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Synthesis and Preliminary Biological Evaluation of New Heterocyclic Carboxamide Models

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Abstract: The heterocyclic system is a promising core nucleus in many bioactive compounds. This work describes our effort to synthesize and characterize a set of new biphenyl, benzofuran and benzothiophene carboxamide derivatives. Our biological studies showed that compounds **10** and **17** have antifungal activity against *C. galabrate* more potent than fluconazole compounds **9**, **10**, and **17** exerted cytotoxic activities in immortalized embryonic mouse fibroblast cells (3T3) and a human cervical cancer cell line (HeLa); in particular, the cyclic amidine derivative **17** showed selective toxicity against HeLa. This study showed that the tested compounds have the potential to be useful as antitumor drugs after further optimization.

Keywords: Antifungal activity, cervical cancer cell, cytotoxicity, heterocyclic compounds, NMR, Synthesis.

INTRODUCTION

Heterocyclic systems are initial starting point in designing of new compounds acting at different targets for the treatment of wide-ranging diseases. Benzofuran, benzothiophene and biphenyl systems have evolved as important heterocyclic structures due to their presence in a wide range of bioactive compounds. The benzofuran nucleus was recently reported as the basic system in the serotonergic antidepressant drug, Vilazodone [1]; the antifungal drug, Griseofulvin [2]; the entactogenic drug, 6-MAPB (1-(benzofuran-6-yl)-N-methylpropan-2-amine) [3]; the antiarrhythmic agent, Budiodarone [4]; and many others. On the other hand, benzothiophene has been a part of the chemical structure of many drugs such as Raloxifene [5], zileuton [6], sertaconazole [7], and BTCP (N -(1-(2-benzo[*b*]thiophenyl)cyclohexyl)-piperidine) [8].

The carboxamide functional group has an important role in many drugs [9-12]. It is highly used as a linker between a heterocyclic system and other substituents in many agents. For example, the benzofuran carboxamide is the major nucleus in the serotonergic antidepressant drug, Vilazodone [1] and the psychoactive drug, Befuraline [13]; the benzothiophene carboxamide on the other hand is the basic nucleus in the anti-asthma drug, Zileuton [6] and the drug Ibodutant [14] against irritable bowel syndrome. Carboxamide is also the key linking moiety in protein and peptide drugs such as anti-diabetic drugs Insulin [15] and Exenatide [16]. The presence of carbonyl groups allows amides to act as hydrogen-bond acceptors; in primary and secondary amides, they can serve as hydrogen-bond donors [17].

Biphenyls are important intermediates in organic chemistry, which give rise to various bioactive organic derivatives [18]. For example, some derivatives of biphenyl carboxamides (Fig. **1, I**) are commercial anti hypertension drugs such as Valsartan [19], while other derivatives are used as non-steroidal anti-inflammatory drug (NSAID) such as Diflunisal [20]. Many efforts have been conducted to explore new derivatives that exhibit biological activity such as diuretic and antibacterial effects; *N*-{substituted-1,3-benzothiazol-2-yl}-1,1'-biphenyl-4-carboxamides (Fig. **1, II**) [21] show anti-inflammatory activity; biphenyl-4-carboxylic acid 5-(arylidene)-2-(aryl)-4-oxothiazolidine-3-yl amides (Fig. **1, III**) [22] have antimicrobial activity and biphenyl-4-carboxylic acid-2-(aryl)-4-oxo-thiazolidin-3-yl amides [23] exhibit antidiabetic activity.

Additional research efforts are also being conducted to explore new derivatives of heterocyclic systems including benzofuran and benzothiophene (Fig. **2**); a number of their organic derivatives have been prepared that display a wide spectrum of bioactivities. For instance, benzofuran-2-carboxamides exhibit an excellent antiemetic action, antidopamine action and low toxicity [24] and new derivatives of pyridylbenzo[b]thiophene-2-carboxamides exhibit a strong

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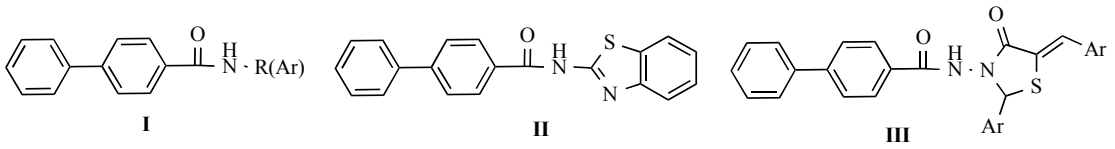


Fig. (1). Structures of representative biphenyl carboxamides.

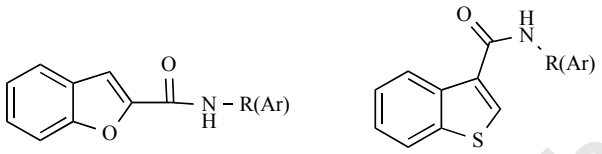
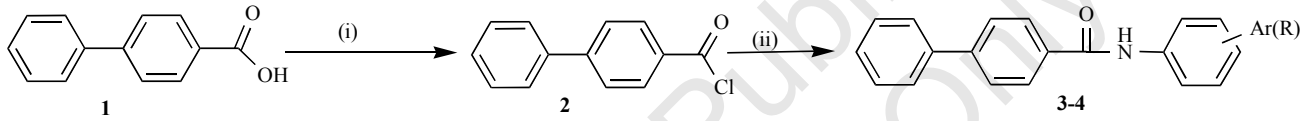


Fig. (2). Structures of representative benzofuran-2-carboxamides and benzothiophene-3-carboxamides.

Table 1. Synthesis of 4-biphenyl carbonyl chloride and its carboxamide derivatives.



Reactions and conditions: (i) $SOCl_2$, DCM, reflux, 6 h; (ii) Ar-NH_2, base, DMF, reflux, 24 h

Product	Ar (R)
3a	2-C(O)Ph
3b	3-C(O)Ph
3c	4-C(O)Ph
4a	2-Ac
4b	3-Ac
4c	4-Ac

growth inhibitory effect on several human cancer cell lines [25] and prominent antiproliferative effect [26].

In continuation of the current research for preparation of new high-impact antihyperlipidimic, antifungal and anticancer molecules related to fused heterocyclic molecules, our group has already reported the synthesis of {substituted}-1*H*-indole-2-carboxamide [27] and anthraquinone benzofuran-2-carboxamide [28]. Herein, we present the synthetic routes and spectroscopic characterization of various benzofuran/benzothiophene and biphenyl carboxamide derivatives followed by preliminary testing towards antifungal and antibacterial. Further, three representative compounds are examined for cytotoxic effects in tumor and immortalized cell lines *in vitro*.

Such rationale is defensible since the carboxamide derivatives of these systems have been reported to act against cervical cancer [29], as novel PARB anticancer compounds [30] and as new benzothiophene anticancer derivatives [31].

RESULTS AND DISCUSSION

Chemistry

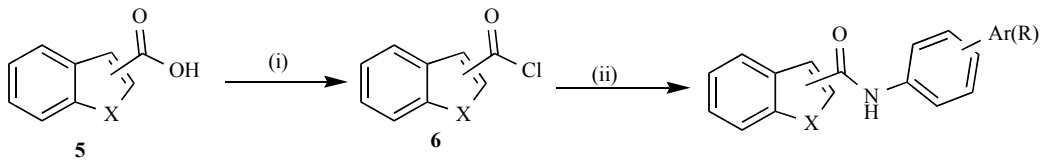
Aryl carbonyl chlorides **2**, **6a** (**2-O**) and **6b** (**3-S**) were prepared in good yield by the reaction of the corresponding carboxylic acid with an excess of thionyl chloride under reflux in anhydrous conditions (Tables **1-2**).

The target compounds **3a**, **4a**, **7a**, **7b**, **7c**, **8a**, **7f**, **7g** and **8d** were prepared by reacting the respective carbonyl chloride (**2**, **6a** and **6b**) with the corresponding 2-amino (*ortho*) aromatic derivative in the presence of strong base (ethoxide) in dry *N*,*N*-dimethylformamide under reflux. The role of sodium ethoxide is to catalyze the rate of the nucleophilic substitution reaction since the nucleophilicity strength of aromatic amines is relatively weak; in particular when the amino group is located at the *ortho* position [27, 28].

The other carboxamide derivatives **3b**, **3c**, **4b**, **4c**, **7d**, **7e**, **7h**, **7i**, **8b**, **8c**, **8e** and **8f** were prepared by applying the former methodology but using a soluble weak liquid base, triethylamine, instead of sodium ethoxide for the 3-amino and 4-amino aromatic derivatives (Tables **1-2**). The reaction progress was monitored throughout the experiment by thin layer chromatography (TLC) technique that was based on the disappearance of the starting material spot (aryl carbonyl chloride).

The use of sodium borohydride as a simple and selective reducing agent of ketones to the corresponding secondary alcohols is well known [32]. The aromatic alcohols **9-12** were prepared by the reduction of the corresponding aromatic ketones with sodium borohydride under mild condi-

Table 2. Synthesis of benzofuran/benzothiophene carbonyl chloride and their carboxamide derivatives.



5 **6** **7-8**

X: a = O, b = S

Reactions and conditions: (i) $SOCl_2$, DCM, reflux, 6 h; (ii) Ar-NH_2, base, DMF, reflux, 24 h

Product	2- or 3- /X	Ar (R)
7a	2/O	2-C(O)Ph
7b	2/O	2-[5-chloro]-C(O)Ph
7c	2/O	2-[4'-methyl]-C(O)Ph
7d	2/O	3-C(O)Ph
7e	2/O	4-C(O)Ph
7f	3/S	2-C(O)Ph
7g	3/S	2-[5-chloro]-C(O)Ph
7h	3/S	3-C(O)Ph
7i	3/S	4-C(O)Ph
8a	2/O	2-Ac
8b	2/O	3-Ac
8c	2/O	4-Ac
8d	3/S	2-Ac
8e	3/S	3-Ac
8f	3/S	4-Ac

tions. The respective alcohol products were obtained after acidic aqueous workup in excellent yields (Table **3**). Normally, a slight excess of the reducing agent is employed to counter the slight consumption by reaction with the incompletely dried solvent.

The reaction of aromatic ketones **7i**, **8c** and **8e** with either 2,4-dinitrophenylhydrazine (2,4-DNP) or thiosemicarbazide yielded the imine bond in **13**, **14**, **15** and **16** *via* the nucleophilic addition-elimination reaction; these reactions were conducted in alcoholic solution in the presence of small amounts of acid as catalyst. Due to the poor solubility of these products in the solvent, they precipitated from the reaction mixture and were separated by suction filtration.

Interestingly, the reaction of **16** with chloroacetic acid produced the novel 11-membered heterocyclic compound **17** that was characterized by NMR, MS and elemental analysis. The sulfur atom in **16** attacked the alpha (α) carbon in chloroacetic acid by the nucleophilic substitution (S_N^2) mechanism releasing the chlorine atom. In parallel, under these reaction conditions, a further intramolecular condensation took place *in situ* between the carbonyl group of the amide in **16** and the primary amine group, which led to amidine cyclization without the employment of any catalyst. Various heterocyclic amides react with primary or secondary amines in the presence of $TiCl_4$ under microwave to produce cyclic amidine products [33]. It is worth mentioning that the amidine functional group is important in medicinal chemistry as some amidine-containing molecules may act as potent antifungal prodrugs [34].

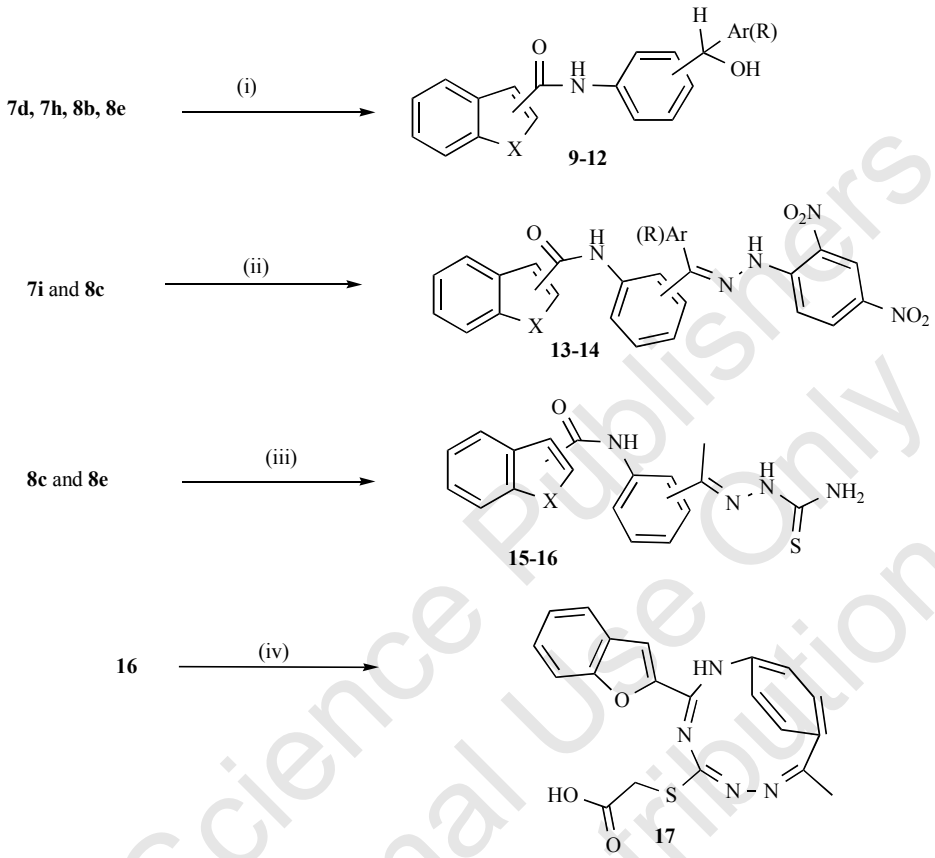
Biological Activity

Antifungal Activity

In order to examine the antifungal activity of some of the prepared compounds against a set of albicans and non-albicans strains, we conducted a preliminary study to determine the minimal inhibitory concentrations (MICs) using the adopted protocol according to the EUCAST [35].

Further, we compared their activities to that of a reference drug, namely fluconazole, with well established antifungal activity. MICs were determined for a collection of fungi consisting of strains (*C. albicans* DSMZ 11949, *C. glabrata* ATCC 90030, and *C. parapsilosis* ATCC 22019) as

Table 3. Synthesis of various benzofuran/benzothiophene carboxamide derivatives by reduction of ketone, formation of imine bond and intra-cyclization reaction.



Reactions and conditions: (i) $NaBH_4$, dry CH_3OH, DCM, r.t, 5 h; (ii) 2,4-DNP, HCl, CH_3OH, r.t, 6 h; (iii) thiosemicarbazide, glacial CH_3COOH, aq. EtOH, reflux, 6 h; (iv) $ClCH_2COOH$, AcONa, EtOH, reflux, 24 h

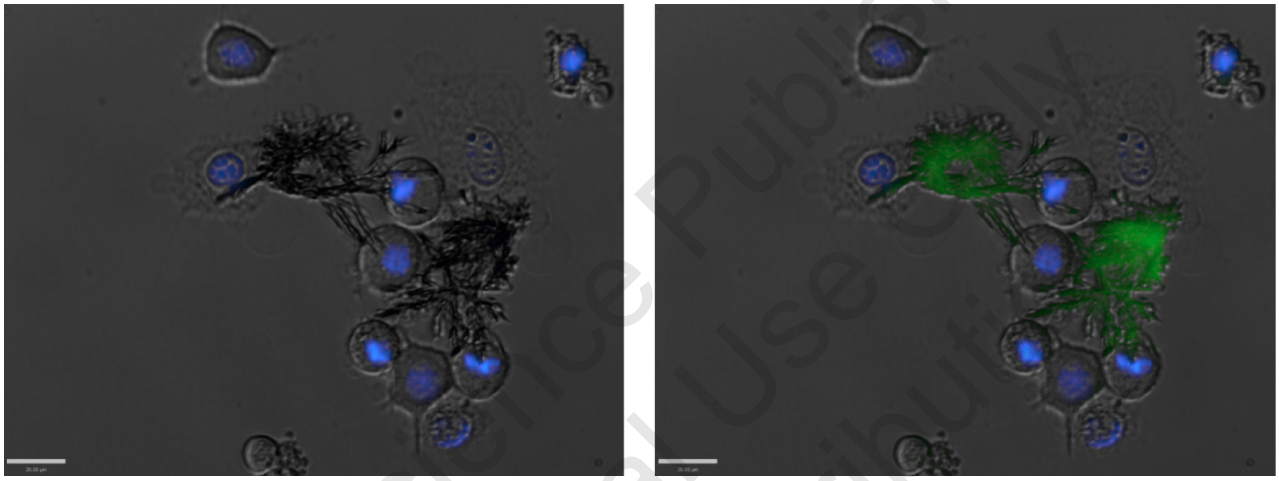
Product	X	Ar (R)
9	S	Methyl
10	O	Ph
11	S	Ph
12	O	Methyl
13	S	(3)-Ph
14	O	(4)-methyl
15	S	At position 3'
16	O	At position 4'

Table 4. *In Vitro* antifungal activity of some of the prepared compounds, expressed as MIC_{avs} (µM).

Compound	*C. albicans*	*C. galabrate*	*C. parapsilosis*	MIC_{av} for species
7g	327	262	262	284
7i	225	356	356	312
8f	16	128	128	90.7

Table 4. contd…

Compound	*C. albicans*	*C. galabrate*	*C. parapsilosis*	MIC_{av} for species
9	54	432	432	306
10	54	27	27	54
13	182.8	228.5	228.5	213.3
17	26	13	13	17.3
Fluconazole	8.4	33.4	5.2	15.7



A **B**

Fig. (3). Microscopic images of HeLa cells treated with 38 µM **17** for 18 h. Overlay of DIC image and blue fluorescence (Hoechst 33342, cell nuclei) (**A**). Additional overlay of the green channel displaying green fluorescence emission of the precipitated crystals of **17** (**B**).

described (Table **4**). The MIC_{av} values mentioned in the table were specifically for active compounds that reinforced the growth pattern of the albicans and non-albicans strains, while compounds **3b**, **4a**, **7f**, **8d**, **8e**, **11**, **15**, and **16** did not show any significant antifungal activity in the tested range of concentration. We measured a range of MICs from 0.125 to128 µg ml^{-1} for the strains with a median of 102.4 µg ml^{-1}. In particular, the MIC_{av} of the three strains was 47.25 µM, in which it was 48.32 µg ml^{-1} for the non albicans strains and was 45.18 µg ml^{-1} for *C. albicans* strains.

C. glabrata was the most susceptible non-albicans species (MIC_{av}, 43.7 µg ml^{-1}), whereas the rarely isolated *C. parapsilosis* was the most resistant species in our collection (MIC_{av}, 53.4 µg ml^{-1}). Most interestingly, the activity of compound **17** was higher than that of fluconazole against *C. glabrata* whereas it was in the two-half folds.

Antibacterial Activity

In order to elucidate the antibacterial activity of the prepared compounds a testing protocol was performed as described in the National Committee for Clinical Laboratory Standards (NCCLS) guidelines [36]. None of the anticipated compounds showed any significant antibacterial activity neither for gram positive nor for gram negative tested strains.

Cytotoxicity Study

The prepared compounds **9**, **10**, and **17** were selected to evaluate their cyctotoxic effect in vitro. Two cell lines were used, immortalized embryonic mouse fibroblast cells (3T3) and the human cervical cancer cell line HeLa. All three compounds showed cytotoxic effects in both cell lines after an incubation period of 18 h. However, precipitation of insoluble crystals was observed at higher concentrations of **10** and **17** (≥230 µM and ≥38 µM, respectively) (Fig. **3**). Interestingly, the precipitated crystals of **17** showed a strong green fluorescence on excitation with blue light (525 nm).

For both **10** and **17**, the cytotoxic effect is saturable at the respective highest achievable concentration in solution making it even impossible to estimate an EC_{50} for **10** in HeLa cells. Only compound **9** was completely soluble over the whole range of the applied concentrations thus showing a typical growth inhibition curve (Fig. **4**). Nevertheless, compound **17** with EC_{50} values of 10 and 31 µM in HeLa and 3T3 cells, respectively, displayed a significantly higher cytotoxicity compared to **9** and **10** (Fig. **4** and Table **5**). Only for this compound, the effect was about three times higher in the tumor cell line HeLa compared to the immortalized 3T3 cells, indicating a slight preferential toxic effect in cancer cells. No comparable preference could be observed for **9** and **10**.

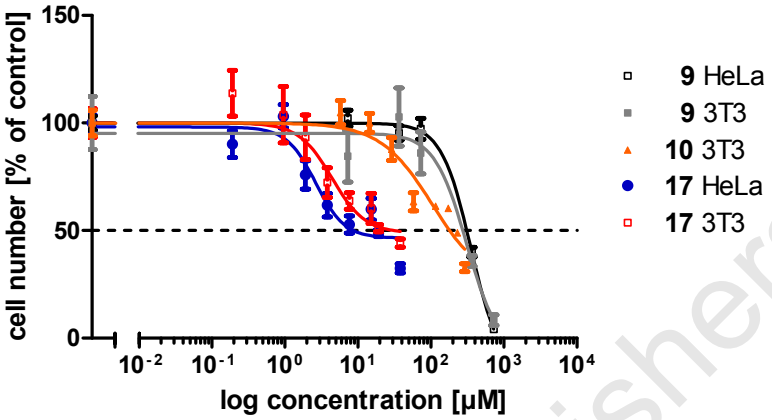


Fig. (4). Cytotoxic action of **9**, **10**, and **17** on HeLa and 3T3 cells.

Table 5. EC_{50} values for the cytotoxic action of 9, 10, and 17 on HeLa and 3T3 cells.

Compound	Cell Line	EC_{50} [µM]
9	HeLa	340
	3T3	267
10	HeLa	n.d.
	3T3	180
17	HeLa	10
	3T3	31

Values were determined using the non-linear fitting of the growth inhibition curves where applicable. Data represent mean of 1 – 3 independent experiments, n.d. not determinable; no EC_{50} could be determined because in none of the experiments the number of cells was reduced to near or below 50 % even at the highest possible concentration and therefore no reliable fit could be achieved.

The comparable low active concentration in case of **17** and its higher toxicity in tumor cells makes it a potential candidate for future evaluation of cytotoxic and cytostatic action in other cancer cell lines.

Cells were incubated as described in the experimental. Typical curves of single experiments are shown. Data represent mean ± SEM (n=6). No curve is presented for **10** in HeLa cells because in none of the experiments the number of cells was reduced to near or below 50 % even at the highest possible concentration and therefore, no reliable fit could be achieved.

CONCLUSION

We developed a new diverse library of heterocyclic carboxamide compounds with potential biological activity. Compounds **9**, **10**, and **17** showed *in vitro* inhibitory effect in immortalized embryonic mouse fibroblast cell (3T3) and human cervical cancer cell (HeLa) lines. Interestingly, **17** exerted a selective cytotoxic activity against HeLa cells.

The obtained data indicate that compound **17** might be a good candidate for further optimization toward a specific antitumor drug. In addition, heterocyclic carboxamides such as **8f, 9**, **10**, and **17** could pose a starting point for the development of potentially antifungal molecules. However, this requires further studies on the biological activity as well as computational studies that go beyond these preliminary evaluations.

EXPERIMENTAL

Materials and Instruments

All starting materials and reagents were purchased from Sigma-Aldrcih Co. and used without further purification. Experiments were performed in purified solvents and the progress of reactions was monitored by TLC using pre-coated silica gel glass plates (E. Merck Kiesegel 60 F_{254}, layer thickness 0.25 mm). Melting points were determined using a Stuart Scientific electrothermal melting point apparatus and were uncorrected. Nuclear magnetic resonance (NMR) ^{1}H NMR and ^{13}C spectra were acquired by a Bruker DRX 400 MHz instrument operating at 400.13 (^{1}H) and 100.61 MHz (^{13}C) relative to TMS as reference standard. Infrared (IR) spectra were recorded on an Avatar Thermo Nicolet Impact 400 FT-IR spectrophotometer using the Smart Omni-Transmission software; all samples were prepared as potassium bromide (Acros, Belgium) discs. The EI-Mass spectra were acquired on a Finnigan TQS 70, at 70 eV and 200°C or by electrospray ionization (ESI) in positive

mode on a Bruker APEX-4 (7 Tesla) instrument. Elemental analyses were obtained using Elemental analyzer model EA3000 A, Italy. Differential Interference Contrast (DIC) as well as fluorescence images (excitation wavelength 465 nm for Hoechst 33342, Ex 535 nm for green fluorescence) were made on a Zeiss Axiovert 200 M microscope (Zeiss, Goettingen, Germany) with a LD Plan NeoFluor 40X objective.

General Procedure for the Synthesis of Arylcarbonyl chlorides (2 and 6)

A mixture of the corresponding carboxylic acid (12.3 mmol) and thionyl chloride (6 ml, 83 mmol) in 40 ml of dry dichloromethane (DCM) was stirred under reflux for 6 h. After cooling to room temperature, DCM and excess thionyl chloride were evaporated under reduced pressure. The residue was treated without further purification.

Biphenyl-4-carbonyl Chloride 2 [37]

Yield: 85%; M.p 108-110 °C; ^{1}H-NMR ($CDCl_3$) δ (ppm): 7.36-7.40 (5H, Ar), 8.19-8.30 (4H, Ar'); IR (KBr): (cm^{-1}) = 3110, 1801, 1706.

Benzofuran-2-carbonyl Chloride 6a

Yield: 88%; M.p 55-57 °C; ^{1}H-NMR ($CDCl_3$) δ (ppm): 7.31-7.80 (4H, Ar), 8.21 (1H, H-3); IR (KBr): (cm^{-1}) = 3108, 1791, 1702.

Benzothiophene-3-carbonyl Chloride 6b

Yield: 90%; M.p 40-42 °C; ^{1}H-NMR ($CDCl_3$) δ (ppm): 7.21-8.38 (4H, Ar), 8.81 (1H, H-2); IR (KBr): (cm^{-1}) = 3112, 1799, 1711.

General Procedure for the Synthesis of Biphenyl-4-Carboxamide Derivatives 3a & 4a

A solution of **2** (4.4 mmol) was added to a solution of 2-aminobenzophenone or 2-aminoacetophenone (4.4 mmol) and sodium ethoxide (8.8 mmol) in dry *N*,*N*-dimethylformamide (DMF). The reaction mixture was refluxed for 24 h, and then cooled to room temperature. DMF was evaporated under reduced pressure and the residue was stirred for 10 min in chloroform and flashed through a short chromatography column (chloroform:methanol) in a ratio of (99.3:0.7). The solvent was removed under reduced pressure and the product dried in vacuo.

N-(2-Benzoylphenyl)biphenyl-4-carboxamide 3a

Yield: 36%; M.p 171-174 °C; ^{1}H-NMR ($CDCl_3$) δ (ppm): 7.11-7.20 (m, 3H), 7.38 – 7.54 (m, 8H), 7.61-7.70 (m, 5H), 7.73 (d, 1H, *J* = 8.0 Hz), 8.10 (d, 1H, *J* = 8.0 Hz), 11.93 (s, 1H, NH,); ^{13}C-NMR ($CDCl_3$) δ (ppm): 120.4, 122.5, 126.2, 126.9, 127.1, 127.4, 127.9, 128.0, 128.4, 128.9, 129.0, 129.1, 132.3, 133.4, 135.0, 138.9, 139.9, 143.7, 161.5, 199.0; IR (KBr) cm^{-1}: 1678, 1716 (CO), 3399 (NH); MS (EI / 70 eV): *m/z* (%) = 152 (51) $[M\text{-}CONHPhCOPh]^+$, 181 (100) $[M\text{-}NHPhCOPh]^+$, 378 (31) $[M+H]^+$; Anal. Calcd for $C_{26}H_{19}NO_2$ (377.14 g/mol): C, 82.74; H, 5.07; N, 3.71. Found: C, 82.44; H, 5.18; N, 3.34 %.

N-(2-Acetylphenyl)biphenyl-4-carboxamide 4a

Yield: 32%; M.p 179-181 °C; ^{1}H-NMR ($CDCl_3$) δ (ppm): 2.50 (s, 3H, CH_3), 7.15 – 7.21 (m, 1H), 7.41-7.47 (m, 1H), 7.48-7.55 (m, 2H), 7.64-7.69 (m, 3H), 7.76 (d, 2H, *J* = 8.0 Hz), 7.98 (d, 2H, *J* = 8.0 Hz), 8.16 (dd, 2H, *J* = 2.4, 8.0 Hz), 12.81 (s, 1H, NH,); ^{13}C-NMR ($CDCl_3$) δ (ppm): 28.6, 120.9, 122.0, 122.5, 127.3, 127.5, 128.1, 128.9, 131.9, 133.5, 135.4, 140.0, 141.5, 144.8, 165.9, 203.3; IR (KBr) cm^{-1}: 1673, 1720 (CO), 3339 (NH); MS (EI / 70 eV): *m/z* (%) = 152 (50) $[M\text{-}CONHPhCOCH_3]^+$, 181 (100) $[M\text{-}NHPhCOCH_3]^+$, 316 (33) $[M+H]^+$; Anal. Calcd for $C_{21}H_{17}NO_2$ (315.13 g/mol): C, 79.98; H, 5.43; N, 4.44. Found: C, 79.54; H, 5.31; N, 4.21 %.

General Procedure for the Synthesis of Biphenyl-4-carboxamide Derivatives 3(b-c) & 4 (b-c)

A solution of **2** (4.4 mmol) was added to a solution containing the specified aminobenzophenone (4.4 mmol) and triethylamine (1.3 ml, 8.8 mmol) in dry DMF. The reaction mixture was refluxed for 24 h, and then cooled to rt. DMF and excess triethylamine was evaporated under reduced pressure. To the residue, 20 ml of DCM was added and the solution was stirred for 10 min. at room temperature. The filtered solution was extracted with 10 ml of water. The organic layer was dried over sodium sulfate and evaporated to dryness. The residue was recrystallized from DCM/diethylether.

N-(3-Benzoylphenyl)biphenyl-4-carboxamide 3b [38]

Yield: 72%; M.p 156-158 °C; ^{1}H-NMR ($CDCl_3$) δ (ppm): 7.38 – 7.45 (m, 1H), 7.48-7.53 (m, 5H), 7.55-7.65 (m, 4H), 7.67 (d, 2H, *J* = 8.1 Hz), 7.79 (d, 2H, *J* = 7.9 Hz), 7.96-8.02 (dd, 2H, *J* = 4.1, 8.0 Hz), 7.96-8.02 (t, 1H, *J* = 2 Hz), 8.18 (qd, 1H, *J* = 1.0, 8.0 Hz), 10.41 (s, 1H, NH); ^{13}C-NMR ($CDCl_3$) δ (ppm): 121.5, 124.5, 126.2, 127.2, 127.4, 127.7, 128.2, 128.4, 129.0, 129.1, 130.1, 132.6, 133.2, 137.3, 138.3, 138.4, 139.8, 144.8, 165.8, 196.5; IR (KBr) cm^{-1}: 1673, 1711 (CO), 3411 (NH); MS (EI / 70 eV): *m/z* (%) = 152 (53) $[M\text{-}CONHPhCOPh]^+$, 181 (100) $[M\text{-}NHPhCOPh]^+$, 378 (28) $[M+H]^+$; Anal. Calcd for $C_{26}H_{19}NO_2$ (377.14 g/mol): C, 82.74; H, 5.07; N, 3.71. Found: C, 82.92; H, 5.29; N, 3.39 %.

N-(4-Benzoylphenyl)biphenyl-4-carboxamide 3c [39]

Yield: 84%; M.p 179-182 °C; ^{1}H-NMR ($CDCl_3$) δ (ppm): 7.41-7.49 (m, 2H), 7.51 – 7.59 (m, 6H), 7.64 (d, 2H, *J* = 8.0 Hz), 7.71 (d, 2H, *J* = 8.0 Hz), 7.74-7.82 (m, 2H), 7.84 (d, 2H, *J* = 8.0 Hz), 8.01 (d, 2H, *J* = 8.0), 10.62 (s, 1H, NH); ^{13}C-NMR ($CDCl_3$) δ (ppm): 117.3, 121.9, 127.2, 127.4, 128.5, 129.1, 129.8, 130.1, 130.8, 131.3, 131.8, 136.2, 137.2, 138.8, 146.0, 154.8, 193.3; IR (KBr) cm^{-1}: 1681, 1719 (CO), 3401 (NH); MS (EI / 70 eV): *m/z* (%) = 152 (55) $[M\text{-}CONHPhCOPh]^+$, 181 (100) $[M\text{-}NHPhCOPh]^+$, 378 (32) $[M+H]^+$; Anal. Calcd for $C_{26}H_{19}NO_2$ (377.14 g/mol): C, 82.74; H, 5.07; N, 3.71 Found: C, 82.41; H, 5.01; N, 3.29 %.

N-(3-Acetylphenyl)biphenyl-4-carboxamide 4b [38]

Yield: 75%; M.p 160-162 °C; ^{1}H-NMR ($CDCl_3$) δ (ppm): 2.51 (s, 3H, CH_3), 7.42 (d, 1H, *J* = 8.0 Hz), 7.43 – 7.55 (m, 3H), 7.58-7.64 (m, 2H), 7.67-7.75 (m, 2H), 7.77 (dd, 1H, *J* = 2.4, 8.1 Hz), 7.99 (d, 2H, *J* = 7.9 Hz), 8.12 (d, 1H, *J* = 8.0 Hz), 8.22 (s, 1H), 10.45 (s, 1H, NH); ^{13}C-NMR ($CDCl_3$) δ (ppm): 26.7, 119.8, 124.4, 125.0, 127.2, 127.4, 127.7, 128.2, 129.0, 129.4, 133.1, 137.8, 138.7, 139.8, 144.9, 165.8, 198.1; IR (KBr) cm^{-1}: 1669, 1728 (CO), 3339 (NH); MS (EI / 70 eV): *m/z* (%) = 152 (49) $[M\text{-}CONHPhCOCH_3]^+$, 181 (100) $[M\text{-}NHPhCOCH_3]^+$, 316 (31) $[M+H]^+$; Anal. Calcd for $C_{21}H_{17}NO_2$ (315.13 g/mol): C, 79.98; H, 5.43; N, 4.44. Found: C, 79.62; H, 5.41; N, 4.10 %.

N-(4-Acetylphenyl)biphenyl-4-carboxamide 4c [39]

Yield: 85%; M.p 185-187 °C; ^{1}H-NMR ($CDCl_3$) δ (ppm): 2.51 (s, 3H, CH_3), 7.21-7.29 (m, 2H), 7.40 – 7.52 (m, 3H), 7.54-7.67 (m, 2H), 7.69-7.79 (m, 2H), 7.82 (d, 2H, *J* = 8.1 Hz), 7.85-8.09 (m, 2H), 10.64 (s, 1H, NH); ^{13}C-NMR ($CDCl_3$) δ (ppm): 26.1, 119.0, 126.2, 127.4, 127.9, 128.0, 129.2, 129.7, 131.1, 134.9, 138.0, 143.8, 148.0, 166.8, 197.5; IR (KBr) cm^{-1}: 1665, 1716 (CO), 3323 (NH); MS (EI / 70 eV): *m/z* (%) = 152 (49) $[M\text{-}CONHPhCOCH_3]^+$, 181 (100) $[M\text{-}NHPhCOCH_3]^+$, 316 (28) $[M+H]^+$; Anal. Calcd for $C_{21}H_{17}NO_2$ (315.13 g/mol): C, 79.98; H, 5.43; N, 4.44. Found: C, 79.41; H, 5.82; N, 4.22 %.

General Procedure for the Synthesis of Benzofuran/benzothiophene Carboxamide Derivatives 7 (a, b, c, f, g) and 8 (a, d)

A solution of **6a** or **6b** (4.4 mmol) was added to a solution containing specified 2-aminobenzophenones or 2-aminoacetophenone (4.4 mmol) and sodium ethoxide (0.6 g, 8.8 mmol) in dry DMF. The reaction mixture was refluxed for 36 h, and then cooled to room temperature. DMF was evaporated under reduced pressure and the residue was stirred for 10 min in chloroform followed by purification using silica gel column chromatography (chloroform: methanol) in a ratio of (993:7) unless otherwise indicated.

N-(2-benzoylphenyl)benzofuran-2-carboxamide 7a [28]

Yield: 39%; M.p 168-170 °C; ^{1}H-NMR ($CDCl_3$) δ (ppm): 7.33-7.49 (m, 10H), 7.65 (d, 1H, *J* = 8.5 Hz), 7.70 (s, 1H), 7.73 (d, 1H, *J* = 8.1 Hz), 8.10 (d, 1H, *J* = 8.0 Hz), 12.62 (s, 1H, NH); ^{13}C-NMR ($CDCl_3$, 100.61MHz) δ (ppm): 112.4, 114.9, 117.1, 124.9, 125.2, 126.7, 127.3, 128.2, 129.4, 130.3, 131.8, 132.5, 133.2, 134.7, 138.2, 139.1, 150.3, 159.2, 160.1, 199.2; IR (KBr) cm^{-1}: 1669, 1709 (CO), 3381 (NH); MS (EI / 70 eV): *m/z* (%) = 118 (44) $[M\text{-}CONHPhCOPh]^+$, 146 (100) $[M\text{-}NHPhCOPh]^+$, 342 (25) $[M+H]^+$; Anal. Calcd for $C_{22}H_{15}NO_3$ (341.11 g/mol): C, 77.41; H, 4.43; N, 4.10. Found: C, 77.01; H, 4.78; N, 3.84.

N-(2-benzoyl-4-chlorophenyl)benzofuran-2-carboxamide 7b

Yield: 37%; M.p 194-196 °C; ^{1}H-NMR ($CDCl_3$) δ (ppm): 7.31-7.52 (m, 8H), 7.71 – 7.79 (m, 2H), 7.91 (s, 1H), 8.11 (d, 1H, *J* = 4.0 Hz), 8.92 (d, 1H, *J* = 8.1 Hz), 12.58 (s, 1H, NH,); ^{13}C-NMR ($CDCl_3$) δ (ppm): 112.1, 115.0, 119.1, 124.0, 124.9, 126.3, 128.1 130.2, 132.1, 132.7, 134.6, 135.2, 136.7, 139.0, 150.1, 159.0, 160.7, 203.2; IR (KBr) cm^{-1}: 1679, 1715 (CO), 3388 (NH); MS (EI / 70 eV): *m/z* (%) = 118 (40) $[M\text{-}CONHPhCOPhCl]^+$, 146 (100) $[M\text{-}NHPhCOPhCl]^+$, 376, 378 (37, 11) $[M+H]^+$; Anal. Calcd for $C_{22}H_{14}ClNO_3$ (375.07 g/mol): C, 70.31; H, 3.75; N, 3.73. Found: C, 70.11; H, 4.14; N, 3.46.

N-(2-(4-methylbenzoyl)phenyl)benzofuran-2-carboxamide 7c [28]

Yield: 45%; M.p 163-165 °C; ^{1}H-NMR ($CDCl_3$) δ (ppm): 7.23-7.51 (m, 9H), 7.64 (d, 1H, *J* = 8.5 Hz), 7.73 (s, 1H), 7.83 (d, 1H, *J* = 8.1 Hz), 8.15 (d, 1H, *J* = 8.0 Hz), 12.53 (s, 1H, NH); ^{13}C-NMR ($CDCl_3$) δ (ppm): 23.1, 112.7, 114.5, 117.3, 124.7, 125.3, 126.1, 127.9, 128.1, 129.1, 129.7, 130.1, 133.0, 138.0, 138.3, 139.6, 140.4, 141.2, 150.6, 159.2, 160.2, 199.7; IR (KBr) cm^{-1}: 1673, 1713 (CO), 3383 (NH); MS (EI / 70 eV): *m/z* (%) = 118 (39) $[M\text{-}CONHPhCOPhCH_3]^+$, 146 (100) $[M\text{-}NHPhCOPhCH_3]^+$, 356 (29) $[M+H]^+$; Anal. Calcd for $C_{23}H_{17}NO_3$ (355.12 g/mol): C, 77.73; H, 4.82; N, 3.94. Found: C, 77.41; H, 5.08; N, 3.63.

N-(2-benzoylphenyl)benzo[b]thiophene-3-carboxamide 7f

Yield: 42%; M.p 129-132 °C; Column-Chromatography: $CHCl_3$; ^{1}H-NMR ($CDCl_3$) δ (ppm): 7.09-7.25 (m, 7H), 7.35 (d, 1H, *J* = 9.0 Hz), 7.43 (d, 1H, *J* = 9.0 Hz), 7.48 (d, 1H, *J* = 9.0 Hz), 7.53 (d, 1H, *J* = 9.0 Hz), 7.75 (d, 1H, *J* = 9.0 Hz), 8.53 (s, 1H), 8.78 (d, s, d, 1H, *J* = 9.0 Hz), 12.02 (s, 1H, NH); ^{13}C-NMR δ (ppm): 119.0, 120.5, 122.1, 123.0, 123.5, 124.9, 127.8, 128.6, 132.6, 134.8, 135.5, 137.1, 140.4, 140.8, 141.3, 142.8, 163.3, 196.0; IR (KBr) cm^{-1}: 1665, 1713 (CO), 3388 (NH); MS (EI / 70 eV): *m/z* (%) = 134 (41) $[M\text{-}CONHPhCOPh]^+$, 162 (100) $[M\text{-}NHPhCOPh]^+$, 358 (31) $[M+H]^+$; Anal. Calcd for $C_{22}H_{15}NO_2S$ (357.08 g/mol): C, 73.93; H, 4.23; N, 3.92. Found: C, 73.61; H, 4.58; N, 3.64.

N-(2-benzoyl-4-chlorophenyl)benzo[b]thiophene-3-carboxamide 7g

Yield: 35%; M.p 125-127 °C; Column-Chromatography: $CHCl_3$; ^{1}H-NMR ($CDCl_3$) δ (ppm): 7.41-7.68 (m, 7H), 7.70-7.80 (m, 2H), 7.89 (s, 1H), 7.92-7.99 (m, 2H), 8.55 (s, 1H), 12.04 (s, 1H, NH); ^{13}C-NMR ($CDCl_3$) δ (ppm): 119.4, 120.3, 121.5, 123.0, 123.7, 124.8, 127.1, 128.4, 132.1, 135.6, 136.0, 137.0, 140.6 141.1, 141.5, 142.9, 162.1, 196.3; IR (KBr) cm^{-1}: 1669, 1713 (CO), 3391 (NH); MS (EI / 70 eV): *m/z* (%) = 134 (32) $[M\text{-}CONHPhCOPhCl]^+$, 162 (100) $[M\text{-}NHPhCO\text{-}PhCl]^+$, 393, 395 (29, 11) $[M+H]^+$; Anal. Calcd for $C_{22}H_{14}ClNO_2S$ (391.04 g/mol): C, 67.43; H, 3.60; N, 3.57. Found: C, 67.01; H, 4.01; N, 3.36.

N-(2-acetylphenyl)benzofuran-2-carboxamide 8a [28]

Yield: 44%; M.p 204-206 °C; Column-Chromatography: $CHCl_3$; ^{1}H-NMR ($CDCl_3$) δ (ppm): 2. 51(s, 3H, $COCH_3$), 7.31 – 7.40 (m, 5H), 7.68 (d, 1H, *J* = 8.4 Hz), 7.78 (d, 1H, *J* = 8.1 Hz), 7.85 (s, 1H), 8.10 (d, 1H, *J* = 8.0 Hz), 12.54 (s, 1H, NH); ^{13}C-NMR ($CDCl_3$) δ (ppm): 27.1, 112.0, 113.8, 116.9, 124.5, 125.2, 127.1, 128.2, 129.9, 130.1, 136.1, 138.0,

140.1, 150.5, 159.0, 162.1, 204.2; IR (KBr) cm^{-1}: 1661, 1715 (CO), 3370 (NH); MS (EI / 70 eV): *m/z* (%) = 118 (48) $[M-CONHPhCOCH_3]^+$, 146 (100) $[M-NHPhCOCH_3]^+$, 280 (29) $[M+H]^+$; Anal. Calcd for $C_{17}H_{13}NO_3$ (279.09 g/mol): C, 73.11; H, 4.69; N, 5.02. Found: C, 72.67; H, 4.98; N, 4.71.

N-(2-acetylphenyl)benzo[b]thiophene-3-carboxamide 8d

Yield: 44%; M.p 147-149 °C; Column-Chromatography: $CHCl_3$:CH_3OH (98:2); ^{1}H-NMR ($CDCl_3$) δ (ppm): 2.52(s, 3H, CH_3), 7.03 – 7.11 (m, 2H), 7.22-7.39 (m, 2H), 7.61 (d, 1H, *J* = 9 Hz), 7.81 (d, 1H, *J* = 9 Hz), 8.10 (d, 1H, *J* = 9.0 Hz), 8.41 (d, 1H, *J* = 9 Hz), 8.54 (s, 1H), 12.01 (s, 1H, NH); ^{13}C-NMR ($CDCl_3$) δ (ppm): 28.0, 118.1, 119.4, 120.2, 124.5, 125.0, 125.6, 129.1, 130.5, 131.7, 138.0, 138.2, 1395, 140.1, 144.6, 162.1, 198.8.; IR (KBr) cm^{-1}: 1669, 1710 (CO), 3373 (NH); MS (EI / 70 eV): *m/z* (%) = 134 (60) $[M-CONHPhCOCH_3]^+$, 162 (100) $[M-NHPhCOCH_3]^+$, 296 (25) $[M+H]^+$; Anal. Calcd for $C_{17}H_{13}NO_2S$ (295.07 g/mol): C, 69.13; H, 4.44; N, 4.74. Found: C, 68.77; H, 4.65; N, 4.31.

General Procedure for the Synthesis of Benzofuran/benzothiophene Carboxamide Derivatives 7 (d, e, h, i) and 8 (b, c, e, f)

A solution of **6** (4.4 mmol) was added to a solution containing 3- or 4-aminobenzophenone or (3- or 4- aminoacetophenone) (4.4 mmol) and triethylamine (1.3 ml, 8.8 mmol) in dry DMF. The reaction mixture was refluxed for 24 h and then cooled to room temperature. DMF and excess triethylamine were evaporated under reduced pressure. The residue was extracted with dichloromethane and water; the organic layer was separated then dried over anhydrous sodium sulfate, filtered and evaporated to dryness. The residue was purified using silica gel column chromatography (dichloromethane/diethylether) in a ratio of (993:7) unless otherwise indicated.

N-(3-benzoylphenyl)benzofuran-2-carboxamide 7d [28]

Yield: 81%; M.p 119-121 °C; Column-Chromatography: $CHCl_3$:CH_3OH (98:2); ^{1}H-NMR ($CDCl_3$) δ (ppm): 7.36 – 7.42 (m, 6H), 7.59 (d, 1H, *J* = 8.1 Hz), 7.63-7.69 (m, 2H), 7.71(d, 1H, *J* = 8.4), 7.79 (s, 1H), 7.92 (d, 1H, *J* = 8.0 Hz), 8.02 (dd, 1H, *J* = 2.4, 8.1 Hz), 10.38 (s, 1H, NH); ^{13}C-NMR ($CDCl_3$) δ (ppm): 113.1, 114.8, 119.5, 121.1, 121.7, 124.5, 125.1, 126.0, 126.8, 128.9, 130.5, 131.7, 132.4, 134.7, 136.0, 137.9, 140.8, 143.3, 150.2, 158.3, 159.0, 197.1; IR (KBr) cm^{-1}: 1672, 1711 (CO), 3378 (NH); MS (EI / 70 eV): *m/z* (%) = 118 (43) $[M-CONHPhCOPh]^+$, 146 (100) $[M-NHPhCOPh]^+$, 342 (19) $[M+H]^+$; Anal. Calcd for $C_{22}H_{15}NO_3$ (341.11 g/mol): C, 77.41; H, 4.43; N, 4.10. Found: C, 77.10; H, 4.82; N, 3.72.

N-(4-benzoylphenyl)benzofuran-2-carboxamide 7e [40]

Yield: 51%; M.p 258-260 °C; ^{1}H-NMR ($CDCl_3$) δ (ppm): 7.30-7.58 (8H, m, Ar-H), 7.62 (1H, s, H-3), 7.70 (2H, d, *J* = 2.6 Hz, Ar-H), 7.79 (1H, d, *J* = 8.6 Hz Ar-H), 7.87 (2H, m, Ar-H) 10.63 (1H, s, NH);); ^{13}C-NMR ($CDCl_3$) δ (ppm): 111.2, 116.7, 118.4, 121.9, 123.4, 125.1, 125.8, 127.0, 127.4, 128.7, 131.5, 131.9, 132.9, 135.1, 135.9, 138.3, 141.4, 142.5, 149.2, 157.5, 160.2, 198.0; IR (KBr) cm^{-1}: 1681, 1729 (CO), 3395 (NH); MS (CI/ESI -ve): *m/z* (%) = 341 (18), 340 (100), 237 (34); Anal. Calcd for $C_{22}H_{15}NO_3$ (341.11 g/mol): C, 77.41; H, 4.43; N, 4.10. Found: C, 77.01; H, 4.80; N, 3.72 %.

N-(3-benzoylphenyl)benzo[b]thiophene-3-carboxamide 7h

Yield: 62%; M.p 146-148 °C; ^{1}H-NMR ($CDCl_3$) δ (ppm): 7.33 – 7.53 (m, 6H), 7.59 (d, 1H, *J* = 9.0 Hz), 7.63 (d, 1H, *J* = 8.6 Hz), 7.72 (s, 1H), 7.80-8.04 (m, 3H), 8.27 (dd, 1H, *J* = 3.1, *J* = 9.0 Hz), 8.52 (s, 1H), 10.48 (s, 1H, NH); ^{13}C-NMR ($CDCl_3$) δ (ppm): 119.1, 120.9, 121.8, 122.1, 123.1, 123.7, 124.0, 124.7, 128.0, 128.5, 130.2, 131.4, 133.0, 135.1, 135.8, 136.0, 140.3, 140.9, 141.9, 142.3, 162.2, 195.9; IR (KBr) cm^{-1}: 1675, 1719 (CO), 3380 (NH); MS (EI / 70 eV): *m/z* (%) = 134 (39) $[M-CONHPhCOPh]^+$, 162 (100) $[M-NHPhCOPh]^+$, 358 (25) $[M+H]^+$; Anal. Calcd for $C_{22}H_{15}NO_2S$ (357.08 g/mol): C, 73.93; H, 4.23; N, 3.92. Found: C, 73.60; H, 4.51; N, 3.73.

N-(4-benzoylphenyl)benzo[b]thiophene-3-carboxamide 7i

Yield: 75%; M.p 201-203 °C; ^{1}H-NMR ($CDCl_3$) δ (ppm): 7.30 – 7.53 (m, 7H), 7.88 (dd, 2H, *J* = 3.1, *J* = 9.0 Hz), 7.96 (dd, 2H, *J* = 3.1, *J* = 9.0 Hz), 8.04 (d, 1H, *J* = 9.0 Hz), 8.31 (d, 1H, *J* = 8.6 Hz), 8.53 (s, 1H), 10.61 (s, 1H, NH); ^{13}C-NMR ($CDCl_3$) δ (ppm): 119.3, 120.5, 122.4, 123.7, 124.2, 124.9, 125.4, 126.6, 128.9, 129.4, 130.4, 131.5, 132.3, 133.2, 136.5, 139.6, 140.4, 141.2, 145.2, 164.1, 197.5; IR (KBr) cm^{-1}: 1679, 1717 (CO), 3377 (NH); MS (EI / 70 eV): *m/z* (%) = 134 (37) $[M-CONHPhCOPh]^+$, 162 (100) $[M-NHPhCOPh]^+$, 358 (22) $[M+H]^+$; Anal. Calcd for $C_{22}H_{15}NO_2S$ (357.08 g/mol): C, 73.93; H, 4.23; N, 3.92. Found: C, 73.63; H, 4.55; N, 3.67.

N-(3-acetylphenyl)benzofuran-2-carboxamide 8b [28]

Yield: 79%; M.p 125-127 °C; ^{1}H-NMR ($CDCl_3$) δ (ppm): 2.50 (s, 3H, CH_3), 7.30 -7.39 (m, 3H), 7.60 (d, 1H, *J* = 8.1 Hz), 7.69 (d, 1H, *J* = 8.5 Hz), 7.75 (s, 1H), 7.90 (d, 1H, *J* = 8.1 Hz), 8.05 (dd, 1H, *J* = 2.4, 8.1 Hz), 8.31 (s, 1H), 10.45 (s, 1H, NH); ^{13}C-NMR ($CDCl_3$) δ (ppm): 26.2, 113.9, 114.9, 119.8, 120.3, 122.9, 123.5, 124.1, 129.9, 130.4, 138.2, 141.1, 142.1, 150.1 158.0, 164.1, 202.1; IR (KBr) cm^{-1}: 1678, 1719 (CO), 3381 (NH); MS (EI / 70 eV): *m/z* (%) = 118 (47) $[M-CONHPhCOCH_3]^+$, 146 (100) $[M-NHPhCOCH_3]^+$, 280 (21) $[M+H]^+$; Anal. Calcd for $C_{17}H_{13}NO_3$ (279.09 g/mol): C, 73.11; H, 4.69; N, 5.02. Found: C, 72.72; H, 4.91; N, 4.80.

N-(3-acetylphenyl)benzofuran-2-carboxamide 8c [28]

Yield: 80%; M.p 199-201 °C; ^{1}H-NMR ($CDCl_3$) δ (ppm): 2.51 (s, 3H, CH_3), 7.21-7.28 (m, 2H), 7.31-7.45 (m, 3H), 7.70 (d, 1H, *J* = 8.5 Hz), 7.76 (s, 1H), 7.85-7.96 (m, 2H), 10.64 (s, 1H, NH); ^{13}C-NMR ($CDCl_3$) δ (ppm): 26.1, 113.1, 114.6, 118.2, 123.2, 123.9, 127.4, 129.1, 130.1, 132.6, 134.8, 140.8, 143.9, 148.1, 158.2, 166.1, 196.1; IR (KBr) cm^{-1}: 1679, 1715 (CO), 3372 (NH); MS (EI / 70 eV): *m/z* (%) = 118 (46) $[M-CONHPhCOCH_3]^+$, 146 (100) $[M-NHPhCOCH_3]^+$, 280 (24) $[M+H]^+$; Anal. Calcd for $C_{17}H_{13}NO_3$ (279.09 g/mol): C, 73.11; H, 4.69; N, 5.02. Found: C, 72.83; H, 4.85; N, 4.91.

N-(3-acetylphenyl)benzo[b]thiophene-3-carboxamide 8e

Yield: 79%; M.p 79-82 °C; ^{1}H-NMR ($CDCl_3$) δ (ppm): 2.50 (s, 3H, CH_3), 7.36 -7.44 (m, 2H), 7.47 (s, 1H), 7.62 (d, 1H, *J* = 9.0 Hz), 7.91-7.99 (m, 2H), 8.29 (dd, 1H, *J* = 3.1, *J* = 9.0 Hz), 8.34 (d, 1H, *J* = 9.0 Hz), 8.51 (s, 1H), 10.51 (s, 1H, NH); ^{13}C-NMR ($CDCl_3$) δ (ppm): 27.2, 118.4, 119.5, 120.1, 124.7, 125.4, 125.6, 129.7, 131.0, 132.8, 137.4, 137.7, 139.7, 140.0, 147.7, 162.7, 198.8; IR (KBr) cm^{-1}: 1670, 1711 (CO), 3379 (NH); MS (EI / 70 eV): *m/z* (%) = 134 (55) [M-$CONHPhCOCH_3$]$^+$, 162 (100) [M-$NHPhCOCH_3$]$^+$, 296 (29) [M+H]$^+$; Anal. Calcd for $C_{17}H_{13}NO_2S$ (295.07 g/mol): C, 69.13; H, 4.44; N, 4.74. Found: C, 68.76; H, 4.78; N, 4.50.

N-(4-acetylphenyl)benzo[b]thiophene-3-carboxamide 8f

Yield: 80%; M.p 154-157 °C; ^{1}H-NMR ($CDCl_3$) δ (ppm): 2.30 (s, 3H, CH_3), 7.42-7.50 (m, 2H), 7.88 (dd, 1H, *J* = 3.1, *J* = 9.0 Hz), 7.96 (dd, 1H, *J* = 3.0, *J* = 9.0 Hz), 8.04 (d, 1H, *J* = 9.1 Hz), 8.38 (d, 1H, *J* = 9.0 Hz), 8.45 (s, 1H), 10.64 (s, 1H, NH); ^{13}C-NMR ($CDCl_3$) δ (ppm): 27.2, 120.3, 124.9, 125.5, 126.0, 130.2, 131.2, 132.9, 133.4, 137.7, 140.2, 144.2, 163.1, 198.2; IR (KBr) cm^{-1}: 1674, 1719 (CO), 3379 (NH); MS (EI / 70 eV): *m/z* (%) = 134 (53) [M-$CONHPhCOCH_3$]$^+$, 162 (100) [M-$NHPhCOCH_3$]$^+$, 296 (30) [M+H]$^+$; Anal. Calcd for $C_{17}H_{13}NO_2S$ (295.07 g/mol): C, 69.13; H, 4.44; N, 4.74. Found: C, 69.83; H, 4.63; N, 4.39.

General procedure for the synthesis of 10, 11, 12 and 9

A known portion of sodium borohydride (1.2 mmol) was added to a solution containing the corresponding carboxamide (**7d**, **7h**, **8b** and **8e)** (1.0 mmol) in dry methanol (15 ml). The reaction mixture was stirred for 5 h at room temperature then excess methanol was evaporated under reduced pressure. The residue was treated with diluted solution of hydrochloric acid and a precipitate was formed. Suction filtration of the precipitate was done followed by drying under reduced pressure.

N-(3-(1-hydroxyethyl)phenyl)benzo[b]thiophene-3-carboxamide 9

Yield: 84%; M.p 109-111 °C; ^{1}H-NMR ($CDCl_3$) δ (ppm): 1.33 (d, 3H, *J* = 9 Hz), 2.52 (s br, 1H, OH), 4.71 (q, 1H, *J* = 9.0 Hz), 7.37 -7.48 (m, 2H), 7.52 (s, 1H), 7.71 (d, 1H, *J* = 9.1 Hz), 7.85-7.93 (m, 2H), 8.38 (dd, 1H, *J* = 3.1, *J* = 9.0 Hz), 8.48 (d, 1H, *J* = 9.0 Hz), 8.51 (s, 1H), 10.50 (s, 1H, NH); ^{13}C-NMR ($CDCl_3$) δ (ppm): 26.2, 68.5, 118.0, 119.3, 121.5, 123.4, 124.7, 125.6, 129.0, 131.5, 132.1, 137.5, 139.2, 139.9, 141.4, 148.3, 162.6; IR (KBr) cm^{-1}: 1670, 1711 (CO), 3379 (NH), 3445 (OH); (MS-ESI): *m/z* = 320 calculated for [M+Na]$^+$ = 320; Anal. Calcd for $C_{17}H_{15}NO_2S$ (297.09 g/mol): C, 68.66; H, 5.08; N, 4.71. Found: C, 68.36; H, 4.92; N, 4.43.

N-(3-(hydroxy(phenyl)methyl)phenyl)benzofuran-2-carboxamide 10

Yield: 87%; M.p 134-135 °C; ^{1}H-NMR ($CDCl_3$) δ (ppm): 5.64 (s, 1H, CH), 5.79 (s, 1H, OH), 7.30 – 7.38 (m, 6H), 7.47 (d, 1H, *J* = 8.1 Hz), 7.52-7.66 (m, 2H), 7.61(d, 1H, *J* = 8.4 Hz), 7.67 (s, 1H), 7.82 (d, 1H, *J* = 8.0 Hz), 8.01 (dd, 1H, *J* = 2.4, 8.1 Hz), 10.38 (s, 1H, NH); ^{13}C-NMR ($CDCl_3$) δ (ppm): 72.4, 113.1, 114.8, 119.7, 121.1, 121.7, 124.5, 125.1, 126.0, 126.8, 128.9, 130.5, 131.7, 132.4, 134.7, 136.0, 137.9, 143.3, 146.2, 150.2, 158.3, 159.0; IR (KBr) cm^{-1}: 1672, 1711 (CO), 3378 (NH) , 3440 (OH); (MS-ESI): *m/z* = 366 calculated for [M+Na]$^+$ = 366; Anal. Calcd for $C_{22}H_{17}NO_3$ (343.12 g/mol): C, 76.95; H, 4.99; N, 4.08. Found: C, 76.61; H, 5.28; N, 3.83.

N-(3-(hydroxy(phenyl)methyl)phenyl)benzo[b]thiophene-3-carboxamide 11

Yield: 82%; M.p: 159-161 °C; ^{1}H-NMR ($CDCl_3$) δ (ppm): 5.69 (s, IH, CH), 6.03 (s, 1H, OH), 7.29 – 7.50 (m, 6H), 7.57 (d, 1H, *J* = 9.1 Hz), 7.69 (d, 1H, *J* = 8.6 Hz), 7.77 (s, 1H), 7.82-8.05 (m, 3H), 8.31 (dd, 1H, *J* = 3.1, *J* = 9.0 Hz), 8.60 (s, 1H), 10.31 (s, 1H, NH); ^{13}C-NMR ($CDCl_3$) δ (ppm): 74.3, 119.1, 120.9, 121.8, 122.1, 123.1, 123.7, 124.0, 124.7, 128.0, 128.5, 130.2, 131.4, 133.0, 135.1, 135.8, 136.0, 140.3, 140.9, 141.9, 142.3, 162.2; IR (KBr) cm^{-1}: 1675, 1719 (CO), 3380 (NH), 3449 (OH); (MS-ESI): *m/z* = 382 calculated for [M+Na]$^+$ = 382; Anal. Calcd for $C_{22}H_{17}NO_2S$ (359.09 g/mol): C, 73.51; H, 4.77; N, 3.90. Found: C, 73.22; H, 4.97; N, 3.65.

N-(3-(1-hydroxyethyl)phenyl)benzofuran-2-carboxamide 12

Yield: 89 %; M.p 129-132 °C; ^{1}H-NMR ($CDCl_3$) δ (ppm): 1.43 (d, 3H, *J* = 9 Hz), 2.50 (s br, 1H, OH), 4.75 (q, 1H, *J* = 9.1 Hz), 7.33 -7.40 (m, 3H), 7.62 (d, 1H, *J* = 8.1 Hz), 7.68 (d, 1H, *J* = 8.5 Hz), 7.74 (s, 1H), 7.89 (d, 1H, *J* = 8.1 Hz), 8.06 (dd, 1H, *J* = 2.4, 8.1 Hz), 8.29 (s, 1H), 10.41 (s, 1H, NH); ^{13}C-NMR ($CDCl_3$) δ (ppm): 26.2, 64.2, 113.8, 114.7, 119.6, 121.0, 122.6, 123.5, 124.2, 129.7, 130.8, 137.2, 141.3, 142.4, 149.8, 158.1, 164.0; IR (KBr) cm^{-1}: 1678, 1719 (CO), 3381 (NH), 3440 (OH); (MS-ESI): *m/z* = 304 calculated for [M+Na]$^+$ = 304; Anal. Calcd for $C_{17}H_{15}NO_3$ (343.12 g/mol): C, 72.58; H, 5.37; N, 4.98. Found: C, 72.22; H, 5.61; N, 4.65.

General Procedure for the Synthesis of 13 and 14

The corresponding carboxamide (**7i** and **8c**) (1.0 mmol) was added slowly to a solution of 2,4-dinitrophenylhdrazine (0.198 g, 1.0 mmol) and 1 ml of concentrated hydrochloric acid in 15 ml of methanol. After 6 h, an orange precipitate was obtained which was filtered, washed with methanol and dried in air.

N-(3-((2-(2,4-dinitrophenyl)hydrazono)(phenyl)methyl)phenyl)benzo[b]thiophene-3-carboxamide 13

Yield: 79%; M.p 93-96°C (decomp.); ^{1}H-NMR (DMSO-d_6) δ (ppm): 7.28 – 7.49 (m, 7H), 7.80 (dd, 2H, *J* = 3.0, *J* = 9.1 Hz), 7.96-8.09 (m, 6H), 8.31 (d, 1H, *J* = 8.6 Hz), 8.56 (s, 1H), 10.58 (s, 1H, NH_{amide}), 10.91 (s, 1H, NH); ^{13}C-NMR (DMSO-d_6) δ (ppm): 115.3, 119.3, 120.5, 121.3, 122.4, 123.7, 124.2, 124.9, 125.4, 126.6, 127.8, 128.9, 129.4, 130.4, 131.1, 131.5, 132.3, 133.2, 136.5, 137.3, 139.6, 140.4, 141.2,

145.2, 164.1, 197.5; IR (KBr) cm^{-1}: 1330, 1510 (NO_2), 1670, 1711 (CO), 1618 (C=N), 3377 (NH_{amide}), 3450 (NH); (MS-ESI): *m/z* = 560 calculated for $[M+Na]^+$ = 560; Anal. Calcd for $C_{28}H_{19}N_5O_5S$ (537.11 g/mol): C, 62.56; H, 3.56; N, 13.03. Found: C, 62.22; H, 3.84; N, 13.00.

N-(4-(1-(2-(2,4-dinitrophenyl)hydrazono)ethyl)phenyl)benzofuran-2-carboxamide 14

Yield: 70 %; M.p 165-168°C (decomp.); ^{1}H-NMR (DMSO-d_6) δ (ppm): 2.55 (s, 3H, CH_3), 7.20-7.29 (m, 2H), 7.34-7.49 (m, 3H), 7.65-7.96 (m, 7H), 10.52 (s, 1H, NH_{amide}), 11.01 (s, 1H, NH); ^{13}C-NMR (DMSO-d_6) δ (ppm): 26.2, 113.5, 114.2, 117.8, 119.2, 120.7, 122.4, 123.2, 123.9, 125.9, 127.4, 129.1, 130.1, 132.5, 133.3, 134.8, 140.6, 143.1, 148.8, 157.8, 165.0, 195.0; IR (KBr) cm^{-1}: 1335, 1512 (NO_2), 1671, 1720 (CO), 1623 (C=N), 3381 (NH_{amide}), 3440 (NH); (MS-ESI): *m/z* = 482 calculated for $[M+Na]^+$ = 482; Anal. Calcd for $C_{23}H_{17}N_5O_6$ (459.12 g/mol): C, 60.13; H, 3.73; N, 15.24. Found: C, 59.83; H, 3.89; N, 15.00.

General Procedure for the Synthesis of 15 and 16

Two ml of glacial acetic acid was added to a solution of **8e** or **8c** (1.0 mmol) and thiosemicarbazide (1.3 mmol) in water-ethanol (1:1) solvent. The resulting solution was refluxed for 6 h. After cooling, the precipitate was formed, then filtered using suction filtration; the precipitate was washed with water and dried under reduced pressure.

N-(3-(1-(2-carbamothioylhydrazono)ethyl)phenyl)benzo[b]-thiophene-3-carboxamide 15

Yield: 55%; M.p 199-202 °C; ^{1}H-NMR (DMSO-d_6) δ (ppm): 2.29 (s, 3H, CH_3), 7.37 -7.54 (m, 3H), 7.62 (d, 1H, *J* = 9.0 Hz), 7.80-7.85 (m, 2H), 8.03 (dd, 1H, *J* = 3.1, *J* = 9.0 Hz), 8.10 (s, 1H), 8.20 (s, 1H), 8.36 (dd, 1H, *J* = 3.0, *J* = 9.1 Hz), 8.50 (s, 1H), 10.21 (s, 1H, NH), 10.37 (s, 1H, NH_{amide}); ^{13}C-NMR (DMSO-d_6) δ (ppm): 14.6, 119.0, 121.5, 122.7, 123.4, 124.7, 125.7, 125.8, 129.5, 131.4, 132.5, 137.5, 138.6, 139.3, 140.0, 149.0, 162.8, 179.3; IR (KBr) cm^{-1}: 1251 (C=S), 1674, 1719 (CO), 3160 (NH), 3370 (NH), 3310, 3406 (NH_2); (MS-ESI): *m/z* = 391 calculated for $[M+Na]^+$ = 391; Anal. Calcd for $C_{18}H_{16}N_4OS_2$ (368.08 g/mol): C, 58.67; H, 4.38; N, 15.20. Found: C, 58.26; H, 4.81; N, 15.01.

N-(4-(1-(2-carbamothioylhydrazono)ethyl)phenyl)benzofuran-2-carboxamide 16

Yield: 61%; M.p 115-118 °C; ^{1}H-NMR (DMSO-d_6) δ (ppm): 2.23 (s, 3H, CH_3), 7.37-7.47 (m, 2H), 7.74 (d, 1H, *J* = 9.0 Hz), 7.82-7.95 (m, 4H), 8.01 (dd, 1H, *J* = 3.1, *J* = 9.0 Hz), 8.08 (s, 1H), 8.32 (dd, 1H, *J* = 3.0, *J* = 9.0 Hz), 8.46 (s, 1H), 10.07 (s, 1H, NH), 10.44 (s, 1H, NH_{amide}); ^{13}C-NMR (DMSO-d_6) δ (ppm): 14.2, 120.0, 120.3, 123.4, 124.7, 125.7, 127.7, 130.0, 131.4, 131.9, 132.6, 133.1, 137.4, 139.9, 140.5, 148.7, 162.6, 178.9; IR (KBr) cm^{-1}: 1250 (C=S), 1680, 1720 (CO), 3154 (NH), 3369 (NH), 3314, 3409 (NH_2); (MS-ESI): *m/z* = 375 calculated for $[M+Na]^+$ = 375; Anal. Calcd for $C_{18}H_{16}N_4O_2S$ (352.09 g/mol): C, 61.35; H, 4.58; N, 15.90. Found: C, 61.03; H, 4.70; N, 15.59.

Procedure for the Synthesis of 17

To a solution of chloroacetic acid (0.22 g, 2.3 mmol) and sodium acetate (0.41 g, 5.0 mmol) in 15 ml ethanol, **16** (0.3 g, 0.81 mmol) was added and the resulting solution was refluxed for 24 h. After cooling, the precipitate was collected by suction filtration, washed with water and then dried under reduced pressure.

S-{[3-(1-benzofuran-2-yl)-8-methyl-2,4,6,7-tetraazabicyclo-[7.2.2]trideca 1(11),3,5,7,9,12-hexaen 5-yl]methyl} hydrogen thiocarbonate 17

Yield: 41%; M.p 145-148 °C; ^{1}H-NMR (DMSO-d_6) δ (ppm): 2.24 (s, 3H, CH_3), 4.01 (s, 2H, CH_2), 7.43-7.50 (m, 2H), 7.78-7.86 (m, 4H), 8.03 (d, 1H, *J* = 6.0 Hz), 8.38 (d, 1H, *J* = 6.1 Hz), 8.50 (s, 1H), 10.50 (s, 1H, NH), 12.56 (s, 1H, COOH); ^{13}C-NMR (DMSO-d_6) δ (ppm): 15.0, 33.4, 120.3, 123.4, 124.7, 125.7, 127.5, 131.2, 132.6, 133.5, 137.4, 139.9, 140.9, 160.6, 162.6, 164.2, 174.9; ^{13}C-NMR (dept) δ (ppm): 33.4 (CH_2, down), 15.0, 120.3, 123.4, 124.7, 125.7, 127.5, 131.2, 132.6 (CH, up); IR (KBr) cm^{-1}: 1251 (C=S), 1679, 1715, 1722 (CO), 2909 (br, OH), 3151 (NH), 3366 (NH), 3319, 3412 (NH_2); (MS-ESI): *m/z* = 415 calculated for $[M+Na]^+$ = 415; Anal. Calcd for $C_{20}H_{16}N_4O_3S$ (392.09 g/mol): C, 61.21; H, 4.11; N, 14.28. Found: C, 61.43; H, 4.21; N, 14.09.

Biological Activity

Antifungal Activity

For the determination of MIC_s, we applied the standard protocol EDef 7.1 *Antifungal Susceptibility Testing of Yeasts* according to the EUCAST reference method [35]. It was modified to our strain collection consisting of *C. albicans* DSMZ 11949, *C. glabrata ATCC* 90030, and *C. parapsilosis* ATCC 22019 which were used also as reference control strains confirming that the MIC values were within the limits of the EUCAST procedure. According to the classical EUCAST protocol, prior to the test, strains were grown at 37 °C for 24 hours on solid sabouraud (Sab) plates. On each microdilution plate, row 1 was used as a growth control for viability of fungal cells. Rows 2 to 12 contained an increasing amount of the test compound in a 2- fold titration scheme resulting in a range of final concentrations from 0.125 to 128μg ml^{-1}. Fluconazole was used as a control for antifungal activity of the test compounds. Microtiter plates were incubated at 35 °C and the growth of yeast cells was evaluated after 22 ± 2 hours by measuring the optical density at 450 nm using a TECAN microtiter plate reader and analysed with Magellan software. MICs were defined as the lowest drug concentration giving rise to an inhibition of growth of more than 50% of that of the drug free control. The MIC values were evaluated in three distinct trials and the mean value was adopted for further calculations. The average MIC (MIC_{av}) for a subset of test strains was calculated as the geometric mean of the MIC test results of strains included in the subset.

Antibacterial Tests

We applied the standard protocol described in the NCCLS and the National Center for Infectious Diseases, Center for Disease Control and prevention reference method [36] with some modifications to our strain collection consisting of two standard strains of *Staphylococcus aureus* ATCC 6538 and *E. coli* ATCC 8739. According to the classical NCCLS protocol, prior to the test, strains were grown at 37 °C for 24 hours on nutrient broth plates. On each microdilution plate row 1 was used as a growth control for viability of bacterial cells. Rows 2 to 12 contained an increasing amount of the test compound in a 2-fold titration scheme resulting in a range of final concentrations from 0.125- 128 $\mu g\ mL^{-1}$.

Ciprofloxacin was used as a control substance for the antibacterial activity of the test compounds. Microtiter plates were incubated at 37 °C and the growth of bacterial cells was evaluated after 22 ± 2 hours by observing wells for turbidity or growth.

Cytotoxic Activity

HeLa human cervical cancer cells (ATCC, USA) were grown in Minimal Essential Medium (MEM), NIH-3T3 embryonic mouse fibroblast cells (DSMZ, Germany) in Dulbecco's Modified Eagle Medium (DMEM), both supplemented with 10 % fetal bovine serum, 4 mM L-glutamine, 100 µg/ml streptomycin and 100 U/ml penicillin (all from Biochrom AG, Germany) at 37 °C with 10 % CO_2. Confluent cultures were split using trypsin/EDTA 0.05/0.02 % (w/v) in phosphate-buffered saline (PBS; Biochrom AG, Germany).

Stock solutions of **9**, **10** and **17** were prepared in DMSO (57.7, 72.7, and 38.0 mM, respectively). For the evaluation of cytotoxicity cells were inoculated into 96 well plates and treated 24 h later with various concentrations of **9**, **10** and **17** (2.9 – 577 µM, 7.3 – 727 µM, and 0.2 – 38 µM, respectively) in complete culture medium for an additional 18 h. The final concentration of DMSO did not exceed 1 % to avoid toxic effects of the vehicle alone. Two types of controls were prepared, one incubated with culture medium alone and one with the respective highest DMSO concentration (1 % for **9**, **10** or 0.1 % **17**). Afterwards, the supernatant was removed and the cells were incubated with Bisbenzimid 33342 (Hoechst 33342), a nuclear stain, in order to assess the DNA content per well (correlating with the number of cells) [41,42]. Cells were washed with HBSS, 200 µL/well HBSS was added and the cell number-related fluorescence (Ex 346 nm/ Em 460 nm) of the DNA dye was measured in a multiplate reader (BMG Labtech, Germany).

One to three independent experiments with six replicates per concentration were performed for each cell line. Data are displayed as mean±SEM and the curves were fitted using Prism 5.03 (GraphPad Software, Inc., USA). The effective concentration at which the number of cells is reduced to 50 % of control (EC_{50}) was determined by nonlinear regression if applicable. For a subset of experiments, microscopy was performed after fluorescence spectroscopy.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

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