Tetrahedron Letters 51 (2010) 6037–6040

Contents lists available at ScienceDirect

Tetrahedron Letters

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

Microwave-assisted synthesis of 3-aryl-pyrimido[5,4-*e*][1,2,4]triazine-5,7-(1*H*,6*H*)-dione libraries: derivatives of toxoflavin

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ARTICLE INFO

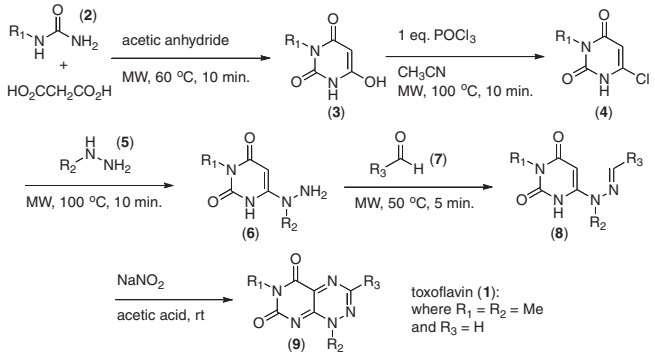
Article history:
Received 16 August 2010
Revised 9 September 2010
Accepted 13 September 2010
Available online 17 September 2010

ABSTRACT

The parallel synthesis of a library of toxoflavin derivatives is described. The microwave-assisted approach involves the de novo generation of the heterocyclic scaffold and allows for facile introduction of a variety of fragments.

The alkaloid toxoflavin (**1**), first isolated in 1933 from *Pseudomonas cocovenenans*,[1] has been shown to possess antimicrobial activity and has been the target of a number of total syntheses.[1–4] Derivatives of toxoflavin have also been described with these compounds possessing a variety of biological properties. Recently, work in our laboratories required a series of 3-aryl-pyrimido[5,4-*e*][1,2,4]triazine-5,7(1*H*,6*H*)-diones for biological studies and as a result, a synthetic scheme appropriate for the generation of a library of toxoflavin derivatives was developed (Scheme 1). The route was designed with an eye to maximizing structural diversity (with variation at N1 and N6 and arylation at C3). The present paper describes the development of a microwave-assisted methodology that allows for facile preparation of this heterocyclic family.

Compounds of the general type **4** were identified as ideal scaffolds for our libraries. The requisite barbituric acid (**3**) was prepared via the condensation of a substituted urea (**2**) and malonic acid (Scheme 1).[5] Microwave irradiation at 60 °C for 10 min allowed for the preparation of **3** (with R_1 = H, methyl or ethyl) in good yields. Furthermore, isolation of these barbituric acids is achieved by simple recrystallization.



Scheme 1. Synthesis of 3-aryl-pyrimido[5,4-*e*][1,2,4]triazine-5,7(1*H*,6*H*)-diones.

A number of protocols have been described for the chlorination of barbituric acid derivatives[6,7] and these generally involve refluxing in neat $POCl_3$ over a few hours. However, the treatment of **3** in acetonitrile with 1 equiv of $POCl_3$ at 100 °C for 10 min in a microwave allowed for an excellent yield of the desired chloride scaffold (**4**).

The synthesis of the requisite hydrazone (**8**) involved treatment of the chloride scaffold (**4**) with a substituted hydrazine (**5**, used as a free base) followed by microwave irradiation at 100 °C for 10 min in ethanol. Compound **6** was not isolated but treated with an aldehyde (**7**) and irradiated in a microwave for a further 5 min at 50 °C to form imine (**8**). The hydrazones generated using this 'one-pot' approach precipitated from ethanol and were easily isolated by filtration. A series of examples are provided in Table 1.

Given that our biological studies required methyl substitution at N1, methylhydrazine was frequently employed in the reaction converting **4**–**6**. While the conjugate addition/displacement sequence involving methylhydrazine could deliver two regioisomers, the major product (in a ratio of >95:5 when compared to the minor regioisomer) was the system formed via the nucleophilic attack with the secondary nitrogen. The regiochemistry was confirmed with the X-ray crystallographic analysis of one of the hydrazone compounds generated, namely 6-(1-methyl-2-(4-iodobenzyl)hydrazinyl)-3-methylpyrimidine-2,4(1*H*,3*H*)-dione (**8**, where $R_1 = CH_3$, $R_2 = CH_3$, and $R_3 = p\text{-}C_6H_4I$) shown in Figure 1 as structure A.[8] The regioselectivity observed provides an excellent illustration of the soft/hard acid/base principle[9] wherein, the softer secondary nitrogen of the hydrazine reacts preferentially at the softer C–Cl site (in contrast with the harder primary nitrogen reacting at the harder C=O of the benzaldehyde). It should be noted that other substituted hydrazines could be incorporated using the same procedure. For

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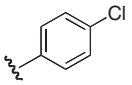
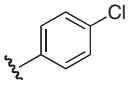
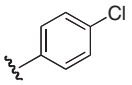
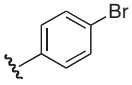
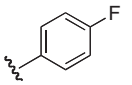
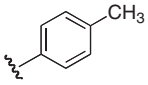
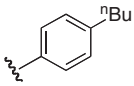
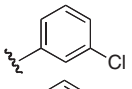
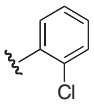
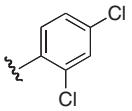
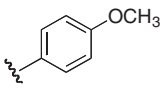
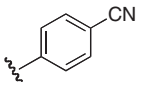
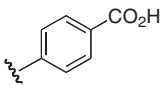
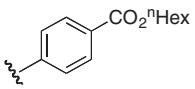
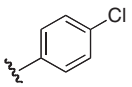
doi:10.1016/j.tetlet.2010.09.044

Table 1
Isolated yields of synthesized hydrazones (**8**) and corresponding 3-aryl-pyrimido[5,4-*e*][1,2,4]triazine-5,7(1*H*,6*H*)-diones (**9**)

Entry	R_1	R_2	R_3	% Yield (**8**)	% Yield (**9**)
1	H	CH_3		70	81
2	CH_2CH_3	CH_3		59	43
3	CH_3	CH_3		91	50
4	CH_3	CH_3		76	69
5	CH_3	CH_3		62	68
6	CH_3	CH_3		53	40
7	CH_3	CH_3		22	53
8	CH_3	CH_3		16	30
9	CH_3	CH_3		18	49
10	CH_3	CH_3		63	16
11	CH_3	CH_3		89	13
12	CH_3	CH_3		75	25
13	CH_3	CH_3		80	86
14	CH_3	CH_3		50	55
15	CH_3	Ph		45	10

example, utilization of phenyl hydrazine followed by treatment with *p*-chlorobenzaldehyde allowed for the formation of 6-(1-phenyl-2-(4-chlorobenzyl)hydrazinyl)-3-methylpyrimidine-2,4(1*H*,3*H*)-dione (Table 1, entry 15). In this case, however, the regiochemistry of the conjugate addition/displacement reaction involving phenylhydrazine is less selective since roughly equal amounts of both isomers were generated.

Finally, a mixture of hydrazone (**8**) with $NaNO_2$ in acetic acid was stirred for 18 h to allow for ring closure. Addition of Et_2O allowed for precipitation of the desired toxoflavin derivative (**9**) and its N-oxide. Filtration was followed by separation via column chromatography.

The synthetic method developed[10] allowed for the parallel production of a library of derivatives (with isolated yields presented in Table 1). Confirmation of the final structures was provided by NMR and MS. In the case of 3-(4-bromophenyl)-1,6-dimethyl-1,2-dihydropyrimido [5,4-*e*][1,2,4]triazine-5,7(6*H*,8*H*)-dione (Table 1, entry 4), X-ray crystallographic analysis allowed for the determination of the structure provided in Figure 1 (structure B).[11]

In conclusion, we have developed a practical synthesis of 3-aryl-pyrimido[5,4-*e*][1,2,4]triazine-5,7(1*H*,6*H*)-diones derivatives suitable for parallel approaches. The use of microwave irradiation allows for rapid reaction times, products in good to moderate yields, and avoids the use of metal salts.[12]

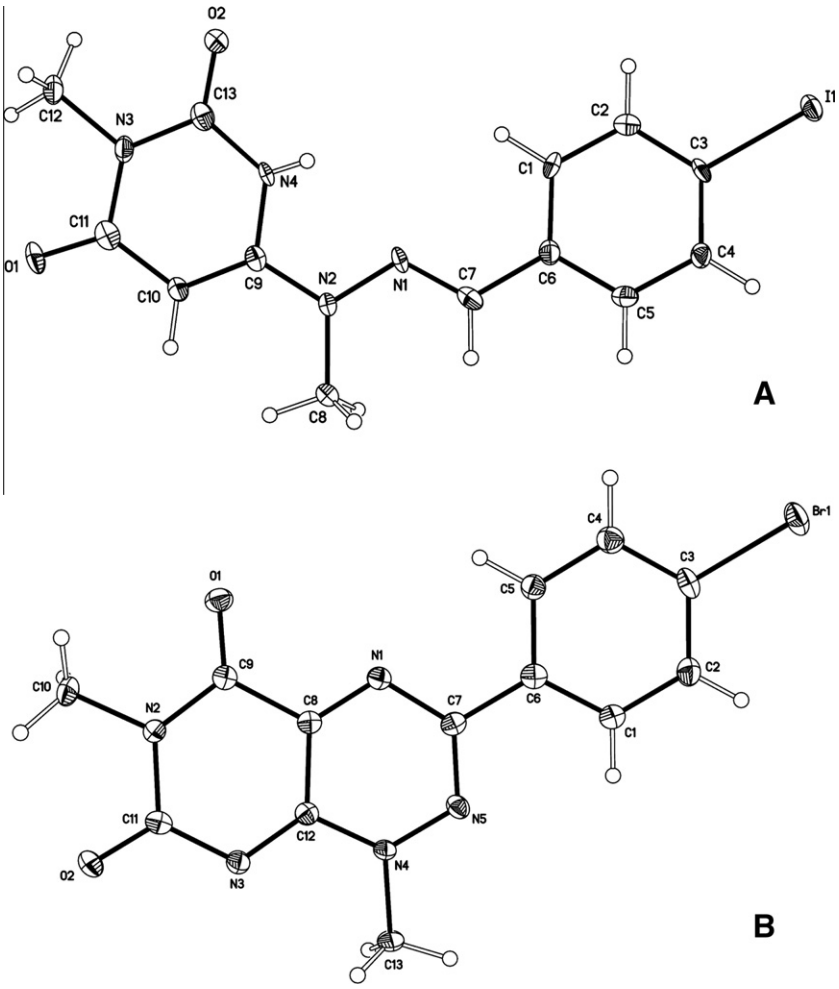


Figure 1. X-ray crystal structures of (A) 6-(1-methyl-2-(4-iodobenzyl)hydrazinyl)-3-methylpyrimidine-2,4(1*H*,3*H*)-dione and (B) 3-(4-bromophenyl)-1,6-dimethyl-1,2-dihydropyrimido [5,4-*e*][1,2,4]triazine-5,7(6*H*,8*H*)-dione.

Acknowledgments

The authors thank the Institute for Infectious Disease Research (McMaster University), the Natural Sciences and Engineering Research Council of Canada, the Canadian Foundation for Innovation, and the Ontario Innovation Trust for their financial support.

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10. *Typical experimental procedures.* Microwave reactions were performed using a CEM Discover microwave equipped with an IR sensor which allowed for monitoring and control of both temperature and pressure. *6-Hydroxy-3-methylpyrimidine-2,4(1H,3H)-dione* (**3**, with $R_1 = CH_3$): A mixture of malonic acid (5.0 g, 48.0 mmol) and methyl urea (3.6 g, 48.0 mmol) was dissolved in acetic anhydride (9.08 mL, 96.1 mmol). The solution was microwave heated at 60 °C for 15 min and the resulting acetic acid was removed under a reduced pressure. The viscous liquid was crystallized in ethanol and filtered to afford the product as a pale peach solid in 80% yield (5.86 g, 43.3 mmol); mp 125–130 °C; ^{1}H NMR (200 MHz, DMSO-d_6) δ 11.32 (s, 1H), 3.58 (s, 2H), 3.05 (s, 3H); ^{13}C NMR (50 MHz) δ 167.1, 166.6, 151.9, 39.8, 26.9; MS [EI+] *m/z* (RI%) 142 $[M]^+$ (100); HRMS for $C_5H_6N_2O_3$ calcd *m/e* 142.0378, observed *m/e* 142.0377. *6-Chloro-3-methylpyrimidine-2,4(1H,3H)-dione* (**4**, with $R_1 = CH_3$): A mixture of 6-hydroxy-3-methylpyrimidine-2,4(1*H*,3*H*)-dione (0.2 g, 1.4 mmol) and phosphorous oxychloride (0.26 mL, 2.8 mmol) in 5 mL acetonitrile was microwave heated at 120 °C for 20 min. The solution was cooled to room temperature and methanol was added. The resulting precipitate was filtered and dried to afford the product as an orange solid in 80% yield (0.18 g, 1.1 mmol); ^{1}H NMR (200 MHz, DMSO-d_6) δ 12.36 (s, 1H), 5.89 (s, 1H), 3.09 (s, 3H); ^{13}C NMR (50 MHz) 161.8, 150.6, 143.3, 99.2, 26.7; MS [EI+] *m/z* (RI%) 160 $[M]^+$ (100); HRMS for $C_5H_5ClN_2O_2$ calcd *m/e* 160.0040, observed *m/e* 160.0030. *3-Methyl-6-(1-methylhydrazinyl)pyrimidine-2,4(1H,3H)-dione* (**6**, with $R_1 = R_2 = CH_3$): To a mixture of 6-chloro-3-methylpyrimidine-2,4(1*H*,3*H*)-dione (1.56 g, 9.9 mmol) dissolved in ethanol (25 mL) was added methylhydrazine (1.15 mL, 21.9 mmol) and microwave heated at 100 °C for 10 min. The solution was cooled to room temperature and stirred overnight. The resulting precipitate was filtered and dried to afford the product as a pale orange solid in 60% yield (1.01 g, 5.9 mmol); ^{1}H NMR (200 MHz, $CDCl_3$) δ 8.93 (s, 1H), 4.78 (s, 1H), 3.29 (s, 3H), 3.17 (s, 3H); ^{13}C NMR (50 MHz, DMSO) δ 162.9, 153.9, 150.4, 72.3, 40.6, 26.1; MS [EI+] *m/z* (RI%) 170 $[M]^+$ (100); HRMS for $C_6H_{10}N_4O_2$ calcd *m/e* 170.0804, observed *m/e* 170.0808. *6-(2-(4-Chlorobenzylidene)-1-methylhydrazinyl)-3-methylpyrimidine-2,4(1H,3H)-dione* (**8**, with $R_1 = CH_3$, $R_2 = CH_3$, and $R_3 = p$-C_6H_4Cl; Table 1, entry 3): A mixture of 3-methyl-6-(1-methylhydrazinyl)pyrimidine-2,4(1*H*,3*H*)-dione (0.22 g, 1.3 mmol) and 4-chlorobenzaldehyde (0.3 g, 2.1 mmol) in 5 mL ethanol was microwave heated at 50 °C for 10 min and cooled to room temperature. The resulting precipitate was filtered and dried to afford the product as a yellow solid in 91% yield (0.35 g, 1.2 mmol); ^{1}H NMR (200 MHz, $CDCl_3$) δ 9.18 (s, 1H), 7.69 (s, 1H), 7.62 (d, *J* = 8.8 Hz, 2 H), 7.41 (d, *J* = 8.5 Hz, 2 H), 5.14 (d, *J* = 2.5 Hz, 1H), 3.34 (s, 3H), 3.32 (s, 3H); ^{13}C NMR (50 MHz) 164.4, 150.7, 149.5, 138.0, 136.3, 132.0, 129.4, 128.5, 77.9, 31.4, 27.1; MS [EI+] *m/z* (RI%) 292 $[M]^+$ (20); HRMS for $C_{13}H_{13}ClN_4O_2$ calcd *m/e* 292.0727, observed *m/e* 292.0726. *3-(4-Chlorophenyl)-1,6-dimethylpyrimido[5,4-e][1,2,4]triazine-5,7(1H,6H)-dione* (**9**

with $R_1 = CH_3$, $R_2 = CH_3$ and $R_3 = p\text{-}C_6H_4Cl$; Table 1, entry 3): 6-(2-(4-Chlorobenzylidene)-1-methylhydrazinyl)-3-methylpyrimidine-2,4(1*H*,3*H*)-dione (0.05 g, 0.15 mmol) was dissolved in 3 mL acetic acid and 0.3 mL water. The solution was cooled to 5 °C and $NaNO_2$ (0.016 g, 0.23 mmol) was added and stirred for 10 min. The mixture was slowly warmed to room temperature and stirred overnight. Diethyl ether was added to the mixture and the resulting precipitate was filtered and purified via column chromatography (1:1 EtOAc/DCM) to afford the product as a yellow solid in 50% yield (0.023 g, 0.08 mmol); ^{1}H NMR (200 MHz, $CDCl_3$) δ 8.27 (d, J = 8.7 Hz, 2H), 7.50 (d, J = 8.6 Hz, 2 H), 4.24 (s, 3H), 3.53 (s, 3 H); ^{13}C NMR (50 MHz) δ 158.6, 156.4, 154.3, 152. 5, 138.4, 131.1, 130.4, 129.5, 128.7, 43.8, 29.3; MS [EI+] *m/z* (RI%) 303 $[M]^+$ (100); HRMS for $C_{13}H_{10}ClN_5O_2$ calcd *m/e* 303.0523, observed *m/e* 303.0521.

11. The structure has been deposited with the Cambridge Crystallographic Data Centre (deposition number 788118).
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