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Synthesis of novel β-lactam fused spiroisoxazolidine chromanones and tetralones as potent antimicrobial agent for human and plant pathogens

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ABSTRACT

Synthesis of novel β-lactam fused spiroisoxazolidine chromanones and tetralones ring systems has been achieved by intermolecular 1,3-dipolar cycloaddition reaction of bicyclic nitrone with unusual dipolarophiles, arylidene chromanones/tetralones under different reaction conditions. The synthesized compounds were evaluated for antimicrobial activities. It was observed that two of the synthesized compounds exhibited relatively good antibacterial and antifungal activities.

β-Lactams form a class of antibiotics characterized by the presence of an azetidine-2-one ring, which is the core structure responsible for biological activity.[1] The β-lactam ring system is a common structural feature found in a number of broad spectrum β-lactam antibiotics, like penicillins,[2] cephalosporins,[3] carbapenems, nocardicins and monobactams, which have been widely used as chemotherapeutic agents for treating microbial diseases.[4] It also shows many other interesting biological properties, such as cholesterol absorption inhibitors,[5] human cytomegalovirus protease inhibitors,[6] thrombin inhibitors,[7] anti-hyperglycemic,[8] anti-tumor,[9] anti-HIV,[10] anti-inflammatory, analgesic activities,[11] and serine-dependent enzyme inhibitors.[12,13] However, microorganisms have built up resistance against the most traditional β-lactam antibiotics due to excess use of antibiotics. Therefore there arises a need to modify the structure of known active compounds and the development of new ones.

Functionalized isoxazolidine derivatives are known to posses antifungal,[14] anti-inflammatory,[15] antiviral,[16] herbicidal[17–19] properties. In particular, modified isoxazolidines have been proved to efficiently inhibit in vitro and in vivo virus infections caused by HIV, HBV, and HTLV-1.[20,21] Spiro compounds represent an important class of naturally occurring substances characterized by highly pronounced biological properties.[22] In particular, spiroisoxazolidine derivatives showed anti-mycobacterial and anti-invasive activitives.[23,24] Furthermore, spiroisoxazolidine nucleoside is reported to possess anti-HIV activity[25,26] (Fig. 1). The derivatives of chromanone display a wide range of biological application such as, antioxidant activity[27] and have significant activity against anti-human immuno deficiency virus type HIV.[28] Likewise, tetralone derivatives have shown considerable bioactivity and have been utilized for the synthesis of benzophenanthridine antitumor alkaloids and ring B of tetracyclins.[29]

Our research group has been largely involved in the synthesis of β-lactam, chromanone, tetralone and pyrrolidine substituted spiroheterocycles[30–34] by 1,3-dipolar cycloaddition reaction which are found in many naturally occurring alkaloids with significant biological activity. Recently, we reported[35] the synthesis of β-lactam substituted spiropyrrolidine and pyrrolizidine derivatives through 1,3-dipolar cycloaddition and macrocyclic bis β-lactam derivatives by [2+2] cycloaddition reaction.[36]

In continuation of our research in the area of 1,3-dipolar cycloaddition reaction and in view of the importance of isoxazolidines and β-lactam derivatives in medicinal chemistry, we focused our attention in synthesizing isoxazolidines by intermolecular [3+2] cycloaddition reaction of β-lactam fused bicyclic nitrones with

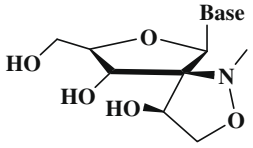

Figure 1. Representation of spiroisoxazolidine compound.

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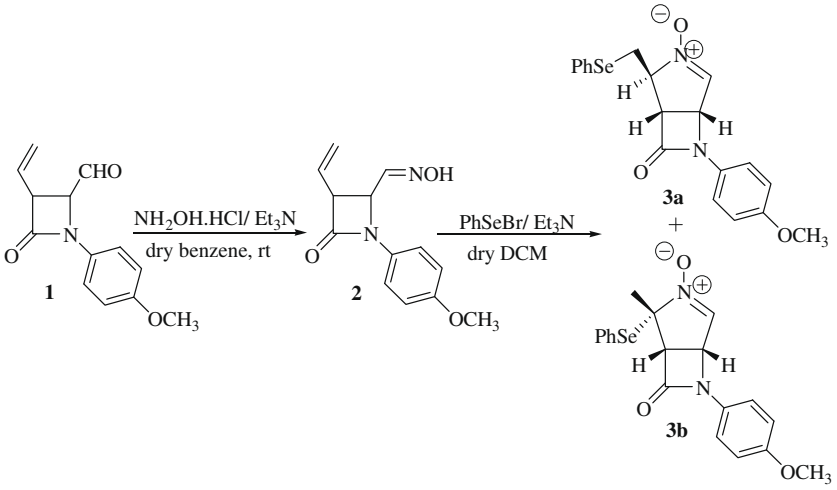


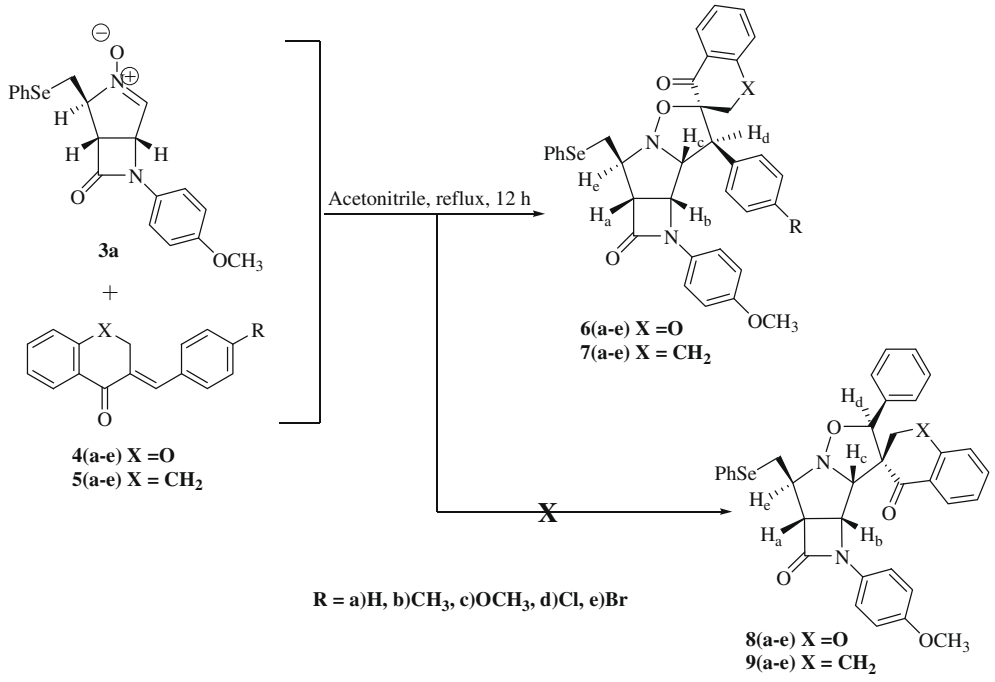
Scheme 1.

various substituted arylidene chromanones/tetralones. Although there are reports available for the synthesis of substituted isoxazolidine, there seems to be no reports for the synthesis of β-lactam fused spiroisoxazolidine having chromanone/tetralones skeleton. Because of their remarkable activities, significant efforts have been devoted to the synthesis of spiroisoxazolidines.

The bicyclic nitrone **3** was prepared,[37] as regioisomeric mixture (**3a** and **3b**) in good yields, by the reaction of vinyl β-lactam aldehyde **1** with hydroxylamine hydrochloride in the presence of triethylamine to afford oxime **2**. which was then treated with phenyl selenyl bromide in dry dichloromethane at room temperature followed by the addition of triethylamine to give the bicyclic nitrones **3a** and **3b** (67:33). They were easily separated by flash column chromatography (Scheme 1).

The required dipolarophiles, arylidene chromanones and arylidene tetralones were prepared as per the literature procedures.[38,39] When the bicyclic nitrone **3a** was reacted with various substituted (*E*)-3-arylidene chroman-4-ones **4a–e** in acetonitrile under reflux conditions afforded β-lactam fused spiroisoxazolidine chromanones (**6a–e**) with moderate yields (45–55%) (Scheme 2).

The structure and the regiochemistry of the cycloadduct was confirmed by spectral and elemental analysis.[40] The ^{1}H NMR spectrum of **6a**, exhibited a doublet at δ 4.81 (J = 8.1 Hz) for the benzylic proton. If the other isomer **8a** was formed, one would expect a singlet instead of a doublet. H_a and H_b protons showed triplets at δ 3.80 (J = 5.4 Hz) and δ 4.74 (J = 5.4 Hz), the H_c proton showed a doublet of doublet in the region δ 4.47–4.50 (J = 5.4 Hz, 8 Hz) and multiplet for H_e proton appeared in the region δ 4.28–4.33. The ^{13}C NMR spectrum of **6a** exhibited a peak at 164.2 ppm due to β-lactam carbonyl carbon and a peak at 186.4 ppm is due to chromanone carbonyl carbon. The spirocarbon showed a peak at δ 84.6 ppm. Identical results were obtained for compounds **6b–e**. The stereochemistry of the cycloadduct **6a** was further unambiguously corroborated by an X-ray (Fig. 2) single crystal analysis,[41] which also proved the regiochemistry of the cycloadduct.



Scheme 2.

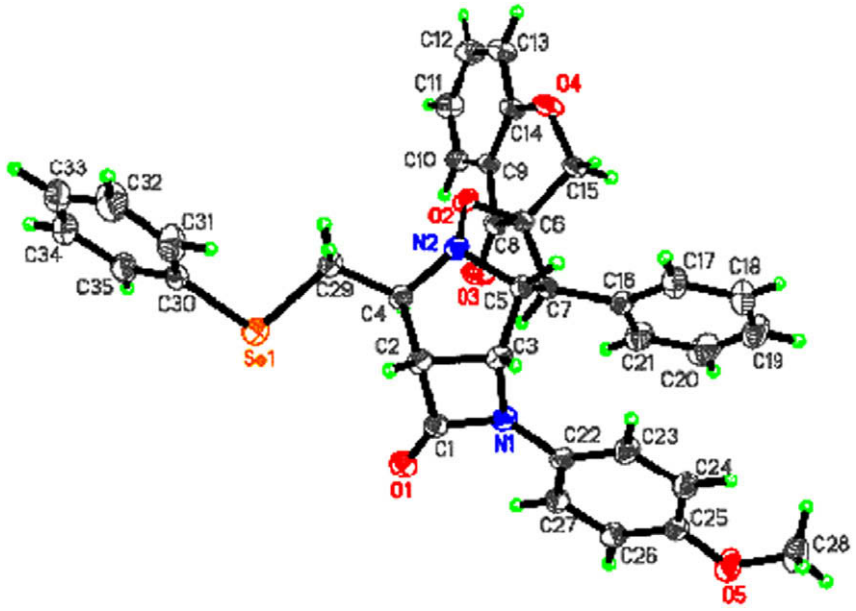


Figure 2. ORTEP diagram of **6a**.

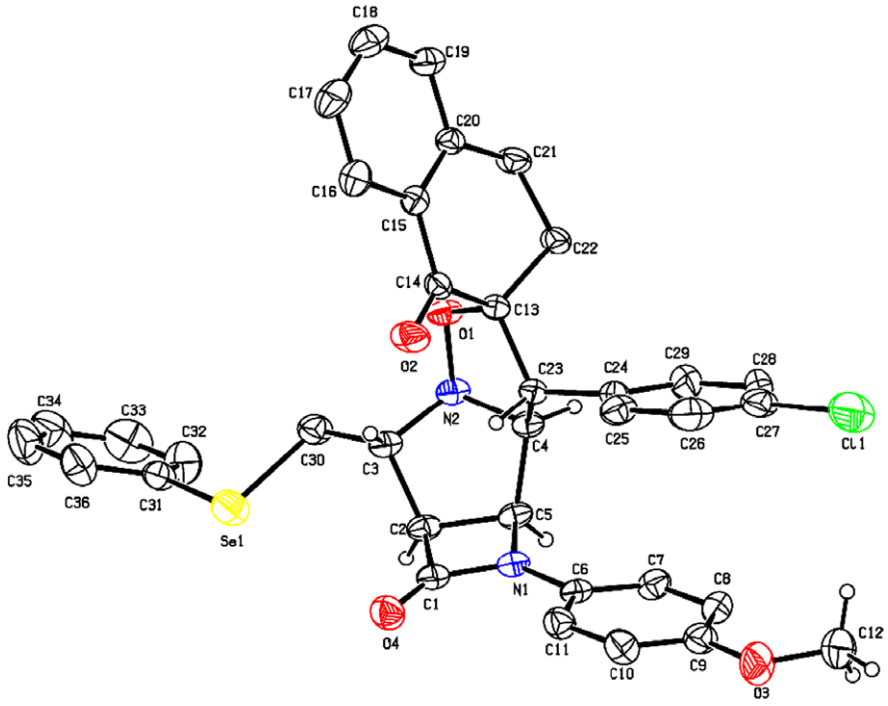


Figure 3. ORTEP diagram of **7d**.

Table 1
Synthesis of spiroisoxazolidine chromanones/tetralones (**6a–e**) and (**7a–e**)

Entry	Compound	R	Conventional heating (4 h) Yield (%)	Microwave heating in CH_3 CN (10 min) Yield (%)
1	**6a**	H	60	89
2	**6b**	Me	62	85
3	**6c**	OMe	55	88
4	**6d**	Cl	65	92
5	**6e**	Br	64	94
6	**7**°	H	62	90
7	**7b**	Me	60	82
8	**7c**	OMe	56	86
9	**7d**	Cl	64	92
10	**7e**	Br	63	93

In the molecular structure of **6a**, the isoxazolidine ring is in a twist conformation and the pyrrolidine ring adopts an envelope conformation. The tetrahydropyran ring adopts a half-chair conformation. In the crystal structure, intermolecular C–H···O interactions link the molecules into a two-dimensional network.

The same reaction was carried out with (*E*)-3-arylidene tetralones (**5a–e**) to obtain β-lactam fused spiroisoxazolidine tetralones (**7a–e**) in moderate yields (45–55%). The structure of the cycloadducts was confirmed by spectroscopic techniques and by single crystal X-ray analysis of **7d**.[42] (Fig. 3).

To improve the yield of the products, we have carried out the reaction in various organic solvents. We found that using methanol and benzene did not give fruitful result and gave a mixture of inseparable byproducts. The cycloaddition with other solvents like

Table 2
Effect of spiroisoxazolidine chromanones/tetralones (**6a–e**) and (**7a–e**) on the growth of human pathogens

Compound	Minimum inhibitory concentration[a] (MIC) (μg/ml)			
	Proteus vulgaris	*Proteus mirabilis*	*Streptococcus aureus*	*Salmonella typhi*
6a	30	55	45	20
6b	40	65	45	35
6c	15	25	40	45
6d	10	20	20	25
6e	25	30	30	45
7	40	55	25	45
7b	45	50	40	35
7c	35	50	45	40
7d	45	40	35	30
7e	35	50	45	35
Tetracycline	15	20	10	15

[a] The values are means of triplicate with ±SD.

Table 3
Effect of spiroisoxazolidine chromanones/tetralones (**6a–e**) and (**7a–e**) on mycelial growth of plant fungal pathogens

Fungal pathogens	Minimum inhibitory concentration (MIC)[a] Compound (μg/ml)										
	6a	**6b**	**6c**	**6d**	**6e**	**7a**	**7b**	**7c**	**7d**	**7e**	Carbendazim
Fusarium oxysporum	125	150	125	75	150	125	75	125	50	150	18
Macrophomena phaseolina	50	75	75	50	75	75	25	50	25	75	15

[a] The values are means of triplicate with ±SD.

dioxane and toluene under the same reaction condition did not give the expected products. However, when the above reaction was carried out in acetonitrile under microwave irradiation (600 W), we found that the products were obtained in good yields. (82–96%) (Table 1).

In the present antimicrobial study, minimum inhibitory concentration[43] of 10 different newly synthesized spiroisoxazolidines were evaluated against four human pathogens. *Proteus vulgaris, Proteus mirabilis, Staphylococcus aureus*, *Salmonella typhi*, by well diffusion method[44] and two plant fungal pathogens *Fusarium oxysporum*, *Macrophomena phaseolina*, by poison food technique.[45] The compounds tetracycline and carbendazim at the concentration range 5–200 μg/ml in 0.25% DMSO were used as control for bacteria and fungi respectively.

*Effect of spiroisoxazolidine chromanones/tetralones (**6a–e**) and (**7a–e**) on the growth of human pathogens*: The MIC of the tested compounds (**6a–e**) and (**7a–e**) against bacterial pathogen ranged from 10 to 65 μg/ml (Table 2). Compound **6d** showed higher activity against *P. vulgaris* than the standard drug tetracycline, equally active against *P. mirabilis* and is not much active than the standard drug with respect to microorganisms, *S. aureus* and *S. typhi*. The minimum inhibitory concentration of the compound **6d** was found to be 10–25 μg/ml followed by **6c** and **6e** with 15–45 μg/ml and 25–45 μg/ml and control having a MIC value of 10–20 μg/ml. The MIC values are relatively high for **6a**, **6b**, **7a**, **7b**, **7c**, **7d**, and **7e** as compared to control. However, for the compound **6d**, its activity against *P. mirabilis* was equal to that of tetracycline.

*Effect of spiroisoxazolidine chromanones/tetralones (**6a–e**) and (**7a–e**) on the mycelial of plant fungal pathogens*: The MIC of the tested compounds (**6a–e**) and (**7a–e**) against plant fungal pathogens ranged from 25 to 150 μg/ml (Table 3). The synthesized compound **7d** was effective in controlling both fungal pathogens namely *F. oxysporum* and *M. phaseolina* with MIC values of 50 and 25 μg/ml, respectively, followed by **6d** (75 and 50 μg/ml) and **7b** (75 and 25 μg/ml). Compounds **6a**, **6b**, **6c**, **6e**, **7a**, **7c**, and **7e** inhibited mycelial growth at higher concentration (50–150 μg/ml) compared to control, carbendazim.

In conclusion, for the first time, we have successfully synthesized a series of novel β-lactam fused spiroisoxazolidine chromanones and tetralones by intermolecular 1,3-dipolar cycloaddition. The microwave irradiation method was found to be synthetically useful in achieving high yields of the products with reduced reaction time compared to conventional heating. The compounds **6a–e** and **7a–e** exhibited good antibacterial and antifungal activity. In particular, compound **6d** showed significant activity against some tested pathogens. Similarly it has been observed that **7d** exhibited good antifungal activity against used fungal pathogens.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.04.084.

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40. *General procedure for the synthesis of spiroisoxazolidine chromanones/tetralones* **6a–e** and **7a–e**: To a solution of bicyclic nitrone (**3a**) (200 mg, 0.5 mmol) in dry acetonitrile (20 ml) was added the arylidene chromanones (**4a–e**) (117 mg, 0.5 mmol) or arylidene tetralones (**5a–e**) (118 mg, 0.5 mmol) at room temperature. The reaction mixture was refluxed for 12 h and after completion of the reaction, the solvent was distilled off under reduced pressure and the crude product was purified by column chromatography using hexane/ethyl acetate mixture (8:2) as an eluent. The product was crystallized from ethyl acetate by slow evaporation method.
Hexahydro-3-(4-chlorophenyl)-6-(phenylselenylmethyl)-pyrrolo-[1,2-b]-isoxazol-1′-(4-methoxyphenyl)-[3′,4′-a]-azetidin-2′-one-spiro[4.2″] chromanone (**6d**): white fluffy solid, mp 196 °C; ν_{max} (KBr) 1746, 1680 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 3.06–3.11 (m, 1H); 3.15–3.20 (m, 1H); 3.47 (d, 1H, J = 12.6 Hz); 3.67 (s, 3H); 3.73 (t, 1H, J = 5.4 Hz); 4.20–4.25 (m, 1H); 4.36–4.40 (dd, 1H, J = 5.4, 8.0 Hz); 4.43 (d, 1H, J = 12.6 Hz); 4.65 (t, 1H, J = 5.4 Hz); 4.66 (d, 1H, J = 8.0 Hz); 6.54–7.70 (m, 17H, Ar-H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 30.9, 49.2, 55.4, 55.9, 58.8, 62.7, 70.6, 70.8, 84.7, 113.9, 117.8, 119.8, 121.5, 127.0, 128.0, 128.8, 129.2, 129.3, 129.9, 131.0, 132.1, 132.4, 133.7, 136.5, 156.1, 160.8, 163.9, 186.3; EI-MS m/z 672.09 (M^+). Anal. Calcd for $C_{35}H_{29}ClN_2O_5Se$: C, 62.55; H, 4.35; N, 4.1. Found: C, 62.67; H, 4.40; N, 4.19.
Hexahydro-3-(4-chlorophenyl)-6-(phenylselenylmethyl)-pyrrolo-[1,2-b]-isoxazol-1′-(4-methoxyphenyl)-[3′,4′-a]-azetidin-2′-one-spiro[4.2″]tetralone (**7d**): colorless crystals, mp 185 °C; ν_{max} (KBr) 1748, 1681 cm^{-1};^1H NMR (400 MHz, $CDCl_3$): δ 2.23–2.27 (m, 1H); 2.56–2.61 (m, 1H); 3.12 (d, 2H); 3.28–3.35 (m, 1H); 3.71 (s, 3H); 3.72 (t, 1H, J = 5.4 Hz); 3.73–3.79 (m, 1H); 4.12–4.18 (m, 1H); 4.47–4.51 (dd, 1H, J = 5.4, 8.0 Hz); 4.69 (t, 1H, J = 5.4 Hz); 4.95 (d, 1H, J = 8.0 Hz); 6.53–7.92 (m, 17H, Ar-H). ^{13}C (100 MHz, $CDCl_3$): δ 23.8, 28.9, 30.6, 31.2, 48.6, 54.7, 57.3, 61.7, 69.5, 88.4, 113.2, 117.2, 125.9, 126.2, 127.7, 127.8, 127.9, 128.4, 129.2, 129.5, 130.3, 130.6, 131.9, 132.3, 133.1, 143.1, 155.4, 163.2, 191.4; EI-MS m/z 670.1 (M^+). Anal. Calcd for $C_{36}H_{31}ClN_2O_4Se$: C, 64.53; H, 4.66; N, 4.18. Found: C, 64.61, H, 4.77, N, 4.25.
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