Bioorganic & Medicinal Chemistry 18 (2010) 1054–1061

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

Salicylanilide carbamates: Antitubercular agents active against multidrug-resistant *Mycobacterium tuberculosis* strains

Juana M. Férriz [a], Kateřina Vávrová [a], Filip Kunc [b,c], Aleš Imramovský [d], Jiřina Stolaříková [e], Eva Vavříková [a], Jarmila Vinšová [a,*]

[a] Charles University, Faculty of Pharmacy, Department of Inorganic and Organic Chemistry, Heyrovského 1203, 500 05 Hradec Králové, Czech Republic
[b] Charles University, Faculty of Medicine in Hradec Králové, Department of Medicinal Biology, Šimkova 870, 500 38 Hradec Králové, Czech Republic
[c] Generi Biotech, Machkova 587, 500 11 Hradec Králové, Czech Republic
[d] Institute of Organic Chemistry and Technology, Faculty of Chemistry and Chemical Technology, University of Pardubice, Studentská 573, 532 10 Pardubice, Czech Republic
[e] Laboratory for TBC, Health Institute in Ostrava, 702 00 Ostrava, Czech Republic

ARTICLE INFO

Article history:
Received 21 July 2009
Revised 15 December 2009
Accepted 20 December 2009
Available online 29 December 2009

Keywords:
Salicylanilide carbamates
Multidrug-resistant tuberculosis
In vitro antimycobacterial activity
Cytotoxicity
Chemical hydrolysis

ABSTRACT

A series of 27 salicylanilide-based carbamates was prepared as a part of our ongoing search for new antituberculosis drugs. These compounds exhibited very good in vitro activity against *Mycobacterium tuberculosis, Mycobacterium kansasii* and *Mycobacterium avium* and, in particular, against five multidrug-resistant strains, with MIC values between 0.5–2 μmol/L. Moreover, they displayed moderate toxicity against intestinal cells with the selectivity index being up to 96. Furthermore, acid stability and a half-life of 43 h at pH 7.4 were shown. Thus, these novel salicylanilide derivatives are drug candidates which should be seriously consider for further screening.

1. Introduction

The recent worldwide emergence of drug-resistant tuberculosis (TB) is alarming, especially the increase of multidrug-resistant tuberculosis (MDR-TB),[1] and most recently, extensively drug-resistant tuberculosis (XDR-TB).[2] Every year around 9 million people develop the active and contagious pulmonary TB and 20% of them die of their infection.[3] More than four percent of all the worldwide tuberculosis patients are resistant to at least one of the current first line medications.[4] Furthermore, every year almost 500,000 people are infected with MDR-TB and there are estimated 40,000 new cases of XDR-TB annually.[5,6] TB in co-occurrence with the spread of human immunodeficiency virus infection[7] belong amongst the most serious worldwide health threats. Therefore, effective new drugs[8] and strategies[9] to treat the TB bacilli as well as its resistance pattern are an urgent demanding task.

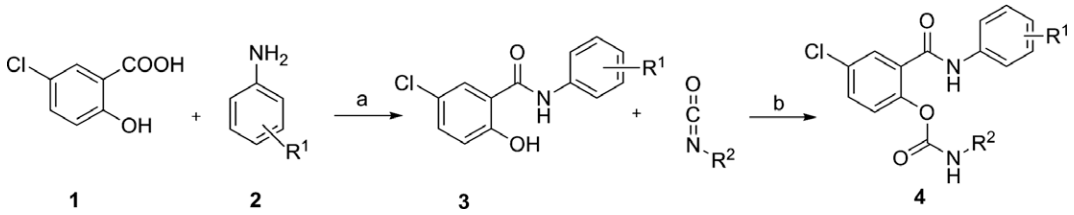
Salicylanilides (SAL) are an important class of aromatic compounds with a wide range of pharmacological activities, such as antibacterial,[10–12] antifungal[13] and anti-inflammatory,[14] among others. Furthermore; several studies reported their potent antimycobacterial effect.[15] Their activity results from multiple mechanisms. SAL were identified as inhibitors of the two-component regulatory systems[16] of bacteria[17,18] by a mechanism related to the effects on uncoupling oxidative phosphorylation. In recent studies, they were also found to be selective inhibitors of interleukin-12p40 production that play specific role in the initiation, expansion and control of the cellular response to TB as well.[19,20]

In the recent past, a number of organic carbamates have been found as potential antibacterial and antiviral agents.[21] The carbamate residue present in these new molecules contributes as a core component[22] or incorporated into a known molecule, contributes to the improvement of its pharmacodynamic and pharmacokinetic properties.[23] In particular, carbamate was successfully used to protect phenolic drugs.[24]

Thus, we hypothesised that masking the phenolic hydroxyl in SAL by carbamate formation may protect the molecule against extensive first-pass metabolism following oral administration, broaden its activity profile and improve its physicochemical and pharmacokinetic properties. In this context, the aim of this article was to describe the synthesis, antimycobacterial activity and cytotoxicity of a series of SAL carbamates as well as to evaluate their stability against chemical hydrolysis at various pH values.

* Corresponding author.
E-mail address: vinsova@faf.cuni.cz (J. Vinšová).

0968-0896/$ - see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmc.2009.12.055



Scheme 1. Synthesis of 4-chloro-2-(R^1-chlorophenylcarbamoyl)phenyl alkylcarbamates (R^1 = 3-Cl, 3,4-diCl, 4-Cl, R^2 = ethyl, butyl, pentyl, hexyl, heptyl, oktyl, nonyl, decyl, undecyl). Reagents and conditions: (a) PCl_3, chlorobenzene, microwave irradiation (530 W); (b) TEA, ACN, rt.

2. Results and discussion

2.1. Chemistry

The preparation of the carbamates is outlined in Scheme 1. The starting SAL **3** were selected according to previous results showing high in vitro activity against *Mycobacterium tuberculosis*[25] They were routinely prepared by the reaction of 5-chlorosalicylic acid **1** with the appropriate aniline **2** in chlorobenzene with PCl_3.[26] By using microwave irradiation, the reaction time was shortened from several hours to minutes.

For the synthesis of the corresponding carbamates **4**, a suspension of SAL **3** in acetonitrile (ACN) was treated with one equivalent of triethylamine (TEA), adding then the corresponding isocyanate. This reaction was performed at room temperature due to thermal instability of the products. The prepared carbamates **4a**–**zz** belong into three series: those having chlorine at position 4 of salicylic part, and at positions 3 and 4, respectively, of the anilide ring (see Table 1). The yields of the synthesized compounds varied in the interval of 35–80%. The carbamates **4** were characterised by means of infrared, NMR spectroscopy and elemental analyses.

2.2. Antimycobacterial activities

The prepared carbamates **4** were tested in vitro for their antimycobacterial activity in the *Laboratory for TBC, Health Institute in Ostrava*, against *M. tuberculosis* 331/88 and against some non-TB strains such as *Mycobacterium avium* (330/88) and *Mycobacterium kansasii* (235/80 and 6509/96), where the first line drug isoniazid (INH) shows no activity. The anti-TB screening results of the compounds **4** are summarized in Table 1.

In general, there are two factors influencing the activity of these compounds. Firstly, the most active derivatives with minimal inhibition concentration (MIC) values from 0.5 to 2 μmol/L possess two chlorines at positions 3 and 4 of the aniline moiety (**4j**–**4r**). Such presence of an electron acceptor substituent in the aniline moiety was previously found to play an important role in the activity of SAL.[25] Focusing our attention on the alkyl chain, the compounds with 5, 6 or 7 carbons (**4d**, **4l**, **4m**, **4n** and **4u**) showed the highest activity with MIC of 0.5–1 μmol/L. This suggests that the lipophilicity of the carbamates plays an important role in their biological activity. The Log *P* values of these compounds (Table 1), that is, the logarithm of the partition coefficient for *n*-octanol/water, calculated

Table 1
Antimycobacterial activity and calculated lipophilicity of SAL alkylcarbamates **4**

	R^1	R^2	MIC (μmol/L)								log *P*/*C* log *P*[a]
			M. tuberculosis 331/88		*M. avium* 330/88		*M. kansasii* 235/80		*M. kansasii* 6509/96		
			14 d	21 d	14 d	21 d	14 d	21 d	14 d	21 d	
4a	3-Cl	Ethyl	2	2	8	8	8	8	8	8	3.79/4.19
4b	3-Cl	Butyl	2	4	8	16	16	16	8	16	4.69/5.25
4c	3-Cl	Pentyl	2	4	8	8	8	8	8	8	5.11/5.78
4d	3-Cl	Hexyl	0.5	1	8	8	4	4	4	4	5.53/6.31
4e	3-Cl	Heptyl	1	2	8	8	4	8	8	8	5.94/6.84
4f	3-Cl	Octyl	2	2	4	4	8	8	4	8	6.36/7.37
4g	3-Cl	Nonyl	1	2	2	4	4	4	4	4	6.78/7.89
4h	3-Cl	Decyl	1	2	8	8	8	8	8	8	7.19/8.42
4i	3-Cl	Undecyl	2	2	8	8	8	8	8	8	7.61/8.95
4j	3,4-diCl	Ethyl	0.5	1	16	32	2	4	2	4	4.35/4.82
4k	3,4-diCl	Butyl	0.5	1	8	16	2	2	2	4	5.25/5.88
4l	3,4-diCl	Pentyl	0.5	0.5	8	16	2	2	2	4	5.67/6.41
4m	3,4-diCl	Hexyl	0.5	0.5	4	8	2	2	1	2	6.08/6.93
4n	3,4-diCl	Heptyl	0.5	1	4	8	2	2	2	4	6.50/6.46
4o	3,4-diCl	Octyl	0.5	1	4	8	2	4	2	4	6.92/7.99
4p	3,4-diCl	Nonyl	2	2	8	8	8	8	4	4	7.34/8.52
4q	3,4-diCl	Decyl	2	2	8	8	4	4	2	4	7.77/9.05
4r	3,4-diCl	Undecyl	2	2	16	16	4	4	4	4	8.17/9.58
4s	4-Cl	Ethyl	1	2	8	8	4	8	4	8	3.79/4.19
4t	4-Cl	Butyl	2	2	4	8	4	4	2	4	4.69/5.25
4u	4-Cl	Pentyl	0.5	0.5	8	8	2	4	4	4	5.11/5.78
4v	4-Cl	Hexyl	1	2	4	8	4	4	2	4	5.53/6.31
4w	4-Cl	Heptyl	2	2	8	8	8	8	4	4	5.94/6.84
4x	4-Cl	Octyl	2	2	8	8	8	8	4	4	6.36/7.37
4y	4-Cl	Nonyl	2	2	8	16	4	4	4	4	6.78/7.89
4z	4-Cl	Decyl	2	2	8	8	8	8	8	8	7.19/8.42
4zz	4-Cl	Undecyl	2	2	8	8	8	8	8	8	7.61/8.95
INH	—	—	0.5	0.5	>250	>250	>250	>250	4	4	—

[a] The Log *P* values were calculated using the program CS ChemOffice Ultra ver. 9.0 (CambridgeSoft, Cambridge, MA, USA).

Table 2
Activity of selected carbamates against MDR-TB strains

	MIC (μmol/L)												EC_{50} (μmol /L)	SI*	SI*
	M. tuberculosis 331/88		*M. tuberculosis* 7357/98[a]		*M. tuberculosis* 9449/06[b]		*M. tuberculosis* 2092/05[c]		*M. tuberculosis* Praha 1[d]		*M. tuberculosis* Praha 128[e]			For MTB	For MDR-TB
	14 d	21 d	14 d	21 d	14 d	21 d	14 d	21 d	14 d	21 d	14 d	21 d			
4d	0.5	1	1	1	1	1	1	2	1	2	1	2	14.9	14.9	7.4–14.9
4j	1	1	0.5	1	0.5	0.5	0.5	0.5	0.5	1	0.5	0.5	31.0	31.0	31–62
4k	0.5	1	0.5	1	1	0.5	0.5	1	0.5	1	0.5	1	38.0	38.0	38–76
4l	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	1	0.5	0.5	40.0	80.0	40–80
4m	0.5	0.5	0.5	1	0.5	0.5	0.5	0.5	0.5	1	0.5	0.5	27.9	55.8	28–56
4n	0.5	1	0.5	0.5	0.5	0.5	0.5	1	0.5	1	0.5	1	43.4	43.4	43.4–86.8
4o	1	1	1	1	1	0.5	0.5	1	1	2	0.5	1	48.5	48.5	24–97
4u	0.5	0.5	1	2	1	1	1	1	1	2	1	1	17.4	34.8	8.7–17.4
INH	0.5	0.5	16	16	16	16	16	16	16	16	16	16	>100	>200	>6.25

[a] Resistant to INH, RMP, ETA, STM, OFX and ansamycin.
[b] Resistant to INH, STM, RMP and ansamycin.
[c] Resistant to INH, RMP, ETA, STM, OFX and ansamycin.
[d] Resistant to INH, RMP, ETA, STM, CFZ and ansamycin.
[e] Resistant to INH, RMP, ETA, STM, GTM, CFZ, ansamycin and AK.
* SI = EC_{50}/MIC.

using the program CS ChemOffice Ultra ver. 9.0 (CambridgeSoft, Cambridge, MA, USA), are from 5 to 6.4, which does not correlate with Lipinski rule of five. However, we can speculate that due to high lipophilicity, the molecule presents higher membrane permeability, making more effective the delivery of the parent drug. This hypothesis is supported by the results of our previous research,[26] where we presented a series of 30 N-protected amino acid esters of salicylanilides.[27] In this study, the most active derivatives resulted to be those with log *P* values of approximately 6.

Although higher lipophilicity may be the reason for the increase in the activities of the carbamates **4** against *M. tuberculosis, kansasii* and *avium* compared to the starting SAL,[25] it cannot explain their higher potency compared to the corresponding N-protected amino acid ester derivatives, whose lipophilicities are comparable.[27] For example, compound **4m** was 16 times more potent that the parent SAL and four times more active that the most active corresponding N-protected amino acid ester derivative against *M. tuberculosis* (331/88).

The most active derivatives of this series were tested against five clinically isolated MDR-TB strains as well, including *M. tuberculosis* 7357/98, resistant to INH, rifampicine (RMP), ethambutol (ETA), streptomycin (STM), ofloxacin (OFX) and ansamycin, *M. tuberculosis* 9449/06, resistant to INH, STM, RMP and ansamycin, *M. tuberculosis* 2092/05, resistant to INH, RMP, ETA, STM, OFX and ansamycin, *M. tuberculosis* Praha 1, resistant to INH, RMP, ETA, STM, clofazimine (CFZ) and ansamycin, and *M. tuberculosis* Praha 128 resistant to IHN, RMP, ETA, STM, gentamicin (GTM), CFZ, ansamycin and amikacin (AK). All the studied compounds exhibited high activity against the MDR-TB strains, with MIC values between 0.5–2 μmol/L (Table 2). These activities are comparable with that presented by compounds undergoing PhaseII clinical trials such a nitroimidazopyran PA-824,[28,29] with MIC values between 0.1–0.7 μmol/L against mono and MDR-TB strains or the diamine analogue of ETA, SQ109,[30,31] with MIC values between 0.5–1.8 μmol/L against strains resistant to ETA, INH and RMP.

2.3. Cytotoxicity of the most active compounds

The in vitro mammalian cell toxicity of the most active compounds **4d**, **4j**, **4k**, **4l**, **4m**, **4n**, **4o** and **4u** was assessed on human intestinal cell line HCT-8 (ECACC, UK) using the XTT assay.[32] The cytotoxicity results presented in Table 2 are expressed as the concentration inhibiting 50% of the cell growth (EC_{50}). The studied compounds show moderate cytotoxicity in comparison to the standard INH with the EC_{50} values in the range of 14.9–48.5 μmol/L. Nevertheless, all the studied compounds displayed the selectivity index (SI) value, defined as a ratio of EC_{50} to MIC, higher than 10 for both TB and MDR-TB strains (Table 2), what means that they should be seriously considered for further screening.[33]

2.4. Stability of compound 4m

The chemical hydrolysis of compound **4m** was studied in aqueous buffer solutions at pH from 3 to 8 at 37 °C. The decomposition of **4m** and the release of the parent SAL were monitored by HPLC for 48 h. The carbamate bond was stable at acidic pH values but decomposed in alkaline environment (Table 3, Fig. 1).

Table 3
Half-lives ($t_{1/2}$) of the carbamate **4m** at various pH values

pH	$t_{1/2}$ (h)
3	nd
4	nd
5	nd
6	117.5 ± 19.5
7	64 ± 8
7.4	43 ± 6
8	5 ± 2

Mean ± SEM, $n = 3$.
nd = no significant decomposition observed ($p < 0.05$).

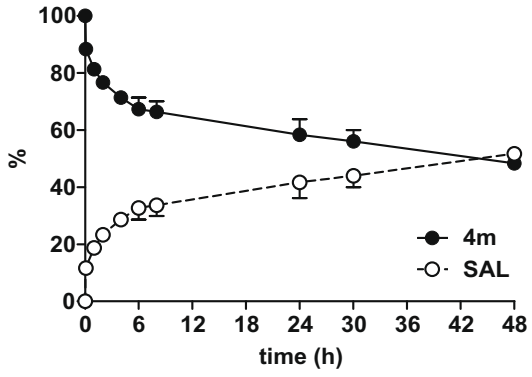


Figure 1. Hydrolysis of the carbamate **4m** to the corresponding SAL in 100 mM phosphate buffer at pH 7.4 at 37 °C.

At physiological pH of 7.4, the carbamate **4m** showed a $t_{1/2}$ of almost 2 days. Although these data describe chemical hydrolysis only, we hypothesize that these compounds may also be stable in plasma for sufficient time to reach its site of action, that is, the mycobacteria. This assumption is supported by the relative stability of the carbamate bond towards esterases.[34] Moreover, in a previous study, a carbamate of a similar structure displayed a $t_{1/2}$ of 15.9 and 2.7 h in a buffer and plasma, respectively.[35] In addition, due to the stability in acidic environment, these compounds may be good candidates for oral administration. Furthermore, protection of the phenolic hydroxyl may render the compound less susceptible towards first-pass metabolism. However, the stability of the synthesized SAL carbamates in biological environments and their bioavailability warrants further investigation.

3. Conclusion

The results obtained in this study show that the derivatization of the phenolic group of SAL in the form of carbamate appears to be a useful approach to increase their antimycobacterial activity. Whether this is a simple effect of an increased hydrophobicity and therefore better permeability through the lipophilic mycobacterial cell wall or a direct effect of the carbamate moiety remains to be elucidated. However, the high potencies of these newly prepared compounds, particularly those against the MDR-TB strains, together with their favorable selectivity makes these SAL-derived carbamates promising drug candidates.

4. Experimental

4.1. General

All reagents and solvents were purchased from commercial sources (Sigma–Aldrich, Merck). Reactions were monitored by thin layer chromatography plates coated with 0.2 mm silica gel 60 F_{254} (Merck). During the measurement of melting point, carbamates undergo thermal degradation.[36] Melting points of degradated products were in the range of 212–248 °C and are not presented. Infrared spectra (KBr pellets) were evaluated on FT-IR spectrometer Nicolet 6700 FT-IR in the range of 400–4000 cm^{-1}. NMR spectra were measured in THF solutions at room temperature on a Varian Mercury-Vxbb 300 (300 MHz for ^1H and 75.5 MHz for ^{13}C; Varian Comp. Palo Alto, CA, USA). Elemental analyses (C, H, N) were performed on an automatic micro analyser CHNS-OCE instrument (Fisons EA 1110, Milano, Italy).

4.1.1. General procedure for the preparation of 4-chloro-2-(R^1-chlorophenylcarbamoyl)-phenyl alkylcarbamates 4

To a suspension of the corresponding SAL **3** (1 mmol) in ACN (7 mL) was added one equivalent of TEA (1 mmol, 101 mg). After SAL dissolved, the appropriate isocyanate (1 mmol) was added in four fractions during 2 h at room temperature under stirring. After the complete addition of the isocyanate, the reaction mixture was stirred for two more hours at room temperature. The resulting crystals were filtered off and washed with methanol.

4.1.1.1. 4-Chloro-2-(3-chlorophenylcarbamoyl)phenyl ethylcarbamate (4a). (35%) as a white solid; ν_{max} (KBr) 1660 (CO), 1715 (CO), 3279 (NH), 3343 (NH) cm^{-1}; ^1H NMR (THF-d_8, 300 MHz): δ ppm 9.57 (s, 1H, NH), 7.88 (s, 1H, H2′), 7.65 (s, 1H, H3), 7.55 (d, J = 8.0 Hz, 1H, H4′), 7.45 (d, J = 8.6 Hz, 1H, H5), 7.29–7.21 (m, 2H, H6, H5′), 7.16–6.94 (m, 2H, NH, H6′), 3.21–3.05 (m, 2H, CH_2), 1,10 (t, J = 7.1 Hz, 3H, CH_3); ^{13}C NMR (THF-d_8, 75 MHz): δ ppm 163.9, 154.4, 148.5, 141.7, 134.9, 132.9, 131.5, 130.7, 129.4, 125.7, 124.2, 120.2, 118.4, 36.7, 15.2. Anal. Calcd for $C_{16}H_{14}Cl_2N_2O_3$ (353.20): C, 54.41; H, 4.00; N, 7.93. Found: C, 54.23; H, 3.89; N, 7.90.

4.1.1.2. 4-Chloro-2-(3-chlorophenylcarbamoyl)phenyl butylcarbamate (4b). (40%) as a white solid; ν_{max} (KBr) 1654 (CO), 1723 (CO), 3279 (NH), 3343 (NH) cm^{-1}; ^1H NMR (THF-d_8, 300 MHz): δ ppm 9.58 (s, 1H, NH), 7.89 (dd, J = 2.0, 2.0 Hz, 1H, H2′), 7.65 (d, J = 2.6 Hz, 1H, H3), 7.56 (ddd, J = 8.2, 2.0, 0.9 Hz, 1H, H4′), 7.45 (dd, J = 8.7, 2.6 Hz, 1H, H5), 7.30–7.20 (m, 2H, H6, H5′), 7.06 (ddd, J = 8.0, 2.0, 0.9 Hz, 1H, H6′), 3.11 (dt, J = 6.8, 5.6 Hz, 2H, CH_2), 1.49–1.24 (m, 4H, CH_2), 0.85 (t, J = 7.2 Hz, 3H, CH_3); ^{13}C NMR (THF-d_8, 75 MHz): δ ppm 163.9, 154.5, 148.5, 141.7, 134.9, 133.0, 131.5, 130.7, 130.6, 129.3, 125.7, 124.2, 120.2, 118.4, 41.6, 32.8, 20.6, 14.1. Anal. Calcd for $C_{18}H_{18}Cl_2N_2O_3$ (381.25): C, 56.71; H, 4.76; N, 7.35. Found: C, 56.83; H, 4.59; N, 7.49.

4.1.1.3. 4-Chloro-2-(3-chlorophenylcarbamoyl)phenyl pentylcarbamate (4c). (63%) as a white solid; ν_{max} (KBr) 1655 (CO), 1713 (CO), 3257 (NH) cm^{-1}; ^1H NMR (THF-d_8, 300 MHz): δ ppm 9.58 (s, 1H, NH), 7.89 (dd, J = 2.0, 2.0 Hz, 1H, H2′),7.65 (d, J = 2.6 Hz, 1H, H3), 7.56 (ddd, J = 7.9, 2.0, 0.9 Hz, 1H, H4′), 7.45 (dd, J = 8.7, 2.6 Hz, 1H, H5), 7.31–7.29 (m, 2H, H6, H5′), 7.13 (t, J = 5.5 Hz, 1H, NH), 7.07 (ddd, J = 7.5, 2.0, 0.9 Hz, 1H, H6′), 3.10 (dt, J = 6.1, 5.5 Hz, 2H, CH_2), 1.51–1.40 (m, 2H, CH_2), 1.31–1.23 (m, 4H, CH_2), 0.86 (t, J = 6.7 Hz, 3H, CH_3); ^{13}C NMR (THF-d_8, 75 MHz): δ ppm 163.8, 154.4, 148.4, 141.6, 134.8, 132.8, 131.4, 130.6, 130.5, 129.2, 125.6, 124.1, 120.1, 118.3, 41.8, 30.2, 29.6, 23.1, 14.2. Anal. Calcd for $C_{19}H_{20}Cl_2N_2O_3$ (394.28): C, 57.73; H, 5.10; N, 7.09. Found: C, 57.52; H, 5.29; N, 7.04.

4.1.1.4. 4-Chloro-2-(3-chlorophenylcarbamoyl)phenyl hexylcarbamate (4d). (68%) as a white solid; ν_{max} (KBr) 1657 (CO), 1716 (CO), 3262 (NH) cm^{-1}; ^1H NMR (THF-d_8, 300 MHz): δ ppm 9.57 (s, 1H, NH), 7.88 (dd, J = 2.0, 2.0 Hz, 1H, H2′), 7.65 (d, J = 2.6 Hz, 1H, H3), 7.58–7.53 (m, 1H, H4′), 7.45 (dd, J=8.7, 2.6 Hz, 1H, H5), 7.24 (m, 2H, H6, H5′), 7.13 (t, J = 5.5 Hz, 1H, NH), 7.08–7.05 (m, 1H, H6′), 3.10 (dt, J = 6,18, 5,53 Hz, 2H, CH_2), 1.44 (m, 2H, CH_2), 1.27 (m, 6H), 0.87 (t, J = 6.7 Hz, 3H); ^{13}C NMR (THF-d_8, 75 MHz): δ ppm 163.9, 154.5, 148.5, 141.7, 135.0, 132.9, 131.5, 130.7, 130.6, 129.3, 125.7, 124.2, 120.2, 118.4, 41.9, 32.4, 30.6, 27.3, 23.4, 14.4. Anal. Calcd for $C_{20}H_{22}Cl_2N_2O_3$ (409.31): C, 58.69; H, 5.42; N, 6.84. Found: C, 58.22; H, 5.51; N, 7.00.

4.1.1.5. 4-Chloro-2-(3-chlorophenylcarbamoyl)phenyl heptylcarbamate (4e). (40%) as a white solid; ν_{max} (KBr) 1655 (CO), 1713 (CO), 3267 (NH), 3331 (NH) cm^{-1}; ^1H NMR (THF-d_8, 300 MHz): δ ppm 9.58 (s, 1H, NH), 7.88 (dd, J = 2.0, 2.0 Hz, 1H, H2′), 7.65 (J = 2.6 Hz, 1H, H3), 7.58–7.54 (m, 1H, H4′), 7.45 (dd, J = 8.7, 2.6 Hz, 1H, H5), 7.30–7.20 (m, 2H, H6, H5′), 7.13 (t, J = 5.3 Hz, 1H, NH), 7.07 (ddd, J = 8.1, 1.8, 1.1 Hz, 1H, H6′), 3.10 (dt, J = 6.1, 5.3 Hz, 2H, CH_2), 1.50–1.40 (m, 2H, CH_2), 1.34–1.22 (m, 8H, CH_2), 0.88 (t, J = 6.8 Hz, 3H, CH_3); ^{13}C NMR (THF-d_8, 75 MHz): δ ppm 163.8, 154.4, 148.4, 141.6, 134.8, 132.8, 131.4, 130.6, 130.5, 129.2, 125.6, 124.1, 120.1, 118.3, 41.8, 32.6, 30.5, 29.8, 27.5, 23.4, 14.3. Anal. Calcd for $C_{21}H_{24}Cl_2N_2O_3$ (423.33): C, 59.58; H, 5.71; N, 6.62. Found: C, 59.22; H, 5.43; N, 6.60.

4.1.1.6. 4-Chloro-2-(3-chlorophenylcarbamoyl)phenyl octylcarbamate (4f). (35%) as a white solid; ν_{max} (KBr) 1659 (CO), 1713 (CO), 3325 (NH) cm^{-1}; ^1H NMR (THF-d_8, 300 MHz): δ ppm 9.57 (s, 1H, NH), 7.88 (dd, J = 1.9, 1.9 Hz, 1H, H2′), 7.65 (d, J = 2.6 Hz, 1H, H3), 7.56 (ddd, J = 8.1, 1.9, 0.9 Hz, H4′), 7.45 (dd, J = 8.7, 2.6 Hz, 1H, H5), 7.28–7.20 (m, 2H, H6, H5′), 7.12 (t, J = 5.4 Hz, 1H, NH), 7.06 (ddd, J = 8.1, 1.9, 0.9 Hz, 1H, H6′), 3.09 (dt, J = 6.7, 5.4 Hz 2H, CH_2), 1.51–1.39 (m, 2H, CH_2), 1.31–1.25 (m, 10H), 0.88 (t, J = 6.7 Hz, 3H, CH_3); ^{13}C NMR (THF-d_8, 75 MHz): δ ppm 163.9, 154.5, 148.5, 141.7, 135.0, 132.9, 131.5, 130.7, 130.6, 129.4, 125.7, 124.2, 120.2, 118.4, 41.9, 32.8, 30.7, 30.2, 30.2, 27.6, 23.5,

14.4. Anal. Calcd for $C_{22}H_{26}Cl_2N_2O_3$ (437.36): C, 60.42; H, 5.99; N, 6.41. Found: C, 59.84 H, 5.85; N, 6.38.

4.1.1.7. 4-Chloro-2-(3-chlorophenylcarbamoyl)phenyl nonylcarbamate (4g). (54%) as a white solid; ν_{max} (KBr) 1656 (CO), 1716 (CO), 3259 (NH), 3323 (NH) cm^{-1}; ^1H NMR (THF-d_8, 300 MHz): δ ppm 9.56 (s, 1H, NH), 7.88 (dd, J = 1.9, 1.9 Hz, 1H, H2′), 7.65 (d, J = 2.6 Hz, 1H, H3), 7.59–7.53 (m, 1H, H4′), 7.45 (dd, J = 8.7, 2.6 Hz, 1H, H5), 7.24 (m, 2H, H6, H5′), 7.12 (t, J = 5.2 Hz, 1H, NH), 7.06 (ddd, J = 8.0, 1.9, 1.0 Hz, 1H, H6′), 3.10 (dt, J = 6.6, 5.2 Hz, 2H, CH_2), 1.31–1.24 (m, 12H, CH_2), 0.89 (t, J = 6.7 Hz, 3H, CH_3); ^{13}C NMR (THF-d_8, 75 MHz): δ ppm 163.9, 154.5, 148.5, 141.7, 135.0, 133.0, 131.5, 130.7, 130.6, 129.4, 125.7, 124.2, 120.2, 118.4, 41.9, 32.8, 30.7, 30.6, 30.5, 30.3, 27.6, 23.6, 14.4. Anal. Calcd for $C_{23}H_{28}Cl_2N_2O_3$ (451.39): C, 61.20; H, 6.25; N, 6.21. Found: C, 61.28; H, 5.85; N, 6.18.

4.1.1.8. 4-Chloro-2-(3-chlorophenylcarbamoyl)phenyl decylcarbamate (4h). (63%) as a white solid; ν_{max} (KBr) 1660 (CO), 1713 (CO), 3270 (NH), 3329 (NH) cm^{-1}; ^1H NMR (THF-d_8, 300 MHz): δ ppm 9.57 (s, 1H, NH), 7.88 (dd, J = 2.0, 2.0 Hz, 1H, H2′), 7.65 (d, J = 2.6 Hz, H3), 7.58–7.53 (m, 1H, H4′), 7.45 (dd, J = 8.7, 2.6 Hz, 1H, H5), 7.28–7.20 (m, 2H, H6, H5′), 7.12 (t, J = 5.4 Hz, 1H, NH), 7.06 (ddd, J = 8.0, 2.0, 0.9 Hz, 1H, H6′), 3.09 (dt, J = 6.8, 5.4 Hz, 2H, CH_2), 1.49–1.39 (m, 2H, CH_2), 1.33–1.23 (m, 14H, CH_2), 0.89 (t, J = 6.7 Hz, 3H, CH_3); ^{13}C NMR (THF-d_8, 75 MHz): δ ppm 163.9, 154.5, 148.5, 141.7, 134.9, 132.9, 131.5, 130.7, 129.3, 125.7, 124.2, 120.2, 118.4, 41.9, 32.9, 30.7, 30.6, 30.5, 30.3, 30.3, 27.6, 23.6, 14.4. Anal. Calcd for $C_{24}H_{30}Cl_2N_2O_3$ (465.41): C, 61.94; H, 6.50; N, 6.02. Found: C, 61.88; H, 6.75; N, 6.05.

4.1.1.9. 4-Chloro-2-(3-chlorophenylcarbamoyl)phenyl undecylcarbamate (4i). (82%) as a white solid; ν_{max} (KBr) 1659 (CO), 1714 (CO), 3270 (NH), 3330 (NH) cm^{-1}; ^1H NMR (THF-d_8, 300 MHz): δ ppm 9.57 (s, 1H, NH), 7.88 (dd, J = 2.0 Hz, 1H, H2′), 7.65 (d, J = 2.6 Hz, H3), 7.58–7.53 (m, 1H, H4′), 7.45 (dd, J = 8.7, 2.6 Hz, 1H, H5), 7.28–7.20 (m, 2H, H6, H5′), 7.12 (t, J = 5.5 Hz, 1H, NH), 7.06 (ddd, J = 8.0, 2.0, 0.9 Hz, 1H, H6′), 3.09 (dt, J = 6.81, 5.49 Hz, 2H, CH_2), 1.50–1.40 (m, 2H, CH_2), 1.34–1.23 (m, 16H, CH_2), 0.89 (t, J = 6.7 Hz, 3H, CH_3); ^{13}C NMR (THF-d_8, 75 MHz): δ ppm 163.9, 154.5, 148.5, 141.7, 135.0, 133.0, 131.5, 130.7, 130.6, 129.4, 125.7, 124.2, 120.2, 118.4, 42.0, 32.9, 30.7, 30.6, 30.6, 30.5, 30.3, 30.3, 27.6, 23.6, 14.4. Anal. Calcd for $C_{25}H_{32}Cl_2N_2O_3$ (479.44): C, 62.63; H, 6.73; N, 5.84. Found: C, 62.40; H, 6.76; N, 6.00.

4.1.1.10. 4-Chloro-2-(3,4-dichlorophenylcarbamoyl)-phenyl ethylcarbamate (4j). (64%) as a white solid; ν_{max} (KBr) 1661 (CO), 1716 (CO), 3266 (NH), 3336 (NH) cm^{-1}; ^1H NMR (THF-d_8, 300 MHz): δ ppm 9.68 (s, 1H, NH), 8.04 (d, J = 2.4 Hz, 1H, H2′),7.66 (d, J = 2.6 Hz, 1H, H3), 7.58 (dd, J = 8.8, 2.4 Hz, 1H, H6′), 7.49–7.42 (m, 2H, H5, H5′), 7.23 (d, J = 8.7 Hz, 1H, H6), 7.12 (t, J = 4.9 Hz, 1H, NH), 3.18–3.08 (m, 2H, CH_2), 1.07 (t, J = 7.2 Hz, 3H, CH_3); ^{13}C NMR (THF-d_8, 75 MHz): δ ppm 164.1, 154.4, 148.6, 140.2, 132.9, 132.7, 131.7, 131.2, 130.6, 129.3, 127.0, 125.7, 121.9, 120.0, 36.7, 15.3. Anal. Calcd for $C_{16}H_{13}Cl_3N_2O_3$ (387.00): C, 49.57; H, 3.38; N, 7.23. Found: C, 49.47; H, 3.25; N, 7.03.

4.1.1.11. 4-Chloro-2-(3,4-dichlorophenylcarbamoyl)-phenyl butylcarbamate (4k). (75%) as a white solid; ν_{max} (KBr) 1656 (CO), 1720 (CO), 3266 (NH), 3338 (NH) cm^{-1}; ^1H NMR (THF-d_8, 300 MHz): δ ppm 9.68 (s, 1H, NH), 8.04 (d, J = 2.4 Hz, 1H, H2′), 7.65 (d, J = 2.6 Hz, 1H, H3), 7.58 (dd, J = 8.8, 2.4 Hz, 1H, H6′), 7.48–7.42 (m, 2H, H5, H5′), 7.21 (d, J = 8.7 Hz, 1H, H6), 7.11 (t, J = 5.4 Hz, 1H, H5′), 3.10 (dt, J = 6.7, 5.4 Hz, 2H, CH_2), 1.49–1.23 (m, 4H, CH_2), 1.07 (t, J = 7.2 Hz, 3H, CH_3); ^{13}C NMR (THF-d_8, 75 MHz): δ ppm 164.1, 154.5, 148.6, 140.3, 132.9, 132.8, 131.7, 131.2, 130.6, 129.2, 127.0, 125.7, 121.9, 120.0, 41.6, 32.8, 20.6, 14.0. Anal. Calcd for $C_{18}H_{17}Cl_3N_2O_3$ (415.70): C, 52.01; H, 4.12; N, 6.74. Found: C, 52.13; H, 4.18; N, 7.01.

4.1.1.12. 4-Chloro-2-(3,4dichlorophenylcarbamoyl)-phenyl pentylcarbamate (4l). (75%) as a white solid; ν_{max} (KBr) 1659 (CO), 1715 (CO), 3328 (NH) cm^{-1}; ^1H NMR (THF-d_8, 300 MHz): δ ppm 9.67 (s, 1H, NH), 8.03 (d, J = 2.4 Hz, 1H, H2′), 7.65 (d, J = 2.7 Hz, 1H, H3), 7.57 (dd, J = 8.7, 2.4 Hz, 1H, H6′), 7.49–7.41 (m, 2H, H5, H5′), 7.21 (d, J = 8.8 Hz, 1H, H6), 7.11 (t, J = 5.4 Hz, 1H, H5′), 3.09 (dt, J = 6.7, 5.4, 2H, CH_2), 1.50–1.39 (m, 2H, CH_2), 1.32–1.21 (m, 4H, CH_2), 0.86 (t, J = 6.6 Hz, 3H, CH_3); ^{13}C NMR (THF, 75 MHz): δ ppm 164.1, 154.5, 148.6, 140.2, 132.9, 132.7, 131.6, 131.2, 130.6, 129.2, 127.0, 125.7, 121.9, 120.0, 41.9, 30.3, 29.7, 23.2, 14.3. Anal. Calcd for $C_{19}H_{19}Cl_3N_2O_3$ (429.72): C, 53.10; H, 4.46; N, 6.52. Found: C, 53.01; H, 4.23; N, 6.72.

4.1.1.13. 4-Chloro-2-(3,4-dichlorophenylcarbamoyl)-phenyl hexylcarbamate (4m). (74%) as a white solid; ν_{max} (KBr) 1664 (CO), 1724 (CO), 3286 (NH) cm^{-1}; ^1H NMR (THF-d_8, 300 MHz): δ ppm 9.67 (s, 1H, NH), 8.03 (d, J = 2.4 Hz, 1H, H2′), 7.65 (d, J = 2.6 Hz, 1H, H3), 7.57 (dd, J = 8.8, 2.4 Hz, 1H, H6′), 7.48–7.41 (m, 2H, H5, H5′), 7.21 (d, J = 8.7 Hz, 1H, H6), 7.11 (t, J = 5.5 Hz, 1H, NH), 3.09 (dt, J = 6.9, 5.5 Hz, 2H, CH_2), 1.49–1.38 (m, 2H, CH_2), 1.33–1.21 (m, 6H, CH_2), 0.87 (t, J = 6.7 Hz, 3H, CH_3); ^{13}C NMR (THF-d_8, 75 MHz): δ ppm 164.1, 154.5, 148.6, 140.3, 132.9, 132.8, 131.7, 131.2, 130.6, 129.3, 127.0, 125.7, 121.9, 120.0, 41.9, 32.5, 30.6, 27.3, 23.5, 14.4. Anal. Calcd for $C_{20}H_{21}Cl_3N_2O_3$ (443.75): C, 54.13; H, 4.77; N, 6.31. Found: C, 53.92; H, 4.55; N, 6.42.

4.1.1.14. 4-Chloro-2-(3,4dichlorophenylcarbamoyl)-phenyl heptylcarbamate (4n). (53%) as a white solid; ν_{max} (KBr) 1659 (CO), 1714 (CO), 3268 (NH), 3330 (NH) cm^{-1}; ^1H NMR (THF-d_8, 300 MHz): δ ppm 9.67 (s, 1H, NH), 8.03 (d, J = 2.4 Hz, 1H, H2′), 7.65 (d, J = 2.6 Hz, 1H, H3), 7.58 (dd, J = 8.8, 2.4 Hz, 1H, H6′), 7.48–7.41 (m, 2H, H5, H5′), 7.21 (d, J = 8.7 Hz, 1H, H6), 7.11 (t, J = 5.6 Hz, 1H, NH), 3.09 (dt, J = 6.9, 5.6, 2H, CH_2), 1.49–1.38 (m, 2H, CH_2), 1.33–1.25 (m, 8H, CH_2), 0.88 (t, J = 6.9 Hz, 3H, CH_3); ^{13}C NMR (THF-d_8, 75 MHz): δ ppm 164.1, 154.5, 148.6, 140.3, 132.9, 132.8, 131.7, 131.2, 130.6, 129.3, 127.0, 125.7, 121.9, 120.0, 41.9, 32.7, 30.7, 29.9, 27.6, 23.5, 14.4. Anal. Calcd for $C_{21}H_{23}Cl_3N_2O_3$ (457.78): C, 55.10; H, 5.06; N, 6.12. Found: C, 54.97; H, 4.88; N, 6.03.

4.1.1.15. 4-Chloro-2-(3,4-dichlorophenylcarbamoyl)-phenyl octylcarbamate (4o). (39%) as a white solid; ν_{max} (KBr) 1659 (CO), 1713 (CO), 3266 (NH), 3330 (NH) cm^{-1}; ^1H NMR (THF-d_8, 300 MHz): δ ppm 9.66 (s, 1H, NH), 8.03 (d, J = 2.4 Hz, 1H, H2′), 7.65 (d, J = 2.6 Hz, 1H, H3), 7,57 (dd, J = 8.8, 2.4 Hz, 1H, H6′), 7.48–7.40 (m, 2H, H5, H5′), 7.21 (d, J = 8.7 Hz, 1H, H6), 7.12 (t, J = 5.5 Hz, 1H, NH), 3.09 (dt, J = 6.5, 5.5 Hz, 2H, CH_2), 1.49–1.38 (m, 2H, CH_2), 1.33–1.25 (m, 10H, CH_2), 0.89 (t, J = 6.5 Hz, 3H, CH_3); ^{13}C NMR (THF-d_8, 75 MHz): δ ppm 164.1, 154.5, 148.6, 140.3, 132.9, 132.8, 131.7, 131.2, 130.7, 129.3, 127.0, 125.7, 121.9, 120.0, 41.9, 32.8, 30.7, 30.2, 30.2, 27.6, 23.5, 14.5. Anal. Calcd for $C_{22}H_{25}Cl_3N_2O_3$ (471.80): C, 56.01; H, 5.34; N, 5.94. Found: C, 56.12; H, 5.20; N, 6.05.

4.1.1.16. 4-Chloro-2-(3,4-dichlorophenylcarbamoyl)-phenyl nonylcarbamate (4p). (58%) as a white solid; ν_{max} (KBr) 1659 (CO), 1715 (CO), 3266 (NH), 3329 (NH) cm^{-1}; ^1H NMR (THF-d_8, 300 MHz): δ ppm 9.67 (s, 1H, NH), 8.02 (d, J = 2.4 Hz, 1H, H2′), 7.65 (d, J = 2.5 Hz, 1H, H3), 7.58 (dd, J = 8.6, 2.4 Hz, 1H, H6′), 7.49–7.41 (m, 2H, H5, H5′), 7.21 (d, J = 8.7 Hz, 1H, H6), 7.11 (t, J = 5.4 Hz, 1H, NH), 3.08 (dt, J = 6.0, 5.4 Hz, 2H, CH_2), 1.48–1.37 (m, 2H, CH_2), 1.36–1.20 (m, 12H, CH_2), 0.89 (t, J = 6.4 Hz, 3H, CH_3); ^{13}C NMR (THF-d_8, 75 MHz): δ ppm 164.1, 154.5, 148.6, 140.3, 132.9, 132.8, 131.7, 131.7, 130.6, 129.3, 127.0, 125.7, 121.9, 120.0, 41.9, 32.8, 30.7,

30.5, 30.3, 27.6, 25.8, 23.6, 14.5. Anal. Calcd for $C_{23}H_{27}Cl_3N_2O_3$ (485.83): C, 56.86; H, 5.60; N, 5.77. Found: C, 56.58; H, 5.49; N, 6.00.

4.1.1.17. 4-Chloro-2-(3,4-dichlorophenylcarbamoyl)-phenyl decylcarbamate (4q). (70%) as a white solid; ν_{max} (KBr) 1660 (CO), 1715 (CO), 3266 (NH), 3329 (NH) cm^{-1}; ^1H NMR (THF-d_8, 300 MHz): δ ppm 9.66 (s, 1H, NH), 8.02 (d, J = 2.4 Hz, 1H, H2′), 7.65 (d, J = 2.6 Hz, 1H, H3), 7.58 (dd, J = 8.8, 2.4 Hz, 1H, H6′), 7.48–7.41 (m, 2H, H5, H5′), 7.21 (d, J = 8.7 Hz, 1H, H6), 7.11 (t, J = 5.6 Hz, 1H, NH), 3.09 (dt, J = 6.8, 5.6 Hz, 2H, CH_2), 1.50–1.39 (m, 2H, CH_2), 1.36–1.20 (m, 14H, CH_2), 0.89 (t, J = 6.7 Hz, 3H, CH_3); ^{13}C NMR (THF-d_8, 75 MHz): δ ppm 164.1, 154.5, 148.6, 140.3, 132.9, 132.7, 131.7, 131.2, 130.6, 129.3, 128.1, 127.0, 125.7, 121.9, 41.9, 32.9, 30.7, 30.6, 30.6, 30.4, 30.3, 27.6, 23.6, 14.5. Anal. Calcd for $C_{24}H_{29}Cl_3N_2O_3$ (499.86): C, 57.67; H, 5.85; N, 5.60. Found: C, 57.45; H, 5.73; N, 6.01.

4.1.1.18. 4-Chloro-2-(3,4-dichlorophenylcarbamoyl)-phenyl undecylcarbamate (4r). (78%) as a white solid; ν_{max} (KBr) 1659 (CO), 1714 (CO), 3266 (NH), 3328 (NH) cm^{-1}; ^1H NMR (THF-d_8, 300 MHz): δ ppm 9.66 (s, 1H, NH), 8.03 (d, J = 2.4 Hz, 1H, H2′), 7.65 (d, J = 2.6 Hz, 1H, H3), 7.58 (dd, J = 8.8, 2.4 Hz, 1H, H6′), 7.51–7.40 (m, 2H, H5, H5′), 7.21 (d, J = 8.7 Hz, 1H, H6), 7.11 (t, J = 5.5 Hz, 1H, NH), 3.09 (dt, J = 6.6, 5.5 Hz, 2H, CH_2), 1.48–1.40 (m, 2H, CH_2), 1.36–1.20 (m, 14H, CH_2), 0.89 (t, J = 6.6 Hz, 3H, CH_3); ^{13}C NMR (THF-d_8, 75 MHz): δ ppm 164.2, 154.5, 148.6, 140.2, 132.9, 132.8, 131.7, 131.2, 130.6, 129.3, 127.0, 125.7, 121.9, 120.0, 41.9, 32.9, 30.7, 30.6, 30.6, 30.5, 30.5, 30.3, 27.6, 23.6, 14.4. Anal. Calcd for $C_{25}H_{31}Cl_3N_2O_3$ (513.88): C, 58.43; H, 6.08; N, 5.45. Found: C, 58.66; H, 5.94; N, 5.89.

4.1.1.19. 4-Chloro-2-(4-chlorophenylcarbamoyl)phenyl ethylcarbamate (4s). (46%) as a white solid; ν_{max} (KBr) 1654 (CO), 1714 (CO), 3273 (NH), 3335 (NH) cm^{-1}; ^1H NMR (THF-d_8, 300 MHz): δ ppm 9.52 (s, 1H, NH), 7.74–7.68 (m, 2H, H3′, H5′), 7.65 (d, J = 2.6 Hz, 1H, H3), 7.44 (dd, J = 8.7, 2.6 Hz, 1H, H5), 7.32–7.26 (m, 2H, H2′, H6′), 7.21 (d, J = 8.7 Hz, 1H, H6), 7.11 (t, J = 5.2 Hz, 1H, NH), 3.18–3.07 (m, 2H, CH_2), 1.06 (t, J = 7.2 Hz, 3H, CH_3); ^{13}C NMR (THF-d_8, 75 MHz): δ ppm 163.7, 154.4, 148.5, 139.2, 133.0, 131.4, 130.6, 129.6, 129.4, 129.0, 125.7, 121.7, 36.7, 15.2. Anal. Calcd for $C_{16}H_{14}Cl_2N_2O_3$ (353.20): C, 54.41; H, 4.00; N, 7.93. Found: C, 54.20; H, 3.79; N, 7.56.

4.1.1.20. 4-Chloro-2-(4-chlorophenylcarbamoyl)phenyl butylcarbamate (4t). (48%) as a white solid; ν_{max} (KBr) 1656 (CO), 1714 (CO), 3286 (NH), 3335 (NH) cm^{-1}; ^1H NMR (THF-d_8, 300 MHz): δ ppm 9.54 (s, 1H, NH), 7.74–7.68 (m, 2H, H3′, H5′), 7.64 (d, J = 2.6 Hz, 1H, H3), 7.44 (dd, J = 8.7, 2.6 Hz, 1H, H5), 7.31–7.26 (m, 2H, H2′, H6′), 7.20 (d, J = 8.7 Hz, 1H, H6), 7.10 (t, J = 5.4 Hz, 1H, NH), 3.10 (dt, J = 6.7, 5.4 Hz, 2H, CH_2), 1.48–1.23 (m, 4H, CH_2), 0.85 (t, J = 7.2 Hz, 3H, CH_3); ^{13}C NMR (THF-d_8, 75 MHz): δ ppm 163.8, 154.6, 148.5, 139.2, 133.1, 131.4, 130.6, 129.6, 129.4, 129.0, 125.7, 121.7, 41.6, 32.8, 20.6, 14.1. Anal. Calcd for $C_{18}H_{18}Cl_2N_2O_3$ (381.25): C, 56.71; H, 4.76; N, 7.35. Found: C, 56.42; H, 4.83; N, 7.22.

4.1.1.21. 4-Chloro-2-(4-chlorophenylcarbamoyl)phenyl pentylcarbamate (4u). (63%) as a white solid; ν_{max} (KBr) 1657 (CO), 1717 (CO), 3282 (NH), 3336 (NH) cm^{-1}; ^1H NMR (THF-d_8, 300 MHz): δ ppm 9.53 (s, 1H, NH), 7.74–7.68 (m, 2H, H3′, H5′), 7.65 (d, J = 2.6 Hz, 1H, H3), 7.44 (dd, J = 8.7, 2.6 Hz, 1H, H5), 7.32–7.26 (m, 2H, H2′, H6′), 7.21 (d, J = 8.7 Hz, 1H, H6), 7.10 (t, J = 5.6 Hz, 1H, NH), 3.09 (dt, J = 6.8, 5.6 Hz, 2H, CH_2), 1.51–1.40 (m, 2H, CH_2), 1.32–1.23 (m, 4H), 0.87 (t, J = 6.7 Hz, 3H, CH_3); ^{13}C NMR (THF-d_8, 75 MHz): δ ppm 163.7, 154.5, 148.5, 139.2, 133.1, 131.4, 130.6, 129.4, 129.4, 129.0, 125.7, 121.7, 41.9, 30.3, 29.8, 23.2, 14.3. Anal. Calcd for $C_{19}H_{20}Cl_2N_2O_3$ (395.28): C, 57.73; H, 5.10; N, 7.09. Found: C, 57.55; H, 4.89; N, 7.15.

4.1.1.22. 4-Chloro-2-(4-chlorophenylcarbamoyl)phenyl hexylcarbamate (4v). (36%) as a white solid; ν_{max} (KBr) 1657 (CO), 1720 (CO), 3285 (NH), 3332 (NH) cm^{-1}; ^1H NMR (THF-d_8, 300 MHz): δ ppm 9.53 (s, 1H, NH), 7.73–7.68 (m, 2H, H3′, H5′), 7.65 (d, J = 2.6 Hz, 1H, H3), 7.44 (dd, J = 8.7, 2.6 Hz, 1H, H5), 7.31–7.26 (m, 2H, H2′, H6′), 7.20 (d, J = 8.7 Hz, 1H, H6), 7.11 (t, J = 5.5 Hz, 1H, NH), 3.09 (dt, J = 6.8, 5.5 Hz, 2H, CH_2), 1.49–1.38 (m, 2H, CH_2), 1.33–1.20 (m, 6H), 0.88 (t, J = 6.6 Hz, 3H, CH_3); ^{13}C NMR (THF-d_8, 75 MHz): δ ppm 163.8, 154.6, 148.5, 139.2, 133.1, 131.4, 130.6, 129.4, 129.4, 129.0, 125.7, 121.7, 41.9, 32.5, 30.6, 27.3, 23.5, 14.4. Anal. Calcd for $C_{20}H_{22}Cl_2N_2O_3$ (409.31): C, 58.69; H, 5.42; N, 6.84. Found: C, 58.74; H, 5.67; N, 6.75.

4.1.1.23. 4-Chloro-2-(4-chlorophenylcarbamoyl)phenyl heptylcarbamate (4w). (67%) as a white solid; ν_{max} (KBr) 1654 (CO), 1712 (CO), 3274 (NH), 3332 (NH) cm^{-1}; ^1H NMR (THF-d_8, 300 MHz): δ ppm 9.52 (s, 1H, NH), 7.73–7.68 (m, 2H, H3′, H5′), 7.65 (d, J = 2.6 Hz, 1H, H3), 7.44 (dd, J = 8.7, 2.6 Hz, 1H, H5), 7.31–7.26 (m, 2H, H2′, H6′), 7.20 (d, J = 8.7 Hz, 1H, H6), 7.11 (t, J = 5.5 Hz, 1H, NH), 3.09 (dt, J = 6.8, 5.5 Hz, 2H, CH_2), 1.49–1.38 (m, 2H, CH_2), 1.34–1.22 (m, 8H), 0.89 (t, J = 6.7 Hz, 3H, CH_3); ^{13}C NMR (THF-d_8, 75 MHz): δ ppm 163.7, 154.6, 148.5, 139.2, 133.1, 131.4, 129.6, 129.4, 129.3, 129.1, 125.7, 121.7, 41.9, 32.8, 30.7, 30.0, 27.6, 23.5, 14.4. Anal. Calcd for $C_{21}H_{24}Cl_2N_2O_3$ (423.33): C, 59.58; H, 5.71; N, 6.84. Found: C, 59.28; H, 5.54; N, 6.93.

4.1.1.24. 4-Chloro-2-(4-chlorophenylcarbamoyl)phenyl octylcarbamate (4x). (46%) as a white solid; ν_{max} (KBr) 1655 (CO), 1716 (CO), 3276 (NH), 3334 (NH) cm^{-1}; ^1H NMR (THF-d_8, 300 MHz): δ ppm 9.53 (s, 1H, NH), 7.74–7.68 (m, 2H, H3′, H5′), 7.65 (d, J = 2.6 Hz, 1H, H3), 7.44 (dd, J = 8.7, 2.6 Hz, 1H, H5), 7.31–7.25 (m, 2H, H2′, H6′), 7.20 (d, J = 8.7 Hz, 1H, H6), 7.11 (t, J = 5.4 Hz, 1H, NH), 3.09 (dt, J = 6.8, 5.4 Hz, 2H, CH_2), 1.51–1.39 (m, 2H, CH_2), 1.35–1.19 (m, 10H), 0.89 (t, J = 6.5 Hz, 3H, CH_3); ^{13}C NMR (THF-d_8, 75 MHz): δ ppm 163.7, 154.5, 148.5, 139.2, 133.1, 131.4, 130.6, 129.4, 129.4, 129.0, 125.7, 121.7, 41.9, 32.8, 30.7, 30.2, 30.2, 27.6, 23.5, 14.5. Anal. Calcd for $C_{22}H_{26}Cl_2N_2O_3$ (437.36): C, 60.42; H, 5.99; N, 6.41. Found: C, 60.34; H, 5.88; N, 6.27.

4.1.1.25. 4-Chloro-2-(4-chlorophenylcarbamoyl)phenyl nonylcarbamate (4y). (71%) as a white solid; ν_{max} (KBr) 1657 (CO), 1712 (CO), 3282 (NH), 3342 (NH) cm^{-1}; ^1H NMR (THF-d_8, 300 MHz): δ ppm 9.52 (s, 1H, NH), 7.73–7.68 (m, 2H, H3′, H5′), 7.65 (d, J = 2.6 Hz, 1H, H3), 7.44 (dd, J = 8.7, 2.6 Hz, 1H, H5), 7.31–7.25 (m, 2H, H2′, H6′), 7.20 (d, J = 8.7 Hz, 1H, H6), 7.10 (t, J = 5.2 Hz, 1H, NH), 3.09 (dt, J = 6.7, 5.2 Hz, 2H, CH_2), 1.49–1.39 (m, 2H, CH_2), 1.34–1.20 (m, 12H), 0.89 (t, J = 6.7 Hz, 3H, CH_3); ^{13}C NMR (THF-d_8, 75 MHz): δ ppm 163.8, 154.6, 148.5, 139.2, 133.1, 131.4, 130.6, 129.6, 129.4, 129.0, 125.7, 121.7, 42.0, 30.7, 30.5, 30.3, 30.3, 23.6, 14.5. Anal. Calcd for $C_{23}H_{28}Cl_2N_2O_3$ (451.39): C, 61.20; H, 6.25; N, 6.21. Found: C, 61.07; H, 6.22; N, 6.35.

4.1.1.26. 4-Chloro-2-(4-chlorophenylcarbamoyl)phenyl decylcarbamate (4z). (51%) as a white solid; ν_{max} (KBr) 1656 (CO), 1713 (CO), 3278 (NH), 3330 (NH) cm^{-1}; ^1H NMR (THF-d_8, 300 MHz): δ ppm 9.53 (s, 1H, NH), 7.74–7.68 (m, 2H, H3′, H5′), 7.65 (d, J = 2.6 Hz, 1H, H3), 7.44 (dd, J = 8.7, 2.6 Hz, 1H, H5), 7.32–7.25 (m, 2H, H2′, H6′), 7.20 (d, J = 8.7 Hz, 1H, H6), 7.11 (t, J = 5.5 Hz, 1H, NH), 3.09 (dt, J = 6.7, 5.5 Hz, 2H, CH_2), 1.48–1.39 (m, 2H, CH_2), 1.34–1.20 (m, 14H), 0.89 (t, J = 6.7 Hz, 3H, CH_3); ^{13}C NMR (THF-d_8, 75 MHz): δ ppm 163.8, 154.6, 148.5, 139.2, 133.1, 131.4, 130.6, 129.4, 129.4, 129.0, 125.7, 121.7, 41.9, 32.9, 30.7, 30.6, 30.5, 30.3, 30.3, 27.6, 23.6, 14.5. Anal. Calcd for $C_{24}H_{30}Cl_2N_2O_3$ (465.41): C, 61.94; H, 6.50; N, 6.02. Found: C, 61.87; H, 6.74; N, 5.87.

4.1.1.27. 4-Chloro-2-(4-chlorophenylcarbamoyl)phenyl undecylcarbamate (4zz). (80%) as a white solid; ν_{max} (KBr) 1656 (CO), 1713 (CO), 3278 (NH), 3338 (NH) cm^{-1}; ^1H NMR (THF-d_8, 300 MHz): δ ppm 9.52 (s, 1H, NH), 7.74–7.68 (m, 2H, H3′, H5′), 7.65 (d, J = 2.6 Hz, 1H, H3), 7.44 (dd, J = 8.7, 2.6 Hz, 1H, H5), 7.31–7.26 (m, 2H, H2′, H6′), 7.20 (d, J = 8.7 Hz, 1H, H6), 7.11 (t, J = 5.6 Hz, 1H, NH), 3.09 (dt, J = 6.8, 5.6 Hz, 2H, CH_2), 1.48–1.38 (m, 2H, CH_2), 1.36–1.22 (m, 16H), 0.89 (t, J = 6.6 Hz, 3H, CH_3); ^{13}C NMR (THF-d_8, 75 MHz): δ ppm 163.8, 154.6, 148.5, 139.2, 133.1, 131.4, 130.6, 129.6, 129.4, 129.0, 125.7, 121.7, 41.9, 32.9, 30.7, 30.6, 30.6, 30.5, 30.3, 30.3, 27.6, 23.6, 14.4. Anal. Calcd for $C_{25}H_{32}Cl_2N_2O_3$ (479.44): C, 62.63; H, 6.73; N, 5.84. Found: C, 62.75; H, 6.57; N, 6.58.

4.2. Materials and methods

4.2.1. In vitro antimycobacterial assay

In vitro antimycobacterial activity of the synthesized compounds was evaluated against *Mycobacterium tuberculosis* My 331/88, *M. avium* My 330/88, *M. kansasii* My 235/80 and *M. kansasii* 6509/96. The most active compounds **4d**, **4j**, **4k**, **4l**, **4m**, **4n**, **4o**, **4u** were tested as well against five MDR-TB strains: 7357/98, 9449/06, 2092/05, Praha 1 and Praha 128. For this purpose, the micromethod[37] for the determination of the minimum inhibitory concentration (MIC) was used. All the strains were obtained from the Czech National Collection of Type Cultures (CNCTC) with the exception of *M. kansasii* 6509/96 and the five MDR-TB strains which were clinically isolated. The antimycobacterial activities of the compounds were determined in a Šula semisynthetic medium (SEVAC, Prague). The compounds were added to the medium in DMSO solutions. The following concentrations were used: 1000, 500, 250, 125, 62, 32, 16, 8, 4, 2, 1 and 0.5 μmol/L. MICs values were determined after incubation at 37 °C for 14 and 21 days, for *M. kansasii* for 7, 14, and 21 days. MIC was the lowest concentration of a substance, at which the inhibition of the growth of mycobacterium occurred. INH was used as a standard.

4.2.2. Cytotoxicity assay

The cytotoxic effect of the compounds was tested by XTT assay[33] on human intestinal cell line HCT-8 (ECACC, UK). The cells were grown in Eagle's minimal essential medium (EMEM) supplemented by 5% fetal bovine serum at 37 °C in a humidified atmosphere of 5% CO_2. For the experiments, the cells were harvested with trypsin, resuspended in a fresh medium to a final concentration of 2×10^5 cells/ml and seeded in aliquots (100 μl) onto 96-well tissue culture plates (TPP AG, Switzerland). The medium was removed after 24 h of cell incubation and replaced by EMEM culture medium containing the tested compounds dissolved in DMSO. In control wells, the cells were incubated in a medium containing DMSO without the tested compound (positive control for cell viability) and in the medium containing 20% DMSO (positive control for cytotoxic effect). The ability of the compounds to inhibit cellular growth was determined after 72 h by adding XTT solution (20 μl, 1 mg/ml, F. Hoffmann-La Roche Ltd, Switzerland)) to each well. After incubation for 4 h absorbance was read at 450 nm using multiplate spectrometer LM 01 A (Beckmann Coulter Inc., USA). Each concentration of the compounds was tested in triplicate.

4.2.3. Hydrolysis assay

The stability of **4m** at pH from 3 to 8 was determined by HPLC using a Shimadzu Prominence (Kyoto, Japan) instrument consisting of LC-20AD pumps with DGU-20A3 degasser, SIL-20A HT autosampler, CTO-20AC column oven, SPD-M20A diode array detector and CBM-20A communication module. Data were analyzed using LCsolutions 1.22 software. 50 μl of **4m** in acetonitrile (1 mg/ml, prepared immediately before use) was added to 4.95 ml of 100 mM phosphate (pH 3, 6, 7, 7.4 and 8) or acetate buffer (pH 4 and 5). The reactions were stirred at 37 °C and at predetermined time intervals, 300 μl of each solution was withdrawn and analyzed for **4m** and the hydrolysis product. 20 μl of the sample was injected into a LiChroCART 125-4 column (LichroSpher RP-18e, 5 μm; Merck, Darmstadt, Germany) equipped with a LiChroCART 4-4 precolumn with the same sorbent. Acetonitrile/20 mM phosphate buffer at pH 6.5 (7:3 v/v) was used as the mobile phase at a flow rate of 2 mL/min and was monitored at 263 nm. The retention of **4m** and the hydrolysis product was 4.0 and 1.6 min, respectively. The calibration curves of **4m** and the hydrolysis product were linear (p <0.0001, R^2 = 0.9997 and p <0.0001, R^2 = 0.996, respectively) in the range of concentrations of 0.5–100 μg/ml. The accuracy ranged from 93.13% to 108.55% and relative standard deviation was less than 6.0%. Half-lives for the hydrolysis of the carbamates were calculated from the linear slopes of plots of the logarithm of remaining carbamate against time.

Acknowledgment

This work was financially supported by the Research project MSM 0021620822, GAUK 76807/2007 and IGA NS 10367-3.

References and notes

1. Espinal, M. A. *Tuberculosis (Edinb)* **2003**, *83*, 44.
2. Shenoi, S.; Friedland, G. *Annu. Rev. Med.* **2009**, *60*, 307.
3. http://www.who.int/tb/en/.
4. Janin, Y. L. *Bioorg. Med. Chem.* **2007**, *15*, 2479.
5. Dye, C. F. K.; Gunneberg, C.; Hosseini, M.; Lonnroth, K.; Nathanson, E.; Pantoky, A.; Piatek, M.; Uplekar, M.; Watt, C.; Williams, B.; Wright, A. *Zignol*, 1st. ed.; World Health Organization: Geneva, 2007.
6. http://www.who.int/tb/publications/2008/factsheet_april08.pdf.
7. Breen, R. A.; Swaden, L.; Ballinger, J.; Lipman, M. C. *Drugs* **2006**, *66*, 2299.
8. Zhang, Y.; Post-Martens, K.; Denkin, S. *Drug Discovery Today* **2006**, *11*, 21.
9. Brown, E. D.; Wright, G. D. *Chem. Rev.* **2005**, *105*, 759.
10. Vinsova, J.; Imramovsky, A. *Ceska Slov. Farm.* **2004**, *53*, 294.
11. De la Fuente, R.; Sonawane, N. D.; Arumainayagam, D.; Verkman, A. S. *Br. J. Pharmacol.* **2006**, *149*, 551.
12. Dahlgren, M. K.; Kauppi, A. M.; Olsson, I. M.; Linusson, A.; Elofsson, M. *J. Med. Chem.* **2007**, *50*, 6177.
13. Vinsova, J.; Imramovsky, A.; Buchta, V.; Ceckova, M.; Dolezal, M.; Staud, F.; Jampilek, J.; Kaustova, J. *Molecules* **2007**, *12*, 1.
14. Daidone, G.; Raffa, D.; Plescia, S.; Matera, M.; Caruso, A.; Leone, V.; Amico-Roxas, M. *Farmaco* **1989**, *44*, 465.
15. Waisser, K.; Matyk, J.; Divisova, H.; Husakova, P.; Kunes, J.; Klimesova, V.; Kaustova, J.; Mollmann, U.; Dahse, H. M.; Miko, M. *Arch. Pharm. (Weinheim)* **2006**, *339*, 616.
16. Stephenson, K.; Yamaguchi, Y.; Hoch, J. A. *J. Biol. Chem.* **2000**, *275*, 38900.
17. Macielag, M. J.; Demers, J. P.; Fraga-Spano, S. A.; Hlasta, D. J.; Johnson, S. G.; Kanojia, R. M.; Russell, R. K.; Sui, Z.; Weidner-Wells, M. A.; Werblood, H.; Foleno, B. D.; Goldschmidt, R. M.; Loeloff, M. J.; Webb, G. C.; Barrett, J. F. *J. Med. Chem.* **1998**, *41*, 2939.
18. Kamath, S.; Buolamwini, J. K. *Med. Res. Rev.* **2006**, *26*, 569.
19. Brown, M. E.; Fitzner, J. N.; Stevens, T.; Chin, W.; Wright, C. D.; Boyce, J. P. *Bioorg. Med. Chem.* **2008**, *16*, 8760.
20. Boyce, J. P.; Brown, M. E.; Chin, W.; Fitzner, J. N.; Paxton, R. J.; Shen, M.; Stevens, T.; Wolfson, M. F.; Wright, C. D. *Bioconjugate Chem.* **2008**, *19*, 1775.
21. Ray, S.; Pathak, S. R.; Chaturvedi, D. *Drugs Future* **2005**, *30*, 161.
22. Agouridas, C.; Denis, A.; Auger, J. M.; Benedetti, Y.; Bonnefoy, A.; Bretin, F.; Chantot, J. F.; Dussarat, A.; Fromentin, C.; D'Ambrieres, S. G.; Lachaud, S.; Laurin, P.; Le Martret, O.; Loyau, V.; Tessot, N. *J. Med. Chem.* **1998**, *41*, 4080.
23. Meng, Q.; Luo, H.; Liu, Y.; Li, W.; Zhang, W.; Yao, Q. *Bioorg. Med. Chem.* **2009**, *19*, 2908.
24. Thorberg, S.; Berg, S.; Lundstrom, L.; Pettersson, B.; Wijkstrom, A.; Sanchez, D.; Lindberg, P.; Nilsson, J. G. *J. Med. Chem.* **1987**, *30*, 2008.
25. Waisser, K.; Bures, O.; Holy, P.; Kunes, J.; Oswald, R.; Jiraskova, L.; Pour, M.; Klimesova, V.; Kubicova, L.; Kaustova, J. *Arch. Pharm. (Weinheim)* **2003**, *336*, 53.
26. Imramovsky, A.; Vinsova, J.; Ferriz, J. M.; Buchta, V.; Jampilek, J. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 348.
27. Imramovsky, A.; Vinsova, J.; Ferriz, J. M.; Dolezal, R.; Jampilek, J.; Kaustova, J.; Kunc, F. *Bioorg. Med. Chem.* **2009**, *17*, 3572.
28. Lenaerts, A. J.; Gruppo, V.; Marietta, K. S.; Johnson, C. M.; Driscoll, D. K.; Tompkins, N. M.; Rose, J. D.; Reynolds, R. C.; Orme, I. M. *Antimicrob. Agents Chemother.* **2005**, *49*, 2294.
29. Stover, C. K.; Warrener, P.; VanDevanter, D. R.; Sherman, D. R.; Arain, T. M.; Langhorne, M. H.; Anderson, S. W.; Towel, J. A.; Yuan, Y.; Mc Murray, D. N.; Kreiswirth, B. N.; Barry, C. E.; Baker, W. R. *Nature* **2000**, *405*, 962.
30. Nikonenko, B. V.; Protopopova, M.; Sarnala, R.; Einck, L.; Nacy, C. A. *Antimicrob. Agents Chemother.* **2007**, *51*, 1563.

31. Jia, L.; Tomaszewski, J. E.; Hanrahan, C.; Coward, L.; Noker, P.; Gorman, G.; Nikonenko, B.; Protopopova, M. *Br. J. Pharmacol.* **2005**, *144*, 80.
32. Scudiero, D. A.; Shoemaker, R. H. *Cancer Res.* **1988**, *48*, 4827.
33. http://www.taacf.org/Process-text.htm.
34. D'Souza, A. J.; Topp, E. M. *J. Pharm. Sci.* **2004**, *93*, 1962.
35. Savolainen, J.; Leppanen, J.; Forsberg, M.; Taipale, H.; Nevalainen, T.; Huuskonen, J.; Gynther, J.; Mannisto, P. T.; Jarvinen, T. *Life Sci.* **2000**, *67*, 205.
36. Dyer, E.; Wright, G. C. *J. Am. Chem. Soc.* **1959**, *81*, 2138.
37. Beargie, R. A.; Bracken, E. C.; Riley, H. D., Jr. *Appl. Microbiol.* **1965**, *13*, 279.