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Searching for new agents active against *Candida albicans* biofilm: A series of indole derivatives, design, synthesis and biological evaluation

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Abstract

Candida albicans biofilm represents a major clinical problem due to its intrinsic tolerance to anti-fungal compounds and it has been highly related to infections in catheterized patients. Few compounds are described as able to inhibit biofilm formation or to interfere with preformed biofilm of *C. albicans*. Here we report the *in vitro* evaluation of anti-biofilm activity on *C. albicans* ATCC 10231 of a series of new and already known amine and amide indole derivatives. Among the studied compounds, fifteen resulted active on *C. albicans* ATCC 10231 biofilm, with $BMIC_{50} \leq 16$ μg/mL. Three of them (**7**, **23** and **33**) showed a selectivity towards mature biofilm and the most active of them was the compound **23** ($BMIC_{50} = 4$ μg/mL). On the other hands, two different compounds (**21** and **22**) were selective towards biofilm formation with $BMIC_{50}$ values of 8 μg/mL. Otherwise, compounds **16** and **17** resulted active on biofilm formation $BMIC_{50}$ of 8 μg/mL and 2 μg/mL respectively and on mature biofilm with $BMIC_{50}$ of 2 μg/mL. These two last compounds also showed an interesting activity towards the planktonic cells of *C. albicans*. A selection of the more active compounds was also evaluated on different *C. albicans* strains (PMC1042, PMC1083 and ATCC 10261), showing a comparable or higher anti-biofilm activity, especially on mature biofilm. *In vivo* toxicity studies using the

Galleria mellonella larvae, were finally carried out on more active indole derivatives, showing that they are poorly toxic even at the highest concentrations tested (500-1000 µg/mL).

Keywords

Indole derivatives; *Candida albicans* biofilm; *Galleria mellonella.*

1. Introduction

Microorganisms, in their natural habitats, are found attached to surfaces and not only as free-floating (planktonic) organisms. *Candida albicans* is a part of the normal microbiota of humans, which allows the contact with most medical devices and host surfaces, permitting formation of robust biofilms [1]. *Candida* biofilms lead to recurring infections and in some cases death [2] and it has been estimated that it may be responsible for up to 65% of infections in catheterized patients [3]. *C. albicans* biofilm presents a significant clinical problem, with current treatment options severely limited by the intrinsic tolerance of fungal biofilms for anti-fungal [4]; as a matter of fact, biofilms of *C. albicans* are less susceptible to many antifungal drugs, including fluconazole [5]. Few molecules were reported in literature to inhibit or to prevent the *C. albicans* biofilm formation; therefore, there is a strong need for the development of novel antifungal biofilm strategies. In previous works, we focused our attention on antifungal compounds, particularly, we studied compounds active against *C. albicans* planktonic cells [6, 7] and biofilm [8]; studying a set of cinnamic acid derivatives, we identified a new indole compound which was active against biofilm formation of *C. albicans* with a MIC of 8 µg/mL [9].

The analysis of literature on the anti-biofilm activity of indolic compounds evidenced few data, such as the paper of Oh and co-workers [10] that reports the ability of indole and indole-3-acetonitrile to repress the biofilm formation in *C. albicans* interfering with quorum sensing and to inhibit the yeast filamentation. Recently Vila and Lopez-Ribot have identified the indole derivative MMV688768, as the most potent compound belonging to a synthetic library, which was able to reduce the metabolic cell activity within the *C. albicans* preformed biofilm [11].

On these bases we decided to explore the anti-biofilm activity of the indole moiety and, therefore, we have selected a set of indole amine and amide derivatives with the aim to identify new compounds able to interfere with the *C. albicans* biofilm. We have selected small indole molecules already known in the literature or commercially available and furthermore, we have designed and synthesized some new original compounds. In particular, tryptamine has been chosen as starting compound and its structure was modified as outlined in Chart 1. A set of amine derivatives was obtained by binding the amine function of the tryptamine to an aromatic group, such as naphthyl or variously substituted benzylic group or to a heterocycle such as pyridine, nitrofuran and indole. A further series of amines was obtained by molecular duplication starting from tryptamine, coupled with terephthalaldehyde or from 1*H*-indole-3-carbaldehyde coupled with linear alkyl-diamines.

Moreover, we synthesized a series of amide derivatives, containing a set of compounds analogous to some of the amine derivatives and a set of amides obtained by conjugation with some representative nonsteroidal antiinflammatory drugs (NSAIDs), as some authors reported the inhibitory activities of NSAIDs against *C. albicans* biofilm [12, 13].

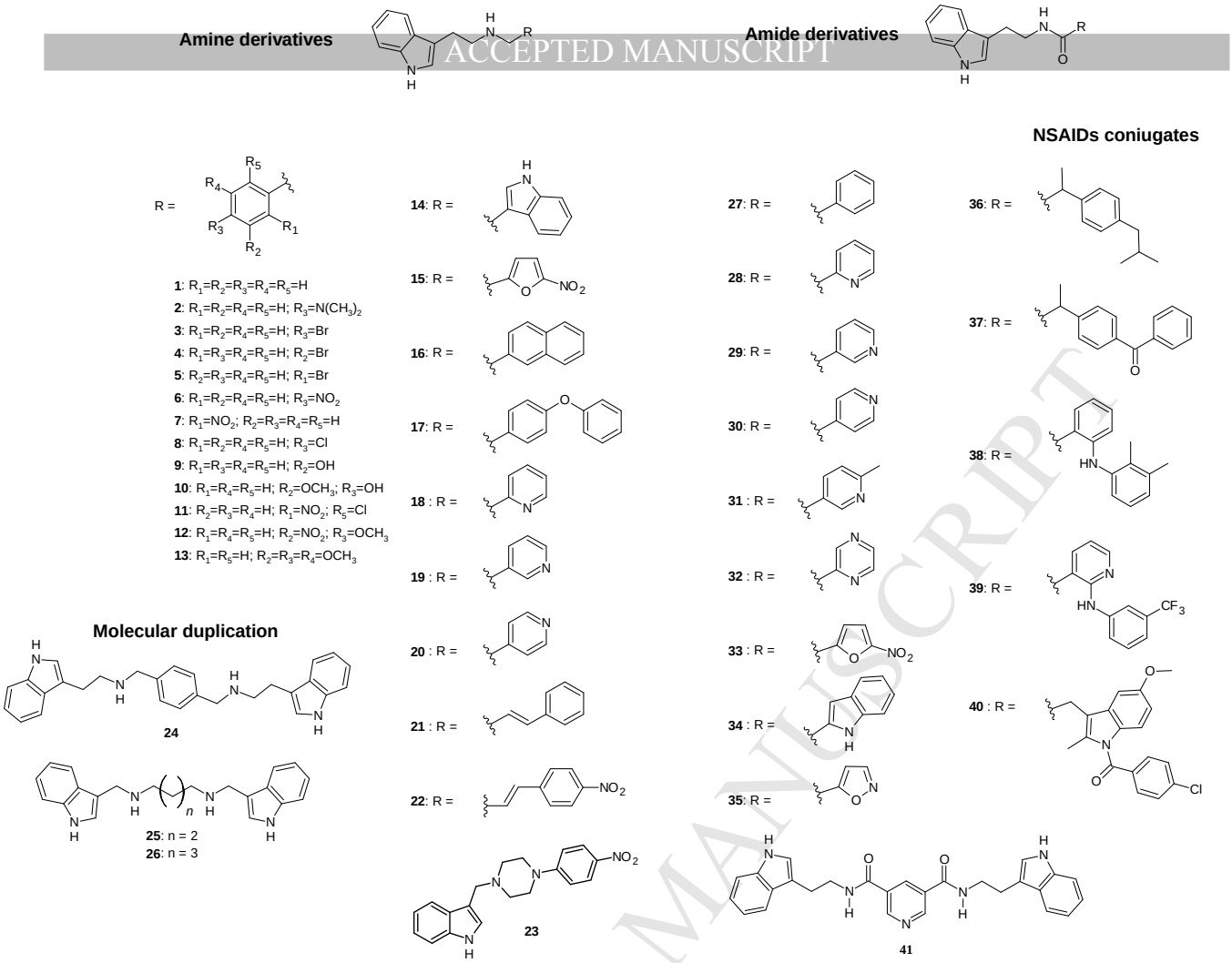


Chart 1: designed indole derivatives

All synthesized compounds were evaluated for the activity against planktonically grown cells of *C. albicans* as well as on preformed and forming *C. albicans* biofilm. Finally, the most active compounds were selected in order to evaluate the in vivo toxicity using the larvae of the greater wax moth, *Galleria mellonella*.

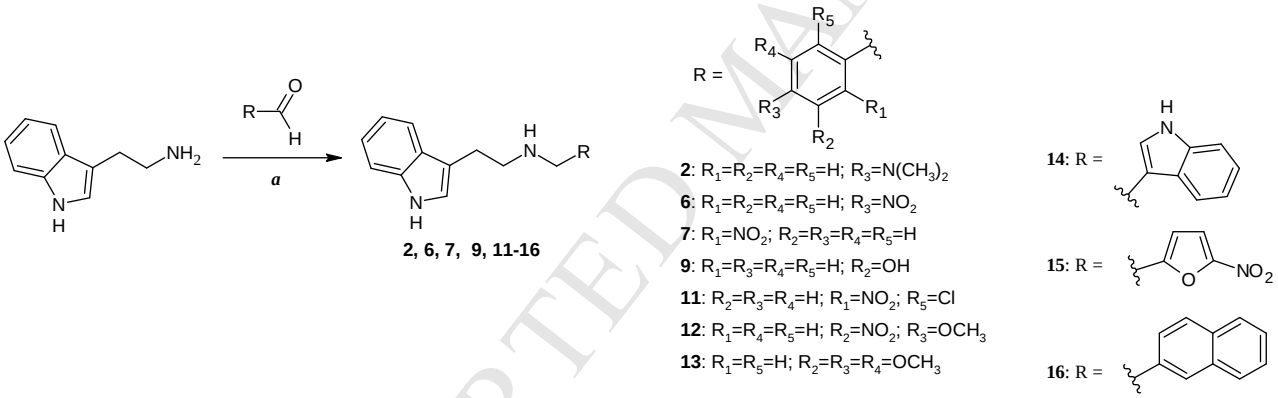
2. Results and discussions

2.1 Chemistry

In the group of studied compounds, some are known in the literature, others are commercially available but synthesized on request and not easily purchased through our usual suppliers, others are not known or described in literature. All the compounds have been re-synthesized, in some cases according to the procedures described in the literature, as for the amine derivatives **1** [14], **3** [15], **10** [16], **17** [16], **18** [17], **21**

[18], **22 [**18**],** and **23** [19]; in the other cases the target compounds were obtained by alternative methods or

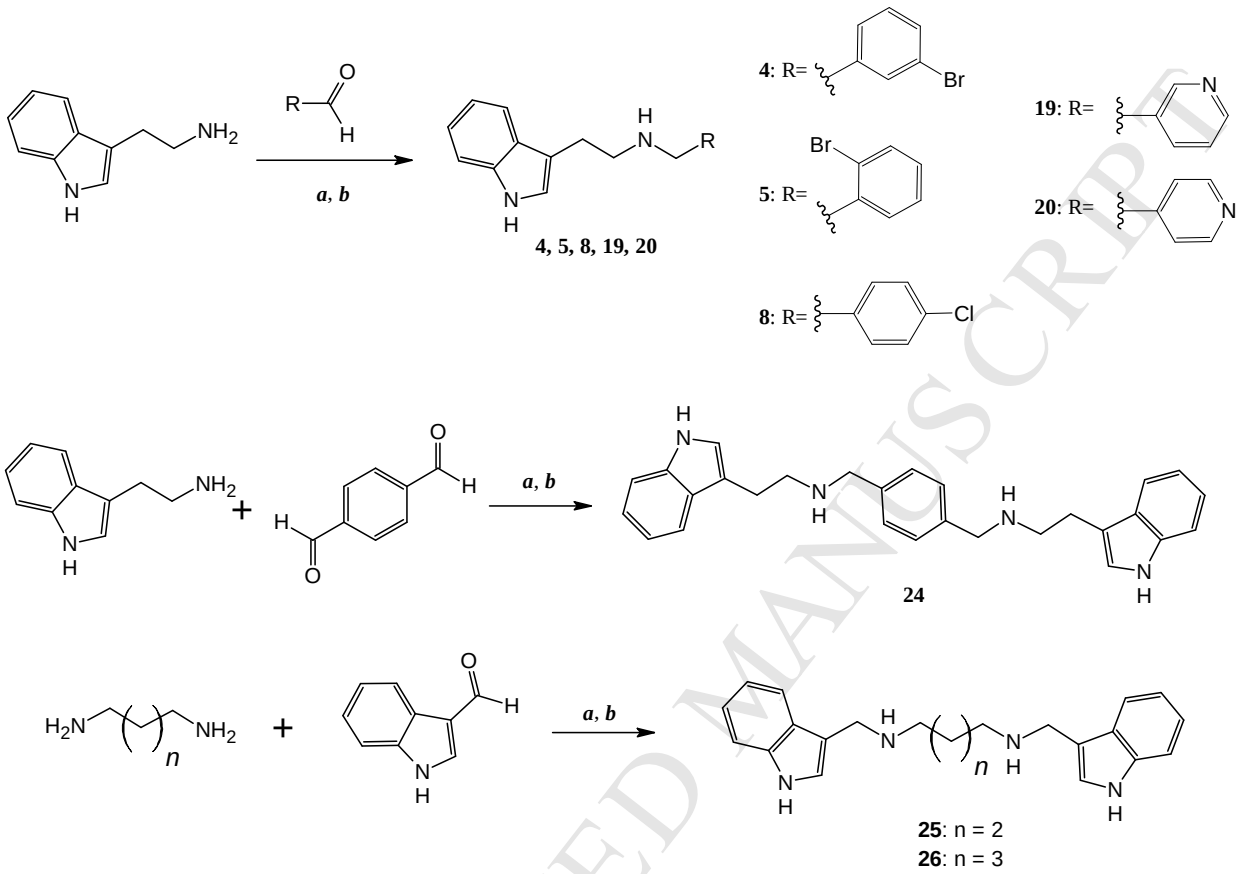
by modification of literature procedure, as specified for each compound. The amine derivatives were prepared following two synthetic methods, illustrated in the Schemes 1 and 2. The compounds **2**, **6**, **7**, **9**, and **11-16**, were synthesized by direct reductive amination modifying the procedures reported in literature [20]. The reaction has been carried out in MeOH using a small amount of acetic acid as catalyst and α-picoline-borane as reductive agent. Equimolar amounts of amine, aldehyde, and α-picoline-borane were generally used and the reaction was monitored by IR spectroscopy to evaluate the disappearance of the carbonyl stretching band of the aldehyde function around at 1690 cm^{-1} and by TLC. At this point 10% aqueous HCl was added and the obtained aqueous acid solution was neutralized to pH = 7 by the addition of saturated aqueous Na_2CO_3. The obtained residues were purified by chromatography on a silica gel column and/or by crystallization. The structure of synthesized compounds was confirmed by spectroscopic analysis, as the appearance of the benzyl methylene singlet between 4.05 and 3.64 ppm in the ^{1}H-NMR spectra.



Scheme 1. Synthetic procedure for amines **2**, **6**, **7**, **9**, **11-16** (Method A). *Reagents and conditions*: ***a***) pic-BH_3, AcOH, MeOH, 2h, rt.

The amine derivatives **4**, **5**, **8**, **19**, **20**, **24-26** were synthesized following the method B procedure. Amine reagent and appropriate aldehyde were dissolved in the required amount of dichloromethane and molecular sieves were added as desiccant agents. The reaction was monitored by IR spectroscopy to confirm the imine formation, with stretching absorption band around 1640 cm^{-1}, and the disappearance of the aldehyde band at about 1700 cm^{-1}. The obtained imine intermediate, after removal of molecular sieves and solvent, was

reduced without further purification, by treatment with $NaBH_4$ in methanol. The obtained crude residues were purified by chromatography on a silica gel column and/or by crystallization. The structure of the synthesized compounds was confirmed by spectroscopic analysis, as the appearance of the benzyl methylene singlet between 3.80 and 3.74 ppm in the ^{1}H-NMR spectra.

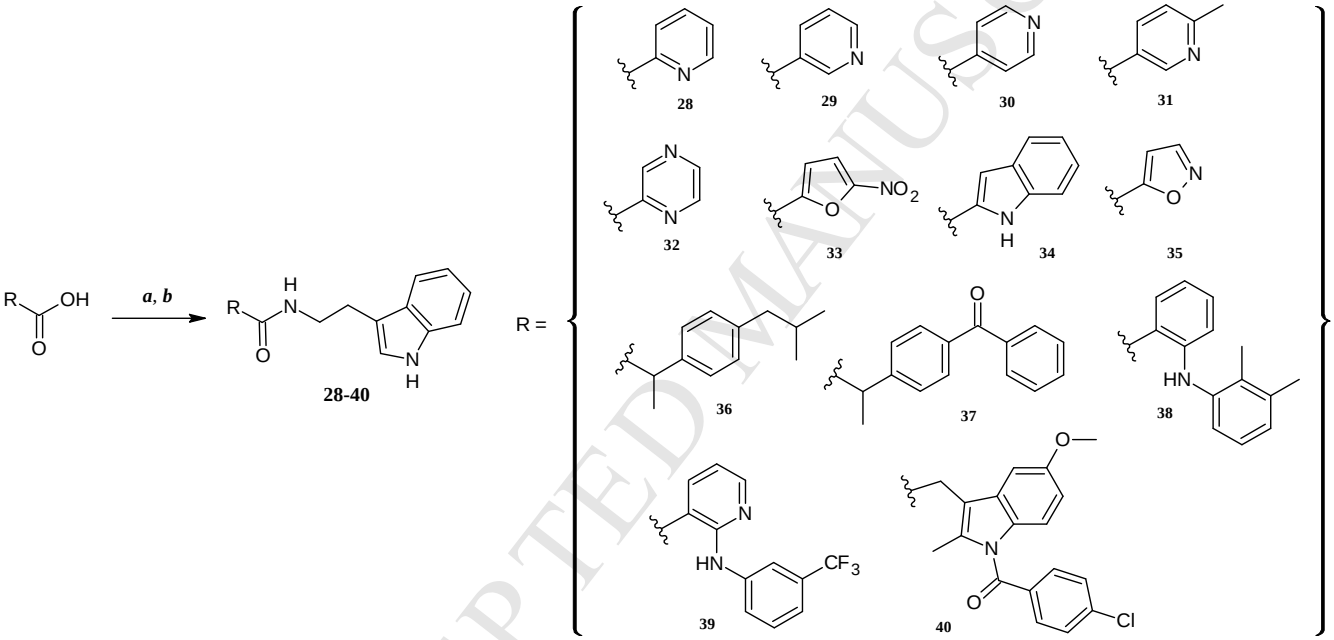


Scheme 2. Synthetic procedure for amines **4**, **5**, **8**, **19, 20**, **24-26** (Method B). *Reagents and conditions*: ***a***) molecular sieves (4Å), CH_2Cl_2, 12h, rt; ***b***) $NaBH_4$, MeOH, 2h, rt.

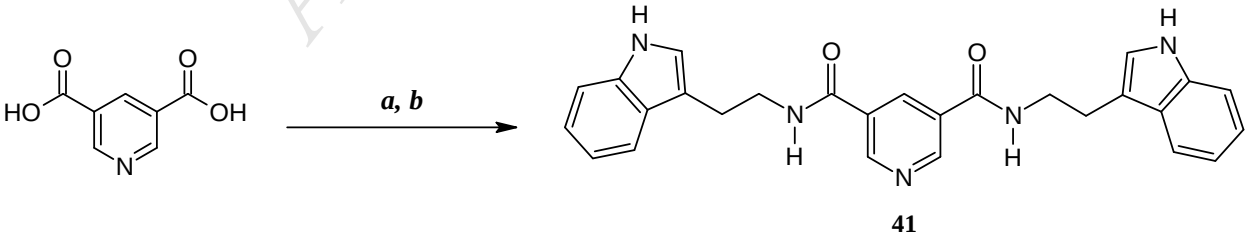
The amide derivative **27** was prepared as described by Honegr and coll. [21] and amides **29**, **30** and **34**, already described in the literature [22, 23] were prepared in higher or equivalent yields than those described but using milder reagents and conditions. The amides **28-41** were prepared following the procedure illustrated in the Schemes 3 and 4. The synthesis was carried out using carbonyldiimidazole (CDI) as activating agent of the carboxylic acid function. Although the reactivity of the CDI-carboxylic acid intermediate is lower than acid chlorides, CDI is more controllable and allows to avoid the use of more reactive activating agents, such as thionyl chloride, which may cause cross reactions. Therefore, CDI was

suspended in ethyl acetate and the opportune carboxylic acid was added, the reaction was heated to reflux to improve solubility of the reagents and it was monitored by IR spectroscopy evaluating the appearance of two carbonyl stretching bands around 1735 and 1700 cm^{-1}, concerning the CDI-carboxylic acid intermediate.

At this point tryptamine was added and the reaction was refluxed until the appearance of the amide carbonyl stretching band in the IR spectra between 1634 and 1650 cm^{-1}. The compounds were purified by chromatography on a silica gel or alumina column and/or by crystallization. Also in this case, the structure of final compounds was confirmed by spectroscopic analysis; as an example in the ^{1}H-NMR spectra of amide derivatives was observed the shift of the signal due to the -CH_2-NH- group from 2.67 ppm (tryptamine) to 3.62-3.55 ppm (amide derivatives).



Scheme 3. Synthesis of amide compounds (**28-40**). *Reagents and conditions*: ***a***) CDI, AcOEt, 5h, reflux; ***b***) Tryptamine, 12h, reflux.



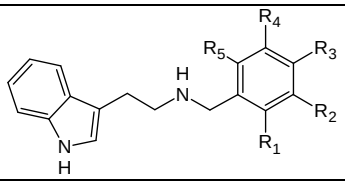
Scheme 4. Synthesis of amide **41**. *Reagents and conditions*: ***a***) CDI, AcOEt, 5h, reflux; ***b***) Tryptamine, 12h, reflux.

The detailed synthetic procedures, the analytical and spectroscopic data of the new and the re-synthesized compounds are reported in the experimental section and are in agreement with the proposed structures.

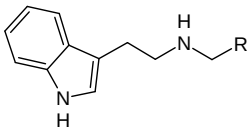
2.2 Microbiological assays

The synthesized compounds **1**–**41** have been screened against *C. albicans* planktonic cells and against *C. albicans* biofilm, in formation and mature (24 h), according to the literature procedures [24, 25], using *C. albicans* ATCC 10231, a strain sensitive to fluconazole on planktonic cells (0.5 µg/mL) and resistant in the different phases of biofilm formation ($BMIC_{50}$ 128 µg/mL on biofilm formation and >128 µg/mL on mature biofilm). All the compounds showing anti-biofilm and anti-fungal activity with $BMIC_{50}$ and MIC_{50} values lower than 64 µg/mL were reported in the Table 1.

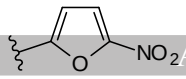
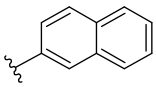
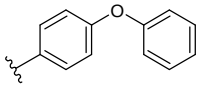
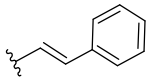
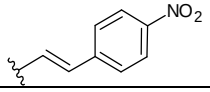
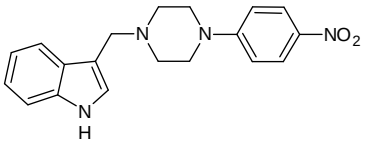
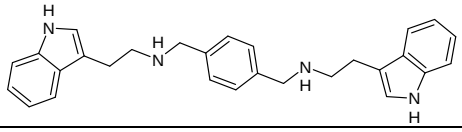
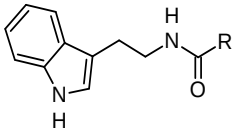
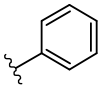
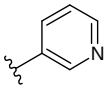
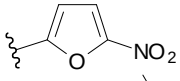
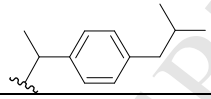
Table 1. Antifungal activity of the amine and amide derivatives against *C. albicans* ATCC 10231 biofilms and planktonic cells.



Compound	R_1	R_2	R_3	R_4	R_5	$BMIC_{50}$ (µg/mL) Mature biofilm	$BMIC_{50}$ (µg/mL) Biofilm formation	MIC_{50} (µg/mL) Planktonic cells
1	H	H	H	H	H	32	32	>128
2	H	H	$N(CH_3)_2$	H	H	16	32	>128
3	H	H	Br	H	H	8	8	64
4	H	Br	H	H	H	32	4	64
5	Br	H	H	H	H	16	16	128
6	H	H	NO_2	H	H	32	32	>128
7	NO_2	H	H	H	H	8	64	>128
8	H	H	Cl	H	H	32	16	32
11	NO_2	H	H	H	Cl	8	16	>128
12	H	NO_2	OCH_3	H	H	32	128	>128



R

15		16	16	>128
16		2	8	16
17		2	2	4
21		64	8	64
22		>128	8	>128
23		4	>128	>128
24		8	16	>128
				
	R			
27		32	32	>128
28		32	32	>128
29		64	>128	>128
33		16	128	>128
36		8	16	>128
Tryptamine		>128	128	>128
Fluconazole		>128	128	0.5

The inhibition of biofilm formation and destruction of pre-formed biofilm were evaluated by measuring the metabolic activity of cells within the biofilm (XTT assay). The BMIC end point for biofilm is based on the lowest drug concentration producing a decrease of 50% metabolic activity relative to the untreated growth control. MIC end point for planktonic cells is based on lowest drug concentration that prevented 50% of growth with respect to the untreated control. At least two experiments were performed on two separate dates for each compound tested in triplicate. The results were expressed as median. Compounds showing $BMIC_{50}$ and MIC_{50} values higher than 64 μg/mL are not reported in the table.

In the first set of amine derivatives of the tryptamine compounds (**1-13**) we identified ten active compounds (**1-8, 11** and **12**) which differ for the presence of various substituent on the phenyl ring. The compound **1**, that does not present substituents on the benzyl group, has a good activity both on biofilm formation and on

mature biofilm ($BMIC_{50}$ = 32 µg/mL); taking it as reference, we try to outline structure-activity relationships

(SAR). The presence of a halogen, as bromine or chlorine, on the phenyl ring produces an increase of the anti-biofilm activity, in particular on forming biofilm, as can be observed for compounds **3, 4, 5**, and **8**. Furthermore, bromine was slightly more effective than chlorine in increasing the anti-biofilm activity, as shown by the comparison between **3** and **8**. The introduction of a nitro group in *para* position on phenyl (compound **6**) retains the same activity of compound **1**, but, if the nitro group is placed in *ortho* position, the activity on mature biofilm enhances as the $BMIC_{50}$ value decreases from 32 to 8 µg/mL (compound **7**). Combining the presence of the nitro group and chlorine on the phenyl ring (**11**) also the anti-biofilm activity was combined; indeed, **11** possess the same activity of **7** on mature biofilm ($BMIC_{50}$ = 8 µg/mL) and the same activity of **8** on biofilm formation ($BMIC_{50}$ = 16 µg/mL).

On the other hand, the introduction of a hydroxy and/or methoxy groups on the phenyl ring (**9**, **10** and **13**) dramatically reduced the anti-biofilm activity ($BMIC_{50}$ > 128 µg/mL), compared to the compound **1**; in the same way, the simultaneous presence of the nitro group and a methoxy group (**12**) decreases the activity on biofilm formation ($BMIC_{50}$ = 128 µg/mL). Finally, compound **2**, containing a dimethylamino group on the phenyl ring, shows an interesting activity on mature biofilm, with a $BMIC_{50}$ value of 16 µg/mL. In general, these amine derivatives do not show activity on planktonic cells, except for **3**, **4** (MIC_{50} = 64 µg/mL both) and **8** (MIC_{50} = 32 µg/mL), consequently it is reasonable to imagine that these compounds could interfere with some specific biofilm mechanisms.

Tryptamine, tested as reference molecule, does not show any activity, either on planktonically grown cells or in formation and mature biofilms, as reported in Table 1. This evidence suggests that the structural modification of the primary amine function of tryptamine is a key requirement to obtain the anti-biofilm activity.

In a second set of amine derivatives (**14-23**) the phenyl group was replaced with various aromatic and heteroaromatic rings (**14-20**), with cinnamic or 4-nitrocinnamic groups (**21**, **22**) or modified by introducing a piperazine spacer (**23**). The activity data showed in the Table 1 indicate that the nitrofurane derivative (**15**) has a good activity on mature biofilm and on biofilm formation with $BMIC_{50}$ of 16 µg/mL. Moreover, the naphthyl (**16**) and the 4-phenoxybenzyl derivatives (**17**) are very active against biofilm in formation ($BMIC_{50}$

= 8 and 2 µg/mL, respectively) as well as on mature biofilm ($BMIC_{50}$ = 2 µg/mL, both) and they are also active on planktonic cells (16 and 4 µg/mL, respectively), though less than fluconazole (0.5 µg/mL). These data also suggest that the anti-biofilm activity of compounds **16** and **17** could be due to a combined mechanism of action directed on planktonic cells and on the biofilm. The replacement of benzyl group of the compound **1** with cinnamic moiety, to obtain compounds **21** and **22**, has produced an enhancement of the anti-biofilm activity, selectively against biofilm in formation, with the reduction of $BMIC_{50}$ value from 32 µg/mL to 8 µg/mL.

The activities of compounds **14**, **18-20** on biofilm mature and in formation, as well on planktonic cells, ranging from 64 to >128 µg/mL, indicates that the replacement of the phenyl group of the reference compound **1** with other heterocyclic groups, as indole (**14**) and pyridines (**18-20**), resulted in a significant reduction of the anti-biofilm activity. The introduction of a piperazine group between indole and nitrophenyl functions to obtain compound **23** has produced an enhancement of the anti-biofilm activity, selectively against mature biofilm, with $BMIC_{50}$ value of 4 µg/mL.

In the set of amide derivatives (**27-35**) only four compounds showed MIC_{50} and $BMIC_{50}$ values lower than 64 µg/mL (Table 1). It can be observed that the amide derivative **27** corresponding to amine compounds **1** retains the activity both on biofilm formation and on mature biofilm with a $BMIC_{50}$ value of 32 µg/mL. Among the pyridine derivatives (**28-30**) only compound **28** has the same activity of **27**, also significantly better than to the analogous amine derivative (**18**); the others pyridines present $BMIC_{50}$ value range from 64 to >128 µg/mL on mature biofilm, on biofilm formation, as well on planktonic cells. The replacement of phenyl group with nitrofurane moiety to obtain compound **34** have produced an enhancement of the anti-biofilm activity, selectively against biofilm in formation, with the reduction of $BMIC_{50}$ value from 32 µg/mL to 16 µg/mL.

Moreover, the substitution of phenyl with pyrazine (**32**), indole (**34**), or isoxazole (**35**) rings resulted in a significant reduction of the anti-biofilm activity.

The amide bond was useful to conjugate the tryptamine moiety to NSAIDs, as ibuprofen, ketoprofen, mefenamic acid, niflumic acid and indomethacin to obtain the amide derivatives **36-40**. In literature, as described above, it is reported the bacterial and fungal anti-biofilm activity of some NSAIDs, but only the

hybrid between tryptamine and ibuprofen (**36**) shows a good activity on mature and in formation biofilm, with $BMIC_{50}$ value of 8 and 16 μg/mL, respectively. Finally, all the amide compounds do not show activity against *C. albicans* planktonic cells.

The duplication of the indole moiety to obtain diamine derivatives (**24**-**26**), induced different effects on anti-biofilm activity that depend on the nature of the spacer. The presence of an aromatic linker increased the activity, as can be noticed for compound **24**, that possess $BMIC_{50}$ values of 8 and 16 μg/mL on mature and formation biofilm respectively; otherwise, an aliphatic spacer decreases significantly the activity as in the cases of compounds **25** and **26** ($BMIC_{50}$ > 128 μg/mL).

Lastly, the duplication of indole moiety with insertion of a pyridine to obtain diamide derivative **41**, on the contrary, does not produce activity on mature and formation biofilm and on planktonic cells ($BMIC_{50}$ > 128 μg/mL).

The most active compounds were also tested on different strains of *C. albicans*, coming from the American Type Culture Collection (*C. albicans* ATCC 10261) and from the Pharmaceutical Microbiology Culture Collection (*C. albicans* PMC 1042 and PMC 1082) (Table 2).

Table 2. Antifungal activity of selected amine and amide derivatives against biofilm formation, mature biofilm and planktonic cells of *C. albicans* PMC1042, PMC1083 and ATCC 10261.

	***C. albicans* PMC1042**			***C. albicans* PMC1082**			***C. albicans* ATCC10261**		
	$BMIC_{50}$ (μg/mL)		**MIC_{50} (μg/mL)**	**$BMIC_{50}$ (μg/mL)**		**MIC_{50} (μg/mL)**	**$BMIC_{50}$ (μg/mL)**		**$BMIC_{50}$ (μg/mL)**
Compound	**Mature biofilm**	**Biofilm formation**	**Planktonic cells**	**Mature biofilm**	**Biofilm formation**	**Planktonic cells**	**Mature biofilm**	**Biofilm formation**	**Planktonic cells**
3	2	8	16	4	8	32	8	8	32
16	2	8	128	2	8	32	8	8	32
17	2	8	64	2	8	16	8	8	16
23	2	32	>256	2	32	>256	8	32	>256
24	2	8	>256	4	8	>256	4	4	256
36	2	8	>256	4	8	>256	8	8	>256
Fluconazole	>64	>64	8	>64	>64	0.125	>64	>64	2

The inhibition of biofilm formation and destruction of pre-formed biofilm were evaluated by measuring the metabolic activity of cells within the biofilm (XTT assay). The BMIC end point for biofilm is based on the lowest drug concentration producing a decrease of 50% metabolic activity relative to the untreated growth control. MIC end point for planktonic cells is based on lowest drug concentration that prevented 50% of growth with respect to the untreated control. At least two experiments were performed on two separate dates for each compound tested in triplicate. The results were expressed as median.

The compounds **3**, **16**, **17**, **23**, **24** and **36** were active also towards biofilm from *C. albicans* ATCC 10261, PMC 1042 and PMC 1082 strains, and showed, in most cases, $BMIC_{50}$ values lower than those observed on ATCC 10231 strain. The obtained results indicate that these indole derivatives are more effective on mature biofilm than on biofilm formation, suggesting that they could act more selectively in the advanced stages of biofilm formation, rather than in the early stages.

The evaluation of selective toxicity is an extremely important and preliminary phase in the development of new compounds of pharmaceutical interest; for this reason, we decided to study in vivo toxicity, as described by Dolan et al. [26], using the larvae of the Greater wax moth, *Galleria mellonella*. McCann et al. have demonstrated that the toxicity exhibited in *G. mellonella* was similar to that observed in Swiss mice [27]. A selection of the synthesized derivatives that exhibited good anti-biofilm activity (**3**, **7**, **16**, **17**, **23**, **24** and **36**) was chosen for the evaluation of their toxicity. Almost all compounds tested displayed a 100% survival rate of *G. mellonella* larvae. Compounds **3** and **23** at the concentration range of 5-500 µg/mL and compound **36** at the concentration range of 10-1000 µg/mL, displayed a 100% survival rate after 72 h. Compounds **16** and **24** at the concentration of 50 µg/mL and compound **7** at the concentration of 50 and 100 µg/mL displayed a 90% survival rate after 72 h and 100% survival at the other concentrations after 72 h (statistically not significant, Student's t test, P> 0.1). Compound **17** at the concentration of 500 µg/mL displayed a 90% survival rate after 24 h, a 70% survival rate after 72 h (Student's t test, P<0.05) and 100% survival for the other concentrations after 72 h. (Table 3).

Table 3. Survival rate of *Galleria mellonella* larvae (expressed as %) at 24, 48 and 72 h after the injection of the indole derivatives **3**, **7**, **16**, **17**, **23**, **24** and **36**.

Cmpd	Dosage concentration (µg/mL)[a]	*G. mellonella* survival (%)[b]			Cmpd	Dosage concentration (µg/mL)[a]	*G. mellonella* survival (%)[b]		
		24h	48h	72h			24h	48h	72h
3	500	100	100	100	**23**	500	100	100	100
	100	100	100	100		100	100	100	100
	50	100	100	100		50	100	100	100
	10	100	100	100		10	100	100	100
	5	100	100	100		5	100	100	100
7	500	100	100	100	**24**	500	100	100	100
	100	90 *	90 *	90 *		100	100	100	100
	50	90 *	90 *	90 *		50	100	90 *	90 *

	10	100	100	100		10	100	100	100
	5	100	100	100		5	100	100	100
16	500	100	100	100	**36**	1000	100	100	100
	100	100	100	100		500	100	100	100
	50	90 *	90 *	90 *		100	100	100	100
	10	100	100	100		50	100	100	100
	5	100	100	100		10	100	100	100
17	500	90 **	70 **	70 **					
	100	100	100	100					
	50	100	100	100					
	10	100	100	100					
	5	100	100	100					

[a] Compounds were dissolved in sterile water (containing less than 1% v/v of DMSO) and the volume injected into the last, left pro-leg, of the *G. mellonella* larvae was 20 µL; [b] The results represent the mean percentage of survival, of two independent experiments, of *G. mellonella* larvae, as a function of the administered dosage; * Student's t test, $P > 0.1$; ** Student's t test, $P < 0.05$.

3. Conclusions

The synthesis of indole derivatives has allowed to identify new compounds endowed of anti-biofilm activity. In general, it was observed that the halogen derivatives have shown higher inhibition activity both of mature and of in formation biofilms, in particular the bromine derivatives are more effective than chlorine ones. For almost all nitro derivatives, except for **22**, a higher activity against mature biofilm was observed; this could be related to the ability of these compounds to generate ROS. In fact, it is known from the literature that compounds able to induce the production of ROS possess a potential anti-biofilm activity [28, 29].

Many of the synthesized compounds showed higher activity towards biofilm (both mature and in formation) if compared with planktonic cells. This fact could be related to a different susceptibility due to the phenotypic and physiologic differences between biofilm (sessile cells) and non-adhered (planktonic cells). Moreover, this selectivity could be explained considering that the molecules could act by preventing the adhesion of the cells to form biofilm and/or favoring the release of the cells in planktonic form which would result in a lesser amount of experimentally determined biofilm.

Finally other interesting compounds are the naphthyl (**16**) and the 4-phenoxybenzyl (**17**) derivatives, that showed high activity against *C. albicans* ATCC 10231 mature biofilm ($BMIC_{50}$ 2 µg/mL) and biofilm in formation ($BMIC_{50}$ 8 µg/mL and 2 µg/mL respectively) as well as on planktonic cells (MIC_{50} 16 and 4 µg/mL respectively) though less than fluconazole (MIC_{50} 0.5 µg/mL). These data also suggest that the anti-

biofilm activity of compounds **16** and **17** could be due to a combined action on planktonic cells and on the biofilm; indeed, they could be considered good lead compounds to develop new antifungal drugs.

Promising anti-biofilm compounds have been identified and the combination of these compounds with established antifungal agents could have additive or synergistic action that should be studied.

Further investigations into the development of efficient anti-biofilm agents against other microorganisms based on these results are still in progress.

4. Experimental section

4.1 Chemistry

4.1.1 Materials and methods

Reagents and solvents were of analytical grade and were purchased from Sigma-Aldrich (Milano, Italy). Melting points were determined on Tottoli apparatus (Buchi) and are uncorrected. Infrared spectra were recorded on a Perkin Elmer Spectrum One FT-IR spectrometer equipped with an ATR system. ^{1}H-NMR and ^{13}C-NMR spectra were acquired on a Bruker AVANCE-400 spectrometer at 9.4 Tesla, in DMSO-d_6, CD_3OD, $CDCl_3$, CD_2Cl_2 and Acetone- d_6 at 27 °C; chemical shift values are given in δ (ppm) with respect to the solvent residual peak (DMSO = 2.50 ppm, CD_3OD = 3.31 ppm, $CDCl_3$ = 7.26, CD_2Cl_2 = 5.32 ppm, Acetone = 2.05 ppm). Coupling constants are given in Hz. The following abbreviation were used: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, s *br* = singlet broad, dd = doublet of doublets, dt = doublet of triplets. Mass spectra were recorded on a ThermoFinnigan LCQ Classic LC/MS/MS ion trap equipped with an ESI source and a syringe pump. Samples (10^{-4}-10^{-5} M in MeOH/H_2O 90:10) were infused in the electrospray system at a flow rate of 5-10 µl min^{-1}. When necessary, 50 µL of 10^{-2} M aqueous HCOOH or 10^{-2} M aqueous NH_3 were added to the sample solutions, in order to promote the analyte ionization. Elemental analyses were obtained by a PE 2400 (Perkin-Elmer) analyser; analyses indicated by the symbols of the elements or functions were within ± 0.4 % of the theoretical values.

4.1.2 General procedure for the synthesis of amine compounds

Method A: Tryptamine and the opportune aldehyde (1:1 molar ratio) were dissolved in 10 mL of MeOH–AcOH (10:1), pic-BH_3 was added, and the reaction mixture was stirred for 2 h at room temperature. Then MeOH was evaporated under reduce pressure and HCl (10 %, 10 mL) was added to the residue. The aqueous solution was stirred for 0.5 h at room temperature, then cooled in an ice bath and Na_2CO_3 (≈ 2.5 g) and H_2O (10 mL) were added until pH = 7. The aqueous solution was extracted with AcOEt (3 x 30 mL) and the combined organic layers were washed with brine (15 mL), dried over Na_2SO_4 and concentrated under reduced pressure.

Method B: Tryptamine, the opportune aldehyde (1:1 or 2:1 molar ratio) and molecular sieves (4 Å) in CH_2Cl_2 (20 mL) were stirred at rt for 12 h. The mixture was then filtered on a celite pad, concentrated and treated with $NaBH_4$ in MeOH (30 mL) at rt for 2 h. After reaction MeOH was evaporated under reduced pressure, H_2O (15 mL) was added and the mixture was extracted with CH_2Cl_2 (3 x 20 mL). The organic layers were dried over Na_2SO_4 and the solvent was removed under vacuum.

4.1.2.1 4-(((2-(1*H*-indol-3-yl)ethyl)amino)methyl)-*N*,*N*-dimethylaniline (2)

Compound **2** was prepared using tryptamine (0.208 g, 1.30 mmol), 4-(dimethylamino)benzaldehyde (0.194 g, 1.30 mmol) and pic-BH_3 (0.139 g, 1.30 mmol), following the method A procedure. The obtained residue was subjected to silica gel column chromatography using AcOEt/MeOH (1:1) to give a yellow-orange solid, mp 98-99°C (R_f = 0.24, 80.5 mg, 21 % yield). IR (cm^{-1}): ν_{N-H} 3281, finger print 1215, 1170, 1097. ^{1}H-NMR (DMSO-d_6) δ (ppm): 10.75 (s *br*, 1H, -NH- indole); 7.52 (d, *J* = 8.0 Hz, 1H, indole proton), 7.31 (d, *J* = 8.0 Hz, 1H, indole proton); 7.12-7.10 (m, 3H, aromatic protons); 7.04 (t, *J* = 7.2 Hz, 1H, indole proton); 6.94 (t, *J* = 7.1 Hz, 1H, indole proton); 6.65 (d, *J* = 8.4 Hz, 2H, aromatic protons); 4.10 (bs, 1H, NH amine); 3.60 (s, 2H, Ph-CH_2-NH-); 2.84 (s, 6H, -N(CH_3)$_2$); 2.84-2.74 (m, 4H, -CH_2- CH_2-). ^{13}C-NMR (MeOD) δ (ppm): 151.6, 138.2, 130.4, 128.6, 128.5, 123.4, 122.4, 119.6, 119.3, 114.2, 113.4, 112.3, 53.7, 50.0, 41.1, 26.0. ESI-MS (m/z): $(M+H)^+$ = 293.47. Anal. C19H23N3 (C, H, N).

4.1.2.2 *N*-(3-bromobenzyl)-2-(1*H*-indol-3-yl)ethanamine (4)

Compound **4** was prepared using tryptamine (0.160 g, 1.00 mmol), 3-bromobenzaldehyde (117 μL, 1.00 mmol) and $NaBH_4$ (0.076 g, 2.00 mmol), following the method B procedure. The obtained residue was

subjected to silica gel column chromatography using CH_2Cl_2/MeOH (9:1) to give a pink solid, mp 64-66°C (R_f = 0.37, 198 mg, 60 % yield). IR (cm^{-1}): ν_{N-H} 3289, finger print 1232, 1110, 1076, 1068. ^{1}H-NMR (DMSO-d_6) δ (ppm): 10.77 (s, 1H, -NH- indole); 7.55 (s, 1H, aromatic proton); 7.49 (d, *J* = 7.8 Hz, 1H, indole proton); 7.41 (dd, *J* = 7.8 Hz, 1H, aromatic proton); 7.32 (d, *J* = 7.9 Hz, 2H, aromatic protons); 7.25 (t, *J* = 7.7 Hz, 1H, aromatic proton); 7.12 (d, *J* = 2.1 Hz, 1H, indole proton); 7.05 (t, *J* = 7.2 Hz, 1H, indole proton); 6.96 (t, *J* = 7.3 Hz, 1H, indole proton); 3.74 (s, 2H, Ph-CH_2-NH-); 2.84 (t, J = 6.8 Hz, -CH_2-CH_2-); 2.77 (t, J = 6.4 Hz, 2H, -CH_2- CH_2). ^{13}C-NMR ($CDCl_3$) δ (ppm): 142.8, 136.4, 131.1, 130.0, 129.9, 127.4, 126.7, 122.5, 122.1, 122.0, 119.4, 118.9, 113.8, 111.2, 53.3, 49.3, 25.8. ESI-MS (m/z): $(M+H)^+$ = 328.67 (85); 330.80 (100). Anal. C17H17BrN2 (C, H, N).

4.1.2.3 *N*-(2-bromobenzyl)-2-(1*H*-indol-3-yl)ethanamine (5)

Compound **5** was prepared using tryptamine (0.160 g, 1.00 mmol), 2-bromobenzaldehyde (116 μL, 1.00 mmol) and $NaBH_4$ (0.076 g, 2.00 mmol), following the method B procedure. The obtained residue was subjected to silica gel column chromatography using CH_2Cl_2/MeOH (9:1) to give a pink solid, mp 95-96°C (R_f = 0.43, 124 mg, 38 % yield). IR (cm^{-1}): ν_{N-H} 3265, finger print 1321, 1218, 1087, 926. ^{1}H-NMR (DMSO-d_6) δ (ppm): 10.78 (s, 1H, -NH- indole); 7.56 (d, *J* = 7.9 Hz, 1H, indole proton); 7.52 – 7.49 (m, 2H, aromatic protons); 7.36-7.32 (m, 2H, aromatic protons); 7.18 (td, J_1 = 7.7 Hz, J_2 = 1.6 Hz, 1H, aromatic proton); 7.14 (d, *J* = 2.1 Hz, 1H, indole proton); 7.05 (t, *J* = 7.2 Hz, 1H, indole proton); 6.96 (t, *J* = 7.4 Hz, 1H, indole proton); 3.80 (s, 2H, Ph-CH_2-NH-); 2.89-2.82 (m, 4H, -CH_2- CH_2- NH-). ^{13}C-NMR (MeOD) δ (ppm): 138.1, 136.9, 132.5, 130.3, 128.7, 127.3, 127.2, 123.5, 122.2, 121.0, 118.2, 117.9, 111.8, 110.9, 52.7, 48.5, 24.8. ESI-MS (m/z): $(M+H)^+$ = 328.80 (100); 330.87 (80). Anal. C17H17BrN2 (C, H, N).

4.1.2.4 2-(1*H*-indol-3-yl)-*N*-(4-nitrobenzyl)ethanamine (6)

Compound **6** was prepared using tryptamine (0.301 g, 1.88 mmol), 4-nitrobenzaldehyde (0.284 g, 1.88 mmol) and pic-BH_3 (0.201 g, 1.88 mmol), following the method A procedure. The obtained residue was subjected to silica gel column chromatography using initially $CHCl_3$ and subsequently $CHCl_3$/MeOH (9:1) to give a yellow solid, mp 55-57°C (R_f = 0.27, 453 mg, 82 % yield). IR (cm^{-1}): ν_{N-H} 3416, ν_{NO2} 1513 and 1345,

finger print 1231, 1106, 1073. ^{1}H-NMR (DMSO-d_6) δ (ppm): 10.77 (s *br*, 1H, -NH- indole); 8.16 (d, *J* = 8.7 Hz, 2H, aromatic protons); 7.61 (d, *J* = 8.6 Hz, 2H, aromatic protons); 7.49 (d, *J* = 7.8 Hz, 1H, indole proton); 7.32 (d, *J* = 8.1 Hz, 1H, indole proton); 7.13 (d, *J* = 1.7 Hz, 1H, indole proton); 7.05 (t, *J* = 7.0 Hz, 1H, indole proton); 6.95 (t, *J* = 7.7 Hz, 1H, indole proton); 3.88 (s, 2H, Ph-CH_2-NH-); 2.8 (t, *J* = 6.8 Hz, 2H, -CH_2- CH_2- NH-); 2.79 (t, *J* = 6.8 Hz, 2H, -CH_2- CH_2- NH-). ^{13}C-NMR (DMSO-d_6) δ (ppm): 150.1, 146.7, 136.7, 129.2, 127.7, 123.7, 123.0, 121.3, 118.7, 118.5, 112.9, 111.8, 52.6, 50.0, 26.0. ESI-MS (m/z): $(M+H)^+$ = 295.87. Anal. C17H17N3O2 (C, H, N).

4.1.2.5 2-(1*H*-indol-3-yl)-*N*-(2-nitrobenzyl)ethanamine (7)

Compound **7** was prepared using tryptamine (0.160 g, 1.00 mmol), 2-nitrobenzaldehyde (0.151 g, 1.00 mmol) and pic-BH_3 (0.107 g, 1.00 mmol), following the method A procedure. The obtained residue was subjected to silica gel column chromatography using AcOEt/MeOH (9:1) to give a brownish solid, mp 70-73°C (R_f = 0.58, 53 mg, 18 % yield). IR (cm^{-1}): ν_{N-H} 3288, ν_{NO2} 1515 and 1340, finger print 1230, 1105, 1073. ^{1}H-NMR (MeOD) δ (ppm): 7.94 (d, *J* = 8.1 Hz, 1H, aromatic proton); 7.60 (t, *J* = 7.2 Hz, 1H, aromatic proton); 7.53-7.44 (m, 3H, aromatic protons); 7.33 (d, *J* = 8.1 Hz, 1H, indole proton); 7.09-7.04 (m, 2H, indole protons); 6.94 (t, *J* = 7.4 Hz, 1H, indole proton); 4.01 (s, 2H, Ph-CH_2-NH-); 3.00-2.92 (m, 4H, -CH_2-CH_2-). ^{13}C-NMR (MeOD) δ (ppm): 149.0, 136.9, 133.9, 133.0, 131.6, 128.2, 127.2, 124.5, 122.1, 121.0, 118.2, 117.8, 111.8, 110.9, 49.7, 48.7, 24.8. ESI-MS (m/z): $(M+H)^+$ = 295.80. Anal. C17H17N3O2 (C, H, N).

4.1.2.6 *N*-(4-chlorobenzyl)-2-(1*H*-indol-3-yl)ethanamine (8)

Compound **8** was prepared using tryptamine (0.160 g, 1.00 mmol), 4-chlorobenzaldehyde (0.141 g, 1.00 mmol) and $NaBH_4$ (0.076 g, 2.00 mmol), following the method B procedure. The obtained residue was subjected to silica gel column chromatography using CH_2Cl_2/MeOH (8:2) to give a white solid, mp 83-85°C (R_f = 0.53, 219 mg, 77 % yield). IR (cm^{-1}): ν_{N-H} 3416, finger print 1218, 1092, 1013. ^{1}H NMR (DMSO-d_6) δ (ppm): 10.77 (s, 1H, -NH- indole); 7.48 (d, *J* = 7.8 Hz, 1H, indole proton); 7.35 – 7.31 (m, 5H, aromatic protons); 7.12 (d, *J* = 2.1 Hz, 1H, indole proton); 7.05 (t, *J* = 7.3 Hz, 1H, indole proton); 6.96 (t, *J* = 7.3 Hz, 1H, indole proton); 3.74 (s, 2H, Ph-CH_2-NH-); 2.87-2.76 (m, 4H, -CH_2- CH_2- NH-). ^{13}C NMR (MeOD) δ

(ppm): 137.6, 136.9, 132.6, 129.7, 128.1, 127.2, 122.1, 121.1, 118.3, 117.9, 111.9, 111.0, 51.9, 48.7, 24.6.

ESI-MS (m/z): $(M+H)^+$ = 284.93 (100); 286.87 (30). Anal. C17H17ClN2 (C, H, N).

4.1.2.7 3-(((2-(1*H*-indol-3-yl)ethyl)amino)methyl)phenol (9)

Compound **9** was prepared using tryptamine (0.528 g, 3.30 mmol), 3-hydroxybenzaldehyde (0.402 g, 3.30 mmol) and pic-BH_3 (0.353 g, 3.30 mmol), following the method A procedure. The obtained residue was subjected to silica gel column chromatography using AcOEt/MeOH (1:1) to give a yellowish solid, mp 132-134°C (R_f = 0.36, 100 mg, 11 % yield). IR (cm^{-1}): ν_{N-H} 3445, ν_{OH} 3326, finger print 1275, 1160, 1084. ^{1}H-NMR (DMSO-d_6) δ (ppm): 10.82 (s *br*,1H, -NH- indole); 7.53 (d, *J* = 7.8 Hz, 1H, indole proton); 7.36 (d, *J* = 7.9 Hz, 1H, indole proton); 7.16-7.07 (m, 3H, aromatic protons); 6.99 (t, *J* = 7.2 Hz, 1H, indole proton); 6.80-6.76 (m, 2H, aromatic protons); 6.65 (d, *J* = 7.5 Hz, 1H, aromatic proton); 3.71 (s, 2H, Ph-CH_2-NH-); 2.89-2.93 (m, 4H, -CH_2- CH_2-). ^{13}C-NMR (DMSO-d_6) δ (ppm): 157.7, 142.5, 136.7, 129.4, 127.7, 123.0, 121.3, 119.0, 118.8, 118.6, 115.3, 114.0, 112.9, 111.8, 53.2, 49.9, 25.7. ESI-MS (m/z): $(M+H)^+$ = 265.13. Anal. C17H18N2O (C, H, N).

4.1.2.8 *N*-(2-chloro-6-nitrobenzyl)-2-(1*H*-indol-3-yl)ethanamine (11)

Compound **11** was prepared using tryptamine (0.160 g, 1.00 mmol), 2-chloro-6-nitrobenzaldehyde (0.185 g, 1.00 mmol) and pic-BH_3 (0.107 g, 1.00 mmol), following the method A procedure. The obtained residue was subjected to silica gel column chromatography using CH_2Cl_2/MeOH (9.5:0.5) and then crystallized from methanol to give a yellow solid, mp 127-129°C (R_f = 0.40, 66 mg, 20 % yield). IR (cm^{-1}): ν_{N-H} 3283, ν_{NO2} 1525 and 1349, finger print 1109, 1080, 1006. ^{1}H-NMR (DMSO-d_6) δ (ppm): 10.78 (s, 1H, -NH- indole); 7.81-7.74 (m, 2H, aromatic proton), 7.49 (t, *J* = 8.0 Hz, 1H, aromatic proton); 7.43 (d, *J* = 7.6 Hz, 1H, indole proton); 7.30 (d, *J* = 8.0 Hz, 1H, indole proton); 7.08 (s, 1H, indole proton); 7.03 (t, 1H, *J* = 7.2 Hz, indole proton); 6.93 (t, *J* = 7.2 Hz, 1H, indole proton); 4.03 (s, 2H, Ph-CH_2-NH-); 2.75 (m, 4H, -CH_2- CH_2-). ^{13}C-NMR (DMSO-d_6) δ (ppm): 152.1, 136.7, 135.5, 133.8, 132.7, 129.8, 127.6, 123.6, 123.0, 121.3, 118.6, 118.5, 112.6, 111.8, 50.3, 46.4, 25.9. ESI-MS (m/z): $(M+H)^+$ = 329.73. Anal. C17H16ClN3O2 (C, H, N).

4.1.2.9 2-(1*H*-indol-3-yl)-*N*-(4-methoxy-3-nitrobenzyl)ethanamine (12)

Compound **12** was prepared using tryptamine (0.160 g, 1.00 mmol), 4-methoxy-3-nitrobenzaldehyde (0.181 g, 1.00 mmol) and pic-BH_3 (0.107 g, 1.00 mmol), following the method A procedure. The obtained residue was subjected to silica gel column chromatography using CH_2Cl_2/MeOH (9:1) to give an off-white solid, mp 71-73°C (R_f = 0.30, 193 mg, 59 % yield). IR (cm^{-1}): ν_{N-H} 3413, ν_{NO2} 1526 and 1344, finger print 1280, 1259, 1018. ^{1}H-NMR (DMSO-d_6) δ (ppm): 10.78 (s, 1H, -NH- indole); 7.83 (d, *J* = 1.6 Hz, 1H, indole proton); 7.60 (dd, J_1 = 2.0 Hz, J_2 = 8.8 Hz, 1H, aromatic proton); 7.48 (d, *J* = 7.6 Hz, 1H, indole proton); 7.33-7.29 (m, 2H, aromatic protons); 7.12 (s, 1H, indole proton); 7.05 (t, *J* = 7.6 Hz, 1H, indole proton); 6.95 (t, *J* = 8.0 Hz, 1H, indole proton); 5.76 (s, 1H, -NH- amine); 3.90 (s, 3H, –OCH_3); 3.74 (s, 2H, Ph-CH_2-NH-); 2.84 (t, *J* = 6.8 Hz, 2H, CH_2- CH_2- NH-); 2.76 (t, *J* = 6.4 Hz, 2H, CH_2- CH_2- NH-). ^{13}C-NMR (MeOD) δ (ppm): 151.7, 139.6, 136.8, 134.0, 131.1, 127.2, 124.8, 122.2, 121.1, 118.3, 117.9, 113.5, 111.7, 110.9, 55.7, 51.2, 48.6, 24.5. ESI-MS (m/z): $(M+H)^+$ = 325.80. Anal. C18H19N3O3 (C, H, N).

4.1.2.10 2-(1*H*-indol-3-yl)-*N*-(3,4,5-trimethoxybenzyl)ethanamine (13)

Compound **13** was prepared using tryptamine (0.327 g, 2.04 mmol), 3,4,5-trimethoxybenzaldehyde (0.400 g, 2.04 mmol) and pic-BH_3 (0.218 g, 2.04 mmol), following the method A procedure. The obtained residue was subjected to silica gel column chromatography using AcOEt/MeOH (1:1) to give a white solid, mp 69-71°C (R_f = 0.35, 383 mg, 55 % yield). IR (cm^{-1}): ν_{N-H} 3386, finger print 1237, 1128, 1000. ^{1}H-NMR (DMSO-d_6) δ (ppm): 10.77 (s *br*, 1H, -NH- indole); 7.49 (d, *J* = 7.8 Hz, 1H, indole proton); 7.31 (d, *J* = 8.1 Hz, 1H, indole proton); 7.13 (s, 1H, indole proton); 7.04 (t, *J* = 7.2 Hz, 1H, indole proton); 6.95 (t, *J* = 7.7, 1H, indole proton); 6.61 (s, 2H, phenyl protons); 3.71 (s, 6H, *m*-OCH_3); 3.68 (s, 2H, Ph-CH_2-NH-); 3.61 (s, 3H, *p*-OCH_3); 2.87-2.77 (m, 4H, CH_2- CH_2-). ^{13}C-NMR (DMSO-d_6) δ (ppm): 153.1, 137.2, 136.7, 136.4, 127.8, 123.0, 121.3, 118.8, 118.5, 113.1, 111.8, 105.3, 60.4, 56.1, 53.4, 50.0, 25.9. ESI-MS (m/z): $(M+H)^+$ = 340.70. Anal. C20H24N2O3 (C, H, N).

4.1.2.11 2-(1*H*-indol-3-yl)-*N*-(1*H*-indol-3-ylmethyl)ethanamine (14)

Compound **14** was prepared using tryptamine (0.301 g, 1.88 mmol), 1*H*-indole-3-carbaldehyde (0.273 g, 1.88 mmol) and pic-BH_3 (0.201 g, 1.88 mmol), following the method A procedure. The obtained residue was subjected to silica gel column chromatography using AcOEt/MeOH (1:1) to give a yellow solid, mp 78-80°C

(R_f = 0.35, 148 mg, 27 % yield). IR (cm^{-1}): ν_{N-H} 3395, finger print 1225, 1092, 1007. ^{1}H-NMR (DMSO-d_6) δ (ppm): 10.81 (s *br*, 1H, -NH- indole); 10.75 (s *br*, 1H, -NH- indole); 7.56 (d, *J* = 7.8 Hz, 1H, indole proton); 7.50 (d, *J* = 7.8 Hz, 1H, indole proton); 7.34-7.31 (m, 2H, indole protons); 7.20 (d, *J* = 2.4 Hz 1H, indole proton); 7.11 (d, *J* = 2.0 Hz 1H, indole proton); 7.05 (t, *J* = 7.2 Hz 2H, indole protons); 6.95 (t, *J* = 7.8 Hz, 2H, indole protons); 4.11 (bs, 1H, NH amine); 3.89 (s, 2H, Ph-CH$_2$-NH-); 2.87 (s, 4H, CH$_2$- CH$_2$-). ^{13}C-NMR (MeOD) δ (ppm): 147.7, 145.6, 136.9, 130.9, 129.8, 127.2, 122.1, 121.0, 118.3, 117.9, 114.7, 114.3, 112.2, 111.8, 111.7, 110.9, 54.9, 52.5, 24.4. ESI-MS (m/z): $(M+H)^+$ = 289.73. Anal. C19H19N3 (C, H, N).

4.1.2.12 2-(1*H*-indol-3-yl)-*N*-((5-nitrofuran-2-yl)methyl)ethanamine (15)

Compound **15** was prepared using tryptamine (0.248 g, 1.55 mmol), 5-nitrofuran-2-carbaldehyde (0.220 g, 1.55 mmol) and pic-BH_3 (0.166 g, 1.55 mmol), following the method A procedure. The obtained residue was subjected to silica gel column chromatography using CH_2Cl_2 to give a yellow solid, mp 98-100°C (R_f = 0.27, 69 mg, 16 % yield). IR (cm^{-1}): ν_{N-H} 3432, ν_{NO2} 1494 and 1354, finger print 1241, 1019. ^{1}H-NMR (DMSO-d_6) δ (ppm): 10.76 (s, 1H, -NH- indole); 7.65 (m, 1H, indole proton); 7.32 (d, *J* = 7.7 Hz, 1H, indole proton); 7.14 (s, 1H, indole proton); 7.04 (t, *J* = 7.8 Hz, 1H, indole proton); 6.95 (t, *J* = 7.8 Hz, 1H, indole proton); 6.76 (m, 2H, furane protons); 3.74 (s, 2H, Ph-CH$_2$-NH-); 2.82 (m, 4H, CH$_2$- CH$_2$-). ^{13}C-NMR (DMSO-d_6) δ (ppm): 157.8, 151.8, 136.6, 127.6, 123.2, 121.3, 118.7, 118.6, 114.4, 113.3, 112.4, 111.8, 54.6, 50.2, 23.3. ESI-MS (m/z): $(M+H)^+$ = 285.93. Anal. C15H15N3O3 (C, H, N).

4.1.2.13 2-(1*H*-indol-3-yl)-*N*-(naphthalen-2-ylmethyl)ethanamine (16)

Compound **16** was prepared using tryptamine (0.160 g, 1.00 mmol), naphthalene-2-carbaldehyde (0.156 g, 1.00 mmol) and pic-BH_3 (0.107 g, 1.00 mmol), following the method A procedure. The obtained residue was subjected to silica gel column chromatography using CH_2Cl_2/MeOH (9.5:0.5) to give a white solid, mp 97-99°C (R_f = 0.81, 140 mg, 46 % yield). IR (cm^{-1}): ν_{N-H} 3417, finger print 1230, 1104, 1076. ^{1}H-NMR (MeOD) δ (ppm): 7.77-7.69 (m, 3H, aromatic protons); 7.60 (s, 1H, aromatic protons); 7.50 (d, *J* = 8.0 Hz, 1H, indole proton); 7.42-7.39 (m, 2H, aromatic protons); 7.35 (d, *J* = 8.4 Hz, 1H, indole proton); 7.31 (dd, J_1 = 1.6 Hz, J_2 = 8.4 Hz, 1H, aromatic proton); 7.19-7.07 (m, 1H, indole proton); 7.03 (s, 1H, indole proton); 6.99-6.95 (m, 1H, indole proton); 3.84 (s, 2H, Ph-CH$_2$-NH-); 2.97 (t; *J* = 6.4 Hz, 2H, CH$_2$- CH$_2$- NH-); 2.91 (t, *J* = 6.8

Hz, 2H, C<u>H</u>$_2$- CH$_2$- NH-). ^{13}C-NMR (MeOD) δ (ppm): 136.9, 136.2, 133.5, 132.8, 127.8, 127.4, 127.3, 127.2, 126.6, 126.2, 125.7, 125.4, 122.1, 121.1, 118.3, 118.0, 111.9, 110.9, 52.7, 48.7, 24.6. ESI-MS (m/z): $(M+H)^+$ = 300.88. Anal. C21H20N2 (C, H, N).

4.1.2.14 2-(1*H*-indol-3-yl)-*N*-(pyridin-3-ylmethyl)ethanamine (19)

Compound **19** was prepared using tryptamine (0.160 g, 1.00 mmol), pyridine-3-carbaldehyde (94 µL, 1.00 mmol) and $NaBH_4$ (0.076 g, 2.00 mmol), following the method B procedure. The obtained product was an orange oil (247 mg, 98 % yield). IR (cm^{-1}): ν_{N-H} 3406, finger print 1231, 1100, 1029, 1010. ^{1}H-NMR (DMSO-d_6) δ (ppm): 10.78 (s, 1H, -N<u>H</u>- indole); 8.52 (d, *J* = 1.7 Hz, 1H, pyridine proton); 8.43 (dd, J_1 = 71.6 Hz, J_2 = 4.8 Hz, 1H pyridine proton); 7.73 (d, *J* = 7.8, 1H, pyridine proton); 7.49 (d, *J* = 7.8 Hz, 1H, indole proton); 7.32 (dd, J_1 = 2.4 Hz, J_2 = 5.6 Hz, 2H, pyridine proton); 7.13 (d, *J* = 2.1 Hz, 1H, indole proton); 7.05 (t, *J* = 7.3 Hz, 1H, indole proton); 6.95 (t, *J* = 7.4 Hz, 1H, indole proton); 3.76 (s, 2H, Py-C<u>H</u>$_2$-NH-); 2.86-2.76 (m, 4H, -C<u>H</u>$_2$- C<u>H</u>$_2$- NH-). ^{13}C-NMR (MeOD) δ (ppm): 148.8, 147.4, 137.0, 136.8, 135.5, 127.2, 123.8, 122.1, 121.0, 118.2, 117.8, 111.9, 110.9, 49.9, 48.9, 24.7. ESI-MS (m/z): $(M+H)^+$ = 252.00. Anal. C16H17N3 (C, H, N).

4.1.2.15 2-(1*H*-indol-3-yl)-*N*-(pyridin-4-ylmethyl)ethanamine (20)

Compound **20** was prepared using tryptamine (0.160 g, 1.00 mmol), pyridine-4-carbaldehyde (95 µL, 1.00 mmol) and $NaBH_4$ (0.076 g, 2.00 mmol), following the method B procedure. The obtained product was an ocra yellow solid (128 mg, 51 % yield). IR (cm^{-1}): ν_{N-H} 3301, finger print 1220, 1119, 1062, 1002. ^{1}H-NMR (DMSO-d_6) δ (ppm): 10.79 (s, 1H, -N<u>H</u>- indole); 8.47 (d, *J* = 5.7 Hz, 2H, pyridine protons); 7.49 (d, *J* = 7.8 Hz, 1H, indole proton); 7.34-7.31 (m, 3H, aromatic protons); 7.13 (d, *J* = 1.9 Hz, 1H, indole proton); 7.05 (t, *J* = 7.5 Hz, 1H, indole proton); 6.96 (t, *J* = 7.4 Hz, 1H, indole proton); 3.77 (s, 2H, Py-C<u>H</u>$_2$-NH-); 2.87-2.76 (m, 4H, -C<u>H</u>$_2$- C<u>H</u>$_2$- NH-). ^{13}C-NMR (MeOD) δ (ppm): 150.0, 148.5, 136.9, 127.3, 123.4, 122.2, 121.1, 118.3, 117.9, 112.0, 111.0, 51.4, 49.0, 24.8. ESI-MS (m/z): $(M+H)^+$ = 252.07. Anal. C16H17N3 (C, H, N).

4.1.2.16 *N*,*N'*-(1,4-phenylenebis(methylene))bis(2-(1*H*-indol-3-yl)ethanamine) (24)

Compound **24** was prepared using tryptamine (0.320 g, 2.00 mmol), benzene-1,4-dicarbaldehyde (0.134 g, 1.00 mmol) and $NaBH_4$ (0.114 g, 3.00 mmol), following the method B procedure. The obtained residue was subjected to silica gel column chromatography using CH_2Cl_2/MeOH (8:2) to give an off-white solid, mp 144-145°C (R_f = 0.32, 114 mg, 26 % yield). IR (cm^{-1}): ν_{N-H} 3314, finger print 1241, 1019. ^{1}H-NMR (DMSO-d_6) δ (ppm): 10.76 (s, 2H, -NH- indole); 7.48 (d, *J* = 7.5 Hz, 2H, aromatic protons); 7.32 (d, *J* = 8.2 Hz, 2H, aromatic protons); 7.23 (s, 4H, indole protons); 7.11 (s, 2H, indole protons); 7.04 (t, *J* = 6.2 Hz, 2H, indole protons); 6.94 (t, *J* = 6.6 Hz, 2H, indole protons); 3.70 (s, 4H, Ph-CH_2-NH-); 2.84-2.78 (m, 8H, -CH_2- CH_2-). ^{13}C-NMR (DMSO-d_6) δ (ppm): 139.6, 136.7, 128.1, 127.7, 123.0, 121.3, 118.8, 118.6, 113.1, 111.8, 53.2, 50.0, 25.9. ESI-MS (m/z): $(M+H)^+$ = 422.93. Anal. C28H30N4 (C, H, N).

4.1.2.17 *N*^{1},*N*^{4}-bis((1*H*-indol-3-yl)methyl)butane-1,4-diamine (25)

1*H*-indole-3-carbaldehyde (0.290 g, 2 mmol), butane-1,4-diamine (0.088 g, 1 mmol), molecular sieves (4 Å) in CH_2Cl_2 (15 mL) and MeOH (5 mL) were stirred at rt for 12 h. The mixture was then filtered on a celite pad, concentrated and treated with $NaBH_4$ (0.302 g, 8 mmol) in MeOH (60 mL) at rt for 2 h. After reaction MeOH was evaporated under reduced pressure, H_2O (15 mL) was added and the mixture was extracted with CH_2Cl_2 (3 x 20 mL). The organic layers were dried over Na_2SO_4 and the solvent was removed under vacuum. The yellow solids obtained were washed with diethyl ether (5x1 mL) to give the pure **25** as a white solid, mp 125-126°C (94 mg, 25 % yield). IR (cm^{-1}): ν_{N-H} 3170, finger print: 1235, 1124, 1109, 1009. ^{1}H-NMR (DMSO-d_6) δ (ppm): 10.83 (s, 2H, -NH- indole); 7.59 (d, *J* = 7.8 Hz, 2H, indole protons); 7.34 (d, *J* = 8.0 Hz, 2H, indole protons); 7.20 (d, *J* = 1.9 Hz, 2H, indole protons); 7.06 (t, *J* = 7.1 Hz, 2H, indole protons); 6.96 (t, *J* = 7.4 Hz, 2H, indole protons); 3.82 (s, 4H, Ar-CH_2-NH-); 2.57-2.54 (m, 4H, -NH-CH_2-CH_2-CH_2-CH_2-NH-); 1.49-1.47 (m, 4H, -NH-CH_2-CH_2-CH_2-CH_2-NH-). ^{13}C-NMR (MeOD) δ (ppm): 136.7, 127.0, 123.7, 121.3, 118.7, 117.7, 111.4, 111.1, 48.0, 43.0, 26.7. ESI-MS (m/z): $(M+H)^+$ = 346.47. Anal. C22H26N4 (C, H, N).

4.1.2.18 *N*^{1},*N*^{5}-bis((1*H*-indol-3-yl)methyl)pentane-1,5-diamine (26)

1*H*-indole-3-carbaldehyde (0.290 g, 2 mmol), pentane-1,5-diamine (117 µL, 1 mmol), molecular sieves (4 Å) in CH_2Cl_2 (15 mL) and MeOH (5 mL) were stirred at rt for 12 h. The mixture was then filtered on a celite pad, concentrated and treated with $NaBH_4$ (0.302 g, 8 mmol) in MeOH (60 mL) at rt for 2 h. After reaction

MeOH was evaporated under reduced pressure, H_2O (15 mL) was added and the mixture was extracted with CH_2Cl_2 (3 x 20 mL). The organic layers were dried over Na_2SO_4 and the solvent was removed under vacuum. The yellow solids obtained were washed with diethyl ether (5x1 mL) to give the pure **26** as a yellowish solid, 104-105 °C (93 mg, 26 % yield). IR (cm^{-1}): ν_{N-H} 3404, finger print: 1225, 1105, 1010. ^{1}H-NMR (DMSO-d_6) δ (ppm): 10.82 (s, 2H, -NH- indole); 7.59 (d, *J* = 7.8 Hz, 2H, indole protons); 7.34 (d, *J* = 8.0 Hz, 2H, indole protons); 7.20 (s, 2H, indole protons); 7.06 (t, *J* = 7.4 Hz, 2H, indole protons); 6.96 (t, *J* = 7.4 Hz, 2H, indole protons); 3.82 (s, 4H, Ar-CH_2-NH-); 2.56-2.54 (m, 4H, –NH-CH_2-CH_2-CH_2-CH_2-CH_2-NH- partially covered by DMSO signal); 1.47-1.40 (m, 4H, –NH-CH_2-CH_2-CH_2-CH_2-CH_2-NH-); 1.35-1.30 (m, 2H, –NH-CH_2-CH_2-CH_2-CH_2-CH_2-NH-). ^{13}C-NMR (MeOD) δ (ppm): 136.7, 127.0, 123.4, 121.1, 118.6, 117.8, 112.0, 111.0, 48.2, 43.4, 28.6, 24.7. ESI-MS (m/z): $(M+H)^+$ = 360.87. Anal. C23H28N4 (C, H, N).

4.1.2.19 *N*-(2-(1*H*-indol-3-yl)ethyl)pyridine-2-carboxamide (28)

Compound **28** was prepared using 1,1-carbonyldiimidazole (0.162 g, 1 mmol), pyridine-2-carboxylic acid (0.123 g, 1 mmol) and tryptamine (0.160 g, 1 mmol), following the same procedure described for **27**. The obtained residue was subjected to silica gel column chromatography using CH_2Cl_2/MeOH (9.8:0.2) to give a yellowish solid, mp 132-134 °C (R_f = 0.54, 224 mg, 84 % yield). IR (cm^{-1}): ν_{N-H} 3373 and 3247, $\nu_{c=o}$ 1662, finger print 1431, 1339, 1228. ^{1}H-NMR (DMSO-d_6) δ (ppm): 10.84 (s, 1H, -NH- indole); 8.90 (t, *J* = 5.9 Hz, 1H, -NH- amide); 8.63 (d, *J* = 4.5 Hz, 1H, pyridine proton); 8.06 (d, *J* = 7.7 Hz, 1H, pyridine proton); 7.99 (td, J_1 = 7.6 Hz, J_2 = 1.6 Hz, 1H, pyridine proton); 7.63-7.58 (m, 2H, aromatic protons); 7.34 (d, *J* = 8.1 Hz, 1H, indole proton); 7.20 (d, *J* = 8.1 Hz, 1H, indole proton); 7.07 (t, *J* = 7.30 Hz, 1H, indole proton); 6.98 (t, *J* = 7.3 Hz, 1H, indole proton); 3.61 (m, 2H, -CH_2-CH_2-NH-CO-); 2.97 (t, *J* = 7.5 Hz, 2H, -CH_2-CH_2-NH-CO-). ^{13}C-NMR ($CDCl_3$) δ (ppm): 164.4, 149.9, 148.0, 137.5, 136.5, 127.4, 126.2, 122.25, 122.20, 122.0, 119.3, 118.8, 112.9, 111.3, 39.8, 25.6. ESI-MS (m/z): $(M+H)^+$ = 265.87. Anal. C16H15N3O (C, H, N).

4.1.2.20 *N*-(2-(1*H*-indol-3-yl)ethyl)pyridine-3-carboxamide (29)

Compound **29** was prepared using 1,1-carbonyldiimidazole (0.162 g, 1 mmol), pyridine-3-carboxylic acid (0.123 g, 1 mmol) and tryptamine (0.160 g, 1 mmol), following the same procedure described for **27**. The obtained residue was subjected to aluminum oxide column chromatography using CH_2Cl_2/MeOH (9:1) to

give a pinkish solid, mp 148-150 °C (R_f = 0.62, 216 mg, 82 % yield). IR (cm^{-1}): ν_{N-H} 3278, $\nu_{c=o}$ 1652, finger print 1420, 1310, 1109. ^{1}H-NMR (DMSO-d_6) δ (ppm): 10.84 (s, 1H, -NH- indole); 9.00 (d, *J* = 2.0 Hz, 1H, pyridine proton); 8.82 (t, *J* = 5.4 Hz, 1H, -NH- amide); 8.70 (dd, J_1 = 4.80 Hz, J_2 = 1.40 Hz, 1H, pyridine proton); 8.18 (td, J_1 = 7.90 Hz, J_2 = 1.70 Hz, 1H, pyridine proton); 7.58 (d, *J* = 7.80 Hz, 1H, indole proton); 7.51 (dd, J_1 = 7.9 Hz, J_2 = 4.8 Hz, 1H, pyridine proton); 7.35 (d, *J* = 8.1 Hz, 1H, indole proton); 7.20 (d, *J* = 1.8 Hz, 1H, indole proton); 7.07 (t, *J* = 7.4 Hz, 1H, indole proton); 6.98 (t, *J* = 7.4 Hz, 1H, indole proton); 3.59-3.54 (m, 2H, -CH_2-CH_2-NH-CO-); 2.97 (t, *J* = 7.5 Hz, 2H, -CH_2-CH_2-NH-CO-). ^{13}C-NMR (MeOD) δ (ppm): 166.4, 151.1, 147.6, 136.8, 135.6, 130.9, 127.5, 123.7, 122.1, 121.0, 118.2, 117.9, 111.9, 110.9, 40.9, 24.7. ESI-MS (m/z): $(M+H)^+$ = 265.80. Anal. C16H15N3O (C, H, N).

4.1.2.21 *N*-(2-(1*H*-indol-3-yl)ethyl)pyridine-4-carboxamide (30)

Compound **30** was prepared using 1,1-carbonyldiimidazole (0.162 g, 1 mmol), pyridine-4-carboxylic acid (0.123 g, 1 mmol) and tryptamine (0.160 g, 1 mmol), following the same procedure described for **27**. The obtained residue was subjected to aluminum oxide column chromatography using CH_2Cl_2/MeOH (9:1) to give a yellow brilliant solid, mp 166-168 °C (R_f = 0.67, 230 mg, 87 % yield). IR (cm^{-1}): ν_{N-H} 3163, $\nu_{c=o}$ 1654, finger print 1402, 1304, 1213. ^{1}H-NMR (DMSO-d_6) δ (ppm): 10.84 (s, 1H, -NH- indole); 8.92 (t, *J* = 5.4 Hz, 1H, -NH- amide); 8.72 (dd, J_1 = 4.5 Hz, J_2 = 1.5 Hz, 2H, pyridine protons); 7.75 (dd, J_1 = 4.5 Hz, J_2 = 1.5 Hz, 2H, pyridine protons); 7.58 (d, *J* = 7.80 Hz, 1H, indole proton); 7.35 (d, *J* = 8.1 Hz, 1H, indole proton); 7.19 (d, *J* = 2.0 Hz, 1H, indole proton); 7.07 (t, *J* = 7.4 Hz, 1H, indole proton); 6.98 (t, *J* = 7.3 Hz, 1H, indole proton); 3.59-3.53 (m, 2H, -CH_2-CH_2-NH-CO-); 2.97 (t, *J* = 7.5 Hz, 2H, -CH_2-CH_2-NH-CO). ^{13}C-NMR (MeOD) δ (ppm): 166.3, 149.3, 142.9, 136.8, 127.5, 122.1, 121.6, 121.0, 118.2, 117.9, 111.8, 110.9, 40.9, 24.7. ESI-MS (m/z): $(M+H)^+$ = 265.80. Anal. C14H11NO7 (C, H, N).

4.1.2.22 *N*-(2-(1*H*-indol-3-yl)ethyl)-6-methylpyridine-3-carboxamide (31)

Compound **31** was prepared using 1,1-carbonyldiimidazole (0.162 g, 1 mmol), 6-methylpyridine-3-carboxylic acid (0.137 g, 1 mmol) and tryptamine (0.160 g, 1 mmol), following the same procedure described for **27**. The obtained residue was subjected to aluminum oxide column chromatography using CH_2Cl_2/MeOH

(9:1) to give a pinkish solid, mp 127-130 °C (R_f = 0.75, 275 mg, 98 % yield). IR (cm^{-1}): ν_{N-H} 3163, $\nu_{c=o}$ 1654, finger print 1213, 1063, 1002. ^{1}H-NMR (DMSO-d_6) δ (ppm): 10.83 (s, 1H, -NH- indole); 8.88 (d, *J* = 2.1 Hz, 1H, pyridine proton); 8.73 (t, *J* = 5.5 Hz, 1H, -NH- amide); 8.08 (dd, J_1 = 8.1 Hz, J_2 = 2.3 Hz, 1H, pyridine protons); 7.58 (d, *J* = 7.8 Hz, 1H, indole proton); 7.36 (d, *J* = 3.3 Hz, 1H, indole proton); 7.34 (d, *J* = 3.3 Hz, 1H, pyridine proton); 7.19 (d, *J* = 2.0 Hz, 1H, indole proton); 7.07 (t, *J* = 7.2 Hz, 1H, indole proton); 6.98 (t, *J* = 7.3 Hz, 1H, indole proton); 3.57-3.52 (m, 2H, -CH_2-CH_2-NH-CO-); 2.96 (t, *J* = 7.5 Hz, 2H, -CH_2-CH_2-NH-CO-); 2.52 (s, partially covered by DMSO signal -CH_3). ^{13}C-NMR (MeOD) δ (ppm): 166.4, 161.0, 147.1, 136.8, 135.9, 128.0, 127.5, 123.2, 122.1, 121.0, 118.3, 118.0, 111.9, 110.9, 40.8, 24.8, 22.5. ESI-MS (m/z): $(M+H)^+$ = 279.93. Anal. $C_{17}H_{17}N_3O$ (C, H, N).

4.1.2.23 *N*-(2-(1*H*-indol-3-yl)ethyl)pyrazine-2-carboxamide (32)

Compound **32** was prepared using 1,1-carbonyldiimidazole (0.162 g, 1 mmol), pyrazine-2-carboxylic acid (0.124 g, 1 mmol) and tryptamine (0.160 g, 1 mmol), following the same procedure described for **27**. The obtained residue was subjected to silica gel column chromatography using CH_2Cl_2/MeOH (9:1) to give a light orange solid, mp 184-186 °C (R_f = 0.62, 66 mg, 25 % yield). IR (cm^{-1}): ν_{N-H} 3381 and 3253, $\nu_{c=o}$ 1655, finger print 1225, 1094, 1020. ^{1}H-NMR (DMSO-d_6) δ (ppm): 10.84 (s, 1H, -NH- indole); 9.21 (d, *J* = 1.1 Hz, 1H, pyrazine proton); 9.05 (t, *J* = 5.8 Hz, 1H, -NH- amide); 8.87 (d, *J* = 2.4 Hz, 1H, pyrazine proton), 8.73 (m, 1H, pyrazine proton), 7.61 (d, *J* = 7.8 Hz, 1H, indole proton); 7.34 (d, *J* = 8.1 Hz, 1H, indole proton); 7.20 (d, *J* = 1.9 Hz, 1H, indole proton); 7.07 (t, *J* = 7.3 Hz, 1H, indole proton); 6.98 (t, *J* = 7.4 Hz, 1H, indole proton); 3.64-3.59 (m, 2H, -CH_2-CH_2-NH-CO-); 2.98 (t, *J* = 7.50 Hz, 2H, -CH_2-CH_2-NH-CO-). ^{13}C-NMR (DMSO-d_6) δ (ppm): 163.2, 147.9, 145.3, 143.9, 143.8, 136.7, 127.7, 123.1, 121.4, 118.8, 118.7, 112.1, 111.8, 40.2*, 25.6. *partially covered by DMSO signal. ESI-MS (m/z): $(M+H)^+$ = 266.73. Anal. C15H14N4O (C, H, N).

4.1.2.24 *N*-(2-(1*H*-indol-3-yl)ethyl)-5-nitrofuran-2-carboxamide (33)

Compound **33** was prepared using 1,1-carbonyldiimidazole (0.162 g, 1 mmol), 5-nitrofuran-2-carboxylic acid (0.157 g, 1 mmol) and tryptamine (0.160 g, 1 mmol), following the same procedure described for **27**. The

obtained residue was subjected to silica gel column chromatography using CH_2Cl_2/MeOH (9:1) to give an orange solid, mp 157-159 °C (R_f = 0.77, 116 mg, 39 % yield). IR (cm^{-1}): ν_{N-H} 3420 and 3335, $\nu_{c=o}$ 1657, ν_{NO2} 1353, finger print 1270, 1017. ^{1}H-NMR (DMSO-d_6) δ (ppm): 10.84 (s, 1H, -NH- indole); 9.03 (t, *J* = 5.7 Hz, 1H, -NH- amide); 7.76 (d, *J* = 3.9 Hz, 1H, furane proton); 7.58 (d, *J* = 7.8 Hz, 1H, indole proton); 7.39 (d, *J* = 3.9 Hz, 1H, furane proton); 7.34 (d, *J* = 8.1 Hz, 1H, indole proton); 7.19 (d, *J* = 2.1 Hz, 1H, indole proton); 7.07 (t, *J* = 7.1 Hz, 1H, indole proton); 6.99 (t, *J* = 7.1 Hz, 1H, indole proton); 3.57-3.52 (m, 2H, -CH_2-CH_2-NH-CO-); 2.95 (t, *J* = 7.5 Hz, 2H, -CH_2-CH_2-NH-CO-). ^{13}C-NMR (DMSO-d_6) δ (ppm): 156.5, 151.8, 149.0, 136.7, 127.7, 123.2, 121.4, 118.7, 118.7, 115.9, 114.0, 111.9, 111.9, 40.3* 25.4, *partially covered by DMSO signal. ESI-MS (m/z): $(M-H)^-$ = 297.93. Anal. ($C_{15}H_{13}N_3O_4$ (C, H, N).

4.1.2.25 *N*-(2-(1*H*-indol-3-yl)ethyl)-1*H*-indole-2-carboxamide (34)

Compound **34** was prepared using 1,1-carbonyldiimidazole (0.162 g, 1 mmol), 1*H*-indole-2-carboxylic acid (0.161 g, 1 mmol) and tryptamine (0.160 g, 1 mmol), following the same procedure described for **27**. The obtained residue was subjected to silica gel column chromatography using CH_2Cl_2/MeOH (9:1) to give a white solid, mp 202-204 °C (R_f = 0.68, 152 mg, 50 % yield). IR (cm^{-1}): ν_{N-H} 3412 and 3247, $\nu_{c=o}$ 1637, finger print 1257, 1100. ^{1}H-NMR (DMSO-d_6) δ (ppm): 11.61 (s, 1H, -NH- indole); 10.83 (s, 1H, -NH- indole); 8.64 (t, *J* = 5.6 Hz, 1H, -NH- amide); 7.61 (d, *J* = 7.9 Hz, 2H, indole protons); 7.43 (d, *J* = 8.2 Hz, 1H, indole proton); 7.35 (d, *J* = 8.0 Hz, 1H, indole proton); 7.20-7.15 (m, 2H, indole protons); 7.12-6.94 (m, 4H, indole protons); 3.61-3.56 (m, 2H, -CH_2-CH_2-NH-CO-); 2.98 (t, *J* = 7.5 Hz, 2H, -CH_2-CH_2-NH-CO-). ^{13}C-NMR (DMSO-d_6) δ (ppm): 161.6, 136.9, 136.7, 132.5, 127.7, 127.6, 123.6, 123.1, 121.9, 121.4, 120.1, 118.8, 118.7, 112.8, 112.3, 111.9, 102.7, 40.3*, 25.8. *partially covered by DMSO signal. ESI-MS (m/z): $(M+H)^+$ = 303.93. Anal. $C_{19}H_{17}N_3O$ (C, H, N).

4.1.2.26 *N*-(2-(1*H*-indol-3-yl)ethyl)-1,2-oxazole-5-carboxamide (35)

Compound **35** was prepared using 1,1-carbonyldiimidazole (0.107 g, 0.66 mmol), 1,2-oxazole-5-carboxylic acid (0.075 g, 0.66 mmol) and tryptamine (0.106 g, 0.66 mmol), following the same procedure described for **27**. The obtained residue was subjected to aluminum oxide column chromatography using AcOEt/MeOH

(9:1) and then crystallized from methanol to give a light orange solid, mp 127-129 °C (R_f = 0.83, 61 mg, 24 % yield). IR (cm^{-1}): ν_{N-H} 3383 and 3255, $\nu_{c=o}$ 1656, finger print 1296, 1205. ^{1}H-NMR (DMSO-d_6) δ (ppm): 10.84 (s, 1H, -NH- indole); 9.09 (t, *J* = 5.5 Hz, 1H, -NH- amide); 8.74 (d, *J* = 1.8 Hz, 1H, oxazole proton); 7.57 (d, *J* = 7.9 Hz, 1H, indole proton); 7.34 (d, *J* = 8.0 Hz, 1H, indole proton); 7.19 (d, *J* = 2.0 Hz, 1H, indole proton); 7.08-7.04 (m, 2H, oxazole + aromatic protons); 6.98 (t, *J* = 7.4 Hz, 1H, indole proton); 3.57-3.52 (m, 2H, -CH_2-CH_2-NH-CO-); 2.96 (t, *J* = 7.5 Hz, 2H, -CH_2-CH_2-NH-CO-). ^{13}C-NMR (DMSO-d_6) δ (ppm): 163.4, 156.0, 152.1, 136.7, 127.7, 123.2, 121.4, 118.74, 118.69, 111.94, 111.87, 106.2, 40.2*, 25.3. *partially covered by DMSO signal. ESI-MS (m/z): $(M+H)^+$ = 255.87. Anal. C14H13N3O2 (C, H, N).

4.1.2.27 *N*-(2-(1*H*-indol-3-yl)ethyl)-2-(4-isobutylphenyl)propanamide (36)

Compound **36** was prepared using 1,1-carbonyldiimidazole (0.081 g, 0.50 mmol), 2-(4-(2-methylpropyl)phenyl)propanoic acid (0.103 g, 0.50 mmol) and tryptamine (0.080 g, 0.50 mmol), following the same procedure described for **27**. The obtained residue was subjected to aluminum oxide column chromatography using CH_2Cl_2/MeOH (9.5:0.5) to give a white solid, mp 102-105 °C (R_f = 0.90, 116 mg, 67 % yield). IR (cm^{-1}): $\nu_{c=o}$ 1648, finger print 1234, 1213. ^{1}H-NMR (DMSO-d_6) δ (ppm): 10.76 (s *br*, 1H, -NH- indole); 8.02 (bs, 1H, NH amide); 7.49 (d, *J* = 7.6 Hz, 1H, indole proton); 7.31 (d, *J* = 7.8 Hz, 1H, indole proton); 7.20 (d, *J* = 8.0 Hz, 2H, aromatic protons); 7.08-7.03 (m, 4H, aromatic + indole protons); 6.95 (t, *J* = 7.6 Hz, 1H, indole proton); 3.53 (q, *J* = 7.1 Hz, 1H, -CH-CH_3); 3.31-3.27 (m, partially obscured by HDO signal -CH_2-CH_2-NH-); 2.77 (t, *J* = 7.6 Hz, 2H, -CH_2-CH_2-NH-); 2.40 (d, *J* = 7.2 Hz, 2H, -CH_2-CH-$(CH_3)_2$); 1.83-1.76 (m, 1H, -CH_2-CH-$(CH_3)_2$); 1.30 (d, *J* = 7.0 Hz, 3H, -CH-CH_3); 0.85 (d, *J* = 6.6 Hz, 6H, -CH_2-CH-$(CH_3)_2$). ^{13}C-NMR (DMSO-d_6) δ (ppm): 173.7, 140.1, 139.6, 136.7, 129.2, 127.7, 127.4, 123.1, 121.3, 118.7, 118.6, 112.2, 111.8, 45.2, 44.7, 44.2 *, 30.0, 25.6, 22.6, 19.1, *partially covered by DMSO signal. ESI-MS (m/z): $(M+H)^+$ = 348.93. Anal. $C_{23}H_{28}N_2O$ (C, H, N).

4.1.2.28 *N*-(2-(1*H*-indol-3-yl)ethyl)-2-(4-benzoylphenyl)propanamide (37)

Compound **37** was prepared using 1,1-carbonyldiimidazole (0.107 g, 0.66 mmol), 2-(3-benzoylphenyl)propanoic acid (0.150 g, 0.59 mmol) and tryptamine (0.095 g, 0.59 mmol), following the

same procedure described for **27**. The obtained residue was subjected to aluminum oxide column chromatography using $CHCl_3$/MeOH (9.5:0.5) and then it was washed with diethyl ether (3x1 mL) to give a yellowish oil (R_f = 0.92, 80 mg, 27 % yield). IR (cm^{-1}): $\nu_{c=o}$ 1649, finger print 1285, 1064. ^{1}H-NMR (MeOD) δ (ppm): 7.75-7.73 (m, 3H, aromatic + indole protons); 7.66 - 7.44 (m, 7H, aromatic protons); 7.31 (d, *J* = 8.2 Hz, 1H, indole proton); 7.07 (t, *J* = 7.2 Hz, 1H, indole proton); 6.97 (t, *J* = 7.2 Hz, 1H, indole proton); 6.90 (s, 1H, aromatic proton); 3.68 (q, *J* = 7.1 Hz, 1H, -C<u>H</u>-CH_3); 3.55-3.39 (m, 2H, -CH_2-C<u>H_2</u>-NH-); 2.90 (t, *J* = 7.2, 2H, -C<u>H_2</u>-CH_2-NH-); 1.45 (d, *J* = 7.1, 3H, -C<u>H_3</u>). ^{13}C-NMR (MeOD) δ (ppm): 194.3, 171.1, 140.2, 138.4, 136.5, 136.1, 133.3, 132.4, 131.1, 130.3, 129.2, 128.9, 128.4, 127.4, 123.0, 121.7, 119.8, 118.8, 113.0, 111.2, 42.9, 42.1, 25.6, 15.4. ESI-MS (m/z): $(M+H)^+$ = 397.16. Anal. C26H24N2O2 (C, H, N).

4.1.2.29 *N*-(2-(1*H*-indol-3-yl)ethyl)-2-((2,3-dimethylphenyl)amino)benzamide (38)

Compound **38** was prepared using 1,1-carbonyldiimidazole (0.162 g, 1.00 mmol), 2-(2,3-dimethylanilino)benzoic acid (0.241 g, 1.00 mmol) and tryptamine (0.160 g, 1.00 mmol), following the same procedure described for **27**. The obtained residue was subjected to silica gel column chromatography using CH_2Cl_2/MeOH (9.5:0.5) to give a pinkish solid, mp 162-164 °C (R_f = 0.87, 320 mg, 84 % yield). IR (cm^{-1}): ν_{N-H} 3410, 3379, 3302, $\nu_{c=o}$ 1625, finger print 1222, 1063. ^{1}H-NMR (DMSO-d_6) δ (ppm): 10.84 (s, 1H, -N<u>H</u>-indole); 9.62 (s, 1H, -N<u>H</u>- aniline) 8.67 (t, *J* = 5.6 Hz, 1H, -N<u>H</u>- amide); 7.63-7.57 (m, 2H, aromatic protons); 7.34 (d, *J* = 8.0 Hz, 1H, indole proton); 7.24 (t, *J* = 7.3Hz, 1H, aromatic proton); 7.20 (d, *J* = 2.0 Hz, 1H, indole proton); 7.14-7.03 (m, 3H, indole + aromatic protons); 7.02-6.90 (m, 2H, aromatic protons); 6.84 (d, *J* = 8.3 Hz, 1H, aromatic proton); 6.73 (d, *J* = 7.4 Hz, 1H, indole proton); 3.58-3.53 (m, 2H, -CH_2-C<u>H_2</u>-NH-CO-); 2.97 (t, *J* = 7.4 Hz, 2H, -C<u>H_2</u>-CH_2-NH-CO-); 2.28 (s, 3H, *o*-C<u>H_3</u>); 2.12 (s, 3H, *m*-C<u>H_3</u>). ^{13}C-NMR ($CDCl_3$) δ (ppm): 169.8, 147.0, 139.6, 138.1, 136.5, 132.2, 130.8, 127.4, 127.3, 125.8, 125.6, 122.3, 122.2, 120.8, 119.6, 118.8, 117.3, 116.9, 115.0, 112.9, 111.4, 40.0, 25.3, 20.7, 13.9. ESI-MS (m/z): $(M+H)^+$ = 383.79. Anal. C25H25N3O (C, H, N).

4.1.2.30 *N*-(2-(1*H*-indol-3-yl)ethyl)-2-((3-(trifluoromethyl)phenyl)amino)nicotinamide (39)

Compound **39** was prepared using 1,1-carbonyldiimidazole (0.162 g, 1.00 mmol), 2-(3-(trifluoromethyl)anilino)pyridine-3-carboxylic acid (0.282 g, 1.00 mmol) and tryptamine (0.160 g, 1.00 mmol), following the same procedure described for **27**. The obtained residue was subjected to silica gel column chromatography using CH_2Cl_2/MeOH (9.5:0.5) and then it was crystallized from methanol to give a white solid, mp 160-162 °C (R_f = 0.78, 270 mg, 64% yield). IR (cm^{-1}): ν_{N-H} 3399, 3359, $\nu_{c=o}$ 1635, finger print 1156, 1110, 1066. ^{1}H-NMR (DMSO-d_6) δ (ppm): 11.20 (s, 1H, -N<u>H</u>- aniline); 10.84 (s, 1H, -N<u>H</u>- indole); 8.98 (t, *J* = 5.5 Hz, 1H, -N<u>H</u>- amide); 8.37 (dd, J_1 = 4.8 Hz, J_2 = 1.7 Hz, 1H, pyridine proton); 8.31 (s, 1H, aromatic proton); 8.12 (dd, J_1 = 7.7 Hz, J_2 = 1.7 Hz, 1H, pyridine proton); 7.82 (d, *J* = 8.0 Hz, 1H, aromatic proton); 7.59 (d, *J* = 7.8 Hz, 1H, indole proton); 7.52 (t, *J* = 8.0 Hz, 1H, aromatic proton); 7.35 (d, *J* = 8.1 Hz, 1H, indole proton); 7.29 (d, *J* = 8.2 Hz, 1H, aromatic proton); 7.21 (d, *J* = 2.3 Hz, 1H, indole proton); 7.07 (t, *J* = 7.3 Hz, 1H, indole proton); 6.98 (t, *J* = 7.1 Hz, 1H, indole proton); 6.95 (dd, J_1 = 7.9 Hz, J_2 = 4.8 Hz, 1H, pyridine proton); 3.61-3.57 (m, 2H, -CH_2-C<u>H</u>$_2$-NH-CO-); 3.00 (t, *J* = 7.4 Hz, 2H, -C<u>H</u>$_2$-CH_2-NH-CO-). ^{13}C-NMR (DMSO-d_6) δ (ppm): 167.8, 154.6, 150.8, 141.5, 137.5, 136.7, 130.2, 130.1, 129.8, 127.7, 124.8 (q, *J* = 270.6 Hz), 123.4, 123.3, 123.2, 121.4, 118.7, 115.5, 114.8, 112.4, 112.2, 111.9, 40.8, 25.4. ESI-MS (m/z): $(M+H)^+$ = 425.00. Anal. C23H19F3N4O (C, H, N).

4.1.2.31 *N*-(2-(1*H*-indol-3-yl)ethyl)-2-(1-(4-chlorobenzoyl)-5-methoxy-2-methyl-1*H*-indol-3-yl)acetamide (40)

Compound **40** was prepared using 1,1-carbonyldiimidazole (0.097 g, 0.60 mmol), [1-(4-chlorobenzoyl)-5-methoxy-2-methyl-1*H*-indol-3-yl]acetic acid (0.225 g, 0.60 mmol) and tryptamine (0.096 g, 0.60 mmol), following the same procedure described for **27**. The obtained residue was subjected to silica gel column chromatography using CH_2Cl_2/MeOH (9:1) to give a white solid, mp 164-166 °C (R_f = 0.83, 290 mg, 58 % yield). IR (cm^{-1}): ν_{N-H} 3261, $\nu_{c=o}$ 1688, 1625, finger print 1217, 1150, 1088, 927. ^{1}H-NMR (DMSO-d_6) δ (ppm): 10.81 (s, 1H, -N<u>H</u>- indole); 8.15 (t, *J* = 5.7 Hz, 1H, -N<u>H</u>- amide); 7.69 (d, *J* = 8.4 Hz ,2H, aromatic protons); 7.64 (d, *J* = 8.4 Hz ,2H, aromatic protons); 7.52 (d, *J* = 7.9 Hz, 1H, indole proton); 7.33 (d, *J* = 8.1 Hz, 1H, indole proton); 7.13 (d, *J* = 6.4 Hz, 1H, aromatic proton); 7.12 (d, *J* = 2.1 Hz, 1H, indole proton); 7.06 (t, *J* = 7.5 Hz, 1H, indole proton); 6.98 - 6.94 (m, 2H, aromatic + indole protons); 6.72 (dd, J_1 = 9.0, J_2 =

2.5 Hz, 1H, aromatic proton); 3.75 (s, 3H, -OC<u>H</u>$_3$), 3.52 (s, 2H, -C<u>H</u>$_2$-CO-NH-); 3.39-3.34 (m, partially obscured by HDO signal, -CH$_2$-C<u>H</u>$_2$-NH-CO-); 2.83 (t, *J* = 7.4 Hz, 2H, -C<u>H</u>$_2$-CH$_2$-NH-CO-); 2.21 (s, 3H, -C<u>H</u>$_3$). ^{13}C-NMR ($CDCl_3$) δ (ppm): 169.7, 168.5, 156.4, 139.6, 136.4, 135.8, 133.6, 131.1, 131.0, 130.3, 129.2, 126.8, 122.2, 122.2, 119.4, 118.5, 115.1, 113.0, 112.5, 112.1, 111.3, 100.9, 55.8, 39.2, 32.2, 24.9, 13.1. ESI-MS (m/z): $(M+H)^+$ = 500.00 (100); 501.93 (40). Anal. C29H26ClN3O3 (C, H, N).

4.1.2.32 *N*^{3}, *N*^{5}-bis(2-(1*H*-indol-3-yl)ethyl)pyridine-3,5-dicarboxamide (41)

Compound **41** was prepared using 1,1-carbonyldiimidazole (0.218 g, 1.34 mmol), pyridine-3,5-dicarboxylic acid (0.100 g, 0.60 mmol) and tryptamine (0.192 g, 1.20 mmol), following the same procedure described for **27**. The obtained residue was subjected to silica gel column chromatography using CH_2Cl_2/MeOH (9:1) and then it was crystallized from AcOEt to give a white solid, mp 204-205 °C (R_f = 0.50, 215 mg, 80 % yield). IR (cm^{-1}): $\nu_{c=o}$ 1634, finger print 1297, 1224. ^{1}H-NMR (DMSO-d_6) δ (ppm): 10.82 (s *br*, 2H, -N<u>H</u>- indole); 9.09 (d, *J* = 2.0 Hz, 2H, pyridine proton); 8.95 (t, *J* = 5.6 Hz, 2H, indole protons); 8.61 (t, *J* = 2.1 Hz, 1H, pyridine proton), 7.58 (d, *J* = 7.8 Hz, 2H, indole protons); 7.34 (d, *J* = 8.1 Hz, 2H, indole protons); 7.20 (d, *J* = 2.0 Hz, 2H, indole protons); 7.07 (t, *J* = 7.1 Hz, 2H, indole protons); 3.60-3.56 (m, 4H, -CH$_2$-C<u>H</u>$_2$-NH-); 2.98 (t, *J* = 7.6 Hz, 4H, -C<u>H</u>$_2$-CH$_2$-NH-). ^{13}C-NMR (DMSO-d_6) δ (ppm): 164.7, 150.7, 136.7, 134.4, 130.3, 127.7, 123.2, 121.4, 118.7, 112.2, 111.9, 40.8, 25.5. ESI-MS (m/z): $(M+H)^+$ = 452.15. Anal. C27H25N5O2 (C, H, N).

4.2 Biological activity

4.2.1 Antifungal susceptibility testing

To evaluate the minimal inhibitory concentration (MIC), strains coming from the American Type Culture Collection (*C. albicans* ATCC 10231 and ATCC 10261) (Rockville, MD, USA) and from the Pharmaceutical Microbiology Culture Collection (*C. albicans* PMC 1042 and PMC 1082) (Department of Public Health and Infectious Diseases, Sapienza, Rome, Italy) were tested. The broth microdilution method to evaluate the susceptibility *in vitro* on *C. albicans* strains was performed according to standardized method for yeasts (CLSI M27-A3. 2008; CLSI M27-S42012). *C. albicans* strains were grown on sabouraud dextrose agar (Sigma Aldrich, St. Louis, Missouri, U.S.A.) at 35°C for 24 h. The final concentration of the inoculum was

1×10^3-5×10^3 cells/mL. The studied compounds were dissolved in dimethyl sulfoxide (Sigma Aldrich, St. Louis, Missouri, U.S.A.) at concentrations at least 100 times higher than the highest desired test concentration, in order to obtain a final DMSO concentration lower than 1%. DMSO at 1% concentration without compound has been used in the test as a control (CLSI M27-A3. 2008; CLSI M27-S42012). The final concentration of compounds ranged from 256 to 0.25 µg/mL. The MIC_{50} was the lowest concentration of compounds or reference drugs that caused ≥ 50% growth inhibition. The antifungal activities are the result of three independent experiments performed in duplicate. The data were presented as median. Fluconazole was used as reference drug (Acofarma, Madrid, Spain; lot n° 140024, purity > 99%)

4.2.2 In vitro activity of compounds against *Candida albicans* biofilms

The anti-biofilm activity was evaluated as described by Pierce et al. [16]. The BMIC end point for biofilm is based on the lowest drug concentration producing a decrease of 50% metabolic activity relative the untreated growth control (XTT reduction assay). At least two experiments were performed on two separate dates for each compound tested in triplicate.

4.2.3 In vivo toxicity assay

Galleria mellonella (Lepidoptera: Pyralidae, the Greater Wax Moth) (Island Paradise, Rome, Italy) were stored in wood shavings in the dark at 15 °C. The experiments were carried out using ten healthy *G. mellonella* larvae (between 0.20-0.30 g in weight) placed in sterile 9 cm petri dishes containing a sheet of Whatman filter paper and wood shavings. The compounds used were **3**, **7**, **16**, **17**, **23**, **24** at the concentration 5-10-50-100-500 µg/mL and the compound **36** at the concentration 10-50-100-500-1000 µg/mL. The solutions of tested compound were freshly prepared on the day of testing, prior to administration. Each compound was dissolved in DMSO and added to sterile, distilled water to give stock solutions consisting of less than 1% (v/v) DMSO. The injection was carried out using a sterile insulin syringe (Unimed, Rome Italy) and the amount being injected was 20 µL. Injections were made into the last, left pro-leg, of the *G. mellonella* larvae, directly into the haemocoel.

After injection, the larvae were incubated at 30°C for a total of four days. Larvae were monitored for survival and melanisation, at 24 hour intervals. Death was assessed based on the lack of movement in response to

stimulation together with discolouration of the cuticle. Three controls were employed for the assay: i) untreated larvae maintained under the same conditions as the treated larvae; ii) larvae pierced with an inoculation needle into the last, left pro-leg, but no solution injected; iii) larvae treated with 20 µL of sterile water/DMSO solution, in concentrations analogous to those of the test compounds. The results are presented as the mean percentage survival, of two independent experiments, of *G. mellonella* larvae, as a function of the test compounds administered dosage.

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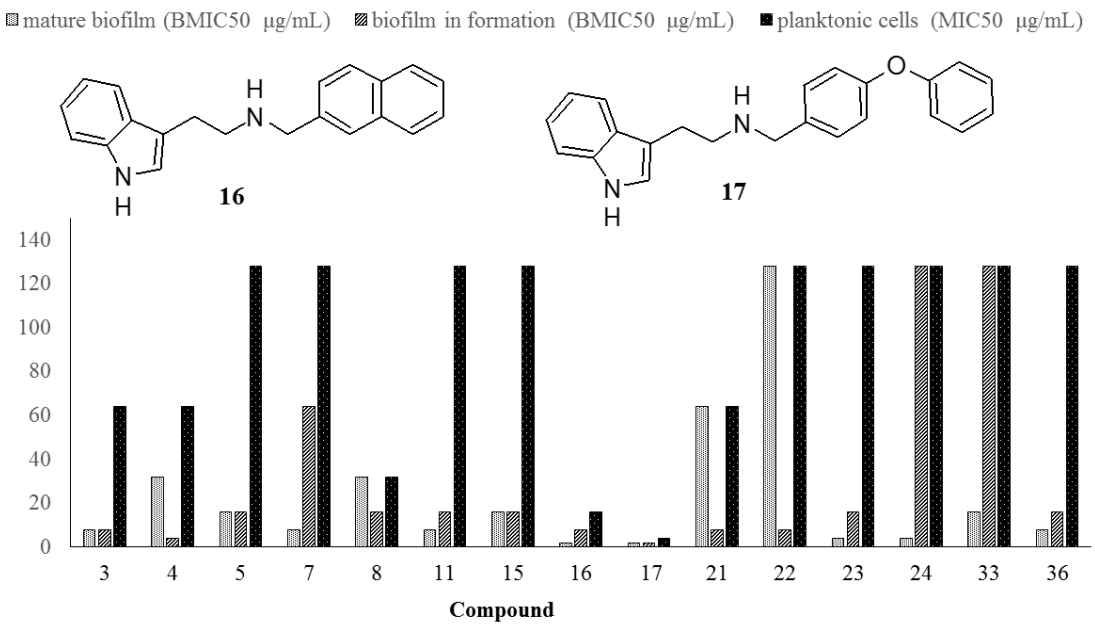

Highlights

- A series of indole was selected and evaluated against *C. albicans* biofilm.
- Fifteen compounds possess $BMIC_{50} \leq 16$ µg mL on *C. albicans* biofilm.
- The most active has $BMIC_{50} = 2$ µg/mL both on mature and in formation biofilm.
- Tested compounds were poorly toxic *in vivo* on *Galleria mellonella* larvae.