Egyptian Journal of Chemistry

http://ejchem.journals.ekb.eg/

2-Cyano-N-(2,5-dioxopyrrolidin-1-yl) acetamide as a building block for developing new azole and azine derivatives and assessing their biological properties

Mohamed Ahmed Mahmoud Abdel Reheim, Ibrahim Saad Abdel Hafiz, Reem Mustafa Darweesh, Mohamed Ahmed Elian Sophy *

Department of Chemistry, Faculty of Science, Arish University, Arish 45511, Egypt.

Abstract

The following new compounds have been produced: iminopyran, dihydropyridine, thiophene, tetrahydrobenzo[b]thiophene, and dihydrothiazole. The specific compound structures were verified by elemental analysis, spectral approaches, and the production mechanisms were explored. The newly synthesized compounds showed encouraging inhibitory efficacy against bacteria and fungi, according to the data that were reported. Two bacterial species that are gram-positive (Staphylococcus aureus and Bacillus subtilis) and two bacterial species that are gram-negative (Escherichia coli and Pseudomonas aeuroginosa) were used in the test of the synthesized compounds' anti-microbial activities. The compounds were evaluated for their antifungal properties against two fungi, Aspergillus flavus and Candida albicans, in order to ascertain their minimum inhibitory concentration (MIC). Generally, the newly synthesized substances had strong antibacterial action against the microorganisms listed before.

Keywords: Cyanoacetanilide, Heterocyclic compounds, Biological evaluation

1. Introduction

In recent years, our research programs have aimed to create unique procedures for the production of various unique heterocyclic rings with biological assessment by using cyanoacetic acid hydrazides as raw precursors, a lot of heterocyclic rings were synthesized through cyanoacetic acid hydrazides like benzothiazoles, thiazoles, thiophenes, pyrazoles, pyridines, pyrans and other rings. According to a literature review, we found that the biological effects of heterocyclic compounds, specifically benzothiazoles, thiazoles, thiophenes, pyrazoles, pyridines, and pyrans, play a crucial role in organic and medicinal chemistry. Derivatives of benzothiazole represent a significant class of heterocycles when searching for novel medications .They work well in the clinic as anticancer [1–3], antidiabetic agent [4], antiviral [5,6], anti-microbial [7,8], anti-inflammatory [9], anticonvulsant [10], antitumor [11]. Thiazole derivatives have been created in medicinal chemistry for a variety of uses, including antimicrobial [12–15], anticancer [16,17], antioxidant [18,19], anti-diabetes [20], antiviral [21], thiophenes exhibit a wide range of pharmacological actions, including antimicrobial [22,23], anti-inflammatory [24,25], neuraminidase [26], anticancer [27–29], anti-proliferative [30], antioxidant [31]. Multiple compounds with pyrazole nucleus have been commercialized as anti-inflammatory [32–34], antimicrobial [35–37], antiviral [38], anticancer [39,40], antitumor [41]. Pyridines have drawn a lot of attention because many of these substances have proven essential in the study of medicinal chemistry, including as antibacterial [42,43], antiviral [44], anticancer [45]. The biological properties of pyrans are described in numerous articles as being anti-Alzheimer disease [46], antioxidant [47]. This prompted us to prepare a series of novel structural analogues of these heterocycles.

*Corresponding author e-mail:.; mohamedahmedelian@gmail.com; (Mohamed Ahmed Elian).
Received date 20 July 2024; revised date 03 October 2024; accepted date 12 October 2024
DOI: 10.21608/ejchem.2024.305844.10048

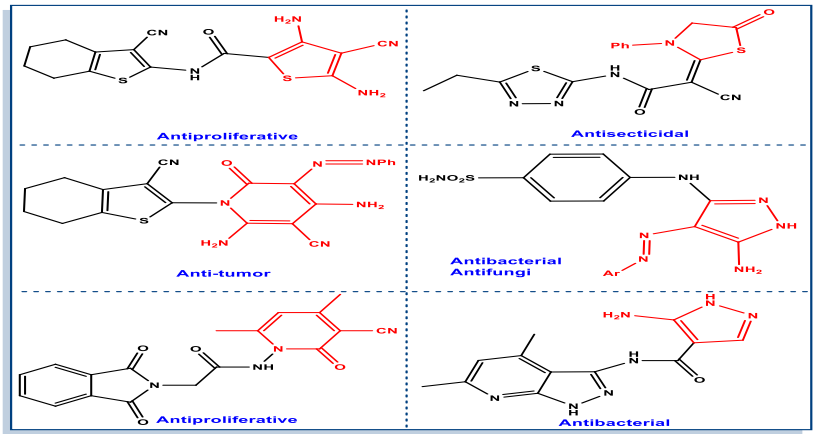


Fig. (1): Examples of biologically active cyanoacetamide derivatives.

2. Result and discussion

2-cyano-N-(2,5-dioxopyrrolidin-1-yl) acetamide **1** is a particularly active substrate because it contains electrophilic sites that are represented in cyano and carbonyl function clusters. Furthermore, nucleophilic sites are represented in the nitrogen atom and the acidic hydrogen at position two as shown in figure 2. These function groups allow this substrate to impact different condensation and substitution reactions, making it an important building block in the synthesis of unique heterocycles. As a result, we reacted succinic anhydride with cyanoacetic acid hydrazide in ethanol in the presence of acetic acid, which resulted in the formation of 2-cyano-N-(2,5-dioxopyrrolidin-1-yl) acetamide **1**. The structure of the product was provided using the spectral data. IR of compound **1** exhibited absorption bands at υ 3215, 2966-2931, 2264, and 1696-1607 cm^{-1}, attributed to NH, CH-sp^3, cyano, and carbonyl functional groups. The ^{13}C-NMR spectrum showed signals for methylene, cyano, and carbonyl moieties at δ 26.32, 116.05, 170.63, and 174.03 ppm, respectively. The ^{1}H-NMR spectrum of Compound **1** indicated the presence of an NH proton at δ 10.2 ppm and a significant singlet integration of two protons at δ 3.72 ppm.

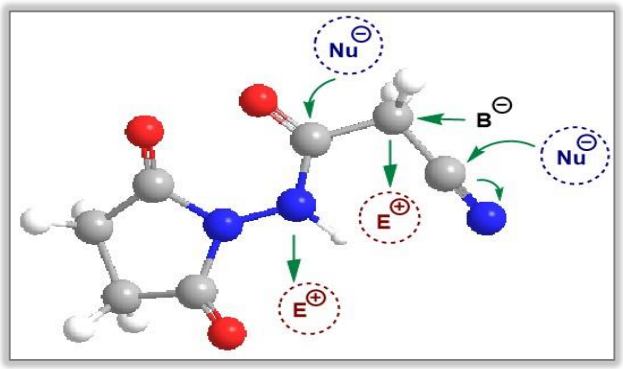
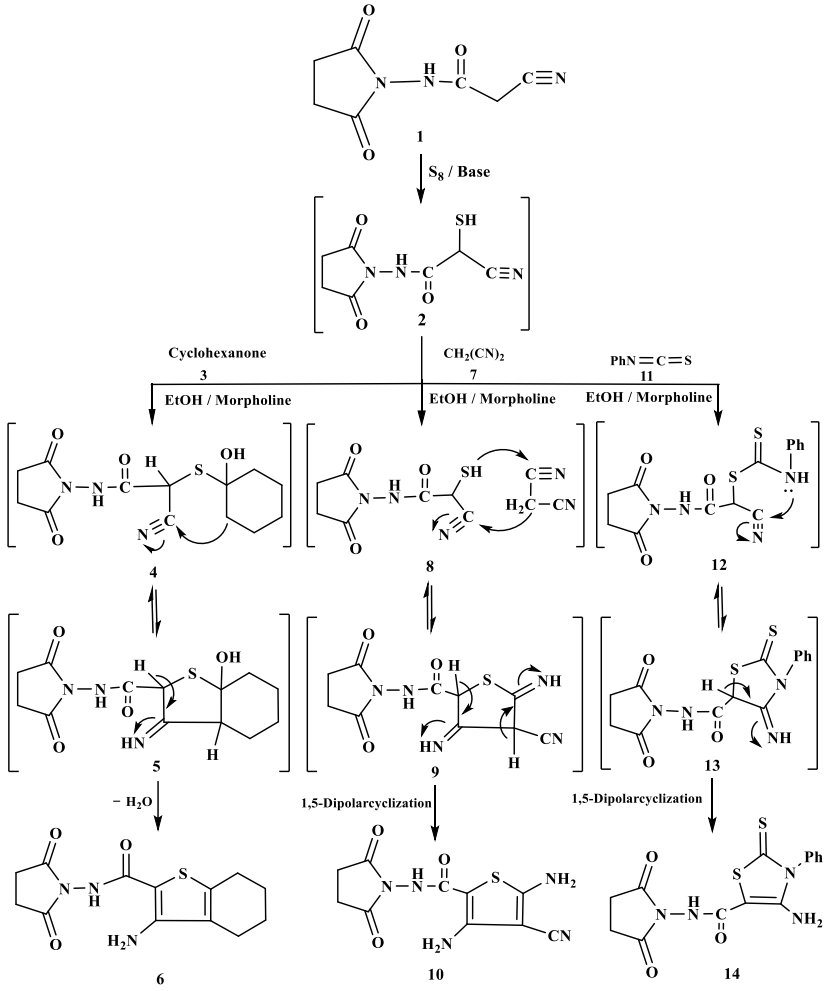


Fig.2. Active sites of 2-cyano-N-(2,dioxopyrrolidin-1-yl) acetamide 1

The important precursor 2-cyano-N-(2,5-dioxopyrrolidin-1-yl) acetamide **1** was employed to produce novel heterocyclic compounds. Thus, the reaction of cyanoacetanilide **1** with cyclohexanone and elemental sulfur in boiling ethyl alcohol in the presence of a catalytic quantity of morpholine yields the appropriate fused thiophene derivative **6** [48]. Formation of **6** is considered as an extension of the Gewald process for synthesizing thiophene and fused thiophene **6**. The spectral and analytical results support the suggested structure. For example the infrared spectrum of compound **6** showed no absorption band that could be assigned to a cyano group and absorption bands for amino groups at 3417–3400 cm^{-1} and carbonyl groups at 1700–1627 cm^{-1}. The ^{1}H-NMR spectrum of the same compound revealed a multiplet signal at δ 1.21-3.80 ppm corresponding to SP^3 protons, a signal at δ 7.47 ppm corresponding to amino protons, and a signal at δ 8.97 ppm corresponding to NH function group. In addition to this, the disappearance of a signal characteristic to active methylene protons in the starting material **1**. The mass spectrum of the same compound showed a molecular ion peak at m/z 293 (M^+), confirming the existence of the molecular formula $C_{13}H_{15}N_3O_3S$. Similarly, compound **6** reacts with elemental sulfur and malononitrile in refluxing ethanol, producing thiophene derivative **10**. The ^{1}H-NMR spectrum of compound **10** showed two signals corresponding to two amino groups at δ 6.52 and 6.98 ppm. The mass spectrum of the same product confirms the proposed structure.

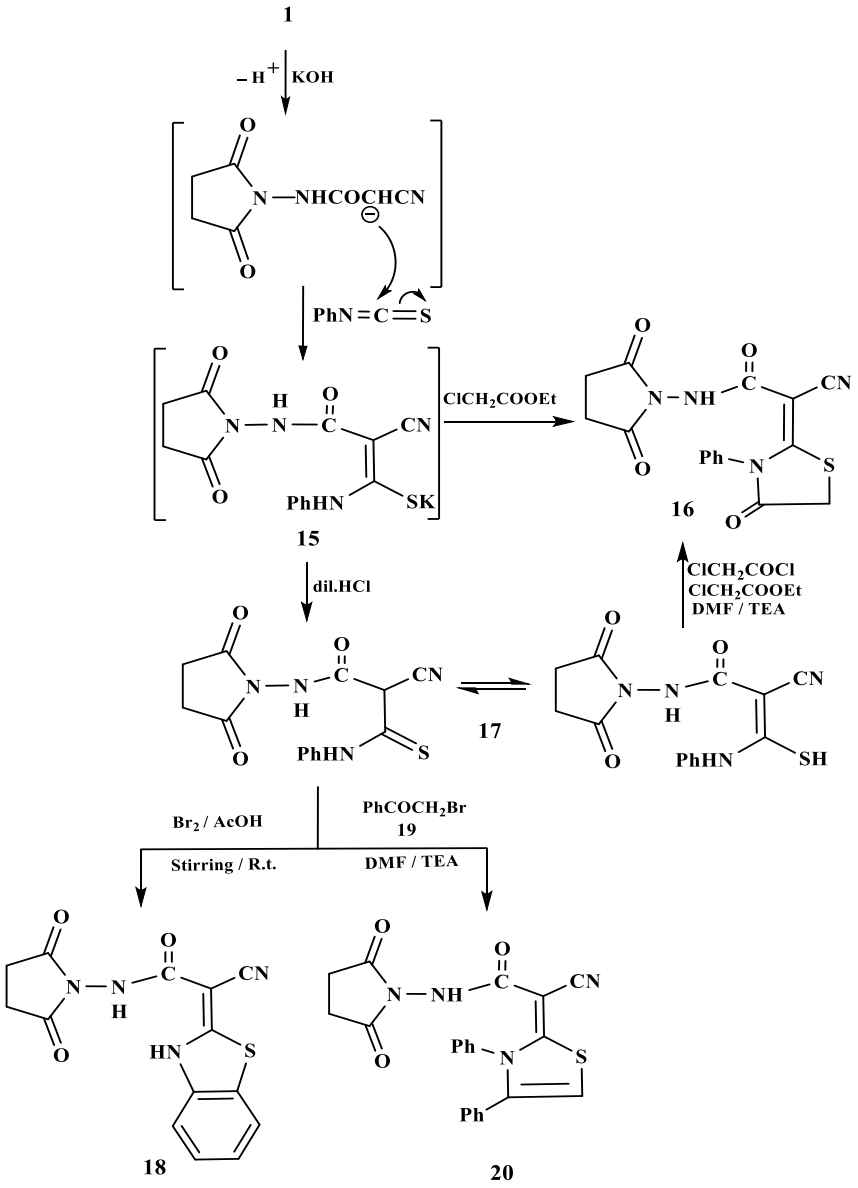
In conjunction to Gewald approach a mixture of **1**, elemental sulphur and phenyl isothiocyanate is allowed to react in refluxing ethanol to afford thiazole derivative **14**. Establishing structure of thiazole **14** was based on elemental and spectroscopic analyses. For example, the IR spectrum of **14** revealed the absence of the peak corresponding to cyano group and emergence of new peaks at 3217, 3118 cm^{-1}supporting the proposed reaction mechanism.



Scheme 1

The ^{1}H-NMR spectrum of the same product revealed the presence of a signal at δ 11.05 ppm for the NH group and another new signal at δ 6.99 ppm. The structure of compound **14** was confirmed further by combination of both ^{13}C-NMR and mass spectroscopic analyses [49–50] **scheme(1)**.

As a part of the present study, the utility of 2-Cyano-N-(2,5-dioxopyrrolidine-1-yl)acetamide **1** as a nucleophile was also investigated. Thus, when cyanoacetamide derivative **1** is stirred with KOH/DMF solution followed by adding phenyl isothiocyanate with continuous stirring then adding equimolar amount of ethyl chloroacetate and finally the solution mixture is allowed to boil under reflux for about 6 hrs., afforded the phenyl thiazolidine derivative **16** in a quantitative yield. Compound **16** is believed to be formed from **1** *via* initial creation of carbanion using KOH followed by nucleophilic attack to phenyl isothiocyanate to afford the non-isolable intermediate potassium salt **15**. The potassium salt **15** reacts readily with ethyl chloroacetate in the same reaction condition to afford **16** through elimination of ethanol and hydrogen chloride as demonstrated in **scheme (2)**.

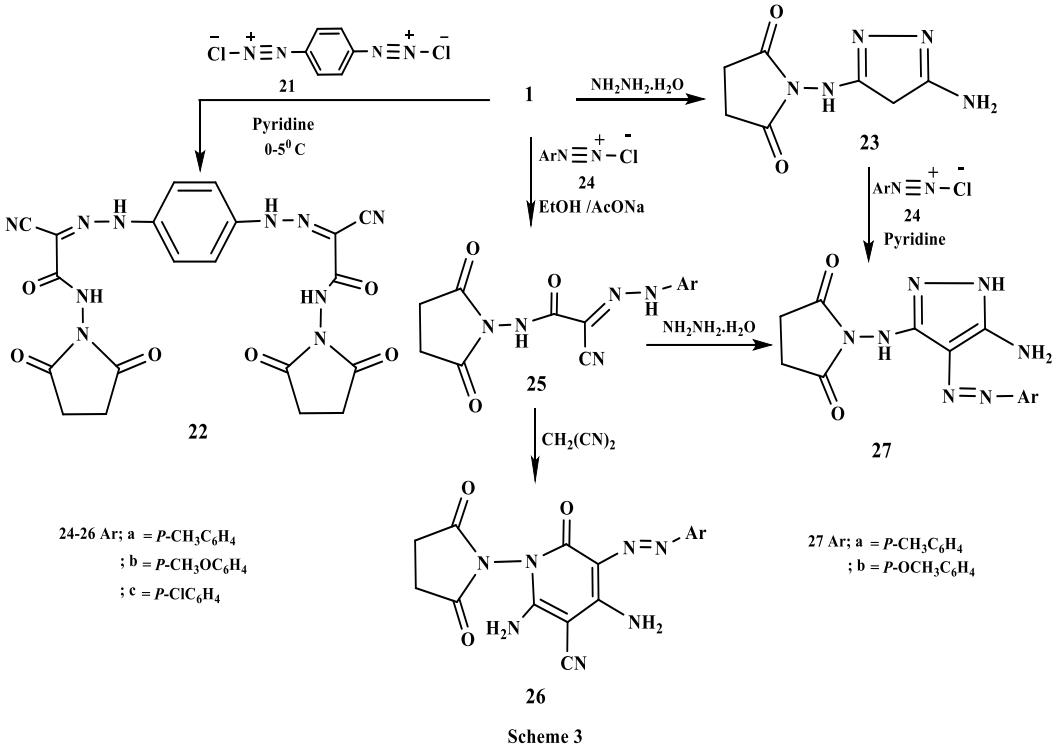


Scheme 2

Establishing compound **16** was based on its elemental analysis and spectral data. The IR spectrum revealed the presence of peaks corresponding to carbonyl and nitrile functions at 2200 cm^{-1} and at 1750 cm^{-1} respectively, both of them support the proposed reaction mechanism. The ^{1}H-NMR of the same compound revealed an intense signal at δ 4.17 ppm corresponding to 5H-thiazole. Further confirmation of the structure of phenyl thiazolidine derivative **16** based on chemical evidence, which can be summarized in treatment of the mixture which contains the potassium salt **15** with dilute HCl acid and left overnight at room temperature with continuous stirring to produce cyanothioxopropanamide derivative **17** further treatment of **17** with ethyl chloroacetate and chloroacetylchloride, a new product is formed in refluxing DMF that is exactly the same as compound **16** based on melting and mixed melting point of compound **16** and its authentic sample. Establishing structure of compound **17** was based on its spectral analyses in parallel with its synthetic potentiality for preparation of some thiazole and benzothiazole derivatives **18** and **20**. Thus, benzo[d]thiazole derivative **18** can be synthesized from **17** by treatment of **17** with bromine solution in acetic acid at 0-5^{0}C [51]. Establishing structure **18** was based on its spectral data. Similarly, thiazole derivative **20** is obtained from **17** by treating it with phenacylbromide **19** in refluxing DMF containing TEA as a catalyst. The reaction is believed most

likely to proceed by nucleophilic substitution of halogen to produce S-alkylated accompanying by losing water to produce thiazole **20**. Establishing of structure 20 was based on spectroscopic analyses [52–55].

The reactivity of active methylene in cyanoacetanilide **1** towards a variety of diazonium salts was also investigated. Thus, when **1** is coupled with aryl diazonium salt **21** in pyridine afforded the coupled product **22**. The suggested structure is fully supported by the IR, ^{1}H-NMR, and mass spectroscopy as well. Similarly, arylhydrazone derivatives **25a-c** are produced by coupling cyanoacetanilide **1** with aryl diazonium chlorides **24a-c** in ethyl alcohol and sodium acetate at 0-5° C. The data from the infrared spectrum, ^{1}H-NMR, and mass spectroscopy supported the proposed structures. The behaviour of compounds **25** towards some active methylene reagents was also investigated. Thus, when compound **25** is allowed to fuse with malononitrile in the presence of ammonium acetate afforded the dihydropyridines **26a-c**. Structures of **26a-c** could be confirmed by incorporated their spectral data. For instance, the IR spectrum of **26a** revealed the presence of many peaks at 3313-3185 cm^{-1}coresponding to amino functions and a peak at 2197 cm^{-1}coresponding to cyano function. The ^{1}H-NMR and mass spectra of the same product further support the proposed structure. Similarly, **25a-c** react with bi-nucleophilic reagent (i-e hydrazine hydrate) to produce the pyrazole derivatives **27a,b**.

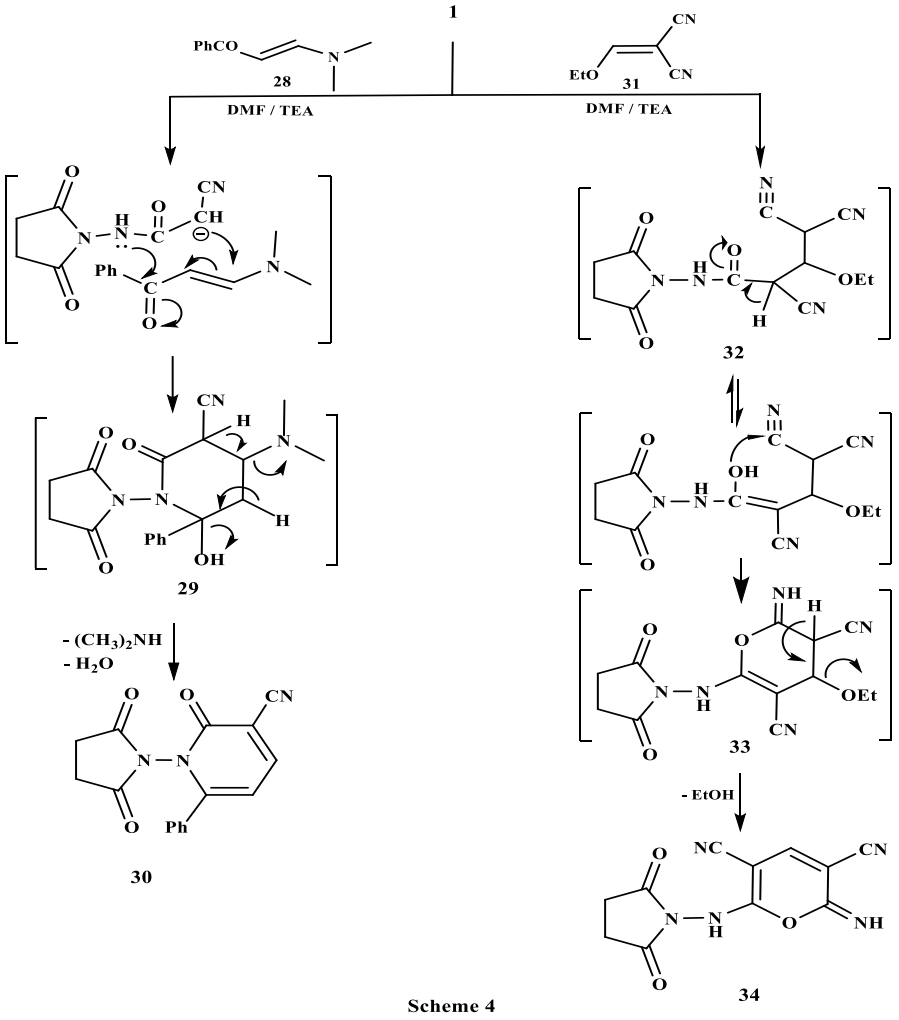


Scheme 3

Establishing structure **27** was based on its spectral data and the authentic sample prepared from the reaction of cyanoacetamide derivative **1** with hydrazine hydrate in refluxing ethanol to give aminopyrazole derivative **23** [56] and further treatment of **23** by aryl diazonium salt **24** in pyridine. Confirmation structure **23** was based on elemental analyses and spectral data [57]

scheme (3).

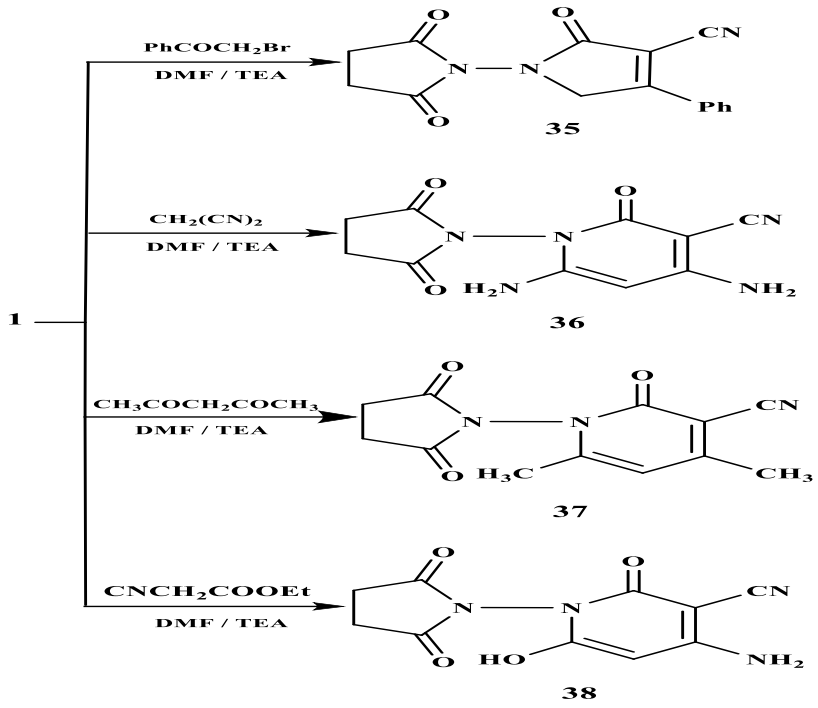
The novel heterocycles obtained from **1** prompted us to investigate further the reactivity of cyano function toward enaminone and its analogues. Thus, compound **1** was treated with 3-(dimethylamino)-1-phenylprop-2-en-1-one **28** in DMF with TEA acting as a catalyst afforded 1-(2,5-dioxopyrrolidin-1-yl)-2-oxo-6-phenyl-1,2-dihydropyridine-3-carbonitrile **30**. The identity of structure **30** was based on the infrared spectrum which shows absorption band at 2222 cm^{-1} assignable for CN group, absorption bands at 1731, 1669, 1639(C=O). cm^{-1} assignable for carbonyl groups and in addition to the disappearance of the NH function group. On the other hand, in dimethylformamide containing TEA at reflux temperature, cyanoacetanilide **1** and 2-(ethoxymethylene) malononitrile **31** were reacted to furnish 6-((2,5-dioxopyrrolidin-1-yl) amino)-2-imino-2H-pyran-3,5-dicarbonitrile **34**.



Scheme 4

Formation of final product **34** involving Michael addition to afford intermediate **32** followed by intermolecular cyclization of enol form **33** to give the final product **34**. The structure of **34** was clarified using spectral and analytical data. (see experimental section) [57,58] **scheme(4)**.

A novel and straightforward method for synthesis of pyrrole derivative is represented by the reaction of the cyanoacetanilide moiety with phenacylbromide. Triethylamine acts as a catalyst in the reaction between compound **1** and phenacyl bromide, which results in the production of the pyrrole derivative **35**. The structure of the later product was confirmed using analytical and spectral information. Its infrared emission spectrum showed carbonyl groups at 1705 and 1666 cm^{-1} and the nitrile specific absorbance band at 2214 cm^{-1}. The methylene of the pyrrole ring was responsible for the singlet signal seen in the ^{1}H-NMR spectrum at δ 5.50 ppm. Additionally, because of the molecular formula $C_{15}H_{11}N_3O_3$, the mass spectrum showed the molecular ion peak at m/z 281 (M^+). The pyridine derivative **36** was produced by allowing the cyanoacetanilide **1** to interact with malononitrile in dimethylformamide containing triethylamine under reflux. The suggested structure for compound **36** was supported by all of the spectral data. The ^{1}H-NMR spectrum showed that the pyridine 5-H proton was responsible for the signals at δ 7.50 ppm and amino protons at δ 6.97 and 7.09 ppm. A common peak at m/z 247 (M^+) in its mass spectrum was found to match the molecular ion peak. The reactivity of cyanoacetanilide **1** towards 1,3-diketone was also investigated. Thus, treatment of cyanoacetanilide **1** with acetylacetone in dry dimethylformamide catalyzed with two drops of TEA as a catalyst produced pyridinone derivative **37**. The structure of **37** was confirmed using spectral and analytical data. Three absorption bands for CH-aliphatic, cyano, and carbonyl groups were visible in the infrared spectrum at 2996–2932, 2220, and 1692, 1666 cm^{-1}, respectively, ^{1}H-NMR exhibited three singlet signals at δ 2.19, 2.22 and 6.14 ppm corresponding to two methyl groups beside CH-pyridine. Similar to this, a new pyridine derivative **38** was created when cyanoacetanilide **1** was treated with ethyl cyanoacetate in dimethylformamide containing triethylamine. The analyses and spectroscopic data of compound **38** support the proposed structure [59–61] **scheme(5)**.



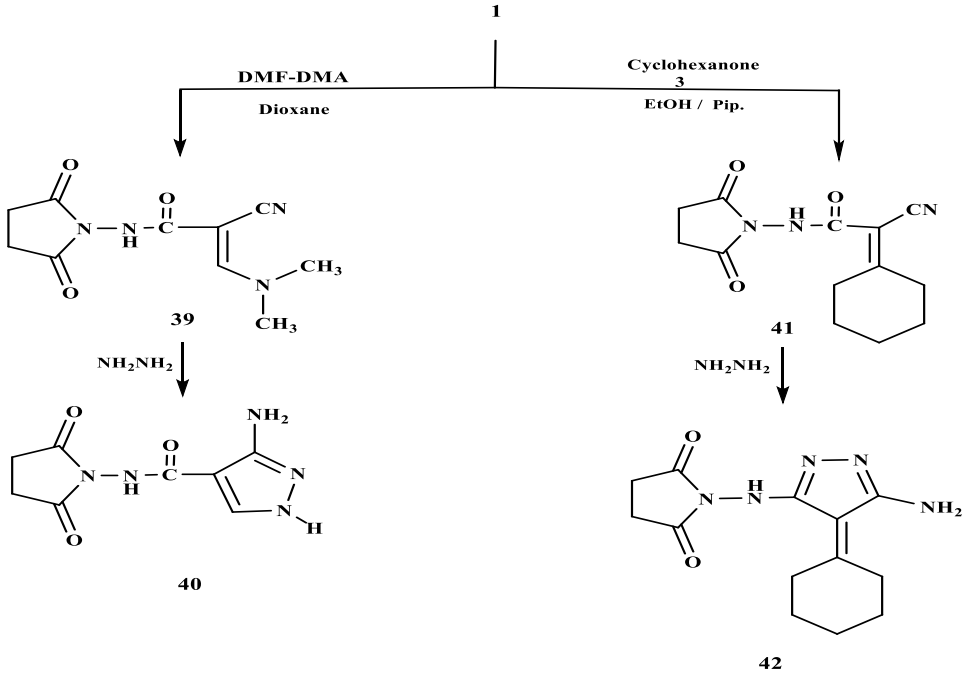
Scheme 5

The reaction of cyanoacetanilide **1** with (DMF-DMA) in refluxing dioxane, 2-Cyano-3-(dimethylamino)-N-(2,5-dioxopyrrolidin-1-yl) acrylamide **39** was produced. Enaminonitrile **39** was converted into the new 2-cyano-2-cyclohexylidene-N-(2,5-dioxopyrrolidin-1-yl) acetamide **40** when hydrazine hydrate was added in refluxing ethanol. Based on its spectral data, the resulting yield's structure was identified as chemical **40**. For instance, the IR spectra revealed three absorbance bands for amino, CH-aliphatic, and carbonyl groups at 3232–3213, 2962–2931, and 1650 cm^{-1}·respectively. Thus, the chemical reactivity of cyanoacetanilide **1** towards cyclohexanone has been investigated, cyanoacetanilide **1** was treated with cyclohexanone under reflux in ethyl alcohol catalyzed with two drops of piperidine to produce compound **41**. The typical bands for NH, cyano, and carbonyl groups are visible in the infrared spectra of the reaction result at 3303 cm^{-1}, 2182 cm^{-1}, and 1730, 1666 cm^{-1}, respectively. The ^{1}H-NMR spectra of compound **41**, which show up-field signals at δ 1.57–2.30 ppm for SP^3 protons, were primarily responsible for clarifying its structure. Furthermore, the anticipated singlet at δ 9.49 ppm for the NH group was present.. The new pyrazolone derivative **42** was produced by the reaction between enaminonitrile **41** and a binucleophilic reagent like hydrazine hydrate in refluxing ethanol. Based on its spectral data, this product's structure was determined. IR spectrum of compound **42** was free of cyano group and showed the absorption band for NH_2 and NH in the range 3350-3201 cm^{-1}. The ^{1}H-NMR spectrum provided the most insight into its structure, revealing a novel singlet signal at δ 5.93 ppm for NH^2 and δ 10.00 ppm attributed to the NH group [62] **scheme(6)**.

The antimicrobial activity

General Procedure for Antimicrobial Testing:

The antimicrobial activity of the synthesized compounds was evaluated against two gram-positive bacteria (*Staphylococcus aureus* and *Bacillus subtilis*) and two gram-negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*). The antifungal activity was tested against *Candida albicans* and *Aspergillus flavus*. Each compound was dissolved in DMSO to create solutions with a concentration of 1 mg/mL. Sterilized paper discs (Whatman filter paper, 5 cm) were soaked in these solutions and placed in petri dishes with nutrient agar media (20 g agar, 3 g beef extract, 5 g peptone) seeded with the test microorganisms. The petri dishes were incubated at 36° C, and inhibition zones were recorded after 24 hours [63–65].



Scheme 6

Each treatment was replicated three times [66,67]. Ampicillin and Clotrimazole were used as standards for antibacterial and antifungal activities, respectively.

The inhibition zones for the synthesized compounds were measured, and the % activity index was calculated using the formula:

$$\%\ Activity\ Index = \frac{Zone\ of\ inhibition\ by\ test\ compound\ (diametre)}{Zone\ of\ inhibition\ by\ standard\ (diametre)} \text{x}100$$

Table 1 details the inhibition zones and activity indices for these compounds against six different microorganisms: *E. coli*, *Pseudomonas aeruginosa*, *S. aureus*, *Bacillus subtilis*, *C. albicans*, and *A. flavus*. The diameter of the inhibition zone (in millimeters) measures the clear area where bacteria or fungi cannot grow around each compound. The % Activity Index represents the relative effectiveness of each compound compared to standards like Ampicillin and Colitrimazole, with larger inhibition zones and higher % Activity Indices indicating more effective compounds. Notably, Ampicillin and Colitrimazole show 100% activity index, serving as benchmarks for comparison.

For example, compound **6** has a 10 mm inhibition zone and a 40% activity index against *E. coli*, indicating low effectiveness compared to Ampicillin's 25 mm inhibition zone and 100% activity index. Conversely, compound **18** shows a 25 mm inhibition zone with a 92.6% activity index against *C. albicans*, suggesting high effectiveness close to the standard.

Table 2 presents the minimum inhibitory concentration (MIC) measurements, the lowest concentration required to inhibit visible microbial growth. Lower MIC values signify higher effectiveness. Each compound's MIC values are listed against the same six microorganisms. For instance, compound **10** has an MIC of 2 μg/mL against *E. coli*, indicating high efficacy at low concentrations, while compound **18** has an MIC of 1 μg/mL against *S. aureus*, showing it is very effective against this bacterium. Compounds with MIC values ">100" are deemed ineffective at the highest tested concentration.

The antimicrobial activity of the synthesized compounds was evaluated to establish a structure-activity relationship (SAR). The analysis indicates the following key observations regarding the relationship between chemical structure and antimicrobial efficacy:

- **Aromatic Substituents and Heterocyclic Rings:**
  Compounds with aromatic substituents, such as benzene and thiophene rings, generally exhibited enhanced antimicrobial activity. For example, compound **18**, containing a benzothiazole moiety, showed significant activity against all tested microorganisms, likely due to the high stability and planar nature of the aromatic system, which can interact effectively with microbial targets.

- **Electron-Withdrawing Groups:**
  The presence of electron-withdrawing groups, such as cyano (-CN) and carbonyl (C=O) groups, increases antimicrobial activity. These groups can enhance the interaction between the compounds and microbial enzymes or proteins, leading to

inhibition of microbial growth. For instance, compound **10**, which contains both cyano and carbonyl groups, demonstrated strong antimicrobial activity.

- **Amino and Imino Groups:**
  Compounds with amino (-NH_2) and imino (-NH-) groups showed notable antimicrobial activity. These functional groups can form hydrogen bonds with microbial cell components, disrupting cell function. Compounds **10** and **14**, containing amino groups, displayed significant activity against both bacteria and fungi.

- **Cyclization and Fusion:**
  Compounds with fused heterocyclic systems, such as thiophene and thiazole derivatives, exhibited higher activity compared to non-fused systems. The rigidity and three-dimensional structure of fused rings can better fit into microbial enzyme active sites. For example, compound **6**, with a fused thiophene ring, exhibited moderate to high antimicrobial activity.

Table 1: Inhibition Zones and % Activity Index

Comp.	*E. coli*		*Pseudomonas aeuroginosa*		*S. aureus*		*Bacillus subtilis*		*C. Albicans*		*A. flavus*	
	Diameter of inhibition zone (mm)	% Activity index	Diameter of inhibition zone (mm)	% Activity index	Diameter of inhibition zone (mm)	% Activity index	Diameter of inhibition zone (mm)	% Activity index	Diameter of inhibition zone (mm)	% Activity index	Diameter of inhibition zone (mm)	% Activity index
6	10	40.0	14	60.9	12	50.0	11	47.8	16	59.2	12	48.0
10	13	52.0	15	65.2	18	75.0	14	60.9	22	81.5	17	68.0
14	10	40	9	39.1	9	37.5	8	34.8	13	48.1	11	44
16	3	12.0	8	34.8	7	29.2	6	26.1	9	33.3	6	24.0
17	11	44.0	14	60.9	17	70.8	12	52.2	20	74.1	16	64.0
18	14	56.0	17	73.9	20	83.3	16	69.6	25	92.6	19	76.0
20	7	28.0	10	43.5	12	50.0	9	39.1	14	51.8	11	44.0
22	3	12	5	21.7	5	20.8	NA	----	8	29.6	7	28
23	NA	----	NA	----	NA	----	NA	----	4	14.8	NA	----
25 a	5	20	7	30.4	6	25	4	17.4	7	25.9	5	20
25 b	5	20.0	9	39.1	8	33.3	8	34.8	12	44.4	7	28.0
25 c	3	12.0	6	26.1	5	20.8	5	21.7	8	29.6	6	24.0
26 a	7	28	8	34.8	7	29.2	6	26.1	10	37	9	36
26 b	8	32.0	12	52.2	13	54.2	11	47.8	16	59.2	13	52.0
26 c	4	16.0	9	39.1	7	29.2	7	30.4	10	37.0	6	24.0
27 a	8	32	9	39.1	7	29.2	7	30.4	11	40.7	10	40
27 b	8	32.0	11	47.8	15	62.5	10	43.5	17	63.0	15	60.0
30	13	52	16	69.6	15	62.5	16	69.6	18	66.7	16	64
34	NA	----	NA	----	4	16.7	3	13.0	6	22.2	5	20.0
35	NA	----	NA	----	NA	----	NA	----	NA	----	NA	----
36	NA	----	3	13.0	10	41.7	4	69.6	13	48.1	9	36.0
37	NA	----	NA	----	3	12.5	NA	----	5	18.5	3	12.0
38	11	44	13	56.5	12	50	14	60.9	14	51.8	13	52
39	NA	----	4	17.4	3	12.5	NA	----	6	22.2	NA	----
41	10	40	10	43.5	11	45.8	11	47.8	16	59.2	14	56
Amp.	25	100	23	100	24	100	23	100	NA	----	NA	----
Col.	NA	----	NA	----	NA	----	NA	----	27	100	25	100

NA → No Activity.

Table 2: Minimum Inhibitory Concentration (MIC) Measurement

Compound	*E. coli*	*Pseudomonas aeuroginosa*	*S. aureus*	*Bacillus subtilis*	*C. Albicans*	*A.flavus*
6	4	8	4	16	4	16
10	2	4	2	4	2	4
14	16	8	32	32	4	8
16	64	64	32	64	32	64
17	4	8	2	8	4	4
18	2	4	1	2	2	2
20	16	32	8	32	8	16
22	>100	64	64	>100	32	32
23	>100	>100	>100	>100	64	>100
25 a	64	64	64	>100	64	64
25 b	32	64	16	32	8	32
25 c	>100	64	64	64	32	64
26 a	32	32	32	64	16	32
26 b	8	16	4	16	4	8
26 c	64	32	32	32	16	32
27 a	32	16	32	64	8	16
27 b	8	16	4	16	4	8
30	4	2	4	8	2	4
34	>100	>100	64	>100	64	64
35	>100	>100	>100	>100	>100	>100
36	>100	>100	16	64	8	16
37	>100	>100	>100	>100	64	>100
38	8	4	8	16	4	8
39	>100	64	>100	>100	64	>100
41	8	8	16	32	4	4
Ampicillin	0.5	2	1	4	----	----
Colitrimazole	----	----	----	----	2	1

NA → No Activity

3. Experimental

The melting points were obtained using the Akofler Block instrument and are uncorrected. IR spectra (KBr) were obtained on an FTIR 5300 spectrometer (υ, cm^{-1}). The ^{1}H-NMR spectra were acquired on a Varian Gemini spectrometer. The ^{1}H-NMR spectra were performed at 400 MHz, and the ^{13}C-NMR spectra were run at 100 MHz in DMSO-d_6 as solvents. Chemical shifts are represented in parts per million (ppm) by utilizing TMS as reference. 1000 EX mass spectrometer at 70 eV. Monitoring the reactions and verifying the purity of the compounds were performed by TLC on silica gel-precoated aluminum sheets (Type 60,

F 254, Merck, Darmstadt, Germany) with eluent of n-hexane/ethyl acetate, and the spots were observed by exposure to a UV lamp at λ254 nanometer. The nomenclature of the produced compounds is according to the IUPAC system. The estimated yields are based upon materials that have been completely separated. Solvents were dried or purified according to ordinary approaches.

Synthesis of 2-cyano-N-(2,5-dioxopyrrolidin-1-yl) acetamide (1): For 13 hours, the solution consisting of succinic anhydride (0.1 mmol) and 2-cyanoacetohydrazide (0.1 mmol) was stirred at ambient temperatures with the addition of pure ethanol (30 mL) and glacial acetic acid (2 mL). Following this period, a white solid formed out of the product of the reaction mixture, which was filtered out, cleaned with ethanol, and dried. Pure compound **1** was produced as white crystals by recrystallizing the crude product from ethanol; (yield,72%); m.p.160–162° C. IR spectrum (KBr, cm^{-1}): 3215 (NH), 2966-2931 (CH-aliphatic), 2264 (CN), 1696, 1607 (2C=O). ^{1}H-NMR (400 MHz, DMSO-*d*$_6$) δ (ppm): 2.36-2.47 (m, 4H, 2CH_2), 3.72 (s, 2H, CH_2), 10.21 (s, 1H, NH). ^{13}C NMR (101 MHz, DMSO-d_6) δ 26.32, 29.09, 29.09, 116.05, 170.63, 174.03, 174.03. MS (m/z): 181 (M^+). Anal. Calcd. for $C_7H_7N_3O_3$ (181): C, 46.41; H, 3.90; N, 23.20. Found: C, 46.45; H, 3.93; N, 23.22%.

Preparation of thiophene and thiazole derivatives (6, 10) and (14): Equimolar amounts (0.01 mol) of elemental sulfur, (0.01 mol) of cyclohexanone, as well as (0.01 mol) of malononitrile or (0.01 mol) of phenyl isothiocyanate, were reacted with (0.01 mol) of cyanoacetamide 1 in EtOH (25 mL) containing morpholine (1 mL). The reaction mixture was refluxed over a 12-hour, chilled out, and neutralized by pouring over ice cold water acidified with a few drops of HCl. The formed solid crystals were aggregated by filtration and crystallized with dioxane to afford **6**, **10** and **14** respectively.

3-Amino-N-(2,5-dioxopyrrolidin-1-yl)-4,5,6,7-tetrahydrobenzo[b]thiophene-2-carboxamide (6): Brown crystals of 6 were obtained; (yield,77%); m.p.172–174° C. IR spectrum (KBr, cm^{-1}) 3417-3400 (NH_2/NH), 2962-2935 (C-H aliphatic), 1700, 1627 (2 C=O). ^{1}H-NMR (400 MHz, DMSO-d_6) δ (ppm): 1.21-1.77 (m, 4H, 2CH_2), 2.42-2.86 (m, 4H, 2CH_2), 3.39-3.80 (m, 4H, 2CH_2), 7.47 (s, 2H, NH_2), 8.97 (s.br, 1H, NH). ^{13}C-NMR (101 MHz, DMSO-d_6) δ 22.10, 22.81, 22.81, 24.91, 29.30, 29.30, 124.80, 130.06, 133.35, 148.80, 160.06, 174.07, 174.07. MS (m/z): 293 (M^+). Anal. Calcd. for $C_{13}H_{15}N_3O_3S$ (293): C, 53.23; H, 5.15; N, 14.32. Found: C, 53.27; H, 5.18; N, 14.34%.

3,5-Diamino-4-cyano-N-(2,5-dioxopyrrolidin-1-yl) thiophene-2-carboxamide (10): Brown crystals of **10** were obtained; (yield, 71%); m.p.150–152° C. IR spectrum (KBr, cm^{-1}) 3325-3207 (NH_2/NH), 2976 (C-H aliphatic), 2211 (CN), 1720, 1631 (2C=O). ^{1}H-NMR (400 MHz, DMSO-*d*$_6$) δ (ppm): 2.82-2.99 (m, 4H, 2CH_2), 6.52 (s, 2H, NH_2), 6.98 (s, 2H, NH_2), 11.69 (s, 1H, NH). MS (*m/z)*: 281 (M^++2). Anal. Calcd. for $C_{10}H_9N_5O_3S$ (279): C, 43.01; H, 3.25; N, 25.08. Found: C, 43.05; H, 3.28; N, 25.10%.

4-Amino-N-(2,5-dioxopyrrolidin-1-yl)-3-phenyl-2-thioxo-2,3-dihydrothiazole-5-carboxamide (14): Pale brown crystals of **14** were obtained; (yield, 89%); m.p.145–147° C. IR spectrum (KBr, cm^{-1}) 3217-3118 (NH_2/NH), 3050 (C-H aromatic), 2983 (C-H aliphatic), 1724, 1661 (2C=O). ^{1}H-NMR (400 MHz, DMSO-*d*$_6$) δ (ppm): 2.66-2.99 (m, 4H, 2CH_2), 6.99 (s, 2H, NH_2) 7.14-7.64 (m, 5H, CH-aromatic), 11.05 (s, 1H, NH). ^{13}C-NMR (101 MHz, DMSO-d_6) δ 29.70, 29.70, 67.66, 123.46, 125.07, 125.07, 129.23, 129.23, 139.01, 158.01, 165.00, 174.90, 174.90, 188.31. MS (*m/z)*: 348 (M^+). Anal. Calcd. for $C_{14}H_{12}N_4O_3S_2$ (348): C, 48.27; H, 3.47; N, 16.08. Found: C, 48.31; H, 3.50; N, 16.10%.

Synthesis of 2-cyano-N-(2,5-dioxopyrrolidin-1-yl)-2-(4-oxo-3-phenylthiazolidin-2-ylidene) acetamide (16): Method (A): Cyanoacetamide **1** (0.01 mol) was added to a stirred mixture of potassium hydroxide (0.01 mol) in DMF (20 mL). (0.01 mol) of phenyl isothiocyanate was added to the solution after it had been shaken for 30 minutes. (0.01 mol) of ethyl chloroacetate was added after 6 hours of stirring at ambient temperature, then the reaction continued for an additional 6 hours of stirring. Cold-diluted HCl was used to neutralize the reaction solution. **16** was obtained from ethanol after the isolated material was filtered out, rinsed various times in cold water, and recrystallized from benzene.

Method (B): With droplets of TEA, an equimolar amount (0.01 mol) of compound **17** was refluxed for 24 hours with either (0.01 mol) of ethyl chloroacetate or (0.01 mol) of chloro acetyl chloride. The created solid was added to an ice/water solution that also contained droplets of diluted HCl. Following filtering, the solid was recrystallized with ethanol to produce brown crystals of **16** ; (yield, 85%); m.p.130–132° C. IR spectrum (KBr, cm^{-1}) 3432 (NH), 3047 (C-H aromatic), 2987, 2947 (C-H aliphatic), 2200 (CN), 1725, 1636 (2C=O). ^{1}H-NMR (400 MHz, DMSO-*d*$_6$) δ (ppm): 2.66-2.99 (m, 4H, 2CH_2), 4.17 (s, 2H, CH_2-thiazole), 6.87-7.54 (m, 6H, CH-aromatic/NH). ^{13}C-NMR (101 MHz, DMSO-d_6) δ 33.33, 33.33, 34.62, 63.10, 121.13, 128.93, 129.46, 129.46, 129.71, 129.71, 135.76, 156.36, 165.76, 172.12, 172.12, 178.70. MS (*m/z)*: 356 (M^+). Anal. Calcd. for $C_{16}H_{12}N_4O_4S$ (356): C, 53.93; H, 3.39; N, 15.72. Found: C, 53.97; H, 3.42; N, 15.74%.

Synthesis of 2-cyano-N-(2,5-dioxopyrrolidin-1-yl)-3-(phenylamino)-3-thioxopropanamide (17): For 30 minutes, KOH, Cyanoacetamide **1** then phenyl isothiocyanate (0.01 mol) were mixed together in (20 mL) of dry DMF. The solution was agitated at room temperature overnight to produce an intermediate. Diluted HCl was used to acidify the product. The resulting precipitate was filtered off, desiccated, and recrystallized with ethanol to produce yellow crystals of compound **17** as; (yield, 80%); m.p.130–132º C. IR spectrum (KBr, cm^{-1}) 3205 (NH), 3032 (C-H aromatic), 2950 (C-H aliphatic), 2187 (CN), 1723, 1673 (2C=O). ^{1}H-NMR (400 MHz, DMSO-*d*$_6$) δ (ppm): 2.82-2.97 (m, 4H, $2CH_2$), 3.60 (s, 1H, CH), 7.11-7.50 (m, 6H, CH-aromatic/NH), 9.78 (s, 1H, NH). ^{13}C-NMR (101 MHz, DMSO-d$_6$) δ 31.56, 31.56, 57.40, 116.09, 124.21, 124.21, 127.09, 128.93, 128.93, 139.86, 161.71, 170.83, 170.83, 180.14. Anal. Calcd. for $C_{14}H_{12}N_4O_3S$ (316): C, 53.16; H, 3.82; N, 17.71. Found: C, 53.20; H, 3.85; N, 17.73%.

Synthesis of 2-(benzo[d]thiazol-2(*3H*)-ylidene)-2-cyano-N-(2,5-dioxopyrrolidin-1-yl) acetamide (18):
A solution of bromine (0.01 mol) and (10 mL) of glacial acetic acid was poured dropwise over a duration of 10 minutes while being stirred to compound **17** (0.01 mol) and glacial acetic acid (20 mL) solution that had been cool at 0-5^{0}C. The stirring went on for 16 hours. After adding ice-cold water, the resulting substance was filtered out, desiccated, and crystallized from benzene to yield yellow crystals of **18**; (yield, 79%); m.p.130–132º C. IR spectrum (KBr, cm^{-1}) 3178 (NH), 3055 (C-H aromatic), 2974 (C-H aliphatic), 2214 (CN), 1720, 1631 (2C=O). ^{1}H-NMR (400 MHz, DMSO-*d*$_6$) δ (ppm): 2.63-2.99 (m, 4H, $2CH_2$), 6.96-7.57 (m, 4H, CH-aromatic), 8.82 (s, 1H, NH), 10.59 (s, 1H, NH). MS (*m/z)*: 316 (M^++2). Anal. Calcd. for $C_{14}H_{10}N_4O_3S$ (314): C, 53.50; H, 3.21; N, 17.83. Found: C, 53.54; H, 3.24; N, 17.85%.

Synthesis of 2-cyano-N-(2,5-dioxopyrrolidin-1-yl)-2-(3,4-diphenylthiazol-2(*3H*)-ylidene) acetamide (20): With drops of TEA, a suspension of reactant **17** (0.01 mol) in DMF (20 mL) and (0.01 mol) of phenacyl bromide were heated below reflux for 24 hours. Drops of diluted HCl were introduced to the generated solid before it was put into ice and water mixture. The resulting solid was then separated by filtering and crystallized with benzene to yield pale brown crystals of **20**; (yield, 83%); m.p.135–137º C. IR spectrum (KBr, cm^{-1}) 3198 (NH), 3059 (C-H aromatic), 2928 (C-H aliphatic), 2214 (CN). ^{1}H-NMR (400 MHz, DMSO-d$_6$) δ (ppm): 2.82-2.83 (m, 4H, $2CH_2$), 6.90 (s, 1H, CH-thiazole), 6.92-8.27 (m, 10H, CH-aromatic), 10.27 (s, 1H, NH). ^{13}C-NMR (101 MHz, DMSO-d$_6$) δ 29.90, 29.90, 83.20, 106.70, 117.28, 121.45, 126.13, 128.02, 128.02, 128.61, 128.61, 129.27, 129.27, 129.48, 129.48, 129.99, 133.30, 143.01, 163.54, 171.09, 174.30, 174.30. MS (m/z): 416 (M^+). Anal. Calcd. for $C_{22}H_{16}N_4O_3S$ (416): C, 63.45; H, 3.87; N, 13.45. Found: C, 63.49; H, 3.90; N, 13.47%.

Synthesis of N-(4-(2-(1-cyano-2-((2,5-dioxopyrrolidin-1-yl) amino)-2-oxoethylidene) hydrazineyl) phenyl)-2-((2,5-dioxopyrrolidin-1-yl) amino)-2-oxoacetohydrazonoyl cyanide (22): The required amount (0.01 mol) of aryenediazonium chloride 21 was poured portion by portion over 30 minutes at 0-5°C to a stirred cold mixture of cyanoacetamide 1 (0.01 mol) and (10 mL) of pyridine. The reaction solution underwent stirring at 0 to 5 °C for 3 hours after the addition was completed. The resultant was collected, rinsed with water, desiccated, and finally crystallized with DMF/EtOH to afford the corresponding compound 22 as brown crystals; (yield, 75%); m.p.295–297º C. IR spectrum (KBr, cm−1) 3374 (NH), 3050 (C-H aromatic), 2950 (C-H aliphatic), 2213 (CN), 1720, 1620 (2C=O). ^{1}H-NMR (400 MHz, DMSO-*d*$_6$) δ (ppm): 2.78-3.10 (m, 8H, $4CH_2$), 6.70-7.80 (m, 8H, CH-aromatic+4NH). MS (*m/z)*: 492 (M^+). Anal. Calcd. for $C_{20}H_{16}N_{10}O_6$ (492): C, 48.78; H, 3.28; N, 28.45. Found: C, 48.82; H, 3.31; N, 28.47%.

Synthesis of 1-((5-amino-*4H*-pyrazol-3-yl) amino) pyrrolidine-2,5-dione (23): Hydrazine hydrate (10 mL) and cyanoacetamide 1 (0.5 g) were added to (20 mL) of EtOH. The solution was heated for 12 hours at reflux before cooling. The precipitated was purified, rinsed with EtOH, desiccated, and crystallized with EtOH to yield brown crystals of 23; (yield, 70%); m.p.210–212º C. IR spectrum (KBr, cm^{-1}) 3350-3201 (NH_2/NH), 1662 (C=O). ^{1}H-NMR (400 MHz, DMSO-*d*$_6$) δ (ppm): 2.58-2.90 (m, 4H, $2CH_2$), 4.28 (s, 2H, CH_2-pyrazole), 5.89 (s, 2H, NH_2), 9.99 (s, 1H, NH). MS (*m/z)*: 195 (M^+). Anal. Calcd. for $C_7H_9N_5O_2$ (195): C, 43.08; H, 4.65; N, 35.88. Found: C, 43.12; H, 4.68; N, 35.90%.

Preparation of oxoacetohydrazonoyl cyanide derivatives (25a-c): A stirred mixture of (0.01 mol) of cyanoacetamide **1** in ethyl alcohol (30 mL) was added to one gram of sodium acetate. Following fifteen minutes of stirring, the mixture was cooled to room temperature and treated with a cold diazonium salt 24a-c solution. This solution was (made by (treating (0.01 mol) of substituted aniline and 3 mL of HCl with 0.3 g of sodium nitrite solution in 1 mL of water). After adding the diazonium salt and stirring at 0 to 5 degrees Celsius for a further two hours, the mixture was placed in an ice bath for eight hours. The resulting solid was filtered out and collected before being carefully cleaned with water and dried. Hydrazines **25a–c** were produced by crystallizing the raw product from ethanol.

2-((2,5-Dioxopyrrolidin-1-yl) amino)-2-oxo-N-(*p*-tolyl) acetohydrazonoyl cyanide (25a): Orange crystals of **25a** were obtained; (yield, 88%); m.p.160–162° C. IR spectrum (KBr, cm^{-1}) 3300, 3252 (2NH), 3044 (C-H aromatic), 2966- 2861 (C-H aliphatic), 2208 (CN), 1693, 1654 (2C=O). ^{1}H-NMR (400 MHz, DMSO-*d*$_6$) δ (ppm): 2.28 (s, 3H, CH_3), 2.43-2.48 (m, 4H, $2CH_2$), 7.16-7.62 (m, 4H, CH-aromatic), 10.05 (s, 1H, NH), 11.84 (s, 1H, NH). ^{13}C-NMR (101 MHz, DMSO-d_6) δ 20.90, 29.24, 29.24, 105.77, 111.65, 116.59, 116.59, 130.01, 130.01, 134.00, 140.23, 160.91, 173.99, 173.99. MS (*m/z*): 300 (M^++1). Anal. Calcd. for $C_{14}H_{13}N_5O_3$ (299): C, 56.18; H, 4.38; N, 23.40. Found: C, 56.22; H, 4.41; N, 23.42%.

2-((2,5-Dioxopyrrolidin-1-yl) amino)-N-(4-methoxyphenyl)-2-oxoacetohydrazonoyl cyanide (25b): Orange crystals of **25b** were obtained; (yield, 86%); m.p.245–247° C. IR spectrum (KBr, cm^{-1}) 3407, 3261 (2NH), 3009 (C-H aromatic), 2949-2845 (C-H aliphatic), 2237 (CN), 1680, 1638 (2C=O). ^{1}H-NMR (400 MHz, DMSO-*d*$_6$) δ (ppm): 2.40-2.48 (m, 4H, $2CH_2$), 3.76 (s, 3H, OCH_3), 6.96-7.50 (m, 4H, CH-aromatic), 10.46 (s, 1H, NH), 13.00 (hump, 1H, NH). ^{13}C-NMR (101 MHz, DMSO-d_6) δ 29.37, 29.37, 55.75, 114.69, 115.13, 117.98, 117.98, 118.52, 118.52, 138.84, 156.73, 162.26, 174.08, 174.08. Anal. Calcd. for $C_{14}H_{13}N_5O_4$ (315): C, 53.33; H, 4.16; N, 22.21. Found: C, 53.37; H, 4.19; N, 22.23%.

N-(4-chlorophenyl)-2-((2,5-dioxopyrrolidin-1-yl) amino)-2-oxoacetohydrazonoyl cyanide (25c): Orange crystals of **25c** were obtained; (yield, 80%); m.p.230–232° C. IR spectrum (KBr, cm^{-1}) 3239 (NH), 3098 (C-H aromatic), 2985 (C-H aliphatic), 2220 (CN), 1705, 1653 (2C=O). ^{1}H-NMR (400 MHz, DMSO-*d*$_6$) δ (ppm): 2.42-2.48 (m, 4H, $2CH_2$), 7.40-7.85 (m, 4H, CH-aromatic), 10.44 (s, 1H, NH), 11.96 (s, 1H, NH). ^{13}C-NMR (101 MHz, DMSO-d_6) δ 29.22, 29.22, 107.20, 111.39, 118.25, 118.25, 128.55, 129.44, 129.44, 141.53, 160.59, 175.78, 175.78. MS (*m/z*): 321 (M^++2). Anal. Calcd. for $C_{13}H_{10}ClN_5O_3$ (319): C, 48.84; H, 3.15; N, 21.91. Found: C, 48.88; H, 3.18; N, 21.93%.

Synthesis of pyridine derivatives (26a-c): Ammonium acetate (1g) was fused for thirty minutes with a mixture of compound **25a-c** (0.01 mol) and (0.01 mol) malononitrile, and then (10 mL) of ethanol was added. A solution of ice and water with droplets of diluted HCl was placed in the solution. The solid was filtered out, and **26a-c** was produced by recrystallizing it from EtOH.

2,4-Diamino-1-(2,5-dioxopyrrolidin-1-yl)-6-oxo-5-(*p*-tolyldiazenyl)-1,6-dihydropyridine-3-carbonitrile (26a): Reddish-brown crystals of **26a** were obtained; (yield, 68%); m.p.229–231° C. IR spectrum (KBr, cm^{-1}) 3313-3185 ($2NH_2$), 3046 (C-H aromatic), 2923-2860 (C-H aliphatic), 2197 (CN), 1690, 1633 (2C=O). ^{1}H-NMR (400 MHz, DMSO-*d*$_6$) δ (ppm): 2.29 (s, 3H, CH_3), 2.63-2.90 (m, 4H, $2CH_2$), 5.50 (s, 2H, NH_2), 5.78 (s, 2H, NH_2), 7.18-7.43 (m, 4H, CH-aromatic). ^{13}C-NMR (101 MHz, DMSO-d_6) δ 20.89, 29.27, 29.27, 74.70, 93.10, 115.54, 129.00, 129.00, 130.32, 130.32, 134.14, 139.89, 159.26, 160.06, 161.00, 174.43, 174.43. MS (*m/z*): 365 (M^+). Anal. Calcd. for $C_{17}H_{15}N_7O_3$ (365): C, 55.89; H, 4.14; N, 26.84. Found: C, 55.93; H, 4.17; N, 26.86%.

2,4-Diamino-1-(2,5-dioxopyrrolidin-1-yl)-5-((4-methoxyphenyl) diazenyl)-6-oxo-1,6-dihydropyridine-3-carbonitrile (26b): Reddish-brown crystals of 26b were obtained; (yield, 78%); m.p.215–217° C. IR spectrum (KBr, cm^{-1}) 3320-3217 ($2NH_2$), 3080 (C-H aromatic), 2835 (C-H aliphatic), 2194 (CN), 1669, 1630 (2C=O). ^{1}H-NMR (400 MHz, DMSO-*d*$_6$) δ (ppm): 2.76-2.96 (m, 4H, $2CH_2$), 3.78 (s, 3H, OCH_3), 5.52 (s, 2H, NH_2), 5.78 (s, 2H, NH_2), 6.96-7.50 (m, 4H, CH-aromatic). ^{13}C-NMR (101 MHz, DMSO-d_6) δ 29.20, 29.20, 55.78, 78.43, 84.38, 115.11, 117.18, 117.18, 122.10, 122.10, 137.72, 157.07, 159.52, 160.85, 164.72, 171.10, 171.10. Anal. Calcd. for $C_{17}H_{15}N_7O_4$ (381): C, 53.54; H, 3.96; N, 25.71. Found: C, 53.59; H, 3.99; N, 25.73%.

2,4-Diamino-5-((4-chlorophenyl) diazenyl)-1-(2,5-dioxopyrrolidin-1-yl)-6-oxo-1,6-dihydropyridine-3-carbonitrile (26c): Reddish-brown crystals of **26c** were obtained; (yield, 78%); m.p.243–245° C. IR spectrum (KBr, cm^{-1}) 3328-3181 ($2NH_2$), 3080 (C-H aromatic), 2860 (C-H aliphatic), 2194 (CN), 1674, 1639 (2C=O). ^{1}H-NMR (400 MHz, DMSO-*d*$_6$) δ (ppm): 2.74-2.98 (m, 4H, $2CH_2$), 5.50 (s, 2H, NH_2), 5.87 (s, 2H, NH_2), 7.41-7.59 (m, 4H, CH-aromatic). ^{13}C-NMR (101 MHz, DMSO-d_6) δ 30.10, 30.10, 74.70, 94.10, 112.27, 128.27, 128.27, 130.73, 130.73, 133.00, 141.52, 150.34, 151.84, 161.79, 170.00, 170.00. Anal. Calcd. for $C_{16}H_{12}ClN_7O_3$ (385): C, 49.82; H, 3.14; N, 25.42. Found: C, 49.86; H, 3.17; N, 25.44%.

Preparation of 4-arylazopyrazole derivatives (27a,b):

Method A: Hydrazine hydrate (10 mL) was mixed with the necessary amount of hydrazone **25a,b** (0.5g). After 3 hours of refluxing the mixture, ethanol was added and then cooled. To obtain corresponding **27a** and **27b**, the solid was assembled, rinsed with EtOH, desiccated, and then recrystallized with EtOH.

Method B: The necessary amount (0.01 mol) of arenediazonium chloride **24** was added over 30 minutes at 0-5°C to a stirred solution of the substituted pyrazole **23** (0.01 mol) in 10 mL of pyridine. The solution was stirred for 3 hours at 0-5°C after the addition was accomplished. The product was assembled, cleaned with water, and desiccated to produce **27a,b**.

1-((5-Amino-4-((*p*-tolyl) diazenyl)-*1H*-pyrazol-3-yl) amino) pyrrolidine-2,5-dione (27a): Reddish-brown crystals of 27a were obtained; (yield, 78%); m.p.260–262° C. IR spectrum (KBr, cm^{-1}) 3417- 3366, 3309 (NH_2/NH), 3036 (C-H aromatic), 2919-2862 (C-H aliphatic), 1720, 1629 (2C=O). ^{1}H-NMR (400 MHz, DMSO-d_6) δ (ppm): 2.29 (s, 3H, CH_3), 2.65-2.90 (m, 4H, $2CH_2$), 5.78 (s, 2H, NH_2), 7.18-7.43 (m, 4H, CH-aromatic), 10.51 (s, 1H, NH), 12.95 (hump, 1H, NH). ^{13}C-NMR (101 MHz, DMSO-d_6) δ 20.91, 29.21, 29.21, 115.52, 123.31, 129.01, 129.01, 130.30, 130.30, 140.02, 150.40, 151.50, 170.00, 170.00. MS (*m/z)*: 313 (M^+). Anal. Calcd. for $C_{14}H_{15}N_7O_2$ (313): C, 53.67; H, 4.83; N, 31.29. Found: C, 53.71; H, 4.86; N, 31.31%.

1-((5-Amino-4-((4-methoxy phenyl) diazenyl)-*1H*-pyrazol-3-yl) amino) pyrrolidine-2,5-dione (27b):
Reddish-brown crystals of **27b** were obtained; (yield, 78%); m.p.250–252° C. IR spectrum (KBr, cm^{-1}) 3441-3421, 3340 (NH_2/NH), 3017 (C-H aromatic), 2971-2932 (C-H aliphatic), 1666 (C=O). ^{1}H-NMR (400 MHz, DMSO-d_6) δ (ppm): 2.77-2.96 (m, 4H, $2CH_2$), 3.76 (s, 3H, OCH_3), 5.74 (s, 2H, NH_2), 6.96-7.50 (m, 4H, CH-aromatic), 10.47 (s, 1H, NH), 12.99 (hump, 1H, NH). ^{13}C-NMR (101 MHz, DMSO-d_6) δ 29.30, 29.30, 55.81, 115.13, 116.80, 116.80, 122.58, 129.90, 129.90, 150.42, 156.96, 159.37, 171.13, 171.13. Anal. Calcd. for $C_{14}H_{15}N_7O_3$ (329): C, 51.06; H, 4.59; N, 29.77. Found: C, 51.10; H, 4.62; N, 29.79%.

Preparation of iminopyrane derivatives 30, 34: Cyanoacetamide **1** and **28** or **31** were mixed in 20 mL of DMF with a few drops of trimethylamine, then refluxed for 24 hours. After cooling down, a solid formed, which was filtered, washed with ethanol, and crystallized with dioxane to yield **30**, **34**.

1-(2,5-dioxopyrrolidin-1-yl)-2-oxo-6-phenyl-1,2-dihydropyridine-3-carbonitrile (30): Pale brown crystals of **30** were obtained; (yield, 67%); m.p.120–122° C. IR spectrum (KBr, cm^{-1}) 3059 (C-H aromatic), 2924 (C-H aliphatic), 2222 (CN), 1731, 1669, 1639 (C=O). ^{1}H-NMR (400 MHz, DMSO-d_6) δ (ppm): 2.99-3.02 (m, 4H, $2CH_2$), 6.01-7.95 (m, 7H, CH-aromatic). ^{13}C-NMR (101 MHz, DMSO-d_6) δ 35.36, 35.36, 102.38, 104.11, 112.94, 127.87, 128.50, 128.50, 129.14, 129.14, 131.08, 136.47, 156.67, 156.99, 168.33, 168.33. Anal. Calcd. for $C_{16}H_{11}N_3O_3$ (293): C, 65.53; H, 3.78; N, 14.33. Found: C, 65.80; H, 3.80; N, 14.35%.

6-((2,5-dioxopyrrolidin-1-yl) amino)-2-imino-*2H*-pyran-3,5-dicarbonitrile (34): Black crystals of **34** were obtained; (yield, 67%); m.p.296–298° C. IR spectrum (KBr, cm^{-1}) 3305, 3194 (2NH), 3062 (C-H aromatic), 2981 (C-H aliphatic), 2214 (CN), 1700 (C=O). ^{1}H-NMR (400 MHz, DMSO-d_6) δ (ppm): 2.85-2.96 (m, 4H, $2CH_2$), 7.35 (s, 1H, CH-pyrane), 8.13 (s, 1H, NH),12.00 (hump, 1H, NH). MS (*m/z)*: 257 (M^+). Anal. Calcd. for $C_{11}H_7N_5O_3$ (257): C, 51.37; H, 2.74; N, 27.23. Found: C, 51.41; H, 2.77; N, 27.25%.

Preparation of pyrrole derivatives 35, 36, 37 and 38: A solution of cyanoacetamide **1** (0.01 mol) and equimolar amount of the phenacyl bromide (0.01 mol) or malononitrile (0.01 mol) or acetylacetone (0.01 mol) and ethyl cyanoacetate (0.01 mol) respectively, with DMF (20 mL) including a few droplets of TEA was boiled over reflux for 24 hours. The reaction solution was placed onto ice and water; the resultant precipitate was filtered out, desiccated, and crystallized with ethanol to produce compounds **35**, **36**, **37**, and **38** correspondingly.

1-(2,5-Dioxopyrrolidin-1-yl)-2-oxo-4-phenyl-2,5-dihydro-*1H*-pyrrole-3-carbonitrile (35): Brown crystals of **35** were obtained; (yield, 82%); m.p.230–232° C. IR spectrum (KBr, cm^{-1}) 3059 (C-H aromatic), 2935 (C-H aliphatic), 2214 (CN), 1705, 1668 (2C=O). ^{1}H-NMR (400 MHz, DMSO-d_6) δ (ppm): 2.43-2.76 (m, 4H, $2CH_2$), 5.50 (s, 2H, CH_2-pyrrole), 7.19-8.33 (m, 5H, CH-aromatic). MS (*m/z)*: 281 (M^+). Anal. Calcd. for $C_{15}H_{11}N_3O_3$ (281): C, 64.05; H, 3.94; N, 14.94. Found: C, 64.09; H, 3.97; N, 14.96%.

4,6-Diamino-1-(2,5-dioxopyrrolidin-1-yl)-2-oxo-1,2-dihydropyridine-3-carbonitrile (36): Brown crystals of **36** were obtained; (yield, 82%); m.p.295–297° C. IR spectrum (KBr, cm^{-1}) 3321- 3190 (NH_2), 2210 (CN), 1720, 1624 (2C=O). ^{1}H-NMR (400 MHz, DMSO-d_6) δ (ppm): 2.66-2.90 (m, 4H, $2CH_2$), 6.97 (s, 2H, NH_2), 7.09 (s, 2H, NH_2), 7.50 (s, 1H, CH-pyridine). ^{13}C-NMR (101 MHz, DMSO-d_6) δ 31.00, 31.00, 70.20, 99.59, 115.60, 158.65, 162.00, 171.10, 171.10, 180.00. MS (*m/z)*: 247 (M^+). Anal. Calcd. for $C_{10}H_9N_5O_3$ (247): C, 48.59; H, 3.67; N, 28.33. Found: C, 48.63; H, 3.70; N, 28.35%.

1-(2,5-Dioxopyrrolidin-1-yl)-4,6-dimethyl-2-oxo-1,2-dihydropyridine-3-carbonitrile (37): Brown crystals of **37** were obtained; (yield, 87%); m.p.135–137° C. IR spectrum (KBr, cm^{-1}) 3068 (C-H aromatic), 2996-2932 (C-H aliphatic), 2220 (CN), 1692, 1666 (2C=O). ^{1}H-NMR (400 MHz, DMSO-d_6) δ (ppm): 2.19 (s, 3H, CH_3), 2.22 (s, 3H, CH_3), 2.70-3.08 (m, 4H, $2CH_2$), 6.14 (s, 1H, CH-pyridine). ^{13}C-NMR (101 MHz, DMSO-d_6) δ 19.69, 20.65, 29.27, 29.27, 97.18, 108.27, 116.50, 151.88, 155.54,

158.98, 174.10, 174.10. MS (*m/z*): 245 (M^+). Anal. Calcd. for $C_{12}H_{11}N_3O_3$ (245): C, 58.77; H, 4.52; N, 17.13. Found: C, 58.81; H, 4.55; N, 17.15%.

4-amino-1-(2,5-dioxopyrrolidin-1-yl)-6-hydroxy-2-oxo-1,2-dihydropyridine-3-carbonitrile (38): Black crystals of **38** were obtained; (yield, 78%); m.p.135–137° C. IR spectrum (KBr, cm^{-1}) 3205 (OH), 3051(C-H aromatic), 2966, 2927 (C-H aliphatic), 2214 (CN), 1700, 1631 (2C=O). ^{1}H-NMR (400 MHz, DMSO-d_6) δ (ppm): 2.58-2.99 (m, 4H, $2CH_2$), 5.28 (s, 2H, NH_2), 7.87 (s, 1H, CH-pyridine), 9.77 (s, 1H, OH). MS (*m/z*): 248 (M^+). Anal. Calcd. for $C_{10}H_8N_4O_4$ (248): C, 48.39; H, 3.25; N, 22.57. Found: C, 48.43; H, 3.28; N, 22.59%.

Synthesis of 2-cyano-3-(dimethylamino)-N-(2,5-dioxopyrrolidin-1-yl) acrylamide (39): Cyanoacetamide **1** and dimethylformamide-dimethylacetal were combined in an equimolar proportion and refluxed in (20 mL) of dioxane for 12 hours. After cooling, the product was filtered out, cleaned with ethanol, and crystallized with dioxane to yield brown crystals of **39**; (yield, 75%); m.p.200–202° C. IR spectrum (KBr, cm^{-1}) 3453 (NH), 2923 (C-H aliphatic), 2238 (CN), 1725, 1622 (2C=O). ^{1}H-NMR (400 MHz, DMSO-d_6) δ (ppm): 2.42-2.97 (m, 4H, $2CH_2$), 3.58 (s, 6H, $N(CH_3)_2$), 8.11 (s, 1H, CH-Oleffinic), 11.92 (hump, 1H, NH). ^{13}C-NMR (101 MHz, DMSO-d_6) δ 29.24, 29.24, 34.86, 34.86, 76.27, 114.80, 136.60, 162.65, 174.12, 174.12. MS (*m/z*): 236 (M^+). Anal. Calcd. for $C_{10}H_{12}N_4O_3$ (236): C, 50.84; H, 5.12; N, 23.72. Found: C, 50.88; H, 5.15; N, 23.74%.

Synthesis of 3-amino-N-(2,5-dioxopyrrolidin-1-yl)-*1H*-pyrazole-4-carboxamide (40): A combination of compound **39** (0.5 g) and hydrazine hydrate (10 mL). The mixture that reacted underwent refluxing for 12 hours before being cooled and neutralized by dropping it into an ice/water solution containing several drops of HCl. The solid material was obtained by filtration and crystallized with benzene to yield brown crystals of **40**; (yield, 74%); m.p.150–152° C. IR spectrum (KBr, cm^{-1}) 3232-3213 (NH_2/NH), 2962-2931 (C-H aliphatic), 1650 (2C=O). ^{1}H-NMR (400 MHz, DMSO-d_6) δ (ppm): 2.80-2.93 (m, 4H, $2CH_2$), 6.00 (s, 2H, NH_2), 7.96 (s, 1H, CH-pyrazole), 9.10 (s, H, NH).), 10.00 (s, H, NH). Anal. Calcd. for $C_8H_9N_5O_3$ (223): C, 43.05; H, 4.06; N, 31.38; Found:): C, 43.11; H, 4.11; N, 31.41%.

Synthesis of 2-cyano-2-cyclohexylidene-N-(2,5-dioxopyrrolidin-1-yl) acetamide (41): A 25-mL solution of cyanoacetamide **1** (0.01 mol) in ethanol with a few droplets of piperidine and cyclohexanone **3** (0.01 mol). The resulting solution was heated over reflux for 12 hours before being cooled and neutralized by dumping it into an ice/water combination containing HCl drops. The solid substance was produced by filtration and crystallized with benzene, yielding pale brown crystals of **41**; (yield, 70%); m.p.110–112° C. IR spectrum (KBr, cm^{-1}) 3303 (NH), 2932-2856 (C-H aliphatic), 2182 (CN), 1730, 1666 (2C=O). ^{1}H-NMR (400 MHz, DMSO-d_6) δ (ppm):0.88-2.30 (m, 10H, cyclohexene), 2.73-2.87 (m, 4H, $2CH_2$), 9.49 (s, 1H, NH). MS (*m/z*): 261 (M^+). Anal. Calcd. for $C_{13}H_{15}N_3O_3$ (261): C, 59.76; H, 5.79; N, 16.08. Found: C, 59.80; H, 5.82; N, 16.10%.

Synthesis of 1-((5-amino-4-cyclohexylidene-*4H*-pyrazol-3-yl)amino)pyrrolidine-2,5-dione (42): compound **41** and 0.5 g of hydrazine hydrate were combined then the mixture was heated over a 12-hour reflux period, cooled, and neutralized by being poured over ice and water solution that had been diluted with a few droplets of HCl. Each time, the solid was extracted by filtering and crystallized with n-hexane to produce brown crystals of **42**; (yield, 74%); m.p.150–152° C. IR spectrum (KBr, cm^{-1}) 3350-3201 (NH_2/NH), 2935 (C-H aliphatic), 1654 (C=O). ^{1}H-NMR (400 MHz, DMSO-d_6) δ (ppm): 1.23-2.20 (m, 10H, cyclohexene), 2.72-2.87 (m, 4H, $2CH_2$), 5.93(s, 2H, NH_2, 10.00 (s, 1H, NH). ^{13}C-NMR (101 MHz, DMSO-d_6) δ 22.70, 28.20, 28.20, 29.80, 29.80, 33.01, 33.01, 104.83, 134.71, 134.71, 171.08, 171.08, 172.00. Anal. Calcd. for $C_{13}H_{17}N_5O_2$ (275): C, 56.71; H, 6.22; N, 25.44. Found: C, 56.75; H, 6.25; N, 25.46%.

4. Conclusion

The research study reveals the effective synthesis and antimicrobial activity of novel tetrahydrobenzo[b]thiophene, dihydropyridine, dihydrothiazole, thiophene, and iminopyrane derivatives. The chemical structure of the generated substances was confirmed by elemental analysis. The antimicrobial activity investigation demonstrated that all the investigated substances exhibited moderate to excellent antibacterial and antifungal properties against pathogenic organisms.

5. Conflict of Interest

The authors state that there is no conflict of interests.

6. References

[1] S. Dhadda, A.K. Raigar, K. Saini, Manju, A. Guleria, Benzothiazoles: From recent advances in green synthesis to anti-cancer potential, Sustain. Chem. Pharm. 24 (2021). https://doi.org/10.1016/j.scp.2021.100521.

[2] A.E. et al., Evaluation of some new synthesis benzothiazole and benzimidazole Derivatives as potential antimicrobial and anticancer agents, Int. J. Adv. Appl. Sci. 7 (2020) 69–77. https://doi.org/10.21833/ijaas.2020.02.010.
[3] A. Irfan, F. Batool, S.A. Zahra Naqvi, A. Islam, S.M. Osman, A. Nocentini, S.A. Alissa, C.T. Supuran, Benzothiazole derivatives as anticancer agents, J. Enzyme Inhib. Med. Chem. 35 (2020) 265–279. https://doi.org/10.1080/14756366.2019.1698036.
[4] K. Bhagdev, S. Sarkar, Benzothiazole: As An Antidiabetic Agent, 2021. http://annalsofrscb.ro.
[5] Y.I. Asiri, A. Alsayari, A.B. Muhsinah, Y.N. Mabkhot, M.Z. Hassan, Benzothiazoles as potential antiviral agents, J. Pharm. Pharmacol. 72 (2020) 1459–1480. https://doi.org/10.1111/jphp.13331.
[6] K. Bhagdev, S. Sarkar, Benzothiazole Moiety and Its Derivatives as Antiviral Agents, in: MDPI AG, 2022: p. 9. https://doi.org/10.3390/ecms2021-10839.
[7] K. Gupta, A.K. Sirbaiya, V. Kumar, M.A. Rahman, Current Perspective of Synthesis of Medicinally Relevant Benzothiazole based Molecules: Potential for Antimicrobial and Anti-Inflammatory Activities, Mini-Reviews Med. Chem. 22 (2022) 1895–1935. https://doi.org/10.2174/1389557522666220217101805.
[8] P. Kashyap, S. Verma, P. Gupta, R. Narang, S. Lal, M. Devgun, Recent insights into antibacterial potential of benzothiazole derivatives, Med. Chem. Res. (2023). https://doi.org/10.1007/s00044-023-03077-z.
[9] K.S. Mohamed, E.E. Elbialy, A.A. Fadda, Synthesis of Novel Heterocycles Comprising Benzothiazole Moiety and Their Antimicrobial Evaluations, Polycycl. Aromat. Compd. 42 (2022) 5585–5598. https://doi.org/10.1080/10406638.2021.1947332.
[10] A. Kale, R. Kakde, S. Pawar, R. Thombare, Recent Development in Substituted Benzothiazole as an Anticonvulsant Agent, Mini-Reviews Med. Chem. 21 (2020) 1017–1024. https://doi.org/10.2174/1389557521666201222145236.
[11] A.K. Pathak, CLINICAL EFFICACY OF BENZOTHIAZOLE IN ANTITUMOR ACTIVITY: A RECENT TRENDS Adaptation and Development of Medicinal Plant Technologies for Income Generation, Agricultural Diversification and Export Promotion View project clinical pharmacy. View project, (n.d.). https://doi.org/10.20959/wjpps20205-16151.
[12] A.E. EVREN, S. DAWBAA, D. NUHA, Ş.A. YAVUZ, Ü.D. GÜL, L. YURTTAŞ, Design and synthesis of new 4-methylthiazole derivatives: In vitro and in silico studies of antimicrobial activity, J. Mol. Struct. 1241 (2021). https://doi.org/10.1016/j.molstruc.2021.130692.
[13] M.A. Salem, S.Y. Abbas, M.A.M.S. El-Sharief, M.H. Helal, M.A. Gouda, M.A. Assiri, T.E. Ali, Novel Structural Hybrids of Pyrrole and Thiazole Moieties: Synthesis and Evaluation of Antibacterial and Antifungal Activities, Acta Chim. Slov. 68 (2021) 990–996. https://doi.org/10.17344/acsi.2021.6980.
[14] D. Malūkaitė, B. Grybaitė, R. Vaickelionienė, G. Vaickelionis, B. Sapijanskaitė-Banevič, P. Kavaliauskas, V. Mickevičius, Synthesis of novel thiazole derivatives bearing β-amino acid and aromatic moieties as promising scaffolds for the development of new antibacterial and antifungal candidates targeting multidrug-resistant pathogens, Molecules 27 (2022). https://doi.org/10.3390/molecules27010074.
[15] P. Mohanty, S. Behera, R. Behura, L. Shubhadarshinee, P. Mohapatra, A.K. Barick, B.R. Jali, Antibacterial activity of thiazole and its derivatives: A review, Biointerface Res. Appl. Chem. 12 (2022) 2171–2195. https://doi.org/10.33263/BRIAC122.21712195.
[16] R. Raveesha, A.M. Anusuya, A.V. Raghu, K.Y. Kumar, M.G.D. Kumar, S.B.B. Prasad, M.K. Prashanth, Synthesis and characterization of novel thiazole derivatives as potential anticancer agents: Molecular docking and DFT studies, Comput. Toxicol. 21 (2022) 100202. https://doi.org/https://doi.org/10.1016/j.comtox.2021.100202.
[17] P.C. Sharma, K.K. Bansal, A. Sharma, D. Sharma, A. Deep, Thiazole-containing compounds as therapeutic targets for cancer therapy, Eur. J. Med. Chem. 188 (2020). https://doi.org/10.1016/j.ejmech.2019.112016.
[18] S. Abu-Melha, Molecular modeling and docking of new 2-acetamidothiazole-based compounds as antioxidant agents, J. Saudi Chem. Soc. 26 (2022). https://doi.org/10.1016/j.jscs.2022.101431.
[19] M.A. Sofan, A. El-Mekabaty, A.M. Hasel, S.B. Said, Synthesis, cytotoxicity assessment and antioxidant activity of some new thiazol-2-yl carboxamides, J. Heterocycl. Chem. 58 (2021) 1645–1655. https://doi.org/10.1002/jhet.4301.
[20] K. Chen, X. Yao, T. Tang, L.M. Chen, C. Xiao, J.Y. Wang, H.F. Chen, Z.X. Jiang, Y. Liu, X. Zheng, Thiazole-based and thiazolidine-based protein tyrosine phosphatase 1B inhibitors as potential anti-diabetes agents, Med. Chem. Res. 30 (2021) 519–534. https://doi.org/10.1007/s00044-020-02668-4.
[21] I. Stankova, K. Chuchkov, R. Chayrov, L. Mukova, A. Galabov, D. Marinkova, D. Danalev, Adamantane Derivatives Containing Thiazole Moiety: Synthesis, Antiviral and Antibacterial Activity, Int. J. Pept. Res. Ther. 26 (2020) 1781–1787. https://doi.org/10.1007/s10989-019-09983-4.
[22] Y.I. Asiri, A. Bin Muhsinah, A. Alsayari, K. Venkatesan, M. Al-Ghorbani, Y.N. Mabkhot, Design, synthesis and antimicrobial activity of novel 2-aminothiophene containing cyclic and heterocyclic moieties, Bioorganic Med. Chem. Lett. 44 (2021). https://doi.org/10.1016/j.bmcl.2021.128117.

[23] H.M. Metwally, N.A. Khalaf, E. Abdel-Latif, M.A. Ismail, Synthesis, DFT investigations, antioxidant, antibacterial activity and SAR-study of novel thiophene-2-carboxamide derivatives, BMC Chem. 17 (2023). https://doi.org/10.1186/s13065-023-00917-2.

[24] N.A. Qandeel, A.K. El-Damasy, M.H. Sharawy, S.M. Bayomi, N.S. El-Gohary, Synthesis, in vivo anti-inflammatory, COX-1/COX-2 and 5-LOX inhibitory activities of new 2,3,4-trisubstituted thiophene derivatives, Bioorg. Chem. 102 (2020). https://doi.org/10.1016/j.bioorg.2020.103890.

[25] R.M.D. Da Cruz, F.J.B. Mendonça-Junior, N.B. De Mélo, L. Scotti, R.S.A. De Araújo, R.N. De Almeida, R.O. De Moura, Thiophene-based compounds with potential anti-inflammatory activity, Pharmaceuticals 14 (2021). https://doi.org/10.3390/ph14070692.

[26] Z.J. Zhong, X.T. Hu, L.P. Cheng, X.Y. Zhang, Q. Zhang, J. Zhang, Discovery of novel thiophene derivatives as potent neuraminidase inhibitors, Eur. J. Med. Chem. 225 (2021). https://doi.org/10.1016/j.ejmech.2021.113762.

[27] A.M. Atta, E. Abdel-Latif, Synthesis and Anticancer Activity of New Thiophene, Thiazolyl-Thiophene, and Thienopyridine Derivatives, Russ. J. Gen. Chem. 91 (2021) 456–463. https://doi.org/10.1134/S1070363221030154.

[28] Š. Gubič, L.A. Hendrickx, X. Shi, Ž. Toplak, Š. Možina, K.M. Van Theemsche, E.L. Pinheiro-Junior, S. Peigneur, A.J. Labro, L.A. Pardo, J. Tytgat, T. Tomašič, L.P. Mašič, Design of New Potent and Selective Thiophene-Based KV 1.3 Inhibitors and Their Potential for Anticancer Activity, Cancers (Basel). 14 (2022). https://doi.org/10.3390/cancers14112595.

[29] R. Elrayess, Y.M. Abdel Aziz, M.S. Elgawish, M. Elewa, A.S.A. Yassen, S.S. Elhady, H.A. Elshihawy, M.M. Said, Discovery of potent dual egfr/her2 inhibitors based on thiophene scaffold targeting h1299 lung cancer cell line, Pharmaceuticals 14 (2021) 1–21. https://doi.org/10.3390/ph14010009.

[30] M.I. Alahmdi, R.M. Mohareb, M.A. Abdelaziz Mahmoud, K. Alkhamis, N.E. Abo-Dya, N.S. Zidan, S. Khasim, M.A. Alsharif, Anti-proliferative Activities of Thiophenes, Pyrans and PyridinesDerived from 1,3-Dicarbonyl Compounds, ChemistrySelect 6 (2021) 12094–12100. https://doi.org/10.1002/slct.202102868.

[31] N.A. Abed, M.M. Hammouda, M.A. Ismail, E. Abdel-Latif, Synthesis of new heterocycles festooned with thiophene and evaluating their antioxidant activity, J. Heterocycl. Chem. 57 (2020) 4153–4163. https://doi.org/10.1002/jhet.4122.

[32] S.M. TK, A. Das, S.C. C, R. BC, S. K, S. KV, Synthesis, molecular docking, and in-vivo anti-inflammatory screening of novel substituted pyrazole analogues, J. Mol. Struct. 1255 (2022). https://doi.org/10.1016/j.molstruc.2022.132415.

[33] W. A. A. Fadaly, Y. A. M. M. Elshaier, M. T. M. Nemr, K. R. A. Abdellatif, Design, synthesis, modeling studies and biological evaluation of pyrazole derivatives linked to oxime and nitrate moieties as nitric oxide donor selective COX-2 and aromatase inhibitors with dual anti-inflammatory and anti-neoplastic activities, Bioorg. Chem. 134 (2023). https://doi.org/10.1016/j.bioorg.2023.106428.

[34] D. Priya, P. Gopinath, L.S. Dhivya, A. Vijaybabu, M. Haritha, S. Palaniappan, M.K. Kathiravan, Structural Insights into Pyrazoles as Agents against Anti-inflammatory and Related Disorders, ChemistrySelect 7 (2022). https://doi.org/10.1002/slct.202104429.

[35] M. Arshad, Heterocyclic compounds bearing pyrimidine, oxazole and pyrazole moieties: design, computational, synthesis, characterization, antibacterial and molecular docking screening, SN Appl. Sci. 2 (2020). https://doi.org/10.1007/s42452-020-2243-0.

[36] S.A. Ibrahim, H.F. Rizk, M.A. El-Borai, M.E. Sadek, Green routes for the synthesis of new pyrazole bearing biologically active imidiazolyl, pyridine and quinoxaline derivatives as promising antimicrobial and antioxidant agents, J. Iran. Chem. Soc. 18 (2021) 1391–1404. https://doi.org/10.1007/s13738-020-02119-2.

[37] E.M. Mohi El-Deen, E.S. Nossier, E.A. Karam, New Quinazolin-4(3H)-one Derivatives Incorporating Hydrazone and Pyrazole Scaffolds as Antimicrobial Agents Targeting DNA Gyraze Enzyme, Sci. Pharm. 90 (2022). https://doi.org/10.3390/scipharm90030052.

[38] M.M.S. Wassel, W.M. Gamal El-Din, A. Ragab, ; Gameel, A.M.E. Ali, Y.A. Ammar, Antiviral Activity of Adamantane-Pyrazole Derivatives Against Foot and Mouth Disease Virus Infection in vivo and in vitro With Molecular Docking Study, J. Appl. Vet. Sci. 5 (2020) 37–46. https://doi.org/10.21608/ja.

[39] G.D. Kumar, B. Siva, S. Vadlamudi, S.R. Bathula, H. Dutta, K. Suresh Babu, Design, synthesis, and biological evaluation of pyrazole-linked aloe emodin derivatives as potential anticancer agents, RSC Med. Chem. 12 (2021) 791–796. https://doi.org/10.1039/d0md00315h.

[40] M.J. Alam, O. Alam, M.J. Naim, F. Nawaz, A. Manaithiya, M. Imran, H.K. Thabet, S. Alshehri, M.M. Ghoneim, P. Alam, F. Shakeel, Recent Advancement in Drug Design and Discovery of Pyrazole Biomolecules as Cancer and Inflammation Therapeutics, Molecules 27 (2022). https://doi.org/10.3390/molecules27248708.

[41] N.E. Santos, A.R.F. Carreira, V.L.M. Silva, S.S. Braga, Natural and biomimetic antitumor pyrazoles, a perspective, Molecules 25 (2020). https://doi.org/10.3390/molecules25061364.

[42] N.P. Mishra, S. Mohapatra, T. Das, S. Nayak, Imidazo[1,2-a]pyridine as a promising scaffold for the development of antibacterial agents, J. Heterocycl. Chem. 59 (2022) 2051–2075. https://doi.org/10.1002/jhet.4534.
[43] M.B. Islam, M.I. Islam, N. Nath, T. Bin Emran, M.R. Rahman, R. Sharma, M.M. Matin, Recent Advances in Pyridine Scaffold: Focus on Chemistry, Synthesis, and Antibacterial Activities, Biomed Res. Int. 2023 (2023). https://doi.org/10.1155/2023/9967591.
[44] M. Marinescu, C.V. Popa, Pyridine Compounds with Antimicrobial and Antiviral Activities, Int. J. Mol. Sci. 23 (2022). https://doi.org/10.3390/ijms23105659.
[45] R. Sahu, R. Mishra, R. Kumar, C. Majee, A. Mazumder, A. Kumar, Pyridine Moiety: Recent Advances in Cancer Treatment Sahu et al.: A Review: An Insight on Recent Advancement of Pyridine Moiety in Treatment of Cancer, n.d. www.ijpsonline.com.
[46] F.A. Almalki, An overview of structure-based activity outcomes of pyran derivatives against Alzheimer's disease, Saudi Pharm. J. 31 (2023) 998–1018. https://doi.org/10.1016/j.jsps.2023.04.030.
[47] M. Aghajani, S. Asghari, G.F. Pasha, M. Mohseni, Study of three-component reaction of α-ketoesters and active methylenes with OH-acids to synthesize new 2-amino-4H-pyran derivatives and evaluation of their antibacterial and antioxidant activities, Res. Chem. Intermed. 46 (2020) 1841–1855. https://doi.org/10.1007/s11164-019-04066-x.
[48] A. A. Fadda, M. M. Mukhtar, H. M. Refat, Utility of Activated Nitriles in the Synthesis of Some New Heterocyclic Compounds, Am. J. Org. Chem. 2 (2012) 32–40. https://doi.org/10.5923/j.ajoc.20120202.06.
[49] A.S. Salman, N.A. Mahmoud, A. Abdel-Aziem, M.A. Mohamed, D.M. Elsisi, Synthesis, Reactions and Antimicrobial Activity of Some New 3-Substituted Indole Derivatives, Int. J. Org. Chem. 05 (2015) 81–99. https://doi.org/10.4236/ijoc.2015.52010.
[50] A.A. Fadda, N.M. Bayoumy, N.N. Soliman, D.M. Eldiasty, Cyanoacetamide Intermediate in Heterocyclic Synthesis: Synthesis and Biological Evaluation of Hitherto New Dioxoisoindoline Heterocyclic Derivatives, J. Heterocycl. Chem. 56 (2019) 597–607. https://doi.org/10.1002/jhet.3436.
[51] M.A. Gouda, M. Al-Ghorbani, N. Al-Zaqri, Synthesis and cytotoxic activity of some new heterocycles incorporating cyclohepta[b]thiophene-3-carboxamide derivatives, J. Heterocycl. Chem. 57 (2020) 3664–3672. https://doi.org/10.1002/jhet.4085.
[52] K.M. El-Gaml, Synthesis and Antimicrobial Evaluation of New Polyfunctionally Substituted Heterocyclic Compounds Derived from 2-cyano-N-(3-cyanoquinolin-2-yl)acetamide, Am. J. Org. Chem. 5 (2015) 1–9. https://doi.org/10.5923/j.ajoc.20150501.01.
[53] M.R. Mahmoud, A.K. El-Ziaty, F.S.M.A. El-Azm, M.F. Ismail, S.A. Shiba, Utility of Cyano- N -(2-oxo-1,2-dihydroindol-3-ylidene)acetohydrazide in the Synthesis of Novel Heterocycles, J. Chem. Res. 37 (2013) 80–85. https://doi.org/10.3184/174751912X13567100793191.
[54] M. A. Khalil, S. M. Sayed, M. A. Raslan, Reactivity of 2-Cyano-N-(4-(1-Methyl-1H-benzo[d]imidazol-2-yl)-3-(Methylthio)-1-Phenyl-1H-Pyrazol-5-yl)Acetamide: A Facile Synthesis of Pyrazole, Thiazole, 1,3,4-Thiadiazole and Polysubstituted Thiophene Derivatives, Am. J. Org. Chem. 2 (2012) 161–170. https://doi.org/10.5923/j.ajoc.20120206.06.
[55] E. Darwish, A. Fattah, F. Attaby, O. Al-Shayea, Synthesis and Antimicrobial Evaluation of Some Novel Thiazole, Pyridone, Pyrazole, Chromene, Hydrazone Derivatives Bearing a Biologically Active Sulfonamide Moiety, Int. J. Mol. Sci. 15 (2014) 1237–1254. https://doi.org/10.3390/ijms15011237.
[56] R.E. Khidre, I.A.M. Radini, Synthesis and Antimicrobial Activity of Novel Heterocycles Utilizing 3-(1,4-Dioxo-3,4-dihydrophthalazin-2(1H)-yl)-3-oxopropanenitrile as Precursors, J. Heterocycl. Chem. 56 (2019) 850–858. https://doi.org/10.1002/jhet.3463.
[57] A.A. Fadda, M.A. El Salam, E.H. Tawfik, E.M. Anwar, H.A. Etman, Synthesis and insecticidal assessment of some innovative heterocycles incorporating a thiadiazole moiety against the cotton leafworm,: Spodoptera littoralis, RSC Adv. 7 (2017) 39773–39785. https://doi.org/10.1039/c7ra06087d.
[58] S. Bondock, S. Adel, H.A. Etman, Synthesis and antioxidant evaluation of some new 2-benzoylamino-5-hetaryl-1,3,4-oxadiazoles, Res. Chem. Intermed. 42 (2016) 1845–1861. https://doi.org/10.1007/s11164-015-2121-3.
[59] S. Abu-Melha, Synthesis and Antimicrobial Activity of Some New Heterocycles Incorporating the Pyrazolopyridine Moiety, Arch. Pharm. (Weinheim). 346 (2013) 912–921. https://doi.org/10.1002/ardp.201300195.
[60] H.Z. Shams, R.M. Mohareb, M.H. Helal, A.E. Mahmoud, Novel synthesis and antitumor evaluation of polyfunctionally substituted heterocyclic compounds derived from 2-cyano-N-(3-cyano-4,5,6,7- tetrahydrobenzo[b]thiophen-2-yl)-acetamide, Molecules 16 (2011) 52–73. https://doi.org/10.3390/molecules16010052.

[61] M.H. Hekal, Y.M. Ali, F.S.M. Abu El-Azm, Utilization of cyanoacetohydrazide and 2-(1,3-dioxoisoindolin-2-yl) acetyl chloride in the synthesis of some novel anti-proliferative heterocyclic compounds, Synth. Commun. 50 (2020) 2839–2852. https://doi.org/10.1080/00397911.2020.1786125.

[62] A.M. Fahim, A.M. Farag, M.R. Shaaban, E.A. Ragab, Regioselective synthesis and DFT study of novel fused heterocyclic utilizing Thermal heating and Microwave Irradiation, n.d.

[63] R.R. Zaky, T.A. Yousef, K.M. Ibrahim, Co(II), Cd(II), Hg(II) and U(VI)O2 complexes of o-hydroxyacetophenone[N-(3-hydroxy-2-naphthoyl)] hydrazone: Physicochemical study, thermal studies and antimicrobial activity, Spectrochim. Acta Part A Mol. Biomol. Spectrosc. 97 (2012) 683–694. https://doi.org/http://dx.doi.org/10.1016/j.saa.2012.05.086.

[64] I. Das, Medical Microbiology Illustrated, 2000. https://doi.org/10.1099/0022-1317-49-8-769.

[65] H.M. Refat, A.A. Fadda, Synthesis and antimicrobial activity of some novel hydrazide, benzochromenone, dihydropyridine, pyrrole, thiazole and thiophene derivatives, Eur. J. Med. Chem. 70 (2013) 419–426. https://doi.org/http://dx.doi.org/10.1016/j.ejmech.2013.09.003.

[66] P.R. Murray, E.J. Baron, M.A. Pfaller, F.C. Tenover, R.H. Yolken, in: G.L. wood, J.A. Washington (Eds.), Manual of Clinical Microbiology, Am. Soc. Microbiol., Washington, DC, 1995.

[67] R.N. Jones, A.L. Barry, T.L. Gavan, I.I. A. Washington, in: E.H. Lennette, A. Ballows, W. J. HauslerJr, H.J. Shadomy (Eds.), Manual of Clinical Microbiology, fourth edn, Am. Soc. Microbiol. (1972), Washington DC, 1985