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Convenient one-pot synthesis, anti-mycobacterial and anticancer activities of novel benzoxepinoisoxazolones and pyrazolones

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ABSTRACT

Series of new benzoxepinoisoxazolones **4a**–**d** and pyrazolones **6a**–**t** were prepared by the cyclocondensation of substituted (*E*)-ethyl 3-oxo-2,3-dihydrobenzo[*b*]oxepine-4-carboxylates **3a**–**d** with hydroxylamine hydrochloride and phenylhydrazine hydrochlorides **5a**–**k**. Synthesized compounds were screened for their *in vitro* anti-mycobacterial activity and anticancer activity. Ten compounds displayed good anti-mycobacterial activity, among these; compound **4d** and **6b** found to be potent when compared to standard drug isoniazid. Eleven compounds displayed good anticancer activity and compounds **4b**–**d** displayed equipotent activity on HeLa cell lines when compared to standard drug doxorubicin. Activation of caspase-3 and caspase-9 has been measured for compounds **4b**–**d** on HeLa cell lines (apoptosis). This is the first report assigning *in vitro* anti-mycobacterial, anticancer and structure–activity relationship for this new class of benzoxepinoisoxazolones and pyrazolones.

1. Introduction

Isoxazolones and pyrazolones are an important heterocyclic compounds and drug substances in pharmaceutical industry due to their potential biological activities [1]. Phenazone, propyphenazone, ampyrone and metamizole sodium are useful pyrazolone drugs as antipyretic and analgesic [2], whilst edaravone (MCI-186) has been used for brain disorders [3] and myocardial ischemia [4] (Fig. 1). Few isoxazolones were also reported as effective p38 MAP kinase inhibitors [5].

Tuberculosis [6] is chronic infective disease caused by *Mycobacterium tuberculosis*, *Mycobacterium bovis*, *Mycobacterium avium*, *Mycobacterium microti*, *Mycobacterium africanum*, *Mycobacterium leprae* and Gram-positive pathogens of higher vertebrates. Rifampicin, isoniazid, pyrazinamide, ethambutol and streptomycin are the first line drugs used for tuberculosis and the treatment involves two months of isoniazid, pyrazinamide, ethambutol, and rifampicin; followed by rifampicin and isoniazid for four months. Increasing of drug resistant associated with HIV infection which compromises host defense and allows latent infection to reactivate or render individuals more susceptible to tuberculosis. New drugs are very much needed to reduce time therapy for cure and to treat multidrug resistant (MDR) *M. tuberculosis* strains. Cancer [7], the uncontrolled and rapid proliferation of abnormal cells is the leading cause of human deaths, despite of its progress in chemical biology and pharmacology. Therefore, there is an urgent need to develop more potent new chemical entities for *M. tuberculosis* and cancer.

Our studies on biologically active heterocyclic compounds [8] and recent focus on feasible reactions of carbonyl compounds/salicylaldehydes with 3-oxobutanoates bearing chloro or trifluoro substituents. In this context, we studied the reactivity of carbonyl compounds with ethyl 4,4,4-trifluoro-3-oxobutanoate using piperidine in CH_2Cl_2 at room temperature to provide a series of (*E*)-α,β-unsaturated esters and ketones [9a]. We also studied the reactivity of various salicylaldehydes with ethyl 4-chloro-3-oxobutanoate using piperidine to provide 2*H*-chromenes [9b], interim these derivatives were successfully converted to corresponding 2,3-dihydrobenzoxepine-4-carboxylates [9c]. Further, 2*H*-chromenes were utilized for the preparation of chromenopyrrolones and 1*H*-1,2,3-triazolylchromenopyrrolones in very good yields [10]. In this study we report series of substituted new benzoxepinoisoxazolone, pyrazolone derivatives, their anti-

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mycobacterial and anticancer activities as well as structure–activity relationship in relation to various substitutions present on benzoxepine/phenylhydrazines for the first time.

2. Chemistry

Scheme 1 illustrates the synthesis of (*E*)-ethyl 3-oxo-2,3-dihydrobenzo[*b*]oxepine-4-carboxylates **3a**–**d** [9c]. Salicylaldehydes **1a**–**d** with ethyl 4-chloro-3-oxobutanoate under optimized conditions provided 2*H*-chromenes **2a**–**d**. Wittig reaction of **2a**–**d** with (1-ethoxycarbonylethylidene)triphenylphosphorane provided benzoxepinecarboxylates **3a**–**d**. These derivatives are an important synthons having an olefin ester and carbonyl functional group similar to 1,3-dicarbonyl compounds, hence, these functional groups can be utilized for the preparation of various new heterocyclic compounds. Accordingly, the initial reaction was performed with benzoxepinecarboxylate **3a** and hydroxylamine hydrochloride in presence of acetic acid in ethanol under reflux conditions for 3 h (Scheme 2) and to provide 3*H*,10*H*-benzo[6,7]oxepino[3,4-*c*]isoxazol-3-one **4a** as pale yellow color solid in 72% yield. The reaction proceeds initial formation of an oxime intermediate followed by in situ cyclization with the elimination of ethanol afforded **4a**. The IR spectrum of compound **4a** has shown absorption bands at $\nu_{max} = 1710$ and 1560 cm^{-1} corresponds to carbonyl group and an imine double bond. The ^1H NMR spectrum shown a characteristic singlet resonated at $\delta = 7.74$ ppm corresponds to C-5 of the benzoxepine; another singlet at $\delta = 5.02$ ppm corresponds to methylene protons. The multiplet at $\delta = 7.20–7.32$ ppm and doublet at $\delta = 7.54$ ppm correspond to aromatic protons. Compound **4a** was further analyzed by ^{13}C NMR spectroscopy, signal at $\delta = 168.84$ ppm corresponds to carbonyl ester group and another characteristic signal at $\delta = 66.78$ ppm corresponds to OCH_2 of the benzoxepinoisoxazolone. Compound **4a** was shown molecular ion MS (ESI) *m/z* 202 $[M + H]^+$. Finally, the compound **4a** was crystallized [11] in ethanol and its structure was confirmed by X-ray crystallography (Fig. 2). Under optimized conditions, benzoxepinoisoxazolone derivatives **4b**–**d** was prepared from the corresponding benzoxepinones **3b**–**d** with hydroxylamine hydrochloride in good yields. Electron withdrawing groups have an advantage over electron donating groups in terms of yields (Scheme 2).

After having achieved the synthesis of benzoxepinoisoxazolones **4a**–**d**, next we extended this protocol to prepare benzoxepinopyrazolones **6a**–**t**. Condensation of **3a** with phenylhydrazine hydrochloride **5a** in presence of acetic acid in ethanol under reflux conditions provided 2-phenyl-2,10-dihydro-3*H*-benzo[6,7]oxepino[3,4-*c*]pyrazol-3-one **6a** as orange color solid in 80% yield. The reaction was preceded by the formation of phenylhydrazone intermediate followed by in situ cyclization afforded **6a**. Next, the reactions were carried out with substituted benzoxepinecarboxylates **3a**–**d** and phenylhydrazine hydrochlorides **5a**–**k** under optimized conditions to provide series of benzoxepinopyrazolones **6b**–**t** (Scheme 3). Thus synthesized compounds are new and well characterized by spectral data (Supporting Information).

3. Biology

The benzoxepinoisoxazolones **4a**–**d** and pyrazolones **6a**–**t** were screened for their *in vitro* anti-mycobacterial activity against *Mycobacterium smegmatis* ATCC 14468 (MC^2) by broth dilution method [12] and compared with the standard drugs rifampicin and isoniazid. GI_{50} values were calculated (concentration of compounds inhibiting growth by 50%) in triplicate. Further, anticancer activity was also screened for all the synthesized compounds against a panel of three human cancer cell lines such as lung (A549), prostate (DU-145), cervical (HeLa) and non cancerous human embryonic kidney cells HEK 293 by the standard MTT assay method and compared with the standard drug doxorubicin [13].

4. Results and discussion

4.1. Anti-mycobacterial activity of benzoxepinoisoxazolones and pyrazolones

The GI_{50} values for compounds **4a**–**d** and **6a**–**t** were tabulated in Table 1; based on GI_{50} values the structure–activity relationships were discussed below. The compound having phenyl substitution

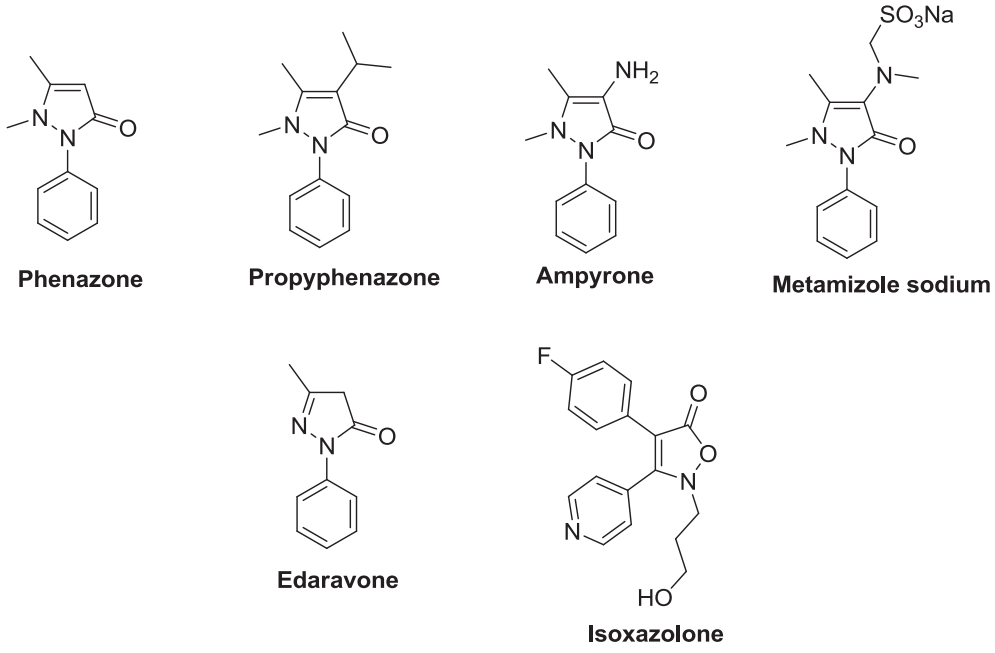

Fig. 1. Structures of isoxazolones and pyrazolones drugs.