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Research Article

Synthesis and Pharmacological Evaluation of Few 2-(Substituted Aryl)-4-(Heteroaryl)-2,3-Dihydro-1*h*-1,5-Benzodiazepine Derivatives

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ABSTRACT

Benzodiazepines represent a class of privilege scaffold in the modern era of medicinal chemistry as central nervous system active agents. The present work reported synthesis of a series of 2-(substituted aryl)-4-(heteroaryl)-2,3-dihydro-1H-1,5-benzodiazepines. Structural characterization of title compounds was done by using analytical techniques such as IR and ^{1}H-NMR. An acute toxicity study was done to determine the LD_{50} of the compounds. The compounds were subjected to pharmacological studies to evaluate anticonvulsant potential in mice by strychnine-induced convulsions method. The pharmacological evaluation of the compounds showed an increase in latency (onset time) to induce convulsions and a decrease in convulsions compared to control. The compounds B3, B6 and B8 showed the highest percentage protection as 80% with latency to induce convulsions as 6.46, 6.35 and 6.12 minutes, respectively compared to control at 3 mg/kg. The structural features analysis revealed that the phenyl ring's second position substituted with halogen, hydroxy group of 2-(substituted phenyl)-4-(furan-2-yl/thiophene-2-yl)-2,3-dihydro-1*H*-1,5-benzodiazepine enhanced the anticonvulsant potential of the compounds.

Introduction

Benzodiazepines are a crucial class of organic molecules with a good array of biological activities and therapeutic functions. Hoffmann-La Roche chemist Leo Sternbach identified the first benzodiazepine, chlordiazepoxide in 1955. By 1960, Hoffmann-La Roche marketed it as 'Librium'; therefore, the research team pursued molecular modifications for enhanced activity. Valium (Diazepam), stamped with its trademark 'V' and named supported the Latin Valere (be strong), followed in 1963. Both drugs were tremendously successful, largely replacing older sedatives and hypnotics by 1970. Medical professionals greeted benzodiazepines enthusiastically initially, skyrocketing their popularity and patient demand.[1] There are various reports of the synthesis of benzodiazepines derivatives, few of these have been mentioned here. 2,3-dihydro-1*H*-1,5-benzodiazepines have been synthesized in solvent-free conditions from *o*-phenylenediamines and ketones in the presence of a catalytic amount of acetic acid, under microwave irradiation. This method is a very easy, rapid and high yielding reaction for the synthesis of 1,5-benzodiazepine derivatives.[2] The reaction of *o*-phenylenediamines with both acyclic and cyclic ketones in the ionic liquid 1,3-di-*n*-butylimidazolium bromide

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afforded 1,5-benzodiazepines in excellent isolated yields in the absence of a catalyst at ambient temperature.[3] Novel 1,5-benzodiazepine derivatives were rationally designed and synthesized according to the principle of superposition of bioactive substructures by the combination of 1,5-benzodiazepines, thiophene or thiazole and ester group. A preliminary study of the structure–activity relationship revealed that substituents in the phenyl ring and the thiophene ring greatly affected the antimicrobial activity of these compounds. In addition, the thiazole ring at C_2 may be a pharmacophore of these compounds, and the $COOC_2H_5$ group at C3 is the best substituent for maintaining antimicrobial activities at low concentrations.[4] A crystalline iron-basedmetal–organic framework MOF-235 was synthesized. The MOF-235 was used as a heterogeneous catalyst for the synthesis of 1,5-benzodiazepines by the cyclocondensation of 1,2-diamines with ketones. Excellent conversions to 1,5-benzodiazepines were achieved in the presence of 5 mol% MOF-235 catalyst using molecular oxygen in air as the stoichiometric oxidant.[5] Pharmacodynamic studies of 1,5-benzodiazepin-2,4-diones and allyl derivatives have sedative, myorelaxant and anxiolytic actions and anticonvulsant properties.[6] A one-pot protocol involving nitrile-derived amidoxime of 1,5-benzodiazepine to synthesize its pyrimidine derivatives using dimethyl acetylenedicarboxylate and 1,4-diazabicyclo [2.2.2] octane catalyst under microwave conditions has been described. The antibacterial activity of the synthesized compounds was examined against gram-positive *S. aureus* and Gram-negative *E. coli* using broth micro-dilution assay.[7]

Benzodiazepines usually occur within the diimine form rather than within the conjugated amidine form. Within the diimine form some extra stabilization arises because of the conjugation of the imine group with the benzene ring. Protonation of benzodiazepines cause the successive formation of monocations. The conjugated form which might have 8-pi- electrons related to a 7-membered ring is electronically an analogue of benzocyclo-octatetraene. Annular conjugation around either the diazepine ring or the general periphery makes no positive contribution to the stableness of the system, whereas electronic interaction between the benzene formula and two imino groups within the imino form does.[8]

Inspired from the therapeutic potential of Benzodiazepines, in the present work a series of 2-(substituted aryl)-4-(heteroaryl)-2,3-dihydro-1*H*-1,5-benzodiazepines was synthesized by condensing substituted chalcones with o-phenylenediamine or substituted o-phenylenediamine in the presence of piperidine as a catalyst with the aim to identify 'leads' with better anticonvulsant potential.

Material and Methods

All of the materials used in the experiment were purchased locally and are of laboratory quality. The course of the reaction is monitored using thin layer chromatography (TLC), and the products are purified by recrystallization from ethanol. On a silica gel G covered plate with a thickness of 0.5 mm, the purity of the chemicals was evaluated using TLC solvent system as n-hexane and Ethyl acetate (8:2). The spectral investigations, IR, and ^{1}H-NMR were determined using standard techniques. Infrared spectra were recorded using a Shimadzu IR Affinity-1 device. Brucker Avance II 400 MHz NMR Spectrometer in DMSO-d6 was used to produce the ^{1}H-NMR spectra at Central Instrumentation Facility, Savitribai Phule Pune University, Pune.

General Procedure

Synthesis of 3-(substituted phenyl)-1-(furan-2-yl/thiphene-2-yl) prop-2-en-1-one

An equimolar portion of the appropriately substituted aryl aldehydes and 2-acetyl furan or 2-acetyl thiophene were dissolved in 25 mL of ethanol. The mixture was allowed to stir for 30 minutes at 5–10°C. 1-mL aliquot of a 10% methanolic sodium hydroxide solution was added dropwise to the reaction mixture. The reaction solution was allowed to stir at room temperature for approximately 4 to 10 hours. Progress of the reaction was monitored via TLC by using solvent system A. After the reaction was completed, excess solvent was evaporated from the mixture using rotary evaporator. The reaction mixture was poured into crushed ice and acidified with HCl. Precipitate was filtered dried and recrystallized from ethanol (Fig. 1).[9]

Synthesis of 2-(substituted phenyl)-4-(furan-2-yl/thiophene-2-yl)-2,3-dihydro-1H-1,5-benzodiazepines.

An equimolar mixture of substituted chalcone and o-phenylene diamine was dissolved in absolute ethanol (30 mL) in the presence of piperidine, and the reaction mixture was refluxed for about 8 to 10 hours. Progress of the reaction was monitored via TLC. After completion of the reaction, the reaction mixture was poured into crushed ice. The product obtained was filtered, washed with cold water. The compounds were obtained as yellow, brown, and dark brown crystals and it was recrystallized from ethanol (Fig. 2).

Pharmacological evaluation

Ethical Approval

Institutional Animal Ethical Committee approved the animal study protocol.

Acute Toxicity Studies

According to OECD guidelines (no. 425), the compounds' median Lethal Dose (LD_{50}) was determined. Each animal was closely observed for symptoms of toxicity and mortality in the first 30 minutes following dosage, as well as periodically for a further 4 hours and then every day after that for a period of 14 days. The number of mice that died over a 48 hours period was counted.

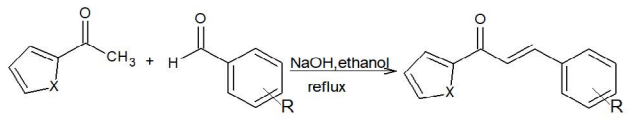

Fig. 1: Synthesis of 3-(substituted phenyl)-1-(furan-2-yl/thiphene-2-yl) prop-2-en-1-one (chalcones)

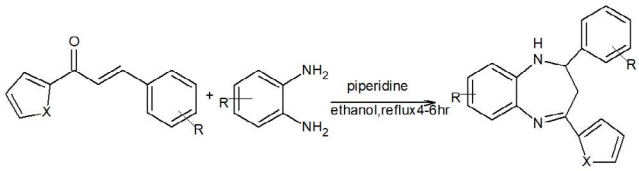

Fig. 2: 2-(substituted phenyl)-4-(furan-2-yl/ thiophene-2-yl)-2,3-dihydro-1H-1,5-benzodiazepines

Anticonvulsant Activity

The drugs and chemical solutions were freshly prepared. Strychnine hydrochloride (Dose: 3 mg/kg, i.p.), a stock solution containing 0.3 mg/mL in distilled water. Diazepam (Dose: 2 mg/kg, i.p.), a stock solution containing 0.2 mg/mL in distilled water. Test compounds (3 mg/kg, i.p.) a suspension in Tween 80 containing 0.3 mg/mL.

The animals were first weighed and those within the 18 to 22 gm range were selected for the experiment. The animals were then divided into ten groups of six animals each. One group is used for studying the effects of strychnine hydrochloride alone (Control) and the other for studying the protection effects of diazepam (Standard). The remaining groups were used to study the synthesized compound's effects (Test). Strychnine hydrochloride (which caused clonic seizures in 100% of the animals) was injected after 30 minutes of administration of the diazepam or test compound, the animals were placed in individual cages and observed for 30 minutes. The latency of onset of convulsion, number of convulsions and percentage protection as compared to the control were recorded[10]. In case of diazepam treated animals complete abolition of convulsions was noted. The results are shown in Table 1.

Results and Discussion

2-(2-chlorophenyl)-4-(thiophen-2-yl)-2,3-dihydro-1H-1,5- benzodiazepine (B1)

White crystalline solid in 68% yield; mp: 180-184°C; R_f 0.63 ; IR Vmax (KBr cm^{-1}): 3367 (N-H str), 1516 (N=C str), 1354 (C-N str), 725 (C-Cl); ^{1}H-NMR (400MHz, δ ppm, DMSO-d6): 1.8 (d, 2H, CH_2), 3.4 (t, 1H, C-H), 4.4 (S, 1H, N-H), 6.89-7.22(m, 4H, BZD), 6.93-7.75(m, 3H, Thio), 7.23-7.72 (m, 4H, Ar-H), MS: m/z =337.25 [M + 1]

2-(4-hydroxyphenyl)-4-(thiophen-2-yl)-2,3-dihydro-1H-1,5- benzodiazepine (B2)

Off White solid in 72% yield; mp: 192–194°C; R_f 0.65 ; IR Vmax (KBr cm^{-1}): 3367 (N-H str), 1566 (N=C str), 1473 (C=C str), 1354(C-N str), 1225 (C-O), 3132 (O-H); ^{1}H-NMR (400MHz, δ ppm, DMSO-d6): 1.7 (d, 2H, CH_2), 3.4 (t, 1H, C-H), 4.34 (S, 1H, N-H), 5.2 (S, 1H, O-H), 6.8-7.28(m, 4H, BZD), 6.9-7.72(m, 4H, Ar-H), 6.93-7.75(m, 3H, Thio); MS: m/z = 321.74[M + 1]

2-(4-chlorophenyl)-4-(thiophen-2-yl)-2,3-dihydro-1H-1,5- benzodiazepine (B3)

White solid in 79% yield; mp: 96–98°C; R_f 0.68 ; IR Vmax (KBr cm^{-1}): 3363 (N-H str), 1593 (N=C str), 1465(C=C str), 1354(C-N str), 721(C-Cl); ^{1}H-NMR (400MHz, δ ppm, DMSO-d6): 1.9 (d, 2H, CH_2), 3.42 (t, 1H, C-H), 3.9 (S, 1H, N-H), 6.89-7.22 (m, 4H, BZD), 7.1-7.6 (m, 3H,furan); 7.23-7.72 (m, 4H, Ar-H); MS: m/z = 335.78 [M + 1]

4-(furan-2-yl)-2-phenyl-2,3-dihydro-1H-1,5-benzodiazepine (B4)

Brownish solid in 70% yield; mp: 162–164°C; R_f 0.61 ; IR Vmax (KBr cm^{-1}): 3356 (N-H str), 1563 (N=C str), 1411 (C=C str), 1273 (C-N str), 1161 (C-O); ^{1}H-NMR (400MHz, δ ppm, DMSO-d6): 1.92 (d, 2H, CH_2), 3.89 (S, 1H, N-H), 3.42 (t, 1H, C-H), 6.89-7.22(m, 4H, BZD), 6.93-7.6 (m, 3H, furan); 7.29-7.40 (m, 5H, Ar-H); MS: m/z = 286.56 [M + 1]

2-phenyl-4-(thiophen-2-yl)-2,3-dihydro-1H-1,5-benzodiazepine (B5)

Off White solid in 74% yield; mp: 86–90°C; R_f 0.58 ; IR Vmax (KBr cm^{-1}): 3363 (N-H str), 1600 (N=C str), 1469 (C=C str), 1273 (C-N str); ^{1}H-NMR (400MHz, δ ppm, DMSO-d6): 1.84 (d, 2H, CH_2), 3.45 (t, 1H, C-H), 3.89 (S, 1H, N-H), 6.89-7.22(m, 4H, BZD), 7.1-7.6 (m, 3H,thio), 7.23-7.72 (m, 5H, Ar-H) ; MS: m/z = 306.65 [M + 1]

2-(2-chlorophenyl)-4-(furan-2-yl)-2,3-dihydro-1H-1,5- benzodiazepine (B6)

White solid in 83% yield; mp: 98–102°C; R_f 0.64 ; IR Vmax (KBr cm^{-1}): 3356 (N-H str), 1670 (N=C str), 1454 (C=C str), 1273 (C-N str), 750 (C-Cl), 1273 (C-O); ^{1}H-NMR (400MHz, δ ppm, DMSO-d6): 1.8 (d, 2H, CH_2), 3.42 (t, 1H, C-H), 4.32 (S, 1H, N-H), 6.52-7.76 (m, 3H,furan); 6.77-7.23 (m, 4H, BZD), 7.23-7.72 (m, 4H, Ar-H); MS: m/z = 323 [M + 1]

2-(2-chlorophenyl)-4-(furan-2-yl)-7-nitro-2,3-dihydro-1H-1,5- benzodiazepine (B7)

Yellow solid in 68% yield; mp: 130–134°C; R_f 0.62 ; IR Vmax (KBr cm^{-1}): 3348 (N-H str), 1662 (N=C str), 1454 (C=C str), 1338 (C-N str), 748 (C-Cl), 1519 (N=O); ^{1}H-NMR (400MHz, δ ppm, DMSO-d6): 1.9 (d, 2H, CH_2), 3.42 (t, 1H, C-H), 3.89 (S, 1H, N-H), 6.89-7.22 (m, 4H, BZD), 7.1-7.6 (m, 3H,furan), 7.23-7.72 (m, 5H, Ar-H); MS: m/z = 366.78 [M + 1]

2-(2-hydroxyphenyl)-4-(thiophen-2-yl)-2,3-dihydro-1H-1,5- benzodiazepine (B8)

White solid in 81% yield; mp: 146–150°C; R_f 0.67 ; IR Vmax (KBr cm^{-1}): 3178 (N-H str); 1577 (N=C str), 1469 (C=C str), 1354 (C-N str), 3440 (O-H); ^{1}H-NMR (400MHz, δ ppm, DMSO-d6): 1.7 (d, 2H, CH_2), 3.4 (t, 1H, C-H), 4.54 (S, 1H,

Table 1: Anticonvulsant effect of 2-(substituted phenyl)-4-(furan-2-yl/ thiophene-2-yl)-2,3-dihydro-1H-1,5-benzodiazepine derivatives in mice using Strychnine induced convulsions method

Code	*Dose (mg/Kg, i.p)*	*Latency to induce convulsions (Minutes ± SEM)*	*No. of convuls-ions*	*Percentage Protection*
Strychnine hydrochloride (Control)	3	2.20 ± 0.05	5	0
Diazepam (Standard)	2	-	0	100
B1	3	2.96 ± 0.11	4	20
B2	3	4.20 ± 0.09	3	40
B3	3	6.46 ± 0.19	1	80
B4	3	3.98 ± 0.22	3	40
B5	3	3.07 ± 0.12	4	20
B6	3	6.35 ± 0.20	1	80
B7	3	4.18 ± 0.22	3	40
B8	3	6.12 ± 0.14	1	80

N=6, in each group; *:p< 0.0001: S: significant; One Way ANOVA followed Dunnett's test. Value expressed as Mean ± SEM

N-H), 5.4 (S, 1H, O-H), 6.89-7.22 (m, 4H, BZD), 7.1-7.6(m, 3H,Thio), 7.23-7.72 (m, 3H, Ar-H); MS: m/z = 321.09 [M + 1]

Spectral Analysis

The structure of benzodiazepine nucleus was confirmed from IR and ^{1}H-NMR spectra of N-H. The FTIR spectra of the compounds displayed the presence of typical absorption bands (cm^{-1}) in the region at 3310–3350 (N-H str, secondary amine), near 1475 (C=C Ar-), 1430–1600 (N=C str), 1000–1350 (C-N str), 650–900 (C-H Ar-), The ^{1}H-NMR spectrum showed -CH_2 (2H) as doublet at 1.70-1.92 δ ppm; N-H (1H) as singlet at 3.89-4.54 δ ppm; -CH (1H) as triplet at 3.40-3.45 δ ppm. The molecular weight is confirmed from the molecular ion peak in Mass spectra

Anticonvulsant Activity Study

2-(substituted phenyl)-4-(furan-2-yl/ thiophene-2-yl)-2,3-dihydro-1*H*-1,5-benzodiazepine derivatives were subjected to acute oral toxicity (AOT) to determine the LD_{50} of compounds based on the OECD 425 guidelines. The doses were calculated by using the AOT425 instate software. Each animal was monitored for signs of toxicity and mortality in the first 30 minutes after dosing, then every 4 hours for the next 14 days. The LD_{50} was 29.57 mg/Kg. The dose 3 mg/Kg (approx. $1/10^{th}$ that of LD_{50}) was selected for evaluation. The pharmacological evaluation of the compounds revealed that they delayed the onset of convulsions and reduced the number of episodes of convulsions when compared to a control group. As well as the survival or mortality of the animals was also recorded to calculate percentage protection. When compared to the control, compounds B3, B6 and B8 provided 80% protection with the latency to induce convulsions was 6.12-6.46 minutes compared to 2.20 minutes that of the control.

Conclusion

The synthesis of novel 2-(substituted phenyl)-4-(furan-2-yl/ thiophene-2-yl)-2,3-dihydro-1*H*-1,5-benzodiazepine derivatives comprises of two steps viz. first Step, synthesis of 3-(substituted phenyl)-1-(furan-2-yl) prop-2-en-1-one and 3- (substituted phenyl)-1-(thiophene-2-yl) prop-2-en-1-one (Chalcones) from substituted aromatic aldehydes and 2-acteyl furan or 2-acetyl thiophene in presence of sodium hydroxide at cold conditions; second step, condensation of the chalcones with o-phenylene diamine or substituted o-phenylene diamine in presence piperidine as catalyst afforded the title compounds. The completion of reaction was monitored by TLC method. Structural characterization of title compounds was done by using analytical techniques such as IR and ^{1}H-NMR. Acute toxicity study was done to determine the LD_{50} of the compounds. The compounds were subjected to pharmacological studies to evaluate anticonvulsant potential in mice by strychnine-induced convulsions method. The pharmacological evaluation of the compounds showed an increase in latency (onset time) to induce convulsions and a decrease in convulsions compared to control. On the basis of the present investigation following conclusions are outlined: The compounds B3, B6 and B8 showed highest percentage protection as 80% with latency to induce convulsion upto 6.46 minutes compared to control the dose 3 mg/kg. The pharmacological evaluation of the compounds showed an increase in latency (onset time) to induce convulsions and a decrease in convulsions compared to control. The structural feature analysis revealed that the phenyl ring's second position substituted with halogen, hydroxy group of 2-(substituted phenyl)-4-(furan-2-yl/ thiophene-2-yl)-2,3-dihydro-1*H*-1,5-benzodiazepine enhanced the anticonvulsant potential of the compounds.

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Conflict of Interest

There is no any conflict of interest.

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