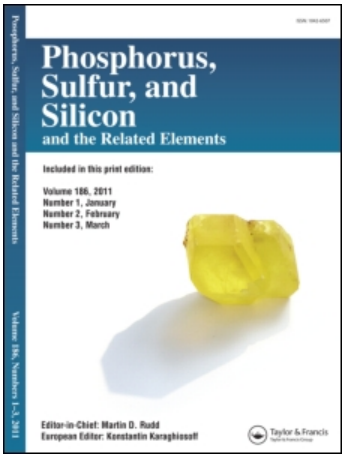

Phosphorus, Sulfur, and Silicon and the Related Elements

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Synthesis and Antiproliferative Activity in vitro of New 2-Thioxo-1*H*, 3*H*-imidazo[4,5-*b*]pyridine Derivatives

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SYNTHESIS AND ANTIPROLIFERATIVE ACTIVITY IN VITRO OF NEW 2-THIOXO-1*H*, 3*H*-IMIDAZO[4,5-*b*]PYRIDINE DERIVATIVES

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*S-Alkylation of 2-thioxo-1*H,*3*H-*imidazo[4,5-*b*]pyridine **2** with α-halogenoketones furnished unsymmetrical sulfides (**3–11**) with an imidazo[4,5-*b*]pyridine moiety. In some cases the two 1*H- *and 3*H-*tautomers were observed. All new compounds were examined for their antiproliferative activity in vitro against the cells of human cancer cell lines, using SRB technique. Preliminary screening data indicated that three out of all tested compounds revealed cytotoxic activity.*

Keywords: 1-(3*H*-imidazo[4,5-*b*]pyridin-2-ylthio)-1-phenyl-ethanone (**B**); 1-(3*H*-imidazo[4,5-*b*]-pyridin-2-ylthio)propan-2-one; 2-Thioxo-1*H*,3*H*-imidazo[4,5-*b*]pyridine derivatives; Antiproliferative activity; tautomers 1-(1*H*-imidazo[4,5-*b*]pyridin-2-ylthio)-1-phenyl-ethanone (**A**)

Compounds incorporating the imidazo[4,5-*b*]pyridine ring system have shown a broad range of biological and pharmacological activities. Among others their activity includes antibacterial,[1] antiinflammatory,[2] antiulcer,[3] cardiotonic,[4] and anticancer[5] action. Such diversity of bioactivities depends on the nature of the substituents on the heterocyclic ring system. Furthermore, since organosulfur compounds play a pivotal role in biology and nonsymmetrical sulfides have been reported to be the simplest and yet most potent antitumor drugs,[6] it was anticipated that the combined presence of imidazopyridine ring system and sulfur in a compound may enhance their biological activity.

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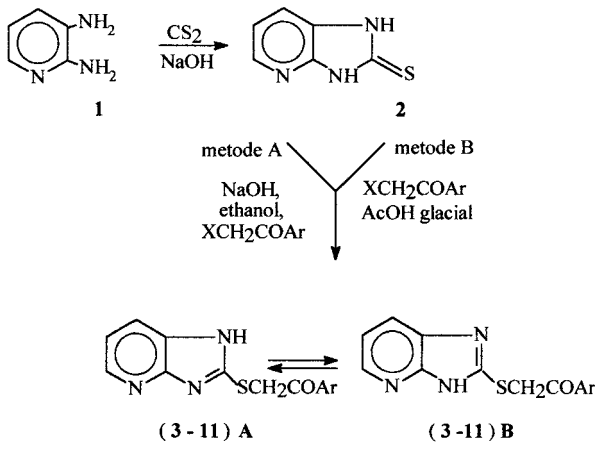
The aim of this work was the synthesis of compound with the toxophoric (—N=C—S—) group and its reaction with α-halogenoketones under different reaction conditions. In view of this, 2-thioxo-1*H*,3*H*-imidazo[4,5-*b*]pyridine (**2**) was prepared in the reaction of 2,3-diaminopyridine (**I**) with carbon disulfide.[7] Compound **2** proved to be a versatile starting material in synthesis of unsymmetrical sulfides with an imidazo[4,5-*b*]pyridine moiety (**3–11**) both in acidic and basic reaction environment.

RESULTS AND DISCUSSION

Chemistry

Treatment of **2** with appropriate α-halogenoketones: chloroacetone, phenacyl bromide and p-chloro-, p-bromo-, p-fluoro-, o-nitro-, p-nitro-, p-methyl-, p-methoxyphenacyl bromide in ethanol solution both in the presence of equimolar amount of sodium hydroxide (method A) and in glacial acetic acid (method B) gave mixtures of tautomers



3 Ar = CH_3
4 Ar = Ph
5 Ar = Ph-Cl(p)
6 Ar = Ph-Br(p)
7 Ar = Ph-F(p)
8 Ar = Ph-NO_2(o)
9 Ar = Ph-NO_2(p)
10 Ar = Ph-CH_3(p)
11 Ar = Ph-OCH_3(p)

SCHEME 1

2-(1*H*-imidazo[4,5-*b*]pyridin-2-ylthio)-1-alkyl(aryl)ethanones **3–11** (**A**) and 2-(3*H*-imidazo[4,5-*b*]pyridin-2-ylthio)-1-alkyl(aryl)ethanones **3–11** (**B**) (Scheme 1). Route **A** offered a higher yield of reaction product (10 ÷ 15%), suggesting that the S-alkylation reaction is base-catalyzed. All these newly synthesized compounds gave satisfactory elemental analyses. Confirmation of their structure was based on spectral data (IR, ^{1}H NMR and MS) which are presented in Table I Imidazo[4,5-*b*]-pyridines can exist in tautomeric forms.[8] In DMSO-d_6 solution, the ^{1}H NMR spectrum of 2-thioxo-1H,3*H*-imidazo[4,5-*b*]pyridine (**2**) showed the presence of two low-field resonance signals assigned to H-1 and H-3 protons at $\delta = 12.7$ and 13.1 ppm respectively. This spectrum is very valuable for comparison purposes in order to determine the presence of tautomeric forms and their relative populations for corresponding imidazopyridines. In DMSO-d_6 solution of compounds **4**, **5**, **9**, **10**, and **11**, a dynamic behavior was observed resulting in line broadening of the imidazole proton and indicating a rapid tautomerization. It was possible to distinguish individual tautomers for compounds **3**, **6**, **7**, and **8** with the 3*H* tautomer being the major species. The rate of tautomerization was shown to be dependent on a substituent attached to the carbonyl moiety in studied ethanones **3–11** and this can lead to the conclusion that sulfur participates in the transmission of the substituent effects from substituted phenyl through ethanone sidechain to imidazopyridine ring system. The capability of sulfur to trasmit the substituent effect was also found for 6-substituted 2-naphthyl methyl sulfides[9] and 4-substituted phenylthiol cinnamates.[10]

Pharmacology

Antiproliferative Activity in vitro

The results of experiments, expressed as ID_{50} (inhibitory dose 50%) values, determined for a given cancer cell line for three compounds tested are summarized in Table II. As shown in Table II, the compound **2** revealed weak cytotoxic effect in vitro against the cells of SW707 rectal cancer cell line only. Compounds **9** and **10** revealed weak activity only against human breast cancer cell line T47D. All three compounds should be further studied in vitro, applying larger panel of human cancer cell lines of different tissue origin and in vivo in the models of transplantable mouse tumor. It cannot be excluded that this type of compound may require more than direct contact with cancer cells to reveal antitumor properties, e.g., participation of tumor host's immunological and/or hormonal systems.

TABLE I Physical and Analytical Data of Compounds **2–11**

No.	Formula molecular weight	m.p. °C Method A Yield% (solvent)	IR (KBr) ν [cm^{-1}]	^{1}H NMR (DMSO-d_6, 300 MHz) δ [ppm] MS (70 eV): m/z (%)
2	$C_6H_5N_3S_1$ (151.19)	315–7 76 DMF	3400, 3050, 2800, 1620, 1520, 1430, 1380, 1250, 1180	13.10 (s, 1H, H-3), 12.70 (s, 1H, H-1), 8.08 (dd, J = 4.80 Hz, J = 1.20 Hz, 1H, H-5), 7.48 (dd, J = 7.80 Hz, J = 1.20 Hz, 1H, H-7), 7.12 (dd, J = 7.80 Hz, J = 4.80 Hz, 1H, H-6)
3	$C_9H_9N_3O_1S_1$ (207.25)	216–8 81 ethanol	3500, 3030, 2600, 1720, 1580, 1530, 1405, 1150	13.19 (0.7 H, s, H-3), 12.85 (0.3 H, s, H-1 of tautomer), 8.25 (dd, J = 4.80 Hz, J = 1.47 Hz, 1H, H-5), 7.95 (dd, J = 7.92 Hz, J = 1.47 Hz, 1H, H-7), 7.15 (dd, J = 7.92 Hz, J = 4.80 Hz, 1H, H-6), 4.36 (s, 2H, CH_2), 2.30 (s, 3H, CH_3)
4	$C_{14}H_{11}N_3O_1S_1$ (269.36)	199–202 85 ethanol	3350, 3200, 3020, 2600, 1705, 1610, 1580, 1525, 1440, 1405, 1210	13.20 (total 1H, br, s, H-3, H-1 of tautomer), 8.19 (dd, J = 4.85 Hz, J = 1.40 Hz, 1H, H-5), 8.08 (d, J = 7.11 Hz, 2H, Ar-H), 7.79 (dd, J = 7.90 Hz, J = 1.40 Hz, 1H, H-7), 7.66 (dd, J = 7.36 Hz, J = 7.36 Hz, 1H, Ar-H), 7.57 (dd, J = 7.36 Hz, J = 7.11 Hz, 2H, Ar-H), 7.13 (dd, J = 7.90 Hz, J = 4.85 Hz, 1H, H-6), 5.10 (s, 2H, CH_2) 270 (11), 269 (70), 251 (7), 241 (18), 227 (22), 165 (6), 164 (65), 151 (16), 150 (8), 135 (4), 134 (6), 123 (22), 120 (10), 106 (27), 105 (100), 93 (7), 92 (8), 91 (16), 78 (25), 77 (94), 76 (5), 51 (32)
5	$C_{14}H_{10}N_3O_1S_1Cl_1$ (303.77)	207–8 72 ethanol	3440, 3040, 2940, 1700, 1600, 1590, 1500, 1425, 1210	13.12 (total 1H, br, s, H-3 + H-1 of tautomer), 8.19 (d, J = 4.80 Hz, 1H, H-5), 8.09 (d, J = 8.60 Hz, 2H, Ar-H), 7.79 (d, J = 7.92 Hz, 1H, H-7), 7.64 (d, J = 8.60 Hz, 2H, Ar-H), 7.13 (dd, J = 7.92 Hz, J = 4.80 Hz, 1H, H-6), 5.07 (s, 2H, CH_2) 305 (7), 304 (4), 303 (22), 165 (4), 164 (25), 152 (3), 151 (19), 150 (4), 142 (3), 141 (41), 140 (9), 139 (100), 127 (10), 123 (14), 113 (9), 112 (4), 111 (31), 93 (7), 92 (4), 85 (6), 84

6	$C_{14}H_{10}N_3O_1S_1Br$ (348.22)	192–4 64 ethanol	3420, 3050, 2960, 1715, 1605, 1580, 1440, 1195	13.24 (0.7 H, s, H-3), 12.90 (0.3 H, s, H-1 of tautomer), 8.18 (br, s, 1H, H-5), 8.00 (d, J = 7.56 Hz, 2H, Ar-H), 7.80-7.78 (m, 3H, H-7 + Ar-H), 7.13 (dd, J = 7.92 Hz, J = 4.86 Hz, 1H, H-7), 5.07 (s, 2H, CH_2) 349 (21), 347 (33), 186 (7), 185 (86), 184 (7), 183 (100), 166 (3), 165 (5), 164 (48), 157 (25), 156 (3), 155 (25), 152 (6), 151 (66), 150 (9), 124 (6), 123 (19), 105 (8), 104 (7), 93 (17), 92 (8), 91 (5), 90 (6), 78 (10), 77 (9), 76 (18), 75 (14), 51 (9), 50 (16)
7	$C_{14}H_{10}N_3O_1S_1F_1$ (287.24)	205–6 66 ethanol	3380, 3070, 2940, 2610, 1700, 1650, 1590, 1430, 1270, 1170	13.13 (0.7 H, s, H-3), 12.71 (0.3 H, s, H-1 of tautomer), 8.17 (dd, J = 8.90 Hz, J = 5.52 Hz, 2H, Ar-H), 8.10 (dd, J = 5.05 Hz, J = 1.37 Hz, 1H, H-5), 7.47 (dd, J = 7.87 Hz, J = 1.37 Hz, 1H, H-7), 7.41 (dd, J = 8.90 Hz, 2H, Ar-H), 7.13 (dd, J = 7.87 Hz, J = 5.05 Hz, 1H, H-6), 5.08 (s, 2H, CH_2) 287 (14), 164 (15), 151 (100), 150 (4), 124 (14), 123 (77), 97 (4), 96 (5), 95 (19), 93 (26), 92 (7), 78 (3), 77 (3), 76 (4), 51 (4), 50 (3)
8	$C_{14}H_{10}N_4O_3S_1$ (314.32)	196–8 53 n-butanol	3420, 3090, 2920, 2600, 1715, 1640, 1580, 1540, 1400, 1350, 1310, 1200	13.22 (0.7 H, s, H-3), 12.90 (0.3 H, s, H-1 of tautomer), 8.17-8.14 (m, 2H, H-5 + Ar-H), 7.90-7.74 (m, 2H, H-5 + Ar-H), 7.90-7.74 (m, 4H, H-7 + Ar-H), 7.16 (dd, J = 7.78 Hz, J = 4.88 Hz, 1H, H-6), 4.86 (s, 2H, CH_2) 315 (6), 314 (27), 255 (10), 165 (7), 164 (50), 153 (6), 152 (18), 151 (100), 150 (37), 147 (18), 146 (4), 135 (8), 134 (20), 124 (7), 123 (18), 121 (5), 120 (14), 119 (13), 105 (9), 104 (21), 103 (5), 93 (25), 92 (20), 91 (7), 90 (6), 79 (7), 78 (31), 77 (12), 76 (29), 75 (6), 65 (15), 51 (22), 50 (19)

(Continued on next page.)

TABLE I Physical and Analytical Data of Compounds **2–11** (Continued)

No.	Formula molecular weight	m.p. °C Method A Yield% (solvent)	IR (KBr) ν [cm^{-1}]	^{1}H NMR (DMSO-$d_{|kern1pt6}$, 300 MHz) δ [ppm] MS (70 eV): m/z (%)
9	$C_{14}H_{10}N_4O_3S_1$ (314.32)	213–4 59 ethanol	3425, 3000, 2920, 2640, 1695, 1620, 1580, 1470, 1400, 1350, 1200	13.13 (total 1H, br, H-3 + H-1 of tautomer), 8.40-8.28 (m, 4H, Ar-H), 8.18 (d, J = 4.80 Hz, 1H, H-5), 7.78 (d, J = 7.80 Hz, 1H, H-7), 7.13 (dd, J = 7.80 Hz, J = 4.80 Hz, 1H, H-6), 5.13 (s, 2H, CH_2) 315 (7), 314 (44), 286 (10), 273 (3), 272 (19), 166 (7), 165 (18), 164 (100), 151 (69), 150 (91), 135 (5), 134 (5), 124 (6), 123 (22), 120 (25), 119 (7), 105 (13), 104 (47), 93 (19), 92 (24), 91 (5), 90 (3), 78 (22), 77 (10), 76 (24), 75 (8), 65 (11), 64 (13), 51 (8), 50 (12)
10	$C_{15}H_{13}N_3O_1S_1$ (283.35)	187–8 72 ethanol	3320, 3000, 2800, 1760, 1680, 1585, 1500, 1420, 1270, 1180	13.11 (total 1H, br, s, H-3 + H-1 of tautomer), 8.18 (dd, J = 4.83 Hz, J = 1.35 Hz, 1H, H-5), 7.97 (d, J = 8.50 Hz, 2H, Ar-H), 7.78 (dd, J = 7.94 Hz, J = 1.35 Hz, 1H, H-7), 7.38 (d, J = 8.50 Hz, 2H, Ar-H), 7.13 (dd, J = 7.94 Hz, J = 4.83 Hz, 1H, H-6), 5.06 (s, 2H, CH_2), 2.40 (s, 3H, CH_3) 285 (4), 284 (12), 283 (70), 255 (16), 241 (15), 164 (16), 151 (20), 150 (6), 123 (14), 120 (34), 119 (74), 105 (10), 93 (7), 92 (13), 91 (100), 90 (5), 78 (10), 77 (9), 51 (8), 50 (3)
11	$C_{15}H_{13}N_3O_2S_1$ (299.35)	209–10 65 ethanol	3400, 2900, 2600, 1740, 1680, 1550, 1460, 1420, 1280, 1190	13.12 (total 1H, br, s, H-3 + H-1 of tautomer), 8.19 (d, J = 4.70 Hz, 1H, H-5), 8.06 (d, J = 8.92 Hz, 2H, Ar-H), 7.79 (d, J = 7.80 Hz, 1H, H-7), 7.13 (dd, J = 7.80 Hz, J = 4.70 Hz, 1H, H-6), 7.09 (d, J = 8.92 Hz, 2H, Ar-H), 5.05 (s, 2H, CH_2), 3.86 (s, 3H, OCH_3) 301 (4), 300 (8), 299 (46), 271 (7), 152 (3), 151 (28), 150 (14), 149 (3), 136 (29), 135 (100), 123 (10), 108 (3), 107 (19), 93 (10), 92 (27), 91 (6), 78 (14), 77 (40), 76 (6), 51 (6), 50 (4)

TABLE II The Cytotoxic Activity in vitro (ID_50 in μg/ml) of the Tested Compounds Against the Cells of Various Tumor Cell Lines

	2	**9**	**10**
HCV29T (bladder)	77,8 ± 1,6	—*	—
SW707 (rectal)	—	—	—
T47D (breast)	—	55,6 ± 1,4	41,5 ± 1,1
A549 (lung)	—	—	—

*—negative.

EXPERIMENTAL

Chemistry

Melting points (uncorrected) were measured with a Boethius melting point apparatus. Analyses were performed on a Perkin Elmer 2400 analyzer and satisfactory results within ±0.4% calculated values were obtained for the new compounds. IR spectra (in KBr) were recorded with an IR 75 spectrophotometer. ^{1}H NMR spectra was measured on a Bruker ARX 300 MHz using DMSO-d_6 as solvents at room temperature and chemical shifts are referred to the residual solvent signal at δ 2.50 ppm, respectively. Mass spectra were determined on a GCMS-LK 82091 spectrometer at the ionization anergy 70 eV. The course of reaction and the purity of products were checked by TLC (kieselgel G, Merck) in diethyl ether:ethanol =5:1 for eluation.

*Reactions of 2-Thioxo-1*H,*3*H-*imidazo[4,5-*b*]pyridine (2) with α-Halogenoketones*

Method A: A mixture of 0.01 mmol of 2-thioxoimidazo[4,5-*b*]pyridine (**2**) and 0.01 mmol of NaOH in 50 ccm of absolute ethanol was refluxed for 0.5 h, after cooling 0.01 mmol appropriate α-halogenoketone was added. The mixture was refluxed for 2–3 h. The precipitate was filtered of, washed with water, dried, and recrystallized.

Method B: To a solution of 0.01 mmol of compound **2** in 30 ccm of glacial acetic acid, 0.01 mmol of the appropriate α-halogenoketones was added at room temperature and stirred. After 20 h of stirring, the precipitate was collected by filtration and neutralized with 5% solution of $NaHCO_3$. The final product was separated by filtration, washed with water, dried, and recrystallized.

Biology

Antiproliferative Assay in Vitro

Test solutions of the compounds (1 mg/ml) were prepared ex tempore for each test by dissolving them in 100 μl of DMSO + 900 μl of culture medium. After that, the compounds were diluted in culture medium (described below) to reach final concentrations 100, 10, 1, and 0.1 μg/ml.

Cell lines: Cells of the following human cancer lines were used: SW707 (colon adenocarcinoma), T47D (breast carcinoma), A549 (non-small cell lung carcinoma). All lines were obtained from American Type Culture Collection (Rockville, MD, USA) and cultured in the Cell Culture Collection of Department of Tumor Immunology, Institute of Immunology and Experimental Therapy (Wroclaw, Poland). Human uroepithelial cell line HCV29T established in Fibiger Institute (Copenhagen, Denmark), were obtained from Dr. J. Kieler in 1982.

Twenty-four hours before addition of the tested agents, the cells were plated in 96-well plates (Sarstedt, USA) at a density of 10^4 cells per well. The cells were cultured in the opti-MEM medium supplemented with 2 mM glutamine (Gibco, Warsaw, Poland), streptomycin (50 μg/ml), penicillin (50 U/ml) (both antibiotics from Polfa, Tarchomin, Poland) and 5% fetal calf serum (Gibco, Grand Island, USA). The cell cultures were maintained at 37°C in humid atmosphere saturated with 5% CO_2.

SRB (Sulphorodamine B) Assay

The cytotoxic assays were performed after 72-hour exposure of the cultured cells to varying concentrations (from 0.1 to 100 μg/ml) of the tested agents. The SRB method was used as described by Skehan et al.[11] The optical densities of the samples were measured on a Multiskan RC photometer (Labsystems, Helsinki, Finland) at 570 nm. The results were calculated as an ID_{50} (inhibitory dose 50%)—the dose of compound that inhibits proliferation rate of the tumor cells by 50% as compared to control untreated cells. Each compound in every concentration was tested in triplicates per experiment. Every experiment was repeated 3 times.

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