Synthesis of Bioactive Heterocycles from 6-amino-4-(2-chloro-5-nitrophenyl)-3-methyl-1, 4-dihydropyran[2,3-c] pyrazole-5-carbonitrile

A.K. Elziaty, G. Bassioni, A.M.A. Hassan and H.A. Derbala

1Department of Chemistry, Faculty of Science, Ain shams university, Abbassia, Cairo Egypt 11566
2Department of Chemistry, Faculty of Engineering, Ain shams University, Abbassia, Cairo Egypt 11566

Abstract: Enaminonitrile derivative, 6-amino-4-(2-chloro-5-nitrophenyl)-3-methyl-1,4-dihydropyran [2,3-c] pyrazole-5-carbonitrile 1 was synthesized. This compound was utilized as a building block for the synthesis of new 3-methylpyrazolopyran moiety incorporated with different heterocycles involving pyrimidinone2, oxazinone 4a,b and iminopyrimdine 8, in addition to novel derivatives including diacetyl derivative 5, benzoyl derivative 6, carbamodithioic acid 10 and urea derivative 13. Spectral techniques, FTIR, H-NMR and Mass spectroscopy and elemental analysis were used to characterize the synthesized compounds. Screening and evaluation of these products as antimicrobial agents showed that the derivatives 5, 6, 10 and 13 possess a potent activity.

Key words: Enaminonitrile • Pyranopyrazole • Pyrimidinone • Oxazinone • Antimicrobial agents

INTRODUCTION

It has been reported that pyran derivatives possess hypotensive effect [1], anticancer activity [2], antifungal effect [3, 4], plant growth regulation activity [5]. Pyranopyrazoles are important compounds for the preparation of many biological active heterocyclic compounds [6] and they proved to have useful properties as therapeutics in clinical application [7-9]. A literature survey revealed that pyrazole derivatives have received much attention during the recent years on account of their utilization as antioxidant [10] antihypertensive [11] antifungal [12, 13] and vasodilator [14]. As well as, pyrimidinone derivatives have extensive applications as structural units of various biologically important molecules and as useful intermediates in medicinal chemistry [15] and pyranopyrimidinones compounds showed considerable pharmaceutical and biological activities, including anticancer, antitumor, antimalarial, antibacterial, antihypertensive, anti-inflammatory, hepatoprotective, cardiotonic, vasodilator, bronchodilator, antifolate and antiallergic activities [16-28]. They are also used in the preparation of dyes and pigments flavoring agents [29-30] and in luminescence chemistry [31]. Over the past decades, significant efforts have been devoted to develop the synthesis of pyrimidinethione derivatives [32, 33] as they are considered versatile synthons for the construction of many heterocycles of synthetic and biological importance [34-37]. Thus, in view of the above facts and in continuation of our efforts to construct heterocyclic compounds from pyran derivatives and to study their biological potency [38-45] it was of interest to synthesize a ring system combine both the pyrazole and the pyran moieties which might have good biological activity.

RESULT AND DISCUSSION

Chemistry: The previously reported pyranopyrazole derivative1 [46] was allowed to react with different reagents aiming to synthesize antimicrobial heterocycles. Reaction of 1 with formic acid afforded the pyrimidinone derivative 2 whose structure was confirmed from IR spectral data which revealed the absence of absorption bands of C=N and NH groups and the appearance of bands characteristic to carbonyl and NH activities, including anticancer, antitumor, antimalarial, antibacterial, antihypertensive, anti-inflammatory, hepatoprotective, cardiotonic, vasodilator, bronchodilator, antifolate and antiallergic activities [16-28]. They are also used in the preparation of dyes and pigments flavoring agents [29-30] and in luminescence chemistry [31]. Over the past decades, significant efforts have been devoted to develop the synthesis of pyrimidinethione derivatives [32, 33] as they are considered versatile synthons for the construction of new heterocyclic compounds. Acid hydrolysis of the cyano functionality was carried out by addition of concentrated sulphuric acid onto pyranopyrazole derivative 1 at room temperature to give the amide derivative 3. The structure of the amide 3 was elucidated by the FTIR spectra which showed no absorption band of C=N and appearance of a new band due to C=O group at $\nu$ 1685 cm$^{-1}$.
Scheme 1: Construction of pyrimidinone, amide, pyrazolopyranooxazinones, diacetyl and benzoyl derivatives

In our previously work for the synthesis of oxazinone derivatives [46], the pyranopyrazole derivative 1 was allowed to react with acetic anhydride and/or benzoyl chloride in neat reactions afforded the pyrazolopyranooxazinones 4a,b. On contrary, herein, the reaction of 1 with acetic anhydride in pyridine gave the diacetyl derivative 5 and benzoylation with benzoyl chloride in dry toluene as a solvent afforded the benzoyl derivative 6. The IR spectra of both products 5 & 6 revealed the presence of cyano group absorption that proved no cyclization has occurred (Scheme 1).

To make use of the beneficial role of nucleophilic character of the amino group, it was subjected to react with various electrophiles. Thus, when enaminonitrile 1 was treated with trie thy or tho formate, it gave the imidoformate derivative 7. The latter product was utilized as a precursor for the synthesis of pyrazolo pyranopyrimidine 8 by reaction with hydrazine hydrate in ethanol. The structure of 7 was confirmed from IR spectrum that showed no absorption frequency of NH₂ group appeared, in addition to the appearance of C=N group band at \(1632 \text{ cm}^{-1}\). The H-NMR spectrum showed a singlet at \(12.36 \text{ ppm}\) which disappeared by a triplet peak at \(4.34-4.28 \text{ ppm}\) and a triplet peak at \(1.31-1.28 \text{ ppm}\) due to CH protons. The structure of 8 has been elucidated on the basis of IR spectrum which showed a coupling band at \(3188, 3119 \text{ cm}^{-1}\) due to NH group and two peaks for NH pyrazole and NH imino at \(3349\) and \(3309 \text{ cm}^{-1}\), respectively. The H-NMR spectrum showed a singlet at \(12.57 \text{ ppm}\) (NH) pyrazole group and 10.25 ppm for C=NH. However, when the imidoformate derivative 7 was subjected to react with ammonium hydroxide in methanol, hydrolysis of the imidoformate functionality to the formamide derivative 9 occurred instead of the formation of the pyrimidinone derivative 2. Further, treatment of the enaminonitrile 1 with carbondisulfide afforded carbamodithioic acid 10 instead of pyrimidenedithione derivative 11. The IR spectrum of 10 revealed the absorption band attributable for C=N group at \(2215 \text{ cm}^{-1}\) and a sharp band at \(1390 \text{ cm}^{-1}\) due to C=S group. Recently, reaction of the assigned compound 1 with phenylisocyanate in pyridine provided the urea derivative 13 instead of the pyrimidinone derivative 12, the structure of 13 was confirmed from its elemental and spectral analysis (Scheme 2).
Antimicrobial Study: The antibacterial activity of the synthesized compounds 2, 3, 7, 8, 10 and 13 was tested against a panel of two gram positive bacteria (Staphylococcus aureus, Bacillus subtilis) and two Gram-negative bacteria (Escherichia coli, Pseudomonas aeruginosa). The antifungal activities of the compounds were tested against two fungi (Candida albicans, Aspergillus flavus).

Each compound was dissolved in DMSO and solution of the concentration 1 mg/ml were prepared separately paper discs (5cm) were cut and sterilized in an autoclave. The paper discs soaked in the desired concentration of the complex solution were placed aseptically in the petri dishes containing nutrient agar media (agar 20g + beef extract 3g + peptone 5g) seeded with Staphylococcus aureus, Bacillus subtilis, Escherichia coli, Pseudomonas aeruginosa, Candida albicans and Aspergillus flavus. The petri dishes were incubated at 36°C and the inhibition zones were recorded after 24 h of incubation. Each treatment was replicated three times. The antibacterial activity of a common standard antibiotic ampicillin and antifungal colitrimazole was also recorded using the same procedure as above at the same concentration and solvents.

The activity index for the complex was calculated by the formula as shown below:

\[
\text{Activity Index} = \frac{\text{zone of inhibition by test}}{\text{zone of inhibition by standard}} \times 100
\]

The antimicrobial activity of the synthesized heterocycles was shown in Table 1 and Fig 1.

Minimum Inhibitory Concentration (MIC) Measurement: The MIC was determined using the disc diffusion technique by preparing discs containing 1.9-1000 µg/ml of each compound against gram positive Staphylococcus aureus, Bacillus subtilis and gram negative Escherichia coli, Pseudomonas aeruginosa. The antifungal activities of the compounds were tested against two fold fungi Candida albicans, Aspergillus flavus and applying the protocol. The two fold dilutions of the solution were prepared. The microorganism suspensions at 10 CF-U/ml (colony forming unit/ml) concentration were inoculated to the corresponding wells. The plates were incubated at 36°C for 24 h for the bacteria. The standard antibiotic
ampicillin and antifungal colitrimazole was also recorded using the same procedure as above at the same concentration and solvents. At the end of the incubation period, the minimum inhibitory concentration (MIC) values were recorded as the lowest concentration of the substance that had no visible turbidity. Control experiments with DMSO and uninoculated media were run parallel to the test compounds under the same condition. The MIC measurement of the synthesized heterocyclic compounds was shown in Table 2:

Experimental: All melting points were determined on an electro-thermal apparatus and are uncorrected. The infrared spectra were recorded in potassium bromide disks on PyeUnicam SP3-300 and Shimdazu FTIR 8101PC Infrared spectrophotometers. The $^1$H-NMR was recorded on a Varian Mercury VX-300 NMR spectrometer. $^1$H-NMR spectra were run at 300MHz and on a Varian Gemini 200 MHz, Bruker AC 200 MHz using TMS as internal standard in deuterated chloroform (CDCl$_3$) or deuterated dimethyl sulfoxide (DMSO-d$_6$). Chemical shifts are quoted in ppm.

Table 1: Diameter of inhibition zone (mm) and Activity index (%) measurements of synthesized heterocycles compounds

| Compound | E. coli (mg/ml) | Pseudomonas aeruginosa (mg/ml) | S. aureus (mg/ml) | B. subtilis (mg/ml) | C. Albicans (mg/ml) | A. flavus (mg/ml) |
|----------|----------------|-------------------------------|------------------|-------------------|-------------------|------------------|
|          | Diameter of inhibition zone (mm) | % Activity index | Diameter of inhibition zone (mm) | % Activity index | Diameter of inhibition zone (mm) | % Activity index | Diameter of inhibition zone (mm) | % Activity index |
| 2        | 9              | 36.0                         | 16               | 60.9              | 16                | 69.6             | 8                 | 30.8              |
| 3        | 13             | 52.0                         | 20               | 86.9              | 14                | 69.6             | 16                | 78.3              |
| 7        | NA             | ----                         | 15               | 65.2              | 18                | 78.3             | 21                | 80.8              |
| 8        | NA             | ----                         | 5                | 7.8               | 2                 | 8.7              | 2                 | 30.8              |
| 10       | 5              | 21.7                         | 17.4             | 26.1              | 8                 | 19.2             | 9                 | 28.0              |
| 13       | 7              | 28.0                         | 47.8             | 56.5              | 10                | 38.5             | 18                | 72.0              |
| Ampicillin | 25             | 100                          | 100              | 100               | NA                | 26               | 100               | 25                |

Table 2: Antimicrobial and Antimycotic Activities in terms of MIC (µg/mL)

| Compound | E. coli | Pseudomonas aeruginosa | S. aureus | B. subtilis | C. Albicans | A. flavus |
|----------|---------|------------------------|-----------|------------|-------------|-----------|
|          | 250     | 187.5                  | 93.7      | 187.5      | 93.7        | 46.9      |
| 3        | 187.5   | 125                    | 62.5      | 187.5      | 23.4        | 7.8       |
| 7        | NA      | 750                    | 500       | NA         | NA          | NA        |
| 8        | NA      | 500                    | 375       | 750        | NA          | 250       |
| 10       | 750     | 375                    | 250       | 375        | 187.5       | 62.5      |
| 13       | 375     | 250                    | 187.5     | 250        | 46.9        | 23.4      |
| Ampicillin | 125     | 187.5                  | 93.7      | 187.5      | ----        | ----      |
| Colitrimazole | ---- | ---- | ---- | ---- | 7.8 | 5.8 |
Fig. 2: Minimum inhibitory concentration (MIC) of the synthesized heterocyclic compounds and were related to that of the solvents. The mass spectra were recorded on a Shimadzu GC-MS QP1000 EX mass spectrometer at 70eV. Elemental analyses were carried out at the Micro analytical Center of Cairo University. All the reactions and the purity of the new compounds were followed and checked by TLC.

Chemistry:

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

A mixture of 1 (5 mmol, 1.66 gm) and formic acid (20 ml) was refluxed for 2h, the reaction mixture was poured after cooling into water and crushed ice, the solid formed was filtered off, washed with cold water and crystallized from ethanol to give compound 2, Pale yellow color, m.p. 229-230 °C, yield 72%. Anal.Calcd.for C$_{19}$H$_{13}$N$_5$O$_7$Cl (359.73): C, 20.08; H, 2.80; Cl, 9.85; N, 19.47. Found: C, 20.06; H, 2.82; Cl, 9.83; N, 19.48. FTIR (KBr, cm$^{-1}$): 3403(NH) pyrazole, 3182 (NH) pyrim, 1682 (CO).$^1$H-NMR (DMSO-d$_6$) (ppm): 11.4 (s, 1H, NH), 11.08 (s, 1H, NH), > 300°C, yield 50%. Anal.Calcd.for C$_{19}$H$_{14}$N$_5$O$_7$Cl (415.79): C, 52; H, 3.39; Cl, 8.52; N, 16.85. FTIR (KBr, cm$^{-1}$): 3355(NH) pyrazole, 1787, 1739 (C-O), 2223(C = N).$^1$H-NMR (DMSO-d$_6$) (ppm): 11.28 (s, 1H, NH, pyrazole, exch. with D$_2$O), 62.6, 68.55, 70.05, 72.15. MS m/z (%): 415(M$^+$;100), 417(32), 419(19.5).

6-amino-4-(2-chloro-5-nitrophenyl)-3-methyl-1,4-dihydropyra[2,3-c]pyrazole-5-carboxamide (3)

Compound 1 (5 mmol, 1.66 gm) was added dropwise with stirring to concentrated cold sulphuric acid at (20°C) (6 ml), the temperature does not exceed (40°C) then the solution was stirred for further an hour at room temperature and poured onto ice cold water (10 ml). The reaction mixture was left overnight in the refrigerator. The yellow precipitate was filtered off and crystallized from water to give compound 3, Pale yellow color, m.p. 175-176 °C, yield 68%. Anal.Calcd.for C$_{19}$H$_{13}$N$_5$O$_7$Cl (350.72): C, 47.95; H, 3.16; Cl, 10.11; N, 15.98. Found: C, 47.93; H, 3.15; Cl, 10.11; N, 15.99. FTIR (KBr, cm$^{-1}$): 3588(NH) pyrazole, 3567-3370 (NH), 3191-3108(amide NH), 1685 (CO).

8.49 (s, 1H, CH, N = C2-H), 8.07–7.70 (m, 3H, ArHs), 4.64 (s, 1H, benzyl), 2.12 (s, 3H, CH$_3$). MS m/z (%): 350(M$^+$;1.36), 351(4.93), 307 (67.39), 230(70.44), 151 (43.39), 43(100).

N-acetyl-

N-acetyl-N-[4-(2-chloro-5-nitrophenyl)-5-cyano-3-methyl-1,4-dihydropyra[2,3-c]pyrazol-6-yl]acetamide (5)

A mixture of 1 (5 mmol, 1.66 gm) in acetic anhydride-pyridine mixture (30 ml, 2:1 v/v) was heated on a water bath for 8h, then cooled and poured into ice/ water mixture. The precipitate thus formed was filtered off, washed several times with water, dried and crystallized from dioxane to give compound 5, deep brown color, m.p. > 300°C, yield 50%. Anal.Calcd.for C$_{21}$H$_{16}$N$_5$O$_4$Cl (415.79): C, 52; H, 3.39; Cl, 8.52; N, 16.85. FTIR (KBr, cm$^{-1}$): 3355(NH) pyrazole, 1787, 1739 (C-O), 2223(C = N).$^1$H-NMR (DMSO-d$_6$) (ppm): 11.28 (s, 1H, NH, pyrazole, exch. with D$_2$O), 7.38- 6.67 (s, 4H, C2-NH$_2$, CONH$_2$, exch. with D$_2$O), 8.63–7.60 (m, 3H, ArHs), 4.68 (s, 1H, benzylic), 2.1 (s, 3H, CH$_3$). MS m/z (%): 505(M$^+$;1.36), 517(4.93), 307 (67.39), 230(70.44), 151 (43.39), 43(100).
was crystallized from (ethanol: dioxane (1:1)) to give compound 6, brown color, m.p. 261-262°C, yield 53%. Anal.Calcd.for C₆H₆N₂O₂Cl (539.93): C, 62.29; H, 3.36; Cl, 5.67; N, 12.97. Found: C, 62.27; H, 3.35; Cl, 5.65; N, 12.98. 

**Ethyl-4-(2-chloro-5-nitrophenyl)-5-cyano-3-methyl-1,4-dihydropyrazolo[2,3-c]pyrazol-6-yl formamide (7)**

A mixture of 1 (5 mmol, 1.66 gm) and triethylorthofomate (20 ml) was refluxed for 24h. After reaction completion the excess of triethylorthofomate was removed under vacuum. The solid remained was washed with n-hexane several times and crystallized from benzene to give compound 7, pale brown color, m.p. 233-234 °C, yield 60%. Anal.Calcd.for C₁₅H₁₂N₂O₂Cl (387.78): C, 52.66; H, 3.64; Cl, 9.14; N, 18.06. Found: C, 52.64; H, 3.62; Cl, 9.13; N, 18.08. FTIR (KBr, cm⁻¹): 3180 (NH) pyrazole, 1632 (C-N), 2215 (C=N). H-NMR (DMSO-d₆) (ppm): 12.36 (s, 1H, NH, pyrazole, exch. with D₂O), 8.59 (s, 1H, N-CH), 8.17–7.77 (m, 3H, Har), 5.47 (s, 1H, benzylic), 4.34–4.28 (q, 2H, CH₃), 1.77 (s, 3H, CH₃, pyrazole), 1.31–1.28 (t, 3H, CH₃). MS m/z (%): 386.87(M⁺); 11.08, 389.567, 283(25.01), 259(34.62), 202(32.17), 146 (82.33), 82 (100).

**4-(2-chloro-5-nitrophenyl)-5-imino-3-methyl-1,4-dihydropyrazolo[4’,3’:5,6]pyrano[2,3-d]pyrimidin-6-yl,1,4-dihydropyrazolo[2,3-c]pyrazol-6-yl formamide (9)**

Compound 7 (2 mmol, 0.78 gm) was added to a mixture of methanol (15 ml) and 25% aqueous ammonia solution (15 ml). The reaction mixture was stirred for 24h, cooled and the precipitated solid filtered off and crystallized from toluene to give compound 9, pale brown color, m.p. > 300°C, yield 51%. Anal.Calcd.for C₁₅H₁₈N₂O₂SCl (407.85): C, 44.17; H, 2.47; Cl, 8.69; N, 17.17. Found: C, 44.16; H, 2.46; Cl, 8.68; N, 17.18. FTIR (KBr, cm⁻¹): 3261 (NH) pyrazole, 3151(NH), 2966 (SH), 2215 (C=N), 1390 (C=S). H-NMR (DMSO-d₆) (ppm): 12.02 (s, 1H, NH, pyrazole), 7.70–7.14 (m, 3H, Har), 5.47 (s, 1H, benzylic), 4.34–4.28 (q, 2H, CH₃), 1.77 (s, 3H, CH₃, pyrazole), 1.31–1.28 (t, 3H, CH₃). MS m/z (%): 366.8 (M⁺), 11.08, 389.567, 283(25.01), 259(34.62), 202(32.17), 146 (82.33), 82 (100).
1745 (C=O), 2210 (C=N). 3H-NMR (DMSO- d$_6$) (ppm): 11.5 (s, 1H, NH, pyrazole), 8.58-7.11 (m, 8H, Har), 4.7(s, 1H, benzylic), 8.9 (s, 1H, NH), 6.7 (s, 1H, NH), 1.9 (s, 3H, CH$_3$).MS m/z (%): 450 (M$^+$; 3.47), 451.85 (4.90), 244 (22.11), 219 (41.50), 198 (79.99).

REFERENCES

1. Goel, A. and F.V. Singh, 2005. Regioselective synthesis of functionally congested biaryl by a novel C–C bond formation reaction. Tetrahedron Lett, 46: 5585.
2. EL-Far, M., F.V.G.A. EL megeed, E.F. Eskander, H.M. Rady and M.A. Tantawy, 2009. Novel modified steroid derivatives of androstanolone as chemotherapeutic anti-cancer agents. Eur. J. med. Chem, 44(10): 3936.
3. Pasternak, P.V., Averkiev B.B.M.Y. Antipin, A.S. Perepogodov and N. D. J. Chikanikov, 2004. Synthesis and some heterocyclization reactions of new diethyl (1, 1-difuoro-3, 3-dicyano-2-trifluoromethylallyl) phosphonate and ethyl 3, 3-dicyano-2-[(diethoxyphosphoryl) difluoromethyl] acrylate. Fluorine Chem, 125, 1853.
4. Ballini, R., G. Sartori and R. Sartorio, 2004. Utiliry of 4-formylantipyrine in heterocyclic synthesis Basic alumina catalysed synthesis of substituted 2-amino-2-chromenes via three-component reaction. Tetrahedron Lett, 45: 2297.
5. Abdel hamid, A.O. and M.A.M. Afifi, 2010. Novel Pyranopyrazoles: Synthesis and Theoretical Studies. Journal of Advanced Research, 1: 137.
6. AI-Amiry, A.A., R.I. Al-Bayati, F.M. Saed, W.B. Ali, A.A.H. Kadhum and A.B. Mohamed, 2012. Synthesis, analgesic, anti-inflammatory and antimicrobial activity of some novel pyrimido[4,5-b]quinolin-4-ones. Molecules, 17: 10377.
7. El-Gazzar, A.B.A., M.M. El-Enany and M.N. Mahmoud, 2008. Bioorg. Med. Chem, 16: 3261.
8. Fayed, A.A., H.M. Hosni, E.M. Flefel and A.E.E. Amr, 2009. Synthesis and pharmacological activity of some new thieno[2,3-d]pyrimidine and pyrimidopyrazolothieno pyrimidine derivatives. World J. Chem, 4: 58.
9. Sondhi, S.M., N. Singh, M. Johar and A. Kumar, 2005. Synthesis, anti-inflammatory and analgesic activities evaluation of some mono, bi and tricylic pyrimidine derivatives. Bioorg. Med. Chem, 13: 6158.
10. Abu-Hashem, A.A., M.F. El-Shehry and F.A. Badria, 2010. Design and synthesis of novel thiophenecarbohydrazide, thienopyrazole and thienopyrimidine derivatives as antioxidant and antitumor agents. Acta Pharm, 60: 311.
11. Svetlik, J., L. Veizerova, T. Liptaj and J. Kubista, 2009. Transformation of oxygen-bridged pyrimidines with nitrogen nucleophiles and characterization of resulting products ARKIVOC, 2009, 79.
12. Fathalla, O.A., I.F. Zeid, M.E. Haiba, A.M. Soliman, S.I. Abd-Elmoez and W.S. Serwy, 2009. Synthesis, antibacterial and anticancer evaluation of some pyrimidine derivatives. World J. Chem, 4: 127.
13. El-Assiery, S.A., G.H. Sayed and A. Fouda, 2004. Synthesis of some new annulated pyrazolo-pyrido (or pyrano) pyrimidine, pyrazolopyridine and pyranopyrazole derivatives. Acta Pharm, 54: 143.
14. Li, M., S.W. Wang, L.R. Wen, W.Y. Qi and H.Z. Yang, 2005. An Unexpected and Green Synthetic Protocol for Ethyl 1-Aroyl/Aroylmethyl-5-methyl-3-methylthiopyrazole-4-carboxylates: High Regioselectivity in Alkylation and Acylation Reactions between N-1 and N-2 of a Pyrazole Ring. Chin. J. Struct. Chem, 24: 64.
15. Wannberg, J., D. Dallinger, C.O. Kappe and M.J. Larhed, 2005. Microwave-enhanced and metal-catalyzed functionalizations of the 4-aryl-dihydropyrimidone template. Comb. Chem, 7: 574.
16. Alqasoumi, S.I., A.M. Al-Taweel, A.M. Alafeefy, E. Noaman and M.M. Ghorab, 2010. Novel quinolines and pyrimido [4, 5-b] quinolines bearing biologically active sulfonamide moiety as a new class of antitumor agents. Eur. J. Med. Chem, 45: 738.
17. Broom, A.D., J.L. Shim and G.L. Anderson, 1976. Pyrido[2,3-d]pyrimidines. IV. Synthetic studies leading to various oxopyrido[2,3-d]pyrimidines. J. Org. Chem, 41: 1095.
18. Joshi, A.A. and C.L. Viswanathan, 2006. Recent developments in antimalarials drug discovery Anti-Infect. Agents Med. Chem, 5: 105.
19. Ghorab, M.M. and A.Y. Hassan, 1998. Synthesis and antibacterial properties of new dithienyl containing pyran, pyrano [2,3-b] pyridine, pyrano [2,3-d] pyrimidine and pyridine derivatives. Phosphorus, Sulfur Silicon Relat. Elem., 141: 251.
20. Sharma, P., N. Rane and V.K. Gurram, 2004. Synthesis and QSAR studies of pyrimido[4,5-d]pyrimidine-2,5-dione derivatives as potential antimicrobial agents. Bioorg. Med. Chem. Lett, 14: 4185.
21. El-Gazzar, A. B. A., H. N. Hafez and G. A. M. Nawwar, 2009. New Acyclic Nucleosides Analogue as Potential Analgesic, Anti-Inflammatory, Anti-Oxidant and Anti-Microbial Derived from Pyrimido[4,5-b]quinolines. Eur. J. Med. Chem., 44: 1427.

22. de la Cruz, J.P., A. Moreno, F. Mérida, J. García-Campos and F.S. de la Cuesta, 1994. The pyrimido-pyrimidine derivatives, dipyrindamole and RA-642, reduce opacification of crystalline lens in diabetic rats. Pharmacol. Toxicol, 75: 250.

23. Gupta, R., A. Jain, R. Joshi and M. Jain, 2011. Eco friendly solvent less synthesis of 5-indolyl pyrimido[4,5-d]pyrimidinones. Bull. Korean Chem. Soc, 32: 899.

24. Hanna, M.M., 2012. New pyrimido[5,4-e]pyrrolo[1,2-c]pyrimidines: Synthesis, 2D-QSAR, anti-inflammatory, analgesic and ulcerogenicity studies. Eur. J. Med. Chem, 55: 12.

25. Shinde, S.V., W.N. Jadhav and N.N. Karade, 2010. Three component solvent-free synthesis and fungicidal activity of substituted pyrimido [4,5-d] pyrimidine-2-(1H)-one. Orient. J. Chem, 26: 307.

26. Lichtner, R.B., G. Hutchinson and K. Hellmann, 1989. The pyrimido-pyrimidine derivatives RA233 and RX-RA85 affect cell cycle distribution of two murine tumour cell lines. Eur. J. Cancer Clin. Oncol., 25: 945.

27. DeGraw, J.I., P.H. Christie, W.T. Clowell and F.M. Sirotnak, 1992. Synthesis and antifolate properties of 5,10-ethano-5,10-dideazaaminopterin. J. Med. Chem, 35: 320.

28. Debrah, K.D., K. Saravanan, L.Z. Wang, M.J. Lin, J.S. Northen, H. Barlow, M. Barton, D.R. Newell, R.J. Griffin, B.T. Golding and N.J. Curtin, 2009. Preclinical evaluation of a novel pyrimidopyrimidine for the prevention of nucleoside and nucleobase reversal of antifolate cytotoxicity. Mol. Cancer Ther, 8: 1828.

29. Petal, N.C. and A.G. Mehta, 2001. Synthesis and Bisazo Acid Dyes Based on 4-Hydroxy-1-Phenylquinoline-2(1H)-one System and Their Dyeing Performance on Various Fabrics Asian J. Chem, 13, 1385.

30. Holla, B.S., M. Mahalinga, M.S. Karthikeyan, P.M. Akberali and N.S. Shetty, 2006. Synthesis of some novel pyrazolo[3,4-d]pyrimidine derivatives as potential antimicrobial agents. Bioorg. Med. Chem., 14: 2040.

31. Çağus, S., E. Gondek, A. Daniel, B. Jarosz, M. Pokładko and A.V. Kityk, 2007. Electroluminescence of 6-R-1,3-diphenyl-1H-pyrazolo[3,4-b]quinoline-based organic light-emitting diodes (R = F, Br, Cl, CH₃, C₂H₅ and N(C₆H₅)). Mater. Lett, 61: 3292.

32. Yousif, N.M., F.A. Gad and H.H. Sayed, 1992. Synthesis and reactions of some arylmethylenem-1- indanone derivatives of expected biological activity. Egypt. J. Chem, 35: 101.

33. Yousif, N.M., F.A. Gad and H.H. Sayed, 1996. Synthesis and reactions of β-ketoanilides for biological activity. Egypt. J. Pharm. Sci, 37: 145.

34. Sayed, H.H., 1998. Reactions with α,β- spiroepoxyalkanes part – VI. Reactions of spirononanones with amines and thiourea. Indian J. Chem, 37B: 1054.

35. Sayed, H.H. and F.A. Fahmy, 2001. Uses of 2-aryl-3-(tetral-1-one) oxirane in synthesis of spiro and condensed heterocyclic compounds. Egypt. J. Chem, 44: 365.

36. Rashad, A.E., H.H. Sayed, A.H. Shamroukh and H.M. Awad, 2005. Preparation of some fused pyridopyrimidine and pyridothienotriazine derivatives for biological evaluation. Phosphorus Sulfur, 180: 2767.

37. Abdel-Mageid, F.M.E., N.A. Hassan, M.A. Zahran and A.E. Rashad, 1998. Synthesis of 5,6-dihydropyrido[1′,2′:4,5]thieno[2,3-d] pyrimidines, 5,6-dihydro naphtho[1′,2′:4,5] thieno [3,2-c] [1,2,4]triazolo [1,5-c] pyrimidines and some of their nucleosides. Sulfur Lett, 21: 269.

38. El-Ziaty, A.K., A.A. Abdalha, A.A. Hamed, S.A. Shiba and A.A. Abdulahi, 2012. Synthesis of novel 2-propenyl amides, esters, heterocyclic compounds and their screening as antifungal and antibacterial agents. European Journal of Chemistry, 3(1): 65.

39. El-Ziaty, A.K. and S. Shiba, 2007. Antibacterial activities of new (E) 2-cyano-3-(3`,4`-dimethoxyphenyl) -2-propenoylamide derivatives. A Synthetic Communications, 27: 4043.

40. Abou-Elmagd, W.S.I., A.K. El-Ziaty and A.A. Abdalha, 2015. Ring transformation and antimicrobial activity of indolyl-substituted 2(3H)-furanones. Heterocyclic Communications, 21(13): 179.

41. Elziaty, A.K., O.E.A. Mostafa, E.A. El-Bordany, M. Nabil and H.M.F. Madkour, 2014. Access to new pyranopyrazoles and related heterocycles. Int. J. Sci. Eng. Res, pp: 5.
42. Ismail, M.F., M.R. Mahmoud, S.A. Shiba, A.K. El-Ziaty and F.S.M. Abu El-Azm, 2014. Synthesis and Reactions of Novel 2, 5-Disubstituted 1, 3, 4-Thiadiazoles. Synthetic Communications, 44: 1094.

43. El-Ziaty, A.K., A.M. Hussein and M.R. Mahmoud, 2012. Utility of S-benzylthiuronium chloride in the synthesis of heterocyclic systems. World Applied Science Journal, 17(1): 101.

44. Morsy, J.M., A.F. Aly, M.M. Elshahawi and A.K. El-Ziaty, 2016. Synthesis and Insecticidal Efficacy of Novel Bis Quinazolinone Derivatives. Journal of Heterocyclic Chemistry, 53(5): 1443.

45. El-Ziaty, A.K. and M.M. El-Shahawi, 2017. Enaminonitrile as Building Block in Heterocyclic Synthesis: Synthesis of Novel 4H-Furo[2,3-d][1,3]oxazin-4-one and Furo[2,3-d]pyrimidin-4(3H)-one Derivatives. Journal of Chemistry.

46. EL-Ziaty, A.K., M.S. Abdel-Aziz, G. Bassioni, A.M.A. Hassan and H.A. Derbala, 2016. A Synthetic Approach to Pyrazolopyranopyrimidinones and Pyrazolopyranoaxazinones as Antimicrobial Agents. Journal of Chemistry.