Bioorganic & Medicinal Chemistry Letters 18 (2008) 5800–5803

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

One-pot synthesis and antibacterial activities of pyrazolo[4′,3′:5,6]pyrido[2,3-*d*]pyrimidine-dione derivatives

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ARTICLE INFO

Article history:
Received 2 July 2008
Revised 11 August 2008
Accepted 15 September 2008
Available online 18 September 2008

Keywords:
1*H*-Pyrazol-5-amines
Barbituric acid
Pyrimidopyrimidine
Thiobarbituric acid
Pyrazolopyrimidine

ABSTRACT

A one-pot and efficient method for the synthesis of pyrazolo[4′,3′:5,6]pyrido[2,3-*d*]pyrimidine-dione derivatives by condensation reaction of barbituric acids, 1*H*-pyrazol-5-amines and aldehydes under solvent-free conditions is reported. These products were evaluated in vitro for their antibacterial activities.

Polyfunctionalized heterocyclic compounds play important roles in the drug discovery process, and analysis of drugs in late development or on the market shows that 68% of them are heterocycles.[1,2] Therefore, it is not surprising that research on the synthesis of polyfunctionalized heterocyclic compounds has received significant attention.

Pyrimidopyrimidine, a condensed heterocycles have attracted considerable interest in the recent years. Its derivatives have been known to display a wide range of pharmacological activities as regards the tyrosine kinase domain of epidermal growth factor receptor,[3] 5-phosphoribosyl-1-pyrophosphate synthetase[4] and dihydrofolate reductase[5] have been fully demonstrated. Numerous reports delineate the antitumour,[6] antiviral,[7] antioxidant,[8] and hepatoprotective[9] activity of these compounds. Similarly, in recent years, considerable attention has been focused on the development of new methodologist to synthesize many kinds of pyrazolopyrimidine ring system.[10] Indeed, these compounds are, by now widely recognized as important organic materials showing interesting biological activities.[11] In addition, fused heterocyclic systems like pyrazolopyridopyrimidines, pyrazoloquinolines, and pyrazolopyridines present interesting biological properties such as virucidal, anticancer, fungicidal, bactericidal, and vasodilatory activities.[12]

Multicomponent reactions of 1*H*-pyrazol-5-amines, aldehydes and CH-acid compounds have recently attracted the interest of the synthetic community because the formation of different condensation products can be expected depending on the specific conditions and structure of the building blocks.[13–16] On the other hand, synthesis of benzopyrimidoquinoline-diones by reaction of naphthalene-2-amine, aldehyde and barbituric acid is reported.[17]

As part of our program aimed at developing new selective and environmentally friendly methodologies for the preparation of heterocyclic compounds,[18–24] we report an efficient, one-pot, and three-component method for the preparation of 1*H*-pyrazolo[4′,3′:5,6]pyrido[2,3-*d*]pyrimidine-dione derivatives by condensation of 1*H*-pyrazol-5-amines **1**, barbituric acid **2**, and aromatic aldehydes **3** under solvent-free conditions (Scheme 1).

We found that a mixture of 1*H*-pyrazol-5-amines **1a,b**, barbituric acid **2** and aromatic aldehydes **3a–e**, in the presence of a catalytic amount of *p*-toluenesulfonic acid (*p*-TSA) as an inexpensive and readily available catalyst at 100 °C for 4 h under solvent-free conditions, afforded 4,9-dihydro-1*H*-pyrazolo[4′,3′:5,6]pyrido[2,3-*d*]pyrimidine-5,7 (6*H*,8*H*)-diones **4a–j** in good yields (Table 1).

The results were good in terms of yields and product purity in the presence of *p*-TSA, while without *p*-TSA and over long period of time (12 h) the yields of products were low (<30%).

When this reaction was carried out with aliphatic aldehyde such as butanal or pentanal, TLC and ^{1}H NMR spectra of the reaction mixture showed a combination of starting materials and numerous products, the yield of the expected product was very poor.

The formation of products **4a–j** can be rationalized by initial formation of heterodiene **5** by standard Knoevenagel condensation of barbituric acid **2** and aromatic aldehyde **3**. Subsequent Michael-

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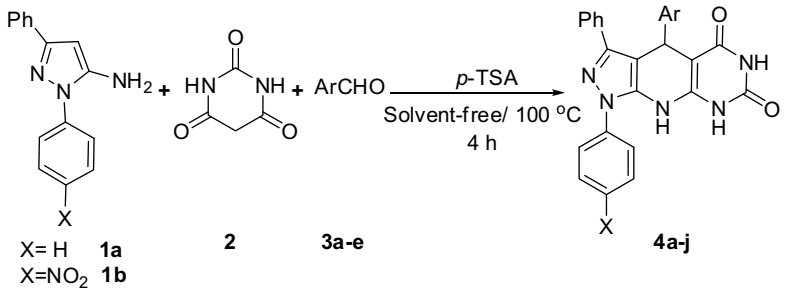
0960-894X/$ - see front matter © 2008 Published by Elsevier Ltd.
doi:10.1016/j.bmcl.2008.09.057

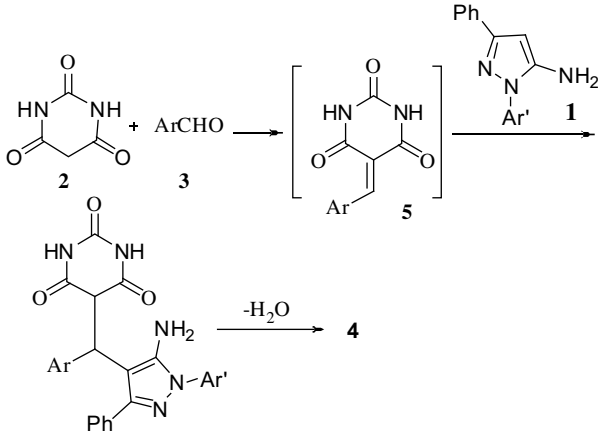


Scheme 1.

Table 1
Synthesis of 1*H*-pyrazolo[4′,3′:5,6]pyrido[2,3-*d*]pyrimidinones **4a–j**

Product **4**	Ar	X	Yield[a] (%)
a	C_6H_5	H	83
b	4-Cl-C_6H_4	H	80
c	4-Br-C_6H_4	H	85
d	4-Me-C_6H_4	H	92
e	3-NO_2-C_6H_5	H	80
f	C_6H_5	NO_2	83
g	4-Cl-C_6H_4	NO_2	90
h	4-Br-C_6H_4	NO_2	84
i	4-Me-C_6H_4	NO_2	95
j	3-NO_2-C_6H_5	NO_2	84

[a] Isolated yields.



Scheme 2.

type addition of 1*H*-pyrazol-5-amines **1** to the heterodienes **5**, followed by cyclization afforded the corresponding products **4a–j** and water (Scheme 2).

Table 2
Synthesis of 7*H*-pyrazolo[4′,3′:5,6]pyrido[2,3-*d*]pyrimidine-7-diones **7a–f**

Product **7**	Ar	X	Yield[a] (%)
a	C_6H_5	H	90
b	4-Cl-C_6H_4	H	86
c	4-NO_2-C_6H_5	H	83
d	C_6H_5	NO_2	81
e	4-Cl-C_6H_4	NO_2	89
f	4-NO_2-C_6H_5	NO_2	84

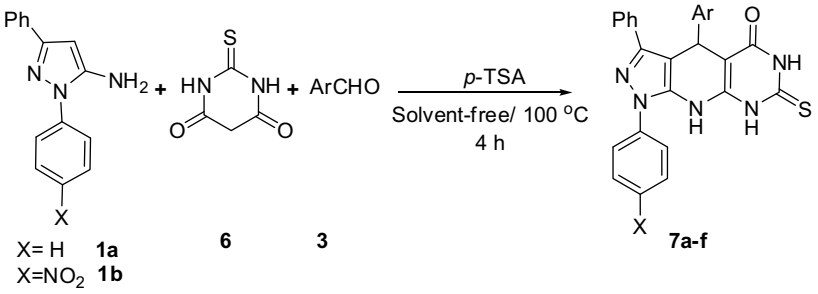
[a] Isolated yields.

Encouraged by these results, we replaced the thiobarbituric acid **6** instead of barbituric acid **2** in same conditions (Scheme 3). The reaction of 1*H*-pyrazol-5-amines **1a,b**, and thiobarbituric **6** was carried out with various aromatic aldehydes **3** under solvent-free conditions at 100 °C, which also afforded 5-thioxo-1,4,5,6,8,9-hexahydro-7*H*-pyrazolo[4′,3′:5,6]pyrido[2,3-*d*]pyrimidine-7-diones **7a–f** in good yields (Table 2).

The nature of these compounds as 1:1:1 adducts was apparent from their mass spectra, which displayed, in each case, the molecular ion peak at appropriate *m/z* values. Compounds **4a–j** and **7a–f** are stable solids whose structures are fully supported by IR, ^{1}H, and ^{13}C NMR spectroscopy, mass spectrometry, and elemental analysis.[25]

Finally, all synthesized compounds were screened for antimicrobial activity. The microorganisms used in this study were *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 85327 (Gram-negative bacteria) *Enterococcus faecalis* ATCC 29737, *Bacillus subtilis* ATCC 465, *Bacillus pumilus* PTCC 1114, *Micrococcus luteus PTCC* 1110, *Staphylococcus aureus* ATCC 25923, *Staphylococcus epidermidis* ATCC 12228, and Sterptococcus mutans PTCC 1601 (Gram-positive bacteria). The minimum inhibitory concentration (MIC) of the synthesized compounds determined by microdillution method[26] and compared to two commercial antibiotics (Table 3).

As can be seen from Table 3, good to improved antibacterial activity was observed for most of the compounds against all



Scheme 3.

Table 3
MIC (μg/ml) values of products **4** and **7**

	Products																Standard	
	4a	**4b**	**4c**	**4d**	**4e**	**4f**	**4g**	**4h**	**4i**	**4j**	**7a**	**7b**	**7c**	**7d**	**7e**	**7f**	Tetracycline	Gentamicin
Bacillus subtilis	2	4	2	2	2	6	8	8	6	6	4	4	4	3	4	4	4	*
Bacillus pumilus	<2	2	2	2	2	4	6	4	6	4	4	4	4	4	4	4	8	*
Micrococcus luteus	<2	<2	2	<2	2	2	4	2	2	2	8	4	3	4	3	4	4	*
Staphylococcus aureus	2	64	58	2	2	4	4	4	2	2	8	4	4	3	6	6	4	*
Staphylococcus epidermidis	2	4	2	2	2	18	28	32	24	26	4	8	8	7	6	8	<2	*
Sterptococcus mutans	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2	8	6	8	8	8	2	*
Escherichia coli	<2	<2	<2	<2	<2	4	6	4	4	6	4	4	6	5	6	6	*	4
Enterococcus faecalis	2	32	30	2	2	4	2	4	2	2	4	16	14	13	12	10	8	*
Pseudomonas aeruginosa	2	2	2	2	2	20	28	32	28	30	32	16	12	14	16	14	*	8

*Not active.

species of Gram-positive and Gram-negative bacteria used in the study. Compounds **4a–j** were found to be more active than Tetracycline against *B. pumilus*, *M. luteus*, *S. mutans*, *E. coli*, and *P. aeruginosa*. Almost, all of the compounds were found to be more active than Gentamicin against all tested strains. Compounds **7a–f** were found to be more active than Tetracycline against *B. pumilus*, *E. coli*, and *P. aeruginosa*. The results indicate that introduction of **–Cl** and **–Br** at aldehyde moiety, thereby producing analogues **4b** and **4c**, respectively, decrease the activity against *E. faecalis* and *S. aureu* (MIC: 30, 32, 58, 64 μg/ml). On the other hand, in other cases of each class of products, types of substitution on aldehyde moiety have not affected the antibacterial activity. 1,3-Diphenyl-4,9-dihydro-1*H*-pyrazolo[4′,3′:5,6]pyrido[2,3-*d*]pyrimidine-5,7(6*H*,8*H*)-diones **4a–e** were more active than the corresponding ones of the series **7a–c**, reinforcing the pharmacophoric contribution of carbonyl moiety relative to thiocarbonyl moiety to the mechanism of action against the all of the tested bacteria except **4b,c** against *E. faecalis* and *S. aureus*. Decreasing of the activity was observed in the case of **4f–g** relative to **4a–e** due to replacing H by NO_2 on *N*-phenyl ring in pyrazole moiety except **4b,c** against *E. faecalis* and *S. aureus*.

In summary, the synthesis and screening of antibacterial activity for a novel series of pyrazolo[4′,3′:5,6]pyrido[2,3-*d*]pyrimidine-dione derivatives was investigated. Almost most of the compounds exhibited good to excellent antibacterial activity against all the tested strains.

Acknowledgment

We gratefully acknowledge financial support from the Research Council of Shahid Beheshti University.

References and notes

1. Dömling, A.; Ugi, I. *Angew. Chem. Int., Ed. Engl.* **2000**, *39*, 3168.
2. Dömling, A. *Chem. Rev.* **2006**, *106*, 17.
3. Rewcastle, G. W.; Bridges, A. J.; Fry, D. W.; Rubin, J. R.; Denny, W. A. *J. Med. Chem.* **1997**, *40*, 1820.
4. Fry, D. W.; Becker, M. A.; Switzer, R. L. *Mol. Pharm.* **1995**, *47*, 810.
5. Gready, J. E.; McKinlay, C.; Gebauer, M. G. *Eur. J. Med. Chem.* **2003**, *38*, 719.
6. Sanghvi, Y. S.; Larson, S. B.; Matsumoto, S. S.; Nord, L. D.; Smee, D. F.; Willis, R. C.; Avery, T. H.; Robins, R. K.; Revankar, G. R. *J. Med. Chem.* **1989**, *32*, 3629.
7. Tenser, R. B.; Gaydos, A.; Hay, K. A. *Antimicrob. Agents Chemother.* **2001**, *45*, 3657.
8. Nizamuddin-Mishra, M.; Srivastava, M. K.; Khan, M. H. *Indian J. Chem.* **2001**, *40*, 49.
9. Ram, V. J.; Goel, A.; Sarkhel, S.; Maulik, P. R. *Bioorg. Med. Chem.* **2002**, *10*, 1275.
10. Elnagdi, M. H.; Al-awadi, N.; Erian, A. N. In *Compensative Heterocyclic Chemistry II*, 1st ed.; Katritzky, A. R., Rees, C. W., Scriven, E. F. V. Eds.; Pergamon Press: Oxford, 1996; Vol. 7, p 431.
11. Elnagdi, M. H.; Elmoghayar, M. R. H.; Elgemeie, G. F. *Adv. Heterocycl. Chem.* **1984**, *41*, 319.
12. Ahluwalia, V. K.; Dahiya, A.; Garg, V. J. *Indian J. Chem.* **1997**, *36B*, 88. and references sited therein.
13. Quiroga, J.; Portilla, J.; Serrano, H.; Abonía, R.; Insuasty, B.; Nogueras, M.; Cobo, J. *Tetrahedron Lett.* **2007**, *48*, 1987.
14. Shi, C.-L.; Shi, D.-Q.; Kim, S. H.; Huang, Z.-B.; Ji, S.-B.; Ji, M. *Tetrahedron* **2008**, *64*, 2425.
15. Quiroga, J.; Insuasty, B.; Hormaza, A.; Saitz, C.; Jullian, C. *J. Heterocycl. Chem.* **1998**, *35*, 575.
16. Quiroga, J.; Mejía, D.; Insuasty, B.; Abonía, R.; Nogueras, M.; Sánchez, A.; Cobo, J.; Low, J. N. *Tetrahedron* **2001**, *57*, 6947.
17. Kozlov, N. G.; Basalaeva, L. I. *Russ. J. Org. Chem.* **2007**, *43*, 432.
18. Bazgir, A.; Seyyedhamzeh, M.; Yasaei, Z.; Mirzaei, P. *Tetrahedron Lett.* **2007**, *48*, 8790.
19. Sayyafi, M.; Seyyedhamzeh, M.; Khavasi, H. R.; Bazgir, A. *Tetrahedron* **2008**, *64*, 2375.
20. Dabiri, M.; Arvin-Nezhad, H.; Khavasi, H. R.; Bazgir, A. *J. Heterocycl. Chem.* **2007**, *44*, 1009.
21. Dabiri, M.; Azimi, S. C.; Arvin-Nezhad, H.; Bazgir, A. *Heterocycles* **2008**, *75*, 87.
22. Dabiri, M.; Delbari, A. S.; Bazgir, A. *Synlett* **2007**, 821.
23. Dabiri, M.; Arvin-Nezhad, H.; Khavasi, H. R.; Bazgir, A. *Tetrahedron* **2007**, *63*, 1770.
24. Dabiri, M.; Delbari, A. S.; Bazgir, A. *Heterocycles* **2007**, *71*, 543.
25. *Procedure for the preparation of 1,3,4-triphenyl-4,9-dihydro-1H-pyrazolo[4′,3′:5,6] pyrido[2,3-d]pyrimidine-5,7(6H,8H)-dione (**4a**):* A mixture of barbituric acid (1 mmol), 1,3-diphenyl-1*H*-pyrazol-5-amine (1 mmol), benzaldehyde (1.2 mmol) and *p*-TSA (0.1 g) was heated at 100 °C for 4 h (TLC). After cooling, the reaction mixture was washed with water (15 ml) and residue recrystallized from EtOH to afford the pure product **4a**. White powder (83%); mp: 297 °C dec. IR (KBr) (ν_{max}/cm^{-1}): 3212, 3064, 1706, 1636. MS (EI, 70 eV) *m*/*z* (%): 433 (M^+, 90), 356 (100), 313 (10). ^{1}H NMR (300 MHz, DMSO-d_6): δ_H (ppm) 5.33 (1H, s, CH), 7.04–7.74 (15H, m, H–Ar), 9.15 (1H, br s, NH), 10.08 (1H, br s, NH), 10.74 (1H, s, NH). ^{13}C NMR (DMSO-d_6): δ_C (ppm) 35.9, 89.8, 102.0, 123.5, 126.5, 127.1, 128.3, 128.8, 130.3, 133.2, 137.7, 138.2, 145.4, 146.5, 147.7, 150.1, 163.3. Anal. Calcd for $C_{26}H_{19}N_5O_2$: C, 72.04; H, 4.42; N, 16.16%. Found: C, 72.09; H, 4.37; N, 16.10%. Selected characterization data.
*4-(4-Chlorophenyl)-1,3-diphenyl-4,9-dihydro-1H-pyrazolo[4′,3′:5,6]pyrido[2,3-d] pyrimidine-5,7 (6H,8H)-dione (**4b**):* White powder (80%); mp: 320 °C dec. IR (KBr) (ν_{max}/cm^{-1}): 3212, 3060, 1710, 1635. MS (EI, 70 eV) *m*/*z* (%): 467 (M^+, 60), 356 (100), 77 (20). ^{1}H NMR (300 MHz, DMSO-d_6): δ_H (ppm) 5.37 (1H, s, CH), 7.17–7.71 (14H, m, H–Ar), 9.16 (1H, br s, NH), 10.09 (1H, br s, NH), 10.76 (1H, s, NH). ^{13}C NMR (DMSO-d_6): δ_C (ppm) 35.6, 89.3, 101.4, 123.5, 127.1, 128.2, 128.4, 128.8, 130.2, 131.0, 133.0, 137.6, 138.1, 145.3, 145.5, 147.8, 150.1, 163.3. Anal. Calcd for $C_{26}H_{18}ClN_5O_2$: C, 66.74; H, 3.88; N, 14.97%. Found: C, 66.69; H, 3.82; N, 14.90%.
*4-(4-Methylphenyl)-1,3-diphenyl-4,9-dihydro-1H-pyrazolo[4′,3′:5,6]pyrido[2,3-d]pyrimidine-5,7 (6H,8H)-dione (**4d**):* White powder (92%); mp: 314 °C dec. IR (KBr) (ν_{max}/cm^{-1}): 3231, 3058, 1730, 1623. MS (EI, 70 eV) *m*/*z* (%): 447 (M^+, 40), 445 (100), 356 (50). ^{1}H NMR (300 MHz, DMSO-d_6): δ_H (ppm) 2.15 (3H, s, CH_3), 5.33 (1H, s, CH), 6.94–7.75 (14H, m, H–Ar), 9.11 (1H, br s, NH), 10.09 (1H, br s, NH), 10.75 (1H, s, NH). ^{13}C NMR (DMSO-d_6): δ_C (ppm) 21.0, 35.6, 90.1, 102.1, 123.5, 127.1, 128.2, 128.3, 128.8, 128.9, 130.3, 133.3, 135.5, 137.7, 138.2, 143.6, 145.3, 147.7, 150.1, 163.3. Anal. Calcd for $C_{27}H_{21}N_5O_2$: C, 72.47; H, 4.73; N, 15.65%. Found: C, 72.42; H, 4.69; N, 15.59%.
*1-(4-Nitrophenyl)-3,4-diphenyl-4,9-dihydro-1H-pyrazolo[4′,3′:5,6]pyrido[2,3-d]pyrimidine-5,7 (6H,8H)-dione (**4f**):* Yellow powder (83%); mp: 324 °C dec. IR (KBr) (ν_{max}/cm^{-1}): 3216, 3064, 1727, 1624. MS (EI, 70 eV) *m*/*z* (%): 476 (M^+−2, 100), 446 (80), 429 (30). ^{1}H NMR (300 MHz, DMSO-d_6): δ_H (ppm) 5.33 (1H, s, CH), 7.02–7.67 (10H, m, H–Ar), 8.04 (2H, d, *J* = 8.9 Hz, H–Ar), 8.45 (2H, d, *J* = 8.9 Hz, H–Ar), 9.34 (1H, br s, NH), 10.37 (1H, br s, NH), 10.81 (1H, s, NH). ^{13}C NMR (DMSO-d_6): δ_C (ppm) 35.9, 90.4, 103.5, 122.9, 125.7, 126.6, 127.3, 128.3, 128.4, 128.9, 132.6, 138.5, 143.5, 145.4, 145.7, 146.1, 149.4, 150.2, 163.3. Anal. Calcd for $C_{26}H_{18}N_6O_4$: C, 65.27; H, 3.79; N, 17.56%. Found: C, 65.31; H, 3.85; N, 17.48%.
*4-(4-Methylphenyl)-1-(4-nitrophenyl)-3-phenyl-4,9-dihydro-1H-pyrazolo[4′,3′:5,6]-pyrido[2,3-d]pyrimidine-5,7 (6H,8H)-dione (**4i**):* Yellow powder (95%); mp: 330 °C dec. IR (KBr) (ν_{max}/cm^{-1}): 3217, 3007, 1722, 1617. MS (EI, 70 eV) *m*/*z* (%): 492 (M^+−2, 100), 460 (90), 407 (10). ^{1}H NMR (300 MHz, DMSO-d_6): δ_H (ppm) 2.15 (3H, s, CH_3), 5.29 (1H, s, CH), 6.93–7.68 (9H, m, H–Ar), 8.03 (2H, d, *J* = 9.0 Hz, H–Ar), 8.46 (2H, d, *J* = 8.9 Hz, H–Ar), 9.35 (1H, br s, NH), 10.35 (1H, br s, NH), 10.78 (1H, s, NH). ^{13}C NMR (DMSO-d_6): δ_C (ppm) 21.0, 35.4, 90.5, 103.6, 122.9, 125.7,

127.2, 128.2, 128.9, 132.6, 135.6, 138.5, 143.2, 143.5, 145.3, 145.7, 149.3, 150.2, 163.3. Anal. Calcd for $C_{27}H_{20}N_6O_4$: C, 65.85; H, 4.09; N, 17.06%. Found: C, 65.90; H, 4.14; N, 17.11%.

*4-(4-Nitrophenyl)-1,3-diphenyl-5-thioxo-1,4,5,6,8,9-hexahydro-7H-pyrazolo[4′,3′:-5,6] pyrido[2,3-d]pyrimidine-7-dione (**7c**):* Yellow powder (83%); mp: 234 °C dec. IR (KBr) (ν_{max}/cm^{-1}): 3242, 3084, 1733, 1647. MS (EI, 70 eV) *m*/*z* (%): 492 (M^+-2, 100), 392 (60), 77 (90). ^{1}H NMR (300 MHz, DMSO-d_6): δ_H (ppm) 5.57 (1H, s, CH), 7.23–8.07 (14H, m, H–Ar), 9.26 (1H, br s, NH), 10.19 (1H, br s, NH), 10.82 (1H, s, NH). ^{13}C NMR (DMSO-d_6): δ_C (ppm) 36.1, 88.7, 100.9, 121.6, 122.9, 123.6, 127.2, 128.5, 128.8, 129.7, 130.3, 132.8, 135.2, 137.5, 138.1, 145.8, 147.6, 148.3, 150.1, 163.4. Anal. Calcd for $C_{26}H_{18}N_6O_3S$: C, 63.15; H, 3.67; N, 16.99%. Found: C, 63.09; H, 3.71; N, 16.93%.

*1-(4-Nitrophenyl)-3,4-diphenyl-5-thioxo-1,4,5,6,8,9-hexahydro-7H-pyrazolo[4′,3′:5,6] pyrido[2,3-d]pyrimidine-7-dione (**7d**):* Yellow powder (81%); mp: 285 °C dec. IR (KBr) (ν_{max}/cm^{-1}): 3208, 3091, 1786, 1625. MS (EI, 70 eV) *m*/*z* (%): 492 (M^+-2, 100), 462 (60), 172 (30). ^{1}H NMR (300 MHz, DMSO-d_6): δ_H (ppm) 5.32 (1H, s, CH), 7.04–7.71 (10H, m, H–Ar), 8.01 (2H, d, *J* = 8.8 Hz, H–Ar), 8.43 (2H, d, *J* = 8.8 Hz, H–Ar), 9.05 (1H, br s, NH), 11.86 (1H, br s, NH), 12.22 (1H, s, NH). ^{13}C NMR (DMSO-d_6): δ_C (ppm) 35.6, 94.6, 103.0, 122.9, 125.7, 127.2, 128.3, 128.5, 128.6, 128.9, 132.3, 136.8, 143.2, 144.8, 145.4, 145.8, 149.5, 160.8, 173.7. Anal. Calcd for $C_{26}H_{18}N_6O_3S$: C, 63.15; H, 3.67; N, 16.99%. Found: C, 63.20; H, 3.63; N, 16.92%.

*4-(4-Chlorophenyl)-1-(4-nitrophenyl)-3-phenyl-5-thioxo-1,4,5,6,8,9-hexahydro-7H-pyrazolo[4′,3′:5,6] pyrido[2,3-d]pyrimidine-7-dione (**7f**):* Yellow powder (84%); mp: 234 °C dec. IR (KBr) (ν_{max}/cm^{-1}): 3188, 3005, 1712, 1627. MS (EI, 70 eV) *m*/*z* (%): 526 (M^+-2, 100), 496 (90), 77 (70). ^{1}H NMR (300 MHz, DMSO-d_6): δ_H (ppm) 5.38 (1H, s, CH), 7.16–7.64 (9H, m, H–Ar), 8.01 (2H, d, *J* = 9.1 Hz, H–Ar), 8.44 (2H, d, *J* = 9.0 Hz, H–Ar), 9.34 (1H, br s, NH), 11.88 (1H, br s, NH), 12.26 (1H, s, NH). ^{13}C NMR (DMSO-d_6): δ_C (ppm) 35.6, 94.1, 102.4, 122.8, 125.7, 127.2, 128.2, 128.9, 130.4, 131.4, 132.3, 137.7, 143.2, 144.3, 144.7, 148.8, 149.5, 160.8, 173.9. Anal. Calcd for $C_{26}H_{17}ClN_6O_3S$: C, 59.04; H, 3.24; N, 15.89%. Found: C, 58.99; H, 3.19; N, 15.95%.

26. NCCLS, *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria, which Grows Aerobically*, 5th ed., Approved Standard M7-A5, NCCLS, Villanova, PA, 2000.