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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Microwave-assisted synthesis of 11-substituted-3,3-dimethyl-2,3,4,5,10,11-hexahydrodibenzo[*b,e*][1,4]diazepin-1-one derivatives catalysed by silica supported fluoroboric acid as potent antioxidant and anxiolytic agents

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Abstract

Keeping in view the successive attributes of benzodiazepines on the central nervous system, we have synthesized a novel series of benzodiazepine derivatives as antioxidant and anxiolytic agents. All the compounds were obtained in good yield by facile synthesis. To check their antioxidant potential, DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging assay was performed. Among all the synthetics seven derivatives (**SD-3**, **SD-5**, **SD-7**, **SD-14**, **SD-18**, **SD-19**, and **SD-20**) exhibited IC_{50} values ranging from 76 to 489 nM. These seven compounds were further evaluated to check their binding abilities towards the benzodiazepine binding site on $GABA_A$ receptors. Three best-fit ligands (**SD-3**, **SD-14** and **SD-20**) were further evaluated for their anxiolytic effect by using three in vivo mice models i.e. Elevated Plus Maze, Light & Dark box, and Mirror Chamber model. The study revealed that compounds **SD-3** and **SD-20** showed the best anxiolytic effect as compared to standard drug diazepam even at an oral dose of 1.0 mg/kg.

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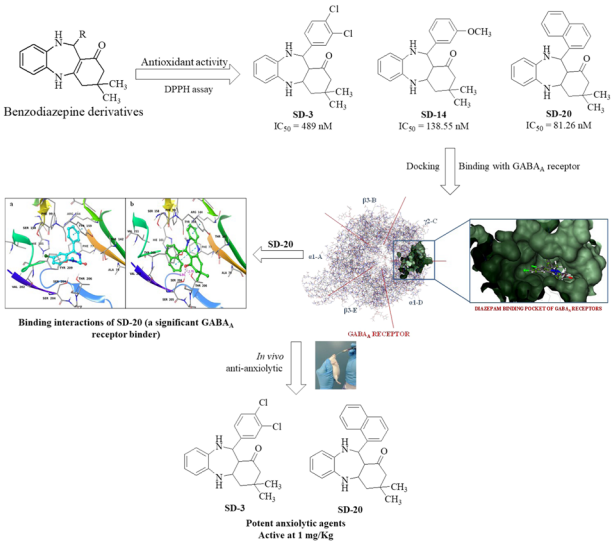
Graphical Abstract



Keywords Benzodiazepines · Antioxidant activity · Molecular modelling studies · In silico studies · In vivo anxiolytic effect

Introduction

Anxiety is a human emotion, commonly experienced by every person in their lives in unique ways such as when the problem is occurring during work, before going for exams and on making important and major decisions (https://www.nimh.nih.gov/health/topics/anxiety-disorders/index.shtml). According to the World Health Organisation (WHO) report 2015, it has been found that the rate of the global population that suffered from an anxiety disorder is estimated to be 3.6%, out of which females were found more prone as compared to males (http://ghdx.healthdata.org/gbd-results-tool). Patient suffering from anxiety disorder shows avoidance of perceived threats from the environment as well as from the internal body (Craske et al. 2009). Pharmacologically, anxiety disorder mainly associated with the dysfunction of various monoamine neurotransmitters (gamma-aminobutyric acid (GABA), dopamine, 5-Hydroxytryptamine (serotonin), and norepinephrine in the central nervous system) (Nash and Nutt 2004; Nutt and Malizia 2001). There are numerous effective psychological (cognitive behavioural therapy, CBT) and pharmacological treatments. Various antidepressants, serotonin noradrenaline-reuptake inhibitors, selective serotonin-reuptake inhibitors, and benzodiazepines are available for the treatment of anxiety.

There is a well-known relation between the increase in oxidative stress and anxiety in rodents (Oliveira et al. 2007; Masood et al. 2008; Salim et al. 2011). It has been reported that elevation in oxidative stress in mice hypothalamus leads to anxiety. Oxidative stress is a cellular or physiological condition of high concentration of ROS i.e free radicals, that cause molecular damage to cellular structures. These free radicals are associated with the development of various chronic disease conditions like cancer, degenerative and neurodegenerative diseases, heart-related complications, as well as can be involved in aging process (Ko et al. 2010), mental disorders (Dean et al. 2009; Salustri et al. 2010), chronic inflammation, arthritis, decrease the function of immune system (Pande and Akoh 2009), fibrosis, cirrhosis, and atherosclerosis (Roesler et al. 2007). Therefore, the compounds having the antioxidant potential could be beneficial to treat these disease conditions.

Benzodiazepines are the elite class of heterocyclic compounds that exhibited a wide range of pharmacological activities and are also considered as one of the most explored classes as 'privileged scaffolds' in both drug discovery and medicinal chemistry (Evans et al. 1988; Pasquale et al. 2013; Horton et al. 2003). In contrast to this, controversy still continues with the use of benzodiazepines due to the high risk of overdose and long-term risk of dementia (Gage et al. 2015; Gray et al. 2016). Nevertheless, they are still recommended for both short-term and long-term treatment of anxiety due to its strong and better efficacy than other anxiolytic agents (Krystal et al. 2015). 1,4-benzodiazepine (psychoactive drugs) involves the augmentation of the inhibitory activity of the gamma-aminobutyric acid (GABA) neurotransmitter at the GABA receptor, further resulting in therapeutic properties like sedation, anxiolytic, alcohol withdrawal, anticonvulsant,

and muscle relaxation (Wang et al. 2017). They have acquired a great reputation in medicinal chemistry for their broad biological and pharmacological activities (Cortes et al. 2007; Makaron et al. 2013). These are broadly prescribed psychotropics due to their exceptional CNS depressant activity (Michelini et al. 1996; Ookura et al. 2008). Diazepam and chlordiazepoxide had set a great example in the class of antianxiety drugs. Further modifications and refinements in these heterocycles have been made and the anxiolytic effect of benzodiazepines has been evaluated. Moreover, their structural significance and the practicality of these organic compounds as anxiolytics have achieved a distinct standard impression in the field of chemistry as a privileged structure. It has also been revealed that benzodiazepines are generally unstable and thus form toxic metabolites in the body. In this quest, researchers around the globe are making continuous efforts for the development of new benzodiazepine compounds with lesser side effects (Skopp et al. 1998; Robertson and Drummer 1998; Coassolo and Aubert 1985). In the recent past, numerous benzodiazepines were approved by Food & Drug Administration (FDA) with their improved efficacy as well as with fewer side effects (Manchester et al. 2017) which include Remimazolam (Wesolowski et al. 2016), 3-hydroxyphenazepam (Garibova and Voronina 1986), 4-chlorodiazepam (Paradis et al. 2013), Adinazolam (Venkatakrishnan et al. 2005), Clonazepam (McLean and Macdonald 1988), Cloniprazepam (Mortele et al. 2018), Desalkyfurazepam (Manchester et al. 2018), Deschloroetizolam (El-Balkhi et al. 2017), Desmethylflunitrazeapm (fonazepam) (Katselou et al. 2017), Diclazepam (Moosmann et al. 2014), Etizolam (Connor et al. 2016), Flubromazepam (Sternbach et al. 1962), Flubromazolam (Lukasik et al. 2016), Phenazepam (Maskell et al. 2014), Pyrazolam (Moosmann et al. 2013), Nifoxipam (Katselou et al. 2017), Meclonazepam (Menezes et al. 2012) (Fig. 1). The seven-membered ring in benzodiazepines has been found to be responsible for their pharmacological activities and also found to display excellent binding ability towards the GABA receptors, due to their conformational enantiomeric property (Constantinos et al. 2010; Zizhao et al. 2012). Since 1977, benzodiazepines have been used globally as the most prescribed medications. Therefore, these derivatives are the compounds of interest among the researchers around the globe. Inspired from these findings, we also have synthesized a novel series of benzodiazepine compounds for the treatment of anxiety. All the synthesized compounds were preliminarily tested for their antioxidant potential by using DPPH assay. Furthermore, the binding ability of the synthesized compounds towards the benzodiazepine binding site on the $GABA_A$ receptor was also determined. The molecules that displayed greater binding potential with the $GABA_A$ receptors were further tested in vivo to check their anxiolytic effect in mice by using three models, Elevated Plus Maze model (EPM), Mirror Chamber model and Light and Dark model.

Experimental

Chemistry

Procurement of the reagents was done from Sigma-Aldrich, CDH, and Loba, India and used without further purification. Purity of the reagents was evaluated by comparison with authentic samples and by ^{1}H NMR and ^{13}C NMR. Avance III HD 500 MHz Bruker Biospin NMR Spectrometer was used to record the NMR spectras. The spectra were recorded in DMSO-d_6 and $CDCl_3$ relative to TMS (0.00 ppm). In ^{1}H NMR chemical shifts were reported in δ values using tetramethyl silane as an internal standard with a number of protons, multiplicities (s-singlet, d-doublet) and coupling constants (J) in Hz in the solvent indicated. Melting points were determined in open capillaries and were uncorrected.

Procedure for the preparation of silica adsorbed tetrafluoroboric acid (HBF_4-SiO_2)

40% aqueous solution of HBF_4 (1.65 g) was added to the suspension of 13.35 g of silica gel (230–400 mesh) in 40 mL of diethyl ether. The mixture was concentrated and the residue thus obtained was dried under vacuum for 72 h at 100 °C to afford 0.5 $mmolg^{-1}$ HBF_4–SiO_2.

Procedure for the synthesis of benzodiazepine analogues

In a 50 mL conical flask *o*-phenylenediamine (1 mmol), dimedone (2 mmol) benzaldehyde (1 mmol) and a catalytic amount of silicate fluoroboric acid (5 mol %) was added. The resulting mixture was placed into the microwave reactor and exposed to microwave radiation in a microwave synthesizer operating at 150 °C with the maximum microwave power of 400 W. After the completion of the reaction, the crude mixture was dissolved in methanol and adsorbed on silica (60–120#). The desired product was purified by column chromatography with an increasing percentage of ethyl acetate in hexane as eluting solvent.

Rest of the compounds were synthesized by the above-mentioned procedure. The characterization data for all the synthesized compounds are given below.

11-phenyl-3,3-dimethyl-2,3,4,5,10,11-hexahydrodibenzo[*b, e*][1,4]diazepin-1-one (SD-1) Yellowish solid; Yield 85%, mp: 174–176 °C, ^{1}H NMR ($CDCl_3$, 500 MHz, δ): 1.05–1.11 (6H, m, -CH_3), 2.16–2.25 (2H, m, -CH_2-), 2.46–2.58 (2H,

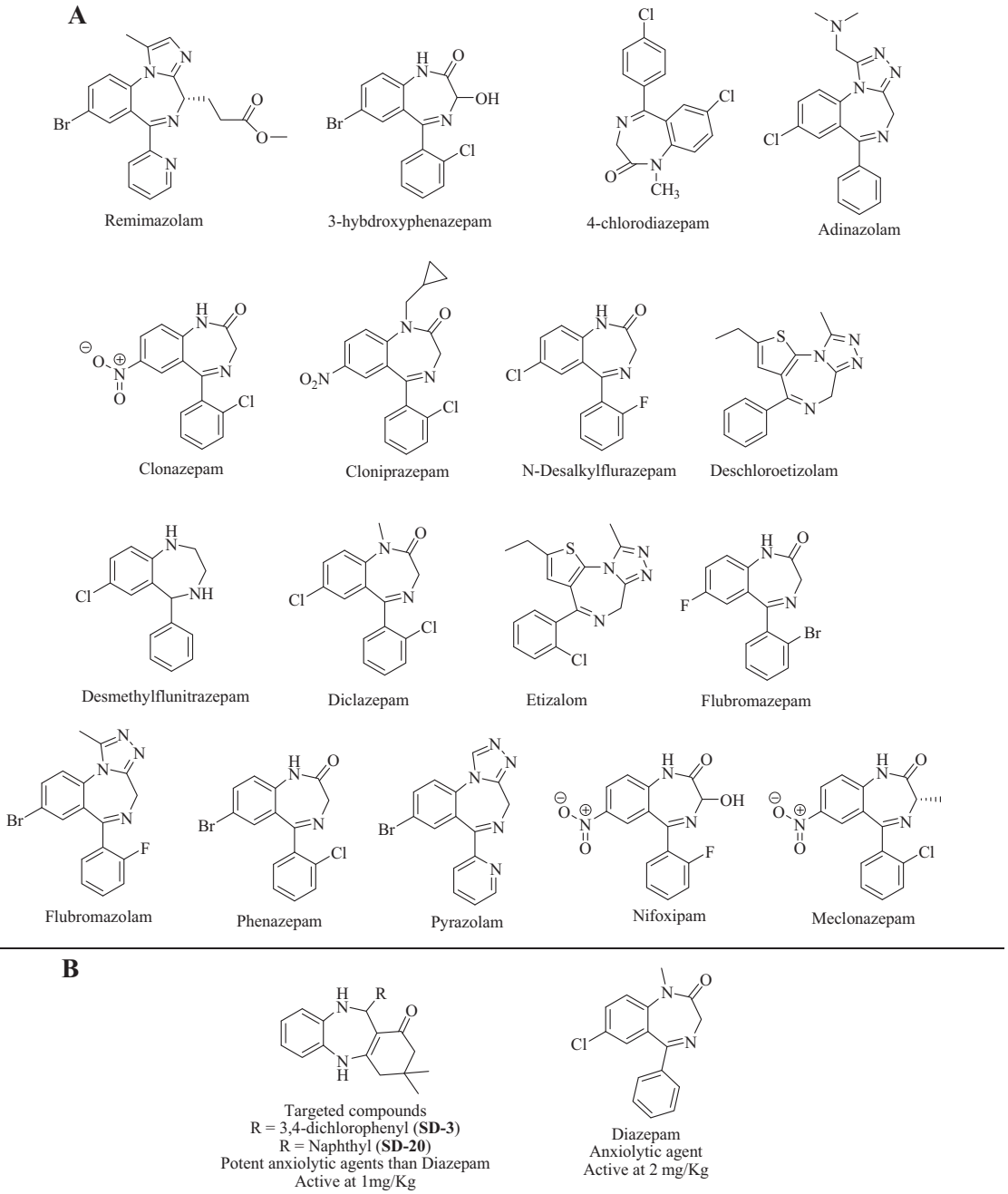


Fig. 1 **a** New benzodiazepine-based drugs for the treatment of various CNS disorders; **b** Comparison of anxiolytic effect of targeted compounds with the existed benzodiazepine derivative (Diazepam)

m, -CH_2-), 5.87 (1H, s, -CH-), 6.48 (1H, s, -CH-), 6.66–6.69 (2H, m, ArH), 6.93–6.95 (2H, m, ArH), 7.05–7.09 (2H, m, ArH), 7.18 (2H, m, ArH), 7.48 (1H, s, ArH). ^{13}C NMR ($CDCl_3$, 125 MHz, δ): 27.7, 28.8, 32.2, 45.8, 49.7, 57.5, 110.4, 120.3, 121.3, 121.3, 121.4, 126.8, 127.0, 128.6, 135.1, 136.9, 146.4, 193.7 (C=O). Anal. Calcd. for $C_{21}H_{21}ClN_2O$: C, 71.48; H, 6.00; Cl, 10.05; N, 7.94; O, 4.53; Found: C, 71.41; H, 5.97; Cl, 10.35; N, 7.54.

11-(4-Chlororphenyl)-3,3-dimethyl-2,3,4,5,10,11-hexahydrodibenzo[*b*,*e*][1,4]diazepin-1-one (SD-2) Yellowish solid; Yield 85%, mp: 174–176 °C, ^{1}H NMR ($CDCl_3$, 500 MHz, δ): 1.06–1.12 (6H, m, -CH_3), 2.15–2.26 (2H, m, -CH_2-), 2.43–2.53 (2H, m, -CH_2-), 5.85 (1H, s, -CH-), 6.41 (1H, bs, -NH-), 6.69–6.72 (2H, m, ArH), 6.86 (1H, m, ArH), 6.92–6.94 (2H, m, ArH), 7.13 (1H, m, ArH), 7.21 (1H, s, ArH), 7.28 (1H, m, ArH), 7.47 (1H, s, ArH). ^{13}C NMR

($CDCl_3$, 125 MHz, δ): 27.7, 28.8, 32.2, 45.8, 49.7, 57.5, 110.4, 120.3, 121.3, 121.3, 121.4, 123.7, 125.7, 129.4, 129.5, 130.4, 131.1, 136.9, 146.4, 154.3, 193.7 (C=O). Anal. Calcd. for $C_{21}H_{21}ClN_2O$: C, 71.48; H, 6.00; Cl, 10.05; N, 7.94; O, 4.53; Found: C, 71.54; H, 5.99; Cl, 10.32; N, 7.56.

11-(3,4-dichlororphenyl)-3,3-dimethyl-2,3,4,5,10,11-hexahydrodibenzo[*b,e*][1,4]diazepin-1-one (SD-3) Yellowish solid; Yield 85%, mp: 174–176 °C, ^{1}H NMR ($CDCl_3$, 500 MHz, δ): 1.05–1.10 (6H, m, -CH_3), 2.13–2.21 (2H, m, -CH_2-), 2.42–2.50 (2H, m, -CH_2-), 5.91 (1H, s, -CH-), 6.69–6.72 (2H, m, ArH), 6.76–6.80 (2H, m,. ArH), 6.98–7.01 (2H, m, ArH), 7.12–7.14 (1H, d, $J = 7.5$ Hz, ArH), 7.28–7.31 (2H, m, ArH). ^{13}C NMR ($CDCl_3$, 125 MHz, δ): 27.7, 28.8, 32.2, 45.8, 49.7, 57.5, 110.4, 120.3, 121.3, 121.3, 121.5, 126.5, 128.2, 128.6, 131.2, 131.4, 133.2, 135.1, 136.9, 146.4, 193.7 (C=O). Anal. Calcd. for $C_{21}H_{20}Cl_2N_2O$: C, 65.12; H, 5.20; Cl, 18.31; N, 7.23; O, 4.13; Found: C, 65.04; H, 5.25; Cl, 17.91; N, 7.41.

11-(4-Bromophenyl)-3,3-dimethyl-2,3,4,5,10,11-hexahydrodibenzo[*b,e*][1,4]diazepin-1-one (SD-4) Yellowish solid; Yield 85%, mp: 174–176 °C, ^{1}H NMR ($CDCl_3$,500 MHz, δ): 1.07–1.14 (6H, m, -CH_3), 2.15–2.22 (2H, m, -CH_2-), 2.42–2.50 (2H, m, -CH_2-), 5.85 (1H, s, -CH-), 6.42 (1H, bs, -NH-), 6.68–6.70 (2H, m, ArH), 6.83 (1H, m, ArH), 6.93–6.95 (2H, m, ArH), 7.12 (1H, m, ArH), 7.20 (1H, s, ArH), 7.28 (1H, m, ArH), 7.45 (1H, bs, ArH). ^{13}C NMR ($CDCl_3$, 125 MHz, δ): 27.7, 28.7, 32.2, 45.8, 49.7, 57.5, 110.4, 120.3, 121.2, 121.2, 121.4, 123.7, 125.7, 129.4, 129.5, 130.4, 131.1, 136.9, 146.4, 154.3, 193.7 (C=O). Anal. Calcd. for $C_{21}H_{21}BrN_2O$: C, 63.48; H, 5.33; Br, 20.11; N, 7.05; O, 4.03; Found: C, 63.54; H, 5.21; Br, 20.35; N, 6.69.

11-(3-Bromophenyl)-3,3-dimethyl-2,3,4,5,10,11-hexahydrodibenzo[*b,e*][1,4]diazepin-1-one (SD-5) Yellowish solid; Yield 85%, mp: 174–176 °C, ^{1}H NMR ($CDCl_3$, 500 MHz, δ): 1.04–1.11 (6H, m, -CH_3), 2.17–2.28 (2H, m, -CH_2-), 2.46–2.58 (2H, m, -CH_2-), 5.84 (1H, m, -CH-), 6.40 (1H, m, ArH), 6.69 (1H, m, ArH), 6.83 (1H, m, ArH), 6.94 (1H, m, ArH), 7.12 (1H, m, ArH), 7.20 (1H, m, ArH), 7.28 (1H, m, ArH), 7.45 (1H, m, ArH). ^{13}C NMR ($CDCl_3$, 125 MHz, δ): 20.9, 27.7, 28.7, 30.8, 32.2, 45.8, 49.7, 57.5, 110.4, 120.3, 121.2, 121.4, 123.7, 125.7, 129.5, 130.4, 146.4, 154.2, 193.7 (C=O). Anal. Calcd. for $C_{21}H_{21}BrN_2O$: C, 63.48; H, 5.33; Br, 20.11; N, 7.05; O, 4.03; Found: C, 63.41; H, 5.38; Br, 20.32; N, 6.65.

11-(4-Fluorophenyl)-3,3-dimethyl-2,3,4,5,10,11-hexahydrodibenzo[*b,e*][1,4]diazepin-1-one (SD-6) Yellowish solid; Yield 85%, mp: 174–176 °C, ^{1}H NMR ($CDCl_3$, 500 MHz, δ): 1.05–1.10 (6H, m, -CH_3), 2.13–2.21 (2H, m, -CH_2-), 2.42–2.50 (2H, m, -CH_2-), 5.87 (1H, s, -CH-), 6.40 (1H, bs, -NH-), 6.69–6.73 (2H, m, ArH), 6.85–6.86 (2H, m, ArH), 6.98–7.03 (3H, m, ArH), 7.08 (1H, m, ArH), 7.14 (1H, m, ArH). ^{13}C NMR ($CDCl_3$, 125 MHz, δ): 27.7, 28.7, 32.2, 45.8, 49.7, 57.5, 110.4, 120.3, 121.3, 121.3, 121.4, 123.7, 125.7, 129.4, 129.5, 130.4, 131.1, 136.9, 146.4, 160.3, 193.7 (C=O). Anal. Calcd. for $C_{21}H_{21}FN_2O$: C, 74.98; H, 6.29; F, 5.65; N, 8.33; O, 4.76; Found: C, 74.87; H, 6.32; F, 5.22; N, 8.68.

11-(4-Nitrophenyl)-3,3-dimethyl-2,3,4,5,10,11-hexahydrodibenzo[*b,e*][1,4]diazepin-1-one (SD-7) Yellowish solid; Yield 95%, mp: 194–195 °C, ^{1}H NMR ($CDCl_3$, 500 MHz, δ): 1.18–1.28 (6H, m, -CH_3), 2.36–2.38 (2H, m, -CH_2-), 2.69–2.75 (2H, m, -CH_2-), 6.02 (1H, s, -CH-), 6.65–6.78 (2H, m, ArH), 7.11 (1H, m, ArH), 7.45 (1H, m, ArH), 8.05 (2H, d, $J = 8.9$ Hz, ArH), 8.94 (2H, m, ArH). ^{13}C NMR ($CDCl_3$, 125 MHz, δ): 32.7, 33.5, 36.9, 49.2, 54.6, 61.4, 114.2, 125.3, 125.50, 125.87, 127.7, 127.8, 128.2, 133.2, 133.3, 136.1, 142.6, 150.8, 157.7, 160.3, 197.7 (C=O). Anal. Calcd. for $C_{21}H_{21}N_3O_3$: C, 69.41; H, 5.82; N, 11.56; O, 13.21; Found: C, 69.48; H, 5.75; N, 11.75.

11-(4-methylphenyl)-3,3-dimethyl-2,3,4,5,10,11-hexahydrodibenzo[*b,e*][1,4]diazepin-1-one (SD-8) Yellowish solid; Yield 85%, mp: 174–176 °C, ^{1}H NMR ($CDCl_3$, 500 MHz, δ): 1.05–1.10 (6H, m, -CH_3), 2.13–2.21 (2H, m, -CH_2-), 2.42–2.50 (2H, m, -CH_2-), 5.86 (1H, s, -CH-), 6.42 (1H, bs, -NH-), 6.68–6.71 (2H, m, ArH), 6.86–6.88 (2H, m, ArH), 6.96–6.99 (2H, m, ArH), 7.10–7.11 (2H, d, $J = 6.5$ Hz, ArH), 7.13–7.14 (2H, d, $J = 6.5$, ArH). ^{13}C NMR ($CDCl_3$, 125 MHz, δ): 24.3, 27.7, 28.7, 32.2, 45.8, 49.7, 57.5, 110.4, 120.3, 121.3, 121.3, 121.4, 123.7, 125.7, 129.4, 129.5, 130.4, 131.1, 136.9, 146.4, 193.7 (C=O). Anal. Calcd. for $C_{22}H_{24}N_2O$: C, 79.48; H, 7.28; N, 8.43; O, 4.81; Found: C, 79.41; H, 7.34; N, 8.02.

11-(4-methoxyphenyl)-3,3-dimethyl-2,3,4,5,10,11-hexahydrodibenzo[*b,e*][1,4]diazepin-1-one (SD-9) Yellowish solid; Yield 85%, mp: 174–176 °C, ^{1}H NMR ($CDCl_3$, 500 MHz, δ): 1.07–1.11 (6H, m, -CH_3), 2.12–2.22 (2H, m, -CH_2-), 2.41–2.49 (2H, m, -CH_2-), 5.90 (1H, s, -CH-), 6.44 (1H, bs, -NH-), 6.68–6.72 (2H, m, ArH), 6.84–6.86 (2H, m, ArH), 6.99–7.03 (2H, m, ArH), 7.01–7.02 (2H, d, $J = 6.5$ Hz, ArH), 7.14–7.15 (2H, d, $J = 6.5$, ArH). ^{13}C NMR ($CDCl_3$, 125 MHz, δ): 27.7, 28.7, 32.2, 45.8, 49.7, 57.5, 61.1, 110.4, 120.3, 121.3, 121.3, 121.4, 123.7, 125.7, 129.4, 129.5, 130.4, 131.1, 136.9, 160.7, 193.7 (C=O). Anal. Calcd. for $C_{22}H_{24}N_2O_2$: C, 75.83; H, 6.94; N, 8.04; O, 9.18; Found: C, 75.89; H, 6.84; N, 7.69.

11-(3,4-dimethoxyphenyl)-3,3-dimethyl-2,3,4,5,10,11-hexahydrodibenzo[*b,e*][1,4]diazepin-1-one (SD-10) Yellowish solid; Yield 85%, mp: 174–176 °C, ^{1}H NMR ($CDCl_3$, 500 MHz, δ): 1.05–1.10 (6H, m, -CH_3), 2.13–2.21 (2H, m, -CH_2-), 2.42–2.50 (2H, m, -CH_2-), 3.73 (6H, s, -OCH_3), 5.86 (1H, s, -CH-), 6.46 (1H, bs, -NH-), 6.68–6.72 (2H, m, ArH), 6.85–6.88 (2H, m, ArH), 7.01–7.03 (3H, m, ArH) 7.09 (1H, m, ArH). ^{13}C NMR ($CDCl_3$, 125 MHz, δ): 27.7, 28.7, 32.2, 45.8, 49.7, 57.5, 61.1, 61.5, 110.4, 118.3, 121.3, 121.3, 121.5, 123.7, 125.7, 125.8, 129.4, 133.4, 134.5, 136.9, 152.6, 155.6, 193.7 (C=O). Anal. Calcd. for $C_{23}H_{26}N_2O_3$: C, 72.99; H, 6.92; N, 7.40; O, 12.68; Found: C, 73.11; H, 6.98; N, 7.70

11-(2,3-dimethoxyphenyl)-3,3-dimethyl-2,3,4,5,10,11-hexahydrodibenzo[*b,e*][1,4]diazepin-1-one (SD-11) Yellowish solid; Yield 85%, mp: 174–176 °C, ^{1}H NMR ($CDCl_3$, 500 MHz, δ): 1.08–1.13 (6H, m, -CH_3), 2.14–2.24 (2H, m, -CH_2-), 2.44–2.53 (2H, m, -CH_2-), 3.74 (6H, s, -OCH_3), 5.92 (1H, s, -CH-), 6.47 (1H, bs, -NH-), 6.66–6.70 (2H, m, ArH), 6.86–6.88 (2H, m, ArH), 7.03–7.06 (2H, m, ArH), 7.09 (1H, m, ArH), 7.50 (1H, s, ArH). ^{13}C NMR ($CDCl_3$, 125 MHz, δ): 27.7, 28.7, 32.2, 45.8, 49.7, 57.5, 61.1, 61.5, 110.4, 118.3, 121.3, 121.3, 121.5, 123.7, 125.7, 125.8, 129.4, 133.4, 134.5, 136.9, 152.6, 155.6, 193.7 (C=O). Anal. Calcd. for $C_{23}H_{26}N_2O_3$: C, 72.99; H, 6.92; N, 7.40; O, 12.68; Found: C, 73.14; H, 6.85; N, 7.74.

11-(3,4,5-trimethoxyphenyl)-3,3-dimethyl-2,3,4,5,10,11-hexahydrodibenzo[*b,e*][1,4]diazepin-1-one (SD-12) Yellowish solid; Yield 85%, mp: 174–176 °C, ^{1}H NMR ($CDCl_3$, 500 MHz, δ): 1.05–1.10 (6H, m, -CH_3), 2.13–2.21 (2H, m, -CH_2-), 2.42–2.50 (2H, m, -CH_2-), 3.74 (6H, s, -OCH_3), 3.82 (3H, s, -OCH_3), 5.86 (1H, s, -CH-), 6.64–6.67 (2H, m, ArH), 6.83 (1H, s, ArH), 6.86–6.88 (2H, m, ArH), 6.94–6.97 (2H, m, ArH), 7.50 (1H, s, ArH). ^{13}C NMR ($CDCl_3$, 125 MHz, δ): 27.7, 28.7, 32.2, 45.8, 49.7, 57.6, 61.1, 61.2, 61.5, 110.4, 118.3, 121.3, 121.3, 121.5, 123.7, 125.7, 125.8, 133.4, 134.5, 136.9, 143.3, 153.6, 160.5, 193.7 (C=O). Anal. Calcd. for $C_{24}H_{28}N_2O_4$: C, 70.57; H, 6.91; N, 6.86; O, 15.67; Found: C, 70.45; H, 6.98; N, 7.12.

11-(2-methoxyphenyl)-3,3-dimethyl-2,3,4,5,10,11-hexahydrodibenzo[*b,e*][1,4]diazepin-1-one (SD-13) Yellowish solid; Yield 85%, mp: 174–176 °C, ^{1}H NMR ($CDCl_3$, 500 MHz, δ): 1.04–1.12 (6H, m, -CH_3), 2.11–2.23 (2H, m, -CH_2-), 2.41–2.52 (2H, m, -CH_2-), 3.76 (3H, s, -OCH_3), 5.84 (1H, s, -CH-), 6.45 (1H, bs, -NH-), 6.68–6.71 (2H, m, ArH), 6.88–6.90 (2H, m, ArH), 7.12–7.17 (2H, m, ArH) 7.31–7.36 (2H, m, ArH), 7.52 (1H, s, ArH). ^{13}C NMR ($CDCl_3$, 125 MHz, δ): 27.7, 28.7, 32.2, 45.7, 49.7, 57.5, 61.1, 110.4, 120.3, 121.3, 121.3, 121.4, 123.7, 125.7, 125.8, 128.4, 129.5, 135.1, 136.9, 160.7, 193.7 (C=O). Anal. Calcd. for $C_{22}H_{24}N_2O_2$: C, 75.83; H, 6.94; N, 8.04; O, 9.18; Found: C, 75.77; H, 7.05; N, 7.85.

11-(3-methoxyphenyl)-3,3-dimethyl-2,3,4,5,10,11-hexahydrodibenzo[*b,e*][1,4]diazepin-1-one (SD-14) Yellowish solid; Yield 85%, mp: 174–176 °C, ^{1}H NMR ($CDCl_3$, 500 MHz, δ): 1.05–1.10 (6H, m, -CH_3), 2.13–2.21 (2H, m, -CH_2-), 2.42–2.50 (2H, m, -CH_2-), 3.74 (3H, s, -OCH_3), 5.86 (1H, s, -CH-), 6.68–6.71 (2H, m, ArH), 6.89–6.92 (2H, m, ArH), 7.15–7.19 (3H, m, ArH), 7.31–7.45 (2H, m, ArH), 7.52 (1H, s, ArH). ^{13}C NMR ($CDCl_3$, 125 MHz, δ): 27.7, 28.7, 32.2, 45.8, 49.7, 57.5, 61.1, 110.4, 120.3, 121.3, 121.3, 121.4, 123.8, 125.7, 125.8, 128.4, 129.5, 135.1, 136.9, 160.7, 193.7 (C=O). Anal. Calcd. for $C_{22}H_{24}N_2O_2$: C, 75.83; H, 6.94; N, 8.04; O, 9.18; Found: C, 75.95; H, 6.88; N, 7.24.

11-(N,N-dimethylaminophenyl)-3,3-dimethyl-2,3,4,5,10,11-hexahydrodibenzo[*b,e*][1,4]diazepin-1-one (SD-15) Yellowish solid; Yield 85%, mp: 174–176 °C, ^{1}H NMR ($CDCl_3$, 500 MHz, δ): 1.03–1.11 (6H, m, -CH_3), 2.11–2.19 (2H, m, -CH_2-), 2.44–2.51 (2H, m, -CH_2-), 2.92 (6H, s, -CH_3), 5.93 (1H, s, -CH-), 6.46 (1H, bs, -NH-), 6.68–6.72 (2H, m, ArH), 6.84–6.86 (3H, m, ArH), 6.96–6.99 (3H, m, ArH) 7.16–7.18 (2H, d, $J = 6.5$ Hz, ArH). ^{13}C NMR ($CDCl_3$, 125 MHz, δ): 27.7, 28.7, 32.2, 44.4, 45.8, 49.7, 57.5, 110.4, 120.3, 121.3, 121.3, 121.4, 123.7, 129.4, 129.8, 134.4, 135.1, 136.9, 155.7, 193.7 (C=O). Anal. Calcd. for $C_{23}H_{27}N_3O$: C, 76.42; H, 7.53; N, 11.62; O, 4.43; Found: C, 76.32; H, 7.59; N, 11.98.

11-methyl-3,3-dimethyl-2,3,4,5,10,11-hexahydrodibenzo[*b,e*][1,4]diazepin-1-one (SD-16) Yellowish solid; Yield 85%, mp: 174–176 °C, ^{1}H NMR ($CDCl_3$, 500 MHz, δ): 1.03 (3H, s, -CH_3), 1.05–1.10 (6H, m, -CH_3), 2.13–2.21 (2H, m, -CH_2-), 2.42–2.50 (2H, m, -CH_2-), 5.86 (1H, s, -CH-), 6.47 (1H, s, ArH), 6.68–6.70 (2H, m, ArH), 6.82–6.85 (2H, m, ArH), 7.52 (1H, s, ArH). ^{13}C NMR ($CDCl_3$, 125 MHz, δ): 20.0, 27.7, 28.8, 32.2, 45.8, 45.9, 57.5, 117.4, 121.3, 123.3, 124.3, 125.4, 131.0, 135.8, 136.0, 193.7 (C=O). Anal. Calcd. for $C_{16}H_{20}N_2O$: C, 74.97; H, 7.86; N, 10.93; O, 6.24; Found: C, 75.05; H, 7.76; N, 10.76.

11-(thiophen-2-yl)-3,3-dimethyl-2,3,4,5,10,11-hexahydrodibenzo[*b,e*][1,4]diazepin-1-one (SD-17) Yellowish solid; Yield 85%, mp: 174–176 °C, ^{1}H NMR ($CDCl_3$, 500 MHz, δ): 1.07–1.13 (6H, m, -CH_3), 2.11–2.22 (2H, m, -CH_2-), 2.41–2.50 (2H, m, -CH_2-), 5.95 (1H, s, -CH-), 6.46 (1H, s, ArH), 6.67–6.69 (2H, m, ArH), 6.83–6.86 (2H, m, ArH), 7.13–7.17 (2H, m, ArH), 7.31 (1H, m, ArH), 7.50 (1H, s, ArH). ^{13}C NMR ($CDCl_3$, 125 MHz, δ): 27.7, 28.8, 32.2, 45.8, 49.7, 57.5, 110.4, 120.3, 121.3, 122.3, 123.4, 125.6, 126.8, 126.9, 127.0, 128.6, 136.9, 145.9, 193.7

(C=O). Anal. Calcd. for $C_{19}H_{20}N_2OS$: C, 70.34; H, 6.21; N, 8.63; O, 4.93; S, 9.88; Found: C, 70.24; H, 6.28; N, 8.83.

11-(3-methylthiophen-2-yl)-3,3-dimethyl-2,3,4,5,10,11-hexahydrodibenzo[*b,e*][1,4]diazepin-1-one (SD-18) Yellowish solid; Yield 85%, mp: 174–176 °C, ^{1}H NMR ($CDCl_3$, 500 MHz, δ): 1.08–1.12 (6H, m, -CH_3), 2.22–2.28 (2H, m, -CH_2-), 2.30–2.37 (3H, m, -CH_3), 2.50–2.62 (2H, m, -CH_2-), 4.41 (1H, bs, -NH-), 6.21 (1H, s, -CH-), 6.47 (1H, m, ArH), 6.67 (1H, m, ArH), 6.76–6.83 (4H, m, ArH). ^{13}C NMR ($CDCl_3$, 125 MHz, δ): 14.1, 27.4, 27.5, 29.0, 29.1, 32.3, 46.2, 49.7, 51.5, 53.1, 113.0, 119.2, 120.0, 120.1, 121.3, 121.6, 123.7, 123.8, 127.0, 129.9, 131.5, 133.0, 136.6, 137.0, 141.3, 153.3. 193.5 (C=O). Anal. Calcd. for $C_{20}H_{22}N_2OS$: C, 70.97; H, 6.55; N, 8.28; O, 4.73; S, 9.47; Found: C, 70.99; H, 6.43; N, 7.92.

11-cinnamyl-3,3-dimethyl-2,3,4,5,10,11-hexahydrodibenzo[*b,e*][1,4]diazepin-1-one (SD-19) Yellowish solid; Yield 85%, mp: 174–176 °C, ^{1}H NMR ($CDCl_3$, 500 MHz, δ): 1.05–1.10 (6H, m, -CH_3), 2.13–2.21 (2H, m, -CH_2-), 2.42–2.50 (2H, m, -CH_2-), 5.85 (1H, s, -CH-), 6.49 (1H, s, ArH), 6.67–6.72 (3H, m, ArH), 6.93–6.98 (3H, m, ArH), 7.18–7.22 (3H, m, ArH), 7.36–7.38 (2H, m, ArH), 7.48 (1H, s, ArH). ^{13}C NMR ($CDCl_3$, 125 MHz, δ): 27.7, 28.7, 30.9, 32.3, 46.4, 54.6, 111.8, 120.0, 121.1, 121.2, 123.8, 126.3, 127.1, 128.2, 129.9, 130.1, 130.8, 137.1, 137.2, 152.2, 193.5 (C=O). Anal. Calcd. for $C_{23}H_{24}N_2O$: C, 80.20; H, 7.02; N, 8.13; O, 4.64; Found: C, 80.03; H, 7.22; N, 8.45.

11-(naphthalen-1-yl)-3,3-dimethyl-2,3,4,5,10,11-hexahydrodibenzo[*b,e*][1,4]diazepin-1-one (SD-20) Yellowish solid; Yield 85%, mp: 174–176 °C, ^{1}H NMR ($CDCl_3$, 500 MHz, δ): 1.17–1.27 (6H, m, -CH_3), 2.26–2.38 (2H, m, -CH_2-), 2.54–2.74 (2H, m, -CH_2-), 5.96 (1H, m, -CH-), 6.76–6.85 (2H, m, ArH), 6.98–7.05 (2H, m, ArH), 7.04–7.08 (2H, m, ArH), 7.51–7.65 (3H, m, ArH), 7.81–7.83 (2H, m, ArH), 8.37 (1H, d, $J = 10$ Hz, ArH). ^{13}C NMR ($CDCl_3$, 125 MHz, δ): 27.7, 28.6, 32.2, 44.5, 49.8, 53.0, 110.9, 120.2, 122.8, 123.2, 125.6, 127.2, 128.7, 129.3, 131.3, 132.0, 133.5, 133.7, 138.0, 139.7, 155.5, 192.4 (C=O). Anal. Calcd. for $C_{25}H_{24}N_2O$: C, 81.49; H, 6.57; N, 7.60; O, 4.34; Found: C, 81.34; H, 6.65; N, 7.38.

Antioxidant activity (DPPH assay)

Antioxidant potential of all the synthesized compounds was tested by using 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay (Tang et al. 2016). DPPH solution (0.004% w/v) and different concentrations of test compounds were prepared in methanol solvent. During the assay, methanol alone was taken as blank while 1.5 mL DPPH solution with 1 mL methanol was taken as a control. The reaction mixture consisted of 1.5 mL of DPPH solution and 1 mL of test compounds in a test tube. The reaction mixture was mixed gently at 37 °C for 1 min and stored in dark conditions for 30 min. The intensity of colour was measured by using spectrophotometer SYSTRONICS-Serial no. 2231 at 517 nm. The % inhibition (DPPH scavenging) was calculated by using the following equation:

$$\%\,\text{inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100\,.$$

Concentration of antioxidant substance causing 50% (IC_{50}) decrease in the concentration of free radicals was also determined. All experiments were performed in triplicate and values were then expressed as mean.

Molecular modelling studies with $GABA_A$ receptor

Molecular docking was performed by using Schrödinger 2015-14 Software. All the molecules were prepared using Ligprep tool of Schrödinger, which convert 1D/2D structures to 3D and also perform optimization. Finally, OPLS 2005 force field was used to minimize prepared ligand molecules. Before docking, the least energy state of ligand was needed to be prepared. The homology model of $GABA_A$ receptor was used for molecular docking studies (Wagner et al. 1998). The protein model was already refined by developers using protein preparation wizard of Schrödinger known as Prepwiz. For docking, prepared protein binding site was used for generating the grids which had a default set of options with van der Waals radius of 1.0, thereby allowing the rotation of the receptor thiol and hydroxyl groups in the receptor site. These grids were then used to calculate the interaction of the individually prepared ligand against the receptor using the extra precision ligand docking in the glide with nitrogen inversion, flexible ligand sampling, epik state penalties and ligand van der Waals scaling radii. We considered only poses having low energy conformations and good H-bond geometries.

In vivo anti-anxiety activity

Animals

Animals were approved by the respective university committees, 'Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA)' for animal experiments with a proposal no. 226/CPCSEA-2016/17. Swiss albino male mice weighing 25–30 g (three-week-old) were purchased from Central Research Institute, Kasauli, Himachal Pradesh, India. Animals were divided into five experimental groups ($n = 5$). The first group received vehicle control, second one received 2 mg/kg diazepam and remaining groups received three doses (0.5, 1.0 and 2.0 mg/kg) of test compounds. Animals were kept

in a plastic cage and acclimatized for 1 week for adapting to the environmental conditions before study. Animals were housed on a 12 h/12 h light/dark cycle at a maintained temperature of 24 ± 2 °C. The standard animal feed and RO water was provided during the study. All experiments were carried out by following the CPSCEA guidelines.

Mice were administered orally with either standard or test compound dissolved in 0.5% carboxymethylcellulose (CMC) (<0.2 mL/animal). After 30 min of treatment, animals were tested as described in the following sections.

Elevated Plus Maze Model

The wooden elevated plus-maze model was used to evaluate the anxiolytic effect of the synthesized compounds. The plus-maze consisted of two open arms of size 30 × 10 cm without any walls and two closed arms of the same size with 5 cm side walls. The central area of the plus-maze interconnecting the four arms was of the size 10 × 10 cm. The elevation of the plus-maze was kept 30 cm from the floor. During the experiment, the animal was placed initially with four legs in the central arena facing towards one of the closed arms. The time spent and a number of entries by the mice were recorded in the open arm during 5 min of the interval. When the mouse had clearly crossed the central arena with its four legs, it was considered to be in the open part.

Light and Dark Box

The box used in this model was made up of glass, sized 25 × 50 × 20 cm having one-third portion as a dark and two-third portion as a light compartment. Initially, mice were placed in the light compartment. A 40-W light bulb was positioned in the light compartment 10 cm above the centre. The mice could move freely between both the compartments through the opening at the centre. The time spent and the entries made by the animals in the light compartment were recorded for 5 min. The mouse was considered to be in the light compartment when its four legs were clearly in the light compartment.

Mirror chamber model

The mirror chamber apparatus consisted of two open-top boxes made up of black Plexiglas. The larger box consisted of one wall made up of mirror and four walls which were black. The inner surfaces of the five panels of the smaller box were lined with mirror panels. The smaller box of size 28 × 28 × 28 cm was fitted inside the larger box of size 38 × 38 × 29 cm with the open side facing towards the mirrored panel of the larger box. The smaller box was placed exactly in the centre of the larger box so that a wall (4.75 cm) was created around the periphery of the smaller box. (4.75 cm wide). In one of the corners of the enclosure, mice were placed as far as possible from the mirrored panel and the mirrored chamber. The time spent and the number of entries in the mirrored chamber were recorded during the interval of 5 min. Entry into the chamber was considered only when all the four paws were placed on the floor of the mirrored chamber.

Statistical analysis

All the in vivo experimental results are expressed as means ± S.E.M. Statistical comparisons were made using one-way analysis of variance (ANOVA). Tukey's test was used to analyse the significant differences between the vehicle control and treated mice.

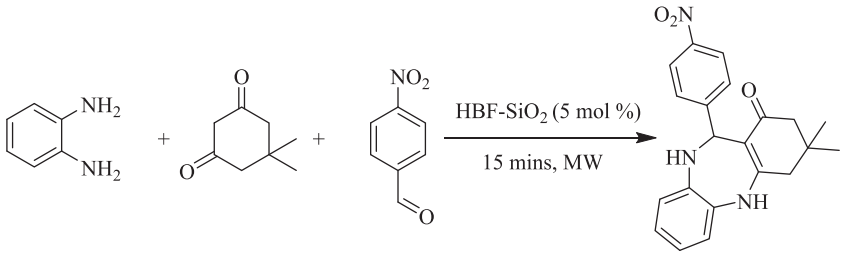
Results and discussion

Chemistry

All the synthetics were synthesized via three-component condensation of *o*-phenylenediamine, dimedone and variously substituted aldehydes, by using silicated fluoroboric acid in a catalytic amount under microwave

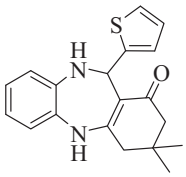
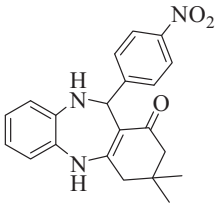
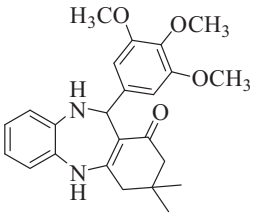
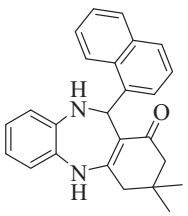
Table 1 Percentage yield of benzodiazepine analogue with various catalysts

Entry	Catalyst (BA-SiO_2)	% age yield
1	HNO_3-SiO_2	40
2	$H_2SO_4-SiO_2$	55
3	$HClO_4-SiO_2$	45
4	HBF_4-SiO_2	71
5	SiO_2	—



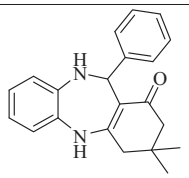
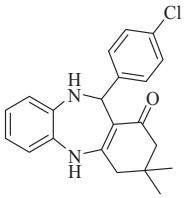
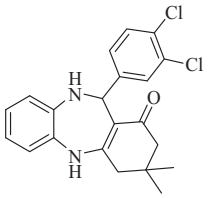
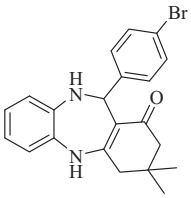
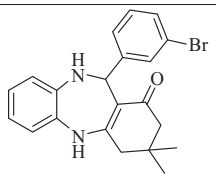
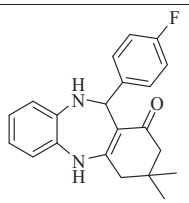
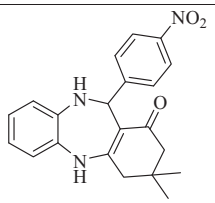
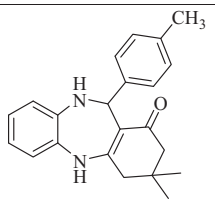
Scheme 1 Model reaction for the synthesis of benzodiazepine derivatives

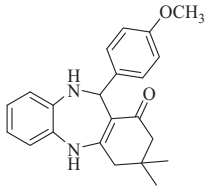
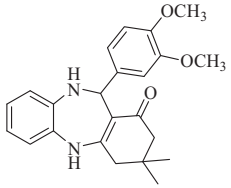
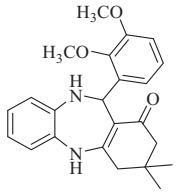
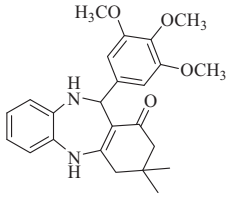
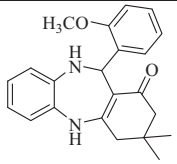
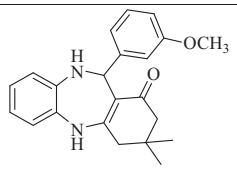
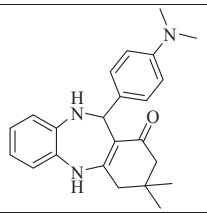
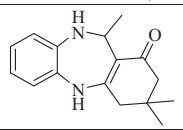
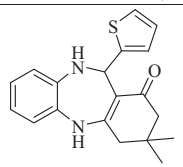
Table 2 Percentage yield with varying mol % of the catalyst and time of exposure to microwave irradiation of some selected benzodiazepines derivatives

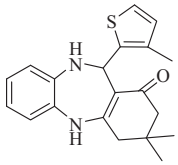
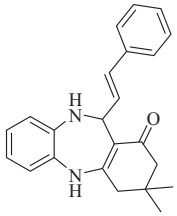
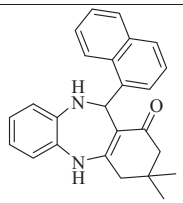
Structure	Loading (mol%)	Time (min)	Yield (%age)
	1	10	65
		15	**69**
		20	67
	5	10	72
		15	**92**
		20	89
	10	10	73
		15	**82**
		20	76
	1	10	63
		15	**79**
		20	73
	5	10	75
		15	**95**
		20	90
	10	10	69
		15	**77**
		20	73
	1	10	63
		15	**68**
		20	73
	5	10	75
		15	**83**
		20	79
	10	10	69
		15	**77**
		20	73
	1	10	53
		15	**61**
		20	58
	5	10	63
		15	**76**
		20	69
	10	10	58
		15	**68**
		20	63

The bold values point to the most applicable conditions

Table 3 Docking scores ($GABA_A$ receptor) of all the synthesized benzodiazepine derivatives

Compound	Structure	Antioxidant IC_{50} (nM)	Dock Score (kcal/mol)
SD-1		>500	ND
SD-2		>500	ND
SD-3		**489**	**-9.025**
SD-4		>500	ND
SD-5		233	-8.662
SD-6		>500	ND
SD-7		470	-8.249
SD-8		>500	ND

SD-9		>500	ND
SD-10		>500	ND
SD-11		>500	ND
SD-12		>500	ND
SD-13		>500	ND
SD-14		**138.55**	**-8.799**
SD-15		>500	ND
SD-16		>500	ND
SD-17		>500	ND

SD-18		83.35	-7.352
SD-19		76	-5.953
SD-20		**81.26**	**-9.996**
Diazepam			-8.153

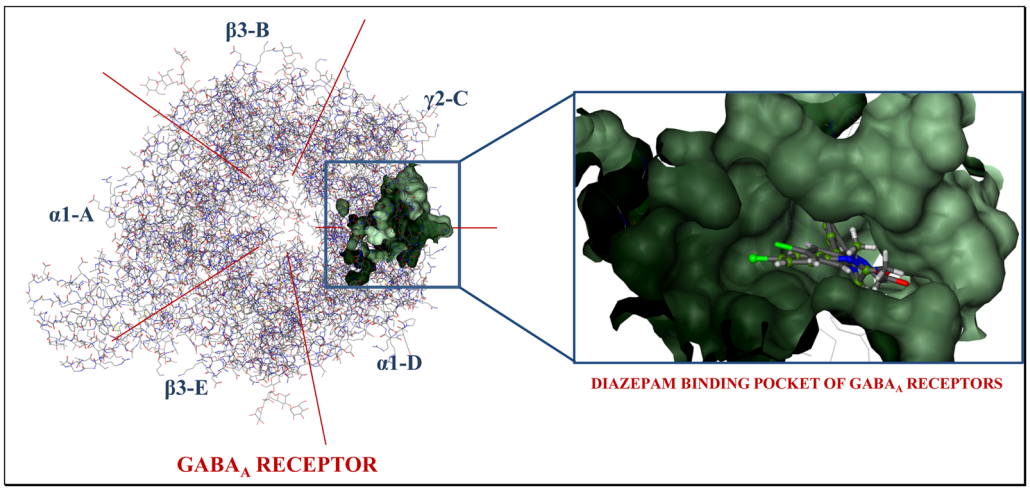


Fig. 2 Structure of a α1β3γ2 GABA$_A$ receptor with diazepam binding site

irradiation. It has been previously reported that these types of reactions are very proficient and eco-friendly to form the desired products.

Initially, for the synthesis of benzodiazepine derivatives, a model reaction was performed in order to check the catalytic effectiveness of various silica adsorbed bronsted acids (BA–silica), (Scheme 1 and Table 1).

From the careful examination of Table 1, it was observed that fluoroboric acid adsorbed on silica was highly efficient to catalyse this type of reaction. The high catalytic impact of HBF_4–SiO_2 could be explained owing to its weak protic acidic nature which might have by-pass the side reaction problems. It was also found that, when the reactions were catalysed by strong bronsted acids like, perchloric acid, sulfuric acid and nitric acid, the final product thus formed was not in such a good yield. From these particular findings, it was concluded that silica performed a critical role as an activator to estimate its catalytic power, in the absence of which reaction did not proceed well.

Furthermore, to improve the %age yield of the final product, reaction optimization was carried out by

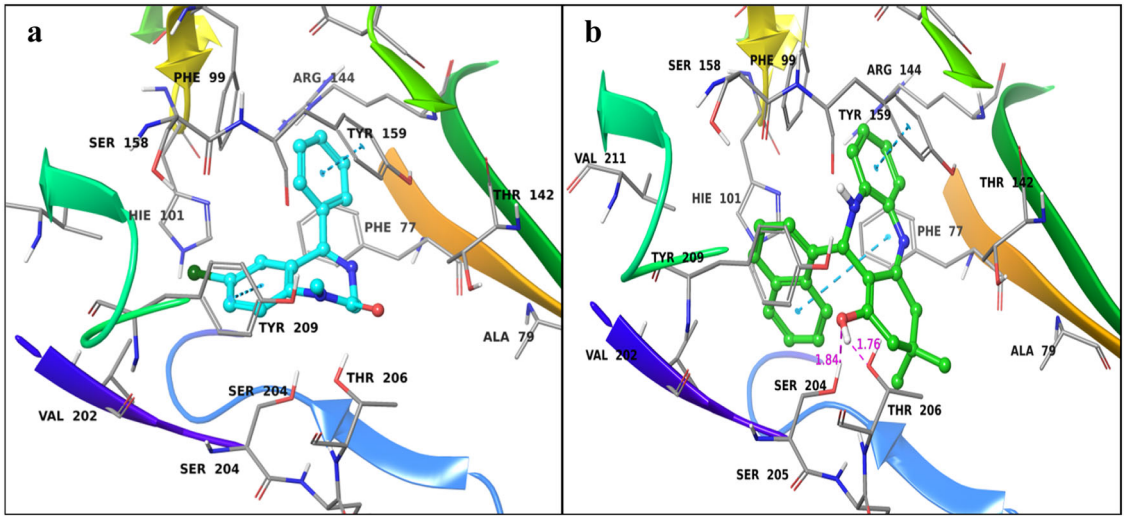


Fig. 3 **a** Diazepam (blue) in its binding pocket of $GABA_A$ receptor showing π−π interactions (blue lines): **b** Compound **SD-20** (green) docked in the binding pocket of Diazepam showing various H-bonding (pink dotted lines) and π−π interactions (blue dotted lines) (colour figure online)

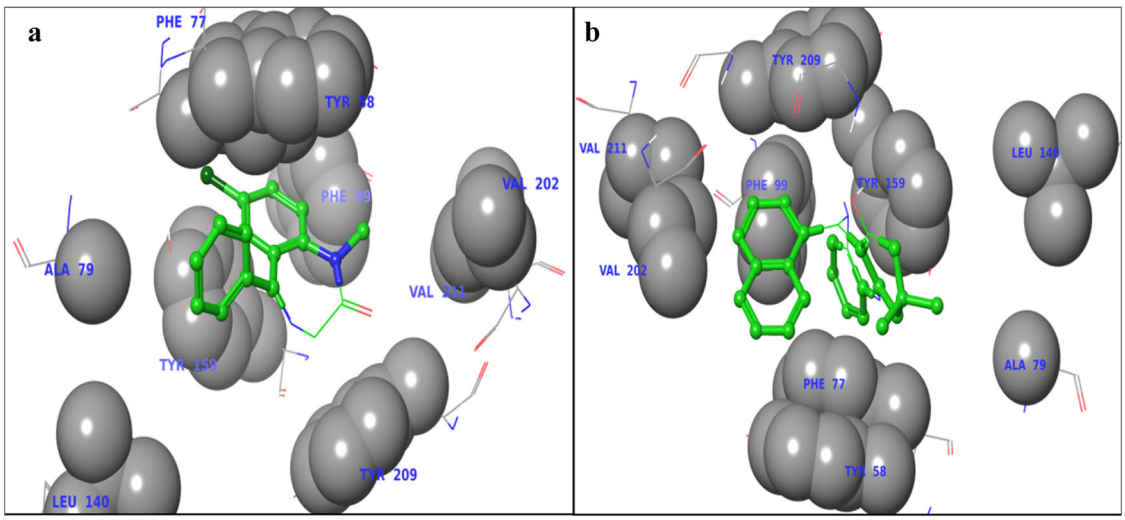


Fig. 4 Diazepam (**a**) and compound **SD-20** (**b**) showing hydrophobic interactions with various binding site residues

using different amounts of silicate fluoroboric acid under similar conditions, for a 100% conversion of reactants. A significant improvement was observed in the yield with 5 mol% of catalyst in 15 min. The amount more than 5 mol% of catalyst did not improve the results (Table 2). Therefore, 5 mol% HBF_4–silica was capable to catalyse the synthesis of benzodiazepines in good to high yields ranging from 67 to 87%.

The structures of synthesized compounds were confirmed by using ^1H NMR, ^{13}C NMR and elemental analysis and the spectral data were found in accordance with the assumed structures.

Antioxidant activity (DPPH assay)

It has been reported that during the compounds with antioxidant potential could treat the anxiety disorder. In this respect, all the synthesized benzodiazepine derivatives were evaluated to check their antioxidant potential by using 1,1-Diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay. Results of DPPH assay (Table 3) revealed that seven compounds **SD-3**, **SD-5**, **SD-7**, **SD-14**, **SD-18**, **SD-19** and **SD-20** have significant antioxidant potential with the IC_{50} values in the nanomolar range. Among all the synthetics, compound **SD-19** exhibited higher antioxidant activity with

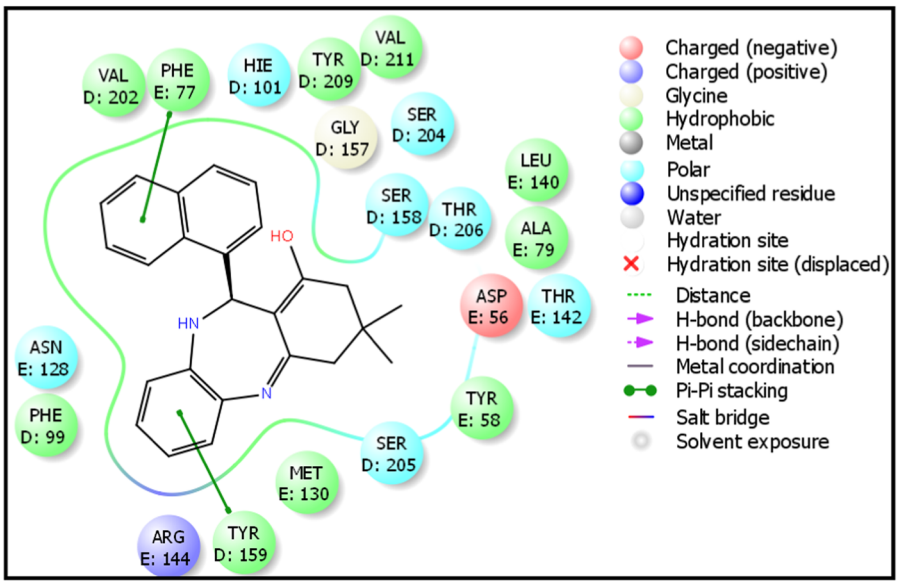


Fig. 5 2D view of the docked structure of compound **SD-20** in $GABA_A$ receptor binding site

Table 4 Physicochemical parameters of compounds **SD-3**, **SD-14** and **SD-20**

Compound	Molecular weight	No. of H-bond donors	No. of H-bond acceptors	Molar Refractivity	Log *P*	No. of Lipinski Violation
SD-3	387	2	3	110.36	4.78	0
SD-14	348	2	4	107.21	3.41	0
SD-20	424	2	3	127.52	5.17	0

the IC_{50} value of 76 nM whereas compounds **SD-18** and **SD-20** also possessed significant activity with their IC_{50} values of 83.35 and 81.26 nM, respectively. The compounds that displayed IC_{50} values above 500 nM are summarized in the table as >500 nM.

Molecular modelling studies with $GABA_A$ receptor

$GABA_A$ receptor is a pentameric ligand-gated ion channel consisting of five main binding domains viz. two α1, two β3, and one γ2. Among these binding sites benzodiazepines have been found to bind on the interface of α1 and γ2 domains (Fig. 2). After the binding of benzodiazepines (Positive Allosteric Modulators) with GABA receptor, the central chloride pore opens up and causes an influx of chloride ions which is associated with the anxiolytic effect. Most commonly used benzodiazepines are the diazepam and lorazepam as positive allosteric modulators of GABA (Simonas et al. 2019).

To predict the binding affinity of the synthesized benzodiazepine molecules, diazepam-bound $GABA_A$ receptor homology model was used in Schrödinger 2015-14 Software and dock scores were calculated (Cottrell et al. 1987). Seven compounds that exhibited significant antioxidant potential were only selected to analyse by docking studies i.e **SD-3**, **SD-5**, **SD-7**, **SD-14**, **SD-18**, **SD-19** and **SD-20**. Among the seven compounds, five compounds exhibited the best dock score as compared to diazepam (Table 3). **SD-20** was found to be the best fit with the diazepam binding site with the most prominent dock score of −9.996 kcal/mol, which was further selected to discuss its binding behaviour.

Binding mode of compound SD-20

In order to see the mode of binding of compound **SD-20** into the $GABA_A$ receptor binding site, diazepam was used as a reference for validating the docking procedure. Diazepam in its binding pocket showed π−π interactions with Tyr159 and Tyr209 residues and also exhibited hydrophobic interactions with various binding site residues (Fig. 3a). Compound **SD-20**, when docked in the binding site grid of GABAA receptor, exhibited well-docked pose with a docking score of −9.996 kcal/mol. It showed two

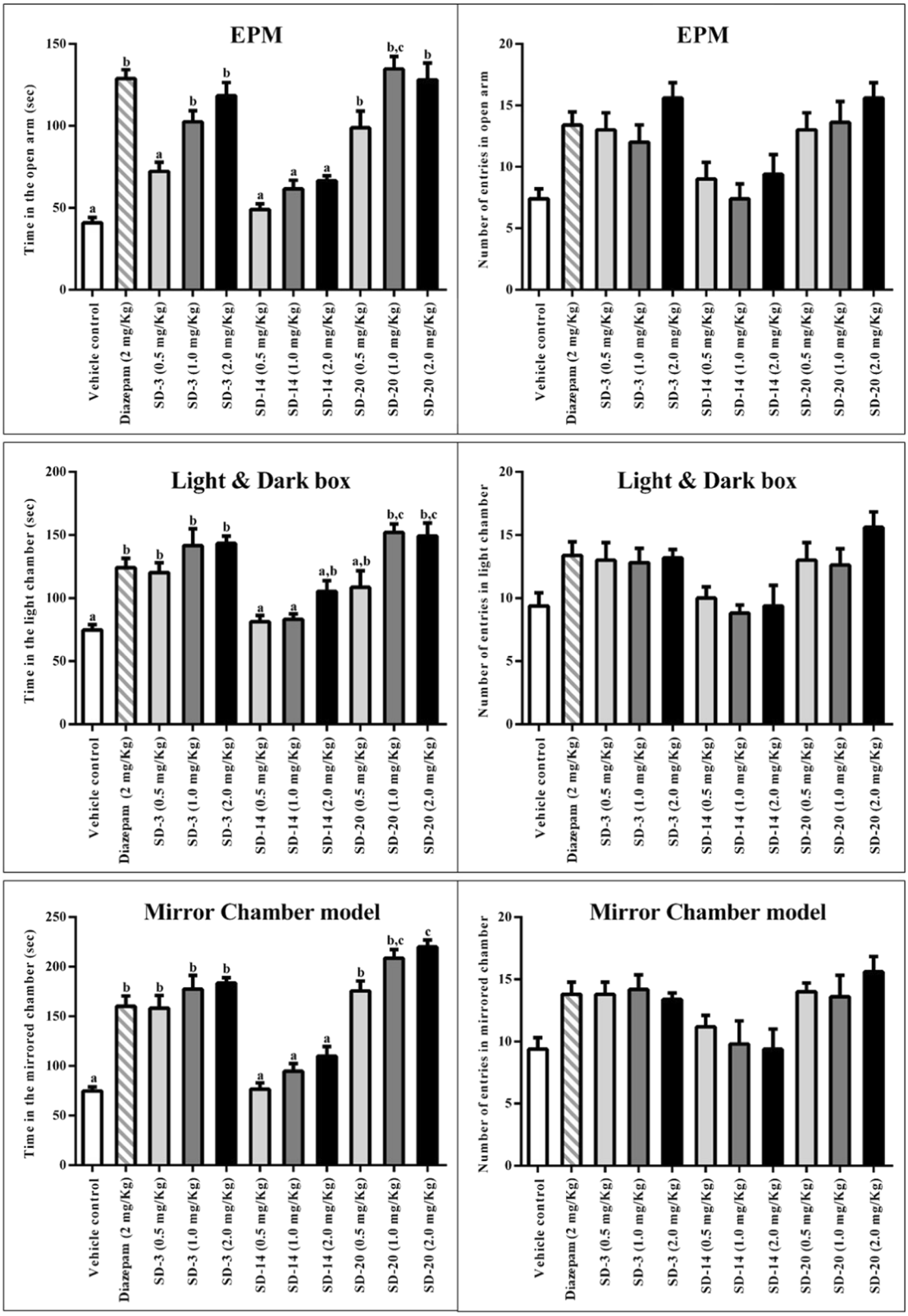


Fig. 6 (Left) Dose−effect relationship of benzodiazepines in three different animal models: (Right) number of positive entries in the open field. The values were shown as the mean ± S.E.M. ($n = 5$). Similar notations define not significant difference between the groups at $p < 0.05$ level, and dissimilar notations define the significant difference between the groups at $p < 0.05$ level

H-bonds through its OH group with Ser204 (1.84 Å) and Thr206 (1.76 Å) and π−π interactions with Phe77 and Tyr159 (Fig. 3b). It also interacted with Val209, Val211, Val202, Phe99, Phe77, Ser158, and Ala79 through various hydrophobic interactions in the same mode as diazepam did (Fig. 4). The two-dimensional view of **SD-20** within the $GABA_A$ receptor is provided in Fig. 5.

In silico study

To determine whether the three potent compounds (**SD-3**, **SD-14** and **SD-20**) fit to the Lipinski rule of five or not, ChemAxon software MarvinSketch was used for the same purpose. The results stated that the compounds well followed all the properties of the rule (Table 4) that indicate: (a) the molecular weight of the synthesized compounds found below 500 Da, i.e. within the range of 348–424; (b) the H-bond donating property of all the compounds lies within the limits (<5); (c) H-bond acceptor sites were found <10; (d) molar refractivities were fallen in the range of 107.21–127.52 that lies well in the permitted range of 40–130 and (e) log *P* of all the synthetics was found in the range of 3.41–5.17 which suggested that the compounds were lipophilic in nature and able to cross the blood brain barrier. These results indicated that all of the synthetics could be pharmacologically efficient to be used further in animal models.

In vivo antianxiety activity

By carefully examining the antioxidant potential as well as docking simulations of the synthesized compounds, we have selected three most prominent molecules **SD-3**, **SD-14** and **SD-20** to check their anxiolytic effect in the three in vivo mice models viz. (1) Elevated Plus Maze Model, (2) Light and Dark box, and (3) Mirror chamber model. The compounds were found to show no signs of acute toxicity, even up to 10 mg/kg in mice.

The three most potent compounds (**SD-3**, **SD-14** and **SD-20**) were tested for their anxiolytic activity by using elevated plus maze model and the time spent by mice in the open arms and the number of entries were observed and the data were displayed in Fig. 6. The results were compared with the diazepam (2 mg/kg) which was used as standard control. The 0.5% carboxymethylcellulose was taken as vehicle control. A significant anxiolytic effect was observed with compounds **SD-3** and **SD-20** at the dose level of 1.0 mg/kg. The total time taken by the animals in the open arm treated with **SD-3** and **SD-20** (1.0 mg/kg) was found almost similar to the time spent by the animals treated with 2 mg/kg of diazepam. Statistically, there was no significant difference between results of 1 mg/kg dose of **SD-3** and **SD-20**, and 2 mg/kg dose of diazepam (Fig. 6). Compound **SD-14** did not show any significant effect even at the dose level of 2 mg/kg. A statistically significant difference was found between the **SD-3** and **SD-20** compounds which suggest that the compound **SD-20** displayed better anxiolytic effect than **SD-3** and diazepam at the dose level of 1.0 mg/kg. Similar results were found with the Light & Dark box and Mirror Chamber model, which are summarized in Fig. 6. Therefore, in vivo results were found in accordance with the molecular docking results which also revealed the **SD-20** compound as best fit molecule with the $GABA_A$ receptor.

Conclusion

In the present study, various benzodiazepine derivatives were synthesized by using silicated fluoroboric acid in good to high yield. All the synthetics were preliminarily evaluated to check their antioxidant potential. Seven compounds with significant antioxidant potential were further streamlined to check their binding ability towards the diazepam binding site of $GABA_A$ receptor. Three compounds **SD-3**, **SD-14** and **SD-20** that significantly bind with $GABA_A$ receptor were tested in vivo to assess their antianxiety activity. Among these three compounds, **SD-3** and **SD-20** at 1.0 mg/kg oral dose displayed better anxiolytic effect as compared to standard drug diazepam. Thus, the overall study distinguished that compounds **SD-3** and **SD-20** could act as hit lead molecules for the further development of anxiolytic agents.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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