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Synthesis, antimicrobial and anti-inflammatory studies of isoxazole analogues of Rosuvastatin

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ABSTRACT

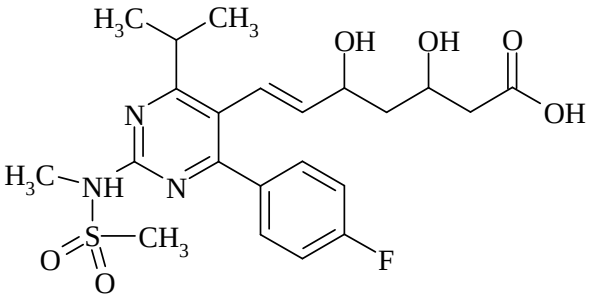
*Novel isoxazole analogues of Rosuvastatin compounds **(14a-j)** were synthesized by the cyclisation of allyl compounds with substituted pyrimidine oxime using chloromine-T in multiple steps. The synthesized compounds were evaluated for their in vitro antmicrobial and anti-inflammatory activity. Some of the newly synthesized compounds showed good antimicrobial and anti-inflammatory activities.*

Keywords: Isoxazole, Rosuvastatin, pyrimidine, antimicrobial, anti-inflammatory.

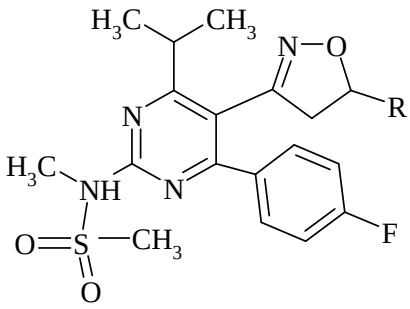
INTRODUCTION

Heterocyclic compounds containing the pyrimidine and isoxazole nucleus possess a diversity of useful biological effects. Rosuvastatin [1] (marketed by AstraZeneca as Crestor) is a member of the drug class of statins, used to treat high cholesterol and related conditions, and to prevent cardiovascular disease. It was reported that the antimicrobial [2] action of Rosuvastatin required high concentrations (>800 mg/l) to achieve a reliable antimicrobial effect**.** In order to increase the antimicrobial activity of Rosuvastatin, we modify the molecule to enhance the antimicrobial activity as shown in Figure 1.

The biological significance of the pyrimidine and isoxazole derivatives has led us to the synthesis of substituted isoxazole-rosuvastation derivatives. As pyrimidine is a basic nucleus in DNA & RNA, it has been found to be associated with diverse biological activities [3].



Rosuvastation



Isoxazole analogues of Rosuvastation

Figure 1. Rosuvastation and isoxazole-rosuvastation analogues

The synthesis of substituted pyrimidine and many detailed reviews have been appeared [4]. Pyrimidines and their derivatives are considered to be important for drugs [5] and agricultural chemicals [6]. Pyrimidine derivatives possess several interesting biological activities such as antimicrobial [7], antitumor [8], antiviral [9], hypnotic sedative [10] and anticonvulsant activities [11]. Many Pyrimidine derivatives are used for thyroid drugs [12] and leukaemia [13]. During the last two decades, several pyrimidine derivatives have been developed as chemotherapeutic [14] agents and have found wide clinical applications such as anti-inflammatory [15], diuretic [16], antimalarial [17] and cardiovascular [18]. Similarly substituted isoxazole are of considerable pharmaceutical interest, which was documented by a steadily increasing number of publications and patents. For instance, isoxazolines were important building blocks in construction of biological active molecules. Substituted isoxazolines have revealed anti-influenza virus [19], antifungal [20], anti-tuberculosis [21], spermicidal and anti-HIV [22], β-adrenergic receptor antagonist properties [23], analgesic and anti-inflammatory [24] properties.

To the best of our knowledge, there have been no previous reports of isoxazole analogous of Rosuvastatin. Herein we reported the synthesis, antimicrobial and in vitro anti-inflammatory activity of novel isoxazole analogues of Rosuvastatin derivatives.

MATERIALS AND METHODS

All chemicals used for the synthesis were of reagent grade and the intermediates prepared as per known literature procedures [25, 26, 27]. ^{1}H and ^{13}C NMR spectra were recorded on AS 400 MHz Varian NMR spectrometer using TMS as an internal standard. IR spectra were recorded by using PerkinElmer Spectrum 100 Series FT-IR spectrometer. Mass spectra were recorded on Agilent 1200 Series LC/MSD VL system. Melting points were determined by using Buchi melting point B-545 instrument and are uncorrected. All the reactions were monitored by thin layer chromatography (TLC) using precoated silica 60 F_{254}, 0.25 mm aluminum plates (Merck). The crude compounds were purified by using CombiFlash® Companion® flash chromatography system, Teledyne Isco, Inc USA using hexane and ethyl acetate as mobile phase and silica gel column.

Preparation of oxime (12). To a solution of compound **11** [25] in methanol was added hydroxylamine hydrochloride and stirred for 5 minutes at 5-10 ^{o}C. Then 1N solution of sodium

hydroxide was added at 5-10 ^{o}C in 10 minutes to the pH~10 and stirred 4 hours. After completion of reaction, the reaction mixture was transferred to ice water and extracted with ethyl acetate (3 X 50 mL). The ethyl acetate layer was dried over anhydrous sodium sulphate and evaporated to get the oxime compound (**12**). White solid; Yield: 73%; m.p: 172 °C; IR(KBr): 3275, 2971, 1592, 1528, 1311, 1172, 810, 525; Mass (ESI) m/z: 367 $(M+H)^+$; ^{1}H NMR (400 MHz, $CDCl_3$): δ 11.24(brs, 1H, =N-OH), 8.47 (s, 1H, = CH), 8.35 – 8.33 (dd, J_{H-F} = 5.2 Hz, J_{H-H} = 8.4 Hz, 2H, Ar-H), 7.62 – 7.58 (dd, J_{H-F} = 8.8 Hz, J_{H-H} = 8.4 Hz, 2H, Ar-H), 3.67 (s, 3H, -N-CH_3), 3.51 (s, 3H, -SO_2CH_3), 3.39 – 3.23 (sep, *J* = 6.8 Hz, 1H, isopropyl –CH), 1.27 (2d, *J* = 6.8 Hz, 6H, isopropyl 2xCH_3).

General method for the preparation of Isoxazole-Rosuvastation analogues (14 a-j). A mixture of oxime (**12**) (10.0 mmol), chloromine-T (11.0 mmol) and potassium carbonate (15.0 mmol) in ethanol (10 mL) were heated to 60 °C for 30 min. Then respective allyl compound (**13a-j**) (8.0 mmol) was added, and the heating was continued for 20-24 h. After completion of the reaction, cooled, diluted with water (50 mL) and extracted with ethyl acetate (3 X 50 mL). After drying with anhydrous sodium sulphate and filtration, the solvent was evaporated to get crude product, which was purified by using flash chromatography eluting with ethyl acetate / hexane (1: 5) to get the corresponding Isoxazole-Rosuvastation analogues (**14 a-j).**

N-(5-(5-cyano-4,5-dihydroisoxazol-3-yl)-4-(4-fluorophenyl)-6-isopropylpyrimidin-2-yl)-N-methylmethanesulfonamide (14a). White solid; Yield: 63%; m.p: 149 °C; IR(KBr): 2975, 2120, 1602, 1549, 1511, 1344, 1154, 802, 575; Mass (ESI) m/z: 418.4 $(M+H)^+$; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.77 - 7.73 (dd, J_{H-F} = 4.8 Hz, J_{H-H} = 8.4 Hz, 2H, Ar-H), 7.25 – 7.20 (dd, J_{H-F} = 8.8 Hz, J_{H-H} = 8.4 Hz, 2H, Ar-H), 5.25 – 5.21 (m, 1H, C-H), 3.61 (s, 3H, -N-CH_3), 3.52 (s, 3H, -SO_2CH_3), 3.34 – 3.29 (sep, *J* = 6.4 Hz, 1H, isopropyl –CH), 3.10 & 2.96 (ABq system, *J* = 17.2 Hz, 2H, CH_2), 1.33 & 1.29 (2d, *J* = 6.4 Hz, 6H, isopropyl 2xCH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ 176.4, 165.5 & 162.9 (J_{C-F} = 250 Hz), 164.0, 159.2, 154.5, 133.21 & 133.18 (J_{C-F} = 3 Hz), 131.2 & 131.1 (J_{C-F} = 10 Hz), 116.6, 116.6 & 116.4 (J_{C-F} = 22 Hz), 111.6, 66.4, 43.9, 42.4, 33.1, 32.6, 22.2, 22.0.

N-(4-(4-fluorophenyl)-5-(5-(hydroxymethyl)-4,5-dihydroisoxazol-3-yl)-6-isopropyl pyrimidin-2-yl)-N-methylmethanesulfonamide (14b). Off white solid; Yield: 60%; m.p: 142 °C; IR(KBr): 3448, 2966, 1726, 1600, 1551, 1349, 1158, 851, 575; Mass (ESI) m/z: 423.5 $(M+H)^+$; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.77 - 7.71 (dd, J_{H-F} = 4.8 Hz, J_{H-H} = 8.2 Hz, 2H, Ar-H), 7.18 – 7.13 (dd, J_{H-F} =9.1 Hz, J_{H-H} = 8.2 Hz, 2H, Ar-H), 4.72 – 4.68 (m, 1H, C-H), 4.08 & 3.75 (ABq system, *J* = 12.0 Hz, 2H, CH_2), 3.60 (s, 3H, -N-CH_3), 3.52 (s, 3H, -SO_2CH_3), 3.32 – 3.29 (sep, *J* = 6.8 Hz, 1H, isopropyl –CH), 2.68 & 2.64 (ABq system, *J* = 16.4 Hz, 2H, CH_2), 1.65 (brs, 1H, OH), 1.31 & 1.26 (2d, *J* = 6.8 Hz, 6H, isopropyl 2xCH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ 178.6, 166.2 & 159.7 (J_{C-F} = 255 Hz), 162.5, 158.5, 135.21 & 133.18 (J_{C-F} = 3.2 Hz), 126.6 & 128.5 (J_{C-F} = 10 Hz), 122.1, 115.2 & 115.0 (J_{C-F} = 22 Hz), 81.8, 63.5, 38.1, 37.8, 35.2 30.2, 21.6, 21.4.

N-(5-(5-(chloromethyl)-4,5-dihydroisoxazol-3-yl)-4-(4-fluorophenyl)-6-isopropyl pyrimidin-2-yl)-N-methylmethanesulfonamide (14c). Off white solid; Yield: 65%; m.p: 132 °C; IR(KBr): 2957, 1624, 1545, 1319, 1175, 864, 524; Mass (ESI) m/z: 441.5 & 443.4 $(M+H)^+$; ^{1}H NMR (400

MHz, $CDCl_3$): δ 7.78 - 7.74 (dd, J_{H-F} = 5.1 Hz, J_{H-H} = 8.2 Hz, 2H, Ar-H), 7.23 – 7.17 (dd, J_{H-F} = 9.1 Hz, J_{H-H} = 8.2 Hz, 2H, Ar-H), 4.89 – 4.83 (m, 1H, C-H), 3.62 (s, 3H, -N-CH_3), 3.59 & 3.48 (ABq system, *J* = 16.4 Hz, 2H, CH_2), 3.55 (s, 3H, -SO_2CH_3), 3.39 – 3.33 (sep, *J* = 6.8 Hz, 1H, isopropyl –CH), 2.80 & 2.67 (ABq system, *J* = 15.8 Hz, 2H, CH_2), 1.32 & 1.27 (2d, *J* = 6.8 Hz, 6H, isopropyl 2xCH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ 176.1, 165.2 & 162.7 (J_{C-F} = 250 Hz), 163.9, 158.8, 133.71 & 133.68 (J_{C-F} = 3.2 Hz), 131.4 & 131.3 (J_{C-F} = 10 Hz), 128.9, 115.9 & 115.7 (J_{C-F} = 21 Hz), 113.7, 79.5, 44.6, 42.3, 41.7, 33.2, 33.1, 22.0.

N-(5-(5-(bromomethyl)-4,5-dihydroisoxazol-3-yl)-4-(4-fluorophenyl)-6-isopropyl pyrimidin-2-yl)-N-methylmethanesulfonamide (14d). Pale brown solid; Yield: 58%; m.p: 167 °C; IR(KBr): 2951, 1629, 1538, 1328, 1121, 878, 598; Mass (ESI) m/z: 485.4 & 487.0 $(M+H)^+$; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.74 - 7.71 (dd, J_{H-F} = 5.4 Hz, J_{H-H} = 8.6 Hz, 2H, Ar-H), 7.19 – 7.15 (dd, J_{H-F} = 9.2 Hz, J_{H-H} = 8.6 Hz, 2H, Ar-H), 4.87 – 4.79 (m, 1H, C-H), 3.60 (s, 3H, -N-CH_3), 3.52 (s, 3H, -SO_2CH_3), 3.44 & 3.24 (ABq system, *J* = 15.8 Hz, 2H, CH_2), 3.36 – 3.32 (sep, *J* = 6.8 Hz, 1H, isopropyl –CH), 2.80 & 2.62 (ABq system, *J* = 16.6 Hz, 2H, CH_2), 1.30 (d, *J* = 6.8 Hz, 6H, isopropyl 2xCH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ 176.1, 165.3 & 162.8 (J_{C-F} = 250 Hz), 163.9, 158.9, 133.71 & 133.68 (J_{C-F} = 3.2 Hz), 131.3 & 131.2 (J_{C-F} = 10 Hz), 129.8, 116.0 & 115.7 (J_{C-F} = 28 Hz), 113.7, 78.4, 42.9, 42.4, 33.1, 32.7, 32.5, 22.2, 22.0.

Methyl 3-(4-(4-fluorophenyl)-6-isopropyl-2-(N-methyl methyl sulfo namido) pyrimidin -5-yl) -4, 5-dihydroisoxazole-5-carboxylate (14e). Off white solid; Yield: 72%; m.p: 151 °C; IR(KBr): 2972, 1725, 1585, 1532, 1372, 1155, 817, 531; Mass (ESI) m/z: 451.1 $(M+H)^+$; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.75 - 7.71 (dd, J_{H-F} = 4.8 Hz, J_{H-H} = 8.1 Hz, 2H, Ar-H), 7.23 – 7.21 (dd, J_{H-F} = 8.8 Hz, J_{H-H} = 8.1 Hz, 2H, Ar-H), 4.85 – 4.79 (m, 1H, C-H), 3.72 (s, 3H, OCH_3), 3.66 (s, 3H, -N-CH_3), 3.51 (s, 3H, -SO_2CH_3), 3.39 – 3.35 (sep, *J* = 6.8 Hz, 1H, isopropyl –CH), 3.27 & 3.22 (ABq system, *J* = 17.1 Hz, 2H, CH_2), 1.20 (d, *J* = 6.8 Hz, 6H, isopropyl 2xCH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ 176.1, 169.8, 168.3, 165.3 & 162.7 (J_{C-F} = 250 Hz), 159.7, 159.4, 133.91 & 133.88 (J_{C-F} = 3.2 Hz), 130.5 & 130.4 (J_{C-F} = 10 Hz), 119.7, 115.3 & 115.1 (J_{C-F} = 22 Hz), 78.2, 51.8, 38.9, 36.5, 36.2, 30.4, 22.3.

Ethyl-3-(4-(4-fluorophenyl)-6-isopropyl-2-(N-methyl methyl sulfo namido) pyrimidin-5-yl)-4,5-dihydroisoxazole-5-carboxylate (14f). White solid; Yield: 55%; m.p: 157 °C; IR(KBr): 2985, 1598, 1515, 1342, 1167, 838, 567; Mass (ESI) m/z: 464.1 $(M+H)^+$; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.71 - 7.65 (dd, J_{H-F} = 5.2 Hz, J_{H-H} = 8.8 Hz, 2H, Ar-H), 7.26 – 7.21 (dd, J_{H-F} = 9.3 Hz, J_{H-H} = 8.8 Hz, 2H, Ar-H), 4.89 – 4.83 (m, 1H, C-H), 4.21 (q, *J* = 7.2 Hz , 2H, -OCH_2), 3.67 (s, 3H, -N-CH_3), 3.51 (s, 3H, -SO_2CH_3), 3.29 – 3.25 (sep, *J* = 6.8 Hz, 1H, isopropyl –CH), 3.19 & 3.13 (ABq system, *J* = 17.5 Hz, 2H, CH_2), 1.27 (t, *J* = 7.2 Hz, 3H, CH_3), 1.22 (d, *J* = 6.8 Hz, 6H, isopropyl 2xCH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ 177.1, 169.9, 165.1 & 162.6 (J_{C-F} = 250 Hz), 159.4, 159.2, 133.81 & 133.78 (J_{C-F} = 3.2 Hz), 130.4 & 130.3 (J_{C-F} = 10 Hz), 119.9, 115.7 & 115.5 (J_{C-F} = 21 Hz), 79.1, 61.6, 38.9, 36.5, 36.1, 30.4, 22.3, 14.3.

N-(4-(4-fluorophenyl)-6-isopropyl-5-(5-phenyl-4,5-dihydroisoxazol-3-yl)pyrimidin-2-yl)-N-methylmethanesulfonamide (14g). Brown solid; Yield: 62%; m.p: 188 °C; IR(KBr): 2964, 1614, 1535, 1511, 1375, 1126, 810, 562; Mass (ESI) m/z: 469.1 $(M+H)^+$; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.75 - 7.71 (dd, J_{H-F} = 5.1 Hz, J_{H-H} = 8.1 Hz, 2H, Ar-H), 7.61 – 7.57 (m, 2H, Ar-H),

7.48 – 7.45 (m, 3H, Ar-H), 7.01 – 6.97 (dd, J_{H-F} = 9.0 Hz, J_{H-H} = 8.1 Hz, 2H, Ar-H), 4.90 – 4.87 (m, 1H, C-H), 3.62 (s, 3H, -N-CH_3), 3.55 (s, 3H, -SO_2CH_3), 3.21 – 3.18 (sep, *J* = 6.8 Hz, 1H, isopropyl –CH), 3.10 & 3.04 (ABq system, *J* = 16.0 Hz, 2H, CH_2), 1.27 (d, *J* = 6.8 Hz, 6H, isopropyl 2xCH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ 176.6, 170.5, 164.9 & 162.4 (J_{C-F} = 250 Hz), 164.2, 160.0, 159.0, 133.48 & 133.45 (J_{C-F} = 3 Hz), 131.8 & 131.7 (J_{C-F} = 10 Hz), 130.9, 126.8, 125.8, 115.4 & 115.2 (J_{C-F} = 20 Hz), 113.6, 82.3, 42.4, 38.9, 36.6, 30.4, 21.9.

N-(4-(4-fluorophenyl)-6-isopropyl-5-(5-p-tolyl-4,5-dihydroisoxazol-3-yl)pyrimidin-2-yl)-N-methylmethanesulfonamide (14h). Pale brown solid; Yield: 68%; m.p: 165 °C; IR(KBr): 2989, 1635, 1521, 1505, 1361, 1175, 885, 591; Mass (ESI) m/z: 483.1 $(M+H)^+$; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.75 - 7.71 (dd, J_{H-F} = 4.8 Hz, J_{H-H} = 8.0 Hz, 2H, Ar-H), 7.54 – 7.49 (dd, J_{H-F} = 8.8 Hz, J_{H-H} = 8.0 Hz, 2H, Ar-H), 7.30 (d, *J* = 8.6 Hz, 2H, Ar-H), 7.21 (d, *J* = 8.6 Hz, 2H, Ar-H), 4.91 – 4.85 (m, 1H, C-H), 3.57 (s, 3H, -N-CH_3), 3.38 (s, 3H, -SO_2CH_3), 3.16 – 3.12 (sep, *J* = 6.8 Hz, 1H, isopropyl –CH), 3.01 & 2.96 (ABq system, *J* = 16.2 Hz, 2H, CH_2), 2.38 (s, 3H, CH_3), 1.24 (d, *J* = 6.8 Hz, 6H, isopropyl 2xCH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ 176.5, 170.0, 165.1 & 162.6 (J_{C-F} = 250 Hz), 163.1, 158.1, 133.25 & 133.22 (J_{C-F} = 3 Hz), 131.7 & 131.6 (J_{C-F} = 10 Hz), 130.1, 129.1, 128.7, 126.1, 119.4, 115.7 & 115.5 (J_{C-F} = 20 Hz), 79.7, 41.0, 38.9, 36.7, 31.2, 22.0, 21.1.

N-(4-(4-fluorophenyl)-6-isopropyl-5-(5-(4-methoxyphenyl)-4,5-dihydroisoxazol-3-yl) pyrimidin-2-yl)-N-methylmethanesulfonamide (14i). Off white solid; Yield: 52%; m.p: 172 °C; IR(KBr): 2971, 1672, 1579, 1525, 1344, 1152, 817, 515; Mass (ESI) m/z: 499.1 $(M+H)^+$; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.77 - 7.73 (dd, J_{H-F} = 4.8 Hz, J_{H-H} = 8.4 Hz, 2H, Ar-H), 7.42 (d, *J* = 8.2 Hz, 2H, Ar-H), 7.25 – 7.21 (dd, J_{H-F} = 8.8 Hz, J_{H-H} = 8.4 Hz, 2H, Ar-H), 6.89 (d, *J* = 8.2 Hz, Ar-H), 4.86 – 4.81 (m, 1H, C-H), 3.80 (s, 3H, -OCH_3), 3.67 (s, 3H, -N-CH_3), 3.56 (s, 3H, -SO_2CH_3), 3.29 – 3.25 (sep, *J* = 6.8 Hz, 1H, isopropyl –CH), 3.17 & 3.08 (ABq system, *J* = 16.4 Hz, 2H, CH_2), 1.25 (d, *J* = 6.8 Hz, 6H, isopropyl 2xCH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ 177.1, 170.1, 165.1 & 162.7 (J_{C-F} = 250 Hz), 164.1, 160.7, 159.4, 133.90 & 133.87 (J_{C-F} = 3 Hz), 131.6, 130.8 & 130.7 (J_{C-F} = 10 Hz), 129.1, 119.1, 115.7 & 115.5 (J_{C-F} = 21 Hz), 113.7, 76.1, 55.2, 40.7, 38.9, 36.2, 30.1, 22.1.

N-(5-(5-(4-chlorophenyl)-4,5-dihydroisoxazol-3-yl)-4-(4-fluorophenyl)-6-isopropyl pyrimidin-2-yl)-N-methylmethanesulfonamide (14j). White solid; Yield: 58%; m.p: 174 °C; IR(KBr): 2971, 1632, 1585, 1524, 1325, 1137, 812, 742, 571; Mass (ESI) m/z: 503.1 : 505.1 $(M+H)^+$; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.79 - 7.75 (dd, J_{H-F} = 5.2 Hz, J_{H-H} = 8.4 Hz, 2H, Ar-H), 7.48 (d, *J* = 8.8 Hz, 2H, Ar-H), 7.22 (d, *J* = 8.8 Hz, Ar-H), 7.12 – 7.08 (dd, J_{H-F} = 9.0 Hz, J_{H-H} = 8.4 Hz, 2H, Ar-H), 4.81 – 4.77 (m, 1H, C-H), 3.61 (s, 3H, -N-CH_3), 3.55 (s, 3H, -SO_2CH_3), 3.31 – 3.27 (sep, *J* = 6.8 Hz, 1H, isopropyl –CH), 3.19 & 3.12 (ABq system, *J* = 16.1 Hz, 2H, CH_2), 1.21 (d, *J* = 6.8 Hz, 6H, isopropyl 2xCH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ 176.9, 171.1, 165.4 & 162.9 (J_{C-F} = 250 Hz), 164.1, 160.7, 133.73 & 133.70 (J_{C-F} = 3 Hz), 132.1, 131.6, 130.6 & 130.4 (J_{C-F} = 10 Hz), 130.5, 128.2, 119.7, 115.2 & 115.0 (J_{C-F} = 21 Hz), 81.4, 40.1, 37.1, 35.2, 31.4, 22.1.

Biological Activity

The antimicrobial activity of newly synthesized compounds **14(a-j)** was determined by well plate method in nutrient agar (antibacterial activity) and Sabouraud dextrose agar (antifungal activity). In this work, *Bacillus subtilis* (MTCC-1789), *Bacillus pumilus* (ATCC-7061), *Escherichia coli* (ATTC-25922), and *Pseudomonas aeruginosa* (ATTC-27853), were used to investigate the antibacterial activities and *Aspergillus niger* (MTCC-1781), *Colletotrichum arachidis* (BCRC-35277) and *Fussarium verticilloids* (FGSC-7600) were used to investigate the antifungal activities. Minimum inhibitory concentration (MIC) of all compounds was determined, which is defined as the lowest concentration of inhibitor at which bacterial growth was not visually apparent.

Investigation on antibacterial screening data (Table 1) showed some of the compounds were active against four human pathogenic bacteria. The results of antimicrobial activity of newly synthesized compounds **14(a-j)** reveals that out of ten compounds, six compounds were found to have good antibacterial activity and only five compounds showed good antifungal activity. Among these compounds the **14a, 14b, 14c, 14d, 14e and 14j** were active against the bacterial strains. The four compounds **14a, 14b, 14c, 14d** were more active against the gram +ve bacterial strains *Bacillus subtilis, Bacillus Pumilus,* where as the compounds **14e** and **14j** were found to be active against the and *Pseudomonas aeruginosa.* The compound **14f** showed moderate activity against all organisms.

From the antifungal activity data it was clear that among the ten tested compounds only three compounds **14a, 14b** and **14d** showed significant antifungal activity against all three fungal strains and the compounds **14c** and **14f** exhibited moderate activity against all three fungal strains**.**

Table 1. Minimum inhibitory concentration data for the compounds 14(a-j)

Compounds Code	Minimum Inhibitory concentration (MIC) in µg / ml						
	Antibacterial activity				Antifungal activity		
	B.subtilis	**B.pumilis**	**E.coli**	**P.aeruginosa**	***A.nigier***	***C.arachidis***	***F.verticilloids***
14a	12.5	25	50	12.5	12.5	12.5	25
14b	12.5	12.5	25	12.5	12.5	12.5	12.5
14c	12.5	25	25	50	12.5	50	50
14d	12.5	25	50	12.5	12.5	12.5	25
14e	25	12.5	>100	12.5	50	100	50
14f	50	50	50	50	25	50	25
14g	>100	>100	>100	>100	>100	>100	>100
14h	>100	25	>100	100	50	>100	100
14i	>100	>100	100	>100	12.5	50	100
14j	50	25	25	12.5	12.5	>100	>100
Ciprofloxacin	6.25	6.25	6.25	6.25	---	---	---
Clotrimazole	---	---	---	---	6.25	6.25	6.25
DMSO	---	---	---	---	---	---	---

2.2. Anti-inflammatory activity:

Anti-inflammatory activity by Carrageenan induced rat paw edema method

Anti-inflammatory activity was assessed by the method described by Winter *et al* [28]. Albino rats of either sex weighing 200 – 250 g were divided in 14 groups (N=6). Group-1 received 2%

acacia gum suspension (control), Group- 2 received 0.1 ml of 1% carrageenan suspension in normal saline (Toxicant control), Group- 3 received Ibuprofen (reference standard 40 mg/kg, P.O) and group 4 to 12 were given the compounds **14(a-j)** (200 mg/kg Paw Oedema) in 2% acacia gum suspension. The standard Ibuprofen and synthesized compounds under study were administered orally to all rats. After 30 minutes 0.1 ml of 1% carrageenan suspension in normal saline was injected into the sub plantar region of the left hind paw of each rat to induce edema. The edema volumes of the injected paw were measured at 1st, 2nd, 3rd and 4th hour. The difference between the paw volumes of treated animals were compared with that of the control group and the mean edema volume was calculated. From the data obtained mean volume of oedema ± SEM and percentage reduction in edema were calculated. Percentage reduction or inhibition in edema volume was calculated by using the formula.

Percentage reduction in edema volume was calculated by using the formula,

$$\text{Percentage reduction} = \frac{V_0 - Vt}{V_0} \times 100$$

Where

V_0 = Volume of the paw of control at time t

V_t = Volume of the paw of drug treated at time t

From the data obtained the mean edema volume and percentage reduction in edema was calculated and the results were summarized in the Table-2.

Statistical analysis

Data analysis was carried out using one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison tests. $P < 0.05$ was considered statistically significant.

The results of carrageenan induced rat paw oedema model indicated that all the synthesized compounds showed moderate to good anti-inflammatory activity. Out of all the synthesized compounds **14a, 14b, 14c** and **14e** showed highly significant good anti-inflammatory activity, whereas the compounds **14d** and **14h** showed moderate activity when compared with that of standard ibuprofen.

Table 3: Anti-inflammatory activity of synthesized compounds (14a-j) in carrageenan induced paw oedema model in Albino rats.

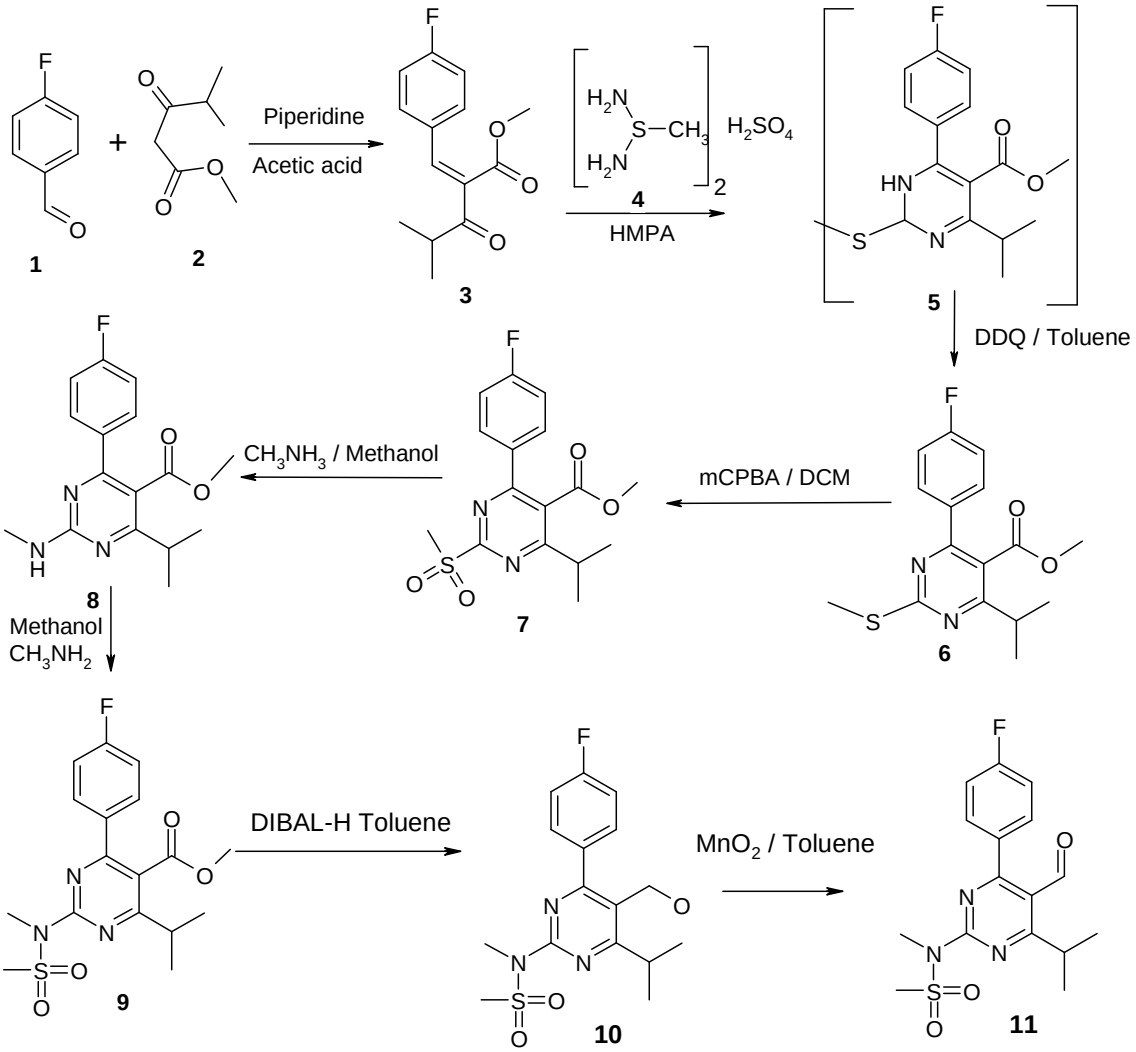
Group	Treatment	Dose mg/kg	Oedema volume and percentage reduction in oedema volume at							
			1 h		2 h		3 h		4 h	
			Mean ± SEM	% ROV	Mean ± SEM	% ROV	Mean ± SEM	% ROV	Mean ± SEM	% ROV
1	Toxicant control (carrageenan)	0.1 ml (1%w/v)	1.18 ±0.05 ns	-	1.22±0.03 ns	-	1.27±0.05*	-	1.40±0.02**	-
2	Standard Ibuprofen.	40	0.96±0.02*	18.64	1.12±0.10 ns	8.91	0.90±0.04**	29.13	0.87±0.01**	39.28
3	**14a**	200	1.06±0.05 ns	10.16	1.05±0.05 ns	13.93	1.00±0.03*	22.25	0.95±0.03**	33.13
4	**14b**	200	1.20±0.04 ns	16.94	1.20±0.04 ns	1.63	1.18±0.05 ns	8.08	0.98±0.08**	31.00
5	**14c**	200	1.08± 0.08 ns	8.47	1.06±0.06 ns	13.11	1.05±0.03*	17.32	0.94±0.04**	32.40
6	**14d**	200	1.23±0.06 ns	4.23	1.12±0.06 ns	8.91	1.15±0.06 ns	10.43	0.99±0.03**	30.28
7	**14e**	200	1.06±0.05 ns	10.16	1.05±0.05 ns	13.93	1.00±0.03*	21.25	0.95±0.03**	32.13
8	**14f**	200	1.16±0.06 ns	1.69	1.13±0.05 ns	7.37	1.05±0.05 ns	17.32	0.99±0.03**	29.28
9	**14g**	200	1.10±0.03 ns	6.77	1.09±0.08 ns	10.65	1.00±0.03*	21.25	1.00±0.01**	28.58
10	**14h**	200	1.20±0.04 ns	16.94	1.20±0.04 ns	1.63	1.18±0.05 ns	7.08	0.98±0.08**	30.00
11	**14i**	200	1.16±0.06 ns	1.69	1.13±0.05 ns	7.37	1.05±0.05 ns	17.32	0.99±0.03**	29.88
12	**14j**	200	1.10±0.03 ns	6.77	1.09±0.08 ns	10.65	1.00±0.03*	21.25	1.00±0.01**	28.78

Animal: Albino rats, Route: p.o.

n=6 ns (non significant) significant at P<0.05 and 0.01** Toxicant control compared with normal control. Standard and Synthesized compounds compared with toxicant control. ROV – Reduction in paw oedema (p.o) volume*

RESULTS AND DISCUSSION

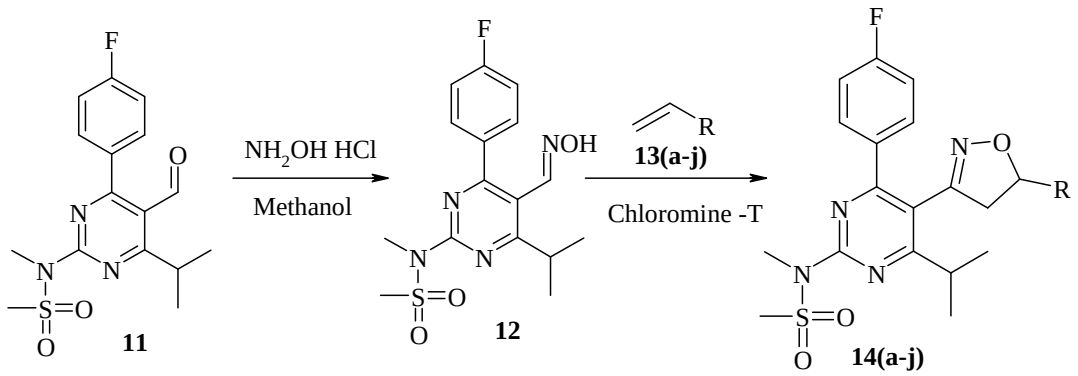
The intermediate for the synthesis of novel isoxazole-rosuvastation analogue is compound **11** which was prepared by the known literature as per the Scheme 1 [25, 26, 27].



Scheme 1. Synthetic method for the preparation of intermediate compound 11.

The aldoxime **12** was prepared by the reaction with hydroxyl amine hydrochloride with corresponding aldehyde in the presence of base.

The oxime (**12**) thus formed undergoes 1,3 dipolar cyclization with corresponding allyl compounds **13(a-j)** via nitrile oxide in presence of chloromine-T, ethanol and potassium carbonate as base gave desired compounds **14(a-j)** (Scheme 2). The novel compounds were characterized by their spectral data like Mass, IR and NMR.



R = **13a, 14a** = CN; **13b, 14b** = CH_2OH; **13c, 14c** = CH_2Cl
13d, 14d = CH_2Br; **13e, 14e** = $COOCH_3$; **13f, 14f** = $COOC_2H_5$
13g, 14g = C_6H_5; **13h,14h** = 4-CH_3-C_6H_5; **13i, 14i** = 4-OCH_3-C_6H_5
13j, 14j = 4-Cl-C_6H_5

Scheme 2. Synthetic method for the preparation of isoxazole-rosuvastation analogues 14(a-j)

Antimicrobial activity

All the synthesized isoxazole-rosuvastatin analogue compounds **14(a-j)** were evaluated for their *in vitro* antimicrobial activity against *Bacillus subtilis* (MTCC-1789), *Bacillus pumilus* (ATCC-7061), *Escherichia coli* (ATTC-25922), and *Pseudomonas aeruginosa* (ATTC-27853), and antifungal activity against *Aspergillus niger* (MTCC-1781), *Colletotrichum arachidis* (BCRC-35277) and *Fussarium verticilloids* (FGSC-7600). Ciprofloxacin and Clotrimazole were used as standard drugs for bacteria and fungi respectively. Preliminary screening for the test compound and standard drugs were performed at fixed concentrations of 400 µg / mL. Inhibition was recorded by measuring the diameter of the inhibition zone at the end of 24 h for bacteria and 72 h for fungi. Each experiment was repeated twice. Based on the results of zone of inhibition, the minimum inhibitory concentration (MIC) of potent compounds **14(a-j)** against all bacterial and fungal strains was determined by two fold dilution method [29]. Stock solutions of tested compounds with 400, 200, 100, 50, 25, 12.5 and 6.25 µg/mL concentrations were prepared with DMSO as solvent. Inoculums of the bacterial and fungal culture were also prepared. To a series of tubes containing 1 mL each of test compound solution with different concentrations and 0.2 mL of the inoculums was added. Further 3.8 mL of the sterile water was added to each of the test tubes. These test tubes were incubated for 24 h at 37 °C and observed for the presence of turbidity. This method was repeated by changing test compounds with standard drugs Ciprofloxacin and Clotrimazole for comparison. The minimum inhibitory concentration at which no growth was observed was taken as the MIC values. The comparison of the MICs (in µg/mL) of potent compounds and standard drugs against tested strains are presented in Table 1. Similarly the MIC for antifungal activity was determined using 72 h old broth culture. The results were compared with Clotrimazole and summarized in Table 1.

CONCLUSION

In conclusion, a series of novel isoxazole-rosuvastatin analogues (**14a-j**) were synthesized and their antimicrobial and anti inflammatory activities were evaluated. The anti microbial screening

suggests that all the newly synthesized compounds showed moderate to good activity against the tested organisms. Among the newly synthesized compounds, **14a, 14b, 14c, 14d, 14e** and **14j** showed the most promising antibacterial activity and the compounds, **14a, 14b** and **14d** showed promising antifungal activity. Whereas the anti inflammatory activity data suggest that the newly synthesized compounds showed moderate to equipotent anti inflammatory activity when compared to standard employed for the study. The compounds **14a, 14b, 14c** and **14e** showed good activity, whereas the compounds **14d** and **14h** showed moderate activity. Hence the fact that the compounds prepared in this study were chemically unrelated to the current medication, suggests that further work with similar analogues is clearly warranted.

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