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Synthesis, Spectral Characterization, *in silico* Molecular Docking and Pharmacological Screening of Some Quinazoline Analogues as Anticonvulsants

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A new analogues of 2-methyl-3-(6-substituted-benzothiazol-2-yl)-3*H*-quinazolin-4-one [**Dm1-Dm5**] and their 6-bromo analogues [**Dm6-Dm10**], similarly a new analogues of 2-methyl-3-(pyridin-4-yl-formamide)-3*H*-quinazolin-4-one [**Em1**] and its 6-bromo analogue [**Em2**] were synthesized and characterized by melting point, elemental and spectral [FTIR, (^{1}H and ^{13}C) NMR and MS] methods. The anticonvulsant activity of selected analogues was assessed against maximal electroshock (MES) induced convulsions model in albino mice. The selected analogues were injected intraperitoneally at dose 20 mg/kg body weight and LD_{50}, ED_{50} and TI values were determined. Among the tested analogues, electron withdrawing groups (-NO_2, -Br) containing analogues (**Dm4**, **Dm8**, **Dm10**) shows better potency than the electron releasing groups containing analogues. The activity was determined to be significant at $p < 0.05$ with regard to standard diazepam. Similarly, the electron withdrawing groups containing compounds shows better docking score against the protein 6X3X.

Keywords: Quinazoline, Benzothiazole, Isonicotinamide, Anticonvulsant, Maximal electroshock, Docking studies, 6X3X protein.

INTRODUCTION

Quinazoline constitutes a unique category of biologically active nitrogen heterocycles with significant anticonvulsant potential [1]. In latest years, many efforts have been committed to the development of new approaches by elucidating the cellular and molecular mechanisms of the hyper excitability to provide specific targets for novel therapies and as a result some drugs like gabapentin, diazepam, vigabatrin, phenobarbital, lamotrigine, phenytoin, tiagabine, felbamate and nitrazepam are available in the market [2]. Literature survey reveals that substituted 4(3*H*)-quinazolinones exhibit potential anticonvulsant activity [3,4]. Similarly, 2-aminobenzothiazole derivatives possessed potent anticonvulsant activity [5]. As per literature, hydrazide of isonicotinic acid possessed antitubercular activity [6].

In continuation of earlier endeavors, this research seeks for the new synthetic anticonvulsant lead compounds. Based on these considerations, new series of quinazoline analogues is designed to accommodate benzothiazole moiety. Herein, reported the synthesis of 2-methyl-3-(6-substituted benzothiazol-2-yl)-3*H*-quinazolin-4-one and their 6-bromo analogues. Another series, 2-methyl-3-(pyridin-4-yl-formamide)-3*H*-quinazolin-4-one and their 6-bromo analogue, is also designed to accomodate pyridine moiety using hydrazide of isonicotinic acid with 6-substituted-2-methyl-4-oxo-4*H*-3,1-benzoxazine. Such structural alteration is expected to enhance the anticonvulsant potency and develop compounds with reasonable therapeutic index (TI). The *in silico* molecular docking analysis of the synthesized compounds were performed using AutoDock Vina against protein 6X3X.

EXPERIMENTAL

All chemicals used were of synthetic grade and procured from Astron Chemicals (India) Pvt. Ltd. and Loba Chemie Pvt. Ltd. Mumbai, India. The melting points of synthesized derivatives were checked by open capillary method and are uncorrected. IR spectra were recorded in a Shimadzu IR spectrophotometer KBr disc method. ^{1}H & ^{13}C NMR spectra were recorded on 500 MHz Bruker NMR spectrometer using DMSO-d_6 as solvent. The molecular weights of the synthesized compounds

were determined by TOF, ESI mass spectrophotometer. The completion of the reaction was monitored by TLC and the spots were visualized by UV lamp.

Synthesis of 2-methyl-4-oxo-4*H*-3,1-benzoxazine [A]: Anthranilic acid (13.7 mL, 0.1 mol) and acetic anhydride (10.2 mL, 0.1 mol) was refluxed on heating mantle for 30 min. The additional acetic anhydride was recovered under vacuum [7,8]. The solid obtained was washed with petroleum ether (60:80), after drying recrystallized using dilute ethanol (m.p.: 79-82 ºC, yield: 56%).

Synthesis of 5-bromo anthranilic acid: Anthranilic acid (20.0 g) was mixed in 250 mL glacial acetic acid and cooled below 16 ºC. After the complete addition of 9.5 mL bromine white glistering crystals were obtained. After filtration, the product was washed with benzene then dried at room temperature. The weighed product (15.7 g) was dissolved by boiling in 500 mL of water containing 25 mL conc. HCl. The half solution was filtered and the undissolved material was extracted with 500 mL of boiling water. The filtrate was mixed and cooled to obtain monobromo anthranilic acid as precipitate [9]. The product after washing with distilled water was recrystallized from hot water (m.p.: 209-210 ºC, yield: 40%).

Synthesis of 6-bromo-2-methyl-4-oxo-4*H*-3,1-benzoxazine: It was synthesized by using 5-bromo anthranilic acid (21.6 g, 0.1 mol) and acetic anhydride (10.2 mL, 0.1 mol) following reported procedure [7,8] (m.p.: 181-184 ºC, yield: 69%).

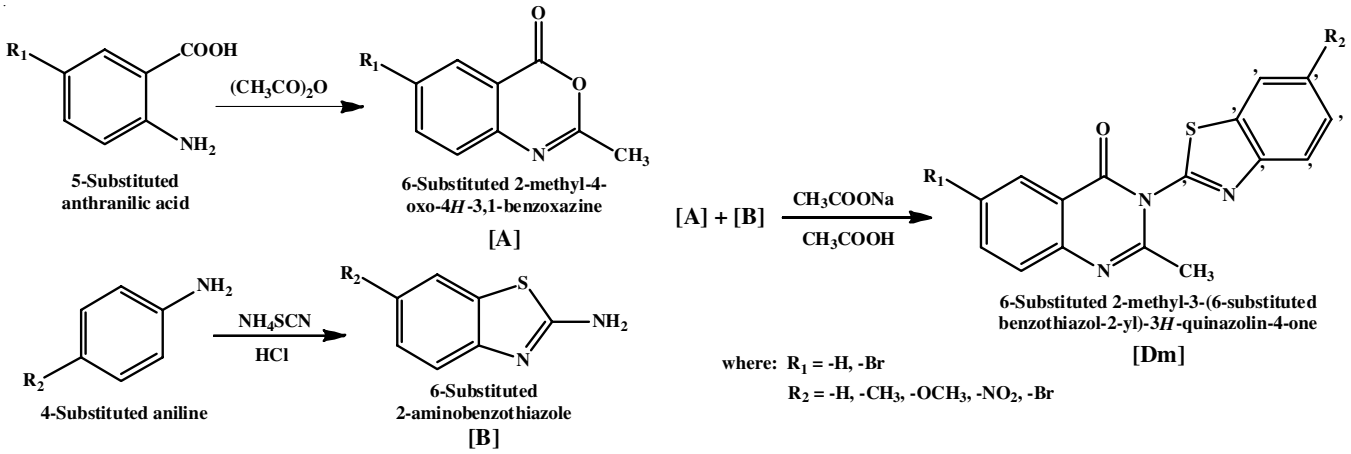
Synthesis of substituted 2-aminobenzothiazole from aniline [B]

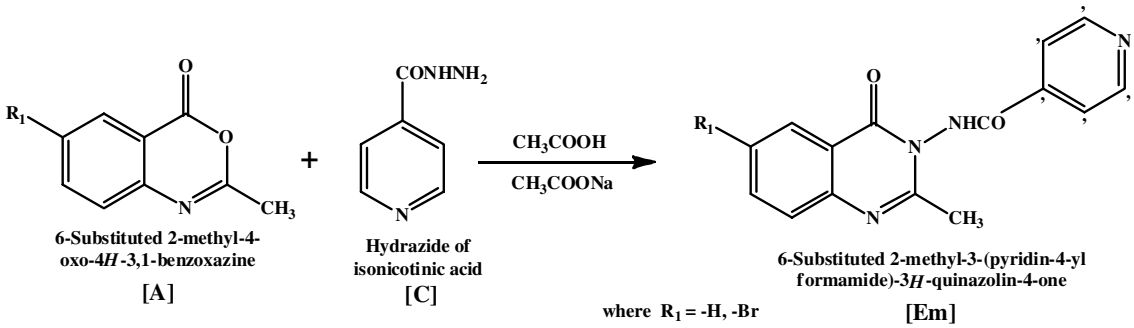
Preparation of phenylthiourea: In a 250 mL round bottom flask, 20 mL of aniline was mixed in 20-25 mL of conc. HCl and solution was warmed. The saturated solution of ammonium thiocyanate (20 g in 50 mL) was poured slowly in above mixture and resulting solution was boiled until the solution gets turbid. The turbid solution was poured on cold ice water, then solid phenylthiourea was obtained [10] (m.p.: 150-153 ºC, yield: 60%).

Synthesis of 2-aminobenzothiazole: Phenylthiourea (19.64 g, 0.1 mol) was added to 150 mL carbon tetrachloride and then bromination was carried out by adding (5% v/v) Br_2 solution in CCl_4 till the orange yellow colour appeared. The resulting slurry stay overnight. The precipitate obtained was filtered, washed with CCl_4 until yellow colour vanished. The resulting precipitate of hydrobromide was dissolved in rectified spirit (200 mL) and basified with conc. NH_4OH solution, which gave the precipitate of 2-aminobenzothiazole [10]. It was washed with water after filtration, dried and then recrystallized by dilute alcohol (70% v/v) (m.p.: 129-131 ºC, yield: 85%).

The remaining 6-substituted-2-aminobenzothiazoles were synthesized by using appropriate quantity of 4-substituted anilines by using above described procedure for the synthesis of 2-aminobenzothiazole (**Scheme-I**).

Synthesis of 2-methyl-3-(benzothiazol-2-yl)-3*H*-quinazolin-4-one (Dm1): In a 250 mL round bottom flask, 2-methyl-4-oxo-4*H*-3,1-benzoxazine (1.61 g, 0.01 mol) [**A**] and 2-aminobenzothiazole (1.5 g, 0.01 mol) [**B**] were mixed in 20 mL glacial CH_3COOH containing 1.5 g sodium acetate. This mixture was refluxed on oil bath for ~25 h at 115-120 ºC, after cooling poured on 100-150 g crushed ice gave a precipitate **Dm1**. It was washed with water after filtration then recrystallized with glacial CH_3COOH [11] (**Scheme-I**).





Scheme-I: Scheme for synthesis of quinazoline analogues

The remaining 2-methyl-3-(6-substituted benzothiazole-2-yl)-3*H*-quinazolin-4-one analogues (**Dm2**-**Dm5**) and their 6-bromo analogues (**Dm6-Dm10**) were synthesized by using 0.025 mol of each of 6-substituted-2-aminobenzothiazoles and 2-methyl-4-oxo-4*H*-3,1-benzoxazine or 6-bromo-2-methyl-4-oxo-4*H*-3,1-benzoxazine using above described procedure. Similarly 2-methyl-3-(pyridin-4-yl-formamide)-3*H*-quinazolin-4-one (**Em1**) and its 6-bromo analogue (**Em2**) were synthesized by using 0.025 mol of hydrazide of isonicotinic acid [**C**] and 2-methyl-4-oxo-4*H*-3,1-benzoxazine or 6-bromo-2-methyl-4-oxo-4*H*-3,1-benzoxazine [A] using above described procedure.

2-Methyl-3-(benzothiazole-2-yl)-3*H*-quinazolin-4-one (Dm1): White solid, yield: 61%, m.p.: 187-190 ºC; R_f value (*n*-hexane:acetonitrile, 4:6): 0.57; FTIR (KBr, ν_{max}, cm^{-1}): 3157, 3093, 3055, 2945, 1681, 1596, 1566, 1504,1448, 1404, 975; ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm: 7.9013-7.4021 (5-H, 6-H, 7-H, 8-H, m, 4H), 0.9232 (CH_3, s, 3H), 8.2354-7.5531 (4′-H, 5′-H, 6′-H, 7′-H, m, 4H); ^{13}C NMR (500 MHz, DMSO-d_6) δ ppm: 160.98, 160.14, 151.73, 149.02, 147.15, 133.57, 128.81, 127.46, 125.95, 125.24, 124.53, 122.42, 121.92, 121.85, 120.94, 22.47; ESI-MS *m/z* for $C_{16}H_{11}N_3OS$: 293.00; found: 294.09 [M+1]; Anal. calcd. (found) %: C, 65.52 (65.28); H, 3.75 (3.74); N, 14.33 (14.28); S, 10.92 (10.88).

2-Methyl-3-(6-methylbenzothiazole-2-yl)-3*H*-quinazolin-4-one (Dm2): White solid, yield: 72%, m.p.: 241-244 ºC; R_f value (*n*-hexane:acetonitrile, 4:6): 0.65; FTIR (KBr, ν_{max}, cm^{-1}): 3070, 3062, 2989, 2938, 1683, 1562,1567, 1494, 1485, 1477, 935; ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm: 7.9124-7.4232 (5-H, 6-H, 7-H, 8-H, m, 4H), 0.9313 (CH_3, s, 3H), 8.1211 (4′-H, d, *J* = 4.50 Hz, 1H), 7.3612 (5′-H, d, *J* = 2.15 Hz, 1H), 7.9233 (7′-H, s, 1H), 2.3573 (CH_3, s, 3H); ^{13}C NMR (500 MHz, DMSO-d_6) δ ppm: 160.94, 160.51, 151.81, 147.81, 146.21, 134.32, 133.21, 128.63, 127.21, 126.68, 124.41, 122.13, 121.74, 121.56, 120.10, 23.91, 22.49; ESI-MS *m/z* for $C_{17}H_{13}N_3OS$: 307.00; found: 308.32 [M+1]; Anal. calcd. (found) %: C, 66.44 (66.16); H, 4.23 (4.21); N, 13.68 (13.62); S, 10.42 (10.37).

2-Methyl-3-(6-methoxybenzothiazol-2-yl)-3*H*-quinazolin-4-one (Dm3): Brown solid, yield: 58%, m.p.: 274-277 ºC; R_f value (*n*-hexane:acetonitrile, 4:6): 0.59; FTIR (KBr, ν_{max}, cm^{-1}): 3161, 3066, 2960, 2910, 1689, 1591, 1544, 1508, 1494, 1473, 993; ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm: 7.9125-7.4224 (5-H, 6-H, 7-H, 8-H, m, 4H), 0.9232 (CH_3, s, 3H), 8.1221 (4′-H, d, *J* = 2.70 Hz, 1H), 7.0622 (5′-H, d, *J*=2.05 Hz, 1H), 7.6633 (7′-H, s, 1H), 3.7342 (OCH_3, s, 3H); ^{13}C NMR (500 MHz, DMSO-d_6) δ ppm: 160.94, 160.53, 157.32, 151.74, 147.21, 141.40, 133.40, 128.94, 127.52, 125.82, 123.12, 122.50, 120.94, 113.70, 105.32, 56.10, 22.50; ESI-MS *m/z* for $C_{17}H_{13}N_3O_2S$: 323.00; found: 324.29 [M+1]; Anal. calcd. (found) %: C, 63.15 (62.90); H, 4.02 (4.00); N, 13.00 (12.95); S, 9.90 (9.86).

2-Methyl-3-(6-nitrobenzothiazol-2-yl)-3*H*-quinazolin-4-one (Dm4): Yellowish brown solid, yield: 63%, m.p.: 289-292 ºC; R_f value (*n*-hexane:acetonitrile, 4:6): 0.74; FTIR (KBr, ν_{max}, cm^{-1}): 3137, 3116, 3031, 2977, 2883, 1695, 1603, 1542, 1506, 1488, 1446, 1528, 1348, 991; ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm: 7.9433-7.4423 (5-H, 6-H, 7-H, 8-H, m, 4H), 0.9316 (CH_3, s, 3H), 8.4922 (4′-H, d, *J* = 4.50 Hz, 1H), 8.4830 (5′-H, d, *J* = 2.70 Hz, 1H), 9.0656 (7′-H, s, 1H); ^{13}C NMR (500 MHz, DMSO-d_6) δ ppm: 160.99, 160.51, 155.22, 151.74, 147.61, 145.94, 133.20, 128.16, 127.41, 125.51, 123.00, 122.13, 120.99, 120.23, 117.82, 22.52; ESI-MS *m/z* for $C_{16}H_{10}N_4O_3S$: 338.00; found: 339.42 [M+1]; Anal. calcd. (found) %: C, 56.80 (56.56); H, 2.95 (2.94); N, 16.56 (16.49); S, 9.46 (9.42).

2-Methyl-3-(6-bromobenzothiazole-2-yl)-3*H*-quinazolin-4-one (Dm5): Whitish grey solid, yield: 51%, m.p.: 278-281 ºC; R_f value (*n*-hexane:acetonitrile, 4:6): 0.82; FTIR (KBr, ν_{max}, cm^{-1}): 3110, 3091, 3066, 2960, 1681, 1596, 1566, 1504, 1473, 1404, 964, 680; ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm: 7.9214 -7.4315 (5-H, 6-H, 7-H, 8-H, m, 4H), 0.9811 (CH_3, s, 3H), 8.1224 (4′-H, d, *J* = 5.00 Hz, 1H), 7.7302 (5′-H, d, *J*=2.10 Hz, 1H), 8.2881 (7′-H, s, 1H); ^{13}C NMR (500 MHz, DMSO-d_6) δ ppm: 161.00, 160.45, 151.85, 148.44, 147.38, 133.61, 128.89, 128.74, 122.48, 127.51, 126.82, 124.41, 124.31, 117.41, 121.01, 22.57; ESI-MS *m/z* for $C_{16}H_{10}BrN_3OS$: 371.00; found: 373.34 [M+2]; Anal. calcd. (found) %: C, 51.75 (51.42); H, 2.69 (2.67); N, 11.32 (11.24); S, 8.62 (8.57).

6-Bromo-2-methyl-3-(benzothiazol-2-yl)-3*H*-quinazolin-4-one (Dm6): Whitish yellow solid, yield: 70%, m.p.: 253-256 ºC; R_f value (chloroform:acetonitrile, 3:7): 0.56; FTIR (KBr, ν_{max}, cm^{-1}): 3157, 3076, 2981, 2935, 1681, 1598, 1566, 1446, 1437, 1413, 964, 530; ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm: 8.1110 (5-H, s, 1H), 7.7432 (7-H, d, *J* = 4.55 Hz, 1H), 7.3345 (8-H, d, *J* = 2.25 Hz, 1H), 0.9762 (CH_3, s, 3H), 8.2344 -7.5563 (4′-H, 5′-H, 6′-H, 7′-H, m, 4H); ^{13}C NMR (500 MHz, DMSO-d_6) δ ppm: 160.96, 160.22, 151.73, 149.31, 146.27, 136.49, 132.36, 125.93, 125.23, 124.64, 124.62, 123.13, 122.03, 121.83, 121.78, 22.48; ESI-MS *m/z* for $C_{16}H_{10}BrN_3OS$: 371.00; found: 373.37 [M+2]; Anal. calcd. (found) %: C, 51.75 (51.42); H, 2.69 (2.67); N, 11.32 (11.24); S, 8.62 (8.57).

6-Bromo-2-methyl-3-(6-methylbenzothiazole-2-yl)-3*H*-quinazolin-4-one (Dm7): White solid, yield: 65%, m.p.: 231-234 ºC; R_f value (chloroform:acetonitrile, 3:7): 0.62; FTIR (KBr, ν_{max}, cm^{-1}): 3074, 2997, 2970, 1687, 1602, 1556, 1473, 1434, 1367, 985, 630; ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm: 8.1220 (5-H, s, 1H), 7.7443 (7-H, d, *J* = 3.50 Hz, 1H), 7.3410 (8-H, d, *J* = 3.05 Hz, 1H), 0.9815 (CH_3, s, 3H), 8.1322 (4′-H, d, *J* = 4.00 Hz, 1H), 7.3661 (5′-H, d, *J* = 2.20 Hz, 1H), 7.9222 (7′-H, s, 1H), 2.3545 (CH_3, s, 3H); ^{13}C NMR (500 MHz, DMSO-d_6) δ ppm: 161.01, 160.49, 151.81, 147.17, 146.78, 136.75, 134.06, 132.39, 126.84, 124.73, 124.62, 124.55, 121.82, 121.73, 121.57, 24.04, 22.52; ESI-MS *m/z* for $C_{17}H_{12}BrN_3OS$: 385.00; found: 387.14 [M+2]; Anal. calcd. (found) %: C, 52.98 (52.69); H, 3.11 (3.09); N, 10.90 (10.84); S, 8.31 (8.26).

6-Bromo-2-methyl-3-(6-methoxybenzothiazol-2-yl)-3*H*-quinazolin-4-one (Dm8): Brown solid, yield: 41%, m.p.: 247-250 ºC; R_f value (chloroform:acetonitrile, 3:7): 0.72; FTIR (KBr, ν_{max}, cm^{-1}): 3193, 3120, 3074, 2993, 2956, 1708, 1600, 1585, 1475, 1448, 1379, 946, 603; ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm: 8.1313 (5-H, s, 1H), 7.7452 (7-H, d, *J* = 4.50 Hz, 1H), 7.3432 (8-H, d, *J* = 2.30 Hz, 1H), 0.9696 (CH_3, s, 3H), 8.1242 (4′-H, d, *J* = 3.35 Hz, 1H), 7.0632 (5′-H, d, *J* = 2.00 Hz, 1H), 7.6431 (7′-H, s, 1H), 3.7342 (OCH_3, s, 3H); ^{13}C NMR (500 MHz, DMSO-d_6) δ ppm: 161.10, 160.73, 157.32, 151.88, 146.68, 141.43, 136.65, 132.99, 125.65, 124.32, 124.24, 123.14, 121.72, 113.87, 105.34, 56.04, 22.48; ESI-MS *m/z* for $C_{17}H_{12}BrN_3O_2S$:

401.00; found: 403.27 [M+2]; Anal. calcd. (found) %: C, 50.87 (50.58); H, 2.99 (2.97); N, 10.47 (10.41); S, 7.98 (7.93).

6-Bromo-2-methyl-3-(6-nitrobenzothiazol-2-yl)-3*H*-quinazolin-4-one (Dm9): Dark brown solid, yield: 74%, m.p.: 278-281 ºC; R_f value (chloroform:acetonitrile, 3:7): 0.69; FTIR (KBr, ν_{max}, cm^{-1}): 3155, 3082, 2969, 2943, 1681, 1598, 1568, 1458, 1444, 1348, 1529, 1349, 975, 686; ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm: 8.1322 (5-H, s, 1H), 7.7562 (7-H, d, *J* = 4.55 Hz, 1H), 7.3457 (8-H, d, *J*= 2.50 Hz, 1H), 0.9572 (CH_3, s, 3H), 8.4933 (4′-H, d, *J*= 4.45 Hz, 1H), 8.4831 (5′-H, d, *J* = 2.00 Hz, 1H), 9.0582 (7′-H, s, 1H); ^{13}C NMR (500 MHz, DMSO-d_6) δ ppm: 160.95, 160.05, 155.25, 151.80, 146.24, 145.73, 136.65, 132.90, 125.45, 124.43, 123.23, 122.80, 121.72, 120.24, 117.83, 22.51; ESI-MS *m/z* for $C_{16}H_9BrN_4O_3S$: 416.00; found: 418.27 [M+2]; Anal. calcd. (found) %: C, 46.15 (45.90); H, 2.16 (2.15); N, 13.46 (13.38); S, 7.69 (7.65).

6-Bromo-2-methyl-3-(6-bromobenzothiazol-2-yl)-3*H*-quinazolin-4-one (Dm10): Whitish yellow solid, yield: 78%, m.p.: 192-195 ºC; R_f value (chloroform:acetonitrile, 3:7): 0.58; FTIR (KBr, ν_{max}, cm^{-1}): 3118, 3066, 2972, 2927, 1676, 1591, 1569, 1498, 1475, 1440, 991, 586; ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm: 8.1300 (5-H, s, 1H), 7.7530 (7-H, d, *J* = 4.50 Hz, 1H), 7.3460 (8-H, d, *J* = 3.00 Hz, 1H), 0.9234 (CH_3, s, 3H), 8.1340 (4′-H, d, *J* = 5.25 Hz, 1H), 7.7351 (5′-H, d, *J* = 2.05 Hz, 1H), 8.2956 (7′-H, s, 1H); ^{13}C NMR (500 MHz, DMSO-d_6) δ ppm: 160.97, 160.32, 151.81, 148.12, 146.63, 136.51, 132.32, 128.81, 126.83, 124.52, 124.43, 124.23, 123.25, 121.72, 117.22, 22.53; ESI-MS *m/z* for $C_{16}H_9Br_2N_3OS$: 449.00; found: 453.20 [M+4]; Anal. calcd. (found) %: C, 42.76 (42.36); H, 2.00 (1.98); N, 9.35 (9.26); S, 7.12 (7.06).

2-Methyl-3-(pyridine-4-yl formamide)-3*H*-quinazolin-4-one (Em1): White solid, yield: 68%, m.p.: 162-165 ºC; R_f value (*n*-hexane:acetonitrile, 2:8): 0.52; FTIR (KBr, ν_{max}, cm^{-1}): 3276, 3157, 3091, 3055, 2995, 2931, 1681, 1616, 1596, 1562, 1560, 1404. ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm: 7.9100-7.4121 (5-H, 6-H, 7-H, 8-H, m, 4H), 0.9741 (CH_3, s, 3H), 8.1224 (NH, s, 1H), 9.0655 (2′-H, 6′-H, d, *J* = 6.75 Hz, 2H), 7.9633 (3′-H, 5′-H, d, *J* = 4.50 Hz, 2H); ^{13}C NMR (500 MHz, DMSO-d_6) δ ppm: 164.92, 164.20, 161.10, 149.86, 149.84, 147.21, 140.93, 133.61, 128.82, 127.53, 122.85, 122.81, 122.51, 120.90, 19.93; ESI-MS *m/z* for $C_{15}H_{12}N_4O_2$: 280.00; found: 281.28 [M+1]; Anal. calcd. (found) %: C, 64.28 (63.99); H, 4.28 (4.26); N, 20.00 (19.90).

6-Bromo-2-methyl-3-(pyridine-4-yl formamide)-3*H*-quinazolin-4-one (Em2): Whitish yellow solid, yield: 72%, m.p.: 190-193 ºC; R_f value (*n*-hexane:acetonitrile, 2:8): 0.68; FTIR (KBr, ν_{max}, cm^{-1}): 3348, 3114, 3062, 3008, 2937, 2869, 1677, 1602, 1579, 1564, 1558, 1444, 584; ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm: 8.1350 (5-H, s, 1H), 7.7351 (7-H, d, *J* = 4.60 Hz, 1H), 7.3573 (8-H, d, *J* = 2.55 Hz, 1H), 0.9694 (CH_3, s, 3H), 8.1242 (NH, s, 1H), 9.0674 (2′-H, 6′-H, d, *J* = 6.70 Hz, 2H), 7.9654 (3′-H, 5′-H, d, *J* = 4.55 Hz, 2H); ^{13}C NMR (500 MHz, DMSO-d_6) δ ppm: 164.95, 164.10, 161.00, 149.85, 149.83, 146.78, 140.96, 136.51, 132.49, 124.63, 123.32, 122.85, 122.83, 121.37, 19.98; ESI-MS *m/z* for $C_{15}H_{11}BrN_4O_2$: 358.00; found: 360.31 [M+2]; Anal. calcd. (found) %: C, 50.27 (49.95); H, 3.07 (3.05); N, 15.64 (15.54).

Molecular docking study: The molecules were docked using AutoDock Vina software [12,13]. The α-1, β-2 and γ-2 sub-type of human $GABA_A$ receptor in complex with diazepam (PDB ID: 6X3X) was selected for the docking [14]. The software predicts the free binding energy with a scoring function based on the linear regression analysis and AMBER force field. The 6X3X structure downloaded from PDB website [15]. The non-amino acid residues, like ions and water molecules existed in downloaded 6X3X structure were removed using MGLTools [16]. Then protein was added with missing hydrogens and subsequently all atoms were provided Kolman charges. The diazepam structure was then removed from the complex 6X3X. Then without diazepam protein was saved in .pdbqt format. All ligand molecules (to be docked) were processed in MGLTools. After identifying rotatable bonds and aromatic carbons, Gasteiger charges were added in the molecules. They were individually saved in .pdbqt format. Then, the GridBox was set around the diazepam binding site. AutoDock Vina software was executed after successfully completion of AutoGrid. The software calculates the interactions in between ligand molecule and amino acids within the GridBox. The co-crystallized ligand *i.e.* diazepam was then docked at the same site using the AutoDock Vina. Afterword, all the designed molecules were allowed to docked individually using a default parameter Lamarckian genetic algorithm (LGA) of AutoDock Vina software.

Animals ethics: Albino mice (25-30 g body weight) of either sex was used. The animals were housed for 7 days before the experiment began. The animals were kept in a 27 ± 10 ºC condition with a light/dark cycle of 12/12 h. Polyacrylic cages with sterile bedding made of rice husks were utilized to house the albino mice rats individually. The subjects were all given the same pellet based nourishment and provided unlimited access to clean water. The experimental protocols were approved by the IAEC of Institute of Pharmaceutical Education and Research, Borgaon (Meghe), Wardha, constituted under the Committee for the Purpose of Control and Supervision of Experimental Animals guidelines. The ethics approval number is 535/PO/ReReBt/S/02/CPCSEA/IPER/IAEC/2023-24/31; dated October 28, 2023.

Anticonvulsant activity

Toxicity study: The OECD guideline 423 was referred for carrying out acute oral toxicity study. The initial dose 2000 mg/kg was administered to 5 animals and the mortality was observed in all 5 animals. The reduced dose (195.31 mg/kg) was administered to the next batch of 5 animals and the mortality was observed in 3 animals. Thus, the lethal dose (LD_{50}) was found to be 195.31 mg/kg. The effective dose (ED_{50}) reduces to 19.07 mg/kg was administered to 5 animals and no mortality observed. Thus, the therapeutic index (TI) was found to be 10.25. Dose consider for checking acute oral toxicity study was 20 mg/kg, for each synthesized analogues.

Anticonvulsant activity using maximal electroshock method (MES): The method of Swinyard *et al.* [17] was used. Albino mice (25-30 g body weight) of either sex were employed. They were fasted overnight but had free access to water. The albino mice were distributed into 8 groups containing 6

animals in each group. The animals were treated with control (PEG-200, dose 1 mL/100 g, ip), standard (diazepam, dose 20 mg/kg, ip) and compounds (**Dm1**, **Dm2**, **Dm4**, **Dm8**, **Dm10** and **Em1**, dose 20 mg/kg each, ip, solutions were prepared in PEG-200). After 30 min all animals were received shock (45 mA for 0.2 sec) and the convulsions induces, the span of increased tone in the extensor muscles was noted. Also the complete abolition of the hindleg extension or reduction in extension time was noted.

In sequence the time in each of the following phases (in second) was noted.

(i) Tonic flexion: The contraction of forelimbs and muscles throughout the body.

(ii) Tonic extension: Extension of forelimbs and hind-limbs.

(iii) Clonus: A stage of relaxation after extension of extremities.

(iv) Stupor: A stage of loss of consciousness before recovery.

RESULTS AND DISCUSSION

In present investigation, 12 quinazoline analogues were synthesized. All the intermediates required for synthesizing different analogues were prepared by the reported procedures. The synthesis of all analogues were achieved by condensing 6-substituted-2-methyl-4-oxo-4*H*-3,1-benzoxazine with 6-substituted-2-aminobenzothiazoles or hydrazide of isonicotinic acid, respectively. The only criteria which changed in the preparation of the title compounds, were the reflux time. This may be due to various substituents present on 2-aminobenzothiazole which can affect the reactivity in present investigation. TLC studies were carried out for all the synthesized compounds. As they gave single spot without tailing after the development of chromatogram in the solvent system *n*-hexane:acetonitrile (4:6, **Dm1-Dm5**); chloroform:acetonitrile (3:7, **Dm6-Dm10**); *n*-hexane:acetonitrile (2:8, **Em1-Em2**), clearly indicated that purity of the synthesized analogues. All analogues were characterized by their physical, spectral and analytical data.

The IR spectra's of intermediates **B** and **C** showed the presence of characteristic absorption peaks in the range of 1640-1580 cm^{-1} (NH deformation) and 3450-3200 cm^{-1} (symmetric and asymmetric NH_2 stretching) while the compounds **Dm1-Dm10** and **Em1-Em2** shows the disappearance of peaks in the range of 1640-1580 cm^{-1} (NH deform.) and 3450-3200 cm^{-1} (symmetric and asymmetric NH_2 stretching) due to the formation of heterocyclic nitrogen of the free primary amino group. The weak absorption peak of NH deformation and NH stretching of formamide observed at 1650-1540 cm^{-1} and 3350-3100 cm^{-1}, respectively for compounds **Em1-Em2**. Halogen containing analogues shows the peaks in between the values of 760-590 cm^{-1} (C-Br stretching) and nitro compounds shows the peaks in between the values of 1600-1300 cm^{-1}. The proton and carbon NMR spectral data and elemental analysis of analogues (**Dm1-Dm10** & **Em1-Em2**) were in conformity with the structure assign. In ESI-MS spectra, molecular ion (M+) peak confirm the molecular weight of the compounds.

The ^{1}H NMR spectra of series **Dm1-Dm10** shows singlet of CH_3 of quinazoline in the range of δ 0.9-2.0 ppm while the multiplate of aromatic rings of quinazoline and benzothiazole shows in range of δ 7.0-9.0 ppm. Similarly, the ^{1}H NMR spectra of **Em1-Em2** shows singlet of CH_3 of quinazoline in the range of δ 0.9-2.0 ppm while the multiplate of quinazoline, formamide and pyridine shows in range of δ 7.0-9.5 ppm. The ^{13}C NMR spectra of **Dm1-Dm10** shows peak of CH_3 of quinazoline in the range of δ 22-23 ppm while multiple peaks of quinazoline and benzothiazole shows in the range of δ 120-161 ppm. Similarly, the ^{13}C NMR spectra of **Em1-Em2** shows peak of CH_3 of quinazoline in the range of δ 19-20 ppm while the multiple peaks of quinazoline, formamide and pyridine is shows in the range of δ 121-165 ppm.

Docking study: As compared to electron releasing groups, electron withdrawing groups (NO_2, Br) containing compounds (**Dm4**, **Dm7**, **Dm8**, **Dm9**, **Dm10**) shows better docking score against the protein 6X3X (Table-1).

TABLE-1
DOCKING SCORE OF SYNTHESIZED ANALOUGES AND STANDARD DIAZEPAM

Compound	Docking score (kcal/mol)	Compound	Docking score (kcal/mol)
Standard	-4.952	**Dm7**	-7.307
Dm1	-6.642	**Dm8**	-7.069
Dm2	-6.966	**Dm9**	-7.375
Dm3	-6.662	**Dm10**	-7.178
Dm4	-7.388	**Em1**	-6.719
Dm5	-6.850	**Em2**	-6.525
Dm6	-6.895		

Anticonvulsant activity: The anticonvulsant activity screening of the six synthesized compounds **Dm1**, **Dm2**, **Dm4**, **Dm8**, **Dm10**, **Em1** and standard was carried out using maximal electroshock method. These compounds exhibited anticonvulsant activity at a dose of 20 mg/kg. The tested analogues shows anticonvulsant activity in between 64% to 86% of standard drug diazepam at a dose of 20 mg/kg. Among the tested analogues, electron withdrawing groups (-NO_2, -Br) containing analogues (**Dm4**, **Dm8**, **Dm10**) shows better potency than the electron releasing groups containing analogues (Table-2).

TABLE-2
SCREENING OF ANTICONVULSANT ACTIVITY BY MAXIMAL ELECTROSHOCK METHOD (MES)

Treatments	Dose	Span of tonic hindleg extension in sec. (Mean ± SEM)	Relative percentage of animal protected (%)
Control	1 mL/100 g	34.000 ± 1.826	23.03
Standard	20 mg/kg	7.833 ± 0.401	100.00
Dm1	20 mg/kg	11.167 ± 0.477	70.14
Dm2	–	10.833 ± 0.749	72.30
Dm4	–	9.167 ± 0.307	85.44
Dm8	–	9.833 ± 0.703	79.66
Dm10	–	9.500 ± 0.428	82.45
Em1	–	12.167 ± 0.601	64.37

*$p < 0.05$ (One way ANOVA followed by Dunnett test).

Conclusion

In this work, newly synthesized quinazoline analogues were constructured and structurally confirmed by spectral and

analytical data. The six synthesized compounds were evaluated for anticonvulsant activity, demonstrating significant pharmacological effects in all six quinazoline analogues (**Dm1**, **Dm2**, **Dm4**, **Dm8**, **Dm10** and **Em1**) as anticonvulsant drugs. Thus, in future, it will be possible to review the exact mechanism of anticonvulsant action of the synthesized analogues. However, further evaluation of quinazoline entity will be undertaken by changing the substitutions on ring for anticonvulsant activity.

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CONFLICT OF INTEREST

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

REFERENCES

1. R.S. Cheke, S.D. Shinde, J.P. Ambhore, S.R. Chaudhari and S.B. Bari, *J. Mol. Struct*., **1248**, 131384 (2022); https://doi.org/10.1016/j.molstruc.2021.131384
2. R.S. Chopade, R.H. Bahekar, P.B. Khedekar, K.P. Bhusari and A.R. Ram Rao, *Arch Pharm*, **8**, 381 (2002); https://doi.org/10.1002/1521-4184(200211)335:8<381:AID-ARDP381>3.0.CO;2-S
3. A.S. El-Azab, K.E.H. El-Tahir and S.M. Attia, *Monatsh. Chem.*, **142**, 837 (2011); https://doi.org/10.1007/s00706-011-0525-3
4. Archana, V.K. Shrivastava, R. Chander and A. Kumar, *Indian J. Chem.*, **41B**, 2371 (2002).
5. J. Mizoule, B. Meldrum, M. Mazadier, M. Croucher, C. Ollat, A. Uzan, J.J. Legrand, C. Gueremy and G. Le Fur, *Neuropharmacology*, **24**, 767 (1985); https://doi.org/10.1016/0028-3908(85)90011-5
6. K.D. Tripathi, Essentials of Medical Pharmacology, Jaypee Brothers Medical Publishers, New Delhi., edn. 7, pp. 766-767 (2018).
7. H.D. Navadiyaa, N.K. Undaviaa and B.S. Patwab, *J. Indian Chem. Soc.*, **86**, 1118 (2009).
8. K. Hemalatha and K. Girija, *Int. J. Pharm. Pharm. Sci.*, **3**, 103 (2011).
9. S. Sharma, V.K. Srivastava and A. Kumar, *Eur. J. Med. Chem.*, **37**, 689 (2002); https://doi.org/10.1016/S0223-5234(02)01340-5
10. T.L. Dadmal, S.D. Katre, M.C. Mandewale and R.M. Kumbhare, *New J. Chem.*, **42**, 776 (2018); https://doi.org/10.1039/C7NJ03776G
11. D.J. Connolly, D. Cusack, T.P. O'Sullivan and P.J. Guiry, *Tetrahedron*, **61**, 10153 (2005); https://doi.org/10.1016/j.tet.2005.07.010
12. M. Bugnon, U.F. Röhrig, M. Goullieux, M.A.S. Perez, A. Daina, O. Michielin and V. Zoete, *Nucleic Acids Res.*, **52W1**, 324 (2021); https://doi.org/10.1093/nar/gkae300
13. J. Eberhardt, D. Santos-Martins, A.F. Tillack and S. Forli, *J. Chem. Inf. Model.*, **61**, 3891 (2021); https://doi.org/10.1021/acs.jcim.1c00203
14. J.J. Kim, A. Gharpure, J. Teng, Y. Zhuang, R.J. Howard, S. Zhu, C.M. Noviello, R.M. Walsh Jr., E. Lindahl and R.E. Hibbs, *Nature*, **585**, 303 (2020); https://doi.org/10.1038/s41586-020-2654-5
15. H.M. Berman, J. Westbrook, Z. Feng, G. Gilliland, T.N. Bhat, H. Weissig, I.N. Shindyalov and P.E. Bourne, *Nucleic Acids Res.*, **28**, 235 (2000); https://doi.org/10.1093/nar/28.1.235
16. S. Dallakyan, MGLTools (2010); http://mgltools.scripps.edu/
17. E.A. Swinyard, W.C. Brown and L.S. Goodman, *J. Pharmacol. Exp. Ther.*, **106**, 319 (1952); https://doi.org/10.1016/S0022-3565(25)05100-6