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SYNTHESIS OF AMINO-ACID DERIVATIVES OF FORMONONETIN AND CLADRIN

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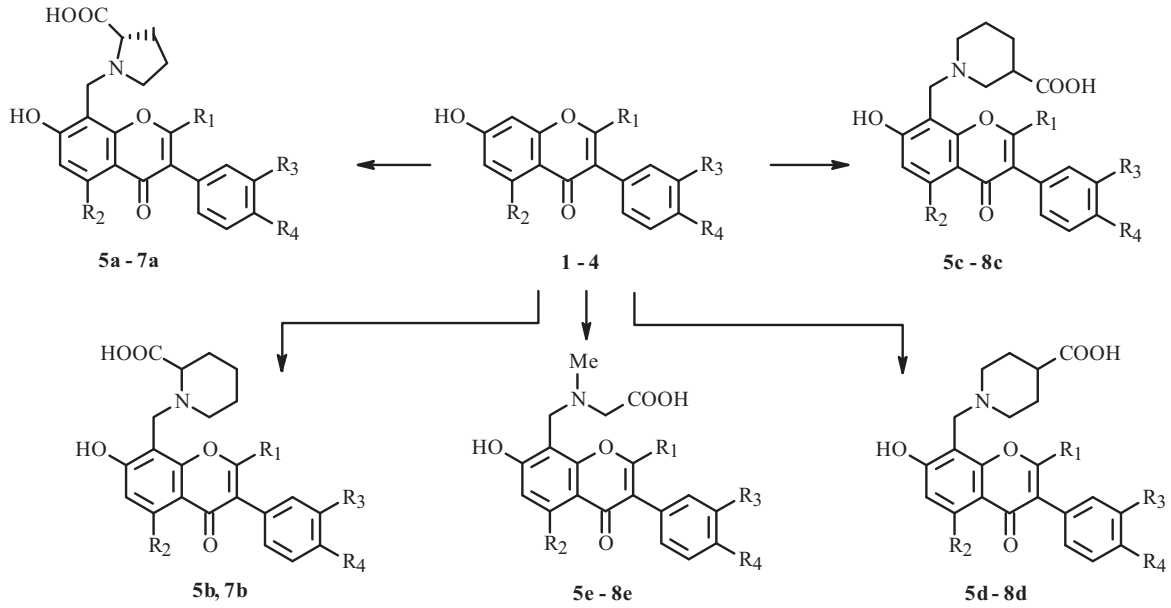
Aminomethylation of natural isoflavones by amino acids was studied. Conditions for selective production of N-(4H-4-oxochromen-8-yl)methyl-substituted amino acids were found.

Keywords: formononetin, cladrin, amino acid, aminomethylation.

Introducing an amino acid into organic compounds while retaining the amine and carboxylic acid is a promising pathway for creating physiologically active compounds that enables water-soluble salts of these compounds in both anionic and cationic forms to be prepared. In this respect the Mannich reaction in addition to reductive amination is an important method for synthesizing derivatives of natural amino acids and their analogs with retention of the asymmetric centers. However, depending on the structures of the starting materials and reagents, a decisive factor for performing aminomethylation is the solution acidity. Thus, catalysis by acids [1–3] and bases [4–9] was used for aminomethylation of phenols involving amino acids. It is well known that *N*,*N*-bis-derivatives of amino acids in addition to the desired mono *N*-substituted amino acids were obtained under acid-catalysis conditions [10–13]. Formation of 1,3-benzoxazine derivatives was observed by carrying out the Mannich reaction in neutral or basic solution [14]. Aminomethylation of 3-formylchromones by amino acids led to their deformylation [15].



1, 5a - e: $R_1 = R_2 = R_3 = H$, $R_4 = OMe$; **2, 6a, 6c - e:** $R_1 = R_2 = H$, $R_3 = R_4 = OMe$
3, 7a - e: $R_1 = Me$, $R_2 = R_3 = H$, $R_4 = OMe$; **4, 8c - e:** $R_1 = R_3 = H$, $R_2 = Me$, $R_4 = OMe$

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Because chemical modification of natural biologically active compounds is a promising pathway to creating new highly effective low-molecular-weight bioregulators, the goal of our work was to synthesize amino-acid derivatives of isoflavones. Introducing amino acids into flavonoid molecules, for which special transport mechanisms exist in the body, improves the permeability of the compounds through the cell membrane. This can enhance the effectiveness of their physiological action [16]. In addition, increasing the solubility of natural isoflavones and their analogs expands the capability for studying their biological activity and increases their bioavailability.

In continuation of research on aminomethylation of natural isoflavones such as formononetin (**1**), 2-methylformononetin (**3**), and cladrin (**2**) in addition to 5-methylformononetin (**4**), we studied the Mannich reaction involving these compounds and the *N*-substituted amino acids L-proline, sarcosine, and piperidinecarboxylic acids.

The previously proposed method for aminomethylation of isoflavones in aqueous alcohol [17–20] turned out in our instance to be unsuitable because of the low solubility of isoflavonoids **1**–**4**. Also, prolonged heating of the reaction mixture facilitated the formation of 8-ethoxymethyl derivatives of the isoflavones as before [18]. This reduced the yields and hindered purification of the target amino-acid derivatives.

We proposed an aminomethylation method using anhydrous EtOH and paraformaldehyde in the presence of a catalytic amount of 4-*N*,*N*-dimethylaminopyridine (DMAP) for the synthesis of Mannich bases of the isoflavones that contained amino acids. Under these conditions, regioselective aminomethylation of the 7-hydroxyisoflavones occurred with formation of their 8-aminomethyl derivatives in high yields and purities.

The structures of **5**–**8** were confirmed by PMR spectroscopy. Thus, PMR spectra of these compounds contained resonances for isoflavone and amino-acid protons. The resonance of the chromone CH_2-8 protons of *N*-substituted nipecotinic acid **5c**, **6c**, **7c**, and **8c**; isonipecotinic acid **5d**, **6d**, **7d**, and **8d**; and sarcosine **5e**, **6e**, **7e**, and **8e** was observed as a 2H singlet at 3.90–4.18 ppm. Peaks for the CH_2-8 protons in spectra of derivatives of L-proline **5a** and **5b** and of D,L-pipecolic acid **7a** and **7b** appeared as a diastereotopic pair of doublets at 4.00–4.18 ppm with SSCC 14.0–14.3 Hz because of the closely situated asymmetric center.

Thus, we synthesized a series of amino-acid derivatives of formononetin, cladrin, and their analogs. The presence of basic and acidic groups in them expands the capability of studying their biological activity. The method for aminomethylation of isoflavones that was developed by us can be used to synthesize amino-acid derivatives of other natural compounds.

EXPERIMENTAL

The course of reactions and purity of products were monitored by TLC on Merck plates (Germany) with elution by $CHCl_3$:MeOH (9:1, 95:5). PMR spectra were measured in DMSO-d_6 with TMS internal standard on the δ-scale on a Mercury M 400 instrument (Varian, 400 MHz). Elemental analyses of all compounds agreed with those calculated.

General Method for Preparing Amino-acid Derivatives of 7-Hydroxyisoflavones 5–8. A solution of 7-hydroxyisoflavone (**1**–**4**, 2 mmol) in anhydrous EtOH (10 mL) was refluxed; treated with the appropriate amino acid (2.4 mmol), paraformaldehyde (2.4 mmol calculated as formaldehyde), and DMAP (5 mg); refluxed for 2–8 h (end of reaction determined by TLC); cooled; and filtered to remove the precipitate. The solid was crystallized from EtOH.

1-{[7-Hydroxy-3-(4-methoxyphenyl)-4-oxo-4*H*-chromen-8-yl]methyl}-L-proline (5a). Yield 46%, $C_{22}H_{21}NO_6$, mp 200–202°C.

PMR spectrum (400 MHz, DMSO-d_6, δ, ppm, J/Hz): 1.65–1.97, 2.14–2.30, 2.52–2.65, 2.98–3.11, 3.39–3.54 (3H, 1H, 1H, 1H, 1H, 5m, proline protons), 3.80 (3H, s, 4′-OCH_3), 4.16, 4.21 (2H, 2d, ^2J = 14.0, CH_2-8), 6.94 (1H, d, ^3J = 9.2, H-6), 6.98 (2H, d, ^3J = 8.2, H-3′, H-5′), 7.52 (2H, d, ^3J = 8.2, H-2′, H-6′), 7.95 (1H, d, ^3J = 9.2, H-5), 8.29 (1H, s, H-2).

1-{[7-Hydroxy-3-(4-methoxyphenyl)-4-oxo-4*H*-chromen-8-yl]methyl}piperidine-2-carboxylic Acid (5b). Yield 89%, $C_{23}H_{23}NO_6$, mp 220–222°C.

PMR spectrum (400 MHz, DMSO-d_6, δ, ppm, J/Hz): 1.28–2.12, 2.69–3.34 (6H, 3H, 2m, pipecolic acid protons), 3.79 (3H, s, 4′-OCH_3), 4.00, 4.10 (2H, 2d, ^2J = 14.3, CH_2-8), 6.89 (1H, d, ^3J = 9.2, H-6), 6.98 (2H, d, ^3J = 8.2, H-3′, H-5′), 7.51 (2H, d, ^3J = 8.2, H-2′, H-6′), 7.92 (1H, d, ^3J = 9.2, H-5), 7.31 (1H, s, H-2).

1-{[7-Hydroxy-3-(4-methoxyphenyl)-4-oxo-4*H*-chromen-8-yl]methyl}piperidine-3-carboxylic Acid (5c). Yield 95%, $C_{23}H_{23}NO_6$, mp 215–216°C.

PMR spectrum (400 MHz, DMSO-d_6, δ, ppm, J/Hz): 1.41–1.96, 2.26–2.40, 2.68–2.80, 2.91–3.02, 3.33–3.53 (5H, 1H, 1H, 1H, 1H, 5m, nipecotinic acid protons), 3.80 (3H, s, 4′-OCH_3), 3.98 (2H, s, CH_2-8), 6.90 (1H, d, ^3J = 8.9, H-6), 6.98 (2H, d, ^3J = 8.9, H-3′, H-5′), 7.52 (2H, d, ^3J = 8.5, H-2′, H-6′), 7.93 (1H, d, ^3J = 8.9, H-6), 8.29 (1H, s, H-2).

1-{[7-Hydroxy-3-(4-methoxyphenyl)-4-oxo-4*H*-chromen-8-yl]methyl}piperidine-4-carboxylic Acid (5d). Yield 86%, $C_{23}H_{23}NO_6$, mp 160–162°C.

PMR spectrum (400 MHz, DMSO-d_6, δ, ppm, J/Hz): 1.50–1.66, 1.81–1.91, 2.23–2.35, 2.85–2.95 (2H, 2H, 3H, 2H, 4m, isonipecotinic acid protons), 3.78 (3H, s, 4′-OCH_3), 3.95 (2H, s, CH_2-8), 6.89 (1H, d, ^3J = 9.2, H-6), 6.98 (2H, d, ^3J = 8.5, H-3′, H-5′), 7.49 (2H, d, ^3J = 8.5, H-2′, H-6′), 7.90 (1H, d, ^3J = 9.2, H-5), 8.34 (1H, s, H-2).

***N*-{[7-Hydroxy-3-(4-methoxyphenyl)-4-oxo-4*H*-chromen-8-yl]methyl}-*N*-methylglycine (5e).** Yield 94%, $C_{20}H_{19}NO_6$, mp 225–226°C.

PMR spectrum (400 MHz, DMSO-d_6, δ, ppm, J/Hz): 2.39 (3H, s, NCH_3), 3.38 (2H, s, NCH_2), 3.81 (3H, s, 4′-OCH_3), 4.01 (2H, s, CH_2-8), 6.92 (1H, d, ^3J = 9.2, H-6), 6.98 (2H, d, ^3J = 8.5, H-3′, H-5′), 7.52 (2H, d, ^3J = 8.5, H-2′, H-6′), 7.95 (1H, d, ^3J = 9.2, H-5), 8.27 (1H, s, H-2).

1-{[7-Hydroxy-3-(3,4-dimethoxyphenyl)-4-oxo-4*H*-chromen-8-yl]methyl}-L-proline (6a). Yield 46%, $C_{23}H_{23}NO_7$, mp 216–217°C.

PMR spectrum (400 MHz, DMSO-d_6, δ, ppm, J/Hz): 1.66–1.97, 2.13–2.22, 2.52–2.62, 2.99–3.10, 3.43–3.55 (3H, 1H, 1H, 1H, 1H, 5m, proline protons), 3.79 (6H, s, 3′-OCH_3, 4′-OCH_3), 4.18 (2H, s, CH_2-8), 6.95 (1H, d, ^3J = 8.9, H-6), 7.00 (1H, d, ^3J = 8.2, H-5′), 7.15 (1H, dd, ^3J = 8.2, ^4J = 1.4, H-6′), 7.21 (1H, d, ^4J = 1.4, H-2′), 7.96 (1H, d, ^3J = 8.9, H-5), 8.33 (1H, s, H-2).

1-{[7-Hydroxy-3-(3,4-dimethoxyphenyl)-4-oxo-4*H*-chromen-8-yl]methyl}piperidine-3-carboxylic Acid (6c). Yield 92%, $C_{24}H_{25}NO_7$, mp 227–229°C.

PMR spectrum (400 MHz, DMSO-d_6, δ, ppm, J/Hz): 1.43–1.95, 2.27–2.42, 2.51–2.59, 2.70–2.80, 2.93–3.02 (5H, 1H, 1H, 1H, 1H, 5m, nipecotinic acid protons), 3.80 (6H, s, 3′-OCH_3, 4′-OCH_3), 3.99 (2H, s, CH_2-8), 6.90 (1H, d, ^3J = 8.5, H-6), 7.00 (1H, d, ^3J = 8.2, H-5′), 7.15 (1H, dd, ^3J = 8.2, ^4J = 1.4, H-6′), 7.23 (1H, d, ^4J = 1.4, H-2′), 7.94 (1H, d, ^3J = 8.5, H-6), 8.32 (1H, s, H-2).

1-{[7-Hydroxy-3-(3,4-dimethoxyphenyl)-4-oxo-4*H*-chromen-8-yl]methyl}piperidine-4-carboxylic Acid (6d). Yield 86%, $C_{24}H_{25}NO_7$, mp 170–172°C.

PMR spectrum (400 MHz, DMSO-d_6, δ, ppm, J/Hz): 1.48–1.97, 2.23–2.42, 2.83–3.08 (5H, 2H, 2H, 3m, isonipecotinic acid protons), 3.80 (6H, s, 3′-OCH_3, 4′-OCH_3), 3.98 (2H, s, CH_2-8), 6.88 (1H, d, ^3J = 9.2, H-6), 6.99 (1H, d, ^3J = 9.2, H-5′), 7.15 (1H, dd, ^3J = 9.2, ^4J = 2.4, H-6′), 7.23 (1H, d, ^4J = 2.4, H-2′), 7.93 (1H, d, ^3J = 9.2, H-5), 8.30 (1H, s, H-2).

***N*-{[7-Hydroxy-3-(3,4-dimethoxyphenyl)-4-oxo-4*H*-chromen-8-yl]methyl}-*N*-methylglycine (6e).** Yield 95%, $C_{21}H_{21}NO_7$, mp 214–215°C.

PMR spectrum (400 MHz, DMSO-d_6, δ, ppm, J/Hz): 2.39 (3H, s, NCH_3), 3.39 (2H, s, NCH_2), 3.80 (6H, s, 3′-OCH_3, 4′-OCH_3), 4.08 (2H, s, CH_2-8), 6.92 (1H, d, ^3J = 8.5, H-6), 7.00 (1H, d, ^3J = 8.5, H-5′), 7.15 (1H, dd, ^3J = 8.5, ^4J = 2.4, H-6′), 7.22 (1H, d, ^4J = 2.4, H-2′), 7.95 (1H, d, ^3J = 8.5, H-5), 8.33 (1H, s, H-2).

1-{[7-Hydroxy-3-(4-methoxyphenyl)-2-methyl-4-oxo-4*H*-chromen-8-yl]methyl}-L-proline (7a). Yield 81%, $C_{23}H_{23}NO_6$, mp 194–195°C.

PMR spectrum (400 MHz, DMSO-d_6, δ, ppm, J/Hz): 1.68–2.00, 2.13–2.23, 2.56–2.68, 3.01–3.11, 3.43–3.55 (3H, 1H, 1H, 1H, 1H, 5m, proline protons), 2.28 (3H, s, 2-CH_3), 3.81 (3H, s, 4′-OCH_3), 4.17, 4.22 (2H, 2d, ^2J = 14.0, CH_2-8), 6.90 (1H, d, ^3J = 8.5, H-6), 6.98 (2H, d, ^3J = 9.2, H-3′, H-5′), 7.19 (2H, d, ^3J = 9.2, H-2′, H-6′), 7.85 (1H, d, ^3J = 8.5, H-5).

1-{[7-Hydroxy-3-(4-methoxyphenyl)-2-methyl-4-oxo-4*H*-chromen-8-yl]methyl}piperidine-2-carboxylic Acid (7b). Yield 96%, $C_{24}H_{25}NO_6$, mp 242–253°C.

PMR spectrum (400 MHz, DMSO-d_6, δ, ppm, J/Hz): 1.28–2.11, 2.68–3.37 (6H, 3H, 2m, pipecolic acid protons), 2.25 (3H, s, 2-CH_3), 3.81 (3H, s, 4′-OCH_3), 4.00, 4.08 (2H, 2d, ^2J = 14.3, CH_2-8), 6.84 (1H, d, ^3J = 8.5, H-6), 6.97 (2H, d, ^3J = 8.5, H-3′, H-5′), 7.18 (2H, d, ^3J = 8.5, H-2′, H-6′), 7.82 (1H, d, ^3J = 8.5, H-5).

1-{[7-Hydroxy-3-(4-methoxyphenyl)-2-methyl-4-oxo-4*H*-chromen-8-yl]methyl}piperidine-3-carboxylic Acid (7c). Yield 75%, $C_{24}H_{25}NO_6$, mp 159–161°C.

PMR spectrum (400 MHz, DMSO-d_6, δ, ppm, J/Hz): 1.36–1.87, 2.28–2.38, 2.65–2.81, 2.88–3.02 (6H, 1H, 1H, 1H, 4m, nipecotinic acid protons), 2.25 (3H, s, 2-CH_3), 3.78 (3H, s, 4′-OCH_3), 3.96 (2H, s, CH_2-8), 6.86 (1H, d, ^3J = 8.9, H-6), 6.97 (2H, d, ^3J = 8.5, H-3′, H-5′), 7.18 (2H, d, ^3J = 8.5, H-2′, H-6′), 7.81 (1H, d, ^3J = 8.9, H-5).

1-{[7-Hydroxy-3-(4-methoxyphenyl)-2-methyl-4-oxo-4*H*-chromen-8-yl]methyl}piperidine-4-carboxylic Acid (7d). Yield 57%, $C_{24}H_{25}NO_6$, mp 182–184°C.

PMR spectrum (400 MHz, DMSO-d_6, δ, ppm, J/Hz): 1.48–1.68, 1.79–1.94, 2.27–2.37, 2.85–2.98 (2H, 2H, 3H, 2H, 4m, isonipecotinic acid protons), 2.24 (3H, s, 2-CH_3), 3.78 (3H, s, 4′-OCH_3), 3.97 (2H, s, CH_2-8), 6.84 (1H, d, ^3J = 8.9, H-6), 6.97 (2H, d, ^3J = 8.9, H-3′, H-5′), 7.18 (2H, d, ^3J = 8.9, H-2′, H-6′), 7.81 (1H, d, ^3J = 8.9, H-5).

***N*-{[7-Hydroxy-3-(4-methoxyphenyl)-2-methyl-4-oxo-4*H*-chromen-8-yl]methyl}-*N*-methylglycine (7e).** Yield 92%, $C_{21}H_{21}NO_6$, mp 219–220°C.

PMR spectrum (400 MHz, DMSO-d_6, δ, ppm, J/Hz): 2.26 (3H, s, 2-CH_3), 2.50 (3H, s, NCH_3), 3.38 (2H, s, NCH_2), 3.81 (3H, s, 4′-OCH_3), 4.08 (2H, s, CH_2-8), 6.88 (1H, d, ^3J = 8.9, H-6), 6.98 (2H, d, ^3J = 8.2, H-3′, H-5′), 7.19 (2H, d, ^3J = 8.2, H-2′, H-6′), 7.84 (1H, d, ^3J = 8.9, H-6).

1-{[7-Hydroxy-3-(4-methoxyphenyl)-5-methyl-4-oxo-4*H*-chromen-8-yl]methyl}piperidine-3-carboxylic Acid (8c). Yield 91%, $C_{24}H_{25}NO_6$, mp 222–224°C.

PMR spectrum (400 MHz, DMSO-d_6, δ, ppm, J/Hz): 1.41–1.88, 2.21–2.37, 2.71–2.81, 2.89–3.02 (6H, 1H, 1H, 1H, 4m, nipecotinic acid protons), 2.70 (3H, s, 5-CH_3), 3.80 (3H, s, 4′-OCH_3), 3.93 (2H, s, CH_2-8), 6.64 (1H, s, H-6), 6.96 (2H, d, ^3J = 8.5, H-3′, H-5′), 7.46 (2H, d, ^3J = 8.5, H-2′, H-6′), 8.16 (1H, s, H-2).

1-{[7-Hydroxy-3-(4-methoxyphenyl)-5-methyl-4-oxo-4*H*-chromen-8-yl]methyl}piperidine-4-carboxylic Acid (8d). Yield 82%, $C_{24}H_{25}NO_6$, mp 223–225°C.

PMR spectrum (400 MHz, DMSO-d_6, δ, ppm, J/Hz): 1.50–1.64, 1.81–1.90, 2.19–2.36, 2.85–2.95 (2H, 2H, 3H, 2H, 4m, isonipecotinic acid protons), 2.67 (3H, s, 5-CH_3), 3.77 (3H, s, 4′-OCH_3), 3.90 (2H, s, CH_2-8), 6.64 (1H, s, H-6), 6.96 (2H, d, ^3J = 8.9, H-3′, H-5′), 7.44 (2H, d, ^3J = 8.9, H-2′, H-6′), 7.22 (1H, s, H-2).

***N*-{[7-Hydroxy-3-(4-methoxyphenyl)-5-methyl-4-oxo-4*H*-chromen-8-yl]methyl}-*N*-methylglycine (8e).** Yield 85%, $C_{21}H_{21}NO_6$, mp 223–225°C.

PMR spectrum (400 MHz, DMSO-d_6, δ, ppm, J/Hz): 2.34 (3H, s, NCH_3), 2.68 (3H, s, 5-CH_3), 3.37 (2H, s, NCH_2), 3.77 (3H, s, 4′-OCH_3), 4.02 (2H, s, CH_2-8), 6.67 (1H, s, H-6), 6.96 (2H, d, ^3J = 8.9, H-3′, H-5′), 7.44 (2H, d, ^3J = 8.9, H-2′, H-6′), 8.22 (1H, s, H-2).

REFERENCES

1. O. Lukin, A. Shivanyuk, V. V. Pirozhenko, I. F. Tsymbal, and V. I. Kalchenko, *J. Org. Chem.*, **63**, 9510 (1998).
2. J. G. Wilson, *Aust. J. Chem.*, **41**, 173 (1988).
3. J. G. Wilson, *Aust. J. Chem.*, **43**, 783 (1990).
4. M. E. Branum, A. K. Tipton, S. Zhu, and L. Que, *J. Am. Chem. Soc.*, **123**, 1898 (2001).
5. L. Que, R. C. Kolanczyk, and L. S. White, *J. Am. Chem. Soc.*, **109**, 5373 (1987).
6. A. E. Martell, R. J. Motekaitis, E. T. Clarke, and J. J. Harrison, *Can. J. Chem.*, **64**, 449 (1986).
7. K. B. Gudasi, M. S. Patil, and R. S. Vadavi, *Eur. J. Med. Chem.*, **43**, 2436 (2008).
8. W. L. Leong, A. Y.-Y. Tam, S. K. Batabyal, L. W. Koh, S. Kasapis, V. W.-W. Yam, and J. J. Vittal, *Chem. Commun.*, **31**, 3628 (2008).
9. J. N. Gadre and P. S. Raote, *Indian J. Chem., Sect. B*, **32**, 679 (1993).
10. E. Safaei, H. Sheykhi, A. Wojtczak, A. Kozakiewicz, and Z. Jaglicic, *Polyhedron*, **30**, 1219 (2011).
11. T. Weyhermueller, R. Wagner, and P. Chaudhuri, *Eur. J. Inorg. Chem.*, **16**, 2547 (2011).
12. J. G. Wilson, *Aust. J. Chem.*, **43**, 1283 (1990).
13. J. H. Short and C. W. Ours, *J. Heterocycl. Chem.*, **12**, 869 (1975).
14. N. V. Gorbulenko, T. M. Tkachuk, T. V. Shokol, V. V. Semenyuchenko, A. V. Turov, and V. P. Khilya, *Khim. Geterotsikl. Soedin.*, **43**, 683 (2007).
15. S. K. Panja, S. Maiti, M. G. B. Drew, and C. Bandyopadhyay, *Tetrahedron*, **65**, 1276 (2009).
16. M. Ninomiya, K. Tanaka, Y. Tsuchida, Y. Muto, M. Koketsu, and K. Watanabe, *J. Med. Chem.*, **54**, 1529 (2011).
17. M. M. Garazd, Ya. L. Garazd, A. S. Ogorodniichuk, V. V. Shilin, A. M. Zhivolup, A. V. Turov, and V. P. Khilya, *Khim. Prir. Soedin.*, 632 (1998).
18. M. M. Garazd, Ya. L. Garazd, A. S. Ogorodniichuk, V. V. Shilin, A. V. Turov, and V. P. Khilya, *Khim. Prir. Soedin.*, 475 (1998).
19. M. M. Garazd, Ya. L. Garazd, S. V. Shilin, and V. P. Khilya, *Khim. Prir. Soedin.*, 383 (1998).
20. M. M. Garazd, Ya. L. Garazd, S. V. Shilin, and V. P. Khilya, *Khim. Prir. Soedin.*, 338 (2002).