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Synthesis and Molecular Docking Study of Novel Heterocyclic Compounds from Cyanoacetohydrazide

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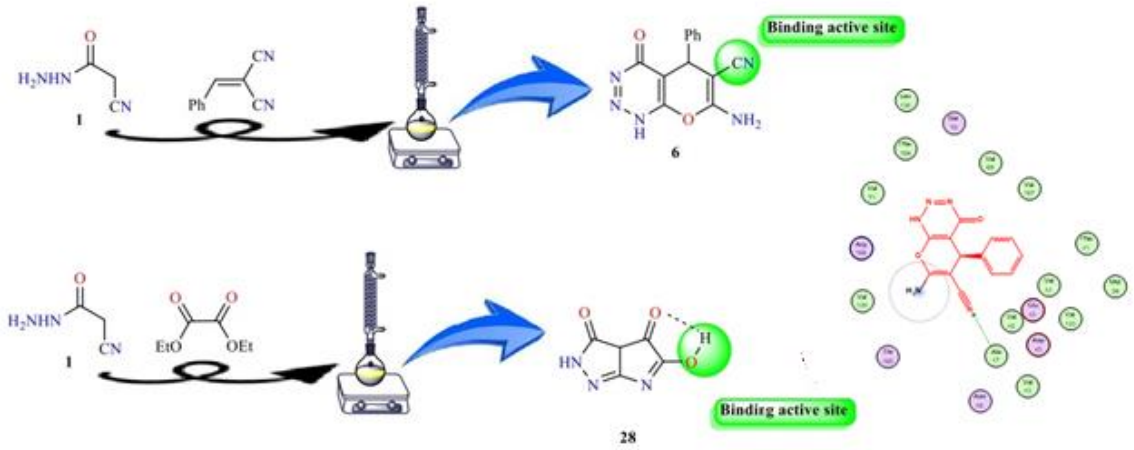

Abstract

The present study reports the synthesis of some novel heterocyclic derivatives by reacting cyanoacetohydrazide in a basic medium with different electrophilic reagents. Initially, its reaction with H_2O_2 afforded 1,2,3-triazine-4,6(1*H*,5*H*)-dione**,** which upon addition to benzylidenemalononitrile under Michael's condition yielded 7-amino-4-oxo-5-phenyl-1,5-dihydro-4H-pyrano[2,3-*d*][1,2,3]triazine-6-carbonitrile. Azidolysis of cyanoacetohydrazide resulted in the formation of 5,8-dihydrotetrazolo[1,5-*c*][1,2,3]triazin-7-ol. On the other hand, heterocyclization of cyanoacetohydrazide with ethyl cyanoacetate and/or chloroacetyl chloride produced 2,5-dioxo-2,4,5,6-tetrahydro-1*H*-pyrazolo[1,5-*b*]pyrazole-3-carbonitrile and 3,5-dihydroxy-1,2-dihydropyridazine-4-carbonitrile, respectively. The later undergoes [2+3] intermolecular cycloaddition to the heteroallene system produced **4-(1,2,4-thiadiazol-3-yl)-1,2-dihydropyridazine-3,5-diol**. Furthermore, Knoevenagel condensation and intramolecular heterocyclization of cyanoacetohydrazide with benzaldehyde and acetylacetone afforded 3-hydroxy-5-phenyl-4*H*-pyrazole-4-carbonitrile and 5,6a-dimethyl-3-oxo-1,2,3,6a-tetrahydro-3a*H*-furo[3,2-*c*]pyrazole-3a-carbonitrile, respectively. Intermolecular heterocyclization of cyanoacetohydrazide with diethyl oxalate and carbon disulphide gave **5-Hydroxy-2,3a-dihydropyrrolo[2,3-*c*]pyrazole-3,4-dione and 4-hydroxy-1,3a-dihydro-3*H*-pyrazolo[3,4-*c*]isothiazole-3-thione**, respectively. All reactions proceeded in good to excellent yields and the synthesized compounds were identified by different spectroscopic techniques. All the obtained compounds are virtually screened by molecular docking on the target protein 1KZN by the MOE for potency as antibacterial agent. Also, pharmacophore and ADME studies were applied. Efficient binding to the target protein was found for some of the synthesized compounds.

Keywords: Cyanoacetohydrazide, heterocyclization, triazine, pyridazine, pyrazole, Molecular docking, ADME.

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INTRODUCTION

Many heterocyclic compounds may be synthesised using cyanoacetohydrazide, which is a flexible and convenient intermediate. The molecule's β-functional nitrile [1-4] group may be suitable for addition followed by heterocyclization or cycloaddition with various chemicals to create heterocyclic compounds of various sizes containing one or more heteroatoms, which are highly valuable as pharmaceutical drugs [5,6], weedkillers [7], medicinal drugs [8], and colourants [9,10]. Due to their various biological actions, several azole compounds have attracted a lot of attention in recent years [11-16]. These compounds are also known for their anticancer properties [17-19]. Furthermore, because of their therapeutic relevance, the chemistry of coalescing pyrazole- and thieno-pyrazole analogues has received much interest [20-22]. Because it contains both a nitrogen and a carbon nucleophile, cyanoacetohydrazide **1** can operate as an ambident nucleophile (Figure 1). Thus, when **1** is treated with various reagents, the attack may occur at five different positions: nucleophiles may attack the carbonyl carbon (position 3), or the nitrile carbon (position 5). Furthermore, the active methylene carbon (position 4) in a basic medium or the amino group (position 1 in a neutral or acidic medium) can attack electrophiles, while position 2 can attack electrophiles during the reaction. [23-25].

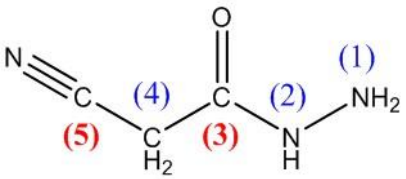


Figure 1: Sites of chemical reactivity of cyanoacetohydrazide **1.**

Furthermore, the addition of the pyrazole moiety improves the molecule's lipophilicity, neutrality, and in vivo penetration of biological membranes, as well as various pharmacological properties such as antibacterial [26,27]. In this paper, we prepared a new class of azole derivatives and tested their bioactivity. Molecular docking investigations were used to prove the interactions between the obtained compounds and the bacterial protein' active sites [28,29].

EXPERIMENTAL

The melting points were determined using a Stuart melting point instrument and were not adjusted. As KBr pellets, the IR spectra were obtained using an FTIR Bruker-vector 22 spectrophotometer. On a Varian Gemini NMR spectrometer, the ^{1}H and ^{13}C NMR spectra were acquired at 100 and 400 MHz, respectively, in a solvent DMSO-d_6 as, with tetramethylsilane as an internal standard. Chemical shifts are expressed in parts per million (ppm). At Cairo University's Micro-analytical Center, elemental analysis was done by a Perkin-Elmer 240 microanalyzer.

1,2,3-Triazine-4,6(1H,5H)-dione (4).

A mixture of hydrazide **1** (50 mmol), hydrogen peroxide (50 mmol), sodium hydroxide (50 mmol), and sodium ethoxide (50 mmol) in absolute ethyl alcohol (20 mL) was refluxed for 12 hours. The mixture was allowed to be cool then poured into crushed ice. The product was filtered and recrystallized from DMF to yield compound **4** as brown crystals. Yield 4.8 g (85%), mp: 195-200 °C. IR spectrum, ν, cm^{-1}: 3332 (NH),1909 (N=N), 1651 (C=O), ^{1}H NMR spectrum, δ, ppm: 9.85 (s, 1H, NH, D_2O exchangeable), 5.85 (s, 2H, CH_2). ^{13}C NMR spectrum, δ, ppm: 24.46 (CH_2), 172.50, 175.48 (2 C=O). Found, %: C, 31.84; H, 2.67; N, 37.19. $C_3H_3N_3O_2$**.** Calculated, %: C, 31.87; H, 2.67; N, 37.16.

7-Amino-4-oxo-5-phenyl-1,5-dihydro-4H-pyrano[2,3-d][1,2,3]triazine-6-carbonitrile (6).

Benzylidenemalononitrile (9 mmol) was added to a solution of **4** (9 mmol) and sodium ethoxide (9 mmol) in absolute ethanol (20 mL). The reaction mixture was heated under reflux for 10 h, then the content of the flask was put into ice/cold water, then acidified with HCl (10%). The obtained solid was filtered off, dried, and recrystallized from ethanol to produce **6** as black crystals. Yield (90%), mp: >300 °C. IR spectrum, ν, cm^{-1}: 3332, 3213 (NH_2), 2353 (CN), 1897 (N=N), 1670 (C=O). ^{1}H NMR spectrum, δ, ppm: 9.87 (s, 1H, NH), 7.83 (s, 2H, NH_2), 7.51-7.35 (m, 5H, ArH), 5.51 (s, 1H, CH). ^{13}C NMR spectrum, δ, ppm: 39.5, 125.55, 127.26, 127.44, 127.18, 128.03, 128.21, 128.48, 128.57, 129.11, 129.54, 166.83. Found, %: C, 58.41; H, 3.38; N,

26.23. $C_{13}H_9N_5O_2$**.** Calculated, %: C, 58.43; H, 3.39; N, 26.21.

5,8-Dihydrotetrazolo[1,5-c][1,2,3]triazin-7-ol (9).

To a mixture of compound **1** (30 mmol) in absolute ethanol (20 ml) and sodium ethoxide (30 mmol), sodium azide (30 mmol) was added and heated under reflux for 10 h. The reaction mixture was cooled at r.t, then poured into an acidified ice/water mixture. The produced product filtered off, dried, and recrystallized from DMF to gives compound **9** as black crystals. Yield (90%), M.P.: 200 °C. IR spectrum, ν, cm^{-1}: 3325 (OH), 3155 (NH), 1682 (C=O), 1651 (C=N), 1596 (N=N). ^{1}H NMR spectrum, δ, ppm: 9.85 (s, 1H, OH), 5.85 (s, 1H, NH), 3.1 (s, 2H, CH_2). ^{13}C NMR spectrum, δ, ppm: 22.2, 157.15,159.33. Found, %: C, 25.70; H, 2.91; N, 59.96. $C_3H_4N_6O$**.** Calculated, %: C, 25.72; H, 2.88; N, 59.98.

2,5-Dioxo-2,4,5,6-tetrahydro-1H-pyrazolo[1,5-b]pyrazole-3-carbonitrile (13).

A mixture of ethyl cyanoacetate (5 mmol), compound **1** (5 mmol), and sodium ethoxide (0.57 mmol) in abs. EtOH (20 mL) was refluxed for 9h. The mixture was then concentrated and then placed into ice/water, which was subsequently neutralised with HCl (10 %). The obtained solid product was collected by filteration, dried, and recrystallized from ethanol to afford **4** as black crystals. Yield (85%), mp: 170-171 °C. IR spectrum, ν, cm^{-1}: 3321 (NH), 2214 (CN), 1681 (C=O), 1519 (C=C). ^{1}H NMR spectrum, δ, ppm: 6.04 (s, 2H, D_2O exchangeable, 2NH), 4.6-4.3 (m, 2H, CH_2). Found, %: C, 43.90; H, 2.49; N, 34.13. $C_6H_4N_4O_2$**.** Calculated, %: C, 43.91; H, 2.46; N, 34.14.

3,5-Dihydroxy-1,2-dihydroazine-4-carbonitrile (16).

A mixture of hydrazide **1** (60.6 mmol), chloroacetyl chloride (65.5 mmol), and sodium acetate in acetic acid (20 mL) was stirred for 4h at r.t. The solid product is obtained by pouring the reaction mixture into an ice/water mixture, filtering it, and recrystallizing it from AcOH to give **16** as brown crystals. Yield= 80%, mp: 62-65 °C. IR spectrum, ν, cm^{-1}: 3450 (OH), 3174 (NH), 2225 (CN), 1612 (C=C). ^{1}H NMR spectrum, δ, ppm: 10.39, 10.20 (s, 2H, 2 NH), 8.5 (s, 2H, 2 OH), 7.08 (s, 1H, CH). Found, %: C, 43.14; H, 3.61; N, 30.24. $C_5H_5N_3O_2$**.** Calculated, %: C, 43.17; H, 3.62; N, 30.21.

4-(1,2,4-Thiadiazol-3-yl)-1,2-dihydropyridazine-3,5-diol (17).

A mixture of **16** (10.7 mmol), ammonium thiocyanate (10.5 mmol), and sodium ethoxide (10.5 mmol) in absolute ethyl alcohol (20 mL) was refluxed for 12h. Then, the mixture was kept to be cooled poured onto crushed ice and neutralised with dil. HCl. The solid product was separated, dried, and recrystallized from ethanol and DMF, yielding **17** as black crystals. Yield 1.9 g (91%), mp: 318-320 °C. IR spectrum, ν, cm^{-1}: 3134 (NH), 1595 (C=C), 1244 (C=N). ^{1}H NMR spectrum, δ, ppm: 11.77 (s, 2H, 2NH), 10.5 (s, 2H, 2 OH), 7.4- 6.95 (m, 2H, 2 CH). ^{13}C NMR spectrum, δ, ppm: 158.75, 173.32 (sp^2). Found, %: C, 36.35; H, 3.03; N, 28.29; S, 16.19. $C_6H_6N_4O_2S$**.** Calculated, %: C, 36.36; H, 3.05; N, 28.27; S, 16.18.

3-Hydroxy-5-phenyl-4H-pyrazole-4-carbonitrile (21).

To a solution of hydrazide **1** (30 mmol) in DMF (30 ml) and triethylamine (5 drops), benzaldehyde (30 mmol) was added and refluxed for 6 h. Then concentrated, allowed to be cooled. Precipitate obtained upon addition to ice/water was separated, dried, and recrystallized from ethyl alcohol to afford **21** as yellow crystals. Yield=80%, M.P.: 200-202 °C. IR spectrum, ν, cm^{-1}: 3330 (OH), 2225 (CN). ^{1}H NMR spectrum, δ, ppm: 11.5 (s, 1H, OH), 7.8-7.1 (m, 5H, ArH), 2.5 (s, 1H, CH). Found, %: C, 64.88; H, 3.82; N, 22.66. $C_{10}H_7N_3O$**.** Calculated, %: C, 64.86; H, 3.81; N, 22.69.

5,6a-Dimethyl-3-oxo-1,2,3,6a-tetrahydro-3a*H*-furo[3,2-*c*]pyrazole-3a-carbonitrile (25).

In absolute ethanol (20 mL), a mixture of hydrazide 1 (10 mmol), acetylacetone (10 mmol), and sodium ethoxide (10 mmol) was refluxed for 10 hours. The mixture was then concentrated, cooled, and poured into ice/water before being neutralised with dilute HCl. Filtration was used to collect the solid product, which was then dried and recrystallized from ethyl alcohol to produce compound **25** as black crystals. Yield (90%), mp: 210-211 °C. IR spectrum, ν, cm^{-1}: 3321, 3290 (2NH), 2222 (CN), 1693 (CO). ^{1}H-NMR spectrum, δ, ppm: 7.03, 6.32 (s, 2H, D_2O exchangeable, 2NH), 4.23 (s, 1H, CH_{furan}), 2.32, 1.45 (s, 6H, $2CH_3$). Found, %: C, 53.60; H, 5.08; N, 23.46. $C_8H_9N_3O_2$**.** Calculated, %: C, 53.63; H, 5.06; N, 23.45.

5-Hydroxy-2,3a-dihydropyrrolo[2,3-c]pyrazole-3,4-dione (28).

In abs. ethanol (20 mL), a mixture of hydrazide **1** (30 mmol), diethyl oxalate (30 mmol), and sodium ethoxide (30 mmol) was kept at r.t. for 6h with continuous stirring. The mixture was then placed into ice cold water and acidified with dil. HCl. Compound **28** brownish-yellow crystals were formed after the product was filtered and it was recrystallized from a mixture of [ethanol+DMF]. Yield (90%), mp.: 303-305 °C. IR spectrum, ν, cm^{-1}: 3337 (OH), 1629 (C=O), 1322 (C=N). ^{1}H-NMR spectrum, δ, ppm: 10.23 (s, 1H, OH), 8.17 (s, 1H, NH), 3.07 (s, 1H, CH). Found, %: C, 39.25; H, 1.96; N, 27.47. $C_5H_3N_3O_3$**.** Calculated, %: C, 39.23; H, 1.98; N, 27.45.

4-Hydroxy-1,3a-dihydro-3H-pyrazolo[3,4-c]isothiazole-3-thione (33).

To a mixture of acetohydrazide **1** (5 mmol) and carbon disulfide (5.2 mmol) in butanol (50 mL) was added triethylamine (5 drops), and refluxed for 12h, then concentrated and cooled. The obtained product was separated, dried, and recrystallized from a mix. of [EtOH+DMF] to produce **33** brown crystals. Yield (90%), mp: 190-191 °C. IR spectrum, ν, cm^{-1}: 3322 (OH), 3215 (NH), 1320 (C=S). ^{1}H-NMR spectrum, δ, ppm: 10.81 (s, 1H, OH), 7.21 (s, 1H, NH), 1.55 (s, 1H, CH). ^{13}C NMR spectrum, δ, ppm: 65.46, 146.3, 148.38, 215.2. Found, %: C, 27.72; H, 1.76; N, 24.28; S, 37.01. $C_4H_3N_3OS_2$**.** Calculated, %: C, 27.74; H, 1.75; N, 24.26; S, 37.02.

Molecular docking

The protein data bank was used to get the proteins crystal structures discovered for *Escherichia Coli* (1KZN). Around at the duplex, H_2O molecules were eliminated and H-atoms were introduced. The MMFF94x force field was used to assign the parameters and charges. Our molecules has been docked in the active site using the MOE DOCK module after alpha-site spheres has been created using the MOE site finder module. The London dG scoring algorithm in MOE software was used to produce the Dock score, which was then adjusted in two distinct ways. The system's planarity was preserved, and the best poses were examined for the best result [30-33].

Pharmacokinetics

Molinspiration online property calculation toolkit and QikProp3.2 tool available in Schrödinger 9.0 version (USA) were used to analyse ADME properties of selenium compounds in order to determine whether the molecule has maximum pharmacokinetic characteristic for entering higher phases of the development process of the drug or not.

Molinspiration is the delicate balance of molecular characteristics and structural elements that determines whether a molecule is linked to recognised medications. H-bonding, Hydrophobicity, molecule size, electronic distribution and flexibility, plus, of course, the existence of multiple pharmacophoric functionalities all have an effect on the effectiveness of molecules in a living organism, which include bioavailability, transport features, attraction to proteins, reactivity, toxic effect, metabolic stability, and other factors. Furthermore, the variety of therapeutic targets (each of which requires a unique set of molecular features) is so great that a common denominator may be found for all of them, allowing molecule drug-likeness to be expressed by a single "magic number". Simple count criteria (such as M. Wt. limitations, log P, or the number of H-bond donors or acceptors) have limited application and are only helpful for weeding out apparent non-drugs. A universal drug-likeness score is a successful Molinspiration method that focuses on certain classes of drugs and the production of distinct activity scores for each of these classes. The approach compares the structures of typical ligands active on a given target against the structures of inactive compounds and identifies substructure features (which dictate physicochemical features) that are representative of active molecules using sophisticated Bayesian statistics [34-36].

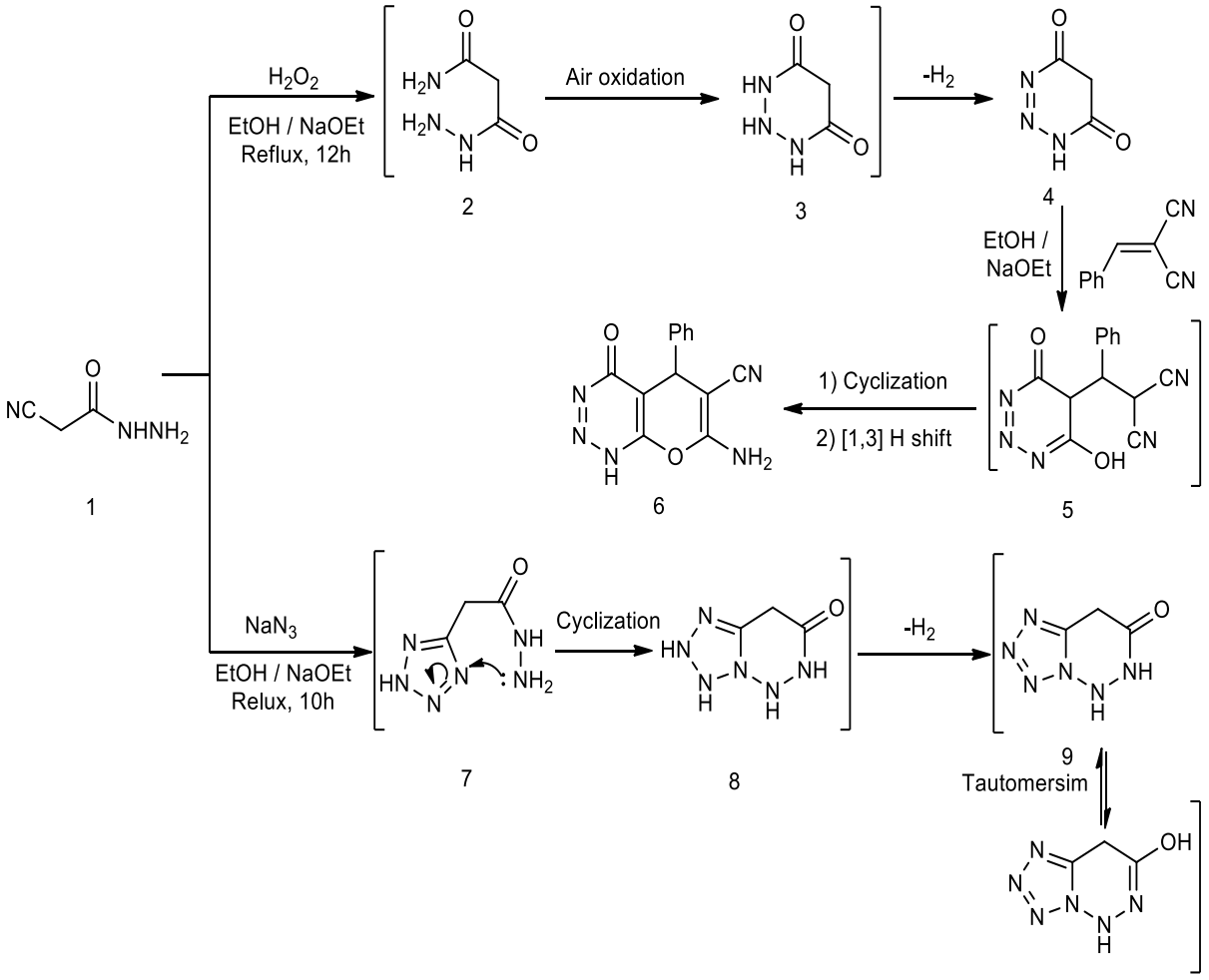
RESULTS AND DISCUSSION

Synthesis and characterization

Cyanoacetohydrazide with its multinucleophilic centers can be functionalized and undergoes heterocyclization using different types of electrophilic reagents. Cyanoacetohydrazide **1** can react through its cyano functionality. They were initially refluxing cyanoacetohydrazide **1** with H_2O_2 in a basic medium for 12 h afforded triazinedione derivative **4** in 85% yield. As depicted in scheme 1, it

is thought that cyanohydrazide **1** undergo cyano oxidative hydrolysis to produce non-isolable amide **2** followed by air oxidation and subsequent dehydrogenation yielded **4**. The reaction product **4** was proven by the absence of cyano function, while NH and C=O appeared at 3332 and 1651 cm^{-1}, respectively, according to IR data. ^{1}H NMR spectrum of **4** exhibited deshielded D_2O exchangeable singlet at 9.85 ppm assigned for NH, together with a singlet at 5.85 ppm for cyclic methylene protons. ^{13}C NMR spectrum of **4** showed carbon signals for two C=O groups at 175.48 and 172.50 ppm, while the methylene carbon signal was detected at 24.46 ppm.

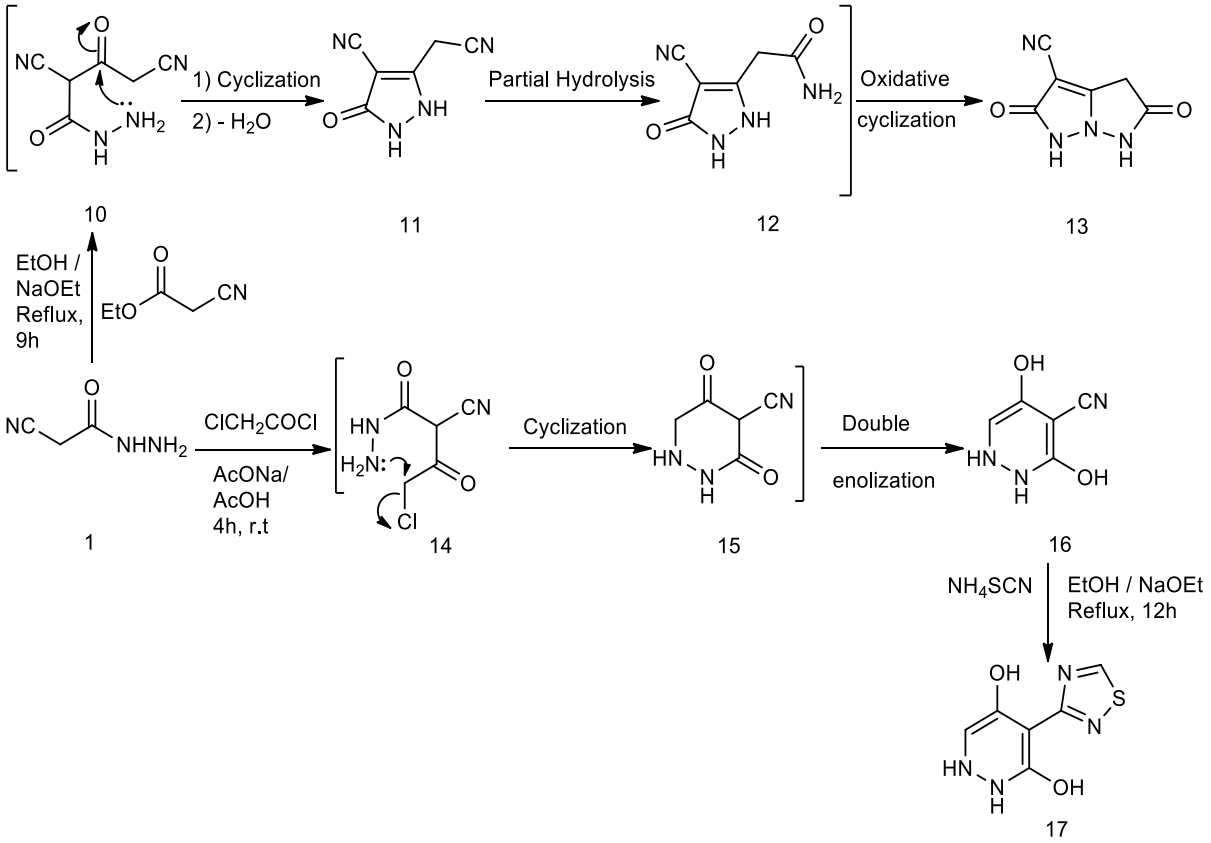
Further evidence for the structure of **4** was obtained through studying their chemical reactivity through some chemical reagents. Thus, the triazinedione **4** undergo conjugate addition to benzylidene malononitrile under Michael's addition condition to furnish pyranotriazinone derivative **6** in excellent yield as displayed in Scheme 1. Presumbly, the reaction may start with the production of an acyclic Michael adduct **5**, which upon intramolecular cyclization followed by [1,3] H-shift affords **6**. IR spectra of **6** exhibited stretching frequencies cm^{-1} at 3332, 3213 for NH_2, 2353 (CN), and 1670 (CO), respectively. In addition, ^{1}H NMR spectra of **6** displayed a singlet at 7.83 ppm for NH_2 protons, together with a multiplet at 7.51-7.37 ppm for phenyl protons and a singlet at 5.51 ppm for CH pyran proton. Furthermore, ^{13}C NMR of **6** exhibited a deshielded carbon signal at 166.83 ppm devoted for C=O carbon and 125.55 ppm for CN carbon plus other peaks for the aromatic and CH carbons.



Scheme 1: Cyano Oxidation and azidolysis of cyanoacetohydrazide **1**

Azidolysis of cyano function of **1** resulted in the formation of non-isolable tetrazole **7** via polarized [3+2] cycloaddition, which undergoes intramolecular cyclization to produce **8** followed by dehydrogenation yielded **9** in excellent yield (Scheme 1). The IR spectra of **9** showed peaks at 3325, 3155, and 1682 cm^{-1} assigned to OH, NH, and C=O groups, respectively, while no absorption band was observed for the CN group**.** Therefore, it was assumed that compound **9** undergo tautomerism and exists in amide-iminol form. In addition, ^{1}H NMR of **9** displays a singlet at 9.85 ppm for OH proton, a singlet at 5.85 ppm for NH proton in addition to singlet at 3.1 ppm for methylene protons. Furthermore, ^{13}C NMR of **9** displayed signals at 22.2, 157.15, and 159.33 correspondings to triazine, iminol, and tetrazole carbons.

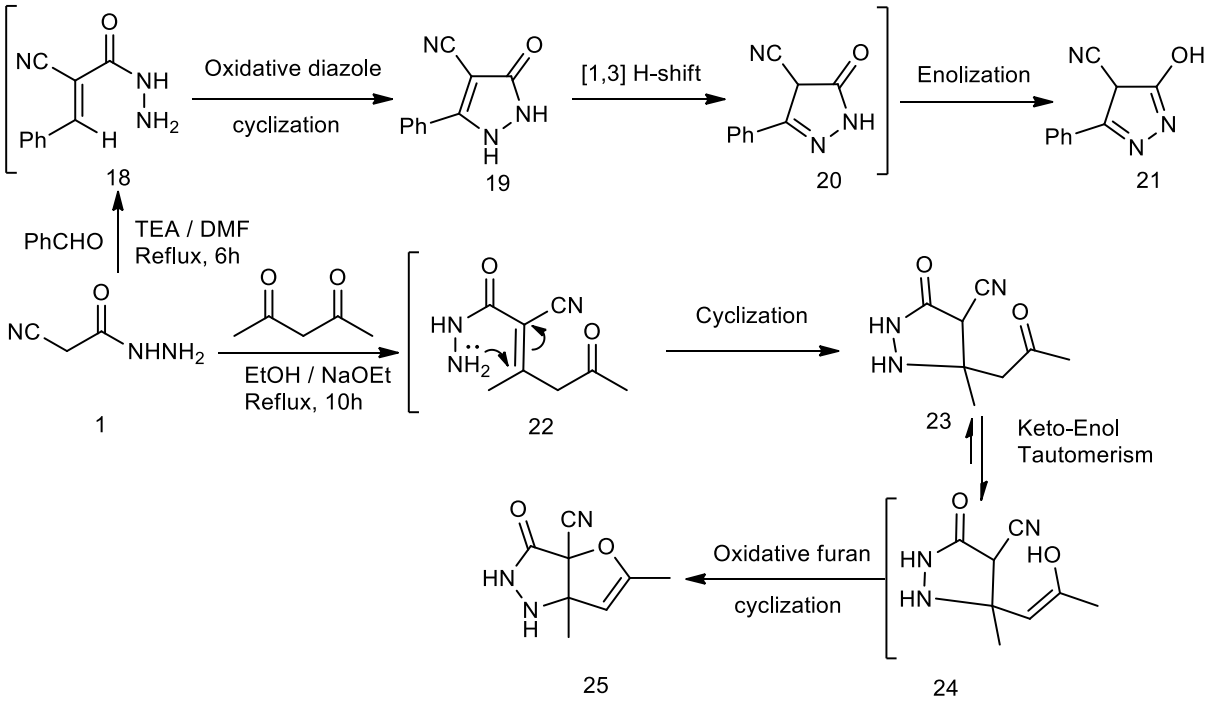
On the other hand, compound **1** could also react through the active methylene group in a basic medium. As shown in scheme 2, refluxing **1** with ethyl cyanoacetate in basic medium afforded cyanopyrazolone derivative **13** in 85% yield. The reaction may be started with the formation of non-isolable cyanoacetyl derivative **10** (by nucleophilic attack of the anion of active methylene carbon of **1** to the electrophilic carbonyl carbon of ethyl cyanoacetate**)** followed by cyclo condensation to give pyrazolone **11,** which undergo basic partial cyano hydrolysis to give amide **12** and subsequent oxidative cyclization to form **13**. The IR spectra of **13** showed stretching bands at 3321, 2214, and 1681 cm^{-1} devoted for NH, CN, and carbonyl groups, respectively. Furthermore, the ^{1}H-NMR spectra of **13** exhibited deshielded D_2O exchangeable singlet at 6.04 ppm assigned for two NH groups proton, together with a multiplet at 4.6-4.3 assigned for nonequivalent CH_2 protons.



Scheme 2: Heterocyclization of compound **1** in basic medium**.**

As shown in scheme 2, when chloroacetyl chloride reacts with an anion of **1** results in the acylated intermediate **14** followed by pyridazine cyclization via intramolecular nucleophilic substitution to form **15,** and subsequent double enolization produces the target **16**. The IR spectral analysis of **16** displayed stretching frequencies at 3174 cm^{-1} as a broad peak assigned for the NH group,

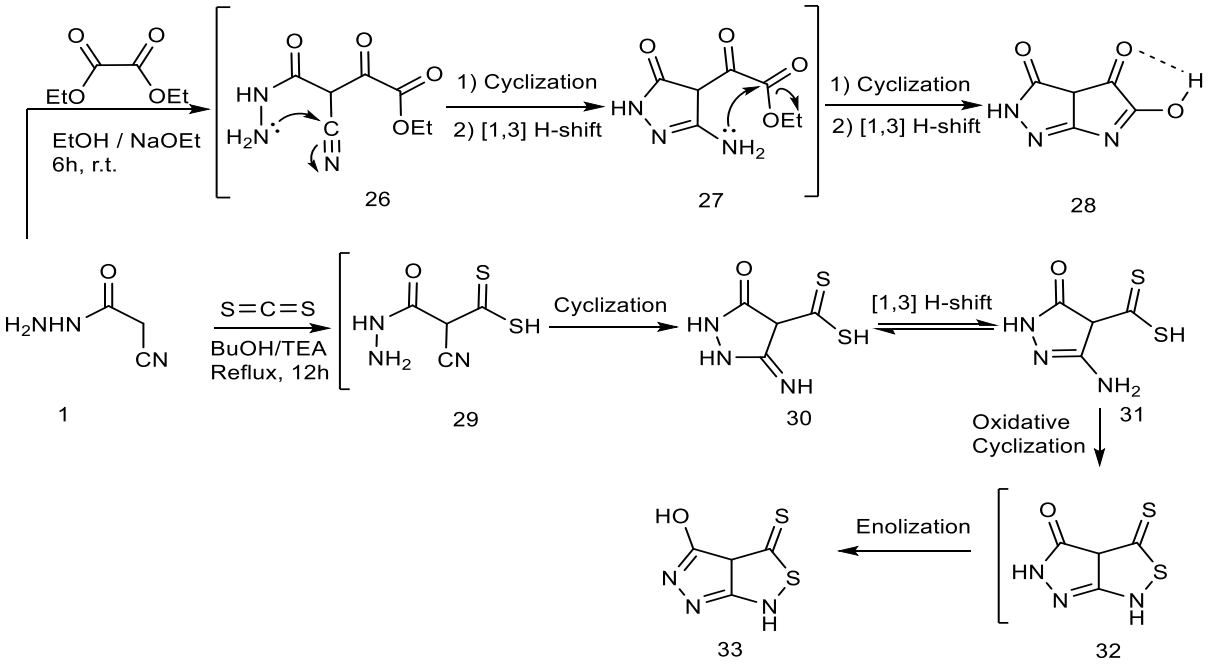
while the conjugated cyano function was observed at 2225 cm^{-1}. The ^1H NMR spectrum of **16** showed the deshielded signals for NH at 10.20 and 10.39 ppm, in addition to a singlet at 8.5 ppm for two OH protons and a singlet at 7.08 ppm for CH proton. Azine **16,** with its cyano function, undergo [2+3] intermolecular cycloaddition to the heteroallene system produced thiadiazole derivative **17** in excellent yield as shown in Scheme 2. Compound **17** was identified by the absence of the cyano group by IR data. ^1H NMR spectra of **17** exhibits two singlets at 11.77 and 10.5 ppm assigned for two NH and two OH protons, and multiplet at 7.4-6.95 ppm assigned for two CH protons. Furthermore, the ^{13}C NMR spectrum of **17** displayed the expected sp^2 carbon signals.



Scheme 3: Knoevenagel condensation and heterocyclization of **1**

Acetohydrazide **1** could undergo Knoevenagel condensation when allowed to react with benzaldehyde produced the non-isolable intermediate **18,** which upon oxidative pyrazole cyclization followed by [1,3] hydrogen shift subsequent enolization afforded diazole derivative **21** in 80% yield (Scheme 3). The IR of **21** showed bands at 3330 and 2225 cm^{-1} assigned for OH and cyano groups, respectively, while there were no peaks for NH or C=O groups. In addition, ^1H-NMR of **21** displayed a singlet at 11.50 ppm for OH proton, a multiplet at 7.8-7.1 ppm for the aromatic protons, in addition to a singlet at 2.5 ppm for CH proton.

On the other hand, refluxing acyclic active methylene **1** and acetylacetone in a basic medium for 10 hr afforded furopyrazolone derivative **25** in excellent yield (Scheme 3). The reaction may be started with the formation of condensed intermediate **22**, followed by intramolecular [1,4] cycloaddition to provide **23,** which undergo keto-enol tautomerism and subsequent intramolecular oxidative furan cyclization afforded **25**. IR spectrum of **25** exhibits absorption frequencies at 3321, 3290 cm^{-1} for two NH groups, additionally 2222 and 1693 cm^{-1} for CN and C=O groups, respectively. Furthermore, ^1H-NMR of **25** displayed two deshielded exchangeable singlets at 7.03 and 6.32 ppm assigned for two NH protons, together with a singlet at 4.23 ppm for CH proton and two singlets at 3.67 and 2.32 ppm for two methyl protons.



Scheme 4: Synthesis of compounds **28** and **33**.

Base mediated cyclo condensation of diethyl oxalate, and **1** yielded pyrrolopyrazolone derivative **28** in excellent yield (Scheme 4). The formation of **28** is assumed to occur via intermediate **26**, followed by pyrazole heterocyclization and [1,3] H-shift gives **27.** intramolecular pyrrole cyclization of **27** and subsequent [1,3] H-shift yields **28,** which stabilized by intramolecular H-bond. According to IR data, the broad band of OH involved in intramolecular hydrogen bond was observed at 3337 while C=O was located at a low value of 1629 cm^{-1}. In addition, the ^{1}H NMR spectrum of **28** exhibits the OH, NH proton signals at 10.23 and 8.17 ppm, respectively.

Finally, refluxing compound **1** with carbon disulfide in butanol in the presence of TEA resulted in fused system **33** (Scheme 4). The formation of **33** may be started with the addition of **1** through its active methylene group on the electrophilic carbon of CS_2 to give the acyclic intermediate **29**, which upon pyrazole cyclization gives pyrazolone derivative **30**, followed by [1,3] H-shift gives **31** and subsequent isothiazole heterocyclization via air oxidation afford **32**. Finally, enolization of **32** affords dihydropyrazoloisothiazolethione derivative **33**. In the IR of **33** appear peaks at 3322, 3215, and 1320 cm^{-1} devoted for OH, NH, & C=S groups, respectively. Additionally, the ^{1}H NMR of **33** displayed two singlets at 10.81 and 7.21 ppm assigned for OH and NH protons, respectively, and another singlet at 1.55 ppm for CH proton. Furthermore, ^{13}C NMR spectrum of **33**: sp^3 carbon signal was observed at 65.46 ppm while C=S at 215.2 ppm and C=N at 148.38 ppm.

Molecular docking

On an Intel Core i5 CPU 1.9 GHz, an 8 GB RAM with Windows 10, and a 64-bit OS., molecular docking investigations were done using the Molecular Operating Environment (MOE, 2019) software. The MOE was utilised to perform energy minimizations using an RMSD gradient of 0.05 kcal/mol and an MMFF94X force field. In addition, the partial charges were determined automatically. The X-ray crystallographic structures were derived using the PDB file 1KZN from the Protein Data Bank (PDB). Deleting the ligand from the enzyme active site and completing the structure by adding hydrogen atoms while keeping their standard geometry in mind, and finding the enzyme active site using the MOE Alpha Site Finder were all used to ready the target enzyme for docking. 1KZN is a code that refers to a 24 kDa gyrase fragment while DNA gyrase is the major protein engaged in bacterial circular DNA replication and transcription. Antibacterial

medications that target DNA gyrase are known to cause bacterial death [30-33].

The docking interactions are represented in table 1, while figure 2 represents the 2D and 3D interaction of the synthesized compounds with 1KZN. As per docking investigation, the most interactive behavior seen between the obtained molecules and receptors is the H-donor/acceptors interaction between the *O*-atom of ALA47, VAL71, and GLU131, for compounds **6**, **28** and **33**, respectively (table 1). Table 2 displays the docking scores of the compounds studied. It was found that compounds **6**, **17** have the highest docking score, although compound **17** has no measurable interactions.

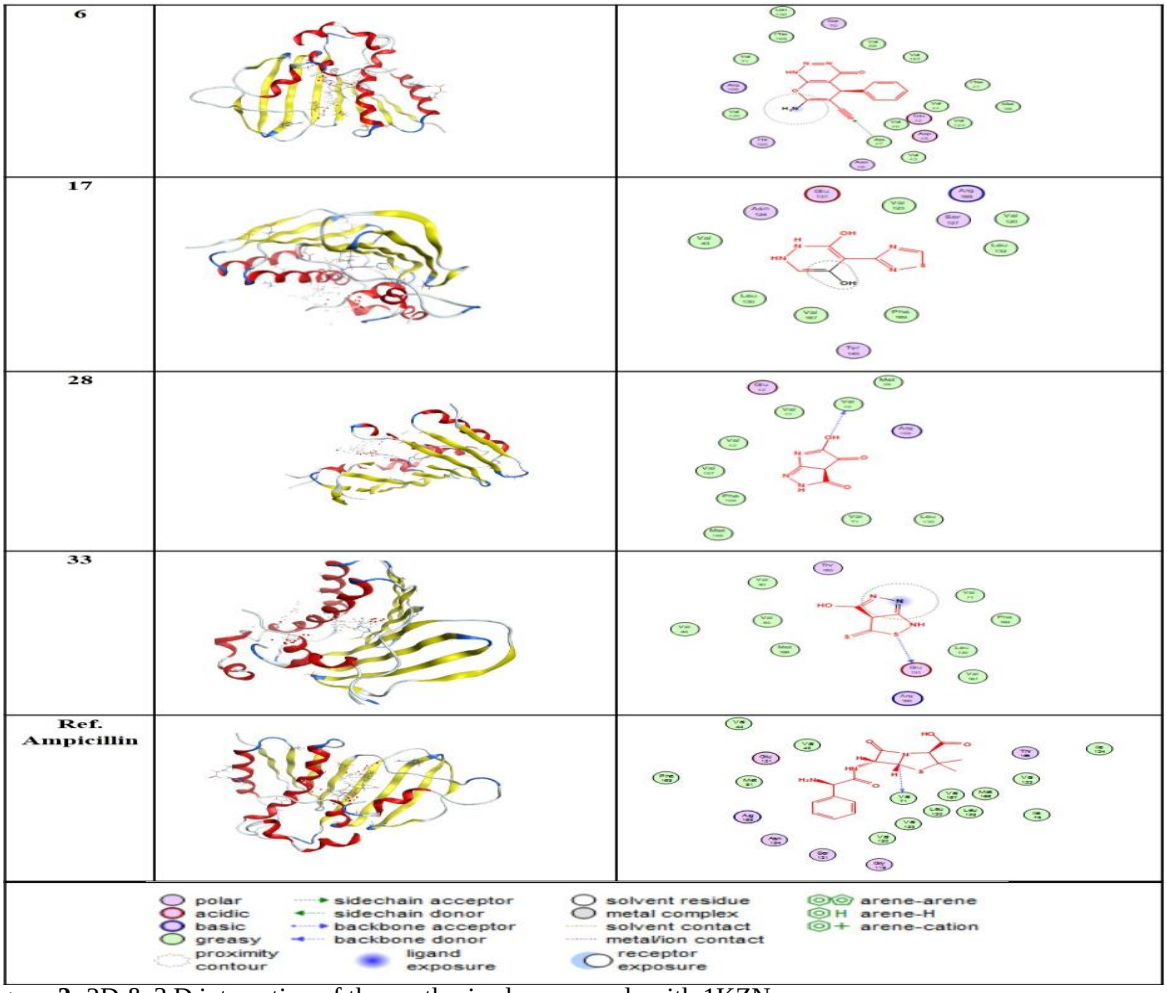


Figure 2: 2D & 3 D interaction of the synthesized compounds with 1KZN.

Table 1: **Docking interaction of all compounds with 1KZN protein**

No.	Ligand	Receptor	Interaction	Distance	(kcal/mol)
6	N	29E CB	ALA 47 (A) H-acceptor	2.98	-0.9
9	No mesurable interaction is observed				
13	No mesurable interaction is observed				
17	No mesurable interaction is observed				
21	No mesurable interaction is observed				
25	No mesurable interaction is observed				
28	O	13 O	VAL 40 (A) H-donor	3.50	-0.6
33	S	9 O	GLU 131 (A) H-donor	3.70	-0.5
Ref.	C	20 O	VAL 71 (A) H-donor	2.67	-1.5

Table 2: Docking score and energies of All compounds with 1KZN							
Comp.	S	rmsd_refine	E_conf	E_place	E_score1	E_refine	E_score2
6	-5.9526	0.8163	-66.181	-69.316	-9.2662	-24.1820	-5.9526
	-5.6149	1.8361	-73.984	-84.254	-9.0063	-25.3443	-5.6149
	-5.5992	1.3219	-74.413	-83.96	-9.1204	-28.1975	-5.5992
	-5.5959	1.7381	-74.602	-88.008	-9.8552	-27.9335	-5.5959
	-5.5493	2.7068	-74.628	-65.186	-8.9856	-27.5206	-5.5493
17	-5.3593	1.5878	10.8137	-71.5425	-11.0626	-23.1453	-5.3593
	-5.2438	1.3528	11.3177	-63.9171	-10.0168	-22.2626	-5.2438
	-5.2176	0.7980	11.8727	-62.7975	-9.7301	-24.6489	-5.2176
	-5.2004	1.6833	12.1326	-80.8569	-11.2346	-22.4946	-5.2004
	-5.1137	1.0547	11.9359	-77.8895	-9.4798	-25.1543	-5.1137
28	-5.2153	0.5273	-29.5604	-47.0012	-8.2303	-16.5512	-5.2153
	-5.1483	0.8569	-29.7482	-51.5029	-7.6634	-21.2170	-5.1483
	-4.5216	0.6433	-29.4959	-47.8682	-8.0403	-15.1184	-4.5216
	-4.4759	1.1012	-30.0264	-47.2142	-8.2652	-17.2843	-4.4759
	-4.4663	2.3344	-29.0240	-55.5576	-7.5355	-15.9631	-4.4663
33	-4.4948	4.1498	-35.6114	-55.1619	-7.8855	-20.9611	-4.4948
	-4.4755	1.3451	-31.8657	-56.2628	-8.3933	-17.8683	-4.4755
	-4.4382	1.4133	-34.4450	-44.9748	-7.7563	-19.2925	-4.4382
	-4.4259	1.6364	-35.7293	-57.5759	-8.4822	-17.4376	-4.4259
	-4.3938	2.1982	-34.8895	-47.9033	-8.2813	-15.8879	-4.3938
Reff	-6.1983	3.3891	92.1193	-30.3920	-9.6207	-33.5876	-6.1983
	-6.1332	2.9885	71.7084	-28.8534	-9.5802	-34.3001	-6.1332
	-6.0373	2.2864	69.0299	-56.2658	-9.3975	-34.3898	-6.0373
	-5.9999	1.9289	68.6877	-57.8640	-9.4327	-33.1946	-5.9999
	-5.8422	2.2781	70.6984	-49.7994	-10.9601	-33.3201	-5.8422

Pharmacokinetics predictions ADME studies

The predicted pharmacokinetic/Molinspiration properties [34-36] of compounds **6**, **17**, **28** and **33** are illustrated in tables 3 and 4. Using Molinspiration virtual screening, the majority of the synthesised molecules had potential bioactivity as indicated by docking parameters, indicating drug-like characteristics incomparison to kinase, protease, and enzyme inhibitors (Table 4).

Table 3. Molinspiration property engine of derivatives 6, 17, 28, 33

Sample ID	MW (g/mol)	MolecularFormula	miLog*P*
6	267.25	$C_{13}H_9N_5O_2$	0.44
17	198.21	$C_6H6N_4O_2S$	0.70
28	153.10	$C_5H_3N_3O3$	-2.13
33	173.22	$C_4H_3N_3OS_2$	0.30

Table 4: Molinspiration bioactivity scores of derivatives 6, 17, 28, 33

Compound	GPCR ligand	Ion channel modulator	Kinase inhibitor	Nuclear receptor ligand	Protease inhibitor	Enzyme inhibitor
6	-1.36	-1.18	-1.16	-1.09	-1.43	-0.68
17	-0.27	-0.34	-0.15	-0.59	-1.03	0.02
28	-1.48	-1.04	-2.16	-1.67	-1.28	-0.81
33	-1.70	-1.54	-2.57	2.20	-1.85	-0.97

CONCLUSIONS

We described a simple and efficient base-mediated preparation of a new target of heterocyclic and condensed heterocyclic systems upon cyclization of cyanoacetohydrazide with various electrophilic reagents. The approach opens the way for synthesizing different heterocyclic compounds that will efficiently provides a route to new biologically active compounds. The molecular docking studies indicating that compounds **6** and **28** are the most potent. This research can be used as a reference for upcoming medical and pharmaceutical chemistry.

CRediT authorship contribution statement

W. Shehta, M. G. Assy and E. O. Hamed conceived, designed the work. Magda H.Abdellattif did the molecular docking studies, editing English, writing draft, funding. W. Shehta, Mohamed G. Assy wrote the manuscript and analyzed the data. Eman El-Said performed the experiments. All authors have read and agreed to the published version of the manuscript.

Declaration of Competing 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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Conflict of interest

Authors declare no competing financial interest.

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