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PII: S0223-5234(12)00694-0

DOI: 10.1016/j.ejmech.2012.11.025

Reference: EJMECH 5851

To appear in: *European Journal of Medicinal Chemistry*

Received Date: 7 May 2012

Revised Date: 14 November 2012

Accepted Date: 16 November 2012

Please cite this article as: S. Botros, N.A. Khalil, B.H. Naguib, Y. El-Dash, Synthesis and Anticonvulsant Activity of New Phenytoin Derivatives, *European Journal of Medicinal Chemistry* (2012), doi: 10.1016/j.ejmech.2012.11.025.

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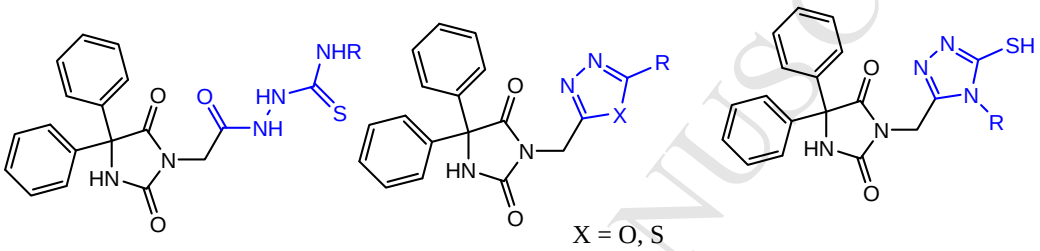
Graphical abstract

Synthesis and Anticonvulsant Activity of New Phenytoin Derivatives

Hybrid approach was used to design and synthesize hybrids between phenytoin and thiosemicarbazide, 1,3,4-oxadiazole, 1,3,4-thiadiazole or 1,2,4-triazole pharmacophores to examine their anticonvulsant activity using MES and scPTZ screens in mice.



5a-e **4, 6a-e** **7a-e**

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Research Highlights

- Synthesis of phenytoin derivatives.
- Anticonvulsant activity of all new synthesized compounds was determined.
- Neurotoxicity of the most active compounds was estimated.
- Compounds **4**, **5b** and **5d** demonstrated marked anticonvulsant effect.
- Elemental analysis and spectroscopic characterization of all new compounds.

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Synthesis and Anticonvulsant Activity of New Phenytoin Derivatives

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Abstract

Hybrids between phenytoin and thiosemicarbazide, 1,3,4-oxadiazole, 1,3,4-thiadiazole or 1,2,4-triazole were synthesized and tested for anticonvulsant activity. Preliminary anticonvulsant screening was performed using standard maximal electroshock (MES) and subcutaneous pentylenetetrazole (scPTZ) screens in mice. The neurotoxicity was determined applying the rotarod test. Among these compounds, **4** and **5d** showed the highest protection (80%) in the scPTZ test at a dose of 100 mg/kg, whereas the compound **5b** displayed promising anticonvulsant effect in the MES model.

Key words: Phenytoin/ Oxadiazole/ Thiadiazole/ Triazole/ Anticonvulsant activity

1. Introduction

Epilepsy is the most prevalent neurological disorder, affecting approximately 50 million people worldwide [1]. It is characterized by the periodic and unpredictable occurrence of seizures that are caused by abnormal discharge of cerebral neurons [2]. Despite the significant advances have been made in epilepsy research, several of the currently available antiepileptic drugs (AEDs) have been associated with severe side effects, that range in harshness from minimal brain impairment and megaloblastic anaemia, to death from aplastic anaemia or hepatic failure [3,4]. In addition, standard drug therapy fails to control seizures in about 30% of epileptic patients [5-7]. These limitations observed with conventional antiepileptic drugs, demand the need for the development of more effective and safer candidates.

A rational drug design process for a new anticonvulsant could be achieved by the traditional screening approach as well as the hybrid pharmacophore approach, which is considered “the wave of drug research furnishing multitarget agents” [8].

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Hybrid pharmacophore approach relies on the use of two or more pharmacophores, each with potential pharmacological activity to be combined in one molecule, aiming that this hybridization could produce compounds with better pharmacological profile.

Phenytoin (Fig. 1), a well-established antiepileptic drug that act through inhibition of brain sodium channels [9], has been chosen for its efficacy against partial and generalized seizures [10].

Previous studies showed that compounds including thiosemicarbazide pharmacophore in their structure, constitute a class of compounds with potential anticonvulsant properties [11, 12]. Furthermore, many compounds bearing 1,3,4-oxadiazole [13-15], 1,3,4-thiadiazole [16-18] or 1,2,4-triazole ring [19,20] in their structure have been demonstrated anticonvulsant activity.

Taking in consideration the potential anticonvulsant properties observed by certain thiosemicarbazides, oxadiazoles, thiadiazoles and triazoles, hybrids between phenytoin and either of these pharmacophores were synthesized and evaluated as anticonvulsant agents (Fig. 1).

Figure 1.

Despite the diversity of models that could potentially be used to screen their anticonvulsant activity, the maximal electroshock model (MES) and the subcutaneous pentylenetetrazole model (sc PTZ) remain the ‘‘gold standards’’ in the early stages of testing. Most clinically significant AEDs have shown protection against seizures in at least one of these two models.

The present work comprises the synthesis and anticonvulsant activity of 3-[(5-sulphanyl-1,3,4-oxadiazol-2-yl)methyl]-5,5-diphenylimidazolidine-2,4-dione (**4)**, 1-[2-(2,5-dioxo-4,4-diphenylimidazolidin-1-yl)acetyl]-4-substituted thiosemicarbazides (**5a-e)** 3-[(5-(alkyl/aryl)-1,3,4-thiadizol-2-yl)methyl]-5,5-diphenylimidazolidine-2,4-diones (**6a-e)** and 3-[(4-alkyl/aryl-5-sulphanyl-4*H*-1,2,4-triazol-3-yl)methyl]-5,5-diphenyl-imidazolidine-2,4-diones (**7a-e)**. Their chemical structures were characterized using IR, ^{1}HNMR, ^{13}CNMR, MS and elemental analysis techniques. The anticonvulsant activity was evaluated by using MES and scPTZ experimental epilepsy screens in mice. The rotarod assay was performed in mice to evaluate the neurotoxicity of the most effective compounds.

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2. Results and discussion

2.1. Chemistry

The title compounds were prepared according to the synthetic strategy described in Scheme 1. Ethyl 2-(2,5-dioxo-4,4-diphenylimidazolidin-1-yl)acetate **2** was synthesized by alkylation of phenytoin sodium **1** with ethyl chloroacetate in anhydrous DMF as a solvent [21]. Reaction of **2** with hydrazine hydrate in absolute ethanol, resulted in the formation of the corresponding hydrazide **3** [22]. The target 3-[(5-sulphanyl-1,3,4-oxadiazol-2-yl)methyl]-5,5-diphenylimidazolidine-2,4-dione **(4)** was obtained in 66 % yield by the cyclization of the hydrazide **3** with carbon disulphide in ethanol in the presence of potassium hydroxide. It is interesting to note that thiocarbonyl compounds are present in their thion-thiol tautomeric forms in solution as indicated by their IR and ^{1}H NMR spectra [23-25]. ^{1}HNMR spectrum of **4** showed the presence of D_2O exchangeable signals corresponding to NH and SH at δ 9.92 and 14.60 ppm respectively. In addition, ^{13}CNMR spectrum showed the appearance of two signals of the oxadiazole ring at δ 158.65 ppm and δ 177.70 ppm, assignable to C-2 and C-5 respectively. Besides, the signal due to CH_2 carbon was observed at δ 33.25 ppm.

The intermediate thiosemicarbazides **5a-e** were synthesized in 60-90 % yields through the reaction of **3** with different isothiocyanates in absolute ethanol. A special feature in the structure of thiosemicarbazides **5a-e** is the presence of thiourea residue (NH-CS-NH), which can be identified by IR spectral data. Compounds **5a-e** exhibited strong absorption in the range of 1188-1203 cm^{-1} attributable to C=S. The amidic carbonyl absorption was observed as a broad band in the range of 1650-1680 cm^{-1}. ^{1}HNMR spectra for **5a-e** showed the presence of a singlet in the range of δ 4.21-4.29 ppm assignable to CH_2 protons flanked between the imidazolidine nitrogen and carbonyl function. The four D_2O exchangeable signals for the NH functions were displayed in the range of δ 7.97-10.41 ppm.

On the other hand, the thiadiazole derivatives **6a-e** were prepared through cyclization of the thiosemicarbazides **5a-e,** using concentrated sulphuric acid as a dehydrating agent.

The structure of the target compounds **6a-e** was confirmed by elemental analyses and spectroscopic data (IR, ^{1}HNMR, ^{13}CNMR and MS). IR spectra showed broad band in the range of 3471-3217 cm^{-1} corresponding to the two NH stretchrs.. ^{1}HNMR spectra of all compounds **6a-e,** displayed a singlet signal in the range of δ 4.26-4.91

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ppm assignable to CH_2 protons.^{13}CNMR spectrum of the compound **6d** showed the presence of the C-2 and C-5 signals of the thiadiazole ring at δ 165.69 and 167.25 ppm respectively. The signal corresponding to OCH_3 carbon was observed at 55.14 ppm.

Furthermore, the triazole derivatives **7a-e** were obtained in 60-70 % yields via cyclization of **5a-e** in the presence of 2N NaOH solution. The characterization of the isolated new compounds was based on elemental analyses and spectroscopic data (IR, ^{1}HNMR, ^{13}CNMR and MS).

Compounds **7a-e** showed the presence of a possible thion-thiol tautomerism, as indicated by their IR and ^{1}H NMR spectra. A singlet signal in the range of δ 13.65-13.90 ppm indicated the existence of SH. Besides, the characteristic singlet signal corresponding to CH_2 was observed in the range of δ 3.97-4.03 ppm. ^{13}CNMR of the compound **7c** displayed the signal due to CH_3 carbon at δ 20.75 ppm, in addition to the C-3 and C-5 signals of the triazole ring at δ 150.56 and 167.84 ppm respectively.

2.2. Anticonvulsant activity

Phase I studies of the investigated compounds **(4**, **5a-e**, **6a-e** and **7a-e)** involved three tests: maximal electroshock seizure (MES), subcutaneous pentylenetetrazole (scPTZ), and rotarod test for neurological toxicity. The MES test is a proven method of generalized tonic-clonic seizures and identifies clinical candidates that prevent seizure spread, whereas scPTZ test indicates their effectiveness against absence of seizures and was used to identify compounds that elevate seizure threshold.

A valuable property of a candidate anticonvulsant is its ability to inhibit seizures upon oral administration. The anticonvulsant activity of the test compounds was established at a dose of 100 mg/Kg in the MES, scPTZ and neurotoxicity assays [23-26]. Compounds found to be effective in these seizure challenges are generally regarded to be potentially useful candidates in treatment of partial, generalized and even absence seizures. The results of the *in vivo* tests are summarized in Table 2, Figs. 2, 3.

Analyzing the activities of synthesized compounds **4**, **5a-e**, **6a-e** and **7a-e**, the following results were obtained:

Preliminary anticonvulsant screening revealed that all new compounds showed some degree of protection in MES screen, which was indicative of the good ability of these compounds to prevent the seizure spread at a certain dose level. It was observed that phenytoin-1,2,4-triazole conjugates **7a-e** were generally more effective than the

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corresponding 1,3,4-oxadiazole derivative **4** and the 1,3,4-thiadiazole analogues **6a-e**. Moreover, compounds **7b-e**, containing aromatic ring at the triazole *N*-3, displayed higher protection against electrically induced seizures than the ethyl substituted analogue **7a**. On the other hand, among the thiosemicarbazide derivatives **5a-e**, only the phenyl substituted derivative, **5b** showed protection from seizures superior to phenytoin, indicating its possible efficacy in protection against generalized seizures of the tonic–clonic (grandmal) type. Additionally, the anti-MES activity of the most active compound, **5b,** was further estimated at a lower dose (30 mg/ Kg). The results revealed remarkable anticonvulsant activity of **5b** at the tested dose, (Table 1). Other test compounds displayed from good to moderate activity against electrically induced seizures.

Concerning scPTZ screen, all test compounds showed varying degree (20-80 %) of anticonvulsant activity except the thiosemicarbazide derivative **5e** and the 1,2,4-thiadiazole derivative **6e.** Both compounds, bearing the 4-chlorophenyl substituent, did not exhibit any anticonvulsant activity. On the other hand, the strongest anti-scPTZ activity was observed by **4** and **5d,** which were equipotent to the reference drug, phenytoin **(**80 % protection of seizures).

Furthermore, the compounds showing significant activity in one or both of the seizure models **(4, 5b, 5d, 7b, 7d)** were selected for evaluation of neurotoxicity using rotarod test in mice. None of the compounds showed significant neurotoxic effect. Compound **5b** was found to be devoid of sedative effect, indicating that this compound could possess a promising protective activity against electroshock-induced convulsions at a given dose without affecting locomotor activity and coordination.

Table 2.

Figure 2.

Figure 3.

3. Conclusion

In summary, compounds **4** and **5d** showed anticonvulsant activity comparable to the reference drug, phenytoin in the scPTZ model. On the other hand, compound **5b** displayed anticonvulsant activity superior to phenytoin in the MES screen. In

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addition, it was devoid of neurotoxic properties in the neurotoxicity screen. These preliminary results suggest further studies to really estimate their *in vivo* potency.

4. Experimental

4.1. Chemistry

All chemicals and reagents were obtained from Aldrich (Sigma–Aldrich, St. Louis, MO, USA), and were used without further purification. Reactions were monitored by TLC, performed on silica gel glass plates containing 60 GF-254, and visualization on TLC was achieved by UV light or iodine indicator. IR spectra were determined on Shimadzu IR 435 spectrophotometer (KBr, cm^{-1}). ^{1}HNMR spectra were carried out using a Mercury 300-BB 300 MHz using TMS as an internal standard. Chemical shifts (δ) were recorded in ppm on δ scale, Micro analytical Center, Cairo University, Egypt. ^{13}CNMR spectra were carried out using a Mercury 300-BB 300 MHz using TMS as an internal standard. Chemical shifts (δ) were recorded in ppm on δ scale, Micro analytical Center, Cairo University, Egypt. Mass spectra were recorded on Shimadzu Qp-2010 plus Spectrometer, Micro analytical Center, Cairo University, Egypt. Elemental analyses were carried out at the Micro analytical Center, Cairo University, Egypt. Their results corresponded to the calculated values within experimental error. Melting points were determined with Stuart apparatus and are uncorrected.

The starting materials, phenytoin sodium (5,5-diphenylimidazolidine-2,4-dione sodium salt) **(1)** commercially available, ethyl 2-(2,5-dioxo-4,4-diphenylimidazolidin-1-yl)acetate **(2)** [21] and 2-(2,5-dioxo-4,4-diphenylimidazolidin-1-yl)acetohydrazide **(3)** [22] were prepared according to the reported procedures.

*4.1. 3-[(5-Sulphanyl-1,3,4-oxadiazol-2-yl)methyl]-5,5-diphenylimidazolidine-2,4-dione **(4)***

A mixture of the hydrazide **3** (0.32 g, 0.001 mol), carbon disulphide (2 mL) and potassium hydroxide (0.5 g, 0.009 mol) in ethanol (5 mL, 95 %) was heated under reflux for 12 h. The reaction mixture was filtered while hot, and the solvent was distilled off under reduced pressure. The residue obtained was dissolved in water (10 mL) then neutralized with dil HCl. The resulting white precipitate was filtered, washed with water, dried and crystallized from dioxane/ water.

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Yield 66 %; mp 225-226 ^{o}C; IR (KBr) (cm^{-1}): 3360 (N-H), 3032 (C-H aromatic), 2943, 2840 (C-H aliphatic), 1766, 1716 (C=O), 1639 (C=N), 1153 (C=S str.); ^{1}HNMR (300 MHz, DMSO-d_6) δ: 4.83 (s, 2H, CH_2), 7.32-7.44 (m, 10H, Ar-H), 9.92 (s, 1H, NH, D_2O exchangeable), 14.60 (s, 1H, SH, D_2O exchangeable); ^{13}CNMR (DMSO-d_6 ppm) δ: 33.25 (CH_2), 69.52 (C-5 imidazolidine), 126.60, 128.29, 128.53, 139.01 (aromatic C's), 153.80 (C=O), 158.65 (oxadiazole C-2), 172.47 (C=O), 177.70 (oxadiazole C-5); EIMS (% rel. abundance): 367 ($M+H^{\square}$, 15.42), 366 ($M^{\cdot+}$, 76.23), 180 (100). Anal. Calcd for $C_{18}H_{14}N_4O_3S$ (366.39): C, 59.01; H, 3.85; N, 15.29, Found, C, 58.96; H, 3.96; N, 15.43.

*4.2. 1-[2-(2,5-Dioxo-4,4-diphenylimidazolidin-1-yl)acetyl]-4-substituted thiosemicarbazides **(5a-e)***

To a solution of the hydrazide **3** (0.32 g, 0.001 mol) in absolute ethanol (10 mL), the appropriate isothiocyanate (0.0015 mol) was added and the reaction mixture was heated under reflux for 10 h. The solvent was concentrated under reduced pressure then diluted with an ice-cold water (10 mL). The precipitated solid product was filtered then crystallized from the appropriate solvent.

*4.2.1. 1-[2-(2,5-Dioxo-4,4-diphenylimidazolidin-1-yl)acetyl]-4-ethylthiosemicarbazide **(5a)***

Crystallized from benzene/ absolute ethanol mixture; yield 60 %; mp 189-190 ^{o}C; IR (KBr) (cm^{-1}): 3433-3209 (N-H), 3040 (C-H aromatic), 2990, 2850 (C-H aliphatic), 1774, 1724-1680 (C=O), 1200 (C=S); ^{1}HNMR (300 MHz, DMSO-d_6) δ: 1.06 (t, 3H, CH_2-$\underline{CH_3}$), 3.48 (q, 2H, $\underline{CH_2}$-CH_3), 4.21 (s, 2H, CH_2-N), 7.34-7.43 (m, 10H, Ar-H), 7.97, 9.32, 9.67, 10.13 (each s, 4H, 4 NH, D_2O exchangeable); EIMS (% rel. abundance): 412 ($M+H^{\square}$, 0.53), 411 ($M^{\cdot+}$, 1.80), 252 (100); Anal. Calcd for $C_{20}H_{21}N_5O_3S$ (411.48): C, 58.38; H, 5.14; N, 17.02, Found, C, 58.51; H, 5.23; N, 17.16.

*4.2.2 . 1-[2-(2,5-Dioxo-4,4-diphenylimidazolidin-1-yl)acetyl]-4-phenylthiosemicarbazide **(5b)***

Crystallized from benzene/ absolute ethanol mixture; yield 66 %; mp 186-187 ^{o}C; IR (KBr) (cm^{-1}): 3406-3271 (N-H), 3062 (C-H aromatic), 2920, 2850 (C-H aliphatic),

1766, 1716-1680 (C=O), 1597 (C=N), 1195 (C=S); ^{1}HNMR (300 MHz, DMSO-d_6) δ: 4.29 (s, 2H, CH_2-N), 7.18-7.44 (m, 15H, Ar-H), 9.65, 9.69, 9.77, 10.40 (each s, 4H, 4N-H, D_2O exchangeable); EIMS (% rel. abundance): 460 ($M+H^+$, 11.56), 459 ($M^{+\cdot}$, 10.54), 366 (74), 104 (100); Anal. Calcd for $C_{24}H_{21}N_5O_3S$ (459.52): C, 62.73; H, 4.61; N, 15.24, Found, C, 62.80; H, 4.72; N, 15.39.

4.2.3. 1-[2-(2,5-Dioxo-4,4-diphenylimidazolidin-1-yl)acetyl]-4-(4-methylphenyl) thiosemicarbazide ***(5c)***

Crystallized from benzene/ pet. ether mixture; yield 87 %; mp 110-111 ^oC; IR (KBr) (cm^{-1}): 3400-3282 (N-H), 3059 (C-H aromatic), 2920, 2865 (C-H aliphatic), 1774, 1716-1665 (C=O), 1593 (C=N), 1192 (C=S); ^{1}HNMR (300 MHz, DMSO-d_6) δ: 2.28 (s, 3H, CH_3), 4.28 (s, 2H, CH_2-N), 7.13 (d, 2H, Ar-H), 7.24 (d, 2H, Ar-H), 7.35-7.44 (m, 10H, Ar-H), 9.60, 9.68, 9.70, 10.37 (each s, 4H, 4 NH, D_2O exchangeable); EIMS (% rel. abundance): 474 ($M+H^+$, 18.65), 473 (M^+, 7.74), 308 (26.11), 251 (M-(27.38); Anal. Calcd for $C_{25}H_{23}N_5O_3S$ (473.55): C, 63.41; H, 4.90; N, 14.79, Found, C, 63.48; H, 5.01; N, 14.88.

4.2.4 1-[2-(2,5-Dioxo-4,4-diphenylimidazolidin-1-yl)acetyl]-4-(4-methoxyphenyl) thiosemicarbazide ***(5d)***

Crystallized from benzene/ petroleum ether mixture; yield 90 %; mp 139-140 ^oC; IR (KBr) (cm^{-1}): 3468-3302 (N-H), 3155 (C-H aromatic), 2927, 2839 (C-H aliphatic), 1770, 1724-1680 (C=O), 1593 (C=N), 1188 (C=S); ^{1}HNMR (300 MHz, DMSO-d_6) δ: 3.74 (s, 3H, OCH_3), 4.28 (s, 2H, CH_2-N), 6.92 (d, 2H, Ar-H), 7.25 (d, 2H, Ar-H), 7.39-7.44 (m, 10H, Ar-H), 9.42, 9.58, 9.68, 10.35 (each s, 4H, 4 NH, D_2O exchangeable); EIMS (% rel. abundance): 489 (M^+, 1.55), 180 (100); Anal. Calcd for $C_{25}H_{23}N_5O_4S$ (489.55): C, 61.34; H, 4.74; N, 14.31, Found, C, 61.44; H, 4.79; N, 14.48.

4.2.5. 1-[2-(2,5-Dioxo-4,4-diphenylimidazolidin-1-yl)acetyl]-4-(4-chlorophenyl) thiosemicarbazide ***(5e)***

Crystallized from benzene/ petroleum ether mixture; yield 88 %; mp 99-100 ^oC; IR (KBr) (cm^{-1}): 3464-3240 (N-H), 3055 (C-H aromatic), 2924, 2850 (C-H aliphatic), 1774, 1716-1675 (C=O), 1597 (C=N), 1203 (C=S); ^{1}HNMR (300 MHz, DMSO-d_6) δ: 4.28 (s, 2H, CH_2-N), 7.33-7.43 (m, 14H, Ar-H), 9.32, 9.68, 9.87, 10.41 (each s, 4H, 4

NH, D_2O exchangeable); EIMS (% rel. abundance): 495 (M+2, 0.03), 493 (M^{+}, 0.02); Anal. Calcd for $C_{24}H_{20}ClN_5O_3S$ (493.97): C, 58.36; H, 4.08; N, 14.18, Found, C, 58.39; H, 4.13; N, 14.27.

4.3. 3-{[5-(Alkyl/arylamino)-1,3,4-thiadiazol-2-yl]methyl}-5,5-diphenylimidazolidine-2,4-diones ***(6a-e)***

To an ice cooled and stirred solution of an appropriate thiosemicarbazide **5a-e** (0.0013 mol) in absolute ethanol (10 mL), concentrated sulphuric acid (5 mL) was slowly added over a period of 15 min. The reaction mixture was stirred at room temperature for 5 h then the mixture was poured onto equal volume of ice-cold water. The white precipitate formed was filtered, washed with water, dried and crystallized from DMF / water mixture.

4.3.1. 3-[(5-Ethylamino-1,3,4-thiadiazol-2-yl)methyl]-5,5-diphenylimidazolidine-2,4-dione ***(6a)***

Yield 60 %; mp 165-166 ^{o}C; IR (KBr) (cm^{-1}): 3450-3221 (N-H), 3105 (C-H aromatic), 2980, 2852 (C-H aliphatic), 1774, 1716 (C=O), 1635 (C=N); ^{1}HNMR (300 MHz, DMSO-d_6) δ: 1.15 (t, 3H, CH_2-$\underline{CH_3}$), 4.12 (q, 2H, $\underline{CH_2}$-CH_3), 4.26 (s, 2H, CH_2), 7.35-7.44 (m, 10H, Ar-H), 9.77 (s, 1H, NH, D_2O exchangeable), 10.23 (s, 1H, NH, D_2O exchangeable); EIMS (% rel. abundance): 393 (M^{+}, 0.01), 265 (18.38), 209 (100); Anal. Calcd for $C_{20}H_{19}N_5O_2S$ (393.46): C, 61.05; H, 4.87; N, 17.80, Found, C, 61.13; H, 4.96; N, 18.11.

4.3.2. 3-[(5-Phenylamino-1,3,4-thiadiazol-2-yl)methyl]-5,5-diphenylimidazolidine-2,4-dione ***(6b)***

Yield 63 %; mp 255-256 ^{o}C; IR (KBr) (cm^{-1}): 3471-3290 (N-H), 3089 (C-H aromatic), 2920, 2850 (C-H aliphatic), 1774, 1716 (C=O), 1595 (C=N); ^{1}HNMR (300 MHz, DMSO-d_6) δ: 4.88 (s, 2H, CH_2), 6.90-7.52 (m, 15H, Ar-H), 9.85 (s, 1H, NH, D_2O exchangeable), 10.33 (s, 1H, NH, D_2O exchangeable); EIMS (% rel. abundance): 442 (M+H, 31.17), 441 (M^{+}, 100); Anal. Calcd for $C_{24}H_{19}N_5O_2S$ (441.50): C, 65.29; H, 4.34; N, 15.86, Found, C, 65.38; H, 4.51; N, 15. 98.

4.3.3. 3-{[5-(4-Methylphenylamino)-1,3,4-thiadiazol-2-yl]methyl}-5,5-diphenylimidazolidine-2,4-dione ***(6c)***

Yield 66 %; mp 234-235 ^{o}C; IR (KBr) (cm^{-1}): 3394-3278 (N-H), 3062 (C-H aromatic), 2920, 2854 (C-H aliphatic), 1774, 1716 (C=O), 1620 (C=N); ^{1}HNMR (300 MHz, DMSO-d_6) δ: 2.24 (s, 3H, CH_3), 4.90 (s, 2H, CH_2), 7.12-7.45 (m, 14H, Ar-H), 9.85 (s, 1H, NH, D_2O exchangeable), 10.25 (s, 1H, NH, D_2O exchangeable); EIMS (% rel. abundance): 456 (M+H, 31.20), 455 ($M^{\cdot+}$,100); Anal. Calcd for $C_{25}H_{21}N_5O_2S$ (455.53): C, 65.92; H, 4.65; N, 15.37, Found, C, 66.14; H, 4.80; N, 15.44.

4.3.4. 3-{[5-(4-Methoxyphenylamino)-1,3,4-thiadiazol-2-yl]methyl}-5,5-diphenylimidazolidine-2,4-dione ***(6d)***

Yield 62 %; mp 220-221 ^{o}C; IR (KBr) (cm^{-1}): 3309-3217 (N-H), 3059 (C-H aromatic), 2943, 2835 (C-H aliphatic), 1774, 1716 (C=O), 1624 (C=N); ^{1}HNMR (300 MHz, DMSO-d_6) δ: 3.73 (s, 3H, OCH_3), 4.89 (s, 2H, CH_2), 6.90-7.48 (m, 14H, Ar-H), 9.87 (s, 1H, NH, D_2O exchangeable), 10.15 (s, 1H, NH, D_2O exchangeable); ^{13}CNMR (DMSO-d_6 ppm) δ: 36.97 (CH_2), 55.14 (OCH_3), 69.36 (C-5 imidazolidine), 114.18, 119.18, 126.59 , 128.21, 128.52, 133.81, 139.21, 154.23)aromatic C's), 154.47 (C=O), 165.69 (thiadiazole C-2), 167.25 (thiadiazole C-5), 172.53 (C=O); EIMS (% rel. abundance): 472 (M+2, 20.14), 471 ($M^{\cdot+}$, 100); Anal. Calcd for $C_{25}H_{21}N_5O_3S$ (471.53): C, 63.68; H, 4.49; N, 14.85, Found, C, 63.85; H, 4.58; N, 14.98.

4.3.5. 3-{[[5-(4-Chlorophenylamino)-1,3,4-thiadiazol-2-yl)methyl]-5,5-diphenylimidazolidine-2,4-dione ***(6e)***

Yield 60 %; mp 273-274 ^{o}C; IR (KBr) (cm^{-1}): 3468-3267 (N-H), 3062 (C-H aromatic), 2927, 2850 (C-H aliphatic), 1774, 1716 (C=O), 1597 (C=N); ^{1}HNMR (300 MHz, DMSO-d_6) δ: 4.91 (s, 2H, CH_2), 7.35-7.59 (m, 14H, Ar-H), 9.86 (s, 1H, NH, D_2O exchangeable), 10.48 (s, 1H, NH, D_2O exchangeable); EIMS (% rel. abundance): 477 (M+2, 9.78), 475 ($M^{\cdot+}$, 8.09), 209 (100); Anal. Calcd for $C_{24}H_{18}ClN_5O_2S$ (475.95): C, 60.56; H, 3.81; N, 14.71, Found, C, 60.46; H, 3.90; N, 14.96.

4.4. 3-[(4-Alkyl/aryl-5-sulphanyl-4H-1,2,4-triazol-3-yl)methyl]-5,5-diphenylimidazolidine-2,4-diones ***(7a-e)***

The appropriate thiosemicarbazide **5a-e** (0.005 mol) in sodium hydroxide solution (2N, 10 mL) was heated under reflux for 4 h. After cooling, dil HCl was added to the

reaction mixture till pH=2-3. The resulting precipitate was filtered, washed with water, dried, and crystallized from benzene-absolute ethanol mixture.

4.4.1. 3-[(4-Ethyl-5-sulphanyl-4H-1,2,4-triazol-3-yl)methyl]-5,5-diphenylimidazolidine-2,4-dione ***(7a)***

Yield 60 %; mp 183-184 ^{o}C; IR (KBr) (cm^{-1}): 3375-3290 (N-H), 3089 (C-H aromatic), 2924, 2854 (C-H aliphatic), 1774, 1724 (C=O), 1624 (C=N); ^{1}HNMR (300 MHz, DMSO-d_6) δ: 1.11 (t, 3H, CH_2-C<u>H</u>$_3$), 4.01 (s, 2H, CH_2), 4.34 (q, 2H, C<u>H</u>$_2$-CH_3), 7.24-7.39 (m, 10H, Ar-H), 9.56 (s, 1H, NH, D_2O exchangeable), 13.65 (s, 1H, SH, D_2O exchangeable); EIMS (% rel. abundance): 393 ($M^{\ddagger}$, 0.17), 265 (36.04); Anal. Calcd for $C_{20}H_{19}N_5O_2S$ (393.46): C, 61.05; H, 4.87; N, 17.80, Found, C, 61.15; H, 4.94; N, 18.07.

4.4.2. 3-[(4-Phenyl-5- sulphanyl-4H-1,2,4-triazol-3-yl)methyl]-5,5-diphenylimidazolidine-2,4-dione ***(7b)***

Yield 66 %; mp 162-163 ^{o}C; IR (KBr) (cm^{-1}): 3379 (N-H), 3059 (C-H aromatic), 2924, 2854 (C-H aliphatic), 1774, 1720 (C=O), 1595 (C=N); ^{1}HNMR (300 MHz, DMSO-d_6) δ: 3.98 (s, 2H, CH_2), 7.24-7.55 (m, 15H, Ar-H), 9.72 (s, 1H, NH, D_2O exchangeable), 13.87 (s, 1H, SH, D_2O exchangeable); EIMS (% rel. abundance): 441 ($M^{\ddagger}$, 2.24); Anal. Calcd for $C_{24}H_{19}N_5O_2S$ (441.50): C, 65.29; H, 4.34; N, 15.86, Found, C, 65.32; H, 4.39; N, 16.12.

4.4.3. 3-{[4-(4-Methylphenyl)-5-sulphanyl-4H-1,2,4-triazol-3-yl]methyl}-5,5-diphenylimidazolidine-2,4-dione ***(7c)***

Yield 70 %; mp 151-152 ^{o}C; IR (KBr) (cm^{-1}): 3371 (N-H), 3059 (C-H aromatic), 2924, 2854 (C-H aliphatic), 1774, 1720 (C=O), 1624 (C=N); ^{1}HNMR (300 MHz, DMSO-d_6) δ: 2.32 (s, 3H, CH_3), 3.97 (s, 2H, CH_2), 7.21-7.41 (m, 14H, Ar-H), 9.78 (s, 1H, NH, D_2O exchangeable), 13.83 (s, 1H, SH, D_2O exchangeable); ^{13}CNMR (DMSO-d_6 ppm) δ: 20.75 (CH_3), 35.34 (CH_2), 68.48 (C-5 imidazolidine), 126.63, 126.75, 128.25, 128.37, 129.77, 130.52, 139.03, 141.69 (aromatic C's), 150.56 (triazole C-3), 156.43 (C=O), 167.84 (triazole C-5), 172.56 (C=O); EIMS (% rel.

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abundance): 456 (M+H, 1.59), 455 ($M^{+\cdot}$, 4.77); Anal. Calcd for $C_{25}H_{21}N_5O_2S$ (455.53): C, 65.92; H, 4.65; N, 15.37, Found, C, 66.04; H, 4.73; N, 15.49.

4.4.4. 3-{[4-(4-Methoxyphenyl)-5-sulphanyl-4H-1,2,4-triazol-3-yl]methyl}-5,5-diphenyl imidazolidine-2,4-dione ***(7d)***

Yield 68 %; mp 260 (dec.) ^oC; IR (KBr) (cm^{-1}): 3383 (N-H), 3059 (C-H aromatic), 2931, 2805 (C-H aliphatic), 1774, 1724 (C=O), 1624 (C=N); ^{1}HNMR (300 MHz, DMSO-d_6) δ: 3.81 (s, 3H, OCH_3), 3.98 (s, 2H, CH_2), 7.01-7.43 (m, 14H, Ar-H), 9.80 (s, 1H, NH, D_2O exchangeable), 13.81 (br, 1H, SH, D_2O exchangeable); EIMS (% rel. abundance): 472 (M+H, 7.48), 471 ($M^{+\cdot}$, 23.93); Anal. Calcd for $C_{25}H_{21}N_5O_3S$ (471.53): C, 63.68; H, 4.49; N, 14.85, Found, C, 63.77; H, 4.46; N, 15.03.

4.4.5. 3-{[4-(4-Chlorophenyl)-5-sulphanyl-4H-1,2,4-triazol-3-yl]methyl}-5,5-diphenyl imidazolidine-2,4-dione ***(7e)***

Yield 63 %; mp 149-150 ^oC; IR (KBr) (cm^{-1}): 3387 (N-H), 3093 (C-H aromatic), 2924, 2810 (C-H aliphatic), 1774, 1720 (C=O), 1597 (C=N); ^{1}HNMR (300 MHz, DMSO-d_6) δ: 4.03 (s, 2H, CH_2), 7.22-7.63 (m, 14H, Ar-H), 9.74 (s, 1H, NH, D_2O exchangeable), 13.90 (s, 1H, SH, D_2O exchangeable); EIMS (% rel. abundance): 477 (M+2, 13.09), 475 ($M^{+\cdot}$, 31.66); Anal. Calcd for $C_{24}H_{18}ClN_5O_2S$ (475.95): C, 60.56; H, 3.81; N, 14.71, Found, C, 60.68; H, 3.92; N, 14.93.

4.5. Anticonvulsant screening

4.5.1. Materials and methods

All the compounds prepared herein were screened for their potential *in vivo* anticonvulsant activity. The Phase I pharmacological screening involves MES, scPTZ and neurotoxicity assays.

Adult albino mice weighing 20–25 g of both sexes (obtained from the animal house colony in the National Research Center) were used throughout this study. Animals were housed in groups of 4–5 and were allowed free access to food pellets (vit mix 1 %, mineral mix 4 % corn oil 10 %, sucrose 20 %, cellulose 0.2 %, casein (95% pure) 10.5 %, starch 54. 3%) and water except for the short time that animals were removed from their cages for testing. All behavioural experiments were

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conducted during the period between 10:00 and 13:00 with normal room light (12 h regular light/dark cycle) and temperature (22 ± 18°C). Animals' housing and rearing had been followed optimum standard rules. Unnecessary disturbance of animals was avoided. Animals were treated gently; squeezing, pressure and tough manoeuvre is avoided. Instruments used for drug preparation and animal injection were previously sterilized. Anaesthesia had been used whenever applicable. The dose of each drug was calculated accurately. Animals' cadavers and parts of tissues were handled with care by following the principles of healthy hygiene; dead bodies were incinerated in the National Research Center incineration. All procedures were carried out in accordance with the (National Research Center, Cairo, Egypt) institutional guidelines for animal care and use as follows:

4.5.1.1. Pentylynetetrazole (PTZ) induced seizure test

The test compound or the reference drug (100 mg/Kg) was given orally to groups of 6 mice. Another group of 6 mice have been taken as a control. Sixty min after oral administration, pentylenetetrazole (PTZ) (85 mg/Kg) was injected subcutaneously. Each animal was placed into an individual plastic cage for observation over a period of 20 min. The incidence of tonic-clonic convulsions lasting for at least 5 sec was recorded [27,28].

Animals devoid of generalized convulsions were considered to be protected and the results were represented as percentage protection. Standard drug used was phenytoin sodium at a dose of 100 mg/Kg (Table 2, Fig. 2).

4.5.1.2. MES-maximal electroshock seizure pattern test

The procedure was carried out as described by Krall, *et al.* [29] and Kitano *et al.* [30]. A compound under investigation and the reference drug suspended in a vehicle (Tween 80) was administered orally to a group of six mice (100 mg/Kg in 1% Tween 80) 60 min before the test. The control animals were administered the vehicle. An increased electric current was applied via an ear electrode, initial current of 1 mA, increment of 0.1 mA/0.2 s at 50 Hz *(Ugo Basil, ECT Unit, 57800).* Tonic extension of hind limbs was taken as the end point. The mean convulsion threshold was considered as the mean of the maximal electric current which could be tolerated by the animal at the end point [31]. Results of the mean electrical stimulation (MES) test were expressed in *milli*-Ampere of the mean convulsion threshold for each compound. The

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mean threshold current for electroshock-induced tonic hind limb extensor seizure was calculated for each drug (Table 2, Fig. 3).

The mean convulsion threshold of compounds under investigation as well as the standard error were calculated using chi-squared test with the aid of Graphpad Prism software, version 5 (inc., San Diego, USA).

4.5.1.3. Neurotoxicity screening

Minimal motor impairment was measured in mice or rats by using standardized rotarod test [32]. Prior to the experiment, male albino mice were placed on 3 cm rod (*Ugo-Basile Accele. ROTA-ROD for mice, 7650*) rotating at 6 rpm, in two training sessions that last 10 and 15 minutes respectively. The candidates under investigation were injected *i.p.* (100 mg/Kg in 1% Tween 80) in four groups each of 6 mice. The control group received 1% Tween 80 as a vehicle. One hour later, the animals were again tested on the rotarod to assess the locomotor coordination and neurological deficit (*e.g*., ataxia, sedation, hyper-excitability), which are reflected by the inability of the animal to maintain equilibrium on the rod after the administration of a selected candidate. The end point for minimal neurotoxicity assessment was reflected by the inability of mice to maintain their equilibrium for at least 1 min in each of the four trials.

ACKNOWLEDGEMENTS

The authors are grateful to Dr. Wafaa El-Eraky, Associate Professor of Pharmacology and Toxicology Department, National Research Center, Cairo. Egypt for carrying out the biological testing.

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Figure captions

Figure 1. Phenytoin and basic structures of the designed compounds.

Figure 2. Anticonvulsant activity of test compounds administrated orally to mice (sc PTZ screen).

Figure 3. Anticonvulsant activity of test compounds administrated orally to mice (MES screen).

Table 1. Anticonvulsant activity and neurotoxicity (NT) of compounds **4**, **5a-e**, **6a-e** and **7a-e** administered orally to mice (100 mg/Kg).

Scheme 1. Reagents and conditions: **a)** $ClCH_2COOC_2H_5$, DMF/ 2h **b)** N_2H_4, abs. C_2H_5OH/ 25 ^oC/ 24 h **c)** CS_2, KOH, C_2H_5OH/ 12 h **d)** RNCS, abs. C_2H_5OH/ 10 h **e)** conc. H_2SO_4/ 5 h **f)** 2N NaOH/ 4h.

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Table 1. Anticonvulsant activity and neurotoxicity (NT) of compounds **4**, **5a-e**, **6a-e** and **7a-e** administered orally to mice (100 mg/Kg).

Compound	PTZ	MES	NT[a]
	%protection	mean convulsion threshold	
4	80*	5.5±0.2887	40
5a	40	5.2±0.3742	-
5b[b]	50	8.4±0.5099*	0
5c	60	5.8±0.3742	-
5d	80*	4.0±0.3162	16.67
5e	0	5.4±0.5099	-
6a	60	5.0±0.3162	-
6b	60	5.0±0.4082	-
6c	40	5.2±0.3742	-
6d	20	4.0±0.3162	-
6e	0	4.25±0.25	-
7a	40	5.8±0.3742	-
7b	40	6.6±0.400*	33.33
7c	20	6.6±0.5099*	-
7d	40	6.6±0.5099*	50
7e	40	6.2±0.5831*	-
Phenytoin	80*	7.25±0.2500*	-

Values represent the mean ± S.E. of 5-6 animals for each group

Values with * are statistically significant ($p<0.05$) from the control group by using one way ANOVA (followed by Tukey's as *p*ost tests).

[a]Percentage of animals that fail to maintain equilibrium on the accelerating rod for 300 seconds.

[b]This compound was tested at a dose 30 mg/Kg in the MES screen, and the mean convulsion threshold was found to be 6.143±0.1429*.

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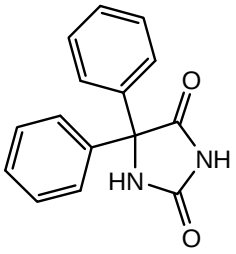
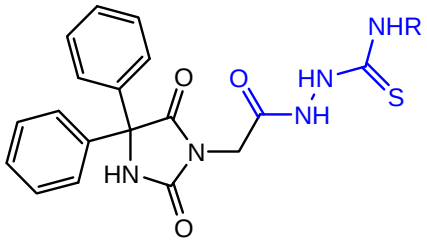
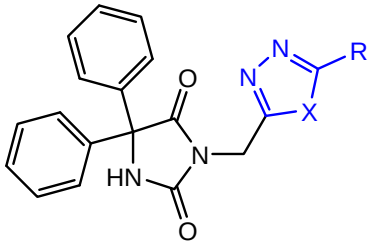


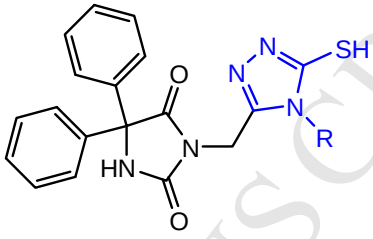
Phenytoin



5a-e



4, 6a-e



7a-e

X = O, S

R= C_2H_5, C_6H_5, 4-$CH_3C_6H_4$, 4-$OCH_3C_6H_4$, 4-ClC_6H_4

Figure 1. Penytoin and basic structures of the designed compounds

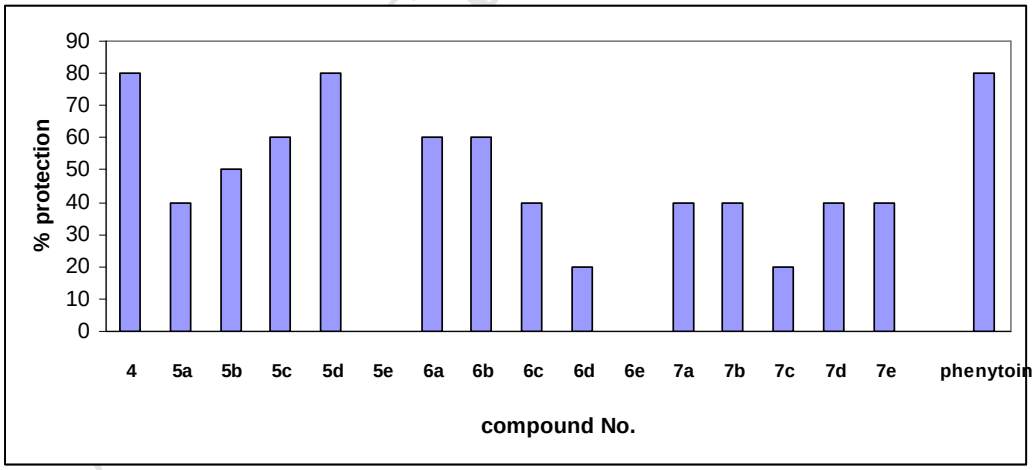


Figure 2. Anticonvulsant activity of test compounds administrated orally to mice (sc PTZ screen).

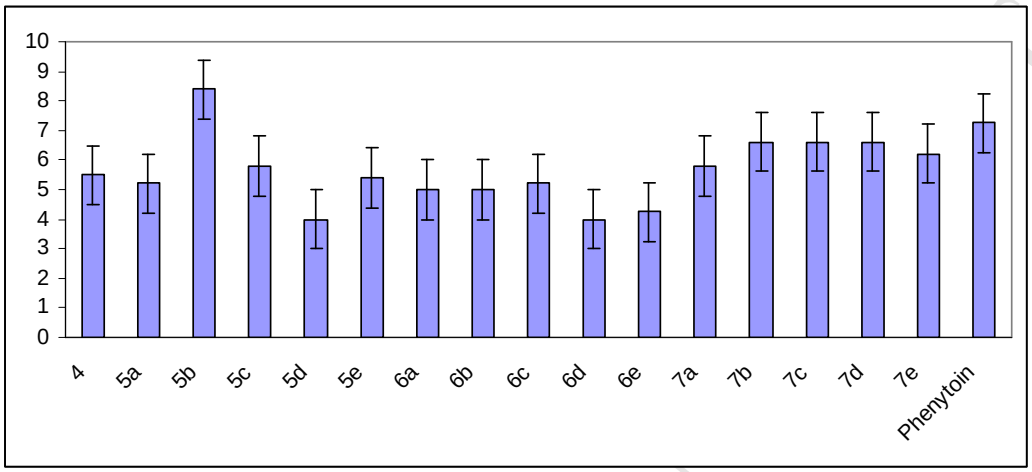


Figure 3. Anticonvulsant activity of test compounds administrated orally to mice (MES screen).

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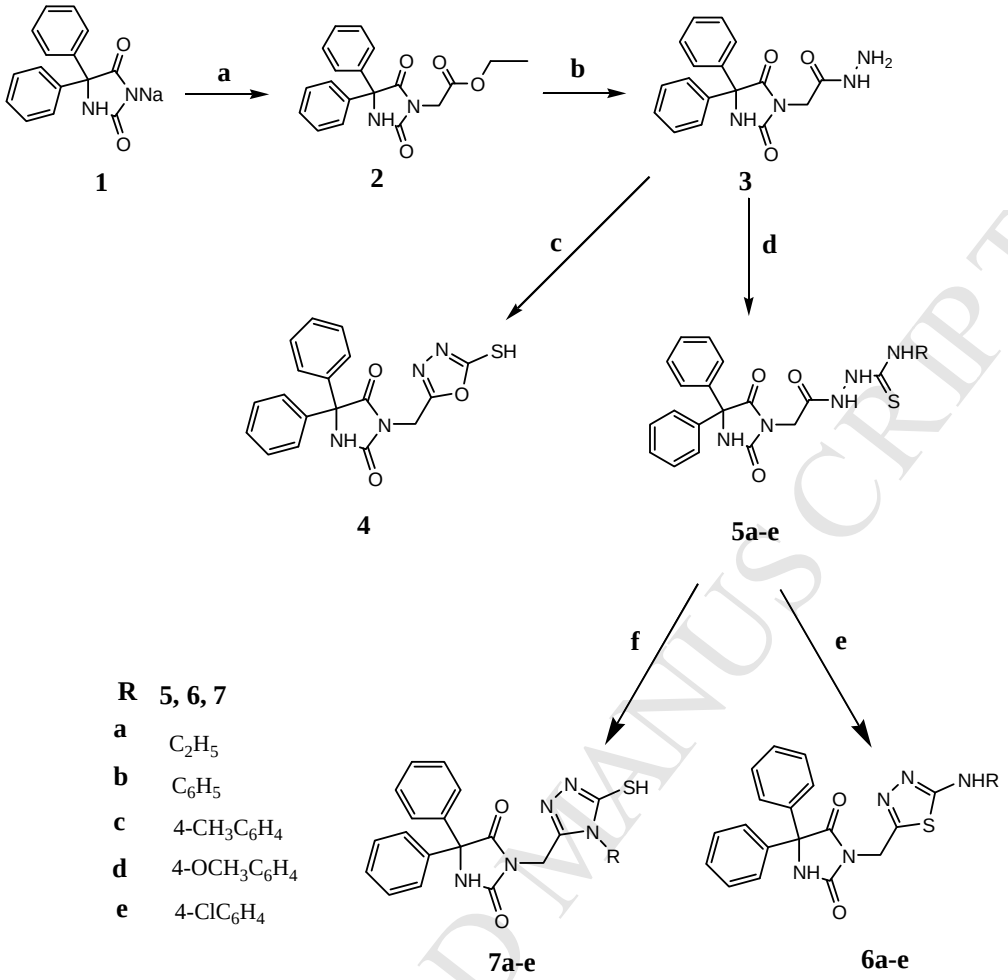

Scheme 1. Reagents and conditions:**a)** $ClCH_2COOC_2H_5$, DMF/ 2 h **b)** N_2H_4, abs.C_2H_5OH/ 25^oC/ 24 h
c) CS_2, KOH, C_2H_5OH/ 12 h **d)** RNCS, abs. C_2H_5OH/ 10 h **e)** conc H_2SO_4/ 5 h **f)** 2N NaOH/ 4 h

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