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Original Article

SYNTHESIS, CHARACTERIZATION AND ANTICONVULSANT ACTIVITY OF SUBSTITUTED 4-CHLORO-2-(4-PIPERAZIN-1-YL) QUINAZOLINES

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ABSTRACT

Objective: Substituted 4-chloro-2-(4-piperazin-1-yl) quinazolines: Synthesis and anticonvulsant activity.

Methods: In the present study, the 2, 4-dichloroquinazoline (5) was synthesized and the compound was reacted with different N-substituted piperazines to obtain a series of title compounds [6(A-G)]. All the new title compounds were characterized by spectral data and were screened for anticonvulsant activity.

Results: The reported compounds were synthesized using the process disclosed by us in U.S.Pat.No.8, 410,268B2. In our present work, we have achieved substantially good yields and purity.

Conclusion: Aryl substituted piperazines exhibited better protection against subcutaneous (s.c.) Pentylenetetrazol induced seizures.

Keywords: Synthesis, Quinazolines, Piperazines, Characterization, Anticonvulsant activity.

INTRODUCTION

Quinazoline heterocycle consists of two fused six membered aromatic rings benzene & pyrimidine. The research on biological activity of quinazoline compounds started when the compound 2-methyl-1,3-aryl-4-quinazoline derivative was synthesized. In 1968 only two derivatives were used, Methaqualone as soporific & anticonvulsant and Quinathazone as diuretic. By 1980, about 50 kinds of derivatives of this class with different medicinal and biological actions like soporific, sedative, tranquilizing, analgesic, anticonvulsant, antitussive, myorelexant, antirheumatic, hypotensive, antiallergic, bronchodilating, antidiabetic, cholagogue, diuretic, cystatic, antimalarial, spermicidal etc [1] were identified . The anticonvulsant activity was attributed to its ability to bind the non-competitive site of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors. In a previous report [2], compounds were synthesized and tested for their anticonvulsant activity, which was comparable to that of diazepam. As a result, these compounds are potential leads for further design of more active compounds. Since the discovery of methaqualone as a sedative hypnotic [3-4], the search for new anticonvulsant drugs with reduced toxicity and fewer side effects has been continuous. It has been reported that replacement of the methyl group by some other functionalities such as alkylthiomethyl or alkyloxymethyl groups reportedly yielded structural analogues which retained the anticonvulsant activity [5-6].

Piperazines are a broad class of chemical compounds with many important pharmacological properties. Piperazine and substituted piperazine nuclei had constituted an attractive pharmacological scaffold present in various potent marketed drugs. The incorporation of piperazine is an important synthetic strategy in drug discovery due to its easy modifiability, proper alkalinity, water solubility, the capacity to form hydrogen bonds and adjustment of molecular physicochemical properties. This di-nitrogen moiety has been an inseparable component of plethora of drugs. A number of substituted piperazines possess significant pharmacological action such as antihistamic [7-8], antimicrobial [9], acetylcholinesterae inhibitors [10], antimalarial [11], dopamine transporter [12-13], D2/D4 antagonist [14], MC4Receptor [15], and HIV-protease inhibitor [16-17]. It has been reported that several of piperazine derivatives showed anticonvulsant properties in several models of seizures. Some piperazine derivatives displayed protection against electroshock (MES) induced seizures, low neurotoxicity (TOX) and little protection in subcutaneous pentylenetetrazole induced seizures (ScPTZ). Some of them, i.e. 1, 4-bis[(4-chloro-3-methyl)-phenoxyethyl]-piperazine dihydrochloride prevent maximal electroshock seizures in mice with an ED50 of 115.9 mg/kg and protective index PI = 2.05 in the MES test in mice which is higher than that of valproate (PI = 1.7) [18]. The present study is a continuation to the various efforts aiming to locate novel synthetic anticonvulsant lead compound(s). Some new quinazoline analogues prepared in our study possessed remarkable anticonvulsant activity. The new series of quinazoline analogues is designed to accommodate N -substituted piperazines and benzoisothiazole rings at C-2. These structure alterations and modifications are expected to contribute to the anticonvulsant activity of the quinazoline nucleus.

MATERIALS AND METHODS

Chemicals and Instrument

Anthranilic acid, potassium cyanate, N, N-dimethyl aniline, Phosphorous oxychloride were obtained from local dealer. Piperazine, 3-(piperazinyl-1-yl) benzo[d] isothiazole, 2-(piperazin-1-yl) phenol, 2-(piperazin-1-yl) ethanol, 2-[(piperazin-1-yl) methoxy] ethanol, 1-(2, 3-dichlorophenyl) piperazine and Morwet D425 were provided by Alkem Laboratories limited. Analytical TLC was performed on Silica plates- GF254 (Merck) with visualization by UV or in iodine. Melting points were determined by MP50 (Mettler Toledo) and are uncorrected. The IR spectra (KBr, λ Max, cm^{-1}) were run on Perkin Elmer FTIR Spectrophotometer. 1H-NMR (in $CDCl_3$ / DMSO-*d*6) spectra were recorded using Bruker -400 with TMS as internal standard. MS spectra were recorded on Brucker DPX 200. Elemental analyses were performed on Carlo Erba 1108 elemental analyzer and were within ± 0.4% of theoretical values. All the chemicals used were of Laboratory grade.

Synthesis of Quinazoline -2,4(1H,3H)-dione[Benzoylene urea] (4)

In a 3-l. round bottom reactor, a mixture of 20 g (0.146 mole) of anthranilic acid, 700 ml of warm water (35°C) and 11 ml (11.6 g., 0.19 mole) of glacial acetic acid were stirred mechanically and allowed to cool to room temperature. A freshly prepared solution of 15 g (0.185 mole) of potassium cyanate in 50 ml of water was then added drop wise with stirring over a period of fifteen to twenty minutes. The resulting pasty mixture was stirred for

twenty minutes and then 200 g (5 moles) of flaked sodium hydroxide was added slowly in small portions. During this addition the reaction mixture was kept below 40°C by cooling in a cold-water bath. A clear solution was obtained momentarily, but in a short time a fine granular precipitate of the hydrated Benzoylene urea precipitated. The mixture was cooled overnight in an ice box. The precipitated sodium salt was collected on a Büchner funnel, using a hardened filter paper. The colourless salt was dissolved in 1 l. of hot water (90–95°C), and the solution was filtered and heated to boiling in a 3-l. beaker. The Benzoylene urea was precipitated by adding dilute sulphuric acid (1:1) with vigorous stirring until the liquor was acid to litmus. The product separates as a hydrate which forms small, lustrous, colourless needles. The material was collected on a Büchner funnel, washed with 200 ml of water, and dried in an oven at 100°C. The yield was 19.5–20.5 g. Melting point: Above 300°C

Synthesis of 2, 4-dichloroquinazoline (5)

2,4-dichloroquinazoline was obtained by refluxing 10.0g (0.061 mole) of quinazoline-2,4(1H,3H)-dione(Benzoylene urea) in 14.2g(0.092 mole) of Phosphorous oxychloride with 7.4g(0.061mole)N,N-dimethylaniline at 108°C . The progress of the reaction was monitored by TLC (Eluent: ethyl acetate: hexane=8:2). After the completion, the reaction mass was cooled to room temperature and hence poured onto ice water under stirring. An off white viscous precipitate formed. The resultant mass was basified with Aqueous 20% w/v of Potassium carbonate to ph 8.0. After reaching the mentioned pH, the reaction mass was extracted with 200.0 ml Dichloromethane. The dichloromethane layer was given a water wash, dried over sodium sulphate and hence distilled to obtain 7.0 g 2, 4-dichloroquinazoline. Melting point: 118-120°C

Synthesis of substituted 4-chloro-2-(4-piperazin-1-yl) quinazoline derivatives [6(A-G)]

0.0075 mole of substituted piperazines, 0.005 mole of 2, 4-dichloroquinazoline, 0.017 mole of Sodium carbonate, water 5.2 times based on 2,4-dichloroquinazoline weight and 1% of dispersing agent MORWET® D-425 were charged in to a round bottom flask and refluxed under nitrogen, under stirring for 12-16 hr. The progress of the reaction was checked by thin layer chromatography (Eluent: ethyl acetate: hexane=8:2). After the completion of the reaction, the reaction mass was cooled to room temperature and the resulting mass was filtered. It was slurried in water and then in isopropyl alcohol (IPA) and isolated by filtration. The solid was dried at 95-100°C. The isolated solids were recrystalized from tetrahydrofuran (THF) to obtain pure compounds.

2-[4-(1, 2-benzoisothiazol-3-yl) piperazin-1-yl]-4-chloroquinazoline, 6A

M.P.272°C; Yield: 89%; MS: 381.08(100.0%), 383.08(37.1%), 382.08(23.2%), 384.08(8.4%); IR max cm^{-1}: 3074.32 (Ar-CH); 1490.56 (Ar C=C); 1668.42 (HC=N); 720.15(C-Cl);

^{1}HNMR(DMSO D^{6}): δ=8.30 (2H,m,quinazoline aromatic CH); 8.16 (1H,d,quinazoline aromatic CH); 7.86 (1H,m,quinazoline aromatic CH); 7.74 (1H,d, benzisothiazole aromatic CH); 7.55 (2H,m, benzisothiazole aromatic CH); 7.48 (1H,d, benzisothiazole aromatic CH);4.08(4H,t,piperazine-CH_2);3.70 (4H,t,piperazine CH_2); ^{13}CNMR: 50.2(4C, piperazine -CH_2); 120-131(9C, Aromatic CH); 138.2(1C, quinazoline aromatic CH); 152.9(1C, quinazoline C-N); 157.4(1C, benzisothiazole N=C-N); 161.6(1C, quinazoline C-Cl); 165.4(1C, benzisothiazole C-S); 185.0(1C, quinazoline N=C-N); Elemental analysis:C-59.76%,H-4.22%,Cl-9.28%,N-18.34%,S-8.40%

2-[4-(4-chloroquinazolin-2-yl) piperazin-1-yl] phenol, 6B

M.P.202°C; Yield: 81%; MS: 340.1(100.0%), 342.1(32.5%), 343.1(6.2%); IR max cm^{-1}: 3415.12(-OH); 3014.12 (Ar-CH); 1470.56 (Ar C=C); 1678.12 (HC=N); 1215(C-O); 750.15(C-Cl)

^{1}HNMR(DMSO D^{6}): δ=7.95(1H,d,quinazoline aromatic CH);7.8-7.85 (2H,m,quinazoline aromatic CH); 7.58(1H,t,quinazoline aromatic CH); 6.4-6.6 (4H,4, aromatic CH);5.2(1H,s,-OH);3.64 (4H,m,piperazine CH_2); 3.34 (4H,m,piperazine CH_2)

^{13}CNMR: 49.2(4C, piperazine -CH_2); 115-123(4C, Aromatic CH); 116.2(1C, quinazoline aromatic CH); 125-139 (4C, quinazoline aromatic CH); 142.4(1C, C-OH); 152.8(1C, quinazoline C-N); 146.8(1C, phenylring C-N); 160.6(1C, quinazoline C-Cl); 184.2(1C, quinazoline N=C-N); Elemental analysis:C-63.44%,H-5.03%,Cl-10.40%,N-16.44%,O-4.69%

2-[4-(4-chloroquinazolin-2-yl) piperazin-1-yl] ethanol, 6C

M.P198°C; Yield: 84%; MS: 292.1(100.0%), 294.1(32.5%), 293.11(16.7%), 295.11(4.9%); IR max cm^{-1}: 3315.12(-OH); 3014.12 (Ar-CH); 1470.56 (Ar C=C); 1678.12 (HC=N); 1065(C-O); 750.15(C-Cl)

^{1}HNMR(DMSO D^{6}): δ=8.13 (1H, d, quinazoline aromatic CH); 7.85 (1H, t, quinazoline aromatic CH); 7.75(1H, d, quinazoline aromatic CH); 7.58(1H, t, quinazoline aromatic CH); 3.82(2H,m,-CH_2); 3.14 (4H,m,piperazine CH_2); 2.64 (4H,m,piperazine CH_2); 2.44 (2H,m,-CH_2); 2.0(1H,s,-OH)

^{13}CNMR: 50.0-53.6 (8C, piperazine -CH_2); 57.0-59.4 (4C, -CH_2); 116.2(1C, quinazoline aromatic CH); 125-139 (4C, quinazoline aromatic CH); 142.4(1C, C-OH); 152.8(1C, quinazoline C-N); 160.6(1C, quinazoline C-Cl); 184.2(1C, quinazoline N=C-N); Elemental analysis: C-57.44%,H-5.85%,Cl-12.11%,N-19.14%,O-5.46%

2-{[4-(4-chloroquinazolin-2-yl) piperazin-1-yl] methoxy} ethanol, 6D

M.P.201°C; Yield: 81%; MS: 336.14(100.0%), 338.13(32.0%), 337.14(17.7%), 339.14(5.7%); IR max cm^{-1}: 3321.12(-OH); 3023.12 (Ar-CH); 1466.56 (Ar C=C); 1678.12 (HC=N); 1084(C-O); 761.15(C-Cl)

^{1}HNMR(DMSO D^{6}): δ=8.15 (1H,d,quinazoline aromatic CH); 7.82 (1H,t,quinazoline aromatic CH); 7.7(1H,d,quinazoline aromatic CH); 7.53 (1H,m,quinazoline aromatic CH);3.55-3.82(6H,m,-CH_2);3.14 (4H,m,piperazine CH_2); 2.64 (4H,m,piperazine CH_2); 2.44 (2H,m,-CH_2);2.0(1H,s,-OH)

^{13}CNMR: 50.6-55.6 (4C, piperazine -CH_2); 54.8 (1C, -CH_2); 61.4 (1C, C-OH); 68.4 (1C, C-O); 72.6 (1C, C-O)116.2(1C, quinazoline aromatic CH); 125-139 (4C, quinazoline aromatic CH);152.8(1C,quinazoline C-N);160.6(1C,quinazoline C-Cl);184.2(1C,quinazoline N=C-N);Elemental analysis:C-57.06%,H-6.28%,Cl-10.53%,N-16.63%,O-9.50%

4-chloro-2-[4-(2, 3-dichlorophenyl) piperazin-1-yl] quinazoline, 6E

M.P.218°C; Yield: 84%; MS: 392.04 (100.0%), 394.03(30.9%)

; IR max cm^{-1}: 3023.12 (Ar-CH); 1466.56 (Ar C=C); 1678.12 (HC=N); 759.15(C-Cl)

^{1}HNMR(DMSO D^{6}): δ=8.1(1H,d,quinazoline aromatic CH); 7.85 (1H, t, quinazoline aromatic CH); 7.73 (1H, d, quinazoline aromatic CH); 7.55 (1H, t, quinazoline aromatic CH); 7.35 (2H, d, phenyl aromatic CH); 7.2 (1H, m, -aromatic -CH); 4.0 (4H, m, piperazine CH_2); 3.35 (4H, m, piperazine CH_2);

^{13}CNMR: 49.1-49.6 (4C, piperazine -CH_2);113.8,119.8,129.2(3C,Phenyl ring)116.2(1C, quinazoline aromatic CH);123.8(1C,C-Cl);134.3(1C,C-Cl); 125-139 (4C, quinazoline aromatic CH); 152.1(1C, C-N); 152.8(1C, quinazoline C-N); 160.6(1C, quinazoline C-Cl); 184.2(1C,quinazoline N=C-N);Elemental analysis:C-54.91%,H-3.84%,Cl-27.02%,N-14.23%

4-chloro-2-(4-methylpiperazin-1-yl) quinazoline, 6F

M.P.188°C; Yield: 84%; MS: 262.10 (100.0%), 264.10(32.2%); IR max cm^{-1}: 3028.52 (Ar-CH); 1476.56 (Ar C=C); 1678.02 (HC=N); 756.15(C-Cl)

^{1}HNMR(DMSO D^{6}): δ=8.01(1H,d,quinazoline aromatic CH);7.8-7.85 (2H,m,quinazoline aromatic CH); 7.61 (1H,m,quinazoline aromatic CH);3.14 (4H,m,piperazine CH_2); 2.64 (4H,m,piperazine CH_2); 2.3(3H,s,-CH_3)

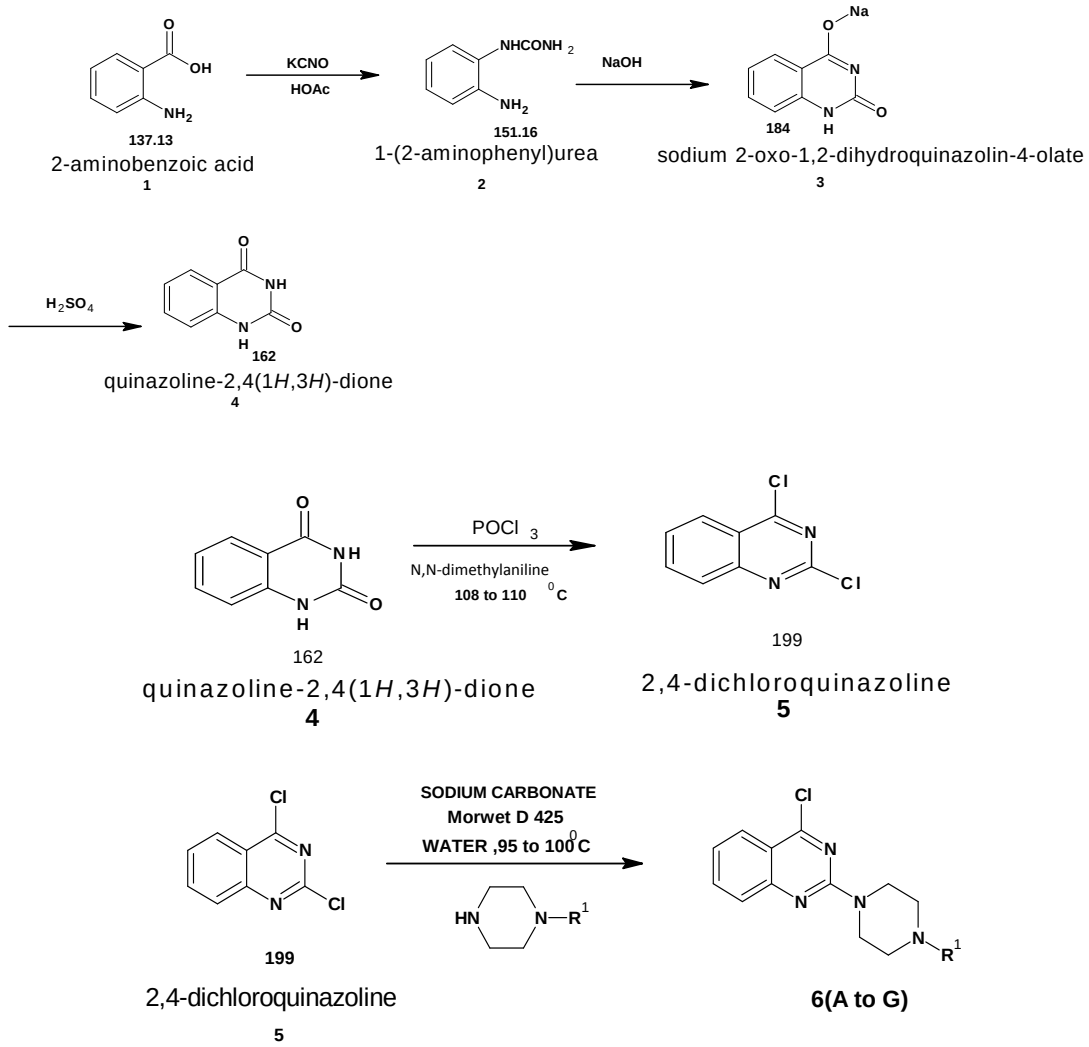
^{13}CNMR: 43.1(1C,-CH_3)49.1-49.6 (4C, piperazine -CH_2);116.2(1C, quinazoline aromatic CH);123.8(1C,C-Cl);134.3(1C,C-Cl); 125-139 (4C, quinazoline aromatic CH);152.1(1C,C-N);152.8(1C,quinazoline C-N);160.6(1C,quinazoline C-Cl);184.2(1C,quinazoline N=C-N);Elemental analysis:C-59.43%,H-5.75%,Cl-13.49%,N-21.32%,

4-chloro-2-(4-(4-chloroquinazolin-2-yl) piperazin-1-yl) quina zoline, 6G: M.P.249°C; Yield: 80%; MS: 410.08 (100.0%); ; IR max cm^{-1}: 3028.52 (Ar-CH); 1476.56 (Ar C=C); 1678.02 (HC=N); 756.15(C-Cl)

^{1}HNMR(DMSO D^6): δ=8.1(2H,d,quinazoline aromatic CH);7.8-7.9 (4H,m,quinazoline aromatic CH); 7.58 (2H,m,quinazoline aromatic CH);3.24 (8H,m,piperazine CH_2);

^{13}CNMR: 49.6 (4C, piperazine -CH_2);116.5(2C, quinazoline CH); 125-139 (8C, quinazoline aromatic CH);

152.8(2C,quinazoline C-N);161.0(2C, quinazoline C-Cl);184.1(2C,quinazoline N=C-N);Elemental analysis:C-58.41%,H-3.92%,Cl-17.24%,N-20.43%,



R^1=3-(piperazinyl-1-yl)benzo[d]isothiazole,2-(piperazin-1-yl)phenol,2-(piperazin-1-yl) nethanol, 2-[(piperazin-1-yl) methoxy] ethanol,1-(2,3-dichlorophenyl) piperazine,N-Methyl piperazine, Piperazine

Scheme 1: Synthesis of substituted 4- chloro-2-(4-piperazin-1-yl) quinazolines 6A-G

Evaluation of anticonvulsant activity

Experimental animals

Male albino Swiss mice, weighing 20 - 25 g, were used to study the effect of the synthesized compounds on subcutaneous (s.c.) Pentylenetetrazole induced seizures. Female animals were excluded because of the fact that estrous cycle could influence their activity threshold.

The animals were housed in a standard cage at room temperature in a12 /12 light dark cycles. The animals were fed on standard mice pellet and water *ad libitum.* All experiments were conducted in accordance with animal use ethics as accepted internationally.

Acute toxicity studies

Compounds were administered subcutaneously (s.c.) in doses of 50, 100, 200, 500, 1000, 1500 and 2000 mg/kg to different groups of mice, each group consisting of six animals (n = 6). The mice were also observed for 24 hours. The final LD50 was calculated as the square root of the product of the lowest lethal dose and the highest non-lethal dose *i.e.* the geometric mean of consecutive doses for which 0 and 100% survival rates were recorded [19].

2 -Pentylenetetrazole (s.c.PTZ) induced seizure test

Forty five adult albino mice were randomly divided into five mice each. Group one (Standard) received 40 mg/kg, body weight of Rufinamide intraperitonealy (i.p.), and group two (Control) was given Pentylenetetrazole subcutaneously. **(**Dose: 40 mg/kg, body weight, s .c**).** The synthesized compounds 6(A-G) were administered to group's three to nine (treated groups) intraperitonealy, 50 mg/kg of body weight. Thirty minutes later, 40 mg/kg of freshly prepared solution of Pentylenetetrazole was administered subcutaneously to each mouse. The onset of action, number of rats showing tonic convulsion as well as mortality where recorded in each group [20].

Table 1: Effect of the compounds (6A-6G) on Pentylenetetrazole (PTZ) – induced seizures in mice

Compound name	Dose in mg Mean± SD	Dilution in ml Mean± SD	Latency of tonic convulsion (sec) Mean± SD	Duration of clonus (sec) Mean± SD	No. of animals convulsed/ No. of animals used	Animals protected (%)
6A	1.29±0.19	0.26±0.04	No onset	No clonus	0/5	100%
6B	1.33±0.14	0.27±0.03	No onset	No clonus	0/5	100%
6C	1.20±0.19	0.24±0.04	No onset	No clonus	0/5	100%
6D	1.41±0.13	0.28±0.02	No onset	No clonus	0/5	100%
6E	1.25±0.07	0.25±0.01	No onset	No clonus	0/5	100%
6F	1.26±0.25	0.25±0.05	82.6±49.92	No clonus	0/5	100%
6G	1.54±0.11	0.30±0.02	45.6±22.26	5.2±5.21	3/5	40%
Rufinamide	1.29±0.19	0.26±0.04	No onset	No clonus	0/5	100%
PTZ	1.04±0.06	0.21±0.01	46.4±14.43	79.2±46.9	5/5	0%

RESULTS

Acute toxicity study

The subcutaneously (s.c.) LD50 of the drugs was found to be 100 mg Kg^{-1}.

Study on the effects of the synthesized compounds on the convulsive activity of 40 mg/Kg of subcutaneous Pentylene tetrazole in mice.

All the control animals exhibited threshold seizures. The synthesized compounds exhibited some anticonvulsant effect on seizure induced by subcutaneous Pentylenetetrazole. It also protected 100% of the animals from death compared to the control group where mortality of 100% was recorded.

The observations are tabulated in Table 1.

DISCUSSION

The 2-chloro position of the 2, 4-dichloroquinazoline was replaced by substituted piperazines. The reaction was facilitated by the use of Morwet-D425. The major problems faced in this type of reactions were the incompletion of the reaction, the formation of sticky material and difficult stirrability of the reaction mass. These problems are evident in smaller scales but especially acute in large scale manufacturing. This results in lesser purity and lower yields. In U.S.Pat.No.8,410,268B2 [21] we have disclosed a process for the preparation of Ziprasidone, which involves the same procedure. In our present work, we have achieved substantially good yields and purity. All the compounds were screened for anticonvulsant activity. The substitutions involving aryl substituted piperazines showed better protection against subcutaneous (s.c.) Pentylenetetrazole induced seizures.

CONCLUSION

The protection offered by the synthesized compounds 6A, 6B, 6C and 6D against subcutaneous (s.c.) Pentylenetetrazole induced threshold seizure, the prevention of the onset of seizure and its protective effect against mortality in mice suggests that the synthesized compounds may be effective in the management of petit mal epilepsy since all antiepileptic drugs that are effective in the treatment of petit mal epilepsy exhibit dose dependent suppression of seizure induced by subcutaneous (s.c.) Pentylenetetrazole [22].

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