ISSN: 0975-833X

Available online at http://www.journalcra.com

International Journal of Current Research
Vol. 11, Issue, 07, pp.5563-5570, July, 2019

DOI: https://doi.org/10.24941/ijcr.36015.07.2019

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

RESEARCH ARTICLE

SYNTHESIS, CHARACTERIZATION, AND ANTIMICROBIAL ACTIVITY OF PYRAZOL-3-YL-PYRIMIDINE, PYRAZOLE AND PYRAN DERIVATIVES

[1]Mohamed A. El Sekily, [2]Sohila H. Mancy and [2,*] Nagwa M. M. Hamada

[1]Department of Chemistry, Faculty of Science, University of Alexandria, 21321 Alexandria, Egypt
[2]Department of Physics and Chemistry, Faculty of Education, University of Alexandria, 21526 Alexandria, Egypt

ARTICLE INFO

Article History:
Received 29th April, 2019
Received in revised form
10th May, 2019
Accepted 15th June, 2019
Published online 31st July, 2019

Key Words:

Pyrimidine,
Pyrazole,
Pyran,
Antibacterial,
Antifungal.

Corresponding author:* **Nagwa M. M. Hamada

ABSTRACT

A series of novel pyrazol-3-yl-pyrimidine, pyrazole, and pyran derivatives have been synthesized in good yields. Thus, the reaction of 5-oxo-1-phenyl-4-(2-phenylhydrazono)-4.5-dihydro-1H-pyrazole-3-carbaldehyde(1) with malononitrile mixture with thiourea, guanidine hydrochloride, barbituric acid, thiosemicarbazide, or phenyl thiosemicarbazide in one-pot synthesis produced the corresponding compounds (2-6). treatment of (2) with o-bromobenzoic acid gave compound (7). Similarly, treatment of (1), malononitrile mixture with hydrazine hydrate, o-nitrophenyl hydrazine, isonicotinoylhydrazine, p-sulphamylphenylhydrazine, or phenyl hydrazine yielded the corresponding (8-12). In the same time, treatment of (1), malononitrile mixture with cyclohexanone, dimedone or acetophenone, afforded the pyrazolyl-pyran derivatives (13-15). In addition, treatment of (15) with methylhydrazine gavemethylaminopyridine-5-carbonitrilederivative (16). The Reaction of (1), malononitrile mixture with hydroxylamine hydrochloride gave 2, 3-dihydroisoxazole-4-carbonitrile derivative (17) and with oxamic acid gave5, 6-dioxo-5, 6-dihydropyridine-5-carbonitrilederivative (18). The structure of all target molecules (2-18) have been confirmed by various spectral techniques and elemental analyses. The synthesized compounds were screened for antimicrobial activity.

Citation: Mohamed A. El Sekily, Sohila H. Mancy and Nagwa M. M. Hamada, **2019.** "Synthesis, characterization, and antimicrobial activity of pyrazol-3-yl-pyrimidine, pyrazole and pyran derivatives", *International Journal of Current Research*, 11, (07), 5563-5570.

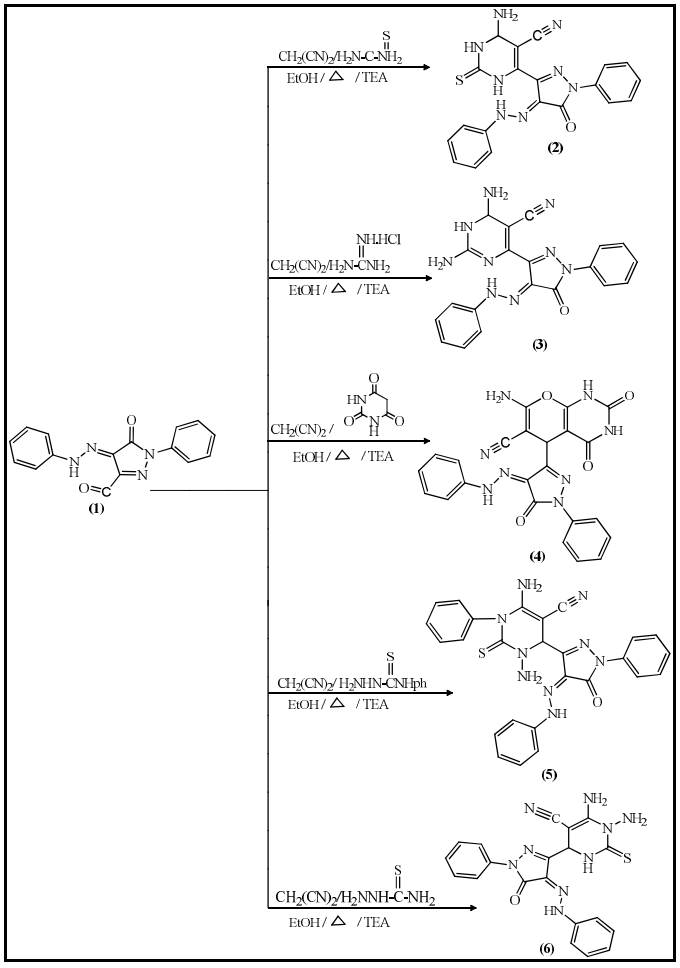
INTRODUCTION

Nitrogen, oxygen and Sulphur containing heterocyclic compounds are of biological interest due to their potential physical and chemical properties (Aziza *et al.,* 1996; Vishnu *et al.,* 2003; Clark *et al.,* 1993; Brown, 1998). Among these the pyrimidine compounds which occupy a unique position in pharmaceutical chemistry, as they are compounds of nucleic acids. The important pyrimidine compounds have diverse applications as bactericidal (Pershin *et al.,* 1972), fungicidal (Metolcsy, 1971) analgesics (Regnier *et al.,* 1972), anti-inflammatory (Winter *et al.,* 1962), and antitumor (Suguira *et al.,* 1973) agents. In the same time pyrazole nucleus has pronounced pharmacological applications as anti-anxiety (Haufel *et al.,* 1974), antimicrobial (Gilbert *et al.,* 2006; Prakash *et al.,* 2009; Isloor *et al.,* 2009), anticancer (Mayedov *et al.,* 2007), anti-inflammatory (Szabo *et al.,* 2008; Benaamane *et al.,* 2008), antidepressant (Prasad *et al.,* 2005), anticonvulsant (Ozdemir *et al.,* 2007), antipyretic (Sener *et al.,* 2002), and selective enzyme inhibitory activity (Wachter *et al.,* 1996). Encouraged by the above observations and in continuation of our research work on synthesis of heterocyclic biologically active molecules (El Sekily *et al.,* 2017 & 2018; Hamada *et al.,* 2018), we have designed and synthesized a series of novel pyrazolyl pyrimidine, 2-7 and pyrazolyl pyrazole 8-12 and pyrazolyl pyran (13-15) derivatives via one step multi component reaction using 5-oxo-1-phenyl-4-(2-phenylhydrazone)-4, 5-dihydro-1H-pyrazol-3-yl)-3-carbaldehyde 1, (El Sekily *et al.,* 1999 & 2000) with the hope to get antimicrobial agents. In the present time, one pot synthesis involving three components condensation using different reagents and catalysis (Patil *et al.,* 2010; Malladia *et al.,* 2012) are popular in the synthesis of heterocyclic compounds. These single step methods are more convenient as compared with two steps strategies as they require shorter reaction times and give higher yields. The newly synthesized compounds have been structurally established by modern analytical tools, FTIR, NMR spectroscopy mass spectrometry and elemental analysis methods.

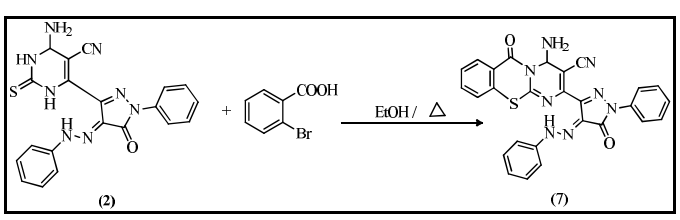
RESULTS AND DISCUSSION

Chemistry: Reaction of 5-oxo-1-phenyl-4-(2-phenylhydrazoneo)-4, 5-dihdro-1H-pyrazole-3-paraldehyde 1 (one mole), malononitrile (one mole) and few drops of triethylamine (TEA) in absolute ethanol in one-step synthesis with thiourea, guanidine hydrochloride, barbituric acid, phenyl thiosemicarbazide or thiosemicarbazide and heating at reflux, afforded the corresponding pyrazolyl pyrimidine derivatives (2-6, Scheme 1). The crude obtained were purified by

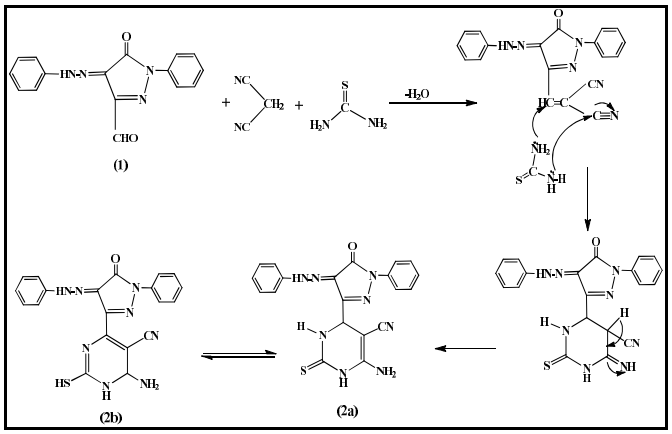
recrystallization from ethanol in 70-80% yield. The reaction of 2 with o-bromobenzoic acid afforded 7. The structure of compounds 2-7 were confirmed by IR, ^{1}H-NMR, MS spectroscopy and elemental analyses. The IR of 2-7 are characterized by the presence of absorption bands within the region 3360-3100 cm^{-1} corresponding to the stretching vibrations of the NH groups. The band at 2222-2225 cm^{-1} were corresponding to the C≡N groups. The structures of compounds (2, 3, 5 and 7) get further support from mass spectroscopy.It has been observed that electron impact (El) spectra have many common features. The first of which is that the highest recorded peak representing the molecular ion peaks at m/z 416, 399, 431& 518 respectively. The second is the other different fragments in their El fragmentation patterns. represented in (Schemes S1, S2, S3, and S4) as supplementary materials. Mechanistic pathway for the formation of compound 2, as an example of the synthetic routes of our compounds, showed that during the progress of reaction, the activated malononitrile is likely to be formed via a Knoevenagel condensation reaction of aromatic aldehydes andmalononitrile, followed by further reaction with thiourea viasimultaneousnucleophilic cycloaddition reaction to give compound 2 (Scheme 2).



Scheme 1. Synthetic routes for compounds (2-6)



Equation 1. Synthesis of compound (7)



Scheme 2. A plausible mechanistic pathway to explain the formation of compound (2)

The IR spectrum of compound 2 confirms the presence of the C=S group at ν=1216 cm^{-1} (1226 calculated) cm^{-1}.Tautomeric structures for compound 2 was confirmed by its ^{1}H-NMR which showed the presence of thiol proton at δ=1.93 (s, 1H, SH), the formation of the pyrimidine ring was confirmed by the presence of singlet proton at δ= 7.89 (s, 1H, pyrimidine C4-H) and the imino proton at δ= 9.2 ppm (s, 1H, NH).Mass spectrum of compound 2also confirm the presence the three tautomers (2a, 2b, 2c, Figure 1), specific fragmentations must be evaluated, from the interpretation of the mass spectrum of compound 2 the data shows the peak at m/z=383 (32.45%) [M-SH] was assigned to the thiol form 2b and the peak at m/z=384 (14.32%) [M-S] was assigned to the thione form 2aand the ion at m/z=383 (M-SH) loss of HCN to give the ion m/z=356 (11.42%) which confirm the structure of tautomer 2c.

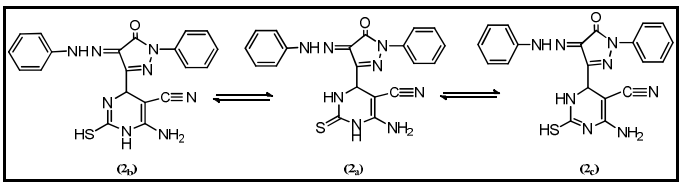
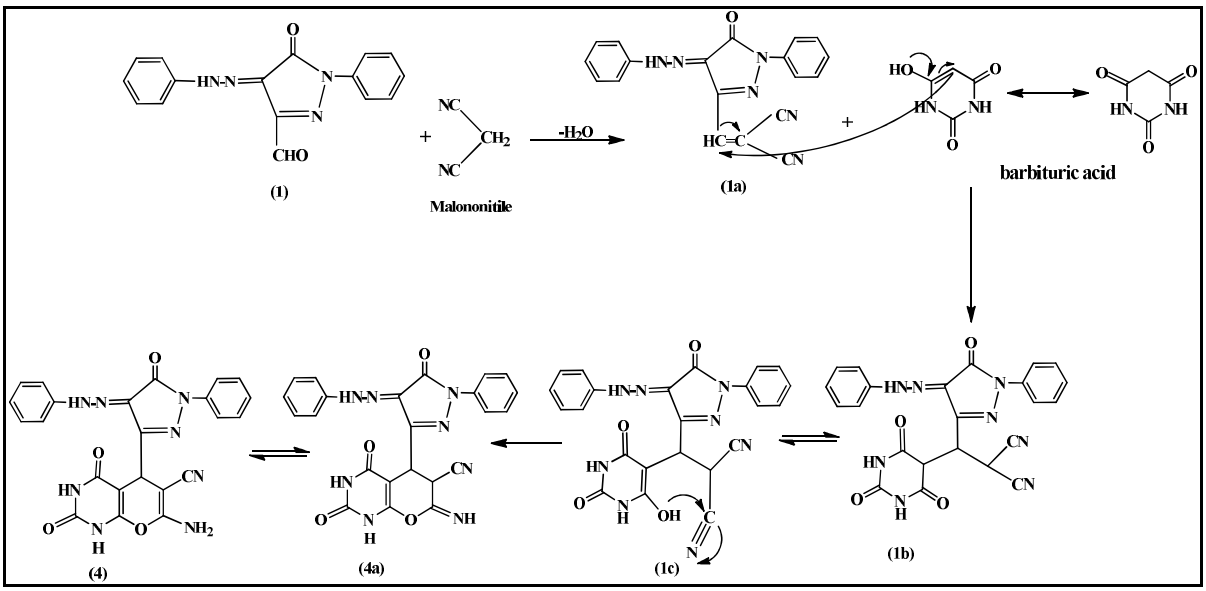


Figure 1. Tautomers of compound (2)

Another example for the mechanistic path ways of our synthesized compounds was the formation of compound 4 via the reaction of the pyrazol aldehyde 1, malononitrile and barbituric acid A possible mechanism is outlined in Scheme 3. The reaction may proceed at first via a Knoevenagel condensation of aldehyde 1 with malononitrile to afford the Michael acceptor 1a. The active methylene of barbituric acid reacted then via its enol form with 1a in a Michael addition reaction to give the intermediate 1b, which is then tautomerized to 1c. Intramolecular cyclative condensation of 1c gave 4a. Finally, the tautomerization of 4a afforded the expected product 4. The infrared spectrum of compound 4 showed different characteristic bands at ν = 3218, 3182 (NH_2), 2224 (C≡N), 1746 (C=O), 1659 (OCN), 1600 cm-1 (C=N). The ^{1}H-NMR showed the presence of the hydrazone NH at δ= 13.64 , the C5-H of the pyran ring appear at δ=8.22 which confirm the *syn* Michael addition and the *Z*-conformer of compound 4. Furthermore, treatment of 1, malononitrile mixture with hydrazine hydrate, p-nitrophenyl hydrazine, isonicotinoyl hydrazine, p-sulphamylphenylhydrazine or phenyl hydrazine and few drops of TEA then heating the mixture at reflux for 4h, afforded the corresponding pyrazolyl pyrazole derivatives 8-12 (Scheme 4) in 70-80% yield.



Scheme 3. A plausible mechanistic pathway to explain the formation of compound (4)

The crude products were purified by recrystallization from dioxane–ethanol mixture. The structure of 8-12 was achieved from their elemental analyses and spectral data. Their IR showed the appearance of C≡N bands at 2222-2225 cm^{-1} in addition to NH bands between 3340-3100 cm^{-1}. The mass spectra of compounds (8-12) showed molecular ion peaks in agreement with the assigned structures, for example the fragmentation pattern routes for compound (9) are shown in (Scheme S5) as supplementary materials. The reaction of compound 1, with malononitrile one molar each with cyclohexanone, dimeclone or acetophenone and drops of (TEA) in absolute ethanol and heating at reflux, gave compounds (13-15, Scheme 5) where their structure was also confirmed by elemental analyses and spectral data. The mass spectra of 13-15 showed the molecular ion peaks in agreement with the assigned structures, as an example, the mass spectral fragmentation of compound 15 is shown in Scheme S6 as supplementary materials. Moreover, the reaction of 2-Amino-4-(5-oxo-1-phenyl-4-(2-phenylhydrazono)-4, 5-dihydro-1H-pyrazol-3-yl)-6-phenyl-4H-pyran-3-carbonitrile 15 with methylhydrazine, gave compound2-amino-4-(5-oxo-1-phenyl-4-(2-phenyl-hydrazone) -4, 5-dihydro-1H-pyrazol-3-yl)-6-phenyl-1, 4-dihydro-1-methylaminopyridine-5-cabonitrile 16 whose structure was also confirmed by elemental analysis and spectral data. Its MS show a molecular ion peak in agreement with the assigned structure. The reaction of 1, malononitrile mixture with hydroxylamine hydrochloride, gave 5-amino-3-(5-oxo-1-phenyl-(2-phenylhydrazono)-4, 5-dihydro-1H-pyrazol-3-yl)-1, 4-dihydro-2, 3-isoxazole-4-carbonitrile 17 whose IR revealed an NH bands at 3360, 3152 cm^{-1} and C≡N at 2222 cm^{-1}. Finally, reaction of 1, malononitrile mixture with oxamic acid, gave 2-amino-4-(5-oxo-1-phenyl-4-(2-phenylhydrazono)-4, 5-dihydro-pyrazol-3-yl)-5, 6-dioxopyridine-3- carbonitrile 18. All the target compounds (2-18) were screened for their antimicrobial activity against various microorganisms.

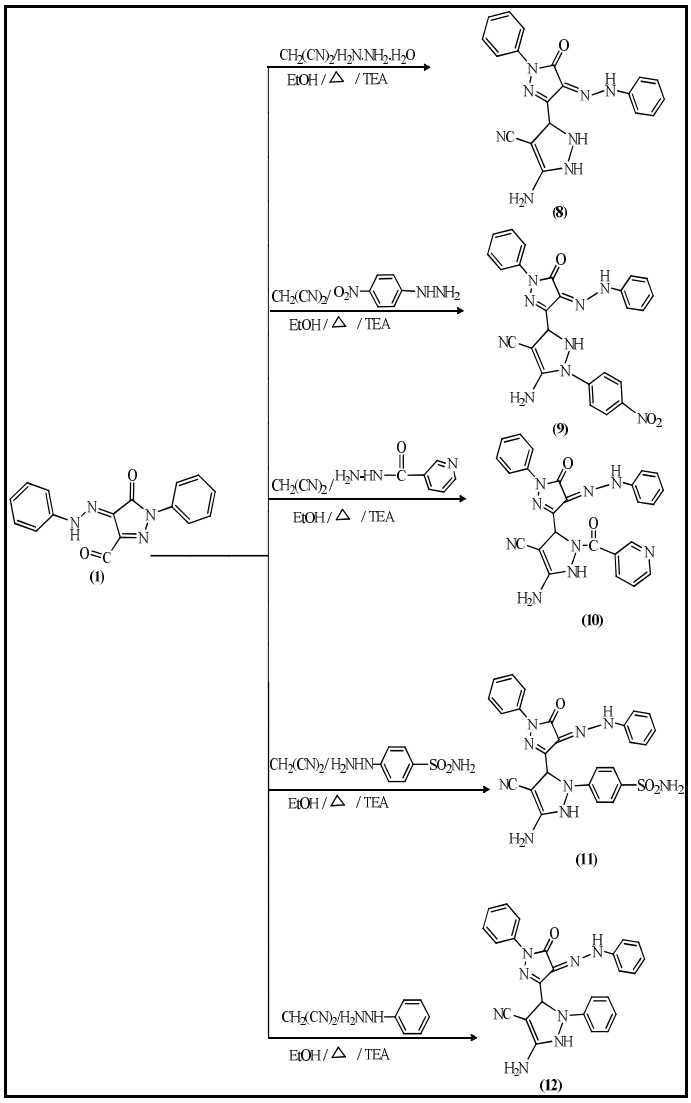
Antimicrobial activity

The antimicrobial activity of some of the synthesized pyrazol-3-yl-pyrimidine, pyrazole and pyran derivatives 3-6, 8-18 were tested against Gram-negative and Gram-positive bacterial strains *Escherichia coli, Staphylococcus aureus* and *Candida albicans* as a fungi strain. The activities were determined *in vitro* using The agar well-diffusion method (Clinical and Laboratory Standards Institute, 2006). Also, a comparison between the activity of our synthesized compounds and Ciprofloxacin and Clotrimazole as standard drugs was discussed. Antibacterial results (Table 1) indicated that the synthesized componds 3, 4, 5, 11, 14, 16 and 18 showed a slight activity against Gram-positive bacteria due to the presence of the cyano and the amino groups in all the studied pyrazole, pyrimidine and pyran derivatives. Furthermore compound 4showed the more active towards both *Staphyllococcus aureus* bacteria and *Candidaalbicans* due to the presence of two fused pyran and pyrimidine rings.

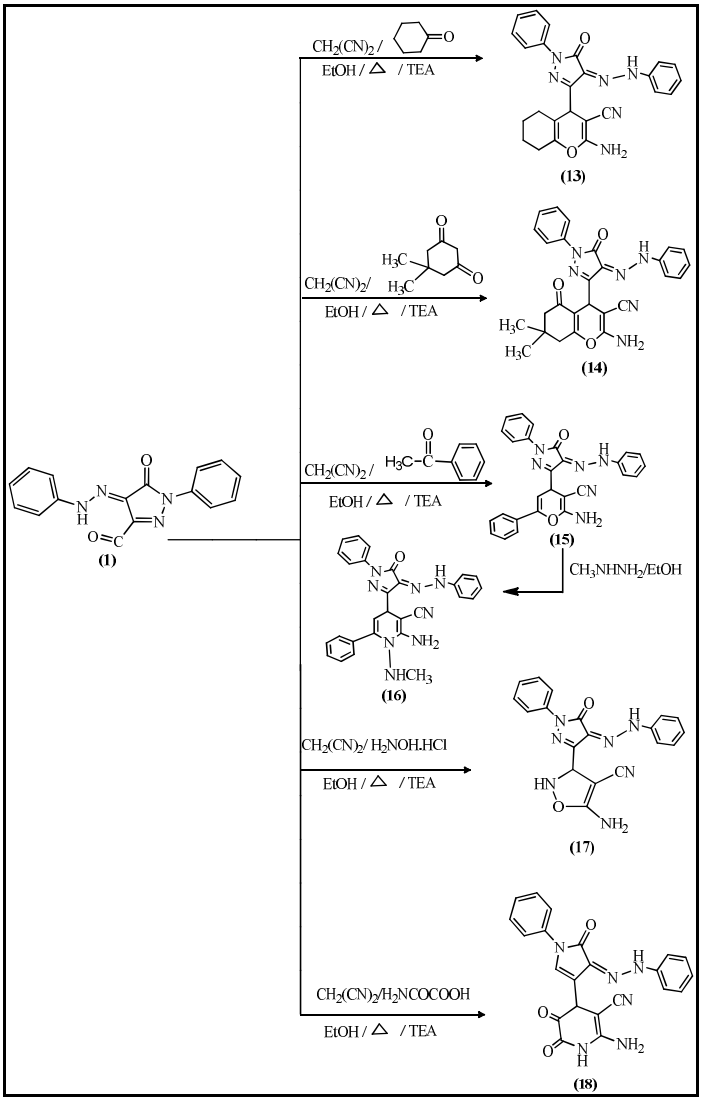
MATERIALS AND METHODS

General Methods: Melting points were carried out on a Tottoli (Büchi) apparatus and are not corrected. IR (KBr) were recorded on Perkin-Elmer 580 VB spectrophotometer and ^{1}H- NMR spectra ($CDCl_3$) and (DMSO-d_6) on Camica 250 Hz spectrometer using TMS as an internal standard. Microanalyses were performed in micro analytical units, department of Chemistry, Faculty of Science, Cairo University, Cairo, Egypt. Mass spectra were performed at the Regional Center for Mycology and Biotechnology Al-Azhar University, Cairo, Egypt., they were recorded with an LKB model 2091 mass spectrometer and intensities are given in parentheses as percentage of the base peaks, 5-oxo-1-phenyl-4-(2-phenylhydrazono)-4, 5-dihydro-1H-pyrazol-3-yl)-3-carbaldehyde was prepared according to the literature procedure, m.p. 140-141^{o}C ; Lit. m.p. 140-141^{o}C[25, 26].The antimicrobial activities were determined at the Department of PharmaceuticalMicrobiology, Faculty of Pharmacy, Alexandria University. Reaction progress was monitored by thin-layer chromatography (TLC) on silica gel 60 f 254 plates.

General Procedure: A mixture of 5-oxo-1-phenyl-4-(2-phenylhydrazono)-4, 5-dihydro-1H-pyrazol-3-yl)-1H-kpyrazole-3-carbaldehyde (1) (1 mmol), malononitrile (1 mmol) and few drops of (TEA) in absolute ethanol (40 ml) was treated with thiourea, guanidine hydrochloride, barbituric acid, phenyl thiosemicarbazide or thiosemicarbazide (1 mmol) each.



Scheme 4. Synthetic routes for compounds (8-12)



Scheme 5. Synthetic routes for compounds (13-18)

Table 1. Antimicrobial activity of the synthesized compounds* (3-6, 8-18)

Cpd.NO	Staphyllococcs aureus *ATCC6538P*		Escherischia coli *ATCC8739*		Candida albicans *ATCC2091*	
	DMF	Cpd.	DMF	Cpd.	DMF	Cpd.
3	13	16	13	13	13	13
4	13	14	13	13	13	17
5	13	16	13	13	13	13
6	13	13	13	13	13	15
8	13	13	13	13	13	15
9	13	13	13	13	13	15
10	13	13	13	13	13	15
11	13	15	13	13	13	15
12	13	13	13	13	13	15
13	13	13	13	13	13	15
14	13	15	13	13	13	13
15	13	13	13	13	13	15
16	13	14	13	13	13	17
17	13	13	13	13	13	15
18	13	14	13	13	13	15
Ciprofloxacin	13	30	13	30	-	-
Clotrimazole	-	-	-	-	12	22

The resulting reaction mixture was heated at reflux using water bath for 4h and evaporated under reduced pressure and left to cool. The resulting solid was collected by filtration, washed with ethanol, dried and recrystallized from dioxane-ethanol mixture to give needles. The physical characters and spectral data of compounds (2-7) are listed below.

6-amino-4-(5-oxo-1-phenyl-4-(2-phenylhydrazono)-4, 5-dihydro-1H-pyrazol-3-yl)-2-thioxo-1, 2- dihydropyrimidine-5-carbonitrile (2): red needles, (86%): m.p. 258-259°C; ^{1}H-NMR (DMSO-d_6): δ= 13.54 (s, 1H, hydrazone NH), 7.89 (s, 1H, pyrimidine C_4H), 7.47-7.87 (m, 10H, ArH), 3.35 (br.s, 2H, NH_2), 1.93 (s, 1H, SH); IR (KBr); ν = 3162 (NH), 3076 (CH, Ar), 2223 (C≡N), 1680 (OCN), 1216 cm^{-1}(CS); MS: *m/z* (%); 416 (4.51), 411(30.03), 410 (100), 384 (14.32), 383 (32.45), 356 (11.42), 411 (30.03), 355 (25.58), 309 (19.29), 265 (11.47), 148 (13.66), 93 (11.75), 77 (32.74); elemental analysis: calcd (%) for C_{20} H_{16} SN_8 O (416.4): C 57.68, H 3.87, N 26.41(%), found: C 57.49, H 3.61, N 26.74.

2, 6-diamino-4-(5-oxo-1-phenyl-4-(2-phenylhydrazono)-4, 5-dihydro-1*H*-pyrazol-3-yl)-1, 6-dihydropyrimidine-5-carbonitrile (3): red needles, (72%): m.p. 240-241°C; ^{1}H NMR (DMSO-d_6): δ= 13.68 (s, 1H, hydrazone NH), 9.14 (s, 1H, C_6H), 9.02 (s, 1H, NH), 7.39-8.10 (m, 10H, ArH), 6.22 (br.s, 2H, NH_2), 3.36 (br.S, 2H, NH_2); IR (KBr); ν = 3280 (NH), 2222 (C≡N), 1680 (OCN), 1610 cm^{-1} (C=N). MS: *m/z* (%); 401(M^++2, 13.99), 399 (M^+, 20.06), 391 (81.84), 386 (100), 372 (35.43), 350 (16.26). 328 (30.09), 309 (19.39), 265 (11.74), 149 (13.66), 93 (15.75), 77 (32.74); elemental analysis: calcd (%) for C_{20} H_{17} N_9 O (399.4): C 60.14, H 4.29, N 31.56 (%), found: C 60.26, H 4.38, N 31.35.

7-amino-2, 4-dioxo-5-(5-oxo-1-phenyl-4-(2-phenylhydrazono)-4, 5-dihydro-1*H*-pyrazol-3-yl)-2, 3, 4, 5-tetrahydro-1H-pyrano[2, 3-d]pyrimidine-6-carbonitrile (4); red needles, (72%): m.p. > 270 °C; ^{1}H NMR (DMSO-d_6): δ= 13.64 (s, 1H, hydrazone NH), 8.22 (s, 1H, C_5H), 7.32-8.02 (m, 10H, ArH), 6.64 (s, 1H, NH), 5.82 (s, 1H, NH), 3.84 (br.s, 2H, NH_2); IR (KBr); ν= 3218, 3182 (NH_2), 2224 (C≡N), 1746 (C=O), 1659 (OCN), 1600 cm^{-1} (C=N). MS: *m/z* (%); 465 (M^++1, 61.49), 462 (70.30), 451 (43.26), 426 (100), 383 (49.43), 377 (58.99). 252 (29.28), 186 (40.07), 170 (51.15), 117 (35.52), 90 (60.61); elemental analysis: calcd (%) for C_{23} H_{16} N_8 O_4 (468.47): C 58.96, H 3.44, N 23.92 (%), found: C 58.76, H 3.32, N 23.74.

-3, 6-diamino-4-(5-oxo-1-phenyl-4-(2-phenylhydrazono)-4, 5-dihydro-1H-pyrazol-3-yl)-1-phenyl-2-thioxo-1, 2, 3, 4-tetrahydropyrimidine-5-carbonitrile (5); red needles, (76%): m.p. 218-220°C; ^{1}H NMR (DMSO-d_6): δ= 13.24 (s, 1H, hydrazone NH), 8.06 (s, 1H, C_4H), 7.24-7.88 (m, 15H, Ar), 4.88 (br.s, 2H, NH_2), 3.82 (br.s, 2H, NH_2); MS: *m/z* (%); 508 (M^++1, 3.84), 505 (5.81), 499 (15.77), 498 (34.36), 487 (25.08), 485 (13.62), 475 (77.51), 454 (15.078), 439 (100), 431 (18.0), 409 (59.13), 394 (39.60), 367 (52.0)m 354 (55.09), 327 (29.60), 228 (14.20), 93 (59.23), 77 (27.56), 64 (51.39). elemental analysis: calcd (%) for C_{26} H_{21} N_9SO (507.5): C 61.53, H 4.17, N 24.84 (%), found: C 61.41, H 4.32, N 24.26.

1, 6-diamino-4-(5-oxo-1-phenyl-4-(2-phenylhydrazono)-4, 5-dihydro-1H-pyrazol-3-yl)-2-thioxo-1, 2, 3, 4-tetrahydropyrimidine-5-carbonitrile(6); red needles, (70%): m.p. 200-201°C. ^{1}H NMR (DMSO-d_6): δ= 13.62 (s, 1H, hydrazone NH), 8.21 (s, 1H, C_6H), 7.36-8.12 (m, 10H, ArH), 4.18 (br. s, 2H, NH_2), 3.82 (br. s, 2H, NH_2), 1.62 (2, 1H, SH). IR (KBr); ν = 3242, 3116 (NH_2), 2222 (C≡N), 1660 (OCN), 1602 cm^{-1} (C=N). MS: *m/z* (%); 431 (3.43), 430 (8.10), 395 (11.86), 379 (10.0). 363 (40.18), 340 (100), 338 (14.47), 178 (8.71), 92 (12.85), 77 (33.99). elemental analysis: calcd (%) for C_{20} H_{17} N_9 OS (431.3): C 55.68, H 3.97, N 29.22 (%), found: C 55.46, H 3.72, N 29.40.

2-Amino-6-oxo-4-(5-oxo-1-phenyl-4-(2-phenylhydrazono)-4, 5-dihydro-1H-pyrazol-3-yl)-4H, 6H-benzo[e] pyrimido [2, 1-b][1, 3]thiazine-3-carbonitrile (7); A mixture of compound **2** (0.208g, 0.5 mmol) in absolute ethanol (30 ml) was treated with o-bromobenzoic acid (0.1g, 0.5 mmol) was heated at reflux for 4h on a steam bath. The mixture was concentrated and left to cool, and the solid that separated was filtered off, washed with ethanol and dried, (76%). It was recrystallized from ethanol in red needles, m.p. 150-151°C. ^{1}H NMR (DMSO-d_6): δ= 13.46 (s, 1H, hydrazone NH), 8.21 (s, 1H, C_4H), 7.33-7.89 (m, 14H, ArH), 3.68 (br. s, 2H, NH_2). IR (KBr); ν = 3218, 3120 (NH_2), 2224 (C≡N), 1680 (OCN), 1600 cm^{-1} (C=N). MS: *m/z* (%); 518 (5.47), 477 (9.48), 461 (26.06), 446 (26.74). 411 (31.13), 383 (100), 377 (30.81), 581 (12.42), 152 (9.42), 93 (8.24), 77 (9.42); elemental analysis: calcd (%) for C_{27} H_{18} N_8 O_2 S (518.46): C 62.54, H 3.50, N 21.61 (%), found: C 62.40, H 3.46, N 21.82.

Synthesis of pyrazol-3-yl-pyrazole derivatives

A mixture of 5-oxo-1-phenyl-4-(2-phenylhydrazono)-4, 5-dihydro-1H-pyrazol-3-yl)-1H-pyrazole-3-carbaldehyde (1) (1 mmol) and malononitrile (1 mmol) in absolute ethanol (40 ml) was treated with hydrazine hydrate (1 mmol), p-nitrophenyl hydrazine (1 mmol), isonicotinoylhydrazine (1 mmol), p-sulphamylphenylhydrazine (1 mmol), or phenyl hydrazine (1 mmol) and few drops of TEA, and the mixture was heated on a steam bath for 4h, evaporated under reduced pressure and left to cool. The solid that separated was filtered off, washed with ethanol and recrystallized from ethanol to give needles. The physical characters and spectral data of compounds (8-12) are listed below.

3-Amino-5-(5-oxo-1-phenyl-4-(2-phenylhydrazono)-4, 5-dihydro-1H-pyrazol-3-yl)-1H-pyrazole-5-carbonitrile (8): Red needles (76%): m.p. 210-211°C; ^{1}H NMR (DMSO-d_6): δ = 13.22 (s, 1H, hydrazone NH), 8.61 (s, 1H, C_5H), 7.31-8.32 (m, 10H, ArH), 7.54 (s, 1H, NH), 6.16 (s, 1H, pyrazole NH). 5.32 (br. s, 2H, NH_2). IR(KBr); ν = 3236, 3018 (NH_2), 2222(C≡N), 1682 (OCN), 1610 cm^{-1} (C=N), MS: m/z(%) 372 (7.41), 371 (17.86), 366 (13.19), 355 (100), 354 (10.91), 352 (18.46), 345 (14.92), 340 (22.82), 322 (4.22), 306 (12.40), 282 (40.32), 196 (12.42), 132 (3.22), 93 (14.62), 77 (36.4); elemental analysis: calcd (%) for C_{19} H_{16} N_8O(372.38): C 61.28, H 4.33, N 30.09 (%), found: C 61.32, H 4.41, N 30.22.

3-Amino-5-(5-oxo-1-phenyl-4-(2-phenylhydrazono)-4, 5-dihydro-1H-pyrazol-3-yl)-1-p-nitrophenyl-1, 5-dihydro-1H-pyrazole-4-carbonitrile (9): Red needles (73%); m.p. 251-252°C; ^{1}H NMR (DMSO-d_6): δ = 13.86 (s, 1H, hydrazone NH). 9.21 (s, 1H, NH), 8.84 (s, 1H, C_5H). 7.70-8.12 (m, 10H, ArH), 7.78-7.86 (dd, 2H, ArH), 7.52-7.56 (dd, 2H, ArH), 4.21 (br.s, 2H, NH_2), MS: *m/z* (%) 493 (5.22), 479 (8.83), 461 (11.29), 446 (15.65), 431 (13.20), 424 (27.10), 410 (100), 308

(30.35) 280 (20.16), 128 (13.42), 93 (12.50), 77 (34.00). elemental analysis: calcd (%) for C_{25} H_{19} N_9 O_3 (493.47): C 60.84, H 3.08, N 25.55 (%), found: C 60.62, H 3.16, N 25.38.

3-Amino-5-(5-oxo-1-phenyl-4-(2-(phenylhydrazono)-4, 5-dihydro-1H-pyrazol-3-yl)-1, 2-dihydro-1-isonicotinoylpyrazole-5-carbonitrile (10): Red needles (72%); m.p. 178-179°C; ^{1}H-NMR (DMSO-d_6): δ = 13.88 (s, 1H, hydrazone NH), 8.14 (s, 1H, C_5H), 7.24-7.96 (m, 14H, ArH), 6.84 (s, 1H, NH), 3.84 (br.s, 2H, NH_2) IR (KBr); ν = 3228, 3116 (NH_2), 2224 (C≡N), 1704 (C=O), 1668 (OCN), 1596 cm^{-1} (C=N). MS: *m/z* (%) 477 (19.50), 468 (23.23), 445 (36.22), 437 (41.28). 408(100), 354 (80.11), 334 (29.42), 304 (38.05), 247 (44.44), 211 (13.22). 103 (42.16), 90 (92.22), 76 (41.63); elemental analysis: calcd (%) for C_{25} H_{19} N_9 O_2 (477.47): C 62.88, H 4.28, N 26.40 (%), found: C 62.67, H 4.42, N 26.55.

3-Amino-5-(5-oxo-1-phenyl-4-(2-phenylhydrazono)-4, 5-dihydro-1H-pyrazol-3-yl)-1-p-sulphamylphenyl-1, 5-dihydro-1H-pyrazole-4-carbonitrile (11): Red needles (80%), m.p. 235-236°C: ^{1}H NMR (DMSO-d_6) δ = 13.62 (s, 1H, hydrazone NH), 7.24-7.92 (m, 10H, ArH), 6.84-6.86 (dd, 2H, J=8.16Hz, ArH), 7.12-7.16 (dd, 2H, J=8.36, ArH), 6.72 (s, 1H, NH), 3.96 (br.s, 2H, NH_2). IR (KBr); ν = 3322, 3136 (NH_2), 2225 (C≡N), 1678 (OCN), 1612 cm^{-1} (C=N). MS: *m/z* (%) 527 (6.07), 526 (15.58), 501 (47.67), 495 (19.19), 445 (100), 460 (22.53), 427 (23.03), 357 (25.67), 311 (39.70), 185 (37.22), 124 (23.67), 91 (49.57), 77 (131.82). elemental analysis: calcd (%) for C_{25} H_{21} N_5 O_3S (527.49): C 56.92, H 4.02, N 24.02 (%), found: C 56.74, H 4.21, N 24.28.

5-Amino-3-(5-oxo-1-phenyl-4-(2-phenylhydrazono)-4, 5-dihydro-1H-pyrazol-3-yl)-2-phenyl-2, 3-dihydro-1H-pyrazole-4-carbonitrile (12): Red needles (78%) m.p. 158-159°C; ^{1}H NMR (DMSO-d_6): δ = 13.86 (s, 1H, hydrazone NH), 8.24 (s, 1H, C_3H), 12.2 (s, 1H, NH), 7.14-7.88 (m, 15H, ArH), 4.32 (br. s, 2H, NH_2). IR (KBr); ν = 3280, 3124 (NH_2), 2224 (C≡N), 1680 (OCN), 1602 cm^{-1} (C=N). MS: *m/z* (%) 450 (32.67), 448 (46.94), 405 (12.37), 365 (63.48), 340 (32.67), 448 (46.94), 405 (12.37), 365 (63.48), 340 (100), 185 (58.28), 144 (28.28), 102 (23.95), 102 (73.95), 92 (43.56), 80 (12.21), 77 (34.28). elemental analysis: calcd (%) for C_{25} H_{20} N_8O (448.47): C 66.95, H 4.50, N 24.99 (%), found: C 66.75, H 4.32, N 24.74.

Synthesis of pyrazol-3-yl-pyran derivatives

A mixture of 5-oxo-1-phenyl-4-(2-phenylhydrazine)-4, 5-dihydro-1H-pyrazol-3-yl)-1H-pyrazole-3-carbaldehyde (1) (1 mmol) and malononitrile (1 mmol) in absolute ethanol (40 ml) was treated with cyclohexanone, (1 mmol), dimedone (1 mmol) or acetophenone (1 mmol) and few drops of (TEA), and the mixture was heated on a water bath for 3h, evaporated under reduced pressure and left to cool. The solid separated was filtered off, washed with ethanol and recrystallized from ethanol to give needles. The physical characters and spectral data of compounds **(13-15)**, are listed below.

5-amino-4-(5-oxo-1-phenyl-4-(2-phenylhydrazono)-4, 5-dihydro-1H-pyrazol-3-yl)-4, 5, 6, 7, 8-pentahydro[b] pyran-5-carbonitrile (13): Red needles (70%); m.p. 240-241°C; ^{1}H NMR (DMSO-d_6): δ = 13.66 (s, 1H, hydrazone NH) 8.32 (s, 1H, C_4H), 7.42-8.02 (m, 10H, ArH), 6.28 (br.s, 2H, NH_2), 1.64 (dd, 2H, J=12.4Hz, CH_2), 1.74 (dd, 2H, J=12.6Hz, CH_2), 1.96 (t, 2H, J=7.6Hz, CH_2), 1.94 (t, 2H, J=7.6Hz, CH_2). MS: *m/z* (%) (438 (13.66). 384 (41.48), 320 (24.25), 284 (44.06), 260 (38.06), 238 (37.24), 184 (36.08), 121 (30.26), 98 (42.88) 91 (41.60), 82 (16.82), 77 (12.43. elemental analysis: calcd (%) for C_{25} H_{22} N_6 O_2 (438.47): C 68.48, H 5.06, N 19.17 (%), found: C 68.29, H 5.24, N 19.36.

2-Amino-4-(5-oxo-1-phenyl-4-(2-phenylhydrazono)-4, 5-dihydro-1H-pyrazol-3-yl)-5-oxo-7-dimethyl-4, 5.6, 7, 8-pentahydrobenzo[b] pyran-3-carbotitrile (14): Red needles (80%): m.p. > 270°C; ^{1}H NMR (DMSO-d_6): δ = 13.84 (s, 1H, hydrazone NH), 8.16 (s, 1H, C_4H), 7.40-7.98 (m, 10H, ArH), 5.21 (br.s, 2H, NH_2), 4.22 (s, 2H, CH_2), 4.12 (s, 2H, CH_2), 2.14 (d, 6H, 2 CH_3), MS: *m/z* (%) 480 (34.68), 462 (31.05), 452 (37.20), 403 (20.80), 382 (85.32), 322 (88.18), 294 (100), 238 (30.22), 203 (90.68), 92 (34.22), 83 (45.20). elemental analysis: calcd (%) for C_{27} H_{24} N_6 O_3 (480.51): C 67.48, H 5.03, N 17.49 (%), found: C 67.33, H 5.24, N 17.32.

2-Amino-4-(5-oxo-1-phenyl-4-(2-phenylhydrazono)-4, 5-dihydro-1H-pyrazol-3-yl)-6-phenyl-4H-pyran-3-carbonitrile (15): Red needles (70%): m.p. 233-234°C; ^{1}H NMR (DMSO-d_6): δ = 13.82 (s, 1H, hydrazone NH), 8.12 (d, 1H, J=7.4Hz, C_4H), 7.22-7.93 (m, 15H, ArH), 6.84 (d, 1H, J=7.2Hz, C_5H), 4.36 (br.s, 2H, NH_2), IR (KBr); ν = 3186, 3120 (NH_2), 2225 (C≡N), 1682 (OCN), 1590 cm^{-1} (C=N). MS: *m/z* (%) 460 (14.90), 450 (26.64), 437 (26.00), 427 (50.83), 365 (16.39), 355 (100), 318 (23.22), 280 (16.22), 271 (13.39), 171 (15.35), 77 (16.63). elemental analysis: calcd (%) for C_{27} H_{20} N_6 O_2 (460.48): C 70.42, H 4.38, N 18.25 (%), found: C 70.36, H 4.18, N 18.43.

2-Amino-4-(5-oxo-1-phenyl-4-(2-phnylhydrazono)-4, 5-dihydro-1H-pyrazol-3-yl)-6-phenyl-1, 4-dihydro-1-methylaminopyridine-5-carbonitrile (16). A solution of compound **(15)** (0.1g, 0.3 mmol) in absolute ethanol (30 ml) was treated with methylhydrazine (0.2 ml, 0.5 mmol) and heated on a water bath for 3h, It was concentrated and left to cool, and the solid that separated was filtered off, washed with ethanol and dried (71%). It was recrystallized from ethanol to give red needles, m.p. 190-191°C; ^{1}H NMR (DMSO-d_6): δ = 13.63 (s, 1H, hydrazone NH), 7.12-7.68 (m, 15H, ArH). 6.72 (s, 1H, J = 9.2Hz, C_5H), 6.18 (s, 1H, NH), 3.96 (br. s, 2H, NH_2), 2.46 (s, 3H, CH_3). IR (KBr); ν = 3284, 3106 (NH_2), 2225 (C≡N), 1676 (OCN), 1608 cm^{-1} (C=N). MS *m/z*: (%) 488 (10.84), 456 (12.48), 446 (17.57), 408 (10.05), 352 (34.66), 318 (100), 306 (26.23), 235 (51.22), 224 (19.0), 164 (20.29), 164 (20.85), 98 (37.70), 87 (19.19), 77 (62.42). elemental analysis: calcd (%) for C_{28} H_{24} N_8 O (488.5): C 68.84, H 4.95, N 22.94 (%), found: C 68.61, H 4.76, N 22.70.

5-Amino-3-(5-oxo-1-phenyl-4-(2-phenylhydrazono)-4, 5-dihydro-1H-pyrazol-3-yl)-2, 3-dihydroisoxazole-4-carbonitrile (17): Red needles (72%); m.p. > 270°C ; ^{1}H NMR (DMSO-d_6) : δ = 13.58 (s, 1H, hydrazone NH), 9.02 (s, 1H, isoxazole NH), 7.54 (s, 1H, C_3H), 7.52-8.14 (m, 10H, ArH), 5.46 (br.s, 2H, NH_2). IR (KBr); ν = 3360, 3096 (NH_2), 2222 (C≡N), 1678 (OCN), 1600 cm^{-1} (C=N). MS: *M/z* (%) 373 (100), 332 (30.02), 321 (18.92), 307 (69.85), 304 (39.10), 296 (20.11), 277 (28.84), 214 (10.02), 125 (18.62), 93 (85.67), 82 (7.12), 60 (23.03); elemental analysis: calcd (%) for C_{19} H_{15} N_7

O_2 (372.36): C 16.12, H 4.05, N 26.26 (%), found: C 61.30, H 4.19, N 26.80.

2-Amino-4-(5-oxo-1-phenyl-4-(2-phenylhydrazono)-4, 5-dihydropyrazol-3-yl)-5, 6-dioxo-5, 6-dihydropyridine-5-carbonitrile (18): Red needles (82%); m.p. 210-211°C ; ^{1}H NMR (DMSO-d_6) : δ = 13.68 (s, 1H, hydrazone NH), 8.06 (s, 1H, C_5H), 7.28-7.92 (m, 10H, ArH), 3.88 (br.s, 2H, NH_2). IR (KBr); ν = 3354, 3132 (NH_2), 2225 (C≡N), 1746 (C=O), 1666 (OCN), 1594 cm^{-1} (C=N). MS: *M/z* (%) 411 (4.12), 399 (28.68), 368 (18.17), 339 (56.17), 326 (14.67), 256 (11.56), 206 (10.84), 104 (18.79, 97 (18.38), 92 (26.99), 77 (100); elemental analysis: calcd (%) for C_{21} H_{13} N_7 O_3 (411.41): C 61.30, H 3.19, N 23.83 (%), found: C 16.42, H 3.32, N 23.62.

Determination of antimicrobial activity

All compounds were tested against three different microorganisms *Staphylococcus aureus*, *Escherichia coli, Candida albicans*. The agar well-diffusion method was applied for the determination of inhibition zone

Preparation of the agar plate

The sterile nutrient agar was poured aseptically as 40 ml portions into sterile Petri dishes (15 cm in diameter) onto a level surface to obtain a layer of about 4 mm thickness and the plates were then left to solidify. After solidification, the plates were incubated in an inverted position at 37ºC for 18 h to be over dried before use.

Preparation of the inoculum

Each tested organism was sub-cultured in 3 ml sterile nutrient broth and the resultant microbial growth was firstly compared with 0.5 'McFarland Opacity Standard' which was equivalent to approximately 108 CFU/ml and properly diluted; if necessary, to achieve the same turbidity of the standard. The turbidity standard "0.5 McFarland Opacity Standard" was prepared by transferring 0.5 ml of 1.175 % solution of barium chloride to 100 ml-graduated cylinder and completing to 100 ml with 1% sulfuric acid. This standard was placed in a tube identical to the one used for both cultures, sealed then kept in the dark at room temperature and used within one month.

Procedure of the test

Sterile cotton swabs were separately dipped into each of the adjusted organism cultures and excess inoculum was removed by pressing and rotating the swab firmly several times against the wall of the tube above the level of the liquid. The swab was streaked all over the surface of the nutrient agar in three dimensions at an angle of 60º to obtain an even distribution of the inoculum. The plates were then left to dry at room temperature for few minutes. A sterile cork porer (8 mm in diameter) is used to make wells in the solid nutrient agar plates, so that the distance between the edges of each two wells is not less than 24 mm. Fill each well with 75 µl of the test compound and another well with same volume of DMF as a vehicle control. Allow a period of free diffusion for 2 h, then incubate at 37º C for 18-24 h.

Reading and interpretation of results

After incubation, the diameters of inhibition zones around the wells were measured, to the nearest mm, in three different directions using a ruler and the average diameter was recorded and compared to that of the control.

Conclusion

In the present work, a series of novel pyrazol-3-yl-pyrimidine, pyrazole and pyran derivatives were synthesized by one step synthesis involving three component condensation. All the synthesized compounds are characterizing by modern spectral data (FT-IR, H-NMR, Mass spectra) and elemental analysis and the structures were consistent with the data. Some of the synthesized compounds were tested for their antimicrobial activity against three strains *Staphyllococcus aureus* ATCC6538P, *Escherischia coli* ATCC8739 and *Candida albican*ATCC2091. Antibacterial results indicated that thecompounds 3, 4, 5, 11, 14, 16 and 18 showed slight activity towards *Staphyllococcus aureus* as Gram positive strain whereas, all the tested compoundsshowed moderate antifungalactivity towards *Candida albican*.

REFERENCES

Aziza, M. A., Nassar, M. W., Abdel-Hamide, S. G., El-Hakim, A. E., El Azab, A. S. 1996. Synthesis and antimicrobial activities of some new 3-heteroaryl-quinazolin-4-ones. *Ind. J. Heterocycl. Chem., 6*, 25-30.

Benaamane, N., Nedjar-Kolli, B., Bentarzi, Y., Hammal, L., Geronikaki, A., Eleftheriou, P., Langunin, A. 2008. Synthesis and in silico biological activity evaluation of new N-substituted pyrazolo-oxazin-2-one systems. *Bioorg. Med. Chem., 16*, 3059-3066.

Brown, R. C. D. 1998. Recent development in solid phase organic synthesis. *J. Chem. Soc. Perkin. Trans 1*, 3293-3320.

Clark, J., Shahhet, M. S., Korakas, D., G. Varvounis, 1993. Synthesis of thieno[2, 3-d]pyrimidines from 4, 6-dichloropyrimidine-5-carbaldehydes *J. Heterocycl. Chem., 30*, 1065–1072.

Clinical and Laboratory Standards Institute. Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically, Approved Standard, 7th ed., CLSI: Wayne, PA, USA, 2006.

El Sekily, M. A., Elba, M. E., Foad, F. S. 1999. Studies on Dehydro-L-ascorbic Acid and Dehydro-D-isoascorbic Acid Hydrazones. *J. Chem. Res. (S), (4),* 296-297.

El Sekily, M. A., Elba, M. E., Foad, F. S. 2000. Some Heterocycles from Dehydro-L-Ascorbic Acid and Dehydro-D-Isoascorbic Acid.*J. Indian Chemical Society*, 77(3), 168-171.

El Sekily, M. A., Mancy, S. H., El Zekely, M. Kh, 2018. Synthesis and characterisation of triazolo- and pyrazol-3-yl quinoxaline derivatives from dehydro-L-ascorbic acid hydrazone and their antimicrobial activity. *J. Indian Chem. Soc*., *95*, 1-9.

El Sekily, M. A., Mancy, S. H., El-Ghannam, A. M., Leon, G. A. 2018. Synthesis and characterization of pyrazol-3-ylthiadiazole and thiadiazoline derivatives from dehydro-L-ascorbic acid.*International Journal of current Research, 10(3)*, 66368-66372.

El Sekily, M. A., Mancy, S. H., El-Ghannam, A. M., Leon, G. A. 2017. Some New Heterocycles from the Reaction of 4-Carboxaldehyde-2-Phenyl-2H-1, 2, 3-Triazole Malononitrile. *Natural Science, 9(12),* 406-411.

Gilbert, A. M., Faille, A., Shumsky, J., Yang, Y., Severin, A., Singh, G., Keeney, W. Hu. D., Petersen, P. J., Katz, A. H. 2006. Pyrazolidine-3, 5-diones and 5-Hydroxy-1*H*-pyrazol-3(2*H*)-ones, Inhibitors of UDP-*N*-acetylenolpyruvyl Glucosamine Reductase. *J. Med. Chem., 49*, 6027–6036.

Hamada, N. M. M. 2018. Synthesis, Spectroscopic Characterization, and TimeDependent DFT Calculations of 1-Methyl-5-phenyl-5Hpyrido[1, 2-a]quinazoline-3, 6-dione and Its StartingPrecursor in Different Solvents. *Chemistry Open*, *7*, 814-823.

Haufel, J., Breitmaier, E. 1974. Synthesis of Pyrazolo Heteroaromatic Compounds by Means of 5-Amino-3-methyl-1-phenylpyrazole-4-carbaldehyde.*Ange w. Chemie*, *13*(9), 604-604.

Isloor, A. M., Kalkuraya, B., Shetty, P. 2009. Regioselective reaction: synthesis, characterization and pharmacological studies of some new Mannich bases derived from 1, 2, 4-triazoles.*Eur. J Med. Chem*., *44,* 3784-3787.

Malladia, S., Isloora, A. M., Peethambar, S. Kb., Ganesh, B. Ma; Sanathkumar, G. 2012. Synthesis and antimicrobial activity of some new pyrazole containing cyanopyridone derivatives. *Der pharma chemical*, *4(1),* 43-52.

Mayedov, I. V., Mampadi, M; Slambrouck, S. V., Steelant, W.R.A., Rozhkova, E., Prheval'skii, N.M., Rogelj, S., Kornienko, A. 2007. Discovery and Investigation of Antiproliferative and Apoptosis-Inducing Properties of New Heterocyclic Podophyllotoxin Analogues Accessible by a One-Step Multicomponent Synthesis. *J. Med. Chem.,* 50(21), 5183-5192.

Metolcsy, G. 1971. Structure-activity correlations and mode of action of some selected types of antifungal compounds. *World Rev. Pest Contr., 10*, 50-59; *Chem. Abstr.,* (1972, 76, 82031 s).

Ozdemir, Z., Kandilioi, H. B., Gum Used, B., Calis, U., Bilgin, A. A. 2007. Synthesis and studies on antidepressant and anticonvulsant activities of some 3-(2-furyl)-pyrazoline derivatives.*Eur. J. Med. Chem., 42(3*), 373-379.

Patil, D. R., Salunkhe, S. M., Deshmukh, M. B., Anbhule, P. V. 2010. One Step Synthesis of 6-Amino-5-Cyano-4-Phenyl-2-Mercapto Pyrimidine Using Phosphorus Pentoxide. The *Open Catalysis Journal*, *3*, 83-86.

Pershin, G. N., Sherbakova, L.I., Zykova, T. N., Sakolova, V. N. Sokolova, 1972. Anticacterial activity of pyrimidine and pyrrolo[3, 2-d] pyrimidine derivatives. *Farmakol Toksikol,* 1972, *35*, 466-471; *Chem. Abstr., 77*, 135580z).

Prakash, O., Pundeer, R., Ranjan, P., Pannu, K., Dhingra, Y., Aneja, K. R. 2009. Synthesis and antibacterial activity of 1, 3-diaryl-4-cyanopyrazoles. *Indian. J. Chem., 48B*, 563-568.

Prasad, Y. R., Lakshmara Rao., A., Prasoona, I., Murali, K., Ravi, K. P. 2005. Synthesis and antidepressant activity of some 1, 3, 5-triphenyl-2-pyrazolines and 3-(2″-hydroxy naphthalen-1″-yl)-1, 5-diphenyl-2-pyrazolines. *Bioorg. Med. Chem. Lett., 15(22),* 5030-5034.

Regnier, G. L., Canevar, R. J., Canevar, J. C., Douarec, L., Halstop, S., Daussy, J. 1972. Triphenylpropylpiperazine derivatives as new potent analgetic substances. *J. Med. Chem., 15,* 295-301.

Sener, A., Sener, M. K., Bildmci, I., Kasimogullari, R., Akcamur, Y. 2002. Studies on the reactions of cyclic oxalyl compounds with hydrazines or hydrazones: Synthesis and reactions of 4-benzoyl-1-(3-nitrophenyl) -5-phenyl-1 H-pyrazole-3-carboxylic acid. *J. Heterocycl. Chem.*, *39*(5), 869-875.

Suguira, K., Schmid, F.A., Schmid, M.M., Brown, G.F. 1973. Effect of compounds on a spectrum of rat tumors. *Cancer Chemother. Rep. Part, 3*, 231-238.

Szabo, G., Fischer, J., Kis-Varga, A., gylres., K. New 2008. Celecoxib Derivatives as Anti-Inflammatory Agents. *J. Med. Chem., 51(1),* 142-147.

Vishnu Ji Ram., Farhanullah; Brajendra K Tripathi, Arvind K Srivastava, 2003. Synthesis and antihyperglycemic activity of suitablyfunctionalized 3H-quinazolin-4-ones. *Bioorg. Med. Chem., 11*, 2439-2444.

Wachter, G. A., Hantmann, R. W., Sergejew, T., Grum., G. L., Ledergerber, D. 1996. Tetrahydronaphthalenes: Influence of Heterocyclic Substituents on Inhibition of Steroidogenic Enzymes P450 arom and P450 17. *J. Med. Chem., 39(4)*, 834- 841.

Winter, A. G., Fisler, E. A., Nuss, G. W. 1962. Carrageenin-induced edema in hind paw of the rat as an assay for antiinflammatory drugs. *Proc. Soc. Exp. Biol. Med., 111*, 544-47.

****