Synthesis and Fungistatic Activity of New Groups of 2,4-Dihydroxythiobenzoyl Derivatives against Phytopathogenic Fungi

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Twenty-six compounds, derivatives of amides, hydrazines, hydrazides, hydrazones, and semicarbazides, with a 2,4-dihydroxythiobenzoyl moiety, were synthesized from sulfinyl-bis(2,4-dihydroxythiobenzoyl). The compositions and chemical structures of these compounds were confirmed by IR, ^{1}H NMR, EI-MS, and elemental analysis. Antifungal properties of chemicals under in vitro conditions against five phytopathogenic fungi were estimated. In vivo studies against *Erisiphe graminis* were also carried out. The compounds N-substituted with an 2,4-dihydroxythiobenzamide group proved to be the most active. *N*-2-(1-Cinnamylbenzene ester)-2,4-dihydroxythiobenzamide, under in vitro conditions, showed activity at the level of 80−100% development of most pathogens at a concentration of 20 μg/mL and partially at a concentration of 200 μg/mL. For compounds with −HN−NH− or −NH−N═ moiety, weak or no fungistatic properties were found at the concentrations studied.

KEYWORDS: Sulfinyl-bis(2,4-dihydroxythiobenzoyl); 2,4-dihydroxythiobenzoyl derivatives; fungistatic activity; phytopathogenic fungi; in vitro and in vivo studies

INTRODUCTION

Fungicides remain vital for effective control of plant diseases, which are estimated to cause yield reductions by almost 20% in the major food crops worldwide. During the past few years, fungicide research has produced a diverse range of products with novel modes of action, which are expected to have a significant impact on disease control in the next decade. These new classes of fungicides include anilinopyrimidines, phenoxyquinolines, oxazolidinediones, spiroketalamines, scytalone dehydratase inhibitors, phenylpyrroles, strobilurines, and activators of systemic acquired resistance (*1*).

Another interesting group is the arylamides. Many compounds of this group, including fenhexamid (hydroxyanilide) (*2*), iprovalicarb (amino acid amide carbamate) (*3*), zoxamide (benzamide) (*4*), AC 382042 (phenoxyamide), carpropamid (cyclopropanamide), diclocymet (cyanoacetamide) (*5*), and MON 65500 (hindered silyl amide) (*6*), are of great interest to many agrochemical firms around the world (*7*). Recently, many sulfur analogues with linear ═NC(═S)− or cyclic −S−N═N− groups have been prepared, among others, tolnaftate and tolciclate (thiocarbamates) (*8*). Acibenzolar-*S*-methyl (benzothiadiazole, CGA 245704, commercialized as Bion) is a new activator of systemic acquired resistance (SAR). It activates SAR against a unique spectrum of diseases. This includes fungi belonging to the classes of oomycetes, ascomycetes, and deuteromycetes as well as bacteria and certain viruses (*1*).

The activity of many linear and cyclic thiocarbonyl combinations, confirmed by us, indicates the appropriateness of searching for new fungicides in the group of sulfur analogues of amides. Aryl and heterocyclic amides from the group of 2,4-dihydroxythiobenzoyl derivatives studied by us possess inhibitory properties against molds, yeasts, and dermatophytes (*9−12*). Similar activity is found in aryl derivatives of *N*-1-(2,4-dihydroxybenzenecarbothio)amidrazones and the corresponding cyclic systems, 5-(2,4-dihydroxybenzene)-1,3,4-thiadiazoles (*13*). For thiobenzanilides, activity against *Botritis cinerea* and *Rhizoctonia solani* was also confirmed (*14*). Therefore, starting with the 2,4-dihydroxythiobenzoyl moiety, leading compounds in the N-substituted (aryl, heterocyclic) amides or properly modified azine systems were studied. Synthesis of the combinations obtained from the reaction of the new thioacylating agent E⊕ (*15*) with nucleophilic compounds, including nitrogen atoms of different electron density like heterocyclic amines, heterocyclic compounds with the amine atom of nitrogen, hydrazines, semicarbazides, etc., was carried out (**Scheme 1**).

This paper presents our results on the preparation and biological activity of 26 compounds with 2,4-dihydroxythiobenzoyl moiety belonging to amide, hydrazine, hydrazide, hydrazone, and semicarbazide groups. The investigations were carried out under in vitro conditions against five strains of phytopatho-

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Scheme 1.   Synthesis of the Compounds with 2,4-Dihydroxythiobenzoyl Moiety
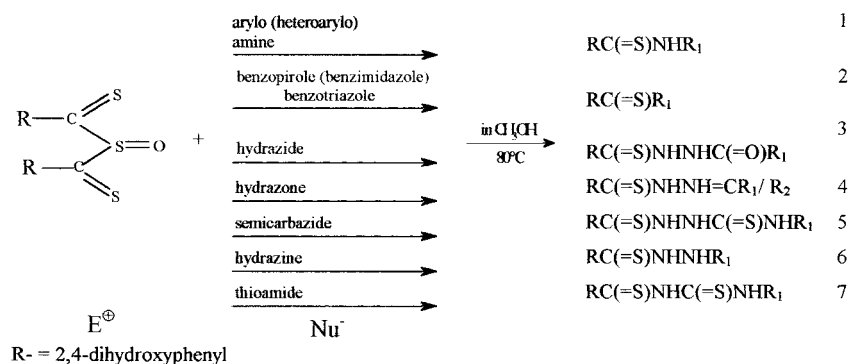
genic fungi and in vivo in relation to *Erysiphe graminis*. Structure−activity relationship studies were also performed.

MATERIALS AND METHODS

Synthesis of Compounds. First, 0.01 mol of $E^{\oplus}$ and 0.025 mol of Nu^{-} (**Scheme 1**) were put into 50 mL of CH_3OH and heated for 3 h. They were then filtered while heated. The remainder of the procedure for individual compounds is described in detail below.

N-2-(Biphenyl)-2,4-dihydroxythiobenzamide (1a) [Reaction of 2-Aminobiphenyl, Aldrich]. The filtrate was left for 24 h to remove the compound (room temperature). Crystallization was carried out from diluted (2:1) methanol (60 mL).

EI-MS: *m/z* (relative intensity) 321 (M^+, 49.17). ^1H NMR: δ (ppm) 6.27−6.37 (sextet, Ar−H, 2H), 7.30−7.49 (m, Ar−H, 8H), 7.77−7.86 (d, Ar−H, 2H), 10.14 (s, HOC-4), 11.38 (s, NH), 11.56 (s, HOC-2). IR: ν (cm^{-1}) 1498 NHC=S), 1033 C=S.

N-4-(Phenyl phenyl ether)-2,4-dihydroxythiobenzamide (1b) [Reaction of 4-Aminodiphenyl Ether, Aldrich]. To the filtrate was added 50 mL of water. The removed compound was crystallized from methanol (60 mL).

EI-MS: *m/z* (relative intensity) 37 (M^+, 41.97). ^1H NMR: δ (ppm) 6.30−6.42 (sextet, Ar−H, 2H), 7.00−7.51 (m, Ar−H, 8H), 7.68−7.89 (m, Ar−H, 2H), 10.04 (s, HOC-4), 11.19 (s, NH), 11.53 (s, HOC-2). IR: ν (cm^{-1}) 1508 NHC=S), 1023 C=S.

N-4-(Benzyloxyphenyl)-2,4-dihydroxythiobenzamide (1c) [Reaction of 4-(Benzyloxy)aniline Hydrochloride, Fluka]. To the filtrate was added 50 mL of water. The removed compound was crystallized from methanol (50 mL).

EI-MS: *m/z* (relative intensity) 351 (M^+, 66.19). ^1H NMR: δ (ppm) 5.12 (s, OCH_2, 2H), 6.32−6.41 (sextet, Ar−H, 2H), 7.01−7.66 (m, Ar−H, 8H), 7.82−7.92 (m, Ar−H, 2H), 10.08 (s, HOC-4), 11.45 (s, NH), 11.68 (s, HOC-2). IR: ν (cm^{-1}) 1511 NHC=S), 1026 C=S.

N-2-(Phenyl-4-chlorophenylsulfide)-2,4-dihydroxythiobenzamide (1d) [Reaction of 2-Aminophenyl-4-chlorophenylsulfide, Fluka]. The filtrate was left for 24 h to remove the compound (room temperature).The removed compound was crystallized from diluted (4:1) methanol (100 mL).

EI-MS: *m/z* (relative intensity) 387 (M^+, 10). ^1H NMR: δ (ppm) 6.34−6.41 (s, Ar−H, 2H), 7.22−7.76 (m, Ar−H, 8H), 8.01−8.10 (d, Ar−H, 2H), 10.26 (s, HOC-4), 11.71 (s, NH); 11.91 (s, HOC-2). IR: ν (cm^{-1}) 1500 NHC=S), 1032 C=S.

N-2-(Benzoic acid cinnamyl ester)-2,4-dihydroxythiobenzamide (1e) [Reaction of 2-Aminobenzoic Acid Cinnamyl Ester, Lancaster]. To the filtrate was added 100 mL of water. The removed compound was crystallized from diluted (2:1) methanol (60 mL).

EI-MS: *m/z* (relative intensity) 403 (M^+, 28.16). ^1H NMR: δ (ppm) 4.10 (t, CH_2, 2H), 4.93 (s, OCH_2, 2H), 6.31−6.40 (sextet, Ar−H, 2H), 6.45−6.70 (m, Ar−H, 4H), 7.58−7.74 (m, Ar−H, 4H), 7.84−8.17 (m, Ar−H, 2H), 10.22 (s, HOC-4), 11.63 (s, NH), 12.07 (s, HOC-2). IR: ν (cm^{-1}) 1687 C=O, 1511 NHC=S), 1087 C=S.

N-3-[4-(4-Morpholino)benzotrifluoride]-2,4-dihydroxythiobenzamide (1f) [Reaction of 3-Amino-4-(4-morpholino)benzotrifluoride, Lancaster]. To the filtrate was added 100 mL of water. The removed compound was crystallized from diluted (2:1) methanol (60 mL).

EI-MS: *m/z* (relative intensity) 349 (M^+, 63.58). ^1H NMR: δ (ppm) 2.96 0 (t, NCH_2, 4H), 3.80 (t, OCH_2, 4H), 6.37−6.46 (sextet, Ar−H, 2H), 7.31−7.62 (m, Ar−H, 2H), 8.32−8.41 (m, Ar−H, 1H), 8.80−8.83 (s, Ar−H, 1H), 9.80 (s, NHC(=O)), 10.27 (s, HOC-4), 11.75 (s, NH), 12.14 (s, HOC-2). IR: ν (cm^{-1}) 1516 NHC(=S), 1469 NCH_2 (δ), 1334 CF_3, 1023 C=S.

N-4-[(2-Benzoyl)-2′-chloroacetanilide]-2,4-dihydroxythiobenzamide (1g) [Reaction of *N*-2-(4-Aminobenzoyl)-2¢-chloroacetamide, Aldrich]. To the filtrate was added 100 mL of water. The removed compound was crystallized from methanol (50 mL).

EI-MS: *m/z* (relative intensity) 287, 244, 153, 127, 120 (100), 92, 77. ^1H NMR: δ (ppm) 4.26 0 (s, CH_2, 2H), 6.24−6.41 (sextet, Ar−H, 2H), 7.10−7.55 (m, Ar−H, 4H), 7.78−8.04 (m, Ar−H, 5H), 9.80 (s, NHC(=O)), 10.07 (s, HOC-4), 11.04 (s, NHC(=S)), 11.78 (s, HOC-2). IR: ν (cm^{-1}) 1686 C=O, 1516 NHC=S, 1057 C−Cl, 1023 C=S.

N-2-(Benzanilide)-2,4-dihydroxythiobenzamide (1h) [Reaction of 2-Aminobenzanilide, Lancaster]. The filtrate was concentrated until it became dry. The removed compound was crystallized from diluted (2:1) methanol (60 mL).

LSIMS: *m/z* (relative intensity) 565 [2M + Na]$^+$, 301 (100), 294 [M + Na]$^+$, 272 [M + H]$^+$. ^1H NMR: δ (ppm) 6.36−6.52 (sextet, Ar−H, 2H), 6.71−8.09 (m, Ar−H, 8H), 8.49−8.55 (m, Ar−H, 2H), 10.16 (s, HOC-4), 10.54 (s, NHC=O), 11.64 (s, NHC(=S)), 11.81 (s, HOC-2). IR: ν (cm^{-1}) 1595 C=O, 1506 NHC=S, 1020 C=S.

N-(Ethylthiazol-2-yl acetate)-2,4-dihydroxythiobenzamide (1i) [Reaction of Ethyl 2-Amino-4-thiazoleacetate, Aldrich]. To the filtrate was added 100 mL of water. The removed compound was crystallized from diluted (2:1) methanol (75 mL).

EI-MS: *m/z* (relative intensity) 338 (M^+, 28.56). ^1H NMR: δ (ppm) 1.22 (CH_3, 3H), 3.85 (t, CH_2, 2H), 4.11 (t, $OCH_2C(=O)$, 2H), 6.26−6.43 (sextet, Ar−H, 2H), 7.00 (s, $C_{thiazole}$−H), 8.25 (d, Ar−H, 1H), 10.15 (s, HOC-4), 11.83 (s, NH), 11.98 (s, HOC-2). IR: ν (cm^{-1}) 1707 C=O, 1506 NHC=S, 1020 C=S.

N-(4,5-Dihydro-1-phenylpyrazol-3-yl)-2,4-dihydroxythiobenzamide (1j) [Reaction of 3-Amino-4,5-dihydro-1-phenylpyrazole, Lancaster]. The filtrate was left for 24 h (at room temperature). The removed compound was crystallized from diluted (3:1) methanol (60 mL).

EI-MS: *m/z* (relative intensity) 313 (M^+, 24.13). ^1H NMR: δ (ppm) 3.39 (t, CH_2CH_2, 4H), 6.23−6.44 (sextet, Ar−H, 2H), 7.75−8.22 (m, Ar−H, 6H), 10.17 (s, HOC-4), 11.43 (s, NH), 11.83 (s, HOC-2). IR: ν (cm^{-1}) 1490 NHC=S, 1024 C=S.

N-(5-*tert*-Butylisoxazol-3-yl)-2,4-dihydroxythiobenzamide (1k) [Reaction of 3-Amino-5-*tert*-butylisoxazole, Lancaster]. To the filtrate was added 100 mL of water. The removed compound was crystallized from methanol (50 mL).

EI-MS: *m/z* (relative intensity) 292 (M^+, 62.31). ^1H NMR: δ (ppm) 1.18 (s, $C(CH_3)_3$, 9H), 6.39 (s, $C_{isoxazole}$−H), 6.47−6.53 (sextet, Ar−H, 2H), 7.95 (d, Ar−H, 1H), 10.15 (s, HOC-4), 11.15 (s, NH), 11.49 (s, HOC-2). IR: ν (cm^{-1}) 1476 C=S, 1019 C=S.

N-(5-*tert*-Butyl-1,3,4-thiadiazol-2-yl)-2,4-dihydroxythiobenzamide (1l) [Reaction of 2-Amino-5-*tert*-butyl-1,3,4-thiadiazole, Lancaster]. To the filtrate was added 100 mL of water. The removed compound was crystallized from diluted (2:1) methanol (45 mL).

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EI-MS: m/z (relative intensity) 309 (M+, 64.18). ^{1}H NMR: δ (ppm) 1.42 (s, 3 CH$_3$, 9H), 6.22−6.41 (sextet, Ar−H, 2H), 8.30−8.35 (m, Ar−H, 1H), 10.23 (s, HOC-4), 11.82 (s, HOC-2), 13.40 (s, SHN). IR: ν (cm^{-1}) 1566 (1525) NHC═S, 1019 C═S.

N-(Ethyl 4-methylthiophen-2-yl-3-carboxylate)-2,4-dihydroxy-thiobenzamide (1m) [Reaction of Ethyl 2-Amino-4-methylthiophene-3-carboxylate, Lancaster]. To the filtrate was added 100 mL of water. The removed compound was crystallized from diluted (4:1) methanol (50 mL).

EI-MS: m/z (relative intensity) 337 (M+, 100). ^{1}H NMR: δ (ppm) 1.34 (t, CH$_3$, 3H), 3.32 (s, CH$_3$C═O, 3H), 6.36−6.42 (sextet, Ar−H, 2H), 6.73 (s, C$_{thiophene}$−H), 8.07 (d, Ar−H, 2H), 10.19 (s, HOC-4), 11.47 (s, HOC-2), 14.06 (s, C(═O)SH). IR: ν (cm^{-1}) 1660 C═O, 1513 NHC(═S), 1026 C═S.

N-(4-Cyano-3-cyanomethyl-1-phenylpyrazol-5-yl)-2,4-dihydroxy-thiobenzamide (1n) [Reaction of 5-Amino-3-cyanomethyl-1-phen-ylpyrazolecarbonitrile, Lancaster]. To the filtrate was added 100 mL of water. The removed compound was crystallized from diluted (2:1) methanol (60 mL).

EI-MS: m/z (relative intensity) 223 (100), 184, 153, 91, 77. ^{1}H NMR: δ (ppm) 4.19 (t, CH$_2$, 2H), 6.35−6.43 (sextet, Ar−H, 2H), 6.84−7.91 (m, Ar−H, 6H), 10.72 (s, HOC-4), 11.85 (s, HOC-2), 13.17 (s, CSHNC). IR: ν (cm^{-1}) 1540 NHC═S, 1049 C═S.

2a, 2b, and 2c. In all cases, after the reaction the filtrate was concentrated until it became dry. The removed compound was crystallized from diluted (2:1) methanol (75 mL).

1-(2,4-Dihydroxythiobenzoyl)-5-benzyloxyindole-2-carboxylic acid (2a) [Reaction of 5-Benzyloxyindole-2-carboxylic Acid, Lancaster]. LSIMS: m/z (relative intensity) 393.1 [M + Na]+, 371.1 [M + H]+, 343, 301.1 (100). ^{1}H NMR: δ (ppm) 4.19 (s, OCH$_2$, 2H), 6.36−6.46 (sextet, Ar−H, 2H), 7.00−7.44 and 7.81−7.92 (m, 9H), 10.73 (s, HOC-4), 11.86 (s, HOC-2), 13.04 (s, CO$_2$H). IR: ν (cm^{-1}) 1701 C═O, 1048 C═S.

1-(2,4-Dihydroxythiobenzoyl)-2-chlorobenzimidazole (2b) [Reaction of 2-Chlorobenzimidazole, Lancaster]. EI-MS: m/z (relative intensity) 252 (100), 184, 164, 153, 137, 124. ^{1}H NMR: δ (ppm) 6.35−6.46 (sextet, Ar−H, 2H), 7.14−7.31 and 7.47−7.61 (m, Ar−H, 5H), 10.72 (s, HOC-4), 11.83 (s, HOC-2). IR: ν (cm^{-1}) 1230 C−Cl, 1052 (1009) C═S.

1-(2,4-Dihydroxythiobenzoyl)-5-chlorobenztriazole (2c) [Reaction of 5-Chlorobenztriazole, Lancaster]. LSIMS: m/z (relative intensity) 329.1 [2M + Na]+, 307 [2M + H]+, 176 [M + Na]+, 154.1 [M + H]+. ^{1}H NMR: δ (ppm) 4.19 (s, OCH$_2$, 2H), 6.33−6.45 (sextet, Ar−H, 2H), 7.39−7.53 (m, Ar−H, 2H), 8.06−8.82 (m, Ar−H, 2H), 10.72 (s, HOC-4), 11.83 (s, HOC-2). IR: ν (cm^{-1}) 1701 C═O, 1048 C═S.

N-(2,4-Dihydroxythiobenzoyl)-4-methoxybenzyl Carbazate (3a) [Reaction of 4-Methoxybenzyl Carbazate, Lancaster]. To the filtrate was added 100 mL of water. The removed compound was crystallized from diluted (3:1) methanol (80 mL).

EI-MS: m/z (relative intensity) 314, 302 (100), 167, 153, 136, 121. ^{1}H NMR: δ (ppm) 3.75 (d, CH$_3$, 3H), 5.07 (s, CH$_2$, 2H), 6.31−6.48 (sextet, Ar−H, 2H), 6.88−8.01 (m, Ar−H, 5H), 10.01 (s, OC(═O)-NHN*H*C(═S)), 10.16 (s, HOC-4), 11.04 (s, OC(═O)N*H*NHC(═S)), 11.82 (s, HOC-2). IR: ν (cm^{-1}) 1691 C═O, 1519, 1503, 1465 C(═O)-NHNHC(═S), 1028 C═S.

N-(2,4-Dihydroxythiobenzoyl)isonicotynic Acid Hydrazide (3b) [Reaction of Isonicotinic Acid Hydrazide, Aldrich]. To the filtrate was added 50 mL of water. The removed compound was crystallized from diluted (2:1) DMSO (60 mL).

EI-MS: m/z (relative intensity) 278 (M+, 16.97), 260 (100), 167, 153, 135, 97, 70, 39. ^{1}H NMR: δ (ppm) 6.35−6.46 (sextet, Ar−H, 2H), 6.88−8.12 (m, 5H), 10.07 (s, HOC-4), 10.27 (s, C(═O)NHN*H*C(═S)), 11.35 (C(═O)N*H*NHC(═S)), 11.88 (s, HOC-2). IR: ν (cm^{-1}) 1659 C═O, 1535, 1491, 1454, 1051 (1015) C═S.

N^1-(4-Ethoxybenzoyl)-N^2-(2,4-dihydroxythiobenzoyl)hydrazine (3c) [Reaction of 4-Ethoxybenzhydrazide, Aldrich]. To the filtrate was added 100 mL of water. The removed compound was crystallized from diluted (2:1) methanol (75 mL).

EI-MS: m/z (relative intensity) 332 (M+, 2.17). ^{1}H NMR: δ (ppm) 1.39 (m, CH$_3$, 3H), 4.15 (q, CH$_2$, 2H), 6.40−6.51 (sextet, Ar−H, 2H), 7.03−8.07 (m, Ar−H, 5H), 10.18 (s, HOC-4), 10.56 (s, C(═O)-

N*H*NHC(═S)), 10.78 (s, C(═O)NHN*H*C(═S)), 11.09 (s, HOC-2). IR: ν (cm^{-1}) 1741 C═O, 1421, 1395 C(═S)NHNHC(═O), 1046 C═S.

N-(2,4-Dihydroxythiobenzoyl)benzophenone Hydrazone (4a) [Reaction of Benzophenone Hydrazone, Lancaster]. The filtrate was left for 24 h (at room temperature) to remove the compound. The removed compound was crystallized from diluted (2:1) methanol (45 mL).

EI-MS: m/z (relative intensity) 348 (M+, 53.72). ^{1}H NMR: δ (ppm) 6.29−6.44 (sextet, Ar−H, 2H), 7.29−7.51 (m, Ar−H, 10H), 8.02 (s, Ar−H, 1H), 9.88 (s, C═NNH), 10.11 (s, HOC-4), 11.52 (s, HOC-2). IR: ν (cm^{-1}) 1510 C═NNHC(═S), 1027 C═S.

N-(2,4-Dihydroxythiobenzoyl)-9-fluorenohydrazone (4b) [Reaction of 9-Fluorenohydrazone, Lancaster]. To the filtrate was added 30 mL of water. The removed compound was crystallized from methanol (40 mL).

EI-MS: m/z (relative intensity) 346 (M+, 10.89). ^{1}H NMR: δ (ppm) 6.43−6.48 (sextet, Ar−H, 2H), 7.36−7.75 (m, Ar−H, 8H), 7.95 (m, Ar−H, 1H), 9.97 (s, C═NNH), 10.23 (s, HOC-4), 11.04 (s, HOC-2). IR: ν (cm^{-1}) 1466, 1418, 1375 C═NNHC(═S), 1002 C═S.

N-(2,4-Dihydroxythiobenzoyl)-4-fluorophenylthiosemicarba-zyde (5a) [Reaction of 4-Fluorophenylthiosemicarbazide, Lan-caster]. To the filtrate was added 100 mL of water. The removed compound was crystallized from methanol (70 mL).

EI-MS: m/z (relative intensity) 303 (M+, 100). ^{1}H NMR: δ (ppm) 6.36−6.45 (sextet, Ar−H, 2H), 7.10−7.83 (m, Ar−H, 5H), 9.93 (d, NHNH, 2H), 10.84 (s, NH), 10.27 (s, HOC-4), 10.84 (s, HOC-2). IR: ν (cm^{-1}) 1510, 1476, 1449, 1441 NHC(═S)NNHC(═S) (ν and δ), 1052, 1013 C═S.

N-(2,4-Dihydroxythiobenzoyl)-3-chlorophenylthiosemicarba-zide (5b) [Reaction of 3-Chlorophenylothiosemicarbazide, Lan-caster]. To the filtrate was added 100 mL of water. The removed compound was crystallized from diluted (4:1) methanol (50 mL).

EI-MS: m/z (relative intensity) 353 (M+, 6.41). ^{1}H NMR: δ (ppm) 6.41−6.50 (sextet, Ar−H, 2H), 6.99−7.90 (m, Ar−H, 4H), 7.98 (s, Ar−H, 1H), 10.00 (s, NH), 10.13 (s, HOC-4), 10.51 (s, NHNH, 2H), 11.28 (s, HOC-2).

N-2,4-Dihydroxythiobenzoylhydralazine (6) [Reaction of Hy-dralazine Hydrochloride, Aldrich]. To the filtrate was added 100 mL of water. The removed compound was crystallized from diluted (2:1) methanol (75 mL).

EI-MS: m/z (relative intensity) 312 (M+, 11.27). ^{1}H NMR: δ (ppm) 6.34−6.50 (sextet, Ar−H, 2H), 7.73−8.58 (m, Ar−H, 6H), 9.15 (s, C(═S)NHN*H*), 10.02 (s, HOC-4), 11.07 (s, HOC-2), 14.39 (s, CSHN ring). IR: ν (cm^{-1}) 1534, 1474, 1418, 1441 NHC(═S), 1016 C═S.

N-(2,4-Dihydroxythiobenzoyl)thionicotinamide (7) [Reaction of Thionicotinamide, Lancaster]. To the filtrate was added 100 mL of water. The removed compound was crystallized from 150 mL water.

EI-MS: m/z (relative intensity) 184 (M+, 100), 168, 153, 138, 124, 105, 78. ^{1}H NMR: δ (ppm) 6.32−6.40 (s, Ar−H, 2H), 7.44 (s, C$_{pyridine}$−H, 1H), 7.80−7.85 (d, Ar−H), 8.15−8.20 (m, C$_{pyridine}$−H, 1H), 8.63−8.89 (m, C$_{pyridine}$−H, 2H), 10.04 (s, HOC-4), 10.69 (s, NH), 11.81 (s, HOC-2). IR: ν (cm^{-1}) 1463, 1436 C(═S)NHC(═S), 1045 (1028) C═S.
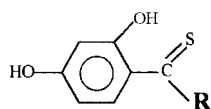
Biological Investigations: Estimation Criteria. Two tests were made for fungistatic action:

(A) The test "in vitro" estimated inhibition of mycelium in the agar culture medium caused by the compound under investigation. The bioindicators presented in **Table 2** (below) were used in the test. The solutions (suspensions) were prepared at the concentrations needed to obtain 200 and 20 μg/mL of the studied substance after dilution with the agar culture medium (PDA). Petri scale pans were used, into which the agar culture medium and the studied substance were poured. When the culture medium set, the infectious material of the tested fungus, in the form of agar disks overgrown with mycelium, was placed at three sites on its surface. After 4−8 days, depending on the mycelium culture, the linear growth of the mycelium colony was measured. The compound's action was determined as the percentage of mycelium growth inhibition compared with the control using the equation

$$J = [(C - T)/C] \times 100$$

where J is the percentage of colony growth inhibition, C is the zone of fungus colony growth in the control combination (millimeters), and

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Table 1. Structure and Physicochemical Properties of Compounds

no.	R	formula	MW	mp (°C)	elemental analysis calcd/found			log k (HPLC)
					C	H	N	
1a		$C_{19}H_{15}NO_2S$	321.40	200–201	70.94/ 71.12	4.66/ 4.53	4.35/ 4.10	0.118
1b		$C_{19}H_{15}NO_3S$	337.40	205–206	67.57/ 67.41	4.44/ 4.37	4.14/ 4.27	1.305
1c		$C_{20}H_{17}NO_3S$	351.23	195–196	68.38/ 68.51	4.84/ 4.90	3.98/ 3.86	0.423
1d		$C_{19}H_{14}ClNO_2S$	387.92	202–204	58.78/ 58.89	3.61/ 3.52	3.59/ 3.66	0.446
1e		$C_{23}H_{17}NO_4S$	405.35	104–105	68.15/ 68.36	4.69/ 4.57	3.46/ 3.65	0.488
1f		$C_{18}H_{17}F_3N_2O_3S$	398.43	264–265	54.14/ 54.29	4.51/ 4.40	7.01/ 6.75	0.035
1g		$C_{22}H_{17}ClN_2O_4S$	440.92	224–225	59.96/ 60.03	3.86/ 3.75	6.35/ 6.43	−0.290
1h		$C_{20}H_{16}N_2O_3S$	364.43	245–246	65.85/ 65.98	4.39/ 4.22	7.68/ 7.62	−0.370
1i		$C_{14}H_{14}N_2O_4S$	338.41	124–126	49.64/ 50.01	4.14/ 4.09	8.27/ 8.44	0.048
1j		$C_{16}H_{13}N_3O_2S$	313.39	95–97	61.27/ 61.15	4.79/ 4.88	13.40/ 13.25	0.643
1k		$C_{14}H_{16}N_2O_3S$	292.36	177–178	57.46/ 57.28	5.47/ 5.33	9.58/ 9.80	−0.302
1l		$C_{13}H_{15}N_3O_2S_2$	309.42	249–250	50.42/ 50.36	4.85/ 5.01	13.57/ 13.45	−0.131
1m		$C_{15}H_{15}NO_4S_2$	337.43	211–212	53.34/ 53.52	4.45/ 4.29	4.15/ 4.27	−0.280
1n		$C_{19}H_{13}N_5O_2S$	375.42	146–147	60.73/ 60.91	3.46/ 3.37	18.65/ 18.95	0.373

Table 1 (Continued)

no.	R	formula	MW	mp (°C)	elemental analysis calcd/found			log *k* (HPLC)
					C	H	N	
2a		$C_{23}H_{17}NO_5S$	419.35	78–80	65.82/ 65.91	4.05/ 3.96	3.34/ 3.17	0.405
2b		$C_{14}H_9ClN_2O_2S$	304.79	230–231	55.12/ 54.96	2.95/ 2.83	9.19/ 9.34	0.378
2c		$C_{13}H_8ClN_3O_2S$	306.75	125–127	50.98/ 51.17	2.61/ 2.69	13.72/ 13.51	0.936
3a		$C_{16}H_{16}N_2O_5S$	348.39	135–136	55.11/ 54.94	4.59/ 4.63	8.04/ 7.82	0.103
3b		$C_{13}H_{11}N_3O_3S$	289.32	305–307	53.98/ 53.88	3.81/ 3.90	14.51/ 14.42	−0.210
3c		$C_{16}H_{16}N_2O_4S$	332.39	251–252	57.76/ 58.02	4.81/ 4.85	8.42/ 8.60	0.352
4a		$C_{20}H_{16}N_2O_2S$	348.43	145–147	68.88/ 68.79	4.59/ 4.62	8.04/ 7.84	0.642
4b		$C_{20}H_{16}N_2O_2S$	346.43	327–238	69.28/ 69.13	3.75/ 3.66	8.08/ 7.78	0.232
5a		$C_{14}H_{12}FN_3O_2S$	337.34	279–280	49.85/ 50.03	3.56/ 3.44	12.45/ 12.66	0.240
5b		$C_{14}H_{12}ClN_3O_2S$	353.86	265–266	47.48/ 46.64	3.39/ 3.31	11.87/ 11.53	0.229
6		$C_{15}H_{12}N_4O_2S$	312.36	283–284	57.62/ 57.72	3.84/ 3.90	17.94/ 17.62	0.228
7		$C_{13}H_{10}N_2O_2S$	290.37	168–170	53.72/ 53.78	3.44/ 3.50	9.64/ 9.85	0.751

T is the zone of fungus colony growth in the combination with the compound (millimeters).

Sarfun 500SC (a.s. carbendazime, Organica Chemical Co. S.A., Nowa Sarzyna, Poland) and Sumilex 500SC (s.a. procymidone,

Table 2. Estimation of Fungistatic Action According to the Four-Degree Scale[a] for Individual Tests

| | estimation of mycelium growth inhibition (μg/mL) | | | | | | | | | | inhibition of infection with *Erysiphe graminis* (μg/mL) | |
| | *Alternaria alternata* | | *Botrytis cinerea* | | *Rhizoctonia solani* | | *Fusarium culmorum* | | *Phytohtora cactorum* | | | |
no.	200	20	200	20	200	20	200	20	200	20	1000	500
1a	3	0	3	0	3	0	3	0	4	2	1	0
1b	2	0	0	0	1	0	0	0	1	0	0	0
1c	3	0	3	1	2	0	1	0	2	0	1	0
1d	2	0	0	0	1	0	1	0	0	0	0	0
1e	3	2	3	1	4	1	4	2	4	2	1	1
1f	1	0	1	0	0	0	1	0	2	0	0	0
1h	3	0	2	0	3	1	3	0	3	0	2	0
1i	1	0	0	0	0	0	0	0	2	0	1	0
1k	3	1	2	0	2	0	3	1	3	0	1	0
1l	0	0	0	0	0	0	0	0	0	0	1	0
1n	2	0	1	0	1	0	0	0	3	0	1	0
2a	1	0	0	0	0	0	1	0	2	0	1	0
2b	4	1	4	0	4	1	4	0	4	1	2	0
2c	4	0	4	1	4	1	4	0	4	1	1	0
4a	2	0	0	0	0	0	0	0	2	0	1	0
4b	2	0	1	0	0	0	0	0	2	0	0	0
5a	1	0	0	0	0	0	0	0	0	0	0	0
5b	0	0	0	0	0	0	0	0	3	0	0	0
7	3	0	4	0	3	0	4	0	4	0	1	0
Sarfun	*b*	*b*	*b*	*b*	4	4	4	4	4	4	*c*	*c*
Sumilex	4	4	4	4	*b*	*b*	*b*	*b*	*b*	*b*	*c*	*c*

[a] The results are given in the four-degree scale determining the percent of mycelium growth inhibition compared with the control: 0 = 0–20%, 1 = 21–40%, 2 = 41–60%, 3 = 61–80%, 4 = 81–100%. [b] The activity was above the concentrations studied. [c] Test was not performed.

Sumitomo Chemical Co. Ltd., Japan) were used as the reference substances, tested under the same experimental conditions. The results are given in the four-degree scale, determined as the percentage of mycelium growth inhibition compared with the control (**Table 2**).

(B) The "in vivo" test was carried out on living plants with contribution of the tested fungus *Erysiphe graminis*. It was carried out under greenhouse conditions on healthy wheat seedlings. In the phase of well-developed first leaf, the plants were sprayed with solutions of the studied compound at 1000 and 500 μg/mL concentration, using 10 mL of solution for three repeated procedures (paraffin cups of 9 cm diameter). The first leaves of 10 plants were estimated after 7 and 14 days (depending on the intensity of plant infection with fungus in the studied combination). The results are given as the percentage of plant infection inhibition, using the same scale and criteria of estimation as in the "in vitro" test. The results are presented in **Table 2**.

Biological studies were done in the Department of Pesticides Application, IPO, Warsaw, with the SPR/FA$_2$/11 procedures (certificate GLP Compliance No. G 013)

Analytical Investigation. ^{1}H NMR spectra were recording using a Varian 400 MHz apparatus, standard TMS, solutions in DMSO-d_6, shift δ (ppm). The position of these signals, defining the interaction strength of induction systems, determines indirectly the ability of compounds to undergo tautomeric rearrangement, associated molecules formation, and change of combination lipophilicity.

The oscillation spectra were recorded with a Perkin-Elmer apparatus (in KBr) in the range of 600–4000 cm^{-1}.

The spectra MS (EI-70 eV) were recorded using the apparatus AMD-604. The spectra of compounds **1h**, **2a**, and **2c** were obtained by means of the LSIMA ionization method using the resolving power HR for M$^+$, M + H$^+$, and M + Na$^+$.

The purity of the compounds was checked by liquid chromatography (Knauer) with a dual pump, a 20 μL simple injection valve, and a UV–visible detector (320 nm). A Hypersil BDS C18 (5 μm, 150 × 4.6 mm) column was used as the stationary phase. The samples were prepared as solutions in methanol. The mobile phase consisted of different volume fractions of methanol in 10 mM acetate buffer at pH 4 as the aqueous phase. The flow rate was 1 mL/min at room temperature. The column dead time was determined by the injection of a small amount of acetone dissolved in water.

RESULTS AND DISCUSSION

The synthetic pathway for the compounds described is illustrated in **Scheme 1**. Sulfinyl-bis(2,4-dihydroxythiobenzoyl) was obtained according to the patent method (*15*). The structures and analytical data of the compounds are summarized in **Table 1**. They were in agreement with the proposed structures. The purity of the compounds was confirmed by HPLC in the reversed-phase system (RP-18, methanol–water). Log *k* values for the methanol–water (7:2 v/v) mobile phase are also presented in **Table 1**.

The signals of molecular ions (M$^+$) are not visible in the spectra of compounds **1a**, **1g**, **3a**, and **7**. Their bands come mainly from complex rearrangements; however, the kinds of fragmentation, like in the case of cluster ions (LSIMS), in the spectra of compounds **1h**, **2a**, and **2c** do not leave any doubts about the mass of the studied molecules. In most ^{1}H NMR spectra, proton lines for the heteroatoms are sharp singlets. Only in a few cases are protons weakly exchangeable and described by broad, low bands. The suspected tautomeric transformations are confirmed by the band systems in the low field, often presenting a number of protons different from that expected from the structure. However, identification of isomerization forms, even in the most readable *cis*-imido-thiol systems, is difficult. Similarly, in the IR spectra, the position of complex bands of coupled systems —C(SH)=N—N= and probable structures of =C=N$^+$—N— of heterocumulene cannot be explicitly ascribed.

The results of in vitro screening against five strains of phytopathogenic fungi (*Alternaria alternata*, *Botrytis cinerea*, *Rhizoctonia solani*, *Fusarium culmorum*, and *Phytohtora cactorum*) and against *Erysiphe graminis* under the in vivo conditions are given in **Table 2**. Sarfun 500SC (a.s. carbendazime) and Sumilex 500SC (s.a. procymidone) were used as reference systems. In the laboratory studies at the concentration of 200 μg/mL, compounds **1a**, **1e**, **1h**, **2b**, **2c**, and **7** revealed significant fungistatic action (at the level 61–100%) against all bioindicators. Compounds **2b** and **2c** showed a particularly

strong inhibitory action, inhibiting growth of five pathogens. Compounds **1c** and **1k** inhibited development of all pathogens but at a slightly lower level (61−80%). However, the fungistatic action of the substances **1b**, **1d**, **1f**, **1i**, **1n**, **2a**, **4**, and **5** was selective and quite differentiated. *Alternaria alternata* and *Phytohtora cactorum* seem to be particularly susceptible to these compounds. Compounds **1l** and **1g**, **1j**, **1m**, **3a**, **3b**, **3c**, and **6**, not given in **Table 2**, are characterized by the action at the level 0 on the applied scale.

With the compounds at 20 μg/mL concentration, the activity was insufficient. Only the compound **1e** inhibited growth of all pathogens, at the level 21−60%. Compounds **1k**, **2b**, and **2c** were characterized by weaker activity and only against some fungi. Selective action (single pathogens) was found for compounds **1a**, **1c**, and **1h**. For the other compounds at the concentration of 20 μg/mL under the in vitro conditions, no fungistatic activity was observed (**Table 2**). All compounds showed a lower level of activity in comparison with Sarfun 500SC and Sumilex 500SC.

None of the compounds was characterized by the desired action against the cause of grain powdery mildew, the fungus *Erysiphe graminis* in the greenhouse experiments. In some compounds at the concentration 1000 μg/mL, the growth inhibition was 1−40% (**Table 2**). At the concentration of 500 μg/mL, only compound **1e** retained this level of action. Compound **1e** proved to have the strongest fungistatic action in the group of studied compounds both in vitro and in vivo.

The most active compounds under the laboratory conditions generally possessed the strongest inhibitory property also under the greenhouse conditions. Only compound **1l**, which was inactive in vitro at the level of concentrations studied, was active under the greenhouse conditions.

Our preliminary results suggest some directions for further synthesis. Compounds **1a**, **1e**, and **1h**, modified thiobenzanilides, show higher fungistatic action level than the analogues described previously (*14*, *16*, *17*). The derivatives from the group of N[1]-substituted benzazoles (**2b**, **2c**) are also characterized by strong action. This points to specific functions of the heterocyclic ring in relation to the toxophorous moiety thiocarbonyl (*12*). The weakest fungistatic action is found for the compounds with a hydrazine −NH−NH− or hydrazone −NH−N= moiety. As in the case of other compounds, this may result from unfavorable scattering of the charge (electron density) in the linear arrangement of atoms (*13*). Differentiation of biological activity of the studied connections indicates that the planned studies of lipophilicity and electron potential parameters of the molecules' functional groups can verify further their synthesis and the mechanism of their activity.

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