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Synthesis, molecular docking studies, and antimicrobial evaluation of pyrano[2, 3-c]pyrazole derivatives

Samy A. El-Assaly[a], Abd El-Hamid A. Ismail[b], Hamed Abdel Bary[b] and Mohamed G. Abouelenein[b*]

[a]*Natural and Microbial Products Chemistry Department, National Research Centre (NRC), Dokki, Giza, Egypt*
[b]*Chemistry Department, Faculty of Science, Menofia University, Shebin El-Koam, Menofia, Egypt*

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A sequence of pyrano[2, 3-*c*]pyrazoles was constructed through promoting an eco-friendly, green, and efficient approach. **M1**-**M25** derivatives were developed by a base-catalyzed one-pot reaction involving application of hydrazine hydrate 96%, β-keto ester as ethyl acetoacetate or diethyl malonate, aryl/heteroaryl aldehyde or isatin, and enolizable active methylene compounds with isolation of unexpected compound **M2**. Further on, intramolecular cyclization of compounds **M10**, **M13** with formic acid, acetic anhydride, and formamide leads to the corresponding pyrimidine derivatives **M26**-**M31**. Afterwards, the antimicrobial activity of the compounds was evaluated and fortunately, the vast majority of the compounds showed outstanding anti-bacterial results. Besides, the potential mode of action of the synthesized compounds was determined by employing a molecular-docking study against penicillin-binding protein implicated in anti-bacterial action. Compound **M21** was one of the most promising anti-bacterial agents with potential binding affinity against the penicillin-binding protein. This study shed light on novel compounds for further antimicrobial drug development.

1. Introduction

The main challenge facing scientists today is a pollution-free environment.[1] Thus, synthetic organic chemists centered their attention on developing practical methods, conditions, usage of materials, and the reaction media depending on green chemistry principles.[2] Multicomponent reactions (MCRs) are processes for constructing molecular diversity and complexity through a concerted reaction involving a combination of three or more reactants.[3] The MCRs evolvement has brought about a paradigm shift in current day research in synthetic organic, medicinal, and combinatorial chemistry by complying with the green chemistry principles in terms of frugality of energy and steps and waste pot MCRs allow swift approach to combinatorial libraries of bioactive scaffolds and -lessening.[4] One complex heterocycles, especially in drug discovery.[5] MCRs offer significant features over conventionally linear-type synthesis by dint of their building up various organic molecules from easily pot process with inherent flexibility, selective convergence, good -obtainable reactants in a one

* Corresponding author. Tel: +2-01068628458
E-mail address: moh.gamal89@yahoo.com (M. G. Abouelenein)

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productivity, facile execution, and high yields.[6] The first multicomponent reaction was described in 1850 by Strecker, and subsequently, various reactions of such type have been published.[7, 8]

In recent years, the synthesis of 4-*H* pyran unit bearing heterocyclic compounds has gained a lot of interest, as they are essential precursors to promising biologically active compounds in the field of medicinal chemistry.[9] Pyranopyrazoles are referred to as a fused five-member pyrazole ring with a six-member pyran ring. Pyrano[2, 3-*c*] pyrazoles are even more prominent at the study level because of their high pharmacological importance and due to the various activities of the heterocyclic core. The dihydropyrano[2, 3-*c*] pyrazole scaffolds are comprehended to display a broad spectrum of biological activities as anti-cancer,[10] anti-inflammatories,[11] antimicrobial,[12] antidepressant,[13] anti-tuberculous,[14] anti-tumour,[15] anticonvulsant,[16] anti-platelet,[17] molluscicidal agents,[18] herbicides,[19] insecticides,[20] vasodilators,[21] analgesics,[22] and medicinal ingredients.[23] Furthermore, 6-amino-4-(3,4-dihydroxyphenyl)-3-methyl-1,4-dihydropyrano[2, 3-*c*]pyrazole-5-carbonitrile has been reported as a possible human Chk1 kinase inhibitor.[24] Furthermore, some of these substances are widely used as cosmetics,[25] pigments,[26] lubricant oil antioxidants,[27] effective steel corrosion inhibitors,[28] pharmaceutical ingredients, and biodegradable agrochemical agents.[29] They also play a significant role as crucial synthetic intermediates.[30]

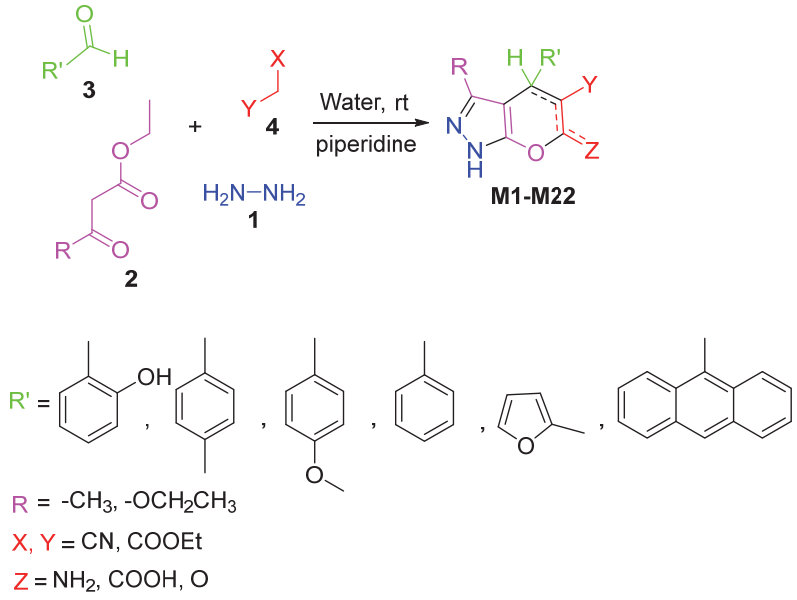
With respect to biological significance, the dihydropyrano[2, 3-*c*]pyrazole derivatives synthesis has experienced a resurgence of interest by the organic chemists. This is reflected in a vast number of synthetic methods through multicomponent reactions of this class of compounds. In 1973, scientist Stolle first synthesized a pyranopyrazole derivative by the condensation of hydrazine hydrate and ethyl acetoacetate.[31] Junek and co-workers synthesized a pyrano pyrazole poly-nitrile derivative by conducting a reaction, in the presence of triethylamine, between 5-methyl-2-phenyl-2,4-dihydro-3*H*-pyrazol-3-one and ethene-1,1,2,2-tetracarbonitrile.[32] In 1974, Otto produced 2-amino-4-substituted pyrano[2, 3-*c*]pyrazole-3-carbonitriles by addition of malononitrile to 4-arylidene-5-methyl-2,4-dihydro-3*H*-pyrazol-3-one.[33] Gradually, a number of synthetic methods including bases has been developed to produce various 6-amino-4-alkyl/aryl-3-methyl-1,4-dihydropyrano[2, 3-*c*]pyrazole-5-carbonitriles by the reaction of arylidienemalononitrile with 3-methylpyrazoline-5-ones or by the condensation of 4-arylidienepyrazoline-5-one with malononitrile.[34] Sharanin and co-workers have developed a three-component reaction between pyrazolone, an aldehyde and malononitrile in ethanol using triethylamine as the catalyst.[35] Shestopalov and co-workers reported the synthesis of pyrazolopyran via a three-component condensation between *N*-methyl-4-piperidone, pyrazoline-5-one, and malononitrile in absolute ethanol.[36] However, this reaction occurred only on heating or when induced by electrochemical methods under an inert atmosphere. Peng and co-workers have developed a two-component reaction involving pyran derivatives and hydrazine hydrate to obtain pyranopyrazoles in water.[37] The reaction was promoted by a combination of microwave and ultrasonic irradiation. In the present work, we report an efficient and ecofriendly four-component reaction protocol in aqueous medium at room temperature for the synthesis of pyranopyrazole derivatives using conventional method. The synthesized compounds were screened for their antimicrobial activity, further molecular docking studies were carried out to recognize the drug–receptor interactions.

2. Results and Discussion

2.1. Chemistry

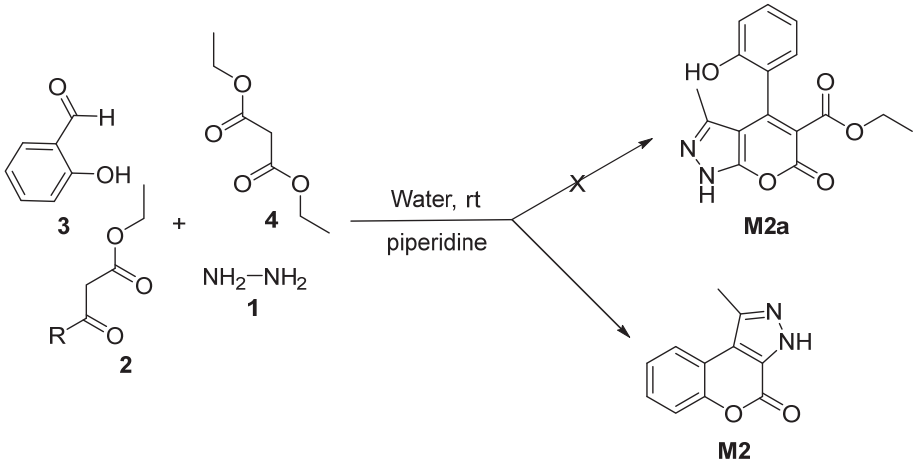
Base-catalyzed one-pot four-component approach was conducted to synthesize various 1,4-dihydro-pyrano[2,3-*c*]pyrazole derivatives using a clean method of simple conditions to adopt the principles of green chemistry. The synthesis of 1,4-dihydro-pyrano [2,3-*c*]pyrazoles derivatives **M1**-**M21** in 85-93% yields could be easily accomplished by tandem Knoevenagel condensation/Michael addition/imine–enamine tautomerism/*O*-cyclization through the reaction of hydrazine hydrate, 96% **1**, β-keto ester as ethyl acetoacetate or diethyl malonate **2**, aryl/heteroaryl aldehyde **3**, and enolizable active methylene compounds as malononitrile or ethyl cyanoacetate or diethyl malonate **4** in the

presence of piperidine as a catalyst (5 mol %) with vigorous stirring in an aqueous medium for 20 min at room temperature under an open atmosphere (**Scheme 1**). The spectral results for all new products are in good agreement with their chemical structures (*cf.* experimental part for details).



Scheme 1. One-pot synthesis of several pyrano[2, 3-*c*]pyrazoles **M1**-**M22**

The reaction sequence (**Scheme 2**) illustrates the construction of the desired compound **M2a** starting from the reaction of hydrazine hydrate, 96% **1**, ethyl acetoacetate **2**, salicylaldehyde **3**, and diethyl malonate **4** in the presence of a catalytic amount of piperidine (5 mol %). Unfortunately, we noticed that the isolated expected product **M2a** was not supported by spectral data, whereas spectral data agrees well with the unexpected compound **M2**. It worthy noted that the reason behind the formation of compound **M2** instead of **M2a** lies in the reaction of salicylaldehyde with diethyl malonate, which does not follow the Knoevenagel condensation reaction as the other aryl/heteroaryl aldehydes, and thus does not form arylidene but rather ethyl 2-oxo-2*H*-chromene-3-carboxylate.[38]



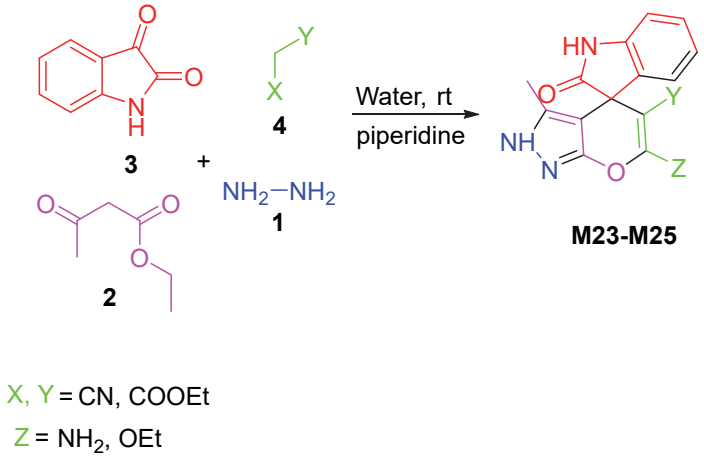
Scheme 2. One-pot synthesis of compound **M2**

IR spectrum shows an intense band at 1708 cm^{-1} due to the lactone carbonyl group. Whereas, pyrazole ring NH stretching band was observed at 3392 cm^{-1}. ^{1}H NMR further confirmed the un expected compound **M2**, wherein the NH proton of the pyrazole ring appeared as a singlet peak at 12.5

ppm; however, the most significant note is the absence of the ester group protons signals from the spectrum which confirms the unexpected compound **M2**. ^{13}C NMR spectra also contributed significantly to confirm the structure of this compound **M2**, as it revealed the presence of a characteristic signal for the lactone carbonyl group at δ 162.55 ppm but also was freed from the ester group carbons signals. The elemental analysis values and the mass spectral data of the unexpected synthesized compound **M2** are in good agreement with the theoretical data.

It should be noted that basic hydrolysis of the ester group of **M6** was observed upon carrying out the reaction involving hydrazine hydrate, 96% **1**, ethyl acetoacetate **2**, 4-Methylbenzaldehyde **3**, and ethyl cyanoacetate **4** in the presence of an excess catalytic amount of piperidine (10 mol %) with vigorous stirring for 20 min in an aqueous medium at room temperature under an open atmosphere to yield the corresponding carboxylic acid derivative **M22**. The structure of compound **M22** was verified by means of IR, ^{1}H NMR, ^{13}C NMR, mass spectra, and elemental analysis. The IR spectrum showed characteristic absorption bands corresponding to the carboxylic acid hydroxyl group and amino groups at 3464-3186, 3413, 3333, 3278 cm^{-1}, respectively, in addition to a very sharp absorption band at 1707 cm^{-1} corresponding to the carboxylic acid carbonyl group. The ^{1}H NMR spectra established the characteristic signal of the amino group protons at δ 7.65 ppm, further to a broad singlet signal at δ 12.88 ppm for the carboxylic acid proton. ^{13}C NMR spectra also highlighted the existence of a characteristic signal for the carboxylic acid carbonyl group at δ 174.55 ppm. Furthermore, the elemental analysis values and mass spectral experimental data of the synthesized compound **M22** have perfect conformity with the theoretical data.

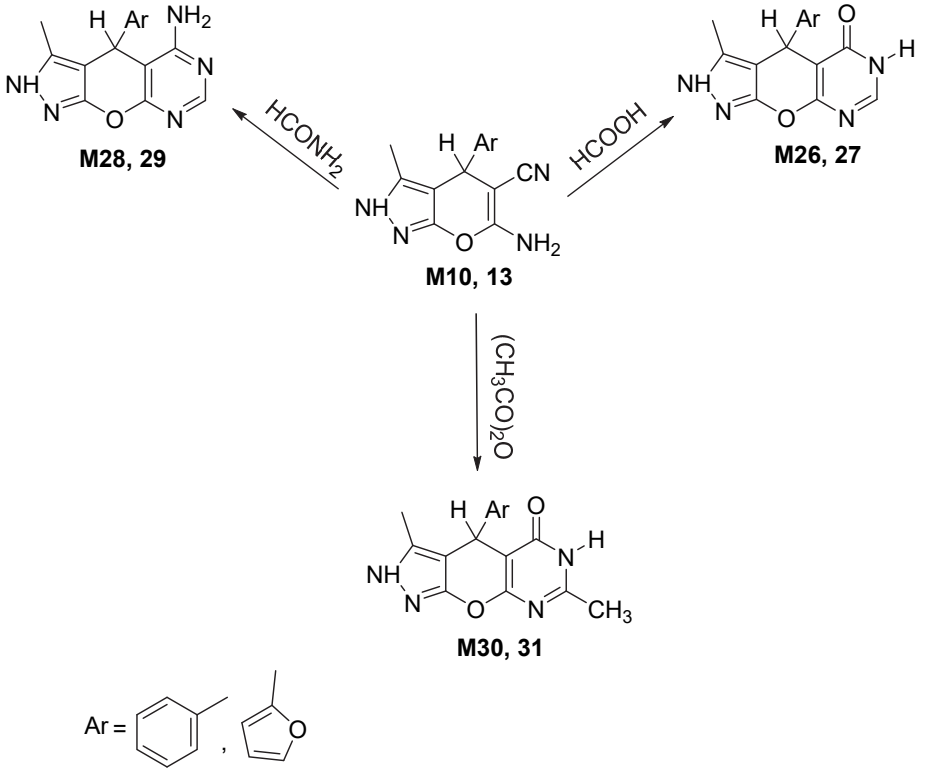
Isatin is a privileged lead molecule for designing potential pharmacological agents as spirooxindole-based organic compounds. The spirooxindole system is the core structure of many pharmacological agents and natural alkaloids.[39] Spirooxindoles, especially those spiro-annulated with heterocycles at the 3-position, have shown good biological activities.[40] Due to the pharmacological activities of spirooxindole motifs and the bioactivity of pharmacophores such as naphthoquinone, indandione, pyrazolone, and pyrimidine derivatives, we sought to develop a single structural framework by combining spirooxindole, pyran, and pyrazole motifs for emergent interest in designing novel hybrid polycyclic heterocycles which may inherit biological properties of both spirooxindole and pharmacophore structures.



Scheme 3. One-pot synthesis of compounds **M23**-**M25**

The synthesis of spiro[indoline-3,4'-pyrano[2,3-*c*]pyrazole] derivatives **M23**-**M25** in 78-82% yields could be easily achieved by tandem Knoevenagel condensation/Michael addition/imine–enamine tautomerism/*O*-cyclization through the reaction of hydrazine hydrate 96% **1**, β-keto ester as ethyl acetoacetate **2**, isatin **3**, and enolizable active methylene compounds as malononitrile or ethyl cyanoacetate or diethyl malonate **4** in the presence of piperidine as a catalyst with vigorous stirring for 25 min in aqueous medium at room temperature under an open atmosphere (**Scheme 4**). An aqueous mixture of hydrazine hydrate and ethyl acetoacetate was firstly stirred at room temperature for about 5 min then, isatin, active methylene compounds, and piperidine as the base catalyst were introduced.

The structures of the target compounds **M23**-**M25** were confirmed by spectral (IR,^{1}H NMR,^{13}C NMR, and Mass) and elemental analyses. According to ^{1}H and ^{13}C NMR spectra, the proton and carbon resonance signals of aliphatic and aromatic groups were observed at the expected regions. The mass spectra of the title compounds were verified by ESI spectra, where the *m*/*z* values of molecular ion peaks were in complete agreement with the calculated molecular weight for each compound. The purity levels of compounds were determined by elemental analysis (C, H, N), and the results were almost identical to the calculated values (*cf*. experimental part for details).



Scheme 4. synthesis of pyrano[2, 3-*c*]pyrazoles **M26**-**M31**

Conducting the four-component reaction in this sequence (**Scheme 1** and **Scheme 4**) removed any risk of aldehyde/isatin reaction with hydrazine hydrate and assured that all amount of aldehyde/isatin would react with the active methylene compounds according to the Knoevenagel condensation reaction. This sequence led to diminishing the side reactions, reducing the amounts of impurities, and increasing the yield percentage. Literature survey revealed that the formal isoelectronic relation between pyrimidine and purine provided much attention to fused pyrimidines, especially pyranopyrimidine derivatives.[41] They play an essential role as antipyretic, analgesic, anti-inflammatory, antihypertensive drugs, herbicides, pesticides, and plant growth regulators.[42] These considerable remarks inspired us for the synthesis of these new compounds with predictable bioefficacy. Various reactions of *o*-aminonitriles attached to aromatic or heterocyclic moieties with the familiar acylating agents and formic acid were stated.[43] The resulting *o*-acylaminonitriles or the *o*-

formyl derivatives are of significant vitality since they can be straightforwardly converted to the corresponding pyrimidine derivatives by acid or base. Accordingly, it was noticed that cyclocondensation of pyrano[2, 3-*c*]pyrazoles **M10**, **M13** with either formic acid or acetic anhydride afforded the corresponding pyrimidine- 4-one derivatives **M26**, **M27**, **M30**, and **M31** (**Scheme 4**). The reaction is proposed to proceed *via* the hydrolysis of the cyano group to amide group and the formylation of the amino group or acetylation accompanied by a ring closure by eliminating water molecule to produce the pyrimidine- 4-one ring. The structures of the products got corroborated from their analytical along with spectral data. IR spectra were devoid of absorption band characteristic to cyano group and experienced the appearance of the absorption bands for the carbonyl group of the pyrimidine- 4-one ring at 1680-1667 cm^{-1}. Besides refluxing the pyrano[2, 3-*c*]pyrazoles **M10**, **M13** with formamide in dimethylformamide, the 4-amino pyrimidine derivatives **M28**, **M29** were isolated via cyclocondensation reaction (**Scheme 4**). Thus, the cyano group absorption band was not perceived in the IR spectrum but revealed the appearance of the absorption bands for an amino group of the 4-amino pyrimidine ring at 3410-2923 cm^{-1}.

2.2. Pharmacology

2.2.1 Antimicrobial Activity

For both their anti-bacterial activity versus Gram-positive (*B. subtilis*, *C. tetani*, and *S. pneumoniae*), Gram-negative (*S. typhi*, *P. aeruginosa*, and *V. cholerae*) bacterial strains, and antifungal efficiency versus three strains of fungi (*C. krusei*, *A. fumigatus*, and *A. niger*), the synthesized compounds **M1**-**M31** were evaluated using the Broth microdilution method.[44, 45] The minimum inhibitory concentration (MIC) was the lowest concentration of the tested compound, which causes no noticeable growth (turbidity). With the like dilutions as those utilized in the experiments, the test medium complemented by DMSO was deemed as a control to guarantee that the solvent did not affect the bacterial or fungal growth. Consequently, DMSO was noted to not influence the microorganisms in the concentrations tested; the attained results are tabularized and discussed in **Table 1**.

The compounds **M1**-**M31** anti-bacterial testing disclosed that nearly the vast majority of pyrano[2, 3-*c*]pyrazoles derivatives were extremely active versus Gram-positive bacterial strains. In case of *B. subtilis*, the synthesised compounds **M1** (CH_3 substitution at C_3 of pyrazole ring and 2-hydroxyphenyl, CN, and NH_2 substitutions on pyran ring), **M2** (CH_3 substitution at C_3 of pyrazole ring fused with chromene ring), **M3** (CH_3 substitution at C_3 of pyrazole ring and 2-hydroxyphenyl, COOEt, and NH_2 substitutions on pyran ring), **M4** (CH_3 substitution at C_3 of pyrazole ring and *p*-tolyl, CN, and NH_2 substitutions on pyran ring), **M5** (CH_3 substitution at C_3 of pyrazole ring and *p*-tolyl, COOEt, and Oxo substitutions on pyran ring), **M6** (CH_3 substitution at C_3 of pyrazole ring and *p*-tolyl, COOEt, and NH_2 substitutions on pyran ring), **M7** (CH_3 substitution at C_3 of pyrazole ring and 4-methoxyphenyl, CN, and NH_2 substitutions on pyran ring), **M8** (CH_3 substitution at C_3 of pyrazole ring and 4-methoxyphenyl, COOEt, and Oxo substitutions on pyran ring), **M10** (CH_3 substitution at C_3 of pyrazole ring and phenyl, CN, and NH_2 substitutions on pyran ring), **M11** (CH_3 substitution at C_3 of pyrazole ring and phenyl, COOEt, and oxo substitutions on pyran ring), **M13** (CH_3 substitution at C_3 of pyrazole ring and furan-2-yl, CN, and NH_2 substitutions on pyran ring), **M14** (CH_3 substitution at C_3 of pyrazole ring and furan-2-yl, COOEt, and oxo substitutions on pyran ring), **M16** (OCH_2CH_3 substitution at C_3 of pyrazole ring and 2-hydroxyphenyl, CN, and NH_2 substitutions on pyran ring), **M17** (OCH_2CH_3 substitution at C_3 of pyrazole ring and *p*-tolyl, CN, and NH_2 substitutions on pyran ring), **M18** (OCH_2CH_3 substitution at C_3 of pyrazole ring and 4-methoxyphenyl, CN, and NH_2 substitutions on pyran ring), **M19** (OCH_2CH_3 substitution at C_3 of pyrazole ring and phenyl, CN, and NH_2 substitutions on pyran ring), **M20** (OCH_2CH_3 substitution at C_3 of pyrazole ring and anthracen-9-yl, CN, and NH_2 substitutions on pyran ring), **M21** (OCH_2CH_3 substitution at C_3 of pyrazole ring and furan-2-yl, CN, and NH_2 substitutions on pyran ring), and **M22** (CH_3 substitution at C_3 of pyrazole ring and *p*-tolyl, COOH, and NH_2 substitutions on pyran ring), **M23** (CH_3 substitution at C_3 of pyrazole ring and NH_2 and CN substitutions on spiro[indoline-3,4'-pyran]-2-one ring), **M24** (CH_3 substitution at C_3 of

pyrazole ring and COOEt and OCH_2CH_3 substitutions on spiro[indoline-3,4'-pyran]-2-one ring), **M25** (CH_3 substitution at C_3 of pyrazole ring and NH_2 and COOEt substitutions on spiro[indoline-3,4'-pyran]-2-one ring), **M26** (CH_3 substitution at C_3 of pyrazole ring and furan-2-yl and oxo substitutions on pyrano[2,3-*d*]pyrimidine ring), **M27** (CH_3 substitution at C_3 of pyrazole ring and phenyl and oxo substitutions on pyrano[2,3-*d*]pyrimidine ring), **M28** (CH_3 substitution at C_3 of pyrazole ring and furan-2-yl and NH_2 substitutions on pyrano[2,3-*d*]pyrimidine ring), **M29** (CH_3 substitution at C_3 of pyrazole ring and phenyl and NH_2 substitutions on pyrano[2,3-*d*]pyrimidine ring), **M30** (CH_3 substitution at C_3 of pyrazole ring and furan-2-yl, CH_3, and oxo substitutions on pyrano[2,3-*d*]pyrimidine ring), and **M31** (CH_3 substitution at C_3 of pyrazole ring and furan-2-yl, CH_3, and oxo substitutions on pyrano[2,3-*d*]pyrimidine ring) exposed, at a lower concentration, higher activity than ciprofloxacin as standard drug. Moreover, compounds **M12** (CH_3 substitution at C_3 of pyrazole ring and phenyl, COOEt, and NH_2 substitutions on pyran ring) and **M15** (CH_3 substitution at C_3 of pyrazole ring and furan-2-yl, COOEt, and NH_2 substitutions on pyran ring) exhibited comparable activity with ciprofloxacin against *B. subtilis*, whereas, at a lower concentration, compound **M9** (CH_3 substitution at C_3 of pyrazole ring and 4-methoxyphenyl, COOEt, and NH_2 substitutions on pyran ring) exhibited lower activity than ciprofloxacin as standard drug. In case of *C. tetani*, the synthesised compounds **M1-M5**, **M8-M31** exposed, at a lower concentration, higher activity than ciprofloxacin as standard drug. Moreover, compound **M6** exhibited comparable activity with ciprofloxacin while, compound **M7** exhibited lower activity than ciprofloxacin as standard drug. In case of *S. pneumoniae*, the synthesised compounds **M2**, **M3**, **M5**, **M7**, **M9**, **M10**, **M11**, **M14**, **M16**, **M8**, **M20**, **M21**, **M22**, **M24**, **M25**, **M26**, **M27**, and **M28** exposed, at a lower concentration, higher activity than ciprofloxacin as standard drug. Moreover, compounds **M4**, **M23**, and **M29** exhibited comparable activity with ciprofloxacin while, the rest of synthetic compounds exhibited lower activity than ciprofloxacin as standard drug. The activity of **M1-M31** compounds was estimated versus three Gram-negative bacterial strains; it was observed that, at a lower concentration, the synthetic compounds exposed selective activities versus ciprofloxacin as standard drug. In case of *S. typhi*, the synthesised compounds **M1**, **M6**, **M8**, **M14**, **M15**, **M19**, **M21**, **M22**, **M23**, **M29**, **M30**, and **M31** exposed, at a lower concentration, higher activity than ciprofloxacin as standard drug. Moreover, compounds **M3**, **M17**, **M27**, and **M28** exhibited comparable activity with ciprofloxacin while, the rest of synthetic compounds exhibited lower activity than ciprofloxacin as standard drug. In case of *P. aeruginosa*, the synthesised compounds **M1**, **M2**, **M3**, **M6**, **M7**, **M10**, **M11**, **M14**, **M18**, **M21**, **M22**, **M23**, and **M31** exposed, at a lower concentration, higher activity than ciprofloxacin as standard drug. Moreover, only compounds **M16**, **M19**, and **M20** exhibited comparable activity with ciprofloxacin while, the rest of synthetic compounds exhibited lower activity than ciprofloxacin as standard drug. In case of *V. cholerae*, the synthesised compounds **M4**, **M6**, **M7**, **M13**, **M15**, **M17**, **M21**, **M24**, and **M25** exposed, at a lower concentration, higher activity than ciprofloxacin as standard drug. Moreover, compounds **M1**, **M12**, **M14**, and **M22** exhibited comparable activity with ciprofloxacin while, the rest of synthetic compounds exhibited lower activity than ciprofloxacin as standard drug. In the antifungal screening of compounds **M1**-**M31** against three strains of fungi (*C. krusei*, *A. fumigatus*, and *A. niger*), at a lower concentration, almost the whole compounds exhibited lower efficiency than nystatin griseofulvin as reference drugs except, compound **M6**, which was almost equally active compared to griseofulvin as a standard drug in case of *C. krusei*; the study outcomes are described in **Table 1**. However, the majority of the synthesized compounds were clearly noted to exhibit lower antifungal activity compared to their anti-bacterial activity, and this is due to the fact that antibiotics are one of the main risk factors for fungal infections as depicted in **Table 1**, where compounds, for instance, **M14**, **M21**, **M22**, and **M23** which exhibited higher anti-bacterial efficacy, but, in contrast, they exhibited lower antifungal efficacy and dramatically allowed fungal growth.[46]

Table 1. *In vitro* antimicrobial efficiency results of the compounds **M1-31**

Entry	Minimum inhibitory concentration in µg/mL (MIC)								
	Gram +ve bacteria			Gram -ve bacteria			Fungai strains		
	B. s.	***C. t.***	***S. p.***	***S. t.***	***P. a.***	***V. c.***	***C. k.***	***A. f.***	***A. n.***
M1	3.57	3.57	5.00	1.87	1.25	3.125	5.00	6.25	7.50
M2	1.25	5.00	0.93	7.50	1.87	3.75	8.75	10.00	7.50
M3	1.87	0.93	1.25	3.125	1.87	5.00	8.75	7.50	6.25
M4	1.25	0.10	3.125	3.57	7.50	1.87	7.50	10.00	12.50
M5	1.87	1.00	2.50	5.00	3.57	6.25	8.75	12.50	6.25
M6	1.87	6.25	3.75	1.87	0.93	2.50	3.125	7.50	6.25
M7	3.125	7.50	2.50	6.25	1.25	1.00	3.75	5.00	7.50
M8	1.87	3.125	6.25	1.87	6.25	7.50	6.25	10.00	3.75
M9	7.50	3.125	1.25	6.25	3.75	5.00	10.00	8.75	12.50
M10	3.57	1.00	1.25	5.00	1.87	6.25	5.00	6.25	7.50
M11	3.125	1.00	2.50	3.75	1.25	5.00	7.50	5.00	8.75
M12	6.25	2.50	5.00	3.57	7.50	3.125	8.75	7.50	5.00
M13	2.50	1.87	5.00	6.25	3.57	1.25	10.00	3.75	7.50
M14	0.75	0.10	1.25	1.87	2.50	3.125	3.75	8.75	12.50
M15	6.25	1.25	7.50	2.50	3.75	1.00	10.00	7.50	6.25
M16	3.125	1.87	2.50	5.00	3.125	6.25	12.50	8.75	6.25
M17	1.00	1.25	3.75	3.125	5.00	0.93	8.75	10.00	7.50
M18	0.75	1.00	1.25	3.75	2.50	5.00	8.75	7.50	6.25
M19	1.00	2.50	3.75	2.50	3.125	6.25	12.50	10.00	8.75
M20	1.25	0.93	1.87	3.75	3.125	7.50	12.5	5.00	7.50
M21	**0.10**	**1.00**	**0.10**	**0.50**	**0.75**	**2.50**	**8.75**	**7.50**	**10.00**
M22	0.50	1.00	1.25	0.75	2.50	3.125	7.50	12.50	8.75
M23	1.87	2.50	3.125	2.50	1.25	3.75	10.00	7.50	8.75
M24	2.50	3.75	1.25	6.25	5.00	2.50	6.25	7.50	10.00
M25	1.87	3.125	2.50	5.00	6.25	1.25	7.50	12.50	8.75
M26	3.75	2.50	1.87	3.75	5.00	7.50	7.50	8.75	10.00
M27	1.87	1.25	1.00	3.125	6.25	3.75	10.00	7.50	12.50
M28	1.87	3.75	2.50	3.125	7.50	5.00	8.75	7.50	10.00
M29	2.50	5.00	3.125	2.50	3.75	6.25	10.00	8.75	12.50
M30	2.50	1.87	6.25	2.50	5.00	7.50	6.25	10.00	5.00
M31	1.25	3.125	7.50	1.00	2.50	6.25	7.50	8.75	12.50
Ciprofloxacin	**6.25**	**6.25**	**3.125**	**3.125**	**3.125**	**3.125**	-	-	-
Griseofulvin	-	-	-	-	-	-	**3.125**	**1.25**	**1.25**
Nystatin	-	-	-	-	-	-	**1.25**	**1.00**	**1.00**

2.2.2 Molecular docking study

In order to take a supplemental step for the determination of the mode of action of the studied compounds, the molecular-docking research was applied for the binding mode determination and the antimicrobial *in silico* study versus penicillin-binding protein (PBPb) that act as drug target for antimicrobial agent. For the orientation and position of the detected ligand in the crystal structure to be represented, the authenticity of the docking parameters and methods should be emphasized, so the cocrystal ligand redocking was necessary. Confirming the approval with the accuracy and parameters of the docking protocols, the RMSD value difference was <2Å among cocrystal ligand to the authentic cocrystal ligand.

Based on antimicrobial assays, compound **M21** was the most promising anti-bacterial agent against both Gram-negative and Gram-positive bacterial strains; therefore, we have investigated its activity against the penicillin-binding protein. Molecular docking results showed that compound **M21** has a tremendous binding affinity with PBPb with a free binding of energy less than the PNM, the co-crystalized ligand of PBPb (-7.3 Kcal/mole, -7.2 Kcal/mole, respectively). Besides, compound **M21** was capable of forming 5 hydrophobic interactions and 4 critical hydrogen bonds with the key amino acid residues in the PBPb binding pocket (**Table 2** and **Fig. 1**).

Table 2. The docking study results of the most auspicious testified compound (**M21**) into PBPb binding pocket (PDB: 3UDI) in comparing with the co-crystallized ligand.

Entry	ΔG_b [a]	Types of interactions			
		Hydrogen bonding			Hydrophobic
		No	Length Å [b]	AA[c]	AA[c]
M21	-7.3	4	3.214	Ser434	Ile710, Leu484, Leu486, Leu526, Val649
			2.279	Ser487	
			2.400	Thr670	
			2.438	Thr670	
RL	-7.2	7	3.019	Asn489	Leu486, Ala676
			2.755	Thr672	
			2.774	Thr672	
			3.377	Ser487	
			3.554	Lys669	
			2.991	Thr670	
			2.634	Thr670	

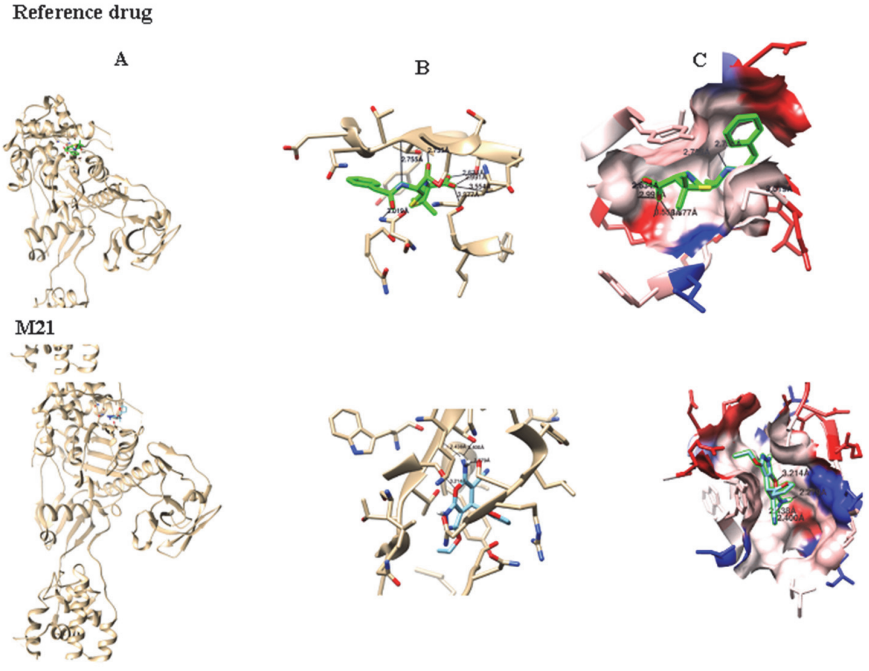


Fig. 1. Compound **M21** interaction with PBPb protein, A) 3D interaction, B) hydrogen bond formation, and C) hydrophobic interaction representation

3. Conclusions

In this study, we have conducted a successful green approach for a sequence of pyrano[2, 3-*c*]pyrazole as well as pyrazolopyranopyrimidine derivatives to be synthesized within a short period with yields 78-93%. The target compounds were elucidated by means of an array of spectral techniques, encompassing IR, ^{1}H NMR, ^{13}C NMR, and mass spectrometry, and the purity levels of the compounds were determined through elemental analysis (C, H, N). Toward antimicrobial activity, the vast majority

of the synthesized compounds manifested excellent efficiency. Moreover, compound **M21** was the highly efficient synthesized scaffold in anti-bacterial and molecular docking simulation studies. Furthermore, the presented results highlighted a newly prospective way of discovering drugs with antimicrobial activity.

4. Experimental

4.1. Materials and Methods

All the chemicals were purchased by commercial sources and used without further purification. The purity of the synthesized compounds and the progress of the reactions were monitored by thin-layer chromatography (TLC), which was performed with Merck pre-coated silica gel 60 F254 aluminum sheets and visualized using UV light at 254 nm. The melting points were measured on the Stuart science melting point apparatus by the open capillary method and are uncorrected.^{1}H NMR and ^{13}C NMR spectra were measured on a JEOL ECA-500 II spectrometer (500 MHz or 100 MHz, respectively) using dimethylsulfoxide (DMSO-d6) as solvent and tetramethylsilane (TMS) as an internal standard. Chemical shifts are reported in δ part per million (ppm) relative to internal tetramethylsilane standard (TMS, δ 0.00). The peak patterns are indicated as follows: s, singlet; d, doublet; t, triplet; m, multiplet; q, quartet; br, broad. The coupling constant, *J*, is reported in Hertz (Hz). The IR spectra were recorded on a Thermo Fisher Nicolet IS10, USA spectrometer (Mansoura University), Egypt, by the KBr disc method, and mass spectra were recorded using GC/MS SHIMADZU spectrophotometer operating at 70 eV at the Micro analytical center (Al-Azhar University). The CHN elemental analyses of all the compounds were recorded by an automatic analyzer (CHNS Vario EL III-Elementar Analyzer, Germany) at the Micro Analytical Unit (Al-Azhar University).

4.2. General procedure

4.2.1 General procedure for preparation of pyrano[2, 3-c]pyrazole derivatives (M1-M15), (M16-M21)

To a well-stirred an aqueous mixture of hydrazine hydrate, 96% **1** (1 mmol) and ethyl acetoacetate or diethyl malonate **2** (1 mmol) for 5 min in a 50 mL rounded bottom flask, aryl/heteroaryl aldehyde **3** (1 mmol), either malononitrile or ethyl cyanoacetate or diethyl malonate **4** (1 mmol), and piperidine (5 mol %) were successively added at room temperature under an open atmosphere with vigorous stirring for 20 min. The reaction progress, in each case, was monitored by TLC (eluent: ethyl acetate/ *n*-hexane, 1:2). After completion of the reaction, the precipitated solid, in each case, was collected by filtration, washed with cold water and then with a mixture of (ethyl acetate/ *n*-hexane) (20:80) and dried well. The obtained products were purified, in each case, by recrystallization from ethanol.

4.2.2 General procedure for preparation of pyrano[2, 3-c]pyrazole derivative (M22)

To a well-stirred an aqueous mixture of hydrazine hydrate, 96% **1** (1 mmol) and ethyl acetoacetate **2** (1 mmol) for 5 min in a 50 mL rounded bottom flask, 4-Methylbenzaldehyde **3** (1 mmol), ethyl cyanoacetate **4** (1 mmol) and piperidine (10 mol%) were successively added at room temperature under an open atmosphere with vigorous stirring for 20 min. The reaction progress was monitored by TLC (eluent: ethyl acetate/ *n*-hexane, 1:2). After completion of the reaction, the precipitated solid was collected by filtration, washed with cold water and then with a mixture of (ethyl acetate/ *n*-hexane) (20:80), and dried well. The obtained product was purified by recrystallization from ethanol.

4.2.3 General procedure for preparation of spiro[indoline-3,4'-pyrano[2, 3-c]pyrazole derivatives (M23-M25)

To a well-stirred an aqueous mixture of hydrazine hydrate, 96% **1** (1 mmol) and ethyl acetoacetate **2** (1 mmol) for 5 min in a 50 mL rounded bottom flask, isatin **3** (1 mmol), either malononitrile or ethyl cyanoacetate or diethyl malonate **4** (1 mmol), and piperidine (5 mol%) were successively added at room

temperature under an open atmosphere with vigorous stirring for 25 min. The reaction progress, in each case, was monitored by TLC (eluent: ethyl acetate/ *n*-hexane, 1:2). After completion of the reaction, the precipitated solid, in each case, was collected by filtration, washed with cold water, and then with a mixture of (ethyl acetate/ *n*-hexane) (20:80), and dried well. The product obtained, in each case, was purified by recrystallization from ethanol.

4.2.4 General procedure for preparation of pyrazolo[4',3':5,6]pyrano[2,3-d]pyrimidine derivatives (M26, *M27)*

A mixture of either compound **M10** or **M13** (5 mmol) and formic acid (20 mL) was refluxed for 4 h. The solvent was evaporated under vacuum, and the formed precipitate, in each case, was collected by filtration, washed several times with cold water, dried well, and purified by recrystallization from ethanol.

4.2.5 General procedure for preparation of pyrazolo[4',3':5,6]pyrano[2,3-d]pyrimidine derivatives (M28, *M29)*

A mixture of either compound **M10** or **M13** (5 mmol) with formamide (10 mL), formic acid (5 mL), and dimethylformamide (5 mL) was refluxed for 6 h. Then, in each case, the reaction mixture was allowed to cool at room temperature, and the formed precipitate, in each case, was collected by filtration, washed several times with cold water, dried well, and purified by recrystallization from ethanol.

4.2.6 General procedure for preparation of pyrazolo[4',3':5,6]pyrano[2,3-d]pyrimidine derivatives (M30*, *M31)

A mixture of either compound **M10** or **M13** (5 mmol) in acetic anhydride (10 mL) and acetic acid (10 mL) was refluxed for 5 h. Then, in each case, the reaction mixture was allowed to cool at room temperature and poured into ice-water (20 mL). The formed precipitate, in each case, was collected by filtration, washed several times with cold water, dried well, and purified by recrystallization from ethanol.

4.3 Physical and Spectral Data

*6-amino-4-(2-hydroxyphenyl)-3-methyl-1,4-dihydropyrano[2, 3-c]pyrazole-5-carbonitrile (**M1**)*

White powder; Yield 92%; m.p: 225-227 °C; IR (KBr) cm^{-1} 3447, 3417, 3351, 2925, 2187, 1659, 1609; ^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm): 11.14 (s, 1H), 9.52 (s, br, 1H), 7.15-7.18 (m, 1H), 6.99-7.41 (m, 2H), 6.93-6.94 (d, *J*= 8.5 Hz, 1H), 6.68 (s, 2H), 4.61 (s, 1H), 1.95 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 160.18, 159.06, 148.36, 136.78, 131.27, 130.18, 124.05, 123.11, 120.36, 115.11, 105.11, 55.11, 35.47, 9.85; EIMS, *m/z* $[M]^+$ calcd: 268.10; found: 268.09; Elemental Analysis for $C_{14}H_{12}N_4O_2$ (%), Calcd: C, 62.68; H, 4.51; N, 20.88; found: C, 62.63; H, 4.47; N, 20.83.

*1-methylchromeno[3,4-c]pyrazol-4(3H)-one (**M2**)*

White powder; Yield 88%; m.p: 189-191 °C; IR (KBr) cm^{-1} 3392, 3052, 2922, 1708, 1619, 1603; ^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm): 12.5 (s, 1H), 7.77-7.80 (m, 1H), 7.61-7.64 (m, 2H), 7.54-7.56 (d, *J*= 8.5 Hz, 1H), 2.13 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 162.55, 151.11, 139.27, 137.82, 130.91, 129.82, 126.2, 118.19, 112.5, 108.55, 13.09; EIMS, *m/z* $[M]^+$ calcd: 200.06; found: 200.05; Elemental Analysis for $C_{11}H_8N_2O_2$ (%), Calcd: C, 66.00; H, 4.03; N, 13.99; found: C, 65.94; H, 3.98; N, 13.92.

*Ethyl 6-amino-4-(2-hydroxyphenyl)-3-methyl-1,4-dihydropyrano[2, 3-c]pyrazole-5-carboxylate (**M3**)*

White powder; Yield 89%; m.p: 224-226 °C; IR (KBr) cm^{-1} 3438, 3313, 3214, 2987, 2921, 1726, 1657, 1618; ^1H NMR (500 MHz, DMSO-d_6) δ (ppm): 12.10 (s, 1H), 10.12 (s, br, 1H), 7.47 (s, 2H), 7.06-7.09 (m, 1H), 6.92-6.94 (m, 2H), 6.85-6.86 (d, *J*= 8.5 Hz, 1H), 5.11 (s, 1H), 4.05-4.11 (q, 2H), 1.82 (s, 3H), 1.23-1.26 (t, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 164.91, 159.64, 158.36, 148.22, 134.90, 130.72, 130.08, 122.91, 120.54, 114.73, 104.06, 62.55, 59.78, 33.27, 13.44, 10.54; EIMS, *m/z* $[M]^+$ calcd: 315.12; found: 315.13; Elemental Analysis for $C_{16}H_{17}N_3O_4$ (%), Calcd: C, 60.94; H, 5.43; N, 13.33; found: C, 60.89; H, 5.39; N, 13.29.

*6-amino-3-methyl-4-(p-tolyl)-1,4-dihydropyrano[2, 3-c]pyrazole-5-carbonitrile (**M4**)*

White powder; Yield 91%; m.p: 205-207 °C; IR (KBr) cm^{-1} 3410, 3378, 3275, 2923, 2869, 2192,1647, 1601; ^1H NMR (500 MHz, DMSO-d_6) δ (ppm): 12.06 (s, 1H), 7.1-7.11 (d, *J*= 8.5 Hz, 2H), 7.02-7.04 (d, *J*= 8.5 Hz, 2H), 6.82 (s, 2H), 4.53 (s, 1H), 2.26 (s, 3H), 1.77 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 160.73, 154.72, 142.54, 135.64, 135.09, 128.90, 127.64, 120.73, 97.82, 57.27, 35.82, 20.55, 9.63; EIMS, *m/z* $[M]^+$ calcd: 266.12; found: 266.12; Elemental Analysis for $C_{15}H_{14}N_4O$ (%), Calcd: C, 67.65; H, 5.30; N, 21.04; found: C, 67.60; H, 5.26; N, 20.98.

*Ethyl 3-methyl-6-oxo-4-(p-tolyl)-1,6-dihydropyrano[2, 3-c]pyrazole-5-carboxylate (**M5**)*

White powder; Yield 85%; m.p: 189-191 °C; IR (KBr) cm^{-1} 3190, 3072, 2925, 1739, 1706, 1610; ^1H NMR (500 MHz, DMSO-d_6) δ (ppm): 12.25 (s, 1H), 7.24-7.26 (d, *J*= 8.5 Hz, 2H), 7.17-7.19 (d, *J*= 8.5 Hz, 2H), 4.26-4.30 (q, 2H), 2.24 (s, 3H), 1.88 (s, 3H), 1.24-1.28 (t, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 164.17, 160.19, 153.81, 153.09, 152.36, 144.54, 136.36, 135.81, 128.72, 127.63, 103.46, 62, 20.73, 13.63, 10.55; EIMS, *m/z* $[M]^+$ calcd: 312.11; found: 312.10; Elemental Analysis for $C_{17}H_{16}N_2O_4$ (%), Calcd: C, 65.38; H, 5.16; N, 8.97; found: C, 65.33; H, 5.12; N, 8.92.

*Ethyl 6-amino-3-methyl-4-(p-tolyl)-1,4-dihydropyrano[2, 3-c]pyrazole-5-carboxylate (**M6**)*

White powder; Yield 86%; m.p: 204-206 °C; IR (KBr) cm^{-1} 3402, 3268, 3138, 3013, 2926, 2859, 1725, 1670, 1603; ^1H NMR (500 MHz, DMSO-d_6) δ (ppm): 12.15 (s, 1H), 7.38 (s, 2H), 6.98-7 (d, *J*= 8.5 Hz, 2H), 6.91-6.92 (d, *J*= 8.5 Hz, 2H), 5.04 (s, 1H), 4.02-4.07 (q, 2H), 2.23 (s, 3H), 1.81 (s, 3H), 1.16-1.20 (t, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 164.87, 159.45, 154.18, 141.81, 135.64, 135.09, 128.55, 127.27, 103.27, 61.82, 60.54, 33.64, 20.55, 13.45, 10.37; EIMS, *m/z* $[M]^+$ calcd: 313.14; found: 313.13; Elemental Analysis for $C_{17}H_{19}N_3O_3$ (%), Calcd: C, 65.16; H, 6.11; N, 13.41; found: C, 65.11; H, 6.05; N, 13.37.

*6-amino-4-(4-methoxyphenyl)-3-methyl-1,4-dihydropyrano[2, 3-c]pyrazole-5-carbonitrile (**M7**)*

White powder; Yield 91%; m.p: 210-212 °C; IR (KBr) cm^{-1} 3482, 3259, 3113, 2960, 2925, 2191, 1643, 1604; ^1H NMR (500 MHz, DMSO-d_6) δ (ppm): 12.07 (s, 1H), 7.04-7.08 (m, 2H), 6.84-6.87 (m, 2H), 6.81 (s, 2H), 4.52 (s, 1H), 3.72 (s, 3H),1.78 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 160.36, 159.73, 155.09, 139.82, 137.09, 136.55, 128.54, 121.27, 114.50, 98.91, 58.21, 55.45, 35.64, 9.93; EIMS, *m/z* $[M]^+$ calcd: 282.11; found: 282.11; Elemental Analysis for $C_{15}H_{14}N_4O_2$ (%), Calcd: C, 63.82; H, 5.00; N, 19.85; found: C, 63.77; H, 4.94; N, 19.81.

*Ethyl 4-(4-methoxyphenyl)-3-methyl-6-oxo-1,6-dihydropyrano[2, 3-c]pyrazole-5-carboxylate (**M8**)*

White powder; Yield 86%; m.p: 195-197 °C; IR (KBr) cm^{-1} 3400, 3086, 2967, 2930, 2841, 1729, 1705, 1601; ^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm): 12.18 (s, 1H), 7.39-7.45 (m, 2H), 7.21-7.22 (m, 2H), 4.34-4.39 (q, 2H), 3.81 (s, 3H), 1.94 (s, 3H), 1.18-1.21 (t, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 164.18, 160.90, 158.18, 157.45, 156.72, 152.72, 142.54, 136.91, 136.36, 128.36, 114.72, 98.73, 61.82, 55.63, 14, 10.91; EIMS, *m/z* $[M]^+$ calcd: 328.11; found: 328.12; Elemental Analysis for $C_{17}H_{16}N_2O_5$ (%), Calcd: C, 62.19; H, 4.91; N, 8.53; found: C, 62.13; H, 4.87; N, 8.48.

*Ethyl 6-amino-4-(4-methoxyphenyl)-3-methyl-1,4-dihydropyrano[2, 3-c]pyrazole-5-carboxylate (**M9**)*

White powder; Yield 87%; m.p: 209-211 °C; IR (KBr) cm^{-1} 3402, 3268, 3180, 3039, 2966, 2931, 1723, 1666, 1601; ^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm): 12.14 (s, 1H), 7.71 (s, 2H), 7.03-7.07 (m, 2H), 6.86-6.87 (m, 2H), 5.12 (s, 1H), 4.28-4.33 (q, 2H), 3.73 (s, 3H), 1.89 (s, 3H), 1.14-1.17 (t, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 164.64, 159.27, 158.44, 155.62, 140.32, 136.62, 136.16, 128.19, 114.29, 98.36, 61.62, 60.36, 55.44, 33.63, 13.83, 10.72; EIMS, *m/z* $[M]^+$ calcd: 329.14; found: 329.13; Elemental Analysis for $C_{17}H_{19}N_3O_4$ (%), Calcd: C, 62.00; H, 5.81; N, 12.76; found: C, 61.95; H, 5.77; N, 12.71.

*6-amino-3-methyl-4-phenyl-1,4-dihydropyrano[2, 3-c]pyrazole-5-carbonitrile (**M10**)*

White powder; Yield 93%; m.p: 243-245 °C; IR (KBr) cm^{-1} 3373, 3311, 3170, 3021, 2925, 2191, 1649, 1599; ^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm): 12.08 (s, 1H), 7.29-7.32 (t, *J*= 7.5 Hz, 2H), 7.2-7.23 (m, 1H), 7.15-7.16 (t, *J*= 7 Hz, 2H), 6.86 (s, 2H), 4.58 (s, 1H), 1.77 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 160.88, 154.77, 144.47, 135.57, 128.45, 127.48, 126.75, 120.82, 97.65, 57.15, 36.23, 9.75; EIMS, *m/z* $[M]^+$ calcd: 252.10; found: 252.10; Elemental Analysis for $C_{14}H_{12}N_4O$ (%), Calcd: C, 66.65; H, 4.79; N, 22.21; found: C, 66.61; H, 4.74; N, 22.17.

*Ethyl 3-methyl-6-oxo-4-phenyl-1,6-dihydropyrano[2, 3-c]pyrazole-5-carboxylate (**M11**)*

White powder; Yield 88%; m.p: 224-226 °C; IR (KBr) cm^{-1} 3384, 3034, 2984, 2938, 1730, 1707, 1604; ^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm): 12.21 (s, 1H), 7.30-7.34 (t, 2H), 7.21-7.24 (m, 1H), 7.16-7.18 (t, 2H), 4.22-4.27 (q, 2H), 2.19 (s, 3H), 1.17-1.21 (t, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 164.74, 161.27, 156.55, 155.82, 155.09, 143.83, 135.28, 128.55, 127.45, 126.73, 97.82, 61.63, 13.81, 10.73; EIMS, *m/z* $[M]^+$ calcd: 298.10; found: 298.09; Elemental Analysis for $C_{16}H_{14}N_2O_4$ (%), Calcd: C, 64.42; H, 4.73; N, 9.39; found: C, 64.35; H, 4.66; N, 9.37.

*Ethyl 6-amino-3-methyl-4-phenyl-1,4-dihydropyrano[2, 3-c]pyrazole-5-carboxylate (**M12**)*

White powder; Yield 89%; m.p: 242-244 °C; IR (KBr) cm^{-1} 3338, 3220, 3126, 3004, 2960, 2926, 2855,1744, 1658, 1600; ^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm): 12.14 (s, 1H), 7.71 (s, 2H), 7.27-7.30 (t, 2H, Ar-H), 7.18-7.21 (m, 1H, Ar-H), 7.13-7.14 (t, 2H, Ar-H), 5.04 (s, 1H), 4.17-4.22 (q, 2H), 1.89 (s, 3H), 1.24-1.28 (t, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 164.82, 159.46, 154.36, 143.63, 135.05, 128.07, 127.09, 126.36, 97.63, 61.27, 60.19, 34.31, 13.63, 10.54; EIMS, *m/z* $[M]^+$ calcd: 299.13; found: 299.12; Elemental Analysis for $C_{16}H_{17}N_3O_3$ (%), Calcd: C, 64.20; H, 5.72; N, 14.04; found: C, 64.15; H, 5.68; N, 13.99.

*6-amino-4-(furan-2-yl)-3-methyl-1,4-dihydropyrano[2, 3-c]pyrazole-5-carbonitrile (**M13**)*

White powder; Yield 90%; m.p: 230-232 °C; IR (KBr) cm^{-1} 3357, 3315, 3172, 2926, 2187, 1649, 1602; ^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm): 12.14 (s, 1H), 7.51-7.52 (d, *J*= 2 Hz, 1H), 6.94 (s, 2H), 6.35-

6.36 (m, 1H), 6.16-6.17 (d, *J*= 2.5 Hz, 1H), 4.76 (s, 1H), 1.96 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 161.49, 155.72, 154.81, 142.29, 135.83, 120.61, 110.25, 105.65, 95.11, 53.95, 29.81, 9.59; EIMS, *m/z* $[M]^+$ calcd: 242.08; found: 242.07; Elemental Analysis for $C_{12}H_{10}N_4O_2$ (%), Calcd: C, 59.50; H, 4.16; N, 23.13; found: C, 59.45; H, 4.12; N, 23.08.

*Ethyl 4-(furan-2-yl)-3-methyl-6-oxo-1,6-dihydropyrano[2, 3-c]pyrazole-5-carboxylate (**M14**)*

White powder; Yield 86%; m.p: 212-214 °C; IR (KBr) cm^{-1} 3388, 3091, 2987, 2938, 1729, 1704, 1607; ^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm): 12.30 (s, 1H), 7.50 (d, *J*= 2 Hz, 1H), 6.63-6.64 (m, 1H), 6.45-6.46 (d, *J*= 2.5 Hz, 1H), 4.15-4.19 (q, 2H), 2.10 (s, 3H), 1.19-1.23 (t, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 164.37, 161.64, 157.82, 157.28, 156.55, 152.36, 143.10, 142.01, 113.10, 108, 95.42, 61.45, 13.63, 10.36; EIMS, *m/z* $[M]^+$ calcd: 288.07; found: 288.05; Elemental Analysis for $C_{14}H_{12}N_2O_5$ (%), Calcd: C, 58.33; H, 4.20; N, 9.72; found: C, 58.27; H, 4.15; N, 9.67.

*Ethyl 6-amino-4-(furan-2-yl)-3-methyl-1,4-dihydropyrano[2, 3-c]pyrazole-5-carboxylate (**M15**)*

White powder; Yield 89%; m.p: 229-231 °C; IR (KBr) cm^{-1} 3419, 3129, 3040, 2988, 2935,1717, 1658, 1622; ^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm): 12.22 (s, 1H), 7.76 (s, 2H), 7.32-7.33 (d, *J*= 2 Hz, 1H), 6.48-6.49 (m, 1H), 6.29 (d, *J*= 2.5 Hz, 1H), 5.05 (s, 1H), 4.08-4.14 (q, 2H), 1.92 (s, 3H), 1.21-1.25 (t, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 164.73, 159.82, 155.45, 154.36, 142.01, 135.80, 110.18, 105.10, 95.02, 61.09, 59.64, 29.78, 13.36, 10.18; EIMS, *m/z* $[M]^+$ calcd: 289.11; found: 289.10; Elemental Analysis for $C_{14}H_{15}N_3O_4$ (%), Calcd: C, 58.13; H, 5.23; N, 14.53; found: C, 58.08; H, 5.17; N, 14.49.

*6-amino-3-ethoxy-4-(2-hydroxyphenyl)-1,4-dihydropyrano[2, 3-c]pyrazole-5-carbonitrile (**M16**)*

White powder; Yield 91%; m.p: 258-260 °C; IR (KBr) cm^{-1} 3398, 3351, 3187, 2926, 2855, 2203, 1619, 1566; ^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm): 11.23 (s, 1H), 9.62 (s, br, 1H), 7.26-7.28 (m, 1H), 7.1-7.12 (m, 2H), 7.03-7.04 (d, *J*= 8.5 Hz, 1H), 6.78 (s, 2H), 4.91 (s, 1H), 4.11-4.17 (q, 2H), 1.38-1.41 (t, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 160.62, 159.63, 158.80, 148.29, 131.09, 130.29, 123.60, 123.01, 120.18, 114.55, 104.91, 63.83, 55.11, 35.27, 13.45; EIMS, *m/z* $[M]^+$ calcd: 298.11; found: 298.10; Elemental Analysis for $C_{15}H_{14}N_4O_3$ (%), Calcd: C, 60.40; H, 4.73; N, 18.78; found: C, 60.35; H, 4.69; N, 18.74.

*6-amino-3-ethoxy-4-(p-tolyl)-1,4-dihydropyrano[2, 3-c]pyrazole-5-carbonitrile (**M17**)*

White powder; Yield 89%; m.p: 238-240 °C; IR (KBr) cm^{-1} 3447, 3417, 3351, 3058, 2986, 2921, 2862, 2182, 1624, 1567; ^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm): 12.15 (s, 1H), 7.16-7.18 (d, *J*= 9.5 Hz, 2H), 7.08-7.10 (d, *J*= 9.5 Hz, 2H), 6.90 (s, 2H), 5.12 (s, 1H), 4.07-4.13 (q, 2H), 2.21 (s, 3H), 1.34-1.38 (t, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 160.54, 154.36, 153.82, 135.45, 134.91, 128.73, 127.46, 120.37, 97.65, 63.40, 57.09, 35.45, 20.35, 13.12; EIMS, *m/z* $[M]^+$ calcd: 296.13; found: 296.11; Elemental Analysis for $C_{16}H_{16}N_4O_2$ (%), Calcd: C, 64.85; H, 5.44; N, 18.91; found: C, 64.79; H, 5.39; N, 18.86.

*6-amino-3-ethoxy-4-(4-methoxyphenyl)-1,4-dihydropyrano[2, 3-c]pyrazole-5-carbonitrile (**M18**)*

White powder; Yield 90%; m.p: 243-245 °C; IR (KBr) cm^{-1} 3373, 3311, 3170, 3021, 2925, 2850, 2191, 1649, 1599; ^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm): 12.06 (s, 1H), 7.05-7.09 (m, 2H), 6.86-6.89 (m, 2H), 6.83 (s, 2H), 5.04 (s, 1H), 4.17-4.22 (q, 2H), 3.71 (s, 3H), 1.35-1.39 (t, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 160.09, 159.07, 158.37, 154.65, 136.91, 136.36, 128.29, 121.09, 114.18, 98.47, 64.52, 58.18, 54.55, 35.11, 13.62; EIMS, *m/z* $[M]^+$ calcd: 312.12; found: 312.11; Elemental Analysis for $C_{16}H_{16}N_4O_3$ (%), Calcd: C, 61.53; H, 5.16; N, 17.94; found: C, 61.49; H, 5.11; N, 17.88.

*6-amino-3-ethoxy-4-phenyl-1,4-dihydropyrano[2, 3-c]pyrazole-5-carbonitrile (**M19**)*

White powder; Yield 91%; m.p: 276-278 °C; IR (KBr) cm^{-1} 3410, 3378, 3269, 3002, 2923, 2869, 2192, 1647, 1601; ^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm): 12.11 (s, 1H), 7.30-7.33 (t, 2H), 7.21-7.24 (m, 1H), 7.16-7.18 (t, 2H), 6.87 (s, 2H), 4.95 (s, 1H), 4.10-4.16 (q, 2H), 1.35-1.39 (t, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 160.70, 154.70, 154.18, 135.27, 128.18, 127.20, 126.47, 120.55, 97.35, 64.42, 57.04, 36.12, 13.46; EIMS, *m/z* $[M]^+$ calcd: 282.11; found: 282.11; Elemental Analysis for $C_{15}H_{14}N_4O_2$ (%), Calcd: C, 63.82; H, 5.00; N, 19.85; found: C, 63.79; H, 4.98; N, 19.82.

*6-amino-4-(anthracen-9-yl)-3-ethoxy-1,4-dihydropyrano[2, 3-c]pyrazole-5-carbonitrile (**M20**)*

Brownish white powder; Yield 86%; m.p: > 300 °C; IR (KBr) cm^{-1} 3456, 3396, 3251, 3050, 2923, 2205, 1671, 1623; ^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm): 11.91 (s, 1H), 8.19-8.21 (m, 2H), 8 (s, 1H), 7.66-7.69 (m, 2H), 7.44-7.49 (m, 2H), 7.38-7.43 (m, 2H), 6.79 (s, 2H), 5.20 (s, 1H), 4.12-4.18 (q, 2H), 1.34-1.37 (t, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 160.54, 154.54, 152.35, 133.46, 131.46, 130.91, 130.18, 127.82, 125.64, 122.31, 120.35, 116.36, 98.21, 63.24, 58.90, 29.45, 12.91; EIMS, *m/z* $[M]^+$ calcd: 382.14; found: 382.12; Elemental Analysis for $C_{23}H_{18}N_4O_2$ (%), Calcd: C, 72.24; H, 4.74; N, 14.65; found: C, 72.18; H, 4.66; N, 14.58.

*6-amino-3-ethoxy-4-(furan-2-yl)-1,4-dihydropyrano[2, 3-c]pyrazole-5-carbonitrile (**M21**)*

White powder; Yield 91%; m.p: 263-265 °C; IR (KBr) cm^{-1} 3434, 3339, 3206, 2929, 2856, 2206, 1630, 1577; ^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm): 12.16 (s, 1H), 7.53-7.54 (d, *J*= 2 Hz, 1H), 6.96 (s, 2H), 6.37-6.38 (m, 1H), 6.18 (d, *J*= 2.5 Hz, 1H), 4.92 (s, 1H), 4.08-4.14 (q, 2H), 1.39-1.43 (t, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 161.46, 156.90, 154.91, 153.64, 142.17, 120.37, 110.13, 105.42, 95.01, 64, 53.28, 29.56, 13.10; EIMS, *m/z* $[M]^+$ calcd: 272.09; found: 272.07; Elemental Analysis for $C_{13}H_{12}N_4O_3$ (%), Calcd: C, 57.35; H, 4.44; N, 20.58; found: C, 57.30; H, 4.38; N, 20.54.

*6-amino-3-methyl-4-(p-tolyl)-1,4-dihydropyrano[2, 3-c]pyrazole-5-carboxylic acid (**M22**)*

White powder; Yield 81%; m.p: > 300 °C; IR (KBr) cm^{-1} 3464-3186, 3413, 3333, 3278, 3006, 2976, 2918, 1707,1661, 1598; ^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm): 12.88 (s, br, 1H), 12.09 (s, 1H), 7.65 (s, 2H), 7.24-7.26 (d, *J*= 8.5 Hz, 2H), 7.17-7.19 (d, *J*= 8.5 Hz, 2H), 5.08 (s, 1H), 2.28 (s, 3H), 1.8 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 174.55, 161.66, 154.33, 142.01, 135.84, 135.34, 128.68, 127.46, 103.33, 60.72, 33.45, 20.67, 10.37; EIMS, *m/z* $[M]^+$ calcd: 285.11; found: 285.09; Elemental Analysis for $C_{15}H_{15}N_3O_3$ (%), Calcd: C, 63.15; H, 5.30; N, 14.73; found: C, 63.08; H, 5.21; N, 14.66.

*6'-amino-3'-methyl-2-oxo-1'H-spiro[indoline-3,4'-pyrano[2, 3-c]pyrazole]-5'-carbonitrile (**M23**)*

White powder; Yield 82%; m.p: 279-281 °C; IR (KBr) cm^{-1} 3389, 3339, 3259, 3141, 2183, 1714, 1641, 1617; ^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm): 12.27 (s, 1H), 10.59 (s, 1H), 7.22-7.25 (m, 3H), 6.97-7.03 (m, 2H), 6.89-6.90 (d, *J*= 7.5 Hz, 1H), 1.52 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 178.04, 162.49, 155.28, 141.52, 134.72, 132.70, 128.91, 124.53, 122.52, 118.76, 109.67, 95.40, 55.15, 47.29, 8.97; EIMS, *m/z* $[M]^+$ calcd: 293.09; found: 293.08; Elemental Analysis for $C_{15}H_{11}N_5O_2$ (%), Calcd: C, 61.43; H, 3.78; N, 23.88; found: C, 61.38; H, 3.72; N, 23.84.

*Ethyl 6'-ethoxy-3'-methyl-2-oxo-1'H-spiro[indoline-3,4'-pyrano[2, 3-c]pyrazole]-5'-carboxylate (**M24**)*

White powder; Yield 78%; m.p: 240-242 °C; IR (KBr) cm^{-1} 3357, 3203, 2992, 2934, 1728, 1705, 1655, 1615; ^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm): 12.18 (s, 1H), 10.38 (s, 1H), 7.01-7.04 (m, 3H), 6.98-6.99 (d, *J*= 6 Hz, 1H), 4.04-4.07 (q, *J*= 7 Hz, 2H), 3.88-3.91 (q, *J*= 7 Hz, 2H), 1.60 (s, 3H), 1.13-1.16

(t, *J*= 7 Hz, 3H), 0.95-0.98 (t, *J*= 7 Hz, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 169.16, 167.27, 163.64, 155, 142.73, 136.84, 134, 128.37, 124, 122.36, 109.46, 97.34, 74.18, 62.83, 61.82, 48.83, 14.17, 13.09, 9.33; EIMS, *m/z* $[M]^+$ calcd: 369.13; found: 369.16; Elemental Analysis for $C_{19}H_{19}N_3O_5$ (%), Calcd: C, 61.78; H, 5.18; N, 11.38; found: C, 61.72; H, 5.12; N, 11.33.

*Ethyl 6'-amino-3'-methyl-2-oxo-1'H-spiro[indoline-3,4'-pyrano[2, 3-c]pyrazole]-5'-carboxylate (**M25**)*

White powder; Yield 80%; m.p: 278-280 °C; IR (KBr) cm^{-1} 3384, 3352, 3152, 3088, 2990, 2915, 1730, 1706, 1680, 1620; ^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm): 12.17 (s, 1H), 10.37 (s, 1H), 8.02 (s, 2H), 7-7.04 (m, 3H), 6.97-6.98 (d, *J*= 6 Hz, 1H), 3.87-3.91 (q, 2H), 1.58 (s, 3H), 0.99-1.26 (t, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 180.15, 168.80, 163.46, 154.84, 142.91, 136, 134.17, 128.18, 123.81, 122.18, 109.28, 97.16, 74.55, 61.09, 49, 13.64, 9.27; EIMS, *m/z* $[M]^+$ calcd: 340.12; found: 340.14; Elemental Analysis for $C_{17}H_{16}N_4O_4$ (%), Calcd: C, 60.00; H, 4.74; N, 16.46; found: C, 59.94; H, 4.69; N, 16.41.

*4-(furan-2-yl)-3-methyl-4,6-dihydropyrazolo[4',3':5,6]pyrano[2,3-d]pyrimidin-5(1H)-one (**M26**)*

Brown powder; Yield 85%; m.p: 250-252 °C; IR (KBr) cm^{-1} 3406, 3227, 3088, 2970, 1680, 1634, 1597; ^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm): 12.20 (s, 1H), 11.16 (s, 1H), 8.52 (s, 1H), 7.41-7.42 (d, *J*= 2 Hz, 1H), 6.24-6.25 (m, 1H), 6.06 (d, *J*= 2.5 Hz, 1H), 5.20 (s, 1H), 2.08 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 162.58, 157.79, 156, 154.55, 150.52, 142.90, 136.35, 111.45, 106.90, 99.61, 98.90, 30.31, 10.15; EIMS, *m/z* $[M]^+$ calcd: 270.08; found: 270.06; Elemental Analysis for $C_{13}H_{10}N_4O_3$ (%), Calcd: C, 57.78; H, 3.73; N, 20.73; found: C, 57.72; H, 3.69; N, 20.68.

*3-methyl-4-phenyl-4,6-dihydropyrazolo[4',3':5,6]pyrano[2,3-d]pyrimidin-5(1H)-one (**M27**)*

White powder; Yield 86%; m.p: 264-266 °C; IR (KBr) cm^{-1} 3193, 3103, 3020, 2915, 1679, 1633, 1595; ^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm): 12.12 (s, 1H), 11.13 (s, 1H), 8.34 (s, 1H), 7.34-7.37 (t, 2H), 7.25-7.28 (m, 1H), 7.20-7.22 (t, 2H), 5.22 (s, 1H), 2.24 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 161.83, 159.27, 155.82, 151.46, 144.91, 135.82, 128.91, 127.82, 127.09, 99.45, 98.72, 36.54, 10.71; EIMS, *m/z* $[M]^+$ calcd: 280.10; found: 280.11; Elemental Analysis for $C_{15}H_{12}N_4O_2$ (%), Calcd: C, 64.28; H, 4.32; N, 19.99; found: C, 64.23; H, 4.27; N, 19.94.

*4-(furan-2-yl)-3-methyl-1,4-dihydropyrazolo[4',3':5,6]pyrano[2,3-d]pyrimidin-5-amine (**M28**)*

Brown powder; Yield 84%; m.p: 268-270 °C; IR (KBr) cm^{-1} 3410, 3245, 2924, 1630, 1591; ^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm): 12.19 (s, 1H), 8.35 (s, 1H), 7.61 (d, *J*= 2 Hz, 1H), 7.04 (s, 2H), 6.36-6.37 (m, 1H), 6.17-6.18 (d, *J*= 2.5 Hz, 1H), 5.21 (s, 1H), 2.16 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 163.64, 159.11, 157.21, 155.82, 152.91, 143.09, 136.54, 112.38, 107.81, 100.54, 99.62, 31.24, 10.34; EIMS, *m/z* $[M]^+$ calcd: 269.09; found: 269.11; Elemental Analysis for $C_{13}H_{11}N_5O_2$ (%), Calcd: C, 57.99; H, 4.12; N, 26.01; found: C, 57.93; H, 4.07; N, 25.97.

*3-methyl-4-phenyl-1,4-dihydropyrazolo[4',3':5,6]pyrano[2,3-d]pyrimidin-5-amine (**M29**)*

White powder; Yield 86%; m.p: 284-286 °C; IR (KBr) cm^{-1} 3378, 3208, 2923, 1627, 1566; ^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm): 12.13 (s, 1H), 8.39 (s, 1H), 7.32-7.35 (t, 2H), 7.21-7.26 (m, 1H), 7.18-7.19 (t, 2H), 6.85 (s, 2H), 5.19 (s, 1H), 2.18 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 163.10, 158.54, 156.54, 155.28, 145.27, 136.19, 129.09, 128.17, 127.46, 100.18, 99.45, 37.45, 10.91; EIMS, *m/z* $[M]^+$ calcd: 279.11; found: 279.08; Elemental Analysis for $C_{15}H_{13}N_5O$ (%), Calcd: C, 64.51; H, 4.69; N, 25.07; found: C, 64.46; H, 4.63; N, 25.02.

*4-(furan-2-yl)-3,7-dimethyl-4,6-dihydropyrazolo[4',3':5,6]pyrano[2,3-d]pyrimidin-5(1H)-one (**M30**)*

Brown powder; Yield 87%; m.p: 280-282 °C; IR (KBr) cm^{-1} 3451, 3123, 3025, 2933, 1674, 1643, 1603; ^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm): 12.21 (s, 1H), 11.51 (s, 1H), 7.52 (d, *J*= 2 Hz, 1H), 6.35-6.36 (m, 1H), 6.16-6.17 (d, *J*= 2.5 Hz, 1H), 5.19 (s, 1H), 2.24 (s, 3H), 1.91 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 162.90, 157.36, 156.37, 155.63, 153.10, 143.10, 138.91, 112.17, 107.45, 99.80, 99.07, 30.52, 20.18, 10.34; EIMS, *m/z* $[M]^+$ calcd: 284.09; found: 284.08; Elemental Analysis for $C_{14}H_{12}N_4O_3$ (%), Calcd: C, 59.15; H, 4.26; N, 19.71; found: C, 59.10; H, 4.21; N, 19.67.

*3,7-dimethyl-4-phenyl-4,6-dihydropyrazolo[4',3':5,6]pyrano[2,3-d]pyrimidin-5(1H)-one (**M31**)*

White powder; Yield 88%; m.p: 275-277 °C; IR (KBr) cm^{-1} 3454, 3225, 3097, 2925, 2851, 1667, 1628, 1601; ^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm): 12.23 (s, 1H), 11.50 (s, 1H), 7.22-7.25 (t, 2H), 7.13-7.16 (m, 1H), 7.08-7.10 (t, 2H), 5.18 (s, 1H), 2.23 (s, 3H), 1.92 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 162.37, 158, 155.82, 155.09, 143.09, 136.01, 129.09, 128, 127.27, 99.64, 98.91, 36.18, 20.90, 10.90; EIMS, *m/z* $[M]^+$ calcd: 294.11; found: 294.08; Elemental Analysis for $C_{16}H_{14}N_4O_2$ (%), Calcd: C, 65.30; H, 4.79; N, 19.04; found: C, 65.25; H, 4.73; N, 18.99.

4.4. Biological evaluation

The diverse biological efficiencies of pyrano[2, 3-*c*]pyrazole derivatives inspired us to evaluate the antimicrobial efficacy of the new synthetic compounds.

4.4.1 Antimicrobial screening

Many antimicrobial agents have now been applied for treatment; In order to get over the highly resistant species of the microorganisms, the medical field still needs widespread efforts to develop new antimicrobial agents. All the synthetic compounds were tested *in vitro* by broth dilution assay, as minimum inhibitory concentration (MIC), for their anti-bacterial and antifungal activity. For every standard strain acquired from Gene Bank and the Microbial Type Culture Collection (MTCC), Institute of Microbial Technology, Chandigarh, India, triplicates were done.

Culture media: For this study, nine microorganisms were chosen: Gram-negative bacteria (*Salmonella typhi* (*S. typhi*) MTCC 98, *Pseudomonas aeruginosa* (*P. aeruginosa*) MTCC 1688, and *Vibrio cholerae* (*V. cholerae*) MTCC 3906), Gram-positive bacteria (*Bacillus subtilis* (*B. subtilis*) MTCC 441, *Clostridium tetani* (*C. tetani*) MTCC 449, and *Streptococcus pneumoniae* (*S. pneumoniae*) MTCC 1936), and three strains of fungi (*Candida krusei* (*C. krusei*) MTCC 3020, *Aspergillus fumigatus* (*A. fumigatus*) MTCC 3008, and *Aspergillus niger* (*A. niger*) MTCC 282) taking ciprofloxacin, griseofulvin, and nystatin as standard antibiotics. Prior to the vehicle testing, all microorganisms were previously subcultured under gaseous conditions and in suitable media (HIMEDIA M210-500) for 48 h at 35 °C to check their purity.

Inoculum preparation: The growth method was carried out as follows. From an agar culture plate, at least three to five well-isolated colonies have been chosen with the same morphology. A loop was used to scoop the top of each colony, followed by transporting the growth into a tube holding 5 mL of Brain Heart Infusion (BHI) broth. At 35 °C, the incubation of the broth culture lasted for 2-6 h, till accomplishing the 0.5 turbidity of McFarland reference. To achieve definitive turbidity optically analogous to the 0.5 turbidity of McFarland reference, the effectively developing turbidity of the broth culture was modified utilizing broth, and this was visually performed by comparing the standard and the inoculum tube versus a white card involving contradicting black lines.

4.4.1.1 Broth dilution assay

A total of 10 tubes were prepared, and nine vehicle dilutions were performed with BHI for MIC. In the first tube, just 200 µL of the vehicle was applied. 200 µL of the broth of BHI was individually applied to the subsequent nine tubes for further dilutions. 200 µL of the vehicle inclusive 200 µL of BHI broth was appended to the second tube and regarded as 10 dilution. 10 dilution was made from the 10 mitigated tube by transfer of 200 µL to the second tube. For each vehicle, the sequent dilution was replicated over until 10 dilution. 5 µL was taken from the required microorganisms preserved stock cultures and applied to 2 mL of the broth of BHI. 200 µL of the preceding culture suspension was inserted in each sequentially diluted tube. Only the culture suspension and the media were included in the last tube as a negative control. For incubation, the tubes were reserved in a bacteriological incubator for 24 h at 37 °C, and the turbidity was detected and observed.

4.4.2 Computational Studies

The structures of the all-tested compounds were modelled utilizing the Chemsketch software (http://www.acdlabs.com/resources/ freeware/). Using VEGA ZZ software, the structures were enhanced and energy minimalized.[47] The optimized compounds were applied to execute molecular docking against both CDK2 and penicillin-binding protein. From Protein Data Bank (PDB) (www.rcsb.org): (PDB: 3UDI, https://www.rcsb.org/structure/3UDI), and (PDB: 1DI8, https://www.rcsb.org/structure/1di8), the molecular target three-dimensional structure was gained. The eradicating of heteroatoms (water and ions), the charge assignment, and adding polar hydrogen were comprised in the receptor preparation steps. The suitable sized-grid boxes round the restrictive cocrystal ligands were used to define the active sites. Autodock vina and Chimera were utilized for visualization in performing the docking study.[48, 49]

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