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Broad spectrum anti-infective properties of benzisothiazolones and the parallels in their anti-bacterial and anti-fungal effects

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ABSTRACT

Various mono- and *bis*-benzisothiazolone derivatives were synthesized and screened against different strains of bacteria and fungi in order to understand the effect of multiple electrophilic sulfur atoms and substitution pattern in the immediate vicinity of reactive sulfur. *Staphyllococcus aureus*-ATCC 7000699, MRSA and *S. aureus*-ATCC 29213 (Quality Control strain) were more susceptible to this class of compounds, and the most potent derivative **1.15** had MIC_{50} of 0.4 μg/mL (cf. Gentamicin = 0.78 μg/mL). CLogP value, optimally in the range of 2.5–3.5, appeared to contribute more to the activity than the steric and electronic effects of groups attached at nitrogen. By and large, their anti-fungal activities also followed a similar trend with respect to the structure and CLogP values. The best potency of IC_{50} = 0.1 μg/mL was shown by N-benzyl derivative (**1.7**) against *Aspergillus fumigatus*; it was also potent against *Candida albicans*, *Cryptococcus neoformans*, *Sporothrix schenckii*, and *Candida parapsilosis* with IC_{50} values ranging from 0.4 to 1.3 μg/mL. Preliminary studies also showed that this class of compounds have the ability to target malaria parasite with IC_{50} values in low micromolar range, and improvement of selectivity is possible through structure optimization.

Introduction

Combating infectious diseases is a major challenge of the present century due to increasing resistance against common therapeutic agents. Among various classes of heterocyclic compounds with anti-infective properties, benzisothiazolones (BITs) are special because of the presence of electrophilic sulfur as part of the bicyclic skeleton.[1,2] Its ability to form disulfide bonds with sulfur nucleophiles in the target cells, or to chelate biologically relevant metals such as zinc from the functional domains of proteins have been correlated with the biological effects.[3–6] Recently we have conducted a series of studies to understand the effect of benzisothiazolones on cancer cells and found that they are capable of inducing apoptosis through intrinsic pathway, and can arrest the cell cycle of HeLa cells at G2/M phase.[7] Although the IC_{50} values were in micromolar range, their ability to induce DNA fragmentation, perturb mitochondrial membrane potential and more importantly, the ability of externally added sulfur nucleophiles like N-acetyl cysteine (NAC) to reverse the inhibitory effects of BITs were indicative of the direct role of sulfur in the biochemical responses.

A literature search revealed that most of the isothiazolones and benzisothiazolones (BITs) investigated thus far are 'monomeric' in the sense that they contain only one electrophilic sulfur atom. These reports showed their effect on whole cell/infectious agent (anti-proliferative effects, inhibition of blood platelet aggregation, antiviral, antibacterial & anti-fungal activities),[7–13] as well as against specific targets like human leukocyte elastase (BIT as 1,1-dioxide),[14–16] RNA polymerase,[5] HIV-NCp-7,[6] macrophage migration inhibitory factor,[17] histone acetyltransferases,[3,18] telomerase[19] and phosphomannose isomerase.[20] Introduction of additional electrophilic sulfur in the molecule could in principle augment the biological response but comparative assessment of their properties remains to be carried out in a systematic manner. The present work primarily aims to compare the activities of monomeric and dimeric BITs (Fig. 1) against different strains of bacteria, fungi and malaria parasite to understand the structural features/molecular properties that are decisive. They were synthesized by reaction of either bromosulfenyl benzoyl chloride or dithiodibenzoyl chloride with appropriate amines in presence of a base such as triethylamine.[7] Experimental procedures and spectral data of these compounds are presented in the supporting material. They differ in the nature of N-substitution and present varying degrees of steric and electronic influences in the vicinity of electrophilic sulfur.

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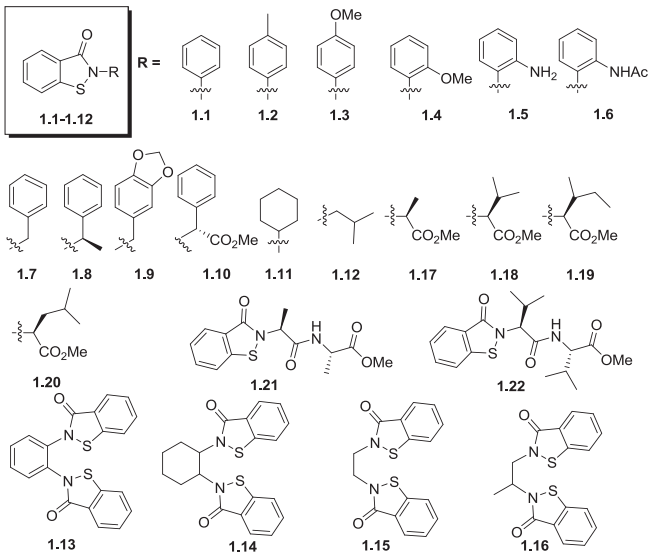

Fig. 1. Benzisothiazolone derivatives used in the present study.

Results and discussion

Antibacterial and antifungal assay results: Compounds **1.1**–**1.22** (Fig. 1) were screened against various bacteria and fungi as per literature protocols. The bacteria were tested by NCCLS method in Mueller Hinton Broth.[21] Gentamicin and Norfloxacin were used as standard drugs to compare antibacterial activity whereas Fluconazole and Amphotericin B were used for antifungal studies as controls. The bacterial strains used were: (1) *Escherichia coli* (ATCC 9637) (2) *Pseudomonas aeruginosa* (ATCC BAA-427) (3) *Klebsiella pneumoniae* (ATCC 27736) (4) *Staphylococcus aureus* (ATCC 25923) (5) *S. aureus* (ATCC 700699, MRSA), and (6) *S. aureus* (ATCC 29213, Quality Control strain).

Compounds **1.1**–**1.22** can be broadly categorized into four groups: (i) N-aryl and N-alkylaryl substituted benzisothiazolone (**1.1**–**1.10**), (ii) those derived from aliphatic or alicyclic amines (**1.11**–**1.12**), (iii) those based on amino acid esters or peptides (**1.17**–**1.22**), and (iv) benzisothiazolone dimers (**1.13**–**1.16**); the alanine-based **1.17** has previously been studied by Dou et al.[10] Among the six bacterial strains tested, *S. aureus* was more sensitive to these compounds (column 4–6, Table 1); for others (strains 1–3, SI), IC_{50} values were >50 μg/mL and are presented in SI. Among *S. aureus* strains, MRSA (ATCC 700699) and the Quality Control strain (ATCC 29213) (Table 1) in general were more susceptible compared to wild type. Although a clear structure-activity relationship in accordance with variation in N-substitution was not obvious, the fact that simple N-aryl benzisothazolones are as active as dimeric systems shows that increase in the number of electrophilic sulfur atoms does not lead to a proportionate increase in activities. Compounds **1.1**–**1.4**, **1.7** and **1.10** had IC_{50} values <2 μg/mL against *S. aureus*, **1.2** being the most potent (0.6 μg/mL). In the case of methicillin resistant *S. aureus*, compounds **1.7** and **1.15** were superior with IC_{50} of 0.4 μg/mL each. Other compounds with IC_{50} value <2 μg/mL against this strain are **1.8**, **1.9**, **1.11**, **1.14**, and **1.16**–**1.19**. The compound **1.15** was found to be the most potent (IC_{50} = 0.3 μg/mL) against the Quality Control *S. aureus* (ATCC 29213), and compounds **1.1**–**1.4**, **1.7** and **1.9** had the IC_{50} value within 2 μg/mL. Simple aryl groups or amino acid units as part of N-substitution gave sub-micro IC_{50} values while introduction of more than one amino acid residue doesn't give any advantage which becomes clear if we compare the activities of **1.17**&**1.18** vs. **1.21**&**1.22**. The fact that a number of these compounds are more active than the standard drugs Gentamycin and Norfloxacin in assays against resistant strains is particularly noteworthy (see Table 2).

After understanding the antibacterial activities of these compounds we continued our studies by evaluating their effect on various fungal strains namely: 1. *Candida albicans,* 2. *Cryptococcus neoformans,* 3. *Sporothrix schenckii,* 4. *Trichophyton mentagrophytes,* 5. *Aspergillus fumigatus* and 6. *Candida parapsilosis* (ATCC-22019). The MICs of compounds were determined by broth microdilution technique as per the guidelines of the National Committee for Clinical Laboratory Standards using RPMI-1640 media buffered with MOPS [3-(N-morpholino)propanesulfonic acid]. Starting inoculums of test culture was $1–5 \times 10^3$ CFU mL^{-1}. Micro titer plates were incubated at 35 °C. The MIC values were recorded after 48 h of incubation.[22,23] Interestingly, there were some parallels in the antifungal and antibacterial assay results. While, most of them were active with low IC_{50} values (**1.7** being one of the most potent with IC_{50} = 0.1 μg/mL against *Aspergillus fumigatus*), the compounds **1.6**, **1.10**, **1.13, 1.14, 1.21** and **1.22**, which were less potent in antibacterial assays, remained so against fungal strains as well. Among the active ones, IC_{50} value of <1 μg/mL was shown by simple N-aryl (**1.1**–**1.4**), N-alkyl (**1.11**–**1.12**) and N-arylalkyl (**1.7**–**1.9**) derivatives.

Similar trend in their antibacterial and antifungal activity prompted us to look for physicochemical properties that are likely decisive. Of various descriptors, partition coefficients (LogP) which reflects organic/aqueous solubility, seemed important as it affects

Table 1
Antibacterial activities of benzisothiazolones **1.1**–**1.22**.

No	4*		5*		6*		No	4*		5*		6*	
	MIC	IC_{50}	MIC	IC_{50}	MIC	IC_{50}		MIC	IC_{50}	MIC	IC_{50}	MIC	IC_{50}
1.1	1.6	1.1	0.77	ND	2.1	1.6	**1.13**	18.0	10.0	10.2	4.7	6.5	6.3
1.2	1.07	0.6	0.75	ND	1.0	0.6	**1.14**	3.4	3.2	2.3	1.4	3.7	2.5
1.3	1.6	1.5	0.73	ND	1.9	1.2	**1.15**	16.7	4.1	0.9	0.4	0.4	0.3
1.4	2.0	1.0	1.07	ND	2.4	1.6	**1.16**	12.4	6.1	2.6	1.5	4.1	2.0
1.5	>50	>50	>50	>50	>50	>50	**1.17**	3.0	2.0	0.87	0.84	8.4	6.4
1.6	>50	>50	>50	>50	>50	>50	**1.18**	2.9	2.4	0.97	0.82	4.6	2.6
1.7	1.6	1.5	0.8	0.4	2.3	1.7	**1.19**	4.2	2.0	0.89	0.43	4.3	2.1
1.8	10.2	6.8	1.9	1.0	8.5	4.6	**1.20**	3.5	2.1	3.5	2.1	6.9	2.7
1.9	2.6	2.2	1.0	0.6	2.5	1.9	**1.21**	7.5	3.6	7.5	3.6	5.6	3.5
1.10	1.5	1.1	4.2	2.6	18.7	13.5	**1.22**	>50	>50	>50	>50	>50	>50
1.11	9.8	6.8	2.5	1.0	7.1	4.6	**Std1**[7]	6.25	–	>50	–	0.78	–
1.12	15.4	10.6	5.2	4.7	9.8	6.3	**Std2**[8]	0.39	–	>50	–	0.78	–

* MIC and IC_{50} values in μg/mL, MIC-Minimum inhibitory concentration; (1) *E. coli* (ATCC 9637), (2) *Pseudomonas aeruginosa* (ATCC BAA-427), (3) *Klebsiella pneumoniae* (ATCC 27736); results from assays involving these strains are given in SI; (4) *Staphylococcus aureus* (ATCC 25923), (5) *Staphylococcus aureus* (ATCC 700699, MRSA), (6) *Staphylococcus aureus* (ATCC 29213 Quality control strain for susceptibility testing), (7) Gentamicin and (8) Norfloxacin.