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Original article

Synthesis and anticonvulsant activity of novel purine derivatives

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ABSTRACT

A series of new purines containing triazole and other heterocycle substituents was synthesized and evaluated for their preliminary anticonvulsant activity and neurotoxicity by using the maximal electroshock (MES), subcutaneous pentylenetetrazole (scPTZ) and rotarod neurotoxicity (TOX) tests. Among the compounds studied, 9-decyl-6-(1*H*-1,2,4-triazol-1-yl)-9*H*-purine (**5e**) was the most potent compound, with a median effective dose of 23.4 mg/kg and a high protective index of more than 25.6 after intraperitoneal administration in mice. Compound **5e** showed significant oral activity against MES-induced seizures in mice, with an ED_{50} of 39.4 mg/kg and a PI above 31.6. These results demonstrate that compound **5e** possesses better anticonvulsant activity and is safer than the commercially available drugs carbamazepine and valproate in MES, scPTZ and TOX models.

1. Introduction

Epilepsy is a heterogeneous neurological disorder characterized by the onset of spontaneous convulsive and non-convulsive seizures. This disorder affects over 50 million people worldwide, and it is estimated that only 50% of patients are adequately treated for their symptoms with currently available antiepileptic drugs (AEDs). Of the remaining patients, approximately 20% are either inadequately treated or treated at the expense of severe side effects, leaving approximately 30% of patients with refractory epilepsy [1,2]. Conventional antiepileptic agents (phenytoin and carbamazepine) and recent antiepileptic drugs (gabapentin, vigabatrin, remacemide, and loreclezole) are clinically effective against different types of seizures. However, many AEDs have serious side effects [3–7]. Therefore, the continued search for safer and more effective AEDs is necessary.

The purine nucleus is one of the most important and widely exploited heterocyclic ring for the development of bioactive molecules. The literature contains many examples of purine derivative synthesis, and these compounds exhibiting a range of biological properties, including anticonvulsant [8,9], antibacterial [10,11], antifungal [12], antimalarial [13], anticancer [14–16], and anti-inflammatory [17] activities.

Previously, we reported the synthesis of several triazole-containing heterocycles, including triazoloquinolines, triazolophthalazines, triazolobenzothiazines, and triazolobenzooxazepines, and evaluated their anticonvulsant activity. The majority of these heterocycles exhibited potent anticonvulsant activity [18–21] (Fig. 1). Here, a series of 7-alkyl-7*H*-[1,2,4]triazolo[4,3-*g*]purine derivatives (**4a**–**o**) was designed to combine both purine and triazole moieties. Based on the anticonvulsant activities of compounds **4a**–**o**, another series of 9-alkyl-6-(1*H*-1,2,4-triazol-1-yl)-9*H*-purine derivatives (**5a**–**p**) was designed and synthesized through the ring-opening of compounds **4a**–**o**. For the sake of having a structure–activity relationship, the triazole ring in compounds **5a**–**p** was replaced by other heterocycles such as imidazole, methylimidazole, or pyrazole rings to give compounds **6**–**8** and **9**, and their structures were characterized using IR, ^{1}H NMR, MS, and ^{13}C NMR techniques. The anticonvulsant activity of the titled compounds was evaluated using the maximal electroshock test in mice, and their neurotoxicity was evaluated using the rotarod test. The activity of compound **5e** against pentylenetetrazole-induced seizures was also established.

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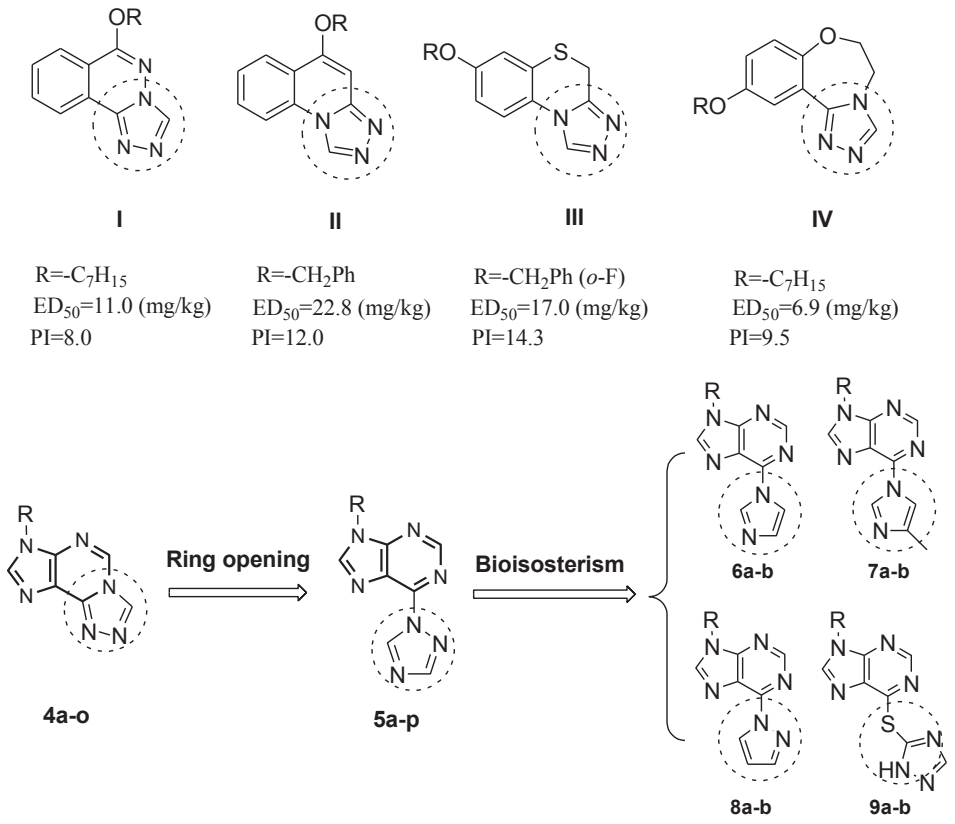


Fig. 1. Structures of some compounds containing triazole and target compounds.

2. Results and discussion

2.1. Chemistry

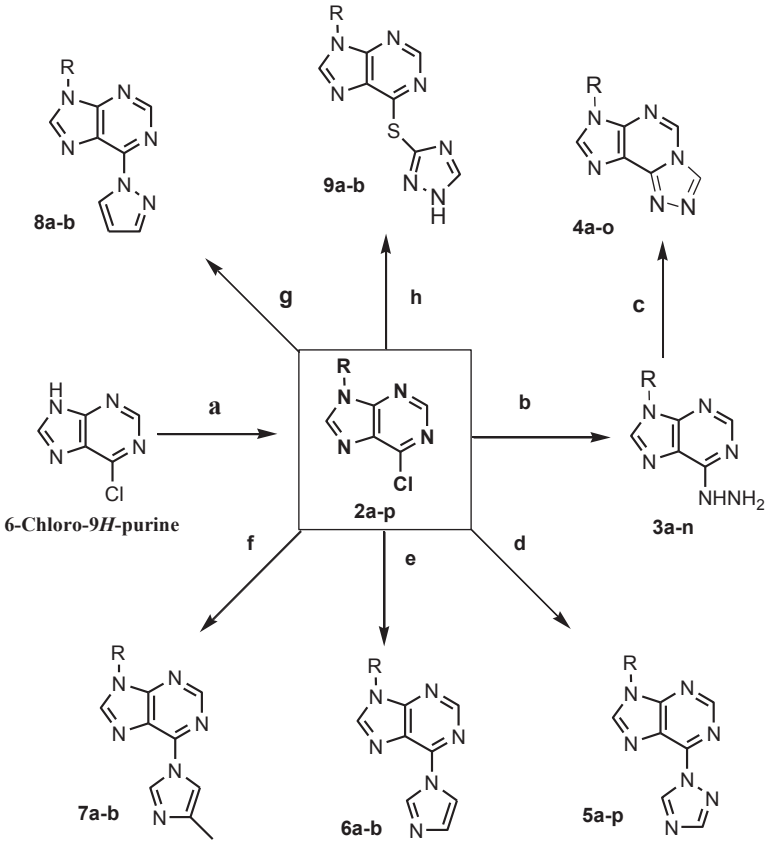
6-Chloro-9-alkyl-9*H*-purines (**2a**–**p**) were prepared by alkylating 6-chloro-9*H*-purine (**1**) using alkyl bromide or benzyl chloride derivatives (Scheme 1) [22]. Treatment of **2a**–**p** with hydrazine hydrate in methanol afforded 1-(9-alkyl-9*H*-purin-6-yl) hydrazines (**3a**–**o)** in a high yield [23]. Cyclization of compounds **3a**–**o** with triethyl orthoformate yielded 7-alkyl-7*H*-[1,2,4]triazolo [4,3-*g*]purines (**4a**–**o)**. When 6-chloro-9-alkyl-9*H*-purines (**2a**–**p**) were allowed to react with different azoles such as triazole, imidazole, methylimidazole and pyrazole in refluxing dimethylformamide (DMF) in the presence of K_2CO_3 [24,25], the 9-chlorine atom of 2 was easily substituted by these heterocylces, producing the corresponding purines: 9-alkyl-6-(1*H*-1,2,4-triazol-1-yl)-9*H*-purine (**5a**–**p)**, 9-alkyl-6-(1*H*-imidazol-1-yl)-9*H*-purine (**6a**–**b)**, 9-alkyl-6-(4-methyl-1*H*-imidazol-1-yl)-9*H*-purine (**7a**–**b)**, and 9-alkyl-6-(1*H*-pyrazol-1-yl)-9*H*-purine (**8a**–**b)**. 9-Alkyl-6-(3*H*-1,2,4-triazol-3-ylthio)-9*H*-purines (**9a**–**b)** were synthesized by reacting compounds **2a**–**p** with 1*H* (2*H*), (4*H*)-1,2,4-triazole-3-thiol in acetonitrile in the presence of sodium methylate. The structures of the targeted compounds were characterized using spectral methods, and all spectral data corroborated the assumed structures.

2.2. Pharmacology

The obtained compounds were submitted for *in vivo* evaluation using the methods described in the Antiepileptic Drug Development Program (ADD) of the National Institutes of Health according to previously described testing procedures (USA) [26,27]. The described pharmacological evaluation was accepted by Ethics Commission of China. Primary studies of anticonvulsants in mice involved two tests: MES and neurotoxicity tests. The most promising derivatives were subjected to quantitative determination of their ED_{50} and TD_{50}. Experiments were performed in KunMing mice (weighing 18–22 g) purchased from the Laboratory of Animal Research, College of Pharmacy, Yanbian University, Yanji, Jilin Province, China. The animals had free access to food and water, except during the testing period. The test compounds were dissolved in DMSO.

The MES seizure model was used for preliminary screening of purine derivatives. All the compounds were administered by intraperitoneal injection in triplicate at doses of 30 and 100 mg/kg. The findings were recorded at 0.5 h, and the results are presented in Table 1 and Table 2 (phase I).

The MES test showed that some derivatives of the series **4a**–**o** were active at a dose of 100 mg/kg (Table 1), indicating their ability to prevent the seizer spread. Four derivatives (**4b**–**e**) out of the six alkyltriazolopurines (**4a**–**f**) showed anticonvulsant activity. The length of the alkyl chain appeared to affect the anticonvulsant activity of the alkyl-substituted derivatives (**4a**–**f**). We observed a correlation between the length of the alkyl chain of compounds (**4a**–**f**) and the corresponding anticonvulsant activity. An increase in the length of the alkyl chain gradually increased the anticonvulsant activity, with the maximum anticonvulsant activity being shown by compound **4c** ($R = C_6H_{13}$), after which a gradual decrease in activity was observed. In this study, the activity curve of the alkyl chain substituted derivatives was bell-shaped with a maximum activity peak. Compound **4c** showed the maximum activity peak, which may reflect the optimal partition coefficient associated with the easiest crossing of biological membranes. Among the benzyl group-substituted derivatives (**4g**–**o**), compounds **4g**, **4j** and **4o**



(**a**) K_2CO_3, RX, DMF, rt, 24h; (**b**) $NH_2NH_2.H_2O$, CH_3OH, reflux, 1h; (**c**) $HC(OC_2H_5)_3$, reflux, 6h; (**d**) K_2CO_3, 1,2,4-triazole, DMF, reflux, 3h; (**e**) K_2CO_3, imidazole, DMF, reflux, 6h; (**f**) K_2CO_3, 4-methylimidazole, DMF, reflux, 10h; (**g**) K_2CO_3, pyrazole, DMF, reflux, 6h; (**h**) 1*H* (2*H*),(4*H*)-1,2,4-triazole-3-thiol, CH_3ONa, acetonitrile, rt, 18h. **R:** **4a** -C_4H_9, **4b** -C_5H_{11}, **4c** -C_6H_{13}, **4d** -C_7H_{15}, **4e** -C_8H_{17}, **4f** -$C_{10}H_{21}$, **4g** -$CH_2C_6H_5$ (*o*-Cl), **4h** -$CH_2C_6H_5$ (*m*-Cl), **4i** -$CH_2C_6H_5$ (*p*-Cl), **4j** -$CH_2C_6H_5$ (*o*-F), **4k** -$CH_2C_6H_5$ (*m*-F), **4l** -$CH_2C_6H_5$ (*p*-F), **4m** -$CH_2C_6H_5$ (*p*-CH_3), **4n** -$CH_2C_6H_5$ (*p*-OCH_3), **4o** -$CH_2C_6H_5$; **5a** -C_5H_{11}, **5b** -C_6H_{13}, **5c** -C_7H_{15}, **5d** -C_8H_{17}, **5e** -$C_{10}H_{21}$, **5f** -$C_{12}H_{25}$, **5g** -$C_{14}H_{29}$, **5h** -$CH_2C_6H_5$ (*o*-Cl), **5i** -$CH_2C_6H_5$ (*m*-Cl), **5j** -$CH_2C_6H_5$ (*p*-Cl), **5k** -$CH_2C_6H_5$ (*o*-F), **5l** -$CH_2C_6H_5$ (*m*-F), **5m** -$CH_2C_6H_5$ (*p*-F), **5n** -$CH_2C_6H_5$ (*p*-CH_3), **5o** -$CH_2C_6H_5$ (*p*-OCH_3), **5p** -$CH_2C_6H_5$, **6a** -$C_{10}H_{21}$, **6b** -$CH_2C_6H_5$ (*o*-F), **7a** -$C_{10}H_{21}$, **7b** -$CH_2C_6H_5$ (*o*-F), **8a** -$C_{10}H_{21}$, **8b** -$CH_2C_6H_5$ (*o*-F), **9a** -$C_{10}H_{21}$, **9b** -$CH_2C_6H_5$ (*o*-F)

Scheme 1. Synthetic route of target compounds.

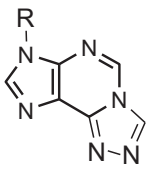
protected against MES-induced seizures to varying degrees at the dose of 100 mg/kg.

Among all the compounds tested, compounds **4c** and **4g** exhibited the most potent anticonvulsant activity against MES-induced seizures, and these compounds were subjected to phase II trials to quantify their preliminary anticonvulsant activity (indicated by ED_{50}) and neurotoxicity (indicated by TD_{50}) in mice (Table 3).

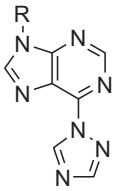
Most of the compounds in series **5a**–**p** were active in the MES test, indicating that they can prevent seizure spread. Four compounds, **5c**–**e** and **5k**, protected against MES-induced seizures at a dose of 30 mg/kg. At 100 mg/kg, most compounds showed protection, except **5g**, **5j**, and **5m**–**o**. Compounds **5b**–**e**, **5i**, **5k**–**l**, and **5p** showed protective effects at 100 mg/kg in all 3 mice tested. Analysis of the activities of the synthesized compounds (**5a**–**p**) showed the following results. In the series of compounds **5a**–**g**, the length of the alkyl chain influenced the anticonvulsant activity of the derivatives. From **5a** to **5e**, as the alkyl chain length increased, anticonvulsant activity increased. From **5e** to **5g**, as the alkyl chain length increased, anticonvulsant activity decreased. Thus, the activity curve of the alkyl chain-substituted derivatives was bell-shaped, displaying a maximum activity peak. Compound **5e** demonstrated the maximum activity peak, which may reflect the optimal partition coefficient associated with the easiest crossing of the biological membranes. In the benzyl-substituted derivatives **5h**–**p**, variation in the substituents on the phenyl group affected activity. The activity of derivatives with different halogen substitution on the benzene ring was in the following order: *m*-Cl > *o*-Cl > *p*-Cl, *o*-F > *m*-F > *p*-F. *p*-CH_3 and *p*-OCH_3 showed low activity owing to their electron-donating moieties. An electron-withdrawing group was a more profitable structural feature than an electron-donating group.

Compounds **6, 7, 8,** and **9** were designed and synthesized using the bioisosterism strategy, replacing the triazole ring of compounds **5e** and **5k** with other heterocycles (i.e., imidazole, methylimidazole, and pyrazole), and their preliminary anticonvulsant activities were

Table 1
Anticonvulsant activities of compounds (**4a**–**o**) and (**5a**–**p**) in MES tests.



4a-o



5a-p

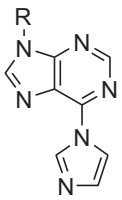
Compound	R	MES (mg/kg)[a]	
		100	30
4a	$-C_4H_9$	0/3[b]	0/3
4b	$-C_5H_{11}$	1/3	0/3
4c	$-C_6H_{13}$	3/3	0/3
4d	$-C_7H_{15}$	2/3	0/3
4e	$-C_8H_{17}$	2/3	0/3
4f	$-C_{10}H_{21}$	0/3	0/3
4g	$-CH_2C_6H_5$ (*o*-Cl)	3/3	0/3
4h	$-CH_2C_6H_5$ (*m*-Cl)	0/3	0/3
4i	$-CH_2C_6H_5$ (*p*-Cl)	0/3	0/3
4j	$-CH_2C_6H_5$ (*o*-F)	1/3	0/3
4k	$-CH_2C_6H_5$ (*m*-F)	0/3	0/3
4l	$-CH_2C_6H_5$ (*p*-F)	0/3	0/3
4m	$-CH_2C_6H_5$ (*p*-CH_3)	0/3	0/3
4n	$-CH_2C_6H_5$ (*p*-OCH_3)	0/3	0/3
4o	$-CH_2C_6H_5$	1/3	0/3
5a	$-C_5H_{11}$	1/3	0/3
5b	$-C_6H_{13}$	3/3	0/3
5c	$-C_7H_{15}$	3/3	1/3
5d	$-C_8H_{17}$	3/3	2/3
5e	$-C_{10}H_{21}$	3/3	2/3
5f	$-C_{12}H_{25}$	1/3	0/3
5g	$-C_{14}H_{29}$	0/3	0/3
5h	$-CH_2C_6H_5$ (*o*-Cl)	2/3	0/3
5i	$-CH_2C_6H_5$ (*m*-Cl)	3/3	0/3
5j	$-CH_2C_6H_5$ (*p*-Cl)	0/3	0/3
5k	$-CH_2C_6H_5$ (*o*-F)	3/3	1/3
5l	$-CH_2C_6H_5$ (*m*-F)	3/3	0/3
5m	$-CH_2C_6H_5$ (*p*-F)	0/3	0/3
5n	$-CH_2C_6H_5$ (*p*-CH_3)	0/3	0/3
5o	$-CH_2C_6H_5$ (*p*-OCH_3)	0/3	0/3
5p	$-CH_2C_6H_5$	3/3	0/3

[a] Maximal electroshock: doses of 30 and 100 mg/kg were administrated intraperitoneally in mice. The animals were examined 0.5 h after administration.
[b] n_1/n_2: the animals protected/the animals tested.

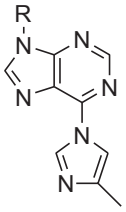
also evaluated. As shown in Table 2, compounds **6a** and **6b** (with imidazole substitution) protected against MES-induced seizures at 100 mg/kg, but not at 30 mg/kg. Among compounds **7a**–**b**, only compound **7a** showed anti-MES-induced seizure activity at 100 mg/kg, and none of the compounds showed protection at the 30 mg/kg dose. Compounds **8a** and **8b,** which contain pyrazole, showed protective effects in 3/3 and 1/3 mice, respectively, at the dose of 100 mg/kg in the MES tests. Introducing a sulfur atom between the purine and triazole resulted in compounds **9a**–**b**, but neither strategy yielded a compound that was protective at a dose of 100 mg/kg.

As a result of preliminary screening (Tables 1 and 2), thirteen compounds were subjected to phase II trials to quantify their preliminary anticonvulsant activity and neurotoxicity in mice. We compared the results of our compounds with the marketed anticonvulsant drugs, carbamazepine and valproate. As shown in Table 3, 9-decyl-6-(1*H*-1,2,4-triazol-1-yl)-9*H*-purine (**5e**) was the most active and promising compound in this study, with an ED_{50} of 23.4 mg/kg and a TD_{50} value of more than 700 mg/kg, resulting in a PI value higher than 25.6. The standard, carbamazepine, showed an ED_{50} value of 11.8 mg/kg and a TD_{50} value of 76.1 mg/kg, resulting in

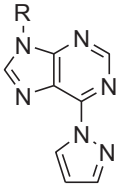
Table 2
Anticonvulsant activities of compounds **6**, **7**, **8** and **9** in MES tests.



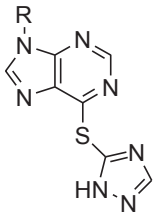
6a-b



7a-b



8a-b



9a-b

Compound	R	MES (mg/kg)	
		100	30
6a	$-C_{10}H_{21}$	1/3	0/3
6b	$-CH_2C_6H_5$ (*o*-F)	3/3	0/3
7a	$-C_{10}H_{21}$	3/3	0/3
7b	$-CH_2C_6H_5$ (*o*-F)	0/3	0/3
8a	$-C_{10}H_{21}$	3/3	0/3
8b	$-CH_2C_6H_5$ (*o*-F)	1/3	0/3
9a	$-C_{10}H_{21}$	0/3	0/3
9b	$-CH_2C_6H_5$ (*o*-F)	0/3	0/3

a PI of 6.4 under the same conditions. Compound **5e** was safer than the anticonvulsant drug carbamazepine. Compounds **5d** and **5i**–**l** also showed higher PI values than those shown by the marketed anticonvulsant drugs in MES and TOX models.

To determine the oral time of peak effect (TPE) of compound **5e**, we conducted a time-course test; compound **5e** reached the TPE at 1.5 h after administering oral medications (Fig 2). Next, we evaluated the oral anticonvulsant activity of compound **5e** against MES-induced seizures and neurotoxicity in mice (Table 4), using carbamazepine as a reference. 9-Decyl-6-(1*H*-1,2,4-triazol-1-yl)-9*H*-purine (**5e**) showed an ED_{50} value of 39.4 mg/kg and a TD_{50} > 1500 mg/kg, resulting in a PI > 38.1. The standard, carbamazepine, showed an ED_{50} value of 27.3 mg/kg and a TD_{50} value of 328.6 mg/kg, resulting in a PI of 12.0 under the same conditions. Thus, compound **5e** was safer than the anticonvulsant drug carbamazepine in MES and TOX models.

Table 3
Quantitative anticonvulsant data in mice (i.p.).

Compound	ED_{50} (MES)[a]	TD_{50} (NT)[b]	PI[c]
4c	51.2 (44.1–59.5)[d]	102.5 (88.2–119.0)	2.0
4g	51.9 (44.6–60.2)	314.6 (234.9–421.5)	6.1
5b	43.9 (37.8–51.0)	209.8 (156.6–281.0)	4.8
5c	31.5 (23.5–42.2)	104.9 (78.3–140.5)	3.3
5d	33.2 (24.8–44.5)	247.4 (184.7–331.5)	7.4
5e	23.4 (17.0–32.2)	>700	>25.6
5i	65.4 (56.3–75.9)	487.7 (419.9–566.5)	7.5
5k	29.9 (25.8–34.8)	448.9 (386.5–521.4)	15.0
5l	45.9 (39.5–53.3)	384.3 (330.8–446.3)	8.3
5p	66.6 (57.3–77.4)	314.6 (234.9–421.5)	4.7
6b	61.4 (52.9–71.3)	115.4 (86.1–154.6)	1.8
7a	67.5 (58.1–78.4)	196.1 (146.4–262.7)	2.9
8a	66.5 (57.3–77.3)	241.2 (180.1–241.2)	3.6
Carbamazepine	11.8 (8.5–16.4)	76.1 (55.8–103.7)	6.4
Valproate	272 (247–338)	426 (369–450)	1.6

[a] ED_{50}: median effective dose affording anticonvulsant protection in 50% of animals, the dose is measured in mg/kg.
[b] TD_{50}: median toxic dose eliciting minimal neurological toxicity in 50% of animals, the dose is measured in mg/kg.
[c] PI: protective index (TD_{50}/ED_{50}).
[d] 95% confidence intervals given in parentheses.

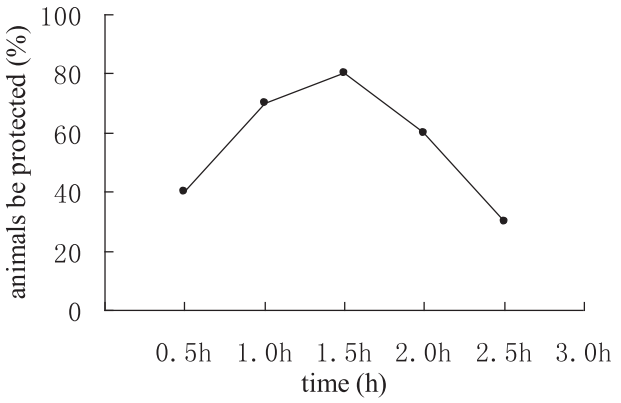


Fig. 2. Time-course of compound **5e** (50 mg/kg) in the MES test (p.o.).

Table 4
Pharmacological evaluation of compound **5e** and carbamazepine administered orally to mice.

Compound	ED_{50} (MES)	TD_{50} (NT)	PI
5e	39.4 (34.0–45.6)	>1500	>38.1
Carbamazepine	27.3 (19.7–37.9)	328.6 (229.9–469.7)	12.0

To further investigate the preliminary anticonvulsant activity of compound **5e** in a chemical model, convulsions were induced using PTZ. Compound **5e** was administered to mice at 50 mg/kg, a dose higher than its ED_{50} value and far below its TD_{50} value. The reference drug, carbamazepine, was also administered at 50 mg/kg. In the sc-PTZ model, carbamazepine inhibited clonic seizures, tonic seizures, and death by 0, 100, and 80%, respectively. Compound **5e** inhibited clonic seizures, tonic seizures, and death by 20, 90, and 70%, respectively (Fig 3). Thus, compound **5e** and carbamazepine can protect the mice from tonic seizures and death in the sc-PTZ model.

In this study, we prepared six series of compounds and evaluated their preliminary anticonvulsant activities, and we established the following SARs.

When the triazole ring of compounds **4a**–**o** and **5a**–**p** was opened, their anticonvulsant activities were stronger and the PIs were higher for **5a**–**p** compared to **4a**–**o**. When the triazole ring in compounds **5a**–**p** was replaced with other heterocycles (i.e., imidazole, methylimidazole, and pyrazole), the resultant compounds, (**6a**–**b**), (**7a**–**b**), and (**8a**–**b**), had slightly decreased activity compared with the compounds containing the triazole ring. These

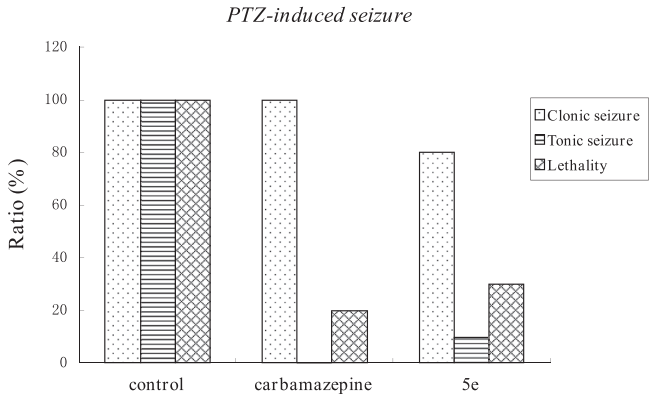


Fig. 3. Effects of compound **5e** on sc-PTZ-induced seizure in mice (i.p.).

modified compounds also showed higher neurotoxicity, which led them to have a lower PI compared to those of compounds **5a**–**p**. When we introduced a sulfur atom in triazole (1*H* (2*H*), (4*H*)-1,2,4-triazole-3-thiol), compounds (**9a**–**b**) showed almost no anticonvulsant activity compared to that shown by compounds containing the triazole ring. These data indicate that separating the triazole from the purine, instead of incorporating the triazole into the purine, displayed the best anticonvulsant activity and most favorable PI. Therefore, the isolated triazole ring may be one of the active centers.

3. Conclusion

Based on our previous work, we designed and synthesized several series of purine derivatives and evaluated their preliminary anticonvulsant activities using the maximal electroshock, subcutaneous pentylenetetrazole and rotarod tests. Among these compounds, 9-decyl-6-(1*H*-1,2,4-triazol-1-yl)-9*H*-purine (**5e**) was the most potent, with an ED_{50} value of 23.4 mg/kg and a PI value above 25.6. Compound **5e** possessed better anticonvulsant activity and higher safety than the marketed drugs, carbamazepine and valproate, as well as previously reported triazole derivatives (**I**, **II**, **III**, and **IV**) in MES, sc-PTZ and TOX models. In addition, Compound **5e** exhibited an ED_{50} value of 39.4 mg/kg and PI > 31.6 by oral administration, indicating that it possesses better anticonvulsant activity and higher safety than carbamazepine in MES and TOX models.

4. Experimental

4.1. Chemistry

Melting points were determined in open capillary tubes and were uncorrected. IR spectra were recorded (in KBr) on an IRPrestige-21 (PerkineElmer, Waltham, MA, USA). ^{1}H NMR and ^{13}C NMR spectra were measured on an AV-300 spectrometer (Bruker, Switzerland), with all chemical shifts given in ppm relative to tetramethylsilane. Mass spectra were measured on an HP1100LC (Agilent Technologies, USA). The purity of all the synthesized compounds was checked by a thin-layer chromatography performed on Merck Silica gel GF_{254} aluminum plates, using the following systems: A. dichloromethane/methanol (40:1), B. ethyl acetate. High resolution mass spectra were measured on an MALDI-TOF/TOF mass spectrometer (Bruker Daltonik, Germany). The major chemicals were purchased from Aldrich Chemical Corporation.

4.1.1. General procedure for the synthesis of 7-alkyl-7H-[1,2,4] triazolo[4,3-g]purine (*4a*–*o*)

In a round-bottomed flask, compound (**3a**–**o**) (5.3 mmol) was dissolved in triethoxymethane (20 ml), the solution was refluxed for 12 h, and the solvent was removed under reduced pressure. The residue was purified by silica gel chromatography using (CH_2Cl_2 : CH_3OH = 60 : 1). The yield, melting point, and spectral data for each compound are shown below.

*4.1.1.1. 7-Butyl-7H-[1,2,4]triazolo[4,3-g]purine (**4a**).* Yield: 57%, mp: 176–177 °C, R_f = 0.15A, R_f = 0.17B, Chemical formula: $C_{10}H_{12}N_6$. ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 0.83 (t, 3H, J = 7.53 Hz, $-CH_3$), 1.19–1.85 (m, 4H, $-CH_2-$), 4.31 (q, 2H, J = 6.90 Hz, $-CH_2-$), 8.40 (s, 1H, Triazole-H), 9.33 (s, 1H, Imidazole-H), 9.41 (s, 1H, Pyrimidine-H). ^{13}C NMR (DMSO-d_6, 75 MHz) δ: 14.40, 22.55, 26.36, 44.36, 120.29, 136.04, 136.50, 139.74, 142.35, 144.85. IR (KBr) cm^{-1}: 1641 1384. MS-EI m/z 217 (M + 1). ESI-HRMS calcd for $C_{10}H_{12}N_6^+$ ($[M + H]^+$): 217.1196; found: 217.1202.

*4.1.1.2. 7-Pentyl-7H-[1,2,4]triazolo[4,3-g]purine (**4b**).* Yield: 49%, mp: 198–200 °C, R_f = 0.14A, R_f = 0.16B, Chemical formula: $C_{11}H_{14}N_6$. ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 0.82 (t, 3H, J = 6.75 Hz, $-CH_3$), 1.25–1.85 (m, 6H, $-CH_2-$), 4.30 (q, 2H, J = 6.60 Hz, $-CH_2-$), 8.41 (s, 1H, Triazole-H), 9.33 (s, 1H, Imidazole-H), 9.41 (s, 1H, Pyrimidine-H). ^{13}C NMR (DMSO-d_6, 75 MHz) δ: 14.22, 22.01, 28.53, 29.81, 44.33, 120.26, 136.07, 136.52, 139.73, 142.34, 144.85. IR (KBr) cm^{-1}: 1643 1381. MS-EI *m/z* 231 (M + 1). ESI-HRMS calcd for $C_{11}H_{14}N_6^+$ ([M + H]$^+$): 231.1353; found: 231.1361.

*4.1.1.3. 7-Hexyl-7H-[1,2,4]triazolo[4,3-g]purine (**4c**).* Yield: 63%, mp: 195–196 °C, R_f = 0.16A, R_f = 0.21B, Chemical formula: $C_{12}H_{16}N_6$. ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 0.81 (t, 3H, J = 6.75 Hz, $-CH_3$), 1.24–1.84 (m, 8H, $-CH_2-$), 4.30 (q, 2H, J = 7.50 Hz, $-CH_2-$), 8.42 (s, 1H, Triazole-H), 9.33 (s, 1H, Imidazole-H), 9.42 (s, 1H, Pyrimidine-H). ^{13}C NMR (DMSO-d_6, 75 MHz) δ: 14.26, 22.37, 26.03, 30.07, 31.06, 44.35, 120.26, 136.06, 136.51, 139.73, 142.34, 144.85. IR (KBr) cm^{-1}: 1642 1384. MS-EI *m/z* 245 (M + 1). ESI-HRMS calcd for $C_{12}H_{16}N_6^+$ ([M + H]$^+$): 245.1509; found: 245.1516.

*4.1.1.4. 7-Heptyl-7H-[1,2,4]triazolo[4,3-g]purine (**4d**).* Yield: 67%, mp: 188–189 °C, R_f = 0.15A, R_f = 0.17B, Chemical formula: $C_{13}H_{18}N_6$. ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 0.80 (t, 3H, J = 6.00 Hz, $-CH_3$), 1.19–1.84 (m, 10H, $-CH_2-$), 4.30 (q, 2H, J = 7.50 Hz, $-CH_2-$), 8.41 (s, 1H, Triazole-H), 9.33 (s, 1H, Imidazole-H), 9.42 (s, 1H, Pyrimidine-H). ^{13}C NMR (DMSO-d_6, 75 MHz) δ: 14.32, 22.41, 26.33, 28.53, 30.12, 31.53, 44.35, 120.27, 136.05, 136.51, 139.72, 142.34, 144.85. IR (KBr) cm^{-1}: 1641 1384. MS-EI *m/z* 259 (M + 1). ESI-HRMS calcd for $C_{13}H_{18}N_6^+$ ([M + H]$^+$): 259.1666; found: 259.1675.

*4.1.1.5. 7-Octyl-7H-[1,2,4]triazolo[4,3-g]purine (**4e**).* Yield: 57%, mp: 189–191 °C, R_f = 0.20A, R_f = 0.19B, Chemical formula: $C_{14}H_{20}N_6$. ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 0.81 (t, 3H, J = 6.00 Hz, $-CH_3$), 1.19–1.85 (m, 12H, $-CH_2-$), 4.30 (q, 2H, J = 6.60 Hz, $-CH_2-$), 8.41 (s, 1H, Triazole-H), 9.33 (s, 1H, Imidazole-H), 9.41 (s, 1H, Pyrimidine-H). ^{13}C NMR (DMSO-d_6, 75 MHz) δ: 14.35, 22.48, 26.36, 28.82, 28.96, 30.09, 31.59, 44.35. 120.27, 136.05, 136.51, 139.73, 142.35, 144.85. IR (KBr) cm^{-1}: 1644 1388. MS-EI *m/z* 273 (M + 1). ESI-HRMS calcd for $C_{14}H_{20}N_6^+$ ([M + H]$^+$): 273.1822; found: 273.1829.

*4.1.1.6. 7-Decyl-7H-[1,2,4]triazolo[4,3-g]purine (**4f**).* Yield: 57%, mp: 184–185 °C, R_f = 0.21A, R_f = 0.22B, Chemical formula: $C_{16}H_{24}N_6$. ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 0.82 (t, 3H, J = 6.00 Hz, $-CH_3$), 1.19–1.85 (m, 16H, $-CH_2-$), 4.31 (q, 2H, J = 5.85 Hz, $-CH_2-$), 8.42 (s, 1H, Triazole-H), 9.33 (s, 1H, Imidazole-H), 9.42 (s, 1H, Pyrimidine-H). ^{13}C NMR (DMSO-d_6, 75 MHz) δ: 14.38, 22.52, 26.34, 28.84, 29.09, 29.30, 29.30, 30.08, 31.71, 44.36, 120.28, 136.06, 136.50, 139.74, 142.36, 144.85. IR (KBr) cm^{-1}: 1641 1384. MS-EI *m/z* 301 (M + 1). ESI-HRMS calcd for $C_{16}H_{24}N_6^+$ ([M + H]$^+$): 301.2135; found: 301.2146.

*4.1.1.7. 7-(2-Chlorobenzyl)-7H-[1,2,4]triazolo[4,3-g]purine (**4g**).* Yield: 55%, mp: 222–224 °C, R_f = 0.13A, R_f = 0.17B, Chemical formula: $C_{13}H_9ClN_6$. ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 5.66 (s, 2H, $-CH_2-$), 7.02–7.54 (m, 4H, Ar-H), 8.47 (s, 1H, Triazole-H), 9.31 (s, 1H, Imidazole-H), 9.42 (s, 1H, Pyrimidine-H). ^{13}C NMR (DMSO-d_6, 75 MHz) δ: 45.62, 120.41, 128.15, 129.65, 130.01, 130.28, 132.40, 134.13, 136.57, 136.64, 139.81, 142.72, 144.78. IR (KBr) cm^{-1}: 1641 1384. MS-EI *m/z* 285 (M + 1). ESI-HRMS calcd for $C_{13}H_9ClN_6^+$ ([M + H]$^+$): 285.0650; found: 285.0642.

*4.1.1.8. 7-(3-Chlorobenzyl)-7H-[1,2,4]triazolo[4,3-g]purine (**4h**).* Yield: 46%, mp: 280–282 °C, R_f = 0.14A, R_f = 0.18B, Chemical formula: $C_{13}H_9ClN_6$. ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 5.58 (s, 2H, $-CH_2-$), 7.29–7.44 (m, 4H, Ar-H), 8.55 (s, 1H, Triazole-H), 9.33 (s, 1H, Imidazole-H), 9.41 (s, 1H, Pyrimidine-H). ^{13}C NMR (DMSO-d_6, 75 MHz) δ: 46.95, 120.49, 126.67, 127.87, 128.38, 131.12, 133.75, 136.62, 136.62, 139.45, 139.60, 142.40, 144.78. IR (KBr) cm^{-1}: 1645 1387. MS-EI *m/z* 285 (M + 1). ESI-HRMS calcd for $C_{13}H_9ClN_6^+$ ([M + H]$^+$): 285.0650; found: 285.0639.

*4.1.1.9. 7-(4-Chlorobenzyl)-7H-[1,2,4]triazolo[4,3-g]purine (**4i**).* Yield: 64%, mp: 198–200 °C, R_f = 0.13A, R_f = 0.16B, Chemical formula: $C_{13}H_9ClN_6$. ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 5.57 (s, 2H, $-CH_2-$), 7.34–7.41 (dd, 4H, Ar-H), 8.55 (s, 1H, Triazole-H), 9.33 (s, 1H, Imidazole-H), 9.41 (s, 1H, Pyrimidine-H). ^{13}C NMR (DMSO-d_6, 75 MHz) δ: 46.92, 120.48, 129.17, 129.17, 129.90, 129.90, 133.05, 136.05, 136.53, 136.61, 139.60, 142.38, 144.77. IR (KBr) cm^{-1}: 1641 1384. MS-EI *m/z* 285 (M + 1). ESI-HRMS calcd for $C_{13}H_9ClN_6^+$ ([M + H]$^+$): 285.0650; found: 285.0641.

*4.1.1.10. 7-(2-Fluorobenzyl)-7H-[1,2,4]triazolo[4,3-g]purine (**4j**).* Yield: 53%, mp: 248–250 °C, R_f = 0.14A, R_f = 0.15B, Chemical formula: $C_{13}H_9FN_6$. ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 5.63 (s, 2H, $-CH_2-$), 7.15–7.39 (m, 4H, Ar-H), 8.50 (s, 1H, Triazole-H), 9.32 (s, 1H, Imidazole-H), 9.41 (s, 1H, Pyrimidine-H). ^{13}C NMR (DMSO-d_6, 75 MHz) δ: 41.85, 115.88, 116.15, 120.36, 123.72, 125.28 (d, $J_{c\text{-}f}$ = 3.75 Hz), 130.40 (d, $J_{c\text{-}f}$ = 3.75 Hz), 130.86 (d, $J_{c\text{-}f}$ = 8.25 Hz), 136.63 (d, $J_{c\text{-}f}$ = 6.75 Hz), 139.68, 142.57, 144.75, 161.99 (d, $J_{c\text{-}f}$ = 244.5 Hz). IR (KBr) cm^{-1}: 1641 1386. MS-EI *m/z* 269 (M + 1). ESI-HRMS calcd for $C_{13}H_9FN_6^+$ ([M + H]$^+$): 269.0945; found: 269.0937.

*4.1.1.11. 7-(3-Fluorobenzyl)-7H-[1,2,4]triazolo[4,3-g]purine (**4k**).* Yield: 43%, mp: 234–235 °C, R_f = 0.14A, R_f = 0.16B, Chemical formula: $C_{13}H_9FN_6$. ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 5.59 (s, 2H, $-CH_2-$), 7.14–7.40 (m, 4H, Ar-H), 8.56 (s, 1H, Triazole-H), 9.33 (s, 1H, Imidazole-H), 9.42 (s, 1H, Pyrimidine-H). ^{13}C NMR (DMSO-d_6, 75 MHz) δ: 47.02, 115.00 (d, $J_{c\text{-}f}$ = 21.7 Hz), 115.39 (d, $J_{c\text{-}f}$ = 21.0 Hz), 120.48, 123.97, 124.01, 131.35 (d, $J_{c\text{-}f}$ = 8.25 Hz), 136.63, 139.64, 139.86 (d, $J_{c\text{-}f}$ = 7.5 Hz), 142.42, 144.79, 164.26 (d, $J_{c\text{-}f}$ = 243.0 Hz). IR (KBr) cm^{-1}: 1641 1386. MS-EI *m/z* 269 (M + 1). ESI-HRMS calcd for $C_{13}H_9FN_6^+$ ([M + H]$^+$): 269.0945; found: 269.0941.

*4.1.1.12. 7-(4-Fluorobenzyl)-7H-[1,2,4]triazolo[4,3-g]purine (**4l**).* Yield: 47%, mp: 218–220 °C, R_f = 0.15A, R_f = 0.17B, Chemical formula: $C_{13}H_9FN_6$. ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 5.56 (s, 2H, $-CH_2-$), 7.15–7.45 (dd, 4H, Ar-H), 8.55 (s, 1H, Triazole-H), 9.33 (s, 1H, Imidazole-H), 9.41 (s, 1H, Pyrimidine-H). ^{13}C NMR (DMSO-d_6, 75 MHz) δ: 46.90, 115.89, 116.17, 120.47, 130.35 (d, $J_{c\text{-}f}$ = 9.0 Hz), 133.33 (d, $J_{c\text{-}f}$ = 3.0 Hz), 136.51, 136.61, 139.58, 142.31, 144.78, 163.80 (d, $J_{c\text{-}f}$ = 242.2 Hz). IR (KBr) cm^{-1}: 1641 1386. MS-EI *m/z* 269 (M + 1). ESI-HRMS calcd for $C_{13}H_9FN_6^+$ ([M + H]$^+$): 269.0945; found: 269.0932.

*4.1.1.13. 7-(4-Methylbenzyl)-7H-[1,2,4]triazolo[4,3-g]purine (**4m**).* Yield: 51%, mp: 192–193 °C, R_f = 0.16A, R_f = 0.18B, Chemical formula: $C_{14}H_{12}N_6$. ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 2.24 (s, 3H, $-CH_3$), 5.50 (s, 2H, $-CH_2-$), 7.12–7.25 (dd, 4H, Ar-H), 8.53 (s, 1H, Triazole-H), 9.32 (s, 1H, Imidazole-H), 9.41 (s, 1H, Pyrimidine-H). ^{13}C NMR (DMSO-d_6, 75 MHz) δ: 21.10, 47.40, 120.39, 127.99, 127.99, 129.71, 129.71, 134.06, 136.40, 136.58, 137.68, 139.61, 142.33, 144.79. IR (KBr) cm^{-1}: 1641 1388. MS-EI *m/z* 265 (M + 1). ESI-HRMS calcd for $C_{14}H_{12}N_6^+$ ([M + H]$^+$): 265.1196; found: 265.1183.

*4.1.1.14. 7-(4-Methoxybenzyl)-7H-[1,2,4]triazolo[4,3-g]purine (**4n**).* Yield: 57%, mp: 201–203 °C, R_f = 0.14A, R_f = 0.16B, Chemical formula: $C_{14}H_{12}N_6O$. ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 3.71 (s, 3H, $-OCH_3$), 5.48 (s, 2H, $-CH_2-$), 6.88–7.35 (dd, 4H, Ar-H), 8.52 (s, 1H, Triazole-H), 9.33 (s, 1H, Imidazole-H), 9.41 (s, 1H, Pyrimidine-H). ^{13}C

NMR (DMSO-d_6, 75 MHz) δ: 47.15, 55.55, 114.54, 114.54, 120.40, 129.00, 129.64, 129.64, 136.38, 136.58, 139.55, 142.21, 144.78, 159.41. IR (KBr) cm^{-1}: 1641 1384. MS-EI *m/z* 281 (M + 1). ESI-HRMS calcd for $C_{14}H_{12}N_6O^+$ ($[M + H]^+$): 281.1145; found: 281.1137.

*4.1.1.15. 7-Benzyl-7H-[1,2,4]triazolo[4,3-g]purine (**4o**).* Yield: 47%, mp: 212–213 °C, R_f = 0.15A, R_f = 0.17B, Chemical formula: $C_{13}H_{10}N_6O$. ^1H NMR (DMSO-d_6, 300 MHz) δ: 5.56 (s, 2H, $-CH_2-$), 7.28–7.34 (m, 5H, Ar-H), 8.54 (s, 1H, Triazole-H), 9.33 (s, 1H, Imidazole-H), 9.41 (s, 1H, Pyrimidine-H). ^{13}C NMR (DMSO-d_6, 75 MHz) δ: 47.60, 120.43, 127.93, 127.93, 128.37, 129.20, 129.20, 136.46, 136.60, 137.07, 139.67, 142.41, 144.81. IR (KBr) cm^{-1}: 1642 1387. MS-EI *m/z* 251 (M + 1). ESI-HRMS calcd for $C_{13}H_{10}N_6^+$ ($[M + H]^+$): 251.1040; found: 251.1029.

*4.1.2. General procedure for the synthesis of 9-alkyl-6-(1H-1,2,4-triazol-1-yl)-9H-purine (**5a**–**p**)*

A solution of **2a**–**p** (2 mmol), 1*H*-1,2,4-triazole (2.2 mmol), and K_2CO_3 (2.2 mmol) in *N*,*N*-dimethylformamide (30 ml) was refluxed for 3 h. After evaporating 2/3 volume of the solution, the residue was poured into ice water. The precipitate was filtered and washed with water to produce a white solid. All the compounds were recrystallized in ethanol to produce the pure compound (**5a**–**p**).

*4.1.2.1. 9-Pentyl-6-(1H-1,2,4-triazol-1-yl)-9H-purine (**5a**).* Yield: 64%, mp: 109–111 °C, R_f = 0.37A, R_f = 0.46B, Chemical formula: $C_{12}H_{15}N_7$. ^1H NMR ($CDCl_3$, 300 MHz) δ: 0.89 (t, 3H, *J* = 6.82 Hz, $-CH_3$), 1.31–2.00 (m, 6H, $-CH_2$), 4.34 (t, 2H, *J* = 7.23 Hz, $-CH_2-$), 8.20 (s, 1H, Triazole-H), 8.29 (s, 1H, Triazole-H), 8.89 (s, 1H, Imidazole-H), 9.70 (s, 1H, Pyrimidine-H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ: 13.83, 22.06, 28.67, 29.55, 44.42, 122.30, 145.00, 145.21, 145.70, 152.02, 153.97, 154.40. IR (KBr) cm^{-1}: 1606 1577. MS-EI *m/z* 258 (M + 1). ESI-HRMS calcd for $C_{12}H_{15}N_7^+$ ($[M + H]^+$): 258.1462; found: 258.1455.

*4.1.2.2. 9-Hexyl-6-(1H-1,2,4-triazol-1-yl)-9H-purine (**5b**).* Yield: 60%, mp: 76–77 °C, R_f = 0.39A, R_f = 0.45B, Chemical formula: $C_{13}H_{17}N_7$. ^1H NMR ($CDCl_3$, 300 MHz) δ: 0.87 (t, 3H, *J* = 6.62 Hz, $-CH_3$), 1.29–1.98 (m, 8H, $-CH_2-$), 4.35 (t, 2H, *J* = 7.24 Hz, $-CH_2-$), 8.21 (s, 1H, Triazole-H), 8.30 (s, 1H, Triazole-H), 8.89 (s, 1H, Imidazole-H), 9.70 (s, 1H, Pyrimidine-H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ: 13.87, 22.38, 26.24, 29.80, 31.08, 44.43, 122.32, 144.99, 145.22, 145.69, 152.01, 153.95, 154.40. IR (KBr) cm^{-1}: 1603 1577. MS-EI *m/z* 272 (M + 1). ESI-HRMS calcd for $C_{13}H_{17}N_7^+$ ($[M + H]^+$): 272.1618; found: 272.1609.

*4.1.2.3. 9-Heptyl-6-(1H-1,2,4-triazol-1-yl)-9H-purine (**5c**).* Yield: 58%, mp: 52–54 °C, R_f = 0.37A, R_f = 0.45B, Chemical formula: $C_{14}H_{19}N_7$. ^1H NMR ($CDCl_3$, 300 MHz) δ: 0.85 (t, 3H, *J* = 6.40 Hz, $-CH_3$), 1.25–2.94 (m, 10H, $-CH_2-$), 4.34 (t, 2H, *J* = 7.20 Hz, $-CH_2-$), 8.20 (s, 1H, Triazole-H), 8.28 (s, 1H, Triazole-H), 8.88 (s, 1H, Imidazole-H), 9.69 (s, 1H, Pyrimidine-H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ: 13.99, 22.47, 26.56, 28.62, 29.87, 31.54, 44.45, 122.31, 144.99, 145.21, 145.72, 152.02, 153.99, 154.41. IR (KBr) cm^{-1}: 1602 1577. MS-EI *m/z* 286 (M + 1). ESI-HRMS calcd for $C_{14}H_{19}N_7^+$ ($[M + H]^+$): 286.1775; found: 286.1769. ESI-HRMS calcd for $C_{14}H_{19}N_7^+$ ($[M + H]^+$): 286.1775; found: 286.1766.

*4.1.2.4. 9-Octyl-6-(1H-1,2,4-triazol-1-yl)-9H-purine (**5d**).* Yield: 45%, mp: 77–79 °C, R_f = 0.38A, R_f = 0.48B, Chemical formula: $C_{15}H_{21}N_7$. ^1H NMR ($CDCl_3$, 300 MHz) δ: 0.86 (t, 3H, *J* = 6.67 Hz, $-CH_3$), 1.25–1.99 (m, 12H, $-CH_2-$), 4.35 (t, 2H, *J* = 7.24 Hz, $-CH_2-$), 8.21 (s, 1H, Triazole-H), 8.31 (s, 1H, Triazole-H), 8.90 (s, 1H, Imidazole-H), 9.71 (s, 1H, Pyrimidine-H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ: 14.03, 22.54, 26.60, 28.91, 29.02, 29.87, 31.64, 44.44, 122.31, 144.99, 145.21, 145.72, 152.02, 153.98, 154.40. IR (KBr) cm^{-1}: 1602 1577. MS-EI *m/z* 300 (M + 1). ESI-HRMS calcd for $C_{15}H_{21}N_7^+$ ($[M + H]^+$): 300.1931; found: 300.1939.

*4.1.2.5. 9-Decyl-6-(1H-1,2,4-triazol-1-yl)-9H-purine (**5e**).* Yield: 55%, mp: 78–79 °C, R_f = 0.40A, R_f = 0.47B. Chemical formula: $C_{17}H_{25}N_7$. ^1H NMR ($CDCl_3$, 300 MHz) δ: 0.87 (t, 3H, *J* = 6.64 Hz, $-CH_3$), 1.25–1.99 (m, 16H, $-CH_2-$), 4.36 (t, 2H, *J* = 7.23 Hz, $-CH_2-$), 8.21 (s, 1H, Triazole-H), 8.31 (s, 1H, Triazole-H), 8.90 (s, 1H, Imidazole-H), 9.71 (s, 1H, Pyrimidine-H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ: 14.10, 22.64, 26.61, 28.96, 29.22, 29.37, 29.42, 29.88, 31.82, 44.46, 122.33, 145.02, 145.21, 145.72, 152.04, 154.02, 154.43. IR (KBr) cm^{-1}: 1604 1577. MS-EI *m/z* 328 (M + 1). ESI-HRMS calcd for $C_{17}H_{25}N_7^+$ ($[M + H]^+$): 328.2244; found: 328.2232.

*4.1.2.6. 9-Dodecyl-6-(1H-1,2,4-triazol-1-yl)-9H-purine (**5f**).* Yield: 72%, mp: 67–68 °C, R_f = 0.39A, R_f = 0.46B, Chemical formula: $C_{19}H_{29}N_7$. ^1H NMR ($CDCl_3$, 300 MHz) δ: 0.88 (t, 3H, *J* = 6.61 Hz, $-CH_3$), 1.25–1.99 (m, 20H, $-CH_2-$), 4.36 (t, 2H, *J* = 7.23 Hz, $-CH_2-$), 8.21 (s, 1H, Triazole-H), 8.31 (s, 1H, Triazole-H), 8.91 (s, 1H, Imidazole-H), 9.71 (s, 1H, Pyrimidine-H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ: 14.14, 22.70, 26.63, 28.98, 29.35, 29.39, 29.49, 29.58, 29.64, 29.67, 31.92, 44.47, 122.35, 145.04, 145.21, 145.71, 152.05, 154.05, 154.44. IR (KBr) cm^{-1}: 1602 1577. MS-EI *m/z* 356 (M + 1). ESI-HRMS calcd for $C_{19}H_{29}N_7^+$ ($[M + H]^+$): 356.2557; found: 356.2549.

*4.1.2.7. 9-Tetradecyl-6-(1H-1,2,4-triazol-1-yl)-9H-purine (**5g**).* Yield: 63%, mp: 66–67 °C, R_f = 0.38A, R_f = 0.47B, Chemical formula: $C_{21}H_{33}N_7$. ^1H NMR ($CDCl_3$, 300 MHz) δ: 0.87 (t, 3H, *J* = 6.00 Hz, $-CH_3$), 1.25–1.96 (m, 24H, $-CH_2-$), 4.35 (t, 2H, *J* = 7.11 Hz, $-CH_2-$), 8.20 (s, 1H, Triazole-H), 8.30 (s, 1H, Triazole-H), 8.90 (s, 1H, Imidazole-H), 9.71 (s, 1H, Pyrimidine-H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ: 14.12, 22.67, 26.44, 26.61, 28.98, 29.33, 29.37, 29.46, 29.57, 29.62, 29.87, 31.88, 44.46, 49.73, 122.32, 145.01, 145.20, 145.70, 152.03, 154.02, 154.42. IR (KBr) cm^{-1}: 1606 1577. MS-EI *m/z* 384 (M + 1). ESI-HRMS calcd for $C_{21}H_{33}N_7^+$ ($[M + H]^+$): 384.2870; found: 384.2861.

*4.1.2.8. 9-(2-Chlorobenzyl)-6-(1H-1,2,4-triazol-1-yl)-9H-purine (**5h**).* Yield: 53%, mp: 157–158 °C, R_f = 0.37A, R_f = 0.42B, Chemical formula: $C_{14}H_{10}ClN_7$. ^1H NMR ($CDCl_3$, 300 MHz) δ: 5.65 (s, 2H, $-CH_2-$), 7.29–7.49 (m, 4H, Ar-H), 8.29 (s, 1H, Triazole-H), 8.31 (s, 1H, Triazole-H), 8.94 (s, 1H, Imidazole-H), 9.72 (s, 1H, Pyrimidine-H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ: 45.51, 122.15, 127.63, 130.19, 130.52, 130.71, 132.11, 133.71, 145.12, 145.76, 152.37, 154.05, 154.45. IR (KBr) cm^{-1}: 1602 1577. MS-EI *m/z* 312 (M + 1). ESI-HRMS calcd for $C_{14}H_{10}ClN_7^+$ ($[M + H]^+$): 312.0759; found: 312.0750.

*4.1.2.9. 9-(3-Chlorobenzyl)-6-(1H-1,2,4-triazol-1-yl)-9H-purine (**5i**).* Yield: 78%, mp: 147–149 °C, R_f = 0.36A, R_f = 0.43B, Chemical formula: $C_{14}H_{10}ClN_7$. ^1H NMR ($CDCl_3$, 300 MHz) δ: 5.50 (s, 2H, $-CH_2-$), 7.23–7.34 (m, 4H, Ar-H), 8.22 (s, 1H, Triazole-H), 8.31 (s, 1H, Triazole-H), 8.94 (s, 1H, Imidazole-H), 9.70 (s, 1H, Pyrimidine-H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ: 47.10, 122.18, 125.91, 127.95, 129.13, 130.60, 135.21, 136.44, 136.56, 145.25, 145.38, 152.52, 154.12., 154.32. IR (KBr) cm^{-1}: 1602 1577. MS-EI *m/z* 312 (M + 1). ESI-HRMS calcd for $C_{14}H_{10}ClN_7^+$ ($[M + H]^+$): 312.0759; found: 312.0747.

*4.1.2.10. 9-(4-Chlorobenzyl)-6-(1H-1,2,4-triazol-1-yl)-9H-purine (**5j**).* Yield: 66%, mp: 185–186 °C, R_f = 0.37A, R_f = 0.41B, Chemical formula: $C_{14}H_{10}ClN_7$. ^1H NMR ($CDCl_3$, 300 MHz) δ: 5.50 (s, 2H, $-CH_2-$), 7.28–7.38 (dd, 4H, Ar-H), 8.20 (s, 1H, Triazole-H), 8.31 (s, 1H, Triazole-H), 8.93 (s, 1H, Imidazole-H), 9.70 (s, 1H, Pyrimidine-H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ: 47.11, 122.18, 129.28, 129.28, 129.48, 129.48, 133.09, 134.91, 145.15, 145.26, 145.36, 152.43, 154.08,

154.29. IR (KBr) cm^{-1}: 1602 1577. MS-EI *m/z* 312 (M + 1). ESI-HRMS calcd for $C_{14}H_{10}ClN_7^+$ ($[M + H]^+$): 312.0759; found: 312.0751.

*4.1.2.11. 9-(2-Fluorobenzyl)-6-(1H-1,2,4-triazol-1-yl)-9H-purine (**5k**).* Yield: 64%, mp: 168–170 °C, R_f = 0.36A, R_f = 0.40B, Chemical formula: $C_{14}H_{10}FN_7$. ^1H NMR ($CDCl_3$, 300 MHz) δ: 5.57 (s, 2H, $-CH_2-$), 7.10–7.43 (m, 4H, Ar-H), 8.28 (s, 1H, Triazole-H), 8.29 (s, 1H, Triazole-H), 8.92 (s, 1H, Imidazole-H), 9.70 (s, 1H, Pyrimidine-H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ: 41.75, 116.15 (d, J_{c-f} = 21.0 Hz), 121.76, 121.95 (d, J_{c-f} = 12.5 Hz), 124.89 (d, J_{c-f} = 3.7 Hz), 130.83, 131.19 (d, J_{c-f} = 8.2 Hz), 145.05, 145.30, 145.73, 152.34, 154.00, 154.31, 162.47 (d, J_{c-f} = 246.0 Hz). IR (KBr) cm^{-1}: 1602 1577. MS-EI *m/z* 296 (M + 1). ESI-HRMS calcd for $C_{14}H_{10}FN_7^+$ ($[M + H]^+$): 296.1054; found: 296.1056.

*4.1.2.12. 9-(3-Fluorobenzyl)-6-(1H-1,2,4-triazol-1-yl)-9H-purine (**5l**).* Yield: 84%, mp: 149–150 °C, R_f = 0.38A, R_f = 0.42B, Chemical formula: $C_{14}H_{10}FN_7$. ^1H NMR ($CDCl_3$, 300 MHz) δ: 5.52 (s, 2H, $-CH_2-$), 7.03–7.35 (m, 4H, Ar-H), 8.22 (s, 1H, Triazole-H), 8.31 (s, 1H, Triazole-H), 8.94 (s, 1H, Imidazole-H), 9.70 (s, 1H, Pyrimidine-H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ: 47.17, 114.79 (d, J_{c-f} = 21.7 Hz), 115.80 (d, J_{c-f} = 21.0 Hz), 122.18, 123.39 (d, J_{c-f} = 3.0 Hz), 131.05, 137.03 (d, J_{c-f} = 6.7 Hz), 145.26, 145.40, 152.49, 154.09, 154.33, 164.68 (d, J_{c-f} = 246.7 Hz). IR (KBr) cm^{-1}: 1602 1577. MS-EI *m/z* 296 (M + 1). ESI-HRMS calcd for $C_{14}H_{10}FN_7^+$ ($[M + H]^+$): 296.1054; found: 296.1044.

*4.1.2.13. 9-(4-Fluorobenzyl)-6-(1H-1,2,4-triazol-1-yl)-9H-purine (**5m**).* Yield: 58%, mp: 187–189 °C, R_f = 0.36A, R_f = 0.41B, Chemical formula: $C_{14}H_{10}FN_7$. ^1H NMR ($CDCl_3$, 300 MHz) δ: 5.49 (s, 2H, $-CH_2-$), 7.04–7.37 (dd, 4H, Ar-H), 8.19 (s, 1H, Triazole-H), 8.29 (s, 1H, Triazole-H), 8.92 (s, 1H, Imidazole-H), 9.69 (s, 1H, Pyrimidine-H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ: 47.11, 116.16, 116.45, 122.23, 129.93 (d, J_{c-f} = 8.2 Hz), 130.50, 145.15, 145.34 (d, J_{c-f} = 6.0 Hz), 152.40, 154.06, 154.30, 164.50 (d, J_{c-f} = 247.5 Hz). IR (KBr) cm^{-1}: 1602 1577. MS-EI *m/z* 296 (M + 1). ESI-HRMS calcd for $C_{14}H_{10}FN_7^+$ ($[M + H]^+$): 296.1054; found: 296.1047.

*4.1.2.14. 9-(4-Methylbenzyl)-6-(1H-1,2,4-triazol-1-yl)-9H-purine (**5n**).* Yield: 53%, mp: 188–190 °C, R_f = 0.39A, R_f = 0.43B, Chemical formula: $C_{15}H_{13}N_7$. ^1H NMR ($CDCl_3$, 300 MHz) δ: 2.35 (s, 3H, $-CH_3$), 5.47 (s, 2H, $-CH_2-$), 7.17–7.25 (dd, 4H, Ar-H), 8.18 (s, 1H, Triazole-H), 8.30 (s, 1H, Triazole-H), 8.93 (s, 1H, Imidazole-H), 9.71 (s, 1H, Pyrimidine-H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ: 21.16, 47.62, 122.26, 127.96, 127.96, 129.94, 129.94, 131.51, 138.87, 145.08, 145.27, 145.56, 152.32, 154.02, 154.40. IR (KBr) cm^{-1}: 1604 1578. MS-EI *m/z* 292 (M + 1). ESI-HRMS calcd for $C_{15}H_{13}N_7^+$ ($[M + H]^+$): 292.1305; found: 292.1296.

*4.1.2.15. 9-(4-Methoxybenzyl)-6-(1H-1,2,4-triazol-1-yl)-9H-purine (**5o**).* Yield: 75%, mp: 174–175 °C, R_f = 0.35A, R_f = 0.39B, Chemical formula: $C_{15}H_{13}N_7O$. ^1H NMR ($CDCl_3$, 300 MHz) δ: 3.81 (s, 3H, $-OCH_3$), 5.45 (s, 2H, $-CH_2-$), 6.89–7.32 (dd, 4H, Ar-H), 8.17 (s, 1H, Triazole-H), 8.30 (s, 1H, Triazole-H), 8.94 (s, 1H, Imidazole-H), 9.71 (s, 1H, Pyrimidine-H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ: 47.40, 55.33, 114.62, 114.62, 122.31, 126.50, 129.54, 129.54, 138.17, 145.05, 145.29, 145.47, 152.28, 153.97, 154.34. IR (KBr) cm^{-1}: 1604 1578. MS-EI *m/z* 308 (M + 1). ESI-HRMS calcd for $C_{15}H_{13}N_7O^+$ ($[M + H]^+$): 308.1254; found: 308.1244.

*4.1.2.16. 9-Benzyl-6-(1H-1,2,4-triazol-1-yl)-9H-purine (**5p**).* Yield: 79%, mp: 165–166 °C, R_f = 0.37A, R_f = 0.41B, Chemical formula: $C_{14}H_{11}N_7$. ^1H NMR ($CDCl_3$, 300 MHz) δ: 5.52 (s, 2H, $-CH_2-$), 7.32–7.39 (m, 5H, Ar-H), 8.19 (s, 1H, Triazole-H), 8.30 (s, 1H, Triazole-H), 8.93 (s, 1H, Imidazole-H), 9.71 (s, 1H, Pyrimidine-H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ: 47.77, 122.20, 127.89, 127.89, 128.87, 129.27, 129.27, 134.58, 145.08, 145.28, 145.58, 152.35, 154.00, 154.39. IR (KBr) cm^{-1}: 1602 1577. MS-EI *m/z* 278 (M + 1). ESI-HRMS calcd for $C_{14}H_{11}N_7^+$ ($[M + H]^+$): 278.1149; found: 278.1142.

*4.1.3. General procedure for the synthesis of 9-alkyl-6-(1H-imidazol-1-yl)-9H-purine (**6a**–**b**)*

A solution of 6-chloro-9-alkyl-9*H*-purine (2 mmol), imidazole (2.2 mmol), and K_2CO_3 (2.2 mmol) in *N*, *N*-dimethylformamide (30 ml) was refluxed for 6 h. After evaporating 2/3 volume of the solution, the residue was poured into ice water, and the product was recrystallized from ethyl acetate with a moderate yield.

*4.1.3.1. 9-Decyl-6-(1H-imidazol-1-yl)-9H-purine (**6a**).* Yield: 56%, mp: 93–94 °C, R_f = 0.42A, R_f = 0.63B, Chemical formula: $C_{19}H_{26}N_6$. ^1H NMR ($CDCl_3$, 300 MHz) δ: 0.88 (t, 3H, *J* = 6.16 Hz, $-CH_3$), 1.25–1.96 (m, 16H, $-CH_2-$), 4.32 (t, 2H, *J* = 7.15 Hz, $-CH_2-$), 7.26–7.28 (d, 1H, Imidazole-H), 8.10 (s, 1H, Imidazole-H), 8.41 (d, 1H, Imidazole-H), 8.80 (s, 1H, Imidazole-H), 9.19 (s, 1H, Pyrimidine-H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ: 14.09, 22.64, 26.64, 28.98, 29.22, 29.38, 29.43, 29.92, 31.82, 44.31, 117.33, 122.43, 130.66, 137.62, 144.38, 145.63, 152.10, 153.72. IR (KBr) cm^{-1}: 1602 1579. MS-EI *m/z* 327 (M + 1). ESI-HRMS calcd for $C_{19}H_{26}N_6^+$ ($[M + H]^+$): 327.2292; found: 327.2285.

*4.1.3.2. 9-(2-Fluorobenzyl)-6-(1H-imidazol-1-yl)-9H-purine (**6b**).* Yield: 64%, mp: 150–152 °C, R_f = 0.44A, R_f = 0.65B, Chemical formula: $C_{15}H_{11}FN_6$. ^1H NMR ($CDCl_3$, 300 MHz) δ: 5.54 (s, 2H, $-CH_2-$), 7.11–7.46 (m, 4H, Ar-H), 7.25 (s, 1H, Imidazole-H), 8.18 (s, 1H, Imidazole-H), 8.40 (s, 1H, Imidazole-H), 8.83 (s, 1H, Imidazole-H), 9.18 (s, 1H, Pyrimidine-H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ: 41.55, 99.99, 115.83 (d, J_{c-f} = 21.0 Hz), 117.34, 122.22, 124.86 (d, J_{c-f} = 3.75 Hz), 130.62, 130.79 (d, J_{c-f} = 3.75 Hz), 131.09, 137.60 (d, J_{c-f} = 7.42 Hz), 144.42, 145.68, 152.38, 153.64, 164.53 (d, J_{c-f} = 247.5 Hz). IR (KBr) cm^{-1}: 1602 1579. MS-EI *m/z* 295 (M + 1). ESI-HRMS calcd for $C_{15}H_{11}FN_6^+$ ($[M + H]^+$): 295.1102; found: 295.1100.

*4.1.4. General procedure for the synthesis of 9-alkyl-6-(4-methyl-1H-imidazol-1-yl)-9H-purine (**7a**–**b**)*

6-Chloro-9-alkyl-9*H*-purine (2 mmol), 4-methylimidazole (2.2 mmol), and K_2CO_3 (2.2 mmol) were placed into a round-bottomed flask containing *N*, *N*-dimethylformamide (25 ml). After stirring for 10 h at 150 °C, the solvent was removed under reduced pressure. The residue was purified by silica gel chromatography (CH_2Cl_2 : CH_3OH = 100 : 1).

*4.1.4.1. 9-Decyl-6-(4-methyl-1H-imidazol-1-yl)-9H-purine (**7a**).* Yield: 36%, mp: 28–29 °C, R_f = 0.47A, R_f = 0.71B, Chemical formula: $C_{19}H_{28}N_6$. ^1H NMR ($CDCl_3$, 300 MHz) δ: 0.86 (t, 3H, *J* = 6.52 Hz, $-CH_3$), 1.24–1.97 (m, 16H, $-CH_2-$), 2.89 (s, 3H, $-CH_3$), 4.31 (t, 2H, *J* = 7.20 Hz, $-CH_2-$), 7.08(s, 1H, Methylimidazole-H), 8.08 (s, 1H, Methylimidazole-H), 8.49 (s, 1H, Imidazole-H), 8.83 (s, 1H, Pyrimidine-H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ: 14.04, 17.77, 22.60, 26.61, 28.95, 29.18, 29.34, 29.39, 29.87, 31.78, 44.29, 120.54, 123.63, 128.21, 144.13, 146.95, 147.45, 151.56, 153.56. IR (KBr) cm^{-1}: 1602 1579. MS-EI *m/z* 341 (M + 1). ESI-HRMS calcd for $C_{19}H_{28}N_6^+$ ($[M + H]^+$): 341.2448; found: 341.2448.

*4.1.4.2. 9-(2-Fluorobenzyl)-6-(4-methyl-1H-imidazol-1-yl)-9H-purine (**7b**).* Yield: 48%, mp: 140–142 °C, R_f = 0.50A, R_f = 0.69B, Chemical formula: $C_{16}H_{14}FN_6$. ^1H NMR ($CDCl_3$, 300 MHz) δ: 2.90 (s, 3H, $-CH_3$), 5.54 (s, 2H, $-CH_2-$), 7.08–7.46 (m, 4H, Ar-H), 7.14 (s, 1H, Methylimidazole-H), 8.17 (s, 1H, Methylimidazole-H), 8.50 (s, 1H, Triazole-H), 8.86 (s, 1H, Imidazole-H), 9.18 (s, 1H, Pyrimidine-H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ: 17.98, 41.54, 116.11 (d, J_{c-f} = 21.0 Hz),

120.54, 122.17 (d, $J_{c\text{-}f}$ = 14.2 Hz), 123.41, 124.85, 128.54, 130.83 (d, $J_{c\text{-}f}$ = 3.0 Hz), 131.07(d, $J_{c\text{-}f}$ = 8.25 Hz), 144.03, 147.00, 147.64, 151.90, 153.50, 162.48 (d, $J_{c\text{-}f}$ = 246.0 Hz). IR (KBr) cm^{-1}: 1604 1579. MS-EI *m/z* 309 (M + 1). ESI-HRMS calcd for $C_{16}H_{14}FN_6^+$ ($[M + H]^+$): 309.1258; found: 309.1251.

*4.1.5. General procedure for the synthesis of 9-alkyl-6-(1H-pyrazol-1-yl)-9H-purine (**8a**–**b**)*

A mixture of 6-chloro-9-alkyl-9*H*-purine (2 mmol), pyrazole (2.2 mmol), and K_2CO_3 (2.2 mmol) in DMF (20 ml) was refluxed for 12 h. After removing half of the solvent, 150 ml of water was poured into the flask, and the precipitate was filtered and recrystallized in ethyl acetate (or ethanol) to produce a white solid.

*4.1.5.1. 9-Decyl-6-(1H-pyrazol-1-yl)-9H-purine (**8a**).* Yield: 54%, mp: 58–59 °C, R_f = 0.43A, R_f = 0.51B, Chemical formula: $C_{18}H_{26}N_6$. ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 0.88 (t, 3H, *J* = 6.45 Hz, $-CH_3$), 1.25–1.95 (m, 16H, $-CH_2-$), 4.33 (t, 2H, *J* = 7.24 Hz, $-CH_2-$), 6.59 (t, 1H, Pyrazole-H), 8.00 (s, 1H, Pyrazole-H), 8.15 (d, 1H, Pyrazole-H), 8.83 (s, 1H, Imidazole-H), 9.04 (s, 1H, Pyrimidine-H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ: 14.10, 22.65, 26.62, 29.00, 29.23, 29.39, 29.43, 29.91, 31.83, 44.28, 108.93, 130.52, 144.64, 144.76, 151.96, 153.53, 154.06, 157.02. IR (KBr) cm^{-1}: 1602 1579. MS-EI *m/z* 327 (M + 1). ESI-HRMS calcd for $C_{18}H_{26}N_6^+$ ($[M + H]^+$): 327.2292; found: 327.2292.

*4.1.5.2. 9-(2-Fluorobenzyl)-6-(1H-pyrazol-1-yl)-9H-purine (**8b**).* Yield: 56%, mp: 179–180 °C, R_f = 0.46A, R_f = 0.53B, Chemical formula: $C_{15}H_{11}FN_6$. ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 5.54 (s, 2H, $-CH_2-$), 6.57 (t, 1H, Pyrazole-H), 7.12–7.38 (m, 4H, Ar-H), 7.98 (s, 1H, Pyrazole-H), 8.20 (s, 1H, Pyrazole-H), 8.85 (s, 1H, Imidazole-H), 9.03 (s, 1H, Pyrimidine-H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ: 41.46, 109.01, 115.74 (d, $J_{c\text{-}f}$ = 21.0 Hz), 121.92, 122.8 (d, $J_{c\text{-}f}$ = 14.4 Hz), 124.79 (d, $J_{c\text{-}f}$ = 3.75 Hz), 130.62, 130.68, 130.80 (d, $J_{c\text{-}f}$ = 8.25 Hz), 144.60, 144.73, 147.35, 152.24, 153.95, 162.45 (d, $J_{c\text{-}f}$ = 247.5 Hz). IR (KBr) cm^{-1}: 1604 1579. MS-EI *m/z* 295 (M + 1). ESI-HRMS calcd for $C_{15}H_{11}FN_6^+$ ($[M + H]^+$): 295.1102; found: 295.1095.

*4.1.6. Synthesis of 9-alkyl-6-(3H-1,2,4-triazol-3-ylthio)-9H-purine (**9a**–**b**)*

CH_3ONa (2.2 mmol), 6-chloro-9-alkyl-9*H*-purine (2 mmol) and 1*H* (2*H*),(4*H*)-1,2,4-triazole-3-thiol were dissolved in acetonitrile (30 ml) and stirred for 18 h at room temperature. After removing the acetonitrile under reduced pressure, the residue was purified by silica gel chromatography (CH_2Cl_2 : CH_3OH = 50 : 1).

*4.1.6.1. 9-Decyl-6-(3H-1,2,4-triazol-3-ylthio)-9H-purine (**9a**).* Yield: 75%, mp: 140–142 °C, R_f = 0.12A, R_f = 0.13B, Chemical formula: $C_{17}H_{25}N_7S^+$. ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 0.87 (t, 3H, *J* = 6.60 Hz, $-CH_3$), 1.25–1.96 (m, 16H, $-CH_2-$), 1.73 (s, 1H, –NH–), 4.34 (t, 2H, *J* = 7.21 Hz, $-CH_2-$), 8.18 (s, 1H, Triazole-H), 8.90 (s, 1H, Imidazole-H), 9.82 (s, 1H, Pyrimidine-H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ: 14.07, 18.42, 22.63, 26.60, 28.95, 29.20, 29.86, 31.80, 44.48, 58.44, 122.16, 144.41, 145.64, 147.36, 152.15, 154.26, 163.24. IR (KBr) cm^{-1}: 1608 1579. MS-EI *m/z* 360 (M + 1). ESI-HRMS calcd for $C_{17}H_{25}N_7S^+$ ($[M + H]^+$): 360.1965; found: 360.1951.

*4.1.6.2. 9-(2-Fluorobenzyl)-6-(3H-1,2,4-triazol-3-ylthio)-9H-purine (**9b**).* Yield: 35%, mp: 156–158 °C, R_f = 0.11A, R_f = 0.14B Chemical formula: $C_{14}H_{10}FN_7S$. ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 1.85 (s, 1H, –NH–), 5.57 (s, 2H, $-CH_2-$), 7.09–7.15 (m, 4H, Ar-H), 8.02 (s, 1H, Triazole-H), 8.66 (s, 1H, Imidazole-H), 9.78 (s, 1H, Pyrimidine-H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ: 41.46, 116.16, 116.45, 122.23, 122.15, 129.93, 130.50, 144.40, 145.15, 145.63, 147.39, 152.17, 154.25, 163.28 (d, $J_{c\text{-}f}$ = 246.0 Hz). IR (KBr) cm^{-1}: 1608 1580. MS-EI *m/z* 328 (M + 1). ESI-HRMS calcd for $C_{14}H_{10}FN_7S^+$ ($[M + H]^+$): 328.0775; found: 328.0764.

4.2. Pharmacology

4.2.1. Anticonvulsant effects in the MES test [26,27]

Seizures were elicited using a 60 Hz alternating current with an intensity of 50 mA in mice. The current was applied via corneal electrodes for 0.2 s. Protection against the spread of MES-induced seizures was defined as the abolition of the hind leg, and the tonic maximal extension component of the seizure. Thirty minutes after administration of the compounds, their activities were evaluated in the MES test.

4.2.2. Neurotoxicity (NT) screening [26,27]

The neurotoxicity of the compounds was measured in mice using the rotarod test. Mice were trained to stay on an accelerating rotarod (diameter, 3.2 cm) rotating at 10 rpm. Trained animals were injected with the test compounds (i.p.) and neurotoxicity was measured as the inability of the animal to maintain equilibrium on the rod for at least 1 min in each of the trials.

4.2.3. sc-PTZ-induced seizures [26,27]

Thirty minutes after the administration of the test compound, 85 mg/kg PTZ dissolved in saline was administered (s.c.). The animals (10 mice/group) were placed in individual cages and observed for 0.5 h. The number of clonic seizure (ranging from exaggerated twitches of the limbs to violent shaking or vibrating of the stiffened extremities), tonic seizure (the extremities pull towards the body or rigidly push away from it, usually with maximal extension of the hind leg) and deaths were recorded.

*4.2.4. Pharmacological evaluation of compound **5e** administered orally to mice*

We determined the effect of compound **5e** over time in the MES test. A suspension of compound **5e** (50 mg/kg) in 0.5% methylcellulose was injected into mice by oral administration (p.o.). The mice were divided into 6 groups (n = 10). Subsequently, the animals were subjected to the MES test at various times: 0.5, 1, 1.5, 2, 2.5, and 3 h. The time of peak effect (TPE) was observed at 1.5 h after p.o. injection. Then, compound **5e** was evaluated for its anticonvulsant activity against MES-induced seizures and neurotoxicity at its TPE, when administered orally. This test involved the same procedures used in the MES test and TOX test screening to determine the ED_{50} and TD_{50} values, except the test drug was administered orally to mice.

Conflict of interest

We declare that we have no conflict of interest with respect to this study.

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