One-Pot Synthesis and Antibacterial Activities of Novel 1*H*-Pyridazino[1,2-*a*]indazole-1,6,9(2*H*,11*H*)-triones

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Received May 6, 2008; accepted June 17, 2008; published online June 18, 2008

Synthesis of novel 1*H*-pyridazino[1,2-*a*]indazole-1,6,9(2*H*,11*H*)-triones using one-pot, three components reaction of 1,2-dihydropyridazine-3,6-dione, dimedone and aldehydes under solvent-free conditions has been reported. These products were evaluated *in vitro* for their antibacterial activities.

Key words dimedone; pyridazino[1,2-*a*]indazole-1,6,9(2*H*,11*H*)-trione; aldehyde; solvent-free

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)] Therefore, it is not surprising that research in the field of synthesis of polyfunctionalized heterocyclic compounds has received special attention.

Pyridazine and fused pyridazine derivatives continue to attract considerable attention, which mainly arises from the large variety of interesting pharmacological activities, herbicides, insecticides and fungicides.[2)] Moreover, pyridazine derivatives are useful as antituberculosis agents[3)] and related to the cardiovascular system.[4)] Similarly, indazole derivatives, which are bioisosteres of indoles, are also an important class of compounds in the medicinal arena.[5)] In fact, compounds containing the indazole skeleton are known to show a variety of biological activities, such as high binding affinity for estrogen receptor,[6)] inhabitation of protein kinase C-β,[7)] 5-HT_2 and 5-HT_3 receptor antagonisms,[8)] human immunodeficiency virus (HIV) protease inhibition,[9)] and anti-tumor activity.[10)]

Considering the above reports and in continuation of our previous works on synthesis of heterocyclic compounds,[11—16)] we wish to report a one-pot and three-component method for the preparation of 11-aryl-3,3-dimethyl-3,4-dihydro-1*H*-pyridazino[1,2-*a*]indazole-1,6,9(2*H*,11*H*)-trione derivatives **4** under solvent-free conditions (Chart 1).

Experimental

Apparatus Melting points were measured on an Electrothermal 9100 apparatus and are uncorrected. Mass spectra were recorded on a FINNIGAN-MAT 8430 mass spectrometer operating at an ionization potential of 70 eV. IR spectra were recorded on a Shimadzu IR-470 spectrometer. ^{1}H- and ^{13}C-NMR spectra were recorded on a BRUKER DRX-300 AVANCE spectrometer at 300.13 and 75.47 MHz, respectively. Elemental analyses were performed using a Heracus CHN-O-Rapid analyzer.

Synthesis of 1*H*-Pyridazino[1,2-*a*]indazole-1,6,9(2*H*,11*H*)-trione (4a—g) A mixture of dimedone (0.14 g, 1 mmol), 1,2-dihydropyridazine-3,6-dione (0.11 g, 1 mmol), benzaldehyde (0.13 g, 1.2 mmol) and *p*-TSA (0.1 g) was heated at 80 °C for 30 min (TLC). After cooling, the reaction mixture was washed with water (15 ml) and residue recrystallized from ethyl acetate : *n*-hexane (1 : 3) to afford the pure product **4a** as a yellow powder.

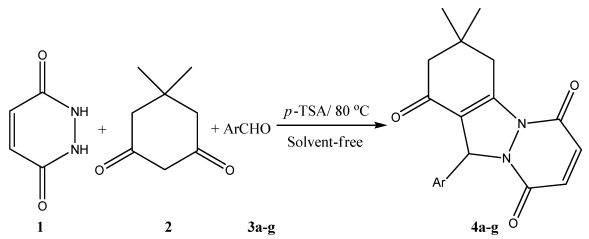

Chart 1. Synthesis of 1*H*-Pyridazino[1,2-*a*]indazole-1,6,9(2*H*,11*H*)-triones

3,3-Dimethyl-11-phenyl-3,4-dihydro-1*H*-pyridazino[1,2-*a*]indazole-1,6,9(2*H*,11*H*)-trione (4a, $C_{19}H_{18}N_2O_3$) Yellow powder; mp 224—226 °C. ^{1}H-NMR (DMSO-d_6) δ: 1.18 (6H, s), 2.31 (2H, s), 3.20 (2H, AB system, ^3J=18.1 Hz), 6.29 (1H, s), 6.90 (2H, s), 7.34 (5H, br s). ^{13}C-NMR (DMSO-d_6) δ: 28.3, 28.6, 34.6, 37.5, 50.9, 65.4, 118.9, 127.1, 128.8, 128.9, 134.8, 135.1, 136.0, 150.3, 153.2, 154.8, 191.9. IR (KBr) cm^{-1}: 3071, 3026, 1666. MS *m*/*z*: 322 (M^+), 245, 217. *Anal.* Calcd for $C_{19}H_{18}N_2O_3$: C, 70.79; H, 5.63; N, 8.69. Found: C, 70.73; H, 5.68; N, 8.60.

11-(4-Chlorophenyl)-3,3-dimethyl-3,4-dihydro-1*H*-pyridazino[1,2-*a*]indazole-1,6,9(2*H*,11*H*)-trione (4b, $C_{19}H_{17}ClN_2O_3$) Yellow powder; mp 196—198 °C. ^{1}H-NMR (DMSO-d_6) δ: 1.17 (3H, s), 1.19 (3H, s), 2.31 (2H, s), 3.21 (2H, AB system, ^3J=18.3 Hz), 6.26 (1H, s), 6.92 (2H, s), 7.27—7.30 (4H, m). ^{13}C-NMR (DMSO-d_6) δ: 28.2, 28.4, 34.6, 37.2, 50.7, 64.4, 117.7, 128.6, 129.8, 133.1, 135.8, 136.1, 151.5, 153.6, 155.2, 192.2. IR (KBr) cm^{-1}: 3051, 2962, 1693. MS *m*/*z*: 356 (M^+), 275, 245, 218. *Anal.* Calcd for $C_{19}H_{17}ClN_2O_3$: C, 63.96; H, 4.80; N, 7.85. Found: C, 63.91; H, 4.76; N, 7.92.

3,3-Dimethyl-11-(4-nitrophenyl)-3,4-dihydro-1*H*-pyridazino[1,2-*a*]indazole-1,6,9(2*H*,11*H*)-trione (4c, $C_{19}H_{17}N_3O_5$) Orange powder; mp 200—202 °C. ^{1}H-NMR (DMSO-d_6) δ: 1.16 (3H, s), 1.20 (3H, s), 2.32 (2H, s), 3.22 (2H, AB system, ^3J=18.2 Hz), 6.36 (1H, s), 6.95 (2H, s), 7.54 (2H, d, ^3J=6.7 Hz), 8.21 (2H, d, ^3J=6.6 Hz). ^{13}C-NMR (DMSO-d_6) δ: 28.3, 28.6, 34.7, 37.4, 50.8, 64.5, 117.7, 124.1, 128.1, 135.4, 135.7, 142.2, 147.9, 151.2, 153.3, 154.7, 191.9. IR (KBr) cm^{-1}: 3051, 2962, 1660. MS *m*/*z*: 367 (M^+), 286, 245. *Anal.* Calcd for $C_{19}H_{17}N_3O_5$: C, 62.12; H, 4.66; N, 11.44. Found: C, 62.17; H, 4.60; N, 11.50.

11-(2-Chlorophenyl)-3,3-dimethyl-3,4-dihydro-1*H*-pyridazino[1,2-*a*]indazole-1,6,9(2*H*,11*H*)-trione (4d, $C_{19}H_{17}ClN_2O_3$) Yellow powder; mp 214—216 °C. ^{1}H-NMR (DMSO-d_6) δ: 1.17 (3H, s), 1.19 (3H, s), 2.29 (2H, s), 3.20 (2H, AB system, ^3J=18.8 Hz), 6.49 (1H, s), 6.88 (1H, d, ^3J=9.7 Hz), 6.93 (1H, d, J=9.8 Hz), 7.28—7.44 (4H, m). ^{13}C-NMR (DMSO-d_6) δ: 28.3, 28.8, 34.6, 37.4, 50.8, 64.6, 116.8, 127.3, 130.1, 130.6, 131.6, 132.2, 135.1, 135.5, 151.4, 153.1, 155.0, 192.0. IR (KBr) cm^{-1}: 3064. 2962, 1668. MS *m*/*z*: 356 (M^+), 321, 275, 245. *Anal.* Calcd for $C_{19}H_{17}ClN_2O_3$: C, 63.96; H, 4.80; N, 7.85. Found: C, 63.92; H, 4.85; N, 7.79.

11-(3-Bromophenyl)-3,3-dimethyl-3,4-dihydro-1*H*-pyridazino[1,2-*a*]indazole-1,6,9(2*H*,11*H*)-trione (4e, $C_{19}H_{17}BrN_2O_3$) Yellow powder; mp 175—177 °C. ^{1}H-NMR (DMSO-d_6) δ: 1.18 (6H, s), 2.32 (2H, s), 3.19 (2H, AB system, ^3J=18.5 Hz), 6.23 (1H, s), 6.93 (2H, s), 7.20—7.44 (4H, m). ^{13}C-NMR (DMSO-d_6) δ: 28.2, 28.4, 34.7, 37.2, 50.7, 64.5, 117.4, 121.9, 127.0, 130.6, 130.8, 131.4, 135.7, 135.9, 139.7, 151.7, 153.7, 155.3, 192.2. IR (KBr) cm^{-1}: 3070, 2959, 1667. MS *m*/*z*: 402 (M^++2), 400 (M^+), 321, 245. *Anal.* Calcd for $C_{38}H_{28}BrN_7O$: C, 67.26; H, 4.16; N, 14.45. Found: C, 67.31; H, 4.11; N, 14.52.

3,3-Dimethyl-11-(3-nitrophenyl)-3,4-dihydro-1*H*-pyridazino[1,2-*a*]indazole-1,6,9(2*H*,11*H*)-trione (4f, $C_{19}H_{17}N_3O_5$) Yellow powder; mp 197—199 °C. ^{1}H-NMR (DMSO-d_6) δ: 1.19 (3H, s), 1.25 (3H, s), 2.33 (2H, s), 3.24 (2H, AB system, ^3J=18.5 Hz), 6.37 (1H, s), 6.92 (1H, d, ^3J=9.5 Hz), 6.99 (1H, d, J=9.4 Hz), 7.54—8.19 (4H, m). ^{13}C-NMR (DMSO-d_6) δ: 28.3,

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28.7, 34.7, 37.7, 50.8, 64.5, 117.5, 121.4, 123.9, 129.8, 134.2, 135.4, 135.7, 137.3, 148.5, 151.3, 153.4, 154.7, 191.9. IR (KBr) cm^{-1}: 3064, 2962, 1661. MS *m/z*: 367 (M^+), 286, 245. *Anal.* Calcd for $C_{19}H_{17}N_3O_5$: C, 62.12; H, 4.66; N, 11.44. Found: C, 62.18; H, 4.61; N, 11.38.

3,3-Dimethyl-11-(4-methylphenyl)-3,4-dihydro-1*H*-pyridazino[1,2-*a*]indazole-1,6,9(2*H*,11*H*)-trione (4g, $C_{20}H_{20}N_2O_3$) Yellow powder; mp 220—222 °C. ^{1}H-NMR (DMSO-d_6) δ: 1.19 (6H, s), 2.32 (5H, s), 3.22 (2H, AB system, ^3J=18.9 Hz), 6.27 (1H, s), 6.90 (2H, s), 7.14—7.27 (4H, m). ^{13}C-NMR (DMSO-d_6) δ: 21.24, 28.3, 28.7, 34.7, 37.5, 50.9, 65.3, 119.1, 127.0, 129.5, 132.1, 134.7, 136.1, 138.8, 150.2, 153.5, 192.1. IR (KBr) cm^{-1}: 3013, 2975, 1666. MS *m/z*: 336 (M^+), 245, 217. *Anal.* Calcd for $C_{20}H_{20}N_2O_3$: C, 71.41; H, 5.99; N, 8.33. Found: C, 71.47; H, 5.38; N, 8.26.

Results and Discussion

To achieve suitable conditions for the above transformation, we investigated the reaction of 1,2-dihydropyridazine-3,6-dione **1**, dimedone **2** and 4-chlorobenzaldehyde **3b** in the presence of *p*-TSA as an inexpensive and available catalyst in various solvents, ionic liquid [bmim]Br and under solvent-free classical heating conditions. In refluxing various solvent and ionic liquid the reaction was very slow and the yield of product was very low. We found that the best result was obtained in the presence of *p*-TSA at 80 °C under solvent-free conditions (Table 1).

To explore the scope and limitation of this reaction, we have extended the reaction of 1,2-dihydropyridazine-3,6-dione **1** and dimedone **2** with a range of aromatic aldehydes **3a**—**g** under similar conditions (solvent-free/*p*-TSA), furnishing the respective 1*H*-pyridazino[1,2-*a*]indazole-1,6,9(2*H*,11*H*)-trione derivatives **4a**—**g**. The optimized results are summarized in Table 2. Varying the substituents on the aldehydes did not detrimentally affect the yields and moderate yields were obtained using aromatic aldehydes carrying electron-donating or electron-withdrawing substituents in the presence of *p*-TSA, while without it for long period of time (5—6 h) and under solvent-free conditions the yields of products were low (<30%).

When this reaction was carried out with aliphatic aldehyde such as butanal or pentanal, the TLC and ^{1}H-NMR spectra of the reaction mixture showed a combination of starting materials and numerous products; the expected product was obtained in only trace amount.

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**—**g** are stable solids whose structures are fully supported by IR, ^{1}H- and ^{13}C-NMR spectroscopy, mass spectrometry, and elemental analysis.

The formation of products **4** can be rationalized by initial formation of intermediate **5** by standard Knoevenagel condensation of the dimedone **2** and aldehyde **3**. Then, the subsequent Michael-type addition of the 1,2-dihydropyridazine-3,6-dione **1** to the intermediate **5**, followed by cyclization and tautomerization affords the corresponding products **4** (Chart 2).

Finally, all synthesized compounds were screened for antimicrobial activity using disc diffusion method.[17] The microorganisms used in this study were *Escherichia coli* ATCC 25922, *Pseudomonas aeruginusa* ATCC 85327 (Gram-negative bacteria), *Bacillus subtilis* ATCC 465, *Staphylococcus aureus* ATCC 25923 (Gram-positive bacteria). All of the compounds were dissolved in DMSO (100 μg/ml) and 25 μl of them were loaded to 6 mm paper discs. One hundred microliters of 10^9 cell/ml suspension of the microorganisms were spread on sterile Muller Hilton Agar plates and the discs were placed on the surface of culture plates. Table 3 shows the inhibition zones of compounds around the discs.

Table 1. Conditions Effect on the Reaction[a]

Entry	Solvent	Yield (%)[b]	Time (h)
1	EtOH (reflux)	Trace	6
2	MeOH (reflux)	Trace	6
3	$CHCl_3$ (reflux)	Trace	6
4	CH_3CN (reflux)	Trace	6
5	[bmim]Br/100 °C	30	5
6	Solvent-free/80 °C	50	0.5
7	Solvent-free/100 °C	48	0.5

a) 1,3-Diphenyl-1*H*-pyrazol-5-amine (1 mmol), dimedone (1 mmol), 4-chlorobenzaldehyde (1.2 mmol) and *p*-TSA (0.1 g). *b*) Isolated yield.

Table 2. Synthesis of 1*H*-Pyridazino[1,2-*a*]indazole-triones **4a**—**g**

Product	R	Time (min)	Yield (%)[a]	mp (°C)
4a	C_6H_5	30	50	224—226
4b	4-Cl–C_6H_4	20	49	196—198
4c	4-NO_2–C_6H_4	25	48	200—202
4d	2-Cl–C_6H_4	40	55	214—216
4e	3-Br–C_6H_4	20	54	175—177
4f	3-NO_2–C_6H_4	20	47	197—199
4g	4-Me–C_6H_4	30	46	220—222

a) Isolated yield.

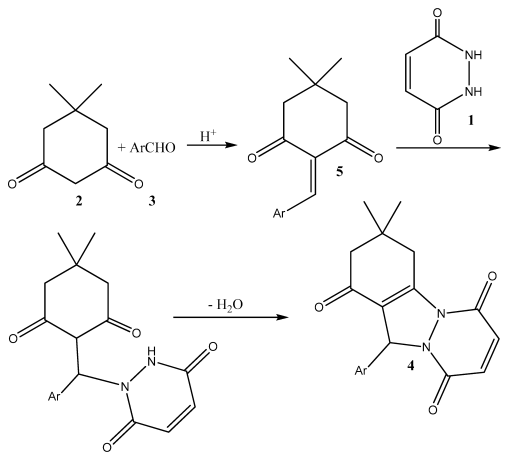

Chart 2. Mechanism for the Synthesis of 1*H*-Pyridazino[1,2-*a*]indazole-triones

Table 3. Antibacterial Activity of Products **4a**—**g**

Product	Zone of inhibition (mm)			
	Escherichia coli	*Pseudomonas aeruginusa*	*Bacillus subtilis*	*Staphylococcus aureus*
4a	[a]	[a]	14	[a]
4b	[a]	7	15	[a]
4c	11	[a]	14	[a]
4d	[a]	[a]	15	[a]
4e	14	8	20	[a]
4f	10	7	20	[a]
4g	[a]	[a]	8	[a]

a) Not active.

Table 4. MIC (μg/ml) Values of Products **4a**—**g**

Product	*Escherichia coli*			
	Escherichia coli	*Pseudomonas aeruginusa*	*Bacillus subtilis*	*Staphylococcus aureus*
4a	*a*)	*a*)	16	*a*)
4b	*a*)	60	8	*a*)
4c	16	*a*)	16	*a*)
4d	*a*)	*a*)	16	*a*)
4e	8	64	8	*a*)
4f	16	60	8	*a*)
4g	*a*)	*a*)	12	*a*)
Amoxycillin	128	—	2	16
Norfloxacin	<2	20	2	16

a) Not active.

The minimum inhibitory concentration (MIC) of the selected compounds which showed antibiotic activity in disc diffusion tests, were also determined by microdillution method[18] and compared to two commercial antibiotics (Table 4).

None of the compounds have antibiotic activity against *S. aureus*. Most of the compounds have a narrow spectrum antimicrobial activity. All the compounds were effective against *B. subtilis* ATCC 465 (MIC: 8—16 μg/ml). Compounds **4e** and **4f** exhibited activity against *P. aeruginusa* (60, 64 μg/ml) and *E. coli* (8, 16 μg/ml). Compounds **4c** (16 μg/ml), **4e** (8 μg/ml) and **4f** (16 μg/ml) were found to be more active than Amoxicillin against *E. coli* (128 μg/ml). Although, all of the compounds were found to be less active than norflaxacin against the screened Gram-positive and Gram-negative bacterial strains.

Conclusions

In summary, we have described a clean and simple method for the preparation of 1*H*-pyridazino[1,2-*a*]indazole-1,6,9(2*H*,11*H*)-trione derivatives in condensation reaction of 1,2-dihydropyridazine-3,6-dione, dimedone and aldehyde under solvent-free conditions. Most of the compounds exhibited moderate antibacterial activity against all the tested strains. These products were evaluated *in vitro* for their antibacterial activities. Most of the compounds have a narrow spectrum antimicrobial activity. All synthesized compounds were found to be more active than Amoxicillin against *P. aeruginusa*.

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

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