Acta Pharmaceutica Sciencia
53: 287-294 (2011)

Synthesis, characterization and antinociceptive activity of some 5-phenyl -1-[5-(substituted phenyl) isoxazol-3-yl]-1H-tetrazole

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Abstract

Nine different derivatives of 5-phenyl-1-(5-substituted phenyl isoxazol-3-yl)-1H-tetrazole (4a-i) were synthesized by reaction of chalcones with hydroxylamine hydrochloride in presence of aq. KOH. Compound (1) was cyclized using sodium azide and benzonitrile. The 5-phenyl tetrazoles on treatment with acetic anhydride forms 5-Phenyl 1-Acetyl Tetrazole (2) which on reaction with different aromatic aldehydes forms chalcones (3a-i). The chemical structures were confirmed by means of FT-IR, ^{1}H-NMR and elemental analysis. The compounds were screened for antinociceptive activity by acetic acid induced writhing method and hot plate method. 5-phenyl-1-(5-phenyl isoxazol-3-yl)-1H-tetrazole (4a) was found to be the most active compound of the series.

Keywords: tetrazole, isoxazole, antinociceptive activity

Introduction

Tetrazoles are an important functionality with wide ranging applications in photography and information recording systems, pharmaceutical and material sciences and appealing ligands in coordination chemistry. All aspects of the chemistry of tetrazoles as well as medicinal application of tetrazoles were covered in the literature. The most direct method to form 5-substituted tetrazoles is via the formal [3 + 2] cycloaddition of azides and nitriles in presence of ammonium chloride (Mohite et al. 2009). Biological activity in tetrazole is encountered due to the special metabolism of the disubstituted tetrazole system and also because, in 5-substituted tetrazole compounds the tetrazole ring is isosteric with a carboxylic acid group and is of comparable acidity. Hence for all biologically active molecules possessing a carboxylic group (CO_2H), there is a theoretical nitrogen analogue possessing a tetrazolic group (CN_4H) and since tetrazole moiety appears to be the metabolically more stable of the two a considerable exploration of these molecules is ongoing. Tetrazole derivatives possess very interesting pharmacological and biological properties and are reported to exhibit variety of biological activities like antibacterial (Kurteshi et al. 2007, Mulwad et al. 2008), antifungal and anticonvulsant (Upadhayaya et al. 2004, Rostom, et al. 2009), analgesic (Rajasekaran et al.

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2004), antibacterial, antifungal and anticonvulsant (Rajasekaran et al. 2006), anti-inflammatory (Mohite et al. 2010), antitubercular activity (Adamec et al. 2005). Similarly 1, 5 disubstituted tetrazoles have long been known for their pharmaceutical activity as stimulants or depressants on the central nervous system and are reported to show oral antidiabetic and antithrombotic and antimicrobial properties. Isoxazoles may show interesting medicinal or crop protection properties or have some other industrial utility or various biological activities (Popat, et al 2003). Various pharmacologically important isoxazoles with antimicrobial (Dabholkar and Ansari et al 2007), anti-inflammatory, analgesic (Karbasanagouda et al. 2009), antitubercular activity (Tangallapally et al. 2007), antitumoral and antimycobacterial activity (De Souza et al. 2005) have already been reported. Isoxazoles are unique in their chemical behavior, not only among heterocyclic compounds in general, but also among related azoles. Isoxazoles functionalized with additional nitrogen-containing groups have seen application in medicinal chemistry.

Encouraged by the intense recent research activity in the tetrazole field and in pursuit of our continuing interest in isoxazole chemistry, we envisioned the combination of these attractive functional groups by the synthesis of various 5-phenyl-1-(5-substituted phenyl lisoxazol-3-yl)-1*H*-tetrazole and the structures of all the various synthesized compounds were assigned on the basis of elemental analysis, IR and ^{1}H NMR spectral data. These compounds were screened for their antinociceptive activity (Kulkarni et al. 2003).

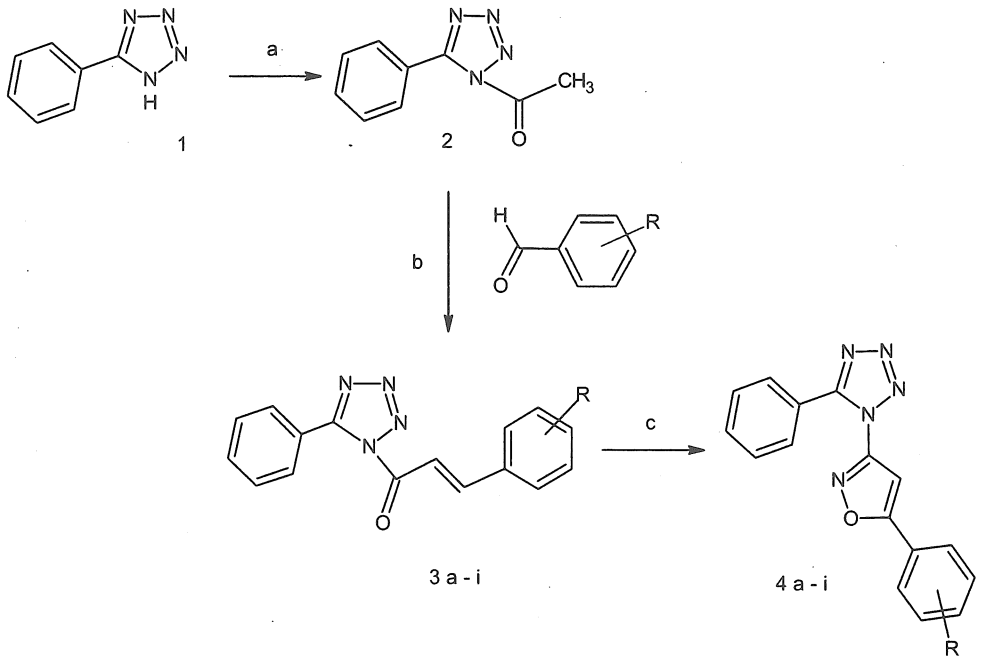
Chemistry

Compounds were prepared as shown in Fig. 1. The 5-substituted tetrazoles can be synthesized by number of methods, viz.reaction of hydrazoic acid or its salts with imidoyl chloride or imino ethers or diazo coupling of heterocyclic hydrazines or hydrocyanic acid. Most of these methods have limited use in preparative organic chemistry because the use of hydrazoic acid presents considerable experimental difficulties due its toxicity and tendency to explode. However, the simple route reported by Mohite et al. was adopted for the preparation of 5-phenyl-1, 2, 3, 4-tetrazoles **(1).** This route replaces the toxic hydrazoic acid by inorganic azide to afford the titled compounds in good yield (59–82%). Compound **1** was cyclized using sodium azide and ammonium chloride and benzonitrile. The 5-phenyl tetrazoles on treatment with acetic anhydride forms 5-Phenyl 1-Acetyl Tetrazole **(2)** which on reaction with different aromatic aldehydes forms chalcones **(3a-i).**The chalcones further undergo cyclisation with hydroxylamine hydrochloride in presence of KOH to form 5-phenyl-1-(5-substituted phenyl lisoxazol-3-yl)-1*H*-tetrazole **(4a-i)**.

Materials and Methods

General procedures

Melting points were determined in open capillaries and were uncorrected. Reactions were monitored by thin layer chromatography using silica gel-G as adsorbent using benzene: ethyl acetate (9:1) as eluent. IR spectra (KBr pellets) were recorded on Shimadzu FT-IR model 8010 spectrophotometer, Japan. ^{1}H NMR spectra (DMSO-d6) were taken a Varian mercury spectrometer (model YH- 300 FT NMR) using TMS as internal standard and chemical shift are expressed in δ ppm.



a. $(CH_3CO)_2O$/GAA

b. Ar-CHO /KOH

c. $NH_2OH.HCL$

4a R=H; 4b R=2-Cl; 4c R=4-Cl; 4d R=4-Br; 4e R=4-OCH3; 4f R=3-NO2; 4g R=4-NO2; 4h R=4-CH3; 4i R=4-N-(CH3)2

Figure 1. Synthetic protocol for synthesis of titled compounds

General procedures for synthesis of 5-phenyl 1-acetyl tetrazole (2)

A solution of 5-phenyl tetrazole (12.8g, 0.08 moles) and acetic anhydride (0.08 moles) and 2-3 drops of concentrated sulphuric acid was warmed for 15-20 min. on water bath. Cooled and poured into ice cold water.The product separated was filtered and dried .It was further purified by crystallization from ethanol and was obtained in 75% yield as a white amorphous solid: m.p. 214-215 °C

IR: 3445 (NH), 3054(Ar-CH), 1608(C=N), 1575(-N==N-), 1569(NH def.), 1164, 1072 (-CN),^{1}H-NMR (DMSO,d)8.80(s,1H,NH),7.08(6H,Ar-H).

General procedures for synthesis chalcones (3a-i)

A solution of 5-phenyl 1-acetyl tetrazole (2g, 0.010 moles) and aromatic aldehydes (0.010 mole) in ethanol (12 mL) was cooled to 5 to 10.°C in an ice bath. The cooled solution was treated with drop wise addition of aqueous potassium hydroxide (2.5 mL, 50%). The reaction mixture was magnetically stirred for 30 min and then left over night. The resulting dark solution was diluted with ice cold water and carefully acidified using diluted hydrochloric acid. The tetrazole analogues of chalcone which crystallized, were collected by filtration by washing with sodium bicarbonate and water. It was further purified by crystallization from ethanol.FT-IR: 1285(N-N=N-), 1108 and 1138 (Tetrazole ring), 1735 (C=O), 1630 (C=C), 3054 (Ar-CH).^{1}H NMR (DMSO) d:6.6.1(1H,d,-CO-CH=),7.05(1H,d,=CH-Ar), 7.14-7.8 0(10H, m, Ar-H).

General procedures for synthesis of isoxazole (4a-i)

A mixture of chalcone (0.01 mol), hydroxylamine hydrochloride (0.01mol, 0.695 g) in ethanol and 40% KOH solution were refluxed for 10 h. Then the reaction mixture was cooled and poured into crushed ice and the product separated out was filtered, washed with water, dried and recrystallised from alcohol to give isoxazole.

5-phenyl-1-(5-phenyl isoxazol-3-yl)-1H-tetrazole (4a)

Yield 78% as a white solid: m.p. 161-162°C. FT-IR:, 3048 (Ar-H)., 1285 (N-N=N-), 1108 and 1138 (Tetrazole ring), 2950 and 1610 (C=N ring stretch), 1580 (C=C), 1540, 1470, 1450 (N-O ring stretch) .^{1}H NMR (DMSO) d: 6.90-7.80 (10H, m, Ar-H) 7.05 (1H, d, =CH in isoxazole). Anal. For $C_{16}H_{11}N_5O$.

1-[5-(2-chloro phenyl) isoxazol-3-yl]-5-phenyl-1H-tetrazole (4b)

Yield 72% as a yellow solid: m.p. 155-156°C. FT-IR, 3055 (Ar-H)., 1285(N-N=N-), 1108 and 1138 (Tetrazole ring), 2950 and 1610 (C=N ring stretch), 1580 (C=C), 1540, 1470, 1450 (N-O ring stretch), 758 (C-Cl). ^{1}H NMR (DMSO) d: 6.92-7.80 (9H, m, Ar-H) 7.05(1H, d,=CH in isoxazole). Anal. For $C_{16}H_{10}ClN_5O$.

1-[5-(4-chloro phenyl) isoxazol-3-yl]-5-phenyl-1H-tetrazole (4c)

Yield 73% as a yellow solid: m.p. 145-146°C. FT-IR, 3055 (Ar-H)., 1285 (N-N=N-), 1108 and 1138 (Tetrazole ring), 2950 and 1610 (C=N ring stretch), 1580 (C=C), 1540, 1470, 1450 (N-O ring stretch),758 (C-Cl). ^{1}H NMR (DMSO) d: 6.92-7.80 (9H, m, Ar-H) 7.05 (1H, d, =CH in isoxazole). Anal. For $C_{16}H_{10}ClN_5O$.

1-[5-(4-bromo phenyl) isoxazol-3-yl]-5-phenyl-1H-tetrazole (4d)

Yield 65% as a brown solid: m.p. 150-151°C. FT-IR, 3056 (Ar-H)., 1285(N-N=N-), 1108 and 1138 (Tetrazole ring), 2950 and 1610 (C=N ring stretch), 1580 (C=C), 1540, 1470, 1450 (N-O ring stretch), 674 (C-Br). ^{1}H NMR (DMSO) d; 6.90-7.80 (9H, m, Ar-H) 7.05 (1H, d, =CH in isoxazole), Anal. For $C_{16}H_{10}BrN_5O$.

1-[5-(4-methoxy phenyl) isoxazol-3-yl]-5-phenyl-1H-tetrazole (4e)

Yield 82% as a redish brown solid: m.p. 125-126°C. FT-IR, 3052(Ar-H)., 1285 (N-N=N-), 1108 and 1138 (Tetrazole ring), 2950 and 1610 (C=N ring stretch), 1580 (C=C), 1540, 1470, 1450 (N-O ring stretch) ,1251 (-OCH_3), 6.91-7.80 (9H, m, Ar-H) 7.05 (1H, d, =CH in isoxazole), 2..37(-OCH3). Anal. For $C_{17}H_{13}N_5O_2$.

1-[5-(3-nitro phenyl) isoxazol-3-yl]-5-phenyl-1H-tetrazole (4f)

Yield 62% as a yellowish amorphous solid: m.p. 167–168°C. FT-IR, 3054(Ar-H)., 1285(N-N=N-), 1108 and 1138(Tetrazole ring), 2950 and 1610 (C=N ring stretch), 1580 (C=C), 1540, 1470, 1450 (N-O ring stretch), 1578 (-NO_2). ^{1}H NMR: 6.80 -7.80 (9H, m, Ar-H) 7.05 (1H, d, =CH in isoxazole). Anal. For $C_{16}H_{10}N_6O_3$.

1-[5-(4-nitro phenyl) isoxazol-3-yl]-5-phenyl-1H-tetrazole (4g)

Yield 64% as a yellowish solid: m.p. 169–170°C. FT-IR, 3054 (Ar-H)., 1285 (N-N=N-), 1108 and 1138 (Tetrazole ring), 2950 and 1610 (C=N ring stretch) , 1580 (C=C), 1540, 1470, 1450 (N-O ring stretch), 1578 (-NO2). ^{1}H NMR: 6.80-7.80 (9H, m, Ar-H) 7.05(1H, d, =CH in isoxazole). Anal. For $C_{16}H_{10}N_6O_3$.

1-[5-(4-methyl phenyl) isoxazol-3-yl]-5-phenyl-1H-tetrazole (4h)

Yield 67% as a colorless solid: m.p. 184-185°C. FT-IR, 3054 (Ar-H)., 1285 (N-N=N-), 1108 and 1138 (Tetrazole ring), 2950 and 1610 (C=N ring stretch), 1580 (C=C), 1540, 1470, 1450 (N-O ring stretch), 1365 (CH_3). ^{1}H NMR: 6.85-7.80 (9H, m, Ar-H) 7.05 (1H, d, =CH in isoxazole), 3.72 (3H, CH_3). Anal. For $C_{17}H_{13}N_5O$

1-[5-(4-dimethylamino phenyl) isoxazol-3-yl]-5-phenyl-1H-tetrazole (4i)

Yield 59% as a yellow crystals: m.p. 152-153°C. FT-IR, 3048 (Ar-H)., 1285 (N-N=N-), 1108 and 1138 (Tetrazole ring), 2950 and 1610 (C=N ring stretch), 1580 (C=C), 1540, 1470, 1450 (N-O ring stretch), 1321 (-$N(CH_3)_2$). ^{1}H NMR: 7.14-7.80 (9H, m, Ar-H) 7.05 (1H, d, =CH in isoxazole), 2.9 (6H, d, CH_3). Anal. For $C_{18}H_{16}N_6O$

Evaluation of antinociceptive activity

Swiss strain albino mice of either sex weighing 25–30 g were used for this study. The test compounds (in 1/10th dose of the average LD_{50} values of titled compounds) were administered intraperitoneally in 10% v/v Tween 80 suspensions. LD_{50} of the newly synthesized compounds were determined by Miller and Tainter method (1944) administering the compounds intraperitoneally.

1. Acetic acid induced writhing method

The method suggested by Witkin et al. (1961) was adopted for the study. The animals were divided into 11 groups of six mice each. The control group of animals was administered with 10% w/v Tween 80 (0.5 mL) suspension. The standard drug ibuprofen (Concept Pharmaceutical Ltd., India) was administered intraperitoneally in a dose of 25 mg kg^{-1}. After 20 min of the administration the test compounds, all the groups of mice were given with the writhing agent 3% v/v aqueous acetic acid in a dose of 2 mL kg^{-1} intraperitoneally. The writhings produced in each animal were recorded visually for 15 min and the numbers of writhings produced in treated groups were compared with those of control group. The results were analyzed statistically by Dunnette's multiple comparison test and recorded in Table 1.

2. Hot plate method

The method of Eddy and Leimbach (1953) was adopted for the study. The pain threshold of the animals was measured on a hot plate before treatment of the test and standard compounds, and the animals that showed more than 10 sec. of reaction time were rejected. The reference compound Pentazocine was administered intraperitoneally in a dose of 25 mg kg^{-1}. After the treatment of test and standard compounds, the pain threshold of the animals was measured at 15, 30 and 60 min. respectively.

Result and Discussion

Tetrazole contains cyclic secondary amino group.All secondary amine undergo acetylation reaction with acetic anhydride and a conc.H_2SO_4. 5-phenyl-1,2,3,4- tetrazoles being a secondary amine was acetylated to compound **2** by acetic anhydride and a conc.H_2SO_4 . The yield of the compound **2** was found to be quantitative and it was readily converted to chalcones by treating them with different aromatic aldehydes and pottasium hydroxide and hence nine different derivatives are synthesized. Infrared spectrum of compound **1** showed absorption bands at 1040, 1108, 1248, 1280 and 1595 cm^{-1} which are attributed to tetrazole ring. An absorption band at 3448 cm−1 is attributed to N–H stretching of the tetrazole ring. Characteristic absorption bands were observed for chloro, nitro group, bromo group, dimethylamino group, methyl group, methoxyl group and aromatic region of the synthesized compounds. ^{1}H-NMR spectra of the

synthesized compounds showed multiplets in the range of d 6.7–7.80 for aromatic protons. The expected signals with appropriate multiplicities for different types of protons such as methyl, methoxy groups were observed for the derivatives within the range.

Antinociceptive activity

1. Acetic acid induced writhing method

Antinociceptive activity was evaluated by acetic acid induced writhing method (Table 1). All compounds tested exhibited significant activity in a dose of 25 mg kg^{-1} .The antinociceptive activity of compound 4a was found to be superior compared to other synthesized compounds. The unsubstituted isoxazole derivative (4a) showed maximum antinociceptive activity in comparison with standard. Introduction of 3-nitro group, 4-nitro group, bromo group, methyl group, showed almost significant antinociceptive activity as that of ibuprofen. 5-phenyl Tetrazole showed moderate antinociceptive activity. Introduction of 4-chloro, 2-chloro, and 4-methoxy and 4-dimethylamino group group in 5-phenyl-1-(5-substituted phenyl isoxazol-3-yl)-1*H*-tetrazole have shown minimum or moderate antinociceptive activity.

Table 1. Evaluation of antinociceptive activity by acetic acid induced writhing method

Compound	Dose (mg kg^{-1})	Writhing episodes in 15 min. (Mean ±SEM)	Percent protection
Control	-	52.83±0.00	-
Ibuprofen	2.5	09.17±0.83**	82.64
4a	25	14.17±0.79**	73.17
4b	25	27.50±0.88**	47.94
4c	25	27.55±0.89**	47.85
4d	25	22.30±0.73**	57.78
4e	25	34.17±0.94**	35.32
4f	25	18.20±0.93**	65.55
4g	25	18.83±0.81**	64.35
4h	25	20.67±0.86**	60.87
4i	25	35.42±0.96**	32.95

*** $p<0.01$ represent the significant difference when compared with control group*

2. Hot plate method

All compounds tested by Eddy's hot plate method (Table 2) exhibited significant activity in a dose of 25 mg kg^{-1}.

Table 2. Evaluation of antinoceptive activity by hot plate method

Compound	Reaction time in sec. at min. (Mean±SEM)			
	0	15	30	60
Control	4.95±0.20	4.95±0.20	4.95±0.20	4.95±0.20
Pentazocine	4.90±0.41**	7.45±0.32**	10.12±0.53**	14.22±0.52**
4a	5.41±0.32**	6.75±0.24**	8.45±0.21**	12.18±0.41**
4b	6.55±0.11**	7.25±0.78**	8.95±0.45**	9.67±0.62**
4c	6.25±0.09**	6.84±0.91**	7.65±0.25**	10.04±0.70**
4d	5.84±0.55**	7.11±0.80**	8.45±0.39**	9.56±0.41**
4e	5.63±0.42**	5.86±0.24**	7.27±0.45**	8.15±0.85**
4f	6.15±0.32**	6.75±0.26**	9.68±0.11**	10.25±0.45**
4g	6.28±0.21**	6.82±0.45**	9.25±0.78**	10.35±0.10**
4h	5.35±.71**	6.55±0.75**	8.48±0.42**	11.15±0.98**
4i	6.12±0.41**	6.68±0.65**	7.25±0.34**	9.05±0.88**

Dose 25 mg/kg for all test compound and 2.5mg/kg for pentazocine
*** $p<0.01$ represent the significant difference when compared control group.*

The analgesic activity of compounds 4a, 4h and 4g, containing 3-nitro group and 4-nitro group were found superior when compared to other synthesized compounds respectively. Compounds 4f and 4d possess moderate analgesic activity.

Conclusions

We prepared a series of some substituted 5-phenyl-1-(5-substituted phenyl isoxazol-3-yl)-1*H*-tetrazole and demonstrated that these compounds possessed good antinociceptive activity tested both by acetic acid induced writhing method and hot plate method. Acetic acid induced writhing method and hot plate method were adopted to assess the peripheral and centrally (narcotic) mediated antinociceptive activities. The most promising compound having potent antinociceptive activity was found to be 5-phenyl-1-(5-phenyl isoxazol-3-yl)-1*H*-tetrazole (4a), 1-[5-(3-nitro phenyl) isoxazol-3-yl]-5-phenyl-1*H*-tetrazole (4f) ,1-[5-(4-nitro phenyl) isoxazol-3-yl]-5-phenyl-1*H*-tetrazole(4h).

Acknowledgments

We are highly thankful to University of Pune for providing the spectral data and Principal MES College of Pharmacy for providing excellent research facilities.

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Received: 21.08.2010

Accepted: 04.05.2011