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Development of new indole-derived neuroprotective agents

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ABSTRACT

There is a great deal of interest in neurotrophin therapy to prevent neuronal degeneration. The present study aimed at synthesizing new functionalized indole derivatives with structures justifying neuroprotective activity using L-tryptophan (TRP)[†] as starting material. The potential neuroprotective effect of these newly synthesized agents against acrylamide (ACR) induced neurotoxicity was investigated in adult female rats. The novel indole derivatives, indolylmethyl pyridine derivatives **9a**,**b**, pyrimidinylindolyl propanone derivatives **12a–c**, pyrazolylindolyl propanone derivatives **14a**,**b**, and indolyl tetrazolopropanoic acid derivative **17** were synthesized and their chemical structures were confirmed by studying their analytical and spectral data. The administration of ACR [ip, 50 mg kg^{-1} body weight (b. wt.)] alone resulted in significant increase in brain malondialdehyde level (MDA) and lactate dehydrogenase (LDH) activity whereas it caused significant decrease in brain monoamines levels and antioxidant enzymes activity. Treatment with the indole derivatives **9b**, **12c**, **14a**, and **17** (ip, 50 mg kg^{-1} b. wt.) prior to ACR produced neuroprotective activity with various intensities depending on the structure of each compound. Compound **17** in which the tetrazole ring was attached to the TRP moiety ranked as the strongest neuroprotective agent. All the tested compounds have been shown to possess antioxidant properties offering promising efficacy against oxidative stress induced by ACR administration.

1. Introduction

The nervous system is considered the most complex organ system in the body, and many toxic chemicals can affect many different parts of this system. Since the nervous system innervates all areas of the body, some toxic effects may be quite specific and others very generalized depending upon where in the nervous system the toxin exerts its effect. There is a great deal of interest in neurotrophin therapy to prevent neuronal degeneration.[1] Acrylamide (ACR) is water soluble, vinyl monomer that has multiple chemical and industrial applications and also used extensively in molecular laboratories for gel chromatography.[2] ACR has been found to cause a distinct central–peripheral, distal neuropathy, it also showed to have neurotoxic effect on the central nervous system in experimental rats.[3] Recent research suggested that the behavioral acrylamide neurotoxicity, a product of nerve damage, was classified as a central–peripheral, distal axonopathy.[4]

It is well known that indole derivatives, extensively present in natural compounds and are very important substances in medicinal and biological aspects. Indole is a highly conserved molecule that it acts as a free radical scavenger and has broad-spectrum antioxidant activity.[5] Moreover, indole and its derivatives exhibit various therapeutic effects, such as regulation of circadian rhythm and immune functions.[6,7] Recent research has proved that the indole ring in the melatonin molecule is the reactive center dealing with oxidants due to its high resonance stability and very low activation energy barrier towards free radical reactions.[8,9] Indole-3-propionamide derivatives exert higher antioxidant activity compared to melatonin.[10] Also, 2-phenylindole derivatives significantly inhibited lipid peroxidation.[11]

L-Tryptophan (TRP) is the precursor amino acid for the synthesis of serotonin (5-HT) and it has been found that 5-HT increases significantly by TRP treatment.[12] Indole ring in the TRP compound acts as ligand of peripheral benzodiazepine receptor (PBR) which improved functional recovery of the brain in neuro-intoxicated animal models due to its neuroprotective effect.[13] In addition, TRP is the precursor of melatonin[14] which could save both dopaminergic and non-dopaminergic neurons from oxidative stress and protect all types of brain damaged neurons.[15]

A large variety of synthetic compounds have been identified as potent neuroprotective agents, yet many of these compounds have not provided great clinical benefits, and some produced side

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† *Abbreviations*: ACR, acrylamide; AD, adrenaline; b.wt., body weight; CK, creatine kinase; DA, dopamine; GPx, glutathione peroxidase, GR, glutathione reductase; GSH, glutathione content; 5-HT, serotonin; ip, intraperitoneal; LDH, lactate dehydrogenase; MDA, malondialdehyde; NA, noradrenaline; PBR, peripheral benzodiazepine receptor; SOD, superoxide dismutase; TRP, L-tryptophan; TLC, thin layer chromatography.

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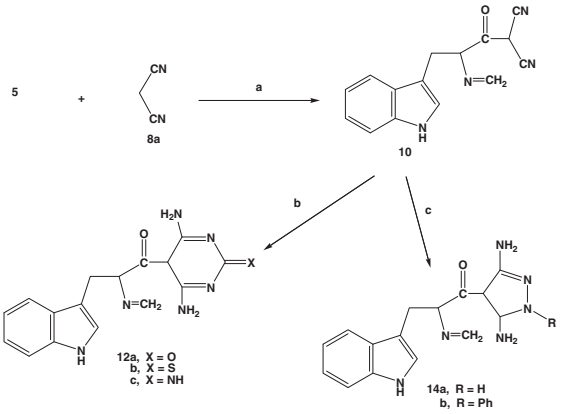
effects. Therefore, the main goal of the present study was to synthesize new indole derivatives with structures justifying neuroprotective activity using TRP as starting material. Furthermore, the potential neuroprotective effects of these newly synthesized agents against acrylamide (ACR) induced neurotoxicity in rats was investigated compared with those of the parent compound.

2. Results and discussion

2.1. Chemistry

In the aim of synthesis of indolyl aminopyridine derivatives of potential neuroprotective activity,[16] the amino group of TRP (**1**) reacted with formaldehyde **2** in the presence of ammonium acetate to afford N*b*-formaltryptophan derivative **3** in 95% yield (Scheme 1). The reaction of compound **3** with thionyl chloride **4** in anhydrous diethyl ether resulted in the corresponding chloride derivative, indolyl methylenaminopropanyl chloride **5**, in 90% yield. The latter reacted with aniline **6** in refluxing dioxane/ triethylamine solution to give the corresponding anilide derivative **7** in 75% yield (Scheme 1). The reaction of compound **7** with either malononitrile **8a** or ethyl cyanoacetate **8b** in refluxing ethanolic/piperidine solution gave the corresponding chromatographically pure products the indolylmethyl aminopyridine derivatives **9a** and **9b** in 76% and 70% yield, respectively (Scheme 1).

The reactivity of indolyl methylenaminopropanyl chloride derivative **5** towards some active methylene reagents was studied. Refluxing of compound **5** with equimolar amount of malononitrile **8a** in 1,4-dioxane/triethylamine solution afforded the corresponding condensate product, indolyl methylenamino-3-oxopentanonitrile derivative **10** (Scheme 2). The reactivity of the α,β-unsaturated nitrile moiety of compound **10** towards some nitrogen nucleophiles was investigated in the aim of synthesis of amino-heterocyclic agents with potential neuroprotective activity.[17,18] Thus, compound **10** reacted with equimolar amount of either urea **11a** or thiourea **11b** in ethanolic sodium ethoxide solution to afford the aminopyrimidinylindolyl propanone derivatives **12a** and **12b**, respectively (Scheme 2). Compound **10** reacted also with guanidine hydrochloride **11c** in ethanolic sodium acetate solution to afford the corresponding aminopyrimidinylindolyl propanone derivative **12c** (Scheme 2). Also, compound **10** reacted with hydrazine hydrate **13a** or phenyl hydrazine **13b** in refluxing absolute ethanol to afford the aminopyrazolylindolyl propanone derivatives **14a** and **14b**, respectively (Scheme 2).

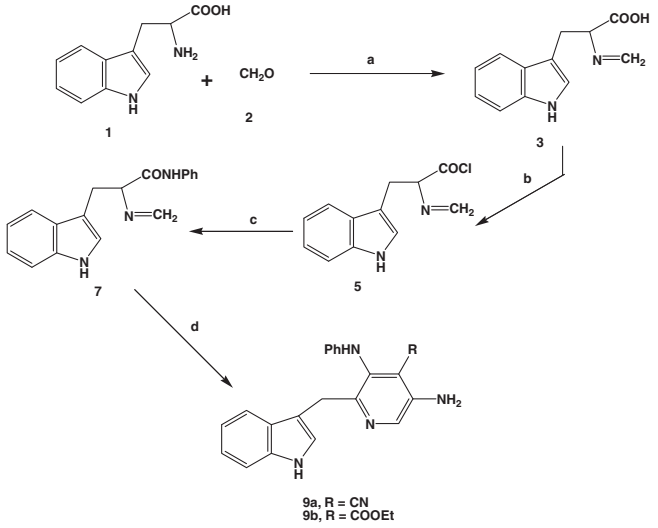


Scheme 2. Reagents and conditions: (a) dioxane, Et_3N, reflux; (b) H_2NCXNH_2 (**11a–c**), EtOH, NaOEt, or NaOAc, reflux; (c) NH_2NHR (**13a**,**b**), EtOH, reflux.

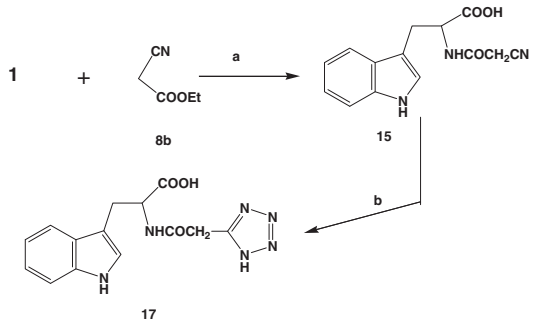
The acetonitrilocarbonyl tryptophan derivative **15** was synthesized via the reaction of TRP with ethyl cyanoacetate in refluxing dimethylformamide (Scheme 3).[19] The reactivity of acetonitrile moiety of compound **15** towards the formation of tetrazole ring was studied. In attempts of having a straightforward synthesis of indolyl tetrazolopropanoic acid, the reaction of compound **15** with sodium azide **16** in dimethylformamide containing a catalytic amount of ammonium chloride has been carried out. Heating of this mixture over oil bath at 125 °C gave one chromatographically isolable product in 75% yield, whose mass spectra and elemental analysis indicated a molecular formula $C_{14}H_{14}N_6O_3$ (m/z = 314). The IR spectrum of the product showed the disappearance of the cyano stretching of compound **15**. The ^{1}H NMR spectrum showed the presence of three singlets at δ = 8.05, 8.62, 9.36 (D_2O-exchangeable) for three NH groups and showed also a singlet signal at δ = 11.02 for carboxylic-OH group. The ^{13}C NMR spectrum confirmed the presence of tetrazole-C peak at δ = 153.9 ppm. Based on all the spectral data, the indolyl tetrazolopropanoic acid derivative **17** proposed to have the structure of this product (Scheme 3). Assignments of structures of all new compounds were obtained on the basis of their correct analytical analyses and compatible spectral data (cf. Section 3).

2.2. Biological assays

The in vivo neuroprotective effect of TRP and the novel synthesized indole derivatives, **9b**, **12c**, **14a**, and **17**, against ACR-induced neurotoxicity in rats was investigated. ACR is a well established agent for induction of neurotoxicity.[20,21] Neuronal damage due to



Scheme 1. Reagents and conditions: (a) (i) NH_4OAc, fusion; (ii) EtOH, heat; (b) $SOCl_2$ (**4**), diethyl ether, ice bath; (c) $PhNH_2$ (**6**), dioxane, Et_3N, reflux; (d) RCH_2CN (**8a**,**b**), EtOH, pip, reflux.



Scheme 3. Reagents and conditions: (a) DMF, reflux; (b) NaN_3 (**16**), DMF, NH_4Cl, heat.

Table 1
Effect of TRP and the new synthesized indole derivatives on brain monoamines levels in ACR-intoxicated rats

Groups	AD (ng mg^{-1} protein)	NA (ng mg^{-1} protein)	DA (ng g^{-1} tissue)
Control	86 ± 1.0	863 ± 14.5	1508 ± 37.1
ACR	52 ± 1.5[a] (−40%)	680 ± 22.7[a] (−21%)	1267 ± 50.3[a] (−16%)
TRP + ACR	61 ± 1.1[b] (17%)	791 ± 18.1[b] (16%)	1531 ± 24.4[b] (21%)
Compound **9b** + ACR	59 ± 1.7[b] (14%)	690 ± 2.8[c] (2%)	1380 ± 14.1[b,c] (9%)
Compound **12C** + ACR	57 ± 1.8[b] (10%)	684 ± 5.6[c] (1%)	1237 ± 27.3[c] (−2%)
Compound **14a** + ACR	60 ± 2.0[b] (15%)	722 ± 16.5[c] (6%)	1460 ± 34.2[b] (15%)
Compound **17** + ACR	87 ± 1.3[b,c] (67%)	823 ± 15.6[b] (21%)	1638 ± 31.1[b,c] (29%)

Within each column means with different superscript letters are significantly different ($p \leqslant 0.05$).
[a] Difference changes in relation to control group.
[b] Difference changes in relation to ACR group.
[c] Difference changes in relation to TRP + ACR group. ACR: acrylamide, TRP: L-tryptophan, AD: adrenaline, NA: noradrenalin, DA: dopamine.

ACR was detected by measuring: (1) the levels of brain monoamines, adrenaline (AD), noradrenalin (NA) and dopamine (DA), (2) the end product of brain lipid peroxidation, malondialdehyde, (MDA), (3) the activity of brain antioxidative enzymes, glutathione peroxidase (GPx), superoxide dismutase (SOD), and glutathione content (GSH), and (4) the activity of brain enzymes lactate dehydrogenase (LDH) and creatine kinase (CK).

The data depicted in Table 1 demonstrated that ACR administration induced degenerative effect on brain tissue as manifested by the significant decrease in the levels of the neurotransmitters, AD, NA, and DA (40%, 21%, and 16%, respectively) as compared to the control group. These results are in agreement with those of LoPachin[22] and Mannaa et al.[23] LoPachin[22] suggested that the inhibition of neurotransmission contributes significantly to the development of corresponding neurological deficits as a potential mechanism of synaptic dysfunction. The level of MDA in the brain was significantly increased (31%) in ACR-intoxicated rats as compared to the control group (Table 2). On the other hand, the brain antioxidant enzymes, SOD, GPx activity, and GSH content significantly decreased in ACR-intoxicated rats, by 32%, 43%, and 27%, respectively, as compared to the control group (Table 2). These results are in consistent with those of Mannaa et al.[23] and Zhu et al.[24] As illustrated in Table 3, administration of ACR increased the brain LDH activity (67%) and decreased brain CK activity (73%) significantly as compared to the control group. These results are in agreement with those of Mannaa et al.[23] and Lüa et al.[25]

In the present study, pre-treatment with TRP in ACR-intoxicated rats resulted in significant increase in brain AD, NA, and DA levels as compared to ACR-intoxicated group (Table 1, Fig. 1). These findings could be attributed to the fact that the indole ring in the TRP acts as ligand of peripheral benzodiazepine receptor (PBR) which improves functional recovery of the brain in this model of neurotoxicity.[13] In addition, TRP is the precursor of melatonin[14] which could rescue both dopaminergic and non-dopaminergic neurons from oxidative stress and preserve all types of brain neurons from neurological damage.[15]

Treatment with TRP prior to ACR intoxication produced significant increase in brain GPx activity accompanied with significant decrease in brain MDA level as compared to ACR-intoxicated group (Table 2, Fig. 2). This result could be explained as that indole possesses antioxidant and free radical scavenging properties. This explanation is in great agreement with Suzen and Buyukbingol[7] and Biradar et al.[26] In addition, indole skeleton has been reported to be a protective agent in different models of oxidative stress both in vivo and in vitro.[27] Furthermore, indoles have been found to induce GSH synthesis and promote GPx activity[28] as well as it could inhibit lipid peroxidation probably by the direct scavenging of the reactive oxygen species.[27] The current results demonstrated that the pre-administration of ACR-intoxicated rats with TRP significantly decreased brain LDH activity (Table 3, Fig. 3). This finding could be attributed to the fact that indole compound exhibits ligand to PBR which promotes neuronal survival and thus preserving LDH activity.[13]

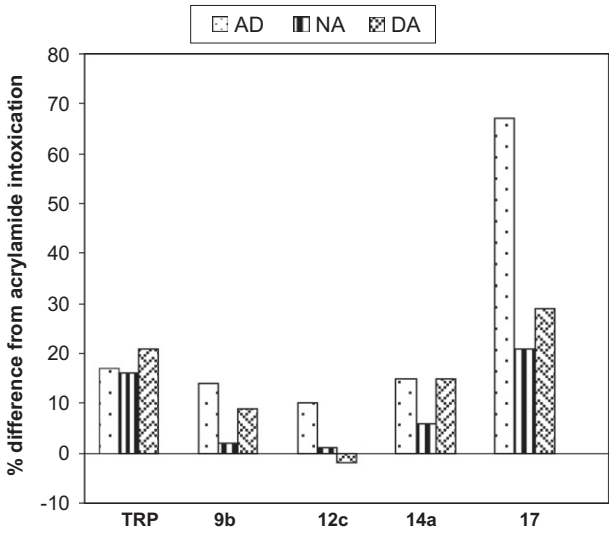


Figure 1. Levels of AD, NA, and DA as % difference from ACR intoxication in TRP and the newly synthesized derivatives-treated groups. ACR: acrylamide, TRP: L-tryptophan, AD: adrenaline, NA: noradrenalin, DA: dopamine.

Table 2
Effect of TRP and the new synthesized indole derivatives on brain oxidant/antioxidant status of ACR-intoxicated rats

Groups	MDA (nmol mg^{-1} protein)	GSH (mmol mg^{-1} protein)	GPx (mU mg^{-1} protein)	SOD (U mg^{-1} protein)
Control	6.2 ± 0.3	1.5 ± 0.14	1.4 ± 0.08	2.5 ± 0.11
ACR	8.1 ± 0.9[a] (31%)	1.1 ± 0.05[a] (−27%)	0.8 ± 0.03[a] (−43%)	1.7 ± 0.22[a] (−32%)
TRP + ACR	6.1 ± 0.3[b] (−25%)	1.2 ± 0.12 (9%)	1.1 ± 0.02[b] (38%)	2.0 ± 0.06 (18%)
Compound **9b** + ACR	7.3 ± 1.1 (−10%)	0.9 ± 0.04[b,c] (−18%)	0.98 ± 0.04[b] (23%)	1.8 ± 0.11 (6%)
Compound **12C** + ACR	7.7 ± 0.75 (−5%)	0.9 ± 0.08[b,c] (−18%)	0.81 ± 0.03[c] (1%)	1.75 ± 0.02 (3%)
Compound **14a** + ACR	6.3 ± 0.6[b] (−22%)	1.2 ± 0.08 (9%)	1.0 ± 0.04[b] (25%)	1.9 ± 0.12 (12%)
Compound **17** + ACR	6.0 ± 0.7[b] (−26%)	1.5 ± 0.07[b,c] (36%)	1.2 ± 0.03[b] (50%)	3.3 ± 0.17[b,c] (94%)

Within each coulmn means with different superscript letters are significantly different ($p \leqslant 0.05$).
[a] Difference changes in relation to control group.
[b] Difference changes in relation to ACR group.
[c] Difference changes in relation to TRP + ACR group. ACR: acrylamide, TRP: L-tryptophan, MDA: malondialdehyde, GSH: glutathione content, GPx: glutathione peroxidase, SOD: superoxide dismutase.

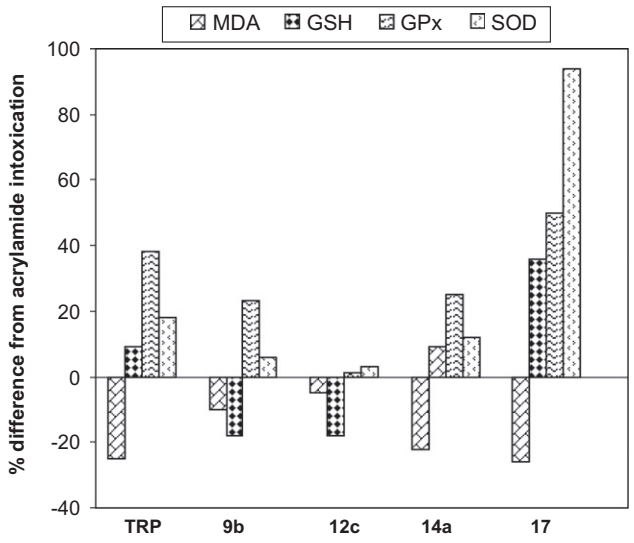


Figure 2. Values of MDA, GSH, GPx and SOD as % difference from acrylamide intoxication in TRP and the newly synthesized derivatives-treated groups. ACR: acrylamide, TRP: L-tryptophan, MDA: malondialdehyde, GSH: glutathione content, GPx: glutathione peroxidase, SOD: superoxide dismutase.

Table 3
Effect of TRP and the new synthesized indole derivatives on brain enzymes activity in ACR-intoxicated rats

Groups	LDH (U mg^{-1}protein)	CK (U mg^{-1}protein)
Control	0.12 ± 0.004	0.27 ± 0.05
ACR	0.2 ± 0.012[a] (67%)	0.073 ± 0.006[a] (−73%)
TRP + ACR	0.13 ± 0.008[b] (−35%)	0.093 ± 0.007 (27%)
Compound **9b** + ACR	0.16 ± 0.005[b,c] (−20%)	0.08 ± 0.005 (14%)
Compound **12C** + ACR	0.18 ± 0.014[b,c] (−10%)	0.078 ± 0.01 (7%)
Compound **14a** + ACR	0.16 ± 0.005[b,c] (−20%)	0.089 ± 0.003 (22%)
Compound **17** + ACR	0.13 ± 0.011[b] (−35%)	0.120 ± 0.007[b] (64%)

Within each column means with different superscript letters are significantly different ($p \leqslant 0.05$).
[a] Difference changes in relation to control group.
[b] Difference changes in relation to ACR group.
[c] Difference changes in relation to TRP + ACR group. ACR: acrylamide, TRP: L-tryptophan, LDH: lactate dehydrogenase, CK: creatine kinase.

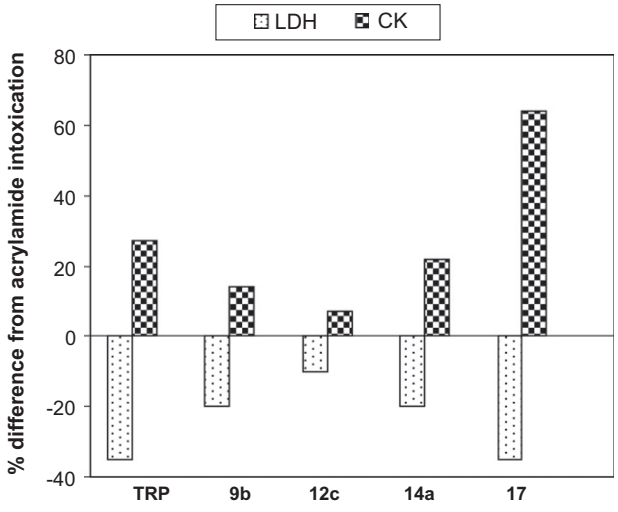


Figure 3. LDH and CK enzymes activity as % difference from acrylamide intoxication in TRP and the newly synthesized derivatives-treated groups. ACR: acrylamide, TRP: L-tryptophan, LDH: lactate dehydrogenase, CK: creatine kinase.

In the present study, pre-administration with the newly synthesized indole derivatives **9b**, **12c**, **14a**, or **17** in ACR-intoxicated rats resulted in significant increase in brain AD level and pre-administration with compounds **9b**, **17**, or **14a** led to significant increase in brain DA level while pre-administration with compound **17** produced significant increase in brain NA level as compared to ACR-intoxicated group (Table 1, Fig. 1). These results indicated that, all the newly tested compounds exhibited neuroprotective effect. In addition to the essential pharmacophoric features of the TRP molecule, compounds **9b** and **14a** containing aminopyridine and aminopyrazole rings, respectively, that behaved as ligand to PBR.[16,29] Therefore, these compounds exerted more or less similar effect as TRP on brain AD level.

Our results demonstrated that the indolyl tetrazolopropanoic acid derivative **17** in which tetrazole ring was attached to the TRP moiety ranked as the strongest neuroprotectant as indicated by the significant increase in each of brain AD, DA, and NA levels. The powerful neuroprotective action of compound **17** might be attributed to the highly antioxidant activity of both the indole and tetrazole moieties.[30] Pyrimidinylindolyl propanone derivative **12c** that contains indole and aminopyrimidine rings has been found to have moderate neuroprotective activity as indicated by the significant increase only in brain DA level.

The current results (Table 2, Fig. 2) demonstrated that pre-administration with the newly synthesized compounds **9b**, **14a**, or **17** induced significant increases in brain GPx activity and compounds **9b**, **17**, or **12c** led to significant increase in brain GSH level. Pre-administration of compound **17** or **14a** resulted in significant decrease in brain MDA level and compound **17** only showed significant increase in brain SOD activity. These findings could be attributed to the indole moiety which has remarkable antioxidant properties. In addition, the newly synthesized compounds **9b**, **17**, **12c**, and **14a** containing pyridine,[31] tetrazole,[30] pyrimidine,[32] and pyrazole[33] rings, respectively, possess antioxidant and free radical scavenging properties. These compounds could suppress oxidative stress with consequent inhibition of lipid peroxidation product (MDA), enhance antioxidant enzyme activities (SOD, GPx) and induce GSH synthesis.

Compounds **9b**, **12c**, **14a**, or **17** administered 10 min before ACR intoxication revealed significant decrease in brain LDH activity and compound **17** only showed significant increase in brain CK activity (Table 3, Fig. 3). These results indicated that, our compounds exhibit neuroprotective effect and confirmed the powerful neuroprotective action of compound **17**. The tetrazole ring of compound **17** acted as protecting agent for neuronal cells from oxidative stress due to its superior pharmacokinetic properties.[34,35]

2.3. Histological examination

Microscopic investigation of brain section of control rat (Fig. 4 A) showed the highly active nerve cells that have huge nuclei with relatively pale-stained (arrow a), disappeared nuclear chromatin and prominent nuclei. The surrounding relatively inactive support cells have small nuclei with densely-stained (arrows b and c), condensed chromatin and no visible nucleoli.

Photomicrograph of brain section of ACR-intoxicated rats showed the dark neurons (arrows d and e) with corkscrew dendrites (Fig. 4 B). Also, the brain section of ACR exposed rats showed neurons with coarse clumping of hyperchromatic nuclear chromatin (arrows f and g) and with granular or amorphous cytoplasm (Fig. 4 C). These findings confirmed the degenerative effect of ACR on brain tissue.[23]

The examination of brain section of rats treated with TRP prior to ACR showed neurons that appeared more or less as the normal control rats (Fig. 4 D). Examination of the brain section of rats gi-

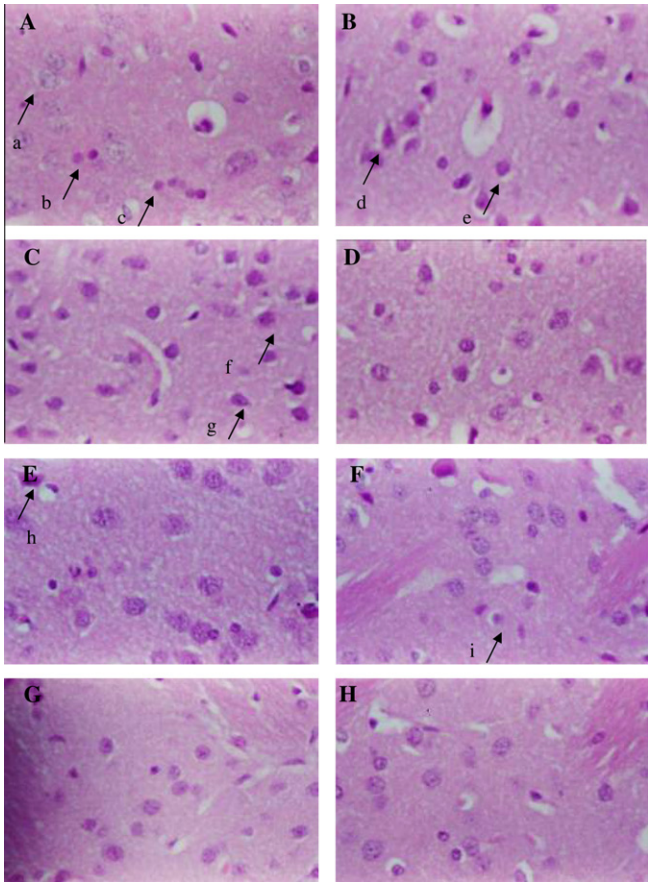


Figure 4. Photomicrograph of a brain section of control rats (A), ACR-intoxicated rats (B and C), TRP pre-treated rats (D), compound **9b** pre-treated rats (E), compound **12c** pre-treated rats (F), compound **14a** pre-treated rats (G), and compound **17** pre-treated rats (H).

ven compound **9b** prior to ACR (Fig. 4 E) indicated that neurons appeared more or less like normal one but some dark neurons (arrow h) were also seen. Examination of brain section of ACR-intoxicated rats pre-treated with compound **12c** (Fig. 4 F) revealed that neurons appeared more or less like normal one with the appearance of some small vacuoles (arrow i) that contain the remaining of dead neurons. Examination of brain section of rats received compounds **14a** (Fig. 4 G) or **17** (Fig. 4 H) prior to ACR indicated that neurons appeared more or less like normal one.

2.4. Conclusion

The present study described a facile synthesis of novel promising neuroprotective indole derivatives and investigated also the importance of incorporating heterocyclic moiety to the TRP nucleus to form new effective hybrid molecules. TRP and its novel indole derivatives **9b**, **12c**, **14a**, and **17** showed neuroprotective activity with various intensities depending on the structure of each compound. Compound **17** in which the tetrazole ring was attached to the TRP moiety ranked as the strongest neuroprotective agent. All the tested compounds have been shown to possess antioxidant properties offering promising efficacy against oxidative stress induced by ACR administration. Finally, the neuroprotective activity displayed by these compounds may be of interest for further study of the toxicity profile of promising tested compounds before application in phase 1 of clinical study in the hope of finding more active and selective neuroprotective agents.

3. Materials and methods

3.1. Chemistry

All chemicals were purchased from commercials suppliers and were used directly. The appropriate precautions in handling moisture-sensitive compounds were undertaken. All melting points were measured using an electro-thermal capillary melting point apparatus (Buchi 535, Switzerland) and were uncorrected; the IR spectra expressed in cm^{-1} and recorded using KBr pellets and a Pa-9721 IR spectrometer. ^1H and ^{13}C NMR spectra were recorded with Jeol instrument (Japan), at 270 and 125 MHz, respectively, in DMSO-d_6 or $CDCl_3$ as solvent and the chemical shifts (δ) were recorded in ppm relative to TMS. Mass spectra were recorded on a GC–MS-QP 1000 Ex spectra mass spectrometer operating at 70 eV. Elemental analyses were carried by the Micro-analytical Data Unit at the National Research Center, Giza, Egypt and the Micro-analytical data Unit at Cairo University. The reactions were monitored by thin layer chromatography (TLC) which was carried out using Merck 60 F254 aluminum sheets and visualized by UV light (254 nm). The mixtures were separated by preparative TLC and gravity chromatography. The starting compound α-amino(acetonitrilocarbamido)-3-indolopropionic acid (**15**) was prepared according to the published procedure.[19]

3.1.1. Synthesis of 3-(1*H*-indol-3-yl)-2-(methylenamino)-propanoic acid (3)

A suspension of TRP **1** (0.2 g, 1 mmol), formaldehyde **2** (0.03 mL, 1 mmol) and ammonium acetate (0.08 g, 1 mmol) was heated in an oil bath at 120 °C for 20 min and then left to cool at room temperature. The reaction mixture triturated with absolute ethanol (10 mL) and heated again under reflux for 15 min. Then the reaction mixture was left to cool at room temperature, poured over ice/water mixture and neutralized with dilute hydrochloric acid. The solid product obtained was collected by filtration, dried, and crystallized from absolute ethanol.

Compound **3**: Yellow crystals yield 0.21 g (95%), mp 207–209 °C. IR (ν/cm^{-1}): 3424 (br, OH, carboxylate), 3396 (NH), 3027 (CH-aromatic), 2984, 2887 (CH-aliphatic), 1728 (C=O), 1660 (C=N), 1645 (C=C). ^1H NMR (DMSO-d_6, δ ppm): 2.68 (t, J = 2.07 Hz, 1H, CH), 2.84 (d, J = 2.07 Hz, 2H, CH_2), 4.56 (s, 2H, N=CH_2), 6.78 (s, 1H, CH-indole), 7.16–7.27 (m, 4H, C_6H_4), 10.40 (s, 1H, NH, D_2O-exchangeable), 11.21 (s, 1H, COOH, D_2O-exchangeable). MS (EI): m/z (%): 215 ($M^{+\cdot}-1$, 42%), 188 (M^+-NCH_2, 18), 171 (M^+-COOH, 50), 116 (100). Anal. Calcd for $C_{12}H_{12}N_2O_2$ (216.236): C, 66.65; H, 5.59; N, 12.96. Found: C, 66.46; H, 5.79; N, 13.21.

3.1.2. Synthesis of 3-(1*H*-indol-3-yl)-2-(methylenamino)-propanoyl chloride (5)

To a solution of compound **3** (0.22 g, 1 mmol) in anhydrous diethyl ether (20 mL) in ice bath, equimolar amount of thionyl chloride **4** (0.119 g, 1 mmol) was added dropwise with stirring. After complete addition, the reaction mixture was stirred in ice bath for 5 h until all starting materials had disappeared as indicated by TLC. Then, the reaction mixture was left at room temperature over night. The solid product that formed was filtered off, dried and crystallized from absolute ethanol.

Compound **5**: Yellow crystals, yield 0.24 g (90%), mp 252–254 °C, IR (ν/cm^{-1}): 3385 (NH), 3035 (CH-aromatic), 2987, 2890 (CH-aliphatic), 1715 (C=O), 1657 (C=N), 1642 (C=C). ^1H NMR ($CDCl_3$, δ ppm): 2.72 (t, J = 3.01 Hz, 1H, CH), 2.89 (d, J = 3.01 Hz, 2H, CH_2), 4.73 (s, 2H, N=CH_2), 6.65 (s, 1H, CH-indole), 7.18–7.29 (m, 4H, C_6H_4), 10.12 (s, 1H, NH, D_2O-exchangeable). MS (EI): m/z (%): 234 ($M^{+\cdot}$, 34%), 206 (M^+-NCH_2, 12), 171 (M^+-COCl, 25), 116 (100).

Anal. Calcd for $C_{12}H_{11}ClN_2O$ (234.682): C, 61.41; H, 4.72; N, 11.94. Found: C, 61.19; H, 4.95; N, 12.15.

3.1.3. Synthesis of 3-(1*H*-indol-3-yl)-2-(methylenamino)-*N*-phenylpropanamide (7)

Equimolar amounts of compound **5** (0.24 g, 1 mmol) and aniline **6** (0.09 mL, 1 mmol) in dioxane (30 mL) containing a catalytic amount of triethylamine (2 mL) were heated under reflux for 6–7 h. The reaction was monitored by TLC, concentrated under vacuum and poured into crushed ice and then left at 4 °C over night. The formed solid product was filtrated off, dried and crystallized from methanol.

Compound **7**: Pale brown crystals, yield 0.22 g (75%), mp 233–235 °C, IR (ν/cm^{-1}): 3435–3376 (2NH), 3043 (CH-aromatic), 2987, 2878 (CH-aliphatic), 1712 (C=O), 1667 (C=N), 1639 (C=C). ^{1}H NMR ($CDCl_3$, δ ppm): 2.57 (t, J = 2.56 Hz, 1H, CH), 2.80 (d, J = 2.56 Hz, 2H, CH_2), 4.69 (s, 2H, N=CH_2), 6.65 (s, 1H, CH-indole), 7.16–7.34 (m, 4H, C_6H_4), 7.60–7.85 (m, 5H, C_6H_5), 8.70, 10.12 (2s, 2H, 2NH, D_2O-exchangeable). ^{13}C NMR ($CDCl_3$, δ ppm): 122.9 (C-2, indole), 110.8 (C-3), 119.0 (C-4), 122.3 (C-5), 120.0 (C-6), 112.0 (C-7), 127.0, 136.2 (fused aromatic-C), 32.2, 96.3 (CH_2, CH), 163.0 (CH_2=N), 139.2, 121.9, 129.7, 124.3 (C-phenyl), 172.2 (C=O). MS (EI): m/z (%): 291 ($M^{+\cdot}$, 74), 199 (M^+−NHPh, 12), 92 (100). Anal. Calcd for $C_{18}H_{17}N_3O$ (291.347): C, 74.20; H, 5.88; N, 14.42. Found: C, 73.95; H, 6.08; N, 14.17.

3.1.4. Synthesis of indolylmethylpyridine derivatives (9a,b)

General procedure: To a solution of compound **7** (0.29 g, 1 mmol) in absolute ethanol (25 mL) containing a catalytic amount of piperidine (0.5 mL) either malononitrile **8a** (0.07 g, 1 mmol) or ethyl cyanoacetate **8b** (0.11 g, 1 mmol) was added. The reaction mixture was heated under reflux for 4–6 h until all the reactants had disappeared as indicated by TLC. The reaction mixture after cooling at room temperature was poured into ice/water mixture and neutralized with dilute hydrochloric acid. The formed solid product, in each case, was filtered off, dried and crystallized from the appropriate solvent.

3.1.4.1. 2-[(1*H*-Indol-3-yl)methyl]-5-amino-3-(phenylamino)-pyridine-4-carbonitrile (9a). Yellow crystals from dioxane, yield 0.26 g (76%), mp 310–312 °C, IR (ν/cm^{-1}): 3438–3396 (2NH, NH_2), 3032 (CH-aromatic), 2985, 2883 (CH-aliphatic), 2228 (CN), 1660 (C=N), 1630 (C=C). ^{1}H NMR ($CDCl_3$, δ ppm): 4.87 (s, 2H, CH_2), 5.23 (s, 2H, NH_2, D_2O-exchangeable), 6.68 (s, 1H, CH-indole), 6.76–7.04 (m, 4H, C_6H_4), 7.16–7.25 (m, 5H, C_6H_5), 8.72 (s, 1H, pyridine_H), 9.80, 10.45 (2s, 2H, 2NH, D_2O-exchangeable). ^{13}C NMR ($CDCl_3$, δ ppm): 123.4 (C-2, indole), 109.2 (C-3), 119.2 (C-4), 121.8 (C-5), 120.2 (C-6), 111.0 (C-7), 127.6, 136.4 (fused aromatic-C), 36.2 (CH_2), 137.4, 146.2, 91.2, 147.0, 126.9 (pyridine-C), 117.2 (CN), 143.2, 116.9, 129.7, 118.0 (phenyl-C). MS (EI): m/z (%): 338 ($M^{+\cdot}$−1, 35), 247 ($M^{+\cdot}$−NHPh, 22), 218 (32), 130 (100), 92 (46). Anal. Calcd for $C_{21}H_{17}N_5$ (339.393): C, 74.32; H, 5.05; N, 20.63. Found: C, 74.54; H, 5.28; N, 20.89.

3.1.4.2. Ethyl 2-[(1*H*-indol-3-yl)methyl]-5-amino-3-(phenylamino)-pyridine-4-carboxylate (9b). Brown crystals from methanol, yield 0.27 g (70%), mp 233–234 °C, IR (ν/cm^{-1}): 3442–3392 (2NH, NH_2), 3036 (CH-aromatic), 2987, 2879 (CH-aliphatic), 1732 (C=O), 1664 (C=N), 1638 (C=C). ^{1}H NMR ($CDCl_3$, δ ppm): 1.38 (t, J = 7.01 Hz, 3H, CH_3-ester), 4.28 (q, J = 7.01 Hz, 2H, CH_2-ester), 4.76 (s, 2H, CH_2), 5.03 (s, 2H, NH_2, D_2O-exchangeable), 6.73 (s, 1H, CH-indole), 6.75–7.07 (m, 4H, C_6H_4), 7.16–7.25 (m, 5H, C_6H_5), 8.67 (s, 1H, pyridine-H), 9.92, 10.25 (2s, 2H, 2NH, D_2O-exchangeable). ^{13}C NMR ($CDCl_3$, δ ppm): 122.9 (C-2, indole), 109.8 (C-3), 119.2 (C-4), 122.3 (C-5), 121.0 (C-6), 111.3 (C-7), 127.4, 136.7 (fused aromatic-C), 30.2 (CH_2), 61.0 (CH_2-ester), 15.0 (CH_3-ester), 167.3 (C=O), 126.4, 143.2, 109.2, 145.0, 135.9 (pyridine-C), 143.2, 116.9, 129.7, 118.0 (phenyl-C). MS (EI): m/z (%): 386 ($M^{+\cdot}$, 65), 371 ($M^{+\cdot}$−CH_3, 28), 294 ($M^{+\cdot}$−NHPh, 20), 130 (100), 92 (56). Anal. Calcd for $C_{23}H_{22}N_4O_2$ (386.446): C, 71.48; H, 5.74; N, 14.50. Found: C, 71.24; H, 5.97; N, 14.22.

3.1.5. Synthesis of 2-cyano-5-(1*H*-indol-3-yl)-4-(methylenamino)-3-oxopentanonitrile (10)

To a solution of compound **5** (0.24 g, 1 mmol) in dioxane (20 mL) containing a catalytic amount of triethylamine (0.5 mL) equimolar amount of malononitrile **8a** (0.07 g, 1 mmol) was added. The reaction mixture was heated under reflux for 3 h until all the reactants had disappeared as indicated by TLC. Then, the reaction mixture was left to cool at room temperature, poured over ice/water mixture, neutralized with dilute hydrochloric acid and extracted with diethyl ether (3 × 20 mL). The organic layer was dried over anhydrous calcium chloride. Removal of the solvent in vacuo afforded the corresponding product, which was crystallized from dioxane.

Compound **10**: Brown crystals, yield 0.21 g (78%), mp 261–263 °C, IR (ν/cm^{-1}): 3382 (NH), 3040 (CH-aromatic), 2987, 2875 (CH-aliphatic), 2232, 2225 (2CN), 1706 (C=O), 1658 (C=N), 1649 (C=C). ^{1}H NMR (DMSO-d_6, δ ppm): 2.60 (d, J = 4.02 Hz, 2H, CH_2), 2.87 (t, J = 4.02 Hz, 1H, CH), 4.35 (s, 1H, CH), 5.06 (s, 2H, N=CH_2), 6.82 (s, 1H, CH-indole), 7.20–7.45 (m, 4H, C_6H_4), 8.43 (s, 1H, NH, D_2O-exchangeable). MS (EI): m/z (%): 265 ($M^{+\cdot}$+1, 32), 199 (38), 116 (23), 84 (100), 65 (12). Anal. Calcd for $C_{15}H_{12}N_4O$ (264.282): C, 68.17; H, 4.58; N, 21.20. Found: C, 68.42; H, 4.29; N, 21.37.

3.1.6. Synthesis of pyrimidinylindolylmethylenamino-propan-1-one derivatives (12a,b)

General procedure: Either urea **11a** (0.12 g, 2 mmol) or thiourea **11b** (0.15 g, 2 mmol) was added to a suspension of compound **10** (0.52 g, 2 mmol) in sodium ethoxide (10 mmol) [prepared by dissolving sodium metal (0.23 g, 10 mmol) in absolute ethanol (35 mL)]. The reaction mixture, in each case, was heated in a boiling water bath for 3–4 h, and then left to cool at room temperature. The reaction was monitored by TLC, concentrated under vacuum, poured into crushed ice and neutralized with dilute hydrochloric acid. The formed solid product, in each case, collected by filtration, dried and crystallized from the appropriate solvent.

3.1.6.1. 1-(4,6-Diamino-2-oxo-5*H*-pyrimidin-5-yl)-3-(1*H*-indol-3-yl)-2-(methylenamino)-propan-1-one (12a). Pale yellow crystals from absolute ethanol, yield 0.24 g (75%), mp 281–283 °C, IR (ν/cm^{-1}): 3385–3348 ($2NH_2$, NH), 3036 (CH-aromatic), 2979, 2864 (CH-aliphatic), 1705, 1698 (2C=O), 1645 (C=N), 1620 (C=C). ^{1}H NMR (DMSO-d_6, δ ppm): 2.35 (s, 1H, pyrimidine-H), 2.63 (d, J = 3.61 Hz, 2H, CH_2), 2.74 (t, J = 3.61 Hz, 1H, CH), 4.03 (s, 4H, $2NH_2$, D_2O-exchangeable), 5.12 (s, 2H, N=CH_2), 6.80 (s, 1H, CH-indole), 7.18–7.35 (m, 4H, C_6H_4), 10.03 (s, 1H, NH, D_2O-exchangeable). ^{13}C NMR (DMSO-d_6, δ ppm): 121.8 (C-2, indole), 111.2 (C-3), 119.0 (C-4), 122.3 (C-5), 120.9 (C-6), 111.7 (C-7), 127.2, 135.3 (fused aromatic-C), 34.2 (CH_2), 74.0 (CH), 164.0 (N=CH_2), 198.5 (C=O), 49.4, 163.2, 164.0, 158.2 (pyrimidine-C). MS (EI): m/z (%): 325 ($M^{+\cdot}$+1, 22), 199 (59), 125 (43), 116 (100). Anal. Calcd for for $C_{16}H_{16}N_6O_2$ (324.337): C, 59.25; H, 4.97; N, 25.91. Found: C, 59.45; H, 4.76; N, 25.63.

3.1.6.2. 1-(4,6-Diamino-2-thioxo-5*H*-pyrimidin-5-yl)-3-(1*H*-indol-3-yl)-2-(methylenamino)-propan-1-one (12b). Yellow crystals from dioxane, yield 0.26 g (75%), mp 302–304 °C, IR (ν/cm^{-1}): 3429–3356 ($2NH_2$, NH), 3037 (CH-aromatic), 2987, 2874 (CH-aliphatic), 1696 (C=O), 1654 (C=N), 1627 (C=C), 1197 (C=S). ^{1}H NMR ($CDCl_3$, δ ppm): 2.30 (s, 1H, pyrimidine-H), 2.58 (d, J = 2.98 Hz, 2H, CH_2), 2.75 (t, J = 2.98 Hz, 1H, CH), 4.23 (s, 4H,

$2NH_2$, D_2O-exchangeable), 5.17 (s, 2H, N=CH_2), 6.80 (s, 1H, CH-indole), 7.18–7.32 (m, 4H, C_6H_4), 10.11 (s, 1H, NH, D_2O-exchangeable). MS (EI): *m/z* (%): 340 ($M^{+\cdot}$, 16), 199 (52), 141 (43), 116 (100). Anal. Calcd for $C_{16}H_{16}N_6OS$ (340.403): C, 56.45; H, 4.74; N, 24.69. Found: C, 56.68; H, 4.53; N, 24.88.

3.1.7. Synthesis of 1-(4,6-diamino-2-imino-5*H*-pyrimidin-5-yl)-3-(1*H*-indol-3-yl)-2-(methylenamino)propan-1-one (12c)

A mixture of compound **10** (0.52 g, 2 mmol), guanidine hydrochloride **11c** (0.2 g, 2 mmol) and sodium acetate (0.16 g, 2 mmol) in absolute ethanol (30 mL) was refluxed for 7 h. The reaction mixture was concentrated in vacuo and the residue was triturated with ethyl acetate. The solid that formed was filtered off, dried and crystallized from DMF.

Compound **12c**: Pale brown powder, yield 0.26 g (80%) mp 247–249 °C, IR (ν/cm^{-1}): 3435–3358 ($2NH_2$, 2NH), 3030 (CH-aromatic), 2985, 2874 (CH-aliphatic), 1698 (C=O), 1645 (C=N), 1595 (C=C). ^{1}H NMR (DMSO-d_6, δ ppm): 2.50 (s, 1H, pyrimidine-H), 2.62 (d, J = 3.04 Hz, 2H, CH_2), 2.84 (t, J = 3.04 Hz, 1H, CH), 5.07 (s, 2H, N=CH_2), 5.53 (s, 4H, $2NH_2$, D_2O-exchangeable), 6.86 (s, 1H, CH-indole), 7.20–7.37 (m, 4H, C_6H_4), 9.76, 10.11 (2s, 2H, 2NH, D_2O-exchangeable). ^{13}C NMR (DMSO-d_6, δ ppm): 122.3 (C-2, indole), 110.8 (C-3), 119.2 (C-4), 122.8 (C-5), 121.6 (C-6), 111.0 (C-7), 127.6, 136.7 (fused aromatic-C), 31.2 (CH_2), 74.0 (CH), 163.5 (N=CH_2), 202.3 (C=O), 50.4, 164.2, 160.2, 164.8 (pyrimidine-C). MS (EI): *m/z* (%): 323 ($M^{+\cdot}$, 62), 199 (24), 116 (100), 84 (76). Anal. Calcd for $C_{16}H_{17}N_7O$ (323.352): C, 59.43; H, 5.30; N, 30.32. Found: C, 59.17; H, 5.53; N, 30.12.

3.1.8. Synthesis of pyrazolylindolylmethylenaminopropan-1-one derivatives (14a,b)

General procedure: To a solution of compound **10** (0.26 g, 1 mmol) in absolute ethanol (20 mL) either hydrazine hydrate **13a** (0.05 g, 1 mmol) or phenyl hydrazine **13b** (0.11 g, 1 mmol) was added. The reaction mixture, in each case, was heated under reflux for 6–8 h until all starting materials had disappeared as indicated by TLC. Then the reaction mixture was concentrated under vacuum, whereby the resulted oily product was triturated with cold water/ice mixture. The solid product so formed in each case was filtered off, dried and crystallized from the appropriate solvent.

3.1.8.1. 1-(3,5-Diamino-4,5-dihydro-1*H*-pyrazol-4-yl)-3-(1*H*-indol-3-yl)-2-(methylenamino)-propan-1-one (14a). Yellow crystals, yield 0.22 g (75%), mp 248–250 °C, IR (ν/cm^{-1}): 3442–3365 ($2NH_2$, 2NH), 3042 (CH-aromatic), 2989, 2869 (CH-aliphatic), 1687 (C=O), 1652 (C=N), 1605 (C=C). ^{1}H NMR ($CDCl_3$, δ ppm): 2.64 (d, J = 4.05 Hz, 2H, CH_2), 2.72 (t, J = 4.05 Hz, 1H, CH), 2.85, 3.86 (2d, J = 5.22 Hz, 2H, pyrazole-2H), 5.12 (s, 2H, N=CH_2), 4.46, 5.23 (2s, 4H, $2NH_2$, D_2O-exchangeable), 6.85 (s, 1H, CH-indole), 7.23–7.38 (m, 4H, C_6H_4), 8.74, 10.00 (2s, 2H, 2NH, D_2O-exchangeable). ^{13}C NMR ($CDCl_3$, δ ppm): 123.0 (C-2, indole), 110.9 (C-3), 119.2 (C-4), 122.5 (C-5), 120.6 (C-6), 111.2 (C-7), 127.6, 137.0 (fused aromatic-C), 30.8 (CH_2), 74.6 (CH), 163.5 (N=CH_2), 208.3 (C=O), 50.4, 64.3, 155.2 (pyrazole-C). MS (EI): *m/z* (%): 300 ($M^{+\cdot}$+2, 32), 199 (26), 182 (17), 116 (83), 99 (100). Anal. Calcd for $C_{15}H_{18}N_6O$ (298.343): C, 60.39; H, 6.08; N, 28.17. Found: C, 60.61; H, 5.86; N, 28.43.

3.1.8.2. 1-(3,5-Diamino-4,5-dihydro-1-phenyl-pyrazol-4-yl)-3-(1*H*-indol-3-yl)-2-(methylenamino)propan-1-one (14b). Brown crystals, yield 0.28 g (75%), mp 236–238 °C IR (ν/cm^{-1}): 3439–3375 ($2NH_2$, NH), 3035 (CH-aromatic), 2987, 2865 (CH-aliphatic), 1695 (C=O), 1658 (C=N), 1614 (C=C). ^{1}H NMR (DMSO-d_6, δ ppm): 2.60 (d, J = 2.68 Hz, 2H, CH_2), 2.74 (t, J = 2.68 Hz, 1H, CH), 2.89, 3.73 (2d, J = 4.92 Hz, 2H, pyrazole-2H), 5.09 (s, 2H, N=CH_2), 4.57, 5.03 (2s, 4H, $2NH_2$, D_2O-exchangeable), 6.58–7.06 (m, 6H, C_6H_5 + CH-indole), 7.23–7.38 (m, 4H, C_6H_4), 10.22 (s, 1H, NH, D_2O-exchangeable). MS (EI): *m/z* (%): 374 ($M^{+\cdot}$, 13), 259 (19), 199 (36), 116 (83), 77 (100). Anal. Calcd for $C_{21}H_{22}N_6O$ (374.439): C, 67.36; H, 5.92; N, 22.44. Found: C, 67.62; H, 6.09; N, 22.72.

3.1.9. Synthesis of 2-(1*H*-tetrazole-5-methylencarboxamido)-3-(1*H*-indol-3-yl)propanoic acid (17)

To a solution of compound **15** (1.35 g, 5 mmol) in dimethyl formamide (10 mL) containing ammonium chloride (0.027 g, 0.5 mmol), sodium azide **16** (0.05 g) was added with stirring. After complete addition, the reaction mixture was heated in an oil bath at 120 °C with stirring for 7 h. The reaction was monitored by TLC, concentrated under vacuum, poured into 100 mL cold water and acidified with dilute hydrochloric acid to pH 2. Then, the reaction mixture was left to cool at 5 °C overnight. The solid product that formed was filtered off, washed with water several times, dried and crystallized from absolute ethanol.

Compound **17**: Dark brown crystals from absolute ethanol, yield 0.23 g (75%), mp 208–210 °C, IR (ν/cm^{-1}): 3392–3353 (3NH), 3042 (CH-aromatic), 2987, 2874 (CH-aliphatic), 1655 (C=N), 1618 (C=C). ^{1}H NMR (DMSO-d_6, δ ppm): 2.84 (d, J = 3.44 Hz, 2H, CH_2), 4.23 (s, 2H, CH_2), 4.85 (t, J = 3.44 Hz, 1H, CH), 6.81 (s, 1H, CH-indole), 7.06–7.20 (m, 4H, C_6H_4), 8.05, 8.62, 9.36 (3s, 3H, 3NH, D_2O-exchangeable), 11.10 (s, 1H, OH, D_2O-exchangeable). ^{13}C NMR ($CDCl_3$, δ ppm): 122.4 (C-2, indole), 110.2 (C-3), 119.2 (C-4), 122.2 (C-5), 120.3 (C-6), 111.2 (C-7), 127.6, 135.7 (fused aromatic-C), 30.4 (CH_2), 55.9 (CH), 162.7, 175.2 (2C=O), 153.9 (tetrazole-C), 143.2, 116.9, 129.7, 118.0 (phenyl-C). MS (EI): *m/z* (%): 300 ($M^{+\cdot}$, 45), 255 ($M^{+\cdot}$−COOH, 38), 231 (23), 69 (100). Anal. Calcd for $C_{14}H_{14}N_6O_3$ (314.299): C, 53.50; H, 4.49; N, 26.74. Found: C, 53.78; H, 4.28; N, 27.01.

3.2. Biological assays

3.2.1. Animals

Sprague–Dawley female rats (70 rats), weighing 160–180 g, aged 16–18 weeks, were supplied by the Animal House Colony of the National Research Centre, Cairo, Egypt, and acclimated for one week in a specific pathogen-free (SPF) barrier area where temperature (25 ± 1 °C) and humidity (55%). Rats were controlled constantly with 12 h light/dark cycle at National Research Centre animal facility breeding colony. Rats were individually housed with ad libitum access to standard laboratory diet and tap water. All animal procedures were performed after approval from the Ethical Committee of the National Research Centre and in accordance with the recommendations for the proper care and use of laboratory animals (NIH publication No. 85–23, revised 1985).

3.2.2. Chemicals

ACR (99%) was purchased from Merck-Schuchardt Chemical Co. (Hohenbrunn, Germany) and the starting pure powder of L-tryptophan was purchased from Sigma Company, USA.

3.2.3. Experimental design

Chart 1 is intended to demonstrate the experimental design. Animals were randomly assigned to seven groups (n = 10) as follows: control, ACR, TRP + ACR, compound **9b** + ACR, compound **12c** + ACR, compound **14a** + ACR, and compound **17** + ACR. ACR dose (50 mg kg^{-1} b. wt.) dissolved in water was injected intraperitoneally.[21,36] TRP and its derivatives were dissolved in 2% Tween 80 and injected intraperitoneally in a dose of 50 mg kg^{-1} b. wt.[37] 3 days/week and 10 min prior to ACR injection for a period of 3 weeks.

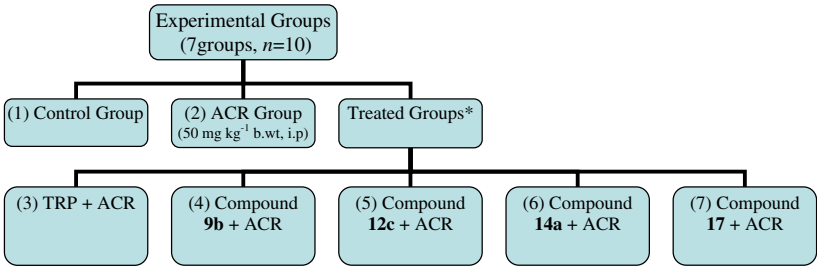


Chart 1. Experimental design for the in vivo neuroprotective effect of TRP and the novel synthesized indole derivatives. ACR: acrylamide, TRP: L-tryptophan, *TRP and all tested compounds injected in a dose of 50 mg kg^{-1} b. wt., ip, 3 days/week and 10 min prior to ACR injection for a period of 3 weeks.

3.2.4. Sample collection

The rats were killed and the whole brain of each animal was rapidly dissected, thoroughly washed with isotonic saline, dried, and then weighed. The first portion of each brain was homogenized immediately to give 10% (w/v) homogenate in ice-cold medium containing 50 mM Tris–HCl (pH 7.4) and 300 mM sucrose.[38] The homogenate was centrifuged at 1800 × *g* for 10 min at 4 °C. The supernatant (10%) was used for the determination of AD, NA, MDA, GSH, GPx, SOD, LDH, CK, and total protein. The second portion was stored at −80 °C for analysis of DA. The third portion of the brain was fixed in formalin buffer 10% for histological examination.

3.2.5. Biochemical analyses

Brain AD and NA levels were determined by enzyme linked immunosorbent assay using kits purchased from Labor Diagnostic Nord Gmbh Company (Germany). Brain DA level was determined spectrofluorimetrically according the method of Ciarlone.[39] Brain MDA, GSH, GPx, and SOD levels were assayed by spectrophotometer using kits purchased from Biodiagnostic Company (Egypt) according the published methods.[40–43] Brain LDH level was estimated by spectrophotometer using kit purchased from Biosystems Company (Spain) according to the method described by Friedman and Young.[44] Brain CK level was determined by spectrophotometer using kit purchased from Biostic Company (Germany) according to the method described by DGKC.[45] Brain total protein determined by spectrophotometer using folin reagent and according to the method described by Lowry et al.[46]

3.3. Histopathology assay

After fixation of brain tissues in buffered formalin solution for 1 week, the brain tissues were washed in tap water for 24 h, dehydrated in ascending series of alcohol. The samples were then cleared in xylene and immersed in paraffin. The paraffin blocks were sectioned at 5 μm thickness and mounted on clean glass slides and the ordinary hematoxylin and eosin stain was used.[47]

3.4. Statistical analysis

In the present study, all results were expressed as mean + SE of the mean. Data were analyzed by one way analysis of variance (ANOVA) using the Statistical Package for the Social Sciences (SPSS) program, version 11 followed by least significant difference (LSD) to compare significance between groups.[48] Difference was considered significant when P value was $\leqslant 0.05$.

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