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Novel *N*-cycloalkylcarbonyl-*N*′-aryl-thioureas: Synthesis, Design, Antifungal Activity and Gene Toxicity

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A synthesis method of novel *N*-cycloalkylcarbonyl-*N*′-aryl-thioureas was developed. It consists of sequential addition of equimolecular amounts of ammonium isothiocyanate and substituted anilines to cycloalkylcarbonyl chlorides. The identity and purity of products were confirmed by LC-MS spectra, their structure by elemental analysis, IR and ^{1}H NMR spectra. Preliminary antimicrobial screening for standard microorganisms and molecular docking allowed to select several structures for antifungal and genetic toxicity studies. Conducted *in vitro* screening of 9 compounds for antifungal potential against 11 phytopathogenic fungi and three *Phytophthora* strains revealed that two *N*-(arylcarbamothioyl)cyclopropane-carboxamides (**2.3**, **2.4**), at a concentration of 50 μg/ml exhibited activities comparable of the standard antifungal agent "Cyproconazole". Analysis of mutagenicity of novel thioureas using the *Salmonella* Reverse Mutagenicity assay ("Ames Test") showed a low gene-toxicity profile.

Keywords: *N*-cycloalkylcarbonyl-*N*′-aryl-thioureas; synthesis; spectral data; anti-phytopathogens; mutagenicity/gene-toxicity

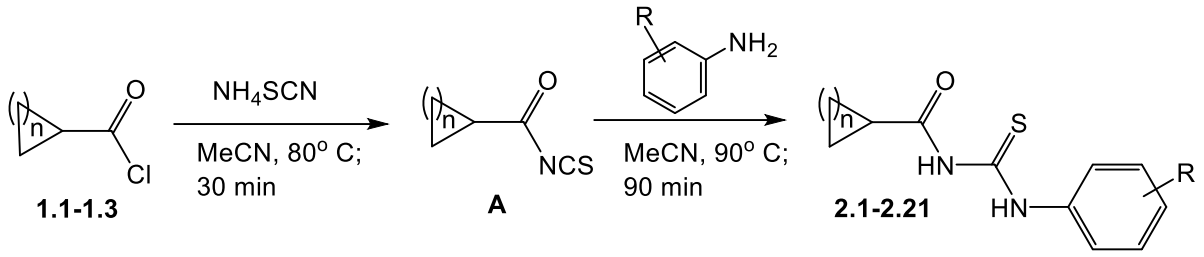
Introduction

Fungi form the largest group among the phytopathogenic microorganisms. Phytopathogenic fungi are capable of damaging wild plants by reducing their competitiveness or survival in natural ecosystems. At the same time they, also affect cultivated plant species, causing diminished yields and reduced qualities of crops, and sometimes bring along complete harvest failures. [1-6] In recent years, synthetic organic fungicides of local and systemic action have become increasingly important to protect plants, as they are more effective and less toxic than inorganic compounds. These agro-chemicals are made from different classes of organic compounds with various mechanisms of action: inhibitors of nucleic acid synthesis (acylalanine, isoxazoles), cellular respiration inhibitors (pyridine carboxamides, pyridine ethylbenzamides, methoxyacrylates, etc.), amino acid and protein synthesis inhibitors (anilino-pyrimidines), mitochondrial election transport inhibitors (qunazolines, phenylpyrroles, dicarboxamides), lipid synthesis inhibitors (carbamates), ergosterol synthesis inhibitors (azoles, morpholines), etc. [2-5] In contrast to the large number of available fungicides, most of them have drawbacks with, a narrow spectrum of activity, significant toxicity to animals, low environmental friendliness, and decreasing effectiveness against emerging resistant pathogens. [2-6] In addition, using the mixtures of fungicides with different mechanisms of action to overcome fungal resistance may add to the deterioration of the environmental situation. Therefore, the search for new agents against pathogenic fungi with improved characteristics, including a wide range of activity, high bioavailability, minimal toxicity and absence of side effects is an urgent task of agro- and medical chemistry.

Thiourea derivatives are one of the promising classes of compounds in the search for antifungal agents. Firstly, they are precursors of the synthesis of many acyclic and heterocyclic compounds, and secondly, they exhibit a wide range of biological activity. [7-20] Therefore, in continuatione of our previous work [21] on antifungal agents, here we present the synthesis of novel *N*-cycloalkylcarbonyl-*N*′-aryl-thioureas. This approach was developed on the basis of a combination of a cycloalkylcarbonyl thioureid moiety in a molecule with a more lipophilic aryl moiety containing substituents with various electronic effects (methyl, methoxy-, trifluoromethyl-, halogens), which can lead to an increased fungicidal activity against phytopathogenic fungi. In our opinion, the replacing of the aryl(hetaryl) amide moiety by a less polar aryl moiety and the variation the of cycloalkyl scaffold size in the previously described compounds[21] will increase the lipophilicity of the molecules. Therefore, it can lead to facilitated transport through biological membranes and to increased biological activity. [22, 23]

Results and Discussion

Novel acylthioureas (**2.1**-**2.21**) were obtained by the «one-pot» method, which consisted of the sequential addition (**1.1**-**1.3**) of equimolecular amounts of ammonium isothiocyanate and substituted anilines to the cycloalkylcarbonyl chlorides (*Scheme 1*). [21] There is no doubt that at the first stage of the reaction, intermediates **A**, namely the corresponding cycloalkylcarbonyl isothiocyanates were formed. These intermediates are highly reactive compounds and easily react with the arylamines by nucleophilic addition mechanism to form substituted acylthioureas (**2.1**-**2.21**). The reaction was carried out in acetonitrile at 80-90º C with constant stirring. The method is preparative and could be characterized by satisfactory yields (52-87%) and high purity of the final products.



1.1, 2.1-2.11 n = 1; **1.2, 2.12-2.14** n = 2; **1.3**, **2.15-2.21** n = 3; **2.1, 2.15** R = H; **2.2, 2.12, 2.16** R = 2-Me; **2.3, 2.17** R = 3-Me; **2.4, 2.13, 2.18** R = 4-Me, **2.5** R = 2-F; **2.6** R = 4-F; **2.7, 2.19** R = 2-Cl; **2.8** R = 3-Cl; **2.9, 2.20** R = 4-Cl; **2.10** R = 3-CF_3; **2.11, 2.14, 2.21** R = 2-MeO.

Scheme 1. Synthesis of target substituted *N*-cycloalkylcarbonyl-*N*′-aryl-thioureas

Analysis with LC-MS using positive-ion atmospheric pressure chemical ionization (APCI) showed the appropriate molecular ions, by which the expected molecular weights of compounds **2.1-2.21** were confirmed. The ^{1}H NMR spectra confirmed compounds **2** formation in which singlet signals of the protons of amide (-C(O)N*H*C(S)-) and thioamide (-C(S)N*H*Ar) groups were recorded at the 12.89-12.30 ppm and at the 11.75-11.02 ppm, respectively. The proton signals of the cyclopropane moiety (compounds **2.1-2.11**) were expressed as a multiplet or triplet of triplets, such H-1 was regestered at the 2.15-2.09 ppm (SCC 8.0-7.9 and 4.6-4.5 Hz) and H-2_{eq}, 3_{eq}, 2_{ax}, 3_{ax} were observed at the 1.17-0.56 ppm as the wide multiplet. Whereas, the proton signals of the cyclobutane moiety (**2.12-2.14**) resonated in the ^{1}H NMR spectrum in the form of sequentially arranged pentet H-1 (SCC 8.3-8.1 Hz), multiplets H-4_{eq}, 2_{eq}, 2_{ax}, 4_{ax} and H-3_{eq}, 3_{ax} at the 3.45-3.43 ppm and at the 2.40-2.09 ppm and at the 2.11-1.67 ppm, respectively. The proton signals of the cyclopentane cycle (**2.15-2.21**) in the ^{1}H NMR spectrum were recorded as a pentet or multiplet of H-1 and a wide multiplet of H-5_{eq}, 2_{eq}, 5_{ax}, 2_{ax}, 3_{eq}, 4_{eq}, 3_{ax}, 4_{ax} at the 3.12-2.78 ppm (SCC 8.0-7.9 Hz) and at the 2.01-1.35 ppm, respectively. The paramagnetic shift of the methine (H-1) and methylene protons in cyclobutane and cyclopentane compared with cyclopropane could be explained by the non-planar structure of the cycle and, as a consequence, by the perpendicular orientation of the ring structure with respect to the magnetic field direction. [24] This leads to a difference between chemical shifts of 1.4-1.2 ppm and specific cleavage of the methine (H-1) proton (triplet of triplets) signal of compounds **2.1-2.11**.

The signals of the phenyl or aryl fragments of compounds **2** could be easily interpreted and were presented in the ^{1}H NMR spectrum as A_2B_2C- (**2.1**, **2.15**), A_2B_2- (**2.4**, **2.6**, **2.9, 2.13, 2.18** and **2.20**), ABCD- (**2.2, 2.5, 2.7, 2.11, 2.12, 2.14, 2.16, 2.19, 2.21**) and ABC- (**2.3**, **2.8**, **2.10**, **2.17**) systems. The presence of fluorine atoms in the compounds (**2.5**, **2.6**) led to a change in the multiplicity of protons due to the additional splitting. [25] In addition, in the ^{1}H NMR spectra of compounds **2.2**-**2.4**, **2.11**-**2.14**, **2.16**-**2.18** and **2.21** proton signals of substituents in the aryl moiety were present. [25]

The characteristic bands of the stretching vibrations of the associated *NH* groups at the range of the 3777-3158 and 3255-3008 cm^{-1} were registered or observed in the IR spectra of compounds **2.1**-**2.21**. In addition, compounds **2.1**-**2.21** were characterized by fluctuations of ν_{CO}-groups (band "Amide I") at the 1693-1651 cm^{-1} and mixed stretching-deformational vibrations of bonds N-H and C-N ("Amide II") at the 1610-1513 cm^{-1}. They indicate the presence of thioamide and amide groups in the molecule. Compounds **2** have characteristic low-intensity vibrations of $\delta_{(CH)}$-bond of the aromatic ring at the 1232-1145 cm^{-1}, non-planar vibration $\gamma_{(CH)}$ at the 746-710 cm^{-1} and intense bands vibration at the 3031-2866 cm^{-1} along with the key stretching bands. The latter refer to symmetric and antisymmetric vibrations of the ν_{CH2}-groups and indicate the presence of cycloalkyl moieties in the molecule. [26]

Previously we evaluated disubstituted thioureas (**2**) with respect to their potential antibacterial activity towards standard test cultures of microorganisms as part of our work strategy. The results of the study show (*Table 1*) that acylthioureas (**2**) were inactive against *E. coli* (MIC = 100 μg/ml; MBC = 200 μg/ml). Compounds (**2.1**-**2.4**, **2.7**, **2.11**, **2.15**-**2.17**, **2.19**) exhibited activity against *S. aureus* (MIC 50 μg/ml) and compounds (**2.1**-**2.6**, **2.10**-**2.14**, **2.16**-**2.20**) against *Pseudomonas aeruginosa* (MIC 50 μg/ml). However, the minimal inhibitory concentration of compounds **2** was higher comparing to the reference drug - nitrofurazone. Whereas studies of antifungal activity against *C. albicans* showed (*Table 1*) that most disubstituted thioureas (**2**) exhibited antifungal activity with a minimal inhibition concentration (MIC) of 25-50 μg/ml. The highest antifungal activity was found for compound **2.4** (MIC 12.5 μg/ml; minimal bactericidal concentration (MBC) 50 μg/ml) and compounds **2.1**-**2.3**, **2.8**, **2.14, 2,15** (MIC 25 μg/ml; MFC 25-50 μg/ml).

Table 1. The antimicrobial and antifungal activity of disubstitued thiourea (**2**) derivatives.

Compound	Strains							
	Escherichia coli ATCC 25922		*Staphylococcus aureus* ATCC 25923		*Pseudomonas aeruginosa* ATCC 27853		*Candida albicans* ATTC (885-653)	
	MIC, μg/ml	MBC, μg/ml	MIC, μg/ml	MBC, μg/ml	MIC, μg/ml	MBC, μg/ml	MIC, μg/ml	MFC, μg/ml
2.1	100	200	50	100	50	100	25	50
2.2	100	200	50	100	50	100	25	50
2.3	100	200	50	100	50	100	25	25
2.4	100	200	50	50	50	100	12.5	50
2.5	100	200	100	200	50	50	100	100
2.6	100	200	100	200	50	100	100	200
2.7	100	200	50	100	100	200	50	50
2.8	100	200	100	200	100	200	25	50
2.9	100	200	100	200	200	>200	50	50
2.10	100	200	100	200	50	100	100	100
2.11	100	200	50	100	50	100	50	50
2.12	100	200	100	200	50	100	50	50
2.13	100	200	100	200	50	100	50	100
2.14	100	200	100	200	50	100	25	25
2.15	100	200	50	100	100	200	25	50
2.16	100	200	50	100	50	100	50	100
2.17	100	200	50	100	50	100	50	100
2.18	100	200	100	200	50	100	50	50
2.19	100	200	50	100	50	100	100	200
2.20	100	200	100	200	50	100	50	100
2.21	100	200	100	200	100	200	50	50
Nitrofurazone	1.5	–	6.25	–	6.25	–	–	–
Ketoconazole	–	–	–	–	–	–	25	50

In a next step molecular docking was used as a tool to predict the affinity of cyproconazole and disubstituted thioureas (**2**) to common antifungal targets (*Table 2*). Sterol 14α-demethylase (CYP51), N-myristoyltransferase (NMT) and secreted aspartic proteinase (SAP2) were choosen as biological targets, since they represent key cellular enzymes in lipid biogenesis, important cell membrane virulence factors and are indispensable for the growth and development for many eukaryotic organisms. [27]

It is shown that sterol 14α-demethylase (CYP51), N-myristoyltransferase (NMT) and secreted aspartic proteinase (SAP2) are possible target enzymes for compounds **2.1**-**2.21** with an average affinity of -6.95, -7.23 and -6.21 kcal/mol, respectively (*Table 2*). This finding agrees with previous studies of antifungal activity for thiosemicarbazides. [21]

Table 2. The calculated affinity of disubstituted thioureas (**2**) to binding sites of sterol 14α-demethylase (CYP51) 5TZ1, N-myristoyltransferase (NMT) 1IYL and secreted aspartic proteinase (SAP2) 1EAG.

Compound	Affinity (kcal/mol) to sterol 14α-demethylase (CYP51) (PDB Id: 5TZ1)	Affinity (kcal/mol) to N-myristoyltransferase (NMT) (PDB Id: 1IYL)	Affinity (kcal/mol) to secreted aspartic proteinase (SAP2) (PDB Id: 1EAG)
Cyproconazole	-6.8	-6.7	-6.0
2.1	-6.5	-6.6	-6.0
2.2	-6.9	-7.1	-6.3
2.3	-6.8	-7.0	-6.3
2.4	-7.6	-7.4	-6.9
2.5	-7.0	-7.4	-5.9
2.6	-7.0	-7.2	-6.5
2.7	-6.3	-7.0	-6.0
2.8	-6.6	-6.8	-6.1
2.9	-6.7	-6.8	-5.8
2.10	-6.9	-7.0	-6.7
2.11	-6.3	-6.3	-5.5
2.12	-6.8	-7.4	-6.7
2.13	-7.7	-7.3	-5.9
2.14	-6.6	-7.0	-5.9
2.15	-7.4	-8.0	-6.3
2.16	-7.6	-7.7	-7.0
2.17	-7.1	-7.8	-6.2
2.18	-7.2	-7.6	-6.7
2.19	-7.0	-7.9	-6.1
2.20	-6.8	-7.4	-6.1
2.21	-7.2	-6.6	-6.0
Mean affinity	-6.95	-7.23	-6.21

The viszualization of molecular docking results (Figure. 1, A) indicates that compound **2.4** with the highest activity against *C. albicans* forms significant interactions with the CYP51 enzyme. Firstly, it is the ionic bond between the nitrogen atom of the thioureid group of compound **2.4** with ASP B: 225 (2.22 Å) and the π-stacking of ASP B: 225 (4.37 Å) with the electron-excess phenyl moiety. Secondly, compound **2.4** is characterized by a significant number of π-alkyl bonds (hydrophobic interactions) of a cyclopropyl moiety with TYR B: 132 and ILE B: 197 (5.21 and 4.29 Å) as with methyl groups and toluene with HIS B: 310 (4.44 Å), ALA B: 313 (3.68 Å), VAL B: 509 (4.44 Å) and LEU B: 511 (4.30 Å). Whereas, analysis of molecular docking of the NMT enzyme with compound **2.4** (*Figure 1, B*) showed that in this case more interactions are predicted: hydrogen bonds between sulfur and TYR A: 225 (3.61Å), GLU A: 109 (3.70 Å), π-sulfur bond with PHE A: 117 (5.11 Å). Also cyclopropyl moiety interacts with PHE A: 339 (5.11 Å), PHE A: 115 (4.95 Å) and PHE A: 240 (4.82 Å), the toluene moiety has a π - π stacked bond between TYR A: 354 (5.13 Å) and an additional π-σ interaction of the methyl group with LEU A: 394 (4.53 Å). Compound **2.4** also fits into the active site of the SAP2 enzyme (*Figure 1, C*) due to the two hydrogen bonds between the NH groups of the thioureid moiety with THR A: 211 (3.28 Å) and GLY A: 220 (2.16 Å), as well as attractive interaction with ASP A: 86 (4.18 Å). Besides, three additional π-σ interactions of the cyclopropyl fragment of compound **2.4** with the SAP2 enzyme at the site of the following amino acids are observed: ILE A: 119 (4.05 Å), ILE A: 123 (5.46 Å) and TYR A: 84 (4.53 Å) and ASP A: 218 (3.37 Å) with the phenyl moiety.

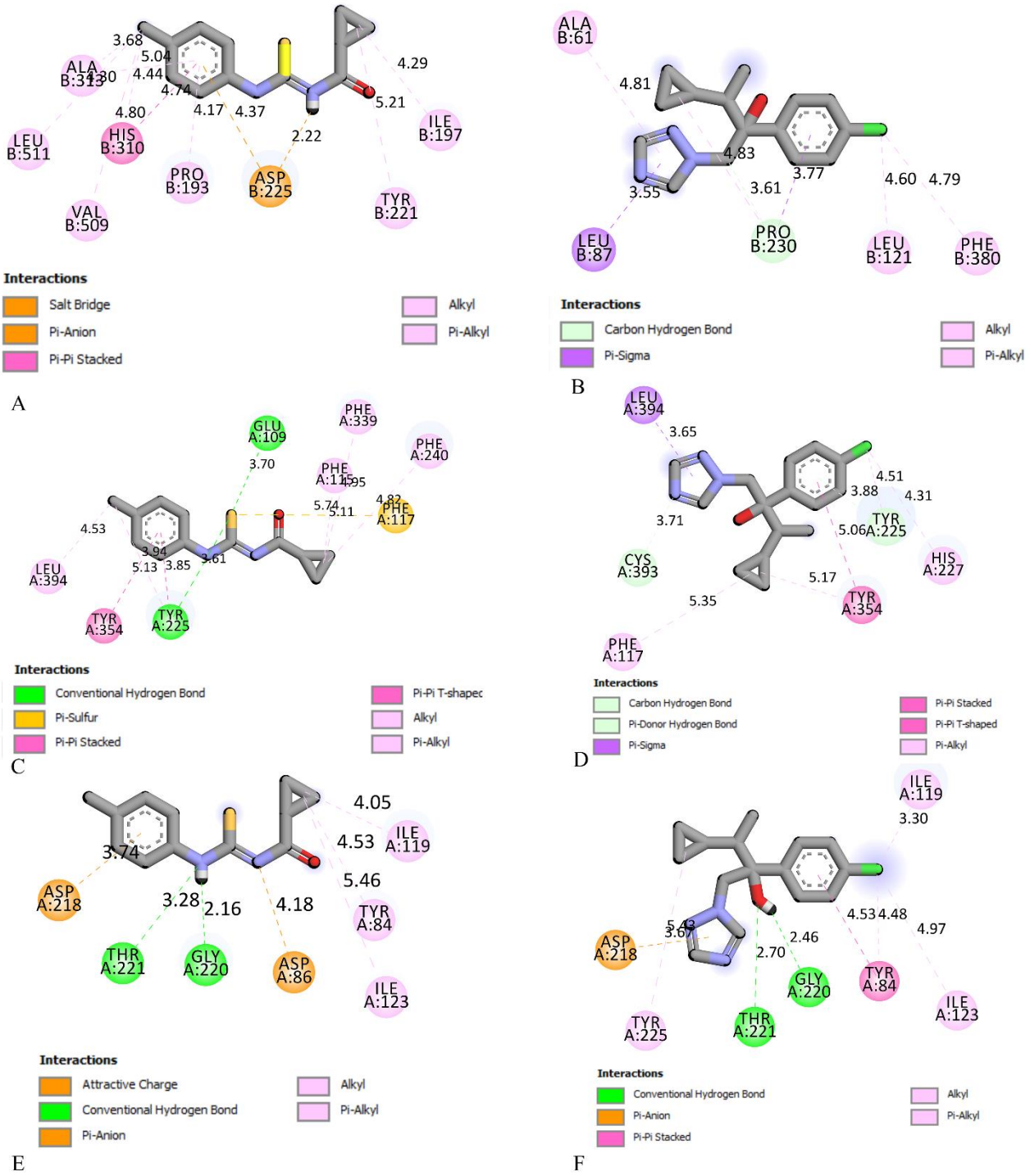


Figure 1. Visualization of the molecular docking results: A) compound **2.4** with CYP51; B) Cyproconazole with CYP51; C) compound **2.4** with NMT; D) Cyproconazole with NMT; E) compound **2.4** with SAP2; F) Cyproconazole with SAP2.

Therefore, the high affinity of compounds **2.1**-**2.21** to key enzymes and visualization of molecular docking of the most active compound **2.4** showed the prospect of further testing for activity against phytopathogenic fungi.

Antifungal activity. Tested disubstituted thioureas (**2.2-2.4**, **2.11-2.14**, **2.16-2.18**, **2.21**) at the concentration 50 μg/ml exhibited an antifungal activity comparable with Cyproconazole, which was used as a positive control (*Table 3*). Generally, the strains revealed different sensitivities towards the tested compounds. The most susceptible strains were *A. alternata, C. higgisianum* and *F. equiseti*, whereas *A. niger, P. infestans* GL-1 01/14 and *P. digitatum* exhibited a distinct resistance. It is obvious, that cyproconazole has the broadest activity profile. On the other hand, this standard antifungal was not active against *M. indicus* and *P. digitatum*, whereas a complete inhibition of these strains could be achieved with compound **2.3** or **2.4**, respectively.

Table 3. Growth inhibition rate (%) of thioureas (numbers refer to *Sheme 1* at the concentration - 50 µg/ml

Compound	AA	AN	BC	CH	FE	FF	FG	FO	P GI-1	P3	P4	MI	PD	VL
2.2	63.9	41.2	76.9	100.0	82.0	47.4	34.5	59.5	56.8	33.7	43.1	29.1	32.2	54.9
2.3	84.0	-0.1	93.3	68.7	99.6	73.9	72.7	28.8	23.8	58.8	68.2	10.1	80.1	82.4
2.4	95.9	42.0	42.4	60.7	100.0	78.7	81.6	58.8	44.2	58.8	69.0	94.5	100.7	100.0
2.11	66.7	-4.8	34.5	47.2	51.2	51.4	22.5	63.3	41.6	25.1	43.1	37.1	6.0	47.1
2.12	76.7	105.8	43.1	106.0	35.3	44.2	30.7	104.8	105.0	36.1	41.6	64.0	43.4	54.9
2.13	74.9	-2.4	9.4	100.0	98.9	45.0	39.0	34.5	29.0	47.1	56.5	49.5	22.5	70.6
2.14	71.2	16.3	18.0	70.2	14.1	34.5	13.5	61.4	49.5	24.3	24.3	16.0	3.0	39.2
2.16	70.3	-8.6	10.2	18.7	20.5	24.1	13.5	25.6	2.0	11.0	10.2	-5.1	0.0	35.3
2.17	91.3	-7.1	64.3	49.6	94.7	62.7	62.9	34.5	41.6	40.8	53.3	8.8	80.9	58.8
2.18	78.5	-2.4	29.8	34.5	67.1	34.5	28.5	39.0	7.9	29.0	35.3	61.1	13.5	51.0
2.21	12.8	19.4	15.7	48.8	2.8	13.7	9.0	62.6	45.5	11.8	13.3	-3.6	0.0	23.5
Cyp	100	100	100	100	100	100	85.4	80.5	79.9	81.6	100	-8.0	0.0	100

Tested strains were *A. alternata* (AA), *A. niger* (AN), *B. cinerea* (BC), *C. higginsianum* (CH), *F. equiseti* (FE), *F. fujikuroi* (FF), *F. graminearum* (FG), *F. oxysporum* (FO), *P. infestans* GL-1 01/14 wild strain (P GL-1), *P. infestans* p-3 (P3), *P. infestans* p-4 (P4), *M. indicus* (MI), *P. digitatum* (PD), *V. lecanii* (VL). (Cyp = Cyproconazole; number/name of acyl thiourea: see *Figure 1* and supplement).

In this study Cyproconazole and substances **2.4**, **2.3** and **2.12** appear to be the most versatile compounds. When activity is averaged over all tested organisms these compounds exhibit the highest antifungal potential (*Figure 2*).

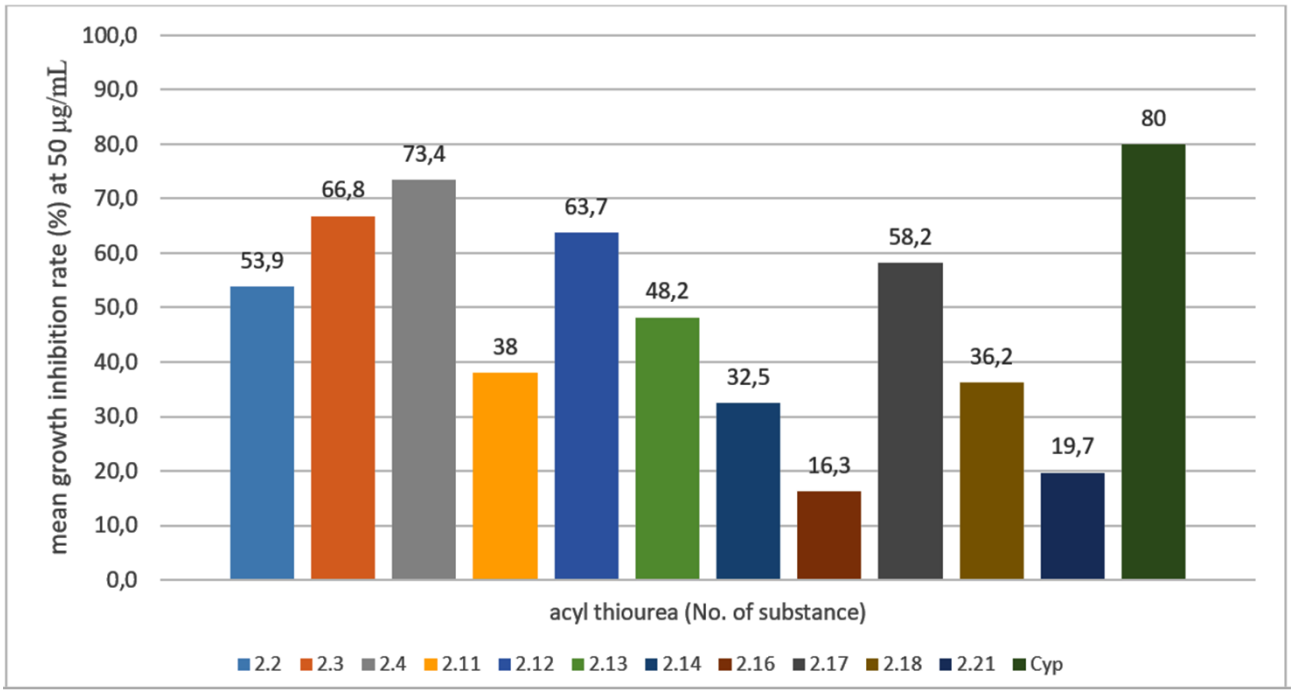


Figure 2. Mean growth inhibition rate (%) at the concentration 50 µg/ml Numbers refer to thioureas in *Scheme 1*; CYP; Cyproconazole.

In addition, compounds **2.4**, **2.12** and cyproconazole were also tested at a lower concentrations (25.0, 12.5, 6.25 µg/ml) against strains (AA, AN, CH, FE, FF, FO, P GI-1, PD) (*Table 4*). The mean growth inhibition activity of compound **2.4** and **2.12** at concentrations (25.0, 12.5, 6.25 µg/ml) was 11.6, 1.8, 3.5% and 4.3, 3.5 and 1.3%, respectively. Namely, these compounds are inferior to the activity of cyproconazole. However, the activity of compound **2.12** at the same concentrations was higher than compound **2.4** and cyproconazole had against *C. higginsianum* (CH) strain.

Table 4. Growth inhibition rate (%) of thioureas

Compound	Conc. µg/ml	AA	AN	CH	FE	FF	FO	P GI-1	PD	Mean growth inhibition rate (%)
2.4	25.0	34.2	-	-	4.2	7.9	-	-	0.0	11.6
	12.5	8.0	-	-	0.0	0.7	-	-	-1.4	1.8
	6.25	1.5	-	-	1.4	2.2	-	-	0.0	3.5
2.12	25.0	13.3	-9.8	10.1	-	-	2.3	5.5	-	4.3
	12.5	-4.4	-19.5	12.1	-	-	-1.5	-4.0	-	3.5
	6.25	-3.0	-17.7	32.3	-	-	-1.5	-9.5	-	1.3
Cyp	25.0	70.1	20.2	6.1	71.8	85.2	42.5	37.9	-0.7	41.6
	12.5	34.9	-2.7	14.1	30.0	66.4	35.5	20.6	-1.4	24.7
	6.25	28.8	-11.6	-2.0	29.3	65.0	19.3	26.1	0.7	19.5

Tested strains were *A. alternata* (AA), *A. niger* (AN), *C. higginsianum* (CH), *F. equiseti* (FE), *F. fujikuroi* (FF), *F. oxysporum* (FO), *P. infestans* GL-1 01/14 wild strain (P GL-1), *P. digitatum* (PD), (Cyp = Cyproconazole; number/name of acyl thiourea: see *Figure 1* and supplement).

Gene toxicity. The development of novel chemicals with the intention to use them on a broad scale in agriculture or industrial applications demands a thorough evaluation of toxicology and environmental risks. In a first attempt to characterize the potentially hazardous profile of novel disubstituted thioureas (**2.2**-**2.4**, **2.11**-**2.14**, **2.16**-**2.18**, **2.21**) they were tested for gene toxicity with the *Salmonella* reverse mutagenicity test (*Table 5*). A potential mutagenicity may be assumed, if mutagenicity index (Mi) is at **2** or higher. [6] Neither with *Salmonella TA 98* (indicator strain for frame shift mutations), nor with *Salmonella TA 100* (indicator strain for base substitution mutations) any of the tested disubstituted thioureas (**2.2**-**2.4**, **2.11**-**2.14**, **2.16**-**2.18**, **2.21**) showed a mutagenic potential. Also with application of metabolic activation by rat liver cell extract (S9-mix) an enhanced Mi was not detected.

Table 5. Mutagenicity (gene toxicity) of derivatives acyl thioureas (**2**).

Pos. controls/compound	TA 98		TA 100	
		metabolic activation (S9)		metabolic activation (S9)
MMS[(a)]	–	–	4.1	3.3
2-NF	52.8	17.0	–	–
2-AF	–	8.2	–	–
2.2	1.1	0.7	1.1	1.0
2.3	1.2	0.7	1.0	0.9
2.4	0.9	0.6	0.9	1.0
2.11	0.9	0.7	1.1	1.0
2.12	1.0	0.8	1.1	1.0
2.13	1.0	1.0	1.0	1.1
2.14	0.6	0.7	1.1	0.9
2.16	1.1	0.7	1.0	1.0
2.17	0.9	0.7	0.8	0.9
2.18	0.9	0.9	1.1	1.1
2.21	1.3	0.8	1.0	1.0

Data refer to Mutagenicity index (Mi, see text), Mi was determined in absence/presence of metabolic activation (S9) for positive controls (MMS, NF, 2-AF) and acyl thioureas (**2**) as described in Materials and Methods. [28]

[a]Controls were: MMS: methyl methane sulfonate; NF: nitrofluorene; 2-AF: 2-aminofluorene; (dosages were as described in Results and Discussion).

Conclusions

The methods of synthesis of novel *N*-cycloalkylcarbonyl-*N'*-aryl-thioureas and their physicochemical properties were discussed. Based on results from antimicrobial screening against standard microorganisms and molecular docking studies compounds were selected for further testing against phytopathogenic fungi. Three compounds with antifungal activity out of 11 novel acyl thiourea derivatives were identified. Antifungal activity of compound **2.12** against *C. higginsianum* (CH) strain in the concentration range of 50.0-6.25 μg/ml competes with cyproconazole. None of the test substances showed mutagenic potential. In addition to our previous work dedicated to disubstituted thiosemicarbazides [21], these results may serve as a basis to exspand the development of antifungals with an acyl thiourea core structure. In this context molecular docking studies and drug likeliness analysis may reveal a roadmap to further enhance the efficiency of this type of drugs. Also synergistic effects may be detected in antifungal test experiments using mixtures of already identified active thioureas.

Experimental Section

General Methods

Melting points were determined in open capillary tubes in a «Mettler Toledo MP 50» apparatus and were uncorrected. The elemental analyses (C, H, N, S) were performed using the ELEMENTAR vario EL cube analyzer (USA). Analyses were indicated by the symbols of the elements or functions within ±0.3% of the theoretical values. IR spectra (4000–600 cm^{-1}) were recorded on a Bruker ALPHA FT-IR spectrometer (Bruker Bioscience, Germany) using a module for measuring attenuated total reflection (ATR). ^{1}H NMR spectra (400 MHz) were recorded on a Varian-Mercury 400 spectrometer (Varian Inc., Palo Alto, CA, USA) with TMS as internal standard in DMSO-d_6 solution. LC-MS were recorded using chromatography/mass spectrometric system which consists of high performance liquid chromatography «Agilent 1100 Series» (Agilent, Palo Alto, CA, USA) equipped with diode-matrix and mass-selective detector «Agilent LC/MSD SL» (atmospheric pressure chemical ionization – APCI). Electron impact mass spectra (EI-MS) were recorded on a Varian 1200 L instrument at 70 eV (Varian, USA).

Cycloalkylcarbonyl chlorides (**1.1-1.3**) were synthesized by known method. [29] Other starting materials and solvents were obtained from commercially available sources and used without additional purification.

*The general method of the N-(R-phenylcarbamothioyl) cycloalkylcarboxamides (**2.1-2.21**) synthesis.*

To a solution of corresponding 0.01 mol cycloalkylcarbonyl chlorides (**1.1-1.3**) in 20 mL of acetonitrile 0.76 g (0.01 mol) of ammonium isothiocyanate was added and stirred at 80ºC for 30 min. The mixture was cooled down to r.t. and 0.01 mol of corresponding aniline was added and stirred at 80ºC for 90 min. The solution was cooled down, poured into the water. The formed precipitate was filtrated, dried and recrystallized from methanol.

N-(Phenylcarbamothioyl)cyclopropanecarboxamide (2.1). Yield: 75.0%; Mp.: 143-146°C; IR (cm^{-1}): 3725 (ν_{NH}), 3176 (ν_{NH}), 3008 (ν_{asCH2}), 1682 (ν_{CO}), 1519 (δ_{NH}), 1145 (δ_{CH}), 722 (γ_{CH}); ^{1}H NMR (400 MHz, DMSO-d_6), δ: 12.71 (s, 1H, -C(O)N<u>*H*</u>C(S)-), 11.61 (s, 1H, -C(S)N<u>*H*</u>Ar), 7.63 (d, *J* = 7.9 Hz, 2H, Ph H-2,6), 7.34 (t, *J* = 7.7 Hz, 2H, Ph H-3,5), 7.19 (t, *J* = 7.4 Hz, 1H, Ph H-4), 2.13 (tt, J = 7.9, 4.6 Hz, 1H, cyclopropyl H-1), 0.99-0.92 (m, 4H, cyclopropyl H-2_{eq}, 3_{eq}, 2_{ax}, 3_{ax}); LC-MS, *m/z* = 221 [M+1]; Anal. Calcd. for $C_{11}H_{12}N_2OS$: C, 59.98; H, 5.49; N, 12.72; S, 14.55; Found: C, 60.02; H, 5.56; N, 12.80; S, 14.59.

N-(o-Tolylcarbamothioyl)cyclopropanecarboxamide (2.2). Yield: 62.3%; Mp.: 141-143°C; IR (cm^{-1}): 3737 (ν_{NH}), 3128 (ν_{NH}), 3011 (ν_{asCH2}), 1681 (ν_{CO}), 1531 (δ_{NH}), 1163 (δ_{CH}), 733 (γ_{CH}); ^{1}H NMR (400 MHz, DMSO-d_6), δ: 12.30 (s, 1H, -C(O)N<u>*H*</u>C(S)-), 11.65 (s, 1H, -C(S)N<u>*H*</u>Ar), 7.65 (d, *J* = 7.5 Hz, 1H, Ar H-6), 7.27-7.07 (m, 3H, Ar H-3, 4, 5), 2.26 (s, 3H, -C<u>*H*</u>$_3$), 2.15 (tt, *J* = 7.9, 4.5 Hz, 1H, cyclopropyl H-1), 1.06-0.72 (m, 4H, cyclopropyl H-2_{eq}, 3_{eq}, 2_{ax}, 3_{ax}); LC-MS, *m/z* = 235 [M+1]; Anal. Calcd. for $C_{12}H_{14}N_2OS$: C, 61.51; H, 6.02; N, 11.96; S, 13.68; Found: C, 61.57; H, 6.12; N, 12.06; S, 13.75.

N-(m-Tolylcarbamothioyl)cyclopropanecarboxamide (2.3). Yield: 60.0%; Mp.: 118-119°C; IR (cm^{-1}): 3717 (ν_{NH}), 3175 (ν_{NH}), 3012 (ν_{asCH2}), 1681 (ν_{CO}), 1536 (δ_{NH}), 1149 (δ_{CH}), 724 (γ_{CH}); ^{1}H NMR (400 MHz, DMSO-d_6) δ: 12.66 (s, 1H, -C(O)N<u>*H*</u>C(S)-), 11.58 (s, 1H, -C(S)N<u>*H*</u>Ar), 7.45 (d, *J* = 8.0 Hz, 1H, Ar H-6), 7.41 (s, 1H, Ar H-2), 7.22 (t, *J* = 7.8 Hz, 1H, Ar H-4), 7.00 (d, *J* = 7.4 Hz, 1H, Ar H-5), 2.36 (s, 3H, -C<u>*H*</u>$_3$), 2.13 (tt, *J* = 8.0, 4.6 Hz, 1H,

cyclopropyl H-1), 1.02-0.87 (m, 4H, cyclopropyl H-2_{eq}, 3_{eq}, 2_{ax}, 3_{ax}); LC-MS, *m/z* = 235 [M+1]; Anal. Calcd. for $C_{12}H_{14}N_2OS$: C, 61.51; H, 6.02; N, 11.96; S, 13.68; Found: C, 61.64; H, 6.09; N, 12.04; S, 13.75.

N-(p-Tolylcarbamothioyl)cyclopropanecarboxamide (2.4). Yield: 70.0%; Mp.: 151-154°C; IR (cm^{-1}): 3714 (ν_{NH}), 3185 (ν_{NH}), 2914 (ν_{asCH2}), 1687 (ν_{CO}), 1530 (δ_{NH}), 1389 (δ_{sCH3}), 1155 (δ_{CH}), 814 (ν_{C-S}), 719 (γ_{CH}); ^{1}H NMR (400 MHz, DMSO-d_6) δ: 12.61 (s, 1H, -C(O)N*H*C(S)-), 11.56 (s, 1H, C(S)N*H*Ar), 7.48 (d, *J* = 8.1 Hz, 2H, Ar H-2,6), 7.14 (d, *J* = 8.1 Hz, 2H, Ar H-3,5), 2.35 (s, 3H, CH_3), 2.13 (tt, *J* = 8.0, 4.5 Hz, 1H, cyclopropyl H-1), 1.07-0.74 (m, 4H, cyclopropyl H-2_{eq}, 3_{eq}, 2_{ax}, 3_{ax}); LC-MS, *m/z* = 235 [M+1]; Anal. Calcd. for $C_{12}H_{14}N_2OS$: C, 61.51; H, 6.02; N, 11.96; S, 13.68; Found: C, 61.47; H, 5.99; N, 11.93, S, 13.58.

N-((2-Fluorophenyl)carbamothioyl)cyclopropanecarboxamide (2.5). Yield: 59.0%; Mp.: 109-113°C; IR (cm^{-1}): 3743 (ν_{NH}), 3196 (ν_{NH}), 3013 (ν_{asCH2}), 1693 (ν_{CO}), 1536 (δ_{NH}), 1452 (ν_{CH}), 1161 (δ_{CH}), 1102(ν_{CF}), 1030, 934, 813 (ν_{C-S}), 710 (γ_{CH}); ^{1}H NMR (400 MHz, DMSO-d_6) δ: 12.68 (s, 1H, -C(O)N*H*C(S)-), 11.74 (s, 1H, -C(S)N*H*Ar), 8.27 (t, *J* = 7.9 Hz, 1H, Ar H-6), 7.26-7.05 (m, 3H, Ar H-3,4,5), 2.10 (tt, *J* = 7.9, 4.6 Hz, 1H, cyclopropyl H-1), 1.07-0.80 (m, 4H, cyclopropyl H-2_{eq}, 3_{eq}, 2_{ax}, 3_{ax}); LC-MS, *m/z* = 239 [M+1]; Anal. Calcd. for $C_{11}H_{11}FN_2OS$: C, 55.45; H, 4.65; N, 11.76; S, 13.45; Found: C, 55.54; H, 4.72; N, 11.83; S, 13.50.

N-((4-Fluorophenyl)carbamothioyl)cyclopropanecarboxamide (2.6). Yield: 58.0%; Mp.: 150-155°C; IR (cm^{-1}): 3747 (ν_{NH}), 3196 (ν_{NH}), 2919 (ν_{asCH2}), 1682 (ν_{CO}), 1610 (δ_{NH}), 1224 (δ_{CH}), 1146 (ν_{CF}), 811 (ν_{C-S}), 719 (γ_{CH}); ^{1}H NMR (400 MHz, DMSO-d_6) δ: 12.57 (s, 1H, -C(O)N*H*C(S)-), 11.62 (s, 1H, -C(S)N*H*Ar), 7.57 (dd, *J* = 8.7, 4.9 Hz, 2H, Ar H-2,6), 7.05 (t, *J* = 8.6 Hz, 2H, Ar H-3,5), 2.09 (tt, *J* = 7.9, 4.6 Hz, 1H, cyclopropyl H-1), 1.04-0.68 (m, 4H, cyclopropyl H-2_{eq}, 3_{eq}, 2_{ax}, 3_{ax}); LC-MS, *m/z* = 238 [M+1]; Anal. Calcd. for $C_{11}H_{11}FN_2OS$: C, 55.45; H, 4.65; N, 11.76; S, 13.45; Found: C, 55.41; H, 4.59; N, 11.73; S, 13.41.

N-((2-Chlorophenyl)carbamothioyl)cyclopropanecarboxamide (2.7). Yield: 77.2%; Mp.: 160-161°C; IR (cm^{-1}): 3233 (ν_{NH}), 3144 (ν_{NH}), 3031 (ν_{asCH2}), 1659 (ν_{CO}), 1515 (δ_{NH}), 1176 (δ_{CH}), 742 (ν_{CCl}); ^{1}H NMR (400 MHz, DMSO-d_6) δ: 12.71 (s, 1H, -C(O)N*H*C(S)-), 11.74 (s, 1H, -C(S)N*H*Ar), 8.22 (d, *J* = 7.8 Hz, 1H, Ar H-6), 7.41 (d, *J* = 7.9 Hz, 1H, Ar H-3), 7.28 (t, *J* = 7.4 Hz, 1H, Ar H-5), 7.18 (t, *J* = 7.1 Hz, 1H, Ar H-4), 2.11 (tt, *J* = 7.9, 4.5 Hz, 1H, cyclopropyl H-1), 1.17 – 0.56 (m, 4H, cyclopropyl); H-2_{eq}, 3_{eq}, 2_{ax}, 3_{ax}); LC-MS, *m/z* = 255 [M+1]; Anal. Calcd. for $C_{11}H_{11}ClN_2OS$: C, 51.87; H, 4.35; N, 11.00; S, 12.59; Found: C, 51.82; H, 4.42; N, 10.97; S, 12.52.

N-((3-Chlorophenyl)carbamothioyl)cyclopropanecarboxamide (2.8). Yield: 54.5%; Mp.: 123-125°C; IR (cm^{-1}): 3744 (ν_{NH}), 3169 (ν_{NH}), 3031 (ν_{asCH2}), 1688 (ν_{CO}), 1532 (δ_{NH}), 1162 (δ_{CH}), 736 (ν_{CCl}), 694; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 12.75 (s, 1H, -C(O)N*H*C(S)-), 11.69 (s, 1H, -C(S)N*H*Ar), 7.84 (s, 1H, Ar H-2), 7.44 (d, *J* = 8.1 Hz, 1H, Ar H-6), 7.30 (t, *J* = 8.0 Hz, 1H, Ar H-5), 7.16 (d, *J* = 7.9 Hz, 1H, Ar H-4), 2.09 (tt, *J* = 7.9, 4.6 Hz, 1H, cyclopropyl H-1), 1.04-0.73 (m, 4H, cyclopropyl H-2_{eq}, 3_{eq}, 2_{ax}, 3_{ax}); LC-MS, *m/z* = 255 [M+1]; Anal. Calcd. for $C_{11}H_{11}ClN_2OS$: C, 51.87; H, 4.35; N, 11.00; S, 12.59; Found: C, 51.90; H, 4.39; N, 11.02; S, 12.61.

N-((4-Chlorophenyl)carbamothioyl)cyclopropanecarboxamide (2.9). Yield: 76.1%; Mp.: 176-180°C; IR (cm^{-1}): 3745 (ν_{NH}), 3152 (ν_{NH}), 3013 (ν_{asCH2}), 1680 (ν_{CO}), 1514 (δ_{NH}), 1152 (δ_{CH}), 822 (ν_{C-S}), 712 (ν_{CCl}), 676; ^{1}H NMR (400 MHz, DMSO-d_6), δ: ^{1}H NMR (400 MHz, DMSO-d_6) δ: 12.72 (s, 1H, -C(O)N*H*C(S)-), 11.68 (s, 1H, -C(S)N*H*Ar), 7.65 (d, *J* = 8.7 Hz, 2H, Ar H-2, 6), 7.33 (d, *J* = 8.7 Hz, 2H, Ar H-3, 5), 2.12 (tt, *J* = 7.8, 4.6 Hz, 1H, cyclopropyl H-1), 1.03-0.82 (m, 4H, cyclopropyl H-2_{eq}, 3_{eq}, 2_{ax}, 3_{ax}); LC-MS, *m/z* = 255 [M+1]; Anal. Calcd. for $C_{11}H_{11}ClN_2OS$: C, 51.87; H, 4.35; N, 11.00; S, 12.59; Found: C, 51.93; H, 4.42; N, 11.08; S, 12.63.

N-((3-(Trifluoromethyl)phenyl)carbamothioyl)cyclopropanecarboxamide (2.10). Yield: 76.3%; Mp.: 109-113°C; IR (cm^{-1}): 3713 (ν_{NH}), 3160 (ν_{NH}), 3015 (ν_{asCH2}), 1677 (ν_{CO}), 1518 (δ_{NH}), 1232 (δ_{CH}), 1161(ν_{CF}), 1120 (ν_{CF}), 1066 (ν_{CF}), 885 (ν_{C-S}), 738 (γ_{CH}), 689; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 12.83 (s, 1H, -C(O)N*H*C(S)-), 11.75 (s, 1H, -C(S)N*H*Ar), 8.09 (s, 1H, Ar H-2), 7.78 (d, *J* = 7.8 Hz, 1H, Ar H-6), 7.52 (t, *J* = 7.9 Hz, 1H, Ar H-5), 7.45 (d, *J* = 7.7 Hz, 1H, Ar H-4), 2.10 (tt, *J* = 8.4, 4.6 Hz, 1H, cyclopropyl H-1), 1.02-0.83 (m, 4H, cyclopropyl H-2_{eq}, 3_{eq}, 2_{ax}, 3_{ax}); Anal. Calcd. for $C_{12}H_{11}F_3N_2OS$: C, 50.00; H, 3.85; N, 9.72; S, 11.12; Found: C, 50.09; H, 3.91; N, 9.77; S, 11.19.

N-((2-Methoxyphenyl)carbamothioyl)cyclopropanecarboxamide (2.11). Yield: 80.0%; Mp.: 186-190°C; IR (cm^{-1}): 3744 (ν_{NH}), 3255 (ν_{NH}), 3014 (ν_{asCH2}), 1666 (ν_{CO}), 1530 (δ_{NH}), 1397 (δ_{sCH3}), 1168 (δ_{CH}), 745 (γ_{CH}), 678; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 12.82 (s, 1H, -C(O)N*H*C(S)-), 11.48 (s, 1H, -C(S)N*H*Ar), 8.63 (d, *J* = 7.9 Hz, 1H, Ar H-6), 7.13 (t, *J* = 7.6 Hz, 1H, Ar H-4), 6.98 (d, *J* = 8.1 Hz, 1H, Ar H-3), 6.92 (t, *J* = 7.7 Hz, 1H, Ar H-5), 3.89 (s, 3H, -OC*H*$_3$), 2.13 (tt, *J* = 7.9, 4.5 Hz, 1H, cyclopropyl H-1), 1.01-0.87 (m, 4H, cyclopropyl H-2_{eq}, 3_{eq}, 2_{ax}, 3_{ax}); LC-MS, m/z = 251 [M+1]; Anal. Calcd. for $C_{12}H_{14}N_2O_2S$: C, 57.58; H, 5.64; N, 11.19; S, 12.81; Found: C, 57.64; H, 5.71; N, 11.24; S, 12.87.

N-(o-Tolylcarbamothioyl)cyclobutanecarboxamide (2.12). Yield: 69.4%; Mp.: 156-160°C; IR (cm^{-1}): 3777 (ν_{NH}), 3160 (ν_{NH}), 2939 (ν_{asCH2}), 1687 (ν_{CO}), 1516 (δ_{NH}), 1155 (δ_{CH}), 725 (γ_{CH}); ^{1}H NMR (400 MHz, DMSO-d_6) δ: 12.35 (s, 1H, -C(O)N*H*C(S)-), 11.20 (s, 1H, -C(S)N*H*Ar), 7.66 (d, *J* = 7.5 Hz, 1H, Ar H-6), 7.25-7.11 (m, 3H, Ar H-3, 4, 5), 3.45 (p, *J* = 8.1 Hz, 1H, cyclobutyl H-1), 2.33-2.10 (m, 7H, -CH_3, cyclobutyl H-4_{eq}, 2_{eq}, 2_{ax}, 4_{ax},), 2.08-

1.80 (m, 2H, cyclobutyl H-3_{eq}, 3_{ax}); LC-MS, *m/z* = 249 [M+1]; Anal. Calcd. for $C_{13}H_{16}N_2OS$: C, 62.87; H, 6.49; N, 11.28; S, 12.91; Found: C, 62.93; H, 6.54; N, 11.33; S, 12.97.

N-(p-Tolylcarbamothioyl)cyclobutanecarboxamide (2.13). Yield: 87.5%; Mp.: 126-129°C; IR (cm^{-1}): 3771 (ν_{NH}), 3151 (ν_{NH}), 2941 (ν_{asCH2}), 1680 (ν_{CO}), 1513 (δ_{NH}), 1159 (δ_{CH}), 695; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 12.66 (s, 1H, -C(O)N*H*C(S)), 11.11 (s, 1H, -C(S)N*H*Ar), 7.51 (d, *J* = 8.0 Hz, 2H, Ar H-2,6), 7.15 (d, *J* = 8.0 Hz, 2H, Ar H-3,5), 3.43 (p, *J* = 8.1 Hz, 1H, cyclobutyl H-1), 2.35 (s, 3H, -C*H*$_3$), 2.33-2.30 (m, 4H, cyclobutyl H-4_{eq}, 2_{eq}, 2_{ax}, 4_{ax}), 2.11-1.83 (m, 2H, cyclobutyl H-3_{eq}, 3_{ax}); Anal. Calcd. for $C_{13}H_{16}N_2OS$: C, 62.87; H, 6.49; N, 11.28; S, 12.91; Found: C, 62.81; H, 6.43; N, 11.22; S, 12.87.

N-((2-Methoxyphenyl)carbamothioyl)cyclobutanecarboxamide (2.14). Yield: 52.0%; Mp.: 153-156°C; IR (cm^{-1}): 3742 (ν_{NH}), 3195 (ν_{NH}), 3031 (ν_{asCH2}), 1692 (ν_{CO}), 1520 (δ_{NH}), 1445 (ν_{CH}), 1359 (δ_{sCH3}), 1145 (δ_{CH}), 848 (ν_{C-S}), 746 (γ_{CH}); ^{1}H NMR (400 MHz, DMSO-d_6) δ: 12.89 (s, 1H, -C(O)N*H*C(S)-), 11.02 (s, 1H, -C(S)N*H*Ar), 8.64 (d, *J* = 7.7 Hz, 1H, Ar H-6), 7.14 (t, *J* = 7.6 Hz, 1H, Ar H-4), 6.99 (d, *J* = 8.0 Hz, 1H, Ar H-3), 6.92 (t, *J* = 7.7 Hz, 1H, Ar H-5), 3.93 (s, 3H, -OC*H*$_3$), 3.43 (p, *J* = 8.3 Hz, 1H, cyclobutyl H-1), 2.40-2.09 (m, 4H, cyclobutyl H-4_{eq}, 2_{eq}, 2_{ax}, 4_{ax}), 2.08-1.67 (m, 2H, cyclobutyl H-3_{eq}, 3_{ax}); LC-MS, *m/z* = 265 [M+1]; Anal. Calcd. for $C_{13}H_{16}N_2O_2S$: C, 59.07; H, 6.10; N, 10.60; S, 12.13; Found: C, 59.16; H, 6.13; N, 10.68; S, 12.18.

N-(Phenylcarbamothioyl)cyclopentanecarboxamide (2.15). Yield: 75.7%; Mp.: 86-89°C; IR (cm^{-1}): 3737 (ν_{NH}), 3163 (ν_{NH}), 2951 (ν_{asCH2}), 1686 (ν_{CO}), 1518 (δ_{NH}), 1149 (δ_{CH}), 685; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 12.73 (s, 1H, -C(O)N*H*C(S)-), 11.32 (s, 1H, -C(S)N*H*Ar), 7.65 (d, *J* = 7.5 Hz, 2H, Ar H-2,6), 7.36 (t, *J* = 7.5 Hz, 2H, Ar H-3,5), 7.21 (t, *J* = 5.8 Hz, 1H, Ar H-4), 3.06-2.89 (m, 1H, cyclopentyl H-1), 1.96-1.52 (m, 8H, cyclopentyl H-5_{eq}, 2_{eq}, 5_{ax}, 2_{ax}, 3_{eq}, 4_{eq}, 3_{ax}, 4_{ax}); Anal. Calcd. for $C_{13}H_{16}N_2OS$: C, 62.87; H, 6.49; N, 11.28; S, 12.91; Found: C, 62.95; H, 6.53; N, 11.33; S, 12.97.

N-(o-Tolylcarbamothioyl)cyclopentanecarboxamide (2.16). Yield: 62.0%; Mp.: 153-155°C; IR (cm^{-1}): 3713 (ν_{NH}), 3160 (ν_{NH}), 2950 (ν_{asCH2}), 1688 (ν_{CO}), 1517 (δ_{NH}), 1160 (δ_{CH}), 689; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.34 (s, 1H, -C(O)N*H*C(S)-), 11.32 (s, 1H, -C(S)N*H*Ar), 7.67 (d, *J* = 7.5 Hz, 1H, Ar H-6), 7.26-7.10 (m, 3H, Ar H-3, 4, 5), 3.01 (p, *J* = 7.9 Hz, 1H, cyclopentyl H-1), 2.28 (s, 3H, -C*H*$_3$), 1.92-1.70 (m, 6H, cyclopentyl H-5_{eq}, 2_{eq}, 5_{ax}, 2_{ax}, 3_{eq}, 4_{eq}), 1.68-1.53 (m, 2H, cyclopentyl H-3_{ax}, 4_{ax}); LC-MS, *m/z* = 263 [M+1]; Anal. Calcd. for $C_{14}H_{18}N_2OS$: C, 64.09; H, 6.92; N, 10.68; S, 12.22; Found: C, 64.14; H, 6.98; N, 10.74; S, 12.29.

N-(m-Tolylcarbamothioyl)cyclopentanecarboxamide (2.17). Yield: 61.0%; Mp.: 86-100°C; IR (cm^{-1}): 3158 (ν_{NH}), 3031 (ν_{NH}), 2915 (ν_{asCH2}), 1683 (ν_{CO}), 1531 (δ_{NH}), 1136 (δ_{CH}), 691; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.70 (s, 1H, -C(O)N*H*C(S)-), 11.24 (s, 1H, -C(S)N*H*Ar), 7.47 (d, *J* = 8.0 Hz, 1H, Ar H-6), 7.43 (s, 1H, Ar H-2), 7.22 (t, *J* = 7.8 Hz, 1H, Ar H-5), 7.00 (d, *J* = 7.4 Hz, 1H, Ar H-3), 3.12-2.91 (m, 1H, cyclopentyl H-1), 2.37 (s, 3H, -C*H*$_3$), 1.98-1.83 (m, 2H, cyclopentyl H-5_{eq}, 2_{eq}); 1.83-1.67 (m, 4H cyclopentyl H-5_{ax}, 2_{ax}, 3_{eq}, 4_{eq}), 1.67-1.49 (m, 2H, cyclopentyl H-3_{ax}, 4_{ax}); LC-MS, m/z = 263 [M+1]; Anal. Calcd. for $C_{14}H_{18}N_2OS$: C, 64.09; H, 6.92; N, 10.68; S, 12.22; Found: C, 64.02; H, 6.88; N, 10.64; S, 12.18.

N-(p-Tolylcarbamothioyl)cyclopentanecarboxamide (2.18). Yield: 65.5%; Mp.: 91-96°C; IR (cm^{-1}): 3756 (ν_{NH}), 3161 (ν_{NH}), 2946 (ν_{asCH2}), 1651 (ν_{CO}), 1513 (δ_{NH}), 1161 (δ_{CH}), 818 (ν_{C-S}), 730 (γ_{CH}); ^{1}H NMR (400 MHz, DMSO-d_6) δ: 12.65 (s, 1H, -C(O)NHC(S)-), 11.23 (s, 1H, -C(S)N*H*Ar), 7.51 (d, *J* = 8.1 Hz, 2H, Ar H-2,6), 7.15 (d, *J* = 8.1 Hz, 2H, Ar H-3,5), 3.06-2.93 (m, 1H, cyclopentyl H-1), 2.35 (s, 3H, -C*H*$_3$), 1.96-1.53 (m, 8H, cyclopentyl H-5_{eq}, 2_{eq}, 5_{ax}, 2_{ax}, 3_{eq}, 4_{eq}, 3_{ax}, 4_{ax}); LC-MS, *m/z* = 263 [M+1]; Anal. Calcd. for $C_{14}H_{18}N_2OS$: C, 64.09; H, 6.92; N, 10.68; S, 12.22; Found: C, 64.16; H, 6.99; N, 10.77; S, 12.31.

N-((2-Chlorophenyl)carbamothioyl)cyclopentanecarboxamide (2.19). Yield: 67.8%; Mp.: 113-115°C; IR (cm^{-1}): 3189 (ν_{NH}), 2866 (ν_{asCH2}), 1688 (ν_{CO}), 1529 (δ_{NH}), 1154 (δ_{CH}), 728 (ν_{CCl}); ^{1}H NMR (400 MHz, DMSO-d_6) δ: 12.76 (s, 1H, -C(O)N*H*C(S)-), 11.41 (s, 1H, -C(S)N*H*Ar), 8.25 (d, *J* = 8.1 Hz, 1H, Ar H-6), 7.42 (d, *J* = 7.9 Hz, 1H, Ar H-3), 7.28 (t, *J* = 7.7 Hz, 1H, Ar H-5), 7.18 (t, *J* = 7.1 Hz, 1H, Ar H-4), 2.98 (p, *J* = 8.0 Hz, 1H, cyclopentyl H-1), 1.96-1.35 (m, 8H, cyclopentyl H-5_{eq}, 2_{eq}, 5_{ax}, 2_{ax}, 3_{eq}, 4_{eq}, 3_{ax}, 4_{ax}); LC-MS, *m/z* = 283 [M+1]; Anal. Calcd. for $C_{13}H_{15}ClN_2OS$: C, 55.22; H, 5.35; N, 9.91; S, 11.34; Found: C, 55.28; H, 5.41; N, 9.97; S, 11.38.

N-((4-Chlorophenyl)carbamothioyl)cyclopentanecarboxamide (2.20). Yield: 74.8%; Mp.: 123-131°C; IR (cm^{-1}): 3182 (ν_{NH}), 2959 (ν_{asCH2}), 1676 (ν_{CO}), 1514 (δ_{NH}), 1159 (δ_{CH}), 823 (ν_{C-S}), 719 (ν_{CCl}); ^{1}H NMR (400 MHz, DMSO-d_6) δ: 12.72 (s, 1H, -C(O)N*H*C(S)-), 11.33 (s, 1H, -C(S)N*H*Ar), 7.64 (d, *J* = 8.7 Hz, 2H, Ar H-2, 6), 7.31 (d, *J* = 8.7 Hz, 2H, Ar H-3,5), 3.02-2.78 (m, 1H, , cyclopentyl H-1), 2.00-1.35 (m, 8H, cyclopentyl H-5_{eq}, 2_{eq}, 5_{ax}, 2_{ax}, 3_{eq}, 4_{eq}, 3_{ax}, 4_{ax}); LC-MS, *m/z* = 283 [M+1]; Anal. Calcd. for $C_{13}H_{15}ClN_2OS$: C, 55.22; H, 5.35; N, 9.91; S, 11.34; Found: C, 55.18; H, 5.30; N, 9.88; S, 11.30.

N-((2-Methoxyphenyl)carbamothioyl)cyclopentanecarboxamide (2.21). Yield: 62.0%; Mp.: 141-143°C; IR (cm^{-1}): 3744 (ν_{NH}), 3195 (ν_{NH}), 2934 (ν_{asCH2}), 1682 (ν_{CO}), 1537 (δ_{NH}), 1350 (δ_{sCH3}), 1146 (δ_{CH}), 843 (ν_{C-S}), 744 (γ_{CH}); ^{1}H NMR (400 MHz, DMSO-d_6) δ: 12.87 (s, 1H, -C(O)N*H*C(S)-), 11.14 (s, 1H, -C(S)N*H*Ar), 8.65 (d, *J* = 7.8 Hz, 1H, Ar H-6), 7.14 (t, *J* = 7.4 Hz, 1H, Ar H-4), 6.99 (d, *J* = 8.0 Hz, 1H, Ar H-3), 6.92 (t, *J* = 7.7 Hz, 1H, Ar H-5),

3.92 (s, 3H, -OC$\underline{H}_3$), 3.12-2.86 (m, 1H, , cyclopentyl H-1), 2.01-1.51 (m, 8H, cyclopentyl H-5_{eq}, 2_{eq}, 5_{ax}, 2_{ax}, 3_{eq}, 4_{eq}, 3_{ax}, 4_{ax}); LC-MS, m/z = 279 [M+1]; Anal. Calcd. for $C_{14}H_{18}N_2O_2S$: C, 60.41; H, 6.52; N, 10.06; S, 11.52; Found: C, 60.48; H, 5.63; N, 10.11; S, 11.60.

Antimicrobial test. The sensitivity of the microorganisms to the synthesized compounds was evaluated according to the described methods. [30] The assay was conducted on Mueller-Hinton agar by two-fold serial dilution of the compound in 1 ml. After which, 0.1 ml of microbial seeding (10^6 cells/ml) was added. Minimal inhibition concentration of the compound was determined by the absence of visual growth in the test tube with a minimal concentration of the substance. Minimal bactericide/fungicide concentration was determined by the absence of growth on agar medium after inoculation of the microorganism from the transparent test-tubes. DMSO was used as a solvent, initial solution concentration was 1 mg/ml. For preliminary screening of the abovementioned standard test cultures were used: *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, and *Candida albicans* ATCC 885-653. All test strains were received from bacteriological laboratory in Zaporizhzhia Regional Laboratory Center of State Sanitary and Epidemiological Service of Ukraine. Nitrofural ((*E*)-2-((5-nitrofuran-2-yl)methylene)hydrazine-1-carboxamide) and Ketoconazole (1-(4-(4-[(2-((1*H*-imidazol-1-yl)methyl)-2-(2,4-dichlorophenyl)-1,3-dioxolan-4-yl)methoxy)phenyl)piperazin-1-yl)ethan-1-one) were used as reference compounds with proved antibacterial/antifungal activity. Additional quality control of the culture media and solvents was conducted by commonly used methods. [30]

Molecular docking. Macromolecular data were downloaded from the Protein Data Bank (PDB) namely, the crystal structures of sterol 14α-demethylase (CYP51) 5TZ1, N-myristoyltransferase (NMT) 1IYL and secreted aspartic proteinase (SAP2) 1EAG. [31]

Ligand preparation. Substances were drawn using MarvinSketch 20.6[32] and were saved in mol format. As reference Cyproconazole (2-(4-chlorophenyl)-3-cyclopropyl-1-(1*H*-1,2,4-triazol-1-yl)-2-butanol) was chosen. [33] Next, they were optimized by program Chem3D using molecular dynamics MM2 algorithm and saved as pdb-files. Molecular mechanics were used to produce more realistic geometry values for most of organic molecules owing to the fact of being highly parameterized. By using AutoDockTools-1.5.6 pdb-files were converted to PDBQT, and number of active torsions was set as default. [34]

Protein preparation. PDB files were downloaded from the protein data bank. [26] Discovery Studio V19.1.0.18287 was used to delete water molecules and ligand from the crystal. The proteins were saved as pdb-files. In AutoDockTools-1.5.6 polar hydrogens were added and saved as PDBQT. Grid box was set as follows: center_x = 70.728, center_y = 65.553, center_z = 3.865, size_x = 20, size_y = 20, size_z = 20. Vina was used to carry out docking. For visualization Biovia Discovery Studio Visualizer (v17.2.0.16349, Accelrys, San Diego, CA, USA) was applied. [35]

Antifungal activity. The mycelial growth rate assay was used for antifungal studies as described. [36] Strains of filamentous fungi were obtained from the following sources: *Asperillus niger* (AN) DSM 246, *Altenaria alternata* (AA) DSM 1102, *Fusarium equiseti* (FE) DSM 21725, *F. graminearum* (FG) DSM 1095 and *F. fujikuroi* (FF) DSM 893, *Verticillium lecanii* (VL), *Mucor indicus* (MI) DSM 2185, *Penicillium digitatum* (PD) DSM 2731 from DSMZ (Braunschweig, Germany); *Fusarium oxysporum* (FO) 39/1201 St. 9336 and *Botrytis cinerea* from the Technische Universität Berlin (Germany); *Colletotrichum higginsianum* (CH) MAFF 305635, originally isolated in Japan, *via* the Department of Biology, Friedrich-Alexander-Universität (Erlangen, Germany). Oomycete strains *Phytophthora infestans* (PI GL-1) GL-1 01/14 wild strain, p-3 (PI p-3) (4/91; R+) and p-4 (PI p-4) (4/91; R-) were kindly donated by Julius Kühn-Institut (Quedlinburg, Germany). Potato Dextrose Agar (PDA) was purchased from C. Roth (Karlsruhe, Germany). Cyproconazole (98 %) was obtained from Sigma-Aldrich (Steinheim, Germany). Strains were cultivated on PDA for 6 d at 25°C. Spores from each strain were gently harvested with a sterile glass rod from plate surfaces with deionized water. Spore concentration numbers in suspension were determined microscopically and adjusted to 7.5×10^6 spores/ml. Clear stock solutions of 5 mg/ml were made of 0.050 g of reference substance Cyproconazole or acyl thiourea in 10 ml of sterile dimethyl sulfoxide (DMSO). 1 mL of each stock solution was mixed *in situ* into 99 ml of PDA prior to solidification to obtain a final concentration of 50 µg/ml. In the same way, series of PDA with tested compounds were prepared with final concentrations of 25, 12.5, and 6.25 µg/ml. 9 ml of each mixture were poured into 6 cm diameter petri dishes. After solidification a central hole (diameter: 2.5 mm) was cut out and inoculated with 6.5 µl spore suspension. Plates were incubated at 25°C (+/- 1°C) for 6 d. Control plates containing only PDA and water were prepared in the same way. Inhibitory effects (I %) were determined by analyzing growth zone diameters and were calculated as described by Tang *et al.*: I % = [(C-T) / (C - 2.5 mm)] × 100, where C (mm) represented the growth zone of control PDA, and T (mm) - the average growth zone in presence of reference or test substances. [36] All growth experiments were carried out in triplicate. Means and standard deviations were calculated with software «Excel 2016» (Microsoft, USA).

***Salmonella* reverse mutagenicity test**. The mutagenicity test was applied as a standard plate incorporation assay with *Salmonella typhimurium* strains TA 98 and TA 100 as described by Maron and Ames. [28] Tested Salmonella strains were obtained from culture collection, University of Göteborg (Göteborg, Sweden). 2-Nitrofluorene (2-NF), dimethylsulfoxide (DMSO), 2-aminofluorene (2-AF), methyl

methanesulfonate (MMS), β-nicotinamide adenine dinucleotide phosphate hydrate (β -NADP) and glucose-6-phosphate were purchased from Sigma–Aldrich (Steinheim, Germany), whereas *D*(+)-biotin, *D*(+)-glucose anhydrous, *L*-histidine and $NaNH_4HPO_4$ were sourced from Carl Roth GmbH & Co. KG (Karlsruhe, Germany). Citric acid monohydrate, NaCl, NaH_2PO_4, K_2HPO_4 anhydrous, $MgCl_2{\times}6H_2O$, KCl were purchased from Applichem GmbH (Darmstadt, Germany). NaOH solution was obtained from Riedel deHaen/Seelze (Hannover, Germany), $MgSO_4$ anhydrous was obtained from Merck (Darmstadt, Germany). Stock solutions of controls and acyl thioureas were solved in DMSO. Their final doses in top agar were adjusted to 50 µg/ml (i.e. 135 µg/plate). The positive controls were 2-NF (10 mg/ml in DMSO; 10 µl/plate) for TA 98 and methyl-methanesulfonate (MMS; 10 % (v/v) in DMSO; 1 µl/plate) for TA100; buffer with 100 µl of DMSO for both strains were used as negative control (i.e. determination of spontaneous reversion rate). In parallel, experiments with metabolic activation were carried out by adding activated rat liver extract (S9-mix, Trinova Biochem, Giessen/Germany) instead of sodium buffer. Activity of S9-mix was confirmed with Salmonella TA 98 and 2-aminofluorene (2-AF, 10 mg/ml in DMSO, 10 µl/plate). All further experimental procedures were as described. [28]

Author Contribution Statement

Olena V. Kholodniak and Fatuma Meyer performed the experiments, analized the data and wrote the article. Sergiy I. Kovalenko, Karl G. Steffens and Maksym S. Kazunin helped to revise and to edit the article.

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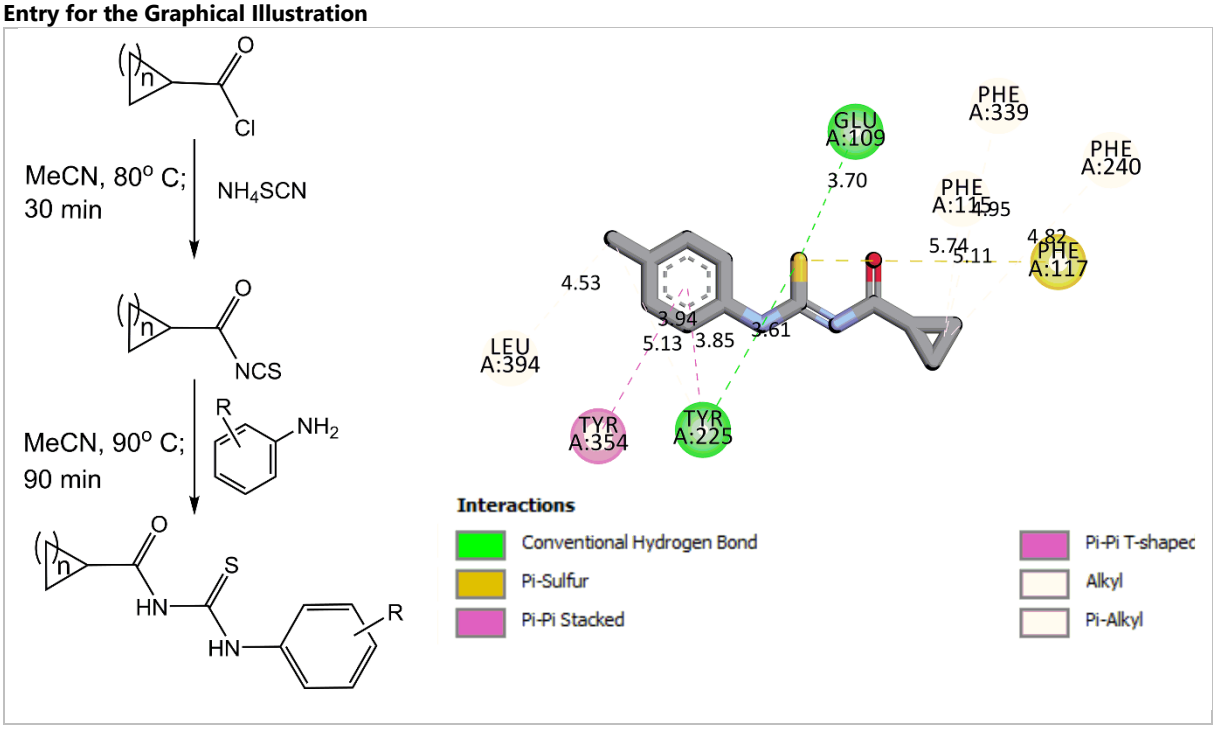

Twitter Text

A synthesis method of novel *N*-cycloalkylcarbonyl-*N'*-aryl-thioureas was developed. It consists of sequential addition of equimolecular amounts of ammonium isothiocyanate and substituted anilines to cycloalkylcarbonyl chlorides. The identity and purity of products were confirmed by LC-MS spectra, their structure by elemental analysis, IR and ^{1}H NMR spectra. Preliminary antimicrobial screening for standard microorganisms and molecular docking allowed to select several structures for antifungal and genetic toxicity studies. Conducted *in vitro* screening of 9 compounds for antifungal potential against 11 phytopathogenic fungi and three *Phytophthora* strains revealed that two *N*-(arylcarbamothioyl)cyclopropanecarboxamides (**2.3**, **2.4**), at a concentration of 50 μg/ml exhibited activities comparable of the standard antifungal agent "Cyproconazole". Analysis of mutagenicity of novel thioureas using the *Salmonella* Reverse Mutagenicity assay ("Ames Test") showed a low gene-toxicity profile.