J Chem Crystallogr (2013) 43:561–567
DOI 10.1007/s10870-013-0461-7

ORIGINAL PAPER

2–Methylpyridinium 2,6-Dioxo-5-(2,4,6–Trinitrophenyl)–1,2,3,6-Tetrahydropyrimidin–4–Olate: Synthesis, Characterization, Single Crystal X-ray Analysis and Evaluation of Anticonvulsant/Hypnotic activity and Toxicity Effects

M. Buvaneswari · D. Kalaivani

Received: 29 January 2013 / Accepted: 3 September 2013 / Published online: 26 September 2013

Abstract A new type of barbiturate (a pyrimidine derivative) has been prepared through one pot synthesis from the ethanolic solution of 1-chloro-2,4,6-trinitrobenzene, barbituric acid and 2-methylpyridine. The mechanism of the formation of the reported barbiturate involves an intermediate sigma complex formation and proton abstraction reactions. The structure of the title barbiturate has been identified from UV–Vis, FT-IR, ^{1}H NMR, ^{13}C NMR and elemental analysis data. Single crystal XRD studies further confirm the formation of the reported barbiturate. The anticonvulsant activity of the synthesized barbiturate has been tested by Maximal Electro Shock method on albino rats. The title barbiturate showed notable anticonvulsant activity even at low concentration (dose, 25 mg/kg). It also induces hypnosis in albino mice (dose, 100 mg/kg) for a duration of 47 min. Acute toxicity studies on albino mice indicate that LD_{50} value is >1,500 mg/kg and the animals do not show any behavioral changes.

Keywords 1-Chloro-2,4,6-trinitrobenzene · 2-Methylpyridine · Barbiturate · Anticonvulsant activity · Hypnotic activity

Essence of the paper The article focuses on the formation of a new type of barbiturate [2–Methylpyridinium 2,6-dioxo-5-(2,4,6–trinitrophenyl)–1,2,3,6–tetrahydropyrimidin–4 –olate) which has notable anticonvulsant activity even at low concentration (25 mg/kg) and high LD_{50} value (>1,500 mg/kg).

M. Buvaneswari · D. Kalaivani (✉)
Post Graduate and Research Department of Chemistry, Seethalakshmi Ramaswami College, Affiliated to Bharathidasan University, Tiruchirappalli 620 002, Tamil Nadu, India
e-mail: kalaivbalaj@yahoo.co.in

Introduction

Convulsion is a common neurological disorder which results due to the spontaneous intermittent abnormal electrical activity in the brain [1–4]. The pharmacotherapy of convulsion has received considerable attention in recent years and conventional antiepileptic drugs such as pyrimidone, phenytoin, carbamazepine, phenobarbital and valproate are mainly available for tonic–clonic seizure (grandmal type). These drugs exhibit unfavourable side effects and they are still used in certain clinical situations [5–7]. Approximately 1 % people of the world population suffer from convulsion [8] and hence it is necessary to search for new anticonvulsant agents with a more selective activity and lower toxicity. In this context, we report in this article a new barbiturate which has been synthesized from 1-chloro-2,4,6-trinitrobenzene (TNCB), barbituric acid (BBA) and 2-methylpyridine. It has notable anticonvulsant activity even at low concentration (25 mg/kg). Unlike the reported barbiturates [9] it has remarkably higher LD_{50} value (>1,500 mg/kg).

Experimental

Materials and Methods

Analar grade reagents and solvents for synthesis and analysis were commercially available and used as received without purification. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker DRX-500 spectrophotometer with ($DMSO\text{-}d_6$) as solvent and tetramethylsilane as internal standard. Chemical shifts are expressed in ppm. IR spectra were recorded using KBr pellets on a FT–IR Perkin-Elmer RXI spectrophotometer.

Preparation of the Title Molecular Salt (Barbiturate)

TNCB was prepared from picric acid and phosphorus oxychloride [10]. TNCB (2.5 g, 0.01 mol) in 20 mL of absolute ethanol was mixed with barbituric acid (1.3 g, 0.01 mol) dissolved in 30 mL of absolute ethanol. 2-Methylpyridine (3 mL, 0.05 mol) was then added and the mixture was shaken well for 2–3 h. On standing reddish orange coloured crystals come out from the solution. The crystals were filtered and ground well. The powder thus obtained was washed with 5 mL of absolute ethanol and then with 50 mL of dry ether to remove the unreacted reactants. The crystals were recrystallized from ethanol. Good quality single crystals for X-ray diffraction studies were obtained by slow evaporation of ethanol at room temperature (m.p. 513 K, yield of the pure product 85 %). Micro anlaysis calcd: C, 44.44; H, 2.78; N, 19.44; found: C, 44.78; H, 2.18; N, 18.91. Solubility: Water (15 g/dm^3), Dimethylsulfoxide (66 g/dm^3), ethanol (20 g/dm^3). The title salt is insoluble in ether.

Spectral Data

Vis (EtOH, λ_{max}): TNCB, 3,600 Å; Barbiturate, 4,600 Å. ^{1}H NMR [500 MHz, (DMSO-d_6)] δ: TNCB, 9.20 (s, 2H, ring protons); BBA, 11.09 (s, 2H, two N–H protons), 3.39 (s, 2H, -CH_2 protons); 2-methylpyridine, 7.14–8.44 (m, 4H, Ring protons), 2.50 (s, 3H, methyl group); Barbiturate, 9.78 (s, 2H, -NH), 8.56 (s, 2H, nitro aromatic ring), 7.82–8.78 (m, 4H, ring protons of methylpyridinium cation), 2.71 (s, 3H, -CH_3 proton of methylpyridinium cation). ^{13}C NMR [500 MHz, (DMSO-d_6)] δ: TNCB, 149, 146, 127, 123; BBA, 168, 152, 40; 2-methylpyridine, 158, 149, 136, 123, 121, 24.49; barbiturate, 163, 151, 149, δ145, 142, 141, 134, 132, 127, 124, 123, 84, 39. IR [KBr, ν (cm^{-1})]: TNCB, 1,538 [NO (asym. str.)], 1,342 [NO (sym. str.)], 718 [C–Cl (str)]; BBA, 3,479 [N–H (str)], 2,879 [-CH_2 (asym. str.)], 2,819 [-CH_2 (sym. str.), 1,716 [C=O (str)], 1,619 [N–H (bend)]; Barbiturate, 3,454 [N–H (str)], 3,200–2,500 [broad, amine salt], 1,707 [C=O (str)], 1,626 [N–H (bend)], 1,527 [NO (asym. str.)], 1,347 [NO (sym. str.)], 542 [torsional oscillation, amine salt]. Qualitative tests [11] on the synthesized barbiturate indicate the presence of nitrogen atom, nitro groups and the absence of chlorine atom. The purity of the sample was checked employing thin layer chromatographic technique.

Crystal Structure Determination

Crystallographic measurements were obtained on a BRUKER AXS KAPPA APEX 2 CCD diffractometer with graphite monochromated MoKα radiation (λ = 0.71073 Å). A single crystal of the dimension 0.30 × 0.20 × 0.20 mm was selected for X-ray diffraction study. Data were collected at 293 (2) K. The structure was solved by direct methods and all non-hydrogen atoms were obtained from the Difference Fourier map and subjected to anisotropic refinement by full-matrix least-squares calculations on F^2. All hydrogen atoms were geometrically fixed by Difference Fourier Synthesis and refined isotropically. The program used for the crystal structure determination are—Data Collection: APEX2 [12]; Cell Refinement: SAINT—plus [12]; Data Reduction: SAINT—plus [12]; Program used to solve structure: SIR92 [13]. Program used to refine structure: SHELXL 97 [14]. Molecular graphics: [15, 16]. Important information concerning crystallographic data are summarized in Table 1. Selected bond distances and angles are listed in Tables 2 and 3. The important N–H···O hydrogen bonds are exhibited in Table 4. The ORTEP diagram and packing view of the barbiturate are presented in Figs. 1 and 2, respectively.

Results and Discussion

In the presence of pyridine (base), a proton is abstracted from the cyclic molecule (barbituric acid) which has an active methylene group. The resulting carbanion readily

Table 1 Crystal data, data collection and refinement details

Chemical formula, Mr	C_{16} H_{12} N_6 O_9, 432.32
Crystal system, Space group, Z	Monoclinic, p2(1)/n, 4
a	8.4450(5) Å
b	11.7887(7) Å
c	18.5181(10) Å
α, β, γ	90°, 97.027(1)°, 90°
Volume	1,829.73(18) A^3
Calculated density	1.569 mg/m^3
Crystal size	0.30 × 0.20 × 0.20 mm
μ	0.132 mm^−1
T	293(2) K
T_{min}, T_{max}	0.9523, 0.9840
$F_{(000)}$	888
Scan range	−10 < = h < = 10
	−14 < = k < = 14
	−22 < = l < = 22
Θ_{min}, Θ_{max}	2.05, 25.00°
Completeness to theta 25	=100 %
Number of measured/Independent reflection, R_{int}	16,234/3,210, 0.0231
Number of refined parameters	290
S	1.042
R[I > 2σ(I), wR_2]	0.0311, 0.0826
R indices (all data)	0.0392, 0.0881
Δρmax, Δρmin	0.199, −0.156e.A^−3

Table 2 Selected bond distances (Å) of title barbiturate

Bond distance	Å	Bond distance	Å
C1–N6	1.335 (2)	C13–C16	1.405 (2)
C1–C5	1.379 (3)	C13–C14	1.4167 (2)
C1–C6	1.481 (3)	C14–O8	1.242 (2)
C2–C3	1.359 (3)	C14–N4	1.386 (2)
C3–C4	1.373 (3)	C15–O9	1.218 (2)
C4–C5	1.356 (3)	C15–N(5)	1.357 (2)
C12–C7	1.374 (2)	C15–N(4)	1.362 (2)
C12–C11	1.375 (2)	C(16)–O(7)	1.261 (2)
C7–C8	1.373 (2)	C(16)–N(5)	1.380 (2)
C7–N2	1.465 (2)	N(1)–O2	1.216 (2)
C8–C9	1.380 (2)	N(1)–O1	1.219 (2)
C9–C10	1.402 (2)	N2–O4	1.221 (2)
C9–N11	1.475 (2)	N2–O3	1.224 (2)
C10–C11	1.413 (2)	N3–O5	1.217 (2)
C10–C13	1.462 (2)	N3–O6	1.222 (2)
C11–N3	1.475 (2)	N6–C2	1.333 (2)

Table 3 Selected bond angles (°) of title barbiturate

Bond angle		Bond angle	
C2–C3–C4	118.4 (2)	C16–C13–C10	120.11 (12)
C5–C4–C3	120.26 (19)	C14–C13–C10	120.50 (12)
C4–C5–C1	120.66 (19)	O8–C14–N4	117.86 (12)
C5–C1–C6	124.54 (19)	O8–C14–C13	125.10 (12)
C2–N6–C1	123.65 (16)	N4–C14–C13	117.04 (12)
C7–C12–C11	117.32 (13)	O9–C15–N5	122.85 (13)
C8–C7–C12	121.90 (13)	O9–C15–N4	122.56 (13)
C8–C7–N2	118.39 (13)	N5–C15–N4	114.58 (12)
C12–C7–N2	119.71 (13)	O7–C16–N5	116.80 (12)
C7–C8–C9	118.09 (13)	O7–C16–C13	125.75 (12)
C8–C9–C10	124.30 (13)	N5–C16–C13	117.45 (12)
C8–C9–N1	113.31 (12)	C15–N5–C16	125.82 (12)
C10–C9–N1	122.10 (12)	O2–N1–O1	125.45 (13)
C9–C10–C11	112.74 (12)	O2–N1–C9	117.54 (12)
C9–C10–C13	124.27 (12)	O1–N1–C9	116.90 (12)
C11–C10– C13	122.96 (12)	O4–N2–O3	124.73 (14)
C12–C11–C10	124.97 (13)	O4–N2–C7	117.44 (14)
C12–C11– N3	114.31 (12)	O3–N2–C7	117.83 (14)
C10–C11–N3	120.60 (12)	C15–N4–C14	125.56 (12)
C16–C13–C14	119.38 (12)	O5–N3–O6	125.00 (14)
		O5–N3–C11	117.64 (14)
		O6–N3–C11	117.25 (13)
		N6–C1–C5	117.06 (18)
		N6–C1–C6	118.39 (18)

forms the molecular salt through steps as specified in Fig. 3. Similar molecular salts have been reported by us involving aliphatic tertiary amines N,N-disubstituted aniline, barbituric acid and TNCB [17–20]. The structure of the synthesized title molecule is verified by spectral and single crystal X-ray diffraction data.

Interpretation of Spectral Data

TNCB absorbs at 3,600 Å, where as the title molecular salt (barbiturate) prepared in the present work from TNCB absorbs at 4,600 Å. This supports the fact that the negative charge of the anion of the molecular salt is delocalised over a large area as indicated in Fig. 3. In TNCB a strong sharp absorption band characteristic of C–Cl stretching mode is observed at 718 cm^{-1}, which is absent in the title molecular salt which reveals the removal of chlorine atom. The strong band of N–O asymmetric stretching mode observed at 1,538 cm^{-1} in TNCB is shifted to lower frequency (1,527 cm^{-1}) which is attributed to the stretching of N–O bond during the delocalization of negative charge in the molecular salt. The broad band at 3,200–2,500 cm^{-1} is reminiscent of amine salt [21]. The sharp strong band at $\sim$542 cm^{-1} corresponds to the torsional oscillation of amine salt [22]. In the pmr spectrum of barbituric acid, the N–H protons resonate at δ 11.09 but in the barbiturate formed, it is shifted to higher field (δ 9.78). In the title barbiturate, the negative charge is delocalised over a large area including the keto functions near to the N–H groups, which causes the signal to appear in higher field as compared to that of barbituric acid. The prepared molecular salt contains nitro aromatic, barbituric acid and 2-methylpyridine moieties. In the molecular salt, the nitro aromatic ring protons appear at a slightly higher field than that of TNCB which supports the development of negative charge in the nitro aromatic moiety. The protons of 2-methylpyridine are shifted to lower field upon the formation of molecular salt which supports the development of positive charge on the nitrogen atom of 2-methylpyridine. However the shifts are not very high because the positive and negative entities are held by electrostatic force of attraction in the molecular

Table 4 Hydrogen bond geometry of title barbiturate

D–H···A (°)	dD–H (Å)	dH···A (Å)	D(D···A) (Å)	<DHA (°)
N4–H4A···07[a]	0.878 (18)	2.016 (18)	2.8932 (16)	177.9 (16)
N5–H5A···08[b]	0.842 (19)	1.961 (19)	2.7994 (16)	173.5 (17)
N6–H2···O7[c]	0.86	1.92	2.7673	169.4

Symmetry code

[a] $-x + 3/2, y - 1/2, -z + 3/2$

[b] $-x + 3/2, y + 1/2, -z + 3/2$

[c] $x + 1, y, z$

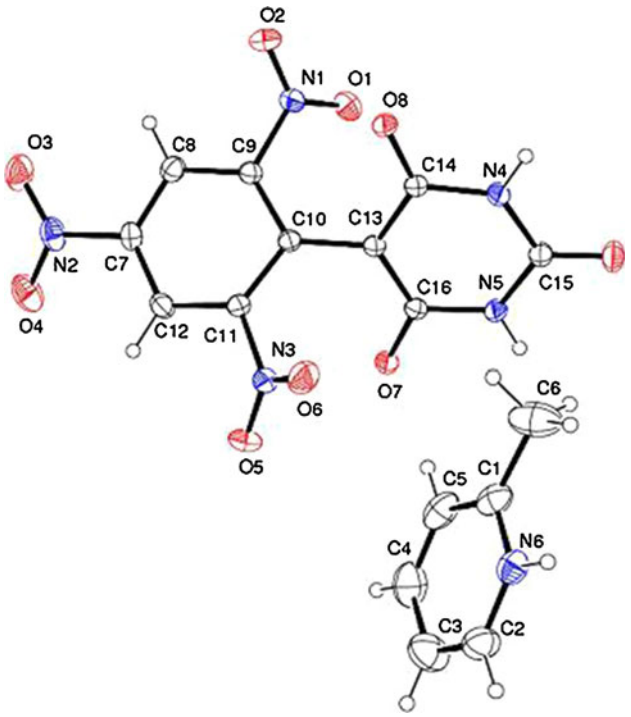

Fig. 1 ORTEP view of 2–Methylpyridinium 2,6-dioxo-5-(2,4,6–trinitrophenyl)–1,2,3,6-tetrahydropyrimidin–4–olate

salt. As expected, thirteen signals corresponding to different carbon environments are noticed in the ^{13}C NMR spectrum of title molecular salt and elemental analysis data also support the proposed structure.

Description of Crystal Structure

Single Crystal XRD results of the product confirm the formation of synthesized barbiturate under the present experimental conditions. The asymmetric unit (Fig. 1) is comprised of 2,4,6-trinitrophenylbarbiturate anion and 2-methylpyridinium cation. In the synthesised barbiturate the adjacent barbiturate anions are linked through two different N–H···O hydrogen bonds (N5–H5A···O8 and N4–H4A···O7) constituting an $R_2^2(8)$ dimer and this one dimensional anionic tape extends infinitely along [010] direction. To the anionic chain the 2-methylpyridinium cations are connected through N6–H2···O7 hydrogen bond resulting in an anionic–cationic supramolecular tape along [010] direction. Similar chains of the tape are further linked through C–H···O interactions generating a three dimensional networks thus building the molecular crystal (Fig. 2). The trinitroaromatic ring which comprises of C_7, C_8, C_9, C_{10}, C_{11} and C_{12} atoms and the barbiturate ring with C_{13}, C_{14}, N_4, C_{15}, N_5 and C_{16} atoms are not co-planar. The dihedral angle between them has been observed to be 41.20 (5)°.

Biological Activity Studies

The experimental procedures adopted for the evaluation of the biological activities have been approved by the Institutional Animal Ethics Committee and conducted according to the guidelines of Indian National Science Academy for the use and care of experimental animals [23]. The animals were maintained at a well ventilated temperature controlled (303 ± 1 K) stopanimal room for 7 days prior to the experimental period and provided with food and water. The animals were acclimatized to laboratory conditions before the test. Each animal was used only once.

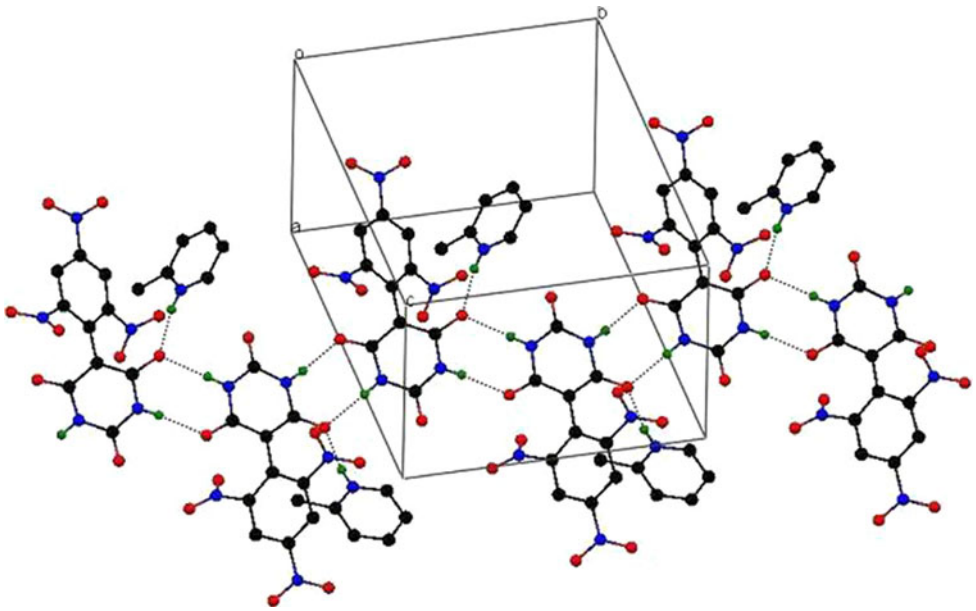

Fig. 2 Packing view of 2–Methylpyridinium 2,6-dioxo-5-(2,4,6–trinitrophenyl)–1,2,3,6-tetrahydropyrimidin–4–olate

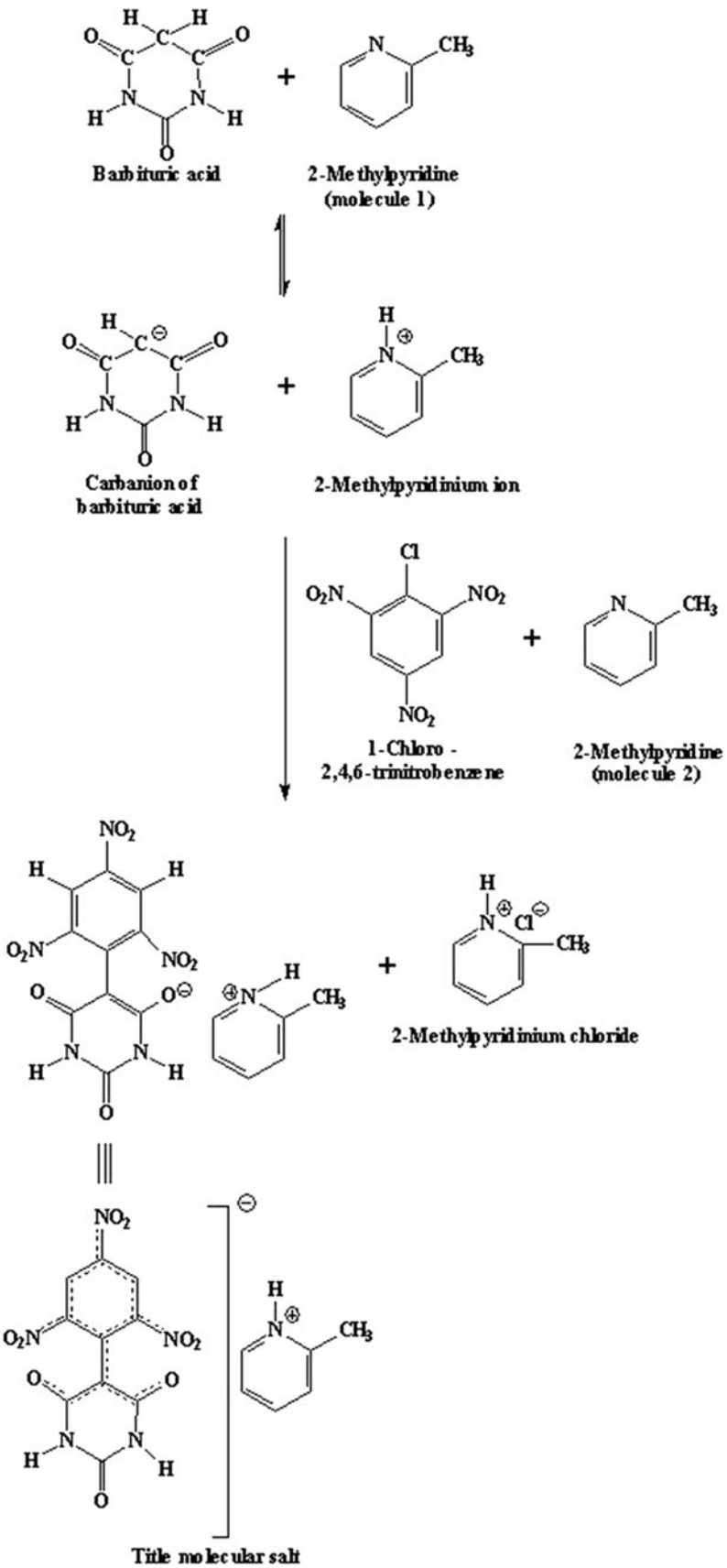

Fig. 3 Schematic representation of the formation of 2–Methylpyridinium 2,6-dioxo-5-(2,4,6–trinitrophenyl)–1,2,3,6-tetrahydropyrimidin–4–olate

Table 5 Anticonvulsant activity of title barbiturate against maximal electro shock induced convulsion in Albino rats

S. no.	Treatment	Time (s)				
		Various phases of convulsion				
		Flexor	Extensor	Clonus	Stupor	Recovery/death
1.	Control (normal saline) (5 mL/kg)	12 ± 1	21 ± 1.5	35 ± 2.5	149 ± 11.0	Recovery
2.	Title barbiturate (25 mg/kg)	0.0	0.0	12.0 ± 2.1	136 ± 4.8	Recovery
3.	Standard phenobarbitone (20 mg/kg)	2 ± 0.2	0.0	15 ± 0.8	57 ± 3.9	Recovery

Mean ± SEM, N = 6, $P < 0.001$ versus standard

Table 6 Hypnotic activity of title barbiturate

S. no.	Treatment	Time of administration (min) (A)	Time of loss of righting reflex (min) (B)	On set of action (min) (B–A)	Recovery (min) (C)	Duration (min) (C–B)
1.	Control (normal saline)	0	–	–	–	–
2.	Title barbiturate (100 mg/kg)	0	42.89 ± 2.3	42.89 ± 2.3	90.13 ± 3.4	47.24
3.	Pentobarbitone (20 mg/kg)	0	26.8 ± 1.42	26.8 ± 1.42	168.36 ± 6.4	141.56

Mean ± S.E.M, N = 6, $P < 0.001$ versus standard

Evaluation of Anticonvulsant Activity

The Maximal Electro Shock (MES) method [24, 25] was followed to study the anticonvulsant activity. Albino rats of either sex weighing 150–200 g were divided into groups of six animals each. The positive control used was phenobarbitone (20 mg/kg). The control group was fed with normal saline (5 mL/kg). The other group received the synthesized molecule (25 mg/kg). The normal saline, standard drug and the synthesised barbiturate were given 1 h before the induction of MES (150 mA/0.2 s). The current was applied to the animals using the corneal electrodes of electro convulsometer (model 100-3, INCO). The different stages of convulsions such as tonic flexion, tonic extensor, clonus convulsion, stupor and recovery/death were noted. The time spent by the animal in each of these phases was noted. Reduction in time in extensor phase of convulsion has been noticed for the title barbiturate (Table 5). The mean value for each group was calculated and compared with the control. The results were expressed as mean ± standard error. The test of significance was analyzed by students' *t* test [26].

Evaluation of Hypnotic Action

Albino mice of either sex weighing 25–30 g were divided into groups of six animals each. The positive control used was pentobarbital 20 mg/kg. The control group was given normal saline (1 mL/kg). The other group received synthesized molecular salt (100 mg/kg). From the time of administration, time of loss of righting reflex, time of on set of action and time of recovery, the hypnotic action of the synthesized molecule has been established (Table 6).

Acute Toxicity Study

LD_{50} of the synthesized barbiturate was determined as per OECD guide lines [27, 28]. The title molecular salt falls under class 4 ($LD_{50} > 1{,}500$ mg/kg). The animals did not show any sign of acute toxicity and behavioral changes.

Conclusion

The title molecular salt (barbiturate) is obtained in high purity with good yield (>85 %) through one pot synthesis using ethanol as solvent. It possesses many unique properties such as (1) good stability at room temperature (2) high solubility in water (3) notable anticonvulsant activity even at low concentration (dose 25 mg/kg) and (4) high LD_{50} value (>1,500 mg/kg), which may probably receive considerable significance in the field of epilepsy research in future.

Supplementary Data

CCDC No.866572 contains the supplementary crystallographic data for 2-Methylpyridinium 2,6-dioxo-5-(2,4,6-trinitrophenyl)-1,2,3,6-tetrahydropyrimidin-4-olate. These data can be obtained free of charge from Cambridge Crystallographic Data Center via www.ccdc.cam.ac.uk/data request/cif.

Acknowledgments The authors thank SAIF, IIT Madras, Chennai-600 036 for IR, NMR and Crystal data and Periyar College of Pharmaceutical Sciences, Tiruchirappalli-620 021 for biological activity results.

References

1. Hardman JG, Limbird LE (2001) The pharmacological basis of therapeutics, 10th edn. Mc.Graw-Hill
2. Jain KS, Chitre TS, Miniyar PB, Kathiravan MK, Bendre VS, Veer VS, Shahane SR, Shishoo CJ (2006) Curr Sci 90:793–803
3. Bell GS, Sander JW (2002) The epidemiology of epilepsy : the size of the problem. Seizure 11(suppl A):306–314
4. Browne TR, Holmes GL (2001) Epilepsy New Engl J Med 344:1145–1151
5. Erickson MK, Pasternak JJ, Weglinski RM, Lanier LW (2009) J Neurosurg Anesth 21:307–317
6. Feldmann ER, Krang G, Praschak RN, Kasper S (2009) Curr Med Res Opin 25:2281–2285
7. Manas KH, Michael DS, Sule N, Srivastava DK, Sanku M (2008) Bioorg Med Chem Lett 18:2373–2376
8. Rout SK, Kar DM (2010) Int J Pharm Sci Rev Res 3:19–23
9. Tripathi KD (2009) Essentials of medical pharmacology, 6th edn. Jaypee brothers medical publishers, Chennai
10. Vogel AI (1978) Text book of practical organic chemistry. Longman, London, p 731
11. Vogel AI (1978) Text book of practical organic chemistry. Longman, London, p 776
12. Bruker (2004) APEX2, SAINT-plus and SADABS. Brunker AXS-INC. Madison, Wisconsin, USA
13. Altomare A, Cascarano G, Giacovazzo C, Guagliardi A (1993) J Appl Cryst 26:343–350
14. Sheldrick GM (2008) Acta Cryst A 64:112–122
15. Macrae CF, Bruno IJ, Chisholm JA, Edgington PR, McCabe P, Pidcock E, Rodriguez- Monge L, Taylor R, Van de Streek J, Wood PA (2008) J Appl Cryst 41:466–470
16. Farrugia LJ (1997) J Appl Cryst 30:565
17. Kalaivani D, Buvaneswari M, Rajeswari S (2012) Acta Cryst E68:o29–o30
18. Buvaneswari M, Kalaivani D (2011) Acta Cryst E67:o3452
19. Buvaneswari M, Kalaivani D (2011) Acta Cryst E67:o1433–o1434
20. Kalaivani D, Buvaneswari M (2010) Recent advances in clinical medicine. WSEAS Publication, UK, pp 255–260
21. Silverstein RM, Webster FX (2004) Spectrometric identification of organic compounds. Wiley, New York, p 103
22. Ramachandran E, Baskaran K, Natarajan S (2007) Cryst Ref Technol 42:73–77
23. Guidelines for care and use of animals in scientific research, Indian National Science Academy, 1st edn., publisher S.K.Sahni, New Delhi (1992/2000)
24. Misra AK, Dandiya PC, Kulkarni SK (1974) Indian J Pharmacol 5:449
25. Bhattacharya SK, Chakrabati A (1998) Indian J Exp Biol 36:112
26. Armitage P (1971) Statistical methods in medical research, 1st edn. Blackwell Scientific Publications, London, p 217
27. Miller LC, Trainter ML (1944) Proc Soc Exp Med Biol 57:261
28. Shetty Akhila J, Deepa S, Alwar MC (2007) Curr Sci 93:917–920