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Designing and synthesis of novel antimicrobial heterocyclic analogs of fatty acids

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ABSTRACT

Novel series of long chain isoxazole derivatives were designed as inhibitors of Cytochrome P450-14DM14a-demethylase from *Candida albicans* and ribosomal subunit of S12 protein from *Escherichia coli*. The novel compounds (**6**–**10**) were synthesized through 1,3-dipolar cycloaddition of nitrile oxide to long chain alkynoic acid and alkenyl/hydroxyalkenyl esters and tested for their antimicrobial activity by disk diffusion assay and MIC by broth micro dilution method. After predicting the hidden potential and drug-likeness of compounds, ADMET-related descriptors were also calculated to predict pharmacokinetic properties. Molecular docking studies have been performed to evaluate possible mode of action of molecules in active site of receptor. Compounds (**9** and **10**) showed excellent antimicrobial activity nearly equivalent to the control compounds.

1. Introduction

The enteric bacterial infections are one of the major factors of morbidity in developing countries including Indian sub-continent, part of South America and tropical part of Africa [1]. The common gram-positive pathogens of nosocomial infections are *Staphylococcus aureus* which may also account for outbreaks [2,3]. The world's most common and serious infectious diseases like invasive dysentery and diarrhea are caused by *Escherichia coli* [4,5]. Amoxicillin, norfloxacin and ciprofloxacin are generally used to treat the infections caused by the *E. coli* but they have some side effects [6]. The important role in the treatment failure is played by toxicity and resistance to the drugs also [7]. Several severe diseases may be caused by gram negative and positive bacteria which may lead to huge damage of host tissues [8]. More than 90% of the cases of vaginitis are of candidiasis, trichomoniasis and bacterial vaginosis [9].

Lipophilicity is the key physicochemical parameter linking membrane permeability and hence drug absorption and distribution with the route of clearance (metabolic or renal) [10,11]. The biological activity spectra of these compounds obtained by PASS online (http://www.pharmaexpert.ru/PASSOnline/index.php) estimates the predicted activity spectrum of a compound as probable activity (Pa) and probable inactivity (Pi) [12,13].

Nitrogen and oxygen containing heterocyclic compounds have received considerable attention due to their wide range of biological and pharmacological activities. Among the family of heterocyclic compounds the five membered isoxazoles and isoxazolines/dihydroisoxazoles provide a valuable scaffold in medicinal chemistry as well as a useful synthon in organic synthesis. Isoxazoles and isoxazolines have been reported to possess antitubulin as well as anti-inflammatory activity [14,15]. Dihydroisoxazole derivatives are reported as antinociceptive compounds [16]. In addition, isoxazoline derivatives have played a crucial role in the theoretical development of heterocyclic chemistry and are also used extensively in organic synthesis [17]. Also, medicinal activity like anxiolytic activity has been reported for isoxazole derivatives [18]. So many methodologies exist towards the synthesis of isoxazoles [19,20] and dihydroisoxazoles [21] and most of them endeavor nitrile oxide cycloaddition as a keystep [22]. The addition of a 1,3-dipole to an alkene/alkyne for the synthesis of 5-membered ring is a classic reaction in organic chemistry. The general application of 1,3-dipoles in organic chemistry was first established by Huisgen in

Abbreviations: MR, molar refractivity; MW, molecular weight; MV, molecular volume; PASS, predicted activity spectrum of substances; PSA, polar surface area; SAR, structure–activity relationship; MIC, minimal inhibitory concentration; MRSA, methicillin resistant *Staphylococcus aureus*; Sa, *Staphylococcus aureus*.

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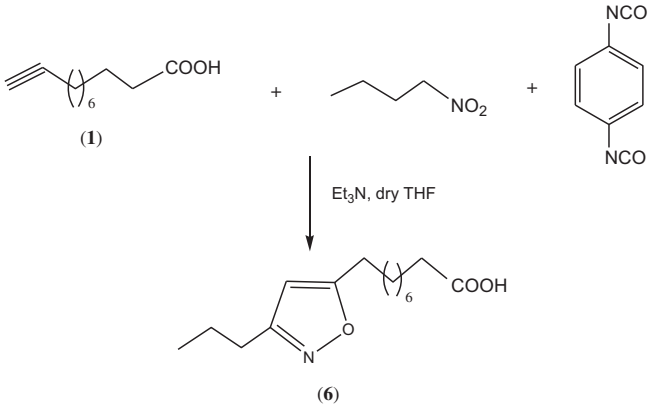
1960s [23]. Isoxazole and isoxazoline heterocycles are derived from the [3 + 2] cycloaddition of a nitrile oxide to an alkene and alkyne, respectively [24]. The nitrile oxide 1,3-dipole is generated in situ in Mukaiyama reaction from the corresponding primary nitroalkane and phenyl isocyanate [25]. Here, nitrile oxide is generated by dehydration of primary nitroalkanes. This method is however the most useful due to its easy set up as described by Mukaiyama in 1960 [25], 1,4-phenylene diisocyanate was used as dehydrating agent [26]. By using 1,4-phenylene diisocyanate, polyurea byproduct was obtained which may be removed from product by simple filtration because this polyurea byproduct was insoluble in the reaction solvent (benzene and tetrahydrofuran). Various biological applications such as antimicrobial [27], pesticidal [28], anticancer [29] and antifungal activities [30] have been reported for seed oils, long chain alkenoic acids and their derivatives. These observations and our interest in the chemistry of heterofatty acids prompted us to synthesize isoxazole derivatives of fatty acids with different substituent at 4- and 5- positions. For this reason, the present strategy for the synthesis of new compounds is aimed in the direction of developing new isoxazole derivatives to inhibit the growth of gram-positive, gram-negative bacteria and most pathogenic fungi. After synthesis the compounds were tested for their antimicrobial activity by disk diffusion assay and MIC by broth micro dilution method against bacterial and fungal strains. Also, molecular docking studies have been performed on Peptide deformylase (PDF) of *E. coli* and CYP 450-14DM of *Candida albicans* to evaluate the possible mode of action of the molecules in the active site of the receptor.

2. Results and discussion

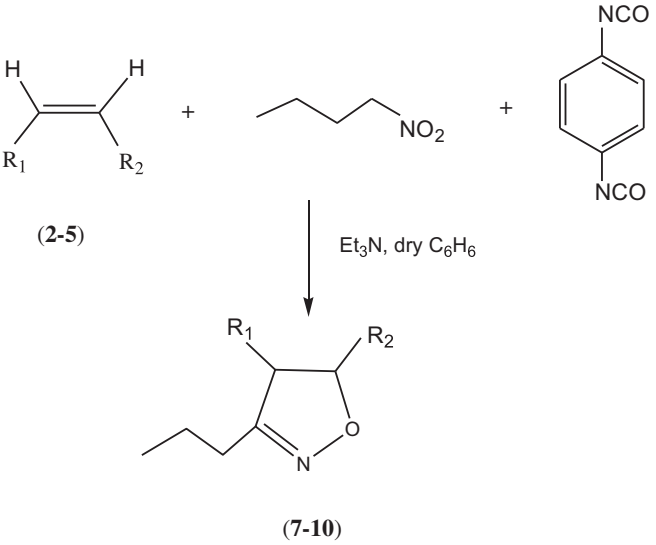
2.1. Chemistry

The long chain alkynoic acid and alkenoates used as the starting materials were prepared from the corresponding long chain alkenoic acids or fatty acids. The long chain alkynoic acid (**1**) was synthesized using the procedure of Kannan et al. [31]. The esters of fatty acids (**2**–**5**) (long chain alkenoates) were prepared by refluxing the fatty acid in methanol in the presence of catalytic amount of sulfuric acid (concentrated). Nitrobutane and 1,4-phenylene diisocyanate were purchased from Sigma Aldrich. Triethylamine, tetrahydrofuran (THF) and benzene were purchased from Merck, Mumbai. Nitrile oxide was generated in situ from nitrobutane employing 1,4-phenylene diisocyanate. The 1,3-dipolar cycloaddition of nitrile oxide to an alekene/alkyne gives an inseparable isomeric mixture of dihydroisoxazole/isoxazole derivatives. This transformation was most effective when excess base (3 equivalents of triethylamine) was employed and nitrobutane added dropwise over 6–8 h while heating. After refluxing, the reaction was quenched with water. The polyurea (polymer) was removed by filtration. Products were purified by column chromatography and identified using different spectral techniques. The signals of products in the ^{1}H and ^{13}C NMR spectra were successfully assigned. The high resolution mass (electron ionization) spectral studies have further confirmed their structures. The reaction sequences are outlined in Schemes 1 and 2.

IR spectrum of compound (**6**), 5-(carboxyoctyl)-3-propylisoxazole, revealed characteristic bands at 3285 (OH stretching), 2918 (CH stretching), 1702 (acid C=O stretching), 1465 (C=N stretching).^{1}H NMR peaks at 12.61 (1H, s, COO*H*), 6.98 (1H, s, C*H* ring), 2.35 (2H, t, J = 7.52 Hz, CH_2COOH), two triplets merged together at δ 2.18 were observed for four CH_2 protons alpha to isoxazole ring, 1.63 (2H, m, CH_2CH_2COOH), 1.54 (2H, m, $CH_2CH_2CH_3$), 1.28 (10H, br.s, CH_2 chain), 0.90 (3H, t, J = 7.21 Hz, CH_3). In ^{13}C NMR peaks at δ 176.18, 167.86, 82.17, 80.80, 44.26, 35.81, 32.68, 29.83, 29.68, 29.39, 29.01, 27.46, 25.61, 22.93, 14.10 were observed. The mass spectra showed characteristic molecular ion peak in accord with the molecular formula.



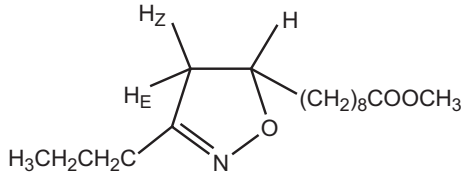
Scheme 1. Synthesis of 3,5-disubstituted isoxazole (**6**).



Compounds	R_1	R_2
2, **7**	H	$(CH_2)_8COOCH_3$
3, **8**	$CH_3(CH_2)_7$	$(CH_2)_7COOCH_3$
4, **9**	$CH_3(CH_2)_5CHOHCH_2$	$(CH_2)_7COOCH_3$
5, **10**	$CH_3(CH_2)_4$	$(CH_2)_2CHOH(CH_2)_7COOCH_3$

Scheme 2. Synthesis of 3,5-disubstituted and 3,4,5-trisubstituted-4,5-dihyroisoxazoles (**7**–**10**).

The structure of (**7**–**10**) is evident from their spectral data. The structure of compound (**7**) is outlined in Scheme 3. Compound (**7**), 5-(carbomethoxyoctyl)-3-propyl-4,5-dihyroisoxazole, showed IR absorption bands at 2931 cm^{-1} (CH stretching), 1740 cm^{-1} (ester C=O stretching), 1457 (C=N stretching). The ^{1}H NMR was more informative in assigning the structure. Diagnostic peaks for cyclic protons were appeared at 4.46 (1H, m, CH_2–*CH* ring), 2.91 (1H, dd,



Scheme 3. Structure of 5-(carbomethoxyoctyl)-3-propyl-4,5-dihydroisoxazole (**7**).