SYNTHESIS AND NEUROPHARMACOLOGY OF BENZFUUSED FIVE MEMBERED HETEROCYCLIC COMPOUNDS

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SUMMARY

In the present study various benzofused five membered heterocyclic moieties (benzimidazole, benzothiazole and isatin) are synthesized using different schemes (1-5) and their neuropharmacological screenings have been performed. A total of 98 compounds have been synthesized and characterized from five different schemes, 16 compounds each from first three schemes and 25 compounds each from last two schemes. Purity of all the newly synthesized compounds was monitored by thin layer chromatography (TLC) technique and elemental analysis. All the melting points were determined in open glass capillary using Kjeldahl flask containing paraffin and are uncorrected. New synthesized compound were characterized by FT-IR, $^1$H-NMR, $^{13}$C NMR and mass spectroscopic techniques. Anticonvulsant activities of all the titled compounds were evaluated by maximal electroshock seizure (MES) and subcutaneous pentylenetetrazole tests. The anticonvulsant testing of the final compounds of series 2 and 3 have been performed by National Institute of Neurological Disorders and Stroke (NINDS), USA under Anticonvulsant screening program (ASP), following the protocol adopted by the Antiepileptic Drug Development (ADD) program. The active compounds of the anticonvulsant screen were further evaluated for their antidepressant and antinociceptive activities. Acute toxicity studies (LD$_{50}$) of the active compounds have been performed by Litchfield and Wilcoxon method. Minimal motor impairment was assessed by rotorod method and the unwanted effects on liver were checked by liver enzyme estimation techniques biochemically. Neurochemical study of active compounds of scheme 4 and 5 were done by Estimation of GABA level in rat brain. Computational studies like three-dimensional structural analysis and distance mapping were also calculated for different series of compounds.
Scheme 1 contained the synthesis of 2-[(1-substituted benzyl-1H-benzo[d]imidazol-2-yl) methyl]-1-arylisothiourea (3a-p), were obtained by refluxing 2-(chloromethyl)-1-(2-substituted benzyl)-1H-benzo[d]imidazole with different arylthioureas in presence of acetone. Synthesized compounds were characterized by elemental analysis, FT-IR and $^1$H-NMR. Analytical and spectral data were in good agreement with the composition of synthesized compounds. In the anticonvulsant screening, almost all the compounds showed encouraging activity. Compounds 3g, 3l and 3o were found to be highly active against MES test at a dose level 30 mg/kg at 0.5 h time interval indicative of their ability to prevent seizure spread at relatively lower dose. Compounds that exhibited moderate protection against MES model at 100 mg/kg include 3b, 3e, 3h, 3j, 3m and 3p at 0.5 h. In chemoshock investigation compounds 3g, 3l, 3m and 3o were found to be active after 0.5 h of the drug administration at a dose of 100 mg/kg. In neurotoxicity study rotorod tests, compounds 3e, 3g, 3h, 3m and 3p were less neurotoxic and the rest of the compounds did not exhibit neurotoxicity. In acute toxicity study (LD$_{50}$) it was found that the tested compounds 3l and 3o did not produce any mortality or gross effect on the central nervous system at any of the dose levels i.e., 5, 25, 125, 250 and 400 mg/kg. However, the animals have exhibited some of the toxic signs such as chewing, licking, salivation followed by brief period of sedation. But at the doses of 250 & 400 mg/kg compound 3g showed mortality (2/6 & 4/6 respectively). In antidepressant activity compound 3j showed extremely significant decrease in the immobility time and was potent comparable to Fluoxetine ($p < 0.001$), whereas, compounds 3g and 3m were found to be highly significant with ($p < 0.01$) and compounds 3e, 3l and 3p were found to be significant with ($p < 0.05$). In antinociceptive activity compounds 3b, 3j and 3o were found to be highly significant with ($p < 0.01$) increase in the reaction time and compound 3e was found to be significant with ($p < 0.05$). Increase in the reaction time due to other compounds was non-significant. In biochemical estimation compound 3o showed moderate but highly significant ($p < 0.01$) while 3l showed significant ($p < 0.05$) change in the SGOT level as compared to the control. Compounds 3l and 3o were also found to alter significant ($p < 0.05$) in the SGPT level. Only compound 3o was found to be significant ($p < 0.05$) in the alkaline phosphatase level. All the other values were not significant indicating the non-toxic nature of the tested compounds.
Scheme 2 contained the synthesis of 2-[(1-(2-substitutedbenzyl)-1H-benzo[d]imidazol-2-yl)methyl]-N-substituted phenyl hydrazine carbothioamides (4a-p), were prepared on refluxing different substituted phenylisothiocyanates with compounds 1-[(1-substituted benzyl-1H-benzo[d]imidazol-2-yl) methyl] hydrazine. Synthesized compounds were characterized by elemental analysis, FT-IR, $^{13}$C NMR, $^1$H-NMR and mass spectrometry. Analytical and spectral data were in good agreement with the composition of synthesized compounds. In the anticonvulsant screening, almost all the compounds showed encouraging activity. In MES test, compounds 4e and 4p were active at dose of 100 mg/kg (1/1 and 2/3 animals protected) 4.0 h after administration, however compound 4p showed some toxicity at a dose of 100 and 300 mg/kg. Interestingly compound 4e was also active at dose of 300 mg/kg (1/1 animal protected) after 4.0 h. In sc.PTZ screening compounds 4f and 4p were active at doses of 100 mg/kg (1/5 and 1/1 animals protected) 4.0 h after administration, interestingly compound 4f did not show any toxicity at any dose. Dramatically compound 4p was found to be most potent at the dose of 100 mg/kg (1/1 animal protected) after 4.0 h and at the dose of 300 mg/kg (4/5 animals protected) after 0.5 h administration, however it showed some toxicity at higher doses of 100 & 300 mg/kg. The compound 4p that displayed a marked MES activity in mice at the dose of 100 mg/kg i.p (2/3 animals protected), was evaluated for oral activity in rats, Test 2 results. Compound 4p did not show any activity in the MES screen at 30 mg/kg (po dose) but tremendously showed far-fetched activity in the scPTZ screen at 30 mg/kg (po dose) (1/4, 1/4 and 2/4 animals protected) 1.0 h, 2.0 h and 4.0 h after administration. It is interesting that compound 4p showed lack of any toxicity at 30 mg/kg (po dose) in any time interval. Compound 4m was evaluated for 6 Hz assay in mice, Test 7 results. Compound 4m showed protection in the Minimal clonic seizure (6 Hz) test at 100 mg/kg (i.p dose) (1/4 animal protected) 0.5 h after administration. Interestingly it
lacks any toxicity in given dose in any time intervals. In acute toxicity study (LD₅₀) it was found that the tested compounds 4f and 4m did not produce any mortality or gross effect on the central nervous system at any of the dose levels i.e., 5, 25, 125, 250 and 400 mg/kg. However, the animals have exhibited some of the toxic signs such as chewing, licking, salivation followed by brief period of sedation. But at the doses of 125, 250 & 400 mg/kg compound 4p showed mortality (1/6, 3/6 & 3/6 respectively). In antidepressant activity compound 4j showed extremely significant decrease in the immobility time and was potent comparable to Fluoxetine (p < 0.001), whereas, compound 4p was found to be highly significant with (p < 0.01) and compounds 4e and 4m were found to be significant with (p < 0.05). In antinociceptive activity compound 4m was found to extremely significant with (p < 0.001) increase in the reaction time. A compound 4p was found to be highly significant with (p < 0.01) while compound 4j and 4l was found to be significant with (p < 0.05). Increase in the reaction time due to other compounds was non-significant.

Scheme 3 contained the synthesis of 1-(amino-N-arylmethanethio)-3-(1-substituted benzyl-2, 3-dioxoindolin-5-yl) urea (5a-p), were prepared on refluxing different substituted phenylisothiocyanates with compounds 1-(1-substituted benzyl-2, 3-dioxoindolin-5-yl) urea. All the final compounds were characterized by different spectral analytical techniques like IR spectroscopy, ¹H NMR, ¹³C NMR and Mass spectrometry and the data confirmed the structures of synthesized compounds. In the anticonvulsant screening, almost all the compounds showed encouraging activity. In MES test, compound 5i was active at dose of 100 mg/kg (1/3 animal protected) 4.0 h after administration; interestingly compound 5i was also active at dose of 300 mg/kg (1/1 & 1/1 animals protected) after 0.5 h & 4.0 h. In 300 mg/kg dose, compounds 5f and 5h were also found to be active (1/1 and 1/1 animals protected) 0.5 h and (1/1 and 1/1 animals protected) 4.0 h after administration. In 300 mg/kg dose, compounds 5k
and 5i were active (1/1 and 1/1 animals protected) 4.0 h after administration. In sc.PTZ screening compounds 5h and 5i were active at dose of 300 mg/kg (1/5 and 2/5 animals protected) 0.5 h after administration. Compounds 5k and 5l were also showed protection at dose of 300 mg/kg (1/1 and 1/1 animals protected) 4.0 h after administration. In the neurotoxicity screening, compounds 5f, 5h, 5i, 5k and 5l that were found to be potent were devoid of minimal motor impairment at any doses. Rest all the compounds, although were not potent showed lack of any neurotoxicity. The compounds 5h and 5i that displayed a marked MES as well as sc.PTZ activity in mice at the dose of 100 & 300 mg/kg i.p were evaluated for 6 Hz assay in mice, Test 7 results. Compound 5h showed protection in the Minimal clonic seizure (6 Hz) test at 100 mg/kg (i.p dose) (1/4 & 1/4 animals protected) 0.25 & 0.5 h after administration. In 6 Hz test compound 5i showed protection at 100 mg/kg (i.p dose) (3/4, 2/4 & 2/4 animals protected) 0.25, 0.5 & 1.0 h after administration. Interestingly it lacks any toxicity in given dose in any time intervals. In acute toxicity study (LD_{50}) it was found that the tested compounds did not produce any mortality or gross effect on the central nervous system at any of the dose levels tested, i.e., 5, 25, 125, 250 and 400 mg/kg. In antidepressant study Compound 5h showed extremely significant decrease in the immobility time and was potent comparable to Fluoxetine \( p < 0.001 \), whereas, compound 5k was found to be highly significant with \( p < 0.01 \) and compound 5f was found to be significant with \( p < 0.05 \). In antinociceptive activity compounds 5k was found to extremely significant with \( p < 0.001 \) increase in the reaction time and compound 5f and 5l were found to be significant with \( p < 0.05 \). Increase in the reaction time due to other compounds was non-significant.

Scheme 4 contained the synthesis of 2-[2-(4- substituted benzylidene)hydrazinyl]-N-(6- substituted benzo[d]thiazol-2-yl) acetamide (4a-y). The compounds N-(6-substituted benzo[d]thiazol-2-yl)-2-hydrazoneacetamide in glacial acetic acid and ethanol were heated to boiling and refluxed with aromatic aldehydes to get the final product.
Summary

Compounds 4a-y. Synthesized compounds were characterized by elemental analysis, FT-IR and $^1$H-NMR. The structures and purity of the final compounds were confirmed on the basis of spectral and elemental analyses. In the anticonvulsant screening, almost all the compounds showed encouraging activity. Compounds 4p, 4q, 4r, 4u, 4v and 4w were found to be highly active against MES test at a dose level 30 mg/kg at 0.5 h time interval indicative of their ability to prevent seizure spread at relatively lower dose. Interestingly compounds 4q, 4r, 4v and 4w also showed protection at the dose level 30 mg/kg after 4.0 h. Compounds that exhibited moderate protection against MES model at 100 mg/kg include 4b, 4c, 4e, 4i, 4j, 4n, 4o, 4s, 4t and 4y at 0.5 h. Thus majority of the compounds showed encouraging anticonvulsant activity at 0.5 h interval indicating that they have rapid onset and shorter duration of action. In chemoshock investigation compounds 4b, 4f, 4j, 4q, 4t, 4x and 4y were found to be active after 0.5 h of the drug administration at a dose of 100 mg/kg. In neurotoxicity study rotorod tests were employed to estimate the undesired effects like sedation and ataxia produced by the compounds. In rotorod test, compounds 4b, 4g, 4h, 4l, 4m and 4n were less neurotoxic and the rest of the compounds did not exhibit neurotoxicity. In acute toxicity study (LD$_{50}$) it was found that the tested compounds did not produce any mortality or gross effect on the central nervous system at any of the dose levels tested, i.e., 5, 25, 125, 250 and 400 mg/kg. In antidepressant study compound 4n and 4u showed extremely significant decrease in the immobility time and were potent comparable to Fluoxetine ($p < 0.001$), whereas, compound 4j, 4o and 4x were found to be highly significant with ($p < 0.01$) and compound 4c, 4f and 4s were found to be significant with ($p < 0.05$). The change in the immobility time due to other compounds was non-significant. In antinociceptive activity compounds 4n and 4v were found to extremely significant with ($p < 0.001$) increase in the reaction time. Compounds 4b, 4e, 4q, 4s, were found to be highly significant with ($p < 0.01$) while compound 4p was found to be significant with ($p < 0.05$). Increase in the reaction time due to other compounds was non-significant. In biochemical estimation compound 4r showed moderate but highly significant ($p < 0.01$) while 4q, 4v and 4w showed significant ($p < 0.05$) change in the SGOT level as compared to the control. Compound 4u showed moderate but highly significant ($p < 0.01$) while compounds 4q and 4w were also found to alter significant ($p < 0.05$) in the SGPT level. Only compound 4r and 4u were found to be significant ($p < 0.05$) in the alkaline
phosphatase level. All the other values were not significant indicating the non-toxic nature of the tested compounds. In estimation of GABA level in rat brain the statistical data showed that, in the olfactory lobe area of rat brain concentration of GABA increased highly significantly \((p < 0.01)\) after administering the compounds \(4q\) and significantly \((p < 0.05)\) after administering the compound \(4p\). Non-significant result was produced by the compound \(4r\). In the mid brain region both the compounds \(4p\) and \(4q\) exhibited significantly highly increased \((p < 0.01)\) concentration of GABA. In the medullary area and cerebellum, compound \(4p\) elevated the GABA concentration significantly \((p < 0.05)\).

![Scheme 5](image)

**Scheme 5**

Scheme 5 contained the synthesis of \(2\)\{\((6\)-substituted benzo\([d]\)thiazol-2-ylcarbamoyl) methyl\}\)-1-(4-substituted phenyl) isothiourea \((4a-y)\). A mixture of compound 2- chloro-\(N\)-(6-substituted benzo\([d]\)thiazol-2-yl) acetamide and arylthiourea in acetone was refluxed for 3 h. It was then poured onto crushed ice to give final compounds \(4a-y\). Synthesized compounds were characterized by elemental analysis, FT-IR and \(^1\)H-NMR. Analytical and spectral data were in good agreement with the composition of synthesized compounds. In the anticonvulsant screening, almost all the compounds showed encouraging activity. In the anticonvulsant screening, almost all the compounds showed encouraging activity. Compounds \(4b, 4c, 4p, 4q, 4r, 4v\) and \(4w\) were found to be highly active against MES test at a dose level 30 mg/kg at 0.5 h time interval indicative of their ability to prevent seizure spread at relatively lower dose. Interestingly compounds \(4b, 4c, 4q, 4r\) and \(4w\) also showed protection at the dose level 100 mg/kg after 4.0 h. Compounds that exhibited moderate protection against MES model at 100 mg/kg include \(4d, 4h, 4i, 4j, 4n, 4u\) and \(4x\) at 0.5 h. Thus majority of the compounds showed encouraging anticonvulsant activity at 0.5 h interval indicating that they have rapid onset and shorter duration of action. In chemoshock investigation compounds \(4b, 4l, 4m, 4n, 4o, 4q\) and \(4u\) were found to be active after 0.5 h of the drug administration at a dose of 100 mg/kg. Interestingly compound \(4l\) and \(4n\) were also found to be active after 4.0 h of the drug administration at a dose of
100 mg/kg. In neurotoxicity study rotorod tests were employed to estimate the undesired effects like sedation and ataxia produced by the compounds. In rotorod test, compounds 4c, 4e, 4g, 4l, 4n, 4s, 4t and 4u were less neurotoxic and the rest of the compounds did not exhibit neurotoxicity. In acute toxicity study (LD₅₀) it was found that the tested compounds 4b, 4q, 4r and 4w did not produce any mortality or gross effect on the central nervous system at any of the dose levels i.e., 5, 25, 125, 250 and 400 mg/kg. However, the animals have exhibited some of the toxic signs such as chewing, licking, salivation followed by brief period of sedation. But at the doses of 250 & 400 mg/kg compound 4c showed mortality (3/6 & 4/6 respectively). In antidepressant study compound 4n showed extremely significant decrease in the immobility time and was potent comparable to Fluoxetine (p < 0.001), whereas, compound 4b and 4w were found to be highly significant with (p < 0.01) and compounds 4h, 4r and 4u were found to be significant with (p < 0.05). The change in the immobility time due to other compounds was non-significant. In antinociceptive activity compounds 4h and 4w were found to extremely significant with (p < 0.001) increase in the reaction time while compounds 4i, 4n, 4v and 4x were found to be highly significant with (p < 0.01). Increase in the reaction time due to other compounds was non-significant. In biochemical estimation compound 4r showed moderate but highly significant (p < 0.01) while 4c and 4w showed significant (p < 0.05) change in the SGOT level as compared to the control. Compound 4b showed moderate but highly significant (p < 0.01) while compound 4c was also found to alter significant (p < 0.05) in the SGPT level. Only compound 4b and 4c were found to be significant (p < 0.05) in the alkaline phosphatase level. All the other values were not significant indicating the non-toxic nature of the tested compounds. In estimation of GABA level in rat brain the statistical data showed that, in the olfactory lobe area of rat brain concentration of GABA increased highly significantly (p < 0.01) after administering the compounds 4w and significantly (p < 0.05) after administering the compound 4b. Non-significant result was produced by the compound 4q. In the midbrain region both the compounds 4b and 4w exhibited significantly highly increased (p < 0.01) concentration of GABA. In the medullary area and cerebellum, compound 4b elevated the GABA concentration significantly (p < 0.05).

The computational analysis of all the synthesized compounds was performed to obtain an overview on their minimum conformation for bioactivity. The synthesized compounds were examined to check whether they reflect the conditions of the derived pharmacophore model. Analyses of the distance relationship showed that synthesized
compounds fulfil the essential demands of pharmacophore when compared with other known anticonvulsant drugs.

Three dimensional structure analyses were performed by the ORTEP diagram (50% probability) using the software Ortep3v2 and the characteristics were studied. The prototype compounds revealed that some compounds had one or more hydrogen bondings present in their structure that could be one of the reasons for their efficacy as better CNS agents.

The dependence of biological activity in the set of congeneric agents or lipophilic character has been shown in many types of drug action in particular, the reports by Lien and co-workers indicate that the anticonvulsant activity of different types of compounds were correlated with lipophilicity. However it has been observed that the maximum potency of the drugs that act on the central nervous system (CNS) is obtained with congeners having an optimum lipophilicity (log Po). In this study, we attempted to correlate the anticonvulsant activity of synthesized compounds of Scheme 1-5 with their log P value. The experimental log P values were determined using octanol-phosphate buffer method and the calculated log P values were taken from the software Chem Draw Ultra 8.0. Most of the synthesized compounds met the criteria for lipophilicity and showed good anticonvulsant activity with lesser neurotoxicity. Due to their higher lipophilicity they are expected to have rapid onset and shorter duration of action.

It may be concluded that the synthesized derivatives of benzfused five membered heterocyclic (benzimidazoles, benzothiazoles and isatins) compounds may be regarded as newer classes of anticonvulsant, antidepressant and antinociceptive agents with less toxicities. The most active compounds from each series can act as leads and further modifications in their structure can be performed to get even better CNS agents.

(Signature of the candidate)  (Signature of the supervisor)