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Synthesis, Characterization, Docking Studies and Antiepileptic Activity of Novel Piracetam Derivatives

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Piracetam is a nootropic drug that has been used in clinical trials for decades, but is still a mystery due to a lack of understanding of its mechanism of action. In this research, sixteen novel piracetam derivatives were synthesized in three steps and characterized by IR, NMR and mass spectroscopic techniques. Based on the docking studies, two derivatives were identified as more active based on the drug receptor interactions studies and were further subjected to animal studies for the evaluation of the activity. Compounds **6** and **10** had shown a strong anticonvulsant activity based on the molecular docking studies. It was hypothesized from the synthesized analogues that the non-substitution with thio moiety at has a major effect on reducing the contagiousness of seizure discharge and increasing the seizure threshold.

Keywords: Piracetam, Docking studies, Nootropic.

INTRODUCTION

Piracetam is a cyclic derivative of 2-oxo-1-pyrrolidine acetamide. GABA is a neurotransmitter belongs to the γ-amino-butyric acid (GABA) family of neurotransmitters. Racetams are a type of medication known as a 'nootropic' and it improves the cognitive function without sedating or stimulating the brain. It's also used in clinical practice to treat ischemia, epilepsy, palatal myoclonus and Parkinson's disease without a well-defined mechanism of action [1]. Different effects on subtypes of glutamate receptors without GABAergic responses have been linked to piracetam's mechanisms of action. At the levels utilized in human research, piracetam has no major adverse effects or acute toxicity. The LD_{50} in rats is 5.6 g/kg while in mice it is 20 g/kg, showing very low acute toxicity [2]. It has initially been utilized in the management of Alzheimer's disease, prominent valve for this drug mainly is the improvement of memory [3]. Its effects on passive avoidance conditioning (PAC) was observed in experiments utilizing compartment modeling [4].

Effects of extract of Ginkgo biloba along with piracetam has been tested in dementia effected by alcohol [5]. Piracetam role in the allosteric modulation of receptor embodying α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid is well established [6]. After the investigation of the brain slices it has been observed that synaptic transmission was enhanced by piracetam which was induced by ketamine and stimulates the antidepressant effect mediated by ketamine [7]. In another study action of piracetam and noopept in neurons of snail on depression induced by acetyl choline was studied [8]. Piracetam has also been utilized in the management and treatment of agenesis concerned with corpus callosum, sickle cell anemia, dyslexia and CNS disorders [9-12].

The mechanism of action of these chemicals is similarly insufficiently comprehended. Even minor modifications in structure can have dramatic effects on the pharmacological profile of drug. For instance, the pyrrolidone derivatives piracetam and levetiracetam have similar chemical structures but different pharmacological characteristics and thus, different clinical usage [13]. Following the above-mentioned research, chirally

substituted morpholine, thiomorpholine and piperazine structures are used to replace the ubiquitous unsubstituted oxopyrrolidone ring. The designed molecules share properties with piracetam and levetiracetam, making them hybrids of sub-groups 1 and 2, and it is anticipated that they will exhibit pharmacological activities similar to those of piracetam and levetiracetam, including the effects of cognitive enhancement and antiepileptic/anti-convulsant action [14].

Based on the biological activities of skeleton containing 2-oxopiperazine base structure conjugated to an amide bond, hereby, 16 novel compounds by introducing the different groups on the nitrogen atoms were synthesized. Further, molecular docking studies were also performed in order to understand how the synthesized compounds, interact with calcium channel (6KZV), GABAA (4COF) and sodium channels (2KAV). The anticonvulsant activity was evaluated by tonic-clonic generalized seizure induced by pentylenetetrazole (PTZ) on male mice.

EXPERIMENTAL

All the chemicals and reagents used in the synthesis of novel compounds were purchased from Sigma-Aldrich and Westin Pharmaceuticals. The progess of the reactions were monitored with the help of TLC. The KBr pellets were placed in a FT-IR spectrophotometer (Perkin-Elmer) to record the FT-IR spectra, while the BRUKER-400 Ultra Shield™ spectrometer were used to record the ^{1}H NMR spectra. The mass spectra was recorded with the help of Agilent Technology India Pvt. Ltd. (model no 6230B).

Synthesis of piperazine-2-one

Ethylenediamine (32 mL, 479.2 mmol) in anhydrous methanol (287 mL, 0.6 mL/mmol), ethylbromo acetate (13 mL, 117.54 mmol) in anhydrous methanol (144 mL, 0.3 mL/mmol) was added dropwise with continuous stirring for 6 h. After removing the volatiles using a vacuum evaporator, the collected filtrate was redissolved in anhydrous methanol (287 mL, 0.6 mL/mmol) and finally the mixture was refluxed for 5 h. After the complete evaporation, the filtrate obtained was purified using the column chromotagraphy in aq. NH_3:methanol:DCM:0.1:0.9:9 to obtain the product (yield: 16 g, 33.33%) (**Scheme-I**).

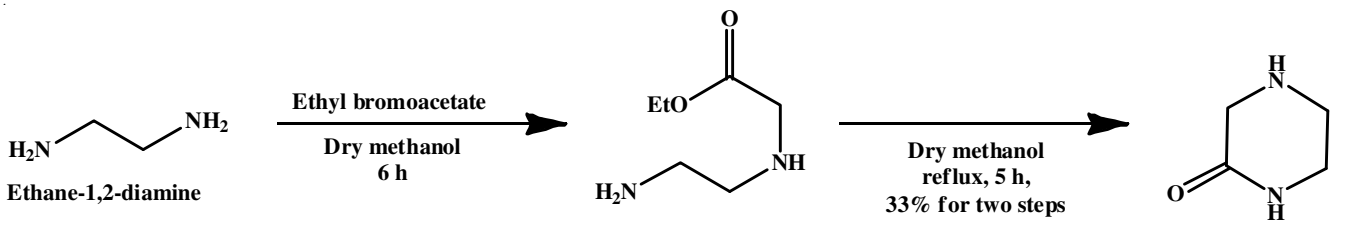
Synthesis of compounds 1-3

Step-1: In a churned solution of piperazin-2-one in methanol (84 mL, 5 mL/mmol), triethylamine (4.68 mL, 33.6 mmol) and alkyl bromide (2.46 mL, 33.6 mmol) were added, then the solution was refluxed for 5 h. After removing volatiles with a vacuum, the remaining residue was purified by means of column filtration using methanol:DCM to get the product.

Step-2: A solution of 4-alkyl piperazin-2-one in anhydrous DMF, 60% NaH was mixed at 0 ºC with continuous stirring for 15 min. Chloroacetamide was added to the above solution mixture then stirring was done for 16 h at room temperature. Saturated NaCl solution was added to the reaction and then it was diluted with ethylacetate (100 mL). Removal of unwanted compound was done by washing with distilled water (2 × 20 mL) and brine (20 mL), whereas the vacuum evaporator was used to remove the volatiles. The obtained filtrate was purified using the column chromatography in methanol:DCM to obtain the final product (**Scheme-II**). The conditions and other details are given in Table-1.

Compound 1: FT-IR (KBr, ν_{max}, cm^{-1}): 3215 (NH), 1681 (C=O); ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.34 (bs, 1H), 5.43 (bs, 1H), 4.02 (s, 2H), 3.48 (t, *J* = 5.2 Hz, 2H), 3.20 (s, 2H), 2.73 (t, *J* = 5.6 Hz, 2H), 2.50 (q, *J* = 7.2 Hz, 2H), 1.11 (t, *J* = 7.2 Hz, 3H); MS (ESI) *m/z*: calculated, $C_8H_{15}N_3O_2$, 185.22 $[M]^+$; found, 186.1 $[M+H]^+$.

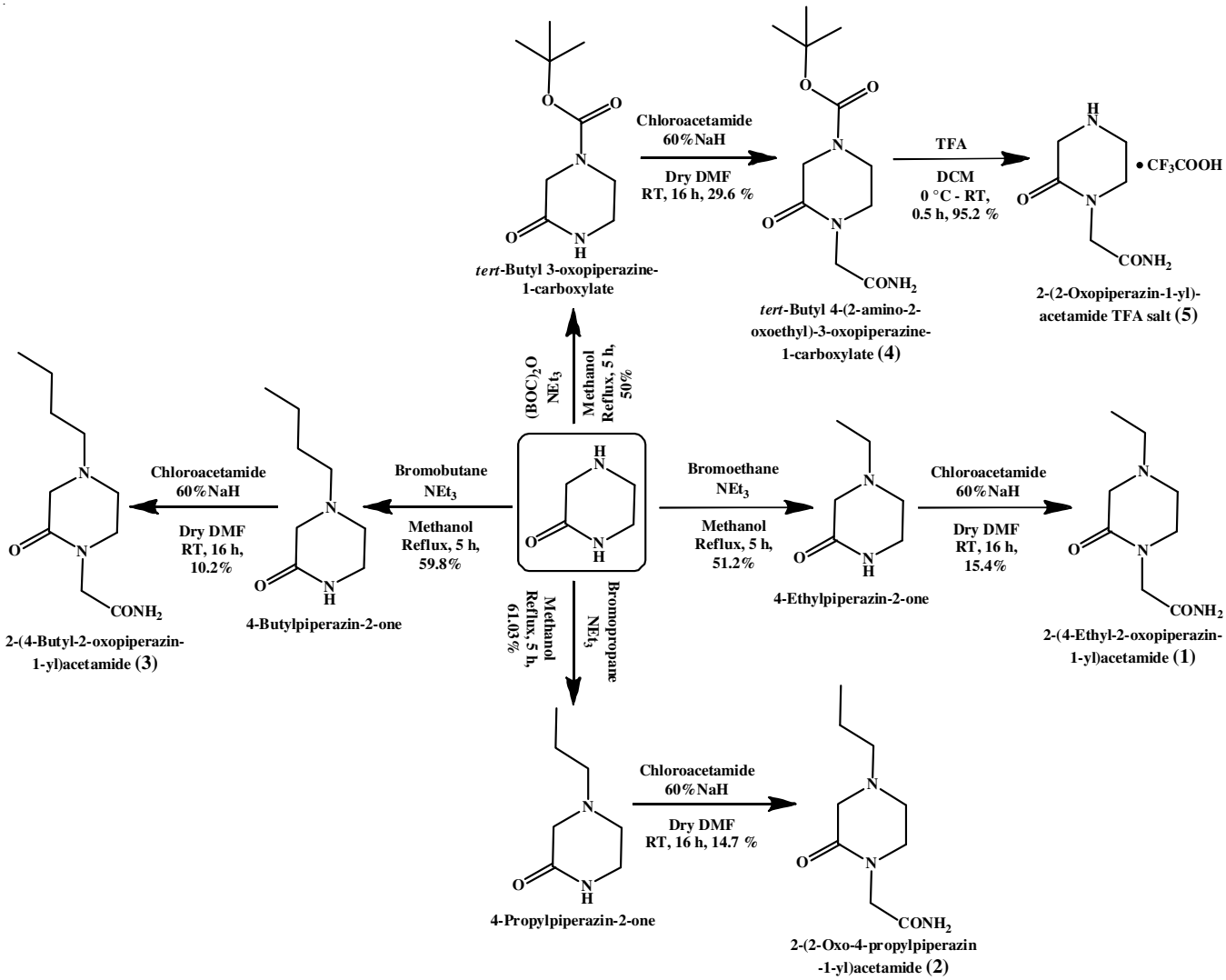
Compound 2: FT-IR (KBr, ν_{max}, cm^{-1}): 3207 (NH), 687 (C=O), ^{1} H NMR (400 MHz, $CDCl_3$): δ 6.50 (bs, 1H), 5.54 (bs, 1H), 4.26-4.18 (m, 2H), 4.03 (s, 2H), 3.49-3.45 (m, 2H), 3.20 (s, 2H), 2.73 (t, *J* = 5.6 Hz, 2H), 2.59 (t, *J* = 7.2 Hz, 2H),



Scheme-I

TABLE-1
CHEMICALS AND ITS QUANTITIES AND CONCENTRATION USED FOR SYNTHESIS OF COMPOUNDS **1-3** AND THERE PERCENTAGE YIELDS AND REFLUX TIME

Compd.	Piperazin-2-one in methanol	Triethylamine	Alkyl bromide	Reflux time (h)	4-Alkyl piperazin-2-one in dry DMF	Chloroacetamide/ 60% NaH	Final product yield (%)
	Step-1				**Step-2**		
1	1.68 g, 16.8 mmol (84 mL, 5 mL/mmol)	4.68 mL, 33.6 mmol	2.46 mL, 33.6 mmol	5	900 mg, 7.02 mmol (10 mL, 1.4 mmol)	788 mg, 8.4 mmol/ 337 mg, 8.4 mmol	200 mg (15.4)
2	3.00 g, 30 mmol (150 mL, 5 mL/mmol)	8.37 mL, 60 mmol	5.47 mL, 60 mmol	5	2.67g, 18.8 mmol (28.2 mL, 1.5 mmol)	2.1 g, 22.5 mmol/ 902 mg, 22.5 mmol)	550 mg (14.7)
3	3.00 g, 30 mmol) (150 mL, 5 mL/mmol)	8.37 mL, 60 mmol	6.47 mL, 60 mmol	5	2.8 g, 17.9 mmol) (26.5 mL, 1.5 mmol	2.0 g, 21.5 mmol/ 861 mg, 21.5 mmol	400 mg (10.7)



Scheme-II

1.57-1.44 (2H), 0.95-0.88 (m, 3H), MS (ESI) *m/z*: calculated, $C_9H_{17}N_3O_2$, 199.25 $[M]^+$; found, 200.2 $[M^+H]^+$.

Compound 3: FT-IR (KBr, ν_{max}, cm^{-1}): 3201 (NH), 1634 (C=O), 1391 (-CH), 1075 (C-O); ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.40 (bs, 1H), 5.47 (bs, 1H), 4.02 (s, 2H), 3.48 (t, *J* = 5.2 Hz, 2H), 3.21 (s, 2H), 2.74 (t, *J* = 5.2 Hz, 2H), 2.43 (t, *J* = 7.2 Hz, 2H), 1.53-1.45 (m, 2H), 1.40-1.30 (m, 2H), 0.93 (t, *J* = 7.6 Hz, 3H); MS (ESI) *m/z*: calculated, $C_{10}H_{19}N_3O_2$, 213.28 $[M]^+$; found, 214.2 $[M^+H]^+$.

Synthesis of compound 4

Step-1: Instead of alkyl bromide, di-*tert*-butyl dicarbonate $(BOC)_2O$ (11.4 mL, 50 mmol) was used while keeping other conditions same.

Step-2: A solution of *tert.*-butyl-3-oxopiperazine-1-carboxylate (5 g, 25.1 mmol) in anhydrous DMF (37.5 mL, 1.5 mL/mmol), 60% NaH (1.2 g, 30.1 mmol) was added at 0 ºC with continuous stirring for 15 min. Chloroacetamide (2.8 g, 30.1 mmol) was added with stirring for 16 h at room temperature. After saturated the reaction with a conc. NaCl solution and then ethylacetate was added (100 mL). Removal of unwanted compound was done by washing with distilled water (2 × 40 mL) and brine (40 mL). Vacuum evaporator was used to remove organic volatiles then the obtained filtrate was purified using column chromatography in methanol:DCM to obtain the final product (Yield: 1.9 g, 29.6%) (**Scheme-II**). FT-IR (KBr, ν_{max}, cm^{-1}): 3207 (NH), 1720 (C=O), 1230 (CN), 1160 (C-O); ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.21 (bs, 1H), 5.52 (bs, 1H), 4.15 (s, 2H), 4.05 (s, 2H), 3.70 (t, *J* = 5.6 Hz, 2H), 3.51 (t, *J* = 5.6 Hz, 2H); MS (ESI) *m/z*: calculated, $C_{11}H_{19}N_3O_4$, 257.29 $[M]^+$; found, 258.2 $[M+H]^+$.

Synthesis of compound 5

In a well stirred solution of compound **4** (200 mg, 0.78 mmol) in dry DCM (1.95 mL, 2.5 mL/mmol) was added to trifluoroacetic acid (1.95 mL, 2.5 mL/mmol) at 0 ºC with continuous stirring for 30 min at room temperature. The vacuum evaporator was used to remove the organic volatiles and then the obtained filtrate was washed with *n*-hexane (5 mL) to obtain the final product (Yield: 200 mg (95.2%) (**Scheme-II**). FT-IR (KBr, ν_{max}, cm^{-1}): 3207 (NH), 1720 (C=O), 1230 (C≡N), 1160 (C-O), H NMR (400 MHz, $CDCl_3$): δ 6.40 (bs, 1H), 5.47 (bs, 1H), 4.02 (s, 2H), 3.48 (t, *J* = 5.2 Hz, 2H), 3.19 (s, 2H), 2.78 (t, *J* = 4.9 Hz, 2H), 2.44 (t, *J* = 6.9 Hz, 2H), 1.53-1.45 (m,

2H), 1.39-1.28 (m, 2H), 0.95 (t, *J* = 7.6 Hz, 3H); MS (ESI) *m/z*: calculated, $C_6H_{11}N_3O_2$, 157.09 $[M]^+$; found, 158.09 $[M^+H]^+$.

Synthesis of compounds 6-12

Step-1: Sodium hydride dissolved in 1,4-dioxane was added to a solution of *tert.*-butyl (2-mercaptoethyl)carbamate in 1,4-dioxane at 0 ºC with continuous stirring for 10 min followed by the addition of substituted 2-bromo ester with continuous stirring for further 3 h at room temperature. Cool the reaction mixture to 0 ºC and saturated the reaction with a conc. NaCl solution and then ethylacetate was added (100 mL). The resulting solution was separated using water (10 mL) and ethyl acetate (20 mL). Brine solution (10 mL) was used to wash the ethyl acetate layer, desiccated over anhydrous Na_2SO_4 and then organic volatiles were dried in a vacuum. By employing ethyl acetate: hexane system in column chromatography, the residue was obtain the product.

Step-2: A solution of ethyl 2-((2-((*tert*-butoxy-carbonyl) substituted acetate in CH_2Cl_2 was added to trifluoroacetic acid at 0 ºC. The resulting mixture was swirled for 1 h at room temperature. The organic volatiles vaporized under reduced pressure then the filtrate obtained was dissolved in saturated $NaHCO_3$ solution (4.18 mL, 1 mL/mmol). Soluble organic layers were rinsed with brine (10 mL) solution after being extracted with dichloromethane (3 × 10 mL). The volatiles were removed by evaporation and the resulting residue was employed directly for the subsequent steps.

Step-3: At 0 ºC, 60% NaH (192.5 mg, 4.81 mmol) was added and stirred for 15 min in thiomorpholin-3-one (470 mg, 4.01 mmol) in dry DMF (5.6 mL, 1.4 mL/mmol). Then chloroacetamide (449 mg, 4.81 mmol) was added and swirled the reaction mixture room temperature for 3 h. Saturated sodium chloride solution was added slowly to quench it and diluted with ethylacetate (25 mL). The organic volatiles were removed by evaporation and the remaining residue was purified in methanol:DCM *via* column chromatography to obtain the final product (**Schemes III-V**). The conditions and other details are given in Table-2.

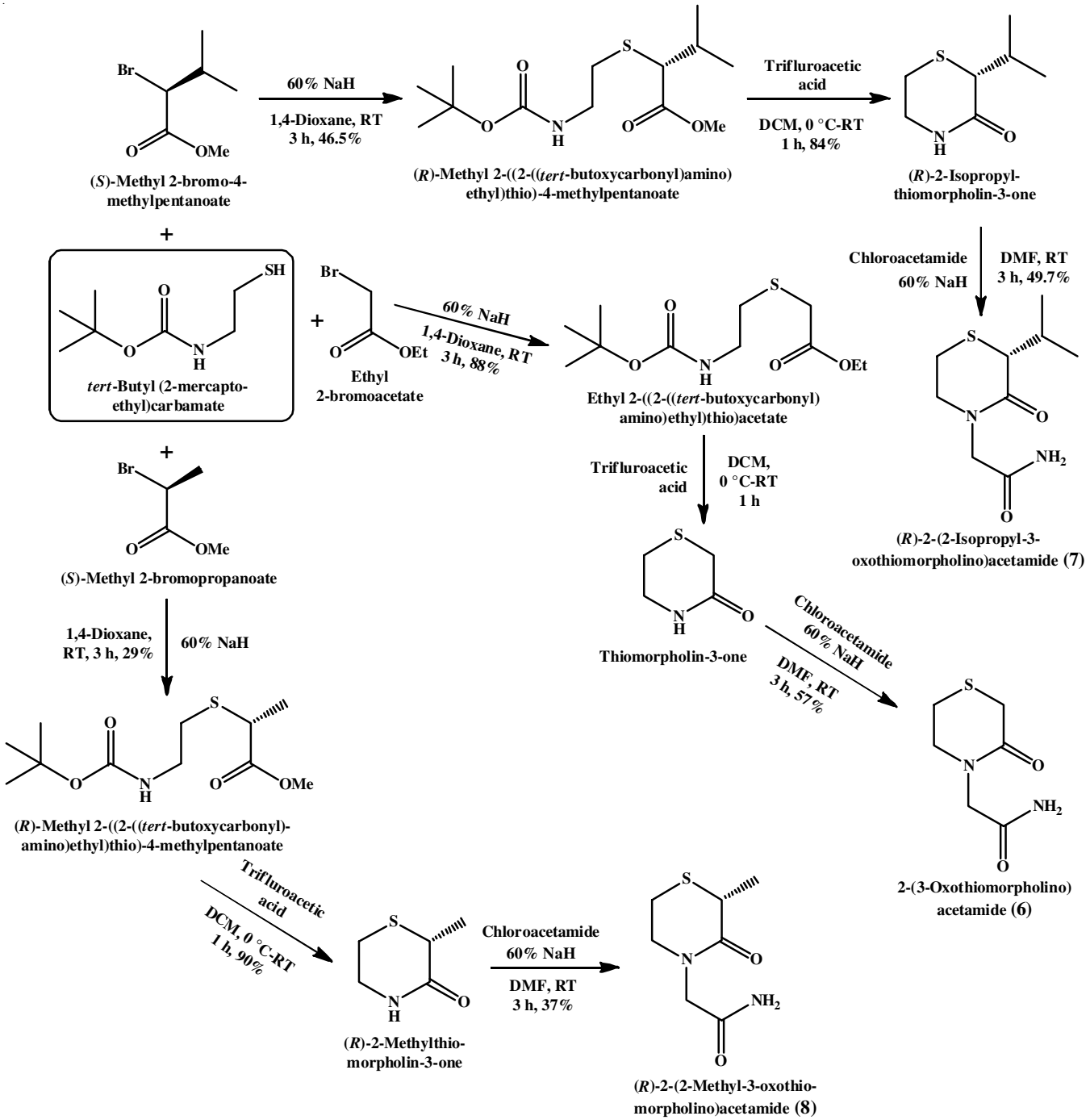
Compound 6: FT-IR (KBr, ν_{max}, cm^{-1}): 3213 (NH), 1687 (C=O), 2550 (S-H); ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.20 (bs, 1H), 5.44 (bs, 1H), 4.06 (s, 2H), 3.76 (t, *J* = 5.6 Hz, 2H), 3.38 (s, 2H), 2.94 (t, *J* = 6.0 Hz, 2H); MS (ESI) *m/z*: calculated, $C_6H_{10}N_2O_2S$, 174.22 $[M]^+$; found, 175.2 $[M+H]^+$.

Compound 7: FT-IR (KBr, ν_{max}, cm^{-1}): 3354, 3190 (NH), 1600 (C=O), 1259 (CN), 1070 (C-O); ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.27 (bs, 1H), 5.49 (bs, 1H), 4.10-4.01 (m, 2H), 3.78-3.75 (m, 2H), 3.44 (d, *J* = 5.2 Hz, 1H), 2.94-2.91 (m, 1H), 1.07 (d, *J* = 6.8 Hz, 3H), 1.03 (d, *J* = 6.8 Hz, 3H); MS (ESI) *m/z*: calculated, $C_9H_{16}N_2O_2S$, 216.30 $[M]^+$; found, 217.1 $[M+H]^+$.

Compound 8: FT-IR (KBr, ν_{max}, cm^{-1}): 3315 (NH), 1828 (C=O, cyclic), 1669 (C=O), 1543 (N-O), 1356 (CN), 1139 (C-O), ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.25 (bs, 1H), 5.54 (bs, 1H), 4.12-4.03 (m, 2H), 3.84-3.74 (m, 2H), 3.64 (d, *J* = 6.8 Hz, 1H), 3.04-2.96 (m, 2H), 1.47 (d, *J* = 6.8 Hz, 3H), MS

TABLE-2
CHEMICALS AND ITS QUANTITIES AND CONCENTRATION USED FOR SYNTHESIS OF COMPOUNDS **6-12** AND THERE PERCENTAGE YIELDS AND STIRRING TIME

Compd.	*tert.*-Butyl (2-mercaptoethyl)-carbamate in 1,4-dioxane	Substituted 2-bromo acetate	60% NaH in 1,4-dioxane	Stirring time	2-((2-((tert-Butoxy-carbonyl) substituted acetate in dichloromethane	Trifluoro acetic acid	60% NaH/ chloro-acetamide	Substituted thiomorpholin-3-one in dry DMF	Final product yield (%)
	Step-1				**Step-2**		**Step-3**		
6	1 g, 5.68 mmol (17 mL, 3 mL/mmol)	0.63 mL, 5.68 mmol	250 mg, 6.25 mmol (17 mL)	3 h	1.1 g, 4.18 mmol (8.35 mL, 2 mL/mmol)	12.54 mL, 3 mL/mmol	192.5 mg, 4.81 mmol/(449 mg, 4.81 mmol)	470 mg, 4.01 mmol (5.6 mL, 1.4 mL/mmol)	400 mg (57)
7	1 g, 5.68 mmol (17 mL, 3 mL/mmol)	3.1 g, 15.89 mmol	250 mg, 6.25 mmol (50 mL)	3 h	0.8 g, 2.75 mmol (5.49 mL, 2 mL/mmol)	8.24 mL, 3 mL/mmol	120 mg, 3.01 mmol/ 281.46 mg, 3.01 mmol	400 mg, 2.51 mmol (3.52 mL, 1.4 mL/mmol)	270 mg (49.7)
8	2.8 g, 15.90 mmol (48 mL, 3 mL/mmol)	8.0 g, 47.9 mmol	17.5 mmol (20 mL)	10 min	1.4 g, 5.32 mmol) (10.63 mL, 2 mL/mmol)	15.95 mL, 3 mL/mmol	256.32 mg, 6.41 mmol/ 600 mg, 6.41 mmol	700 mg, 5.34 mmol (7.5 mL, 1.4 mL/mmol)	370 mg (37)
9	1 g, 5.68 mmol (17 mL, 3 mL/mmol)	3.56 g, 17.03 mmol	250 mg, 6.25 mmol) (17 mL)	3 h	1.7 g, 5.57 mmol (11.13 mL, 2 mL/mmol)	16.70 mL, 3 mL/mol	332.64 mg, 8.32 mmol/ 778 mg, 8.32 mmol	1.2 g, 6.93 mmol) (9.7 mL, 1.4 mL/mmol)	633.69 mg (39.7)
10	2.8 g, 15.90 mmol (48 mL, 3 mL/mmol)	8.0 g, 47.9 mmol	700 mg, 17.5 mmol (20 mL)	10 min	1.38 g, 5.32 mmol) (10.63 mL, 2 mL/mmol)	15.95 mL, 3 mL/mmol	256.32 mg, 6.41 mmol/600 mg, 6.41 mmol	700 mg, 5.34 mmol) (7.5 mL, 1.4 mL/mmol)	310 mg (37)
11	1 g, 5.68 mmol (17 mL, 3mL/mmol)	3.1 g, 15.89 mmol	250 mg, 6.25 mmol (20 mL)	10 min	0.8 g, 2.75 mmol (5.49 mL, 2 mL/mmol)	8.24 mL, 3 mL/mmol	120 mg, 3.01 mmol/281.46 mg, 3.01 mmol	400 mg, 2.51 mmol (3.52 mL, 1.4 mL/mmol)	206 mg (38)
12	1 g, 5.68 mmol (17 mL, 3 mL/mmol)	3.56 g, 17.03 mmol	250 mg, 6.25 mmol (20 mL)	10 min	1.7 g, 5.57 mmol) (11.13 mL, 2 mL/mmol).	16.70 mL, 3 mL/mmol	332.64 mg, 8.32 mmol/778 mg, 8.32 mmol	1.2 g, 6.93 mmol) (9.7 mL, 1.4 mL/mmol)	479 mg (30)



Scheme-III

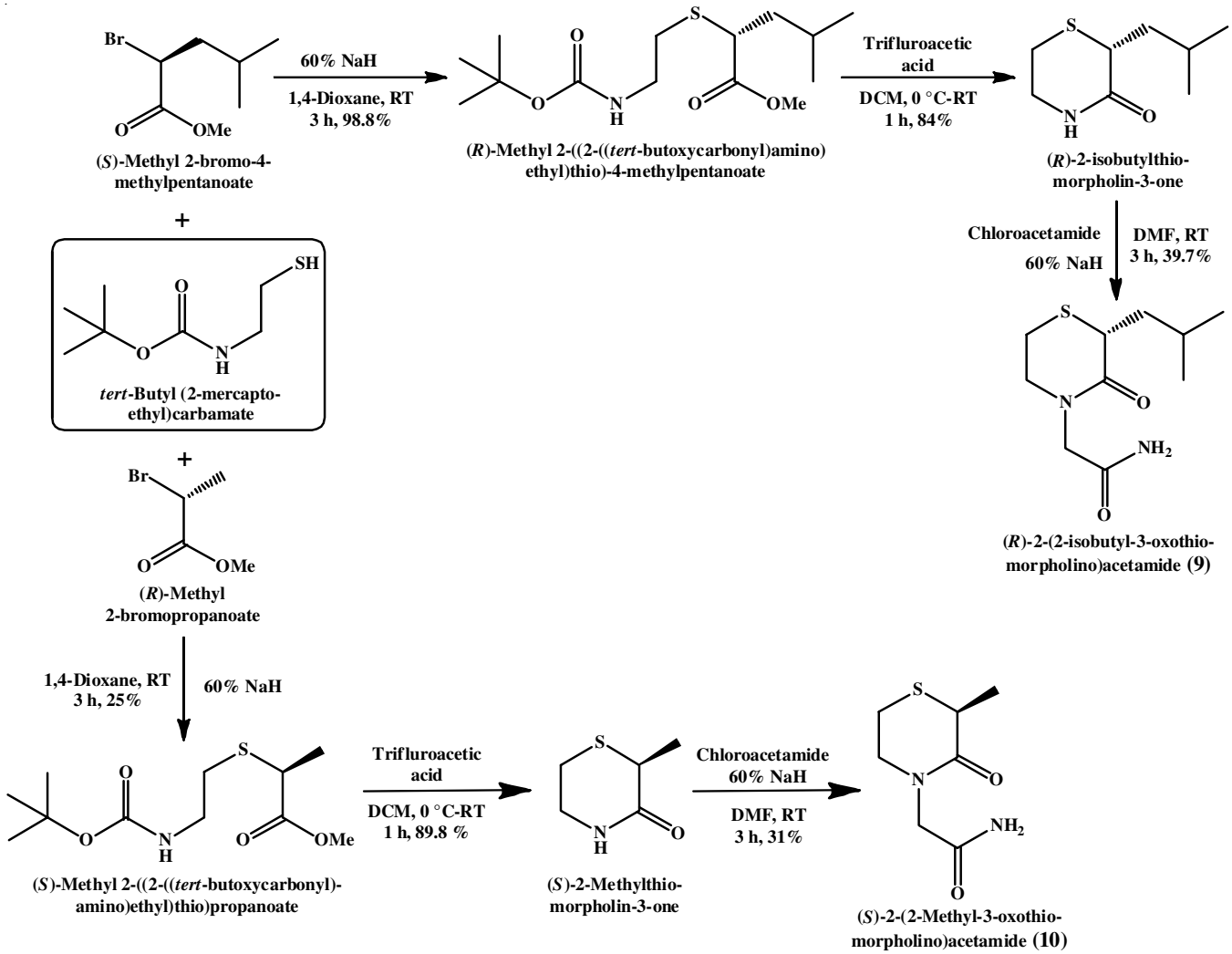
(ESI) *m/z*: calculated, $C_7H_{12}N_2O_2S$, 188.25 $[M]^+$; found, 189.0 $[M+H]^+$.

Compound 9: FT-IR (KBr, ν_{max}, cm^{-1}): 3207 (NH), 1681 (C=O), 1310 (CN), 1091 (C-O), ^1H NMR (400 MHz, $CDCl_3$): δ 6.50-5.55 (m, 2H), 3.81-3.73 (m, 2H), 3.56-3.52 (m, 1H), 3.03-2.90 (m, 2H), 1.98-1.90 (m, 2H), 1.61-1.54 (m, 1H), 0.97-0.90 (m, 6H), MS (ESI) *m/z*: calculated, $C_{10}H_{18}N_2O_2S$, 230.33 $[M]^+$; found, 231.1 $[M+H]^+$.

Compound 10: FT-IR (KBr, ν_{max}, cm^{-1}): 3202 (NH), 1626 (C=O), 1260 (C-O), 1070 (CN), ^1H NMR (400 MHz, $CDCl_3$): δ 6.25 (bs, 1H), 5.54 (bs, 1H), 4.12-4.03 (m, 2H), 3.84-3.74 (m, 2H), 3.64 (d, *J* = 6.8 Hz, 1H), 3.04-2.96 (m, 2H), 1.47 (d, *J* = 6.8 Hz, 3H), MS (ESI) *m/z*: calculated, $C_7H_{12}N_2O_2S$, 188.25 $[M]^+$; found, 189.1 $[M+H]^+$.

Compound 11: FT-IR (KBr, ν_{max}, cm^{-1}): 3420 (NH), 1710 (C=O), 1120 (CN), ^1H NMR (400 MHz, $CDCl_3$): δ 6.27 (bs, 1H), 5.49 (bs, 1H), 4.10-4.01 (m, 2H), 3.78-3.75 (m, 2H), 3.44 (d, *J* = 5.2 Hz, 1H), 2.94-2.91 (m, 1H), 1.07 (d, *J* = 6.8 Hz, 3H), 1.03 (d, *J* = 6.8 Hz, 3H), MS (ESI) *m/z*: calculated, $C_9H_{16}N_2O_2S$, 216.3 $[M]^+$; found, 217.2 $[M+H]^+$.

Compound 12: FT-IR (KBr, ν_{max}, cm^{-1}): 3340 (NH), 1687 (C=O), 1410 (-CH), 1140 (CN), ^1H NMR (400 MHz, $CDCl_3$): δ 6.25 (bs, 1H), 5.51 (bs, 1H), 3.81-3.73 (m, 2H), 3.56-3.52 (m, 1H), 3.03-2.90 (m, 2H), 1.98-1.90 (m, 2H), 1.61-1.54 (m,



Scheme-IV

1H), 0.97-0.90 (m, 6H), MS (ESI) *m/z*: calculated, $C_{10}H_{18}N_2O_2S$, 230.33 $[M]^+$; found, 231.1 $[M+H]^+$.

Synthesis of compounds 13-16

Step-1: At 0 ºC, 60% NaH (3.2 g, 80.0 mmol) was added to a solution of 2-aminoethanol (4.4 mL, 72.76 mmol) in 1,4-dioxane (36 mL, 0.5 mL/mmol) then agitated about 15 min. The mixture was then refluxed for 20 min. A solution of bromoethylacetate (8 mL, 72.76 mmol) in 1,4-dioxane (18 mL, 0.25 mL/mmol) was added and refluxed for 1 h after the reaction mixture had been cooled to room temperature. By adding saturated sodium chloride solution, the reaction was stopped and the volatiles were expelled. The resulting residue was utilized directly in the following step without being further purified.

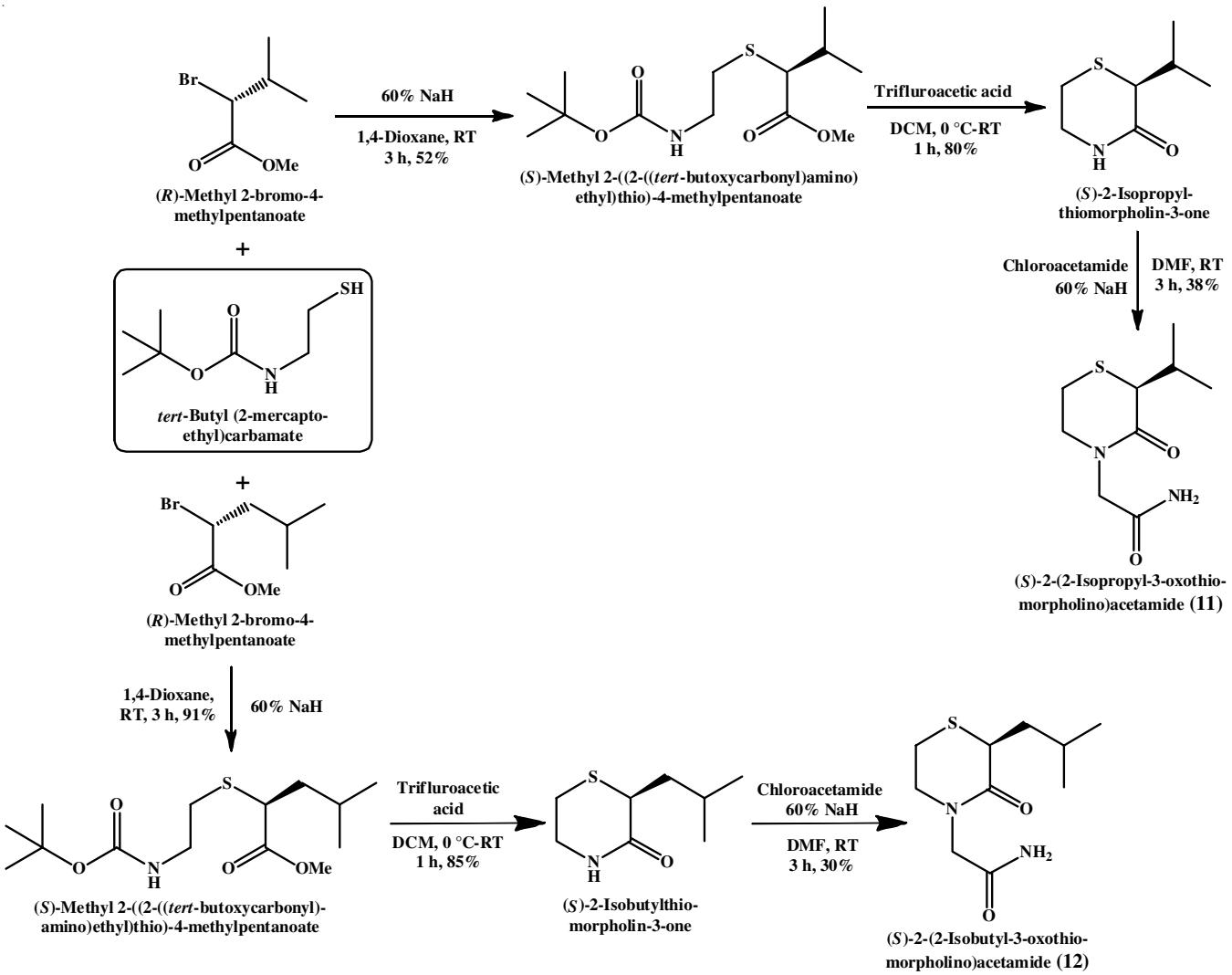
Step-2: Morpholin-3-one (1.3 g, 13.0 mmol) dissolved in dry DMF (26 mL, 2 mL/mmol) before being treated with 60% NaH (620 mg, 15.6 mmol) at 0 ºC for 15 min. The resultant mixture was then mixed with 1.5 g of chloroacetamide (15.6 mmol) and swirled at room temperature for 16 h. The addition of saturated sodium chloride solution stopped the reaction and caused the volatiles to evaporate. The filtrate collected was purified by column chromatography in methanol:DCM to obtain the final product (**Scheme-VI**). The conditions and other details are given in Table-3.

Compound 13: FT-IR (KBr, ν_{max}, cm^{-1}): 3443 (NH), 1624 (C=O), 1350 (CN), ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.20 (bs, 0.5 H), 7.92 (bs, 0.5 H), 7.38 (bs, 0.5H), 7.08 (bs, 0.5H), 4.05 (s, 1H), 3.97 (s, 1H), 3.91 (s, 1H), 3.84-3.82 (m, 1H), 3.68 (d, *J* = 6.0 Hz, 1H), 3.51-3.46 (m, 1H), 3.38-3.35 (m, 1H), 3.29 (q, *J* = 5.6 Hz, 1H), MS (ESI) *m/z*: calculated, $C_6H_{10}N_2O_3$, 158.16 $[M]^+$; found, 157.0 $[M-1]^-$.

Compound 14: FT-IR (KBr, ν_{max}, cm^{-1}): 3413 (NH), 1670 (C=O), 1310 (CN), ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.42 (bs, 1H), 5.58 (bs, 1H), 4.41-3.11 (m, 7H), 1.47 (d, *J* = 6.8 Hz, 6H), MS (ESI) *m/z*: calculated, $C_7H_{12}N_2O_3$, 172.18 $[M]^+$; found, 173.1 $[M+H]^+$.

Compound 15: FT-IR (KBr, ν_{max}, cm^{-1}): 3350 (NH), 1714 (C=O), 1360 (CN), ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.62 (bs, 1H), 6.26 (bs, 1H), 4.12-3.37 (m, 7H), 2.19-2.06 (m, 1H), 1.03 (d, *J* = 6.8 Hz, 3H), 0.95 (d, *J* = 6.8 Hz, 3H), MS (ESI) *m/z*: calculated, $C_9H_{16}N_2O_3$, 200.23 $[M]^+$; found, 201.2 $[M+H]^+$.

Compound 16: FT-IR (KBr, ν_{max}, cm^{-1}): 3410 (NH), 1740 (C=O), 1330 (CN), ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.66 (bs, 1H), 6.32 (bs, 1H), 4.17-3.32 (m, 7H), 1.85-1.78 (m, 1H),



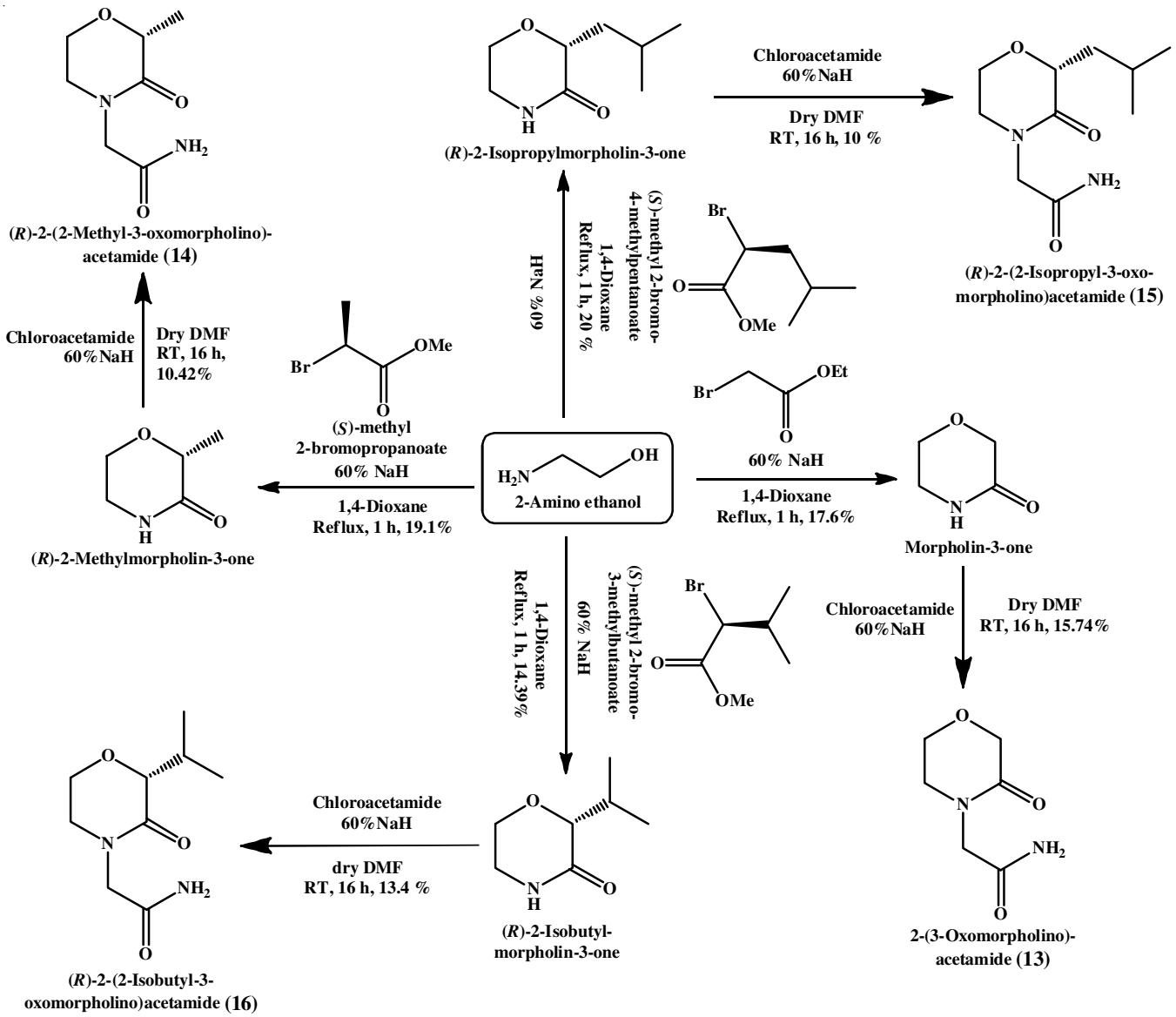
Scheme-V

TABLE-3
CHEMICALS AND ITS QUANTITIES AND CONCENTRATION USED FOR SYNTHESIS OF COMPOUNDS **13-16** AND THERE YEILDS

Compd.	2-Aminoethanol in 1,4-dioxane	60% NaH	Substituted bromo acetate in 1,4-dioxane	Substituted Morpholin-3-one in dry DMF	60% NaH/ chloro-acetamide	Final product yield (%)
	Step-1			**Step-2**		
13	4.4 mL, 72.76 mmol (36 mL, 0.5 mL/mmol)	3.2 g, 80.0 mmol	8 mL, 72.76 mmol (18 mL, 0.25 mL/mmol)	1.3 g, 13.0 mmol (26 mL, 2 mL/mmol)	620 mg, 15.6 mmol/ 1.5 g, 15.6 mmol	320 mg (15.74)
14	4.4 mL, 72.76 mmol (36 mL, 0.5 mL/mmol)	3.2 g, 80.0 mmol	12 g, 72.76 mmol (18 mL, 0.25 mL/mmol)	1.6 g, 13.9 mmol (28 mL, 2 mL/mmol)	667 mg, 16.6 mmol/ 1.56 g, 16.6 mmol	250 mg (10.42)
15	4.95 mL, 81.0 mmol (41 mL, 0.5 mL/mmol)	3.5 g, 89.1 mmol	15 g, 81.0 mmol (21 mL, 0.25 mL/mmol)	1.7 g, 11.87 mmol) (24 mL, 2 mL/mmol)	570 mg, 12.5 mmol/ 1.3 g, 12.5 mmol	320 mg (13.4)
16	5.2 mL, 86.1 mmol (43 mL, 0.5 mL/mmol)	3.78 g, 94.7 mmol	18 g, 86.1 mmol (22 mL, 0.25 mL/mmol)	2.7 g, 17.1 mmol (35.3 mL, 2 mL/mmol)	825 mg, 20.6 mmol/ 1.9 g, 20.6 mmol	365 mg (10.0)

1.69-1.52 (m, 2H), 0.97-0.88 (m, 4H), MS (ESI) *m/z*: calculated, $C_{10}H_{18}N_2O_3$, 214.26 $[M]^+$; found, 215.1 $[M+H]^+$.

Docking studies to study drug receptor interactions for antiepileptic activity: Docking studies were carried out using AutoDock docking software. The docking studies was mainly done to accesses the compound with higher antiepileptic activity and to filter out the compounds with lower potency. The drug receptor interactions was studied on calcium channel, sodium channel and GABAA receptors and based on the binding energy the compound was selected for further activity.



Scheme-VI

Animal ethics: The research is done with prior approval with certificate No. 1275/PO/RE/S/09/CPCSEA. The experimental procedure was approved by IAEC (Institutional animal ethical committee of Deshpande Laboratories-CCP).

Antiepileptic activity: Adult male albino mice, weighing 20-25 g were chosen. During the experiment, the animals were housed in big, spacious, sanitary cages and kept in a well-kept environment with a 12 h day at room temperature (25 ± 1 ºC) maintained at standard experimental conditions. The animals were fasted for 12 h before the experiment, with only normal water available to them. Prior to medication treatment, mice were fasted overnight. A total of five animals were employed, each of which received a single oral dosage of drug. Food was withheld for another 3-4 h after the test extract was administered. Individual animal was monitored at least once during the first 30 min after treatment, on a regular basis for the next 24 h (with specific focus during the first 4 h) and daily for the next 14 days. Changes in skin and fur, eyes and mucous membranes (nasal), as well as respiratory rate, circulatory (heart rate and blood pressure), autonomic (salivation, lacrimation, perspiration, piloerection, urinary incontinence and defecation) and central nervous system (ptosis, drowsiness, gait, tremors and convulsion) changes, were observed once daily in the cage over the course of two weeks, any mortality was determined. The LD_{50} was calculated according to OECD criteria for determining the dosage for biological assessment [15].

Evaluation of antiepileptic activity

Maximal electroshock seizure (MES) studies: Albino mice weighed around 20-25 g were used for the study. Mice were divided into four groups of 4 animals each.

The control group (Group 1) receive an equivalent amount of normal saline *via* the intraperitoneal method. The usual medicine diphenylhydantoin is given to the animals in Group 2. Compounds **6** and **10** low and high doses will be given through I.P in 1% carboxy methyl cellulose solution in Groups 3 and 4, respectively. After 30 min of treatment with the aforesaid compounds, all mice were electroshocked for 0.2 s using

an electro-convulsiometer through ear electrodes (after moistening the animals' ears with a drop of normal saline) at intensity of 50 mA, 60 Hz. Following that, a variety of parameters recorded.

Pentylenetetrazol [PTZ] model: Albino mice weighed around 20-25 g were used for the study. Mice were divided into four groups of 4 animals each.

The control group (Group 1) received an equivalent amount of normal saline *via* the intraperitoneal method. The usual medicine sodium valproate were given to Group 2. Compounds **6** and **10** low and high doses were given through intraperitoneal route in 1% carboxy methyl cellulose solution in Groups 3 and 4, respectively. All of the animals were administered pentylenetetrazol (PTZ) after 30 min after receiving the aforesaid medicines and the relevant parameters were recorded.

RESULTS AND DISCUSSION

A total of sixteen novel piracetam derivatives were successfully synthesized and characterized using NMR, IR and Mass techniques. The docking results of all the synthesized compounds are shown in Table-4. Compounds **6** and **10** were found to be more active based on the drug receptor interactions and binding energy. Results were analyzed by one way annova test with $P < 0.05$, when compared with normal saline and standard drug.

Antiepileptic activity: The synthesized compounds were tested at dosages of 2000, 1000, 900, 850, 800 and 750 mg/kg using acute toxicity test. As a result, the test animal received 2000 mg/kg orally. In addition, three animals perished. Following the observation of mortality following the injection of 2 g/kg body weight, a lower dosage of 1000 mg/kg and 900

TABLE-4
DOCKING SCORE OF THE SYNTHESIZED DERIVATIVES

Compounds	With calcium channel (6KZV)	With GABAA (4COF)	With sodium channels (2KAV)
	kcal/mol affinity		
1	-5.7	-5.1	-4.2
2	-5.8	-5.2	-4.5
3	-5.6	-5.5	-4.5
4	-5.8	-6.4	-4.2
5	-5.4	-4.7	-4.2
6	-3.8	-3.9	-3.2
7	-5.3	-5.3	-4.4
8	-5.3	-4.9	-4.1
9	-5.6	-5.4	-4.6
10	-5.2	-4.8	-3.7
11	-5.6	-5.5	-4.4
12	-5.6	-5.5	-4.5
13	-5.5	-4.6	-3.8
14	-5.1	-5.2	-3.9
15	-5.5	-5.6	-4.5
16	-5.7	-5.6	-4.5

TABLE-5
ACUTE TOXICITY STUDY OF COMPOUNDS **6** AND **10**

Number of animals used	Dose (mg/kg)	Number of animals survived	
		Compd. **6**	Compd. **10**
3	2000	0	0
3	1000	0	0
3	900	0	0
3	850	1	1
3	800	2	2
3	750	3	3

TABLE-6
EFFECT OF COMPOUND **6** IN MES INDUCED SEIZURES MODELS

Treatment	Body weight (g)	Time (s) in various phases of convulsion				
		Flexion	Extensor	Clonus	Stupor	Recovery/death
Control group						
Saline	22.4	2	3	2	4	Recovery
	24.0	2	2	2	4	Recovery
	23.8	3	2	3	4	Recovery
	24.9	3	3	3	4	Recovery
	Mean	2.5	2.5	2.5	4	
Standard group						
Diphenylhydantoin	23.5	0	1	0	3	Recovery
	23.2	1	1	1	1	Recovery
	22.0	1	0	1	2	Recovery
	21.8	0	0	1	2	Recovery
	Mean	0.5	0.5	0.75	2	
Test group with low dose						
Compound **6** (low dose-75 mg/kg)	22.0	1	1	1	3	Recovery
	23.5	0	1	1	2	Recovery
	22.3	1	0	1	2	Recovery
	24.0	1	1	1	3	Recovery
	Mean	0.75	0.75	1	2.5	
Test group with high dose						
Compound **6** (high dose-150 mg/kg)	21.7	1	1	1	2	Recovery
	23.0	0	0	1	3	Recovery
	24.7	0	1	0	2	Recovery
	24.2	1	0	1	3	Death
	Mean	0.5	0.5	0.75	2.5	

mg/kg was administered. For both dosages, mortality was found in all animals. As a result, a reduced dosage of 850 mg/kg was administered. After receiving 850 mg/kg, two animals died and one animal survived. As a result, a reduced dosage of 800 mg/kg was administered. For 800 mg/kg, two animals survived and one died. Then a 750 mg/kg dosage was administered. Following treatment of 750 mg/kg, all animals survived and no symptoms of toxicity were seen. As a result, at 750 mg/kg,

TABLE-7
EFFECT OF COMPOUND **10** IN MES INDUCED SEIZURES MODELS

Treatment	Body weight (g)	Time (s) in various phases of convulsion				
		Flexion	Extensor	Clonus	Stupor	Recovery/death
Control group						
Saline	20.8	3	3	1	5	Recovery
	22.6	2	2	2	4	Recovery
	23.7	3	2	3	4	Recovery
	23.2	3	3	3	4	Recovery
	Mean	2.75	2.5	2.25	4.25	
Standard group						
Diphenylhydantoin	21.5	1	1	1	2	Recovery
	24.2	0	1	0	3	Recovery
	23.1	1	0	1	2	Recovery
	22.8	0	2	0	2	Recovery
	Mean	0.5	1	0.5	2.25	
Test group with low dose						
Compound **10** (low dose-75 mg/kg)	22.0	2	1	2	3	Recovery
	23.5	1	1	1	4	Recovery
	22.3	1	1	2	3	Recovery
	24.0	2	1	2	2	Recovery
	Mean	1.5	1	1.75	3	
Test group with high dose						
Compound **10** (high dose-150 mg/kg)	21.7	1	0	1	2	Recovery
	23.0	1	1	1	2	Recovery
	24.7	2	1	1	3	Recovery
	24.2	1	1	0	3	Recovery
	Mean	1.25	0.75	0.75	2.5	

TABLE-8
EFFECT OF COMPOUND **6** ON PTZ INDUCED SEIZURES MODELS

Treatment	Body weight (g)	Time (s) in various phases of convulsion				
		Flexion	Extensor	Clonus	Stupor	Recovery/death
Control group						
Saline	21.4	3	2	4	5	Recovery
	23.0	2	2	3	4	Recovery
	23.0	3	2	3	5	Recovery
	24.1	3	3	3	5	Recovery
	Mean	2.75	2.25	3.25	4.75	
Standard group						
Sodium valporate	23.5	1	0	1	2	Recovery
	23.2	0	0	1	3	Recovery
	22.0	1	0	0	2	Recovery
	21.8	0	1	1	3	Recovery
	Mean	0.5	0.25	0.75	2.5	
Test group with low dose						
Compound **6** (low dose-75 mg/kg)	22.5	2	1	2	3	Recovery
	23.0	1	0	1	3	Recovery
	24.0	1	1	2	4	Recovery
	22.0	1	2	2	3	Recovery
	Mean	1.25	1	1.75	3.25	
Test group with high dose						
Compound **6** (high dose-150 mg/kg)	23.2	2	1	1	2	Recovery
	23.7	0	1	1	3	Recovery
	24.0	1	0	2	3	Recovery
	24.5	1	1	1	3	Recovery
	Mean	1	0.75	1.25	2.75	

TABLE-9
EFFECT OF COMPOUND **10** ON PTZ INDUCED SEIZURES MODELS

Treatment	Body weight (g)	Time (s) in various phases of convulsion				
		Flexion	Extensor	Clonus	Stupor	Recovery/death
Control group						
Saline	22.0	3	1	3	6	Recovery
	24.6	2	3	3	6	Recovery
	23.0	2	2	2	5	Recovery
	22.9	3	3	3	6	Recovery
	Mean	2.5	2.25	2.75	5.75	
Standard group						
Diphenylhydantoin	21.0	1	0	1	2	Recovery
	24.2	0	1	1	2	Recovery
	23.4	1	0	0	3	Recovery
	22.0	1	1	1	2	Recovery
	Mean	0.75	0.5	0.75	2.25	
Test group with low dose						
Compound **10** (low dose-75 mg/kg)	24.1	2	1	2	3	Recovery
	21.5	2	1	2	3	Recovery
	20.8	1	2	1	2	Recovery
	24.2	2	1	2	4	Recovery
	Mean	1.75	1.25	1.75	3	
Test group with high dose						
Compound **10** (high dose-150 mg/kg)	23.0	2	2	1	3	Recovery
	23.0	1	0	1	2	Recovery
	22.7	1	1	1	2	Recovery
	24.4	0	1	1	2	Recovery
	Mean	1	1	1	2.25	

compounds **6** and **10** were confirmed to be safe. Acute toxicity results of compounds **6** and **10** are given in Table-5. For the assessment of antiepileptic activity, the effect of compounds **6** and **10** in MES and PTZ induced seizures models was assessed and the results are given in Tables 6-9.

Conclusion

A total of sixteen novel compounds were synthesized in the present research which are piracetam and its related compounds and based on the docking results compounds **6** and **10** was found to be more active and their antiepileptic activity was assessed as per the docking studies.

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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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