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Synthesis and antinociceptive activity of pyrazolyl isoxazolines and pyrazolyl isoxazoles

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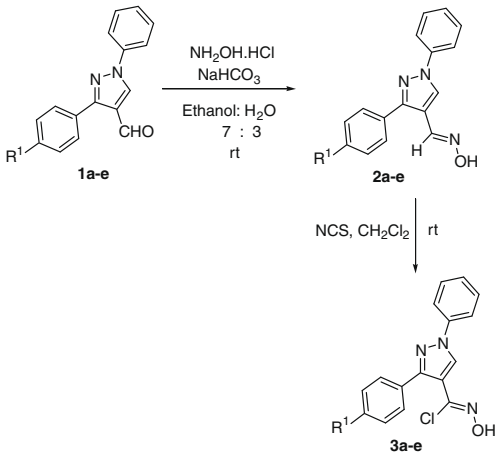
ABSTRACT

Pyrazolyl isoxazolines and isoxazoles were synthesised in moderate to good yields using 1,3-dipolar cycloaddition of pyrazole derived nitrile oxide with various dipolarophiles such as N-substituted maleimide, diethylacetylene dicarboxylate and phenylacetylene. The synthesized compounds were evaluated for antinociceptive activities. The 3-pyrazolyl-4,5-dicarbethoxy isoxazoles (**9a**–**c**) exhibited the maximum antinociceptive activity.

1,3-Dipolar cycloaddition is a versatile synthetic strategy for the construction of five-membered heterocycles.[1] The 1,3-dipolar cycloaddition of nitrile oxides to alkenes and alkynes affords isoxazolines and isoxazoles respectively.[2,3] Isoxazolines (4,5-dihydroisoxazoles) are versatile intermediates in the synthesis of various natural products.[4] The reductive cleavage of isoxazoline ring can lead to many synthetically important compounds, such as β-hydroxy ketones, α,β-unsaturated ketones or γ-amino alcohols.[5] The general and most widely used method for the synthesis of isoxazolines is the 1,3-dipolar cycloaddition of nitrile oxides to activated double bond of alkenes; aliphatic nitrile oxides are predominantly generated in situ from primary nitro compounds in a Mukaiyama reaction,[6] while their aromatic counterparts are prepared by dehydrohalogenation of hydroximoyl chlorides.[7] In most cases, 1,3-dipolar cycloadditions of nitrile oxides to alkenes proceed with high regioselectivity and the relative configuration on the 4- and 5-carbon atoms of the isoxazoline ring depends on the geometry of the alkene.

Pain is widely accepted to be one of the most important determinations of quality of life. A study reported by the World Health Organization demonstrated that individuals who live with persistent pain suffer fourfold more from depression (or) anxiety compared to healthy subjects.[8] The identification of compounds able to treat both acute and chronic pain with limited effects is one of the prominent goals in biomedical research.

Isoxazolines exhibit important biological activities such as antibacterial,[9] antiplatelet,[10] antiviral,[11] anticonvulsant,[12] immunostimulatory[13] and antinociceptive.[14,15] Pyrazole nucleus has pronounced pharmacological applications as anti-anxiety,[16,17] antipyretic, antinociceptive[18] and anti-inflammatory drugs.[19–21] Certain alkyl pyrazoles show significant bacteriostatic, bactericidal



Scheme 1. Preparation of pyrazolyl hydroximoyl chlorides.

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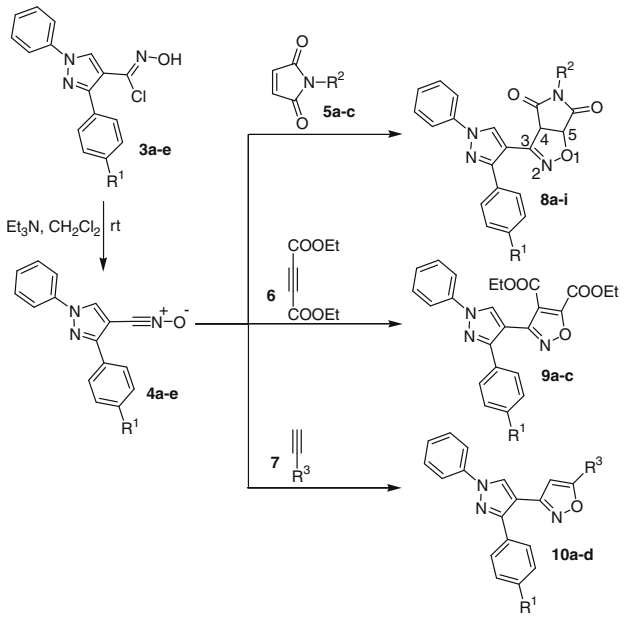

Scheme 2. Synthesis of Isoxazolines and Isoxazoles.

and fungicidal activities.[22] In continuation of our work on the biological activities of pyrazole derivatives[23] and products from 1,3-dipolar cycloaddition reactions,[24] we herein report the synthesis and screening results of the antinociceptive activities of pyrazolyl isoxazoles and pyrazolyl isoxazolines.

We have investigated the 1,3-dipolar cycloaddition of pyrazole derived nitrile oxides with both alkenes and alkynes. Pyrazolyl nitrile oxides were prepared from pyrazolyl oxime, *N*-chlorosuccinimide and triethylamine in dichloromethane at room temperature.

Treatment of pyrazolyl oximes **2a–e** with an equimolar amount of *N*-chlorosuccinimide (NCS) in dichloromethane at room temperature gave the corresponding hydroximoyl chlorides 3a–e. The reaction occured instantaneously upon mixing the substrates as indicated by the appearance of yellow colour in the reaction mixture. Completion of the reaction was finally assessed by TLC. The generality of this reaction was demonstrated by the synthesis of hydroximoyl chlorides with different functionalities (Scheme 1).

Reaction of pyrazolyl oximes **2a–e** with *N*-chlorosuccinimide in dichloromethane at room temperature and subsequent addition of triethylamine facilitated the in situ formation of nitrile oxides. Addition of N-substituted maleimide **5a–c** as the dipolarophile to the reaction mixture resulted in the formation of isoxazolines **8a–i** (Scheme 2). The mixture was stirred at room temperature for 15–30 min for completion. (Table 1) The generality of this reaction was demonstrated by employing dipolarophiles of diverse structures (Table 2). The structure of the compounds was assigned based on the NMR and mass spectral data.[25]

Table 1
Synthesis of isoxazolines

Entry	R^1	R^2	Product[a]	Yield[b] (%)
1	H	Ph	**8a**	85
2	H	Me	**8b**	82
3	Br	Ph	**8c**	78
4	Br	Me	**8d**	79
5	OEt	Ph	**8e**	82
6	OEt	Me	**8f**	81
7	Cl	Ph	**8g**	76
8	Cl	Me	**8h**	80
9	Cl	*p*-Me-Ph	**8i**	77

[a] All the reactions took place in 30 min.
[b] Isolated yield after column chromatography.

Table 2
Synthesis of isoxazoles

Entry	R^1	Alkyne	Product[a]	Yield[b] (%)
1	Br	**6**	**9a**	81
2	OMe	**6**	**9b**	78
3	Cl	**6**	**9c**	76
4	Br	**7a** R^3 = Ph	**10a**	80
5	OMe	**7a** R^3 = Ph	**10b**	81
6	Cl	**7a** R^3 = Ph	**10c**	76
7	Cl	**7b** $R^3 = (CH_2)_3–CH_3$	**10d**	78

[a] All the reactions took place in 30 min.
[b] Isolated yield after column chromatography.

In the present study, two different experimental models tested the potential antinociceptive activity of the synthesized compounds.[26] In the Tail Flick test the acute thermal stimulus was applied and the abdominal constriction test, in which a painful chemical stimulus (acetic acid) was used.

The oral administration 15 mg/kg po and 30 mg/kg po of synthetic compounds induced antinociception in rat Tail Flick test. The effect appeared 15 min after administration, peaked after 60 min and then slowly diminished (Table 3). Antinociceptive efficacy of the synthesized compounds was comparable to that of standard drug pentazocine (5 mg/kg). All compounds at a dose of 15 mg/kg po and 30 mg/kg po exhibited the antinociceptive activity in a dose dependent manner. Among the 16 compounds, compounds **9a–c** showed the maximum antinociceptive effect at 30 mg/kg po which was found to be comparable with that of the standard drug. The compounds **8a**, **8c, 8d, 8e, 8f, 8g, 8h**, **10c**, and **10d** showed moderate antinociceptive effect at 30 mg/kg po. Finally the compounds **8b**, **8i**, **10a**, **10b** have minimal antinociceptive effect at a dose of 30 mg/kg po.

In acetic acid induced abdominal constriction assay all the synthesized compounds were able to reduce the number of abdominal constrictions and showed a potency comparable to that of reference standards (aspirin). In particular **9a**, **9b** and **9c** were able to exhibit a good antinociceptive activity reducing by more than 50% the number of abdominal constrictions with respect to controls (Table 4). Antinociceptive effect induced by the above mentioned compounds was comparable to that shown by standard drug aspirin. Compounds **8a**, **8c**, **8e**, **8f**, **8i**, **10c**, and **10d** reduced by around 50% the number of abdominal constrictions and finally compounds **8b**, **8d**, **8g**, **8h**, **10a**, and **10b** had the least protection in the abdominal constriction test.

For Structure–Activity Relationship (SARs), the data obtained in both the methods clearly suggest that the activity of the series does not depend on the diphenyl pyrazolyl fused system. When the diphenyl pyrazolyl fused system is linked with the isoxazole 4,5-dicarboxylicacid diethyl ester, good results were obtained with different functional groups at position 4 of the phenyl ring.

In summary, we have reported the synthesis of novel pyrazolyl isoxazolines and isoxazoles. Based on the inherent biological activity of pyrazole and isoxazole units we identified a group of antinociceptive agents that are active in the Tail Flick test and acetic acid induced abdominal constriction assay method with an efficacy comparable to that of standard drugs.

Table 3
Antinociceptive activity of synthetic compounds on tail Flick method in rats

Groups	Treatments	Dose mg/kg	Tail Flick latency in seconds (mean ± SEM) At time				
			0 min	30 min	60 min	120 min	180 min
1	Control (saline)	1 ml	3.33 ± 0.21	3.33 ± 0.21	3.16 ± 0.16	3.33 ± 0.21	3.16 ± 0.16
2	Pentazocine	5 mg	3.80 ± 0.19[a]	9.16 ± 0.50[a]	9.33 ± 0.30[a]	9.30 ± 0.50[a]	8.00 ± 0.90[a]
3	**8a**	30 mg	3.33 ± 0.21[a]	3.33 ± 0.57[a]	6.83 ± 0.16[a]	6.66 ± 0.21[a]	5.66 ± 0.21[a]
4	**8b**	30 mg	3.16 ± 0.16[a]	3.33 ± 0.21[a]	5.16 ± 0.16[a]	5.00 ± 0.00[a]	4.83 ± 0.16[a]
5	**8c**	30 mg	3.16 ± 0.16[a]	3.16 ± 0.16[a]	7.00 ± 0.00[a]	6.83 ± 0.21[a]	6.50 ± 0.22[a]
6	**8d**	30 mg	3.33 ± 0.21[a]	3.33 ± 0.21[a]	7.16 ± 0.16[a]	7.00 ± 0.00[a]	6.50 ± 0.22[a]
7	**8e**	30 mg	3.16 ± 0.16[a]	3.16 ± 0.16[a]	6.83 ± 0.16[a]	6.16 ± 0.21[a]	5.83 ± 0.21[a]
8	**8f**	30 mg	3.33 ± 0.21[a]	3.83 ± 0.16[a]	6.16 ± 0.16[a]	6.00 ± 0.00[a]	5.83 ± 0.16[a]
9	**8g**	30 mg	3.16 ± 0.16[a]	3.33 ± 0.16[a]	6.16 ± 0.16[a]	6.00 ± 0.00[a]	5.50 ± 0.22[a]
10	**8h**	30 mg	3.16 ± 0.16[a]	3.33 ± 0.21[a]	6.66 ± 0.21[a]	6.50 ± 0.22[a]	5.50 ± 0.22[a]
11	**8i**	30 mg	3.16 ± 0.16[a]	3.33 ± 0.21[a]	5.33 ± 0.21[a]	5.16 ± 0.16[a]	4.50 ± 0.22[a]
12	**9a**	30 mg	3.33 ± 0.21[a]	3.33 ± 0.21[a]	8.16 ± 0.16[a]	8.00 ± 0.00[a]	7.50 ± 0.22[a]
13	**9b**	30 mg	3.33 ± 0.21[a]	3.83 ± 0.16[a]	7.83 ± 0.16[a]	7.66 ± 0.21[a]	7.00 ± 0.00[a]
14	**9c**	30 mg	3.16 ± 0.16[a]	3.83 ± 0.16[a]	8.66 ± 0.21[a]	8.33 ± 0.21[a]	7.50 ± 0.22[a]
15	**10a**	30 mg	3.33 ± 0.21[a]	3.33 ± 0.21[a]	5.83 ± 0.16[a]	5.66 ± 0.21[a]	5.16 ± 0.16[a]
16	**10b**	30 mg	3.16 ± 0.21[a]	3.33 ± 0.16[a]	6.00 ± 0.00[a]	5.83 ± 0.16[a]	5.33 ± 0.21[a]
17	**10c**	30 mg	3.33 ± 0.16[a]	3.83 ± 0.21[a]	6.83 ± 0.21[a]	6.60 ± 0.90[a]	6.16 ± 0.33[a]
18	**10d**	30 mg	3.16 ± 0.16[a]	3.33 ± 0.28[a]	6.83 ± 0.16[a]	6.60 ± 0.21[a]	6.16 ± 0.33[a]

N = 6.
Data were analysed by one way ANOVA followed by Dunnett's test.
Values are expressed as mean ± SEM.
[a] $P < 0.01$ versus control.

Table 4
Antinociceptive activity of synthesized compounds on acetic acid induced abdominal constriction in MICE

Groups	Treatments	Dose (mg/kg)	No. of abdominal constriction (per 10 min)	% Production
1	Control (saline)	5 mg/kg	42.50 ± 0.428	—
2	Aspirin	100	16.00 ± 0.365[a]	62.35
3	**8a**	30 mg	22.33 ± 0.422[a]	47.39
4	**8b**	30 mg	29.50 ± 0.428[a]	30.58
5	**8c**	30 mg	21.66 ± 0.258[a]	49.04
6	**8d**	30 mg	25.33 ± 0.223[a]	40.40
7	**8e**	30 mg	21.33 ± 0.333[a]	49.51
8	**8f**	30 mg	22.33 ± 0.760[a]	47.46
9	**8g**	30 mg	24.16 ± 0.577[a]	43.15
10	**8h**	30 mg	32.66 ± 0.333[a]	23.15
11	**8i**	30 mg	24.33 ± 0.333[a]	42.75
12	**9a**	30 mg	18.83 ± 0.477[a]	56.87
13	**9b**	30 mg	18.00 ± 0.447[a]	57.65
14	**9c**	30 mg	19.00 ± 0.421[a]	55.29
15	**10a**	30 mg	29.16 ± 0.307[a]	31.39
16	**10b**	30 mg	29.33 ± 0.210[a]	30.99
17	**10c**	30 mg	21.66 ± 0.330[a]	49.04
18	**10d**	30 mg	22.33 ± 0.341[a]	47.09

N = 6.
Data were analysed by one way ANOVA followed by Dunnett's test.
Values are expressed as mean ± SEM.
[a] $P < 0.01$ versus control.

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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.2009.05.055.

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25. A mixture of pyrazolyl oxime (0.5 mmol), *N*-chlorosuccinimide (0.5 mmol) in dichloromethane was stirred at room temperature for 5 min. After formation of hydroximoyl chlorides indicated by TLC, N-substituted maleimide (0.6 mmol) or Phenyl acetylene (2 mmol) or diethyl acetylene dicarboxylate (2 mmol) and Et_3N (0.75 mmol) was added to the reaction mixture. The reaction mixture was stirred at the same temperature for additional 30 min. The reaction mixture was diluted by addition of water (20 mL) and extracted with CH_2Cl_2 (2×20 mL). The combined organic layers were dried over anhydrous Na_2SO_4, concentrated in vacuum. The crude was purified by column chromatography on silica gel (Merck, 100–200 mesh, ethyl acetate–petroleum ether (20:80) to afford pure isoxazolidine and ethyl acetate–petroleum ether (8:92) to afford pure isoxazoles.
3-[3-(4-Bromophenyl)-1-phenyl-1H-pyrazol-4-yl)-5-phenyl-3a,6a-dihydro-pyrrolo[3,4-d]isoxazole-4,6-dione (**8c**): Colourless solid; mp 224–226 °C; R_f: 0.50 (50% ethyl acetate/petroleum ether); IR (cm^{-1}): 3449, 3060, 1727, 1596, 1498, 1372, 1189; ^1H NMR (500 MHz, DMSO-d_6): δ 5.19 (d, 1H, J = 9.9 Hz), 5.68 (d, 1H, J = 9.2 Hz), 7.25 (d, 2H, J = 7.7 Hz), 7.37–7.41 (m, 2H), 7.46 (t, 2H, J = 8.4 Hz), 7.55 (t, 2H, J = 7.7 Hz), 7.64 (d, 2H, J = 8.4 Hz), 7.73 (d, 2H, J = 8.5 Hz), 7.87 (d, 2H, J = 8.4 Hz), 9.11 (s, 1H); ^{13}C NMR (125 MHz, DMSO-d_6): δ 57.6, 80.5, 109.5, 119.3, 122.6, 127.6, 127.8, 129.3, 129.5, 130.3, 131.4, 131.5, 131.9, 132.1, 132.7, 139.2, 147.5, 150.4, 171.4, 172.6; MS m/z = 513 M^++1, 515 M^++3; Anal. Calcd for $C_{26}H_{17}BrN_4O_3$ (512.05): C, 60.83; H, 3.34; N, 10.91. Found: C, 60.72; H, 3.31; N, 10.99.
3-[3-(4-Bromophenyl)-1-phenyl-1H-pyrazol-4-yl)-isoxazole-4,5-dicarboxylic acid diethyl ester (**9a**): Pale green solid; mp 98–100 °C; R_f: 0.55 (30% ethyl acetate/petroleum ether); IR (cm^{-1}): 3429, 3062, 1723, 1670, 1514, 1361, 1181; ^1H NMR (500 MHz, $CDCl_3$): δ 1.14 (t, 3H, J = 6.9 Hz), 1.41 (t, 3H, J = 6.9 Hz), 3.97 (q, 2H, J = 6.9 Hz), 4.46 (q, 2H, J = 6.9 Hz), 7.34 (t, 1H, J = 7.7 Hz), 7.47–7.51 (m, 6H), 7.77 (d, 2H, J = 7.7 Hz), 8.34 (s, 1H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 13.7, 14.0, 62.1, 63.0, 107.6, 115.9, 119.4, 122.8, 127.4, 129.6, 129.7, 130.5, 131.2, 131.9, 139.4, 150.7, 155.5, 156.1, 160.3, 160.4; MS m/z = 510 M^++1, 512 M^++3; Anal. Calcd for $C_{24}H_{20}BrN_3O_5$ (509.06): C, 56.48; H, 3.95; N, 8.23. Found: C, 56.56; H, 3.97; N, 8.41.
3-[3-(4-Bromophenyl)-1-phenyl-1H-pyrazol-4-yl)-5-phenylisoxazole (**10a**): Colourless solid; mp 140–142 °C; R_f: 0.61 (30% ethyl acetate/petroleum ether); IR (cm^{-1}): 3430, 3055, 1585, 1501, 1456, 1364, 1065; ^1H NMR (500 MHz, $CDCl_3$): δ 6.40 (s, 1H), 7.34 (t, 1H, J = 6.9 Hz), 7.43–7.51 (m, 5H), 7.57 (d, 2H, J = 8.4 Hz), 7.64 (d, 2H, J = 8.4 Hz), 7.75 (dd, 2H, J = 7.6, 1.5 Hz), 7.79 (d, 2H, J = 7.7 Hz), 8.38 (s, 1H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 99.2, 110.7, 119.3, 122.9, 125.9, 127.3, 127.9, 129.0, 129.6, 130.3, 130.4, 131.5, 131.7, 139.5, 150.4, 156.5, 170.1; MS m/z = 442 M^++1, 444 M^++3; Anal. Calcd for $C_{24}H_{16}BrN_3O$ (441.05): C, 65.17; H, 3.65; N, 9.50. Found: C, 65.21; H, 3.66; N, 9.55.
26. Materials and methods for the antinociceptive activity: Male albino rats (wistar strain, 150–200 g, Tail Flick method) and Swiss albino mice (25–30 g, Acetic acid induced constriction assay), were used as experimental models. The animals were given food and water ad libitum. The animals were housed under standard conditions of 12 h light and 12 h dark cycle at ambient temperature (35–36 °C). The experiments were carried out during light cycle. The animal care and experimental protocols were in accordance with International Animal Ethical Committee (IAEC), and the experimental proposal number is UCP/IAEC/2008/036.
Tail Flick test: Tail Flick latency was assessed by analgesiometer.[27,28] In this method the animals (rats) were selected by preliminary screening. Those in variation of more than one second between two reaction times at 15 min interval (or) more than three seconds from the group mean were discarded. A cut-off reaction time fixed at 10 s was maintained to avoid damage. The reaction time of animals on the Tail Flick apparatus was recorded at 30, 60, 120, and 180 min after the administration of the drug. The rats were divided in to 34 groups, each group consisting of 6-animals. The test drugs administered at a dose of 15 mg/kg po and 30 mg/kg po and standard drug pentazocine a dose of 5 mg/kg ip and aspirin 100 mg/kg po.
Abdominal constriction test: Mice were injected ip with a 0.6% solution of acetic acid (10 ml/kg), according to the procedure of modified Seigmund technique.[29,30] The number of stretching movements was counted for 10 min, starting 5 min after acetic acid injection.
Statistical analysis: All experimental results are given as the mean ± S.E.M. The data were statistically analyzed by one-way ANOVA followed by Dunnet's test. P value of less than 0.01 were considered significant.
Drugs: The following drugs were used Aspirin (Apex, India), Pentazocine (Ranbaxy, India). Other chemicals were of highest quality commercials available. All drugs were dissolved in isotonic (NaCl 0.9%) saline (or) dispersed in sodium carboxy methyl cellulose 1%. Drug concentrations were prepared in such a way that the necessary dose could be administered in a volume of 0.5 ml/dose for mice and 1.0 ml/dose for rats.
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