Anticonvulsant potential of certain *N*-(6-substituted benzo[*d*] thiazol-2-yl)-2-(4-substituted piperazin-1-yl)acetamides

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Background and objectives
Epilepsy is a chronic neurological disorder. It is characterized by recurrent unprovoked occurrence of seizures that affect people of all ages. Thus, in the current work we undertook the synthesis of the joined structures of both 1, 3-benzothiazole and piperazine through amidic linkage, which will greatly foster the anticonvulsant profile of the new candidates.
Experimental
Synthesis of the target compounds *N*-(6-substituted benzo[*d*]thiazol-2-yl)-2-(4-substitued piperazinyl)acetamide derivatives (**4a–f**) was achieved. The anticonvulsant profile of these compounds at the selected dose of 100 mg/kg was investigated using maximal electroshock seizure and subcutaneous pentylenetetrazole screens as well as neurotoxicity test.
Results and discussion
Most of the synthesized compounds, **4a–f**, displayed 16.67–100% anticonvulsant activity in maximal electroshock seizure screening at a dose range of 0.22–0.31 mmol/kg. The most potent compounds were **4a** (ED_{50} = 58 mg/kg≡0.15 mmol/kg), **4b** (ED_{50} = 64 mg/kg≡0.19 mmol/kg), and **4c** (ED_{50} = 60 mg/kg≡0.19 mmol/kg). Compound **4a** was the only one that exhibited 100% protection in the subcutaneous pentylenetetrazole screen with ED_{50} = 56 mg/kg≡0.15 mmol/kg. It possessed potent activity that was about six-fold more than that of ethosuximide (ED_{50} = 130 mg/kg≡0.92 mmol/kg), which was used as a reference drug, and lower than that of phenobarbital (ED_{50} = 13.20 mg/kg≡0.06 mmol/kg).

Keywords:
anticonvulsants, benzothiazole, epilepsy, synthesis

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Introduction

Epilepsy, one of the oldest chronic neurological disorders, is characterized by fear, discrimination, and social manifestations. It is characterized by periodic and unpredictable occurrence of seizures and affects people of all ages [1]. The WHO, International Bureau for Epilepsy (IBE), and International League Against Epilepsy (ILAE) stated that 1% of the world's population is epileptic. Further, annually 2.4 million new cases are diagnosed with this disorder. Despite the adequacy of the current antiepileptic drugs, such as pregabalin, stiripentol, zonisamide, tiagabine, lamotrigine, levetiracetam, and topiramate, in seizure control, about 30% of patients are estimated to be poorly treated [2,3]. These new drugs have demonstrated high efficacy in seizure control, although they are associated with some undesirable and painful side effects such as headache, nausea, hepatotoxicity, anorexia, ataxia, drowsiness, gastrointestinal disturbances, and hirsutism [4,5]. That is why the search for new antiepileptic compounds with improved activity and lower toxicity continues to be an area of investigation in medicinal chemistry.

2-Aminobenzothiazole is a ubiquitous heterocyclic nucleus prevalent in many marine and natural plant products and has a varied range of biological applications[6–12]. Besides riluzole, which is a clinically available antiepileptic drug [13–15], many other 2-aminobenzothiazoles have been documented as very effective anticonvulsant agents [16–19]. In contrast, piperazine is one of the most widely used heterocyclics for the development of new drug candidates. Its derivatives are driving versatile pharmacological activities [20–23]. Also, many compounds containing piperazine scaffold are endowed with potent anticonvulsant activity [24–27]. In addition, the amide moiety has been

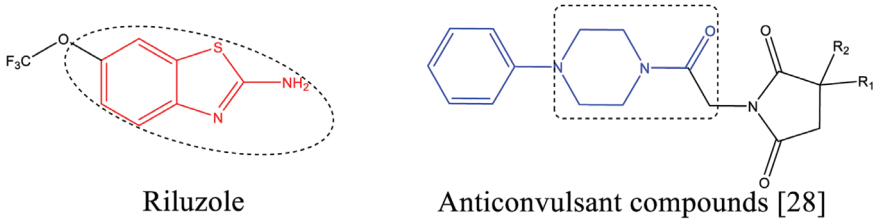
documented as a crucial pharmacophore in anticonvulsant activity [28]. Thus, in the current work we sought to synthesize the joint structures of both 1, 3-benzothiazole and piperazine through amidic linkage with the aim to foster the anticonvulsant profile of new candidates (Fig. 1).

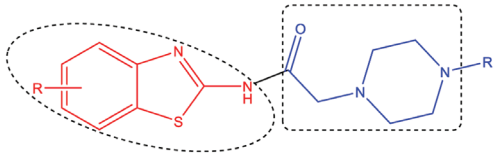
Materials and methods

Chemistry

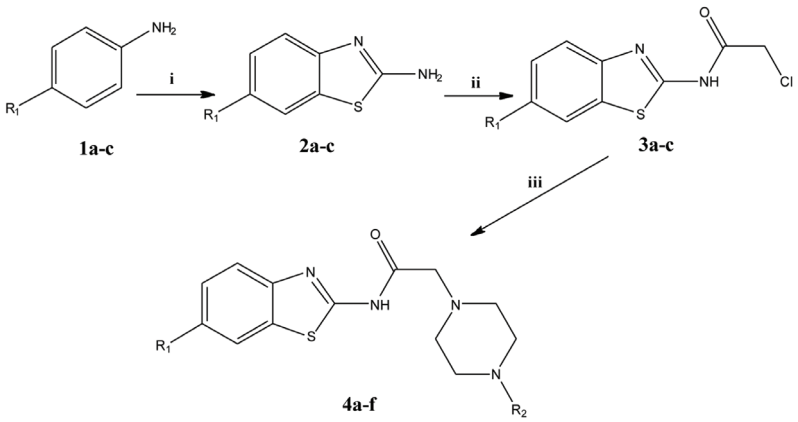
All melting points were uncorrected and were determined with an electrothermal capillary melting point apparatus. Infrared (IR) spectra were recorded as thin film (for oils) in NaCl discs or as KBr pellets (for solids) using a JASCO FT/IR-6100 spectrometer, and values are represented in cm^{-1}. ^{1}H NMR and ^{13}C NMR spectra were determined on a Jeol ECA 500 MHz spectrometer using Tetramethylsilane as internal standard, and chemical shift values were recorded in ppm on scale. The mass spectra were run on Finnigan Mat SSQ-7000 spectrometer and Jeol JMS-AX 500. Elemental analyses were carried out at the Microanalytical Unit, National Research Centre, Cairo, Egypt. Silica gel plates (60F254; Merck, Darmstadt, Germany) were used for thin-layer chromatography. Visualization was performed by illumination with UV light source (254 nm). Column chromatography was performed on silica gel 60 (0.063–0.200) purchased from Merck.

Figure 1





4a-f



	R_1	R_2
4a	Br	CH_2CH_3
4b	Cl	CH_2CH_3
4c	CH_3	CH_2CH_3
4d	Br	$CH_2C_6H_5$
4e	Cl	$CH_2C_6H_5$
4f	CH_3	$CH_2C_6H_5$

Ligand-based design of the target candidates **4a–f**. Scheme 1 1Schematic diagram for the synthesis of compounds **4a–f**. Reagents and conditions: (i): KSCN/Br_2/AcOH, room temperature, 2–4 h; (b): $ClCH_2COCl$/anhyd.K_2CO_3/benzene, reflux, 6–12 h; (c): 4-ethyl or 4-benzyl piperazine hydrochloride/anhyd. K_2CO_3/acetone, reflux, 6–12 h (Method 1) or benzyl piperazine hydrochloride/TEA/DMF, reflux 6 h, then H_2O, room temperature,12 h (Method 2).

General synthetic procedure for 6-substituted benzothiazol-2-amine (2a-c)

A mixture of 0.1 mol of 4-substituted aniline and 0.1 mol of potassium thiocyanate in 100 ml glacial acetic acid was cooled in an ice bath and stirred for 10–20 min; thereafter, 0.1 mol bromine in glacial acetic acid was added dropwise at such a rate to keep the temperature below 10°C throughout the addition. The reaction mixture was stirred at room temperature for 2–4 h. The separated hydrobromide salt was filtered, washed with acetic acid, dried, dissolved in hot water, and neutralized with ammonia solution; and the resulting precipitate was filtered, washed with water, and dried to obtain the desired products **2a–c**. The progress of the reaction was monitored by thin-layer chromatography using toluene: acetone (8: 2) solvent system. The physical characters and spectral data of **2a–c** were in agreement with the reported data [17,29].

6-Bromobenzo[d]thiazol-2-amine (2a)

Light yellow solid, yield: 76%, mp: 215–217°C (Lit. [29] mp: 216–219°C). IR (KBr, cm^{-1}): 3448 (NH), 1526 (C = N). ^1H NMR ($CDCl_3$) δppm: 6.72 (s, 2H, NH_2, D_2O exchangeable), 7.39–7.46 (m, 2H, H_{ar}), 7.72 (d, J = 1.5 Hz, 1H, H_{ar}).

6-Chlorobenzo[d]thiazol-2-amine (2b)

White solid, yield: 72%, mp: 201–203°C (Lit. [29] mp: 202–203°C). IR (KBr, cm^{-1}): 3341 (NH), 1544 (C = N); ^1H NMR ($CDCl_3$) δppm: 6.72 (s, 2H, NH_2, D_2O exchangeable), 7.21–7.35 (m, 2H, H_{ar}), 7.52 (d, J = 1.7 Hz, 1H, H_{ar}).

6-Methylbenzo[d]thiazol-2-amine (2c)

Light yellow solid, yield: 70%, mp: 144–146°C (Lit. [29] mp: 144–145°C). IR (KBr, cm^{-1}): 3343 (NH), 1550 (C = N). ^1H NMR ($CDCl_3$) δppm: 1.96 (s, 3H, CH_3), 5.89 (s, 2H, NH_2, D_2O exchangeable),7.53–7.39 (m, 3H, H_{ar}).

General synthetic procedure for 2-chloro-N-(6-substituted benzo[d]thiazol-2-yl) acetamides (3a–c)

Chloroacetyl chloride (0.06 mol) was added dropwise to a mixture of the appropriate 2-amino-6-substituted benzo[*d*]thiazole (**2a–c**) (0.05 mol) and K_2CO_3 (0.06 mol) in benzene (50 ml) at room temperature. The reaction mixture was refluxed for 6–12 h.

After cooling to room temperature, it was slowly poured into 100 ml of ice cold water. A solid was formed thereafter. The precipitate was separated by filtration and washed successively with water. The product was dried under vacuum to obtain **3a–c**.

The progress of the reaction was monitored by thin-layer chromatography using the toluene: acetone (8: 2) solvent system.

The physical characters and spectral data of **3a–c** were in agreement with the reported data [29–32].

2-Chloro-N-(6-bromobenzo[d]thiazol-2-yl)acetamide (3a)

Light yellow solid, yield: 72%, mp: 194–196°C (Lit. [29] mp: 195–197°C). IR (KBr, cm^{-1}): 3275 (NH), 1667 (C = O). ^1H NMR ($CDCl_3$) δppm: 4.06 (s, 2H, -CH_2-Cl), 7.35–7.43 (m, 2H, H_{ar}), 7.60 (d, J = 1.7 Hz, 1H, H_{ar}), 8.43 (s, 1H, -NH); MS (EI) m/z (%): 305.58 (40) (M^++1), 228 (100).

2-Chloro-N-(6-chlorobenzo[d]thiazol-2-yl)acetamide (3b)

While solid, yield: 71%, mp: 207–209°C (Lit. [29] mp: 207–210°C). IR (KBr, cm^{-1}): 3271 (NH), 1666 (C = O); ^1H NMR ($CDCl_3$) δppm: 4.17 (s, 2H, -CH_2-Cl), 7.27–7.45 (m, 2H, H_{ar}), 7.64 (d, J = 1.6 Hz, 1H, H_{ar}), 8.56 (s, 1H,-NH). MS (EI) m/z (%): 262.03 (20) (M^+ + 1), 260.03 (40), 184.08 (100).

2-Chloro-N-(6-methylbenzo[d]thiazol-2-yl)acetamide (3c)

Light yellow solid, yield: 53%, mp: 174–176 (Lit. [29] 175–177°C). IR (KBr, cm^{-1}): 3291 (NH), 1670 (C = O). ^1H NMR ($CDCl_3$) δppm: 1.81 (s, 3H, CH_3), 4.20 (s, 2H,-CH_2-Cl), 7.63–7.46 (m, 3H, H_{ar}), 8.45 (s, 1H, NH). MS (EI) m/z (%): 239.5 (60) (M^+), 164.11 (100).

General procedure for the synthesis of N-(6-substituted benzothiazol-2-yl)-2-(4- substituted piperazin-1-yl) acetamides (4a–f)

Method 1: A mixture of 0.0025 mol of 2-chloro-*N*-(6-substituted benzo [*d*]thiazol-2-yl) acetamides (**3a–c**) and 0.0025 mol of the suitable piperazine derivative in acetone (50 ml), in the presence of 0.0025 mol anhydrous K_2CO_3, was refluxed for 18 h. The reaction was monitored by thin-layer chromatography with silica gel plate and benzene: methanol (9: 1) mobile phase mixture. Potassium carbonate was removed by filtration. After evaporation of acetone, the precipitated products were recrystallized from absolute ethanol or acetone: distilled water mixture to afford **4a–d** in 30–41% yields.

Method 2: To a solution of 0.003 mol of 2-chloro-*N*-(6-chloro- or 6-methyl-benzo[*d*]thiazol-2-yl) acetamides (**3b or 3c**) and 0.004 mol of benzyl piperazine

hydrochloride in Dimethylformamide (DMF) (15 ml) was added six drops of triethylamine. The reaction mixture was refluxed for 6 h, then diluted with H_2O (20 ml), and stirred at room temperature for 12 h. The solid was filtrated and recrystallized from absolute ethanol to afford **4e** or **4f** in 39% yields.

N-(6-Bromobenzo[d]thiazol-2-yl)-2-(4-ethylpiperazin-1-yl) acetamide (4a)

Yellow solid, yield: 35%, mp: 146–150°C. IR (KBr, cm^{-1}): 3287 (NH), 1702 (C=O); ^{1}H NMR ($CDCl_3$) δppm: 1.05–1.13 (t, J=7.1 Hz, 3H, CH_3), 2.42–2.57 (m, 10H, CH_2-CH_3, 4×CH_2 piperazinyl), 3.31 (s, 2H, O=C-CH_2-N), 7.26–7.74 (m, 3H, H_{ar}), 10.41 (br.s., 1H, NH); ^{13}C NMR ($CDCl_3$) δppm: 12.05 (CH_3), 52.48, 52.66, 53.76 (CH_3-CH_2, 4×CH_2 piperazinyl), 61.30 (O=C-CH_2-N), 121.18, 121.90, 127.08, 129.64 (3×CH_{ar},1×C_{ar}), 133.60 (C_{ar}), 147.17 (C_{ar} -N), 157.71 (C=O), 169.40 (N=C-S, thiazolyl); MS (EI) m/z (%): 383 (5) (M^++1), 228 (10), 127 (100); Anal. Calcd. for $C_{15}H_{19}Br\ N_4OS$: C, 47.00; H, 5.00; N,14.62. Found: C, 47.09; H, 5.05; N, 14.60.

N-(6-Chlorobenzo[d]thiazol-2-yl)-2-(4-ethylpiperazin-1-yl) acetamide (4b)

Light yellow solid, yield: 31%, mp: 176–180°C; IR (KBr, cm^{-1}): 3266 (NH), 1702 (C=O); ^{1}H NMR ($CDCl_3$) δppm: 1.08–1.11 (t, J=7.1 Hz, 3H, CH_3), 2.45–2.68 (m, 10H, CH_2-CH_3, 4×CH_2 piperazinyl), 3.28 (s, 2H, O=C-CH_2-N), 7.37–7.77 (m, 3H, H_{ar}), 10.39 (br.s., 1H, NH); ^{13}C NMR ($CDCl_3$) δppm: 12.01 (CH_3), 52.28, 52.64, 53.72 (CH_3-CH_2, 4×CH_2 piperazinyl), 61.10 (O=C-CH_2-N), 121.18, 121.90, 127.08, 129.64 (3×CH_{ar},1×C_{ar}), 133.60 (C_{ar}), 147.17 (C_{ar} -N), 157.51 (C=O), 169.39 (N=C-S, thiazolyl); MS (EI) m/z (%): 338.5 (10) (M^+), 211.02 (8), 184.03 (9), 127 (100); Anal. Calcd. for $C_{15}H_{19}Cl\ N_4OS$: C, 53.17; H, 5.65; N,16.53. Found: C, 53.20; H, 5.69; N, 16.60.

2-(4-Ethylpiperazin-1-yl)-N-(6-methylbenzo[d]thiazol-2-yl) acetamide (4c)

Yellow white solid, yield: 30%, mp: 98–102°C. IR (KBr, cm^{-1}): 3430 (NH), 1701 (C=O); ^{1}H NMR ($CDCl_3$) δppm: 1.08–1.11 (t, J=7.1 Hz, 3H, CH_3-CH_2), 2.45–2.68 (m, 13H, CH_2-CH_3, CH_3-C_{ar}, 4×CH_2 piperazinyl), 3.37 (s, 2H, O=C-CH_2-N), 7.23–7.66 (m, 3H, H_{ar}), 10.40 (br.s., 1H, NH); ^{13}C NMR ($CDCl_3$) δppm: 11.99 (CH_3-CH_2), 21.58 (CH_3-C_{ar}) 52.28, 52.64, 53.70 (CH_3-CH_2, 4×CH_2 piperazinyl), 61.19 (O=C-CH_2-N),120.63, 121.34, 127.86 (3×CH_{ar}), 132.44, 134.17 (2×C_{ar}), 146.46 (C_{ar}-N), 156.47 (C=O), 169.20 (N=C-S, thiazolyl); MS (EI) m/z (%): 319 (10) (M^++1), 205 (4), 191 (27), 163 (30); Anal. Calcd. for $C_{16}H_{22}N_4OS$: C, 60.35; H, 6.96; N,17.59. Found: C, 60.30; H, 6.89; N, 17.62.

2-(4-Benzylpiperazin-1-yl)-N-(6-bromobenzo[d]thiazol-2-yl) acetamide (4d)

Yellow solid, yield: 41%, mp: 197–200°C. IR (KBr, cm^{-1}): 3292 (NH), 1706 (C=O); ^{1}H NMR ($CDCl_3$) δppm: 2.56 (t, J=5.4 Hz, 4H, 2×CH_2, piperazinyl), 2.66 (t, J=4.8 Hz, 4H, 2×CH_2, piperazinyl), 3.27 (s, 2H, O=C-CH_2-N), 3.54 (s, 2H. CH_2-Ph), 7.25–7.32 (m, 5H, H_{ar}), 7.63–7.93 (m, 3H, H_{ar}), 10.49 (br.s., 1H, NH); ^{13}C NMR ($CDCl_3$) δppm: 52.93, 53.81 (4×CH_2 piperazinyl), 61.12 (O=C-CH_2-N), 62.95 (CH_2-Ph), 117.09 (C_{ar}), 122.31, 124.07, 127.35, 128,42, 129.26, 129.80 (8×CH_{ar}), 134.09 (C_{ar}), 137.80 (C_{ar}), 147.52 (C_{ar}), 157.54 (C=O), 169.52 (N=C-S, thiazolyl); MS (EI) m/z (%): 444 (100) (M^+), 353 (10), 91 (100); Anal. Calcd. for $C_{20}H_{21}BrN_4OS$: C, 53.94; H, 4.75; N, 12.58. Found: C, 53.91; H, 4.79; N, 12.60.

2-(4-Benzylpiperazin-1-yl)-N-(6-chlorobenzo[d]thiazol-2-yl) acetamide (4e)

Light yellow solid, yield: 39%, mp: 118–120°C; IR (KBr, cm^{-1}): 3264 (NH), 1705 (C=O); ^{1}H NMR ($CDCl_3$) δppm: 2.56 (t, J=5.4 Hz 4H, 2×CH_2, piperazinyl), 2.66 (t, J=4.8 Hz, 4H, 2×CH_2, piperazinyl), 3.28 (s, 2H, O=C-CH_2-N), 3.54 (s, 2H. CH_2-Ph), 7.25–7.32 (m, 5H, CH_{ar}), 7.63–7.93 (m, 3H, CH_{ar}), 10.49 (br.s., 1H, NH); ^{13}C NMR ($CDCl_3$) δppm: 52.90, 53.77 (4×CH_2 piperazinyl), 61.11 (O=C-CH_2-N), 62.93 (CH_2-Ph), 121.18 (C_{ar}), 121.90, 127.09, 127.38, 128,44, 129.29, 129.64 (8×CH_{ar}), 133.58 (C_{ar}), 137.70 (C_{ar}), 147.16 (C_{ar}), 157.56 (C=O), 169.51 (N=C-S, thiazolyl); MS (EI) m/z (%): 400.23 (5) (M^+), 309.06 (3), 189.18 (15), 91 (100); Anal. Calcd. for $C_{20}H_{21}ClN_4OS$: C, 59.91; H, 5.28; N, 13.97. Found: C, 59.79; H, 5.30; N, 13.99.

2-(4-Benzylpiperazin-1-yl)-N-(6-methylbenzo[d]thiazol-2-yl) acetamide (4f)

Light yellow solid, yield: 39%, mp: 100–104°C. IR (KBr, cm^{-1}): 3254 (NH), 1706 (C=O); ^{1}H NMR ($CDCl_3$) δppm: 2.26–2.75 (m, 11H, CH_3-C_{ar}, 4×CH_2 piperazinyl), 3.26 (s, 2H, O=C-CH_2-N), 3.54 (s, 2H. CH_2-Ph), 7.24–7.31 (m, 5H, H_{ar}), 7.32–7.59 (m, 3H, H_{ar}), 10.46 (br.s.,1H, NH); ^{13}C NMR ($CDCl_3$) δppm: 21.59 (CH_3), 52.93, 53.77 (4×CH_2 piperazinyl), 61.19 (O=C-CH_2-N), 62.97 (CH_2-Ph), 120.64, 121.35, 127.34, 127.49,127.87,128.42,129.31,

132.44 ($8\times CH_{ar}$, $1\times C_{ar}$), 134.17, 137.77 ($2\times C_{ar}$), 146.44 (C_{ar}-N), 156.48 (C=O), 169.31 (N=C-S, thiazolyl); MS (EI) m/z (%): 381 (25) ($M^{+}+1$), 290.28 (20), 189.30 (30), 191.25 (100); Anal. Calcd. for $C_{21}H_{24}N_4OS$: C, 66.29; H, 6.36; N, 14.72. Found: C, 66.34; H, 6.39; N, 14.70.

Materials and methods

Materials

Animals

Adult male albino mice (18–25 g) were used in this study. The animals were purchased from the Animal House Colony of the National Research Centre, Cairo, Egypt and housed under standardized conditions of room temperature (23 ± C), relative humidity (55 ± 5%), and light (12-h light/dark cycle) and had free access to tap water as well as standard mice chow throughout the experimental period. The procedures on animals, and their care, were performed as per the guidelines of the Ethics Committee of the National Research Centre and the 'Canadian Council on Animal Care Guidelines 1984'. All efforts were made to minimize the suffering of animals and to use only the number of animals necessary to produce reliable data.

Drugs and chemicals

Diphenylhydantoin (Nasr Co., Giza, Egypt), ethosuximide (Pfizer Co., Giza, Egypt), phenobarbital (Memphis Co. for Pharmaceutical and Chemical Industries, Cairo, Egypt), pentylenetetrazole, and Tween 80 (Sigma, St Louis, Missouri, USA) were used. The reference drugs and tested compounds were administered intraperitoneally at a volume of 0.1 ml/10 g body weight.

Methods

Animals were adapted to laboratory conditions for 7 days. They were then randomly assigned to control, reference, and tested groups consisting of six mice each. Every animal was used once. All tested compounds were suspended in 7% Tween 80.

Maximal electroshock seizure screen [33]

The reference group of animals received diphenylhydantoin (45 mg/kg, 0.16 mmol/kg) as the reference drug. A second group received the vehicle and served as the control group. Other groups received the test compounds individually by intraperitoneal injection at a dose level equivalent to 100 mg/kg [34].

Thirty minutes later electroconvulsions were induced through a current (fixed intensity of 25 mA, 0.2 s stimulus duration) delivered through a ear-clip electrode by means of a Rodent Shocker Generator (Type 221; Hugo Sachs Elektronik, Freiburg, Germany). The maximal seizures characterized by a short period of initial tonic flexion and a prolonged period of tonic extension followed by terminal clonus lasted ~22 s. Failure to extend the hind limbs to an angle with the trunk greater than 90° was considered as indicating protection [35].

Subcutaneous pentylenetetrazole-induced seizures screen [36]

The control group of mice was treated with the solvent alone. Meanwhile, the other groups received (intraperitoneal) the reference drugs ethosuximide (150 mg/kg−1.06 mmol/kg), phenobarbital (30 mg/kg−0.13 mmol/kg), or one of the compounds under investigation. Thirty minutes later, pentylenetetrazole was administered subcutaneously in the loose folds of the skin on the back of the neck at a dose of 85 mg/kg [37]. Each animal was observed for 30 min. Failure to observe a threshold seizure (a single episode of clonic spasm of at least 5 s duration) was defined as protection [38].

Neurotoxicity [39]

Neurotoxicity was tested with the rotarod test, which is designed to detect minimal neurological defect. In this test, the animals were trained to maintain equilibrium on a rotating 1-inch-diameter knurled plastic rod at a speed of 6 rpm for at least 1 min in each of three trials using a rotarod device (UGD Basile, Varese, Italy). Only animals that fulfilled this criterion were included in the experiment. The selected animals were then divided between the control group, in which the mice received the vehicle, and the experimental groups, in which the mice received (intraperitoneal) one of the test compounds (in mmol/kg−100 mg/kg in 7% aqueous suspension of Tween 80). Thirty minutes later, the mice were placed again on the rotating rod and the motor performance time was recorded up to 60 s. The neurotoxicity was indicated by the inability of the animal to maintain equilibrium on the rod for at least 60 s.

Median effective dose (ED_{50})

ED_{50} was the dose of the drug required to produce the desired biological response in 50% of animals. Groups of eight mice each were given a range of intraperitoneal doses of the test compound until at least three points were established in the range of 15–84% seizure protection. From the plot of these data, the respective ED_{50} value and the confidence limits were calculated [40].

Results and discussion

Chemistry

Synthesis of the compounds *N*-(6-substituted benzothiazol-2-yl)-2-(4-substituted piperazinyl) acetamide derivatives (**4a–f**) is outlined in Scheme 1. The target compounds were obtained through three stages: first, the substituted 2-amino-benzothiazole derivatives **2a–c** were formed by treating the appropriate substituted anilines **1a–c** with potassium thiocyanate and bromine in acetic acid according to the reported procedure [17,29]. Thereafter, the amines **2a–c** were chloroacetylated in the presence of anhydrous potassium carbonate in benzene to achieve the respective compounds **3a–c** in good yields as described in the literature [30–32]. Reaction of the chloroacetylated compounds **3a–c** and ethyl or benzyl piperazine hydrochlorides under basic conditions using anhydrous potassium carbonate or triethylamine in acetone or DMF (Methods 1 and 2, respectively) afforded the target compounds **4a–f** in 30–41% yields (c.f. experimental).

Pharmacology

The compounds under investigation were subjected to preliminary anticonvulsant screening according to the standard procedure adopted by the Antiepileptic Drug Development (ADD) program [41], which includes the 'gold standard' screens in the early stages of testing (phase 1). These include the following: (a) the maximal electroshock seizure (MES) screen that is indicative of the ability of the test compounds to prevent seizure spreading; and (b) the subcutaneous pentylenetetrazole (sc PTZ) screen that identifies compounds that elevate the seizure threshold. Compounds that exhibited 100% protection against the induced seizures were subjected to median effective dose (ED_{50}) evaluation as well as estimation of the minimal motor impairment (neurotoxicity).

The anticonvulsant activity expressed as % protection of the test compounds *N*-(6-substitutedbenzothiazol-2-yl)-2-(4-substitued piperazinyl)acetamide derivatives (**4a–f**) as well as their neurotoxicity is presented in Table 1. The initial anticonvulsant evaluation indicated that all the tested compounds were effective in intraperitoneal MES and sc PTZ screens as the new entities showed protection in the range of 16.67–100% at the tested dose level equivalent to 100 mg/kg after 30 min from compound administration.

In the MES screen the obtained data indicated the ability of the tested compounds to protect mice from seizure spreading. Compounds **4a**, **4b**, and **4c** were the most potent congeners as they exhibited 100% protection against maximal electric shock at dose levels of 0.26, 0.29, and 0.31 mmol/kg (−100 mg/kg), respectively. Meanwhile, diphenylhydantoin used as a reference drug exhibited 100% protection at a dose level of 0.16 mmol/kg (−45 mg/kg).

In the sc PTZ screen, compound **4a** at a dose of 0.26 mmol/kg (−100 mg/kg) was the most potent congener as it exhibited 100% protection against sc PTZ-induced seizures in mice. Meanwhile, the reference drugs phenobarbital and ethosuximide exhibited 100% protection at dose levels of 0.13 and 1.06 mmol/kg, respectively. Compounds **4b** (0.29 mmol/kg) and **4f** (0.26 mmol/kg) exhibited equipotent activity of 50% protection at the tested dose levels (equivalent to 100 mg/kg).

With regard to the neurotoxicity, acute toxicity from antiepileptic drugs in rodents is shown by neurological deficits, which include ataxia, sedation, impaired righting reflexes, and altered motor activity, collectively are termed 'neurotoxicity'. The standardized test, the rotarod test, can detect the minimal neurological defect, such as impaired motor function [42]. Compounds that exhibited 100% protection in MES and/or sc PTZ screens were subjected to neurotoxicity estimation as well as ED_{50} evaluation.

Results of the neurotoxicity test (Table 1) revealed that compounds **4d** and **4e** were devoid of neurotoxicity. On the other hand, compounds **4a** and **4f** exhibited minimal neurotoxicity. Meanwhile, compounds **4b** and **4c** showed moderate toxicity at the tested dose. Table 2 shows the ED_{50} of the selected compounds. Compound **4a** gave an ED_{50} of 58 mg/kg (−0.15 mmol/kg) in the MES screen. Moreover, in the sc PTZ screen, only compound **4a** showed 100% protection against the induced seizures with ED_{50} = 56 mg/kg (−0.15 mmol/kg), about six-fold more potent than that of the reference drug ethosuximide (ED_{50} = 130 mg/kg−0.92 mmol/kg). Meanwhile, it had lower potency than that of phenobarbital (ED_{50} = 13.20 mg/kg−0.06 mmol/kg).

From the present results we can deduce that compound **4a** was the most potent congener as it exhibited 100% protection against both MES and sc PTZ-induced seizures with minimal neurotoxicity. Meanwhile, compounds **4b** and **4c** exhibited 100% protection against MES-induced seizures only and

Table 1 Anticonvulsant profile (sc PTZ and MES screens) and neurotoxicity of *N*-(6-substituted benzothiazol-2-yl)-2-(4-substituted piperazin-1-yl)acetamides (4a–f) as well as the reference drugs in male albino mice

Compound number	Dose[a] (mmol/kg)	% Protection		Neurotoxicity[b]
		MES	sc PTZ	
4a	0.26	100	100	1/6
4b	0.29	100	50	4/6
4c	0.31	100	33	3/6
4d	0.22	33	16.67	0/6
4e	0.25	16.67	66.67	0/6
4f	0.26	50	50	1/6
Diphenylhydantoin	0.16	100	–	ND
Phenobarbital	0.13	–	100	ND
Ethosuximide	1.06	–	100	ND

–: indicates the absence of anticonvulsant activity at the tested dose level.
[a]Dose equivalent to 100 mg/kg.
[b]Number of animals exhibiting neurotoxicity/number of animals tested.
MES, maximal electroshock seizure; ND, not determined; sc PTZ, subcutaneous pentylenetetrazole.

Table 2 Median effective dose (ED_{50} = mg/kg and mmol/kg) of 2-(4-ethylpiperazin-1-yl)-*N*-(6-substituted benzo[*d*]thiazol-2-yl) acetamides (4a–c) exhibiting 100% protection against MES seizures in adult male albino mice using diphenylhydantoin as the reference standard

Compound number	ED_{50} (confidence limits) (mg/kg mmol/kg)	
4a[a]	58 (70.28–47.87)	0.15 (0.18–0.12)
4b	64 (74.15–55.24)	0.19 (0.22–0.16)
4c	60 (71.23–50.54)	0.19 (0.22–0.16)
Diphenylhydantoin	9.5 (7.80–11.60)	0.04 (0.05–0.03)

[a]ED_{50} in sc PTZ screen = 56 (97.20–32.26) mg/kg; 0.146 (0.25–0.08) mmol/kg; ethosuximide (ED_{50} = 130 mg/kg−0.92 mmol/kg) and phenobarbital (ED_{50} = 13.20 mg/kg−0.06 mmol/kg).
MES, maximal electroshock seizure; sc PTZ, subcutaneous pentylenetetrazole.

demonstrated moderate neurotoxicity. It was observed from the MES screen that the 4-ethyl piperazine derivatives **4a** (ED_{50} = 58 mg/kg−0.15 mmol/kg), **4b** (ED_{50} = 64 mg/kg−0.19 mmol/kg), and **4c** (ED_{50} = 60 mg/kg−0.19 mmol/kg) were the most active congeners, whereas substituting the 4-ethyl piperazine with 4-benzyl piperazine as in compounds **4d**, **4e**, and **4f** decreased the anticonvulsant activity.

Conclusion

Synthesis and determination of the anticonvulsant potential of certain *N*-(6-substitutedbenzo*[d]*thiazol-2-yl)-2-(4-substitued piperazinyl)acetamide derivatives (**4a–f**) were undertaken. Most of the compounds displayed 16.67–100% anticonvulsant activity in the MES screen at a dose range of 0.22–0.31 mmol/kg. The most potent compounds were **4a** (ED_{50} = 58 mg/kg−0.15 mmol/kg), **4b** (ED_{50} = 64 mg/kg−0.19 mmol/kg), and **4c** (ED_{50} = 60 mg/kg−0.19 mmol/kg). Compound **4a** was the only one that displayed 100% protection in the sc PTZ screen with ED_{50} = 56 mg/kg (−0.15 mmol/kg). It possessed potent activity that was about six-fold more than that of ethosuximide (ED_{50} = 130 mg/kg−0.92 mmol/kg) and lower than that of phenobarbital (ED_{50} = 13.20 mg/kg−0.06 mmol/kg), which were used as reference drugs.

Conflicts of interest

The authors declared that there is no conflict of interest.

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