Romanian Biotechnological Letters *Vol. 22, No. 6, 2017*

ORIGINAL PAPER

In vitro evaluation of the antimicrobial activity of N-phenylcarbamothioyl benzamides against planktonic and adherent microbial cells

Received for publication, December 7, 2015
Accepted, August 2, 2016

DIANA CAMELIA NUTA[1], LUMINITA MĂRUȚESCU[2,*], ALEXANDRU VASILE MISSIR[1], LAURENTIU MORUSCIAG[1], CORNEL CHIRITA[3], CARMEN CURUTIU[2], CARMELLINA DANIELA BADICEANU[1], PAPACOCEA MARIUS TOMA[4], CARMEN LIMBAN[1]

[1]*"Carol Davila" University of Medicine and Pharmacy, Department of Pharmaceutical Chemistry, Bucharest, Romania;*

[2]*University of Bucharest, Faculty of Biology, Research Institute of University of Bucharest-ICUB, Department of Botanic-Microbiology, Bucharest, Romania;*

[3]*"Carol Davila" University of Medicine and Pharmacy, Department of Pharmacology and Clinical Pharmacy, Bucharest, Romania*

[4]*"Carol Davila" University of Medicine and Pharmacy, Department of Neurosurgery, "Sf. Pantelimon" Emergency Hospital, Bucharest, Romania*

Address correspondence to: lumi.marutescu@gmail.com

Abstract

*A set of 2-((4-ethylphenoxy)methyl)-N-(substituted-phenylcarbamothioyl)benzamides derivatives (**1a-h**) previously synthesized and characterized by ^{1}H-NMR, ^{13}C-NMR, IR, MS, and elemental analysis have been assessed for their antimicrobial activity against planktonic and adherent microbial cells. Our results demonstrated that the newly synthesized compounds exhibited good antimicrobial activity, especially against adherent cells, so they could be used for the development of novel antimicrobial materials or strategies for fighting medical biofilms frequently implicated in the etiology of chronic infections. Taking into account that the tested compounds proved good anti-inflammatory activity, they might be potential candidates for further pharmacological investigations, as the concept of combining antimicrobial with anti-inflammatory activity in one compound is promising for future medical use in the treatment of bacterial infections, especially those accompanied by pain and inflammation symptoms.*

Keywords: N-phenylcarbamothioylbenzamide, antimicrobial, anti-biofilm.

1. Introduction

Despite many significant progresses made in antimicrobial therapy, infectious diseases caused by bacteria and fungi remain a major health problem due to the rapid development of resistance to the existing antimicrobial drugs [1, 2]. With this background, there is an increasing need to design and develop new antimicrobial agents [3, 4]. It is well known that thiourea derivatives exhibit both antimicrobial activity, such 6-amino-5-nonsubstituted/chloro-3-methyl-2(3*H*)-benzoxazolones with the appropriate isothiocyanates, with antibacterial and antifungal activities, especially on *Escherichia coli* [5]. Reddy *at al.* synthesized novel cell permeable thiourea compounds from economically available anacardic acid and observed moderate to good biological activity by various antibacterial strains [6].

In addition, Doğruer *at al.* synthesized hybrid molecules through the combination of different pharmacophores, such as pyridazine nucleus, thiourea fragment and sulfonamide group, in one structure in order to obtain agents with improved antimicrobial profile [7]. Moreover, thiourea derivatives of 4-azatricyclo[5.2.2.02,6]undec-8-ene-3,5-dione class

showed significant inhibition of Gram- positive cocci [8]. Furthermore, a new series of aliphatic thiourea and various arylurea incorporating 1,3,5-*s*-triazine moiety was developed and screened for its *in vitro* activity against *Staphylococcus aureus*, *Salmonella typhi*, *Escherichia coli* and *Candida albicans* [9]. In an attempt to improve antibacterial activity, the thiourea moiety has been of interest to design new molecules by substituting the acetamide group at 5-position of linezolid [10].

On the other hand, thiourea moiety confers not only antimicrobial, but also anti-inflammatory activity, as previously shown for the 2-((4-ethylphenoxy)methyl)-*N*-(substituted-phenylcarbamothioyl)benzamides, which exhibited significantly higher anti-inflammatory activity when compared with the reference drug indomethacin, by potently inhibiting PGE2 synthesis lowering ulcer incidence [11].

Based on the aforementioned observations, the pharmacological profile of thiourea derivatives drew our interest in studying the activity of 2-((4-ethylphenoxy)methyl)-*N*-(substituted-phenylcarbamothioyl)benzamides against planktonic and adherent microbial cells, in the aim to obtain potent bioactive compounds, exhibiting both antimicrobial and anti-inflammatory activity.

2. Materials and Methods

2.1. Tested compounds

The synthesis, physico-chemical and anti-inflammatory activity of the 2-((4-ethylphenoxy)methyl)-*N*-(substituted-phenylcarbamothioyl)benzamides (encoded **1a-h)** were published by Limban et al. in 2013 [11].

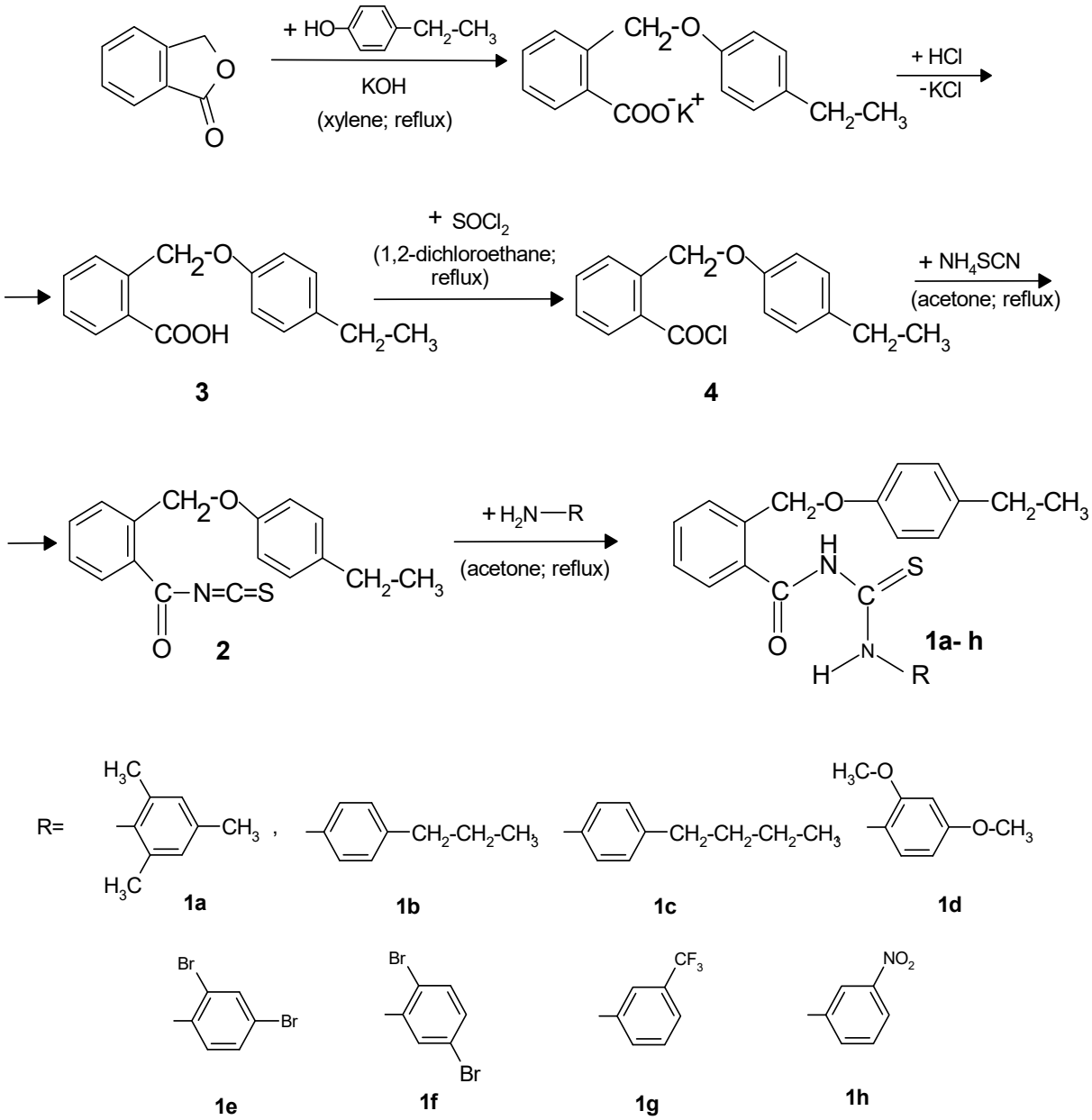
2.2. Antimicrobial and anti-biofilm activity

In vitro antimicrobial tests were carried out on the following microbial strains: *Escherichia coli* 13147, *Escherichia coli* 13529, *Klebsiella pneumoniae* 1204, *Klebsiella pneumoniae* 13420, *Pseudomonas aeruginosa* 1246, *Pseudomonas aeruginosa* 13202, *Bacillus subtilis* 12488, *Staphylococcus aureus* MRSA 1263, *Staphylococcus aureus* 13204, *Candida albicans* 10231, *Candida albicans* 249.

The compounds were solubilized in dimethylsulfoxide to a final concentration of 1 mg/mL. The quantitative assay of the minimal inhibitory concentration (MIC, μg/ mL) was based on liquid medium two-fold microdilutions. For this purpose, serial binary dilutions of the tested compounds (ranging between 1000 and 16.125 μg/ mL) were performed in a 200 μL volume of nutrient broth and each well was seeded with 50 μL microbial inoculum of 0.5 MacFarland density. The plates were incubated for 24 hrs at 37ºC, and MICs were read as the lowest concentration of the tested compound which inhibited the microbial growth. At the end of the MIC experiment the plates were emptied, washed three times with phosphate buffered saline (PBS) to remove the microbial cells grown in suspension (planktonic), the microbial cells adhered to the plastic wells were then fixed with cold methanol for 5 min and then stained with 1% violet crystal solution for 30 minutes. The biomass adhered to the plastic wells was resuspended in 30% acetic acid. The intensity of the colored suspensions was assessed by measuring the absorbance at 490 nm, the intensity of the biofilm development being directly proportional to the values of the measured absorbance and the lowest concentration at which the absorbance was lower than that of the positive control was considered as the minimal biofilm eradication concentration (MBEC) [12-16, 20].

3. Results and discussion

The compounds investigated in the present study are presented in **Scheme 1**.



Scheme 1. Synthetic pathway for the new *N*-phenylcarbamothioylbenzamides 1a-h

The tested compounds (**1a-h)** exhibited low inhibitory activity on the microbial growth, the MIC values exceeding 1mg/mL for all tested variants (Table 1).

Table 1. Minimal inhibitory concentrations (MIC, μg/mL) of the tested compounds

	1a	**1b**	**1c**	**1d**	**1e**	**1f**	**1g**	**1h**
E. coli 13147	>1000	>1000	>1000	>1000	>1000	>1000	>1000	>1000
E. coli 13529	>1000	>1000	>1000	>1000	>1000	>1000	>1000	>1000
K. pneumoniae 1204	>1000	>1000	>1000	>1000	>1000	>1000	>1000	>1000
K. pneumoniae 13420	>1000	>1000	>1000	>1000	>1000	>1000	>1000	>1000
P. aeruginosa 1246	>1000	>1000	>1000	>1000	>1000	>1000	>1000	>1000
P. aeruginosa 13202	>1000	>1000	>1000	>1000	>1000	>1000	>1000	>1000
B. subtilis 12488	>1000	>1000	>1000	>1000	>1000	>1000	>1000	>1000
S. aureus 13204	>1000	>1000	>1000	>1000	>1000	>1000	>1000	>1000
S. aureus MRSA 1263	>1000	>1000	>1000	>1000	>1000	>1000	>1000	>1000
C. albicans 10231	>1000	>1000	>1000	>1000	>1000	>1000	>1000	>1000
C. albicans 249	>1000	>1000	>1000	>1000	>1000	>1000	>1000	>1000

In exchange, the tested compounds exhibited anti-biofilm activity with different minimal biofilm eradication concentrations (MBEC), depending on the compound and the tested strain (Table 2).

Table 2. Minimal biofilm eradication concentrations (MBEC, μg/mL) of the tested compounds

	1a	**1b**	**1c**	**1d**	**1e**	**1f**	**1g**	**1h**
E. coli 13147	250	>1000	>1000	250	250	250	250	250
E. coli 13529	250	>1000	>1000	250	250	250	250	250
K. pneumoniae 1204	>1000	>1000	>1000	>1000	>1000	>1000	>1000	>1000
K. pneumoniae 13420	>1000	>1000	>1000	>1000	>1000	>1000	>1000	>1000
P. aeruginosa 1246	>1000	>1000	>1000	>1000	>1000	>1000	>1000	>1000
P. aeruginosa 13202	>1000	>1000	>1000	>1000	>1000	>1000	>1000	>1000
B. subtilis 12488	500	>1000	>1000	>1000	>1000	>1000	>1000	500
S. aureus 13204	250	>1000	>1000	250	250	250	250	250
S. aureus MRSA 1263	250	>1000	>1000	250	250	250	250	250
C. albicans 10231	250	250	250	250	250	500	250	250

Excepting the compounds **1b** and **1c**, all other tested derivatives exhibited on two different *E. coli* strains, an anti-biofilm activity superior to that of DMSO. Concerning 13420 strains, the derivative **1d** exhibited a slightly higher anti-biofilm efficiency when compared to the control solvent DMSO. None of the tested compounds could inhibit the development of biofilms formed by *K. pneumoniae* and *P. aeruginosa* strains. However, a potent inhibition of pigment production was clearly observed in *P. aeruginosa* strains. The compounds **1a** and **1h** inhibited the biofilm formed by *B. subtilis* strains. Regarding *S. aureus* anti-biofilm activity, the tested derivatives, excepting the compounds **1b** and **1c** were able to inhibit both methicillin resistant *S. aureus* strain (MRSA) 1263 and the methicillin susceptible *S. aureus* 13204 strain. All tested derivatives, i.e. **1a-h** inhibited the two *C. albicans* strains ability to form biofilms.

Among their broad spectrum of applications in medicine, chemistry, industry, and agriculture, thiourea and its derivatives display also anti-inflammatory and antimicrobial activity.

The tested compounds were previously tested for their anti-inflammatory features and during the present study they were screened for their antimicrobial activity against some pathogenic strains, in planktonic and adherent state. All tested derivatives exhibited a relatively low antimicrobial effect against the planktonic strains, with high MIC values, irrespective to the type of present chemical substitution or the tested strain used in the current study. In exchange, the tested compounds exhibited anti-biofilm activity at different concentrations, for different microbial strains. The obtained results showed that the inhibition of the ability of some of the tested microbial strains to colonize the plastic wells, occurred even at sub-inhibitory concentrations, as indicated by the MBEC values which were in some cases lower than the MIC ones. None of the tested compounds interfered with *E. coli* and *P. aeruginosa* biofilm development. Despite the lack of their ability to block *P. aeruginosa* biofilm development, the tested compounds exhibited a potent inhibitory activity on the pyocyanin pigment production.

Hence, the pathogenesis of *P. aeruginosa* infections is multifactorial, as suggested by the large number of cell-associated and extracellular virulence determinants of these bacteria. Among the numerous and redundant adhesions, enzymes, and toxins responsible for extensive tissue damage, bloodstream invasion, and dissemination the pyocyanin production has an important role in protecting bacterial cells from phagocytosis. *In vitro* studies have shown that pyocyanin has multiple deleterious effects on mammalian cells, either epithelial or immune cells [18].

The most susceptible to the tested compounds proved to be the *C. albicans* biofilms, which were inhibited by all derivatives (**1a-h).** The *S. aureus* strains was inhibited by the compounds **1a, 1d, 1e, 1g, 1h,** while *B. subtilis* by **1a** and **1h**.

It could be noticed that the tested derivatives seem to interfere preferentially with the fungal and Gram-positive bacterial cells, and less with the Gram-negative ones. These results could suggest a specific interaction of the tested derivatives with some components of the microbial wall, affecting the consecutive microbial ability to adhere to the inert substratum. The compound exhibiting the strongest spectrum of anti-biofilm activity was the compound **1a,** bearing three methyl and respectively one nitro groups on the phenyl ring.

4. Conclusion

The newly developed 2-((4-ethylphenoxy)methyl)-*N*-(substituted-phenylcarbamothioyl)-benzamides exhibited, in some cases, good antimicrobial activity especially against adherent cells, so they could be used for the development of novel antimicrobial materials or strategies for fighting medical biofilms frequently implicated in the etiology of chronic infections. The compounds **1a,** followed by **1d, 1e, 1f, 1g** and **1h** exhibited important anti-biofilm features, being active against the biofilms formed by the fungal and the Gram-positive strains. Among the current series, the compounds **1e** and **1h,** previously shown to have potent anti-inflammatory activity, also exhibited a significant anti-biofilm activity, proving that the newly developed class includes potential candidates for further pharmacological investigations, since the model compound combining antimicrobial with anti-inflammatory activity is encouraging for future medical use in the treatment of bacterial infections, especially those accompanied by pain and inflammation symptoms.

5. Acknowledgements

The financial support of the research projects TE 373 / 2015 is gratefully acknowledged.

References

1. L. PAGANI, C. COLINON, R. MIGLIAVACCA, M. LABONIA, J.D. DOCQUIER, E. NUCLEO, M., SPALLA, M. LI BERGOLI, G.M. ROSSOLINI. Nosocomial outbreak caused by multidrug-resistant *Pseudomonas aeruginosa* producing IMP-13 metallo-β-lactamase. *J. Clin. Microbiol.* 43: 3824-3828 (2005).
2. European Centre for Disease Prevention and Control/European Medicines Agency ECDC/EMEA joint technical report: The bacterial challenge: time to react: European centre for disease prevention and control & European medicines agency. Stockholm Sweden & London United Kingdom (2009).
3. E. O'FALLON, S. GAUTAM, E.M.C. D'AGATA. Colonization with multidrug-resistant gramnegative bacteria: prolonged duration and frequent cocolonization. *Clin. Infect. Dis.* 48: 1375-1381 (2009).
4. D.L. MAYERS, S.A. LERNER, M. OUELLETTE. Antimicrobial Drug Resistance volume 2: Clinical and Epidemiological Aspects. Humana Press New York (2009).
5. Y. GÜLKOK, T. BIÇER, F. KAYNAK ONURDAG, S. ÖZGEN, M.F. ŞAHIN, D.S. DOĞRUER. Synthesis of some new urea and thiourea derivatives and evaluation of their antimicrobial activities. *Turk J. Chem,* 30: 279-291 (2012).
6. N.S. REDDY, A.S. RAO, M.A. CHARI, V.R. KUMAR, V. JYOTHI, V. HIMABINDU. Synthesis and antibacterial activity of urea and thiourea derivatives at C-8 alkyl chain of anacardic acid mixture isolated from a natural product cashew nut shell liquid (CNSL). *Int. J. Org. Chem, 1:* 167-175 (2011).
7. D. DOĞRUER, S. URLU, T. ÖNKOL, B. ÖZÇELIK, M.F.ŞAHIN. Synthesis of some pyridazine derivatives carrying urea thiourea and sulfonamide moieties and their antimicrobial activity. *Turk. J. Chem,* 34: 57-65 (2010).
8. M. STRUGA, S. ROSOLOWSKI, J. KOSSAKOWSKI, J. STEFANSKA. Synthesis and microbiological activity of thiourea derivatives of 4-azatricyclo[522026]undec-8-ene-35-dione. *Arch. Pharm. Res.* 33: 47-54 (2010).
9. A.D. DESAI, D.H. MAHAJAN, K.H. CHIKHALIA. Synthesis of novel aliphatic thiourea derivatives containing s-triazine moiety as potential antimicrobial agents. *Ind. J. Chem.* 46B:1169-1173, (2007).
10. V.B. LOHRAY, B.B. LOHRAY, B.K. SRIVASTAVA. Novel anti- infective compounds. *Pure Appl. Chem*, 77: 195-200 (2005).
11. C. LIMBAN, A.V. MISSIR, K. MUSTAFA, S. FAHELELBOM, M.M. AL-TABAKHA, M.T. CAPROIU, B. SADEK. Novel N-phenylcarbamothioylbenzamides with anti-inflammatory activity and prostaglandin E_2 inhibitory properties. *Drug Des. Dev. Ther.* 7: 883-892 (2013).
12. C. LIMBAN, L. MARUTESCU, M.C. CHIFIRIUC. Synthesis spectroscopic properties and antipathogenic activity of new thiourea derivatives. *Molecules*, 16: 7593-7607 (2011).
13. C. LIMBAN, M.C. CHIFIRIUC. Antibacterial Activity of New Dibenzoxepinone Oximes with Fluorine and Trifluoromethyl Group Substituents. *Int. J. Mol. Sci.* 12: 6432-6444 (2011).
14. C. LIMBAN, A.V. MISSIR, A.M. GRUMEZESCU, A.E. OPREA, V. GRUMEZESCU, B.Ș. VASILE, G. SOCOL, R. TRUȘCĂ, M.T. CAPROIU, M.C. CHIFIRIUC, B. GĂLĂȚEANU, M. COSTACHE, L. MORUȘCIAG, G. PÎRCĂLĂBIORU, D.C. NUȚĂ. Bioevaluation of Novel Anti-biofilm Coatings Based on PVP/Fe3O4 Nanostructures and 2-((4-Ethylphenoxy)methyl)-N-(arylcarbamothioyl)benzamides. *Molecules*, 19(8): 12011-12030 (2014).
15. C. LIMBAN, A.V. MISSIR, D.C. NUȚĂ, M.T. CĂPROIU, M.T. PAPACOCEA, C. CHIRIȚĂ- synthesis of some new 2-((4-chlorophenoxy)methyl)-N-(arylcarbamothioyl)benzamides as potential antifungal agents, *Farmacia*, 64(5): 774-779 (2016).
16. D.C. NUTA, M.C. CHIFIRIUC, C. DRAGHICI, C. LIMBAN, A.V. MISSIR, L. MORUSCIAG. Synthesis, characterization and antimicrobial activity evaluation of new agents from benzamides class. *Farmacia*, 61(5): 966-074 (2013).
17. R. OLAR, M. BADEA, D. MARINESCU, M.C. CHIFIRIUC, C. BLEOTU, M.N. GRECU, E. IORGULESCU, M. BUCUR, V. LAZAR, A. FINARU. Prospects for new antimicrobials based on N,N-dimethylbiguanide complexes as effective agents on both planktonic and adhered microbial strains. *Eur. J. Med. Chem,* 45: 2868-2875 (2010).
18. G.W. LAU, H. RAN, F. KONG, D.J. HASSETT, D. MAVRODI. *Pseudomonas aeruginosa* Pyocyanin Is Critical for Lung Infection in Mice. *Infect. Immun.* 72: 4275-4278 (2004).
19. K. MALATHUM, K.V. SINGH, B.E. MURRAY. In vitro activity of moxifloxacin a new 8-methoxyquinolone against Gram-positive bacteria. *Diagn. Microbiol. Infect. Dis*, 35: 127-133 (1999).
20. C. LIMBAN, A.V. MISSIR, D.C. NUȚĂ. Synthesis of some new 2-((4-chlorophenoxy)methyl)-n-(arylcarbamothioyl) benzamides as potential antifungal agents . *Farmacia,* 64(5): 775-779 (2016).