

Synthesis and Biological Evaluation of some Novel Pyrano[2,3-*d*]Pyrimidine Derivatives

Pratik K. Talpara, Gaurang G. Dubal, Viresh H. Shah*

Department of Chemistry, Saurashtra University, Rajkot - 360005, India

*drvirehshah@gmail.com

Keywords: Pyrano[2,3-*d*]pyrimidine, domino Knoevenagel-hetero-Diels-Alder reaction, antimicrobial activity

Abstract. The synthesis of novel Pyrano[2,3-*d*]pyrimidine derivatives, had been synthesized by three component domino Knoevenagel hetero Diels-Alder reaction. The products were assayed for their *in vitro* biological assay antibacterial activity against with two Gram-positive bacteria *Staphylococcus aureus* MTCC-96, *Streptococcus pyogenes* MTCC 443, two Gram-negative bacteria *Escherichia coli* MTCC 442, *Pseudomonas aeruginosa* MTCC 441 and three fungal strains *Candida albicans* MTCC 227, *Aspergillus Niger* MTCC 282, *Aspergillus clavatus* MTCC 1323 taking ampicillin, chloramphenicol, ciprofloxacin, norfloxacin, nystatin, and griseofulvin as standard drugs. Among the various synthesized heterocyclic compounds, 1b, 1c and 1g are display broad spectrum antibacterial and antifungal activities against both gram-positive and gram-negative bacteria as compared with standard drugs.

Introduction

The development of a new strategy for the synthesis of complex organic molecules is an important aim in modern organic chemistry. L. F. Tietze introduced the domino-Knoevenagel-hetero-Diels-Alder reaction as a powerful sequential transformation [1]. It has been proven that this reaction is a valuable method for the construction of annulated pyrans [2] and has already been used for the synthesis of several natural products [3]. A family of xanthone natural products with potent antiviral activity has been isolated from plants, and it is a potent inhibitor of human immunodeficiency virus-1 reverse transcriptase [4]. Pyrano[2,3-*d*]pyrimidine is unsaturated six member heterocycle which is formed by fusion of pyran and pyrimidine rings together, consisting of one oxygen atom at position number 8 and two nitrogen atoms at position number 1 and 3 respectively. If pyrano[2,3-*d*]pyrimidine moieties are clubbed into one molecule, then resultant derivative enhances its pharmaceutical activity as abundant in biologically active compounds such as antitumour [5], cardiogenic [6], antibronchitic [7] and antifungal activity [8]. Some of them exhibit antihypertensive activity [9], antimalarial [10], analgesic [10, 11], and antimicrobial and antiviral evaluation [12, 13] properties. Pyrano[2,3-*d*]pyrimidines are building blocks used to evaluate their antimicrobial activities and various derived natural products [14]. Therefore, for the preparation of these complex molecules large efforts have been directed toward the synthetic manipulation of pyrano[2,3-*d*]pyrimidine derivatives. As a result, a number of reports have appeared in the literature that usually requires forcing conditions, long reaction times and complex synthetic pathways. Pyrano[2,3-*d*]pyrimidine synthesis was reported under various conditions such as microwave irradiation [15] and [16], ultrasonic irradiation [17], solvent free condition and in aqueous medium in the absence of catalysts [18].

In order to develop an efficient synthesis of pyrano[2,3-*d*]pyrimidines is of considerable interest in this research to cater short reaction time, short reaction procedure and excellent yields by this proposed route. Methanol was used as a solvent and we describe here a rapid, viable and easy protocol for the synthesis of pyrano[2,3-*d*]pyrimidine derivatives by using piperidine as a catalyst (Scheme 1). The products 1a to 1o were screened for their antibacterial and antifungal evaluation.

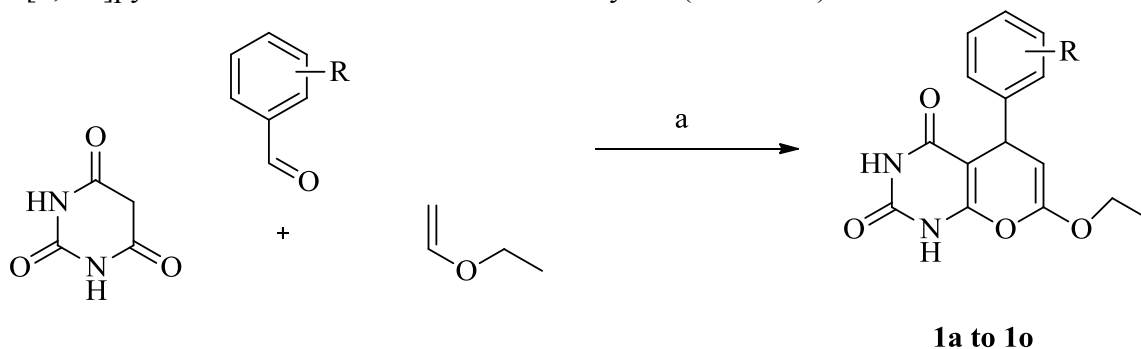
Experimental Section

Materials and Methods

Melting points were determined in open capillary tubes and are uncorrected. Formation of the compounds was routinely checked by TLC on silica gel-G plates of 0.5 mm thickness and spots were located by iodine. IR spectra were recorded on IRAffinity-1S FTIR Spectrophotometer-Shimadzu using 'neat' sample. Mass spectra were recorded on Shimadzu GC-MS-QP-2010 model using Direct Injection Probe technique. ¹H NMR was determined in DMSO-*d*₆ solution on a Bruker Ac 400 MHz spectrometer. Elemental analysis of the all the synthesized compounds was carried out on Elemental Vario EL III Carlo Erba 1108 model and the results are in agreements with the structures assigned.

Chemistry

A synthesis of annulated fused pyrano[2,3-*d*]pyrimidine 1a-1o as a domino Knoevenagel condensation reported in Scheme 1. The experimental procedure is straightforward. Solution of equimolar various aromatic aldehyde and barbituric stirred for 15-20 minutes in the presence of catalytic amount of piperidine in methanol as a solvent to gives knoevenagel adduct which further reacts with equimolar solution of ethyl vinyl ether under reflux condition for 5-6 hours afford pyrano[2,3-*d*]pyrimidinones in medium to excellent yield (Scheme 1).



Reaction condition: (a) Methanol, Reflux 5-6 hr, Piperidine

Where R = CH₃, OCH₃, Cl, F, Br, NO₂ etc.

Scheme 1.

General Procedures

Melting points of all the compounds are uncorrected and have been recorded by open capillary method. Silica gel-G was used for preparing the TLC plates using different solvent systems. Infra red spectra of all the compounds were scanned on SHIMADZU-FOURIER TRANSFORM INFRA RED (FTIR) - 8400 Spectrophotometer using KBr pellet method. PMR Spectra were recorded on BRUKER Spectrophotometer (300 MHz) using TMS as an internal standard and DMSO-*d*₆ as solvent. Electron Impact (EI) mass spectra were recorded on GCMSQP2010 Mass Spectrometer.

Results and Discussion

Preparation of 7-ethoxy-5-aryl-tetrahydro-1H-pyrano[2,3-*d*]pyrimidine-2,4(3H,8aH)-dione (1)

A mixture of Substituted aromatic aldehyde (0.01 M), ethyl vinyl ether (0.01 M), barbiturate (0.01 M) and 2-3 drops of piperidine in Methanol (30 ml) was heated under refluxed condition for 5-6 hrs. Then the reaction mixture was kept at room temperature for 2 hrs. The crystalline product was obtained. The product was isolated and recrystallized from ethanol. Similarly, other compounds (1a-o) were synthesized.

7-ethoxy-5-(p-tolyl)-1H-pyrano[2,3-d]pyrimidine-2,4(3H,5H)-dione (1a)

Yield (87%), mp 170-172 °C; ¹H NMR (DMSO-*d*₆) δ = 1.32 (t, 3H), 2.32 (s, 1H), 4.08 (q, 2H), 4.72 (s, 1H), 4.77 (s, 1H), 5.07 (s, 1H), 5.72 (s, 1H), 7.11 (d, 2H), 7.31 (d, 2H); ¹³C NMR (δ) 14.63, 21.12, 29.83, 61.50, 80.39, 89.31, 127.17, 131.74, 137.22, 139.31, 146.45, 149.85, 153.98, 161.94; Anal. Calcd: C, 63.99; H, 5.37; N, 9.33; Found: C, 63.96; H, 5.35; N, 9.31; MS: m/z 300.

5-(4-chlorophenyl)-7-ethoxy-1H-pyrano[2,3-d]pyrimidine-2,4(3H,5H)-dione (1b)

Yield (68%), mp 167-169 °C; ¹H NMR (DMSO-*d*₆) δ = 2.77 (s, 1H, SH), 4.78 (s, 2H, NH₂), 6.41-6.44 (m, 2H, Ar-H), 7.08 (s, 2H, NH₂), 7.18-7.21 (d, 2H, Ar-H, J=12 Hz), 7.34-7.35 (d, 1H, Ar-H, J=4 Hz), 7.40-7.41 (d, 1H, Ar-H, J=4 Hz), 7.64 (s, 1H, NH) 7.66-7.67 (d, 2H, Ar-H, J=4 Hz); ¹³C NMR (δ) 14.58, 29.79, 61.46, 80.27, 89.20, 127.08, 127.52, 132.12, 139.25, 146.28, 149.61, 153.96, 161.93; Anal. Calcd: C, 56.17; H, 4.09; Cl, 11.05; N, 8.73; Found: C, 56.16; H, 4.05; Cl, 11.04; N, 8.70; MS: m/z 321.

7-ethoxy-5-(4-fluorophenyl)-1H-pyrano[2,3-d]pyrimidine-2,4(3H,5H)-dione (1c)

Yield (65%), mp 145-147 °C; ¹H NMR (DMSO-*d*₆) δ = 1.38 (t, 3H), 4.18 (q, 2H), 4.68 (s, 1H), 4.78 (s, 1H), 5.12 (s, 1H), 5.73 (s, 1H), 6.98 (q, 2H), 7.34 (d, 2H); ¹³C NMR (δ) 14.53, 29.78, 61.43, 80.24, 89.18, 113.27, 127.01, 137.76, 146.23, 149.57, 153.89, 159.03, 162.06; Anal. Calcd: C, 59.21; H, 4.31; F, 6.24; N, 9.21; Found: C, 59.20; H, 4.28; F, 6.21, N, 9.21; MS: m/z 304.

7-ethoxy-5-(4-methoxyphenyl)-1H-pyrano[2,3-d]pyrimidine-2,4(3H,5H)-dione (1d)

Yield (74%), mp 139-141 °C; ¹H NMR (DMSO-*d*₆) δ = 1.33 (t, 3H), 3.78 (s, 1H), 4.12 (q, 2H), 4.75 (s, 1H), 4.78 (s, 1H), 5.09 (s, 1H), 5.81 (s, 1H), 6.95 (d, 2H), 7.37 (d, 2H); ¹³C NMR (δ) 14.48, 29.70, 55.48, 61.34, 80.18, 89.16, 113.11, 126.76, 130.10, 146.19, 149.57, 153.85, 158.43, 161.94; Anal. Calcd: C, 60.75; H, 5.10; N, 8.86; Found: C, 60.75; H, 5.09; N, 8.84; MS: m/z 316.

5-(3-chlorophenyl)-7-ethoxytetrahydro-1H-pyrano[2,3-d]pyrimidine-2,4(3H,8aH)-dione (1e)

Yield (66%), mp 140-142 °C; ¹H NMR (DMSO-*d*₆) δ = 1.36 (t, 3H), 4.17 (q, 2H), 4.73 (s, 1H), 4.77 (s, 1H), 5.11 (s, 1H), 5.86 (s, 1H), 7.22 (m, 1H), 7.28 (d, 2H), 7.30, (s, 1H), 7.37 (d, 2H); ¹³C NMR (δ) 14.56, 29.86, 61.87, 80.21, 89.29, 125.76, 126.47, 127.88, 128.64, 134.12, 143.10, 146.31, 149.62, 153.91, 162.78; Anal. Calcd: C, 56.17; H, 4.09; Cl, 11.05; N, 8.73; Found: C, 56.15; H, 4.08; Cl, 11.04; N, 8.73; MS: m/z 322.

Biological Evaluation**Antimicrobial Evaluation**

All of the synthesized compounds (1a to 1o) were tested for their antibacterial and antifungal activity (MIC) *in vitro* by broth dilution method [19-20] with two Gram-positive bacteria *Staphylococcus aureus* MTCC-96, *Streptococcus pyogenes* MTCC 443, two Gram-negative bacteria *Escherichia coli* MTCC 442, *Pseudomonas aeruginosa* MTCC 441 and three fungal strains *Candida albicans* MTCC 227, *Aspergillus Niger* MTCC 282, *Aspergillus clavatus* MTCC 1323 taking ampicillin, chloramphenicol, ciprofloxacin, norfloxacin, nystatin, and griseofulvin as standard drugs. The standard strains were procured from the Microbial Type Culture Collection (MTCC) and Gene Bank, Institute of Microbial Technology, Chandigarh, India.

The minimal inhibitory concentration (MIC) values for all the newly synthesized compounds, defined as the lowest concentration of the compound preventing the visible growth, were determined by using micro dilution broth method according to NCCLS standards [20]. Serial dilutions of the test compounds and reference drugs were prepared in Muellere-Hinton agar. Drugs (10 mg) were dissolved in dimethylsulfoxide (DMSO, 1 mL). Further progressive dilutions with melted Muellere-Hinton agar were performed to obtain the required concentrations of 1.56, 3.12, 6.25, 10, 12.5, 25, 50, 62.5, 100, 125, 250, 500 and 1000 µg mL⁻¹. The tubes were inoculated with

10^8 cfu mL⁻¹ (colony forming unit/mL) and incubated at 37 °C for 24 h. The MIC was the lowest concentration of the tested compound that yields no visible growth (turbidity) on the plate. To ensure that the solvent had no effect on the bacterial growth, a control was performed with the test medium supplemented with DMSO at the same dilutions as used in the experiments and it was observed that DMSO had no effect on the microorganisms in the concentrations studied. The results obtained from antimicrobial susceptibility testing are depicted in Table 1. Compounds 1c, 1g and 1b are display broad spectrum antibacterial activities against both gram-positive and gram-negative bacteria as compared with ciprofloxacin. Compounds 1b and 1g were found to be 4-fold more active against *S. aureus* and *S. pyogens* (MIC = 25 µg/mL) compared to the standard drug. While compound 1b showed equivalent activity against *E. coli* and *P. aeruginosa* and 4-fold more activity against *S. pyogens* (MIC = 25 µg/mL). Moreover, compound 1b exhibits 4-fold inhibition against *S. aureus* and equivalent inhibition against *P. aeruginosa*. As observed with high antibacterial potency of 1c and 1g a may be attributed to the presence of electron withdrawing substituents such as fluoro and bromo at 4th position of aromatic ring. In comparison to the standard drug griseofulvin, the *in vitro* antifungal activity results indicated that compound 1g substituted with bromo group at 4th position of phenyl ring was found to be the most potent against *A. niger* (MIC = 50 µg/mL), *A. clavatus* (MIC = 25 µg/mL) and *C. albicans* (MIC = 100 µg/mL) respectively.

Conclusions

In the present paper, we report the synthesis, spectral studies and antimicrobial activity of various pyran derivatives. The high bioactivity of these compounds makes them suitable tools for additional *in vitro* and *in vivo* evaluations, in order to develop new class of antimicrobial drugs or prodrugs with potential antimicrobial activity in the treatment of several diseases. Among the various synthesized heterocyclic compounds, 1b, 1c and 1g are found to be potent against both gram-positive and gram-negative bacteria as compared with standard drugs. Further studies and modification in this area are in progress in our laboratory.

Acknowledgments

We acknowledge the support of UGC & CSIR through Grant no. 41-198/ 2012(SR) & 02 (0194)/14/EMR-II, New Delhi, India, for financial support and National Facility for Drug Discovery (NFDD) for providing instrumental support. I also wish to acknowledge Professor and Head, Department of Chemistry, Saurashtra University Rajkot for providing research facility. Pratik K. Talpara is thankful to UGC-BSR for meritorious Fellowship.

Table 1. Antibacterial and antifungal activity of synthesized compounds 1a to 1o.

R	Code	Minimum inhibition concentration ($\mu\text{g mL}^{-1}$)						
		Gram-positive		Gram-negative		Fungal species		
		<i>S.a.</i>	<i>S.p.</i>	<i>E.c.</i>	<i>P.a.</i>	<i>C.a.</i>	<i>A.n.</i>	<i>A.c.</i>
4-CH ₃	1a	500	1000	500	100	1000	>1000	1000
4-Cl	1b	25	25	50	50	500	500	250
4-F	1c	50	50	100	100	500	250	500
4-OCH ₃	1d	100	500	250	250	>1000	1000	>1000
3-Cl	1e	100	100	250	125	500	500	1000
2-OCH ₃	1f	500	1000	250	1000	500	500	>1000
4-Br	1g	25	25	50	25	100	50	25
H	1h	250	100	100	250	1000	500	250
4-NO ₂	1i	100	100	100	250	500	500	250
3,4-(OCH ₃) ₂	1j	100	500	250	100	500	1000	>1000
2,4-dichloro	1k	100	100	500	1000	>1000	500	1000
3-Br	1l	500	250	250	250	500	1000	1000
2-F	1m	100	100	250	250	500	500	250
2-Br	1n	100	250	250	125	250	250	500
2,5-(OCH ₃) ₂	1o	250	250	500	500	1000	>1000	1000
Ampicillin		250	100	100	100	-	-	-
Chloramphenicol		50	50	50	50	-	-	-
Ciprofloxacin		50	50	25	25	-	-	-
Norfloxacin		10	10	10	10	-	-	-
Nystatin		-	-	-	-	100	100	100
Griseofulvin		-	-	-	-	500	100	100

References

- [1] L.F. Tietze, Domino reactions in organic chemistry, Wiley-VCH, Weinheim. 20 (2006) 208–330.
- [2] J.K. Gallos, A.E. Koumbis, Domino Knoevenagel hetero Diels-Alder reactions of sugar derived δ , ϵ -unsaturated aldehydes, Arkivoc. 6 (2003) 135–144.
- [3] L.F. Tietze, U. Beifuss, Sequential transformations in organic chemistry: A synthetic strategy with a future, Angewandte Chemie International Edition in English. 32(2) (1993) 131–163.
- [4] M.E. Fox, I.C. Lennon, G. Meek, A novel synthesis of 5-hydroxy-2, 2-dimethyl-10-propyl-2H-pyrano [2, 3-f] chromen-8-one, Tetrahedron Letters. 43(16) (2002) 2899–2902.
- [5] G.L. Anderson, J.L. Shim, A.D. Broom, Pyrido [2, 3-d] pyrimidines. IV. Synthetic studies leading to various oxopyrido [2, 3-d] pyrimidines, The Journal of Organic Chemistry. 41(7) (1976) 1095–1099.

-
- [6] E.M. Grivsky et al., Synthesis and antitumor activity of 2, 4-diamino-6-(2, 5-dimethoxybenzyl)-5-methylpyrido [2, 3-*d*] pyrimidine, *J. Med. Chem.* 23 (1980) 327–329.
- [7] D. Heber, C. Heers, U. Ravens, Positive inotropic activity of 5-amino-6-cyano-1, 3-dimethyl-1, 2, 3, 4-tetrahydropyrido [2, 3-*d*] pyrimidine-2, 4-dione in cardiac muscle from guinea-pig and man. Part 6: Compounds with positive inotropic activity, *Die Pharmazie.* 48(7) (1993) 537–541.
- [8] Y. Sakuma et al., Fused pyrimidine derivative, process for preparation of same and pharmaceutical preparation comprising same as active ingredient, U.S. Patent 5,378,700, (1995).
- [9] L.R. Bennett et al., Antihypertensive activity of 6-arylprido [2, 3-*d*] pyrimidin-7-amine derivatives, *J. Med. Chem.* 24(4) (1981) 382–389.
- [10] J. Davoll, J. Clarke, F.E. Eislager, Antimalarial substances. 26. Folate antagonists. 4. Antimalarial and antimetabolite effects of 2, 4-diamino-6-[(benzyl) amino] pyrido [2, 3-*d*] pyrimidines, *J. Med. Chem.* 15(8) (1972) 837–839.
- [11] B. Baruah, P.J. Bhuyan, Tertiary amine effect: synthesis of some novel spirosubstituted pyrido [2, 3-*d*] pyrimidines, *Tetrahedron Letters.* 50(2) (2009) 243–245.
- [12] A.H. Shamroukh et al., Synthesis, isomerization, and antimicrobial evaluation of some pyrazolopyranotriazolopyrimidine derivatives, *Archiv der Pharmazie.* 340(7) (2007) 345–351.
- [13] A.H. Shamroukh et al., Synthesis of pyrazolo [4', 3': 5, 6] pyrano [2, 3-*d*] pyrimidine derivatives for antiviral evaluation, *Archiv der Pharmazie.* 340(5) (2007) 236–243.
- [14] C. Musstazza et al., Synthesis of pyrazolo [1, 5-*a*]-, 1, 2, 4-triazolo [1, 5-*a*]-and imidazo [1, 2-*a*] pyrimidines related to zaleplon, a new drug for the treatment of insomnia, *J. Heterocycl. Chem.* 38 (2001) 1119–1130.
- [15] Y. Gao et al., Effective synthesis of 7-amino-6-cyano-5-aryl-5 H-pyrano [2, 3-*d*] pyrimidine-2, 4 (1 H, 3 H)-diones under microwave irradiation, *Synthetic communications.* 34(7) (2004) 1295–1299.
- [16] I. Devi, Kumar, P.J. Bhuyan, A novel three-component one-pot synthesis of pyrano [2, 3-*d*] pyrimidines and pyrido [2, 3-*d*] pyrimidines using microwave heating in the solid state, *Tetrahedron Letters.* 44(45) (2003) 8307–8310.
- [17] T.S. Jin et al., A clean one-pot synthesis of 7-amino-5-aryl-6-cyano-1, 5-dihydro-2H-pyrano [2, 3-*d*] pyrimidine-2, 4 (3H)-diones in aqueous media under ultrasonic irradiation, *J. Chem. Res.* 3 (2005) 162–163.
- [18] H.R. Safaei et al., Glycerol as a biodegradable and reusable promoting medium for the catalyst-free one-pot three component synthesis of 4H-pyrans, *Green Chem.* 14(6) (2012) 1696–1704.
- [19] Vogel's Textbook of practical organic chemistry, Fifth Edition, ELBS, Longman Scientific and Technical: England, 1989, Reprinted, 1994.
- [20] National Committee for Clinical and Laboratory Standards, Method for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically Approved Standard, fourth ed. NCCLS, Villanova, Italy, Document M 100-S7. (1997) S100–S157.