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Synthesis and Molecular Modeling Study of New Pyrimidine-Based Derivatives as Anticonvulsant Agents

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Abstract

Epilepsy is a chronic neurological disorder affecting many people worldwide, however, the low efficacy and the adverse side effects as well as the resistance of many patients to the available antiepileptic drugs have encouraged the persistent endeavor in the discovery of new effective antiepileptic medications. Considering the preceding researches that asserted the efficacy of pyrimidine-based compounds as anticonvulsant agents, the present investigation focused on the synthesis of new substituted thiopyrimidine derivatives in addition to the incorporation of the pyrimidine scaffold with different substituted hydrazinyl moieties and various heterocyclic rings **2-14** to evaluate their anticonvulsant effect utilizing Pentylenetetrazole (PTZ) and Maximal electroshock (MES) tests. Derivatives **8b**, **8c**, **8d**, **10b** and **11** displayed remarkable anticonvulsant efficiency relative to Carbamazepine and Phenytoin as reference drugs. Moreover, the latter derivatives were subjected to further neurochemical studies to determine their effects on various neurotransmitters in the brain such as GABA, norepinephrine, dopamine, serotonin, and glutamate. They displayed a notable elevation of GABA, norepinephrine, dopamine, and serotonin levels (GABA level range 2.62-3.86 µg/g tissue, norepinephrine level range 0.53-0.85 µg/g tissue, dopamine level range 1.80-2.63 µg/g tissue, serotonin level range 0.50-0.73 µg/g tissue) compared to Carbamazepine (3.80, 0.88, 2.85 and 0.82 µg/g tissue, respectively). Otherwise, the investigated candidates effectively reduced the glutamate levels ranging between 3.67-5.70 µg/g tissue comparing to carbamazepine (2.65 µg/g tissue). *In silico* ADMET prediction results revealed that the most potent pyrimidine candidates have good physicochemical and pharmacokinetic properties. Also, the binding interactions of the most prominent candidates within the active sites of GABA-AT enzyme and GluA2 subtype AMPA receptor were illustrated by carrying out a docking study

***Keywords**:* Pyrimidine; Anticonvulsant; GABA; Glutamate; Molecular modeling

1. Introduction

Epilepsy is a prevalent neurological disturbance that is recognized by exaggerated transitory neuronal discharges leading to sudden and recurrence of uncontrolled seizures. It has been estimated that around 65 million individuals suffer from epilepsy worldwide, according to WHO [1,2]. The utilization of the current AEDs is associated with various adverse effects including headaches, drowsiness, ataxia, nausea, megaloblastic anemia, gingival hyperplasia, gastrointestinal disturbances, hepatotoxicity, and attention deficit [3]. Thus, there is an insistent requirement for the discovery of new efficient, and safe AEDs.

AEDs act via boosting Gamma aminobutyric acid (GABA) levels through blocking of GABA aminotransferase (GABA-AT) enzyme which is accountable for GABA metabolism or allosteric modulation of GABAA receptor, ii) suppression of synaptic excitation stimulated by glutamate receptors, iii) adjustment of voltage-gated ion channels [4,5].

GABA is a fundamental suppression neurotransmitter in the central nervous system (CNS) that interacts with GABA receptors and exerts varied physiological functions [6]. The reduction of GABA levels in the brain has a pivotal role in the etiology of varied neurological diseases like epilepsy, parkinson, anxiety, migraine, Alzheimer and depression [7]. Also, glutamate is a considerable excitatory neurotransmitter and the most copious amino acid in the CNS [8]. The glutamate is emitted from the glutamatergic neuron's presynaptic terminal through a calcium-dependent pattern and gets into the extracellular space, which then can bind to both metabotropic and ionotropic glutamate receptors [9]. The balance between neuronal inhibition and excitation has a prominent role in proceeding with normal brain function [10].

There are numerous of evidence that serotonergic neurotransmission adjusts different experimentally stimulated seizures. Accordingly, serotonin reuptake inhibitors which boost extracellular serotonin (5-HT) levels, suppress both generalized and focal seizures, Additionally, the overexpression of 5-HT receptors is implicated in epilepsy, and various AEDs elevate the extracellular 5-HT concentration [11]. Furthermore, norepinephrine (NE) has anticonvulsant effects, the depletion of NE

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raises seizure susceptibility and speeds up the epileptogenesis in almost all the tested animal models [12]. In addition, dopamine is an essential neuromodulator, which has a prominent role in seizure modulation [13]. The pyrimidine scaffold has been received extensive interest due to its versatile pharmacological effects [14-17]. Former studies have identified the outstanding anticonvulsant properties for various pyrimidine derivatives **I-V** (**Fig.1**) [18-23]. Furthermore, the incorporation of the pyrimidine scaffold with various nitrogenous heterocyclic rings such as thiazole, pyrazole and triazole presented significant anticonvulsant agents **VI-VIII** (Fig. 1)[24-26]. In addition, various studies displayed that the anticonvulsant efficiency of the pyrimidine derivatives was exerted via targeting different neurotransmitters such as GABA, glutamate, serotonin and dopamine **IX-XVI** (**Fig.2**) [27-32].

Phenobarbital is the first pyrimidine-based derivative that was extensively used for treating epilepsy and acts as GABA receptor agonist [33]. In addition to the pyrimidine-based anticonvulsant drug primidone which is metabolized to phenobarbital [34]. Moreover, Remeglurant [35] is pyrimidine-based drug and is considered as a selective antagonist of the glutamate receptor (mGlu5) (**Fig.2**). The above-mentioned information prompted us to synthesize new substituted thiopyrimidine derivatives in addition to the incorporation of the pyrimidine scaffold with different substituted hydrazinyl moieties and various heterocyclic rings such as thiazole, pyrazole and triazole to investigate their anticonvulsant efficiency. Moreover, various neurochemical studies were

accomplished for the most prominent candidates to determine their effect on the level of varied neurotransmitters such as GABA, norepinephrine, dopamine, serotonin, and glutamate.

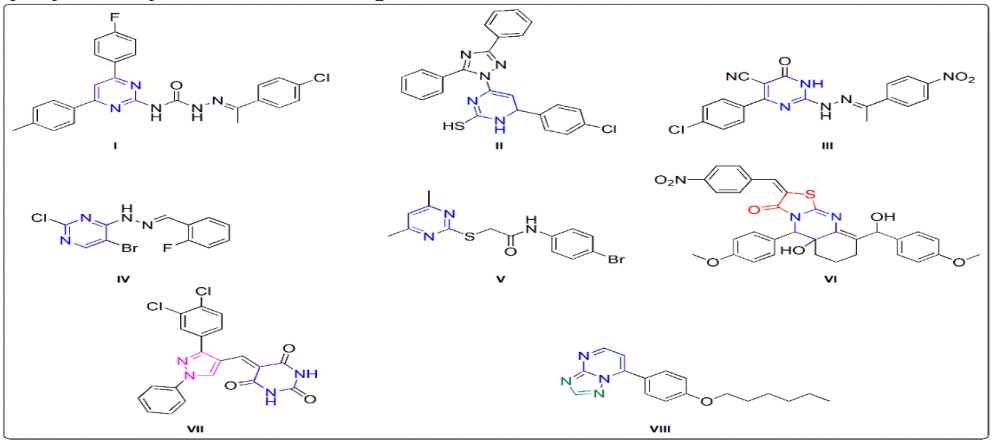


Fig.1. Different pyrimidine-bearing thiazole/pyrazole/triazole based derivatives as anticonvulsant agents.

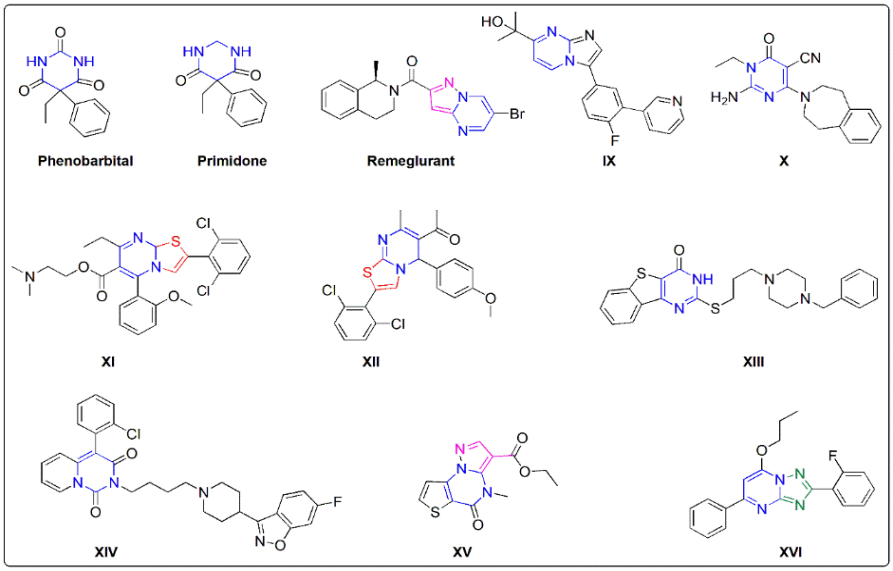


Fig.2. Various reported drugs and synthesized pyrimidine bearing thiazole/pyrazole/triazole based derivatives targeting different neurotransmitters.

2. Material and methods

2.1. Chemistry

Melting points were measured using the Stuart SMP30 apparatus. JASCO 6100 spectrophotometer was used to record IR spectra (KBr). Shimadzu GCMS-QP 1000 EX spectrometer was utilized to assess mass spectra. NMR spectra were carried out by a Mercury 400 MHZ (^{1}H: 400 MHz, ^{13}C: 101 MHz) spectrometer. The purity of the prepared derivatives was checked via elemental microanalyses using a Vario Elementar analyzer. Compounds **1** [36], **2** [37] were prepared following the preceding reported methods.

2.1.1. Method for the preparation of derivatives 3 and 4

To a solution of thiopyrimidine **1** (0.005 mole, 1.6 g) in absolute ethanol (25 mL), methylhydrazine and or phenylhydrazine (0.005 mole) was added and the solution was heated under reflux for 7-8 h. After cooling, the solid formed was filtered, dried and crystallized from ethanol to obtain the required derivatives **3** and **4**, respectively.

2.1.1.1. 2-(2-Methylhydrazinyl)-1,6-dihydro-4-(3,4,5-trimethoxyphenyl)-6-oxopyrimidine-5-carbonitrile (3)

Yellow crystals, mp = 115-117˚C, yield (68%). IR (cm^{-1}): 3385, 3318, 3202 (3NH), 2211(C≡N), 1668 (C=O). ^{1}HNMR (DMSO-d_6): δ 3.74 (s, 3H, OCH_3), 3.85 (s, 6H, $2OCH_3$), 3.89 (s, 3H, CH_3), 7.22 (s, 2H, Ar-Hs), 7.33, 7.44, 8.66 (s, 3H, 3NH, exchangeable with D_2O). MS: m/z (%) 331 (M^+, 35.30). Anal. Calcd for $C_{15}H_{17}N_5O_4$ (331.33): C, 54.38; H, 5.17; N, 21.14. Found: C, 54.20; H, 5.28; N, 21.02.

2.1.1.2. 2-(2-Phenylhydrazinyl)-1,6-dihydro-4-(3,4,5-trimethoxyphenyl)-6-oxopyrimidine-5-carbonitrile (4)

Brown crystals, mp = 247-249˚C, yield (72%). IR (cm^{-1}): 3395, 3325, 3234 (3NH), 2209 (C≡N), 1675 (C=O). ^{1}HNMR (DMSO-d_6): δ 3.78 (s, 3H, OCH_3), 3.84 (s, 6H, 2 OCH_3), 6.28 (s, 1H, NH, exchangeable with D_2O), 6.97-7.47 (m, 8H, Ar-Hs and 1NH), 10.66 (s, 1H, NH, exchangeable with D_2O). MS: m/z (%) 393 (M^+, 24.62). Anal. Calcd for $C_{20}H_{19}N_5O_4$ (393.40): C, 61.06; H, 4.87; N, 17.80. Found: C, 61.29; H, 4.73; N, 17.91.

2.1.2. Method for the preparation of derivatives 5 and 6

A solution of thiopyrimidine **1** (0.005 mole, 1.6 g), 2-chloroacetamide and/or phenacyl bromide (0.005 mole), and potassium carbonate anhydrous (0.01 mole, 1.39 g) in dry acetone (50 mL) was heated for 10-12 h. Upon cooling, the solution was poured onto cold water and neutralized with 2 N HCl. The precipitated product was filtered, desiccated and crystallized from methyl alcohol to obtain the required derivatives **5** and **6**, respectively**.**

2.1.2.1. 2-(5-Cyano-1,6-dihydro-4-(3,4,5-trimethoxyphenyl)-6-oxopyrimidin-2-ylthio)acetamide (5)

Yellow crystals, mp = 123-125˚C, yield (62%). IR (cm^{-1}): 3368, 3316 (NH_2) 3218 (NH), 2223 (C≡N), 1678 (C=O), 1645 (C=O). ^{1}HNMR (DMSO-d_6): δ 3.66 (s, 2H, CH_2), 3.77 (s, 3H, OCH_3), 3.83 (s, 6H, 2 OCH_3), 6.54 (d, 2H, NH_2, exchangeable with D_2O), 7.07 (s, 2H, Ar-Hs), 7.55 (s, 1H, NH, exchangeable with D_2O). MS: m/z (%) 376 (M^+, 31.26). Anal. Calcd for $C_{16}H_{16}N_4O_5S$ (376.39): C, 51.06; H, 4.28; N, 14.89. Found: C, 51.20; H, 4.37; N, 15.00.

2.1.2.2. 6-Oxo-2-((2-Oxo-2-phenylethyl)thio)-4-(3,4,5-trimethoxyphenyl)-1,6-dihydropyrimidine-5-carbonitrile (6)

Orange crystals, mp = 143-145˚C, yield (67%). IR (cm^{-1}): 3380 (NH), 2217(C≡N), 1695 (C=O), 1658 (C=O). ^{1}HNMR (DMSO-d_6): δ 3.75 (s, 3H, OCH_3), 3.83 (s, 6H, 2 OCH_3), 4.67(s, 2H, CH_2), 7.00 (s, 1H, Ar-H), 7.05 (s, 1H, Ar-H), 7.25 (t, 3H, Ar-Hs, *J*=7.0 Hz), 7.34 (d, 1H, Ar-H, *J*=8.0 Hz), 7.79 (d,1H, Ar-H, *J*=7.2 Hz), 8.14 (s, 1H, NH, exchangeable with D_2O). MS: m/z (%) 437 (M^+, 19.67). Anal. Calcd for $C_{22}H_{19}N_3O_5S$ (437.47): C, 60.40; H, 4.38; N, 9.61. Found: C, 60.66; H, 4.20; N, 9.75.

2.1.3. Synthesis of 3,5-Dihydro-7-(3,4,5-trimethoxyphenyl)-3,5-dioxo-2H-thiazolo[3,2-a] pyrimidine-6-carbonitrile (7)

To a solution of thiopyrimidine **1** (0.005 mole, 1.6g) and an anhydrous sodium acetate (0.01 mole, 0.82 g) in acetic acid (25 mL), ethyl bromoacetate (0.005 mole, 0.84 mL) was added and the solution was heated under reflux for 6 h, then cooled and poured onto cold water. The obtained precipitate was filtered, desiccated and crystallized from methanol to obtain the required derivative **7**.

Green crystals, mp = 173-175˚C, yield (70%). IR (cm^{-1}): 2209 (C≡N), 1710 (C=O), 1692 (C=O). ^{1}HNMR (DMSO-d_6): δ 3.72 (s, 3H, OCH_3), 3.84 (s, 6H, 2 OCH_3), 4.12 (s, 2H, CH_2), 6.92 (s, 2H, Ar-Hs). MS: m/z (%) 359 (M^+, 60.32). Anal. Calcd for $C_{16}H_{13}N_3O_5S$ (359.36): C, 53.48; H, 3.65; N, 11.69. Found: C, 53.63; H, 3.81; N, 11.57.

2.1.4. Method for the preparation of derivatives 8a-d and 9

To a solution of thiopyrimidine **1** (0.005 mole, 1.6g), an aryl aldehyde and/or isatin (0.005 mole), and an anhydrous sodium acetate (0.01 mole, 0.82 g) in glacial acetic acid (30 mL), ethyl bromoacetate (0.005 mole, 0.84 mL) was added and the solution was heated under reflux for 3 h. Upon cooling, the reaction solution was poured onto iced water. The obtained precipitate was filtered, desiccated and crystallized from methanol to obtain the required derivatives **8a-d** and **9**, respectively.

2.1.4.1. 2-(4-Chlorobenzylidene)-3,5-dihydro-7-(3,4,5-trimethoxyphenyl)-3,5-dioxo-2H-thiazolo[3,2-a] pyrimidine-6-carbonitrile (8a)

Yellow crystals, mp = 138-140 ˚C, yield (69%). IR (cm^{-1}): 2218(C≡N), 1725 (C=O), 1670 (C=O). ^{1}HNMR (DMSO-d_6): δ 3.78 (s, 3H, OCH_3), 3.85 (s, 6H, 2 OCH_3), 6.92 (s, 2H, Ar-Hs), 7.68 (d, 2H, Ar-Hs, *J*=7.6 Hz), 7.94 (d, 2H, Ar-Hs, *J*=8.0 Hz), 8.02 (s, 1H, =CH-). MS: m/z (%) 481 (M^+, 20.48). Anal. Calcd for $C_{23}H_{16}ClN_3O_5S$ (481.91): C, 57.32; H, 3.35; N, 8.72. Found: C, 57.44; H, 3.21; N, 8.95.

2.1.4.2. 3,5-Dihydro-2-(4-methylbenzylidene)-7-(3,4,5-trimethoxyphenyl)-3,5-dioxo-2H-thiazolo[3,2-a] pyrimidine-6-carbonitrile (8b)

Green crystals, mp = 190-192˚C, yield (72%). IR (cm^{-1}): 2218 (C≡N), 1725 (C=O), 1675 (C=O). ^{1}HNMR (DMSO-d_6): δ 2.24 (s, 3H, CH_3), 3.75 (s, 3H, OCH_3), 3.84 (s, 6H, $2OCH_3$), 7.02 (s, 2H, Ar-Hs), 7.37 (d, 2H, Ar-Hs, *J*=8.0 Hz), 7.57 (d, 2H, Ar-Hs, *J*=8.0 Hz), 7.79 (s, 1H, =CH-).^{13}CNMR (DMSO-d_6): δ 21.56 (CH_3), 56.47 (2 OCH_3), 60.70 (OCH_3), 89.58 (pyrimidine-C5), 108.38 (Ar-C), 125.61(C≡N), 125.84, 126.84, 129.54, 130.45, 130.77, 131.22, 131.41, 135.66, 135.88, 140.13, 141.35,

153.66 (Ar-C), 174.33, 179.12 (2 C=O). MS: m/z (%) 461 (M^+, 34.19). Anal.Calcd for $C_{24}H_{19}N_3O_5S$ (461.49):C, 62.46;H,4.15;N,9.11Found: C, 62.20; H, 4.22; N, 9.25.

2.1.4.3. 2-(4-(Dimethylamino)benzylidene)-3,5-dihydro-7-(3,4,5-trimethoxyphenyl)-3,5-dioxo-2H-thiazolo [3,2-a]pyrimidine-6-carbonitrile (8c)

Green crystals, mp =166-168 °C, yield (75%). IR (cm^{-1}): 2220 (C≡N), 1710 (C=O), 1670 (C=O). ^{1}HNMR (DMSO-d_6): δ 3.03 (s, 3H, CH_3), 3.09 (s, 3H, CH_3), 3.75 (s, 3H, OCH_3), 3.85 (s, 6H, $2OCH_3$), 7.02 (s, 2H, Ar-Hs), 7.51 (d, 2H, Ar-Hs, *J*=8.8 Hz), 7.61 (d, 2H, Ar-Hs, *J*=8.8 Hz), 7.79 (s, 1H, =CH-). MS: m/z (%) 490 (M^+, 15.46). Anal. Calcd for $C_{25}H_{22}N_4O_5S$ (490.53): C, 61.21; H, 4.52; N, 11.42. Found: C, 61.09; H, 4.34; N, 11.31.

2.1.4.4. 3,5-Dihydro-7-(3,4,5-trimethoxyphenyl)-3,5-dioxo-2-((thiophen-2-yl)methylene)-2H-thiazolo[3,2-a] pyrimidine-6-carbonitrile (8d)

Green crystals, mp = 194-196°C, yield (70%). IR (cm^{-1}): 2228 (C≡N), 1695 (C=O), 1668 (C=O). ^{1}HNMR (DMSO-d_6): δ 3.75 (s, 3H, OCH_3), 3.84 (s, 6H, 2 OCH_3), 7.02 (s, 2H, Ar-Hs), 7.29 (t, 1H, Ar-H, *J*= 4.2 Hz), 7.71 (d, 1H, Ar-H, *J*= 3.2 Hz), 7.78 (s, 1H, =CH-), 8.00 (d, 1H, Ar-H, *J*= 4.8 Hz). ^{13}CNMR (DMSO-d_6): δ 56.47 ($2OCH_3$), 60.70 (OCH_3), 86.46 (pyrimidine-C5), 108.39 (Ar-C), 125.84 (C≡N), 128.35, 129.53, 133.69, 135.68, 135.92, 137.46, 138.64, 140.14, 146.73, 150.47, 153.40, 153.66 (Ar-C), 173.62, 175.56 (2 C=O). MS: m/z (%) 453 (M^+, 28.36).Anal.Calcd for $C_{21}H_{15}N_3O_5S_2$ (453.49): C,55.62;H, 3.33;N, 9.27.Found: C,55.83;H,3.50;N, 9.14.

2.1.4.5. 3,5-Dihydro-7-(3,4,5-trimethoxyphenyl)-3,5-dioxo-2-(2-oxoindolin-3-ylidene)-2H-thiazolo[3,2-a] pyrimidine-6-carbonitrile (9)

Orange crystals, mp = 200-202°C, yield (75%). IR (cm^{-1}): 3239 (NH), 2217 (C≡N), 1705 (C=O), 1692 (C=O). ^{1}HNMR (DMSO-d_6): δ 3.75 (s, 3H, OCH_3), 3.83 (s, 6H, $2OCH_3$), 6.91-7.06 (m, 4H, Ar-Hs), 7.34 (t, 1H, Ar-H, *J*= 7.6 Hz), 8.85 (d, 1H, Ar-H, *J*= 7.6 Hz), 11.07 (s, 1H, NH, exchangeable with D_2O). MS: m/z (%) 488 (M^+, 32.68). Anal. Calcd for $C_{24}H_{16}N_4O_6S$ (488.47): C, 59.01; H, 3.30; N, 11.47. Found: C, 59.23; H, 3.11; N, 11.59.

2.1.5. Method for the preparation of derivatives 10a,b

A solution of 2-hydrazinylpyrimidine **2** (0.005 mole, 1.59 g) and an aryl aldehyde (0.005 mole) in glacial acetic (25 mL) was heated for 4h. Upon cooling, the solution was poured onto iced water. The obtained precipitate was filtered, desiccated and crystallized from ethanol to obtain the required derivatives **10a,b**, respectively.

2.1.5.1. 2-((4-Fluorobenzylidene)hydrazinyl)-1,6-dihydro-4-(3,4,5-trimethoxyphenyl)-6-oxopyrimidine-5-carbonitrile (10a)

Buff crystals, mp = 260-262°C, yield (65%). IR(cm^{-1}): 3336, 3281 (2NH), 2224 (C≡N), 1669 (C=O). ^{1}HNMR (DMSO-d_6): δ 3.77 (s, 3H, OCH_3), 3.85 (s, 6H, $2OCH_3$), 7.24 (s, 2H, Ar-Hs),7.27 (d, 2H, Ar-Hs, *J*= 8.8 Hz), 8.11 (d, 2H, Ar-Hs, *J*=8.8 Hz), 8.20 (s, 1H, Ar-H, -N=CH-), 12.44 (s, 1H, NH, exchangeable with D_2O), 12.57 (s,1H, NH, exchangeable with D_2O). ^{13}CNMR (DMSO-d_6): δ 56.49 (2 OCH_3), 60.67 (OCH_3), 86.74 (pyrimidine-C5), 106.61 (Ar-C), 115.95 (C≡N), 116.17, 117.86, 130.84, 130.92, 131.58, 140.40, 146.44, 152.91, 153.76, 162.25, 162.63, 165.10 (Ar-C), 170.43(C=O). MS: m/z (%) 423 (M^+, 52.23). Anal. Calcd for $C_{21}H_{18}FN_5O_4$ (423.40): C, 59.57; H, 4.29; N, 16.54. Found: C, 59.78; H, 4.41; N, 16.36.

2.1.5.2. 2-((4-Chlorobenzylidene)hydrazinyl)-1,6-dihydro-4-(3,4,5-trimethoxyphenyl)-6-oxopyrimidine-5-carbonitrile (10b)

Buff crystals, mp = 223-225°C, yield (72%). IR(cm^{-1}): 3365, 3242 (2NH), 2228 (C≡N), 1678 (C=O). ^{1}HNMR (DMSO-d_6): δ 3.77 (s, 3H, OCH_3), 3.85 (s, 6H, $2OCH_3$), 7.24 (s, 2H, Ar-Hs),7.50 (d, 2H, Ar-Hs, *J*= 8.8 Hz), 8.08 (d, 2H, Ar-Hs, *J*=8.4 Hz), 8.19 (s, 1H, Ar-H, -N=CH-), 12.46 (s, 2H, 2NH, exchangeable with D_2O). ^{13}CNMR (DMSO-d_6): δ 56.50, 56.75 ($2OCH_3$), 60.67 (OCH_3), 86.60 (pyrimidine-C5), 106.05, 106.62 (Ar-C), 117.90 (C≡N), 129.07, 129.45, 130.21, 131.60, 133.13, 135.39, 140.15, 140.38, 146.23, 147.88, 152.92, 153.61, 162.24 (Ar-C), 170.48 (C=O). MS: m/z (%) 439 (M^+, 44.09). Anal. Calcd for $C_{21}H_{18}ClN_5O_4$ (439.85): C, 57.34; H, 4.12; N, 15.92. Found: C, 57.47; H, 4.00; N, 15.79.

2.1.6. Method for the preparation of derivatives 11 and 12

To a solution of 2-hydrazinylpyrimidine **2** (0.005 mole, 1.59 g) in acetic acid (25 mL), acetyl acetone and/or ethyl acetoacetate (0.005 mole) was added and the solution was heated under reflux for 5 h, then cooled and poured onto iced water. The obtained precipitate was filtered, desiccated and crystallized from methanol to afford compounds **11** and **12**, respectively**.**

2.1.6.1. 1,6-Dihydro-4-(3,4,5-trimethoxyphenyl)-2-(3,5-dimethyl-1H-pyrazol-1-yl)-6-oxopyrimidine-5-carbonitrile (11)

Buff crystals, mp =168-170 °C, yield (70%). IR(cm^{-1}): 3225 (NH), 2210 (C≡N), 1667 (C=O). ^{1}HNMR (DMSO-d_6): δ 2.26 (s, 3H, CH_3), 2.69 (s, 3H, CH_3), 3.78 (s, 3H, OCH_3), 3.85 (s, 6H, $2OCH_3$), 6.34 (s, 1H, pyrazole-H), 7.08 (s, 1H, Ar-H), 7.40 (s, 1H, Ar-H), 9.89 (s,1H, NH, exchangeable with D_2O). ^{13}CNMR (DMSO-d_6): δ 13.90, 15.58 (2 CH_3), 56.46, 56.64 (2 OCH_3), 60.68 (OCH_3), 86.32 (pyrimidine-C5), 106.92, 107.19 (Ar-C), 112.68 (C≡N), 124.96, 130.45, 132.02, 140.95, 150.25, 153.06, 153.80, 161.94, 161.99 (Ar-C), 168.23 (C=O). MS: m/z (%) 381 (M^+, 32.43). Anal. Calcd for $C_{19}H_{19}N_5O_4$ (381.39): C, 59.84; H, 5.02; N, 18.36. Found: C, 59.67; H, 5.14; N, 18.51.

2.1.6.2. 1,6-dihydro-2-(5-hydroxy-3-methyl-1H-pyrazol-1-yl)-4-(3,4,5-trimethoxyphenyl)-6-oxopyrimidine-5-carbonitrile (12)

Buff crystals, mp = 183-185°C, yield (66%). IR (cm^{-1}): 3440 (OH), 3263 (NH), 2219 (C≡N), 1675 (C=O). ^{1}HNMR (DMSO-d_6): δ 2.20 (s, 3H, CH_3), 3.75 (s, 3H, OCH_3), 3.83 (s, 6H, $2OCH_3$), 6.89 (s, 1H, pyrazole-H), 7.20 (s, 1H, Ar-H), 7.42 (s, 1H,

Ar-H), 10.72 (s,1H, NH, exchangeable with D_2O), 12.27 (s,1H, OH, exchangeable with D_2O). MS: m/z (%) 383 (M^+, 12.50). Anal. Calcd for $C_{18}H_{17}N_5O_5$ (383.36): C, 56.39; H, 4.47; N, 18.27. Found: C, 56.55; H, 4.63; N, 18.34.

2.1.7. Synthesis of 1,2,3,5-Tetrahydro-7-(3,4,5-trimethoxyphenyl)-3,5-dioxo-[1,2,4]triazolo[4,3-a] pyrimidine-6-carbonitrile (13)

To a mixture of 2-hydrazinylpyrimidine **2** (0.005 mole, 1.59g) in DMF (25 mL), ethyl chloroformate (0.005 mole, 0.54 mL) was added and the solution was heated under reflux for 10 h. The solvent was steamed and the obtained precipitate was filtered, desiccated and crystallized from ethanol to obtain the required compound **13**.

Brown crystals, mp = 210-212˚C, yield (64%). IR (cm^{-1}): 3280 (NH) 3263 (NH), 2219 (C≡N), 1692 (C=O), 1645 (C=O). ^{1}HNMR (DMSO-d_6): δ 3.78 (s, 3H, OCH_3), 3.87 (s, 6H, $2OCH_3$), 7.13 (s, 1H, Ar-H), 7.22 (s, 1H, NH, exchangeable with D_2O), 7.26 (s, 1H, Ar-H), 7.44 (s, 1H, NH, exchangeable with D_2O). MS: m/z (%) 343 (M^+, 18.45). Anal. Calcd for $C_{15}H_{13}N_5O_5$ (343.29): C, 52.48; H, 3.82; N, 20.40. Found: C, 52.63; H, 3.69; N, 20.54.

2.1.8. Synthesis of 1,5-Dihydro-7-(3,4,5-trimethoxyphenyl)-5-oxo-[1,2,4]triazolo[4,3-a]pyrimidine-6-carbonitrile (14)

A mixture of 2-hydrazinylpyrimidine **2** (0.005 mole, 1.59 g) and triethyl orthoformate (0.005 mole, 0.74 mL) in DMF (25 mL) was heated under reflux for 10 h. The solvent was evaporated and the obtained precipitate was filtered, desiccated and crystallized from methanol to obtain the required derivative **14**.

Orange crystals, mp = 181-183˚C, yield (68%). IR (cm^{-1}): 3282 (NH), 2215 (C≡N), 1685 (C=O). ^{1}HNMR (DMSO-d_6): δ 3.74 (s, 3H, OCH_3), 3.85 (s, 6H, $2OCH_3$), 7.23 (s, 2H, Ar-Hs), 8.67 (s, 1H, CH, triazole-H), 9.89 (s, 1H, NH, exchangeable with D_2O). ^{13}CNMR (DMSO-d_6): δ 56.40 ($2OCH_3$), 60.63 (OCH_3), 86.72 (pyrimidine-C5), 106.06, 107.02 (Ar-C), 119.10 (C≡N), 129.73, 133.82, 140.69, 144.89, 153.63, 157.63 (Ar-C), 161.68 (C=O). MS: m/z (%) 327 (M^+, 38.27). Anal. Calcd for $C_{15}H_{13}N_5O_4$ (327.29): C, 55.05; H, 4.00; N, 21.40. Found: C, 55.21; H, 4.13; N, 21.25.

2.2. Biology

The anticonvulsant efficiency of the investigated derivatives was estimated utilizing adult male albino mice weighting 15-25 g (provided from the "Animals House Colony of National Research Centre, Giza, Egypt"), and were housed in cages with the standardized conditions. Animals were permitted to food and water ad libitum. The current study was conducted following the guidelines of the ethical committee of National Research Centre for experimental animal use (Approval No.: 01431023). Mice were allowed to acclimatize to the laboratory conditions at least 7 days before the beginning of the experiment [38]. The screened derivatives were dissolved in Tween 80 solution at 1% concentration.

2.2.1. Maximum electro-shock seizures (MES) test

It is mostly standard model used to evaluate the investigated compounds. Six mice in each group for control and screened compounds were used. Thirty minutes after the intraperitoneally injection of the screened derivatives, each mouse delivered Electric-induced convulsion using device with electrical source for mice (Ugo Basil, ECT Unit, 57,800) which adapted to a fixed current intensity (25 mA), stimulus duration (0.2 s), and the ear attach conductors at (50 Hz). Protection against tonic hind limb extension (THE) for 180◦suggests was considered as positive endpoint. Mice which are freed from any generalized seizures were supposed to be protected. The results were presented as % protection [39].

2.2.2. Subcutaneous pentylenetetrazole (scPTZ)-stimulated seizures test

This model was used to estimate the screened derivatives which are effective in petit mal epilepsy. Intraperitoneal administration (ip) of investigated compounds or the reference were given to mice (n = 6); a control group consisting of an additional six mice was also used. Following the intraperitoneal treatment, a subcutaneous dose of 85 mg/kg PTZ was administered 60 minutes later. Each mouse was individually putted in a cage and monitored for 30 min. Tonic-clonic convulsions incidence were last for nearly 5 seconds as well were registered [38]. Mice which are freed from any generalized seizures, were supposed to be protected. The results were presented as % protection.

2.2.3. Biochemical measurements

2.2.3.1. Neurotransmitters estimation

At the termination of the experiment, rats were sacrificed, and the brain tissue were dissected to GABA, norepinephrine, dopamine, serotonin and glutamate quantification in the brain of the mouse following Heinrikson and Meredith's process [40]. Agilent's (Germany) HPLC system included "a quaternary pump, a column oven, a Rheodine injector, a 20-loop, and a UV detector". The chromatogram and report were achieved utilizing the chemstation application. Estimation of neurotransmitters concentrations were accomplished within the brain homogenate as (µg / g tissue).

2.3. *In silico* ADMET prediction study

Utilizing the Swiss ADME programme and the admet SAR 1.0 internet server(http://lmmd.ecust.edu.cn/admetsa_r1),the promising pyrimidines **8b**, **8c**, **8d, 10b** and **11** were screened for their anticipated toxicity, pharmacokinetic and physicochemical features [41,42].

2.4. Molecular docking studies

The docking simulation of the assessed pyrimidines **8b**, **8c**, **8d, 10b** and **11** against GABA-AT enzyme and GluA2 subtype AMPA receptor were applied following the cited approach in supplementary file [43,44].

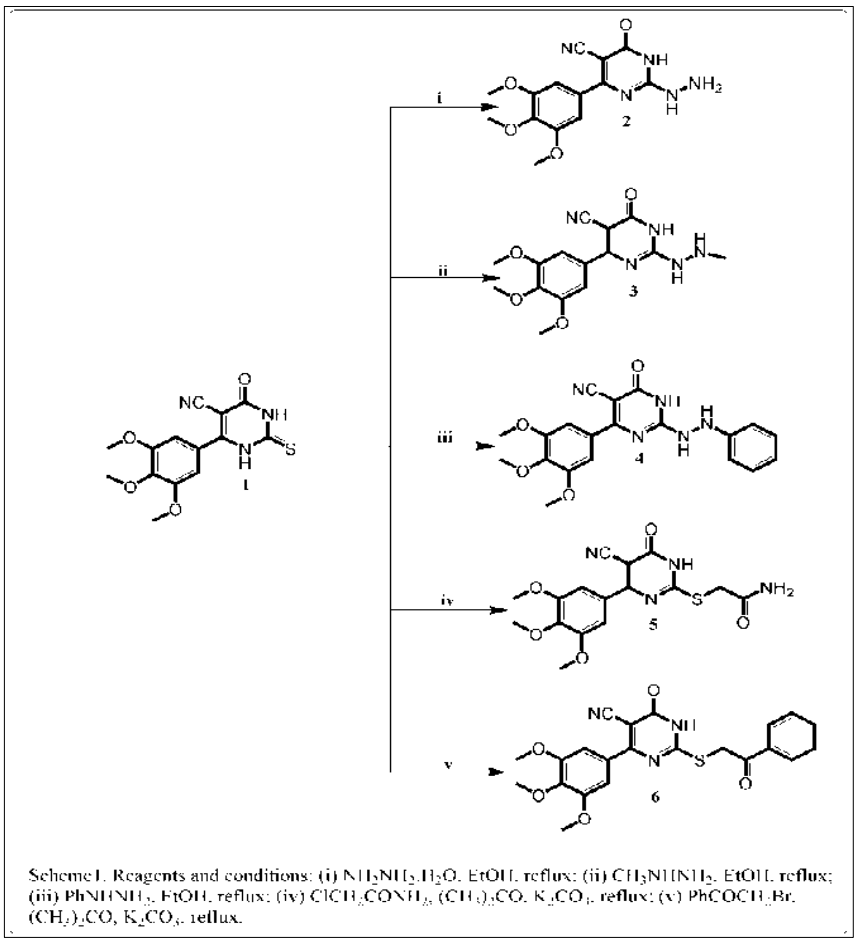
2.5. Statistical Analysis

Data were represented as means ± S.E. values were statistically analyzed using GraphPad Prism software version 9.5.1.733 using one-way ANOVA then Tukey–Kramer Post hoc test. $P < 0.05$ was considered statistically significant.

3. Results and Discussion

3.1. Chemistry

The synthetic routes of the newly prepared pyrimidine derivatives were depicted in **Schemes 1-3**. The reaction of 2-thioxopyrimidine-5-carbonitrile derivative **1** with methylhydrazine and or phenylhydrazine yielded the corresponding methylhydrazinyl **3** and phenylhydrazinyl **4** derivatives, respectively. The ^{1}HNMR spectrum of compound **3** exhibited singlet signal at at δ_H 3.89 ppm attributed to the methyl group, in addition to three D_2O exchangeable signals at δ_H 7.33, 7.44 and 8.66 ppm assignable to three NH groups. Whereas, the ^{1}HNMR spectrum of derivative **4** demonstrated two D_2O exchangeable signals at δ_H 6.28 and 10.66 ppm indicated to two NH groups, alongside, a significant increase of the integration was observed at the aromatic zone δ_H 6.97-7.47 ppm corresponding to the phenyl ring and NH group. Furthermore, the reaction of the 2-thioxopyrimidine-5-carbonitrile derivative **1** with 2-chloracetamide and or phenacyl bromide in acetone containing an anhydrous potassium carbonate furnished compounds **5** and **6**, respectively. The ^{1}HNMR spectrum of compound **5** revealed a singlet signal at δ_H 3.66 ppm assignable to the methylene protons and a doublet signal at δ_H 6.54 ppm attributable to NH_2 functional group, respectively. While the ^{1}HNMR spectrum of compound **6** displayed a singlet signal referring to the methylene protons at δ_H 4.67 ppm, also, a triplet and two doublet signals were detected at H 7.25, 7.3 and 7.79 ppm assignable to the phenyl ring (Scheme 1).

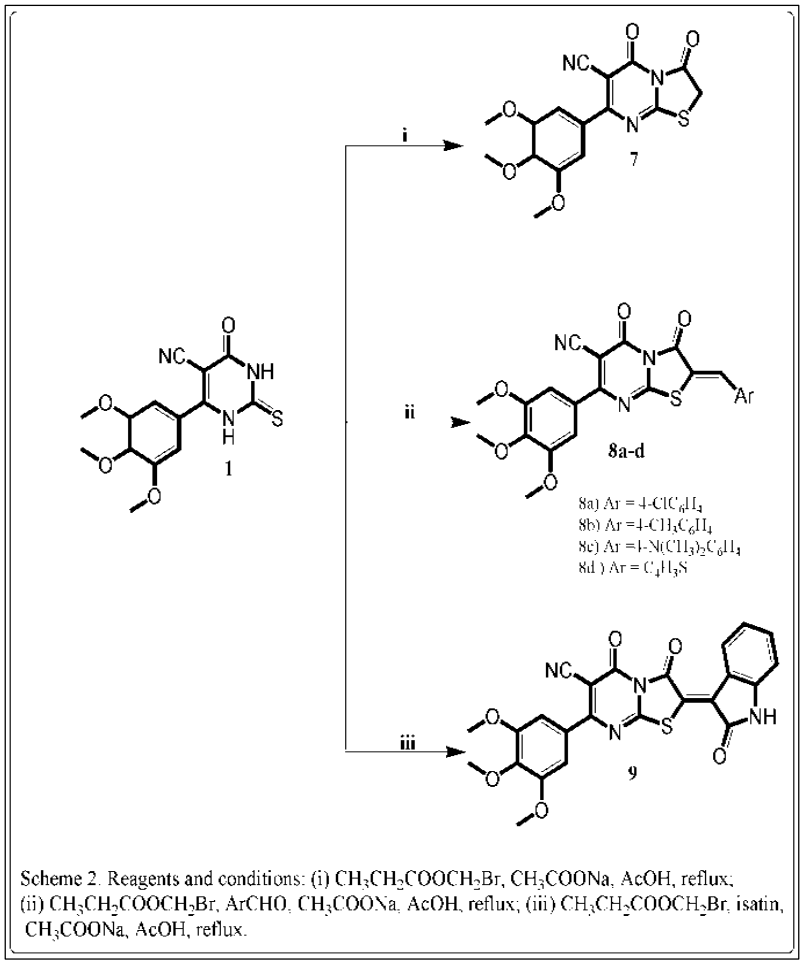


Scheme I. Reagents and conditions: (i) $NH_2NH_2.H_2O$, EtOH, reflux; (ii) CH_3NHNH_2, EtOH, reflux; (iii) $PhNHNH_2$, EtOH, reflux; (iv) $ClCH_2CONH_2$, $(CH_3)_2CO$, K_2CO_3, reflux; (v) $PhCOCH_2Br$, $(CH_3)_2CO$, K_2CO_3, reflux.

Scheme 1. Synthetic approaches for the preparation of 2-hydrazinyl Pyrimidine and 2-thiopyrimidine derivatives 2-6.

Refluxing 2-thioxopyrimidine-5-carbonitrile derivative **1** with ethyl bromoacetate in acetic acid containing sodium acetate gave the corresponding 3,5-dioxo-thiazolo[3,2-a] pyrimidine **7**. The ^{1}HNMR spectrum of derivative **7** showed a characteristic singlet signal at δ_H 4.12 ppm related to the methylene protons. While the one pot reaction of the 2-thioxopyrimidine-5-carbonitrile derivative **1** with ethyl bromoacetate and the appropriate aldehydes and or isatin in acetic acid containing sodium acetate furnished the derivatives **8a-d** and **9**, respectively. The ^{1}HNMR spectral data of derivatives **8a-d** revealed a singlet signal at δ_H 7.78-8.02 ppm referring to =CH, moreover, an additional signals detected in the aromatic zone due to the protons of the arylidene moiety. Otherwise, ^{1}HNMR spectral data of derivative **9** disclosed higher integration values at δ_H 6.91-8.85 ppm due to indolyl moiety, alongside, D_2O exchangeable signal at δ_H 11.07 ppm related to NH group (**Scheme 2**).

The reaction of hydrazinyl derivative **2** with substituted benzaldehydes in glacial acetic acid yielded 2-(4-substituted benzylidene) hydrazinyl derivatives **10a,b.**



Scheme 2. Synthetic approaches for the preparation of thiazolopyrimidine derivatives **7-9**.

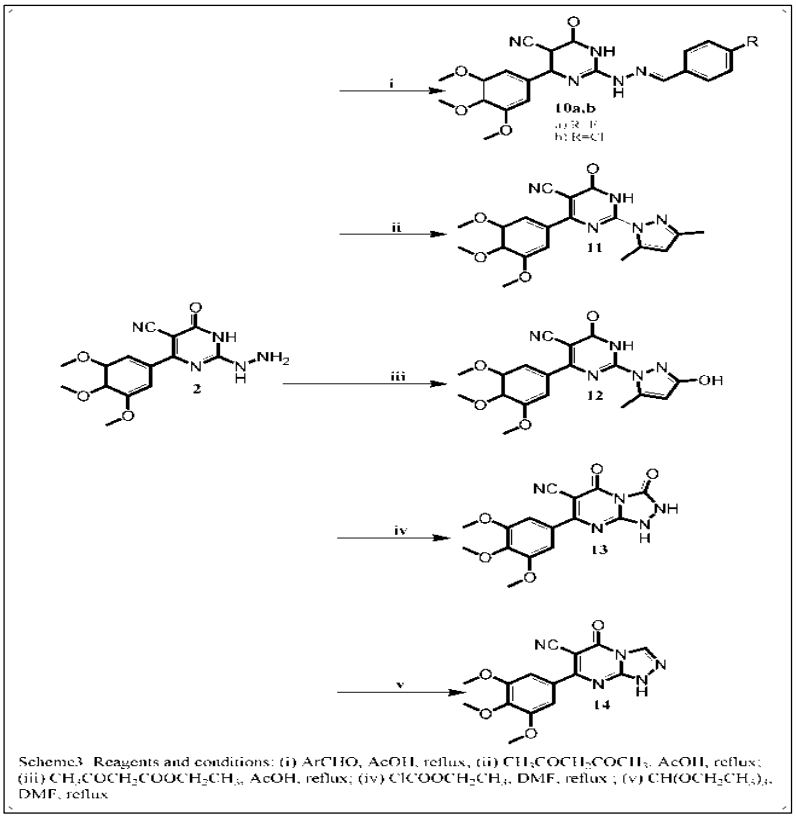
The ^{1}HNMR spectral data of derivatives **10a,b** disclosed a singlet signal at δ_H 8.20 and 8.19 ppm referring to the –N=CH- proton, respectively. Otherwise, the pyrazole derivatives **11** and **12** were accomplished via the reaction of hydrazinyl derivative **2** with various active methylene reagents, like acetylacetone and ethyl-acetoacetate, respectively. The ^{1}HNMR spectrum of compound **11** depicted three singlet signals at δ_H 2.26, 2.69 and 6.34 ppm attributable to the two methyl groups and pyrazole-H, respectively. Moreover, ^{1}HNMR spectrum of compound **12** exhibited a singlet signal at δ_H 6.89 ppm indicated to pyrazole-H, beside, D_2O exchangeable signal at δ_H 12.27 ppm assignable to OH group. On the other hand, 1,2,4-triazolo[4,3-a]pyrimidine derivatives **13** and **14** were obtained *via* the reaction of hydrazinyl derivative **2** with ethyl chloroformate and triethylorthoformate, respectively. The ^{1}HNMR spectral data of derivative **13** demonstrated two D_2O exchangeable signals at δ_H 7.22 and 7.44 ppm assignable to two NH groups. Furthermore, the ^{1}HNMR spectral data of derivative **14** depicted a singlet signal at δ_H 8.67 ppm attributed to CH=N of triazole ring (**Scheme 3**).

3.2. Biological evaluation

3.2.1. Anticonvulsant efficiency

The synthesized pyrimidine derivatives were assessed for their anticonvulsant efficiency via maximal electroshock (MES) and Pentylenetetrazole (PTZ) provoked-seizures methods utilizing Phenytoin and Carbamazepine as reference drugs (**Table 1**). The MES test is usually utilized to characterize the compounds which have significant potency against grand mal seizures, while, in the chemically evoked seizure test (PTZ), the seizure protection by the screened compounds is probably due to GABA-mediated mode of action.

In the MES model screening, compounds **8b**, **8c**, **10b** and **11** displayed the highest anticonvulsant efficacy (protection% =100%) equivalent to that of Phenytoin. Also, compound **8d** presented significant anticonvulsant effect (protection% = 83.33 %). Whereas, compounds **2**, **3**, **5**, **6**, **7**, **8a**, **10a**, **12**, **13** and **14** exhibited moderate effect with protection % ranging from 50 - 66.67 %. Otherwise, the PTZ model screening revealed a modest anticonvulsant efficiency for some of the investigated candidates **4**, **7**, **8a**, **8b**, **8c**, **8d**, **9**, **10b** and **11** (protection % = 60 %) comparing to Carbamazepine (protection% = 100 %). However, weak anticonvulsant effects were detected by compounds **2**, **3**, **5**, **6**, **10a**, **12**, **13** and **14** (protection% = 40 %) relative to Carbamazepine (protection% = 100 %). It could be deduced that compounds **8b**, **8c**, **8d**, **10b** and **11** demonstrated significant protection effects in both MES and PTZ models. Consequently, the latter derivatives could possess efficient potency against grand mal seizures and exerted their effects via GABA-mediated mechanisms.



Scheme3 Reagents and conditions: (i) ArCHO, AcOH, reflux, (ii) $CH_3COCH_2COCH_3$, AcOH, reflux; (iii) $CH_3COCH_2COOCH_2CH_3$, AcOH, reflux; (iv) $ClCOOCH_2CH_3$, DMF, reflux ; (v) $CH(OCH_2CH_3)_3$, DMF, reflux

Scheme 3. Synthetic approaches for the preparation of arylidene hydrazinyl pyrimidine,pyrazolopyrimidine and triazolopyrimidine derivatives **10-14**.

Table 1. Anticonvulsant activity of pyrimidine derivatives **2-14** utilizing MES and PTZ tests

Groups	MES	PTZ
	% protection	% protection
Control	0.00	0.00
2	66	40
3	66.67	40
4	33	60
5	50	40
6	50	40
7	50	60
8a	50	60
8b	100	60
8c	100	60
8d	83.33	60
9	33.33	60
10a	50	40
10b	100	60
11	100	60
12	66.67	40
13	50	40
14	66.67	40
Phenytoin	100	
Carbamazepine		100

3.2.2. Structure activity relationship

According to the results of MES test, it was revealed the 2-hydrazinylpyrimidine 2 showed moderate anticonvulsant effect (protection% = 66%), the anticonvulsant efficiency was retained upon the substitution of 2-hydrazinyl moiety with methyl group (protection% = 66.67%). However, significant reduction in the anticonvulsant potency was noticed by the substitution of 2-hydrazinyl moiety with phenyl group (protection% = 33%). While, the substituted thiopyrimidine derivatives 5 and 6 exhibited moderate protection% =50%. Concerning the thiazolo[3,2-a]pyrimidine derivatives 7-9, the hybridization of the

pyrimidine scaffold with unsubstituted thiazolidinone ring displayed moderate activity (protection% =50%), the activity was retained by the substitution of the thiazolidinone ring with 4-chlorobenzylidene 8a (protection% = 50%), Otherwise, the alteration of the electron withdrawing group with electron donating groups as methyl group 8b or dimethylamino 8c lead to a remarkable anticonvulsant effect (protection % = 100%). Moreover, the replacement of the substituted phenyl ring with thiophene ring 8d demonstrated a prominent anticonvulsant potency (protection% = 83.33%). Conversely, a significant decline in the efficiency was observed by the substitution of the thiazolidinone ring with 2-oxoindole scaffold 9 (protection% =33.33%). Furthermore, the substitution of the 2-hydrazinyl pyrimidine with 4-fluorobenzylidene 10a resulted in moderate protection % = 50%, while the replacement of the fluorine group with the chlorine atom markedly elevated the protection% to 100%. The conjugation of the pyrimidine scaffold with 3,5-dimethyl pyrazole moiety 11 displayed outstanding anticonvulsant efficiency (protection% = 100%). On the other hand, the alteration of one methyl group with hydroxyl group as compound 12 reduced the anticonvulsant potency (protection% = 66.67%). The hybridization of the pyrimidine scaffold with the triazolone ring 13 presented moderate anticonvulsant effect (protection% = 50%). A slight elevation in the anticonvulsant potency was detected by exchanging of the triazolone with the triazole ring 14 (protection% = 66.67%). Thus, the pyrimidine scaffold could be deemed as a principal template to synthesize a promising
Anticonvulsant agent

3.2.3. Various neurotransmitter estimation

The most prominent derivatives **8b, 8c, 8d, 10b** and **11** in the MES and PTZ tests were elected for further neurochemistry study to assess their impact on various neurotransmitters such as GABA, norepinephrine, dopamine, serotonin and glutamate. As depicted in **Table 2**, the level of GABA in the control group recorded 1.80 ± 0.06 µg/g tissue, however, the screened pyrimidine derivatives **8b, 8c, 8d, 10b** and **11** remarkably elevated the GABA levels to 2.82 ± 0.13, 3.63 ± 0.16, 3.86 ± 0.13, 2.62 ± 0.08 and 3.21±0.20 µg/g tissue, respectively, comparing to carbamazepine (3.80 ± 0.20 µg/g tissue). Furthermore, all the screened candidates **8b, 8c, 8d, 10b, 11** and carbamazepine noticeably raised the levels of norepinephrine, dopamine and serotonin neurotransmitters comparing to the control groups as shown in table 2. On the other hand, the investigated pyrimidine derivatives **8b, 8c, 8d, 10b, 11** and carbamazepine reduced the glutamate levels to 5.63 ± 0.37, 4.55 ± 0.27, 3.67 ± 0.03, 5.70 ± 0.12, 4.69 ± 0.33 and 2.65 ± 0.07 µg/g tissue, respectively, comparing to the control group (6.20 ± 0.51 µg/g tissue). It could be noticed that the pyrimidine derivatives probably exerted their anticonvulsant effects via enhancing GABA, norepinephrine, dopamine and serotonin signaling as well as through the suppression of the excitatory neurotransmitter (glutamate).

Table 2. The effect of pyrimidine derivatives **8b**, **8c**, **8d, 10b** and **11** on various neurotransmitters in the brain.

	Average ± SE (µg/g tissue)				
Group	**GABA**	**Norepinephrine**	**Dopamine**	**Serotonin**	**Glutamate**
Control	1.80±0.06$^{@}$	0.30±0.02$^{@}$	1.20±0.11$^{@}$	0.41±0.03$^{@}$	6.20±0.51$^{@}$
8b	2.82±0.13*$^{@}$	0.70±0.01*$^{@}$	2.44±0.12*$^{@}$	0.71±0.02*	5.63±0.37*$^{@}$
8c	3.63±0.16*	0.67±0.04*$^{@}$	1.95±0.10*$^{@}$	0.50±0.02*$^{@}$	4.55±0.27*$^{@}$
8d	3.86±0.13*	0.53±0.01*$^{@}$	1.80±0.04*$^{@}$	0.54±0.03*$^{@}$	3.67±0.03*$^{@}$
10b	2.62±0.08*$^{@}$	0.85±0.03*	2.63±0.07*	0.73±0.03*	5.70±0.12*$^{@}$
11	3.21±0.20*	0.70±0.02*$^{@}$	2.12±0.12*$^{@}$	0.62±0.02*$^{@}$	4.69±0.33*$^{@}$
Carbamazepine	3.80±0.20*	0.88±0.06*	2.85±0.23*	0.82±0.02*	2.65±0.07*

Values are expressed as Mean ± SE (n=6) and analyzed by one-way ANOVA, followed by Tukey's multiple comparisons post-hoc-test. (*) Significant difference from Control, (@) Significant difference from Carbamazepine. $P < 0.05$ was assumed to denote statistical significance.

***3.3. In silico* ADMET prediction study**

Analyzing the pyrimidines **8b**, **8c**, **8d, 10b** and **11**'s absorption, distribution, metabolism, and excretion (ADME) can disclose substantial data concerning the optimal drug choice which was accomplished utilizing the SwissADME application that made this investigation easier to achieve [41,42]. The Veber rule and Lipinski's rule were used to determine the typical drug for oral administration (**Table 3**). With exception of **8d**, which demonstrated TPSA > 140 and one violation to Veber rule, the screened pyrimidines **8b**, **8c**, **10b** and **11** were found to be complied with the earlier rules.

According to the bioavailability radar chart and the six essential criteria (polarity, flexibility, solubility, Lipophilicity, size and saturation), the screened pyrimidine **11** is situated in the ideal pink that provided a reasonable expectation for its oral bioavailability (**Fig.3**). Derivatives **8b**, **8c**, **8d** and **10b** remained far from saturation, while **8d** additionally showed up outside of the solubility optimum zone, suggesting that the latter would not be absorbed orally.

Table 4 provides the pharmacokinetic characteristics of the promising pyrimidine candidates **8b**, **8c**, **8d, 10b** and **11**. All derivatives illustrated an elevated probability of gastrointestinal absorption except **8d**. Indeed, it has been shown that if a given molecule inhibits more CYP enzymes, particularly the isoforms 1A2, 2C19, and 2D6 which account for 90% of oxidative metabolic reactions, then it may be more likely to participate in drug-drug interactions (DDI) with other active compounds [45]. Thus, it was anticipated that all targets would exhibit neither efficacy nor inhibition against these CYPs. Efflux of drugs out of cells through transporter p-glycoprotein (P-gp), is one potential source of drug resistance. The P-gp non-substrates of the screened derivatives imply a slight chance of their outflow leaving the cell with their highest level of activity. It is also not anticipated that these targets will trigger a PAIN warning

Table 3. Prospected physicochemical characteristics of the powerful pyrimidines **8b**, **8c**, **8d, 10b** and **11**.

Compds.	TPSA (Å²)[a]	nRB[b]	MW[c]	nHBA[d]	nHBD[e]	MLogP[f]	violations[g]
8b	131.16	5	461.49	7	0	1.68	0
8c	134.40	6	490.53	7	0	1.38	0
8d	159.40	5	453.49	7	0	1.07	0(Lipinski) 1(Veber)
10b	121.62	7	439.85	7	2	1.50	0
11	115.05	5	381.39	7	1	0.67	0
Veber rule	≤ 140	≤ 10	–	–	–	–	
Lipinski's rule	–	–	≤ 500	≤ 10	≤ 5	≤ 4.15	

[a] Topological Polar Surface Area; [b] Number of Rotatable Bond; [c] Molecular Weight; [d] Number of Hydrogen Bond Acceptor; [e] Number of Hydrogen Bond Donor; [f] Calculated Lipophilicity (MLog Po/w); [g] Violations from Veber and Lipinski Rules.

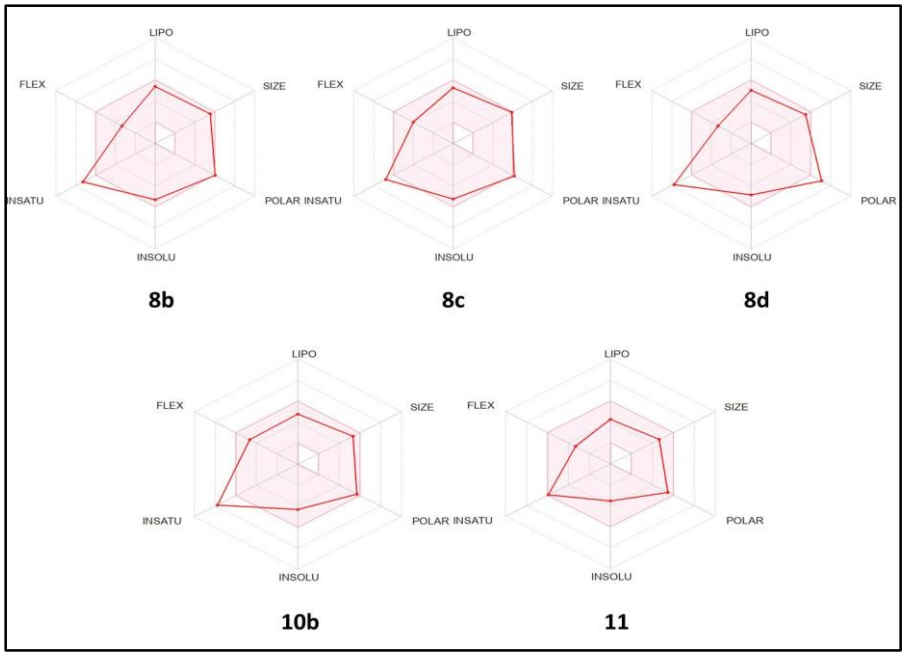


Fig. 3. The bioavailability radar chart of the potent pyrimidines **8b**, **8c**, **8d, 10b** and **11**. The predicted values for the evaluated compounds were displayed as red lines, and the optimal values for each oral bioavailability component were presented in the pink area.

Table 4. Anticipated Pharmacokinetic features of the prominent pyrimidines **8b**, **8c**, **8d, 10b** and **11**.

Comp. No.	GIT absorption	CYP1A2, CYP 2C19, CYP 2D6 inhibitor	P-gp substrate	Log K_p (skin permeation) (cm/s)	Synthetic accessibility	PAINS alert
8b	High	NO	NO	-6.31	3.78	0
8c	High	NO	NO	-6.66	3.92	1
8d	Low	NO	NO	-6.72	3.65	0
10b	High	NO	NO	-6.97	3.45	0
11	High	NO	NO	-7.24	3.33	0

GIT: gastrointestinal; CYP: Cytochrome P450; P-gp substrate: Glycoprotein substrate P.

The potent pyrimidines **8b**, **8c**, **8d, 10b** and **11** in **Table 5** did not inhibit the potassium channel of the human ether-a-go-go-related gene (hERG), as anticipated, which indicates the absence of any cardiac adverse impacts or cardiotoxicity, these are fundamental purposes in the clinical attempts of the promising drug candidates. Otherwise, all the inspected candidates did not disclose any Ames toxicity, which is essential to be detected early in the drug development process to deduce whether the screened candidates has the potential to be genotoxic. Pyrimidines **8b**, **8c**, **8d, 10b** and **11** showed values ranging from 511.1 to 631.7 mg.kg^{-1}, which renders them unharmed compounds and puts them in the third class, according to estimations of the acute oral toxicity. Moreover, these compounds may be categorized as non-carcinogenic and non-required based on the carcinogenicity descriptor (CARC) values, which range from 400.9 to 484.3 mg.kg^{-1} body weight per day. It was assumed that all derivatives of non-biodegradable compounds could be recognized in order to assess biodegradation in the environment.

Table 5. Anticipated toxicity properties of the pyrimidines **8b**, **8c**, **8d, 10b** and **11**.

	Probability				
Toxicity	**8b**	**8c**	**8d**	**10b**	**11**
hERG Inhibition T_hERG_I	0.9923 (Weak inhibitor)	0.9852 (Weak inhibitor)	0.9923 (Weak inhibitor)	0.9738 (Weak inhibitor)	0.9569 (Weak inhibito
hERG Inhibition T_hERG_II	0.7586 (Non-inhibitor)	0.5742 (Non-inhibitor)	0.7586 (Non-inhibitor)	0.7844 (Non-inhibitor)	0.7986 (Non-inhibitor)
AMES Toxicity	0.5700 (Non AMES toxic)	0.5682 (Non AMES toxic)	0.5700 (Non AMES toxic)	0.6297 (Non AMES toxic)	0.5383 (Non AMES toxic)
Carcinogens	0.8644(Non-carcinogens)	0.8213(Non-carcinogens)	0.8644 (Non-carcinogens)	0.7019(Non-carcinogens)	0.8770 (Non-carcinogens)
Acute Oral Toxicity (AO)	0.5401 (III)	0.6317 (III)	0.5401 (III)	0.5724 (III)	0.5111 (III)
Carcinogenicity (Three-class)	0.4843 (non-required)	0.4473 (non-required)	0.4843 (non-required)	0.4796 (non-required)	0.4009 (non-required
Biodegradation	0.9507 (Not ready biodegradable)	0.9837 (Not ready biodegradable)	0.9507 (Not ready biodegradable)	1.0000 (Not ready biodegradable)	0.9925 (Not ready biodegradable

3.4. Molecular docking studies

To illustrate the results of pyrimidines **8b**, **8c**, **8d, 10b** and **11** against GABA-AT enzyme and GluA2 subtype AMPA receptor, MOE-Dock software was employed. The docking of vigabatrin and perampanel (the co-crystallized ligands) were primarily accomplished inside the binding pockets of GABA-AT and GluA2 subtype AMPA (PDB codes: 1OHW and 5L1F, respectively) [43,44] to validate the docking procedure. The energy scores of –10.42 and –11.25 kcal/mol were achieved at RMSD values of 0.79 Å, indicating that the docking process was verified. After that, our pyrimidines **8b**, **8c**, **8d, 10b** and **11** were docked and the acquired results were depicted in **Figs. 4, 5**.

As established in both 2D (**Fig. 4**) and 3D patterns (**Fig. S1**, supplementary data), the binding pocket of GABA-AT contained the extremely promising pyrimidines **8b**, **8c**, **8d, 10b** and **11** with acceptable energy scores ranging from –11.38 to –9.84 kcal/mol (**Table S1**, supplementary data). In all screened derivatives, nitrogen of pyrimidine moiety provided H-bond acceptor with the sidechain of the **Arg192** amino acid. Otherwise, the sidechain of **Lys329** gave H bonding either with carbonyl oxygen of pyrimidine in derivatives **8d, 10b** and **11** or that of thiazole scaffold in **8b** and **8c**. The presence of thiophene moiety in **8d** potentiated binding of sulfur
atom with the backbone of **Glu270** through H-bond donor (distance: 3.26 Å). On the other hand, the dimethylpyrazole at p-2 of pyrimidinone core forced the molecule **11** to form extra H-bonding between the methoxy and cyano groups with **Glu270** and **Arg445** (distance: 2.91 and 3.53 Å, respectively).

Regarding to docking study of pyrimidines **8b**, **8c**, **8d, 10b** and **11** within GluA2 subtype AMPA receptor, the 2D (**Fig. 5**) and 3D patterns (**Fig. S2**, supplementary data) demonstrated the approximately similar interactions with adequate energy scores extended from –10.36 to –10.89 kcal/mol. The carbonyl oxygen of the pyrmidinone scaffold revealed H-bond acceptor with the sidechain of the key amino acid **Ser516** in all screened analogues. Furthermore, cyano nitrogen formed H-bond acceptor with **Tyr616**. From the preceding results, we deduced that the pyrimidine core in **10b** and **11** or fused with thiazole one in **8b**, **8c** and **8d** promoted hydrogen bonding interactions with the key amino acids **Arg192** and **Lys329** within GABA-AT, and **Ser516** and **Tyr616** within GluA2 subtype AMPA. Additionally, the existence of thiophene moiety in **8d** and dimethylpyrazole at p-2 of pyrimidinone core in **11** facilitated extra H-bonds within the active site of GABA-AT confirming their higher and promising *in vitro* results.

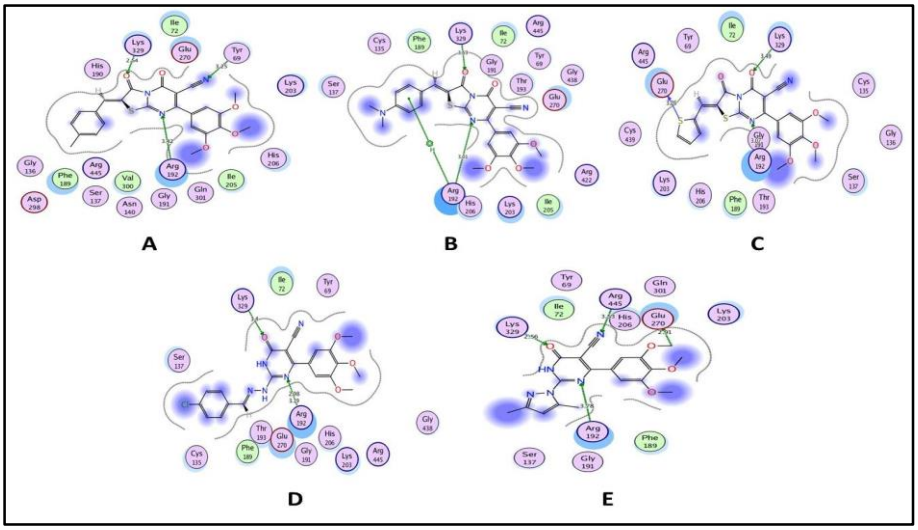


Fig.4. A-E patterns displaying the 2D binding interactions of the pyrimidines **8b**, **8c**, **8d, 10b** and **11** into the GABA-AT binding site, respectively (PDB code: 1OHW).

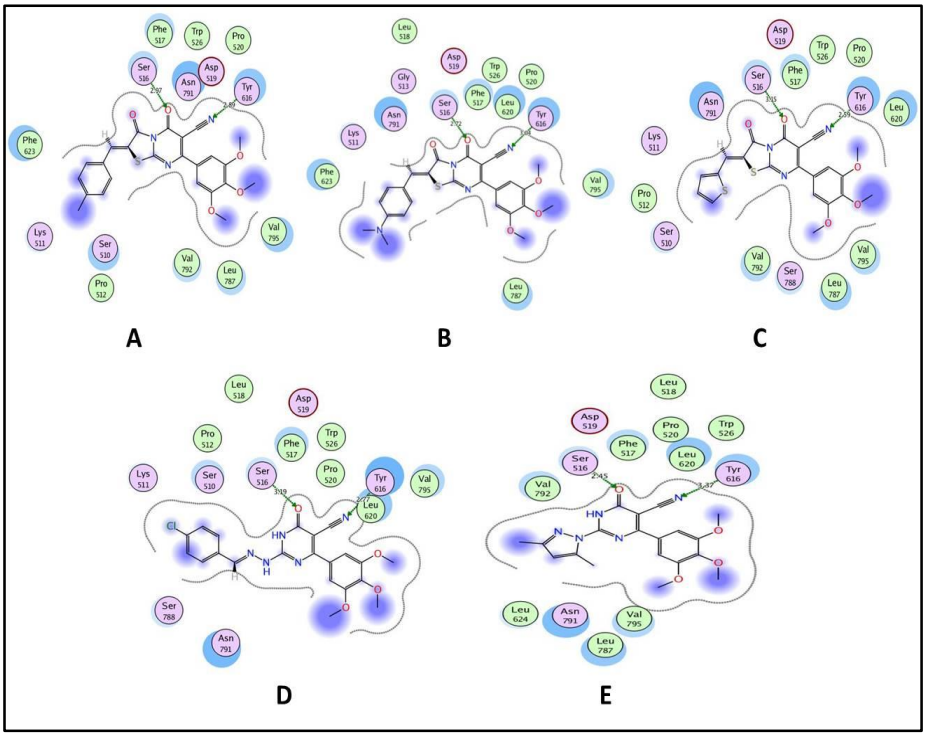


Fig.5. A- E patterns displaying the 2D binding interactions of the pyrimidines **8b**, **8c**, **8d, 10b** and **11** into the GluA2 subtype AMPA binding site, respectively (PDB code: 5L1F)

4. Conclusion

The current investigation focused on the synthesis of new substituted thiopyrimidine derivatives in addition to the conjugation of the pyrimidine scaffold with different substituted hydrazinyl moieties and various heterocyclic rings **2-14** to assess their anticonvulsant efficiency utilizing PTZ and MES tests. Compounds **8b**, **8c**, **8d**, **10b** and **11** presented prominent anticonvulsant effect comparing to Carbamazepine and Phenytoin drugs. In addition, the latter derivatives effectively elevated the GABA, norepinephrine, dopamine, serotonin levels in the brain (GABA level range 2.62-3.86 µg/g tissue, norepinephrine level range 0.53-0.85 µg/g tissue, dopamine level range 1.80-2.63 µg/g tissue, serotonin level range 0.50-0.73 µg/g tissue) comparable to that of Carbamazepine as a reference drug (3.80, 0.88, 2.85 and 0.82 µg/g tissue, respectively). On the other hand, the screened candidates remarkably reduced the glutamate levels ranging between 3.67-5.70 µg/g tissue comparing to Carbamazepine (2.65 µg/g tissue). *In silico* ADMET prediction results demonstrated that the most potent pyrimidine derivatives have good physicochemical and pharmacokinetic properties. Furthermore, the docking simulation study displayed good binding interactions for the most prominent candidates with the active sites of GABA-AT enzyme and GluA2 subtype AMPA receptor. Thus, the potent pyrimidine derivatives may exert their anticonvulsant effect via inhibiting the GABA-AT enzyme which is considered the main reason for GABA metabolism which in turn results in boosting the GABA levels in the brain as well as via blocking GluA2 subtype AMPA receptor to reduce the release of glutamate.

Conflicts of interest

There are no conflicts to declare.

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