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COMMUNICATION

Synthesis and *in vitro* anti-tubercular evaluation of 1,2,3-triazole tethered β-lactam–ferrocene and β-lactam–ferrocenylchalcone chimeric scaffolds†

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Twenty different triazoles were prepared to probe the anti-tubercular structure–activity relationships (SAR) within the β-lactam–ferrocene–triazole conjugate family. The compounds have been synthesized by copper-catalyzed "click chemistry". *In vitro* anti-tubercular activity was determined for each compound but the synthesized hybrids failed to inhibit *Mycobacterium tuberculosis* growth even at high doses. The manuscript assumes significance as this is the first report on the inclusion of ferrocene nucleus in the well established β-lactam family *via* triazole linkers with reputed physicochemical profiles.

Introduction

Tuberculosis (TB), an infectious disease caused by *Mycobacterium tuberculosis*, represents a highly contagious, airborne disease that still remains the leading cause of worldwide death among infectious diseases.[1] World Health Organization (WHO) estimates about 8 million new active cases of TB per year and nearly 2 million deaths each year.[2] The mortality and spread of this disease has further been aggravated because of synergy of this disease with HIV.[3] Even though improved methods of prevention, detection, diagnosis and treatment have greatly reduced the number of people who contract the disease, the emergence of multidrug resistance (MDR) and extensively drug-resistant (XDR) tuberculosis have amplified the incidence of TB which makes the discovery of new molecular scaffolds a priority.[4] Different mechanisms have been put forward for the causes of the development of resistance against existing drugs, such as impermeability of the highly hydrophobic cell envelop to many drugs,[5] a well-developed drug-efflux system, production of certain enzymes to inactivate the drugs (β-lactamases, aminoglycoside acyl transferase),[6] and at the molecular level acquisition of resistance in *M. tuberculosis* because of mutational events in the chromosomes.[7] Indeed, no new drugs have been developed against mycobacteria since the 1960s and there is an urgent need to develop new anti-TB therapeutics.[8]

Over the past few years, bioorganometallic chemistry has developed as a rapidly growing and maturing area which links classical organometallic chemistry to biology, medicine, and molecular biotechnology.[9] The use of metal complexes capable of enhancing the activity of biological compounds has become a relevant strategy of research in both communities of organometallic chemists and biologists. Among metallocenes, ferrocene has attracted special attention as an attractive pharmacophore for drug design[10] as it is a neutral, chemically stable and nontoxic molecule. Many ferrocenyl compounds display interesting cytotoxic,[11] antimalarial,[12] antifungal,[13] antitoxoplasmic[14] and DNA-cleaving activity.[15]

Recent disclosure from our group has revealed the synthesis of quinoline–ferrocene hybrids with significant activity (MIC = 2.5–5 μg ml^{-1}) against *M. tuberculosis*.[16] Moreover, the antimalarial drug candidate ferroquine (FQ, SSR97193) was also evaluated mainly because of its structural similarity with the synthesized hybrids and was found to display moderate inhibitory activity (MIC = 10–15 μg ml^{-1}) against *M. tuberculosis*.[16] Another report by Pélinski *et al.* described the synthesis of ferrocenyl amides derived from nicotinamide and pyrazinamide, ferrocenyl pyridinyl, quinolyl and acridinylhydrazones displaying interesting anti-mycobacterial profiles.[17]

Chalcones (1,3-diaryl-2-propen-1-ones) are open chain flavonoids that are widely biosynthesized in plants.[18] A long-standing scientific research has shown that chalcones also display other interesting biological properties such as antioxidant, cytotoxic, anticancer, antimicrobial, antiprotozoal, antiulcer, antihistaminic and anti-inflammatory activities. Chalcones became an object of continued interest in both academia and industry and nowadays, several chalcones are used for treatment of viral disorders, cardiovascular diseases, parasitic infections, pain, gastritis, and stomach cancer, as well as like food additives and cosmetic formulation ingredients.[19] Chauhan *et al.* have recently reported the synthesis and *in vitro* screenings of quinolinyl chalcones against

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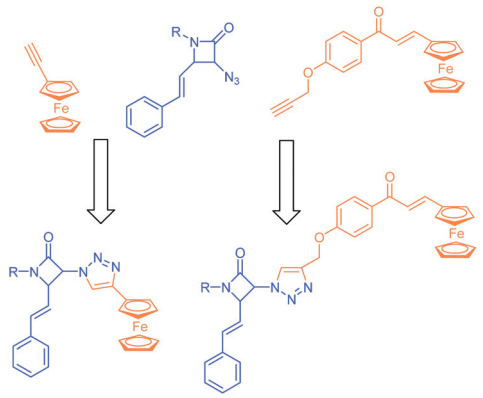
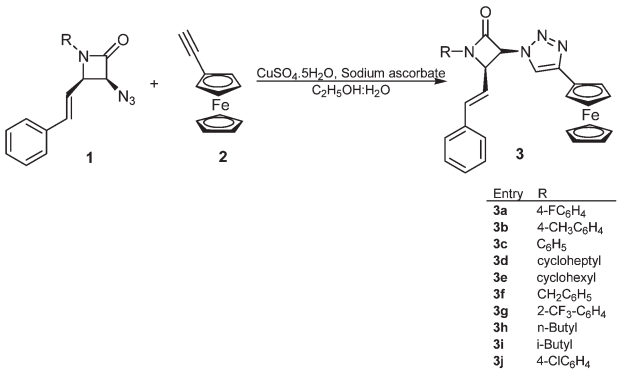
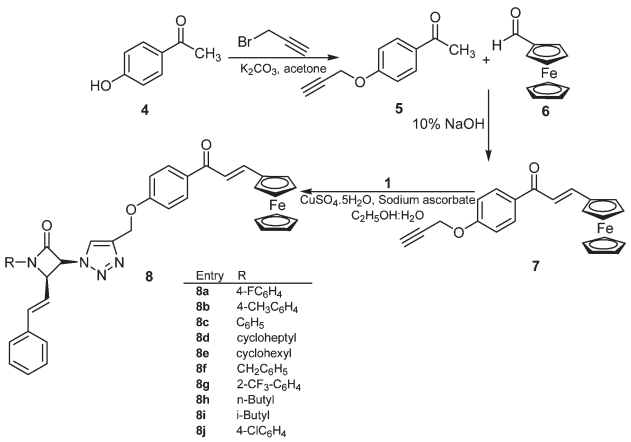


Fig. 1 Designed β-lactam–ferrocene–triazole hybrids.



Scheme 1 Synthesis of β-lactam–ferrocene based hybrids.



Scheme 2 Synthesis of β-lactam–ferrocenylchalcone based hybrids.

M. tuberculosis.[20] The synthesized compounds have shown promising activity with minimal inhibitory concentrations (MIC) in the range of 3.12–12.5 μg ml^{-1}.

β-Lactams are considered as evergreen bioactive molecules with activity ranging from the antimicrobial potency of naturally occurring bicyclic compounds (penicillins and cephalosporins), to the new variants with monocyclic structure (azetidinones) having specific biological activities. Dubey and co-workers have recently reported the synthesis of 2-oxo-4-substituted-2-azetidinone derivatives and evaluated for their anti-tubercular activity against *M. tuberculosis* H37RV with some azetidinone derivatives of benzotriazole displaying good activities.[21] Based on literary rationale, the present work entails the development of a convenient protocol for the synthesis and anti-tubercular evaluation of 1,2,3-triazole tethered ferrocene–β-lactam and ferrocenylchalcone–β-lactam hybrids.

Results and discussion

Recent work conducted in our laboratory has shown the utilization of molecular hybridization as an effective protocol for the synthesis of novel molecular frameworks and their biological evaluation as antimalarial and anticancer agents.[22] Thus, in continuation of our research interest for the synthesis of novel molecular scaffolds with medicinal potential and our recent disclosure on the significant activity exhibited by quinoline–ferrocene hybrid,[16] we report here in the synthesis and anti-tubercular evaluation of 1,2,3-triazole tethered ferrocene–β-lactam and ferrocenylchalcone–β-lactam based chimeric molecules as depicted in Fig. 1 utilizing the Husigen's azide–alkyne cycloaddition reactions and the evaluation of their *in vitro* anti-tubercular activity.

The synthetic protocol involved the Cu-mediated click chemistry of 3-azido-2-azetidinone prepared by Staudinger reaction of 1-azadiene with azidoketene, generated *in situ* from azidoacetic acid and *p*-toluenesulphonyl chloride in the presence of triethylamine, with ethynyl ferrocene (Scheme 1).

The *cis*-stereochemistry to the products **3a–j** was assigned on the basis of observed coupling constant J = 5.7 Hz between H^1 and H^2. The structure assigned to the hybrids **3** was confirmed on the basis of spectral data and analytical evidences. The compound, for example **3j**, showed a molecular ion peak at 535 along with the characteristic peaks in ^{1}H and ^{13}C NMR spectra. The ^{1}H NMR spectrum exhibited a singlet at δ 3.89 corresponding to 5H (cyclopentadiene ring of ferrocene) along with a doublet at δ 4.26 (J = 0.9 Hz, 2H), a doublet at δ 4.60 (J = 1.2 Hz, 2H) along with a doublet at δ 4.69 (J = 0.9 Hz, 1H) because of ferrocene ring protons. The presence of a characteristic singlet at δ 7.61 corresponding to triazole ring proton further ascertains the assigned structure. The assigned structure was further corroborated with the number of carbon atoms in the ^{13}C NMR spectrum.

Ferrocenyl chalcone, prepared by an initial propargylation of 4-hydroxyacetophenone followed by aldol condensation with ferrocene carboxaldehyde, was used as a click chemistry precursor for azide–alkyne cyclization with variedly substituted 3-azido-2-azetidinones in the presence of copper sulphate/sodium ascorbate in ethanol–water mixture (Scheme 2). The reaction resulted in the isolation of desired hybrids **8a–h** in good to excellent yields, the structure to which were assigned on the basis of spectral data and analytical evidences.

The synthesized chimeric scaffolds were then evaluated for their anti-tubercular profiles and the results are summarized in Table 1. Cephalexin, a β-lactam antimicrobial was included as a positive control, and found to exhibit a MIC value of 10–25 μg ml^{-1}, consistent with previous findings.[23]

Table 1 *In vitro* anti-mycobacterial activity of compounds **3a–j** and **8a–j** against *M. tuberculosis* mc^{2}7000

Compound	MIC (μg ml^{-1})
3a	>100
3b	>100
3c	>100
3d	>100
3e	>100
3f	>100
3g	>100
3h	>100
3i	>100
3j	>100
8a	>100
8b	>100
8c	>100
8d	>100
8e	>100
8f	>100
8g	>100
8h	>100
8i	>100
8j	>100
Cephalexin	10–25

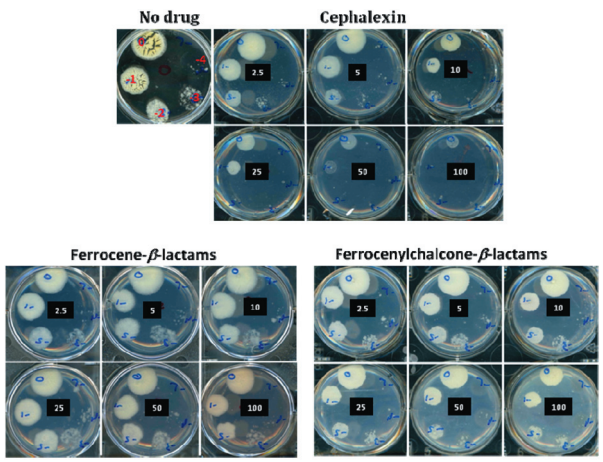


Fig. 2 Cephalexin, but neither β-lactam–ferrocenes hybrids nor β-lactam–ferrocenylchalcone based hybrids, inhibits growth of *M. tuberculosis*. The susceptibility of *M. tuberculosis* was determined on Middlebrook 7H11 solid medium containing OADC enrichment, pantothenic acid with increasing inhibitor concentrations (indicated in white, μg ml^{-1}). Serial 10-fold dilutions (indicated in red on the control plate) of actively growing culture were plated and incubated at 37 °C for 2–3 weeks.

The effect of cephalexin and one representative example of synthesized chimeric molecules **3a–j** and **8a–j** is depicted in Fig. 2.

Conclusions

The evaluation studies clearly revealed that none of the synthesized chimeric scaffolds exhibited any activity to restrict mycobacterial growth, even at high doses. The introduction of various substituents varying from aryl to alkyl on the N-1 position of the β-lactam ring does not have any effect on the anti-tubercular activity profiles. Further, the synthesized ferrocenylchalcone based hybrids also fail to produce any significant enhancement in the activity profiles of these compounds. In conclusion, we have utilized the molecular hybridization protocol for the synthesis of ferrocene–β-lactam and ferrocenylchalcone–β-lactam based structural chimeras *via* Huisgen azide–alkyne cycloaddition and the evaluation of their anti-tubercular activity. The described protocol is the first successful attempt on the inclusion of ferrocene nucleus in the β-lactam family tethered *via* triazole linkers having metabolic stability and physicochemical favourability. The synthesized chimeras will also be explored for their anticancer and antiplasmodial profiles and further work on the scope and extension of the devised protocol is currently under progress.

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