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ORIGINAL PAPER

Novel triazole derivatives as potential rodenticides against the Norway rat, *R. norvegicus*: histology, biochemical alternations, and field application

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Abstract

Economically speaking, rodents possess a serious threat to the agriculture sector. One of these organisms that directly threaten agriculture, stocks, and others is the Norway rat, *Rattus norvegicus* (*R. norvegicus*). The 2-cyano-N-(1H-1,2,4-triazol-3-yl) acetamide **(1)** was used as a precursor to give 2-cyano-3-(dimethylamino)-N-(1H-1,2,4-triazol-3-yl) acrylamide **(2)** and ethyl 2-amino-5-cyano-1,6-dihydro-6-oxo-1-(1H-1,2,4-triazol-3-yl) pyridine-3-carboxylate **(3)**. Infra-red, ^{1}H-NMR, ^{13}C-NMR, MS, and elemental analysis were done for the precise structure elucidation of the applied synthons. The prepared compounds were tested as potential rodenticides against the Norway rat, *Rattus norvegicus*. Toxicity analysis using four serial doses of both prepared compounds revealed that the LD_{50} values were 160.6 and 391.7 mg/kg body weight, for ethyl 2-amino-5-cyano-1,6-dihydro-6-oxo-1-(1H-1,2,4-triazol-3-yl) pyridine-3-carboxylate **(3)** and 2-cyano-N-(1H-1,2,4-triazol-3-yl) acetamide **(1)**, respectively. Several biological variables, such as alanine transaminase (ALT), aspartate transaminase (AST), serum urea, creatinine, and total protein, have been assessed and evaluated as biological response indicators. Analysis revealed a highly significant increase in both AST, ALT, urea, and creatinine levels, while the total protein level showed a considerable reduction in treated rats exposed to 2-cyano-N-(1H-1,2,4-triazol-3-yl) acetamide **(1)** and ethyl 2-amino-5-cyano-1,6-dihydro-6-oxo-1-(1H-1,2,4-triazol-3-yl) pyridine-3-carboxylate **(3)** when compared to the control treatment. Liver histological examination showed structural changes in the form of congestion in the central vein, necrosis in some hepatic regions, and pyknotic nuclei, while kidney histological examination showed vacuolar degeneration of the epithelial cells of some convoluted tubules and the disappearance of some glomeruli and other marked atrophies. Necrosis in some areas was noticed. Field application through bait consumption took place with a satisfactory reduction of 68.4% for ethyl 2-amino-5-cyano-1,6-dihydro-6-oxo-1-(1H-1,2,4-triazol-3-yl) pyridine-3-carboxylate **(3)**, while it was 61.9% for 2-cyano-N-(1H-1,2,4-triazol-3-yl) acetamide **(1)** when compared to the recommended Zinc phosphide commercial rodenticide that poses an 81% reduction.

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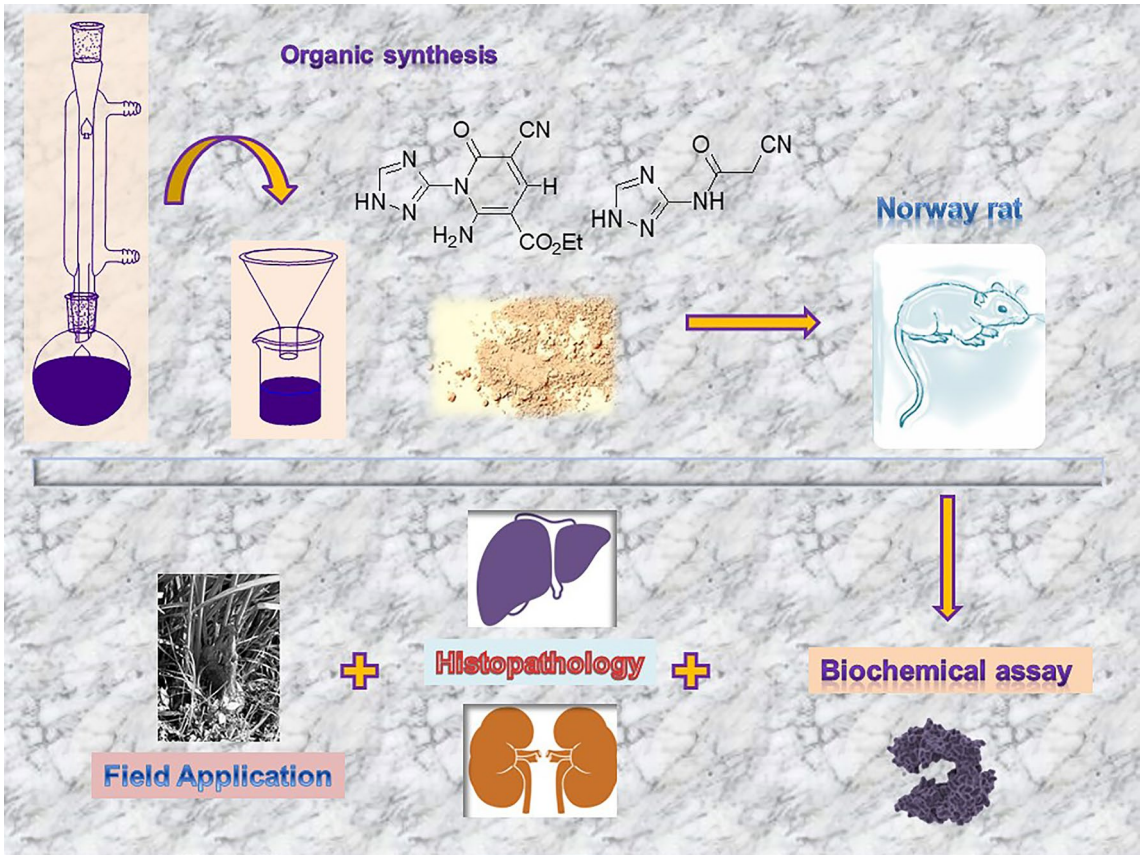
Graphic abstract



Keywords Triazole derivatives · Rodenticide · Histology · Biochemical alternations, field application

Introduction

Rodents have earned a terrible reputation for being one of the most widespread vertebrate pests that seriously impact human populations. They cause economic problems as a result of the damage they can cause to agroecosystems (Neena and Babbar 2010) (Singla and Babbar 2012) environmental problems as a result of the chemicals used to control them (Naidu et al. 2021), social problems as a result of their close proximity to human habitation, and, of course, health problems as zoonotic carriers (Singla et al. 2008) (Singla and Babbar 2012) (Herbreteau et al. 2012). When grains are damaged by rodents in stores, the germ is often destroyed, resulting in germination failure when implantation takes place; because the grain has been contaminated with feces, hair, and even urine, it has a poorer grade and lower marketing value. Contaminated batches are frequently considered unfit for human consumption (Ognakossan 2017). Furthermore, rodents destroy various items, including building structures, packing materials, clothing, and furniture, as a result of their chewing and burrowing behaviors; they cause fires by eating through electrical lines, resulting in severe losses (Belmain et al. 2015). Rodenticides are not exempt from this ongoing change in the agriculture sector, especially with regard to pesticides and pesticide alternatives. In order to successfully reduce the number of these destructive pests, it is therefore needed to look for better alternatives and chemical groups. Despite the fact that triazole compounds were synthesized for the first time decades ago, they continue to attract the interest of organic chemistry scientists (Kumari et al. 2021), (Zhao et al. 2022). To assess the triazole in rat's blood, Li and his team (Li et al. 2013) used ultrasound-enhanced temperature-controlled (UETC) ionic liquid dispersive liquid–liquid micro-extraction (IL-DLLME). The results demonstrated that the approach developed for determining target triazole groups in rat blood samples was effective. 3-Amino-l,2,4-triazole compound was evaluated as an agent of thyroid tumor prevalence in rats when treated with a subeffective dose

of N-bis (2-hydroxypropyl) nitrosamine over a period of twenty weeks and using a triazole diet with a concentration of 2000 ppm, where the incidence of thyroid tumors was 91% in the injected rats (Hiasa et al. 1982). The toxicity effect in rats of hexaconazole, a commonly used fungicide containing a triazole moiety, was well illustrated. For 12 weeks, rats were given 100 mg/kg of hexaconazole orally via diet. A group of biochemical parameters were noticed; creatinine, bilirubin, ALAT, ASAT, and LDH levels were found to be significantly higher in the blood of treated rats, indicating toxicity in various organs. A histopathological examination of the liver was also performed. Several liver abnormalities were observed, including centrilobular vein congestion, necrosis, immune cell infiltration, and microvesicular steatosis. Exposure to hexaconazole causes kidney and liver damage, according to these biochemical and histopathological examinations (Jalal et al. 2020); furthermore, toxigenomic analysis was performed to verify the ability of the genomics to predict the potential toxicity; three compounds used as fungicides, all containing triazole nucleus, were used on male Sprung-Dawley rats via oral serum over one, three, and five days at doses of 300, 175, 20, or 10 mg/kg day. Genomic, clinical chemistry, and hematological analysis showed the induction of triazole compounds on pregnancy X receptors (PXR), the metabolism of xenobiotics, as well as oxidative stress genes (Martin et al. 2007). Biochemical variables like acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE) were investigated, and the effects of 1H-1,2,3-triazole tacrine-chalcone derivatives against mice were well characterized. Additionally, investigations on oxidative stress' effects on behavior and neurochemistry have revealed decreased glutathione levels (GSH) and alterations in lipid peroxidation products (TBARS) in both the brain and hemocytes (Rani et al. 2021). When difenoconazole, a fungicide with a triazole moiety, was applied to the albino rat, many parameters were measured; lipid profile indices and glucose level, as well as thyroid hormonal level and renal parameters. Results revealed a significant increase in blood glucose levels and the lipid profile indices. In addition, a significant increase in renal markers (creatinine and urea) was well noticed (Mohamed El-Sayed et al. 2022).

The current study is an experiment with new synthetic triazole derivatives as novel materials that can be used in *R. norvegicus* control operations as one of the most widely distributed rodent species; it studies the influence of these compounds on biochemical parameters and histological alternations, as well as field experimentation evaluating their ability to reduce the population in the field environment.

Materials and methods

Chemicals

3-Amino-1H-1,2,4-triazole, 2-cyanoacetic acid, and acetic anhydride were obtained from PubChem. N,N-dimethylformamide-dimethylacetal (DMF-DMA) was obtained from Sigma-Aldrich. DMF and ethyl cyanoacetate were obtained from Piochem. Zinc phosphide (94%) was obtained from KZ Pesticides Company, Egypt.

Synthetic procedures

Synthesis of 2-cyano-N-(1H-1,2,4-triazol-3-yl) acetamide) (1)

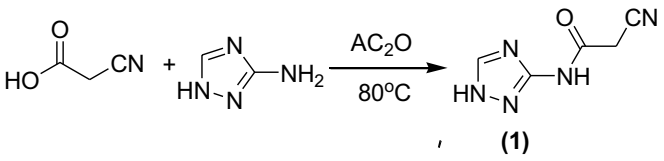
A pre-prepared mixture of cyanoacetic acid (2.55 g, 0.03 mol) in acetic anhydride (25 mL) was heated in a water bath (80 oC) till an orange color appeared; 3-amino-1H-1,2,4-triazole (2.52 g, 0.03 mol) was added; then the mixture was heated for two hours. A faint yellow compound was formed and left to cool at room temperature. The obtained yellow precipitate was filtered, washed with ethanol, and dried to afford 3.6 g of compound **(1)** (79.4%). Yellow powder; mp 260–265 °C; IR [KBr] ν/cm^{-1}: 3255 (NH), 2264 (CN), 1695 (C=O); ^{1}H-NMR [DMSO-d_6]: δ (ppm): 3.93 (s, 2H, CH_2), 7.97 (br., s, 1H, CH-triazole), 11.40 (br., s, NH) and 13.40 (br., s, NH-triazole); ^{13}C-NMR [DMSO-d_6], δ (ppm): 26.56, 115.83, 156.37, 158.28, 163.65 ppm; MS (EI, 70 eV): *m/z*(%) = 151 (M^+, 50.5), 84 (100), 68 (24.5), 57 (12.4); Anal. calculated for $C_5H_5N_5O$ (151.13): C, 39.74; H, 3.33; N, 46.34%. Found: C, 39.69; H, 3.37; and N, 46.40%.

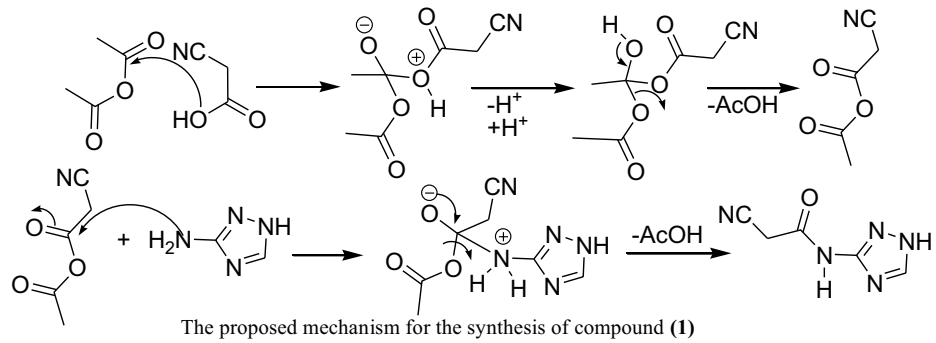
Synthesis of 2-cyano-3-(dimethylamino)-N-(1*H*-1,2,4-triazol-3-yl) acrylamide (2)

A mixture of compound **(1)** (4.53 g, 0.03 mol) and (DMF-DMA) (3.6 mL, 0.03 mol) in dry DMF (30 mL) was stirred for 2 h at room temperature. The precipitated solid was filtered off and dried to afford 4.2 g of enaminonitrile **(2)** (68%).

White powder; mp 210–214 °C; IR [KBr] ν/cm^{-1}: 3305 (NH), 2194 (CN), 1661 (C=O); ^{1}H-NMR [DMSO-d_6]: δ (ppm), 3.27 (s, 6H, $N(CH_3)_2$), 7.77 (s, 1H, α,β-unsaturated carbonyl proton), 7.88 (s, 1H, NH), 7.90 (s, 1H, CH-triazole), and 10.65 (s, 1H, NH); ^{13}C-NMR [DMSO-d_6], δ (ppm), 35.04 ($2CH_3$), 117.30, 119.17, 147.86, 155.95, 156,94 and 164.70; MS (EI, 70 eV): *m/z* (%) = 205.96 (M^+, 24.27), 160 (25.05), 50 (100), 40 (94.01); Anal. calculated for $C_8H_{10}N_6O$ (206.2): C, 46.60; H, 4.89; N, 40.76%. Found: C, 46.75; H, 4.97; and N, 40.79%.

Scheme 1. Synthesis of starting cyanoacetamide derivative **(1)**





The proposed mechanism for the synthesis of compound **(1)**

Synthesis of ethyl 2-amino-5-cyano-1,6-dihydro-6-oxo-1-(1H-1,2,4-triazol-3-yl) pyridine-3-carboxylate (3)

A mixture of the enaminonitrile **(2)** (2.06 g, 0.01 mol) and ethyl cyanoacetate (1.13. mL, 0.01 mol) in ethanol (15 mL) containing piperidine (0.5 mL) was refluxed for 3 h. The reaction mixture was left to cool and poured into ice–water. The obtained precipitated solid was filtered, recrystallized from ethanol, and dried to afford 1.9 g the pyridine-carboxylate derivative **(3)** (69%). Yellow crystals; mp 290–292 °C; IR [KBr] ν/cm^{-1}: 3201 (NH), 3343 (NH_2), 2228 (CN), 1644 and 1699 (2C=O); ^{1}H–NMR [DMSO-d_6]: δ (ppm), 1.30 (*t*, J = 5.0 Hz, 3H, CH_3); 4.26 (q, J = 5.0 Hz, 2H, CH_2), 8.38 (s, 1H, triazole) and 8.77 (s, 1H, pyridine) and 8.99 (br, s, 1H, NH); ^{13}C–NMR [DMSO-d_6], δ (ppm), 14.57, 61.27, 86.94, 90.51, 116.97, 146.93, 149.56, 151.42, 157.83, 159.82 and 165.49 ppm.; MS (EI, 70 eV): *m/z* (%) = 274 (M^+, 48.97), 110 (40.17), 83 (100); Anal. calculated for $C_{11}H_{10}N_6O_3$ (274): C, 48.18; H, 3.68; N, 30.65%. Found: C, 48.32; H, 3.75; and N, 30.78.

Instruments and general remarks

All melting points were determined on Gallenkamp electric melting point apparatus. Elemental analysis was carried out using Thermo Scientific CHNS/O Elemental Analyzer. IR spectra were recorded on a Mattson 5000 spectrometer with a model 550 spectrophotometers using a KBr wafer technique. The ^{13}C- and ^{1}H-NMR spectra were measured on Brucker Wpsy 125 and 500 MHz, deuterated dimethyl sulfoxide (DMSO-d_6) as a solvent, using TMS as an internal reference. The chemical shift (δ) expressed in ppm. The acronyms: (*s*) singlet, (*d*) doublet, (*t*) triplet, (*q*) quartet, and (*m*) multiplet were used to define the type of protons. Mass spectra were determined on a Varian MAT 311 (70 eV).

Rodenticide activity test

Experimental animals

Fields in Aga, Dakahlia Governorate's Norway rat, *R. norvegicus* adults, were captured using traps and then transported to a laboratory. They were kept in individual cages with a diet consisting of 65% crushed maize, 25% ground wheat, 5% sugar, and 5% corn oil. Food and water were available *ad libitum* throughout the whole experimental period. Unhealthy and pregnant animals were not included. Two weeks before the start of the experiments, rats were suitably equipped to adapt to a laboratory environment; the animals under study lived in laboratory conditions of 25 °C in a 12-h light/dark cycle. Each animal weighs between 200 and 230 g. The animals were divided into groups, each group containing five adult rats, and there was also a matching group for the control.

Determination of LD_{50} values

The Norway rat was fasted for approximately 12 h before treatment to estimate the acute oral LD_{50} of the two tested synthetic organic compounds. Serial doses of compound **(1)** were 150, 300, 600, and 800 mg/kg body weight, while those of compound **(3)** were 50, 100, 400, and 500 mg/kg body weight. They were given to the animals orally using stomach tubes, combined with an appropriate amount of vegetable oil for dissolution, and then given water and food two hours later. Plain vegetable oil was used for a comparable control experiment. Up to 7 days after organic synthesized compound dosing, mortality percentages were adjusted using Abbott's method; the LD_{50} and LD_{90} values were calculated using a probit analysis statistical approach (Abbott 1925).

Biochemical analysis

The effects of the lethal dose LD_{50} of the two tested organic synthesized compounds on some biochemical parameters were studied as a physiological response. Animals were orally intubated for 24 h. Animals were sacrificed after 3 days of treatment, and blood samples were taken from each animal, dispensed in clean tubes. Among the results of biochemical analysis of serum samples from the control and treated groups were aspartate aminotransferase and alanine aminotransferase; enzyme activity was quantified colorimetrically for AST and ALT (Huang et al. 2006); serum urea (Wang et al. 2022); creatinine (Imasawa et al. 2021); and total protein (Eaton et al. 2013) of treated and untreated animals.

Histological investigation

After the animals were killed and blood samples were obtained, the animals were dissected to obtain the liver and kidney specimens from the control and treated groups. The specimens were fixed in 10% formalin-saline. The samples were then taken to the histopathology Laboratory at Mansoura University's Faculty of Medicine for analysis. Hematoxylin and eosin were used to stain tissue sections of 5µ thickness (Creasy et al. 2021) for illustration of the histological examination.

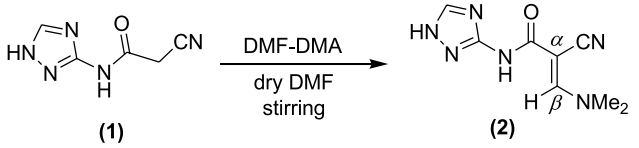
Field experiments

In the field conditions of Aga district, Dakahlia Governorate, a field evaluation of crushed maize bait containing 1% zinc phosphide, compound **(1)**, and compound **(3)** treated groups was performed (Hinds et al. 2023). The whole area is infested with *R. norvegicus*. Each compound was treated in an area equal to one feddan (an area unit used in Egypt equivalent to 4200 m^2), while a comparable area was left untreated as a control. The pre- and post-treatment rat population density was calculated using the food consumption method (Rennison 1977). Each plastic bag containing 100 g (1 g toxicant)synthetic compound /or zinc phosphide(+5 g corn oil and 94 g crushed maize) of the proposed bait weighed 2 kg and was placed in the selected plot for five successive days; each tested bait consumption amount was reported. Calculations were made to determine the population reduction as follows:Population reduction%

$$= \frac{\text{Pre} - \text{treatment consumed} - \text{post} - \text{treatment consumed}}{\text{Pre} - \text{treatment consumed}} \times 100$$

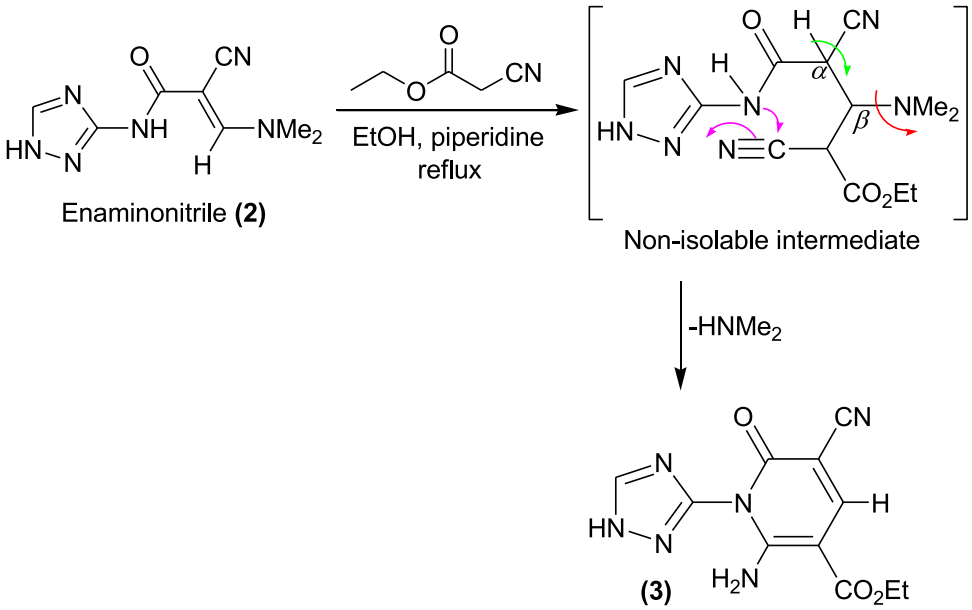
Statistical analysis

LD_{50} values were expressed as mg/kg body weight unit. All data, presented as mean ± SE, were subjected to one way analysis of variance (ANOVA) (St and Wold 1989). Confidence intervals with a 95% simultaneous confidence level were created using Tukey's method. Significant was



Scheme 2: Synthesis of 2-cyano-3-(dimethylamino)-N-(1H-1,2,4-triazol-3-yl) acrylamide **(2)**

Scheme 3. Synthesis of triazolylpyridine derivative **(3)**



considered to be a 0.05 probability. Cohort Software was used for all statistical analyses (Cho et al. 2004).

Results and discussion

Chemistry

In general, our synthons were prepared through the reaction of 3-amino-1H-1,2,4-triazole with cyanoacetic acid to give the 2-cyano-N-(1H-1,2,4-triazol-3-yl) acetamide **(1)**, which reacts with N,N-dimethylformamide dimethyl acetal (DMF-DMA) to give the enaminonitrile **(2)**, which in turn enters into a reaction with ethyl cyanoacetate through a non-isolable intermediate to afford the ethyl 2-amino-5-cyano-1,6-dihydro-6-oxo-1-(1H-1,2,4-triazol-3-yl) pyridine-3-carboxylate **(3)**.

The key starting material, **(1)**, was prepared by cyanoacetylation of 1H-1,2,4-triazol-3-amine through a reaction with a pre-prepared mixture of 2-cyanoacetic acid and acetic anhydride (Scheme 1) (Ibrahim et al. 2011) (Tenor and Kröger 1964). The mechanism of this reaction was preceded via the initial formation of the acetylating agent, which was attacked by the exocyclic amino group of the aminotriazole (Bayazeed and Alnoman 2022), (Mohamed and Mahmoud 2019). The structure of **(1)** has been established via different analytical and spectral data. The IR spectrum showed absorption bands at $\nu = 3260$, 2264 and 1696 cm^{-1} corresponding to (NH), (CN) and amidic carbonyl functions. The ^{1}H-NMR spectrum (DMSO-d_6) showed a singlet signal at δ 3.93 ppm for two protons of CH_2, a singlet signal at δ 7.97 ppm for CH of the triazole ring, and two broad signals for two protons of 2NH groups at δ 11.40 and 13.40 ppm (S1). The ^{13}C-NMR spectrum showed signals for methylene carbon at δ 26.56 ppm, a carbonyl carbon at δ 163.66 ppm, cyano carbon at δ 115.83 ppm, and two carbons at δ 158.28 and 156.37 ppm for C3, C5 triazole carbons (S2). The mass spectrum (S3) showed the molecular ion peak at $m/z = 151$ (M^+), which is well-matched with the molecular formula ($C_5H_5N_5O$).

Table 1 The acute toxicity of the synthesized compounds against the Norway rat *R. norvegicus*

Treatment	LD_{50} (mg/kg body weight)	LD_{90} (mg/kg body weight)	Slope ± S.E
Compound **(1)**	391.7	943.1	3.35 ± 1.02
Compound **(3)**	160.6	665.6	2.07 ± 0.63

The chemistry of enaminonitriles has received significant interest as a precursor to synthesize azoles, azines, and azoloazine ring systems (Madkour et al. 2008) (Bondock et al. 2011) (Fadda et al. 2013) (Fadda et al. 2021). The utility of enaminonitriles in organic synthesis as a reactive precursor for the production of diverse heterocyclic compounds has been reported (Bondock et al. 2009) (Fadda et al. 2012). Treatment of compound **(1)** with DMF-DMA by stirring in dry DMF afforded the predicted enaminonitrile. The enaminonitrile **(2)** structure was elucidated according to analytical and spectral data. The IR spectrum showed an absorption band at 3305 cm^{-1} due to (NH) group, 2194 cm^{-1} for (CN) group and 1661 cm^{-1} due to amidic carbonyl group. The ^{1}H-NMR spectrum (DMSO-d_6) revealed a singlet signal at δ 3.27 ppm analogous to N,N-dimethylamine protons. A characteristic singlet signal at δ 7.77 ppm attributed to β proton in α,β-unsaturated carbonyl system, singlet signal at δ7.88 ppm for the olefinic proton of triazole and two singlet signals (at δ 7.90 and broad at 10.65 ppm) for two NH protons (S4). The ^{13}C-NMR spectrum showed signals at δ 35.04 for two [N–$(CH_3)_2$], and δ 117.30, 119.17, 147.86, 155.95, 156.94, 164.70 ppm (S5). The mass spectrum showed the molecular ion peak at $m/z = 206$ (M^+) for the molecular formula ($C_8H_{10}N_6O$) (Scheme 2).

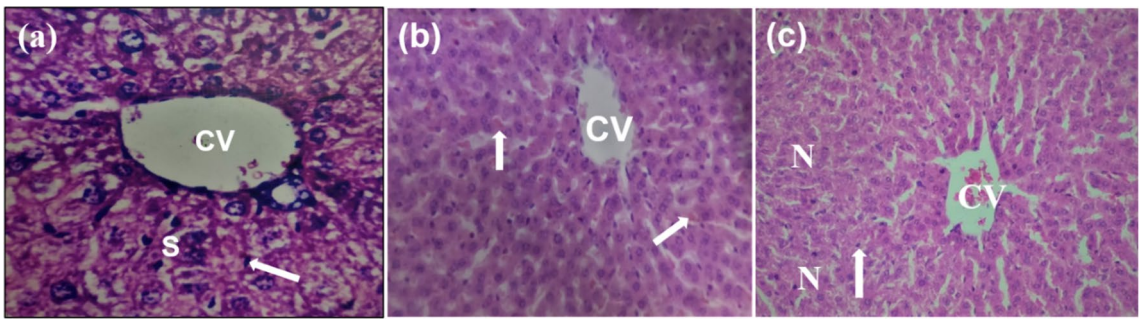


Fig. 1 Photomicrographs of sections in livers of *R. norvegicus*. **a**: Control, showing a central vein (CV), radiating cords of liver cells separated by blood sinusoids (S) and Kupffer cells (arrow). **b**: After treatment with compound **(1)**, displaying sinusoidal hemorrhage (arrow). c: After treatment with compound **(3)**, demonstrating congested central vein (CV), necrosis (N) in some hepatic regions and pyknotic nuclei (arrow). (H & E X400)

Table 2 Effect of the synthesized compounds (LD_{50}) on different biochemical parameters of the Norway rat *R. norvegicus*

Treatment	AST (U/ml)	ALT (U/ml)	Serum urea (mg/dl)	Creatinine (mg/dl)	Total protein (g/dl)
Compound (1)	$47.18^{b} \pm 0.23$	$43.36^{b} \pm 1.27$	$28.50^{a} \pm 0.34$	$0.74^{ab} \pm 0.06$	$5.98^{ab} \pm 0.59$
Compound (3)	$72.82^{a} \pm 0.86$	$60.9^{a} \pm 1.80$	$30.18^{a} \pm 1.77$	$0.88^{a} \pm 0.05$	$4.8^{b} \pm 0.42$
Control	$41.12^{c} \pm 0.56$	$30.62^{c} \pm 0.53$	$24.48^{b} \pm 1.11$	$0.68^{b} \pm 0.05$	$7.44^{a} \pm 0.75$
***P*-value**	0.00	0.00	0.017	0.047	0.029

*Analysis of variance (ANOVA) for means of biochemical parameters using one-way ANOVA

*Grouping information using **"**Tukey**"** method and 95% confidence

*Values of the same column in each biochemical parameter (AST, ALT, serum urea, creatinine, and TP) that do not share the same letter (a, b, c) are significantly different

The reaction of enaminonitrile **(2)** with ethyl cyanoacetate afforded ethyl 2-amino-5-cyano-1,6-dihydro-6-oxo-1-(1*H*-1,2,4-triazol-3-yl) pyridine-3-carboxylate **(3)** through a non-isolable intermediate(Alnajjar et al. 2018) (Bondock et al. 2009). The mechanism of the reaction is proposed through the initial addition of the active methylene group of ethyl cyanoacetate to β-carbon of enaminonitrile to form the non-isolable intermediate, followed by the nucleophilic attack of the NH group on the cyano functional group and the elimination of the dimethylamine molecule (Al-Mousawi et al. 2008) (Scheme 3).

The structure of **(3)** was well confirmed by different elemental analysis and spectral data. The IR spectrum of the pyridine derivative showed absorption bands at $\nu = 1699$, 1664 cm^{-1} due to two carbonyl groups, a band at $\nu = 3343$ cm^{-1} corresponding to the amino functional group, and a band corresponding to the cyano group at $\nu = 2228$ cm^{-1}.

The ^{1}H-NMR spectrum (DMSO–d_6) revealed a singlet signal at δ 8.77 ppm for the CH proton of the triazole ring, a singlet signal at δ 8.38 ppm for the C_4-H proton of the pyridine ring (S6), a quartet at δ 4.26 ppm for the $\underline{CH_2}CH_3$ protons, and a triplet signal at δ 1.30 ppm for the $CH_2\underline{CH_3}$ protons of the ester group (Fig. 1). The ^{13}C-NMR spectrum revealed signals at δ 14.57, 61.27, 86.94, 90.51, 116.97, 146.93, 149.56, 151.42, 157.83, 159.82, and 165.49 ppm, which agree with the proposed structure (S7). The mass spectrum showed the molecular ion peak at $m/z = 274$ (M^+), which is well matched with the molecular formula ($C_{11}H_{10}N_6O_3$).

Toxicity assessment and LD values

We elucidated the rodenticidal activities of **(1)** and **(3)** against the Norway rat; the results are summarized in Table 1. Compound **(3)** shows a higher mortality rate for *R. norvegicus*, followed by compound **(1)**; the LD_{50} and LD_{90} values were (160.6 and 391.7 mg/kg w) and (665.6 and 943.1 mg/kg w) with a 2.07 and 3.35 slope for compounds **(3)** and **(1)**, respectively.

The obtained results showed a considerable toxicity of our synthetically applied compounds against *R. norvegicus*; it obviously shows that **(1)** and **(3)** are toxic to rat*s* in general. Triazoles are a type of fungicide that has recently been evaluated in investigations as a potential rodenticide. Conazoles are a category of azole-based fungicides utilized in pharmaceutical and agricultural applications. Some members of this class have been reported to be hepatotoxic and to cause thyroid follicular cell tumors in Wister rats as well as mice hepatocellular cancers (Allen et al. 2006). Recent studies applying synthetic chemicals or pesticides with triazole nuclei (mefentrifluconazole) on rats revealed LD_{50} values of 2000 mg/kg body weight orally and > 5000 mg/kg body weight by dermal doses; aerosol formulation via inhalation was also used to produce > 5.314 mg/L (Tesh et al. 2019).

Biochemical parameters

Table 2 shows several biological variables (ALT, AST, serum urea, creatinine, and total protein) that were assessed and evaluated as a biological response to compound **(1)** and, **(1)** as potential rodenticidal compounds. The study indicated a significant increase in AST, ALT, serum urea, and creatinine concentration when both **(1)** and **(3)** were applied compared to the control group in the *R. norvegicus* rat. A significant decrease in total protein in treated compound groups is well noticed while compared to the control group in rats.

Amino transferases are a series of enzymes that are contained in the cytoplasm of living cells, with the liver possessing the highest levels of ALT and slightly lower amounts in other tissues such as the brain and muscle (Azimi et al. 2022). AST is a transaminase enzyme that is extensively located in the liver, kidneys, and muscles. Its release into the bloodstream results from an attack on these organs; an

increase in its release into the bloodstream signifies hepatic toxicity (Nirmal et al. 2021). Analysis revealed a highly significant increase ($P = 0.000$) in AST levels in treated rats exposed to **(1)** and, **(3)**; (47.1 ± 0.23, 72.8 ± 0.86 U/ml), respectively, compared to the control (41.1 ± 0.56U/ml); furthermore, ALT enzyme showed a considerable increase ($P = 0.000$) in its level (43.3 ± 1.27 and 60.9 ± 1.8 U/ml) for both prepared **(1)** and **(3)**, respectively, when compared to the control treatment (30.6 ± 0.53U/ml). When animals were treated with LD_{50} values, the AST and ALT, as well as other parameters such as urea and creatinine increasing values, may be interpreted as a reflection of what happens inside the body during breakdown and malfunctioning, and an immediate result of organ damage, particularly liver and kidney damage (Elhamalawy et al. 2022). A clear increase in the concentration of both the ALT and AST enzymes in male albino rats is well considered when liver tissue undergoes histological analysis after exposure to an acetaminophen synthetic compound. There were obvious histological changes and oxidative stress, as well as inflammatory parameters like interleukin-1 beta (IL-1 beta), myeloperoxidase (MPO), and tumor necrosis factor-alpha (TNF-α) (Mohamed Kamel et al. 2022).

The biochemical assay showed a remarkable increase ($P = 0.017$) in urea levels (28.5 ± 0.34 and 30.1 ± 1.7 mg/dl) for **(1)** and **(3)** compounds, respectively, compared to the control treatment (24.4 ± 1.1 mg/dl). Creatinine levels in blood serum ($P = 0.047$) also had a noticeable rise (0.74 ± 0.06 and 0.88 ± 0.05 mg/dl) for **(1)** and **(3)**, respectively, when compared to control (0.68 ± 0.05 mg/dl). Compounds **(1)** and **(3)** in the current investigation cause a considerable rise in serum urea and creatinine levels, which is a result of the body's inability to excrete the metabolic end products of proteins. The byproduct of creatine breakdown; creatinine, is largely filtered out by the kidneys. Creatinine levels rising in the bloodstream are a reliable sign of nephrotoxicity (Wyss and Kaddurah-Daouk 2000). Total protein levels showed a considerable reduction ($P = 0.029$) in their amounts (5.9 ± 0.59 and 4.8 ± 0.42 g/dl) for compounds **(1)** and **(3)**, respectively, when compared to the control (7.44 ± 0.75 g/dl). In general, increasing ALT and AST values were accompanied by a decrease in total protein levels (Helmy et al. 2022). The overall animal metabolism is obviously affected by the toxin's impact; the ALP decrease is well related to the protein synthesis stopping process and is automatically accompanied by a decrease in total protein amount (Anand et al. 2012) (Aryaeian et al. 2021); change in the endoplasmic reticulum that develops the cell membrane is a reasonable factor affecting metabolism through receiving toxins (Zhang et al. 2020a, b) (Zhang et al. 2020a, b).

Histopathology

Liver

Examination of histological sections of the liver of control *R. norvegicus* showed that the classic hepatic lobule was composed of a central vein and masses of liver cells (hepatocytes) arranged in the form of liver cords radiating from the central vein. The hepatocytes were polygonal or rounded in shape, with central and vesicular nuclei. The liver cords were separated from each other by narrow blood sinusoids lined by endothelial cells and Von Kupffer cells (Fig. 1a). Histological examination of a liver section from animals treated with compound **(1)** revealed sinusoidal hemorrhage (Fig. 1b). The histological liver evaluation of the animals treated with compound **(3)** was characterized by structural changes in the form of congestion in the central vein, necrosis in some hepatic regions, and pyknotic nuclei (Fig. 1c).

The most essential organ for the excretion of poisons or any other metabolites is generally agreed to be the liver. It performs a variety of tasks, including transporting and accumulating metabolites, assisting in food digestion, regulating glucose synthesis, and storing glucose. The liver's primary

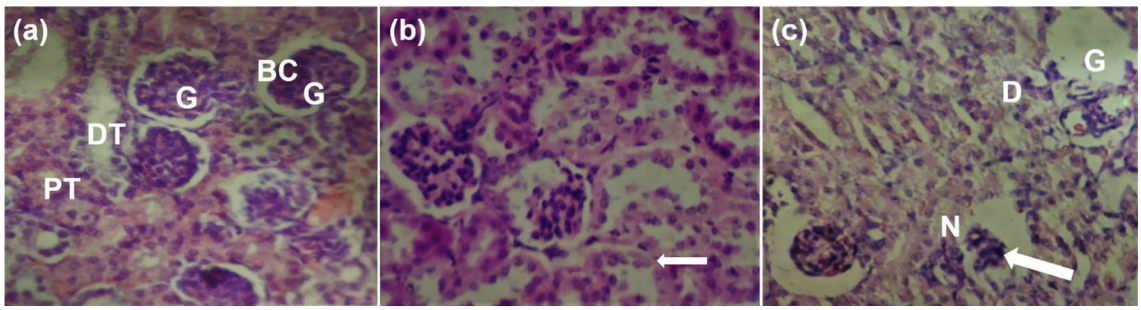


Fig. 2 Photomicrographs of sections in kidney (renal cortex) of *R. norvegicus*. **a**: Control, showing Malpighian renal corpuscles with normal glomerulus (G) and Bowman's capsules (BC), normal proximal convoluted tubules (PCTs) with brush border and distal convoluted tubules (DCTs). **b**: After treated with compound **(1)**, displaying atrophied glomerular capsule and rupture of BC (arrow). **c**: After treated with compound **(3)**, showing atrophied glomerular capsule, vacuolar degeneration (D) of some convoluted tubules and disappearance of some glomeruli (G) and necrosis (N) in some regions. (H & E X400)

Table 3 Field assessment of the synthesized compounds as bait against the Norway rat, *R. norvegicus*, at EL-Dakahlia Governorate

Applied Compound	Bait consumption (gram/feddan)			
	Pre-treatment Mean ± SE	Treatment Mean ± SE	Post-treatment Mean ± SE	Reduction %
Compound (1)	210 ± 44.3[a]	191 ± 39.0[a]	80 ± 5.88[b]	61.90
Compound (3)	222 ± 7.5[a]	198 ± 9.35[a]	70 ± 7.16[bc]	68.46
Zinc phosphide	190 ± 31.5[a]	162 ± 13.7[a]	36 ± 5.47[c]	81.05
Control	225.5 ± 19.1[a]	–	159.6 ± 18.8[a]	–
***P*-Value**	0.822	0.560	0.000	–

*Analysis of variance (ANOVA) for bait consumption using one-way ANOVA

*Grouping information using "Tukey" method and 95% confidence

*Values of the same column that do not share the same letter (a, b and c) are significantly different

* (–) means no result

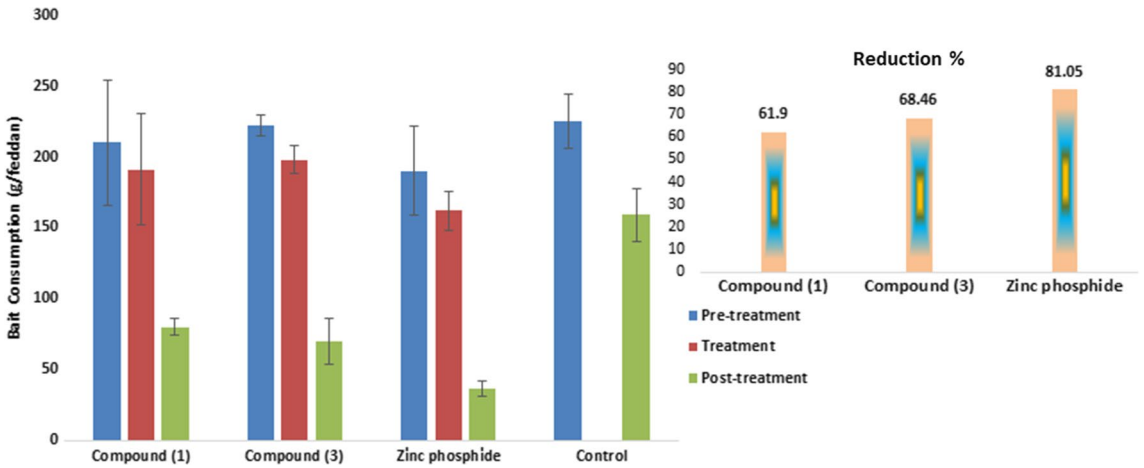


Fig. 3 Zinc phosphide, compound **(1)**, and compound **(3)**; mean bait consumption (g/Feddan) and reduction percent against *R. Norvegicus*

function is to neutralize and remove harmful chemicals from the body (Sun et al. 2021).

Triazole chemicals are used in agricultural control operations, particularly as fungicides. Additionally, these substances have undergone extensive rodenticide evaluation. The hepatotoxic properties of numerous triazole compounds applied to rat control processes have been analyzed in a number of standard toxicity tests (Ku et al. 2021) (Jalal et al. 2020).

Similar investigations were carried out against rodents with high dosages of cyproconazole and epoxiconazole, which definitely increased liver size in addition to causing tumors to appear in sizable portions of hepatocytes (SA 2010); additionally, for cyproconazole, histopathological findings have already been presented (Heise et al. 2015). Treatment-related effects in the liver were mostly limited to the highest dose level (NOAELx10), with cyproconazole displaying the most severe effects. These effects included vacuolization and hypertrophy of hepatocytes. Hepatocyte hypertrophy was noticed in 80–100% of the animals given the highest doses of cyproconazole, whereas vacuolization was detected in 100% of the rats given cyproconazole.

Kidney

Hematoxylin- and eosin-stained sections showed the normal histological structure of the kidney (renal cortex). The cortex showed Malpighian renal corpuscles, proximal convoluted tubules, and distal convoluted tubules. Malpighian renal corpuscles consisting of a normal glomerulus with a thin glomerular basement membrane and Bowman's capsules with normal cellularity and patent capsular space; surrounding tubules (proximal and distal); interstitium; and blood vessels were normal (Fig. 2a). The histological changes in the kidney after treatment with compound **(1)** showed atrophy in some glomeruli capsules and rupture in Bowman's capsules (Fig. 2b). Histological examination of the kidney section after treatment with compound **(3)** showed vacuolar degeneration of the epithelial cells of some convoluted

tubules. Disappearance of some glomeruli and others was markedly atrophied. Necrosis in some areas was noticed (Fig. 2c). When toxic substances are utilized, the kidney is one of the organs that gets attention. The kidney's main tasks include detoxifying metabolic waste products and external substances, including body pigments (Effendy et al. 2006). Pathological alterations and disruptions, including both glomerular and renal tubular activities, will result from exposure to circulation toxins (Wannang, et al. 2005).

At low doses, tebuconazole (TEB), a triazole moiety fungicide, caused an alteration in the biochemical parameters of renal functions, a lesion throughout the renal tissues, and an induced renal toxicity in the male Wistar rat (Othmène et al. 2020).

Field application and population diminution

Data in Table 3 demonstrate the relative effectiveness of our synthons field assessment through bait consumption technique against the Norwegian rat, *Rattus norvegicus*, under natural field conditions. For **(1)** and **(3)** compounds, respectively, the average consumption of untreated crushed maize during the pre-treatment was 210 and 225 g, while it was 80 and 70 g during the post-treatment. When compared to the recommended rodenticide zinc phosphide treatment, there is a significant difference in the consumption of untreated crushed maize (post-treatment). Compound **(3)** bait is highly effective at reducing the rat population in natural field conditions, according to the 68.4% population reduction it achieved, followed by compound **(1)**, and finally an 81% reduction by the recommended rodenticide zinc phosphide. For compounds **(1)** and **(3)**, the average amount of treated bait that was consumed was 191 and 198 g, respectively (Fig. 3). The results from the field and the laboratory were in agreement. Previous data showed an acceptable population diminution under field conditions.

The issue of bait shyness, in addition to the toxicity of zinc phosphide, was one of the guiding and persuasive reasons for carrying out this investigation. Many researchers have dealt with, in not a few studies, the disadvantages of using zinc phosphide in general and its use in rodent control in particular. Studies have been conducted on the toxicity of zinc phosphide on human (Ghasempouri et al. 2022), and others have been conducted to assess its impact on wildlife (Bildfell et al. 2013), as well as investigations that talk about the harm of this substance to the environment in acidic media (Knight 2013). This is in addition to the phenomenon of bait shyness when using zinc phosphide, which would limit its use (Horak et al. 2018).

Bait consumption is regarded as a valuable and effective rodent management technique, as well as a reliable indicator for assessing reduction ratios, particularly in field conditions when different environmental parameters (predators and food competitors) are taken into account (Patergnani et al. 2010). In order to achieve the quality of the bait that achieves the highest percentage of population reduction and also within the control operations in different environmental conditions, the relationship between baits (color, additives, and the percentage of moisture in the total content) and the behavior of four species of rodents, including *R. norvegicus*, toward these different variables has been thoroughly studied (Clapperton 2006). *Rattus sordidus* canefield rats were treated using zinc phosphide bait, which resulted in population declines of 80% and 86%, respectively, for two treatments, while there was no reduction in the control treatment. The results demonstrate that zinc phosphide is effective at suppressing canefield rat populations (Rivera et al. 2008). Through the bait consumption technique, the experiment was done in a clothing store, and the reduction rate was evaluated. Acetylsalicylic acid (0.04%) reduces the rat population by 72% (Kandil et al. 2022).

Conclusion

The present investigation describes the synthesis of cyanoacetamide **(1)** and triazolylpyridine **(3)** derivatives derived from the triazole nucleus compounds. Different analysis techniques confirmed the successful synthesis of cyanoacetamide **(1)** and triazolylpyridine **(3)** derivatives. Both compounds **(1)** and **(3)** were applied as newly synthesized potential rodenticides against *R. norvegicus*. The LD50 values were 391.7 and 160.6 mg/kg body weight for **(1)** and **(3)**, respectively. Both **(1)** and **(3)** instigate a considerable increase in ALT, AST, serum urea, and creatinine enzymes but a significant decrease in TP content activity in treated rats compared to the control group. Histopathological assessment of the liver showed structural changes in the form of congestion in the central vein, necrosis in some hepatic regions, and pyknotic nuclei, while kidney examination showed vacuolar degeneration of the epithelial cells of some convoluted tubules and the disappearance of some glomeruli and other marked atrophies. Necrosis in some areas was noticed. Field application through bait consumption took place with an adequate reduction of 68.4% for compound **(3)**, while it was 61.9% for compound **(1)** when compared to the recommended Zn phosphide commercial rodenticide that poses an 81% reduction; these compounds can be employed in rodent control operations due to their impact on biochemical parameters, histopathological effects, and population reduction rates, according to our results obtained. Among the main reasons for the restrictive use of Zn phosphide, as well as the search for new synthetic acceptable alternatives in rodent control operations, are its high toxicity, the phenomenon of bait shyness, and the fact that Zn phosphide can reach wildlife and the environment.

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Declarations

Conflict of interest The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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