40 μg/ml) against *Mtb* $H_{37}Rv$) and low cytotoxicity, which results in a high selectivity (SI = 25) for toxicity to *Mtb* $H_{37}Rv$ over the RAW 264.7 mouse macrophage cell line.[10] Further characterisation of the effect of carprofen on *Mtb* demonstrated that carprofen is able to inhibit whole-cell efflux

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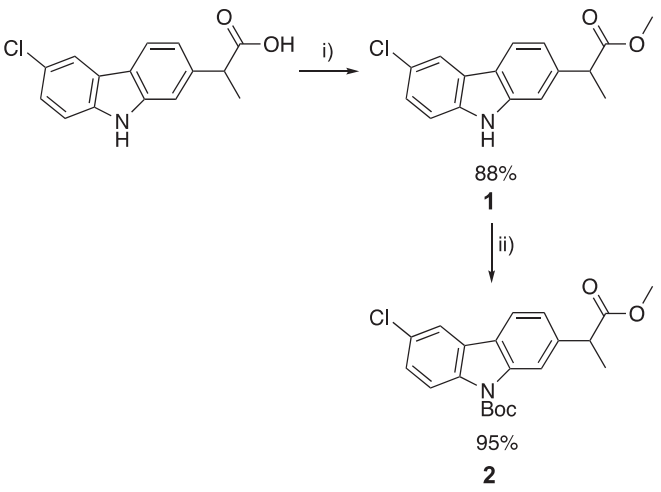
mechanisms and disrupt biofilm formation, two key mechanisms of intrinsic antimicrobial resistance.[11,12] Carprofen also disrupts the membrane potential of mycobacteria and methicillin-resistant *Staphylococcus pseudintermedius* via proton translocation.[13,14]

In the current study we performed SAR-driven optimisation of carprofen to identify structural features which are responsible for its antimycobacterial activity and to develop more potent and selective analogues. Novel carbazoles were synthesised and then screened against the model organisms *Mycobacterium smegmatis* (mc^{2}155) and *Mycobacterium bovis* BCG in the liquid-based resazurin microtiter assay (REMA)[15–17] and agar-based high-throughput spot-culture growth inhibition (HT-SPOTi) assay.[18,19] *M. smegmatis* (mc^{2}155) is a fast-growing mycobacterium with overall ~70 % gene sequence homology with *Mtb*, while *M. bovis* BCG is a slow-growing mycobacterium which has genomic homology of >99.95 % with *Mtb* and can be safely handled in a BSL 2 facility.[20] Promising analogues were further tested against *Mtb* H_{37}Rv and *Mycobacterium abscessus* (ATCC 19977), a causal pathogen for TB-like respiratory diseases (particularly in patients with chronic lung conditions such as chronic obstructive pulmonary disease (COPD), asthma, cystic fibrosis, bronchitis[21,22]).



Scheme 1. Synthesis of protected intermediate **2** for synthesis of carbazoles in **Series 1**. i) *p*TSA, MeOH, reflux, 18 h, ii) Boc_2O, DMAP, MeCN, RT, 1 h.

2. Results and discussion

To aid in the identification of more potent analogues of carprofen, we established structure activity relationships (SAR) around the A-ring and C-ring of the central carbazole scaffold (Fig. 1). Three series were synthesised and evaluated for their antimycobacterial activity and mammalian cell cytotoxicity.

Series 1 was designed to explore the effect of aromatic and aliphatic cyclic substituents at the C-6 position on the A-ring of carprofen. This was achieved through derivatisation of carprofen via Pd-catalysed coupling chemistry. Commercially-sourced carprofen was first converted to the corresponding methyl ester (**1**) by esterification,[23] followed by Boc protection of the carbazole nitrogen to give the fully protected carprofen derivative, **2** (Scheme 1). This was then taken forward into Suzuki-Miyaura coupling reactions with a range of boronic acids to afford compounds (**3**–**7**, Table 1). Similarly, carbazoles **8** and **9** were prepared by the Buchwald-Hartwig amination of **2** with the cyclic amines piperidine and morpholine, respectively (Table 2). Compounds **3**–**9** were then sequentially Boc-deprotected and hydrolysed to give the final compounds **10**–**16** for screening (Table 3).

Compounds **12** and **13** were identified as the most promising analogues in **Series 1** (Table 4), as both had MIC values of 24 μM (7.8 μg/ml) in the HT-SPOTi assay and SI values of 8, based on this data. However, both showed weaker activity in the REMA assay, with MICs of 90 μM (31.3 μg/ml) and 181 μM (62.5 μg/ml), respectively, resulting in reduced SI values of 2 and 1. This represented a significant increase in antimycobacterial activity relative to carprofen, with some small gains in selectivity. That all of the C-6 aryl-substituted carbazoles in this series maintained significant antitubercular activity in both assays suggests that large lipophilic substituents in this position are broadly tolerated. However, the high cytotoxicity observed for this series meant that they were not taken forward for further optimisation. It was nevertheless observed that all of the compounds from this series had a clear selectivity towards the slow-growing *M. bovis* BCG over the fast-growing *M. smegmatis* (Table S1). This trend was observed throughout this study and is consistent with previous observations for the parent compound, carprofen.[13]

To explore the influence of C-ring substitution on the activity of carprofen analogues, **Series 2** was designed and synthesised. First, a Buchwald-Hartwig amination was performed between 4-chloroiodobenzene and the appropriate aniline (**17**, **18**, **19** or **20**) to give diarylamines **21**–**24** (Scheme 2). The substituted anilines (**18**, **19** and **20**) required for this step were prepared from the corresponding nitrophenylacetic acids by Fischer esterification followed by nitro reduction (Scheme S1). Diarylamines **21**–**24** then underwent oxidative cyclisation with $Pd(OAc)_2$ in pivalic acid, under conditions reported by Liegault et al.[24] to afford carbazoles **25**–**29**. The cyclisation of **23** produced regioisomers **27** and **28** which were both isolated. Carbazoles **26**–**29** were then de-esterified with sodium hydroxide in methanol and dichloromethane to afford acids **30**–**33** (Scheme 2).

In **Series 2** (Table 5), it was apparent that the presence of a methyl ester in place of a carboxylic acid was generally beneficial, as the most potent and selective compounds, **26** and **28** were both methyl esters. It was also observed that the presence of a methyl group alpha to the carbonyl of the carboxylic acid/ester either increased or had no effect on cytotoxicity. The increased cytotoxicity was especially pronounced for the methyl esters **1** and **29**, relative to their counterparts **28** and **26**, respectively. C-ring regiochemistry was found to have an important but inconsistent influence on the overall activity. It was clear that C-4 substitution was deleterious to antimycobacterial potency. C-3 substitution was found to confer the most desirable activity to the carboxylic acid-bearing carbazoles, with **30** displaying increased antimycobacterial potency and selectivity compared to **32**. However, for methyl esters the reverse was true, as C-2 substituted carbazole **28** displayed increased potency and selectivity compared to **26**. As was observed for **Series 1**, all compounds from **Series 2** had a clear selectivity for activity against *M. bovis* BCG over *M. smegmatis* mc^{2}155 (Table S2).

Series 3 was designed to build on the observations made in **Series 1** and **2**. Smaller acyclic substituents were explored at the C-6 position of the carbazole ring, with continued exploration of the C-ring substituent

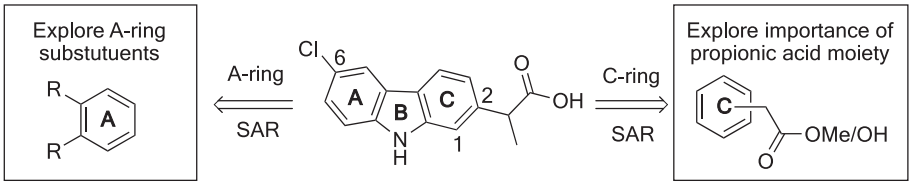
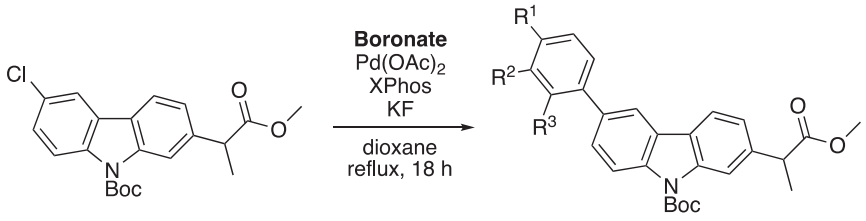


Fig. 1. Overview of antimycobacterial structure activity relationships investigated based on the structure of carprofen.

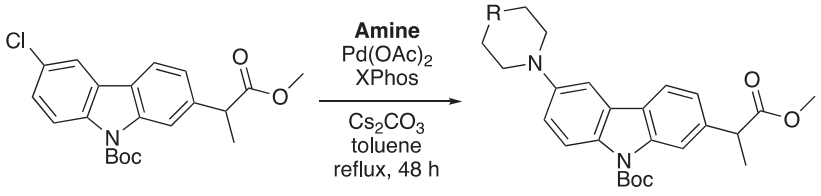
Table 1
Suzuki-Miyaura coupling reactions to afford aryl-carbazoles.



2 **3-7**

Product	R^1	R^2	R^3	Yield
3	H	H	H	95 %
4	Me	H	H	86 %
5	OMe	H	H	75 %
6	H	OMe	H	85 %
7	H	H	OMe	44 %

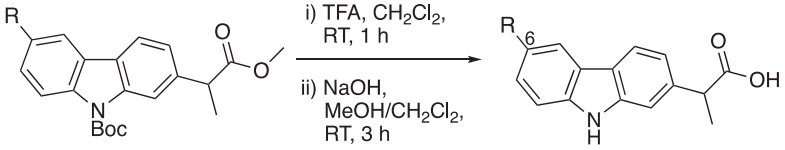
Table 2
Buchwald-Hartwig aminations to afford cyclic-amide substituted carbazoles.



2 **8 & 9**

Product	R =	Yield
8	CH_2	46 %
9	O	12 %

Table 3
Boc-deprotection and ester hydrolysis to afford carprofen analogues. [a] = left to stir for 18 h, [b] = yield after recrystallisation.



3-9 **10-16**

Starting material	Product	Yield
3	**10**	49 %
4	**11**	52 %
5	**12**	63 %
6	**13**	16 %[b]
7	**14**	35 %
8	**15**	6 %[ab]
9	**16**	10 %[b]

at the C-3 position. The compounds in this series were prepared in a similar manner to those in **Series 2**. First, diarylamines **34**–**40** were prepared by Buchwald-Hartwig amination between the appropriate aryl halide and methyl 2-(4-aminophenyl)acetate (Table 6).

Diarylamines **34**–**40** were then cyclised to carbazoles **41**–**47** under modified oxidative cyclisation conditions (Table 7). For these conversions, copper acetate was added to the reaction mixture to increase the rate of re-oxidation of Pd(0) to Pd(II), increasing the reaction rate. This approach has been reported in acetic acid by Schuster *et al.*[42], however to the best of our knowledge has not been applied to oxidative cyclisation with pivalic acid as solvent. This method allowed for the shortening of the reaction time to 6 h and was generally observed to improve the yields obtained. Carbazoles **41**–**47** were then de-esterified to give their carboxylic acid-bearing counterparts **48**–**54** (Table 8).

Table 4
Antimycobacterial and mammalian cell cytotoxicity screening data for Series 1. Antimycobacterial MIC values were obtained by REMA and HT-SPOTi assays with *M. bovis* BCG. THP-1 cytotoxicity data was obtained via a mammalian REMA. SI values were calculated based on MIC values obtained from REMA and HT-SPOTi assays. All assays were performed in biological triplicate. MIC and GIC values are reported in brackets μM.

	MIC vs *M. bovis* BCG		THP-1	Selectivity Index	
Compound	REMA	HT-SPOTi	GIC_{90}	REMA	HT-SPOTi
10	198	52	198	1	4
11	95	25	95	1	4
12	90	24	181	2	8
13	181	24	181	1	8
14	361	24	362	1	8
15	775	203	1551	2	8
16	> 771	> 771	> 771	–	–
CRP	456	114	456	1	4
INH	0.5	0.7	> 3646	> 7000	> 5000

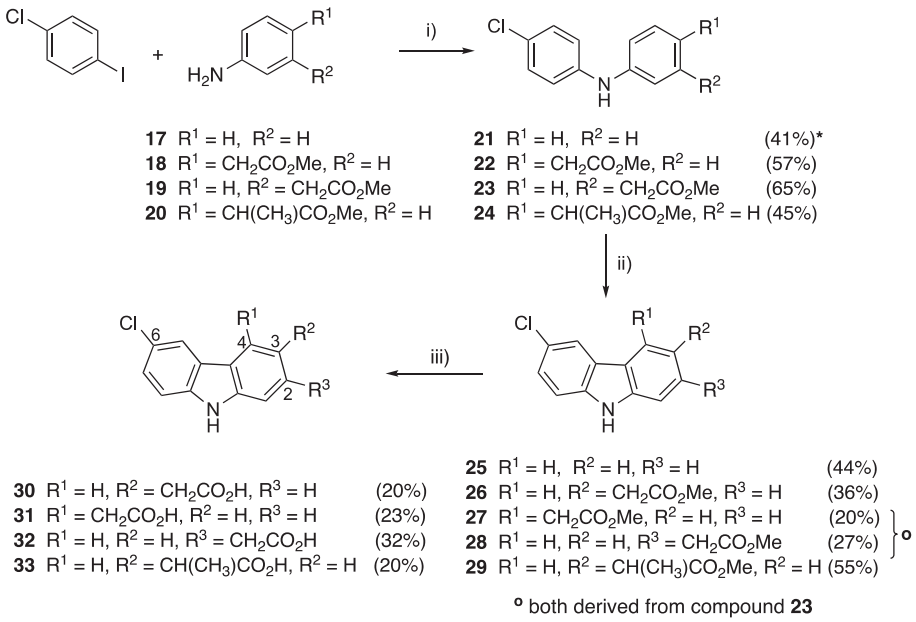
Evaluation of **Series 3** (Table 9) further highlighted that the methyl ester-bearing analogues were more potent and selective than their carboxylic acid counterparts and further confirmed the observation from **Series 2** that the presence of an α-methyl on the C-ring substituent results in a significant increase in mammalian cell cytotoxicity, with little improvement in potency against *M. bovis* BCG. It is notable that the C-7 chloro-substituted carbazole **44** was found to have similar activity to its C-6-substituted regioisomer **26**, with slightly improved potency against *M. bovis* BCG, though at the cost of a slight increase in cytotoxicity, within an acceptable SI range. This suggest that there may be value in further exploration of C-ring regiochemistry to develop more potent analogues of the compounds described here. As observed for **Series 1** and **2**, compounds from **Series 3** had minimal activity against *M. smegmatis* (**Table S3**).

The C-6 trifluoromethyl-substituted carbazole **42**, emerged from **Series 3** as the most potent and selective carprofen analogue identified in this study. This is in line with a general preference observed in this study for lipophilic, electron-withdrawing substituents on the A-ring. The trifluoromethyl group of **42** may increase lipophilic contacts with the as-yet unknown target protein(s) or may increase pi-stacking interactions through the electron withdrawing effect of this group on the carbazole ring system. The increased lipophilicity may also help this compound to penetrate the lipophilic mycobacterial cell well.[26] When potency against *M. bovis* BCG was plotted against ALogP[27] a clear trend was observed whereby increased lipophilicity correlated with increased potency (Fig. 2). As mentioned above, this may be due to the increased permeability of hydrophobic compounds through the waxy mycobacterial cell wall.[28–31] Compounds **26**, **28**, **42**, **44**, **45** and **47**, were found to sit below this trendline, suggesting that their increased potency may not be driven solely by increased lipophilicity and further optimisation should be considered to identify potent analogues which do not have undesirably high lipophilicity.

Due to its promising activity against *M. bovis* BCG, and low toxicity to mammalian cells, compound **42** was further profiled against *Mtb* $H_{37}Rv$ and *M. abscessus* (ATCC 19977), an intrinsically resistant, environmental mycobacterium that causes TB-like respiratory diseases (Table 10). The minimum inhibitory concentration (MIC) of carprofen against *Mycobacterium tuberculosis* $H_{37}Rv$ (*Mtb*) was found to be 228 μM (62.5 μg/mL), whilst **42**, inhibited *Mtb* growth between 13 μM (3.91 μg/mL) and

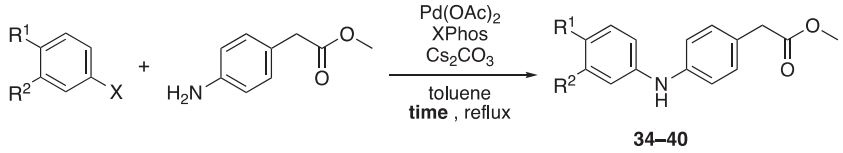
Table 5
Antimycobacterial and mammalian cell cytotoxicity screening data for Series 2. Antimycobacterial MIC values were obtained by REMA and HT-SPOTi assays with *M. bovis* BCG. THP-1 cytotoxicity data was obtained via a mammalian REMA. SI values were calculated based on MIC values obtained from REMA and HT-SPOTi assays. All assays were performed in biological triplicate. MIC and GIC values are reported in μM.

	MIC vs *M. bovis* BCG		THP-1	Selectivity Index	
Compound	REMA	HT-SPOTi	GIC_{90}	REMA	HT-SPOTi
25	> 2480	77	> 2480	–	> 32
26	114	57	1827	16	32
27	> 1827	> 1827	> 1827	–	–
28	57	29	> 1827	> 32	> 64
29	109	54	109	1	2
30	481	120	962	2	8
31	963	963	1925	2	2
32	963	241	962	1	4
1	109	54	220	2	4
33	457	114	457	1	4
CRP	456	114	456	1	4
INH	1.5	1.5	> 500	> 2500	> 2500



Scheme 2. i) $Pd(OAc)_2$, XPhos, Cs_2CO_3, toluene, reflux, 24 h; ii) $Pd(OAc)_2$, K_2CO_3, PivOH, 100–130 °C, 18–24 h; iii) NaOH, $MeOH/CH_2Cl_2$, rt, 20 h; *Conditions: $Pd(OAc)_2$, $P(^tBu)_3.HBF_4$, NaO^tBu, toluene, reflux, 20 h.

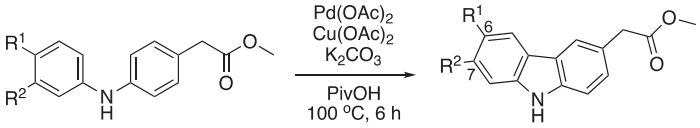
Table 6
Synthesis of diarylamines by Buchwald-Hartwig amination.



34–40

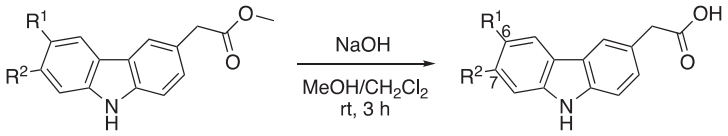
Aryl halide					
X	**R^1**	**R^2**	**Reaction time**	**Product**	**Isolated Yield**
I	F	H	18 h	**34**	67 %[a]
I	CF_3	H	18 h	**35**	86 %[a]
Br	H	H	2 h	**36**	72 %
Br	H	Cl	2 h	**37**	91 %
Br	Me	H	2 h	**38**	86 %
Br	OMe	H	2 h	**39**	76 %
Br	Br	H	18 h	**40**	24 %

Table 7
Assembly of carbazoles via oxidative cyclisation of diarylamines.



Starting Material	R^1	R^2	Product	Isolated Yield
34	F	H	**41**	33 %
35	CF_3	H	**42**	44 %
36	H	H	**43**	63 %
37	H	Cl	**44**	58 %
38	CH_3	H	**45**	63 %
39	OCH_3	H	**46**	68 %
40	Br	H	**47**	22 %

Table 8
Ester hydrolysis of **41–47** to afford the carboxylic acid analogues **48–54**. [a] = yield after recrystallisation.



Starting Material	R^1	R^2	Product	Isolated Yield
41	F	H	**48**	81 %
42	CF_3	H	**49**	66 %
43	H	H	**50**	30 %[a]
44	H	Cl	**51**	64 %
45	CH_3	H	**52**	37 %[a]
46	OCH_3	H	**53**	24 %[a]
47	Br	H	**54**	64 %

51 μM (15.63 μg/mL).

Interestingly, carprofen (MIC = 456 μM (125 μg/mL)) and its analogue **42** (MIC > 1627 μM (> 500 μg/mL)) are less able to inhibit *M. abscessus* growth (Table 10), which suggests a specific mechanism of action (MOA) against non-tubercular mycobacteria (NTM) and/or the intrinsic resistance mechanisms of *M. abscessus* are more efficient in repelling carprofen and **42**'s anti-NTM action. One of carprofen's MOA has been determined as disrupting the proton motive force (PMF)[13,14], which powers mycobacterial efflux pumps, except for the ATP-binding cassette (ABC) family[11]. Due to differences in efflux pump between mycobacterial species[32], it is possible that carprofen and compound **42** are being pumped out differently.

3. Conclusions

Three chemical series were designed, synthesised and evaluated for their antimycobacterial potency and selectivity. **Series 1** highlighted that large substituents were tolerated in the 6-position of the carbazole scaffold, with a preference for aromatic, lipophilic rings observed. However, compounds in this series were not developed further due to their high cytotoxicity and lipophilicity. It was observed from **Series 2** and **3** that the α-methyl of the propionic acid moiety of carprofen increases cytotoxicity without an appreciable gain in potency, that C-4 substitution was detrimental to antimycobacterial potency and that carbazoles bearing methyl acetate substituents had improved potency

Table 9
Antimycobacterial and mammalian cell cytotoxicity screening data for Series 3. Antimycobacterial MIC values were obtained by REMA and HT-SPOTi assays with *M. bovis* BCG. THP 1 cytotoxicity data was obtained via a mammalian REMA. SI values were calculated based on MIC values obtained from REMA and HT-SPOTi assays. All assays were performed in biological triplicate. MIC and GIC values are reported in μM.

	MIC vs *M. bovis* BCG		THP-1	Selectivity Index	
Compound	REMA	HT-SPOTi	GIC_{90}	REMA	HT-SPOTi
41	243	243	486	2	2
42	25	13	814	32	64
43	2090	31	2090	1	16
44	114	28	913	8	32
45	123	62	493	4	8
46	116	116	464	4	4
47	49	49	786	16	16
48	514	128	1028	2	8
49	426	107	426	1	4
50	2220	139	2220	1	16
51	481	12	963	2	8
52	522	131	1045	1	8
53	979	277	1959	2	8
54	822	205	822	1	4
INH	0.5	0.7	3646	> 7000	> 5000

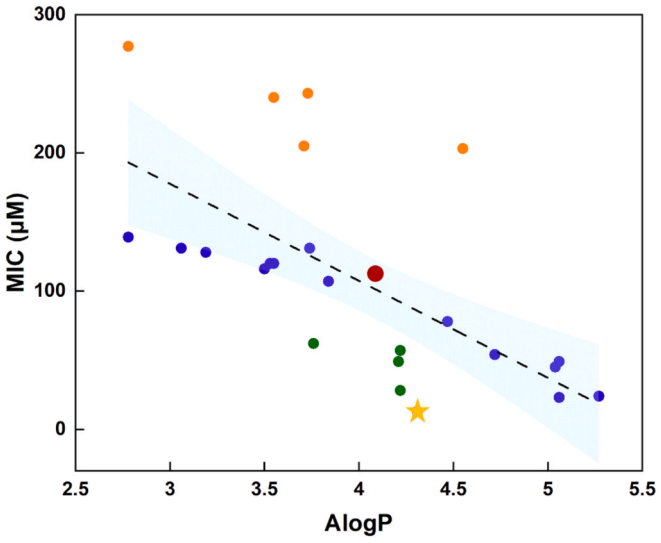


Fig. 2. Plot of MIC against AlogP for compounds from all carbazole screening groups. All compounds from Series 1, 2 and 3 are plotted, with the exception of **16**, **27** and **31**. MIC values are plotted in μM. AlogP values were calculated for each compound using lead-likeness and molecular analysis (LLAMA) software.[27] The dashed line indicates linear regression analysis ($R^2 = 0.44$). The light blue shading indicates the 95 % confidence interval around this trendline. Compounds which fit with the trend are in blue, those which are above the trendline are in orange and those which are below the trendline are in green. Carprofen is shown as a red start while **42** is shown as a yellow star. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

and selectivity when compared to the corresponding acids. Substituents at the C-2, C-3, C-6, and C-7 were all tolerated, with some small changes in antimycobacterial activity and/or mammalian cell cytotoxicity between paired compounds with substituents in either position. This leaves many vectors available for future elaboration, building on the SAR established in this study. The low cytotoxicity observed for **Series 2** and **3** provide some support for the approach taken in this study, whereby a clinically approved compound with a known toxicity profile is repurposed as the starting point a drug discovery campaign for an indication which is distinct from its initial use. This approach may have been helpful in avoiding cytotoxicity which has been observed for some carbazole natural products.[33,34]

The most potent and selective carprofen analogue identified in this study was the C-6 trifluoro-substituted carbazole **42.** Further profiling of this compound in infection models and against drug-resistant TB strains is warranted. However, given that **42** has a low molecular weight (307 g/mol) there is also a significant opportunity to further optimise potency and physicochemical properties to generate a more lead-like anti-tubercular compound. Given that **42** features a methyl ester moiety, which is a potential metabolic liability, future optimisation should include an exploration of bioisosteres of this functional group in order to avoid potential hydrolysis by host esterases, while retaining anti-mycobacterial activity.[35,36]

4. Methods

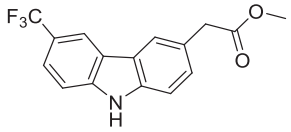
4.1. High-throughput spot culture growth inhibition (HT-SPOTi) assay

HT-SPOTi is a rapid but reliable assay that is principally based on the direct observation of growth of a microorganism on an agar medium containing a range of different concentrations of compounds.[19] Briefly, a two-fold serial dilution of each compound in DMSO was prepared in a master plate and an aliquot (2 μl) was transferred from each well to the equivalent well on the experimental 96-well plate (CELLSTAR® 96-well culture plate, sterile F-bottom with lid). M7H10 agar (200 μl) was dispensed into each well using a Multidrop Combi reagent dispenser (Thermo Scientific) and allowed to solidify. A culture of the bacilli under investigation (*M. smegmatis mc^{2}155* and *M. bovis* BCG) in M7H9 broth was diluted to a concentration of 1×10^6 cells/ml.[37] This culture (2 μl, ~2000 cells) was then dispensed into each well using a Multidrop Combi. The prepared 96-well plates were then wrapped in laboratory film and aluminium foil and placed lid-down in an incubator at 37 °C. For assays with *M. smegmatis* the results were observed after 3 days, for *M. aurum* the results were observed after 5 days, while for *M. bovis* BCG, the results were observed after 14 days. After the incubation period, MICs were visually determined by observing the lowest concentration of each compound at which no bacterial growth could be observed. This HT-SPOTi assay was performed in three biological replicates.

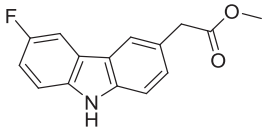
4.2. Resazurin microtiter assay (REMA)

Resazurin is a rapid colourimetric redox indicator. When reduced to resorufin, the solution can be visibly seen as pink, indicating aerobically active bacterial cells. To determine the MIC of compounds against a panel of mycobacterial strains in liquid culture, a REMA[16] was used with further modification and optimisation. Briefly, a two-fold serial dilution of each compound was prepared in a 96-well plate in 100 μL of Middlebrook 7H9 (Difco™) supplemented with 10 % ADC (fast-growing mycobacteria) and OADC (slow-growing mycobacteria). An actively replicating culture ($O.D._{600nm} \sim 1.0$) of the mycobacterial species under investigation (100 μL) was diluted to a cell density of $\sim 1 \times 10^6$ cells/ml was then added to each well for a final density of $\sim 1 \times 10^3$ cells/ml. One row of the 96-well plate was left without cells, as a sterility control, and a DMSO-only control was also included. For our initial screening with *M. smegmatis* mc^{2}155, the plates were then placed in a standing incubator at 37 °C for 24 h (8 generations). For *M. bovis* BCG, the plates were incubated at 37 °C for 7 days (9 generations). After incubation, resazurin solution (0.1 %, 30 μl) was added to each well. The plates were sealed with parafilm and wrapped in aluminium foil and placed in a standing incubator (Genlab) at 37 °C. For *M. smegmatis*, the plates were incubated at 37 °C for 24 h (8 generations). For *M. bovis* BCG, the plates were incubated at 37 °C for 48 h (3 generations). After the incubation period, MICs against *M. smegmatis* mc^{2}155 and M. bovis BCG were visually determined by observing the lowest concentration of each compound at which the resazurin dye was not reduced from blue to pink. Later in our investigation, while recruiting *M. abscessus* (ATCC 19977) and

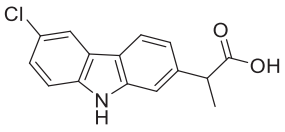
Table 10
Further characterisation of compound 42 (and 41 and carprofen) against *Mtb* H37Rv and *M. abscessus.* MIC values were determined by REMA assay and are given in μM, with values in μg/ml given in brackets.



42



41



CRP

Compound	MIC in μM (μg/ml)			
	***M. smegmatis* mc^2155**	***M. bovis* BCG**	***M. tuberculosis* $H_{37}Rv$**	***M. abscessus* (ATCC 19977)**
42	> 1627 (> 500)	25 (7.8)	13–51 (3.9–15.6)	> 1627 (> 500)
41	> 1944 (> 500)	243 (62.5)	> 1944 (> 500)	> 1944 (> 500)
CRP	1827 (500)	456 (125)	228–456 (62.5–125)	912 (250)

M. tuberculosis $H_{37}Rv$, we validated that 5 min of incubation of mycobacterial cells with compounds and incubating resazurin over 12 generations of mycobacterial growth is optimum for the drug susceptibility assay (REMA) output. MICs against *M. abscessus* (ATCC 19977) and *M. tuberculosis* H37Rv were determined using a plate reader (Biotek Synergy 2) at A_{570} and A_{600}. Relative growth was established by subtraction of media and correction factor to determine MIC. To calculate the correction factor (R_o)[38], the following formula was used:

$$R_o = \frac{A_{570}\ of\ 0.01\%\ Resazurin\ in\ media - A_{570}\ media}{A_{600}\ of\ 0.01\%\ Resazurin\ in\ media - A_{600}\ media}$$

Then to calculate the relative growth, the following formula was used:

$$Relative\ growth = A_{570} - (A_{600} \times R_o)$$

Resazurin Microtiter Assay (REMA) was performed in three biological replicates.

4.3. Mammalian cell cytotoxicity assay

To determine the GIC of compounds against the human monocyte-derived THP-1 cell line in liquid culture, a resazurin-based mammalian-cell cytotoxicity assay was used. This assay was performed according to a previously reported protocol.[39]

Briefly, a two-fold serial dilution of each compound was prepared in a 96-well plate in 100 μL of foetal bovine serum-supplemented Roswell Park Memorial Institute (RPMI) media. A culture of THP-1 cells was diluted to a density of 5×10^5 cells/ml and 100 μl was added to each well of the 96-well plate for a final density of 2.5×10^5 cells/ml. One row of the 96-well plate was left without cells, as a sterility control and a DMSO-only control was also included. The cells were then incubated without shaking (37 °C, 5 % CO_2) for 48 h. The cells were then pelleted by centrifugation of the 96-well plates (270 × g, 2 min) and the spent media was removed by pipetting. The media was replaced with complete RPMI (170 μl) and resazurin solution (30 μl). The plate was then wrapped in aluminium foil and the cells were incubated for a further 24 h (37 °C, 5 % CO_2). After the incubation period, GIC values were visually determined by observing the lowest concentration of each compound at which the resazurin dye was not reduced from blue to pink. Where one value was observed to differ from the others, the value obtained for the two measurements which were concordant was taken as the GIC value. If all three measurements for a given compound were different, or in the case of a difference of greater than 2-fold dilution between results, the assay was repeated for that compound.

4.4. Syntheses

4.4.1. Methyl 2-(6-chloro-9H-carbazol-2-yl)propanoate (1)[40]

To a solution of carprofen (100 mg, 0.37 mmol) in MeOH (5 ml), was added *p*TSA monohydrate (8.00 mg, 0.04 mmol). The reaction mixture was heated to reflux and stirred for 18 h. The reaction was allowed to cool to RT before the solvent was removed from the reaction mixture under reduced pressure. The crude product was dissolved in EtOAc (20 ml) and washed with saturated $NaHCO_{3(aq)}$ (3 x 20 ml). The organic layer was dried over anhydrous Na_2SO_4 and the solvent was removed to afford the product as an off-while solid (93 mg, 88 %). M.p 116–120 °C; IR (neat, ν_{max}/cm^{-1}): 3358, 1715; ^1H NMR (700 MHz, $CDCl_3$) δ 8.16 (1H, s, **NH**), 7.97 (1H, s, **5-ArH**), 7.93 (1H, d, J = 8.0 Hz, **4-ArH**), 7.37–7.31 (2H, m, **2 x ArH**), 7.28 (1H, d, J = 8.5 Hz, **8-ArH**), 7.18 (1H, dd, J = 8.0, 1.6 Hz, **3-ArH**), 3.89 (1H, q, J = 7.2 Hz, **C*H*CH$_3$**), 3.70 (3H, s, **CO$_2$CH$_3$**), 1.59 (3H, d, J = 7.2 Hz, **CHC*H*$_3$**); ^{13}C NMR (176 MHz, $CDCl_3$) δ 175.5, 140.5, 139.3, 138.2, 126.0, 125.0, 124.4, 121.8, 120.8, 120.0, 119.8, 111.7, 109.7, 52.3, 46.0, 19.1; *m/z* [ES+] 288 ([M+H]$^+$, 7 %); 228 ([^{35}M–COOMe]$^+$, 100 %), 230 ([^{37}M–COOMe]$^+$, 35 %); *m/z* [HRMS, ES+] found [M+H]$^+$ 288.0782, $C_{16}H_{15}ClNO_2$ requires 288.0786.

4.4.2. tert-Butyl 3-chloro-6-(1-methoxy-1-oxopropan-2-yl)-9H-carbazole-9-carboxylate (2)

Methyl 2-(6-chloro-9H-carbazol-2-yl)propanoate (660 mg, 2.3 mmol), di-*tert*-butyl dicarbonate (950 mg, 4.36 mmol) and DMAP (280 mg, 2.3 mmol) were dissolved in anhydrous acetonitrile (10 ml) and stirred at RT for 1 h. The solvent was removed from the reaction mixture under reduced pressure and the crude product was purified by flash column chromatography (1–5 % EtOAc in *n*-hexane) to afford the product as a white solid (842 mg, 95 % yield). Mp 131–133 °C; IR (neat, ν_{max}/cm^{-1}): 2979 (weak), 1736, 1708; ^1H NMR (700 MHz, $CDCl_3$) δ 8.27 (1H, s, **1-ArH**), 8.23 (1H, d, J = 9.0 Hz, **8-ArH**), 7.90 (1H, d, J = 2.2 Hz, **5-ArH**), 7.87 (1H, d, J = 7.9 Hz, **4-ArH**), 7.39 (1H, dd, J = 8.8, 2.2 Hz, **7-ArH**), 7.31 (1H, dd, J = 8.1, 1.7 Hz, **3-ArH**), 3.90 (1H, q, J = 7.2 Hz, **C*H*CH$_3$**), 3.68 (3H, s, **CO$_2$CH$_3$**), 1.77 (9H, s, **C(CH$_3$)$_3$**), 1.60 (3H, d, J = 7.2 Hz, **CHC*H*$_3$**); ^{13}C NMR (176 MHz, $CDCl_3$) δ 175.0, 150.9, 140.6, 139.3, 137.3, 128.8, 127.1, 127.0, 124.0, 123.0, 120.0, 119.4, 117.5, 115.7, 84.6, 52.3, 46.1, 28.5, 19.1; *m/z* [ES-] 386 ([^{35}M – H]$^-$, 5 %), 286 ([^{35}M – H – Boc]$^-$, 100 %), 288 ([^{37}M – H – Boc]$^-$, 33 %); *m/z* [HRMS, ES+] found [^{35}M]$^+$ 387.1228, $C_{21}H_{22}NO_4Cl$ requires 387.1232.

4.4.3. tert-Butyl 3-(1-methoxy-1-oxopropan-2-yl)-6-phenyl-9H-carbazole-9-carboxylate (3)

A solution of *tert*-butyl 3-chloro-6-(1-methoxy-1-oxopropan-2-yl)-9H-carbazole-9-carboxylate (200 mg, 0.52 mmol), phenyl boronic acid (94.0 mg, 0.77 mmol), palladium acetate (6 mg, 5 mol%), XPhos (25 mg, 10 mol%), and potassium fluoride (90.0 mg, 1.55 mmol) in anhydrous dioxane (2.5 ml) was stirred at reflux for 18 h. The solvent was removed from the reaction mixture under reduced pressure and the crude product was purified by flash column chromatography (2–20 % EtOAc in *n*-hexane) to afford the product as a white crystalline solid (210 mg, 95 % yield). Mp 49–52 °C; IR (neat, ν_{max}/cm^{-1}): 2976, 1722; ^{1}H NMR (700 MHz, $CDCl_3$) δ 8.34 (1H, d, J = 8.5 Hz, **8-ArH**), 8.31 (1H, s, **1-ArH**), 8.15 (1H, d, J = 2.0 Hz, **5-ArH**), 7.97 (1H, d, J = 7.9 Hz, **4-ArH**), 7.72–7.68 (3H, m, **7-ArH** & **2′-ArH**), 7.50–7.46 (2H, m, **3′-ArH**), 7.38–7.35 (1H, m, **4′-ArH**), 7.32 (1H, dd, J = 7.9, 2.0 Hz, **3-ArH**), 3.92 (1H, q, J = 7.2 Hz, **CH**$_3$), 3.69 (3H, s, **CO$_2$CH$_3$**), 1.79 (9H, s, **C(CH$_3$)$_3$**), 1.61 (3H, d, J = 7.2 Hz, **CHCH$_3$**); ^{13}C NMR (176 MHz, $CDCl_3$) δ 175.2, 151.2, 141.4, 140.1, 139.3, 138.3, 136.5, 129.0, 127.4, 127.2, 126.4, 126.2, 125.1, 122.8, 119.9, 118.1, 116.6, 115.7, 84.3, 52.3, 46.2, 28.6, 19.1; *m/z* [ES+] 429 ([M]$^+$, 14 %); 374 ([MH – ^{t}Bu]$^+$, 100 %), 314 ([M – ^{t}Bu – COOMe]$^+$, 49 %); *m/z* [HRMS, ES+] found [M]$^+$ 429.1933, $C_{27}H_{27}NO_4$ requires 429.1935.

4.4.4. tert-Butyl 2-(1-methoxy-1-oxopropan-2-yl)-6-(p-tolyl)-9H-carbazole-9-carboxylate (4)

This compound was synthesised according to the same method as was reported for **3**, with *p*-tolylboronic acid (131 mg, 0.97 mmol) as the boronate. The product was obtained as a white solid (246 mg, 86 %). Mp 127–132 °C: IR (neat, ν_{max}/cm^{-1}): 2972 br., 1722; ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.31 (2H, m, **1-ArH** & **8-ArH**), 8.13 (1H, s, **5-ArH**), 7.97 (1H, d, J = 8.0 Hz, **4-ArH**), 7.68 (1H, d, J = 8.7 Hz, **7-ArH**), 7.60 (2H, d, J = 8.0 Hz, **2′-ArH**), 7.32 (1H, d, J = 8.0 Hz, **3-ArH**), 7.29 (2H, d, J = 7.6 Hz, **3′-ArH**), 3.92 (1H, q, J = 7.2 Hz, **CH**$_3$), 3.69 (3H, s, **CO$_2$CH$_3$**), 2.42 (3H, s, **ArCH$_3$**), 1.79 (9 h, s, **C(CH$_3$)$_3$**), 1.61 (3H, d, J = 7.2 Hz, **CHCH$_3$**); ^{13}C NMR (151 MHz, $CDCl_3$) δ 175.3, 151.2, 140.0, 139.3, 138.4, 138.1, 137.0, 136.4, 129.7, 127.3, 126.3, 126.1, 125.1, 122.8, 119.9, 117.8, 116.6, 115.7, 84.2, 52.3, 46.1, 28.6, 21.3, 19.2; *m/z* [ES+] 443 ([M]$^+$, 7 %); 388 ([MH – ^{t}Bu]$^+$, 100 %), 328 ([M−^{t}Bu−COOMe]$^+$, 49 %); *m/z* [HRMS, ES+] found [M]$^+$ 443.2086, $C_{28}H_{29}NO_4$ requires 443.2091.

4.4.5. tert-Butyl 2-(1-methoxy-1-oxopropan-2-yl)-6-(4-methoxyphenyl)-9H-carbazole-9-carboxylate (5)

This compound was synthesised according to the same method as was reported for **3**, with 4-methoxyphenylboronic acid (147 mg, 0.97 mmol) as the boronate. The product was obtained as a white solid (223 mg, 75 %). Mp 136–139 °C; IR (neat, ν_{max}/cm^{-1}): 3021, 1730; ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.32 (2H, m, **1-ArH** & **8-ArH**), 8.10 (1H, s, **5-ArH**), 7.96 (1H, d, J = 8.0 Hz, **4-ArH**), 7.68–7.59 (3H, m, **7-ArH** & **2′-ArH**), 7.32 (1H, d, J = 8.0 Hz, **3-ArH**), 7.02 (1H, d, J = 7.2 Hz, **3′-ArH**), 3.92 (1H, q, J = 7.2 Hz, **CH**$_3$), 3.87 (3H, s, **ArOCH$_3$**), 3.69 (3H, s, **CO$_2$CH$_3$**), 1.79 (9H, s, **C(CH$_3$)$_3$**), 1.62 (3H, d, J = 7.2 Hz, **CHCH$_3$**); ^{13}C NMR (151 MHz, $CDCl_3$) δ 175.3, 159.1, 151.2, 140.0, 139.3, 137.9, 136.1, 133.9, 128.4, 126.1, 126.1, 125.1, 122.8, 119.9, 117.6, 116.6, 115.7, 114.4, 84.2, 55.5, 52.3, 46.2, 28.6, 19.2; *m/z* [ES+] 459 ([M]$^+$, 7 %); 404 ([MH – ^{t}Bu]$^+$, 100 %), 344 ([M – ^{t}Bu - COOMe]$^+$, 34 %); *m/z* [HRMS, ES+] found [M]$^+$ 459.2037, $C_{28}H_{29}NO_5$ requires 459.2040.

4.4.6. tert-Butyl 2-(1-methoxy-1-oxopropan-2-yl)-6-(3-methoxyphenyl)-9H-carbazole-9-carboxylate (6)

This compound was synthesised according to the same method as was reported for **3**, with 3-methoxyphenylboronic acid (147 mg, 0.97 mmol) as the boronate. The product was obtained as a colourless oil (252 mg, 85 %). IR (neat, ν_{max}/cm^{-1}): 2977, 1722; ^{1}H NMR (600 MHz, CDCl3) δ 8.38–8.28 (2H, m, **1-ArH** & **8-ArH**), 8.15 (1H, s, **5-ArH**), 7.97 (1H, d, J = 7.9 Hz, **4-ArH**), 7.69 (1H, d, J = 8.7 Hz, **7-ArH**), 7.40 (1H, app. t, J = 7.9 Hz, **5′-ArH**), 7.33 (1H, d, J = 7.9 Hz, **3-ArH**), 7.30 (1H, d, J = 7.6 Hz, **6′-ArH**), 7.23 (1H, s, **2′-ArH**), 6.92 (1H, d, J = 7.3 Hz, **4′-ArH**), 3.90 (4H, m, **CH**$_3$ & **ArOCH$_3$**), 3.69 (3H, s, **CO$_2$CH$_3$**), 1.79 (9H, s, **C(CH$_3$)$_3$**), 1.61 (3H, d, J = 7.2 Hz, **CHCH$_3$**); ^{13}C NMR (151 MHz, $CDCl_3$) δ 175.2, 160.1, 151.1, 142.9, 140.1, 139.3, 138.4, 136.3, 130.0, 126.5, 126.1, 125.1, 122.8, 120.0, 119.9, 118.1, 116.6, 115.7, 113.2, 112.5, 84.3, 55.5, 52.3, 46.2, 28.6, 19.2; *m/z* [HRMS, ES+] found [M]$^+$ 459.2037, $C_{28}H_{29}NO_5$ requires 459.2040.

4.4.7. tert-Butyl 2-(1-methoxy-1-oxopropan-2-yl)-6-(2-methoxyphenyl)-9H-carbazole-9-carboxylate (7)

This compound was synthesised according to the same method as was reported for **3**, with 2-methoxyphenylboronic acid (147 mg, 0.97 mmol) as the boronate. The product was obtained as a colourless oil (129 mg, 44 %). IR (neat, ν_{max}/cm^{-1}): 2977, 1722; ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.35–8.25 (2H, m, **1-ArH** & **8-ArH**), 8.09 (1H, s, **5-ArH**), 7.93 (1H, d, J = 8.0 Hz, **4-ArH**), 7.63 (1H, d, J = 8.7 Hz, **7-ArH**), 7.42 (1H, d, J = 7.4 Hz, **6′-ArH**), 7.35 (1H, app t., J = 7.8 Hz, **4′-ArH**), 7.30 (1H, d, J = 8.0 Hz, **3-ArH**), 7.08 (1H, app. t, J = 7.4 Hz, **5′-ArH**), 7.03 (1H, d, J = 8.3 Hz, **3′-ArH**), 3.91 (1H, q, J = 7.2 Hz, **CH**$_3$), 3.84 (3H, s, **ArOCH$_3$**), 3.69 (3H, s, **CO$_2$CH$_3$**), 1.78 (9H, s, **C(CH$_3$)$_3$**) 1.61 (3H, d, J = 7.2 Hz, **CHCH$_3$**); ^{13}C NMR (151 MHz, $CDCl_3$) δ 175.3, 156.7, 151.2, 139.8, 139.2, 137.9, 133.6, 131.3, 130.8, 128.8, 128.7, 125.6, 125.2, 122.6, 121.0, 120.6, 119.9, 115.8, 115.7, 111.4, 84.1, 55.8, 52.3, 46.1, 28.5, 19.1; *m/z* [ES+] 458 ([M – H]$^-$, 8 %); 358 ([M – H – Boc]$^-$, 100 %); *m/z* [HRMS, ES+] found [M]$^+$ 459.2037, $C_{28}H_{29}NO_5$ requires 459.2040.

4.4.8. tert-Butyl 2-(1-methoxy-1-oxopropan-2-yl)-6-(piperidin-1-yl)-9H-carbazole-9-carboxylate (8)

A solution of *tert*-butyl 3-chloro-6-(1-methoxy-1-oxopropan-2-yl)-9H-carbazole-9-carboxylate (250 mg, 0.64 mmol), piperidine (82.0 mg, 0.97 mmol), palladium acetate (7.2 mg, 5.0 mol%), XPhos (30 mg, 10 mol%) and Cs_2CO_3 (273 mg, 0.84 mmol) in anhydrous toluene (5 ml) was warmed to reflux and stirred for 48 h under an argon atmosphere. The solvent was removed from the reaction mixture under reduced pressure and the crude product was purified by flash column chromatography (5–20 % EtOAc in petroleum ether) to afford the product as a colourless glassy solid (130 mg, 46 %). Mp 121–124 °C; IR (neat, ν_{max}/cm^{-1}): 2948, 2791, 1718; ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.27 (1H, s, **1-ArH**), 8.13 (1H, d, J = 9.2 Hz, **8-ArH**), 7.86 (1H, d, J = 8.0 Hz, **4-ArH**), 7.47 (1H, d, J = 2.4 Hz, **5-ArH**), 7.27 (1H, dd, J = 8.0, 1.6 Hz, **3-ArH**), 7.13 (1H, dd, J = 9.2, 2.4 Hz, **7-ArH**), 3.89 (1H, q, J = 7.2 Hz, **CH**$_3$), 3.68 (3H, s, **CO$_2$CH$_3$**), 3.23–3.18 (4H, m, **2′-H**), 1.76 (13H, m, **3′-H** & **C(CH$_3$)$_3$**), 1.59 (5H, d, J = 7.2 Hz, **CHCH$_3$** & **4′–H**); ^{13}C NMR (151 MHz, $CDCl_3$) δ 175.3, 151.2, 149.2, 139.5, 139.3, 133.0, 126.2, 125.3, 122.3, 119.6, 118.2, 116.7, 115.6, 107.2, 83.7, 52.3, 52.2, 46.1, 28.5, 26.2, 24.4, 19.1; *m/z* [ES+] 436 ([M]$^+$, 100 %); *m/z* [HRMS, ES+] found [M+H]$^+$ 437.2435, $C_{26}H_{33}N_2O_4$ requires 437.2435.

4.4.9. tert-Butyl 2-(1-methoxy-1-oxopropan-2-yl)-6-morpholino-9H-carbazole-9-carboxylate (9)

This compound was synthesised using the same method as for **8**, with morpholine (67.0 mg, 0.77 mmol), as the amine to afford the product as a brown solid (33 mg, 12 %). Mp 153–156 °C; IR (neat, ν_{max}/cm^{-1}): 2979, 2859, 1719, 1121; ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.26 (1H, s, **1-ArH**), 8.17 (1H, app. br s, **8-ArH**), 7.87 (1H, d, J = 7.9 Hz, **4-ArH**), 7.45 (1H, s, **5-ArH**), 7.28 (1H, d, J = 7.9 Hz, **3-ArH**), 7.10 (1H, d, J = 7.9 Hz, **7-ArH**), 4.00–3.86 (5H, m, **CH**$_3$ & **14′-H**), 3.67 (3H, s, **CO$_2$CH$_3$**) 3.24 (4H, t, J = 4.8 Hz, **15′–H**), 1.76 (9H, s, **C(CH$_3$)$_3$**), 1.59 (3H, d, J = 7.2 Hz, **CHCH$_3$**); ^{13}C NMR (151 MHz, $CDCl_3$) δ 175.2, 151.1, 148.0, 139.8, 139.3, 133.4, 126.4, 125.1, 122.4, 119.7, 117.1, 117.0, 115.7, 106.4, 83.9, 67.2, 52.3, 50.9, 46.1, 28.5, 19.1; *m/z* [ES+] 439 ([M+H]$^+$, 100 %); *m/z* [HRMS, ES+] found [M+H]$^+$ 439.2227, $C_{25}H_{31}N_2O_5$ requires 439.2227.

4.4.10. 2-(6-Phenyl-9H-carbazol-2-yl)propanoic acid (10)

To a solution of *tert*-butyl 3-(1-methoxy-1-oxopropan-2-yl)-6-phenyl-9H-carbazole-9-carboxylate (**3**) (125 mg, 0.29 mmol) in CH_2Cl_2 (2 ml) was added TFA (2 ml). The reaction mixture was stirred at RT for 1 h before the solvent was removed under reduced pressure. The crude product was then dissolved in CH_2Cl_2 (4 ml) and methanolic NaOH (2 M, 2 ml, 4 mmol) and stirred at RT for 18 h. The solvent was removed from the reaction mixture and the crude product was dissolved in water (10 ml) and washed with EtOAc (3 x 10 ml). The aqueous layer was acidified with $HCl_{(aq)}$ (1 M) and then extracted with EtOAc (3 x 10 ml). The organic layer was dried over anhydrous Na_2SO_4 and the solvent was removed under reduced pressure to afford the product as an off-white solid (45 mg, 49 %). Mp 188–192 °C; IR (neat, ν_{max}/cm^{-1}): 3420, 2934, 1695; ^{1}H NMR (600 MHz, MeOD) δ 8.26 (1H, d, J = 1.9 Hz, **5-ArH**), 8.06 (1H, d, J = 8.0 Hz, **4-ArH**), 7.70 (2H, dd, J = 8.3, 1.3 Hz, **2-ArH**), 7.64 (1H, dd, J = 8.4, 1.9 Hz, **7-ArH**), 7.49 (1H, d, J = 8.4 Hz, **8-ArH**), 7.46–7.41 (3H, m, **1-ArH** & **3′-ArH**), 7.29 (1H, tt, J = 7.2, 1.3 Hz, **4′-ArH**), 7.15 (1H, dd, J = 8.0, 1.6 Hz, **3-ArH**), 3.87 (1H, q, J = 7.2 Hz, **CH**$\boldsymbol{H_3}$), 1.55 (3H, d, J = 7.2 Hz, **CHCH**$\boldsymbol{_3}$); ^{13}C NMR (151 MHz, MeOD) δ 178.8, 143.7, 142.3, 141.3, 140.3, 133.5, 129.8, 128.1, 127.3, 125.9, 124.7, 123.5, 121.1, 119.7, 119.2, 112.0, 110.7, 47.2, 19.5; *m/z* [ES+] 316 ($[M+H]^+$, 100 %); *m/z* [HRMS, ES+] found $[M+H]^+$ 316.1330, $C_{21}H_{18}NO_2$ requires 316.1332.

4.4.11. 2-(6-(p-Tolyl)-9H-carbazol-2-yl)propanoic acid (11)

This compound was synthesised according to the same method as was reported for **10**, with *tert*-butyl 2-(1-methoxy-1-oxopropan-2-yl)-6-(p-tolyl)-9H-carbazole-9-carboxylate (**4**) (150 mg, 0.34 mmol) as starting material. The product was obtained as a white solid (58 mg, 52 %). Mp 214–219 °C; IR (neat, ν_{max}/cm^{-1}): 3414, 2923, 2543, 1694; ^{1}H NMR (600 MHz, MeOD) δ 8.23 (1H, d, J = 1.9 Hz, **5-ArH**), 8.04 (1H, d, J = 8.0 Hz, **4-ArH**), 7.61 (1H, dd, J = 8.4, 1.9 Hz, **7-ArH**), 7.58 (2H, d, J = 8.3 Hz, **14-Ar'H**), 7.47 (1H, d, J = 8.4 Hz, **8-ArH**), 7.41 (1H, d, 1.6 J = 1.6 Hz **1-ArH**), 7.25 (2H, d, J = 8.3 Hz, **15–Ar'H**), 7.14 (1H, dd, J = 8.0, 1.6 Hz, **3-ArH**), 3.86 (1H, q, J = 7.2 Hz, **CH**$\boldsymbol{CH_3}$), 2.38 (3H, s, **ArCH**$\boldsymbol{_3}$), 1.55 (3H, d, J = 7.2 Hz, **CHCH**$\boldsymbol{_3}$); ^{13}C NMR (151 MHz, MeOD) δ 178.7, 142.3, 141.1, 140.8, 140.2, 137.0, 133.5, 130.4, 127.9, 125.8, 124.7, 123.5, 121.1, 119.6, 119.0, 111.9, 110.6, 47.1, 21.1, 19.5; *m/z* [ES+] 330 ($[M+H]^+$, 100 %); *m/z* [HRMS, ES+] found $[M+H]^+$ 330.1481, $C_{22}H_{20}NO_2$ requires 330.1489.

4.4.12. 2-(6-(4-Methoxyphenyl)-9H-carbazol-2-yl)propanoic acid (12)

This compound was synthesised according to the same method as was reported for **10**, with *tert*-butyl 2-(1-methoxy-1-oxopropan-2-yl)-6-(4-methoxyphenyl)-9H-carbazole-9-carboxylate (**5**) (150 mg, 0.33 mmol) as starting material. The product was obtained as an off-white solid (71 mg, 63 %). Mp 185–187 °C; IR (neat, ν_{max}/cm^{-1}): 3405, 2975, 1695; ^{1}H NMR (600 MHz, MeOD) δ 8.19 (1H, d, J = 1.8 Hz, **5-ArH**), 8.03 (1H, d, J = 8.1 Hz, **4-ArH**), 7.61 (2H, d, J = 8.8 Hz, **14-Ar'H**), 7.58 (1H, dd, J = 8.4, 1.8 Hz, **7-ArH**), 7.45 (1H, d, J = 8.4 Hz, **8-ArH**), 7.41 (1H, s, **1-ArH**), 7.13 (1H, dd, J = 8.1, 1.6 Hz, **3-ArH**), 7.00 (2H, d, J = 8.8 Hz, **15–Ar'H**), 3.86 (1H, q, J = 7.2 Hz, **CH**$\boldsymbol{CH_3}$), 3.83 (3H, s, **ArOCH**$\boldsymbol{_3}$), 1.54 (3H, d, J = 7.2 Hz, **CHCH**$\boldsymbol{_3}$); ^{13}C NMR (151 MHz, MeOD) δ 178.8, 160.0, 142.3, 140.9, 140.2, 136.2, 133.3, 129.0, 125.6, 124.7, 123.5, 121.1, 119.6, 118.7, 115.2, 111.9, 110.6, 55.7, 47.2, 19.5; *m/z* [ES+] 346 ($[M+H]^+$, 100 %); *m/z* [HRMS, ES+] found $[M+H]^+$ 346.1433, $C_{22}H_{20}NO_3$ requires 346.1438.

4.4.13. 2-(6-(3-Methoxyphenyl)-9H-carbazol-2-yl)propanoic acid (13)

This compound was synthesised according to the same method as was reported for **10**, with *tert*-butyl 2-(1-methoxy-1-oxopropan-2-yl)-6-(3-methoxyphenyl)-9H-carbazole-9-carboxylate (**6**) (150 mg, 0.33 mmol) as starting material. The product recrystallised from Et_2O and pentane to give a brown solid (18 mg, 16 %). Mp 159–164 °C; IR (neat, ν_{max}/cm^{-1}): 3443, 2943 br., 2555, 1710; ^{1}H NMR (600 MHz, MeOD) δ 8.24 (1H, d, J = 1.9 Hz, **5-ArH**), 8.03 (1H, d, J = 8.1 Hz, **4-ArH**), 7.61 (1H, dd, J = 8.4, 1.9 Hz, **7-ArH**), 7.46 (1H, d, J = 8.4 Hz, **8-ArH**), 7.41 (1H, s, **1-ArH**), 7.32 (1H, t, J = 7.8 Hz, **5′-ArH**), 7.25 (1H, dt, J = 7.8, 1.4 Hz, **6′-ArH**), 7.24–7.21 (1H, m, **2′-ArH**), 7.13 (1H, dd, J = 8.1, 1.6 Hz, **3-ArH**), 6.85 (1H, dd, J = 8.1, 2.6 Hz, **4′-ArH**), 3.85 (4H, m, **CH**$\boldsymbol{CH_3}$ & **ArOCH**$\boldsymbol{_3}$), 1.54 (3H, d, J = 7.2 Hz, **CHCH**$\boldsymbol{_3}$); ^{13}C NMR (151 MHz, MeOD) δ 178.7, 161.5, 145.1, 142.2, 141.3, 140.3, 133.4, 130.7, 125.9, 124.7, 123.5, 121.2, 120.6, 119.7, 119.3, 113.7, 112.6, 111.9, 110.6, 55.7, 47.1, 19.5; *m/z* [ES+] 346 ($[M+H]^+$, 100 %); *m/z* [HRMS, ES+] found $[M+H]^+$ 346.1432, $C_{22}H_{20}NO_3$ requires 346.1438.

4.4.14. 2-(6-(2-Methoxyphenyl)-9H-carbazol-2-yl)propanoic acid (14)

This compound was synthesised according to the same method as was reported for **10**, with *tert*-butyl 2-(1-methoxy-1-oxopropan-2-yl)-6-(2-methoxyphenyl)-9H-carbazole-9-carboxylate (**7**) (100 mg, 0.22 mmol) as starting material. The product was obtained as a golden oil (53 mg, 35 %). IR (neat, ν_{max}/cm^{-1}): 3404, 2978 br., 1703; ^{1}H NMR (600 MHz, MeOD) δ 8.10 (1H, d, J = 1.8 Hz, **5-ArH**), 7.98 (1H, d, J = 8.0 Hz, **4-ArH**), 7.49 (1H, dd, J = 8.4, 1.8 Hz, **7-ArH**), 7.44–7.40 (2H, m, **1-ArH** & **8-ArH**), 7.36 (1H, dd, J = 7.4, 1.7 Hz, **6′-ArH**), 7.29 (1H, ddd, J = 8.3, 7.4, 1.7 Hz, **4′-ArH**), 7.12 (1H, dd, J = 8.0, 1.6 Hz, **3-ArH**), 7.07 (1H, dd, J = 8.3, 1.2 Hz, **3′-ArH**), 7.02 (1H, app. td, J = 7.4, 1.2 Hz, **5′-ArH**), 3.86 (1H, q, J = 7.1 Hz, **CH**$\boldsymbol{CH_3}$), 3.80 (3H, s, **ArOCH**$\boldsymbol{_3}$), 1.54 (3H, d, J = 7.1 Hz, **CHCH**$\boldsymbol{_3}$); ^{13}C NMR (151 MHz, MeOD) δ 178.8, 158.1, 142.1, 140.8, 140.0, 133.2, 132.1, 130.8, 129.0, 128.5, 124.0, 123.5, 121.9, 121.7, 121.0, 119.5, 112.6, 111.0, 110.6, 56.1, 47.1, 19.5; *m/z* [ES+] 346 ($[M+H]^+$, 100 %); *m/z* [*HRMS*, ES+] found $[M+H]^+$ 346.1438, $C_{22}H_{20}NO_3$ requires 346.1438.

4.4.15. 2-(6-(Piperidin-1-yl)-9H-carbazol-2-yl)propanoic acid (15)

This compound was synthesised according to the same method as was reported for **10**, with *tert*-butyl 2-(1-methoxy-1-oxopropan-2-yl)-6-(piperidin-1-yl)-9H-carbazole-9-carboxylate (**8**) (75.0 mg, 0.17 mmol) as starting material. The product recrystallised from Et_2O and pentane to give a white solid (4.8 mg, 6 %). IR (neat, ν_{max}/cm^{-1}): 3221 br., 2868 br., 1666; ^{1}H NMR (600 MHz, MeOD) δ 8.12 (1H, d, J = 2.4 Hz, **5-ArH**), 8.00 (1H, d, J = 8.1 Hz, **4-ArH**), 7.49 (1H, d, J = 8.8 Hz, **8-ArH**), 7.44 (2H, m, **1-ArH** & **7-ArH**), 7.17 (1H, dd, J = 8.1, 1.6 Hz, **3-ArH**), 3.86 (1H, q, J = 7.2 Hz, **CH**$\boldsymbol{CH_3}$), 3.50 (4H, t, J = 5.6 Hz, **2′–H**), 1.98 (4H, app. pentet, J = 6.0 Hz **3′-H**), 1.74 (2H, pentet, J = 6.0 Hz, **4′-H**), 1.53 (3H, d, J = 7.2 Hz, **CHCH**$\boldsymbol{_3}$); ^{13}C NMR (151 MHz, MeOD) δ 178.9, 142.7, 141.5, 140.4, 139.0, 124.6, 122.8, 121.3, 120.2, 119.2, 112.8, 112.5, 111.0, 57.9, 47.5, 25.8, 23.1, 19.5; *m/z* [ES+] 323 ($[M+H]^+$, 100 %); *m/z* [HRMS, ES+] found $[M+H]^+$ 323.1753, $C_{20}H_{23}N_2O_2$ requires 323.1754.

4.4.16. 2-(6-Morpholino-9H-carbazol-2-yl)propanoic acid (16)

This compound was synthesised according to the same method as was reported for **10**, with *tert*-butyl 2-(1-methoxy-1-oxopropan-2-yl)-6-morpholino-9H-carbazole-9-carboxylate (**9**) (50 mg, 0.11 mmol) as starting material. The product was then recrystallised from a mixture of Et_2O and pentane to afford the product as a white solid (3.7 mg, 10 %). Mp 229–234 °C; IR (neat, ν_{max}/cm^{-1}): 3249 br., 2923 br., 1707; ^{1}H NMR (600 MHz, MeOD) δ 7.95 (1H, d, J = 8.1 Hz, **4-ArH**), 7.65 (1H, d, J = 2.4 Hz, **5-ArH**), 7.36 (1H, s, **1-ArH**), 7.34 (1H, d, J = 8.7 Hz, **8-ArH**), 7.13 (1H, dd, J = 8.7, 2.4 Hz, **7-ArH**), 7.09 (1H, dd, J = 8.1, 1.6 Hz, **3-ArH**), 3.89–3.81 (5H, m, **CH**$\boldsymbol{CH_3}$ & **3′-H**), 3.13 (4H, t, J = 4.8 Hz, **2′-H**), 1.52 (3H, d, J = 7.2 Hz, **CHCH**$\boldsymbol{_3}$); ^{13}C NMR (151 MHz, MeOD) δ 178.8, 145.7, 142.4, 140.0, 137.7, 124.5, 123.4, 121.0, 119.3, 119.1, 112.3, 110.6, 109.1, 68.0, 53.6, 47.1, 19.5; *m/z* [ES+] 325 ($[M+H]^+$, 100 %); *m/z* [HRMS, ES+] found $[M+H]^+$ 325.1544, $C_{19}H_{21}N_2O_3$ requires 325.1547.

4.4.17. 4-Chloro-N-phenylaniline (21)[41]

Aniline (0.90 ml, 9.95 mmol), 1-chloro-4-iodobenzene (3.58 g, 15.0 mmol), palladium acetate (35 mg, 1.5 mol%), tri-*tert*-butyl phosphonium tetrafluoroborate (90 mg, 0.3 mol%) and sodium *tert*-butoxide

(1.25 g, 13.0 mmol) were dissolved in anhydrous toluene (30 ml) under an argon atmosphere. The reaction mixture was heated to reflux and stirred for 20 h. The reaction mixture was filtered through Celite® with EtOAc and the solvent was removed from the filtrate under reduced pressure. The crude product was then purified by flash column chromatography (10 % EtOAc in petroleum ether) to afford the product as a purple solid (658 mg, 32 %). Mp 72–73 °C (lit. 71–73 °C);[41] IR (neat, ν_{max}/cm^{-1}): 3400, 1584, 1305; ^{1}H NMR (700 MHz, $CDCl_3$) δ 7.28 (2H, t, $J = 7.9$ Hz, **3′-ArH**), 7.21 (2H, d, $J = 8.8$ Hz, **3-ArH**), 7.05 (2H, d, $J = 8.7$ Hz, **2′-ArH**), 6.99 (2H, d, $J = 8.7$ Hz, **2-ArH**), 6.96 (1H, t, $J = 7.4$ Hz, **4′-ArH**); ^{13}C NMR (176 MHz, $CDCl_3$) δ 142.8, 142.0, 129.6, 129.4, 125.6, 121.7, 119.0, 118.2; *m/z* [ES+] 204 ([^{35}M+H]$^+$, 100 %); 206 ([^{37}M+H]$^+$, 35 %).

4.4.18. Methyl 2-(4-((4-chlorophenyl)amino)phenyl)acetate (22)[13]

Methyl 2-(4-aminophenyl)acetate (**18**) (300 mg, 1.82 mmol), 1-chloro-4-iodobenzene (541 mg, 2.27 mmol), palladium acetate (20 mg, 5 mol%), XPhos (86 mg, 10 mol%) and Cs_2CO_3 (858 mg, 2.63 mmol) were dissolved in anhydrous toluene (10 ml) under an argon atmosphere. The reaction mixture was warmed to reflux and stirred for 48 h. The solvent was removed from the reaction mixture under reduced pressure and the crude product was purified by flash column chromatography (5–30 % EtOAc in petroleum ether) to afford the product as a pale-yellow solid (286 mg, 57 %). Mp 94–96 °C; IR (neat, ν_{max}/cm^{-1}): 3375, 2949, 1720, 1592; ^{1}H NMR (700 MHz, $CDCl_3$) δ 7.20 (2H, d, $J =$ 8.8 Hz, **3′-ArH**), 7.18 (2H, d, $J = 8.4$ Hz, **3-ArH**), 7.00 (2H, d, $J = 8.4$ Hz, **2-ArH**), 6.97 (2H, d, $J = 8.8$ Hz, **2′-ArH**), 5.66 (1H, s, **NH**), 3.70 (3H, s, **CO_2CH_3**), 3.57 (2H, s, **CH_2**); ^{13}C NMR (176 MHz, $CDCl_3$) δ 172.5, 142.0, 141.8, 130.4, 129.4, 127.1, 125.7, 118.9, 118.4, 52.2, 40.6; *m/z* [ES+] 276.5 ([^{35}M+H]$^+$, 100 %); 276.5 ([^{37}M+H]$^+$, 35 %); *m/z* [HRMS, ES+] found [^{35}M+H]$^+$ 276.0786, $C_{15}H_{15}ClNO_2$ requires 276.0790.

4.4.19. Methyl 2-(3-((4-chlorophenyl)amino)phenyl)acetate (23)

This compound was synthesised using the same method as **22**, with methyl 2-(3-aminophenyl)acetate (**19**) (350 mg, 2.12 mmol) as starting material, to afford the product as a colourless crystalline solid (452 mg, 77 %). Mp 72–73 °C; IR (neat, ν_{max}/cm^{-1}): 3369, 1733, 1322; ^{1}H NMR (700 MHz, $CDCl_3$) δ 7.24–7.19 (3H, m, **3-ArH** & **5′-ArH**), 7.01–6.97 (2H, m, **2-ArH**), 6.97–6.93 (2H, m, **2′-ArH** & **6′-ArH**), 6.85 (1H, d, $J = 7.6$ Hz, **4′-ArH**), 5.75 (1H, s, **NH**), 3.70 (3H, s, **CO_2CH_3**), 3.58 (2H s, **CH_2**); ^{13}C NMR (176 MHz, $CDCl_3$) δ 172.1, 143.1, 141.7, 135.5, 129.8, 129.4, 125.9, 122.4, 119.2, 118.9, 116.7, 52.2, 41.3; *m/z* [ES+] 276 ([^{35}M+H]$^+$, 100 %); 278 ([^{37}M+H]$^+$, 35 %); *m/z* [HRMS, ES+] found [^{35}M+H]$^+$ 276.0783, $C_{15}H_{15}ClNO_2$ requires 276.0791.

4.4.20. Methyl 2-(4-((4-chlorophenyl)amino)phenyl)propanoate (24)

This compound was synthesised using the same method as **22**, with methyl 2-(4-aminophenyl)propanoate (**20**) (500 mg, 2.79 mmol) as the starting material, to afford the product as a brown oil (344 mg, 45 % yield). IR (neat, ν_{max}/cm^{-1}): 3374, 1718, 1593; ^{1}H NMR (700 MHz, $CDCl_3$) δ 7.20 (4H, m, **3-ArH** & **3′-ArH**), 6.99 (2H, d, $J = 8.8$ Hz, **2′-ArH**), 6.97 (2H, d, $J = 8.8$ Hz, **2-ArH**), 5.76 (1H, s, **NH**), 3.69 (4H, m, **C*H*CH_3** & **CO_2CH_3**), 1.50 (3H, d, $J = 7.2$ Hz, **CHC*H*$_3$**); ^{13}C NMR (176 MHz, $CDCl_3$) δ 175.5, 142.0, 141.9, 133.5, 129.4, 128.6, 125.6, 118.9, 118.3, 52.2, 44.8, 18.7; *m/z* [ES+] 290 ([^{35}M+H]$^+$, 100 %), 292 ([^{37}M+H]$^+$, 40 %); *m/z* [HRMS, ES+] found [^{35}M+H]$^+$ 290.0940, $C_{16}H_{17}ClNO_2$ requires 290.0942.

4.4.21. 3-Chloro-9H-carbazole (25)[25]

4-Chloro-*N*-phenylaniline (**21**) (102 mg, 0.50 mmol), palladium acetate (22.4 mg, 0.10 mmol) potassium carbonate (14.0 mg, 0.10 mmol) and pivalic acid (450 mg, 4.4 mmol) were added to a round-bottomed flask. The reaction mixture was then heated to 130 °C and stirred for 18 h. The reaction mixture was dissolved in CH_2Cl_2 (20 ml) and washed with saturated $NaHCO_{3(aq)}$ (3 x 20 ml). The organic layer was then filtered through Celite® with EtOAc. The solvent was removed from the filtrate under reduced pressure and the crude product was purified by flash column chromatography (5 % EtOAc in petroleum ether) to afford the product as light pink solid (44 mg, 44 %). Mp 194–197 °C; IR (neat, ν_{max}/cm^{-1}): 3401, 1436; ^{1}H NMR (700 MHz, $CDCl_3$) δ 8.06 (1H, br. s, **NH**), 8.04–8.01 (2H, m, **4-ArH** & **5-ArH**), 7.47–7.41 (2H, m, **7-ArH** & **8-ArH**), 7.39–7.33 (2H, m, **1-ArH** & **2-ArH**), 7.26–7.23 (1H, m, **6-ArH**); ^{13}C NMR (176 MHz, $CDCl_3$) δ 140.1, 137.9, 126.7, 126.1, 125.1, 124.7, 122.7, 120.7, 120.2, 120.0, 111.7, 110.9; *m/z* [HRMS, ES+] found [^{35}M]$^+$ 201.0341, $C_{12}H_8NCl$ requires 201.0340.

4.4.22. Methyl 2-(6-chloro-9H-carbazol-3-yl)acetate (26)[13]

This compound was synthesised using the same method as **25**, with methyl 2-(4-((4-chlorophenyl)amino)phenyl)acetate (**22**) (170 mg, 0.62 mmol) as starting material to afford the product as a yellow solid (61 mg, 36 %). Mp 139–143 °C; IR (neat, ν_{max}/cm^{-1}): 3347, 1717; ^{1}H NMR (700 MHz, $CDCl_3$) δ 8.07 (1H, br. s, **NH**), 7.99 (1H, s, **5-ArH**), 7.91 (1H, s, **4-ArH**), 7.37–7.31 (4H, m, **1-ArH**, **2-ArH**, **7-ArH** & **8-ArH**), 3.79 (2H, s, **CH_2**), 3.72 (3H, s **CO_2CH_3**); ^{13}C NMR (176 MHz, $CDCl_3$) δ 172.8, 139.9, 138.2, 128.0 126.2, 125.5, 125.1, 124.4, 122.9, 121.3, 120.3, 111.7, 111.0, 52.2, 41.3; *m/z* [ES+] 274 ([^{35}M + H]$^+$, 7 %); 214 ([^{35}M – COOMe]$^+$, 100 %), 216 ([^{37}M – COOMe]$^+$, 30 %); *m/z* [HRMS, ES+] found [^{35}M]$^+$ 273.0561, $C_{15}H_{12}ClNO_2$ requires 273.0551.

4.4.23. Methyl 2-(6-chloro-9H-carbazol-2-yl)acetate and Methyl 2-(6-chloro-9H-carbazol-4-yl)acetate (27 and 28)

These compounds were synthesised using the same method as **25**, with methyl 2-(3-((4-chlorophenyl)amino)phenyl)acetate (**23**) (300 mg, 1.09 mmol) as starting material to afford two products:

4.4.24. Methyl 2-(6-chloro-9H-carbazol-2-yl)acetate (27)

Yellow crystalline solid (80 mg, 27 %); Mp 186–189 °C; IR (neat, ν_{max}/cm^{-1}): 3360, 1716; ^{1}H NMR (700 MHz, $CDCl_3$) δ 8.18 (1H, br. s, **NH**), 8.07 (1H, s, **5-ArH**), 7.41–7.34 (2H, m, **2-ArH** & **7-ArH**), 7.30 (2H, m, **1-ArH** & **8-ArH**), 7.10 (1H, d, $J = 7.2$ Hz, **3-ArH**), 4.21 (2H, s, **CH_2**), 3.75 (3H, s, **CO_2CH_3**); ^{13}C NMR (176 MHz, $CDCl_3$) δ 171.8, 140.5, 138.0, 129.1, 126.6, 125.8, 125.1, 124.1, 122.1, 122.0, 121.3, 111.6, 110.2, 52.4, 39.9; *m/z* [ES+] 274 ([^{35}M+H]$^+$, 4 %); 214 ([^{35}M – COOMe]$^+$, 100 %), 216 ([^{37}M – COOMe]$^+$, 35 %); *m/z* [HRMS, ES+] found [^{35}M + H]$^+$ 274.0630, $C_{15}H_{13}ClNO_2$ requires 274.0629.

4.4.25. Methyl 2-(6-chloro-9H-carbazol-4-yl)acetate (28)

Off-white crystalline solid (60 mg, 20 %); Mp 165–167 °C; IR (neat, ν_{max}/cm^{-1}): 3389, 1726; ^{1}H NMR (700 MHz, $CDCl_3$) δ 8.08 (1H, br. s, **NH**), 7.98 (1H, d, $J = 2.1$ Hz, **5-ArH**), 7.94 (1H, d, $J = 8.6$ Hz, **4-ArH**), 7.35–7.31 (3H, m, **1-ArH**, **7-ArH** & **8-ArH**), 7.15 (1H, dd, $J = 7.9$ Hz, **3-ArH**), 3.79 (2H, s, **CH_2**), 3.72 (3H, s, **CO_2CH_3**); ^{13}C NMR (176 MHz, $CDCl_3$) δ 172.4, 140.4, 138.1, 132.6, 126.0, 125.1, 124.5, 121.8, 121.4, 120.7, 120.1, 111.7, 111.6, 52.3, 41.8; *m/z* [ES+] 214 ([^{35}M – COOMe]$^+$, 45 %), 216 ([^{37}M – COOMe]$^+$, 15 %); *m/z* [HRMS, ES] found [^{35}M]$^+$ 273.0555, $C_{15}H_{12}ClNO_2$ requires 273.0551.

4.4.26. Methyl 2-(6-chloro-9H-carbazol-3-yl)propanoate (29)

This compound was synthesised using the same method as **25**, with methyl 2-(4-((4-chlorophenyl)amino)phenyl)propanoate (**24**) (200 mg, 0.69 mmol) as the starting material to afford the product as a brown waxy solid (109 mg, 55 % yield). IR (neat, ν_{max}/cm^{-1}): 3354, 1710; ^{1}H NMR (600 MHz, MeOD) δ 8.02 (1H, d, $J = 1.6$ Hz, **5-ArH**), 7.94 (1H, d, $J = 1.8$ Hz, **4-ArH**), 7.39 (2H, app. dd, $J = 8.5$, 3.5 Hz, **1-ArH** & **8-ArH**), 7.33 (1H, dd, $J = 8.5$, 1.8 Hz, **2-ArH**), 7.31 (1H, dd, $J = 8.6$, 2.1 Hz, **7-ArH**), 3.91 (1H, q, $J = 7.2$ Hz, **C*H*CH_3**), 3.65 (3H, s, **CO_2CH_3**), 1.55 (3H, d, $J = 7.2$ Hz, **CHC*H*$_3$**); ^{13}C NMR (151 MHz, MeOD) δ 177.4, 141.3, 140.2, 132.8, 126.8, 126.6, 125.4, 125.1, 123.6, 120.6, 120.0, 112.9, 112.1, 52.4, 46.5, 19.5; *m/z* [ES–] 286 ([^{35}M + H]$^+$, 100 %), 288 ([^{37}M + H, 35 %); *m/z* [HRMS, ES-] found [^{35}M – H]$^-$ 286.0640, $C_{16}H_{13}ClNO_2$ requires 286.0630.

4.4.27. 2-(6-Chloro-9H-carbazol-3-yl)acetic acid (30)[13]

To a solution of methyl 2-(6-chloro-9H-carbazol-3-yl)acetate (**26**) (35.0 mg, 0.13 mmol) in a mixture of CH_2Cl_2 (4 ml) and MeOH (0.5 ml) was added NaOH in MeOH (2 M, 2 ml, 0.4 mmol). The reaction mixture was stirred at RT for 36 h. The solvent was removed from the reaction mixture under reduced pressure. The crude product was dissolved in water and washed with Et_2O (3 x 20 ml). The aqueous layer was then acidified by addition of $HCl_{(aq)}$ (1 M) and then extracted with Et_2O (3 x 20 ml). The organic layer was then dried over anhydrous Na_2SO_4 and the solvent was removed under reduced pressure. The crude product was recrystallised over a dry ice bath from a mixture of Et_2O and pentane to afford the product as an off-white crystalline solid (10.6 mg, 32 %). Slowly decomposes from 215–232 °C; IR (neat, ν_{max}/cm^{-1}): 3404, 3347, 3020, 2948, 2921, 1693; ^{1}H NMR (700 MHz, MeOD) δ 8.01 (1H, d, J = 2.1 Hz, **5-H**), 7.94 (1H, s, **4-ArH**), 7.39 (2H, m, **1-ArH** & **8-ArH**), 7.33 (1H, dd, J = 8.3, 1.7 Hz, **2-ArH**), 7.31 (1H, dd, J = 8.5, 2.1 Hz, **7-ArH**), 3.74 (2H, s, **CH_2**); ^{13}C NMR (176 MHz, MeOD) δ 176.4, 141.0, 140.0, 128.7, 126.6, 126.4, 125.3, 125.0, 123.5, 121.8, 120.5, 112.8, 111.8, 41.9; *m/z* [ES+] 260 ([^{35}M+H]$^+$, 45 %); 262 ([^{37}M+H]$^+$, 10 %); 214 ([^{35}M – COOH]$^+$, 75 %); 216 ([^{37}M+H – COOH]$^+$, 25 %); *m/z* [HRMS, ES+] found [^{35}M]$^+$ 259.0398, $C_{14}H_{10}ClNO_2$ requires 259.0395.

4.4.28. 2-(6-Chloro-9H-carbazol-4-yl)acetic acid (31)

This compound was synthesised using the same method as **30**, with methyl 2-(6-chloro-9*H*-carbazol-4-yl)acetate (35.0 mg, 0.13 mmol) as starting material to afford the product as an off-white crystalline solid (6.6 mg, 20 %). Mp. 150–154 °C; IR (neat, ν_{max}/cm^{-1}): 3397, 3337, 2975, 2900, 1696; ^{1}H NMR (700 MHz, MeOD) δ 8.09 (1H, d, J = 2.0 Hz, **5-ArH**), 7.42 (1H, d, J = 8.6 Hz, **8-ArH**), 7.40–7.31 (3H, m, **2-ArH**, **3-ArH** & **7-ArH**), 7.04 (1H, d, J = 7.3 Hz, **1-ArH**), 4.15 (2H, s, **CH_2**); ^{13}C NMR (176 MHz, MeOD) δ 175.2, 142.3, 139.8, 130.7, 127.1, 126.0, 125.0, 124.9, 122.6, 122.4, 122.1, 112.6, 110.9, 40.8; *m/z* [ES+] 260 ([M+H]$^+$, 10 %); 214 ([^{35}M – COOH]$^+$, 100 %); 216 ([^{37}M – COOH]$^+$, 30 %); *m/z* [HRMS, ES+] found [^{35}M]$^+$ 259.0399, $C_{14}H_{10}ClNO_2$ requires 259.0395.

4.4.29. 2-(6-Chloro-9H-carbazol-2-yl)acetic acid (32)

This compound was synthesised using the same method as **30**, with methyl 2-(6-chloro-9*H*-carbazol-2-yl)acetate (20.0 mg, 0.07 mmol) as starting material to afford the product as a pale brown solid (4.3 mg, 23 %). Slowly decomposes from 221–238 °C; IR (neat, ν_{max}/cm^{-1}): 3402, 2920, 2536, 1695; ^{1}H NMR (700 MHz, MeOD) δ 7.99 (1H, d, J = 2.1 Hz, **5-ArH**), 7.97 (1H, d, J = 8.0 Hz, **4-ArH**), 7.41–7.35 (2H, m, **1-ArH** & **8-ArH**), 7.30 (1H, dd, J = 8.5, 2.1 Hz, **7-ArH**), 7.10 (1H, dd, J = 8.0, 1.5 Hz, **3-ArH**), 3.74 (2H, s, **CH_2**); ^{13}C NMR (176 MHz, MeOD) δ 176.0, 142.2, 139.9, 134.4, 126.2, 125.3, 125.0, 122.2, 121.5, 121.0, 120.4, 112.7, 112.6, 42.6; *m/z* [ES+] 260 ([^{35}M+H]$^+$, 10 %); 214 ([^{35}M – COOH]$^+$, 100 %); 216 ([^{37}M – COOH]$^+$, 30 %); *m/z* [HRMS, ES+] found [^{35}M]$^+$ 259.0400, $C_{14}H_{10}ClNO_2$ requires 259.0395.

4.4.30. 2-(6-Chloro-9H-carbazol-3-yl)propanoic acid (33)

This compound was synthesised using the same method as **30**, with methyl 2-(6-chloro-9*H*-carbazol-3-yl)propanoate (70.0 mg, 0.24 mmol) as starting material to afford the product as a white solid (13.3 mg, 20 %). Mp 166–170 °C; IR (neat, ν_{max}/cm^{-1}): 3420 br., 2924 br., 1697, 1240; ^{1}H NMR (600 MHz, MeOD) δ 8.02 (1H, d, J = 2.0 Hz, **5-ArH**), 7.98 (1H, s, **4-ArH**), 7.42–7.36 (3H, m, **1-ArH, 2-ArH** & **8-ArH**), 7.31 (1H, dd, J = 8.6, 2.1 Hz, **7-ArH**), 3.87 (1H, q, J = 7.2 Hz, **C*H*CH$_3$**), 1.55 (3H, d, J = 7.2 Hz, **CHC*H*$_3$**); ^{13}C NMR (151 MHz, MeOD) δ 179.2, 141.2, 140.2, 133.3, 127.0, 126.5, 125.4, 125.1, 123.5, 120.6, 120.0, 112.9, 112.0, 46.7, 19.6; *m/z* [ES-] 272 ([^{35}M – H]$^-$, 100 %), 274 ([^{37}M – H]$^-$, 30 %); *m/z* [HRMS, ES-] found [^{35}M – H]$^-$ 272.0468, $C_{15}H_{11}ClNO_2$ requires 272.0484.

4.4.31. Methyl 2-(4-((4-fluorophenyl)amino)phenyl)acetate (34)

Methyl 2-(4-aminophenyl)acetate (200 mg, 1.21 mmol), 4-fluoroiodobenzene (333 mg, 1.50 mmol), palladium acetate (14 mg, 5 mol%), XPhos (58 mg, 10 mol%) and Cs_2CO_3 (512 mg, 1.57 mmol) were dissolved in anhydrous toluene (8 ml) under an argon atmosphere. The reaction mixture was warmed to reflux and stirred for 18 h. The solvent was removed from the reaction mixture under reduced pressure and the crude product was purified by flash column chromatography (5–20 % Et_2O in *n*-hexane) to afford the product as a white solid (211 mg, 67 % yield). Mp: 53–56 °C; IR (neat, ν_{max}/cm^{-1}): 3360, 1719, 1610, 1500; ^{1}H NMR (700 MHz, $CDCl_3$) δ 7.15 (2H, d, J = 8.6 Hz, **3′-ArH**), 7.03 (2H, dd, J = 9.0, 4.7 Hz, **3-ArH**), 7.00–6.95 (2H, m, **3-ArH**), 6.93 (2H, d, J = 8.6 Hz, **2′-ArH**), 5.58 (1H, s, **NH**), 3.70 (3H, s, **CO_2CH_3**), 3.56 (2H, s, **CH_2**); ^{13}C NMR (176 MHz, $CDCl_3$) δ 172.6, 158.9–157.5 (d, J = 241 Hz), 143.1, 139.0, 130.4, 126.1, 120.6 (d, J = 8.8 Hz,), 117.1, 116.1 (d, J = 22.9 Hz) 52.2, 40.5; ^{19}F NMR (282 MHz, $CDCl_3$) δ –122.09 (**1-F**); *m/z* [ES+] 260 ([M+H]$^+$, 100 %); *m/z* [HRMS, ES+] found [M+H]$^+$ 260.1078, $C_{15}H_{15}FNO_2$ requires 260.1081.

4.4.32. Methyl 2-(4-((4-(trifluoromethyl)phenyl)amino)phenyl)acetate (35)

This compound was prepared by the same method as for **34**, with 4-iodobenzotrifluoride (617 mg, 2.27 mmol) as the aryl halide, to afford the product as white crystalline solid (483 mg, 86 % yield). Mp 88–89 °C; IR (neat, ν_{max}/cm^{-1}): 3369, 1718, 1605, 1530; ^{1}H NMR (700 MHz, $CDCl_3$) δ 7.46 (2H, d, J = 8.3 Hz, **3-ArH**), 7.24 (2H, d, J = 8.6 Hz, **3′-ArH**), 7.10 (2H, d, J = 8.6 Hz, **2′-ArH**), 7.03 (2H, d, J = 8.3 Hz, **2-ArH**), 5.90 (1H, s, **NH**), 3.71 (3H, s, **CO_2CH_3**), 3.60 (2H, s, **CH_2**); ^{13}C NMR (151 MHz, $CDCl_3$) δ 172.4, 146.8, 140.3, 130.6, 128.5, 126.9, 124.7 (q, J = 270 Hz) 121.88, 121.66, 121.8 (q, J = 32.2 Hz), 120.2, 115.4, 52.3, 40.6; ^{19}F NMR (282 MHz, $CDCl_3$) δ −61.67 (**CF_3**); *m/z* [ES+] 310 ([M+H]$^+$, 100 %); *m/z* [HRMS, ES+] found [M+H]$^+$ 310.1049, $C_{16}H_{15}F_3NO_2$ requires 310.1049.

4.4.33. Methyl 2-(4-(phenylamino)phenyl)acetate (36)

This compound was prepared by the same method as for **34**, with bromobenzene (0.16 ml, 1.50 mmol) as the aryl halide and a 2 h reaction time, to afford the product as a colourless oil (210 mg, 72 % yield). IR (neat, ν_{max}/cm^{-1}): 3362, 1723, 1592; ^{1}H NMR (700 MHz, $CDCl_3$) δ 7.26 (2H, dd, J = 8.6, 7.4 Hz, **3-ArH**), 7.17 (2H, d, J = 8.7 Hz, **3′-ArH**), 7.06 (2H, dd, J = 8.6, 1.1 Hz, **2-ArH**), 7.03 (2H, d, J = 8.7 Hz, **2′-ArH**), 6.93 (1H, tt, J = 7.4, 1.1 Hz, **1-ArH**), 5.69 (1H, s, **NH**), 3.70 (3H, s, **CO_2CH_3**), 3.57 (2H, s, **CH_2**); ^{13}C NMR (176 MHz, $CDCl_3$) δ 172.5, 143.2, 142.3, 130.3, 129.5, 126.5, 121.1, 118.1, 117.9, 52.2, 40.6; *m/z* [ES+] 242 ([M+H]$^+$, 100 %); *m/z* [HRMS, ES+] found [M+H]$^+$ 242.1171, $C_{15}H_{16}NO_2$ requires 242.1176.

4.4.34. Methyl 2-(4-((3-chlorophenyl)amino)phenyl)acetate (37)

This compound was prepared by the same method as for **34**, with 1-bromo-3-chlorobenzene (0.22 ml, 1.89 mmol) as the aryl halide and a 2 h reaction time to afford the product as a colourless oil (378 mg, 91 % yield). IR (neat, ν_{max}/cm^{-1}): 3371, 1722, 1590, 1515; ^{1}H NMR (700 MHz, $CDCl_3$) δ 7.21 (2H, d, J = 8.6 Hz, **3′-ArH**), 7.15 (1H, app. t, J = 8.1 Hz, **5-ArH**), 7.06 (2H, d, J = 8.6 Hz, **2′-ArH**), 7.04 (1H, app. t, J = 2.1 Hz, **2-ArH**), 6.89 (1H, ddd, J = 8.0, 2.1, 1.0 Hz, **ArH**), 6.86 (1H, ddd, J = 8.0, 2.1, 1.0 Hz, **ArH**), 3.71 (3H, s, **CO_2CH_3**), 3.59 (2H, s, **CH_2**); ^{13}C NMR (176 MHz, $CDCl_3$) δ 172.4, 144.8, 141.0, 135.2, 130.5, 127.8, 120.8, 119.3, 116.9, 115.4, 52.2, 40.6; *m/z* [ES+] 276 ([^{35}M+H]$^+$, 100 %) 278 ([^{37}M+H]$^+$, 30 %); *m/z* [HRMS, ES+] found [^{35}M+H]$^+$ 276.0786, $C_{15}H_{15}ClNO_2$ requires 276.0786.

4.4.35. Methyl 2-(4-(p-tolylamino)phenyl)acetate (38)

This compound was prepared by the same method as for **34**, with 4-bromotoluene (0.24 ml, 1.89 mmol) as the aryl halide and a 2 h reaction time, to afford the product as a colourless oil (314 mg, 86 %). IR (neat, ν_{max}/cm^{-1}): 3379, 1726, 1610, 1505; ^{1}H NMR (700 MHz, $CDCl_3$) δ 7.15 (2H, d, J = 8.6 Hz, **3′-ArH**), 7.09 (2H, d, J = 8.3 Hz, **3-ArH**), 7.00 (1H, d, J = 8.3 Hz, **2-ArH**), 6.98 (1H, d, J = 8.6 Hz, **2′-ArH**), 3.70 (3H, s,

CO_2CH_3), 3.56 (2H, s, **CH_2**), 2.31 (3H, s, **$ArCH_3$**); ^{13}C NMR (176 MHz, $CDCl_3$) δ 172.6, 143.0, 140.3, 131.3, 130.3, 130.0, 125.9, 119.1, 117.2, 52.2, 40.6, 20.8; *m/z* [ES+] 256 ([M+H]$^+$, 100 %); *m/z* [HRMS, ES+] found [M+H]$^+$ 256.1335, $C_{16}H_{18}NO_2$ requires 256.1332.

4.4.36. Methyl 2-(4-((4-methoxyphenyl)amino)phenyl)acetate (39)

This compound was prepared by the same method as for **34**, with 4-bromoanisole (0.24 ml, 1.89 mmol) as the aryl halide and a 2 h reaction time, to afford the product as a yellow oil (295 mg, 76 % yield). IR (neat, ν_{max}/cm^{-1}): 3381, 1724, 1607, 1511; ^{1}H NMR (700 MHz, $CDCl_3$) δ 7.12 (2H, d, J = 8.5 Hz, **3′-ArH**), 7.07 (2H, br. s, 7.07, **3-ArH**), 6.89 (2H, d, J = 7.4 Hz, **2′-ArH**), 6.86 (2H, d, br. d, **2-ArH**), 3.80 (3H, s, **OCH_3**), 3.69 (3H, s, **CO_2CH_3**), 3.54 (2H, s, **CH_2**); ^{13}C NMR (176 MHz, $CDCl_3$) δ 172.6, 155.6, 144.1, 135.6, 130.3, 125.3, 122.4, 116.2, 114.8, 55.7, 52.1, 40.5; *m/z* [ES+] 272 ([M+H]$^+$, 100 %); *m/z* [HRMS, ES+] found [M+H]$^+$ 272.1278, $C_{16}H_{18}NO_3$ requires 272.1282.

4.4.37. Methyl 2-(4-((4-bromophenyl)amino)phenyl)acetate (40)

This compound was prepared by the same method as for **34**, with 1,4-dibromobenzene (2.14 g, 9.08 mmol) as the aryl halide, to afford the product as a brown solid (140 mg, 24 % yield). Mp 94–97 °C; IR (neat, ν_{max}/cm^{-1}): 3363, 1703, 1585; ^{1}H NMR (700 MHz, $CDCl_3$) δ 7.33 (2H, d, J = 8.9 Hz, **3-ArH**), 7.18 (2H, d, J = 8.6 Hz, **3′-ArH**), 7.00 (2H, d, J = 8.6 Hz, **2′-ArH**), 6.91 (2H, d, J = 8.9 Hz, **2-ArH**), 3.70 (3H, s, **CO_2CH_3**), 3.58 (2H, s, **CH_2**); ^{13}C NMR (176 MHz, $CDCl_3$) δ 172.5, 142.5, 141.6, 132.3, 130.4, 127.2, 119.1, 118.6, 112.8, 52.2, 40.6; *m/z* [ES+] 320 ([^{79}M+H]$^+$, 100 %), 322 ([^{81}M+H]$^+$, 100 %); *m/z* [HRMS, ES+] found [^{79}M+H]$^+$ 320.0278, $C_{15}H_{15}BrNO_2$ requires 320.0281.

4.4.38. Methyl 2-(6-fluoro-9H-carbazol-3-yl)acetate (41)

Methyl 2-(4-((4-fluorophenyl)amino)phenyl)acetate (**34**) (125 mg, 0.48 mmol), palladium acetate (22.0 mg, 0.10 mmol), potassium carbonate (13 mg, 0.10 mmol) and pivalic acid (837 mg 8.20 mmol) were added to a RBF with a condenser fitted. The reaction mixture was warmed to 100 °C and stirred for 6 h. The reaction was cooled to RT and filtered through Celite® with EtOAc. The solvent was removed from the eluent under reduced pressure. The crude product was dissolved in EtOAc (20 ml) and the organic layer was washed with saturated $NaHCO_{3\,(aq)}$ (3 x 20 ml). The solvent was removed from the organic layer under reduced pressure and the crude product was purified by flash column chromatography (10–30 % EtOAc in *n*-hexane) to afford the product as a white solid (41 mg, 33 %). Mp 134–140 °C; IR (neat, ν_{max}/cm^{-1}): 3360, 1722; ^{1}H NMR (700 MHz, $CDCl_3$) δ 8.02 (1H, br s, **NH**), 7.91 (1H, s, **4-ArH**), 7.68 (1H, dd, J = 8.8, 2.6 Hz, **5-ArH**), 7.38–7.34 (2H, m, **1-ArH** & **2-ArH**), 7.32 (1H, dd, J = 8.7, 4.6 Hz, **8-ArH**), 7.14 (1H, app. td, J = 9.0, 2.6 Hz, **7-ArH**), 3.79 (2H, s, **CH_2**), 3.72 (3H, s, **CO_2CH_3**); ^{13}C NMR (176 MHz, $CDCl_3$) δ 172.9, 158.3–157.0 (d, J = 235.8 Hz), 139.8, 136.2, 127.8, 125.2, 123.8 (d, J = 8.8 Hz), 123.5 (d, J = 5.3 Hz), 121.3, 113.9 (d, J = 24.6 Hz), 111.3 (d, J = 10.6 Hz), 111.1, 106.1 (d, J = 22.9 Hz), 52.2, 41.3; ^{19}F NMR (282 MHz, $CDCl_3$) δ −124.34 (**6-F**); *m/z* [ES+] 258 ([M + H]$^+$, 100 %); *m/z* [HRMS, ES+] found [M + H]$^+$ 258.0925, $C_{15}H_{13}FNO_2$ requires 258.0925.

4.4.39. Methyl 2-(6-(trifluoromethyl)-9H-carbazol-3-yl)acetate (42)

This compound was prepared by the same method as for **41**, with methyl 2-(4-((4-(trifluoromethyl)phenyl)amino)phenyl)acetate (**35**) (200 mg, 0.65 mmol) as the starting material, to afford the product as a white solid (87 mg, 44 %). Mp 138–142 °C; IR (neat, ν_{max}/cm^{-1}): 3324, 1716, 1612; ^{1}H NMR (700 MHz, $CDCl_3$) δ 8.31 (1H, s, **NH**), 8.27 (1H, s, **5-ArH**), 7.97 (1H, s, **4-ArH**), 7.62 (1H, dd, J = 8.5, 1.2 Hz, **7-ArH**), 7.42 (1H, d, J = 8.4 Hz, **8-ArH**), 7.36 (2H, m, **1-ArH** & **2-ArH**), 3.81 (2H, s, **CH_2**), 3.74 (3H, s, **CO_2CH_3**); ^{13}C NMR (176 MHz, $CDCl_3$) δ 172.9, 141.4, 139.2, 128.2, 125.9, 127.6–123.0 (q, J = 271 Hz), 123.3, 122.9 (q, J = 4.1 Hz) 122.8, 122.12–121.57 (q, J = 32.3 Hz), 121.3, 118.06 (q, J = 4.1 Hz), 111.2, 110.8, 52.3, 41.3; ^{19}F NMR (282 MHz, $CDCl_3$) δ −60.40 (**CF_3**); *m/z* [ES-] 306 ([M – H]$^-$, 100 %); *m/z* [HRMS, ES-] found [M – H]$^-$ 306.0738, $C_{16}H_{11}F_3NO_2$ requires 306.0747.

4.4.40. Methyl 2-(9H-carbazol-3-yl)acetate (43)

This compound was prepared by the same method as for **41**, with methyl 2-(4-(phenylamino)phenyl)acetate (**36**) (150 mg, 0.62 mmol) as the starting material, to afford the product as a white solid (93 mg, 63 %). Mp 127–131 °C; IR (neat, ν_{max}/cm^{-1}): 3389, 1727; ^{1}H NMR (700 MHz, $CDCl_3$) δ 8.06 (1H, br. s, **NH**), 8.05 (2H, app. dt, J = 7.7, 1.0 Hz, **5-ArH**), 7.98 (1H, d, J = 1.6 Hz, **4-ArH**), 7.43–7.38 (2H, m, **7-ArH** & **8-ArH**), 7.34 (2H, m, **1-ArH** & **2-ArH**), 7.23 (1H, ddd, J = 7.9, 6.4, 1.7 Hz, **6-ArH**), 3.81 (2H, s, **CH_2**), 3.72 (3H, s, **CO_2CH_3**); ^{13}C NMR (176 MHz, $CDCl_3$) δ 173.0, 140.0, 138.8, 127.2, 126.1, 125.1, 123.8, 123.2, 121.1, 120.5, 119.6, 110.8, 110.8, 52.2, 41.4; *m/z* [ES+] 240 ([M+H]$^+$, 100 %); *m/z* [HRMS, ES+] found [M + H]$^+$ 240.1016, $C_{15}H_{14}NO_2$ requires 240.1019.

4.4.41. Methyl 2-(7-chloro-9H-carbazol-3-yl)acetate (44)

This compound was prepared by the same method as for **41**, with methyl 2-(4-((3-chlorophenyl)amino)phenyl)acetate (**37**) (300 mg, 1.09 mmol) as starting material, to afford the product as a white solid (172 mg, 58 %). Mp 104–106 °C; IR (neat, ν_{max}/cm^{-1}): 3359, 1724, 1634; ^{1}H NMR (700 MHz, $CDCl_3$) δ 8.07 (1H, s, **NH**), 7.93–7.90 (2H, m, **4-ArH** & **5-ArH**), 7.38 (1H, d, J = 1.8 Hz, **8-ArH**), 7.36–7.32 (2H, m, **1-ArH** & **2-ArH**), 7.18 (1H, dd, J = 8.3, 1.8 Hz, **6-ArH**), 3.79 (2H, s, **CH_2**), 3.72 (3H, s, **CO_2CH_3**); ^{13}C NMR (176 MHz, $CDCl_3$) δ 172.9, 140.4, 139.0, 131.7, 127.5, 125.6, 123.2, 121.9, 121.3, 121.0, 120.2, 111.0, 110.8, 52.2, 41.3; *m/z* [ES-] 272 ([^{35}M – H]$^-$, 100 %), 274 ([^{37}M – H]$^-$, 100 %); *m/z* [HRMS, ES-] found [^{35}M – H]$^-$ 272.0475, $C_{15}H_{11}ClNO_2$ requires 272.0484.

4.4.42. Methyl 2-(6-methyl-9H-carbazol-3-yl)acetate (45)

This compound was prepared by the same method as for **41**, with methyl 2-(4-(*p*-tolylamino)phenyl)acetate (**38**) (200 mg, 0.78 mmol) as starting material, to afford the product as an off-white solid (126 mg, 63 %). Mp 114–116 °C; IR (neat, ν_{max}/cm^{-1}): 3357, 1723; ^{1}H NMR (700 MHz, $CDCl_3$) δ 7.94 (2H, br. s, **4-ArH** & **NH**), 7.84 (1H, s, **5-ArH**), 7.34 (1H, d, J = 8.3 Hz, **1-ArH**), 7.32–7.28 (2H, m, **2-ArH** & **8-ArH**), 7.23 (1H, d, J = 6.3 Hz, **7-ArH**), 3.79 (2H s, **CH_2**), 3.71 (3H, s, **CO_2CH_3**), 2.52 (3H, s, **$ArCH_3$**); ^{13}C NMR (176 MHz, $CDCl_3$) δ 173.0, 139.1, 138.2, 128.9, 127.4, 127.0, 124.9, 123.7, 123.4, 121.0, 120.4, 110.7, 110.4, 52.2, 41.5, 21.6; *m/z* [ES+] 254 ([M + H]$^+$, 100 %); *m/z* [HRMS, ES+] found [M + H]$^+$ 254.1176, $C_{16}H_{16}NO_2$ requires 254.1176.

4.4.43. Methyl 2-(6-methoxy-9H-carbazol-3-yl)acetate (46)

This compound was prepared by the same method as for **41**, with methyl 2-(4-((4-methoxyphenyl)amino)phenyl)acetate (**39**) (200 mg, 0.74 mmol) as starting material, to afford the product as a white crystalline solid (135 mg, 68 %). Mp 57–61 °C; IR (neat, ν_{max}/cm^{-1}): 3391, 1731; ^{1}H NMR (700 MHz, $CDCl_3$) δ 7.96 – 7.93 (m, 1H), 7.91 (s, 1H), 7.52 (d, J = 2.5 Hz, 1H), 7.36 – 7.28 (m, 3H), 7.05 (dd, J = 8.7, 2.5 Hz, 1H), 3.92 (s, 3H), 3.80 (s, 2H), 3.72 (s, 3H). ^{1}H NMR (700 MHz, $CDCl_3$) δ 7.94 (1H, m, **4-ArH**), 7.91 (1H, br. s, **NH**) 7.52 (1H, d, J = 2.4 Hz, **5-ArH**), 7.34–7.27 (3H, m, **1-ArH**, **2-ArH** & **8-ArH**), 7.05 (1H, dd, J = 8.7, 2.4 Hz, **7-ArH**), 3.92 (3H, s, **OCH_3**), 3.80 (2H, s, **CH_2**), 3.72 (3H, s, **CO_2CH_3**); ^{13}C NMR (151 MHz, $CDCl_3$) δ 173.1, 153.9, 139.5, 134.8, 127.1, 124.6, 123.7, 123.6, 120.9, 115.4, 111.5, 111.0, 103.1, 56.1, 52.2, 41.4; *m/z* [ES+] 270 ([M + H]$^+$, 100 %); *m/z* [HRMS, ES+] found [M+H]$^+$ 270.1125, $C_{16}H_{16}NO_3$ requires 270.1125.

4.4.44. Methyl 2-(6-bromo-9H-carbazol-3-yl)acetate (47)

This compound was prepared by the same method as for **41**, with methyl 2-(4-((4-bromophenyl)amino)phenyl)acetate (**40**) (125 mg, 0.39 mmol) as starting material, to afford the product as a brown solid (27 mg, 22 %). Mp 132–138 °C; IR (neat, ν_{max}/cm^{-1}): 3358, 1720, 1607, 1511; ^{1}H NMR (700 MHz, $CDCl_3$) δ 8.14 (1H, d, J = 2.0 Hz, **5-ArH**), 8.09 (1H, s, **NH**), 7.91 (1H, s, **4-ArH**), 7.48 (1H, dd, J = 8.5, 2.0 Hz, **7-ArH**),

7.37–7.33 (2H, m, **1-ArH** & **2-ArH**), 7.28 (1H d, $J = 8.5$ Hz, **8-ArH**), 3.79 (2H, s, **CH_2**), 3.72 (3H, s, **CO_2CH_3**); ^{13}C NMR (176 MHz, $CDCl_3$) δ 172.8, 139.1, 138.5, 128.8, 128.0, 125.6, 125.0, 123.3, 122.8, 121.2, 112.4, 112.2, 111.0, 52.2, 41.3; *m/z* [ES+] 318 ([^{79}M+H]$^+$, 100 %), 320 ([^{81}M+H]$^+$, 100 %); *m/z* [HRMS, ES–] found [^{79}M – H]$^-$ 315.9968, $C_{15}H_{11}BrNO_2$ requires 315.9979.

4.4.45. 2-(6-Fluoro-9H-carbazol-3-yl)acetic acid (48)

To a solution of methyl 2-(6-fluoro-9H-carbazol-3-yl)acetate (**41**) (26.5 mg, 0.10 mmol) in CH_2Cl_2 (1.10 ml) was added NaOH in MeOH (2 M, 0.4 ml, 0.8 mmol). The reaction mixture was stirred at RT for 3 h. The solvent was removed from the reaction mixture under reduced pressure. The crude product was then dissolved in water and washed with EtOAc (3 x 20 ml). The aqueous layer was then acidified by the addition of $HCl_{(aq)}$ (1 M) and extracted with EtOAc (3 x 20 ml). The organic layer was dried over anhydrous Na_2SO_4 and the solvent was removed under reduced pressure to afford the product as an off-white solid (20.2 mg, 81 %). Mp 216–219 °C; IR (neat, ν_{max}/cm^{-1}): 3403, 2849 br., 1690; ^{1}H NMR (600 MHz, MeOD) δ 7.94 (1H, d, $J = 1.0$ Hz, **4-ArH**), 7.73 (1H, dd, $J = 9.2$, 2.5 Hz, **5-ArH**), 7.41–7.36 (2H, m, **1-ArH** & **8-ArH**), 7.33 (1H, dd, $J = 8.4$, 1.8 Hz, **2-ArH**), 7.12 (1H, app. td, $J = 9.1$, 2.6 Hz, **7-ArH**), 3.74 (2H, s, **CH_2**); ^{13}C NMR (151 MHz, MeOD) δ 176.6, 159.3–157.7 (d, $J = 234.1$ Hz), 141.6, 138.2, 128.6, 126.2, 124.5 (d, $J = 10.6$ Hz), 124.2 (d, $J = 3.0$ Hz) 121.9, 114.1 (d, $J = 25.7$ Hz) 112.4 (d, $J = 9$ Hz), 111.9, 106.2 (d, $J = 22.7$ Hz), 42.0; ^{19}F NMR (282 MHz, $CDCl_3$) δ −127.34 (**6-F**); *m/z* [ES+] 244 ([M+H]$^+$, 100 %); *m/z* [HRMS, ES+] found [M+H]$^+$ 244.0772, $C_{14}H_{11}FNO_2$ requires 244.0768.

4.4.46. 2-(6-Trifluoromethyl)-9H-carbazol-3-yl)acetic acid (49)

This compound was prepared by the same method as for **48**, with methyl 2-(6-(trifluoromethyl)-9*H*-carbazol-3-yl)acetate (**42**) (50.0 mg, 0.16 mmol) as starting material to afford the product as a brown solid (31.4 mg, 66 %). Mp 158–162 °C; IR (neat, ν_{max}/cm^{-1}): 3398, 2852 br., 1699; ^{1}H NMR (600 MHz, MeOD) δ 8.33 (1H, s, **5-ArH**), 8.02 (1H, d, $J =$ 1.4 Hz, **4-ArH**), 7.60 (1H, d, $J = 8.5$ Hz, **7-ArH**), 7.52 (1H, d, $J = 8.5$ Hz, **8-ArH**), 7.43 (1H, d, $J = 8.4$ Hz, **1-ArH**), 7.37 (1H, dd, $J = 8.4$, 1.4 Hz, **2-ArH**), 3.77 (2H, s, **CH_2**); ^{13}C NMR (151 MHz, MeOD) δ 176.5, 143.4, 141.2, 129.6–124.3 (q, $J = 270.8$ Hz), 129.1, 127.2, 124.0, 123.7, 123.2 (q, $J = 3.5$ Hz), 122.0, 121.8–121.4 (m), 118.5 (q, $J = 4.0$ Hz), 112.1, 112.0, 41.9; ^{19}F NMR (282 MHz, $CDCl_3$) δ −61.48 (**CF_3**); *m/z* [ES–] 292 ([M – H]$^-$, 100 %); *m/z* [HRMS, ES+] found [M+H]$^+$ 294.0733, $C_{15}H_{11}F_3NO_2$ requires 294.0736.

4.4.47. 2-(9H-Carbazol-3-yl)acetic acid (50)

This compound was prepared by the same method as for **48**, with methyl 2-(9*H*-carbazol-3-yl)acetate (**43**) (50.0 mg, 0.21 mmol) as starting material. The crude product was recrystallised from a mixture of Et_2O and pentane to afford the product as a white solid (14 mg, 30 %). Mp 235–237 °C; IR (neat, ν_{max}/cm^{-1}): 3392, br 0.2850, 1690; ^{1}H NMR (600 MHz, MeOD) δ 8.03 (1H, d, $J = 7.9$ Hz, **5-ArH**), 7.96 (1H, d, $J = 2.0$ Hz, **4-ArH**), 7.41 (1H, d, $J = 8.2$ Hz, **8-ArH**), 7.39 (1H, d, $J = 8.3$ Hz, **1-ArH**), 7.34 (1H, ddd, $J = 8.2$, 7.0, 1.2 Hz, **7-ArH**), 7.30 (1H, dd, $J = 8.3$, 1.8 Hz, **2-ArH**), 7.13 (1H, ddd, $J = 7.9$, 7.0, 1.0 Hz, **6-ArH**), 3.74 (2H, s, **CH_2**); ^{13}C NMR (151 MHz, MeOD) δ 176.7, 141.8, 140.5, 127.9, 126.6, 126.1, 124.5, 124.1, 121.6, 120.9, 119.7, 111.7, 111.6, 42.1; *m/z* [ES+] 226 ([M+H]$^+$, 100 %); *m/z* [HRMS, ES+] found [M+H]$^+$ 226.0863, $C_{14}H_{12}NO_2$ requires 226.0863.

4.4.48. 2-(7-Chloro-9H-carbazol-3-yl)acetic acid (51)

This compound was prepared by the same method as for **48**, with methyl 2-(7-chloro-9H-carbazol-3-yl)acetate (**44**) (100 mg, 0.37 mmol) as starting material to afford the product as a white solid (62.4 mg, 66 %). Mp: 214–218 °C; IR (neat, ν_{max}/cm^{-1}): 3389, 2917 br., 1690; ^{1}H NMR (600 MHz, MeOD) δ 7.98 (1H, d, $J = 8.3$ Hz, **5-ArH**), 7.95 (1H, s, **4-ArH**), 7.43–7.37 (2H, m, **1-ArH** & **8-ArH**), 7.33 (1H, d, $J = 8.3$ Hz, **2-ArH**), 7.12 (1H, d, $J = 8.3$ Hz, **6-ArH**), 3.75 (2H, s, **CH_2**); ^{13}C NMR (151 MHz, MeOD) δ 176.5, 142.4, 140.9, 132.1, 128.4, 126.8, 123.9, 122.9, 122.0, 121.7, 120.0, 111.8, 111.6, 42.0; *m/z* [ES-] 258 ([^{35}M – H]$^-$, 100 %), 260 ([^{37}M – H]$^-$, 35 %); *m/z* [HRMS, ES+] found [^{35}M+H]$^+$ 260.0474, $C_{14}H_{11}ClNO_2$ requires 260.0473.

4.4.49. 2-(6-Methyl-9H-carbazol-3-yl)acetic acid (52)

This compound was prepared by the same method as for **48**, with methyl 2-(6-methyl-9*H*-carbazol-3-yl)acetate (**45**) (100 mg, 0.39 mmol) as the starting material. The crude product was recrystallised from a mixture of Et_2O and pentane to afford the product as a pale-yellow solid (35.3 mg, 37 %). Mp 211–215 °C; IR (neat, ν_{max}/cm^{-1}): 3400, 2859 br., 1693; ^{1}H NMR (600 MHz, MeOD) δ 7.91 (1H, d, $J = 1.0$ Hz, **4-ArH**), 7.82 (1H, d, $J = 0.8$ Hz, **5-ArH**), 7.35 (1H, d, $J = 8.8$ Hz, **1-ArH**), 7.30 (1H, d, $J = 8.2$ Hz, **8-ArH**), 7.27 (1H, dd, $J = 8.2$, 1.7 Hz, **2-ArH**), 7.18 (1H, dd, J = 8.2, 2.3 Hz, **7-ArH**), 3.73 (2H s, **CH_2**), 2.48 (3H, s, **$ArCH_3$**); ^{13}C NMR (151 MHz, MeOD) δ 176.7, 140.8, 140.1, 128.9, 127.9, 127.7, 125.8, 124.4, 124.3, 121.5, 120.8, 111.5, 111.4, 42.1, 21.5; *m/z* [ES+] 240 ([M+H]$^+$, 100 %); *m/z* [HRMS, ES–] found [M – H]$^-$ 240.1019, $C_{15}H_{12}NO_2$ requires 240.1019.

4.4.50. 2-(6-Methoxy-9H-carbazol-3-yl)acetic acid (53)

This compound was prepared by the same method as for **48**, with methyl 2-(6-methoxy-9*H*-carbazol-3-yl)acetate (**46**) (50.0 mg, 0.19 mmol) as starting material. The crude product was then recrystallised over a dry ice bath from a mixture of Et_2O and pentane to afford the product as a pale-yellow solid (11.4 mg, 24 %). Mp 173–177 °C; IR (neat, ν_{max}/cm^{-1}): 3428, 2937 br., 1697; ^{1}H NMR (600 MHz, MeOD) δ 7.93 (1H, d, $J = 1.0$ Hz, **4-ArH**), 7.57 (1H, d, $J = 2.4$ Hz, **5-ArH**), 7.35 (1H, d, $J = 8.3$ Hz, **1-ArH**), 7.32 (1H, d, $J = 8.7$ Hz, **8-ArH**), 7.27 (1H, dd, $J =$ 8.3, 1.7 Hz, **2-ArH**), 7.00 (1H, dd, $J = 8.8$, 2.5 Hz, **7-ArH**), 3.88 (3H, s, **OCH_3**), 3.73 (2H, s, **CH_2**); ^{13}C NMR (151 MHz, MeOD) δ 176.7, 154.9, 141.2, 136.8, 127.8, 125.6, 124.5, 124.4, 121.6, 116.0, 112.4, 111.7, 103.7, 56.4, 42.1; *m/z* [ES+] 256 ([M+H]$^+$, 100 %); *m/z* [HRMS, ES+] found [M – H]$^-$ 238.0862, $C_{15}H_{12}NO_3$ requires 238.073.

4.4.51. 2-(6-Bromo-9H-carbazol-3-yl)acetic acid (54)

This compound was prepared by the same method as for **48**, with methyl 2-(6-bromo-9*H*-carbazol-3-yl)acetate (**47**) (15.0 mg, 0.05 mmol) as the starting material, to afford the product as an off-white solid (9.2 mg, 64 %). Mp 194–197 °C; IR (neat, ν_{max}/cm^{-1}): 3404, 2920, 1691; ^{1}H NMR (600 MHz, MeOD) δ 8.17 (1H, d, $J = 2.0$ Hz, **5-ArH**), 7.95 (1H, s, **4-ArH**), 7.44 (1H, dd, $J = 8.6$, 2.0 Hz, **7-ArH**), 7.40 (1H, d, $J = 8.3$ Hz, **1-ArH**), 7.34 (2H, m, **2-ArH** & **8-ArH**), 3.75 (2H, s, **CH_2**); ^{13}C NMR (151 MHz, MeOD) δ 176.5, 141.0, 140.4, 129.2, 128.8, 126.8, 126.0, 123.7, 123.5, 121.9, 113.4, 112.2, 111.9, 42.0; *m/z* [ES-] 302 ([^{79}M – H]$^-$, 100 %), 304 ([^{81}M – H]$^-$, 100 %); *m/z* [HRMS, ES-] found [^{79}M – H]$^-$ 301.9813, $C_{14}H_9BrNO_2$ requires 301.9822.

Author contribution

S.B. and H.C.H conceived, designed and overall supervised the project. L.M., M.G.A, S.A. and T.M.D.B performed chemical synthesis, SAR analysis, L.M, C.D., S.W. and A.N performed microbiological and cell toxicity assays, analysed the biology results, and H.C.H and S.B. helped in interpreting the results. L.M. wrote the first complete draft of the manuscript. All the authors contributed significantly to improve the clarity and quality of the original research presentation. The funders had no role in study design, data collection and analysis, and the decision to publish or prepare the manuscript.

CRediT authorship contribution statement

Liam T. Martin: Writing – original draft, Visualization, Validation, Software, Methodology, Investigation, Formal analysis, Data curation. **Chris Daniel:** Writing – review & editing, Visualization, Methodology, Investigation, Formal analysis, Data curation. **Marcus Guldberg-Allen:**

Writing – review & editing, Investigation. **Anushandan Navaratnarajah:** Writing – review & editing, Methodology. **Silvia Anselmi:** Writing – review & editing, Investigation. **Tina-Maria D. Burova:** Writing – review & editing, Investigation. **Sam Willcocks:** Writing – review & editing, Supervision, Methodology, Investigation, Formal analysis, Data curation. **Helen C. Hailes:** Writing – review & editing, Validation, Supervision, Resources, Project administration, Methodology, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Sanjib Bhakta:** Writing – review & editing, Validation, Supervision, Resources, Project administration, Methodology, Funding acquisition, Formal analysis, Data curation, Conceptualization.

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Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Liam Martin reports article publishing charges, equipment, drugs, or supplies, and travel were provided by The Wellcome Trust. Silvia Anselmi reports equipment, drugs, or supplies and travel were provided by BBSRC. Helen Hailes reports equipment, drugs, or supplies was provided by EPSRC. Liam T. Martin, Chris Daniel, Sanjib Bhakta, Helen C. Hailes has patent #UK Patent Application No. 2310572.9 (Anti-Tubercular Carbazoles) issued to UCL Business (https://www.uclb.com/). If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bmc.2025.118226.

Data availability

It is available in the supplementary information.

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