Diels Alder reaction strategy to synthesize 1,2,3,6-tetrahydro-1,2,4,5-tetrazines and exploration of their anti-inflammatory potential

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Abstract

The aim of this study was to investigate the anti-inflammatory potential elicited by 1,2,3,6-tetrahydro-1,2,4,5-tetrazines. It was performed on albino mice after syringe administration (500 mg/ Kg, 1000 mg/ Kg, 1500 mg/ Kg) using *carrgeenan induced paw edema* as an acute inflammatory model. In this test 4-(2,3,6-triphenyl-3,6-dihydro-1,2,4,5-tetrazin-1(2*H*)-yl)phenol **7a** and 4-[3,6-diphenyl-2-(4-nitrophenyl)-3,6-dihydro-1,2,4,5-tetrazin-1(2*H*)-yl]phenol **7b** significantly impaired both early and late phases of the inflammatory responses. All the tested 1,2,3,6-tetrahydro-1,2,4,5-tetrazines were synthesized and their structures were corroborated by spectroanalytical analysis (IR, ^{1}H NMR, ^{13}C NMR, Mass spectrometry).

Keywords: Anti-inflammatory activity, 1,2,3,6-tetrahydro-1,2,4,5-tetrazines, hetero Diels–Alder reaction

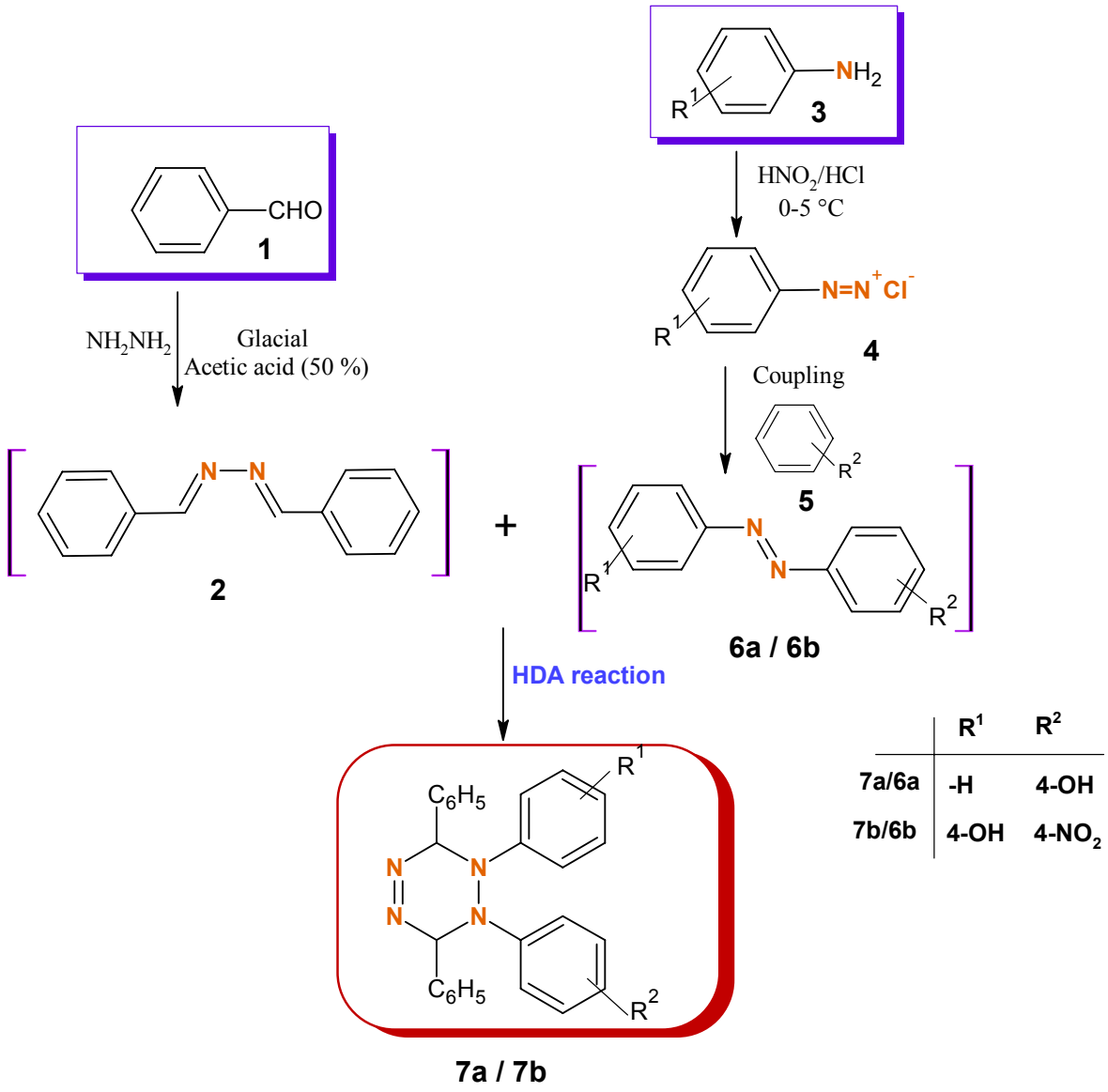
Introduction

The hetero Diels–Alder (HDA) reaction is a powerful methodology for the construction of biologically active six-membered heterocycles. Amongst six-membered heterocyclic systems, tetrazines are of considerable interest not only because of their inherent biological potential[1] but also because of their value as building blocks in organic transformations. Tetrazines have demonstrated powerful synthetic utility through their ability to participate in inverse electron demand Diels–Alder reactions[2,3] providing access to a wide range of other heterocycles and natural products. Azolotetrazinones have been the focus of medicinal chemists in the past decades because of the outstanding antineoplastic activity exhibited by them. Likewise, mitozolomide[4] and temozolomide[5] have attracted remarkable attention owing to their efficiency against malignant melanoma, mycosis fungoides, and brain tumors. Recently it has been reported that the antitumor activity displayed by a new class of pyrrolo[2,1-

d][1,2,3,5]tetrazinones[6] is due to the presence of the deaza skeleton of temozolomide. Tetrazine derivatives were evaluated as active anti-inflammatory agents by Lang *et al*.[7] Thus it is clear that a tetrazine ring can be an effective pharmacophore in various types of medicinal activities. Hence, we set out to develop a route to 4-(2,3,6-triphenyl-3,6-dihydro-1,2,4,5-tetrazin-1(2*H*)-yl)phenol **7a** and 4-[3,6-diphenyl-2-(4-nitrophenyl)-3,6-dihydro-1,2,4,5-tetrazin-1(2*H*)-yl]phenol **7b** to be followed by an exploration of their anti-inflammatory potential using rat *carrageenan induced paw edema* tests. We were encouraged in planning our route by a report of a normal electron demand Diels–Alder reaction between 1,2-diaza-dienes and diethyl azodicarboxylate which was used by Avalos *et al.* to synthesize 1,2,3,6-tetrahydro-1,2,3,4-tetrazines.[8-12]

Chemistry

In order to synthesize 1,2,3,6-tetrahydro-1,2,4,5-tetrazine derivatives **7a** and **7b,** the diazadiene **2** was prepared by condensing hydrazine hydrate and benzaldehyde in the presence of 50% glacial acetic acid in good yield (80%) giving a yellow green crystalline product. To a solution of diazadiene **2** in dry xylene, an equimolar quantity of dienophile 4-[phenyldiazenyl]phenol **6a** or 4-[(4-nitrophenyl)diazenyl]phenol **6b** was added. The solution was then heated at reflux for the required time with constant stirring to afford 1,2,3,6-tetrahydro-1,2,4,5-tetrazines (**7a** and **7b**). The calculated heat of formations at PM3 semi empirical[13] method using MOPAC 2007[14] program for **7a** and **7b** are -153.35 and -144.87 KCal respectively, which are in accordance with the stability of 1,2,3,6-tetrahydro-1,2,4,5-tetrazines. The PM3 optimized geometry of 4-(2,3,6-triphenyl-3,6-dihydro-1,2,4,5-tetrazin-1(2*H*)-yl)phenol **7a** at minimum gradient level is depicted in Figure 1.



Scheme 1. The synthesis of 1,2,3,6-tetrahydro-1,2,4,5-tetrazines.

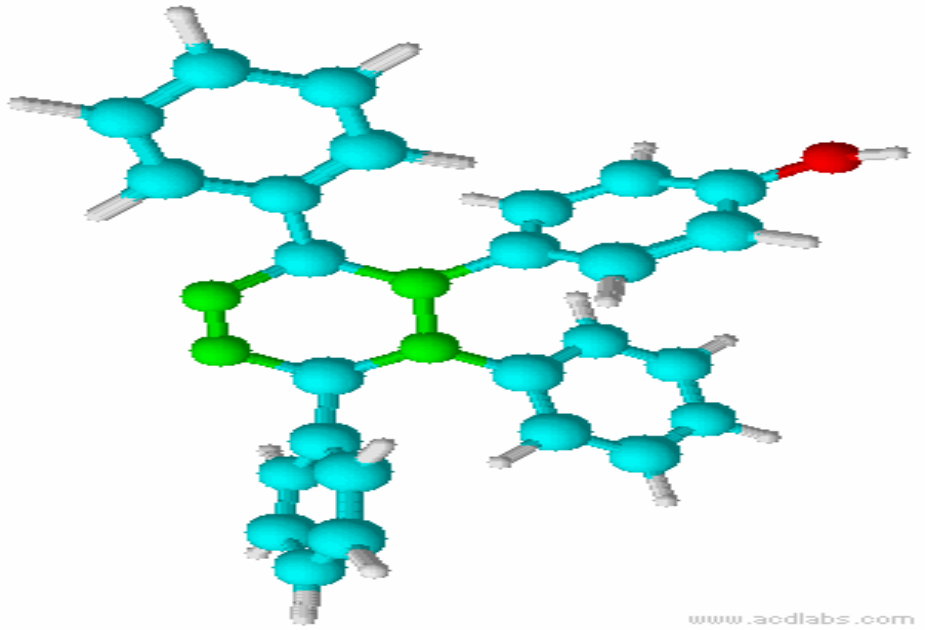


Figure 1. The optimized geometry of 4-(2,3,6-triphenyl-3,6-dihydro-1,2,4,5-tetrazin-1*(2H)*-yl)phenol **7a**.

Material and methods

Healthy adult cross-breed albino male rats (200-250g) were used in the study. The animals were kept in plastic polypropylene cages under standardized conditions viz., temperature (27-31 ^{o}C), relative humidity (50-55%), photoperiod, approximately 12 h natural light per day with a continuous flow of tap water and amrut brand balanced feed except at the time of experimental work. All efforts were made to provide favorable residing conditions to the rats.

Anti-inflammatory activity

Carrageenan-induced paw edema

Fourty-four male rats were selected and randomly divided into five groups. Groups 1, 2, and 3 were treated orally with 500, 1000, 1500 mg/Kg of 1,2,3,6-tetrahydro-1,2,4,5-tetrazines **7a** and **7b** as suspensions respectively. The rats of group 4 were treated with 1 ml of distilled water. The rats in the fifth group were treated with 5 mg/Kg Etrocoxib, the reference drug. After 1 hour 0.05 ml of 1% carrageenan suspension was injected subcutaneously into the hind paw of each rat as described by Winter *et al.* [15] in 1962. The volume of the injected paw of each of these rats were measured using a Plethysmometer method by Harris and Spencer[16] at 1 hour prior to the injection of carrageenan and 1, 2, 3, 4 and 5 h after the injection.

Table 1. Effect of oral administration of 4-(2,3,6-triphenyl-3,6-dihydro-1,2,4,5-tetrazin-1*(2H)*-yl)phenol **7a** on the *carrageenan-induced paw edema* in rats (mean ± SEM)

	Paw volume (ml)				
Treatment	First hour	Second hour	Third hour	Fourth hour	Fifth hour
500 mg/Kg (n=6)	20.62 ± 4.61	28.11 ± 3.22	32.64 ± 6.11	34.28 ± 3.11	34.21 ± 5.65
1000 mg/Kg (n=6)	17.26 ± 4.32	29.84 ± 4.11	38.12 ± 2.41	33.24 ± 4.10	33.41 ± 4.43
1500 mg/Kg (n=6)	22.54 ± 4.30	30.44 ± 4.38	35.68 ± 3.25	28.67 ± 3.42	25.55 ± 3.48
Control (n=6)	30.15 ± 6.05	50.15 ± 3.51	58.32 ± 6.15	60.24 ± 3.75	58.11 ± 4.25
Etrocoxib (n=6)	6.35 ± 3.29	12.16 ± 5.54	10.14 ± 5.22	9.65 ± 3.42	6.88 ± 4.01

Table 2. Effect of oral administration of 4-[3,6-diphenyl-2-(4-nitrophenyl)-3,6-dihydro-1,2,4,5-tetrazin-1(2*H*)-yl]phenol **7b** on the *carrageenan-induced paw edema* in rats (mean ± SEM)

	Paw volume (ml)				
Treatment	First hour	Second hour	Third hour	Fourth hour	Fifth hour
500 mg/Kg (n=6)	22.24 ± 4.21	30.36 ± 4.31	33.18 ± 3.42	36.24 ± 3.54	35.35 ± 5.11
1000 mg/Kg (n=6)	18.26 ± 4.15	30.84 ± 5.21	36.12 ± 5.91	31.24 ± 3.28	29.41 ± 4.78
1500 mg/Kg (n=6)	22.54 ± 3.46	30.44 ± 4.29	35.68 ± 4.55	28.67 ± 6.08	25.55 ± 5.14
Control (n=6)	39.16 ± 3.61	54.42 ± 5.53	59.44 ± 5.12	61.12 ± 5.41	54.11 ± 3.79
Etrocoxib (n=6)	11.18 ± 4.47	17.25 ± 4.91	15.38 ± 4.48	13.44 ± 5.75	10.88 ± 3.45

The level of inhibition (%) of edema was calculated using the relation:
Inhibition (%) = 100[1-(Et/Ec)],
Where,

Et= Average edema of the treated group
Ec=Average edema of the control group

Table 3. Effect and % inhibition of 1,2,3,6-tetrahydro-1,2,4,5-tetrazines **7a** and **7b** on paw-edema

Entry	Drug	Dose	Average Edema Et/Ec	% Inhibition
1	**7a**	1000mg/Kg	30.37	40.90
2	Control-**7a**	-	51.39	-
3	**7b**	1000mg/Kg	29.17	45.63
4	Control-**7b**	-	53.65	-

Statistical analysis

The data are expressed as the mean ± SEM. Statistical analysis was performed using the Mann-Whitney *U* test. Significant values were set at $p \leq 0.05$. Linear regression analysis was performed to assess dose dependencies.

Results

Carrageenan-induced paw edema

The results presented in Table 1 and 2 revealed a marked and significant reduction in paw edema as compared to control at each time in relevant hours. This anti-inflammatory activity was dose-dependant and found to be statistically significant at the medium concentration i.e., 1000mg/ Kg (Table 1 & 2) for 1,2,3,6-tetrahydro-1,2,4,5-tetrazines. The anti-inflammatory activity of Etrocoxib, a standard reference drug, was also found to be significant but this anti-inflammatory effect was much stronger (>75%). The percent inhibition is presented in Table 3.

Experimental Section

General Procedures. All the chemicals used were of AR grade purity. IR spectra were recorded on a Perkin Elmer model 377 spectrophotometer in KBr pellets. ^{1}H and ^{13}CNMR spectra were recorded in $CDCl_3$ solution at 25^{o}C on a Bruker DRX-300 instrument (300 MHz FT NMR with low and high temperature facility -90 ^{o}C to +80 ^{o}C) with deuterium signal as the lock and TMS as internal standard. Chemical shifts were measured in ppm units. The FAB mass spectra were recorded on a JEOLSX102/DA – 6000 Mass Spectrometer using Argon/Xenon (6 kV, 10 mA) as the FAB gas. Analytical thin layer chromatography was performed using E. Merck silica gel G,

0.50 mm plates, (Merck No. 5700). The melting points were determined on an electric melting point apparatus in open capillaries and are uncorrected.

Synthesis of diazadiene 2

The diazadiene **2** was prepared by condensing hydrazine hydrate (0.05 mol) and benzaldehyde (0.10 mol) in the presence of 50% glacial acetic acid (10 ml). Diazadiene **2** was obtained in good yield (80%) as the yellow green crystalline product (mp 94 ^{o}C). It was washed with cold water and recrystallized from ethanol.

Preparation for 1,2,3,6-tetrahydro-1,2,4,5-tetrazines 7a and 7b

To a solution of diazadiene **2** (0.01 mol) in dry xylene was added dienophile 4-[phenyldiazenyl]phenol **6a** or 4-[(4-nitrophenyl)diazenyl]phenol **6b** (0.01 mol) in equimolar quantities. The solution was then refluxed for he required time with constant stirring. At the end of the reaction, solvent was distilled off at reduced pressure and the crude cycloadduct **7a** and **7b** was recrystallized and purified by TLC on silica gel (E Merck) using ethyl acetate-xylene (4:6, v/v).

Adduct **7a**. The reaction was performed according to the general procedure starting with 1.98 g of dienophile **6a**. The cycloadduct was isolated as red brown viscous compound in 65% yield.
mp (^{o}C) dec, IR (KBr) (cm^{-1}) 3570(OH str.), 3033 (=C-H, sp^2), 2986 (C-H, sp^3), 1621 (C=C), 1541 (N=N), 1458, 1371 (C-H, bending, sp^3), 1071 (C-O), 1046 (C-N), 886, 763, 676 (sub. phenyl); ^{1}H NMR (δ ppm) 3.15 (s, 1H, -CH-Ph), 3.37 (s, 1H, -CH-Ph), 6.85 (dd, 2H, -C_6H_4-OH, *J*=8.5 Hz, *J*=2.7 Hz), 7.17 (dd, 2H, -C_6H_4-OH, *J*=8.1 Hz, *J*=2.8 Hz), 7.21-7.67 (m, 15H, -phenyl), 11.75 (s, 1H, -OH); ^{13}C NMR (δ ppm) 77.0 (C-3, C-6), 109.0, 113.2, 116.0, 120.3, 123.8, 129.9, 130.3 (Ph/Ar); FAB-MS *m/z* 407; Anal. Calcd (%) for $C_{26}H_{22}N_4O$: C, 76.83; H, 5.46; N, 13.78. Found: C, 76.78; H, 5.41; N, 13.72.
Adduct **7b**. The reaction was performed according to the general procedure starting with 2.43 gm of dienophile **6b**. The cycloadduct was isolated as reddish brown solid in 78% yield.
mp (^{o}C) 128-130, IR (KBr) (cm^{-1}) 3556 (OH str.), 3028 (=C-H, sp^2), 2987 (C-H, sp^3), 1615 (C=C), 1548 (N=N), 1548 (-NO_2), 1467, 1372 (C-H, bending, sp^3), 1061 (C-O), 1044(C-N), 884, 761, 669 (sub. phenyl); ^{1}H NMR (δ ppm) 3.19 (s, 1H, -CH-Ph), 3.32 (s, 1H, -CH-Ph), 6.78 (dd, 2H, -C_6H_4-OH, *J*=7.9 Hz, *J*=2.9 Hz), 7.31 (dd, 2H, -C_6H_4-OH, *J*=8.1 Hz, *J*=3.0 Hz), 7.40-7.63 (m, 10H, -phenyl), 7.75 (dd, 2H, -C_6H_4-NO_2, *J*=8.5 Hz, *J*=2.8 Hz), 7.91 (dd, 2H, -C_6H_4-NO_2, *J*=8.7 Hz, *J*=2.9 Hz); 77.4 (C-3, C-6), 109.6, 111.3, 112.1, 117.8, 122.6, 128.5, 128.8, 145.9, 147.5 (Ph/Ar); FAB-MS m/z 452; Anal. Calcd (%) for $C_{26}H_{21}N_5O_3$: C, 69.17; H, 4.69; N, 15.51. Found: C, 69.11; H, 4.65; N, 15.49.

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