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Review Article

Recent Structure Activity Relationship Studies of 1,4-Benzodiazepines

Abstract

Structure activity relationship studies of 1,4-benzodiazepines have been discussed especially with their effects as antianxiety and anticonvulsants. The currently available benzodiazepines are associated with various side effects. Nowadays the purpose of these studies is to minimize side effects with these drugs. A very little alteration is possible on the benzene ring while the modification can be done on the diazepine ring. It can adopt the different conformations and in some cases some aromatic and heterocyclic rings have been fused with this part in order to see the effect of these conformation blockers on the pharmacological activity. The structure activity studies are also linked to molecular modeling studies. This is important in adding some information for the interaction of these drugs with the receptors and how this interaction can be improved.

Introduction

Benzodiazepines (BDZs) are the bicyclic heterocyclic compounds in which benzene ring is fused to seven membered diazepine ring containing two nitrogen. There are different types of BDZs such as 1,2-benzodiazepines, 1,3-benzodiazepines, 1,4-benzodiazepines, 1,5-benzodiazepines, 2,3-benzodiazepines. 1,4-BDZs possess anxiolytic, anticonvulsant, muscle relaxant properties. They are widely used as a treatment of anxiety, insomnia, epilepsy, alcohol with drawl, for anaesthesia and about 500 million peoples have been treated with BDZs [1]. There are two types of BDZs receptors, central BDZs receptors and peripheral BDZs receptors. Physiological functions of peripheral BDZs receptors are different from the central BDZs receptors [2]. BDZs produce their effect by binding to the central BDZs receptors which are located at the post and presynaptic membranes [3]. Their binding to γ aminobutyric acid (GABA$_A$) receptors increases the chloride ion conductance by inducing some conformational changes and causes inhibition of action potential [4]. BDZs enhance the effect of neurotransmitter GABA at their receptors.

Use of BDZs is increased from the last 15 and 20 years and now they are widely prescribed. Side effects associated with the use of these drugs such as drowsiness, tolerance, addiction and withdrawal potential causes problems [5]. The discovery of selective ligands for the central BDZs receptors, which have the anxiolytic activity without producing these side effects, is important. Docking of BDZs with the GABA receptors determine the binding analysis of drug with BDZs receptors [6]. The main objective of this is the discovery of product with narrow spectrum of activities.

In this paper some structure modifications studies carried out on different position of the 1,4-BDZs in the last two decades have been summarized. Studies were focused especially on their antianxiety, anticonvulsant and muscular relaxant effects.

Chemistry and biological activity

The discovery of BDZs dates back to 1950 and the first agent which was introduced in the market was chlordiazepoxide. Diazepam, the second member of this series was more active [7]. Introduction of substituent is very important in BDZs especially the position 7. The potency of these compounds also increases by adding the substituent's in ring B and C [8]. Triazolo BDZs which contains triazole ring fused with diazepine nucleus are most potent agents in this class [9,10](Figure 1).

Furan and thiophene are the bioisosteres of the phenyl ring [11]. 5-thienyl and 5-furyl substituted BDZs were synthesized and evaluated for their affinity at the BDZs receptors in order to get information about the binding of lipophilic pocket and its effect on the biological activity. The 5-(2'-thienyl) BDZs showed high affinity for the BDZs receptors which displaced flunitrazepam from rat cortical membrane with high affinity and were anticonvulsant and muscle relaxant. When a bromine atom was substituted at position 4', the compound was less active. The 5-(3'-thienyl) and 5-(2'-furyl) were less potent. The 2'-halo substituted phenyl was more potent than the 2'-thienyl and 2'-furfuryl [12] (Figure 2).
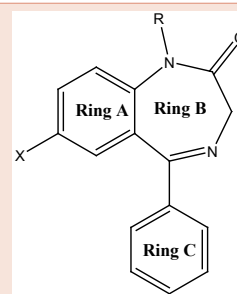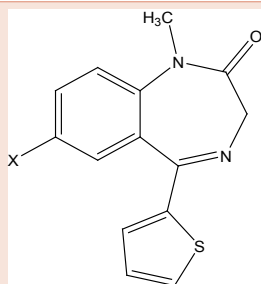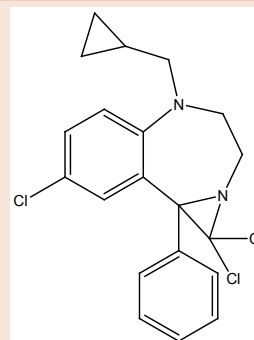

Figure 1:

Figure 2: X=H, F, Cl, N3.

Azirino BDZs are more lipophilic than the BDZs. Some azirino compounds were active as anticonvulsant but failed to displace flumazenil. The activity was evaluated as inhibitor of binding of flumazenil to the cerebral receptors. The less activity may be related to steric hindrance of the azirine nucleus so that the phenyl group cannot adopt the proper conformation for binding or may be due to the less penetration into the central nervous system (CNS) by crossing blood brain barrier [13] (Figure 3).

11-aryl-5H-imidazo [2,1-c][1,4] benzodiazepines and their 10,11 dihydro derivatives were prepared to investigate the effect of additional aromatic ring in the BDZs. The most active compound was with the 11(2'-thienyl) ring as determined for their potency to displace flumazenil from the binding site in rat brain. When the diazepine ring is reduced between positions 10-11, the activity of the compounds decreases because the reduced compound showed less affinity for the central GABA receptors as determined by radioligand binding assay [14] (Figure 4).

4-chloro-pyrimido [4,5-b][1,4] benzodiazepines were synthesized by a new method. The starting compound was the 4,6-dichloro-5-nitropyrimidines, amines and different carboxylic acids. By this method the final compound were obtained in a good yield. Although these compounds were not evaluated for their tranquilizer activity but they contain a 1,4-BDZs core and they can be interesting candidate for the antianxiety and anticonvulsant activity [15] (Figure 5).

Triazole ring system of alprazolam was modified by some aromatic ring such as pyridine, 4- florophenyl, 6-methoxy-2-naphthyl, 2-bromo-5-methoxy phenyl. Some of these compounds showed excellent anticonvulsive activity when compared with diazepam. These compounds also need further evaluation in order to clear their therapeutic profile [16] (Figure 6).

Four different series of 5-aryl-imidazo [2,1-c] [1,4] benzodiazepine were synthesized and evaluated for their central (flumazenil) and peripheral (PK11195) BDZs receptors (PBR) affinity. All compounds were found to be inactive at the central BDZs receptors. For the peripheral receptors compound 1c and 2 b-c were active. 1c, a member of methylated carboxamide derivatives was highly active which indicates the methyl group at position 10 increases the activity. The introduction of chlorine at position 7 significantly increases the activity as compared to the unsubstituted analogues indicating that it might interact with the lipophilic core of PBR. In the oxidized derivatives the activity is associated with the presence of unsaturation between positions 10-11 [17] (Figure 7).
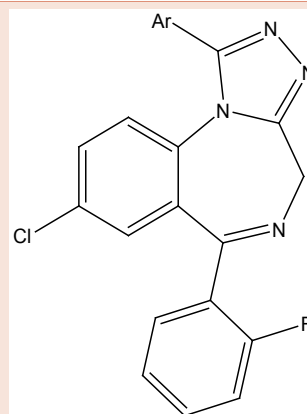

Figure 3:
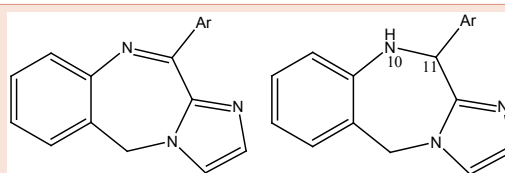
Figure 4: (Ar = phenyl, p-fluoro phenyl, o-fluoro phenyl, p-methoxy phenyl, thienyl, furyl).
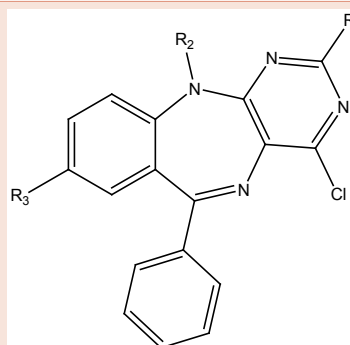
Figure 5: R1=H, R2=CH3, R3=Cl , F, CH3, OH.
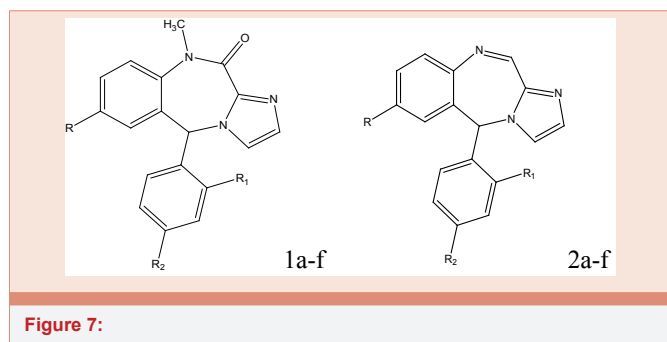

Figure 6:

Figure 7:

Figure 8: R= Cl, Br.

	a	b	c	d	e	f
R	Cl	Cl	Cl	H	H	H
R1	H	H	Cl	H	H	Cl
R2	H	Cl	H	H	Cl	H

Another approach of fusion of heterocyclic ring with the BDZs nucleus was the synthesis of 1-[(*p*-substituted)phenyl]-3a-[o-and *p*-substituted)-phenyl]-5-chloro-9-methylthio-10,3a-dihydro-[1,2,4]-oxadiazolo [2,3-*b*][1,4] benzodiazepines. These compounds are prepared by the reaction of 2-methylthio-5-[(*o*- *p*-substituted)-phenyl]-3*H*-7-chloro-[1,4] benzodiazepine with the benzonitrile oxide amoyl chloride and the resultant mixture were purified by the column chromatography. These compounds also showed the potential for antianxiety, anticonvulsant activity [18] (Figure 8).

Synthesis of ethyl 8-fluoro-6-(4-nitrophenyl) and ethyl 8-fluoro-6-(3-nitrophenyl)-4H-imidazo [1,5-a][1,4] benzodiazepine-3-carboxylates was carried out and their ability to inhibit the binding of flunitrazepam on the bovine and human cerebral BDZs receptors (CBR) was evaluated. The second compound was more active as antianxiety when tested in animals and also did not show side effects of BDZs. The docking result of this compound with the GABA receptor showed that the nitro group is involved in hydrogen bonding with the Thr193 of the receptors [19] (Figure 9).

3-arylamine derivatives of 1,4-benzodiazepine-2-one were prepared by the reaction of 1-methoxycarbonyl methyl-7-bromo-5-phenyl-1,2-dihydro-3H-1,4-benzoidazepines-2-one with the substituted anilines. These compounds were prepared on the basis of quantitative structure activity relationship (QSAR) studies. Affinity of the compounds for the central and peripheral BDZs receptors was determined by radio ligand method by the ability of investigated compounds to displace competitively radioligand central BDZs receptor antagonist (Flumazenil) and peripheral BDZs receptors antagonists (Pk 11195) from their binding sites on BDZs receptors. Ortho substituted nitro aniline was more active than the para and Meta nitro aniline and nitro group was determinant of biological activity [20] (Figure 10).

The introduction of substituted aniline (by imine linkage) at the position 2 of the BDZs showed increased neurotoxicity particularly when aniline is substituted at the para position with halogens atom, the most active in this series was compound with fluoro atom. The biological activity was determined by rota rod animal model and ethanol potentiation animal model [21] (Figure 11).
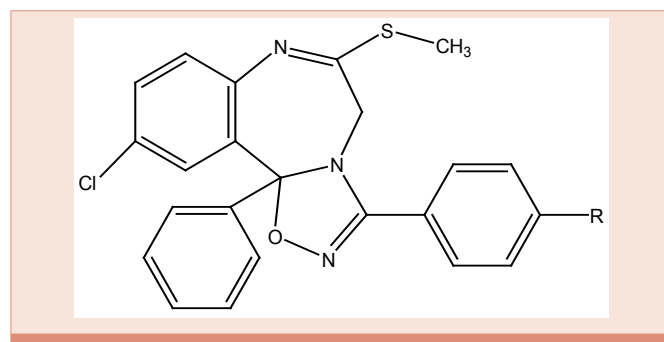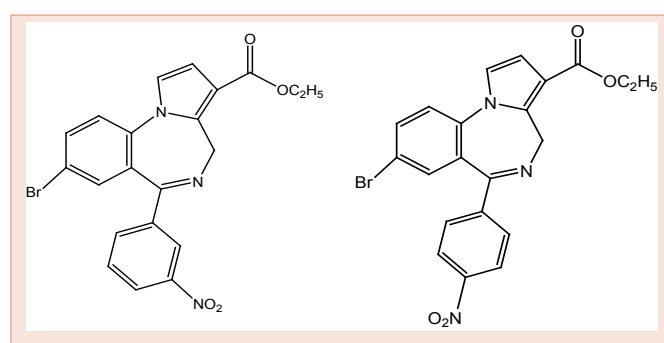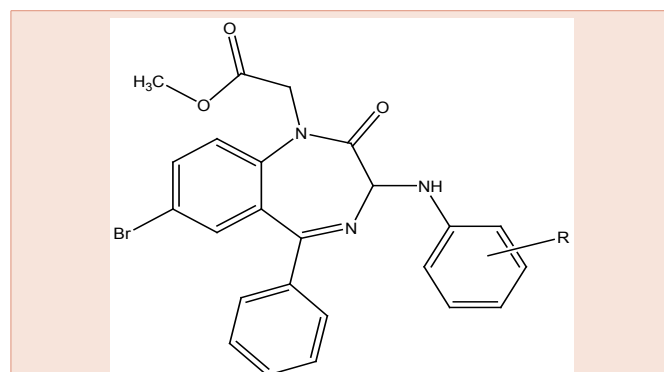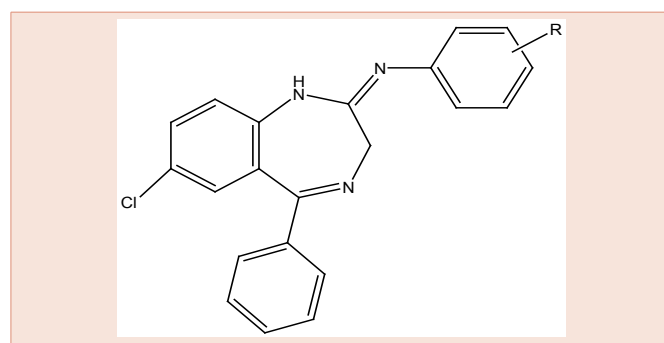

Figure 9:

Figure 10: (R= NO$_2$).

Figure 11: R= H, 3-CH3, 4-F, 2-Cl, 3-Cl 4-Cl,4-Br,2-CH3, 4-NO2, 4-(CH3)2N.

The introduction of benzylidene at the position 3 of the diazepine ring also produced the potent compounds especially when the benzylidene is substituted at para position with the electron withdrawing and lipophilic atom such as halogens. The compounds were evaluated for antianxiety activity via elevated plus maze apparatus [22] (Figure 12).

The diazonium salts derived aromatic group were attached at the position 3 and tested for their anticonvulsant activity. The compounds exhibited good anticonvulsant activity when aromatic ring was substituted with chloro, floro and nitro groups as determined by the maximal electro shock model and inhibition of pentylenetetrazole induced convulsions. This group seems to be affecting the conformation of diazepine ring which is significant for binding with the GABA receptors [23] (Figure 13).

The alkoxy were attached at the position 3 and the resulting compounds were separated into two enantiomers by chiral chromatography. The activity of S enantiomer was found greater than R enantiomer for the cerebral benzodiazepine receptors (CBR) and peripheral benzodiazepine receptors (PBR) of rat. The selectivity of compounds for the CBR was greater than the PBR [24] (Figure 14).

Sugar based pyrrole moiety was connected to the BDZs and evaluated for their GABA$_A$ receptor affinity as an inhibitor of binding of [^{3}H]Flunitrazepam. Different structure variation studies showed that N methylation and halogen substituent are important for the GABA$_A$ receptor binding. Pyrrole ring adopt a rigid conformation around the BDZs and in some cases increases the binding of these compounds. D and L fructose based pyrrole BDZs have different binding capacity. The biological activities of these compounds were less than the traditional GABA receptors ligand but they added some information regarding the conformations of BDZs while binding to the receptors [25] (Figure 15).

3-substituted 6-phenyl-4H-imidazo [1,5-a][1,4]-benzodiazepine are the compound containing the substituent at position 3 such as esters, amides and nitriles. The active compound in this series was having the ester at this position as determined by their activity to displace Flumazenil from their binding sites. Upon *in vivo* evaluation this compound showed prominent effect without the side effects. Molecular dynamics studies of the complex of BDZs with the receptors showed that binding is stable and smaller groups were active at this position while the larger bulkier groups showed less
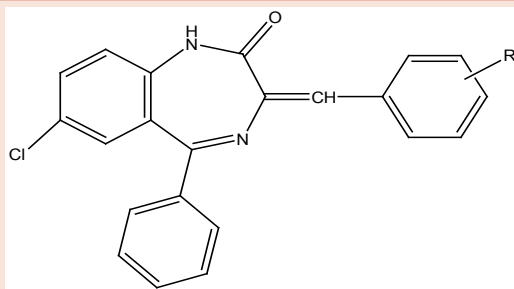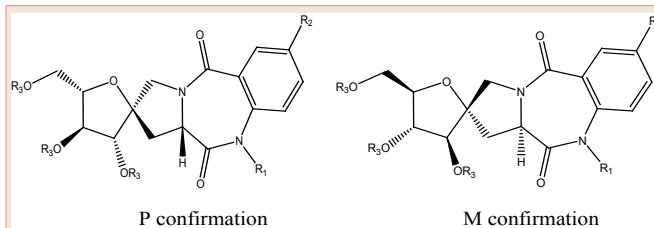

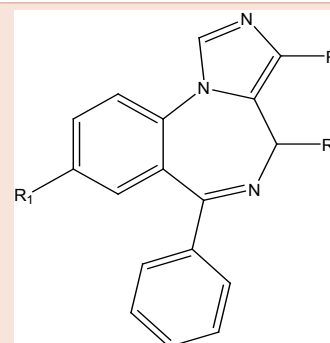**Figure 12:** (R= 4-Cl, 2-Cl, 3,4-OCH3, 3-OH, 4-OH, 2-OH, 4-OCH3, 4-F, 4-Br,4-CH3, 4-(CH3)2N).
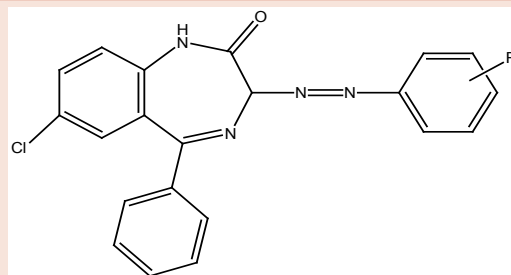
Figure 13: (R= 2-Cl, 3-Cl, 4-Cl, 2,4-di NO2 ,4-NO2 , 4-F, 4-Br, 4-CH3, 4-(CH3)2N, 2-CH3, 3-CH3).

Figure 14:

P confirmation   M confirmation
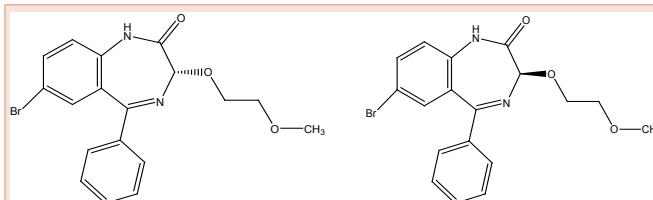
Figure 15: R1=CH3, H R2= Cl, Br, NO2, NH2 R3= OH, O-benzyl.

Figure 16: R=COOC2H5, COONH2, CN, R1=H, F, Cl, R2= H, F, Cl.

binding. The additional imidazo ring in these derivatives is involved in π - π interactions while the phenyl ring increase the hydrophobic interactions [26] (Figure 16).

Conclusion

Lot of compounds in the series of 1,4-BDZs have been synthesized with the substituent's at almost all the positions. The diazepine ring

seems to be most flexible for the various structural modification. Very little alteration is possible in benzene ring. At present structure activity relationship studies of BDZs are also associated with the molecular modeling and docking studies. Enantiomeric separation and QSAR studies of the drugs are also the subject of investigation. These information help in more clear understanding of the physiochemical properties of these drugs and their interaction with receptors at molecular level. The results of these techniques have started to appear as some new compounds exhibited their anxiolytic activity without producing undesirable effects. So in future it might be possible to get some BDZs which are active at the target receptors and without the side effects associated with the currently available BDZs.

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