An Evaluation of Minor Groove Binders as Anti-fungal and Anti-mycobacterial Therapeutics

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Abstract

This study details the synthesis and biological evaluation of a collection of 19 structurally related Minor Groove Binders (MGBs), derived from the natural product distamycin, which were designed to probe antifungal and antimycobacterial activity. From this initial set, we report several MGBs that are worth more detailed investigation and optimisation. **MGB-4**, **MGB-317** and **MGB-325** have promising MIC_{80}s of 2, 4 and 0.25 µg/mL, respectively, against the fungus *C. neoformans.* **MGB-353** and **MGB-354** have MIC_{99}s of 3.1 µM against the mycobacterium *M. tuberculosis*. The selectivity and activity of these compounds is related to their physicochemical properties and the cell wall/membrane characteristics of the infective agents.

1. Introduction

Nearly every disease-causing microbe that infects humans has developed measurable resistance to its respective anti-infective therapies, and worryingly, this has happened much faster than anticipated. The resulting drug-resistant infections give rise to increased patient mortality, hospital

stays, spread of infection and healthcare costs [1]. This paper specifically reports on the discovery of compounds with significant activities against *Cryptococcus neoformans* and *Mycobacterium tuberculosis*. Both of these organisms present difficulties for drug discovery due to the challenges of penetrating their respective cell walls [2].

The fungal pathogen *Cryptococcus neoformans* is capable of causing life-threatening cryptococcal meningitis in patients in an immunocompromised state. It is a particularly significant concern in patients with advanced AIDS, causing 15% to 20% of AIDS-related mortality [3]; however, those on immunosuppressive therapies and those suffering from haematological malignancy are also at risk [4-6]. In general, susceptibility to cryptococcal meningitis is characterised by a failure in pro-inflammatory immune response to primary infection which is likely to occur in the lungs [7-9]. Alveolar macrophages are the first immune cells to encounter cryptococci; however, *C. neoformans* is able to parasitise these macrophages and proliferate within the phagosome [10, 11].

The World Health Organisation (WHO) guidelines recommend that cryptococcal meningitis be treated with a 2-week course of amphotericin B alongside flucytosine which is then followed by a consolidation therapy of fluconazole [12, 13]. Person-to-person transmission of cryptococcal meningitis is rare and so resistance to these antifungal therapies is unlikely to spread rapidly; only a few reports of amphotericin B resistance, the key drug in the regimen, have been documented [3, 14]. However, amphotericin B has a number of significant side-effects, such as anemia, hypokalemia, hypomagnesaemia and nephrotoxicity, making the discovery of alternative therapies particularly relevant [15].

Mycobacterium tuberculosis is the causative agent of tuberculosis (TB) infections and this disease is now recognised as a global emergency. One third of the world's population, that is approximately 6 billion people, are said to be harbouring the tuberculosis bacillus, leading to 1.5 million deaths per year [16]. There is a growing incidence of both multidrug-resistant TB (MDR-TB) and extensively drug-resistant TB (XDR-TB) which has led to difficult and lengthy first line treatments of the infection. A four drug cocktail of isoniazid, rifampin, pyrazinamide and ethambutol is given for the first two months followed by four months of only isoniazid and rifampin – the side effects of such a regimen are notably significant. Moreover, treatment of MDR-TB is characterised by relatively less effective, poorly tolerated, and expensive drugs that may need to be administered for years [17].

There are over 3 million instances of HIV and *Mycobacterium tuberculosis* co-infection which has problematic consequences for treatment options due to the drug-drug interactions between rifampin and many antiretrovirals. Specifically, the cytochrome P450 enzymes that metabolise many antiretrovirals are induced by treatment with rifampin making co-treatment of TB and HIV a significant challenge [18]. Clearly, more effective treatments, with shorter treatment times, are a necessity if the threat of MDR-TB and XDR-TB are to be managed.

At the University of Strathclyde, we have extensively investigated the Minor Groove Binder (MGB) class of compound, based on the natural product distamycin, as anti-infective agents. This distamycin template is built from *N*-methylpyrrole amino acid amides and has an amidine tail group (Figure 1). Our approach has led to some divergence from this structure: less basic functional groups have been introduced to replace the amidine at the C-terminus (usually referred to as the tail group because of its flexibility); larger alkyl side chains have been substituted for methyl groups; thiazole rings have been introduced to the body of the MGB; and, aromatic rings have replaced the formyl group from distamycin [19, 20].

Our extensive investigation of MGBs has resulted in the discovery of significantly active compounds against a range of diseases. We have recently identified MGBs with therapeutically interesting activities against: *Trypanosoma brucei brucei* for the treatment of human African trypanosomiasis [21]; *Trypanosoma vivax* and *T. congolense* for the treatment of animal African trypanosomiasis [unpublished results]; *Plasmodium falciparum* for the treatment of malaria [22]; and, lung cancer [23]. Moreover, our commercial partner, MGB Biopharma, has successfully progressed one compound through phase I clinical trials against the Gram-positive bacterium *Clostridium difficile* [24].

This study focuses on an investigation of a collection of structurally similar MGBs designed to target mycobacteria and fungi, although an examination of their biological activity profiles against a range of infectious organisms, including bacteria, fungi and parasites, is also presented. The compound collection under investigation is comprised of three series which differ in the heterocycle attached to the tail group, either *N*-methylpyrrole, *N*-isopentylpyrrole, or isopentylthiazole (Figure 1). Within each series, the head group position is varied according to the structures indicated in figure 1; however, the isopentylthiazole series also included four additional head groups to further explore the interesting activity of this series discovered during the course of the study. The amidine-linked head group used in this study has been investigated in isolated cases in some of our previous work, but this present study is our first systematic study of MGBs containing it. This amidine head group link might have advantages in terms of solubility and intrinsic activity with pathogens with troublesome cell walls because it generates dicationic MGBs. The systematic structural variations in this study examine whether the inclusion of lipophilic moieties, such as thiazoles and alkyl side chains, are important for penetration of the waxy cell wall of mycobacteria. Similarly, the inclusion of amidine-linked head groups is to investigate their importance in providing an additional source of hydrogen bonding to assist in penetrating the polyglycan cell wall of fungi [2]. Structural features that allow cellular accumulation may be more important for the activity and selectivity of MGBs than their specific DNA binding properties.

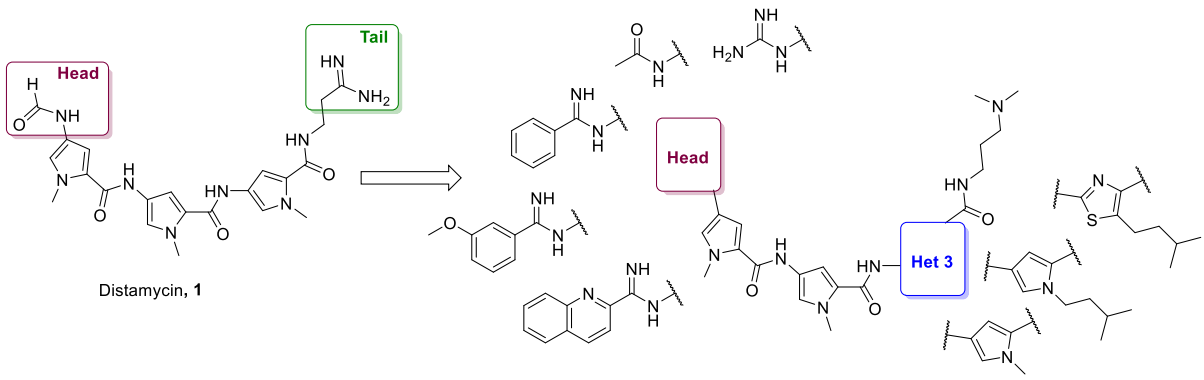


Figure 1. Distamycin and examples of MGBs in this study.

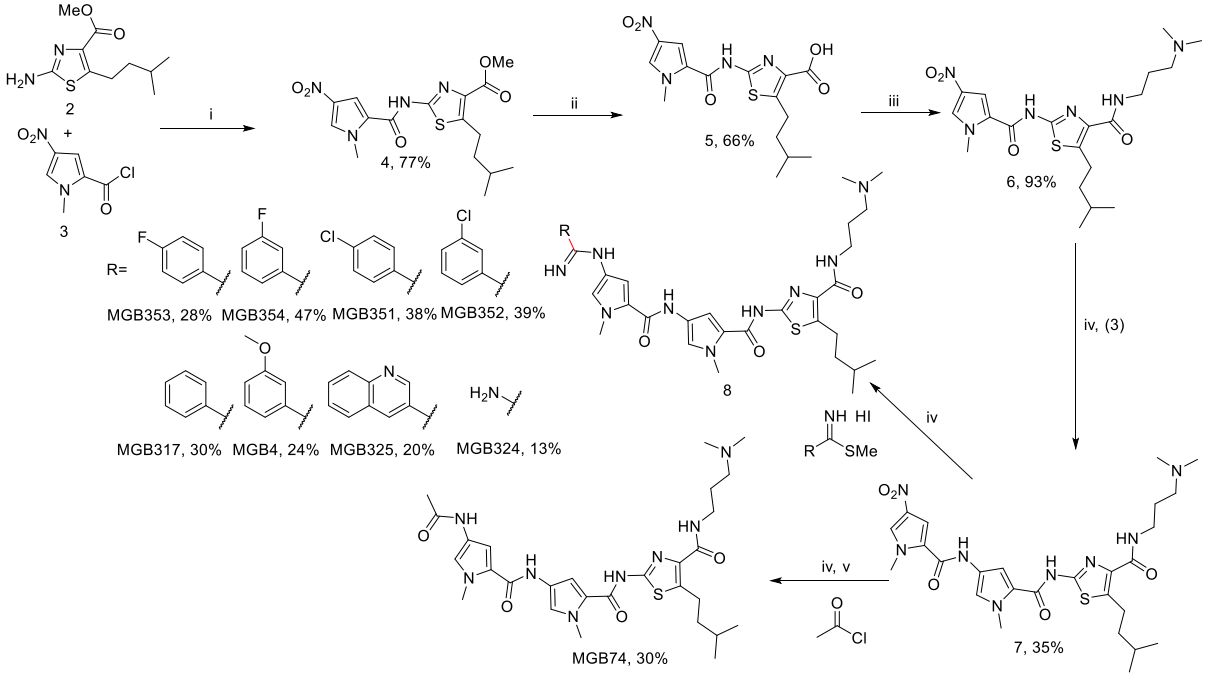
2. Results and Discussion

2. 1 Synthesis

The synthesis of amidine-linked head group or thiazole containing MGBs has not been extensively described in our previous work. To prepare compounds containing isopentyl thiazole(scheme 1), 1-methyl-4-nitro-1*H*-pyrrole-2-carboxylic acid was converted into the corresponding acid chloride **3** in quantitative yield by refluxing in thionyl chloride. The acid chloride so formed was reacted with methyl 2-amino-5-isopentyl-1,3-thiazole-4-carboxylate **2** to give the methyl ester dimer **4** in 77% yield. Hydrolysis of the methyl ester **4** in aqueous lithium hydroxide gave the corresponding

carboxylic acid **5** in 66% yield. HBTU coupling of the dimer carboxylic acid **5** with *N*,*N*-dimethyl-1,3-propanediamine in DCM gave compound **6** in 93% yield as pale yellow solid. Reduction of the nitro group in **6** using 10%-Pd/C in hydrogen gas gave the amine which was coupled with another unit of acid chloride **3** to give the trimer **7** in 35% yield.

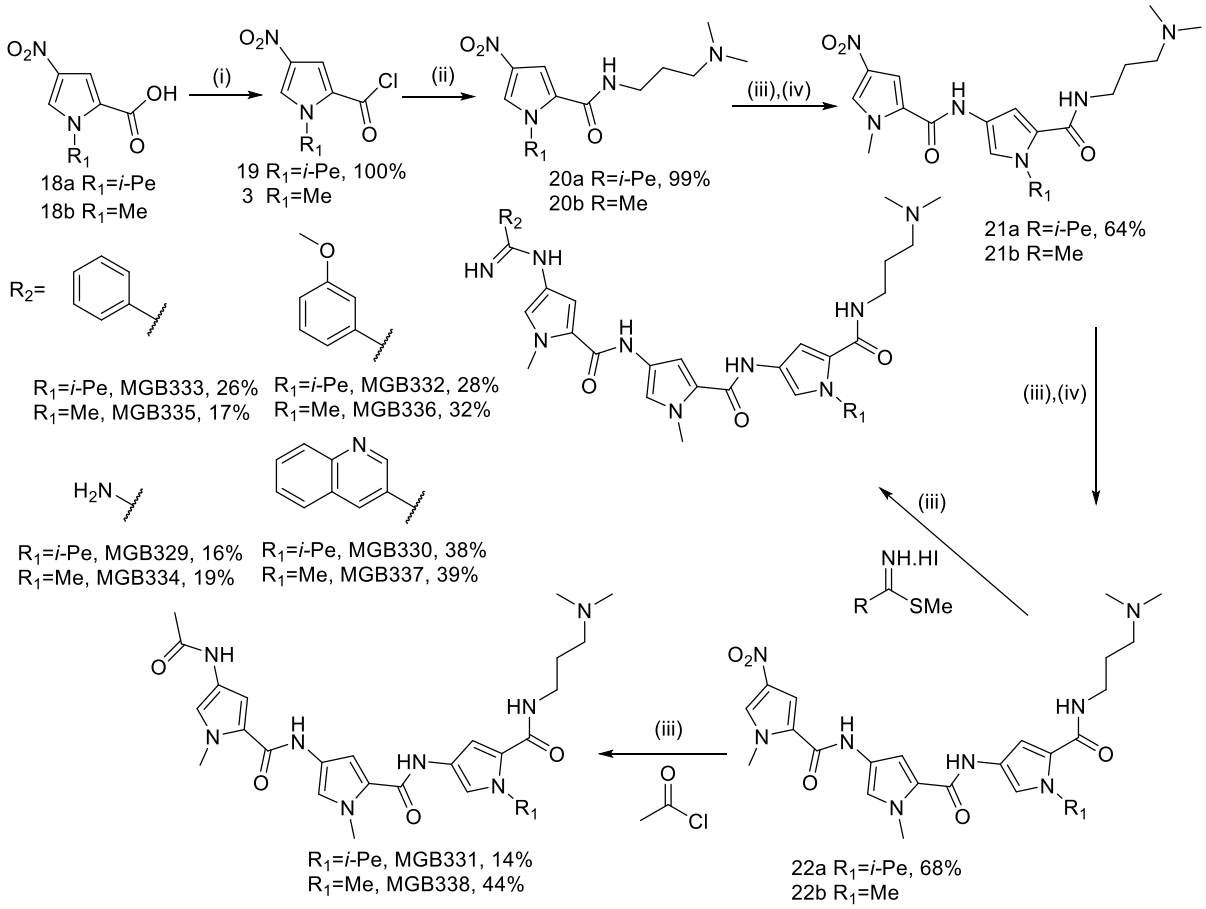
This trimer was used to prepare the final MGBs. To form the amide linked head group MGB, reduction of the trimer **7** as above, followed by reacting the amine so formed with acetyl chloride gave **MGB-74** as white solid in 30% yield, after HPLC purification and lyophilisation. To form the amidine linked head group MGBs, the amine formed from the reduction of nitro-trimer **7** was reacted with the appropriate methyl carbimidothioate hydroiodide at room temperature and then subjected to HPLC purification and lyophilisation to give the required products, **MGB-4**, **MGB-317**, **MGB-324**, **MGB-325**, **MGB-351** through **MGB-354**, in yields ranging from 13-47%.



Scheme 1. Synthesis of the thiazole S-MGB series.

To prepare compounds containing either *N*-isopentylpyrrole or *N*-methylpyrrole (scheme 2), 1-isopentyl-4-nitro-1*H*-pyrrole-2-carboxylic acid **18a** or 1-methyl-4-nitro-1*H*-pyrrole-2-carboxylic acid **18b** were converted into the corresponding acid chloride **19** and **3** in quantitative yields by refluxing in thionyl chloride. The acid chlorides so formed were reacted with *N*,*N*-dimethyl-1,3-propanediamine in DCM to give **20a** in 99% yield and **20b**. Reduction of this monomer **20b** or **20b** using 10%-Pd/C in hydrogen gas gave the amine which was coupled with another unit of acid chloride **3** to give **21a** in 64% yield or **21b**. This process was repeated to give the trimer **22a** in 68% yield or **22b**. This trimer was used to prepare the final MGBs.

To form the amide-linked head group MGBs, reduction of the trimers **22a,b** as above, followed by reacting the amine so formed with acetyl chloride gave **MGB-331 (14%)** and **MGB-338 (44%)** as white solids, after HPLC purification and lyophilisation. To form the amidine linked head group S-MGBs, the amine formed from the reduction of nitro-trimers, **22a,b**, were reacted with the appropriate methyl carbimidothioate hydroiodide at room temperature and then subjected to HPLC purification and lyophilisation to give the required products, **MGB-333**, **MGB-332**, **MGB-335**, **MGB-336**, **MGB-329**, **MGB-330, MGB-334**, **MGB-337** in 16 – 19% yields.



Reagents: (i) $SOCl_2$, reflux; (ii) Dimethylaminopropylamine, DCM; (iii) Pd/C, H_2, methanol; (iv) DCM and 3;

Scheme 2. Synthesis of the *N*-isopentylpyrrole and *N*-methylpyrrole MGB series.

2.2 Physicochemical Property Evaluation

The efficacy of an MGB in these anti-infective applications will depend upon many factors to which physicochemical properties contribute. In this study, the affinity of MGBs for DNA and the lipophilicity of the MGBs were investigated, the latter in particular with respect to cell wall penetration.

2.2.1 DNA Thermal Denaturation

To determine the DNA affinity of all newly synthesised MGBs, thermal denaturation experiments were performed. Salmon sperm DNA (wild-type, sequence unspecific oligonucleotide) was used at a concentration of 20 µg/mL and its melting temperature (T_m) determined to be 71 °C. A 10 µM concentration of MGB was used to evaluate the binding strength with the salmon sperm DNA and ΔT_m values were obtained for each DNA/ligand complex. The results were obtained in at least three independent experiments and are presented in Table 1.

All the MGBs have measurable ΔT_ms, ranging from 2 °C to 17 °C. There are no discernible patterns in these values either when comparing head group modifications. However, in general the inclusion of the larger, more lipophilic side chain appears to decrease binding affinity. Moreover, it is apparent that minor structural modifications can result in significant changes in ΔT_m. Consider **MGB-4**, **MGB-332** and **MGB-336**, in which the change of isopentylthiazole to isopentylpyrrole or methylpyrrole

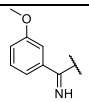
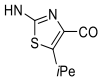
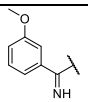
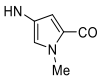
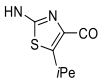
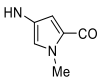
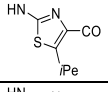
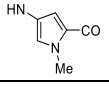
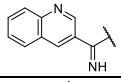
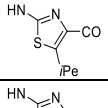
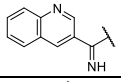
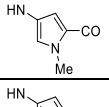
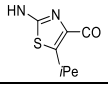
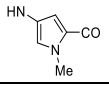
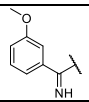
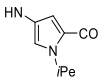
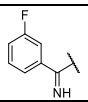
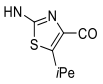
gives ΔT_ms of 7 °C, 5 °C and 11 °C, respectively. Similarly, the change from meta-fluoro, in **MGB-354**, to para-fluoro, in **MGB-353**, results in a ΔT_m change from 4 °C to 9 °C, respectively. The greatest change in ΔT_m is observed in replacing the isopentylthiazole in **MGB-325** with an isopentylpyrrole, to give **MGB-330**, or methylpyrrole, to give **MGB-337**, which results in a significant increase in ΔT_m from 2 °C to 17 °C.

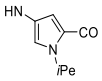
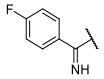
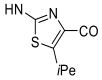
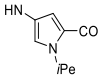
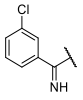
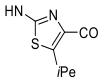
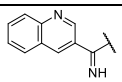
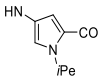
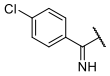
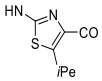
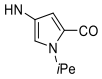
2.2.2 Lipophilicity

In order to glean comparative information regarding the lipophilicity of these MGBs, their retention times were measured using an HPLC system fitted with a C-18 column. A dual solvent system of acetonitrile and water, both containing 0.1% trifluoroacetic acid, was used and the solvent gradient began at 100% water, moving to 100% acetonitrile over 1 hour. It should be noted that the solvent system used was necessarily of very low pH as satisfactory chromatography within reasonable periods could not be achieved at near neutral pHs; the propensity of MGBs to form higher order aggregates at neutral pH has previously been reported [25].

The range of retention time is relatively large, going from 14.2 mins to 27.3 mins, and thus the implied lipophilicity differences could be very significant in terms of biological activity. Observed general trends in lipophilicity were as expected, with compounds from the isopentylthiazole series having the greatest retention times, followed by the *N*-isopentylpyrrole series and finally the *N*-methylpyrrole series. This overall reduction in compound lipophilicity is in line with the reduced lipophilicity of the changing heterocycle. Across the series, the trends in lipophilicity for different head groups were moderately consistent: the isoquinoline head group compounds were the most lipophilic, the simple amidine head group compounds were the least and the others consistently of intermediate lipophilicity although their ordering was variable – this variability is likely due to their similar values of retention time.

Table 1: MGB structures as defined by the framework in figure one. MGB-DNA thermal melting temperature (ΔTm) differences compared to ss-DNA. Lipophilicity measured as the retention time in a reverse phase HPCL method.

MGB ID	Head	Het 3	*ΔTm/°C*	*Lipophilicity/mins*	MGB ID	Head	Het 3	*ΔTm/°C*	*Lipophilicity/mins*
4			7	26.8	**336**			11	17.5
317			7	26.2	**335**			11	17.8
324			4	23.4	**334**			5	14.2
325			2	27.3	**337**			>17	19.7
74			4	27.1	**338**			5	18.3
332			5	23.8	**354**			4	26.2

333			8	22.6	**353**			9	26.2
329			4	20.1	**352**			5	26.9
330			17	24.8	**351**			4	26.8
331			3	22.8					

2.3 Biological Evaluation

All new MGBs that were synthesised as part of this study were evaluated in a panel of biological assays for MIC, EC_{50} or CC_{50} determination. This included a measure of: Gram-positive antibacterial activity against *Staphylococcus aureus* (MRSA); antimycobacterial activity against *Mycobacterium tuberculosis* H37Rv; antifungal activity against *Candida albicans* and *Cryptococcus neoformans*; antiparasitic activity against *Trypanosoma brucei brucei* and *Trypanosoma congolense*; and, cytotoxicity against HEK 293 cells, if sufficient activity warranted further investigation. These data are presented in table 2.

Table 2: Results from panel of biological assays. NA is Not Active, NT is Not Tested due to lack of significant activity against infectious organisms. Strains used: S. aureus (MRSA) ATCC 43300; M. tuberculosis H37Rv; C. albicans ATCC 90028; C. neoformans H99 ATCC 208821; T. congolense IL3000; T.b.brucei Lister 427; HEK 293 ATCC CRL-1573.

MGB ID	***S. aureus MRSA***	***M. tuberculosis H37Rv***	***C. albicans***	***C. neoformans H99***	***T. congolense***	***T. b.brucei***	***HEK-293***
	MIC_{80} (µg/mL)	***MIC_{99} (µM)***	***MIC_{80} (µg/mL)***	***MIC_{70} (µg/mL)***	***EC_{50} (µM)***	***EC_{50} (µM)***	***CC_{50} (µg/mL)***
4	**1**	25	>32	2	5.8	17	>32
317	>32	25	>32	4	5.8	23	>32
324	16	NA	>32	4	6.1	15	20
325	**1**	NA	>32	**0.25**	1.9	3.3	1.2
74	16	NA	>32	16	9.1	28	>32
332	>32	NA	>32	>32	1.4	24	>32
333	>32	NA	>32	>32	1.2	33	NT

329	>32	NA	>32	32	5.2	49	>32
330	32	25	>32	8	0.82	2.7	12
331	>32	NA	>32	>32	6.5	54	NT
336	>32	NA	>32	>32	1.4	44	NT
335	>32	NA	>32	>32	2.2	>100	NT
334	>32	NA	>32	>32	6.2	>100	NT
337	>32	25	>32	8	0.60	5.7	>32
338	>32	NA	>32	>32	7.0	>100	NT
354	8	**3.1**	>32	8	5.5	18	18
353	8	**3.1**	>32	4	10	18	18
352	2	13	>32	2	5.6	13	9.2
351	8	13	>32	4	4.3	13	10

MGBs across all series show a significant absence of activity against both parasitic organisms under investigation. For comparison, recently reported MGBs which have a different structural type, namely alkene or amide-linked head groups, have shown 1000-fold more potent activities against *T. b. brucei* under the same assay conditions [21]. Within this set, MGBs with the isoquinoline head group containing compounds, **MGB-325**, **MGB-330**, **MGB-337**, are the most active within their respective series against *T. b. brucei* and *T. congolense*. Although these compounds are all the most lipophilic within their respective series, this is not necessarily a generic lipophilicity-related trend as **MGB-351** through **MGB-354** are nearly as lipophilic, yet have much lower activities against the parasites. Interestingly, one of the most active *T. b. brucei* MGBs in our recent study contained this head group which may indicate the involvement of a specific biological interaction for this moiety [21].

Within this set of MGBs, only those of the isopentylthiazole series possessed any notable anti-Gram-positive activity against *S. aureus*; **MGB-317** is somewhat anomalous, however, with no measurable activity. In the context of our broader investigations of MGBs as anti-infective agents we have made significant progress towards developing anti-Gram-positive agents and thus, although **MGB-4** and **MGB-325** possess noteworthy activities at 1 μg/mL, we would not consider these active enough for further evaluation in this field [24].

Trends in mycobacterial activity are much less pronounced with most active MGBs coming from the isopentylthiazole series. Other active MGBs, **MGB-330** and **MGB-337** are the most lipophilic of the other two series thus, in contrast to the variation of activities against the parasitic organisms, this perhaps suggests that MGBs' activity against mycobacterial may be associated with a general increase in lipophilicity rather than a specific structural feature. With the highest anti-mycobacterial activities of 3.1 μM, and toxicities against HEK of 18 μg/mL, **MGB-353** and **MGB-354** are the best starting point compounds here for further development. It is worth noting that the anti-mycobacterial activity is given as an MIC_{99}; whereas, the HEK activity is given as a CC_{50}. For comparison, the MIC_{50} of these compounds is approximately 0.8 μM the CC_{50} in molar units is 20.2 μM and thus the selectivity toxicities of these MGBs are about 25 which is in an acceptable range for further study.

Anti-fungal activity is only observed against *C. neoformans* and the trend in activity of MGBs against this fungus generally matches that against *S. aureus*. It is interesting that these compounds

selectively affected *C. neoformans* over *C. albicans*; however, given the differing cell wall compositions between fungal species it is not surprising [26]. For example, the outer chain mannans of *C. albicans* contain negatively charged phosphodiester links, absent from *C. neoformans*, and a complementary bidentate interaction between the positively charged amidine and the phosphodiester anion coupled with an interaction between the cationic tail group and the phosphodiester anion could together sequester the MGB in the cell wall. The single interaction of the cationic tail group with the phosphodiester in **MGB-74** is evidently insufficient. This hypothesis would also explain the lack of activity observed in *C. albicans* [27-29]. Moreover, a recent study by Stocks *et al.* has suggested that pentamidine, an MGB of the diamidine class, which potentiates otherwise non-Gram-negative active drugs against these bacteria, specifically interacts with the phosphodiesters of the cell wall. Bacteria that have a higher phosphodiester composition are less susceptible to the potentiating effects of pentamidine [30].

Across the series, the most active compound from each is that with the isoquinoline head group, again perhaps alluding to a threshold lipophilicity being necessary to achieve notable activity. The most active MGB, **MGB-325**, with an MIC_{80} of 0.25 µg/mL is also the most toxic of the set, with an EC_{50} of 1.2 µg/mL and so **MGB-4** or **MGB-317**, with activities of 2 and 4, respectively, and no measurable toxicity,are more suitable hit compounds for further optimisation against *C. neoformans*. **MGB-317** is interesting as it lacks activity against *S. aureus* and so if this lack of activity is observed against other Gram-positive bacteria then it would be a selective antifungal. For reasons of minimising antimicrobial resistance, we are interested in developing highly selective MGBs.

3. Conclusions

This study investigated a collection of 19 structurally related MGBs and revealed several compounds worth further investigation. **MGB-4** and **MGB-317** have promising MIC_{80}s of 2 and 4 µg/mL, respectively, against *C. neoformans* and good selectivity indices. Although **MGB-325** has a lower MIC_{80} of 0.25 µg/mL than any of the other active examples, its toxicity is too high to warrant further development as it stands. However, our recent developments in antiparasitic MGBs have highlighted a number of structural modifications that significantly reduce toxicity without affecting the activity (unpublished results) and these could be applied to **MGB-325** in further studies.

Of particular interest are **MGB-353** and **MGB-354**, which have MIC_{99}s of 3.1 µM against *M. tuberculosis* and modest mammalian cell toxicity. SAR would suggest that the inclusion of the thiazole unit is involved in providing anti-infective activity to this collection of MGBs. This may be a function of the increased lipophilicity imparted by the replacement of a pyrrole by a thiazole or may be due to a specific molecular interaction such as greater affinity of DNA binding to regions of high GC base pairs density.

Overall, we have identified several members of this class of minor groove binder that are worth progressing to hit to lead optimisation for both antifungal and antimycobacterial indications. Furthermore, we have identified that individual structural features are likely to be of importance in achieving selectivity of uptake through cell walls with evidence suggesting that both charge and overall lipophilicity are important in determining the penetration of an MGB into a target organism.

4. Acknowledgements

The authors would like to thank the Rosetrees Trust for funding and also Scott Brown, Craig Irving, Gavin Bain and Patricia Keating for their support during the course of this research.

5. Experimental

5.1 Chemistry

5.1.1 General Experimental Methods

^{1}H and ^{13}C NMR spectra were measured on a Bruker DPX-400 MHz spectrometer with chemical shifts given in ppm (δ-values), relative to proton and carbon traces in solvent. Coupling constants are reported in Hz. IR spectra were recorded on a Perkin Elmer, FT-IR spectrometer. Mass spectra were obtained on a Jeol JMS AX505. Anhydrous solvents were obtained from a Puresolv purification system, from Innovative Technologies, or purchased as such from Aldrich. Melting points were recorded on a Reichert hot-stage microscope, and are uncorrected. Chromatography was carried out using 200-400 mesh silica gels, or using reverse-phase HPLC on a waters system using a C18 Luna column (Luna Su C18(2), 100A, AXIA, 50x21, 20 mm, 5 micron phenomenex) with the gradient given below and using a detection wavelength of 254 nm.

HPLC method used for the purification of final MGBs:

Time (min)	Flow rate (mL/min)	%Water (0.1%TFA)	%Acetonitrile (0.1%TFA)
0	6	90	10
25	6	60	40
35	6	50	50
40	6	30	70
44	6	90	10

5.1.2 DNA Thermal Denaturation Assays.

Phosphate buffer (1 mM Sodium Phosphate (50:50;Na_2HPO_4:NaH_2PO_4), 13.7 mM NaCl, 0.27 mM KCl, pH7.4) was used as the buffer system. The salmon sperm DNA was purchased from Sigma Aldrich (extinction coefficient ε_{260} = 6600 cm^{-1} M^{-1} base) and used at a final concentration of 20 µg/mL with 10 µM of MGB. UV measurements were obtained with a Shimadzu UV-1800 with an eight-series micro multi-cell, each of which contained a sample volume of 100 µL. The UV spectra were obtained over a range of 20°C–95°C with a ramp speed of 0.5 °C/min and measurement intervals of 0.2 °C. The absorbance was measured at a wavelength of 260 nm. All measurements were performed at least three times to provide an average value which is reported to the nearest degree. The thermal melting temperatures of the DNA or DNA-MGB complex was obtained by fitting a boltzman distribution in OriginPro 9.0.

5.1.3 Lipophilicity

Retention times were measured using an HPLC system fitted with a C-18 column (Luna Su C18(2), 100A, AXIA, 50x21, 20 mm, 5 micron phenomenex). A dual solvent system of acetonitrile and water, both containing 0.1% trifluoroacetic acid, was used and the solvent gradient began at 100% water, moving to 100% acetonitrile over 1 hour.

5.1.4 Synthesis of Thiazole containing MGBs

5.1.4.1 Methyl 5-isopentyl-2-{[(1-methyl-4-nitro-1*H*-pyrrol-2-yl)carbonyl]amino}-1,3-thiazole-4-carboxylate (4) [21]

Prepared as per reference.

5.1.4.2 5-Isopentyl-2-{[(1-methyl-4-nitro-1*H*-pyrrol-2-yl)carbonyl]amino}-1,3-thiazole-4-carboxylic acid (5) [21]

Prepared as per reference.

5.1.4.3 N-[3-(Dimethylamino)propyl]-5-isopentyl-2-{[(1-methyl-4-nitro-1*H*-pyrrol-2-yl)carbonyl]amino}-1,3-thiazole-4-carboxamide (6) [21]

Prepared as per reference.

5.1.4.4 N-[3-(Dimethylamino)propyl]-5-isopentyl-2-{[(1-methyl-4-{[(1-methyl-4-nitro-1*H*-pyrrol-2-yl)carbonyl]amino}-1*H*-pyrrol-2-yl)carbonyl]amino}-1,3-thiazole-4-carboxamide (7) [21]

Prepared as per reference.

5.1.4.5 Synthesis of full MGBs:

N-[3-(Dimethylamino)propyl]-5-isopentyl-2-{[(1-methyl-4-{[(1-methyl-4-nitro-1H-pyrrol-2-yl)carbonyl]amino}-1H-pyrrol-2-yl)carbonyl]amino}-1,3-thiazole-4-carboxamide (368 mg, 0.78 mmol) was dissolved in methanol (50 mL) to which Pd/C-10% (300 mg) was added at 0^{o}C under nitrogen with stirring. The reaction mixture was hydrogenated for 3h at atmospheric pressure and room temperature. The catalyst was removed over Kieselguhr and the solution was divided into nine equal portions:

Portion One:

2-({[4-({[4-(Acetylamino)-1-methyl-1H-pyrrol-2-yl]carbonyl}amino)-1-methyl-1H-pyrrol-2-yl]carbonyl}amino)-N-[3-(dimethylamino)propyl]-5-isopentyl-1,3-thiazole-4-carboxamide trifluoroacetate (MGB-74)

Methanol was removed under reduced pressure and the residue was dissolved in DCM (5 mL, dry) to which TEA (20 µL) was added. A solution of acetyl chloride was prepared as follows: (68 µL) of acetyl chloride was dissolved in DCM (5 mL, dry) and then (0.5 mL) from this solution was added to the reaction mixture dropwise at room temperature with stirring. The reaction mixture was left standing at room temperature overnight. DCM and all volatile reagents were removed under reduced pressure and the residue was dissolved in DMF (1 mL). The solution was subjected to HPLC purification (R_t = 22.0 min). Fractions containing the required product were collected and freeze dried. The required product was obtained as white solid (9.86 mg, 30%), with no distinct melting point.

^{1}H NMR (DMSO-d_6): 12.04(1H, s), 9.99(1H, s), 9.82(1H, s), 9.25(1H, br), 7.98(1H, t, J = 6.2Hz), 7.43(1H, d, J = 1.8Hz), 7.40(1H, d, J = 1.8Hz), 7.16(1H, d, J = 1.8Hz), 6.91(1H, d, J = 1.8Hz), 3.89(3H, s), 3.85(3H, s), 3.22(2H, t, J = 7.7Hz), 3.12-3.06(2H, m), 2.81(6H, d, J = 4.8Hz), 1.98(3H, s), 1.91(2H, qt, J = 7.0Hz), 1.63-1.51(3H, m), 0.94(6H, d, J = 6.4Hz).

IR: 719, 771, 799, 891, 1059, 1126, 1174, 1198, 1263, 1287, 1398, 1437, 1466, 1508, 1547, 1644cm^{-1}

HRESIMS: Found: 585.2965 calculated for $C_{28}H_{41}N_8O_4S$ 585.2966

Portion 2:

N-[3-(Dimethylamino)propyl]-2-{[(4-{[(4-{[imino(phenyl)methyl]amino}-1-methyl-1H-pyrrol-2-yl)carbonyl]amino}-1-methyl-1H-pyrrol-2-yl)carbonyl]amino}-5-isopentyl-1,3-thiazole-4-carboxamide bis(trifluoroacetate) (MGB-317)

Methyl benzenecarbimidothioate hydroiodide (24 mg, 0.087 mmol) was added to the methanolic solution of the amine at room temperature with stirring. The reaction mixture was left standing at room temperature overnight and then the solvent was removed and the residue was dissolved in

DMF (1 mL). HPLC purification (R_t = 21 min) followed by freeze-drying the appropriate fractions gave the required product as white solid (12 mg, 30%) with no distinct melting point.

IR: 715, 799, 829, 893, 1007, 1059, 1128, 1175, 1198, 1275, 1368, 1400, 1429, 1466, 1508, 1547, 1656 cm^{-1}

^1H NMR (DMSO-d_6): 12.11(1H, s), 11.16(1H, s), 10.12(1H, s), 9.81(1H, br), 9.34(1H, br), 8.84(1H, s), 7.97(1H, t, J = 6.0Hz), 7.88(2H, d, J = 7.5Hz), 7.81(1H, t, J = 7.5Hz), 7.70(2H, t, J = 7.5Hz), 7.48(1H, d, J = 1.6Hz), 7.39(1H, d, J = 1.6Hz), 7.31(1H, d, J = 1.6Hz), 7.09(1H, d, J = 1.6Hz), 3.97(3H, s), 3.91(3H, s), 3.21(2H, t, J = 7.8Hz), 3.11-3.07(2H, m), 2.79(6H, d, J = 4.0Hz), 1.90(2H, qt, J = 7.3Hz), 1.62-1.51(3H, m), 0.93(6H, d, J = 6.5Hz).

HRESIMS: Found: 646.3283 calculated for $C_{33}H_{44}N_9O_3S$ 646.3282

Portion 3:

N-[3-(Dimethylamino)propyl]-2-{[(4-{[(4-{[imino(3-methoxyphenyl)methyl]amino}-1-methyl-1H-pyrrol-2-yl)carbonyl]amino}-1-methyl-1H-pyrrol-2-yl)carbonyl]amino}-5-isopentyl-1,3-thiazole-4-carboxamide bis(trifluoroacetate) (MGB-4)

Methyl 3-methoxybenzenecarbimidothioate hydroiodide (28 mg, 0.087 mmol) was added to the methanolic solution of the amine at room temperature with stirring. The reaction mixture was left standing at room temperature overnight and then the solvent was removed and the residue was dissolved in DMF (1 mL). HPLC purification (R_t = 22 min) followed by freeze-drying the appropriate fractions gave the required product as white solid (10 mg, 24%) with no distinct melting point.

IR: 717, 800, 827, 894, 1005, 1059, 1128, 1175, 1198, 1275, 1368, 1400, 1429, 1466, 1508, 1548, 1654 cm^{-1}

^1H NMR (DMSO-d_6): 12.11(1H, s), 11.14(1H, s), 10.12(1H, s), 9.79(1H, br), 9.34(1H, br), 8.84(1H, s), 7.98(1H, t, J = 6.1Hz), 7.48-7.35(5H, m), 7.31(1H, d, J = 1.8Hz), 7.10(1.8Hz), 3.97(3H, s), 3.92(3H, s), 3.89(3H, s), 3.22(2H, t, J = 7.7Hz), 3.11-3.06(2H, m), 2.80(6H, d, J = 4.0Hz), 1.91(2H, qt, J = 7.6), 1.65-1.51(3H, m), 0.94(6H, d, J = 6.4Hz).

HRESIMS: Found: 676.3389 calculated for $C_{34}H_{46}N_9O_4S$ 676.3388

Portion 4:

2-{[(4-{[(4-{[Amino(imino)methyl]amino}-1-methyl-1H-pyrrol-2-yl)carbonyl]amino}-1-methyl-1H-pyrrol-2-yl)carbonyl]amino}-N-[3-(dimethylamino)propyl]-5-isopentyl-1,3-thiazole-4-carboxamide bis(trifluoroacetate) (MGB-324)

To the methanolic solution methyl imidothiocarbamate hydroiodide (19 mg, 0.087 mmol) was added at room temperature with stirring. The stirring was continued overnight at room temperature. Methanol was removed under reduced pressure and the residue was dissolved in DMF (1 mL) and purified by HPLC. Fractions containing the required product (R_t = 19 min) were collected and freeze-dried to give the product as white solid (7.41 mg, 13%) with no distinct melting point.

IR: 714, 796, 831, 890, 1011, 1061, 1125, 1175, 1198, 1285, 1401, 1464, 1547, 1641, 1655 cm^{-1}

^1H NMR (DMDO-d_6): 12.07(1H, s), 10.08(1H, s), 9.28(5H, br), 7.95(1H, t, J = 6.0Hz), 7.42(1H, d, J = 1.8Hz), 7.39(1H, d, J = 1.8Hz), 7.06(1H, s), 6.92(1H, s), 3.89(6H, d, J = 2.1Hz), 3.20(2H, t, J = 7.3Hz), 3.09(2H, t, J = 7.3Hz), 2.80(6H, d, J = 4.0Hz), 1.89(2H, qt, J = 6.5Hz), 1.62-1.49(3H, m), 0.92(6H, d, J = 6.4Hz).

HRESIMS: Found: 585.3077 calculated for $C_{27}H_{41}N_{10}O_3S$ 585.3078

Portion 5:

N-[3-(Dimethylamino)propyl]-2-{[(4-{[(4-{[imino(3-quinolinyl)methyl]amino}-1-methyl-1H-pyrrol-2-yl)carbonyl]amino}-1-methyl-1H-pyrrol-2-yl)carbonyl]amino}-5-isopentyl-1,3-thiazole-4-carboxamide bis(trifluoroacetate) (MGB-325)

To the methanolic solution methyl 3-quinolinecarbimidothioate hydroiodide (29 mg, 0.087 mmol) was added at room temperature with stirring. The stirring was continued overnight at room temperature. Methanol was removed under reduced pressure and the residue was dissolved in DMF (1 mL) and purified by HPLC. Fractions containing the required product (R_t = 21 min) were collected and freeze-dried to give the product as white solid (16 mg, 20%) with no distinct melting point.

IR: 720, 802, 826, 892, 1002, 1061, 1128, 1175, 1198, 1275, 1368, 1401, 1429, 1466, 1508, 1550, 1656 cm^{-1}

^1H NMR (DMSO-d_6): 12.09(1H, s), 11.52(1H, br, s), 10.12(1H, s), 10.02(1H, br, s), 9.61(1H, 1H, s), 9.05(1H, br, s), 8.91(1H, br, s). 8.39(1H, d, J = 7.7Hz), 8.21(1H, d, J = 7.7Hz), 8.05-7.92(3H, m), 7.48(1H, s), 7.40(1H, s), 7.33(1H, s), 7.13(1H, s), 3.98(3H, s), 3.91(3H, s), 3.35-3.34(2H, m), 3.20(2H, t, J = 7.9Hz), 3.08-3.05(2H, m), 2.78(6H, s), 1.89(2H, qt, J = 6.9Hz), 1.61-1.50(3H, m), 0.93(6H, d, J = 6.4Hz).

HRESIMS: Found: 697.3392 calculated for $C_{36}H_{45}N_{10}O_3S$ 697.3391

Portion 6:

2-{[(4-{[(4-{[(4-Chlorophenyl)(imino)methyl]amino}-1-methyl-1H-pyrrol-2-yl)carbonyl]amino}-1-methyl-1H-pyrrol-2-yl)carbonyl]amino}-N-[3-(dimethylamino)propyl]-5-isopentyl-1,3-thiazole-4-carboxamide bis(trifluoroacetate) (MGB-351)

Methyl 4-chlorobenzenecarbimidothioate hydroiodide (27.4 mg, 0.087 mmol) was added at room temperature with stirring. The reaction mixture was left stirring at room temperature overnight and then methanol was removed under reduced pressure. The residue was dissolved in DMF and purified by HPLC. Fractions containing the required product (R_t = 21.0 min) were collected and freeze dried. The required product was obtained as white solid (30 mg, 38%) with no distinct melting point.

IR: 718, 797, 831, 891, 1011, 1061, 1125, 1175, 1198, 1285, 1400, 1464, 1547, 1641, 1655 cm^{-1}

^1H NMR (DMSO-d_6): 12.11(1H, s), 11.21(1H, s), 10.12(1H, s), 9.86(1H, br, s), 8.88(1H, br, s), 7.97(1H, t, J = 6.2Hz), 7.91(2H, d, J = 8.6Hz), 7.80(2H, d, J = 8.6Hz), 7.47(1H, d, J = 1.4Hz), 7.40(1H, d, J = 1.4Hz), 7.30(1H, d, J = 1.4Hz), 7.10(1H, d, J = 1.4Hz), 3.96(3H, s), 3.91(3H, s), 3.21(2H, t, J = 7.8Hz), 3.11(2H, qt, J = 7.8Hz), 2.80(6H, d, J = 4.5Hz), 1.90(2H, qt, J = 7.8Hz), 1.62-1.51(3H, m), 0.93(6H, d, J = 6.5Hz).

HRESIMS: Found: 680.2891 calculated for $C_{33}H_{43}O_3N_9ClS$ 680.2893

Portion 7:

2-{[(4-{[(4-{[(3-Chlorophenyl)(imino)methyl]amino}-1-methyl-1H-pyrrol-2-yl)carbonyl]amino}-1-methyl-1H-pyrrol-2-yl)carbonyl]amino}-N-[3-(dimethylamino)propyl]-5-isopentyl-1,3-thiazole-4-carboxamide bis(trifluoroacetate) (MGB-352)

Methyl 3-chlorobenzenecarbimidothioate hydroiodide (27.4 mg, 0.087 mmol) was added to the methanolic solution at room temperature with stirring. The stirring was continued overnight.

Methanol was removed under reduced pressure and the residue was dissolved in DMF followed by HPLC purification. Fractions containing the required product (R_t = 22 min) were collected and freeze dried. The required product was obtained as white solid (31 mg, 39%) with no distinct melting point.

IR: 718, 797, 829, 889, 1005, 1059, 1126, 1175, 1198, 1287, 1368, 1400, 1429, 1468, 1553, 1647, 1663 cm^{-1}

^{1}H NMR (DMSO-d_6): 12.11(1H, s), 11.27(1H, s), 10.14(1H, s), 9.88(1H, br, s), 9.32(1H, br, s), 8.94(1H, br), 7.99(1H, s), 7.97(1H, t, J = 6.2Hz), 7.88(1H, d, J = 8.1Hz), 7.84(1H, d, J = 8.1Hz), 7.73(1H, t, J = 8.1Hz), 7.48(1H, d, J = 1,4Hz), 7.40(1H, d, J = 1.4Hz), 7.29(1H, d, J = 1.4Hz), 7.09(1H, d, J = 1.4Hz), 3.97(3H, s), 3.92(3H, s), 3.36-3.35(2H, m), 3.21-3.18(2H, t, J = 7.8Hz), 3.10(2H, t, J = 8.0Hz), 2.79(6H, s), 1.90(2H, qt, J = 7.0Hz), 1.62-1.51(3H, m), 0.93(6H, d, J = 6.4Hz).

HRESIMS: Found: 680.2892 calculated for $C_{33}H_{43}O_3N_9ClS$ 680.2893

Portion 8:

N-[3-(Dimethylamino)propyl]-2-{[(4-{[(4-{[(4-fluorophenyl)(imino)methyl]amino}-1-methyl-1H-pyrrol-2-yl)carbonyl]amino}-1-methyl-1H-pyrrol-2-yl)carbonyl]amino}-5-isopentyl-1,3-thiazole-4-carboxamide bis(trifluoroacetate) (MGB-353)

Methyl 4-fluorobenzenecarbimidothioate hydroiodide (26 mg, 0.087 mmol) was added to the methanolic solution at room temperature with stirring. The stirring was continued overnight. Methanol was removed under reduced pressure and the residue was dissolved in DMF followed by HPLC purification. Fractions containing the required product (R_t = 22 min) were collected and freeze dried. The required product was obtained as white solid (21.85 mg, 28%) with no distinct melting point.

IR: 718, 799, 831, 891, 1007, 1061, 1125, 1171, 1198, 1287, 1400, 1427, 1466, 1508, 1549, 1613, 1655 cm^{-1}

^{1}H NMR (DMSO-d_6): 12.11(1H, s), 11.17(1H, s), 10.13(1H, s), 9.82(1H, br, s), 9.37(1H, br, s), 8.84(1H, s), 7.98-7.95(3H, m), 7.58(2H, t, 8.8Hz), 7.48(1H, d, J = 1.4Hz), 7.40(1H, d, J = 1.4Hz), 7.30(1H, d, J = 1.4Hz), 7.09(1H, d, J = 1.4Hz)3.96(3H, s), 3.91(3H, s), 3.21(2H, t, J = 7.8Hz), 3.11(2H, qt, J = 5.2Hz), 2.80(3H, s), 2.79(3H, s), 1.90(2H, qt, J = 6.9Hz), 1.62-1.51(3H, m), 0.93(6H, d, J = 6.5Hz).

HRESIMS: Found: 664.3186 calculated for $C_{33}H_{43}FN_9O_3S$ 664.3188

Portion 9:

N-[3-(Dimethylamino)propyl]-2-{[(4-{[(4-{[(3-fluorophenyl)(imino)methyl]amino}-1-methyl-1H-pyrrol-2-yl)carbonyl]amino}-1-methyl-1H-pyrrol-2-yl)carbonyl]amino}-5-isopentyl-1,3-thiazole-4-carboxamide bis(trifluoroacetate) (MGB-354)

Methyl 3-fluorobenzenecarbimidothioate hydroiodide (26 mg, 0.087 mmol) was added to the methanolic solution at room temperature with stirring. The stirring was continued overnight. Methanol was removed under reduced pressure and the residue was dissolved in DMF followed by HPLC purification. Fractions containing the required product (R_t = 22 min) were collected and freeze dried. The required product was obtained as white solid (36.34 mg, 47%) with no distinct melting point.

IR: 718,797, 831, 889, 1005, 1061, 1126, 1177, 1198, 1285, 1368, 1400, 1435, 1466, 1547, 1586, 1655 cm^{-1}

^{1}H NMR (DMSO-d_6): 12.11(1H, s), 11.16(1H, s), 10.14(1H, s), 9.89(1H, br, s), 9.35(1H, br, s), 8.94(1H, br, s), 7.97(1H, t, J = 6.1Hz), 7.79-7.65(4H, m), 7.48(1H, d, J = 1.4Hz), 7.39(1H, s, J = 1.4Hz), 7.30(1H, d, J = 1.4Hz), 7.09(1H, d, J = 1.4Hz), 3.97(3H, s), 3.92(3H, s), 3.21(2H, t, J = 7.8Hz), 3.10(2H, t, J = 7.8Hz), 2.80(6H, s), 1.90(2H, qt, J = 6.9Hz), 1.62-1.51(3H, m), 0.93(6H, d, J = 6.4Hz).

HRESIMS: Found: 664.3191 Calculated for $C_{33}H_{43}O_3N_9FS$ 663.3104

5.1.5 Synthesis of isopentylpyrrole containing MGBs

***5.1.5.1 N*-[3-(Dimethylamino)propyl]-1-isopentyl-4-nitro-1*H*-pyrrole-2-carboxamide (20a) [32]**

Prepared as per reference.

***5.1.5.2 N*-[3-(Dimethylamino)propyl]-1-isopentyl-4-{[(1-methyl-4-nitro-1*H*-pyrrol-2-yl)carbonyl]amino}-1*H*-pyrrole-2-carboxamide (21a)**

N-[3-(Dimethylamino)propyl]-1-isopentyl-4-nitro-1*H*-pyrrole-2-carboxamide (0.270 g, 0.874 mmol) was dissolved in methanol (25 mL) to which Pd/C-10% (0.200 g) was added at 0^{o}C under nitrogen with stirring. The reaction mixture was hydrogenated for 3h at room temperature and atmospheric pressure. The catalyst was removed over Kieselguhr and the solvent was removed under reduced pressure. The amine so formed was dissolved in DCM (5 mL, dry). 1-Methyl-4-nitro-1*H*-pyrrole-2-carboxylic acid (0.149 g, 0.874 mmol) was dissolved in thionyl chloride (5 mL) and then it was heated under reflux for 2h. Excess thionyl chloride was removed under reduced pressure and the acid chloride so formed was dissolved in DCM (5 mL, dry). The acid chloride solution was added to the amine solution dropwise with stirring at room temperature with stirring under nitrogen. The reaction mixture was left stirring overnight. The reaction mixture was extracted with a saturated solution of sodium hydrogen carbonate and the organic layer was collected, dried (Na_2SO_4), filtered and the solvent removed under reduced pressure. The crude product obtained was purified by silica gel column chromatography and eluted with ethyl acetate/methanol (1/1) containing 1%TEA; R_F = 0.5 [basic TLC]. The required product was obtained as yellow solid (0.240 g, 64%), mp 162-164^{o}C

IR: 748, 777, 810, 858, 1111, 1258, 1306, 1395, 1418, 1452, 1501, 1530, 1645 cm^{-1}

^{1}H NMR (DMSO-d_6): 10.22(1H, s), 8.18(1H, s), 8.10(1H, br), 7.58(1H, s), 7.26(1H, s), 6.78(1H, s), 4.31(2H, t, J = 6.7Hz), 3.96(3H, s), 3.22(2H, q, J = 6.3Hz), 2.29(2H, br), 2.18(6H, s), 1.64(2H, t, J = 6.9Hz), 1.55-1.46(3H, m), 0.89(6H, d, J = 6.3Hz).

^{13}C NMR (DMSO-d_6): 161.6, 157.3, 134.3, 128.7, 126.8, 123.4, 121.9, 117.1, 108.0, 104.5, 57.4, 46.5, 45.5(2xC), 41.0, 37.9, 37.4, 27.5, 25.6, 22.9(2xC)

HRESIMS: Found: 433.2555 calculated for $C_{21}H_{33}O_4N_6$ 433.2558.

***5.1.5.3 N*-[3-(Dimethylamino)propyl]-1-isopentyl-4-{[(1-methyl-4-{[(1-methyl-4-nitro-1*H*-pyrrol-2-yl)carbonyl]amino}-1*H*-pyrrol-2-yl)carbonyl]amino}-1*H*-pyrrole-2-carboxamide (22a)**

N-[3-(Dimethylamino)propyl]-1-isopentyl-4-{[(1-methyl-4-nitro-1*H*-pyrrol-2-yl)carbonyl]amino}-1*H*-pyrrole-2-carboxamide (0.240 g, 0.555 mmol) was dissolved in methanol (25 mL) to which Pd/C-10% (0.200 g) was added at 0^{o}C under nitrogen with stirring. The reaction mixture was hydrogenated for 3h at room temperature and atmospheric pressure. The catalyst was removed over Kieselguhr and the solvent was removed under reduced pressure. The amine so formed was dissolved in DCM (5 mL, dry). 1-Methyl-4-nitro-1*H*-pyrrole-2-carboxylic acid (94 mg, 0.555 mmol) was dissolved in thionyl chloride (5 mL) and then it was heated under reflux for 2h. Excess thionyl chloride was removed

under reduced pressure and the acid chloride so formed was dissolved in DCM (5 mL, dry). The acid chloride solution was added to the amine solution dropwise with stirring at room temperature with stirring under nitrogen. The reaction mixture was left stirring overnight. The reaction mixture was extracted with a saturated solution of sodium hydrogen carbonate and the organic layer was collected, dried (Na_2SO_4), filtered and the solvent removed under reduced pressure. The crude product obtained was purified by silica gel column chromatography and eluted with ethyl acetate/methanol (1/1) containing 1%TEA; R_F = 0.5 [basic TLC]. The required product was obtained as yellow solid (0.210 g, 68%), mp 212-215^oC.

IR: 744, 775, 811, 840, 887, 1059, 1111, 1163, 1202, 1242, 1262, 1289, 1306, 1393, 1424, 1462, 1502, 1546, 1644 cm^{-1}

^1H NMR (DMSO-d_6): 10.28(1H, s), 9.93(1H, s), 8.20(1H, t, J = 4.5Hz), 8.07(1H, t, J = 5.6Hz), 7.60(1H, d, J = 1.8Hz), 7.28(1H, d, J = 1.8Hz), 7.25(1H, d, J = 1.8Hz), 7.04(1H, d, J = 1.8Hz), 6.79(1H, d, J = 1.8Hz), 4.29(2H, t, J = 7.0Hz), 3.97(3H, s), 3.87(3H, s), 3.22(2H, q, J = 6.5Hz), 2.28(2H, t, J = 6.5Hz), 2.16(6H, s), 1.65(2H, qt, J = 7.0Hz), 1.55-1.46(3H, m), 0.89(6H, d, J = 6.3Hz).

^{13}C NMR (DMSO-d_6): 161.6, 158.8, 157.4, 134.3, 128.7, 126.8, 123.5, 123.1, 122.6, 121.9, 119.1, 116.9, 108.1, 105.0, 104.6, 57.6, 45.7(2xC), 41.0, 37.9, 37.6, 36.6, 27.7, 25.6, 23.5, 22.9(2xC)

HRESIMS: Found: 555.3041 calculated for $C_{27}H_{39}O_5N_8$ 555.3038.

5.1.5.4 Synthesis of full MGBs:

N-[3-(Dimethylamino)propyl]-1-isopentyl-4-{[(1-methyl-4-{[(1-methyl-4-nitro-1H-pyrrol-2-yl)carbonyl]amino}-1H-pyrrol-2-yl)carbonyl]amino}-1H-pyrrole-2-carboxamide (0.200 g, 0.36 mmol) was dissolved in methanol (25 mL) to which Pd/C-10% (0.200 g) was added at ^0C with stirring under nitrogen. The reaction mixture was hydrogenated for 4h at room temperature and atmospheric pressure. The catalyst was removed over Kieselguhr and the solution was divided into five equal portions:

Portion 1:

4-{[(4-{[(4-{[Amino(imino)methyl]amino}-1-methyl-1H-pyrrol-2-yl)carbonyl]amino}-1-methyl-1H-pyrrol-2-yl)carbonyl]amino}-N-[3-(dimethylamino)propyl]-1-isopentyl-1H-pyrrole-2-carboxamide bis(trifluoroacetate) (MGB-329)

Methyl imidothiocarbamate hydroiodide (16 mg, 0.072 mmol) was added to the methanolic solution at room temperature with stirring. The reaction mixture was left stirring at room temperature overnight. HPLC purification (R_t = 13 min) gave the required product after freeze-drying as white solid (9 mg, 16%) with no distinct melting point.

IR: 722, 800, 830, 889, 966, 1061, 1123, 1177, 1196, 1290, 1366, 1398, 1429, 1468, 1545, 1645 cm^{-1}

^1H NMR (DMSO-d_6): 10.07(1H, s), 9.96(1H, s), 9.76(1H, s), 9.61(3H, br), 9.38(1H, s), 8.21(1H, t, J = 5.6Hz), 7.29(1H, s), 7.27(1H, s), 7.14(1H, s), 7.12(1H, s), 6.99(2H, s), 6.58(1H, s), 4.36(2H, t, 6.9Hz), 3.96(3H, s), 3.31(2H, q, J = 6.0Hz), 3.15(2H, t, J = 7.7Hz), 2.85(6H, s), 1.92(2H, qt, J = 8.0Hz), 1.62-1.59(3H, m), 0.97(6H, d, J = 6.2Hz).

HRESIMS: Found: 567.3516 calculated for $C_{28}H_{43}N_{10}O_3$ 567.3514

Portion 2:

N-[3-(Dimethylamino)propyl]-4-{[(4-{[(4-{[imino(phenyl)methyl]amino}-1-methyl-1H-pyrrol-2-yl)carbonyl]amino}-1-methyl-1H-pyrrol-2-yl)carbonyl]amino}-1-isopentyl-1H-pyrrole-2-carboxamide bis(trifluoroacetate)(MGB-333)

Methyl benzenecarbimidothioate hydroiodide (20 mg, 0.072 mmol) was added to the methanolic solution at room temperature with stirring. The reaction mixture was left stirring at room temperature overnight. HPLC purification (R_t = 16 min) gave the required product after freeze-drying as white solid (16 mg, 26%) with no distinct melting point.

IR: 798, 831, 893, 1012, 1062, 1124, 1175, 1198, 1285, 1400, 1464, 1547, 1641, 1656 cm^{-1}
^1H NMR (DMSO-d_6): 11.16(1H, s), 10.02(1H, s), 9.92(1H, s), 9.81(1H, s), 9.34(1H, br), 8.84(1H, br), 8.16(1H, t, J = 5.5Hz), 7.88(2H, d, J = 7.5Hz)), 7.80(1H, t, J = 7.5Hz), 7.70(2H, t, J = 7.5Hz), 7.29(1H, d, J = 1.4Hz), 7.26(1H, d, J = 1.4Hz), 7.22(1H, d, J = 1.4Hz), 7.08(1H, d, J = 1.4Hz), 7.07(1H, d, J = 1.4Hz), 6.94(1H, d, J = 1.4Hz), 4.31(2H, 6.7Hz), 3.96(3H, s), 3.87(3H, s), 3.27(2H, q, J = 6.3Hz), 3.09(2H, t, J = 6.5Hz), 2.80(6H, d, J = 4.6Hz), 1.87(2H, qt, J = 6.8Hz), 1.57-1.48(3H, m), 0.91(6H, d, J = 6.3Hz).

HRESIMS: Found: 628.3716 Calculated for $C_{34}H_{46}N_9O_3$ 628.3718

Portion 3:

N-[3-(Dimethylamino)propyl]-4-{[(4-{[(4-{[imino(3-methoxyphenyl)methyl]amino}-1-methyl-1H-pyrrol-2-yl)carbonyl]amino}-1-methyl-1H-pyrrol-2-yl)carbonyl]amino}-1-isopentyl-1H-pyrrole-2-carboxamide bis(trifluoroacetate)(MGB-332)

Methyl 3-methoxybenzenecarbimidothioate hydroiodide (22 mg, 0.072 mmol) was added to the methanolic solution at room temperature with stirring. The reaction mixture was left stirring at room temperature overnight. HPLC purification (R_t = 17 min) gave the required product after freeze-drying as white solid (18 mg, 28%) with no distinct melting point.

IR: 799, 831, 891, 1011, 1064, 1124, 1175, 1198, 1285, 1400, 1464, 1547, 1641, 1655 cm^{-1}
^1H NMR (DMSO-d_6): 11.14(1H, s), 10.02(1H, s), 9.92(1H, s), 9.79(1H, s), 9.36(1H, br), 8.84(1H, br), 8.16(1H, t, J = 5.5Hz), 7.61(1H, t, J = 7.9Hz), 7.45(1H, d, J = 8.4Hz), 7.43(1H, s), 7.37(1H, d, J = 8.4Hz), 7.29(1H, s), 7.26(1H, s), 7.22(1H, s), 7.07(2H, s), 6.94(1H, s), 4.31(2H, t, J = 6.7Hz), 3.96(3H, s), 3.89(3H, s), 3.87(3H, s), 3.27(2H, q, J = 6.2Hz), 3.08(2H, t, J = 6.2Hz), 2.80(6H, d, J = 4.3Hz), 1.86(2H, qt, J = 7.5Hz), 1.57-1.50(3H, m), 0.91(6H, d, J = 6.2Hz).

HRESIMS: Found: 658.3824 calculated for $C_{35}H_{48}N_9O_4$ 658.3824

Portion 4:

N-[3-(Dimethylamino)propyl]-4-{[(4-{[(4-{[imino(3-quinolinyl)methyl]amino}-1-methyl-1H-pyrrol-2-yl)carbonyl]amino}-1-methyl-1H-pyrrol-2-yl)carbonyl]amino}-1-isopentyl-1H-pyrrole-2-carboxamide bis(trifluoroacetate)(MGB-330)

Methyl 3-quinolinecarbimidothioate hydroiodide (24 mg, 0.072 mmol) was added to the methanolic solution at room temperature with stirring. The reaction mixture was left stirring at room temperature overnight. HPLC purification (R_t = 19 min) gave the required product after freeze-drying as yellow solid (25 mg, 38%) with no distinct melting point.

IR: 801, 830, 892, 1012, 1063, 1124, 1175, 1198, 1285, 1400, 1464, 1547, 1642, 1657 cm^{-1}
^1H NMR (DMSO-d_6): 11.54(1H, s), 10.05(2H, br), 9.93(1H, s), 9.62(1H, s), 9.34(1H, br), 9.06(1H, br), 8.92(1H, s), 8.41(1H, d, J = 7.8Hz), 8.22(1H, d, J = 7.8Hz), 8.17(1H, t, J = 5.6Hz), 8.07-7.98(2H, m), 7.34(1H, d, J = 1.8Hz), 7.28(1H, d, J = 1.8Hz), 7.22(1H, d, J = 1.8Hz), 7.12(1H, d, J = 1.8Hz), 7.09(1H, d,

J = 1.8Hz), 6.94(1H, d, J = 1.8Hz), 4.31(2H, t, J = 6.6Hz), 3.98(3H, s), 3.88(3H, s), 3.27(2H, q, J = 6.3Hz), 3.09(2H, t, J = 6.2Hz), 2.80(6H, d, J = 4.7Hz), 1.88(2H, qt, J = 6.6Hz), 1.58-1.48(3H, m), 0.91(6H, d, J = 6.3Hz).

HRESIMS: Found: 679.3829 calculated for $C_{37}H_{47}N_{10}O_3$ 679.3827

Portion 5:

4-({[4-({[4-(Acetylamino)-1-methyl-1*H*-pyrrol-2-yl]carbonyl}amino)-1-methyl-1*H*-pyrrol-2-yl]carbonyl}amino)-*N*-[3-(dimethylamino)propyl]-1-isopentyl-1*H*-pyrrole-2-carboxamide trifluoroacetate (MGB-331)

Methanol was removed under reduced pressure and the residue was dissolved in DCM (2 mL, dry). Acetyl chloride (60 µL) was dissolved in DCM (10 mL, dry) from which (1 mL) was taken and added and added to the reaction mixture with stirring. The stirring was continued overnight. The solvent was removed under reduced pressure and the residue was dissolved in DMF (2 mL). HPLC purification (R_t = 18 min) gave the required product as a white solid (7 mg, 14%) with no distinct melting point.

IR: 719, 771, 799, 894, 1060, 1125, 1173, 1198, 1263, 1287, 1398, 1437, 1466, 1508, 1547, 1646 cm^{-1}

^{1}H NMR (DMSO-d_6): 9.89(2H, s), 9.81(1H, s), 9.24(1H, br), 8.15(1H, t, J = 5.1Hz), 7.22(2H, s), 7.14(1H, s), 7.07(1H, s), 6.93(1H, s), 6.88(1H, s), 4.31(2H, t, J = 6.7Hz), 3.85(6H, d, J = 7.1Hz), 3.27(2H, q, J = 5.6Hz), 3.09(2H, t, J = 5.0Hz), 2.80(6H, d, J = 4.2Hz), 1.98(3H, s), 1.87(2H, qt, J = 6.6Hz), 1.57-1.49(3H, m), 0.91(6H, d, J = 6.2Hz).

HRESIMS: Found: 567.3403 calculated for $C_{29}H_{43}N_8O_4$ 567.3402

5.1.6 Synthesis of N-methylpyrrole MGBs

***5.1.6.1 N*-[3-(Dimethylamino)propyl]-1-methyl-4-{[(1-methyl-4-{[(1-methyl-4-nitro-1*H*-pyrrol-2-yl)carbonyl]amino}-1*H*-pyrrol-2-yl)carbonyl]amino}-1*H*-pyrrole-2-carboxamide (22b) [21]**

Prepared as per reference.

5.1.6.2 Synthesis of full MGBs:

N-[3-(Dimethylamino)propyl]-1-methyl-4-{[(1-methyl-4-{[(1-methyl-4-nitro-1*H*-pyrrol-2-yl)carbonyl]amino}-1*H*-pyrrol-2-yl)carbonyl]amino}-1*H*-pyrrole-2-carboxamide (250 mg, 0.501 mmol) was dissolved in methanol (25 mL) to which Pd/C-10% (170 mg) was added portionwise with stirring at 0^{o}C under nitrogen. The reaction mixture was hydrogenated for 6h. The catalyst was removed over Kieselguhr and the solvent was removed under reduced pressure. The amine so formed was dissolved in methanol (5 mL) and split into five 1 mL portions.

Portion 1:

4-({[4-({[4-(Acetylamino)-1-methyl-1*H*-pyrrol-2-yl]carbonyl}amino)-1-methyl-1*H*-pyrrol-2-yl]carbonyl}amino)-*N*-[3-(dimethylamino)propyl]-1-methyl-1*H*-pyrrole-2-carboxamide trifluoroacetate (MGB-338) [32]

This compound was prepared as per reference.

IR: 3300, 1670, 1647, 1580, 1533, 1502, 1398, 1307 cm^{-1}.

^{1}H NMR (DMSO-d_6): 9.91(1H, s), 9.83(1H, s), 8.18(1H, t, J = 5.6Hz), 7.23(1H, d, J = 1.7Hz), 7.18(1H, d, J = 1.7Hz), 7.15(1H, d, J = 1.7Hz), 7.07(1H, d, J = 1.7Hz), 6.95(1H, d, J = 1.7Hz), 6.88(1H, d, J = 1.7Hz), 3.85(3H, s), 3.84(3H, s), 3.82(3H, s), 3.47-3.42(2H, m), 3.29-3.26(2H, m), 3.14(6H, s), 1.98(3H, s), 1.95-1.91(2H, m).

ESIMS: Found: 511.2774 calculated for $C_{25}H_{35}N_8O_4$ 511.2776

Portion 2:

4-{[(4-{[(4-{[Amino(imino)methyl]amino}-1-methyl-1*H*-pyrrol-2-yl)carbonyl]amino}-1-methyl-1*H*-pyrrol-2-yl)carbonyl]amino}-*N*-[3-(dimethylamino)propyl]-1-methyl-1*H*-pyrrole-2-carboxamide bis(trifluoroacetate) (MGB-334)

The amine solution (1 mL) was added to methyl imidothiocarbamate hydroiodide (22 mg, 0.10 mmol) at room temperature with stirring. The reaction mixture was left standing at room temperature overnight. HPLC purification (R_t = 16 min) gave the required product as white solid (13.64 mg, 19%) with no distinct melting point.

IR: 719, 797, 829, 889, 966, 1061, 1123, 1177, 1196, 1290, 1366, 1398, 1429, 1468, 1545 cm^{-1}

^{1}H NMR (DMSO-d_6): 10.04(1H, s), 9.93(1H, s), 9.69(4H, br), 9.27(2H, br), 8.19(1H, t, J = 5.8Hz), 7.24(1H, d, J = 1.6Hz), 7.17(1H, d, J = 1.6Hz), 7.11(1H, d, J = 1.6Hz), 7.07(1H, d, J = 1.6Hz), 6.97(1H, d, J = 1.6Hz), 6.95(1H, d, J = 1.6Hz), 3.90(3H, s), 3.86(3H, s), 3.82(3H, s), 3.27(2H, q, J = 6.5Hz), 3.07(2H, t, J = 6.5Hz), 2.79(6H, s), 1.87(2H, qt, J = 6.5Hz).

HRESIMS: Found: 511.2885 calculated for $C_{24}H_{35}N_{10}O_3$ 511.2888

Portion 3:

N-[3-(Dimethylamino)propyl]-4-{[(4-{[(4-{[imino(phenyl)methyl]amino}-1-methyl-1H-pyrrol-2-yl)carbonyl]amino}-1-methyl-1H-pyrrol-2-yl)carbonyl]amino}-1-methyl-1H-pyrrole-2-carboxamide bis(trifluoroacetate) (MGB-335) [21]

This compound was prepared as per reference.

IR: 1678, 1638, 1410, 1254, 1200, 1132 cm.$^{-1}$.

^{1}H NMR: (DMSO-d_6): 11.15(1H, s), 10.01(1H, s),9.91(1H, s), 9.79(1H, s), 9.32(1H, br), 8.83(1H, s), 8.14(1H, t, J =5.8Hz), 7.87(2H, d, J =7.2Hz), 7.79(1H, t, J =7.4Hz), 7.67(2H, t, 7.8Hz), 7.28(1H, d, J = 1.8(Hz), 7.25(1H, d, J =1.8Hz), 7.16((1H, d, J =1.8Hz), 7.07(2H, d, J =1.8Hz), 7.06(1H, d, J =1.8Hz), 6.6(1H, d, J =1.8Hz), 3.95(3h, s), 3.86(3H, s), 3.81(3H, s), 3.23(2H, q, 6.0Hz), 3.06(2H, m), 2.79(6H, d, 3.9Hz), 1.84(2H, quintet, J =7.8Hz).

HRESIMS: Found: 572.3124 calculated for $C_{30}H_{38}O_3N_9$ 572.3097.

Portion 4:

***N*-[3-(Dimethylamino)propyl]-4-{[(4-{[(4-{[imino(3-methoxyphenyl)methyl]amino}-1-methyl-1*H*-pyrrol-2-yl)carbonyl]amino}-1-methyl-1*H*-pyrrol-2-yl)carbonyl]amino}-1-methyl-1*H*-pyrrole-2-carboxamide bis(trifluoroacetate) (MGB-336) [33]**

This compound was prepared as per reference.

The product was obtained as a white solid (59 mg, 53%) with no distinct melting point.

IR (KBr): 1683, 1643, 1579, 1467, 1435, 1410, 1270, 1204, 1137 $cm.^{-1}$

^{1}H NMR (DMSO-d_6): 11.14(1H, s), 10.01(1H, s), 9.91(1H, s), 9.79(1H, s), 9.38(1H, br), 8.83(1H, s), 8.15(1H, t, J = 5.7Hz), 7.59(1H, t, J = 7.9Hz), 7.44-7.33(4H,m), 7.28(1H, d, J = 1.8Hz), 7.26(1H, d, J = 1.8Hz), 7.16(1H, d, J = 1.8Hz), 7.06(2H, d, J = 1.8Hz), 6.95(1H,d, J = 1.8Hz), 3.95(3H, s), 3.88(3H, s), 3.86(3H, s), 3.82(3H, s), 3.25(2H, q, J = 6.1Hz), 3.06(2H, m), 2.79(6H, d, J = 4.8Hz), 1.86(2H, quintet, J = 7.7Hz).

HRESIMS: found: 602.3216 calculated for $C_{31}H_{40}O_4N_9$ 602.3203.

Portion 5:

***N*-[3-(Dimethylamino)propyl]-4-{[(4-{[(4-{[imino(3-quinolinyl)methyl]amino}-1-methyl-1*H*-pyrrol-2-yl)carbonyl]amino}-1-methyl-1*H*-pyrrol-2-yl)carbonyl]amino}-1-methyl-1*H*-pyrrole-2-carboxamide bis(trifluoroacetate)(MGB-337)**

The amine solution (1 mL) was added to methyl 3-quinolinecarbimidothioate hydroiodide (32 mg, 0.10 mmol) at room temperature with stirring. The reaction mixture was left standing at room temperature overnight. HPLC purification gave the required product as yellow solid (33.21 mg, 39%) with no distinct melting point.

IR: 803, 832, 890, 1014, 1066, 1124, 1175, 1198, 1285, 1400, 1464, 1547, 1642, 1659 cm^{-1}
^{1}H NMR (DMSO-d_6): 11.55(1H, s), 10.06(2H, br), 9.95(1H, s), 9.62(1H, s), 9.33(1H, br), 9.06(1H, br), 8.92(1H, s), 8.41(1H, d, J = 8.0Hz), 8.21-8.17(2H, m), 8.06-7.98(2H, m), 7.34(1H, d, J = 1.7Hz), 7.29(1H, d, J = 1.7Hz), 7.18(1H, d, J = 1.7Hz), 7.12(1H, d, J = 1.7Hz), 7.09(1H, d, J = 1.7Hz), 6.97(1H, d, J = 1.7Hz), 3.98(3H, s), 3.87(3H, s), 3.82(3H, s), 3.26(2H, q, J = 6.8Hz), 3.06(2H, t, J = 6.8Hz), 2.79(6H, d, J = 4.5Hz), 1.87(2H, qt, J = 6.8Hz).

HRESIMS: Found: 623.3204 calculated for $C_{33}H_{39}N_{10}O_3$ 623.3201

5.2 Biological evaluation

5.2.1 Mtb H37Rv Assay

Using 96-well plates assay (Black, Greiner Bio-One), MGBs were tested for their anti-mycobacterial activity against green fluorescent protein (GFP) labelled H37Rv Mtb strain [34] at a concentration of 1 x 10^5 CFU/well in 7H9 liquid broth + 10% Middlebrook OADC growth supplement (BD BioSciences) and 25 ug/ml kanamycin. The plates were incubated at 37 °C without shaking and the fluorescence (excitation 485 nm/emission 520 nm) was measured at 0, 2, 4, 8, 10, 12, 13 and 14 days in culture to determine the anti-mycobacterial activity of the tested compounds with FLUOstar Optima plate reader (BMG Labtech). Two-fold serial dilutions of the compounds were performed starting from the highest concentration of 25 µM to 0.03 µM to determine the lead compounds with the lowest MIC.

5.2.2 Antibacterial activity against Staphylococcus aureus

The minimum inhibitory concentration (MIC) against *S. aureus* was measured by making two-fold serial dilution of the samples into 384-well non-binding surface plate (NBS; Corning #3640). *Staphylococcus aureus* (MRSA; ATCC 43300) was cultured in Cation-adjusted Mueller Hinton broth (CAMHB) overnight at 37 °C and diluted 40-fold and incubated for a further 1.5-3 h at 37 °C. The resultant mid-log phase cultures were diluted and added to each well of the compound containing

plates, giving a cell density of 5 x 10^5 CFU/mL, measured by absorbance at 600 nm (OD_{600}), and a final compound concentration range of 32 - 0.25 µg/mL. All the plates were covered and incubated at 37 °C for 18 h without shaking. Inhibition of bacterial growth was determined by OD_{600}, using a Tecan M1000 Pro monochromator plate reader. The percentage of growth inhibition was calculated for each well, using the negative control (media only) and positive control (bacteria without inhibitors) on the same plate. The MIC was determined as the lowest concentration at which the growth was fully inhibited, defined by an inhibition ≥ 80%. Each MIC determination was done as duplicates (n=2).

5.2.3 Anti-fungal activity against Candida albicans and Cryptococci neoformans

The minimum inhibitory concentration (MIC) against both yeasts were measured by making two-fold serial dilution of the samples into 384-well non-binding surface plate (NBS; Corning #3640). *Candida albicans* (ATCC 90028) or *Cryptococci neoformans* (ATCC 208821) was cultured for 3 days on Yeast Extract-Peptone Dextrose (YPD) agar at 30 °C . A yeast suspension of 1 x 10^6 to 5 x 10^6 CFU/mL (as determined by OD_{530}) was prepared from five colonies. The suspension was subsequently diluted and added to each well of the compound-containing plates giving a final cell density of fungi suspension of 2.5 x 10^3 CFU/mL and a final compound concentration range of 32 - 0.25 µg/mL. Plates were covered and incubated at 35 °C for 24 h without shaking. Growth inhibition of *C. albicans* was determined measuring absorbance at 530 nm (OD_{530}), while the growth inhibition of *C. neoformans* was determined measuring the difference in absorbance between 600 and 570 nm ($OD6_{00-570}$), after the addition of resazurin (0.001% final concentration) and incubation at 35 °C for a further 2 h. The absorbance was measured using a Biotek Multiflo Synergy HTX plate reader. In both cases, the percentage of growth inhibition was calculated for each well, using the negative control (media only) and positive control (fungi without inhibitors) on the same plate. The MIC was determined as the lowest concentration at which the growth was fully inhibited, defined by an inhibition ≥ 80%. Each MIC determination was done as duplicates (n=2).

5.2.4 Trypanocidal activity against T. congolense

The trypanocidal activity of the S-MGBs against *T. congolense* was assessed by Alamar Blue® assay [35]. Briefly, bloodstream form *T. congolense* IL3000 densities were measured using a cell counter and analyser system (CASY Schärfe System, Reutlingen, Germany) and cells were diluted to obtain a seeding density of 1.25×10^5 trypanosomes/ml. Compounds, containing two times the highest drug concentration, were applied into the empty wells of the first column, corresponding to the required starting concentration of each compound being tested. A threefold serial dilution step was then prepared across the microtitre plate. Thereafter, plates were incubated at 34°C with 5% CO_2, for a total of 69 hours, before addition of 10 µl of Resazurin dye (Aldrich/Fluka, #33934, Buchs, Switzerland) (12.5 mg in 100 ml phosphate buffered saline) into each well. The plates were then further incubated for 3 hours under the same conditions, before being removed and read using a fluorescence reader (SpectraMax, Gemini XS, Bucher Biotec, Basel, Switzerland) at an excitation wavelength of 536 nm and an emission wavelength of 588 nm. The EC_{50} values were determined using SOFTmax Pro 5.2 analysis software. All experiments were conducted in three separate assay runs.

5.2.5 Trypanocidal activity against T. b. brucei

A similar Alamar Blue® assay protocol, with minor modifications from that applied to *T. congolense*, was used to determine S-MGBs EC_{50}s against bloodstream form *T. b. brucei* Lister 427. Cells grown in HMI-9 medium (Gibco) supplemented with 10% heat inactivated FBS (Gibco) were counted using a Neubauer haemocytometer and seeded into 96-well plates containing serial dilutions of test compounds to a final density of 2×10^4 cell/mL. After 48 hours incubation at 37°C, 5% CO_2, 20 µl of resazurin dye (Sigma-Aldrich) solution at 0.49 mM in PBS was added, and cells were incubated for

further 24 hours. The reduction of the resazurin was measured with a fluorimeter (FLUOstar Optima, BMG Labtech) using 544 nm excitation and 590 nm emission wavelengths. Data were plotted using the EC_{50} algorithm of GraphPad Prism 5 software. All experiments were carried out in duplicate and on at least three independent occasions.

5.2.6 Cytotoxicity against HEK-293

The cytotoxicity (CC_{50}) of the samples against human embryonic kidney cells 293 (HEK-293) was measured by making two-fold serial dilution of the samples into 384-well tissue-culture plate (TC; Corning #3712), in duplicates (n=2). HEK-293 (ATCC CRL-1573) cells were counted manually in a Neubauer haemocytometer and plated at a density of 4000 cells/well into each well of the plates, used a Dulbecco's modified eagle medium (DMEM) supplemented with 10% foetal bovine serum (FBS), and resulting in final compound concentration range of 32 - 0.25 µg/mL. Cells were incubated together with the compounds for 20 h at 37 °C, 5% CO_2. Cytotoxicity was measured by adding 5 µL (equals 100 µM final) Resazurin to each well and incubated for further 3 h at 37 °C , 5% CO_2. After final incubation fluorescence intensity was measured at ex:560/10 nm, em:590/10 nm ($F_{560/590}$) using a Tecan M1000 Pro monochromator plate reader. CC_{50} values (concentration at 50% cytotoxicity) were calculated by normalizing the fluorescence readout using 74 µg/ml tamoxifen as negative control (0%) and normal cell growth as positive control (100%). The concentration dependent percentage cytotoxicity was fitted to a dose response function (using Pipeline Pilot) and CC_{50} values determined.

6. References

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Figure 1. *Distamycin and examples of MGBs in this study.*

Scheme 1. *Synthesis of the thiazole S-MGB series.*

Scheme 2. *Synthesis of the N-isopentylpyrrole and N-methylpyrrole MGB series.*

Table 3: *MGB structures as defined by the framework in figure one. MGB-DNA thermal melting temperature (ΔTm) differences compared to ss-DNA. Lipophilicity measured as the retention time in a reverse phase HPCL method.*

Table 4: *Results from panel of biological assays. NA is Not Active, NT is Not Tested due to lack of significant activity against infectious organisms. Strains used: S. aureus (MRSA) ATCC 43300; M. tuberculosis H37Rv; C. albicans ATCC 90028; C. neoformans H99 ATCC 208821; T. congolense IL3000; T.b.brucei Lister 427; HEK 293 ATCC CRL-1573.*