Microwave-Assisted Synthesis and Antimicrobial Evaluation of Novel Spiroisoquinoline and Spiropyrido[4,3-d]pyrimidine Derivatives

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Abstract: Bromination of N-substituted homophthalimides and tetrahydropyrido[4,3-d]pyrimidine-5,7-diones produces 4,4-dibromohomophthalimide and 8,8-dibromo-tetrahydropyrido[4,3-d]pyrimidine-5,7-dione derivatives, respectively, that can be used as precursors for spiro derivatives. The dibromo derivatives react with different binucleophilic reagents to produce several spiroisoquinoline and spirotetrahydropyrido[4,3-d]pyrimidine-5,7-dione derivatives, respectively. Reaction of the dibromo derivatives with malononitrile produces dicyanomethylene derivatives which react with different binucleophiles to produce new spiro derivatives. All new compounds are prepared by using the usual chemical conditions and microwave assisted conditions. The latter conditions improved the reaction yields, reduced reaction times and ameliorated the effects on the surrounding environment as the reactions are carried out in closed systems. Structures of the newly synthesized compounds are proved using spectroscopic methods such as IR, MS, 1H-NMR and 13C-NMR and elemental analyses. Some of the newly synthesized compounds were tested for their antimicrobial activities, whereby four of them showed moderate activities and the rest showed low or no activities towards the investigated species.

Keywords: bromination; homophthalimide; pyrido[4,3-d]pyrimidine; spiro compounds; microwave; antimicrobial
1. Introduction

Spiro compounds constitute a group of generally less investigated compounds, however, recently growing efforts have been made to synthesize and characterize these compounds. Many spiro compounds possess very promising biological activities as anticancer [1,2], antibacterial [3,4], anticonvulsant [5–7], antituberculosis [8], anti-Alzheimer’s [9], pain-relief [10,11] and antidermatitis agents [12]. In addition to their medical uses, some spiro-compounds have found other uses in the agricultural and industrial fields. For example, they are used as antifungal agents [13], pesticides [14], laser dyes [15] and electroluminescent devices [16]. Spiro compounds have also been used as antioxidants [17,18]. Our research group is interested in using the microwave technique [3,19–25], as it has several advantages over conventional methods of synthesis, such as reduced reaction times, fewer effects on the environment and better reactions. In the present research, we used both the microwave technique as well as conventional methods to prepare some new spiro compounds that were then tested for their antimicrobial activities.

2. Results and Discussion

2.1. Chemistry

Homophthalic anhydride (1) was reacted with aromatic amines, namely p-toluidine and p-chloroaniline, to afford N-arylhomophthalimide derivatives 2a,b respectively, which were used as precursors for preparing new spiroisoquinolines (Scheme 1). Compounds 2a,b, having an active methylene group, reacted with two equivalents of bromine in acetic acid to produce 2-aryl-4,4-dibromoisoquinoline-1,3-(2H,4H)dione derivatives 3a,b. The mass spectrum of compound 3a displayed the expected molecular ion isomeric peaks at m/z 407 (4.8%), 409 (10.1%), 411 (5.2%). Compound 3b gave the molecular ion peaks at 427 (4.4%), 429 (9.9%), 431 (6.9%).

Compounds 3a,b underwent direct cyclocondensation when treated with each of o-phenylenediamine (4a) or o-aminophenol (4b) to produce 2'-aryl-1,3-dihydro-1'H-spiro-[benzo[d]imidazole-2,4'-isoquinoline]-1',3'(2'H)-diones 5a,b and 2'-aryl-1'H,3H-spiro[benzo[d]oxazole-2,4'-isoquinoline]-1',3'(2'H)-diones 5c,d, respectively (Scheme 1). The synthesis of compounds 5a–d was carried out under conventional heating conditions. Thus, when the reaction was carried out in a refluxing ethanolic piperidine solution for 5 h under TLC monitoring, the product 5a–d were obtained in 42%–51% yields.

Similarly, compounds 3a,b reacted with thiosemicarbazide under the same reaction conditions and produced 2-aryl-5'-thioxo-1H-spiro[isoquinoline-4,3'-[1,2,4]triazolidine]-1,3(2H)-diones 6a,b (Scheme 1). The analytical and spectral data of 5a–d and 6a,b were in agreement with the proposed structures (Experimental Section).

In a similar manner, when 4-(4-aryl)-6-phenyl-2-thioxo-2,3,4,4a-tetrahydropyrido-[4,3-d]-pyrimidine-5,7(6H,8H)-diones 7a,b [26] were treated with two equivalents of bromine in acetic acid, the 8,8-dibromo derivatives 8a,b were obtained. Elemental analyses as well as the spectroscopic data of 8a,b agreed with the proposed structures (Experimental Section). Heating under reflux compounds 8a,b in absolute ethanol in presence of piperidine with either of ethylenediamine or thiosemicarbazide afforded the corresponding targeted spiro compounds 4'-{(4-aryl)-(6'-phenyl-2'-thioxo-3',4'-dihydro-1'H-spiro- [imidazolidine-2,8'-...
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pyrido[4,3-d]pyrimidine]-5',7'(2'H,6'H)-diones 9a,b and 4-(4-aryl)-6-phenyl-2,5'-dithio-3,4-dihydro-1H-spiro[pyrido[4,3-d]pyrimidine-8,3'{1,2,4]triazolidine]-5,7(2H,6H)-diones 10a,b, respectively.

Scheme 1. Reactions of dibromohomophthalimides with binucleophilic reagents: synthesis of 5 and 6.

Similarly, compounds 8a,b were refluxed with o-phenylenediamine (4a) and o-aminothiophenol (4c) in absolute ethanol, in the presence of piperidine to afford the corresponding cyclized products 4'- (4-aryl)-6'-phenyl-2'-thioxo-1,3,3',4'-tetrahydro-1'H-spiro[benzo[d]imidazole-2,8'-pyrido[4,3-d]pyrimidine]-5',7'(2'H,6'H)-diones 11a,b and 4'- (4-aryl)-6'-phenyl-2'-thioxo-3',4'-dihydro-1'H,3H-spiro-[benzo[d]thiazole-2,8'-pyrido-[4,3-d]pyrimidine]-5',7'(2'H,6'H)-diones 11c,d, respectively (Scheme 2). The produced compounds 9a,b, 10a,b and 11a–d gave fully consistent elemental and spectroscopic analyses data (Experimental Section).

On the other hand, Refluxing compounds 8a,b in ethanol/piperidine with either with malononitrile (12a) or ethyl cyanoacetate (12b) afforded the corresponding 2-(4-(4-aryl)-5,7-dioxo-6-phenyl-2-thioxo-1,2,3,4,6,7-hexahydropyrido[4,3-d]pyrimidin-8(5H)-ylidene)malononitriles (compounds 13a,b) and ethyl 2-(4-(4-aryl)-5,7-dioxo-6-phenyl-2-thioxo-1,2,3,4,6,7-hexahydropyrido-[4,3-d]pyrimidin-8(5H)-ylidene)-2-cyanoacetates 13c,d, respectively.
Scheme 2. Reaction of dibromopyridopyrimidines 8a,b with binucleophilic reagents; formation of 9, 10 and 11.

Scheme 3. Reaction of 8a,b with activated methylene compounds followed by binucleophiles; formation of 14a–d and 15a–d.

Compounds 13a–d were refluxed with hydrazine hydrate in ethanol to obtain the 5-substituted-4′-(4-aryl)-5′,7′-dioxo-6′-phenyl-2′-thioxo-2′,3′,4,4′,5′,6′,7′-octahydro-1′H-spiro[pyrazole-3,8′-pyrido-[4,3-d] pyrimidine]-4-carbonitrile spiro products 14a–d, respectively. Similar treatment of compounds 13a–d
with thiourea in ethanol/piperidine resulted in the formation of the 6'-substituted-4-(4-aryl)-5,7-dioxo-6-phenyl-2,2'-dithioxo-2,3,3',4,5,5',6,7-octahydro-1H,2'H-spiro-[pyrido[4,3-d]pyrimidine-8,4'-pyrimidine]-5'-carbonitriles 15a–d, respectively (Scheme 3).

Trying to take advantage of the benefits of the microwave assisted reaction conditions, compounds 5, 6, 9, 10, 11, 14 and 15 were prepared by using microwave irradiation instead of the conventional heating conditions. The results showed that much less time was needed to prepare these compounds, as well as a considerable increase in the reaction yields upon using the environmentally friendly microwave irradiation conditions. Table 1 shows a comparison in reaction times and yields between the conventional and microwave assisted methods of preparation.

### Table 1. Comparison between conventional methods and microwave assisted methods of synthesis of compounds 5a–d, 6a,b, 9a,b, 10a,b, 11a–d, 14a–d and 15a–d.

| Compound No. | Reaction Times | Reaction Yields (%) |
|--------------|----------------|---------------------|
|              | Conventional Methods * | Microwave ‡ | Conventional Methods * | Microwave ‡ |
| 5a           | 5 h            | 15 min             | 42                   | 91             |
| 5b           | 5 h            | 15 min             | 48                   | 89             |
| 5c           | 5 h            | 15 min             | 47                   | 82             |
| 5d           | 5 h            | 15 min             | 53                   | 92             |
| 6a           | 4 h            | 10 min             | 60                   | 94             |
| 6b           | 4 h            | 10 min             | 55                   | 90             |
| 9a           | 2 h            | 5 min              | 65                   | 93             |
| 9b           | 2 h            | 5 min              | 67                   | 91             |
| 10a          | 2 h            | 5 min              | 61                   | 91             |
| 10b          | 2 h            | 5 min              | 58                   | 89             |
| 11a          | 2 h            | 5 min              | 47                   | 84             |
| 11b          | 2 h            | 5 min              | 50                   | 91             |
| 11c          | 2 h            | 5 min              | 52                   | 88             |
| 11d          | 2 h            | 5 min              | 54                   | 80             |
| 14a          | 3 h            | 7 min              | 37                   | 81             |
| 14b          | 3 h            | 7 min              | 42                   | 84             |
| 14c          | 3 h            | 7 min              | 45                   | 89             |
| 14d          | 3 h            | 7 min              | 44                   | 82             |
| 15a          | 3 h            | 7 min              | 47                   | 88             |
| 15b          | 3 h            | 7 min              | 43                   | 87             |
| 15c          | 3 h            | 7 min              | 51                   | 90             |
| 15d          | 3 h            | 7 min              | 47                   | 91             |

* Conventional reaction conditions: the reactants were heated under reflux in the proper solvent for 2–5 h in open systems (Experimental Section). ‡ Microwave-assisted reaction conditions: the reactants were heated in tightly closed tubes in scientific microwave oven for 5–15 min (Experimental Section).

2.2. Antimicrobial Evaluation

The newly synthesized heterocyclic compounds were tested for their antimicrobial activity against the following microorganisms: *Escherichia coli*, *Pseudomonas putida*, *Bacillus subtilis*, *Streptococcus lactis*, *Aspergillus niger*, *Penicillium* sp. and *Candida albicans*. The filter paper disc diffusion method [27]
was used to perform preliminary screening of the investigated compounds. The most active compounds were 5a, 6b, 9a and 9b, which showed moderate inhibition to the microorganisms. Also, compounds 5c, 6a, 11a, 11c and 15b showed slight inhibitory action. The rest of compounds showed no sensitivity at all to the tested organisms, and the results are summarized in Table 2.

| Comp. No. | Inhibition Zone (mm) |
|-----------|----------------------|
|           | Gram-Negative | Gram-Positive | Fungi | Yeast |
|           | E. coli | P. putida | B. subtilis | S. lactis | A. niger | P. sp. | C. albicans |
| 5a        | 11     | 13     | 8    | 10  | 9   | 8   | 0          |
| 5c        | 4      | 4      | 3    | 2   | 4   | 0   | 0          |
| 6a        | 8      | 6      | 7    | 8   | 6   | 6   | 0          |
| 6b        | 14     | 15     | 13   | 13  | 11  | 9   | 0          |
| 9a        | 10     | 10     | 11   | 10  | 10  | 8   | 0          |
| 9b        | 12     | 10     | 10   | 9   | 6   | 5   | 0          |
| 10a       | 0      | 0      | 0    | 0   | 0   | 0   | 0          |
| 10b       | 0      | 0      | 0    | 0   | 0   | 0   | 0          |
| 11a       | 5      | 5      | 3    | 3   | 5   | 4   | 0          |
| 11c       | 7      | 8      | 7    | 6   | 4   | 0   | 0          |
| 14a       | 0      | 0      | 0    | 0   | 0   | 0   | 0          |
| 14d       | 0      | 0      | 0    | 0   | 0   | 0   | 0          |
| 15b       | 7      | 5      | 5    | 4   | 6   | 3   | 0          |
| 15c       | 0      | 0      | 0    | 0   | 0   | 0   | 0          |
| Chloramphenicol | 22   | 21     | 18   | 19  | 20  | 12  | 0          |
| Ampicillin | 24    | 20     | 19   | 22  | 24  | 14  | 14         |

Chloramphenicol = Escherichia coli; P. putida = Pseudomonas putida; B. subtilis = Bacillus subtilis; S. lactis = Streptococcus lactis; A. niger = Aspergillus niger; P. sp. = Penicillium sp.; C. albicans = Candida albicans. The sensitivity of microorganisms to the tested compounds is identified in the following manner: Highly sensitive = Inhibition zone 15–20 mm; Moderately sensitive = Inhibition zone: 10–15 mm; Slightly sensitive = Inhibition zone: 5–10 mm; Not sensitive = Inhibition zone: 0 mm; Each result represents the average of triplicate readings.

3. Experimental Section

3.1. General

Melting points were determined in open glass capillaries on a Gallenkamp melting point apparatus and are uncorrected. IR spectra (KBr discs) were recorded on Shimadzu FTIR-8201PC spectrophotometer (Giza, Egypt). 1H-NMR and 13C-NMR spectra were recorded on a Varian Mercury 300 MHz or Varian Gemini 200 MHz spectrometers (Giza, Egypt) using TMS as an internal standard and DMSO-d6 as solvent. Microwave reactions were performed with a Millstone Organic Synthesis Unit with touch control terminal (MicroSYNTH, Giza, Egypt) with a continuous focused microwave power delivery system in a pressure glass vessel (10 mL) sealed with a septum under magnetic stirring. The temperature of the reaction mixture was monitored using a calibrated infrared temperature control under the reaction vessel, and control of the pressure was performed with a pressure sensor connected to the septum of the vessel. Elemental analysis was carried out at the Microanalytical Center of Cairo University, Giza, Egypt.
3.1.1. 2-Aryl-4,4-dibromoisoquinoline-1,3-(2H,4H)dione Derivatives 3a, b

A solution of either of 2a (2.37 g, 0.01 mol), 2b (2.51 g, 0.01 mol) or 2c (2.72 g, 0.01 mol) in glacial acetic acid (20 mL) was heated under reflux with bromine (1.1 mL, 3.0 g, 0.02 mole) for 2 h. After cooling, the reaction mixture was poured onto ice-water and the solid that precipitated was filtered off, dried and crystallized from the proper solvent.

4,4-Dibromo-2-p-tolylisoquinoline-1,3-(2H,4H)dione (3a): white crystals after crystallization from acetic acid then washing with ethanol; 66% yield; m.p. 236–238 °C; 1H-NMR: 2.60 (s, 3H, CH₃), 7.10–8.30 (m, 8H, Ar-H); 13C-NMR: 25.3 (CH₃), 80.2 (sp3 C-4), 118.2, 122.7, 125.3, 126.1, 128.7, 130.0, 131.5, 134.6, 135.2, 136.4 (aromatic C), 158.4, 167.6 (2 CO); IR ν: 3066 cm⁻¹ (aromatic CH), 2970 (aliphatic CH), 1645 (broad, 2C=O), 1605, 1500 (aromatic C=C); MS: M⁺ m/z 407 (3.2%), 409 (6.7%), 411 (3.0%); Anal. Calcd. for C₁₆H₁₁Br₂NO₂ (407.07): C (46.98%), H (2.71%), Br (39.07%), N (3.42%); Found: C (46.7%), H (2.9%), Br (38.93%), N (3.1%).

4,4-Dibromo-2-(4-chlorophenyl)isoquinoline-1,3(2H,4H)-dione (3b): white crystals after crystallization from acetic acid then washing with ethanol; 52% yield; m.p. 216–218 °C; 1H-NMR: 7.40–8.50 (m, 8H, Ar-H); 13C-NMR: 80.2 (sp3 C-4), 121.5, 123.9, 125.8, 127.1, 128.7, 130.0, 131.5, 134.6, 135.2, 136.4 (aromatic C), 158.2, 167.5 (2 CO); IR ν: 3060 cm⁻¹ (aromatic CH), 1645 (broad, 2C=O), 1605, 1500 (aromatic C=C); MS: M⁺ m/z 427 (4.7%), 429 (10.3%), 431 (7.2%); Anal. Calcd. for C₁₅H₈Br₂ClNO₂ (429.49): C (41.95%), H (1.88%), Br (37.21%), Cl (8.25%), N (3.26%); Found: C (41.7%), H (1.7%), Br (37.1%), Cl (8.4%), N (3.1%).

3.1.2. Cyclocondensation of 3a with o-Phenylenediamine and o-Aminophenol; Formation of 5a–d

Method A: Compounds 3a, b (0.01 mol) were heated under reflux with either of o-phenylenediamine (1.08 g, 0.01 mol) or o-aminophenol (1.09 g, 0.01 mol) in absolute ethanol (25 mL) and few drops of piperidine for 5 h. The reaction mixture was then cooled, acidified with few drops of conc. hydrochloric acid and the solid that precipitated was filtered at the pump and crystallized from the appropriate solvent.

Method B: The same reactants of method A were heated in microwave oven at 500 W and 140 °C for 15 min. The reaction mixture was treated similar to method A to obtain compounds 5a–d.

2’-(4-Tolyl)-1,3-dihydro-1'H-spiro[benzimidazole-2,4'-isoquinoline]-1’,3’(2'H)-dione (5a): grey crystals after crystallization from acetic acid; 42% yield (Method A) and 91% (Method B); m.p. 229–231 °C; 1H-NMR: 2.40 (s, 3H, CH₃), 3.80 (s, 2H, 2NH, D₂O exchangeable), 6.40–7.70 (m, 12H, Ar-H); 13C-NMR: 25.5 (CH₃), 83.4 (sp3-spiro C), 113.0, 115.9, 120.2, 122.9, 127.3, 127.8, 128.3, 128.9, 131.2, 133.7, 135.1, 135.9, 136.8 (aromatic C), 155.4, 163.6 (2 CO); IR ν: 3180 cm⁻¹ (broad, NH), 3065 (aromatic CH), 2970 (aliphatic CH), 1655, 1640 (2C=O), 1605, 1500 (aromatic C=C); MS: M⁺ m/z 355 (12.3%); Anal. Calcd. for C₂₂H₁₇N₃O₂ (355.39): C (74.35%), H (4.82%), N (11.82%); Found: C (74.1%), H (4.4%), N (12.1%).

2’-(4-Chlorophenyl)-1,3-dihydro-1'H-spiro[benzimidazole-2,4'-isoquinoline]-1’,3’(2'H)-dione (5b): grey crystals after crystallization from acetic acid; 48% yield (Method A) and 89% (Method B); m.p. 215–217 °C; 1H-NMR: 3.90 (s, 2H, 2NH, D₂O exchangeable), 6.90–8.10 (m, 12H, Ar-H); 13C-NMR:
2'-p-Tolyl-1,3-dihydro-1'H-spiro[benzo[d]imidazole-2,4'-isoquinoline]-1',3'(2'H)-dione (5c): grey crystals after crystallization from acetic acid; 47% yield (Method A) and 82% (Method B); m.p. 240–242 °C; 1H-NMR: 2.60 (s, 3H, CH3), 3.90 (s, 1H, NH, D2O exchangeable), 6.70–7.90 (m, 12H, Ar-H); 13C-NMR: 83.4 (sp3-spiro C), 115.4, 118.8, 122.1, 124.9, 125.8, 127.5, 128.9, 129.6, 131.2, 132.9, 134.7, 137.1, 139.9, 146.8 (aromatic C), 155.4, 163.6 (2 CO); IR ν: 3190 cm⁻¹ (broad, NH), 3065 (aromatic CH), 2975 (aliphatic CH), 1655, 1645 (2C=O), 1605, 1500 (aromatic C=C); MS: M⁺ m/z 356 (10.3%); Anal. Calcd. for C22H16N2O3 (356.37): C (74.15%), H (4.53%), N (7.86%); Found: C (74.05%), H (4.43%), N (7.75%).

2'-(4-Chlorophenyl)-1'H,3H-spiro[benzo[d]oxazole-2,4'-isoquinoline]-1',3'(2'H)-dione (5d): grey crystals after crystallization from acetic acid; 53% yield (Method A) and 92% (Method B); m.p. 220–222 °C; 1H-NMR: 3.90 (s, 1H, NH, D2O exchangeable), 6.70–8.00 (m, 12H, Ar-H); 13C-NMR: 93.8 (sp3-spiro C), 119.0, 122.1, 124.9, 125.8, 127.5, 128.9, 129.6, 131.2, 132.9, 134.7, 137.1, 139.9, 146.8 (aromatic C), 155.4, 163.6 (2 CO); IR ν: 3150 cm⁻¹ (broad, NH), 3065 (aromatic CH), 1655,1645 (2C=O), 1605, 1500 (aromatic C=C); MS: M⁺ m/z 376 (8.5%), 378 (2.7%); Anal. Calcd. for C21H13ClN2O3 (376.79): C (66.94%), H (3.48%), Cl (9.41%), N (7.43%); Found: C (66.6%), H (3.6%), Cl (9.1%), N (7.3%).

3.1.3. Cyclocondensation of 3a,b with Thiosemicarbazide; Formation of 6a,b

**Method A:** Each of compounds 3a,b (0.01 mol), was heated under reflux with thiosemicarbazide (0.91 g, 0.01 mol), absolute ethanol (25 mL) and few drops of piperidine for 4 h. The reaction mixture was then cooled, acidified with few drops of conc. hydrochloric acid and the solid that precipitated was filtered at the pump and crystallized from the appropriate solvent.

**Method B:** The same reactants of method A were heated in microwave oven at 500 W and 140 °C for 10 min. The reaction mixture was treated similar to method A to obtain compounds 6a,b.

5'-Thioxo-2-p-tolyl-1H-spiro[isoquinoline-4,3'-[1,2,4]triazolidine]-1,3(2H)-dione (6a): white crystals after crystallization from acetic acid and washing with ethanol; 55% yield (Method A) and 90% (Method B); m.p. 156–158 °C; 1H-NMR: 2.40 (s, 3H, CH3), 3.10 (s, 1H, NH, D2O exchangeable), 3.60 (s, 1H, NH, D2O exchangeable), 4.00 (s, 1H, NH, D2O exchangeable), 7.20–7.90 (m, 8H, Ar-H); 13C-NMR: 23.2 (CH3), 91.7 (sp3 spiro C), 121.1, 123.4, 127.6, 128.4, 128.8, 129.8, 132.1, 134.0, 135.2, 136.8 (aromatic C), 154.9, 159.5 (2 CO), 176.9 (CS); IR ν: 3220, 3185, 3150 cm⁻¹ (NH), 3060 (aromatic CH), 2970 (aliphatic CH), 1670,1650 (2C=O), 1600, 1490 (aromatic C=C); MS (70 eV): M⁺ m/z 338 (10.3%); Anal. Calcd. for C17H14N4O2S (338.36): C (60.34%), H (4.17%), N (16.56%), S(9.48%); Found: C (60.0%), H (3.9%), N (16.8%), S (9.7%).

2-(4-Chlorophenyl)-5'-thioxo-1H-spiro[isoquinoline-4,3'-[1,2,4]triazolidine]-1,3(2H)-dione (6b): white crystals after crystallization from dilute acetic acid and washing with ethanol; 60% yield
(Method A) and 94% (Method B); m.p. 188–190 °C; \(^1\)H-NMR: 3.10 (s, 1H, NH, D\(_2\)O exchangeable), 3.50 (s, 1H, NH, D\(_2\)O exchangeable), 4.00 (s, 1H, NH, D\(_2\)O exchangeable), 7.20–7.90 (m, 8H, Ar-H); \(^1^3\)C-NMR: 92.7 (sp3 spiro C), 127.1, 127.9, 132.6, 133.4, 134.8, 135.6, 136.1, 137.0, 137.9, 138.8 (aromatic C), 156.9, 160.7 (2CO), 180.1 (CS); IR \(\nu\): 3220, 3185, 3150 cm\(^{-1}\) (NH), 3060 (aromatic CH), 1670, 1645 (2C=O), 1600, 1490 (aromatic C=C); MS (70 eV): M\(^+\) \(m/z\) 358 (12.7%) and 360 (4.3%); Anal. Calcd. for C\(_{16}\)H\(_{11}\)N\(_4\)O\(_2\)S (358.80): C (53.56%), H (3.09%), Cl (9.88%), N (15.61%), S (8.94%); Found: C (53.4%), H (2.90%), Cl (9.6%), N (15.5%), S (8.7%).

3.1.4. Bromination of \(7a, b\); Formation of \(8a, b\)

A solution of either of \(7a\) or \(7b\) (0.01 mol) in ethanol (20 mL) was heated under reflux with bromine (1.1 mL, 3.0 g, 0.02 mol) for 2 h. After cooling, the reaction mixture was poured onto ice-water and the solid that precipitated was filtered off, dried and crystallized from the proper solvent.

8,8-Dibromo-4-(4-chlorophenyl)-6-phenyl-2-thioxo-1,2,3,4-tetrahydropyrido[4,3-d]pyrimidine-5,7(6H,8H)-dione (\(8a\)): white crystals after crystallization from absolute ethanol; 55% yield; m.p. 255–257 °C; \(^1\)H-NMR: 3.00 (s, 1H, NH, D\(_2\)O exchangeable), 5.40 (s, 1H, CH), 7.20–7.70 (m, 9H, Ar-H), 11.30 (s, 1H, NH, D\(_2\)O exchangeable); \(^1^3\)C-NMR: 55.7 (pyrimidine C-4), 80.9 (pyridine C-8), 127.1, 127.9, 132.6, 133.4, 134.8, 135.6, 136.1, 137.0, 139.9, 150.8 (aromatic C), 156.9, 160.7 (2CO), 178.0 (CS); IR \(\nu\): 3220, 3180 cm\(^{-1}\) (NH), 3060 (aromatic CH), 2850 (aliphatic CH), 1670, 1640 (2C=O), 1600, 1490 (aromatic C=C); MS (70 eV): M\(^+\) \(m/z\) 539 (4.7%), 541 (10.7%) and 543 (7.1%); Anal. Calcd. for C\(_{19}\)H\(_{12}\)Br\(_2\)ClN\(_3\)O\(_2\)S (541.64): C (42.13%), H (2.23%), Br (29.50%), Cl (6.55%), N (7.76%), S (5.92%); Found: C (42.0%), H (2.3%), Br (29.4%), Cl (6.7%), N (7.9%), S (5.8%).

8,8-Dibromo-4-(4-methoxyphenyl)-6-phenyl-2-thioxo-1,2,3,4-tetrahydropyrido[4,3-d]pyrimidine-5,7(6H,8H)-dione (\(8b\)): white crystals after crystallization from absolute ethanol; 53% yield; m.p. 232–234 °C; \(^1\)H-NMR: 3.00 (s, 1H, NH, D\(_2\)O exchangeable), 3.80 (s, 3H, OCH\(_3\)), 5.40 (s, 1H, CH), 6.90–7.60 (m, 9H, Ar-H), 11.30 (s, 1H, NH, D\(_2\)O exchangeable); \(^1^3\)C-NMR: 53.9 (OCH\(_3\)), 55.5 (pyrimidine C-4), 83.1 (pyridine C-8), 127.4, 128.4, 132.7, 133.7, 134.9, 136.6, 137.1, 138.5, 148.3, 150.8 (aromatic C), 156.9, 160.7 (2CO), 182.0 (CS); IR \(\nu\): 3220, 3180 cm\(^{-1}\) (NH), 3060 (aromatic CH), 2880 (aliphatic CH), 1665, 1645 (2C=O), 1600, 1490 (aromatic C=C); MS (70 eV): M\(^+\) \(m/z\) 535 (6.5%), 537 (13.7%) and 539 (6.1%); Anal. Calcd. for C\(_{20}\)H\(_{15}\)Br\(_2\)N\(_3\)O\(_3\)S (537.22): C (44.71%), H (2.81%), Br (29.75%), N (7.82%), S (5.97%); Found: C (44.8%), H (2.8%), Br (29.9%), N (7.7%), S (6.1%).

3.1.5. Cyclocondensation of \(8a, b\) with Ethylene Diamine and Thiosemicarbazide; Formation of \(9a\) and \(10a\)

**Method A**: Each of compounds \(8a, b\) (0.01 mol) was heated under reflux with either of ethylene diamine (0.67 mL, 0.01 mol) or thiosemicarbazide (0.91 g, 0.01 mol) in absolute ethanol (25 mL) and few drops of piperidine for 2 h. The reaction mixture was then cooled, acidified with few drops of conc. hydrochloric acid and the solid that precipitated was filtered at the pump and crystallized from the appropriate solvent to give \(9a, b\) and \(10a, b\).

**Method B**: The same reactants of method A were heated in microwave oven at 500 W and 140 °C for 5 min. The reaction mixture was treated similar to method A to obtain compounds \(9a, b\) and \(10a, b\).
4′-(4-Chlorophenyl)-6′-phenyl-2′-thioxo-3′,4′-dihydro-1′H-spiroimidazolidine-2,8′-pyrido[4,3-d]pyrimidine]-5′,7′(2′H,6′H)-dione (9a): white crystals after crystallization from absolute ethanol; 65% yield (Method A) and 93% (Method B); m.p. 247–249 °C; 1H-NMR: 2.60 (s, 4H, 2CH2-imidazolidine), 3.10 (s, 1H, NH, D2O exchangeable), 3.80 (s, 2H, 2NH, D2O exchangeable), 5.30 (s, 1H, CH), 7.20–7.70 (m, 9H, Ar-H), 11.30 (s, 1H, NH, D2O exchangeable); 13C-NMR: 51.2 (imidazolidine 2CH2), 55.7 (pyrimidine C-4), 80.9 (pyridine C-8), 127.1, 127.9, 132.6, 133.4, 134.8, 135.6, 136.1, 137.0, 138.5, 147.3 (aromatic C), 156.9, 160.4 (2CO), 181.0 (CS); IR ν: 3280, 3220, 3180 cm−1 (NH), 3060 (aromatic CH), 2850 (aliphatic CH), 2880 (aliphatic CH), 1670, 1645 (2C=O), 1600, 1490 (aromatic C=C); MS (70 eV): M+ m/z 439 (8.7%) and 441 (3.2%). Anal. Calcd. for C21H18ClN5O2S (439.92): C (57.33%), H (4.12%), Cl (8.06%), N (15.92%), S (7.29%); Found: C (57.1%), H (4.2%), Cl (7.9%), N (16.1%), S (7.1%).

4′-(4-Methoxyphenyl)-6′-phenyl-2′-thioxo-3′,4′-dihydro-1′H-spiroimidazolidine-2,8′-pyrido[4,3-d]pyrimidine]-5′,7′(2′H,6′H)-dione (9b): white crystals after crystallization from absolute ethanol; 67% yield (Method A) and 91% (Method B); m.p. 215–217 °C; 1H-NMR: 2.60 (s, 4H, 2CH2-imidazolidine), 3.00 (s, 1H, NH, D2O exchangeable), 3.60 (s, 2H, 2NH, D2O exchangeable), 3.80 (s, 3H, OCH3), 5.40 (s, 1H, CH), 6.90–7.60 (m, 9H, Ar-H), 11.30 (s, 1H, NH, D2O exchangeable); 13C-NMR: 51.0 (imidazolidine 2CH2), 53.9 (OCH3), 55.5 (pyrimidine C-4), 83.1 (pyridine C-8), 116.4, 118.5, 125.7, 128.0, 134.9, 136.6, 137.1, 138.3, 150.8 (aromatic C), 156.9, 160.7 (2CO), 182.0 (CS); IR ν: 3280, 3220, 3180 cm−1 (NH), 3060 (aromatic CH), 2880 (aliphatic CH), 1670, 1645 (2C=O), 1600, 1490 (aromatic C=C); MS (70 eV): M+ m/z 435 (7.1%); Anal. Calcd. for C22H21N5O3S (435.50): C (60.67%), H (4.86%), N (16.08%), S (7.36%); Found: C (60.7%), H (4.9%), N (15.9%), S (7.1%).

4-(4-Chlorophenyl)-6-phenyl-2,5′-dithioxo-3,4-dihydro-1H-spiro[pyrido[4,3-d]pyrimidine-8,3′-[1,2,4]triazolidine]-5,7(2H,6H)-dione (10a): white crystals after crystallization from absolute ethanol; 61% yield (Method A) and 91% (Method B); m.p. 225–227 °C; 1H-NMR: 3.00 (s, 1H, NH, D2O exchangeable), 3.10 (s, 1H, NH, D2O exchangeable), 3.40 (s, 1H, NH, D2O exchangeable), 5.30 (s, 1H, CH), 7.20–7.70 (m, 9H, Ar-H), 8.10 (s, 1H, NH, D2O exchangeable), 11.00 (s, 1H, NH, D2O exchangeable); 13C-NMR: 55.7 (pyrimidine C-4), 80.9 (pyridine C-8), 127.1, 127.9, 132.6, 133.4, 134.8, 135.6, 136.1, 137.0, 138.5, 140.5, 152.3 (aromatic C), 156.9, 160.4 (2CO), 177.3, 181.0 (2CS); IR ν: 3280, 3220, 3180 cm−1 (NH), 3060 (aromatic CH), 2850 (aliphatic CH), 1675, 1640 (2C=O), 1600, 1490 (aromatic C=C); MS (70 eV): M+ m/z 470 (11.3%) and 441 (3.9%). Anal. Calcd. for C20H15ClN6O2S2 (470.96): C (51.01%), H (3.21%), Cl (7.53%), N (13.62%), S (7.29%); Found: C (50.9%), H (3.1%), Cl (7.3%), N (13.4%), S (7.4%).

4-(4-Methoxyphenyl)-6-phenyl-2,5′-dithioxo-3,4-dihydro-1H-spiro[pyrido[4,3-d]pyrimidine-8,3′-[1,2,4]triazolidine]-5,7(2H,6H)-dione (10b): white crystals after crystallization from absolute ethanol; 58% yield (Method A) and 89% (Method B); m.p. 206–208 °C; 1H-NMR: 3.00 (s, 1H, NH, D2O exchangeable), 3.10 (s, 1H, NH, D2O exchangeable), 3.60 (s, 1H, NH, D2O exchangeable), 3.80 (s, 3H, OCH3), 5.40 (s, 1H, CH), 6.90–7.60 (m, 9H, Ar-H), 8.00 (s, 1H, NH, D2O exchangeable), 11.30 (s, 1H, NH, D2O exchangeable); 13C-NMR: 53.3 (OCH3), 55.7 (pyrimidine C-4), 80.9 (pyridine C-8), 114.3, 115.9, 122.6, 126.4, 131.8, 135.6, 136.1, 137.0, 142.5, 150.3 (aromatic C), 160.9, 164.0 (2CO), 173.4, 181.0 (2CS); IR ν: 3280, 3220, 3180 cm−1 (NH), 3060 (aromatic CH), 2850 (aliphatic CH), 1675, 1640
(2C=O), 1600, 1490 (aromatic C=C); MS (70 eV): M⁺ m/z 466 (8.3%). Anal. calcd. for C₂₁H₁₈N₆O₃S₂ (466.54): C (54.06%), H (3.89%), N (18.01%), S (13.75%); Found: C (53.9%), H (3.7%), N (17.9%), S (13.6%).

3.1.6. Cyclocondensation of 8a,b with o-Phenylenediamine (4a) and o-Aminothiophenol (4c);
Formation of 11a–d

Method A: Each of compounds 8a,b (0.01 mol) was heated under reflux with either of o-phenylenediamine (4a; 1.08 g, 0.01 mol) or o-aminothiophenol (4c; 1.25 mL, 0.01 mol) in absolute ethanol (25 mL) and few drops of piperidine for 2 h. The reaction mixture was then cooled, acidified with few drops of conc. hydrochloric acid and the solid that precipitated was filtered at the pump and crystallized from the appropriate solvent.

Method B: The same reactants of method A were heated in microwave oven at 500 W and 140 °C for 5 min. The reaction mixture was treated similar to method A to obtain compounds 11a–d.

4’-(4-Chlorophenyl)-6’-phenyl-2’-thioxo-1,3,3’,4’-tetrahydro-1’H-spiro[benzo[d]imidazole-2,8’-pyrido[4,3-d]pyrimidine]-5’,7’(2’H,6’H)-dione (11a): white crystals after crystallization from absolute ethanol; 47% yield (Method A) and 84% (Method B); m.p. 258–260 °C; ¹H-NMR: 3.10 (s, 1H, NH, D₂O exchangeable), 4.40 (s, 2H, 2NH, D₂O exchangeable), 5.30 (s, 1H, CH), 6.60–7.50 (m, 13H, Ar-H), 12.10 (s, 1H, NH, D₂O exchangeable); ¹³C-NMR: 55.7 (pyrimidine C-4), 80.9 (pyridine C-8), 118.8, 119.2, 127.1, 127.9, 132.6, 133.3, 134.6, 135.6, 136.1, 137.0, 138.5, 145.3, 151.4 (aromatic C), 160.9, 164.4 (2CO), 175.1 (CS); IR ν: 3280, 3220, 3180 cm⁻¹ (NH), 3040 (aromatic CH), 2830 (aliphatic CH), 1665, 1645 (2C=O), 1600, 1490 (aromatic C=C); MS (70 eV): M⁺ m/z 487 (6.3%) and 489 (2.2%). Anal. Calcd. for C₂₅H₁₈ClN₅O₂S (487.96): C (61.54%), H (3.72%), Cl (7.27%), N (14.35%), S (6.57%); Found: C (61.3%), H (3.6%), Cl (7.1%), N (14.3%), S (6.4%).

4’-(4-Methoxyphenyl)-6’-phenyl-2’-thioxo-1,3,3’,4’-tetrahydro-1’H-spiro[benzo[d]imidazole-2,8’-pyrido[4,3-d]pyrimidine]-5’,7’(2’H,6’H)-dione (11b): white crystals after crystallization from absolute ethanol; 50% yield (Method A) and 91% (Method B); m.p. 226–228 °C; ¹H-NMR: 3.10 (s, 1H, NH, D₂O exchangeable), 3.80 (s, 3H, OCH₃), 4.10 (s, 2H, 2NH, D₂O exchangeable), 5.40 (s, 1H, CH), 6.60–7.50 (m, 13H, Ar-H), 12.20 (s, 1H, NH, D₂O exchangeable); ¹³C-NMR: 55.3 (OCH₃), 55.7 (pyrimidine C-4), 80.9 (pyridine C-8), 112.8, 116.2, 123.1, 124.9, 132.6, 133.3, 134.6, 135.6, 136.1, 137.0, 138.5, 145.4, 150.6 (aromatic C), 160.9, 164.4 (2CO), 175.1 (CS); IR ν: 3280, 3220, 3180 cm⁻¹ (NH), 3040 (aromatic CH), 2830 (aliphatic CH), 1665, 1645 (2C=O), 1600, 1490 (aromatic C=C); MS (70 eV): M⁺ m/z 483 (6.8%). Anal. Calcd. for C₂₆H₂₁N₅O₃S (483.54): C (64.58%), H (4.38%), Cl (7.27%), N (14.35%), S (6.63%); Found: C (64.5%), H (4.1%), N (14.6%), S (6.5%).

4’-(4-Chlorophenyl)-6’-phenyl-2’-thioxo-3’,4’-dihydro-1’H-spiro[benzo[d]thiazole-2,8’-pyrido[4,3-d]pyrimidine]-5’,7’(2’H,6’H)-dione (11c): white crystals after crystallization from absolute ethanol; m.p. 230–232 °C, in 52% yield (Method A) and 88% (Method B); ¹H-NMR: 3.10 (s, 1H, NH, D₂O exchangeable), 4.50 (s, 1H, CH), 6.60–7.50 (m, 13H, Ar-H), 11.80 (s, 1H, NH, D₂O exchangeable); ¹³C-NMR: 55.7 (pyrimidine C-4), 82.0 (pyridine C-8), 116.0, 119.6, 121.3, 125.2, 127.4, 128.5, 132.6, 133.3, 134.6, 135.6, 136.1, 137.0, 138.5, 139.3, 145.3, 151.4 (aromatic C),
160.9, 164.4 (2CO), 175.1 (CS); IR \( \nu \): 3280, 3220, 3180 cm\(^{-1}\) (NH), 3040 (aromatic CH), 2830 (aliphatic CH), 1665, 1645 (2C=O), 1600, 1490 (aromatic C=C); MS (70 eV): \( \text{M}^+ \) m/z 504 (9.8%) and 489 (3.7%).

Anal. Calcd. for C\(_{25}\)H\(_{17}\)ClN\(_4\)O\(_2\)S\(_2\) (505.01): C (59.46%), H (3.39%), Cl (7.02%), N (11.09%), S (12.70%); Found: C (59.5%), H (3.2%), Cl (6.9%), N (11.2%), S (12.6%).

4′-(4-Methoxyphenyl)-6′-phenyl-2′-thioxo-3′,4′-dihydro-1′H,3H-spiro[benzo[d]thiazole-2,8′-pyrido[4,3-d]pyrimidine]-5′,7′(2′H,6′H)-dione (11d): white crystals after crystallization from absolute ethanol; 45% yield (Method A) and 80% (Method B); m.p. 210–212 °C; 1H-NMR: 3.10 (s, 1H, NH, D\(_2\)O exchangeable), 3.80 (s, 3H, OCH\(_3\)), 4.00 (s, 1H, NH, D\(_2\)O exchangeable), 5.40 (s, 1H, CH), 6.60–7.50 (m, 13H, Ar-H), 12.20 (s, 1H, NH, D\(_2\)O exchangeable); 13C-NMR: 55.3 (OCH\(_3\)), 55.7 (pyrimidine C-4), 80.9 (pyridine C-8), 116.4, 118.2, 121.2, 125.6, 127.1, 128.5, 132.6, 133.3, 134.6, 135.6, 136.1, 137.0, 138.5, 140.3, 145.1, 151.3 (aromatic C), 160.9, 164.4 (2CO), 175.1 (CS); IR \( \nu \): 3280, 3220, 3180 cm\(^{-1}\) (NH), 3040 (aromatic CH), 2830 (aliphatic CH), 1665, 1645 (2C=O), 1600, 1490 (aromatic C=C); MS (70 eV): \( \text{M}^+ \) m/z 504 (11.3%). Anal. Calcd. for C\(_{26}\)H\(_{20}\)N\(_4\)O\(_3\)S\(_2\) (500.59): C (62.38%), H (4.03%), N (11.19%), S (12.81%); Found: C (62.4%), H (3.9%), N (11.0%), S (12.6%).

3.1.7. Reactions of 8a,b with Malononitrile (12a) and Ethyl cyanoacetate (12b): Formation of 13a–d

To a solution of each of compounds 8a,b (0.01 mol) in absolute ethanol (30 mL) containing a catalytic amount of piperidine was added either of malononitrile (12a; 0.66 g, 0.01 mol) or ethyl cyanoacetate (12b; 1.13 mL, 0.01 mol). The reaction mixture was heated under reflux for 3 h, under TLC monitoring, then cooled and poured onto ice-cold water. The solid product that separated was filtered off, dried and crystallized from ethanol.

2-(4-(4-Chlorophenyl)-5,7-dioxo-6-phenyl-2-thioxo-1,2,3,4,6,7-hexahydropyrido[4,3-d]pyrimidin-8(5H)-ylidene)malononitrile (13a): pale yellow crystals after crystallization from absolute ethanol; 52% yield; m.p. 223–225 °C; 1H-NMR: 3.20 (s, 1H, NH, D\(_2\)O exchangeable), 4.80 (s, 1H, CH), 7.20–7.50 (m, 9H, Ar-H), 12.60 (s, 1H, NH, D\(_2\)O exchangeable); 13C-NMR: 55.5 (pyrimidine C-4), 81.9 (methylidine C), 107.1 (CN), 112.1, 127.1, 127.9, 132.1, 132.9, 133.8, 134.7, 135.1, 137.3, 150.8, 154.3 (sp2 + aromatic C), 156.9, 160.7 (2CO), 178.0 (CS); IR \( \nu \): 3220, 3180 cm\(^{-1}\) (NH), 3060 (aromatic CH), 2850 (aliphatic CH), 2210 (CN), 1670, 1640 (2C=O), 1600, 1490 (aromatic C=C); MS (70 eV): \( \text{M}^+ \) m/z 445 (12.2%) and 447 (4.7%). Anal. Calcd. for C\(_{22}\)H\(_{12}\)ClN\(_5\)O\(_2\)S (445.88): C (59.26%), H (2.71%), Cl (7.95%), N (15.71%), S (7.19%); Found: C (59.2%), H (2.5%), Cl (7.8%), N (15.6%), S (7.0%).

2-(4-(4-Methoxyphenyl)-5,7-dioxo-6-phenyl-2-thioxo-1,2,3,4,6,7-hexahydropyrido[4,3-d]pyrimidin-8(5H)-ylidene)malononitrile (13b): yellow crystals after crystallization from absolute ethanol; 50% yield; m.p. 214–216 °C; 1H-NMR: 3.10 (s, 1H, NH, D\(_2\)O exchangeable), 3.80 (s, 1H, OCH\(_3\)), 4.70 (s, 1H, CH), 6.90–7.50 (m, 9H, Ar-H), 12.60 (s, 1H, NH, D\(_2\)O exchangeable); 13C-NMR: 54.7 (OCH\(_3\)), 55.5 (pyrimidine C-4), 81.8 (methylidine C), 107.1 (CN), 112.1, 114.8, 127.3, 132.1, 132.9, 133.8, 134.6, 135.1, 137.3, 150.8, 154.3 (sp2 + aromatic C), 156.9, 160.7 (2CO), 178.1 (CS); IR \( \nu \): 3220, 3180 cm\(^{-1}\) (NH), 3060 (aromatic CH), 2850 (aliphatic CH), 2210 (CN), 1670, 1640 (2C=O), 1600, 1500 (aromatic C=C); MS (70 eV): \( \text{M}^+ \) m/z 445 (12.2%) and 447 (4.7%). Anal. Calcd. for C\(_{22}\)H\(_{12}\)ClN\(_5\)O\(_2\)S (445.88): C (59.26%), H (2.71%), Cl (7.95%), N (15.71%), S (7.19%); Found: C (59.2%), H (2.5%), Cl (7.8%), N (15.6%), S (7.0%).
Ethyl 2-(4-(4-chlorophenyl)-5,7-dioxo-6-phenyl-2-thioxo-1,2,3,4,6,7-hexahydropyrido[4,3-d]-pyrimidin-8(5H)-ylidene)-2-cyanoacetate (13c): white crystals after crystallization from absolute ethanol; 42% yield; m.p. 188–190 °C; ¹H-NMR: 1.20 (t, 3H, CH₃), 3.00 (s, 1H, NH, D₂O exchangeable), 4.30 (q, 2H, CH₂), 5.10 (s, 1H, CH), 7.20–7.60 (m, 9H, Ar-H), 12.50 (s, 1H, NH, D₂O exchangeable); ¹³C-NMR: 16.7 (CH₃), 55.5 (pyrimidine C-4), 59.1 (CH₂), 93.9 (methylene C), 108.1 (CN), 123.4, 127.1, 127.9, 132.1, 132.9, 133.8, 134.6, 135.1, 137.3, 151.8, 157.1 (sp² + aromatic C), 156.9, 160.7, 165.0 (3CO), 178.0 (CS); IR ν: 3220, 3180 cm⁻¹ (NH), 3060 (aromatic CH), 2850 (aliphatic CH), 2230 (CN), 1710, 1670, 1640 (3C=O), 1600, 1490 (aromatic C=C); MS (70 eV): M⁺ m/z 492 (14.2%) and 494 (5.0%).

Anal. Calcd. for C₂₄H₁₇ClN₄O₄S (492.93): C (58.48%), H (3.48%), Cl (7.19%), N (11.37%), S (6.50%); Found: C (58.3%), H (3.3%), Cl (7.1%), N (11.4%), S (6.4%).

Ethyl 2-cyano-2-(4-(4-methoxyphenyl)-5,7-dioxo-6-phenyl-2-thioxo-1,2,3,4,6,7-hexahydropyrido-[4,3-d]pyrimidin-8(5H)-ylidene)acetate (13d): white crystals after crystallization from absolute ethanol; 42% yield; m.p. 188–190 °C; ¹H-NMR: 1.20 (t, 3H, CH₃), 3.00 (s, 1H, NH, D₂O exchangeable), 3.70 (s, 3H, CH₃), 4.30 (q, 2H, CH₂), 5.10 (s, 1H, CH), 6.90–7.60 (m, 9H, Ar-H), 12.30 (s, 1H, NH, D₂O exchangeable); ¹³C-NMR: 16.7 (CH₃), 53.1 (OCH₃), 55.5 (pyrimidine C-4), 59.0 (CH₂), 91.7 (methylene C), 108.1 (CN), 117.7, 125.1, 126.9, 132.1, 132.9, 133.8, 133.6, 135.1, 137.3, 148.8, 156.6 (sp² + aromatic C), 156.9, 160.7, 165.0 (3CO), 178.0 (CS); IR ν: 3220, 3180 cm⁻¹ (NH), 3060 (aromatic CH), 2850 (aliphatic CH), 2230 (CN), 1710, 1670, 1640 (3C=O), 1600, 1490 (aromatic C=C); MS (70 eV): M⁺ m/z 488 (13.9%); Anal. Calcd. for C₂₅H₂₀N₄O₅S (488.52): C (61.47%), H (4.13%), N (11.47%), S (6.56%); Found: C (61.5%), H (4.0%), N (11.4%), S (6.4%).

3.1.8. Reaction of 13a–d with Hydrazine Hydrate and Thiourea: Formation of 14a–d and 15a–d

Method A: To a solution of each of compounds 13a–d (0.01 mol) in absolute ethanol (30 mL) containing a catalytic amount of piperidine was added hydrazine (0.32 mL, 0.01 mol) or thiourea (0.76 g, 0.01 mol). The reaction mixture was heated under reflux for 3 h, under TLC monitoring, then cooled and poured onto ice-cold water. The solid product that separated was filtered off, dried and crystallized from ethanol.

Method B: The same reactants of method A were heated in microwave oven at 500 W and 140 °C for 7 min. The reaction mixture was treated similar to method A to obtain compounds 14a–d and 15a–d.

5-Amino-4'-(4-chlorophenyl)-5',7'-dioxo-6'-phenyl-2'-thioxo-2,2',3',4,4',5',6',7'-octahydro-1'H-spiro [pyrazole-3,8'-pyrido[4,3-d]pyrimidine]-4-carbonitrile (14a): white crystals after crystallization from absolute dioxane; 37% yield (Method A) and 81% (Method B); m.p. 235–237 °C; ¹H-NMR: 3.20 (s, 1H, NH, D₂O exchangeable), 4.10 (s, 1H, pyrazole H-4), 5.10 (s, 1H, pyrimidine H-4), 6.2 (s, 1H, NH, D₂O exchangeable), 7.20–7.50 (m, 9H, Ar-H), 9.1 (s, 2H, NH₂, D₂O exchangeable), 12.60 (s, 1H, NH, D₂O exchangeable); ¹³C-NMR: 39.5 (pyrazole C-4), 55.4 (pyrimidine C-4), 58.9 (spiro-C), 107.1 (CN), 110.3, 116.1, 126.9, 131.8, 132.7, 133.8, 134.6, 135.1, 137.3, 143.1, 154.3 (sp² + aromatic C), 156.9, 160.7 (2CO), 178.0 (CS); IR ν: 3350, 3220, 3180 cm⁻¹ (broad, NH), 3060 (aromatic CH), 2850 (aliphatic CH), 2200 (CN), 1670, 1640 (2C=O), 1600, 1490 (aromatic C=C); MS (70 eV): M⁺ m/z 477 (9.2%) and 447 (3.5%); Anal. Calcd. for C₂₂H₁₆ClN₄O₂S (477.93): C (55.29%), H (3.37%), Cl (7.42%), N (20.52%), S (6.71%); Found: C (55.1%), H (3.1%), Cl (7.3%), N (20.4%), S (6.6%).
5-Amino-4'- (4-methoxyphenyl)-5', 7'-dioxo-6'-phenyl-2'-thioxo-2, 2', 3', 4, 4', 5, 6', 7'-octahydro-1'H-spiro [pyrazole-3, 8'-pyrido[4, 3-d]pyrimidine]-4-carbonitrile (14b): white crystals after crystallization from dil. dioxane; 42% yield (Method A) and 84% (Method B); m.p. 229–231 °C; 1H-NMR: 3.00 (s, 1H, NH, D2O exchangeable), 3.70 (s, 3H, OCH3), 4.10 (s, 1H, pyrazole H-4), 5.10 (s, 1H, pyrimidine H-4), 6.2 (s, 1H, NH, D2O exchangeable), 7.20–7.50 (m, 9H, Ar-H), 9.4 (s, 2H, NH2, D2O exchangeable), 12.50 (s, 1H, NH, D2O exchangeable); 13C-NMR: 39.5 (pyrazole C-4), 53.7 (OCH3), 55.4 (pyrimidine C-4), 58.9 (spiro-C), 107.1 (CN), 112.7, 114.3, 126.9, 131.8, 132.7, 133.8, 134.6, 135.1, 137.3, 143.1, 154.3 (sp2 + aromatic C), 156.9, 160.7 (2CO), 178.0 (CS); IR ν: 3350, 3220, 3180 cm−1 (broad, NH), 3060 (aromatic CH), 2850 (aliphatic CH), 2200 (CN), 1670, 1640 (2C=O), 1600, 1490 (aromatic C=C); MS (70 eV): M+ m/z 473 (10.8%); Anal. Calcd. for C23H19N7O3S (477.93): C (58.43%), H, 4.04; N, 20.71; S, 6.77; Found: C (58.3%), H (4.1%), N (20.5%), S (6.6%).

4'- (4-Chlorophenyl)-5-hydroxy-5', 7'-dioxo-6'-phenyl-2'-thioxo-2, 2', 3', 4, 4', 5, 6', 7'-octahydro-1'H-spiro [pyrazole-3, 8'-pyrido[4, 3-d]pyrimidine]-4-carbonitrile (14c): white crystals after crystallization from absolute ethanol; 45% yield (Method A) and 89% (Method B); m.p. 258–260 °C; 1H-NMR: 3.40 (s, 1H, NH, D2O exchangeable), 4.50 (s, 1H, pyrazole H-4), 5.40 (s, 1H, pyrimidine H-4), 6.2 (s, 1H, NH, D2O exchangeable), 7.20–7.50 (m, 9H, Ar-H), 11.1 (s, 1H, OH, D2O exchangeable), 12.60 (s, 1H, NH, D2O exchangeable); 13C-NMR: 41.1 (pyrazole C-4), 55.4 (pyrimidine C-4), 58.9 (spiro-C), 108.9 (CN), 110.3, 115.1, 126.9, 128.8, 130.1, 132.5, 134.6, 135.1, 137.3, 143.1, 154.3 (sp2 + aromatic C), 156.9, 160.7 (2CO), 178.0 (CS); IR ν: 3400, 3270, 3180 cm−1 (broad, NH + OH), 3060 (aromatic CH), 2850 (aliphatic CH), 2200 (CN), 1670, 1640 (2C=O), 1600, 1490 (aromatic C=C); MS (70 eV): M+ m/z 478 (10.7%) and 480 (3.8%); Anal. Calcd. for C22H15ClN6O3S (478.91): C (55.17%), H (3.16%), Cl (7.40%), N (17.55%), S (6.70%); Found: C, H, Cl, N, S (6.6%).

5-Hydroxy-4'- (4-methoxyphenyl)-5', 7'-dioxo-6'-phenyl-2'-thioxo-2, 2', 3', 4, 4', 5, 6', 7'-octahydro-1'H-spiro [pyrazole-3, 8'-pyrido[4, 3-d]pyrimidine]-4-carbonitrile (14d): white crystals after crystallization from absolute ethanol; 44% yield (Method A) and 82% (Method B); m.p. 237–239 °C; 1H-NMR: 3.30 (s, 1H, NH, D2O exchangeable), 3.80 (s, 3H, OCH3), 4.10 (s, 1H, pyrazole H-4), 5.10 (s, 1H, pyrimidine H-4), 6.2 (s, 1H, NH, D2O exchangeable), 7.20–7.50 (m, 9H, Ar-H), 10.8 (s, 1H, OH, D2O exchangeable, 12.50 (s, 1H, NH, D2O exchangeable); 13C-NMR: 39.5 (pyrazole C-4), 53.8 (OCH3), 55.4 (pyrimidine C-4), 58.9 (spiro-C), 108.9 (CN), 110.3, 115.1, 126.9, 128.8, 130.1, 132.5, 134.6, 135.1, 137.3, 143.1, 154.3 (sp2 + aromatic C), 156.9, 160.7 (2CO), 178.0 (CS); IR ν: 3350, 3200, 3150 cm−1 (broad, NH + OH), 3060 (aromatic CH), 2850 (aliphatic CH), 2200 (CN), 1670, 1640 (2C=O), 1600, 1490 (aromatic C=C); MS (70 eV): M+ m/z 474 (12.0%) and 480 (3.8%); Anal. Calcd. for C23H18N6O4S (474.49): C (58.22%), H (3.82%), N (17.11%), S (6.76%); Found: C (58.1%), H (3.6%), N (17.5%), S (6.7%).

6'-Amino-4'- (4-chlorophenyl)-5, 7'-dioxo-6-phenyl-2'- thioxo-2,2', 3', 4, 5, 5', 6, 7'-octahydro-1'H-spiro [pyrido[4, 3-d]pyrimidine-8, 4'-pyrimidine]-5'-carbonitrile (15a): white crystals after crystallization from dil. DMF; 47% yield (Method A) and 88% (Method B); m.p. 240–242 °C; 1H-NMR: 3.00 (s, 1H, NH, D2O exchangeable), 3.40 (s, 1H, spiro-pyrimidine H-4), 5.10 (s, 1H, pyrimidine H-4), 5.50 (s, 1H, NH, D2O exchangeable), 7.20–7.50 (m, 9H, Ar-H), 8.70 (s, 2H, NH2, D2O exchangeable), 12.00 (s, 1H, NH, D2O exchangeable); 13C-NMR: 29.0 (spiro-pyrimidine C-5), 55.4 (pyrimidine C-4), 63.9 (spiro-C),
107.8 (CN), 114.3, 119.5, 126.9, 128.8, 132.7, 133.8, 134.6, 136.1, 138.1, 143.1, 154.3 (sp2 + aromatic C), 156.9, 160.7 (2CO), 178.0, 181.0 (2CS); IR ν: 3350, 3220, 3180 cm⁻¹ (broad, NH), 3060 (aromatic CH), 2850 (aliphatic CH), 2200 (CN), 1670, 1640 (2C=O), 1600, 1490 (aromatic C=C); MS (70 eV): M⁺ m/z 522 (10.0%) and 524 (3.8%); Anal. Calcd. for C₂₃H₁₆ClN₇O₂S₂ (522.00): C (52.92%), H (3.09%), Cl (6.79%), N (18.78%), S (12.29%); Found: C (52.8%), H (3.2%), Cl (6.8%), N (18.9%), S (12.1%).

6'-Amino-4-(4-methoxyphenyl)-5,7-dioxo-6-phenyl-2,2'-dithioxo-2,3,3',4,5,5',6,7-octahydro-1H,2'H-spiro[pyrido[4,3-d]pyrimidine-8,4'-pyrimidine]-5'-carbonitrile (15b): white crystals after crystallization from dil. DMF; 43% yield (Method A) and 87% (Method B); m.p. 226–228 °C; ¹H-NMR: 2.80 (s, 1H, NH, D₂O exchangeable), 3.50 (s, 1H, spiro-pyrimidine H-4), 3.80 (s, 1H, OCH₃), 5.00 (s, 1H, pyrimidine H-4), 6.80–7.50 (m, 9H, Ar-H), 8.50 (s, 2H, NH₂, D₂O exchangeable), 12.20 (s, 1H, NH, D₂O exchangeable); ¹³C-NMR: 27.8 (spiro-pyrimidine C-5), 52.6 (OCH₃), 55.7 (pyrimidine C-4), 64.2 (spiro-C), 107.8 (CN), 112.5, 116.2, 125.2, 127.0, 128.4, 129.8, 131.6, 136.1, 138.1, 139.0, 154.2 (sp2 + aromatic C), 156.9, 160.7 (2CO), 178.0, 181.0 (2CS); IR ν: 3350, 3200, 3160 cm⁻¹ (broad, NH), 3080 (aromatic CH), 2850 (aliphatic CH), 1670, 1640 (2C=O), 1600, 1500 (aromatic C=C); MS (70 eV): M⁺ m/z 517 (11.3%); Anal. Calcd. for C₂₄H₁₉N₇O₃S₂ (517.58): C (55.69%), H (3.70%), N (18.94%), S (12.39%); Found: C (55.6%), H (3.5%), N (18.9%), S (12.1%).

4-(4-Chlorophenyl)-6'-hydroxy-5,7-dioxo-6-phenyl-2,2'-dithioxo-2,3,3',4,5,5',6,7-octahydro-1H,2'H-spiro[pyrido[4,3-d]pyrimidine-8,4'-pyrimidine]-5'-carbonitrile (15c): white crystals after crystallization from dioxane; 51% yield (Method A) and 90% (Method B); m.p. 255–257 °C; ¹H-NMR: 3.20 (s, 1H, NH, D₂O exchangeable), 3.50 (s, 1H, spiro-pyrimidine H-4), 5.10 (s, 1H, pyrimidine H-4), 5.80 (s, 1H, NH, D₂O exchangeable), 7.20–7.50 (m, 9H, Ar-H), 11.10 (s, 1H, OH, D₂O exchangeable), 12.40 (s, 1H, NH, D₂O exchangeable); ¹³C-NMR: 29.0 (spiro-pyrimidine C-5), 55.4 (pyrimidine C-4), 63.9 (spiro-C), 107.8 (CN), 113.9, 119.8, 127.2, 128.8, 132.4, 133.8, 134.5, 136.5, 138.6, 152.1, 155.3 (sp2 + aromatic C), 158.9, 162.7 (2CO), 178.0, 180.5 (2CS); IR ν: 3350, 3220, 3180 cm⁻¹ (broad, NH), 3080 (aromatic CH), 2850 (aliphatic CH), 1670, 1640 (2C=O), 1600, 1500 (aromatic C=C); MS (70 eV): M⁺ m/z 522 (14.0%) and 524 (4.7%); Anal. Calcd. for C₂₃H₁₅ClN₆O₂S₂ (522.99): C (52.82%), H (2.89%), Cl (6.78%), N (16.07%), S (12.26%); Found: C (52.6%), H (2.9%), Cl (6.6%), N (15.9%), S (12.1%).

6'-Hydroxy-4-(4-methoxyphenyl)-5,7-dioxo-6-phenyl-2,2'-dithioxo-2,3,3',4,5,5',6,7-octahydro-1H,2'H-spiro[pyrido[4,3-d]pyrimidine-8,4'-pyrimidine]-5'-carbonitrile (15d): white crystals after crystallization from dioxane; 47% yield (Method A) and 91% (Method B); m.p. 238–240 °C; ¹H-NMR: 2.90 (s, 1H, NH, D₂O exchangeable), 3.60 (s, 1H, spiro-pyrimidine H-4), 3.90 (s, 1H, OCH₃), 5.00 (s, 1H, pyrimidine H-4), 5.50 (s, 1H, NH, D₂O exchangeable), 6.80–7.50 (m, 9H, Ar-H), 10.80 (s, 1H, OH, D₂O exchangeable), 12.10 (s, 1H, NH, D₂O exchangeable); ¹³C-NMR: 27.2 (spiro-pyrimidine C-5), 54.4 (OCH₃), 57.1 (pyrimidine C-4), 64.0 (spiro-C), 108.4 (CN), 116.5, 119.6, 125.0, 127.4, 128.8, 130.3, 132.6, 136.1, 138.1, 139.0, 153.1 (sp2 + aromatic C), 158.9, 160.7 (2CO), 178.0, 181.3 (2CS); IR ν: 3350, 3220, 3180 cm⁻¹ (broad, NH), 3080 (aromatic CH), 2850 (aliphatic CH), 1670, 1640 (2C=O), 1600, 1500 (aromatic C=C); MS (70 eV): M⁺ m/z 518 (14.0%), 516 (5.1%); Anal. Calcd. for C₂₄H₁₉N₆O₄S₂ (518.57): C (55.59%), H (3.50%), N (16.21%), S (12.37%); Found: C (55.4%), H (3.4%), N (16.3%), S (12.3%).
3.2. Antimicrobial Screening

The newly synthesized heterocyclic compounds were tested for their antimicrobial activity against the following microorganisms: (a) Gram-negative: *Escherichia coli* and *Pseudomonas putide*; (b) Gram-positive: *Bacillus subtilis* and *Streptococcus lactis*; (c) Fungi: *Aspergillus niger* and *Penicillium* sp.; (d) Yeast: *Candida albicans*. Media: Three types of specific media were used in this study:

- **Medium 1**: Nutrient Medium for bacteria, consisting of (g/L distilled water): peptone, 5 and meat extract, 3. pH was adjusted to 7.0.
- **Medium 2**: Potato Dextrose Medium for fungi, consisting of (g/L distilled water): Infusion from potatoes, 4 and D(+)glucose, 20. pH was adjusted to 5.5.
- **Medium 3**: Universal Medium for yeast, consisting of (g/L distilled water): yeast extract, 3; malt extract, 3; peptone, 5 and glucose, 10. pH was adjusted to 5.5.

For solid media, 2% agar was added. All media were sterilized at 121 °C for 20 min.

3.3. Procedure (Filter Paper Diffusion Method) [27]

Proper concentrations of microbial suspensions were prepared from 1 (for bacteria) to 3 (for yeast and fungi)-day-old liquid stock cultures incubated on a rotary shaker (100 rpm). In the case of fungi, five sterile glass beads were added to each culture flask. The mycelia were then subdivided by mechanical stirring at speed No. 1 for 30 min. Turbidity of microorganisms was adjusted with a spectrophotometer at 350 nm to give an optical density of 1.0. Appropriate agar plates were aseptically surface inoculated uniformly by a standard volume (ca. 1 mL) of the microbial broth culture of the tested microorganism, namely *E. coli*, *P. putida*, *B. subtilis*, *S. lactis*, *A. niger*, *Penicillium* sp. and *C. albicans*. Whatman No. 3 filter paper discs of 10 mm diameter were sterilized by autoclaving for 15 min at 121 °C. Test compounds were dissolved in 80% ethyl alcohol to give final concentration of 5 μg/mL. The sterile discs were impregnated with the test compounds (5 μg/disc). After the impregnated discs have been air dried, they were placed on the agar surface previously seeded with the organism to be tested. Discs were gently pressed with forceps to insure thorough contact with the media. Three discs were arranged per dish, suitably spaced apart, *i.e.*, the discs should be separated by a distance that is equal to or slightly greater than the sum of the diameters of inhibition produced by each disc alone. Each test compound was conducted in triplicate. Plates were kept in the refrigerator at 5 °C for 1 h to permit good diffusion before transferring them to an incubator at 37 °C for 24 h for bacteria and at 30 °C for 72 h for yeast and fungi.

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Author Contributions

Faty designed research; Faty and Rashed performed research and analyzed the data; Faty, Rashed and Youssef wrote the paper. All authors read and approved the final manuscript.
Conflicts of Interest

The authors declare no conflict of interest.

References

1. Reddy, D.M.; Qazi, N.A.; Sawant, S.D.; Bandey, A.H.; Srinivas, J.; Shankar, M.; Singh, S.K.; Verma, M.; Chashoo, G.; Saxena, A.; et al. Design and synthesis of spiro derivatives of parthenin as novel anti-cancer agents. *Eur. J. Med. Chem.* **2011**, *46*, 3210–3217.

2. Erugu, Y.; Sangepu, B.; Varre1, K.; Pamanji, R.; Rao Jonapala, V.; Srinivasarao, V.; Tigulla, P.; Rani Jetti, V. Design, an efficient ecofriendly synthesis of spirooxindole derivatives and their anticancer activity supported by molecular docking studies. *World J. Pharmacy Pharm. Sci.* **2014**, *3*, 1895–1914.

3. Youssef, M.M.; Amin, M.A. Microwave assisted synthesis of some new heterocyclic spiro-derivatives with potential antimicrobial and antioxidant activity. *Molecules* **2010**, *15*, 8827–8840.

4. Miqdad, O.A.; Abunada, N.M.; Hassaneen, H.M.; Abu Samaha, A.S.M. Synthesis and biological activity evaluation of some new heterocyclic spiro-compounds with imidazolinone and pyrazoline moieties. *Int. J. Chem.* **2011**, *3*, 20–31.

5. Kesharwani, S.; Sahu, N.K.; Kohli, D.V. Synthesis and biological evaluation of some new spiro derivatives of barbituric acid. *Pharm. Chem. J.* **2009**, *43*, 315–219.

6. Zaher, A.F.; Khalil, N.A.; Ahmed, E.M. Synthesis and anticonvulsant activity of new 3'-aryl-7-bromo-spiro[[1]benzothiophene-3,2'-[1,3]thiazolidine]-2,4'-dione derivatives. *Orient. J. Chem.* **2010**, *26*, 1241–1248.

7. Obniska, J.; Kamiński, K. Lipophilicity characterization of new N-phenylamino-azaspiranes as potential anticonvulsant agents. *Biomed. Chromatogr.* **2006**, *20*, 1185–1191.

8. Al Houari, G.; Kerba, A.; Bennani, B.; Baba, M.F.; Daoudi, M.; Ben Hadda, T. Drug design of new antitubercular agents: 1,3-dipolar cycloaddition reaction of para-substituted-benzadoximes and 3-para-methoxybenzyliden-isochroman-4-ones. *ARKIVOC 2008*, 42–50.

9. Krzysztof, K.; Jolanta, O.; Malgorzata, D. Synthesis, physicochemical and anticonvulsant properties of new N-phenylamino derivatives of 2-azaspiro[4.4]nonane- and [4.5]decan-1,3-diones: Part V. *Eur. J. Med. Chem.* **2008**, *43*, 53–61.

10. Frank, R.; Reich, M.; Jostock, R.; Bahenberg, G.; Schick, H.; Henkel, B.; Sonnenschein, H. Substituted Spiro Compounds and Their Use for Producing Pain-Relief Medicaments. U.S. Patent 20080269271, 30 October 2008.

11. Schick, H.; Frank, R.; Reich, M.; Jostock, R.; Bahenberg, G.; Fritz, T.; Henkel, B. Substituted Spiro-Compounds and Their Use for Producing Pain-Relief Drugs. Int. Patent WO/2006/122769, 17 May 2006.

12. Nakao, K.; Ikeda, K.; Kurokawa, T.; Togashi, Y.; Umeuchi, H.; Honda, T.; Okano, K.; Mochizuki, H. Effect of trk-820, a selective kappa opioid receptor agonist, on scratching behavior in an animal model of atopic dermatitis. *Nihon Shinkei Seishin Yakurigaku Zasshi* **2008**, *28*, 75–83.

13. Velikorodov, A.; Ionova, V.; Degtyarev, O.; Sukhenko, L. Synthesis and antimicrobial and antifungal activity of carbamate-functionized spiro compounds. *Pharm. Chem. J.* **2013**, *46*, 715–719.
14. Wei R.; Liu Y.; Liang, Y. Advances in spiro compounds as pesticide. Chin. J. Org. Chem. 2009, 12, 476–487.
15. Kreuder, W.; Yu, N.; Salbeck, J. Use of Spiro Compounds as LASER Dyes. Int. Patent WO/1999/040655, 12 August 1999.
16. Kyeom, K.K.; Sehwan, S.; Seokhee, Y.; Jae soon, B.; Youn-Gu, L.; Gap, I.S.; Jieun, K.; Jae, C.L. Organic Electroluminescent Devices Using Double-Spiro Organic Compounds. U.S. Patent 6984462 B2, 10 January 2006.
17. Sarma, B.K.; Manna, D.; Minoura, M.; Mugesh, G. Structure, spirocyclization mechanism and glutathione peroxidase-like antioxidant activity of stable spirodiazaselenurane and spirodiazatellurane. J. Am. Chem. Soc. 2010, 132, 5364–5374.
18. Karali, N.; Güzel, O.; Ozsoy, N.; Ozbey, S.; Salman, A. Synthesis of new spiroindolinones incorporating a benzothiazole moiety as antioxidant agents. Eur. J. Med. Chem. 2010, 45, 1068–1077.
19. Faty, R.M.; Youssef, M.M.; Youssef, A.M.S. Microwave assisted synthesis and unusual coupling of some novel pyrido[3,2-f][1,4]thiazepines. Molecules 2011, 16, 4549–4559.
20. Saad, H.A.; Youssef, M.M.; Mosselhi, M.A. Microwave assisted synthesis of some new fused 1,2,4-triazine bearing thiophene moiety of expected pharmacological activity. Molecules 2011, 16, 4937–4957.
21. Amin, M.A.; Youssef, M.M. Use of modern technique for synthesis of quinoxaline derivatives as potential anti-virus compounds. Der Pharma Chemica 2012, 4, 1323–1329.
22. Amin, M.A.; Youssef, M.M. Microwave assisted synthesis of some new thiazolopyrimidine derivatives with potential biological activity. Org. Chem. Indian J. 2012, 8, 437–446.
23. Amin, M.A.; Youssef, M.M. Microwave assisted synthesis of some new thiazolopyrimidine, thiazolodipyrimidine and thiazolopyrimidothiazolopyrimidine derivatives with potential antioxidant and antimicrobial activity. Molecules 2012, 17, 9652–9667.
24. Amin, M.A.; Youssef, M.M.; Abdel-Hafez, S.H. Microwave-assisted synthesis of benzodiazepine derivatives: As non-nucleoside anti HIV analogue. J. Chem. Acta 2012, 1, 35–39.
25. El Azab, I.H.; Youssef, M.M.; Amin, M.A. Microwave-assisted synthesis of novel 2H-chromene derivatives bearing phenylthiazolidinones and their biological activity assessment. Molecules 2014, 19, 19648–19664.
26. Youssef, M.M. A one pot synthesis of polysubstituted pyrimidines. Facile syntheses of di- and tricyclic systems. Bull. Fac. Sci. Mansoura Univ. 2004, 31, 17–33.
27. Coffen, D.L.; Korzan, D.G. Synthetic quinine analogs. III. Frangomeric and anchimeric processes in the preparation and reactions of α,β-epoxy ketones. J. Org. Chem. 1971, 36, 390–395.

Sample Availability: Samples of the newly synthesized compounds are available from the authors.

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