In vitro biological investigations of novel piperazine based heterocycles

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Eleven *N*-phenyl- and 11 *N*-benzothiazolyl-2-(4-(2,3,4-trimethoxybenzyl)piperazin-1-yl)acetamides have been synthesised by a simple and efficient method. The 22 novel compounds were tested for their *in vitro* biological efficacy against two Gram-positive bacteria, three Gram-negative bacteria, two fungi and *Mycobacterium tuberculosis* H37Rv. The bioassay results revealed that the majority of the *N*-benzothiazole-substituted piperazine derivatives exhibited moderate to good bioefficacies with encouraging MICs. The influence of the presence or absence of various electron-withdrawing or -donating functional groups on the aryl acetamide moiety on the different bioassay results is discussed.

Keywords: benzothiazole, *N*-(2,3,4-trimethoxybenzyl)piperazine, antimicrobial, antimycobacterial

Fighting disease with drugs is a timeless struggle, with the human survival on this planet dependent on its success. Steadily increasing antibiotic resistance and decreasing numbers of newer antibiotics appear to point to a post-antibiotic period during which treatment of infections would become increasingly difficult.[1] *Mycobacterium tuberculosis*, moreover, remains the primary cause of comparatively high mortality worldwide. Due to the quiescent form of mycobacterium tuberculosis strains, many of the currently available antimycobacterial drugs have become ineffective by the imminent onset of multidrug–resistance.[2,3] According to the World Health Organization (WHO), 1.3 million MDR–TB cases will need to be treated in the 27 countries with the highest MDR–TB burden between 2010 and 2015.[4] Thus the necessity to develop newer, potent and unique antibacterial agents is urgent, not least to counteract widespread drug resistance.

Heterocycles play an important role in all spheres of life including pharmaceuticals, natural resources, veterinary products, analytical reagents, agrochemicals and dyes. The development of new approaches for the synthesis of novel heterocycles substituted with unique functional groups forms the basis of an extensive research activity in synthetic organic chemistry.[5] The design of inhibitors that can show potency towards multiple biological targets remains an intriguing scientific endeavour. In the design of new compounds, the developments of hybrid molecules through the combination of different pharmacophores in one structure may lead to increased antimicrobial activity.[6] Indeed, the merging of pharmacophores may offer important medical advances not only to minimise the probability of resistance but to construct scaffolds that would possess multiple biological activities on a range of specific biological targets.[7] In pursuit of this aim, we have synthesised a number of *N*-phenyl- and *N*-benzothiazolyl-substituted quinazoline derivatives and tested them against various microorganisms.

Result and discussion

Synthesis of intermediates and target compounds was accomplished according to the steps illustrated in Scheme **1**. Chloroacetyl chloride was reacted with *para*-(substituted) phenyl amines to give the corresponding 2-chloro-*N*-4-R-phenyl acetamides **2a**–**k**. The 2-amino-6-substituted benzothiazoles **3a**–**k** were synthesised by reacting aryl amines with potassium thiocyanates in a satisfactory yield by a known method. The 2–amino-6-substituted benzothiazoles were converted to the corresponding 2-chloro-*N*-(6-R-benzo[d] thiazol-2-yl)acetamides **4a**–**k** using chloroacetyl chloride in benzene solvent as described in the literature. All the above mentioned intermediate derivatives (**2a**–**k** and **4a**–**k**) were synthesised according to a known procedure reported in the literature and the products were characterised by FTIR and ^{1}H NMR spectroscopy. All the analytical characterisation data of the said intermediates were in good accordance with the proposed structures.

The above acetamide derivatives, **2a**–**k** and **4a**–**k**, were then condensed with *N*-(2,3,4-trimethoxybenzyl)piperazine **5** in acetone solvent at reflux temperature to furnish products **6a**–**k** and **7a**–**k**. The IR and ^{1}H NMR spectral data of compounds **6a**–**k** and **7a**–**k** were in accordance with their assumed structures. The purity of the synthesised compounds was monitored by TLC and confirmed by elemental analysis. The yields, melting points and elemental analysis data of compounds **6a**–**k** and **7a**–**k** are listed in Table 1.

Reagents and conditions: (a) chloroacetyl chloride, acetone, K_2CO_3, reflux; (b) potassium thiocyanate, bromine in glacial acetic acid, room temperature (c) K_2CO_3, acetone, reflux.

Pharmacology

In vitro *antimicrobial evaluation of analogues* **6a**–**k** *and* **7a**–**k**: From the *in vitro* antimicrobial activity bioassay results presented in Table 2 it can be seen that some of the compounds displayed good inhibitory efficacy against several of the panel of seven human pathogenic microorganisms. Compounds with halogen substituents such as chloro, bromo, fluoro or iodo on the *para*-position of the phenyl acetamides moiety condensed to the piperazine ring system **6b**–**e** showed higher inhibitory potential than those compounds possessing alkyl or alkoxy substituents **6h**–**j**. In general, this series of compounds, **6a**–**k** and **7a**–**k**, was found to be more efficacious in inhibiting Gram-positive strains than Gram-negative strains as the level of minimum inhibitory concentration was lowest at 12.5 μg mL^{-1} in the case of the former while in the latter it was found to be between 25 and 50 μg mL^{-1}. Compounds **6d**, **7c**, **7d** and **7j** with a halogen- or an alkoxy-substituted benzylpiperazine moiety showed significant antibacterial activity against Gram-positive strain *Staphylococcus aureus* at 12.5 μg mL^{-1}. The methoxy-substituted *N*-benzothiazolyl analogue **7i** displayed the highest activity against Gram-positive strain *Bacillus cereus* at 12.5 μg mL^{-1}. Analogues **6c**, **6d**, **7b**, **7c**, **7d** and **7j**

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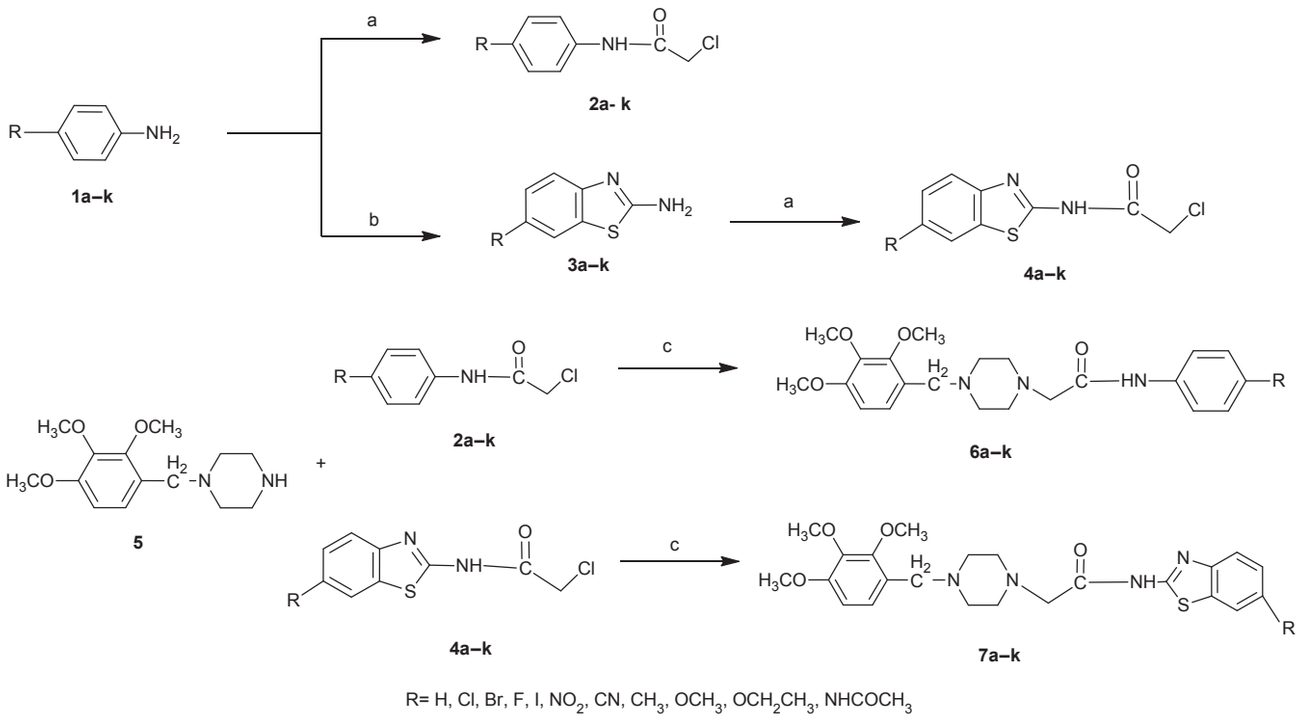

Scheme 1 Synthetic method for the synthesis of **6a–k** and **7a–k**.

with electron-withdrawing halo substituents showed strong inhibitory effects against Gram-positive strain *B. cereus* at 25 μg mL^{-1}. Two of the benzothiazole derivatives, one with a highly electronegative fluorine atom **7d** and the other with a strong electron-withdrawing nitro functional group **7f** exhibited excellent level of activity against Gram-negative bacteria *Escherichia coli* at 25 μg mL^{-1}. In the case of antibacterial efficacy of the final analogue against Gram-negative strain *Pseudomonas aeruginosa* compounds with electron-donating with methoxy and ethoxy functional groups **7i** and **7j**, respectively, displayed a good level of inhibitory efficacy at 25 μg mL^{-1}. The analogues **6f**, **6g**, **7c** and **7g** displayed excellent inhibitory efficacy at 25 μg mL^{-1} against Gram-negative *Klebsiella pneumoniae*. The activities of these compounds against *K. pneumoniae* were equipotent with the control drugs. Some of the compounds also had good inhibitory potential against the above-mentioned Gram-positive and Gram-negative strains at 25–100 μg mL^{-1}, while many of the others were found to be inactive at 200–500 μg mL^{-1}.

In the two *in vitro* antifungal bioassays, it can be seen that compounds with electron withdrawing halo substituents, **6d**, **6e**, **7b** and **7d**, showed good activity against *Aspergillus niger* at

Table 1 Yields and melting points of compounds **6a–k** and **7a–k** and their elemental analysis data

Entry	Yield /%	M.p./°C	Mol. wt	C/%		H/%		N/%	
				Calcd	Found	Calcd	Found	Calcd	Found
6a	72	193–195	399.48	66.14	65.98	7.32	7.54	10.52	10.29
6b	56	204–205	433.93	60.89	61.04	6.50	6.72	9.68	9.51
6c	67	234–236	478.38	55.24	55.09	5.90	5.78	8.78	8.65
6d	75	200–202	417.47	63.29	63.56	6.76	6.68	10.07	9.96
6e	70	221–223	525.38	50.29	50.12	5.37	5.22	8.00	8.26
6f	64	198–199	444.48	59.45	59.58	6.35	6.51	12.60	12.38
6g	72	233–235	424.49	65.08	64.92	6.65	6.47	13.20	13.02
6h	73	256–257	413.51	66.81	66.90	7.56	7.43	10.16	9.98
6i	66	240–242	429.51	64.32	64.48	7.27	7.21	9.78	9.86
6j	68	219–221	443.54	64.99	65.11	7.50	7.73	9.47	9.31
6k	71	195–197	456.53	63.14	62.98	7.06	6.97	12.27	12.21
7a	67	212–214	456.56	60.51	60.33	6.18	5.98	22.27	22.02
7b	60	226–227	491.00	56.26	56.44	5.55	5.76	11.41	11.57
7c	61	251–252	535.45	51.59	51.02	5.08	4.91	10.46	10.25
7d	69	206–208	474.55	58.21	58.40	5.73	5.60	11.81	11.77
7e	74	241–243	582.45	47.73	47.55	4.67	4.79	9.62	9.47
7f	68	210–211	501.56	55.08	55.22	5.43	5.22	13.96	14.09
7g	55	250–251	481.57	59.86	59.99	5.65	5.47	14.54	14.33
7h	66	266–268	470.58	61.26	61.12	6.43	6.66	11.91	12.07
7i	59	233–235	486.58	59.24	59.11	6.21	6.44	11.51	11.33
7j	71	236–238	500.61	59.98	59.76	6.44	6.31	11.19	11.34
7k	65	200–203	513.61	58.46	58.55	6.08	6.23	13.64	13.75

Table 2 Antimicrobial activity of compounds **6a–k** and **7a–k**

Entry	R	MIC/μg mL^{-1} [a]						
		S. aureus	*B. cereus*	*E. coli*	*P. aeruginosa*	*K. pneumoniae*	*A. niger*	*C. albicans*
6a	H	500	200	200	500	100	500	250
6b	Cl	100	50	100	200	50	100	50
6c	Br	25	25	200	200	50	100	100
6d	F	12.5	25	100	100	50	50	100
6e	I	50	50	500	200	100	50	250
6f	NO_2	100	100	200	50	25	200	250
6g	CN	100	250	500	200	25	100	200
6h	CH_3	200	500	250	500	200	500	500
6i	OCH_3	50	100	50	250	100	100	50
6j	OC_2H_5	25	100	50	200	50	100	100
6k	$NHCOCH_3$	250	200	100	500	100	100	200
7a	H	250	200	200	200	100	500	200
7b	Cl	50	25	100	50	50	50	100
7c	Br	12.5	25	50	100	25	100	100
7d	F	12.5	25	25	50	50	50	50
7e	I	50	50	250	200	100	100	500
7f	NO_2	100	50	25	50	50	200	200
7g	CN	100	100	100	200	25	100	200
7h	CH_3	200	250	200	500	200	250	500
7i	OCH_3	25	12.5	50	25	100	50	50
7j	OC_2H_5	12.5	25	50	25	50	100	50
7k	$NHCOCH_3$	100	200	100	100	100	200	100
Amp.		12.5	12.5	6.25	25	25	–	–
Gen.		6.25	6.25	12.5	12.5	25	–	–
Flu.		–	–	–	–	–	6.25	12.5
DMSO		–	–	–	–	–	–	–

[a]Each value is the mean of three independent experiments.

50 μg mL^{-1}, whereas it was compounds with electron donating alkoxy substituents, **6b**, **6i**, **7i** and **7j** that showed good activity against *Candida albicans*, also at 50 μg mL^{-1}. The remainder of the compounds showed moderate activity either of 100 or 200–500 μg mL^{-1}.

In vitro *antimycobacterial activity of analogues* **6a–k** *and* **7a–k**: *In vitro* antimycobacterial activities of compounds **6a–k** and **7a–k** were assessed against *M. tuberculosis* H37Rv and the results are shown in Table 3. Some of the compounds displayed good levels of antimycobacterial activity. The results observed by the L.J. MIC method indicated that the *N*-benzothiazole-substituted piperazine analogue with an electron withdrawing fluoro substituent **7d** exhibited the highest inhibition (99%) at a constant concentration level (12.5 μg mL^{-1}). However, the similar *N*-phenyl derivative **6d** displayed slightly less antimycobacterial activity (25 μg mL^{-1}). Interestingly, both the Methoxy-substituted phenyl and benzothiazolyl compounds, **6i** and **7i**, exhibited good antimycobacterial activity at 25 μg mL^{-1}. However, many of the compounds exhibited only moderate antimycobacterial activity at 50–100 μg mL^{-1} and some were found inactive with values > 100 μg mL^{-1}.

Experimental

Melting points were determined in open capillaries on a Veego electronic apparatus VMP-D and are uncorrected. IR spectra (4000–400 cm^{-1}) were recorded on a Shimadzu 8400-S FTIR spectrophotometer using KBr pellets. TLC was performed on object glass slides (2 × 7.5 cm) coated with silica gel-G and spots were visualised under UV irradiation. 1H NMR spectra were obtained on a Varian 400 MHz model spectrometer in DMSO-d_6 using TMS as internal standard. Chemical shifts (δ) are given in ppm and coupling constants (J) in Hz. Elemental analyses (CHN) were performed using a Heraeus Carlo Erba 1180 CHN analyser (Hanau, Germany).

Table 3 Antimycobacterial activity of compounds **6a–k** and **7a–k**

Entry	R	L.J. MIC Method[a]	
		MIC/μg mL^{-1}	% Inhibition
6a	H	500	92
6b	Cl	100	95
6c	Br	50	97
6d	F	25	98
6e	I	250	93
6f	NO_2	62.5	96
6g	CN	200	94
6h	CH_3	500	92
6i	OCH_3	25	98
6j	OC_2H_5	50	96
6k	$NHCOCH_3$	200	94
7a	H	250	93
7b	Cl	62.5	97
7c	Br	25	98
7d	F	12.5	99
7e	I	250	94
7f	NO_2	100	96
7g	CN	200	95
7h	CH_3	500	92
7i	OCH_3	25	98
7j	OC_2H_5	50	97
7k	$NHCOCH_3$	100	95
Isoniazid		0.20	
Rifampicin		0.25	
Ethambutol		3.12	
Pyrazinamide		6.25	
DMSO	–	–	–

[a]Each value is the mean of three independent experiments.

*Synthesis of 2-chloro-N-aryl acetamides (***2a**–**k***);general procedure*

Chloroacetyl chloride (0.06 mol) was added dropwise to a mixture of the appropriate amine (0.05 mol) and K_2CO_3 (0.06 mol) in acetone (50 mL) at room temperature. The reaction mixture was refluxed for 4 to 8 h, then, after cooling to room temperature, it was slowly poured into 100 mL of ice water. The solid that formed was separated by filtration and washed repeatedly with water. The product was dried under vacuum to obtain **2a**–**k**.[8–12] The progress of the reaction was monitored by TLC using toluene: acetone (8 : 2) solvent system.

*Synthesis of 2-amino-6-substituted benzothiazoles (***3a**–**k***);general procedure*

A mixture of 4–substituted aniline (0.1 mol) and potassium thiocyanate (0.1 mol) in glacial acetic acid (100 mL) was cooled in an ice bath and stirred for 10 to 20 min, and then bromine (0.1 mol) in glacial acetic acid was added dropwise at such a rate as to keep the temperature below 10 °C throughout the addition. The reaction mixture was stirred at room temperature for 2–4 h, and the hydrobromide salt that separated out was filtered, washed with acetic acid, dried, dissolved in hot water and basified to pH 11.0 with ammonia solution. The resulting precipitate was filtered, washed with water and dried to give the products **3a**–**k**.[13–16] The progress of the reaction was monitored by TLC using toluene: acetone (8 : 2) solvent system.

*Synthesis of 2-chloro-N-(6-substitutedbenzo[d]thiazol-2-yl)acetamides (***4a**–**k***); general procedure*

Chloroacetyl chloride (0.06 mol) was added dropwise to a mixture of the appropriate 2-amino-6-substituted benzothiazole, **3a**–**k** (0.05 mol) and K_2CO_3 (0.06 mol) in benzene (50 mL) at room temperature. The reaction mixture was refluxed for 6 to 12 h, then, after cooling to room temperature, it was slowly poured into ice water (100 mL). The solid that formed was separated by filtration, washed successively with water, then dried under vacuum to obtain **4a**–**k**.[17–20] The progress of the reaction was monitored by TLC using toluene: acetone (8 : 2) solvent system.

Synthesis of **6a**–**k** *and* **7a**–**k***;general procedure*

To a solution of **5** (0.01 mol) in acetone (30 mL), the appropriate quantities of **2a**–**k** and **4a**–**k** (0.01 mol) was added and the reaction mixture was refluxed for 15–25 h. Potassium carbonate (0.01 mol) was used for neutralisation of the reaction mixture. Progress of the reaction was monitored by TLC using toluene: acetone (8 : 2) as eluent. The mixture was then treated with crushed ice. The precipitate thus obtained was filtered off, dried and recrystallised from acetone to afford **6a**–**k** and **7a**–**k**.[6]

N-*Phenyl-2-(4-(2,3,4-trimethoxybenzyl)piperazin-1-yl)acetamide* (**6a**): IR (KBr, cm^{-1}): 3328 (N–H), 2990 (C–H), 1676 (C=O); ^{1}H NMR: δ 8.21 (s, 1H, –NH), 7.68–7.32 (m, 7H, ArH), 4.24 (s, 2H, CO–CH_2), 3.88 (br s, 4H, piperazine), 3.51 (s, 2H, N–CH_2), 3.37 (s, 9H, 3OCH_3), 3.28 (br s, 4H, piperazine).

N-*(4-Chlorophenyl)-2-(4-(2,3,4-trimethoxybenzyl) piperazin-1-yl) acetamide* (**6b**): IR (KBr, cm^{-1}): 3287 (N–H), 2938 (C–H), 1688 (C=O), 737 (–Cl); ^{1}H NMR: δ 8.43 (s, 1H, –NH), 7.71–7.26 (m, 6H, ArH), 4.13 (s, 2H, CO–CH_2), 3.80 (br s, 4H, piperazine), 3.59 (s, 2H, N–CH_2), 3.52 (s, 9H, 3OCH_3), 3.34 (br s, 4H, piperazine).

N-*(4-Bromophenyl)-2-(4-(2,3,4-trimethoxybenzyl) piperazin-1-yl) acetamide* (**6c**): IR (KBr, cm^{-1}): 3349 (N–H), 3021 (C–H), 1668 (C=O); ^{1}H NMR: δ 8.15 (s, 1H, –NH), 7.83–7.40 (m, 6H, ArH), 4.38 (s, 2H, CO–CH_2), 3.76 (br s, 4H, piperazine), 3.42 (s, 2H, N–CH_2), 3.23 (s, 9H, 3OCH_3), 3.12 (br s, 4H, piperazine).

N-*(4-Fluorophenyl)-2-(4-(2,3,4-trimethoxybenzyl) piperazin-1-yl) acetamide* (**6d**): IR (KBr, cm^{-1}): 3308 (N–H), 3032 (C–H), 1680 (C=O); ^{1}H NMR: δ 8.39 (s, 1H, –NH), 7.67–7.18 (m, 6H, ArH), 4.08 (s, 2H, CO–CH_2), 3.82 (br s, 4H, piperazine), 3.50 (s, 2H, N–CH_2), 3.34 (s, 9H, 3OCH_3), 3.21 (br s, 4H, piperazine).

N-*(4-Iodophenyl)-2-(4-(2,3,4-trimethoxybenzyl) piperazin-1-yl) acetamide* (**6e**): IR (KBr, cm^{-1}): 3327 (N–H), 2984 (C–H), 1668 (C=O); ^{1}H NMR: δ 8.12 (s, 1H, –NH), 7.61–7.38 (m, 6H, ArH), 4.22 (s, 2H, CO–CH_2), 3.86 (br s, 4H, piperazine), 3.58 (s, 2H, N–CH_2), 3.47 (s, 9H, 3OCH_3), 3.39 (br s, 4H, piperazine).

N-*(4-Nitrophenyl)-2-(4-(2,3,4-trimethoxybenzyl) piperazin-1-yl) acetamide* (**6f**): IR (KBr, cm^{-1}): 3298 (N–H), 3011 (C–H), 1679 (C=O); ^{1}H NMR: δ 8.28 (s, 1H, –NH), 7.70–7.32 (m, 6H, ArH), 4.30 (s, 2H, CO–CH_2), 3.78 (br s, 4H, piperazine), 3.63 (s, 2H, N–CH_2), 3.41 (s, 9H, 3OCH_3), 3.31 (br s, 4H, piperazine).

N-*(4-Cyanophenyl)-2-(4-(2,3,4-trimethoxybenzyl)piperazin-1-yl)acetamide* (**6g**): IR (KBr, cm^{-1}): 3303 (N–H), 2961 (C–H), 2224 (C≡N), 1672 (C=O); ^{1}H NMR: δ 8.15 (s, 1H, –NH), 7.67–7.18 (m, 6H, ArH), 4.21 (s, 2H, CO–CH_2), 3.83 (br s, 4H, piperazine), 3.52 (s, 2H, N–CH_2), 3.48 (s, 9H, 3OCH_3), 3.35 (br s, 4H, piperazine).

N-*(*p*-Tolyl)-2-(4-(2,3,4-trimethoxybenzyl)piperazin-1-yl)acetamide* (**6h**): IR (KBr, cm^{-1}): 3317 (N–H), 3018 (C–H), 1679 (C=O); ^{1}H NMR: δ 8.41 (s, 1H, –NH), 7.84–7.32 (m, 6H, ArH), 4.20 (s, 2H, CO–CH_2), 3.81 (br s, 4H, piperazine), 3.57 (s, 2H, N–CH_2), 3.52 (s, 9H, 3OCH_3), 3.38 (br s, 4H, piperazine), 2.56 (s, 3H, CH_3).

N-*(4-Methoxyphenyl)-2-(4-(2,3,4-trimethoxybenzyl)piperazin-1-yl) acetamide* (**6i**): IR (KBr, cm^{-1}): 3320 (N–H), 2961 (C–H), 1676 (C=O); ^{1}H NMR: δ 8.38 (s, 1H, –NH), 7.67–7.28 (m, 6H, ArH), 4.19 (s, 2H, CO–CH_2), 3.85 (br s, 4H, piperazine), 3.60 (s, 2H, N–CH_2), 3.48 (s, 12H, 4OCH_3), 3.21 (br s, 4H, piperazine).

N-*(4-Ethoxyphenyl)-2-(4-(2,3,4-trimethoxybenzyl)piperazin-1-yl)acetamide* (**6j**): IR (KBr, cm^{-1}): 3345 (N–H), 3008 (C–H), 1680 (C=O); ^{1}H NMR: δ 8.21 (s, 1H, –NH), 7.75–7.31 (m, 6H, ArH), 4.27 (s, 2H, CO–CH_2), 4.06 (q, *J*=6.8 Hz, 2H, –O<u>CH</u>$_2$$CH_3$), 3.91 (br s, 4H, piperazine), 3.58 (s, 2H, N–CH_2), 3.54 (s, 9H, 3OCH_3), 3.29 (br s, 4H, piperazine), 1.48 (t, *J*=7.3 Hz, 3H, –OCH$_2$C<u>H</u>$_3$).

N-*(4-Acetamidophenyl)-2-(4-(2,3,4-trimethoxybenzyl)piperazin-1-yl)acetamide* (**6k**): IR (KBr, cm^{-1}): 3328 (N–H), 2978 (C–H), 1672 (C=O); ^{1}H NMR: δ 8.36 (s, 1H, –NH), 8.25 (s, 1H, N<u>H</u>COCH$_3$), 7.59–7.17 (m, 6H, ArH), 4.20 (s, 2H, CO–CH_2), 3.82 (br s, 4H, piperazine), 3.65 (s, 2H, N–CH_2), 3.58 (s, 9H, 3OCH_3), 3.38 (br s, 4H, piperazine), 2.32 (s, 3H, NHCOC<u>H</u>$_3$).

N-*(Benzo[d]thiazol-2-yl)-2-(4-(2,3,4-trimethoxybenzyl)piperazin-1-yl)acetamide* (**7a**): IR (KBr, cm^{-1}): 3311 (N–H), 2985 (C–H), 1669 (C=O), 1507 (benzothiazole); ^{1}H NMR: δ 8.13 (s, 1H, –NH), 7.67 (d, 1H, benzothiazole), 7.52–6.95 (m, 6H, ArH), 4.14 (s, 2H, CO–CH_2), 3.70 (br s, 4H, piperazine), 3.61 (s, 2H, N–CH_2), 3.35 (s, 9H, 3OCH_3), 3.12 (br s, 4H, piperazine).

N-*(6-Chlorobenzo[d]thiazol-2-yl)-2-(4-(2,3,4-trimethoxybenzyl) piperazin-1-yl)acetamide* (**7b**): IR (KBr, cm^{-1}): 3367 (N–H), 2939 (C–H), 1685 (C=O), 1599 (benzothiazole), 750 (–Cl); ^{1}H NMR: δ 8.22 (s, 1H, –NH), 7.79 (d, 1H, benzothiazole), 7.69–7.18 (m, 5H, ArH), 4.22 (s, 2H, CO–CH_2), 3.86 (br s, 4H, piperazine), 3.65 (s, 2H, N–CH_2), 3.50 (s, 9H, 3OCH_3), 3.38 (br s, 4H, piperazine).

N-*(6-Bromobenzo[d]thiazol-2-yl)-2-(4-(2,3,4-trimethoxybenzyl) piperazin-1-yl)acetamide* (**7c**): IR (KBr, cm^{-1}): 3347 (N–H), 3008 (C–H), 1680 (C=O), 1532 (benzothiazole); ^{1}H NMR: δ 8.54 (s, 1H, –NH), 7.83 (d, 1H, benzothiazole), 7.72–7.34 (m, 5H, ArH), 4.43 (s, 2H, CO–CH_2), 3.81 (br s, 4H, piperazine), 3.63 (s, 2H, N–CH_2), 3.37 (s, 9H, 3OCH_3), 3.29 (br s, 4H, piperazine).

N-*(6-Fluorobenzo[d]thiazol-2-yl)-2-(4-(2,3,4-trimethoxybenzyl) piperazin-1-yl)acetamide* (**7d**): IR (KBr, cm^{-1}): 3302 (N–H), 3085 (C–H), 1678 (C=O), 1564 (benzothiazole); ^{1}H NMR: δ 8.09 (s, 1H, –NH), 7.64 (d, 1H, benzothiazole), 7.49–7.22 (m, 5H, ArH), 4.05 (s, 2H, CO–CH_2), 3.73 (br s, 4H, piperazine), 3.48 (s, 2H, N–CH_2), 3.33 (s, 9H, 3OCH_3), 3.21 (br s, 4H, piperazine).

N-*(6-Iodobenzo[d]thiazol-2-yl)-2-(4-(2,3,4-trimethoxybenzyl) piperazin-1-yl)acetamide* (**7e**): IR (KBr, cm^{-1}): 3289 (N–H), 2982 (C–H), 1667 (C=O), 1585 (benzothiazole); ^{1}H NMR: δ 8.19 (s, 1H, –NH), 7.87 (d, 1H, benzothiazole), 7.72–7.30 (m, 5H, ArH), 4.38 (s, 2H, CO–CH_2), 3.78 (br s, 4H, piperazine), 3.60 (s, 2H, N–CH_2), 3.45 (s, 9H, 3OCH_3), 3.37 (br s, 4H, piperazine).

N-*(6-Nitrobenzo[d]thiazol-2-yl)-2-(4-(2,3,4-trimethoxybenzyl) piperazin-1-yl)acetamide* (**7f**): IR (KBr, cm^{-1}): 3315 (N–H), 3042 (C–H), 1679 (C=O), 1540 (benzothiazole); ^{1}H NMR: δ 8.02 (s, 1H, –NH), 7.69 (d, 1H, benzothiazole), 7.53–7.26 (m, 5H, ArH), 4.29 (s, 2H, CO–CH_2), 3.74 (br s, 4H, piperazine), 3.58 (s, 2H, N–CH_2), 3.43 (s, 9H, 3OCH_3), 3.29 (br s, 4H, piperazine).

N-*(6-Cyanobenzo[d]thiazol-2-yl)-2-(4-(2,3,4-trimethoxybenzyl) piperazin-1-yl)acetamide* (**7g**): IR (KBr, cm^{-1}): 3348 (N–H), 3011 (C–H), 2229 (C≡N), 1684 (C=O), 1560 (benzothiazole); ^{1}H NMR: δ 8.21 (s, 1H, –NH), 7.80 (d, 1H, benzothiazole), 7.67–7.25 (m, 5H, ArH), 4.08 (s, 2H, CO–CH_2), 3.65 (br s, 4H, piperazine), 3.49 (s, 2H, N–CH_2), 3.38 (s, 9H, 3OCH_3), 3.26 (br s, 4H, piperazine).

N-*(6-Methylbenzo[d]thiazol-2-yl)-2-(4-(2,3,4-trimethoxybenzyl) piperazin-1-yl)acetamide* (**7h**): IR (KBr, cm^{-1}): 3360 (N–H), 2987 (C–H), 1669 (C=O), 1587 (benzothiazole); ^{1}H NMR: δ 8.38 (s, 1H, –NH), 7.61 (d, 1H, benzothiazole), 7.48–6.89 (m, 5H, ArH), 4.12 (s, 2H, CO–CH_2), 3.71 (br s, 4H, piperazine), 3.58 (s, 2H, N–CH_2), 3.30 (s, 9H, 3OCH_3), 3.19 (br s, 4H, piperazine), 2.38 (s, 3H, CH3).

N-*(6-Methoxybenzo[d]thiazol-2-yl)-2-(4-(2,3,4-trimethoxybenzyl) piperazin-1-yl)acetamide* (**7i**): IR (KBr, cm^{-1}): 3298 (N–H), 3031 (C–H), 1674 (C=O), 1545 (benzothiazole); ^{1}H NMR: δ 8.32 (s, 1H, –NH), 7.68 (d, 1H, benzothiazole), 7.53–7.28 (m, 5H, ArH), 4.30 (s, 2H, CO–CH_2), 3.92 (br s, 4H, piperazine), 3.60 (s, 2H, N–CH_2), 3.42 (s, 12H, 4OCH_3), 3.27 (br s, 4H, piperazine).

N-*(6-Ethoxybenzo[d]thiazol-2-yl)-2-(4-(2,3,4-trimethoxybenzyl) piperazin-1-yl)acetamide* (**7j**): IR (KBr, cm^{-1}): 3330 (N–H), 2995 (C–H), 1679 (C=O), 1598 (benzothiazole); ^{1}H NMR: δ 7.96 (s, 1H, –NH), 7.67 (d, 1H, benzothiazole), 7.53–7.20 (m, 5H, ArH), 4.25 (s, 2H, CO–CH_2), 4.14 (q, J = 7.6 Hz, 2H, –O$\underline{H}_2$$CH_3$), 3.88 (br s, 4H, piperazine), 3.57 (s, 2H, N–CH_2), 3.39 (s, 9H, 3OCH_3), 3.26 (br s, 4H, piperazine), 1.52 (t, J = 7.3 Hz, 3H, –OCH_2C$\underline{H}_3$).

N-*(6-Acetamidobenzo[d]thiazol-2-yl)-2-(4-(2,3,4-trimethoxybenzyl) piperazin-1-yl)acetamide* (**7k**): IR (KBr, cm^{-1}): 3321 (N–H), 3026 (C–H), 1668 (C=O), 1548 (benzothiazole); ^{1}H NMR: δ 8.28 (s, 1H, –NH), 8.11 (s, 1H, N$\underline{H}$$COCH_3$), 7.86 (d, 1H, benzothiazole), 7.58–7.21 (m, 5H, ArH), 4.35 (s, 2H, CO–CH_2), 3.78 (br s, 4H, piperazine), 3.56 (s, 2H, N–CH_2), 3.49 (s, 9H, 3OCH_3), 3.40 (br s, 4H, piperazine), 2.25 (s, 3H, NHCOC$\underline{H}_3$).

Microbiology

In vitro *antimicrobial activity evaluation:* Compounds **6a**–**k** and **7a**–**k** were examined for their antimicrobial activity against two Gram-positive bacteria (*S. aureus* and *B. cereus*), three Gram-negative bacteria (*E. coli*, *P. aeruginosa* and *K. pneumoniae*) and two fungal species (*A. niger* and *C. albicans*) using the paper disc diffusion technique and the agar streak dilution method.[21] The Mueller–Hinton agar media were sterilised (autoclaved at 120 °C for 30 min), poured at uniform depth of 5 mm and allowed to solidify. The microbial suspension (10^5 CFU/mL) (0.5 McFarland Nephelometry Standards) was streaked over the surface of media using a sterile cotton swab to ensure even growth of the organisms. The test compounds were dissolved in dimethylsulfoxide (DMSO) to give solutions of 3.12–50 μg mL^{-1}. Sterile filter paper discs measuring 6.25 mm in diameter (Whatman no. 1 filter paper), previously soaked in a known concentration of the respective test compound in DMSO were placed on the solidified nutrient agar medium that had been inoculated with the respective microorganism and the plates were incubated for 24 h at (37 ± 1) °C. A control disc impregnated with an equivalent amount of DMSO without any sample was also used and did not produce any inhibition. Ampicillin and gentamicin (100 μg/disc) were used as standard antibacterial drugs while fluconazole (100 μg/disc) was used as an antifungal drug.

The MIC values were determined by the agar streak dilution method.[22] A stock solution of the test compound (100 μg mL^{-1}) in DMSO was prepared and graded quantities of them were incorporated into a specified quantity of molten sterile agar, *i.e.* nutrient agar for evaluation of antibacterial and sabouraud dextrose agar for antifungal activity, respectively. The medium containing the test compound was poured into a Petri dish at a depth of 4–5 mm and allowed to solidify under aseptic conditions. A suspension of the respective microorganism of approximately 10^5 CFU mL^{-1} was prepared and applied to plates with serially diluted compounds with concentrations in the range of 3.12–50 μg mL^{-1} in DMSO and incubated at (37 ± 1) °C for 24 h (bacteria) or 48 h (fungi). The lowest concentration of the substance that prevented the development of visible growth was considered to be the MIC value.

In vitro *evaluation of antitubercular activity evaluation:* The antitubercular screening of test compounds was achieved for *M. Tuberculosis H37Rv* by adopting the L.J. (conventional Lowenstein and Jensen) agar dilution method for the measurement of MIC, and is defined as the lowest concentration of drug, which inhibits ≥ 99% of the bacterial population present at the beginning of the assay. Stock solutions of 100, 62.5, 50, 25, 12.5, 6.25, 3.12 and 1.56 μg mL^{-1} dilutions of each test compound in DMSO were added into the liquid L.J. Medium and then the media were sterilised by the inspissation method. A culture of *M. tuberculosis H37Rv* growing on L.J. Medium was harvested in 0.85% saline in bijou bottles.[23] These tubes were then incubated at 37 °C for 24 h followed by streaking of *M. tuberculosis H37Rv* (5 × 104 bacilli per tube). These tubes were then incubated at 37 °C. Growth of bacilli was assessed after 12 days, 22 days and finally 28 days of incubation. Tubes having test compounds were compared with control tubes where medium alone was incubated with *M. tuberculosis H37Rv*. The concentration at which no development of colonies occurred or < 20 colonies was taken as the MIC concentration of the test compound. The standard strain *M. tuberculosis H37Rv* was tested with the known drugs Isoniazid, Rifampicin, Ethambutol and Pyrazinamide.

Conclusion

In conclusion, this work has focused on the development of new small molecules endowed with a piperazine nucleus bearing various substituents in the form of aryl acetamides moieties. In general, the results of the *in vitro* pharmacological activities are encouraging, as out of two series tested, the compounds containing a *N*-benzothiazolyl acetamide moiety emerged as the class of compounds exhibiting the highest antimicrobial activity. Based on the results described above, such compounds can be recognised as new active leads that provide a powerful incentive for further research in this area.

Received 21 August 2014; accepted 13 September 2014
Paper 1402842 doi: 10.3184/174751914X14116443659287
Published online: 17 October 2014

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