New Isochromans. 1. Synthesis and Antimicrobial Activity of 4-Substituted (±)-1*H*-Spiro[benzo[*c*]pyran-3(4*H*), 1'-cyclohexane]-1-ones

Milen G. Bogdanov[a], Blagovesta T. Gocheva[b], Darina B. Dimitrova[b] and Mariana D. Palamareva[a]*

[a]University of Sofia, Faculty of Chemistry, 1 James Bouchier Blvd., Sofia 1164, Bulgaria
[b]University of Sofia, Faculty of Biology, 8 Dragan Tsankov Blvd., Sofia 1164, Bulgaria
*E-mail: **mpalamareva@chem.uni-sofia.bg**

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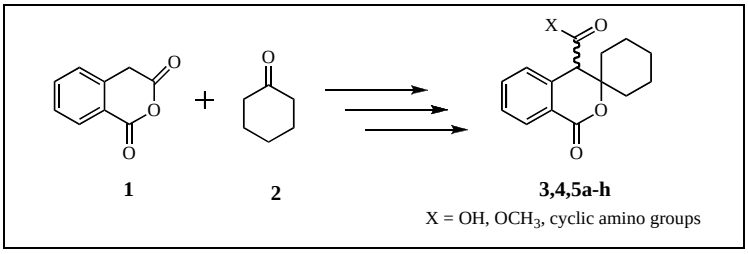

The reaction between homophthalic anhydride and cyclohexanone was examined both in the presence of DMAP or BF_3•Et_2O complex as a catalyst. The latter yielded (±)-1-oxo-1*H*-spiro[benzo[*c*]pyran-3(4*H*), 1'-cyclohexane]-4-carboxylic acid (**3**) in a higher yield (82 %). A series of new (±)-4-(*N*,*N*-disubstituted-1-carbamoyl)-1*H*-spiro[benzo[*c*]pyran-3(4*H*),1'-cyclohexane]-1-ones (**5a**-**h**) were synthesized from the parent acid **3** by a two-step reaction. Differentiating microbial screening was performed for most of the synthesized compounds against twelve microorganisms belonging to different taxonomic groups. The spiro acid **3** was active against all bacterial strains with MIC ≤ 20 μg/ml against *B. subtillis* and *P. vulgaris*. *E. coli* was the most sensitive strain to the antibacterial effect of the tested compounds.

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INTRODUCTION

The naturally occurring 1*H*-benzo[*c*]pyran-1-ones (3,4-dihydroisocoumarins) as well as their synthetic analogues [1,2] present a class of compounds possessing a broad spectrum of pharmacological activities such as antiulcer, [3] antiallergic, [4] antitumor, [5] antibacterial, [6] antifungal [7] *etc*. Thus, this class of compounds can be considered as a challenging target for the development of synthetic strategies and the evaluation of the biological activity of the derivatives.

Several approaches are used for synthesis of compounds containing 3,4-dihydroisocoumarin core. The most important of them are: a) epoxide-opening reaction of *ortho*-metallated benzamide derivatives, [8] b) Diels-Alder/retro-Diels-Alder sequence of acetylenic esters, [9] c) ring-cleavage of phthalides, [10] d) reaction of homophthalic acids with acidic chlorides and subsequent transformations [11] and e) reaction of homophthalic anhydrides with carbonyl compounds [12]. The cyclization proceeds in one step but usually the starting compounds are not easily prepared. Moreover, hard conditions are required for successful performance of the majority of the reactions. The only exception is the reaction between homophthalic anhydride **1** and a commercial carbonyl compound. The reaction proceeds under mild conditions and requires a suitable catalyst. Since now, the reaction has been performed in the presence of different basic or acidic catalysts [12-16]. In contrast to aldehydes, the cyclization of **1** with ketones has been less documented and the only data available are for acyclic and aromatic ketones in the presence of BF_3•Et_2O [13]. Until now, cyclic ketones are not used in this reaction.

Recently, we have demonstrated that the reaction between **1** and a series of aromatic aldehydes proceeds at mild conditions (rt, 2-3 h) in the presence of 4-dimethylaminopyridine (DMAP) as a catalyst, yielding benzopyrano cycloadducts [12]. DMAP has been proved to be a better catalyst than pyridine because of reduction of the by-products. In this paper we continue our attempts to specify further the scope and limitations of the reaction between homophthalic anhydride and compounds containing activated double bonds [17-21]. Our attention was focused on the reaction between homophthalic anhydride and cyclohexanone in the presence of different catalysts. Furthermore, in the context to define the influence of different substituents on the microbial activity, we synthesized some new compounds with a spiro coupling at position 3 and different substituents at position 4 of the dihydroisocoumarin core and carried out a comparative evaluation of their antimicrobial activity against twelve microorganisms belonging to different taxonomic groups.

RESULTS AND DISCUSSION

Trying to perform for the first time the reaction between homophthalic anhydride and cyclic ketones, we first used cyclization conditions similar to those applied for the reaction with aldehydes [12]. Thus, the reaction with cyclohexanone **2** was carried out at room temperature in the presence of the Lewis base DMAP in chloroform for 3 hours (Scheme 1). The consumption of **1** was been monitored by tlc and formation of a complex reaction mixture was detected. It is worth noting that the target acid **3** is already known by another protocol: it is obtained in a lower yield (42 %) and considerably greater reaction time (12 h) by cyclization of a halfester of cyclohexylidene homophthalic acid by refluxing it in a mixture of hydrobromic acid, acetic acid, and water [22]. In our case, the expected spiro acid **3** was isolated in a moderate yield (55 %) after column chromatography. Alternatively, the reaction was carried out in the presence of BF_3•Et_2O as a Lewis acid catalyst. After 8 h, the only product of the reaction (tlc) was the spiro acid **3** isolated in 82 % yield. Consequently, the BF_3•Et_2O complex has been proven to be a better catalyst than DMAP in the reaction of **1** with cyclic ketones. This phenomenon can be explained by the ability of the boron atom to form coordinative bonds [23]. According to the results, the reaction can be mechanicstically considered to proceed *via* initial formation of a six-membered cyclic transition state as expected by the Zimmerman-Traxler model [24,25]. Thus, BF_3 activates both homophthalic anhydride and cyclohexanone through coordination with their Lewis base centers (the oxygen atoms). Alternatively, the Lewis base DMAP activates only the homophthalic anhydride by reaction with its acidic proton in position 4.

Scheme 1 also gives the route of acid **3** to the target compounds **5a**-**h**. The presence of various pharmacophoric groups at position 4 of the isocoumarin moiety gives the possibility to enhance the chemical diversity of this class of compounds and is an additional point that can modify the expected biological activity. Amides of type **5** including **5b** are prepared through a different synthetic strategies. DCC has been used as an activation agent for the conversion of 3,4-dihydroisocoumarin-4-carboxylic acids to amides by Yu *et al*. [13]. Alternatively, compounds of type **5** have been obtained by reaction of 3-N-substituted 1*H*-benzo[*c*]pyran-1-ones and correspondding carbonyl compounds in boiling acetic acid [26,27]. Using the latter method Boyd *et al*. [27] synthesize compound **5b** for 9 days in 18 % yield. In our case, the transformation of **3** to the target compounds **5a**-**h** was performed similarly to the protocol of Haimova *et al*. [28] applied to the tetrahydroisoquinoline series. Thus, the acid **3** was converted into acylchloride in boiling benzene in the presence of thionyl chloride. Treatment of the later with cyclic secondary amines (NuH) gave the target products. The structures of the nucleophiles (Nu) and the yields of the target compounds **5a**-**h** are presented in Table 1. Moreover, we converted acid **3** into the corresponding methyl ester **4** by treatment with an etherial solution of diazomethane, since the traditional esterification lead to compounds with acyclic structure [12]. All synthesized compounds were isolated in solid state after recrystallization and were characterized by spectral methods (^{1}H nmr- and ir-spectra) and elemental analysis. The interpretation of the ^{1}H nmr spectra of the newly synthesized compounds is in agreement with the literature data for this class of compounds [27,29,30] and showed the following characteristic signals: the signals for H-4 proton appear as a singlet within 4.52-4.61 ppm; the signals for aliphatic protons from the spiro coupled chain appear in the region 1.10-2.00 ppm; the signals for proton H-8 appear at lower field (7.90-8.04) compared to the other aromatic protons. This phenomenon is an indication for proximity of H-8 to the lactone carbonyl group.

Scheme 1

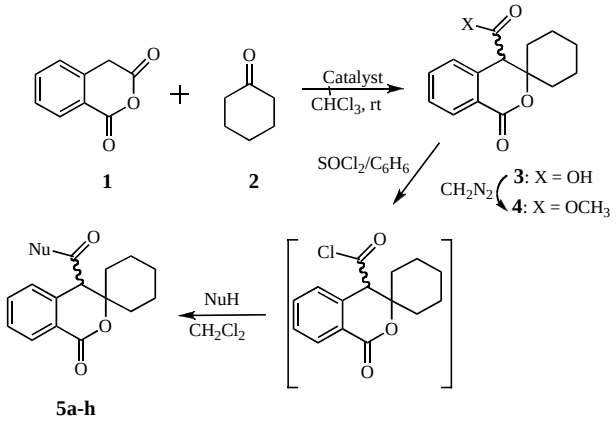
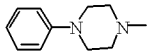
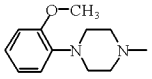
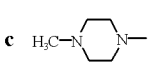
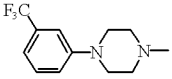
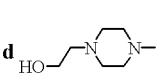
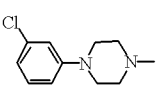


Table 1

Structures of the Nu group and the yields of compounds **5a-h**.

	Nu	Yield (%)		Nu	Yield (%)
a		76	**e**		48
b		68	**f**		52
c		78	**g**		83
d		87	**h**		78

Microbial assay: Seven of the newly synthesized compounds **3**,**4**,**5a**-**d**,**f** were initially screened for their *in*

Table 2

In Vitro Antimicrobial activity. Diameter of inhibition zone [mm] as a criterion for the antimicrobial activity[a] of the tested compounds.

Compounds	Microorganisms											
	Bacterial strains							Fungal strains				
	Gram positive			Gram negative								
						Phytopathogens						
	B. subtilis	*S. aureus*	*S. lutea*	*E. coli*	*P. vulgaris*	*E. amylovora*	*P. syringae*	*C. albicans*	*A. orizae*	*A. niger*	*F. oxysporum*	*P. notatum*
3	13[b]	11	10	12	15[b]	11	10	–	–	–	–	10
4	12	–	13[b]	12	11	–	–	–	–	10	–	–
5a	–	12	–	14[b]	–	–	–	–	–	–	11[b]	11
5b	–	–	–	–	–	–	–	–	–	–	–	–
5c	–	10	–	–	–	–	–	–	–	–	–	–
5d	–	11	–	9	12	–	–	–	–	–	11[b]	10
5f	–	–	–	10	12	–	–	–	–	–	–	11
Penicillin G	32	55	57	20	21	13	nd[c]	nd[c]	nd[c]	nd[c]	nd[c]	nd[c]

[a] Concentration 200μg/disc if not stated otherwise. [b] MIC≤ 20 μg/ml [c] Not Determine.

vitro antibacterial and antifungal activity against various test-microorganisms belonging to different taxonomic groups. The antibacterial activity was tested against the bacterial strains: *Bacillus subtillis* (1049 NBIMCC), *Staphylococcus aureus* (744 NBIMCC), *Sarcina lutea* (Gram-positive); *Esherichia coli* (1752 NBIMCC), *Proteus vulgaris* (1393 NBIMCC) and the phytopathogenic bacteria *Erwinia amylovora* (2331 NBIMCC) and *Pseudomonas syringae patovar siringae* (2420 NBIMCC) (Gram-negative). Antifungal activity was tested against *Candida albicans* (72 NBIMCC), *Aspergillus orizae* (118 NBIMCC), *Aspergillus niger* (1107 NBIMCC), *Fusarium oxysporum* (124 NBIMCC) and *Penicillium notatum*.

The microbial assay was carried out by the agar diffusion method. The compounds were tested both at concentration 20 and 200 μg/disc and the activity was evaluated by the diameter of the inhibition zone in mm. *Penicillin G* was used as a standard drug for comparison of the antibacterial activity. A solvent control was kept. The results obtained are summarized in Table 2.

In general, the tested compounds showed higher antibacterial than antifungal activity. *E. coli* was the most sensitive strain to the antibacterial effect of the tested compounds (5 of 7 compounds active) followed by *P. vulgaris* and *S. aureus* (4 compounds active), *S. lutea* and *B. subtillis* (2 compounds active.) whereas *E. amylovora* and *P. siringae* were the least sensitive organisms (1 compound active). The spiro acid **3** was active against all bacterial strains with MIC ≤ 20 μg/ml against *B. subtillis* and *P. vulgaris*. Also, the antibacterial screening data indicated that only two compounds of **4**,**5a**-**d**,**f** have a higher activity (MIC ≤ 20 μg/ml) than the parent acid **3**, namely, methyl ester **4** against Gram-positive *S. lutea* and amide **5a** against Gram-negative *E. coli*. Thus in the majority of the cases, the presence of an amide or ester group instead of the carboxylic function at position 4 in the dihydroisocoumarin core did not evoke antibacterial activity against the strains tested. It is worth noting that none of the tested compounds showed superior activity to the standard drug *Penicillin G*.

F. oxysporum was the most sensitive fungal test strain which was inhibited by compounds **5a**,**d** with MIC ≤ 20 μg/ml. Compounds **3**,**5a**,**d**,**f** exhibited very weak inhibition effect against *P. notatum* and methyl ester **4** against *A. niger*. None of the tested compounds exhibited any inhibitory effect against *C. albicans* and *A. orizae*. Consequently, the antifungal activity of the tested compounds is limited.

EXPERIMENTAL

Melting points were determined on a Kofler microscope Boetius PHMK 0.5 and are uncorrected. The ir spectra were acquired in chloroform, if not stated otherwise, on a Specord 75 and are reported in reciprocal centimeters. The ^{1}H nmr spectra were obtained on a DRX Bruker Avance NMR spectrometer at 250 MHz in corresponding solvent given in parentheses. The chemical shift is given in ppm (δ) relative to tetramethylsilane as internal standard. Elemental analyses were obtained in the relevant laboratories at the Faculty of Chemistry, University of Sofia. The tlc was done on precoated 0.2 mm Merck silica gel 60F_{254} plates. Merck silica gel 60 (0.040-0.063 mm) was used for column chromatography. The strains *S. lutea* and *P. notatum* were available in the Department of General and Industrial Microbiology, Biological Faculty, Sofia University. The remaining test microorganism strains were taken from National Bank for Industrial Microorganisms and Cell Cultures (NBIMCC), Bulgaria.

(±)-1-Oxo-1*H*-spiro[benzo(c)pyran-3(4*H*),1'-cyclohexane]-4-carboxylic acid (3). *DMAP catalyzed reaction*: To a mixture of homophthalic anhydride (1.24 g, 7.65 mmol) and 1.1 equiv. of cyclohexanone (0.84 g, 8.46 mmol) in dry chloroform (10 ml) DMAP 1 equiv. was added. The reaction mixture was stirred for

3 h at room temperature. At the end of the reaction (tlc) the obtained carboxylic acids were extracted with 10 % sodium hydrogen carbonate. The aqueous layer was acidified (pH = 3) with 10% hydrochloric acid and extracted with ethyl acetate. The organic layer was dried (sodium sulfate), filtered and the solvent was then evaporated under reduced pressure. The carboxylic acid **3** was obtained after column chromatography and crystallization from benzene. Yield: 1.10 g (55 %), mp 165-168° [Lit. mp 183-186° (from ethyl acetate-heptane)] [22]; ir (nujol): 1590-1620 cm^{-1} (ArH), 1710 cm^{-1} (C=O), 1740 cm^{-1} (C=O); ^{1}H nmr (DMSO-d6): δ 1.15-1.90 (m, 10H, *CH_2-aliphatic*), 4.61 (s, 1H, *H-4*), 7.10 (d, 1H, J = 7.5 Hz, *Ph-H*), 7.40-7.50 (dt, 1H, J = 1.5 and 7.5 Hz, *Ph-H*), 7.60 (dt, 1H, J = 1.3 and 7.5 Hz, *Ph-H*), 7.92 (dd, 1H, J = 1.5 and 7.5 Hz, *Ph-H*). *Anal.* Calcd. for $C_{15}H_{16}O_4$: C, 69.22; H, 6.20. Found: C, 69.55; H, 6.14.

BF_3•Et_2O catalyzed reaction: To a mixture of homophthalic anhydride (1.24 g, 7.65 mmol) and cyclohexanone (0.79 ml, 7.65 mmol) in 10 ml dry chloroform BF_3•Et_2O (9.6 ml, 10 equiv.) was added. The reaction mixture was stirred for 8 h at room temperature. At the end of the reaction (tlc) the reaction mixture was diluted with ethyl acetate and was washed with 10 % hydrochloric acid and then with water (pH = 7). The organic layer was dried (sodium sulfate), filtered and the solvent was then evaporated under reduced pressure. The carboxylic acid **3** was obtained as white crystals after crystallization from benzene. Yield: 1.64g (82 %), mp 165-168°.

Methyl ester of (±)-1-Oxo-1*H*-spiro[benzo[*c*]pyran-3(4*H*),1'-cyclohexane]-4-carboxylic acid (**4**). A stirring solution of **3** (0.50 g, 1.92 mmol) in 1 ml chloroform was treated with ether solution of diazomethane (1.13 mmol/ml). The mixture was stirred at room temperature for an hour. The excess of diazomethane and chloroform was removed under reduced pressure giving an oil. The later afforded **4** as colorless crystals (from ethyl acetate). Yield: 0.41 g (78 %), mp 98-100° (Lit. mp 102-103°) [30]; ir (nujol): 1590-1620 cm^{-1} (ArH), 1710 cm^{-1} (C=O), 1740 cm^{-1} (C=O); ^{1}H nmr (DMSO-d6): δ 1.15-1.90 (m, 10H, *CH_2-aliphatic*), 3.65 (s, 3H, *OCH_3*), 4.60 (s, 1H, *H-4*), 7.10 (d, 1H, J = 7.5 Hz, *Ph-H*), 7.40-7.50 (dt, 1H, J = 1.5 and 7.5 Hz, *Ph-H*), 7.60 (dt, 1H, J = 1.3 and 7.5 Hz, *Ph-H*), 7.92 (dd, 1H, J = 1.5 and 7.5 Hz, *Ph-H*). *Anal.* Calcd. for $C_{16}H_{18}O_4$: C, 70.06; H, 6.61. Found: C, 69.89; H, 6.75.

General procedure for the synthesis of compounds 5a-h. To a suspension of acid **3** in dry benzene, thionyl chloride (4 equiv.) was added. The reaction mixture was stirred at 80° for 3 h. Then, the solvents were evaporated under reduced pressure and the residue was dissolved in dichloromethane. The cooled solution (ice bath: 0°) was treated with the corresponding amine (3 equiv.) and was stirred for an hour. At the end of the reaction (tlc), the reaction mixture was diluted with ethyl acetate and was washed with water (pH = 7). The organic layer was dried (sodium sulfate), filtered and the solvent was then evaporated under reduced pressure. The products **5a**-**h** was isolated after recrystallization.

(±)-4-(Piperidine-1-ylcarbonyl)-1*H*-spiro[benzo(c)pyran-3(4*H*),1'-cyclohexane]-1-one (**5a**). This compound was obtained as colorless prisms from methanol (yield: 0.23 g, 58 %), mp 253-255°; NuH: piperidine; ir: 1590-1620 cm^{-1} (ArH), 1640 cm^{-1} (C=O), 1720 cm^{-1} (C=O); ^{1}H nmr (methanol-d4): δ 1.20-2.00 (m, 16H, *CH_2-aliphatic*, *H-11*, *H-12*, *H-13*), 3.35-4.00 (m, 4H, *H-9*, *H-10*), 4.64 (s, 1H, *H-4*), 7.29 (d, 1H, J = 7.5 Hz, *Ph-H*), 7.47 (dt, 1H, J = 1.5 and 7.5 Hz, *Ph-H*), 7.58 (dt, 1H, J = 1.5 and 7.5 Hz, *Ph-H*), 8.02 (dd, 1H, J = 1.5 and 7.5 Hz, *Ph-H*). *Anal.* Calcd. for $C_{20}H_{25}NO_3$: C, 73.37; H, 7.40. Found: C, 73.05; H, 7.75.

(±)-4-(Morpholine-4-ylcarbonyl)-1*H*-spiro[benzo(c)pyran-3(4*H*),1'-cyclohexane]-1-one (**5b**). This compound was obtained as white crystals from methanol (yield: 0.21 g, 37 %), mp 271-273° (Lit. mp 269-271°) [27]; NuH: morpholine; ir: 1590-1620 cm^{-1} (ArH), 1640 cm^{-1} (C=O), 1720 cm^{-1} (C=O); ^{1}H nmr (DMSO-d6): δ 1.15-1.85 (m, 10H, *CH_2-aliphatic*), 3.40-3.60 (m, 4H, *H-11*, *H-12*), 3.62-3.70 (m, 2H, *H-9*), 3.80-3.98 (m, 2H, *H-10*), 4.52 (s, 1H, *H-4*), 7.32 (d, 1H, J = 7.5 Hz, *Ph-H*), 7.45 (dt, 1H, J = 1.5 and 7.5 Hz, *Ph-H*), 7.60 (dt, 1H, J = 1.5 and 7.5 Hz, *Ph-H*), 7.90 (dd, 1H, J = 1.3 and 7.5 Hz, *Ph-H*). *Anal.* Calcd. for $C_{19}H_{23}NO_4$: C, 69.28; H, 7.04. Found: C, 69.36; H, 7.00.

(±)-4-(4-Methylpiperazine-1-ylcarbonyl)-1*H*-spiro[benzo-(c)pyran-3(4*H*),1'-cyclohexane]-1-one (**5c**). This compound was obtained as white crystals from methanol (yield: 0.21 g, 51 %), mp 256-259°; NuH: 4-methylpiperazine; ir: 1590-1620 cm^{-1} (ArH), 1640 cm^{-1} (C=O), 1720 cm^{-1} (C=O); ^{1}H nmr (DMSO-d6): δ 1.15-1.85 (m, 10H, *CH_2-aliphatic*), 2.21 (s, 3H, *NCH_3*), 2.25-2.55 (m, 4H, *H-11*, *H-12*), 3.60-3.70 (m, 2H, *H-9*), 3.70-4.00 (m, 2H, *H-10*), 4.54 (s, 1H, *H-4*), 7.29 (d, 1H, J = 7.5 Hz, *Ph-H*), 7.44 (t, 1H, J = 7.5 Hz, *Ph-H*), 7.60 (t, 1H, J = 7.5 Hz, *Ph-H*), 7.90 (d, 1H, J = 7.5 Hz, *Ph-H*). *Anal.* Calcd. for $C_{20}H_{26}N_2O_3$: C, 70.15; H, 7.65. Found: C, 70.48; H, 7.88.

(±)-4-(4-(2-Hydroxyethyl)-piperazine-1-ylcarbonyl)-1*H*-spiro[benzo(c)pyran-3(4*H*),1'-cyclohexane]-1-one (**5d**). This compound was obtained as yellow crystals from methanol (yield: 0.20 g, 50 %), mp 236-239°; NuH: 2-piperazinoethanol; ir: 1590-1620 cm^{-1} (ArH), 1640 cm^{-1} (C=O), 1720 cm^{-1} (C=O); ^{1}H nmr (methanol-d4): δ 1.20-2.00 (m, 10H, *CH_2-aliphatic*), 2.50-2.76 (m, 8H, *H-11*, *H-12*, *H-13, H-14*), 3.19-3.23 (m, 1H, *-OH*), 3.56-3.65 (m, 2H, *H-9*), 3.85-4.10 (m, 2H, *H-10*), 4.53 (s, 1H, *H-4*), 7.32 (d, 1H, J = 7.5 Hz, *Ph-H*), 7.47 (t, 1H, J = 7.5 Hz, *Ph-H*), 7.61 (t, 1H, J = 7.5 Hz, *Ph-H*), 8.03 (d, 1H, J = 7.5 Hz, *Ph-H*). *Anal.* Calcd. for $C_{20}H_{25}NO_3$: C, 73.37; H, 7.40. Found: C, 73.05; H, 7.75.

(±)-4-(4-Phenylpiperazine-1-ylcarbonyl)-1*H*-spiro[benzo-(c)pyran-3(4*H*),1'-cyclohexane]-1-one (**5e**). This compound was obtained as colorless prisms from ethyl acetate (yield: 0.35g, 70 %), mp 234-236°; NuH: phenylpiperazine; ir: 1590-1620 cm^{-1} (ArH), 1645 cm^{-1} (C=O), 1730 cm^{-1} (C=O); ^{1}H nmr (DMSO-d6): δ 1.15-1.90 (m, 10H, *CH_2-aliphatic*), 3.04-3.16 (m, 2H, *H-11*), 3.24-3.31 (m, 2H, *H-12*), 3.58-3.63 (m, 2H, *H-10*), 3.90-4.10 (m, 2H, *H-9*), 4.61 (s, 1H, *H-4*), 6.83 (t, 1H, J = 7.3 Hz, *Ph-H*), 6.99 (m, 2H, *Ph-H*), 7.21-7.28 (m, 2H, *Ph-H*), 7.34 (d, 1H, J = 7.5 Hz, *Ph-H*), 7.45 (dt, 1H, J = 1.5 and 7.5 Hz, *Ph-H*), 7.60 (dt, 1H, J = 1.5 and 7.5 Hz, *Ph-H*), 7.91 (dd, 1H, J = 1.3 and 7.8 Hz, *Ph-H*). *Anal.* Calcd. for $C_{25}H_{28}N_2O_3$: C, 74.23; H, 6.98. Found: C, 74.11; H, 6.77.

(±)-4-(4-(2-Methoxyphenyl)-piperazine-1-ylcarbonyl)-1*H*-spiro[benzo(c)pyran-3(4*H*),1'-cyclohexane]-1-one (**5f**). This compound was obtained as colorless prisms from methanol (yield: 0.23 g, 58 %), mp 180-183°; NuH: 2-methoxyphenyl-piperazine; ir: 1590-1620 cm^{-1} (ArH), 1645 cm^{-1} (C=O), 1710 cm^{-1} (C=O); ^{1}H nmr (methanol-d4): δ 1.25-1.90 (m, 10H, *CH_2-aliphatic*), 2.85-3.20 (m, 4H, *H-11*, *H-12*), 3.60-3.80 (m, 2H, *H-10*), 3.88 (s, 3H, *OCH_3*), 3.95-4.18 (m, 2H, *H-9*), 4.58 (s, 1H, *H-4*), 6.91-7.05 (m, 4H, *Ph-H*), 7.35 (d, 1H, J = 7.8 Hz, *Ph-H*), 7.48 (dt, 1H, J = 1.5 and 7.5 Hz, *Ph-H*), 7.62 (dt, 1H, J = 1.5 and 7.5 Hz, *Ph-H*), 8.04 (d, 1H, J = 7.8 Hz, *Ph-H*). *Anal.* Calcd. for $C_{26}H_{30}N_2O_4$: C, 71.87; H, 6.96. Found: C, 72.15; H, 7.18.

(±)-4-(4-(3-Chlorophenyl)-piperazine-1-ylcarbonyl)-1*H*-spiro[benzo(c)pyran-3(4*H*),1'-cyclohexane]-1-one (**5g**). This compound was obtained as colorless prisms from methanol (yield: 0.26 g, 67 %), mp 190-192°; NuH: 3-chlorophenyl-piperazine; ir: 1590-1620 cm^{-1} (ArH), 1640 cm^{-1} (C=O), 1730 cm^{-1} (C=O); ^{1}H nmr (methanol-d4): δ 1.25-2.05 (m, 16H, *CH_2-aliphatic*), 3.00-3.15 (m, 2H, *H-11*), 3.20-3.30 (m, 2H, *H-12*), 3.60-3.80 (m, 2H, *H-10*), 3.90-4.20 (m, 2H, *H-9*), 4.57 (s, 1H, *H-4*), 6.82-6.98 (m, 3H, *Ph-H*), 7.20 (t, 1H, J = 7.5 Hz, *Ph-H*), 7.34 (d, 1H, J = 7.5 Hz, *Ph-H*), 7.49 (t, 1H, J = 7.5 Hz, *Ph-H*), 7.62 (dt, 1H, J = 1.5 and 7.5 Hz, *Ph-H*), 8.04 (d, 1H, J = 7.5 Hz, *Ph-H*). *Anal*. Calcd. for $C_{25}H_{27}ClN_2O_3$: C, 68.41; H, 6.20. Found: C, 68.41; H, 6.19.

(±)-4-(4-(3-Trifluoromethylphenyl)-piperazine-1-ylcarbonyl)-1*H*-spiro[benzo(c)pyran-3(4*H*),1'-cyclohexane]-1-one (**5h**). This compound was obtained as colorless prisms from methanol (yield: 0.22 g, 58 %), mp 170-173°; NuH: 3-trifluoromethylphenyl-piperazine; ir: 1590-1620 cm^{-1} (ArH), 1645 cm^{-1} (C=O), 1720 cm^{-1} (C=O); ^{1}H nmr (DMSO-d6): δ 1.15-1.90 (m, 10H, *CH_2-aliphatic*), 3.10-3.32 (m, 2H, *H-11*), 3.35-3.45 (m, 2H, *H-12*), 3.52-3.69 (m, 2H, *H-10*), 4.03-4.10 (m, 2H, *H-9*), 4.61 (s, 1H, *H-4*), 7.10 (d, 1H, *Ph-H*), 7.20-7.38 (m, 3H, *Ph-H*), 7.40-7.50 (m, 2H, *Ph-H*), 7.60 (dt, 1H, J = 1.3 and 7.5 Hz, *Ph-H*), 7.92 (dd, 1H, J = 1.5 and 7.5 Hz, *Ph-H*). *Anal*. Calcd. for $C_{26}H_{27}F_3N_2O_3$: C, 66.09; H, 5.76. Found: C, 66.09; H, 5.75.

Agar diffusion method: Methanol solution was prepared for each compound. The pure culture suspensions of the studied microorganisms in concentration 10^8 CFU/ml are inoculated into 12 ml molten and tempered to 45°C nutrient agar (Difco) for the bacteria, beer agar for the yeast and moulds and potato dextrose agar (Oxoid) for the phytopathogenic bacteria. The inoculated media is poured into sterile petri dishes. After it set, 6 holes have been cut in each plate using a heat-sterilized cork borer. Corresponding quantities of the methanol solutions of each chemical compound have been spilled into the holes. These solutions should diffuse into the agar medium so the petri dishes stay 2 hours in refrigerator at 4°. Afterwards they are put into thermostat for 1 to 5 days at appropriate temperature for growing the test microorganisms. Antimicrobial activity is determined as diameter of the inhibitory zone, obtained after incubating in millimeters.

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