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SYNTHESIS OF AZOLE DERIVATIVES FROM 3-PHENYLAMINOPROPANOHYDRAZIDE AND EVALUATION OF THEIR ANTIMICROBIAL EFFICACY

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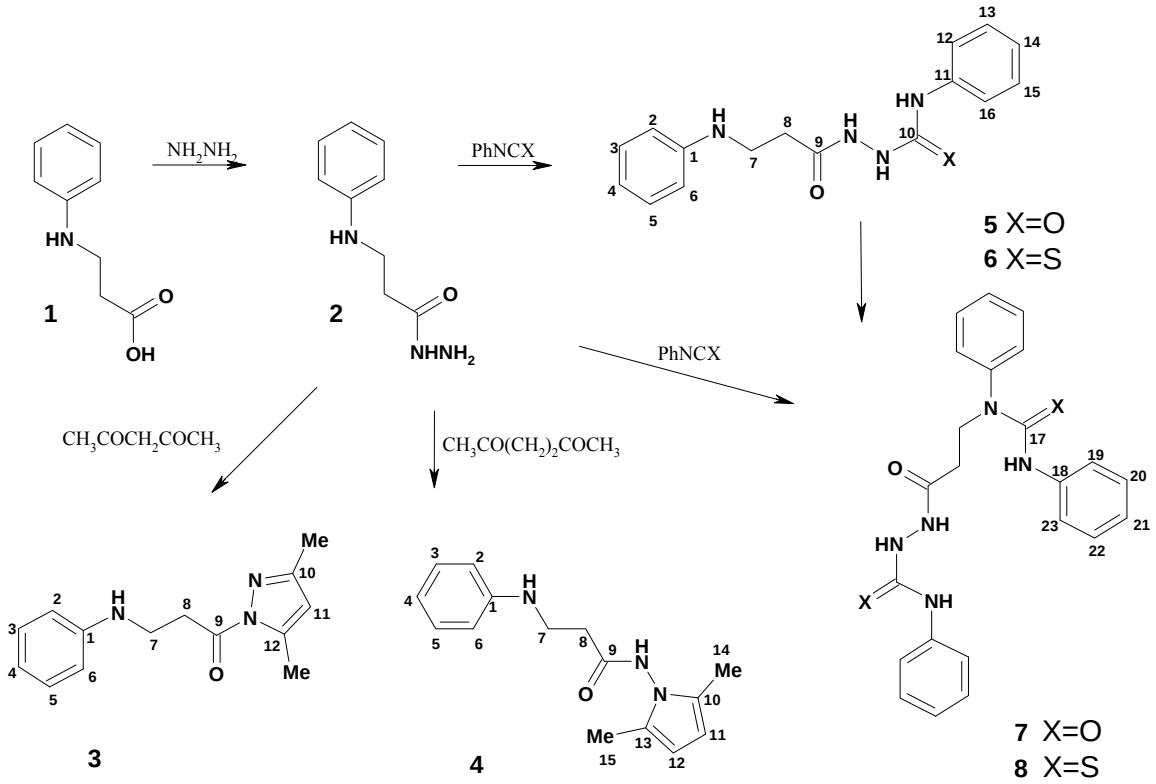
Abstract – Novel pyrrole and pyrazole derivatives containing phenylamino-propanoyl moiety were synthesized from 3-phenylaminopropanohydrazide through its condensation with diketones. The intramolecular cyclization reaction of disubstituted semicarbazides and thiosemicarbazides was used to prepare new 1,3,4-thiadiazole, 1,3,4-oxadiazole and 1,2,4-triazole compounds. Some of the synthesized compounds were evaluated for *in vitro* antimicrobial activity.

INTRODUCTION

Chemical and biological properties of hydrazides, semicarbazides, and the products of their heterocyclization have been widely studied. In particular, some semicarbazides exhibit convincing antimicrobial activity.[1,2] 1,2,4-triazoles and 1,3,4-oxadiazoles are known for their anti-inflammatory,[3] and antibacterial effect.[4,5] Triazole system is a structural element of many drugs that have antimycotic activity such as fluconazol, itraconazol, voriconazol.[6] *N*-substituted 3-aminopropanoic acids and their salts are known to elicit a broad spectrum of biological activities.[7-10] As a part of our study aimed at investigating transformations of *N*-substituted β-amino acid derivatives, in this work we report the synthesis of the five-membered heterocyclic compounds from 3-(phenylamino)pronanohydrazide through direct transformations or via intermediate semicarbazides.

RESULTS AND DISCUSSION

3-(Phenylamino)propanohydrazide[10] was synthesized from *N*-phenyl-β-alanine (**1**) and hydrazine in refluxing toluene (Scheme 1). Condensation of hydrazide **2** with alkanediones was carried out in acidic medium. Heating of **2** with 2,4-pentanedione in the presence of hydrochloric acid gave pyrazole derivative **3**, whereas when **2** was heated with 2,5-hexanedione in the presence of a catalytic amount of acetic acid N-(2,5-dimethyl-1*H*-pyrrol-1-yl)-3-(phenylamino)propanamide (**4**) was obtained since only amino group of hydrazide moiety participated in the cyclization process. Formation of the cycles was supported by NMR spectra. In the ^{1}H NMR spectrum of **3** singlets of methyl group of pyrazole moiety are observed at 2.20 and 2.49 ppm. Singlets of two equivalent methyl groups at 1.96 ppm and two CH groups at 5.62 ppm confirmed the structure of pyrrole **4**.

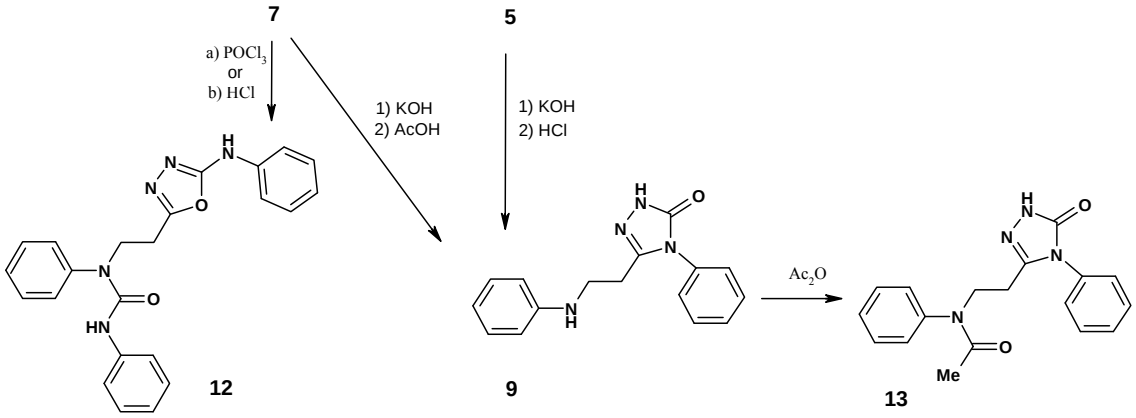


Scheme 1

Depending on the amount of the used isocyanates, semicarbazides **5** and **6** or their *N*-phenylcarbamoyl derivatives **7** and **8** were obtained in the reaction of propanohydrazide **2** with phenyl isocyanate or phenyl isothiocyanate. Strong absorption band of carbonyl group at 1678 cm^{-1} and adsorption band of the C=S group at 1175 cm^{-1} supported the structure of 3-(phenylamino)-*N*-[(phenylthiocarbamoyl)amino]propanamide (**6**). In the IR spectrum of **8** absorption band attributed to CO group was observed at 1704 cm^{-1}, and two C=S group bands were identified at 1159 and 1218 cm^{-1}.

The reaction of cyclization of acyl semicarbazide derivatives depends on the pH of the medium and

substituents present in semicarbazide derivatives. Cyclization of these compounds in an alkaline medium results in 1,2,4-triazole system, whereas reaction in acidic medium affords compounds with 1,3,4-oxadiazole ring.[4,6,11-13]

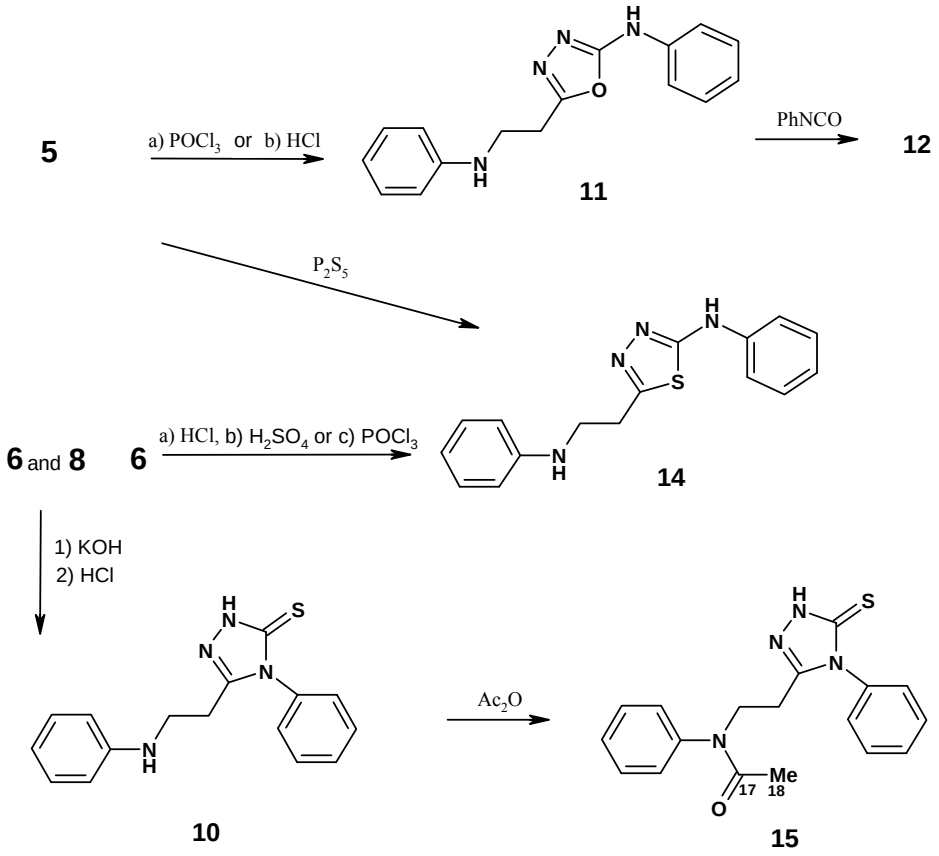


Scheme 2

Refluxing of phenylcarbamoylaminopropanamide **5** in aqueous 20 % KOH with the subsequent acidification of the reaction mixture with hydrochloric acid afforded 4-phenyl-3-[2-(phenylamino)ethyl]-1*H*-1,2,4-triazol-5(4*H*)-one (**9**) in 60-70 % yield (Scheme 2). The same method applied to **7** resulted in the formation of the same triazole **9** because hydrolysis of the phenylcarbamoyl group took place along with the cyclization process. The IR spectrum of **9** showed absorption bands characteristic to 1,2,4-triazolone ring vibrations at 1519, 1367, and 1191 cm^{-1}, and absorption band attributed to one carbonyl group at 1665 cm^{-1}.

^{1}H NMR spectra of **9** and **10** reveal the deshielding effect of triazole ring on the adjacent methylene group. Its triplet was shifted down-field in comparison with the proton signals of methylene group in propanohydrazide moiety and was observed at 2.63 ppm and 2.57 ppm for **9** and **10**, respectively.

Semicarbazide **5** and its phenylcarbamoyl derivative **7** underwent cyclization to oxadiazole derivatives **11** and **12** when heated under reflux with HCl or $POCl_3$ (Schemes 2 and 3). The same method applied to thiosemicarbazide **6** gave **14**. Cyclization of 3-(phenylamino)-*N*-[(phenylcarbamoyl)amino]propanamide (**5**) was more rapid in hydrochloric acid than in phosphoryl chloride. The yield of **11** after heating under reflux in dilute hydrochloric acid (1:1) for 1 h was 64 %, whereas such yield was not achieved even after 36 h of reflux in $POCl_3$. *N*-Phenyl-5-[2-(phenylamino)ethyl]-1,3,4-thiadiazol-2-amine (**14**) was synthesized in good yield by keeping thiosemicarbazide **6** in concentrated sulfuric acid at room temperature. The same thiadiazole **14** was also prepared from propanamide **5** by heating under reflux in xylene with P_2S_5.



Scheme 3

Compound **12** was synthesized by treating oxadiazole **11** with phenyl isocyanate. Heating under reflux of triazoles **9** and **10** with acetic anhydride afforded N-acylated compounds **13** and **15**.

Proton signal of NH group was absent in the ^{1}H NMR spectra of acylated derivatives **13** and **15** in comparison with the respective spectra of **9** and **10**, but singlet attributed to three protons of acetyl group was observed at 1.91, 1.66 ppm.

BIOLOGIGAL ACTIVITY

The potential antimicrobial activity of compounds **4-12** and **14** towards eight bacterial strains and antifungal activity towards *Candida albicans* were investigated.

The experiments have revealed that the presence of oxadiazole and thiadiazole rings stipulates antimicrobial activity of the investigated compounds. Pyrrole **4**, semicarbazides **5–8**, and triazoles **9** and **10** had no inhibitory activity on the tested microorganism strains. Oxadiazole **11** exhibited the widest range of activity (Table 1). It completely inhibited growth of *Staphylococcus aureus* at 100 μg/mL, growth of *Enterococcus faecalis* at 250 μg/mL, and growth of *Bacillus subtilis* and *Bacillus cerius* at 150 μg/mL. MIC of oxadiazole **12** towards *Staphylococcus aureus*, *Bacillus subtilis,* and *Bacillus cerius* were 150 μg/mL, 200 μg/mL, and 250 μg/mL, respectively. Thiadiazole **14** exhibited activity towards *Candida albicans* (300 μg/mL), *Bacillus subtilis* (450 μg/mL) and *Bacillus cerius* (450 μg/mL).

Table 1. Antimicrobial activity of compounds **11**, **12** and **14**

Concentration,	*Staphylococcus aureus*	*Enterococcus faecalis*	*Escherichia coili* ATCC	*Pseudomonas aeruginosa*	*Klebsiella pneumonine* ATCC	*Protus vulgaris* ATCC	*Bacillus subtilis* ATCC	*Bacillus cerius* ATCC	*Candida albicans* ATCC
				Compound **11**					
500	+*	+	–**	–	–	–	+	+	–
450	+	+	–	–	–	–	+	+	–
400	+	+	–	–	–	–	+	+	–
350	+	+	–	–	–	–	+	+	–
300	+	+	–	–	–	–	+	+	–
250	+	+	–	–	–	–	+	+	–
200	+	–	–	–	–	–	+	+	–
150	+	–	–	–	–	–	+	+	–
100	+	–	–	–	–	–	–	–	–
50	–	–	–	–	–	–	–	–	–
25	–	–	–	–	–	–	–	–	–
1	–	–	–	–	–	–	–	–	–
				Compound **12**					
500	+	–	–	–	–	–	+	+	–
450	+	–	–	–	–	–	+	+	–
400	+	–	–	–	–	–	+	+	–
350	+	–	–	–	–	–	+	+	–
300	+	–	–	–	–	–	+	+	–
250	+	–	–	–	–	–	+	+	–
200	+	–	–	–	–	–	+	–	–
150	+	–	–	–	–	–	–	–	–
100	–	–	–	–	–	–	–	–	–
50	–	–	–	–	–	–	–	–	–
25	–	–	–	–	–	–	–	–	–
1	–	–	–	–	–	–	–	–	–
				Compound **14**					
500	–	–	–	–	–	–	+	+	+
450	–	–	–	–	–	–	+	–	+
400	–	–	–	–	–	–	–	–	+
350	–	–	–	–	–	–	–	–	+
300	–	–	–	–	–	–	–	–	+
250	–	–	–	–	–	–	–	–	–
200	–	–	–	–	–	–	–	–	–
150	–	–	–	–	–	–	–	–	–
100	–	–	–	–	–	–	–	–	–
50	–	–	–	–	–	–	–	–	–
25	–	–	–	–	–	–	–	–	–
1	–	–	–	–	–	–	–	–	–

* + – the compound exhibits antimicrobial activity (growth of the microbial strain is inhibited),

** – – the compound does not exhibit antimicrobial activity

CONCLUSIONS

Reactions of 3-(phenylamino)propanohydrazide with aliphatic 2,4-pentanedione and 2,5-hexanedione gave dimethylpyrazole and dimethylpyrrole derivatives. Mono- or diphenylcarbamoyl 3-phenylaminopropanamides were obtained by its reaction with isocyanates. The obtained compounds underwent cyclization into oxadiazole, thiazole, and triazole derivatives. The synthesized compounds containing oxadiazole or thiadiazole moieties exhibit antimicrobial activity.

EXPERIMENTAL

Melting points were determined on a Gallenkamp melting point apparatus and are uncorrected. NMR spectra were recorded on a Varian Unity Inova (300 MHz) spectrometer using DMSO-d_6 as a solvent. Chemical shifts (δ) are reported in parts per million (ppm) calibrated from TMS (0 ppm) for ^{1}H NMR, and DMSO-d_6 (39.5 ppm) for ^{13}C NMR. The IR spectra were measured as potassium bromide pellets using a Perkin-Elmer 1600 series FT-IR spectrometer. All chemicals were obtained from Fluka Chemie AG Buchs (Switzerland). Flash column chromatography was carried out with silica gel 60 (Fluka Chemie AG Buchs (Switzerland)).

3-(Phenylamino)propanohydrazide (2). *N*-Phenyl-*β*–alanine (**1**) (23.7 g, 0.1 mol) and hydrazine hydrate (10 g, 0.2 mol) were stirred under reflux in toluene (500 mL) for 4 h. The solvent was removed on a rotary evaporator, and the product was crystallized from 2-propanol. The crystals were filtered, washed with Et_2O, and recrystallized from 2-propanol. Yield 16.5 g (64.2 %), mp 93–94 ˚C.[14] ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.30 (t, 2H, J = 6.75 Hz; CH_2CO), 3.23 (t, 2H, J = 6.75 Hz, $\underline{CH_2}NH$), 4.22 (s, 2H, NH_2), 5.50 (s, 1H, NH), 6.57 (t, 1H, J = 7.5 Hz, $H_{ar\text{-}4}$), 6.51 (d, 2H, J = 8.1 Hz, $H_{ar\text{-}2,6}$), 7.07 (t, 2H, J = 8.1 Hz, $H_{ar\text{-}3,5}$), 9.05 (s, 1H, $\underline{NH}NH_2$). ^{13}C NMR (75.4 MHz, DMSO-d_6): δ 33.97 ($\underline{CH_2}CO$), 46. 31 (CH_2NH), 106.14 (C-2); 113.85 (C-4), 131.34 (C-3), 146.09 (C-1), 173.43 (CO). MS (ESI, 20 V): m/z 180 $[M+H]^+$ (100 %). Anal. Calcd for $C_9H_{13}N_3O$: C, 60.32; H, 7.31; N, 23.45. Found: C, 60.41; H, 7.29; N, 23.21.

1-(3,5-Dimethyl-1*H*-pyrazol-1-yl)-3-(phenylamino)propan-1-one (3). A mixture of **2** (4.48 g, 25 mmol), 2,4-pentanedione (2 mL, 2.5 g, 25 mmol) and 2M HCl (1 mL) was refluxed in MeOH (50 mL) for 12 h. The liquid fractions were distilled on a rotary evaporator, and the oily residue was crystallized from Et_2O. The product was purified by flash column chromatography (acetone:hexane 1:1). Yield 4.2 g (70 %), mp 39–40 ºC. ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.20, 2.49 (2s, 6H, 2 CH_3), 3.33 (t, 2H, J = 7.2 Hz, $COCH_2$), 3.40 (t, 2H, J = 7.2 Hz, NCH_2), 6.20 (s, 1H, CH=). 6.53 (t, 1H, J = 7.2 Hz, $H_{ar\text{-}4}$). 6.63 (d, 2H, J = 8.1 Hz, $H_{ar\text{-}2,6}$). 7.10 (t, 2H, J = 8.1 Hz; $H_{ar\text{-}3,5}$). ^{13}C NMR (75.4 MHz, DMSO-d_6): δ 171.92 (C-9), 151.26 (C-10), 148.38 (C-1), 143.12 (C-11), 128.84 (C-3, 5), 115.70 (C-4), 111.97 (C-12), 111.06 (C-2, 6), 38.10 (C-7), 34.72 (C-8), 14.03 (C-14), 13.38 (C-13). IR (KBr), ν/cm^{-1}: 1670 (CO). MS (ESI, 20V):

m/z 244 $[M+H]^+$ (40%). Anal. Calcd for $C_{14}H_{17}N_3O$: C, 69.11; H, 7.04; N, 17.27. Found: C, 68.93; H, 7.20; N, 17.08.

***N*-(2,5-Dimethyl-1*H*-pyrrol-1-yl)-3-(phenylamino)propanamide (4).** A mixture of **2** (0.54 g, 3 mmol), 2-propanol (75 mL), 2,5-hexanedione (0.69 g, 6 mmol), and acetic acid (1.5 mL) was refluxed for 20 h. Then cold water (25 mL) was added. The crystals were filtered and recrystallized from EtOH. Yield 0.55 g (71 %), mp. 134–135 ºC. ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.96 (s, 6H, 2 CH_3), 2.55 (t, 2H, J = 6.9 Hz, $COCH_2$), 3.36 (t, 2H, J = 6.9 Hz, NH$\underline{CH_2}$), 5.62 (s, 2H, 2 CH), 5.65 (s, 1H, NH), 6.55 (t, 1H, J = 7.2 Hz, $H_{ar\text{-}4}$), 6.62 (d, 2H, J = 7.2 Hz, $H_{ar\text{-}2,6}$), 7.09 (t, 2H, J = 7.2 Hz, $H_{ar\text{-}3,5}$), 10.61 (s, 1H, NH). ^{13}C NMR (75.4 MHz, DMSO-d_6): δ 170.28 (C-9), 148.50 (C-1), 128.89 (C-3, 5), 126.67 (C-10, 13), 115.77 (C-4), 102.79 (C-11, 12), 112.03 (C-2, 6), 38.38 (C-7), 33.14 (C-8), 10.89 ((C-14, 15). IR (KBr), ν/cm^{-1}: 3368 (NH), 1668 (C=O). MS (ESI, 20V): m/z 258 $[M+H]^+$ (70%). Anal. Calcd for $C_{15}H_{19}N_3O$: C, 70.01; H, 7.44; N, 16.33. Found: C, 69.95; H, 7.9; N, 15.98.

3-(Phenylamino)-*N*-[(phenylcarbamoyl)amino]propanamide (5). To a solution of **2** (4.48 g, 25 mmol) in methanol (50 mL) phenyl isocyanate (2.98 g, 2.71 mL, 25 mmol) was added dropwise. The reaction mixture was refluxed for 1 h, and cooled down. The crystals were filtered, washed with MeOH, and recrystallized from MeOH. Yield 6.0 g (80 %), mp 133–134 ºC. ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.46 (t, 2H, J = 7.2 Hz, CH_2CO), 3.30 (t, 2H, J = 7.2 Hz CH_2N), 6.55 (t, 1H, J = 7.2 Hz, $H_{ar\text{-}4}$), 6.96 (d, 2H, J = 8.5 Hz, $H_{ar\text{-}2,6}$), 7.09 (t, 2H, J = 8.5 Hz, $H_{ar\text{-}3,5}$), 7.23–7.47 (m, 5H, $H_{ar'}$), 8.06 (s, 1H, NH$\underline{NH}$CO), 8.72 (s, 1H, $\underline{NH}$NHCO), 9.76 (s, 2H, NHAr). ^{13}C NMR (75.4 MHz, DMSO-d_6): δ 170.84 (C-9), 155.35 (C-1), 148.50 (C-10), 139.51 (C-11), 128.88 (C-3, 5), 128.58 (C-13, 15), 121.85 (C-14), 118.43 (C-4), 115.83 (C-12, 16),112.09 (C-2, 6), 43.02 (C-7), 33.17 (C-8). IR (KBr), ν/cm^{-1}: 3375, 3344, 3206 (NH); 1638 (CO). MS (ESI, 20 V): m/z 299 $[M+H]^+$ (85 %). Anal. Calcd for $C_{16}H_{18}N_4O_2$: C, 64.41; H, 6.08; N, 18.78. Found: C, 64.04; H, 6.03; N, 18.30.

3-(Phenylamino)-*N*-[(phenylthiocarbamoyl)amino]propanamide (6). To a solution of **2** (4.48 g, 25 mmol) in MeOH (50 mL) phenyl isothiocyanate (3.38 g, 2.99 mL, 25 mmol) was added dropwise. The reaction mixture was refluxed for 5 min. The crystals were filtered, washed with Et_2O, and recrystallized from DMF – water mixture. Yield 5.97 g (65 %), mp 175–176 ^{o}C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.48 (t, 2H, J = 7.2 Hz, CH_2CO), 3.29 (q, 2H, J = 7.2 Hz, CH_2NH), 5.56 (s, 1H, NHAr), 6.51 (t, 1H, J = 7.2 Hz, $H_{ar\text{-}4}$), 6.57 (d, 2H, J = 8.5 Hz, $H_{ar\text{-}2,6}$), 7.08 (t, 2H, J = 7.2 Hz, $H_{ar\text{-}3,5}$), 7.16 (t, 1H, J = 7.2 Hz; $H_{ar'\text{-}4}$), 7.33 (t, 2H, J = 7.2 Hz, $H_{ar'\text{-}3,5}$), 7.43 (d, 2H, J=7.2 Hz, $H_{ar'\text{-}2,6}$), 9.58 (s, 2H, NH$\underline{NH}$CS), 10.00 (s, 1H, NHAr'). ^{13}C NMR (75.4 MHz, DMSO-d_6): δ 175.17 (C-10), 170.72 (C-9), 148.48 (C-1), 138.99 (C-11), 128.85 (C-3, 5), 127.99 (C-13, 15), 125.69 (C-14), 125.06 (C-4), 115.81 (C-12, 16), 112.06 (C-2, 6), 43.68 (C-7), 33.19 (C-8). IR (KBr), ν/cm^{-1}: 3362, 3266, 3137 (NH), 1678 (CO), 1175 (CS). MS (ESI, 20V): 315 $[M+H]^+$ (80%). Anal. Calcd for $C_{16}H_{18}N_4OS$: C, 61.12; H, 5.77; N, 17.82. Found: C, 61.02; H,

5.69; N, 18.08.

3-[Phenyl(phenylcarbamoyl)amino]-*N*-[(phenylcarbamoyl)amino]propanamide (7). To a solution of **2** (4.48 g, 25 mmol) in MeOH (50 mL) phenyl isocyanate (8.93 g, 8.11 mL, 75 mmol) was added dropwise. The reaction mixture was refluxed for 5 min. The crystals were filtered, washed with Et_2O, and recrystallized from MeOH. Yield 8.84 g (85 %), mp 121–122 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.47 (t, 2H, J = 7.2 Hz, CH_2CO), 3.94 (t, 2H, J = 7.2 Hz, CH_2N), 6.92–7.50 (m, 15H, $H_{Ar,Ar',Ar''}$), 8.06, 8.03 (2s, 2H, NHNHCO), 8.72 (s, 1H, NHAr"), 9.80 (s, 1H, NHAr'). ^{13}C NMR (75.4 MHz, DMSO-d_6): δ 170.49 (C-9), 155.21 (C-17), 154.45 (C-10), 141.87 (C-1), 139.83 (C-11), 139.48 (C-18), 129.49 (C-3, 5), 128.51 (C-2, 6), 128.17 (C-20, 22), 127.76 (C-4), 126.63 (C-13, 15), 121.95 (C-14), 121.81 (C-21), 119.71 (C-19, 23), 118.52 (C-12, 16), 45.99 (C-7), 32.19 (C-8). IR (KBr), ν/cm^{-1}: 3412, 3240, 3036 (NH), 1707, 1652 (CO). MS (ESI, 35V): m/z 419 $[M+H]^+$ (80%). Anal. Calcd for $C_{23}H_{23}N_5O_3$: C, 66.17; H, 5.55; N, 16.78. Found: C. 66.06; H, 5.49; N, 16.55.

3-[Phenyl(phenylthiocarbamoyl)amino]-*N*-[(phenylthiocarbamoyl)amino]propanamide (8). To a solution of **2** (4.48 g, 25 mmol) in MeOH (50 mL) phenyl isothiocyanate (10.14 g, 8.97 mL, 75 mmol) was added dropwise. The reaction mixture was refluxed for 6 h. The crystals were filtered, and recrystallized from EtOH. Yield 6.5 g (58 %), mp 152–153 ºC. ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.65 (t, 2H, J = 8.1 Hz, CH_2CO), 4.37 (t, 2H, J = 8.1 Hz, CH_2N), 7.25–7.51 (m, 15H, $H_{Ar,Ar',Ar''}$), 8.86 (s, 1H, NHAr"), 9.52, 9.58 (2s, 2H, NHNHCS), 9.97 (s, 1H, NHAr'). ^{13}C NMR (75.4 MHz, DMSO-d_6): δ 169.95 (C-9), 181.97 (C-17), 180.83 (C-10), 142.95 (C-1), 139.79 (C-11), 139.52 (C-18), 129.50 (C-3, 5), 127.90 (C-2, 6), 128.27 (C-20, 22), 127.78 (C-4), 127.05 (C-13, 15), 121.70 (C-14), 121.79 (C-21), 120.05 (C-19, 23), 119.30 (C-12, 16), 46.21 (C-7), 31.95 (C-8). IR (KBr), ν/cm^{-1}: 3168, 3363 (NH), 1704 (C=O), 1159, 1218 (C=S). MS (ESI, 20V): m/z 450 $[M+H]^+$ (60%). Anal. Calcd for $C_{23}H_{23}N_5OS_2$: C, 61.44; H, 5.16; N, 15.58. Found: C, 61.35; H, 5.01; N, 15.13.

4-Phenyl-3-[2-(phenylamino)ethyl]-1*H*-1,2,4-triazol-5(4*H*)-one (9). a) A mixture of **5** (0.98 g, 3 mmol) and 20% KOH aqueous solution (25 mL) was refluxed for 2 h, and cooled down. Then concentrated HCl was added to pH 4. The crystals were filtered, washed with water, and recrystallized from DMF – water mixture. Yield 0.57 g (62 %), mp 188–189 ºC. **b**) A mixture of **7** (1.25 g, 3 mmol) and 20% KOH aqueous solution (30 mL) was refluxed for 4 h, and cooled down. Acetic acid was added under freezing to pH 4. The crystals were filtered, washed with water, and recrystallized from DMF – water mixture. Yield 0.59 g (70 %), mp 188–189 ºC. ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.63 (t, 2H, J = 7.5 Hz, CH_2C), 3.28 (q, 2H, J = 14.7 Hz, NH$\underline{CH_2}$), 5.67 (t, 1H, J = 6.4 Hz, NHAr), 6.36 (d, 2H, J = 7.5 Hz, $H_{ar\text{-}2,6}$), 6.50(t, 1H, J = 7.5 Hz, $H_{ar\text{-}4}$), 6.99 (dd, 2H, J = 1.2 Hz, J = 7.5 Hz, $H_{ar\text{-}3,5}$), 7.39–7.56 (m, 5H, $H_{Ar'}$), 11.74 (s, 1H, NH). ^{13}C NMR (75.4 MHz, DMSO-d_6): δ 154.28 (C-10), 147.93 (C-1), 145.12 (C-9), 132.79 (C-11), 129.34 (C-13, 15); 128.79 (C-3, 5), 128.44 (C-14), 127.50 (C-12, 16), 115.68 (C-4), 111.73 (C-2, 6), 44.51 (C-7),

33.61 (C-8). IR (KBr), ν/cm^{-1}: 3193, 2920 (NH), 1665 (C=O), 1519, 1367, 1191 (1,2,4-triazole ring), 1157 (N-N). MS (ESI, 20V): 281 $[M+H]^+$ (100%). Anal. Calcd for $C_{16}H_{16}N_4O$: C, 68.55; H, 5.75; N, 19.99. Found: C, 68.28; H, 6.41; N, 19.52.

4-Phenyl-3-[2-(phenylamino)ethyl]-1*H*-1,2,4-triazole-5(4*H*)-thione (10). A mixture of **6** (1.57 g, 5 mmol) and 20% KOH aqueous solution (50 mL) was refluxed for 4 h, and cooled down. Concentrated HCl was added to pH 4. The crystals were filtered, washed with water, and recrystallized from DMF – water mixture. Yield 1.61 g (89 %), mp 128–129 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.57 (t, 2H, CH_2C), 3.11 (q, 2H, J = 14.7 Hz, NH$\underline{CH_2}$), 5.58 (t,1H, J = 6.4 Hz, NHAr), 6.30 (d, 2H, J = 7.5 Hz, $H_{ar\text{-}2,6}$), 6.47 (t, 1H, J = 7.3 Hz, $H_{ar\text{-}4}$), 6.98 (dd, 2H, J = 7.3, 8.6 Hz, $H_{ar\text{-}3,5}$), 7.25 (d, 2H, J=6.9 Hz, $H_{Ar\text{-}12,16}$), 7.38–7.48 (m, 3H, $H_{Ar\text{-}13,14,15}$), 13.52 (s,1H, NH). ^{13}C NMR (75.4 MHz, DMSO-d_6): δ 166.60 (C-10), 148.41 (C-1), 148.16 (C-9), 137.29 (C-11), 128.77 (C-13, 15), 128.47 (C-3, 5), 127.15 (C-12, 15), 115.51 (C-4), 111.175 (C-2, 6), 45.91 (C-7), 33.25 (C-8). IR (KBr), ν/cm^{-1}: 3320; 3392 (NH), 1604, 1497, 1100 (1,2,4-triazole ring), 1101 (CS). MS (ESI, 20V): 297 $[M+H]^+$ (40%). Anal. Calcd for $C_{16}H_{16}N_4S$: C, 64.84; H, 5.44; N, 18.90. Found: C, 64.61; H 5.23; N, 18.70.

***N*-Phenyl-5-[2-(phenylamino)ethyl]-1,3,4-oxadiazol-2-amine (11). a**) A solution of **5** (2.98 g, 0.01 mol) in $POCl_3$ (20 mL) was refluxed for 36 h. A reaction mixture was poured dropwise on ice. The product was washed with water and crystallized from water. Yield 1.62 g (58 %), mp 157–158 °C. **b**) A mixture of **5** (2.98 g, 0.01 mol) and 15 % HCl solution (20 mL) was refluxed for 1 h, and cooled down to rt. The reaction mixture was neutralized with Na_2CO_3. The crystals were filtered, washed with water, and recrystallized from water. Yield 1.80 g (64 %), mp 157–158 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.01 (t, 2H, J = 6.75 Hz, CH_2CN), 3.44 (t, 2H, J = 6.75 Hz, $\underline{CH_2}$NH), 3.47 (s, 1H, $\underline{NH}CH_2$), 6.94 (d, 2H, J = 7.5 Hz, $H_{ar\text{-}2,6}$), 6.59 (t, 1H, J = 7.5 Hz, $H_{ar\text{-}4}$), 7.11 (d, 2H, J = 7.5 Hz, $H_{ar\text{-}3,5}$), 7.30–7.40 (m, 5H, $H_{Ar'}$), 7.42 (s, 1H, $NH_{Ar'}$). IR (KBr), ν/cm^{-1}: 3367; 3366 (NH). MS (ESI, 20V): m/z 281 $[M+H]^+$ (100%). Anal. Calcd for $C_{16}H_{16}N_4O$: C, 68.55; H, 5.75; N 19,99. Found: C, 68.43; H, 5.71; N, 19.89.

1,3-Diphenyl-3-{2-[5-(phenylamino)-1,3,4-oxadiazol-2-yl]ethyl}urea (12). a) A solution of **7** (2.09 g, 5 mmol) in $POCl_3$ (40 mL) was refluxed for 24 h. The reaction mixture was poured dropwise to an ice – water mixture. The crystals were filtered, and recrystallized from water. The filtrate was neutralized with NH_4OH; the crystals were filtered, and washed with water. Yield 1.37 g (69 %), mp 160.1–160.7 °C. **b**) A mixture of **7** (2.09 g, 5 mmol) and dilute HCl (1:1, 15 mL) was refluxed for 1 h, and cooled down to rt. The reaction mixture was neutralized with Na_2CO_3. The crystals were filtered, washed with water, and recrystallized from water. Yield 1.05 g (53 %), mp 160.1–160.7 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.61 (t, 2H, J = 7.2 Hz, CH_2CN), 3.81 (t, 2H, J = 7.2 Hz, CH_2NH), 6.70–6.90 (m, 5H, H_{Ar}), 7.08-7.35 (m, 10H, $H_{Ar',Ar''}$), 7.47, 8.25 (2s, 2H, NHAr, NHAr'). IR (KBr), ν/cm^{-1}: 3285 (NH); 1708 (C=O). MS (ESI, 20V): m/z 400 $[M+H]^+$ (100%). Anal. Calcd for $C_{23}H_{21}N_5O_2$: C, 69.16; H, 5.30; N, 17.53. Found: C,

69.03; H, 5.38; N, 17.51.

***N*-[2-(5-Oxo-4-phenyl-4,5-dihydro-1*H*-1,2,4-triazol-3-yl)ethyl]-*N*-phenylacetamide (13).** A solution of **9** (0.17 g, 0.6 mmol) in acetic anhydride (10 mL) was refluxed for 12 h. The solvent was removed on a rotary evaporator. The product was crystallized from Et_2O, and purified by flash column chromatography (acetone:hexane 1:2). Yield 0.13 g (68 %), mp 132–133 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.91 (s, 3H, $COCH_3$), 2.61 (t, 2H, J = 7.2 Hz; CH_2CN), 3.64 (t, 2H, J = 7.2 Hz, CH_2N), 7.15–7.26 (m, 5H, H_{Ar}), 7.35–7.47 (m, 5H, $H_{Ar'}$), 11.69 (s, 1H, NH). ^{13}C NMR (75.4 MHz, DMSO-d_6): δ 168.92 (C-17), 154.19 (C-10), 144.41 (C-9), 142.45 (C-1), 132.66 (C-11), 129.52 (C-3, 5), 129.22 (C-14), 128.49 (C-13, 15), 127.20 (C-12, 16), 127.92 (C-4), 127.67 (C-2, 6), 45.32 (C-7), 24.31 (C-8), 22.25 (C-18). IR (KBr), ν/cm^{-1}: 3188 (NH), 1691 (C=O). MS (ESI, 20V): m/z 222 $[M+H]^+$ (40%). Anal. Calcd for $C_{18}H_{18}N_4O_2$: C, 67.07; H 5.63; N, 17.38. Found: C, 66.97; H, 5.70; N, 17.18.

***N*-Phenyl-5-[2-(phenylamino)ethyl]-1,3,4-thiadiazol-2-amine (14). a**) A mixture of **6** (1.57 g, 5 mmol) and dilute HCl (1:1, 10 mL) was refluxed for 1 h, and cooled down to rt. The reaction mixture was neutralized with Na_2CO_3 to pH 4–6. The crystals were filtered, and recrystallized from EtOH. Yield 0.79 g (53 %), mp 166–167 °C. **b)** To a concentrated H_2SO_4 (15 mL) **6** (0.79 g, 2.5 mmol) was added in portions, and the reaction mixture was stirred at rt for 3 h. Then it was added dropwise to an ice – water mixture. The crystals were filtered, and recrystallized from EtOH. Yield 0.73 g (97 %), mp 166–167 °C. **c**) A mixture of **5** (1.49 g, 5 mmol), phosphorus pentasulfide (1.33 g, 6 mmol), and xylene (40 mL) was refluxed for 2 h. The solvent was decanted; the product was crystallized from EtOAc, and washed with Et_2O. Yield 1.01 g (68 %), mp 166–167 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.35 (t, 2H, J = 7.35 Hz, NCH_2CH_2), 3.63 (t, 2H, J = 7.35 Hz, $NH\underline{CH_2}$), 6.98 (dt, 1H, J = 1.05, 7.35 Hz, H_{ar-4}), 7.22 (t, 1H, J = 7.05 Hz, H_{ar-14}), 7.41 (d, 2H, J = 7.2 Hz, $H_{ar-2,6}$), 7.32 (t, 2H, J = 7.5 Hz, $H_{ar-3,5}$), 7.63 (dd, 2H, J = 1.2, 7.6 Hz, $H_{ar-12,16}$), 7.38 (t, 2H, J = 7.5 Hz, $H_{Ar-13,15}$), 9.98 (br s, 2H, NH). ^{13}C NMR (75.4 MHz, DMSO-d_6): δ 164.54 (C-10), 155.65 (C-9), 140.57 (C-1), 139.46 (C-11), 129.58 (C-13, 15), 128.96 (C-3, 5), 121.75 (C-14), 120.12 (C-4), 117.33 (C-12, 16), 111.79 (C-2, 6), 46.91 (C-7), 26.91 (C-8). IR (KBr), ν/cm^{-1}: 3275 (NH); 1604 (CN); 693 (CSC). MS (ESI, 20V): m/z 297 $[M+H]^+$ (70%). Anal. Calcd for $C_{16}H_{16}N_4S$: C, 64.84; H, 5.44; N, 18.90. Found: C, 64.52; H, 5.28; N, 18.79.

***N*-Phenyl-*N*-[2-(4-phenyl-5-sulfanylidene-4,5-dihydro-1*H*-1,2,4-triazol-3-yl)ethyl]acetamide (15).** A solution of **10** (0.59 g, 2 mmol) in acetic anhydride (25 mL) was refluxed for 6 h. The solvent was removed on a rotary evaporator. The product was crystallized from EtOH. Yield 0.34 g (68 %), mp. 197–198 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.66 (s, 3H, CH_3), 2.65 (t, 2H, J = 6.9 Hz, CH_2CN), 3.69 (t, 2H, J = 6.9 Hz, CH_2N), 7.18 (d, 2H, J = 6.9 Hz, $H_{ar-2,6}$), 7.33 (t, 1H, J = 6.9 Hz, H_{ar-4}), 7.35 (t, 2H, J = 6.9 Hz, $H_{ar-3,5}$), 7.39–7.53 (m, 5H, $H_{Ar'}$), 13,76 (s, 1H, NH). ^{13}C NMR (75.4 MHz, DMSO-d_6): δ 167.65 (C-17), 173.22 (C-10), 149.71 (C-9), 143.18 (C-1), 142.26 (C-11), 129.59 (C-3, 5), 127.76 (C-14), 129.41

(C-13, 15), 127.94 (C-12, 16), 129.27 (C-4), 128.15 (C-2, 6), 45.35 (C-7), 33.44 (C-8), 22.24 (C-18). IR (KBr), ν/cm^{-1}: 3178 (NH), 1689 (C=O). MS (ESI, 20V): m/z 339 $[M+H]^+$ (30%). Anal. Calcd for $C_{18}H_{18}N_4OS$: C, 63.88; H, 5.36; N, 16.56. Found: C, 63.79; H 5.28; N 16.47.

Antimicrobial susceptibility tests. Antimicrobial and antifungal activity of new compounds was tested *in vitro* in these standard bacterial strains: *Staphylococcus aureus* ATCC 25923, *Enterococcus faecalis* ATCC 29212, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Klebsiella pneumoniae* ATCC 33495, *Proteus mirabilis* ATCC 12459, *Bacillus subtilis* ATCC 6633, and *Bacillus cereus* ATCC 8035, and fungal strain *Candida albicans* ATCC 60193 using a serial agar dilution and broth dilution method (Mueller-Hinton Agar II and Mueller-Hinton Broth II, BBL, Cockeysville, USA).

Standard cultures of nonsporic bacteria *Staphylococcus aureus*, *Enterococcus faecalis, Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae and Proteus mirabilis* were cultivated for 20–24 h at 35–37 °C in Mueller-Hinton Agar. A bacterial suspension was prepared from cultivated bacterial cultures in physiological solution according to turbidity standard 0.5 McFarland (10^7 CFU/mL).

Standard culture of sporic bacteria *Bacillus subtilis* and *Bacillus cereus* was cultivated for 7 days at 35–37 °C in Mueller-Hinton II Agar. After sporic bacteria culture had grown, it was washed away from the surface of the broth with sterile physiological solution, heated for 30 min at 70 °C and diluted till the concentration of spores in 1 mL ranged from $10x10^6$ to $100x10^6$.

The standard fungal culture *Candida albicans* was cultivated for 20–24 h at 30 °C in Mueller-Hinton II Agar. A fungal suspension was prepared from cultivated fungal cultures in physiological solution according to the turbidity standard 0.5 McFarland.

After inoculation with a multi-point inoculator delivering 1–2 µl, the final inoculum on the agar surface should be approximately 10^4 CFU/spot. For broth dilution MICs the final inoculum should be 10^5 CFU/mL.

The main solution of the synthesized compounds was prepared in DMSO. Dilutions of 1, 25, 50, 100, 150, 200, 250, 300, 350, 400, 450 and 500 µg/mL were carried out under aseptic conditions by transferring the necessary amount of the analyzed solution using a sterile pipette to Petri dish filled with 10 mL of Mueller-Hinton agar and to tubes filled with 2 mL of Mueller-Hinton broth.

The antimicrobial effect of the investigated compounds was determined as Minimum Inhibitory Concentration (MIC) in µg/mL.[15,16] The MIC was defined as the lowest concentration that showed no growth.

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