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Microwave Assisted Synthesis of Quinoline Fused Benzodiazepines as Anxiolytic and Antimicrobial Agents

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In the present study, an efficient, facile and green protocol for synthesis of quinoline fused 1,4-benzodiazepine (**4a-j**) by microwave irradiated condensation of 6/7/8-substituted 3-bromomethyl-2-chloro-quinoline (**3a-j**) obtained from 2-chloro 6/7/8-substituted quinoline-3-carbaldehyde (**1a-j**) with 1, 2-phenylenediamine was developed. Surflex docking studies with K^+ channel is one of the physiological targets and inhibition, which plays a role in the pathophysiology of depression revealed that all these compounds show consensus score in the range 2.71-3.68 indicating the summary of all forces of interaction. Further, compounds **4d**, **4g** and **4i** exhibited potent antibacterial activity.

Keywords: Microwave assisted synthesis, Quinoline, Benzodiazepine, Antidepressant.

INTRODUCTION

Depression has become a chronic illness, which has affected a sizable number of people on the globe. However, the mechanism of pathophysiology responsible for the depression is not unearthed completely [1]. It is interesting to note that potassium (K^+) channels play a role in the anxiety related disorders. In this regard, it was observed that 1,4-benzodiazepine analogs (Fig. 1) have been found to act as antidepressant agents. Heterocyclic compounds of both natural and synthetic origin have gained pharmaceutical importance since time immemorial [2,3]. Quinoline containing compounds have been established successfully as antimalarial, antimicrobial and anticancer agents. Interestingly, quinoline containing scaffolds have been used in rubber chemicals and flavouring agents [4-8].1,4-Benzodiazepines are the seven-membered heterocycle fused with benzene moiety and reported to exhibit anticonvulsant, anxiolytic, analgesic, sedative, hypnotic activity and anti-inflammatory agents [9,10]. Also, CNS drugs are showing side effects which include sedation and amnesia [11].

In order to address these issues there is a continued search for new molecules which will have remedial importance. To obtain such molecules, design of eco friendly, benign synthetic route to achieve in a shorter reaction time, increased yield are the added challenges. Literature survey revealed the reports of annulations of 1,2-bifunctional quinolines with pyridine and pyran ring systems, which showed interesting pharmacological activities [12-18]. In this regard, we were successful in constructing 1,4-diazpenine on the quinoline nucleus (**4a-j**) by efficiently condensing 6/7/8-substituted-3-bromomethyl-2-chloroquinolines (**3a-j**) with 1,2-phenylenediamine in presence of anhydrous K_2CO_3 using DMF as solvent under microwave irradiation and thermal conditions as well. Out of the two synthetic approaches microwave assisted synthesis exhibited the products in excellent yield.

Benzodiazepines have a cyclic structure that include one benzene ring with a heterocycles where two nitrogen atoms fused in 1- and 4-positions and sometimes these can also be in 1,5- or 2,3-position. By analyzing the structure, the substitutions and fusion of rings at different positions the consequences

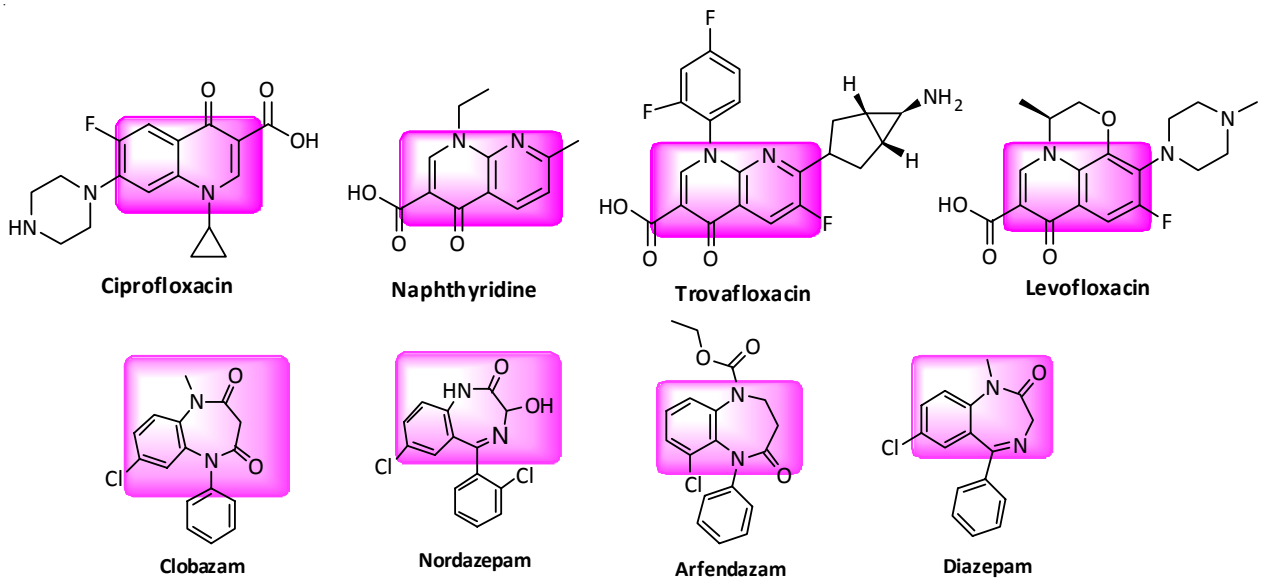


Fig. 1. Derivatives of quinoline and benzodiazepines being

can be seen in Fig. 2 [19]. Several reports [20,21] have indicated that K^+ channels of different types in various tissues can be activated by NO per se or through cGMP production [20,21]. Another study demonstrated that the antidepressant activity elicited by the inhibition of several subtypes of K^+ channels is observed to be dependent on the inhibition of NO-cGMP synthesis [22]. Thus, K^+ channels are considered as one of the physiological targets in the brain and the inhibition of such channels is expected to play an important role in the pathophysiology of depression. Therefore, the aim of this study was to investigate whether the blockade of K^+ channels can contribute to the antidepressant-like effect. The K^+ channel from *Streptomyces lividans* is an integral membrane protein with sequence similarity to all known K^+ channels, particularly in the pore region [23]. In view of all the above, the synthesized compounds were subjected to Surflex-molecular docking with potassium channel (KCSA) from *Streptomyces lividans* PDB: 1BL8.

EXPERIMENTAL

All the reagents and solvents used were of analytical grade and purchased from S.D. Fine, India. Microwave irradiation experiments were carried out using CEM Discover SP microwave synthesizer equipped with IR sensor to monitor reaction temperatures. Thin-layer chromatography was performed on 0.20 mm Aluchrosep silica gel 60 F_{254} plates (S.D. Fine Chemicals, India). Melting points were determined in open capillaries and are uncorrected. CHN analysis was done on Thermoquest CHN analyzer, ^{1}H NMR spectra were recorded at 400 MHz on Bruker Advance FT NMR spectrometer in DMSO-d_6 with TMS as an internal standard.^{13}C NMR spectra were recorded at 100 MHz on Bruker Avance FT-NMR spectrometer in DMSO-d_6 with TMS as internal standard. The mass spectra were recorded on Shimadzu GC-MS instrument operating at 70eV spectrometer. Molecular Osiris property explorer studies were carried out with the help of self consistent field theory level using PM_3 (Hamiltonian Inc.) in MOPAC 6.0 Package. The molecular docking was performed with Surflex-Dock program that is interfaced with Sybyl-X 2.1. Antimicrobial activity has been done with Brain Heart Infusion (BHI) method for MIC estimation. 3-Bromomethyl-2-chloroquinolines (**3a-j**) were synthesized from the reported method [23-25].

Conventional synthetic method of quinoline fused benzodiazepines (4a-j): 3-Bromomethyl-2-chloroquinoline **3a** (0.50

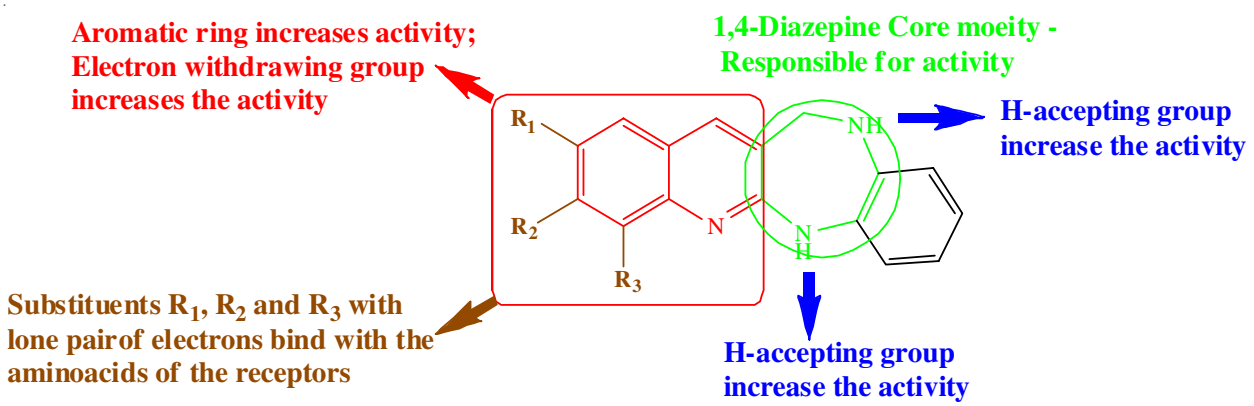


Fig. 2. Structure activity relationship (SAR) study of 1,4-benzodiazepines

g, 1.95 mmol), 1,2-phenylenediamine (1.95 mmol), DMF (10 mL) and anhydrous K_2CO_3 (3.0 mmol) were heated in a round bottom flask at 120 ºC for 3 h (**Scheme-I**). The completion of the reaction was monitored by TLC using ethyl acetate:hexane (3:7) solvent mixture as eluent. The reaction mixture was cooled to room temperature and water (10 mL) was added to get the precipitate. The solid separated was filtered, washed repeatedly with water, dried and recrystallized using ethanol to afford pure 12,13-dihydro-5*H*-5,6,13-triazabenzo[4,5]cyclohepta-[1,2-*b*]naphthalene (**4a**) in good yield. Similarly, compounds **4b-j** were synthesized according to this procedure using different substituted quinoline dihalides **3b-j**.

Microwave assisted synthesis of compounds 4a: A mixture of 3-bromomethyl-2-chloroquinoline (**3a**) (0.78 mmol), 1,2-phenylenediamine (1.95 mmol), anhydrous K_2CO_3 (3.0 mmol) and DMF (6.0 mL) was introduced into a microwave reaction vessel (10 mL) equipped with magnetic stirrer. The reaction vessel was sealed and the reaction mixture was pre-stirred for 1 min at room temperature. Then, the reaction mixture was irradiated with 150 W microwaves for 5 min at 80 ºC.The completion of reaction was monitored by TLC using ethyl acetate:hexane (3:7) solvent mixture as eluent. The reaction mixture was cooled to room temperature and similar work up was followed as mentioned in the conventional method. Other compounds **4b-j** were synthesized accordingly.

12,13-Dihydro-5*H*-5,6,13-triazabenzo[4,5]cyclohepta [1,2-*b*]naphthalene (**4a**)**:** The crude product was recrystallized using ethanol, $R_f = 0.42$); pale yellow solid; m.p.: 150-152 ºC. ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 4.55 (2H, s, CH_2), 5.53 (1H, bs, NH), 6.26-6.63 (4H, m, Ar-H), 7.60-8.39 (5H, m, quin-H); ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 44.84 (CH_2), 105.46, 110.04, 114.33, 117.91, 122.53, 122.51, 128.33, 128.88, 128.92, 131.26, 135.52, 135.46, 141.96, 145.99, 155.89; Mass calcd. (found): *m/z* 247.11 (247 $[M]^+$). Anal. calcd. (found) % for $C_{16}H_{13}N_3$: C, 77.71 (77.81); H, 5.30 (5.40); N, 16.99 (17.06).

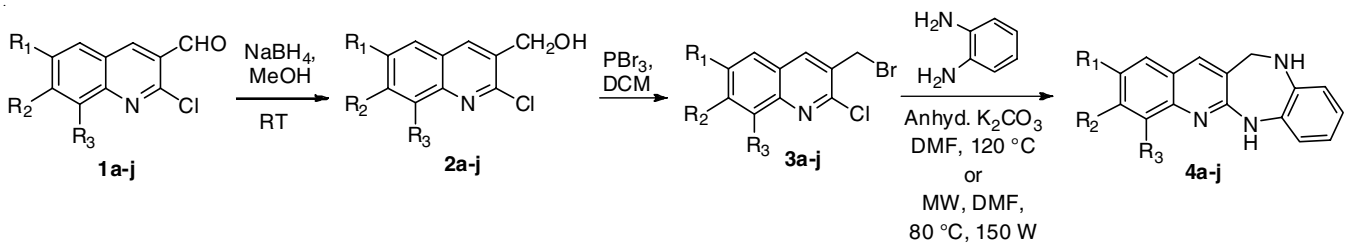
9-Methyl-12,13-dihydro-5*H*-5,6,13-triazabenzo[4,5]-cyclohepta[1,2-*b*]naphthalene (**4b**)**:** The crude product was recrystallized using ethanol ($R_f = 0.40$), pale yellow crystals; m.p.: 159-160 ºC. ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 2.80 (3H, s, Ar-CH_3), 4.53 (2H, s, CH_2), 5.52 (1H, s, NH), 6.20-6.59 (4H, m, Ar-H), 7.59-8.36 (4H, m, quin-H); ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm,): 24.40 (Ar-$\underline{C}H_3$), 44.88 (CH_2), 105.32, 110.01, 113.22, 116.94, 117.12, 122.43, 128.22, 128.62, 128.63, 131.23, 135.42, 135.22, 140.96, 146.00, 155.99; Mass calcd. (found): *m/z* 261.13 (261 $[M]^+$). Anal. calcd. (found) % for $C_{17}H_{15}N_3$: C, 78.13 (78.12); H, 5.79 (5.89); N, 16.08 (16.21).

9-Methoxy-12,13-dihydro-5*H*-5,6,13-triazabenzo[4,5]-cyclohepta[1,2-*b*]naphthalene (**4c**)**:** This compound was obtained from **3c** and 1,2-phenylenediamine. The crude product was recrystallized using ethanol, $R_f = 0.42$, pale yellow crystals; m.p.: 175-177 ºC. ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 3.86 (3H, s, -OCH_3), 4.52 (2H, s, CH_2), 5.56 (1H, bs, NH) 6.39-6.61 (4H, m, Ar-H), 7.35-8.27 (4H, m, quin-H); ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 44.87 (-CH_2), 55.57 (-OCH_3), 105.64, 110.10, 114.36, 117.91, 122.59, 122.54, 128.34, 128.89, 128.92, 131.27, 135.76, 135.46, 141.99, 146.84, 157.79; Mass calcd. (found): *m/z* 277.12 (277 $[M]^+$). Anal. calcd. (found) % for $C_{17}H_{15}N_3O$: C, 73.63 (73.52); H, 5.45 (5.52); N, 15.15 (15.28); O, 5.77 (5.69).

7-Methyl-12,13-dihydro-5*H*-5,6,13-triazabenzo[4,5]-cyclohepta[1,2-*b*]naphthalene (**4d**)**:** The crude product was recrystallized using ethanol, $R_f = 0.40$), pale yellow crystals; m.p.: 165-167 ºC. ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 2.36 (3H, s, Ar-CH_3), 4.48 (2H, s, CH_2), 5.51 (1H, bs, NH), 6.20-6.41 (4H, m, Ar-H), 7.41-8.25 (4H, m, quin-H); ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 23.97 (Ar-$\underline{C}H_3$), 44.62 (CH_2), 104.99, 110.00, 113.21, 116.85, 117.11, 122.32, 122.24, 128.12, 128.42, 128.60, 131.23, 135.32, 135.21, 140.13, 145.98, 155.99; Mass calcd. (found): *m/z* 261.13 (261 $[M]^+$). Anal. calcd. (found) % for $C_{17}H_{15}N_3$: C, 78.13 (78.19); H, 5.79 (5.82); N, 16.08 (16.18).

8-Methyl-12,13-dihydro-5*H*-5,6,13-triazabenzo[4,5]-cyclohepta[1,2-*b*]naphthalene (**4e**)**:** The crude product was recrystallized using ethanol, $R_f = 0.41$, pale yellow crystals; m.p.: 162-164 ºC. ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 2.79 (3H, s, Ar-CH_3), 4.52 (2H, s, CH_2), 5.52 (1H, bs, NH), 6.21-6.61 (4H, m, Ar-H), 7.40-8.23 (4H, m, quin-H); ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm,): 24.27 (Ar-CH_3), 44.81 (CH_2), 105.31, 110.01, 113.27, 116.94, 117.11, 122.36, 122.38, 128.19, 128.56, 128.55, 131.24, 135.43, 135.21, 140.88, 146.09, 155.98; Mass calcd. (found): *m/z* 261.13 (261 $[M]^+$). Anal. calcd. (found) % for $C_{17}H_{15}N_3$: C, 78.13 (78.21); H, 5.79 (5.82); N, 16.08 (16.91).

8-Methoxy-12,13-dihydro-5*H*-5,6,13-triazabenzo[4,5]-cyclohepta[1,2-*b*]naphthalene (**4f**)**:** The crude product was recrystallized using ethanol, $R_f = 0.43$; pale yellow crystals; m.p.:187-189 ºC. ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 3.85 (3H, s, OCH_3), 4.61 (2H, s, CH_2), 5.54 (1H, bs, NH), 6.33-6.61 (4H, m, Ar-H), 7.32-8.21 (4H, m, quin-H); ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm,): 44.81 (CH_2), 55.69 (OCH_3), 105.61, 110.23,



a; R_1= H, R_2 = H, R_3 = H: **b;** R_1= CH_3, R_2 = H, R_3 = H: **c;** R_1 = OCH_3, R_2 = H, R_3= H: **d;** R_1= H, R_2 = H, R_3 = CH_3. **e;** R_1 = H, R_2 = CH_3, R_3 = H: **f;** R_1 = H, R_2 = H, R_3 = OCH_3: **g;** R_1= H, R_2= OCH_3, R_3= H: **h;** R_1 = OCH_3, R_2 = OCH_3, R_3= H: **i;** R_1= Cl, R_2 = H, R_3 = H: **j;** R_1 = Br, R_2 = H, R_3 = H

Schyeme-I: Synthesis of 12,13-dihydro-5*H*-5,6,13-triaza-benzo[4,5]cyclohepta[1,2-*b*]naphthalene (**4a-j**)

114.31, 117.88, 122.58, 122.52, 128.34, 128.86, 128.91, 131.23, 135.84, 135.39, 141.93, 146.81, 157.71; Mass calcd. (found): *m/z* 277.12 (277 $[M]^+$). Anal. calcd. (found) % for $C_{17}H_{15}N_3O$: C, 73.63 (73.70); H, 5.45 (5.48); N, 15.15 (15.21).

8-Methoxy-12,13-dihydro-5*H*-5,6,13-triazabenzo[4,5]-cyclohepta[1,2-*b*]naphthalene (**4g**): The crude product was recrystallized using ethanol, R_f = 0.40, pale yellow crystals; m.p.: 198-200 ºC. ^{1}H NMR (400 MHz, DMSO-d_6, ä ppm): 3.79 (3H, s, -OCH_3), 4.59 (2H, s, CH_2),5.48 (1H, bs, NH), 6.29-6.59 (4H, m, Ar-H), 7.29-8.11 (4H, m, Quin-H); ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 44.73 (CH_2), 55.70 (OCH_3), 105.64, 109.32, 113.93, 117.23, 122.45, 122.78, 128.34, 128.76, 129.07, 130.99, 134.64, 135.20, 142.01, 146.79, 157.84; Mass calcd. (found): *m/z* 277.12 (277 $[M]^+$). Anal. calcd. (found) % for $C_{17}H_{15}N_3O$: C, 73.63 (73.67); H, 5.45 (5.49); N, 15.15 (15.20).

8,9-Dimethoxy-12,13-dihydro-5*H*-5,6,13-triazabenzo-[4,5]cyclohepta[1,2-*b*]naphthalene (**4h**): The crude product was recrystallized using ethanol, R_f = 0.37, pale yellow crystals; m.p.: 210-213 ºC. ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 3.71 (3H, s, -OCH_3), 3.82 (3H, s, -OCH_3), 4.50 (s, 2H, CH_2), 5.53 (1H, bs, NH), 6.26-6.41 (4H, m, Ar-H), 7.29-8.10 (3H, m, quin-H); ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 44.79 (CH_2), 56.12 (OCH_3), 55.99 (OCH_3), 105.41, 110.12, 113.95, 117.45, 122.31, 122.51, 128.32, 128.65, 128.72, 131.27, 135.66, 135.32 (Ph-C), 141.77, 146.61, 157.65; Mass calcd. (found): *m/z* 307.13 (307 $[M]^+$). Anal. calcd. (found) % for $C_{18}H_{17}N_3O_2$: C, 70.34 (70.40); H, 5.58 (5.63); N, 13.67 (13.77).

9-Chloro-12,13-dihydro-5*H*-5,6,13-triazabenzo[4,5]-cyclohepta[1,2-*b*]naphthalene (**4i**): The crude product was recrystallized using ethanol, R_f = 0.42, pale yellow crystals; m.p.: 261-263 ºC. ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 4.56 (2H, s, CH_2), 5.21 (1H, bs, NH), 6.52-6.78 (4H, m, Ar-H), 7.50-8.3 (5H, m, Quin-H); ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 44.82 (CH_2), 105.41, 108.01, 113.83, 117.92, 122.51, 122.52, 128.32, 128.82, 128.67, 131.10, 135.42, 135.37, 141.12, 145.57, 155.76; Mass calcd. (found): *m/z* 281.07 (283 $[M]^{+2}$, 281 $[M]^+$). Anal. calcd. (found) % for $C_{16}H_{12}N_3Cl$: C, 68.21 (68.23); H, 4.29 (4.32); N, 14.91 (14.95).

9-Bromo-12,13-dihydro-5*H*-5,6,13-triazabenzo[4,5]-cyclohepta[1,2-*b*]naphthalene (4j): The crude product was recrystallized using ethanol, R_f = 0.41, pale yellow crystals; m.p.: 272-274 ºC. ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 4.55 (s, 2H, CH_2), 5.22 (bs, 1H, NH), 6.45-6.61 (m, 4H, Ar-H), 7.40-8.20 (m, 5H, quin-H), 6.45-6.61 (m, 4H, Ar-H), 7.40-8.20 (m, 5H, quin-H); ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 44.81 (CH_2), 105.42, 108.01, 113.81, 117.91, 122.53, 122.54, 128.33, 128.82, 128.61, 131.12, 135.30, 135.37, 141.11, 145.53, 155.71; Mass calcd. (found): *m/z* 325.02 (328 $[M]^{+2}$, 326 $[M]^+$). Anal. calcd. (found) % for $C_{16}H_{12}N_3Br$: C, 58.91 (58.91); H, 3.71 (3.74) N, 12.92 (12.88).

Molecular docking: The docking was carried out using Sybyl-X, version 2.1(Tripos Inc., St. Louis, MO), Surflex-Dock algorithm of sybyl-X 2.1 was used to dock designed compounds. The crystal structure of potassium channel (KCSA) from *Streptomyces lividans* was downloaded from the Protein Data Bank (PDB entry code 1BL8: PDB extracted from the Brookhaven Protein Database http://www.rcsb.org/pdb) and used for initial docking studies. Water molecules were removed, essential H atoms were added and side chains were fixed during protein preparation. The structure was then subjected to an energy refinement procedure. Gasteigere-Hückel charges [26] were calculated for the ligand, while Amber7FF02 were used for the K^+ channel. The model was then subjected to energy minimization following the gradient termination of the Powell method for 3000 iterations using Tripos force field with non-bonding cut off set at 9.0 and the dielectric constant set at 4.0 [27]. The binding of the substituted benzodiazepine derivatives was also estimated using a variety of scoring functions that have been compiled into the single consensus score (CScore).

***in vitro* Antimicrobial activity:** Nine dilutions of each drug have been done with Brain Heart Infusion (BHI) for MIC [28]. In the initial tube 20 µL of drug was added into the 380 µL of BHI broth dilutions, 200 µL of BHI broth was added into the next 9 tubes separately. Then from the initial tube 200 µL was transferred to the first tube containing 200 µL of BHI broth. This was considered as 10^{-1} dilution. From 10^{-1} diluted tube 200 µL was transferred to second tube to make 10^{-2} dilution. The serial dilution was repeated up to 10^{-9} dilution for each drug. From the maintained stock cultures of required organisms, 5 µL was taken and added into 2 mL of Brain Heart Infusion (BHI) Broth. In each serially diluted tube 200 µL of above culture suspension was added. The tubes were incubated for 24 h and observed for turbidity.

RESULTS AND DISCUSSION

A conventional synthetic method of substituted quinoline derivatives **1a-j** (**Scheme-I**) resulted in final products **4a-j** to the extent of 62-65%. In our laboratory, a microwave assisted synthesis of benzimidazoles using amides was reported and observed the increase in the yield [29]. This prompted us to conduct the reaction between substituted 3-bromomethyl-2-chloroquinolines (**3a-j**) with 1,2-phenylenediamine under microwave condition to get **4a-j** and found dramatic increase in yield in the range 92-97% within very short time duration of 5 min under irradiation of 150 W (Table-1).

Molecular osiris property explorer studies: Lipinski's rule of five is analyzed while considering a lead molecule as a

TABLE-1
COMPARISON OF YIELDS OF THE SYNTHESIZED COMPOUNDS (**4a-j**) FORMED UNDER THERMAL AND MICROWAVE IRRADIATION

Compd. No.	Thermal		Microwave	
	Time (h)	Yield (%)	Time (min)	Yield (%)
4a	3	62	5	93
4b	3	70	5	94
4c	3	73	5	93
4d	3	75	5	96
4e	3	75	5	96
4f	3	67	5	96
4g	3	67	5	96
4h	3	73	5	93
4i	3	75	5	94
4j	3	73	5	97

drug candidate. Importantly, cLog *P* is a parameter that determines the lipophilicity which is an ability to cross the biological membranes. The cLog *P* values of the title compounds was done at self consistent field theory level using PM_3 (Hamiltonian Inc.) in MOPAC 6.0 Package [30,31]. If a compound exhibits cLog *P* below five, the compound is feasible to be considered as a future drug. Synthesized compounds showed the marginal lipophilicity within the range of 2.68-3.54, which indicate that these molecules have better penetrating power through the cell membrane (Table-2). The *in vivo* administration is directly related to the molecular mass and interesting to note that all these molecules have the molecular mass in the acceptable range (200-500). On the basis of physico-chemical parameters (Table-2), compounds **4f** and **4h** exhibited the higher drug likeliness and drug score. These compounds only were selected for the anxiolytic activity studies.

TABLE-2
OSIRIS PROPERTIES OF THE SYNTHESIZED COMPOUNDS **4a-j**

Compd. No.	clog P	Solubility (log_{10} S)	TPSA	Drug likeliness	Drug score
4a	2.82	-4.72	36.95	0.54	0.60
4b	3.16	-5.07	36.95	-0.60	0.46
4c	2.75	-4.74	46.18	0.73	0.60
4d	3.16	-5.07	36.95	0.72	0.67
4e	3.16	-5.07	36.95	-0.60	0.27
4f	2.75	-4.74	46.18	0.77	0.61
4g	2.75	-4.74	46.18	0.73	0.60
4h	2.68	-4.76	55.41	0.75	0.60
4i	3.42	-5.46	36.95	0.22	0.54
4j	3.54	-5.56	36.95	-1.21	0.36

Molecular docking: Molecular docking study was performed on PDB 1BL8 from *Streptomyces lividans*. The title compounds were docked into the active site of potassium channel (K^+ channel). Potassium channel has adopted a unique binding confirmation and supports the interaction with quinolines (**4a**-**j**). Docked view of all studied molecules (Fig. 3) is represented at the active site of enzyme PDB ID: 1BL8. Computed binding energies of these compounds are listed in Table-3. The molecule **4f** as depicted in Fig. 4 showed hydrogen bonding interactions between NH of diazepine ring with ILE100. Fig. 5 shows the docking of highly active molecule **4h** at the active site of enzyme. Fig. 6 depicts the molecule **4h**, the methoxy group on quinoline ring forming a hydrogen binding with THR107 and two hydrogen bonding interactions exhibited by NH of diazepine ring with ILE100. This has been revealed also in the structure activity relation studies (SAR) of 1,4-benzodiazepine. Fig. 6 showed the oxygen atom of carbonyl group present on the diazepine ring of diazepam making hydrogen bond interaction with hydrogen atom of THR107.

The title compounds have shown the similar kind of interaction with amino acid residue (THR107) as that of diazepam. Consensus score of all the compounds is in the range 2.25-3.68 indicating the summary of all forces of interaction between compounds and the potassium (K^+) channel. Charge and van der Waals interactions between the K^+ channel and ligands varied from -589.65 to -233.52. The Helmholtz free energies of interactions for protein ligands atom pairs range between -22.31 to 13.62. However, its H-bonding, complex (ligand-protein) and internal (ligand-ligand) energies range from -94.77 to -133.79, while those values -25.38 to -29.72 indicate the ligands due to H-bonding, lipophilic contact and rotational entropy, as well as intercept terms [32-35]. These scores indicate that molecules preferentially bind to the K^+ channel as compared to diazepam (Table-3).

Antimicrobial activity: All the synthesized compounds were also screened for their *in vitro* antimicrobial activity at different concentrations *viz.* 100, 50, 25, 12.5, 6.25, 3.125, 1.6, 0.8, 0.4 and 0.2 5 µg/mL broth microdilution method [36]. The minimum inhibitory concentrations (MIC) were determined

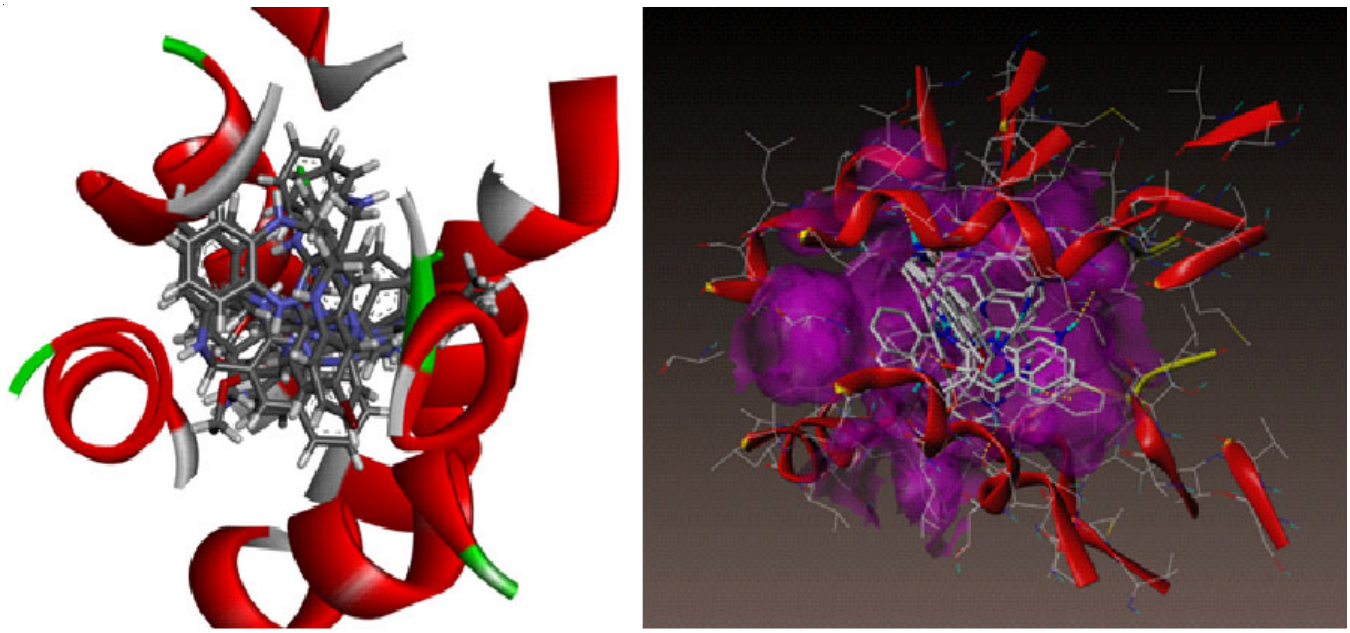


Fig. 3. Docked view of all the 10 inhibitors at the active site of the K^+ channel (KCSA) PDB 1BL8

TABLE-3
SURFLEX DOCKING SCORE (kcal/mol) OF THE SYNTHESIZED COMPOUNDS **4a-j**

Compd. No.	C score[a]	Crash score[b]	Polar score[c]	D score[d]	PMF score[e]	G score[f]	Chem score[g]
4a	3.45	-0.24	1.10	-302.78	-2.95	-111.73	-27.24
4b	3.00	-0.75	0.00	-295.85	-0.37	-133.79	-26.48
4c	3.23	-0.10	1.57	-521.32	-22.31	-102.55	-26.72
4d	3.44	-0.16	1.12	-301.41	-5.54	-113.15	-28.23
4e	3.41	-0.42	1.09	-310.56	-2.76	-123.21	-28.32
4f	3.46	-0.22	0.98	-408.15	-11.96	-111.37	-25.38
4g	3.23	-0.10	1.57	-521.32	-22.31	-102.55	-26.72
4h	3.68	-1.44	1.74	-589.65	13.62	-132.63	-29.26
4i	3.38	-0.16	2.70	-323.37	-17.90	-94.77	-29.24
4j	2.71	-0.11	1.35	-334.27	-17.52	-103.29	-29.15
Diazepam	2.25	-0.47	0.69	-233.52	2.39	-96.68	-29.36

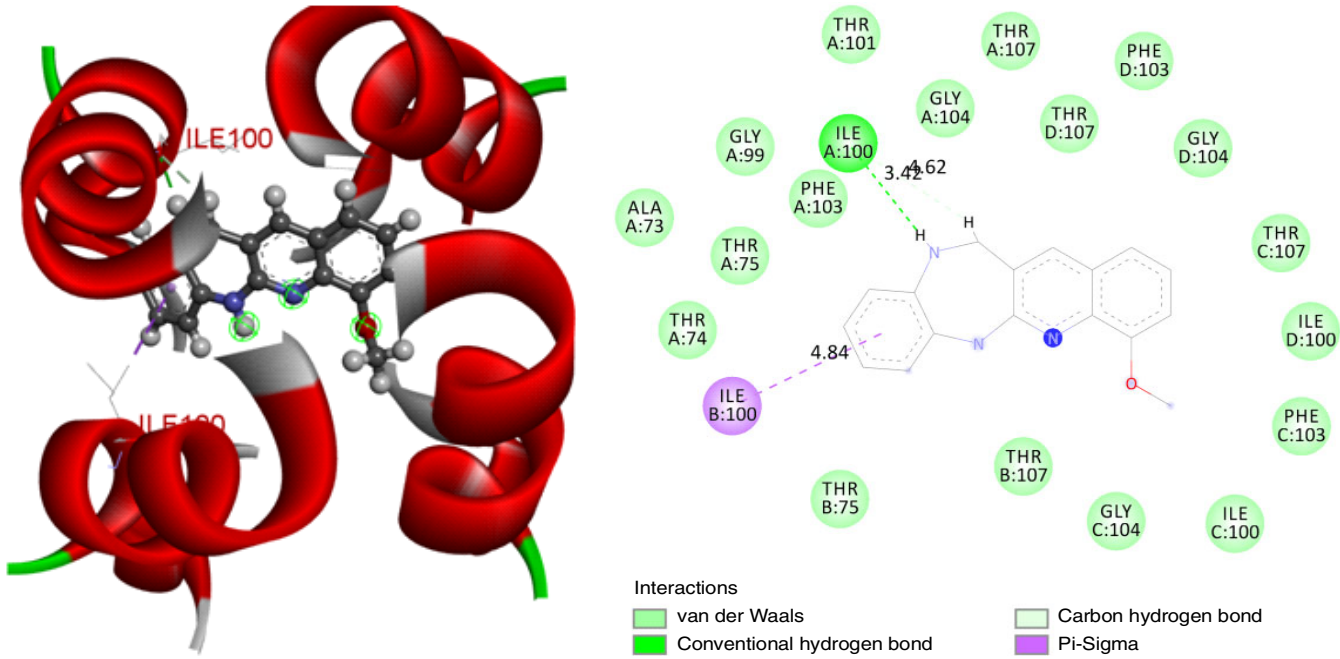


Fig. 4. Binding interaction of compound **4f** at the active site of the K^+ channel (KCSA) PDB 1BL8

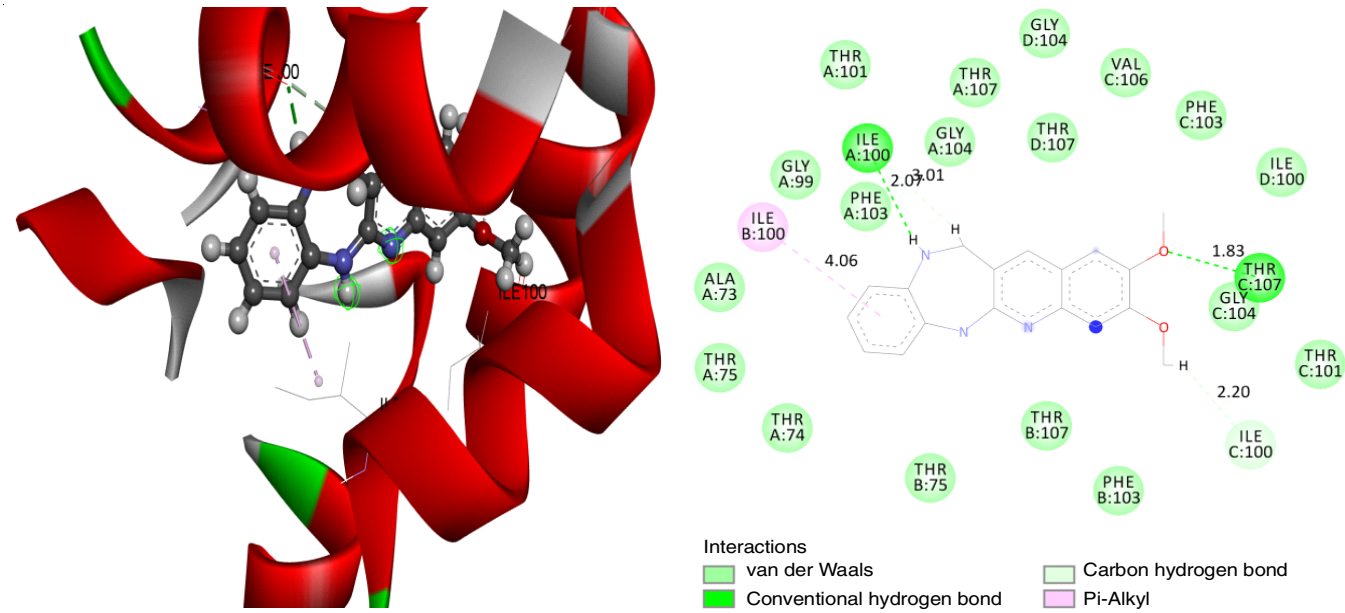


Fig. 5. Binding interaction of **4h** at the active site of the K^+ channel (KCSA) PDB 1BL8

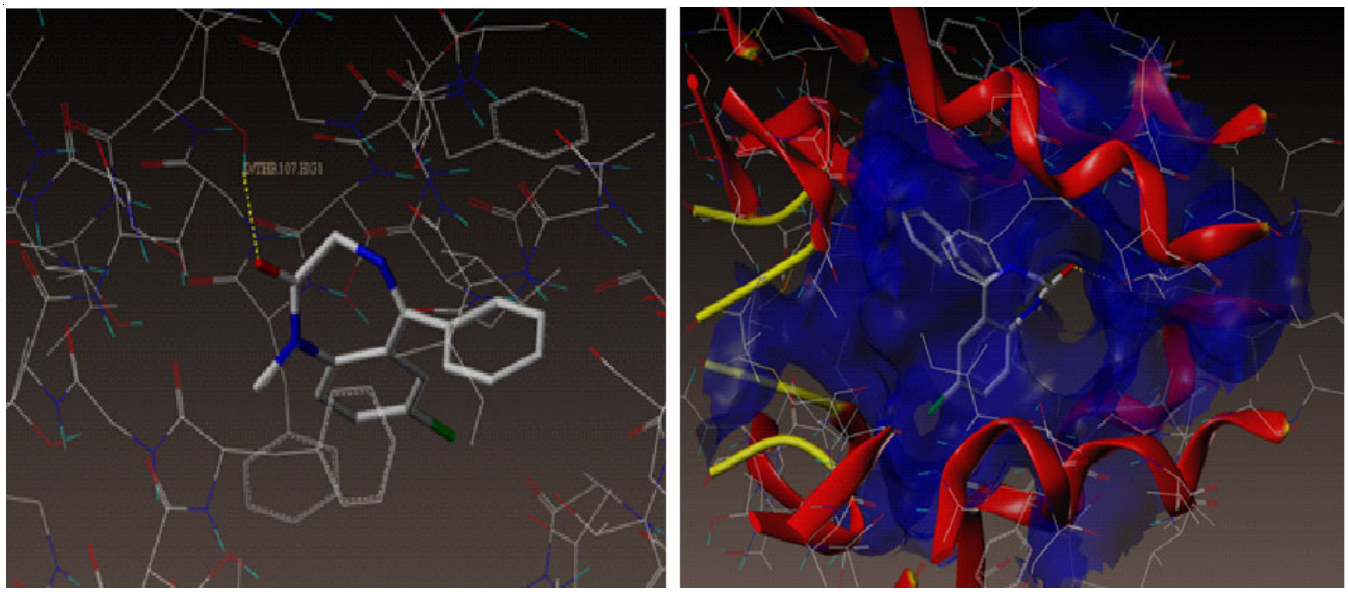


Fig. 6. Binding interaction of diazepam at the active site of the K^+ channel (KCSA) PDB 1BL8

by serial dilution method [37]. Antimicrobial activity was carried out against four different strains *viz*., two Gram-positive bacteria (*Staphylococcus aureus* and *Enterococcus faecalis),* two Gram negative bacteria (*Escherichia coli* and *Klebsiella pneumonea*) and MIC for antifungal activity was carried out against two strains (*Aspergillus fumigatus* and *Candida albicans*). Ciprofloxacin and fluconazole were used as standard antibacterial and antifungal drugs, respectively. The results of antimicrobial screening (Table-4) showed that most of the tested compounds exhibited bacterial and fungal inhibition. Compounds **4b-g** with electron donating substituents were found to be very active against the selected microbes. Compound **4d** with methyl substituent is active against all the microbes with MIC 1.60 µg/mL against *Staphylococcus aureus*, *Enterococcus faecalis*, *Escherichia coli* and 3.12 µg/mL against *Klebsiella pneumoniae*. Its activity is comparable with standard ciprofloxacin. Compounds with electron withdrawing substituents *viz*. **4i** and **4j** with chloro and bromo substitutents were also found to be active against both the types of selected bacteria. Entire series of the title compounds had shown good to moderate antifungal activity (MIC 12.50 to 100 µg/mL) as compared to standard fluconazole.

Conclusion

An inexpensive, novel and facile microwave assisted method to synthesize quinoline fused benzodiazepine derivatives (**4a-j**) is achieved. Molecular docking studies of the compounds indicate the preferential inhibition of K^+ channel one of the target for anxiolytic activity. These molecules also have exhibited moderate to excellent antimicrobial activities. Hence, there is a clear indication from this study that the quinoline fused benzodiazepine derivatives can be lead candidates for further testing.

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TABLE-4
in vitro ANTIMICROBIAL ACTIVITY OF THE SYNTHESIZED COMPOUNDS **4a-j**

Compd. No.	Antimicrobial activity				Antifungal activity	
	Gram-positive		Gram-negative			
	S. aureus	*E. faecalis*	*E. coli*	*K. pneumonea*	*C. albicans*	*A. fumigates*
4a	6.25	3.12	1.60	1.60	12.50	12.50
4b	3.12	1.60	100.00	6.25	12.50	12.50
4c	6.25	3.12	1.60	3.12	25.00	25.50
4d	1.60	1.60	1.60	3.12	12.50	12.50
4e	1.60	3.12	6.25	12.50	25.00	50.00
4f	3.12	1.60	3.12	1.60	25.00	25.00
4g	3.12	3.12	1.60	1.60	50.00	12.50
4h	25.00	100.00	6.25	0.80	25.00	50.00
4i	1.60	1.60	1.60	1.60	100.00	50.00
4j	3.12	3.12	3.12	6.25	25.00	50.00
Ciprofloxacin	2.00	1.00	2.00	1.00	–	–
Fluconazole	–	–	–	–	16.60	8.30

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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