# SEARCH OF ANTIMICROBIAL ACTIVITY OF SELECTED NON–ANTIBIOTIC DRUGS

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**Abstract:** A variety of pharmaceutical preparations, which are applied in the management of non–infectious diseases, have shown *in vitro* some antimicrobial activity. These drugs are called „non–antibiotics".

The aim of this study was to detect and characterise the antimicrobial activity of non–antibiotic drugs, selected from the preparations analysed during state control performed at the Drug Institute in Poland. Over 160 pharmaceutical preparations were randomly chosen from different groups of drugs. The surveillance study was performed on standard ATCC microbial strains used for drug control: *S. aureus*, *E. coli*, *P. aeruginosa* and *C. albicans*.

It was shown that the drugs listed below inhibited growth of at least one of the examined strains: acyclovir (Awirol 5%, cream), alendronate (Alenato 5 mg, tabl.), alverine (Meteospasmyl 20 mg, caps.), butorphanole (Butamidor 10 mg/ml, amp.), clodronate (Sindronat 400 mg, caps), diclofenac (Olfen 75 mg, amp.), emadastine (Emadine 0.05%, eye dr.), etodolac (Febret 200 mg, caps.), fluvastatine (Lescol 40 mg, tabl.), ketamine (Ketamidor 10%, amp.), levocabastine (Histimet 0.5 mg/ml, eye dr.), losartan (Lorista 50 mg, tabl.), matipranolol (Betaman 0.3% eye dr.), mesalazine (Pentasa 1%, susp.), naproxen (Nalgesin 550 mg, tabl.), oxaprosine (Reumax 600 mg, tabl.), oxymethazoline (Nasivin 0.025%, nose dr.), proxymetacaine (Alcaine 0.5%, eye dr.), ribavirin (Rebetol 200 mg, caps.), rutoside with ascorbic acid (Cerutin 20+200 mg, tabl.), sulodexide (Vessel due F, 250 LSU, caps.), tegaserole (Zelmac 50 mg, tabl.), telmisartan (Pritor 20 mg, tabl.), temosolomide (Temodal 100 mg, caps.), ticlopidine (Ticlid 250 mg, tabl.), tolfenamic acid (Migea rapid 200 mg, tabl.), tramadole (Tramundin 100 mg, tabl.), tropicamide (Tropicamidum 1%, eye dr.).

*Staphylococcus aureus* was susceptible to most of the drugs listed above. Ticlopidine showed activity against *S. aureus*, *E. coli* and *C. albicans* (MICs equal to: 0.45; 0.45 and 0.65 mg/ml, respectively). Oxymetazoline showed activity against *S. aureus* and *E. coli* (MICs: 0.005 and 0.025 mg/ml, respectively). *Pseudomonas aeruginosa* was sensitive to alendronate, clondronate, oxaprozine, ribavirin and tramadole (MICs: 10, 63, 60, 3 and 43 mg/ml, respectively).

**Keywords:** non–antibiotics, drugs, antimicrobial activity, *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Candida albicans*

A variety of compounds employed in the management of non–infectious diseases aetiology have shown some antimicrobial activity *in vitro*, against bacteria and other microorganisms (1–4). Such compounds are called „non–antibiotics". By the end of the nineteenth century the dyes were known to possess antimicrobial activity. Paul Ehrlich used methylene blue – one of phenothiazine compounds as an antimicrobial agent (5). So far, a lot of attention has been focused on phenothiazines, thioxanthenes and other agents with affinities to cellular transport systems (6,7).

The search of „non–antibiotics" among drugs distributed on the Polish pharmaceutical market have been performed at the Drug Institute in Warsaw for 3 years. So far, over 220 drugs randomly chosen from different groups of pharmaceutical products were examined. During the previous study (8), it was indicated that some preparations inhibited growth at least one of the examined strains: Acesan 0.075 g tabl., Benuron 500 mg tabl., Chlorchex 0.5 g aerosol, Methotrexat–Ebewe 500 mg amp., Naproxen 500 mg tabl., No Spa Forte 60 mg tabl., Platamine 50 mg amp., Platidiam 50 mg amp., Sensit 50 mg drag., Septofervex 2 mg tabl., Seractil 400 mg tabl., Sermion 4 mg amp., Sinemet 125 mg tabl., Tarproxen 500 mg tabl. and Zyban 150 mg tabl. The surveillance study was performed on standard microbial strains.

The aim of this study was the continuation of searching and characterising the antimicrobial activity expressed by selected non–antibiotic drugs, analysed during state control performed at the Drug Institute in Warsaw.

## EXPERIMENTAL

### Material

The following microorganisms were obtained from the American Type Culture Collection: *Escherichia coli* – ATCC 8739, *Pseudomonas aeruginosa* – ATCC 15442, *Staphylococcus aureus* ATCC – 6538P, *Candida albicans* – ATCC 10231.

The following pharmaceutical products available at the Polish market were randomly chosen for the analysis: ACC 600 mg tabl. (acetocysteine), Adiuretin SD 100 µg/ml drops (desmopresine), Agalin 1% sol. (lindane), Alcaine 0.5% eye dr. (proxymetacaine), Alenato 5 mg tabl. (alendronate), Ambroksol 6 mg/ml syrup (ambroxole), Apo–Acyclovir 400 mg tabl. (acyclovir), Apo–Doxazosin 2 mg (doxazosine), Apo–Mefen 250 mg caps. (mefenamic acid), Apo–Theola 200; 300 mg tabl. (theophylline), Arteoptic 1% eye dr. (carteolol), Atropinum sulfuricum 0.5 mg/ml amp. (atropine), Awirol 5% cream (acyclovir), Azowir 100 mg caps. (zydovudine), Betaman 0.3% eye dr. (matipranolol), Biodylon fortius solut. (mesulphene), Blopress Comp Forte 16 mg tabl. (candesartan), Bodymax tabl. (multivitaminum), Bromhexin syrup (bromhexine), Butamidor 10 mg/ml amp. (butorphanol), Canwerm tabl. (nitroscanate), Cardiamidum 250 mg/ml drops (nicetamide), Cerutin tabl. 20+200 mg (rutinoside + ascorbic acid), Cesol 150 mg tabl. (praziquantel), Clexane 100 mg/ml amp. (enoxaparine), Co–aprovel 300 mg tabl. (irbesartan), Corneregel 5% eye gel

(dexpanthenol), Cusicrom 2% eye dr. (cromoglican acid), Depoprovera 150 mg/ml amp. (medroxyprogesterone), Detrunorm 15 mg tabl. (propiverine), Dexatussin 2 mg/ml syrup (dextrometorphane), Diaklat 80 mg tabl. (gliclazide), Diclofenac 1% powder (diclofenac), Differin 0.1% gel (adapalene), Dihydroergotoxinum 1 mg tabl. (dihydroergotoxin), Diltiazem HCl 60 mg tabl. (diltiazem), Diphereline 3.75 mg amp. (tryptoreline), Diphergan 0.1 g/100 ml syrup (promethazine), Dormicum 5 mg/ml amp. (midazolame), Doxepin 50 mg amp. (doxepin), Elvenal 0.3% caps. (valerenic acid), Emadine 0.05% eye dr. (emadastine), Enap; Epril 2.5 mg tabl. (enalapril), Ergotaminum tartaricum 1 mg tabl. (ergotamine), Famotidine 10 mg coated tabl. (famotidine), Famotidine 20 mg tabl. (famotidine), Febret 200 mg caps. (etodolac), Febrofen 200 mg caps. (ketoprofen), Feloran 1% gel (diclofenac), Fervex 500+25 mg powder (paracetamol + feniramine maleate), Fibrolan ointment (1U. Fibrinolysine + 666j. deoxyribonuclease), Fluanxol 0.5 mg tabl. (flupentyxole),

Table 1. Antimicrobial activity of selected non-antibiotic drugs.

| **Active substance (*Drug*)** | **Strains** | | | |
|---|---|---|---|---|
| | *S. aureus* | *E. coli* | *P. aeruginosa* | *C. albicans* |
| Anaestheticum, Antiasthmaticum* | | | | |
| • ketamine (*Ketamidor*) | 0.45** | –*** | – | – |
| Anaestethicum locale | | | | |
| • proxymetacaine (*Alcaine*) | 0.5 | – | – | – |
| Analgeticum, Antipyreticum, Antiphlogisticum | | | | |
| • diclofenac (*Olfen 75*) | 10 | – | – | 10 |
| • tolphenamic acid (*Migea rapid*) | 5 | – | – | – |
| • naproxen (*Nalgesin*) | 36 | – | – | – |
| • oxaprozine (*Reumax*) | 13 | | 60 | |
| Antianaplasticum | | | | |
| • temosolomide (*Temodal*) | 5 | 5 | – | – |
| Antiarrhythmicum, Antiglaucomicum | | | | |
| • matipranolol (*Betamann*) | 0.14 | – | – | – |
| Antiatheramaticum | | | | |
| • fluvastatine (*Lescol*) | 0.4 | – | – | 0.4 |
| Anticoagulans | | | | |
| • ticlopidine (*Ticlid*) | 0.45 | 0.45 | – | 0.65 |
| • sulodexide (*Vessel due F*) | 25**** | – | – | – |
| Antihistaminicum | | | | |
| • levocabastine (*Histimet*) | 0.02 | – | – | – |
| • emadastine (*Emadine*) | 0.02 | – | – | – |
| Antihypercalcaemicum, Antiosteoporoticum | | | | |
| • clodronate sodium (*Sindronat*) | – | – | 63 | – |
| Antihypertensivum | | | | |
| • losartan (*Lorista*) | 50 | – | – | – |
| • telmisartan (*Pritor*) | 2 | – | – | – |
| Antiphlogisticum | | | | |
| • mesalasine (*Pentasa*) | – | 6.5 | – | – |
| • etodolac (*Febret*) | 4 | 40 | – | – |
| Inhibitor | | | | |
| • alendronate (*Alenato*) | – | 5 | 10 | 5 |
| Mydriaticum, Parasympathicolyticum | | | | |
| • tropicamide (*Tropicamidum*) | 0.2 | – | – | – |
| Narcoticum, Analgeticum | | | | |
| • butorphanole (*Butamidor*) | 0.18 | 0.9 | – | – |
| • tegaserole (*Zelmac*) | 0.08 | – | – | – |
| • tramadole (*Tramundin*) | – | – | 43 | – |
| Spasmolyticum | | | | |
| • alverine (*Meteospasmyl*) | 4 | 4 | – | – |
| Vasoconstrictorium | | | | |
| • oxymetazoline (*Nasivin*) | 0.005 | 0.025 | – | – |
| Vasoprotectivum | | | | |
| • ascorbic acid + rutoside (*Cerutin*) | – | 33.3 + 8.3 | – | – |
| Virustaticum | | | | |
| • ribavirin (*Rebetol*) | – | – | 3 | – |
| • acyclovir (*Awirol*) | – | 5 | – | – |

(*) – the names of pharmaceutical groups according to Podlewski et al. [12]
(**) – minimal inhibitory concentration (MIC) in mg/ml of active substance in drug
(***) – lack of microbial growth inhibition
(****) – lipase activity unit

Flunarizinum 5 mg tabl. (flunarasine), Furosemid 0.04 g tabl. (furosemide), Gensulin M10 (insuline), Glivec 100; 50 mg caps. (imatinib), Glucophage 500 mg tabl. (metphormine), Grofibrat 100 mg caps. (fenofibrate), Histac 75 mg tabl. (ranitidine), Histimet 0.5 mg/ml eye dr. (levocabastine), Holetar 20 mg tabl. (lovastatine), Humulin M3 (insulin 30% + izophane insulin), Hydrocortison Wolff 1% cream (hydrocortisone), Hydroxyzinum 50 mg/ml amp. (hydroxizine), Insulatard HM Penfil (izophane insulin), Keppra 500 mg tabl. (levetiracetam), Ketamidor 10% amp. (ketamine), Ketonal 100 mg/2 ml amp. (ketoprofen), Ketoprofen supp. 100 mg (ketoprofen), Kornam 5 mg tabl. (terazosine), Lansome 30 mg caps. (lansoprasole), Lecitin Nerven Tonikum (lecithin), Leponex 100 mg tabl. (clozapin), Lescol XL 40 mg tabl. (fluvastatine), Leukeran 2 mg tabl. (chlorambucile), Linimer kaps. (Oleum lini), Lipidil–Ter 160 mg tabl. (fenofibrate), Lofenalac C pulver (enz. hydr. caseine), Lorazepamum 1 mg tabl. (lorazepam), Lorista 50 mg tabl. (losartan), Meteospasmyl 20 + 250 mg caps. (alverine + methionine), Meteospasmyl 60 + 300 mg caps. (alverine + simethicone), Metformin 850 mg tabl. (metphormine), Metohexal 200 retard tabl. (metoprolate), Migea rapid 200 mg tabl. (tolfenamic acid), Milocardin 20 mg/ml dr. (α–bromisovale), Minesse tabl. (gestodene, ethynylestradiole), Moexipril 15 mg tabl. (mexipril), Mukolina 5% syrup (carbocysteine), Nalgesin 550 mg tabl. (naproxen), Nasivin 0.025% nose dr. (oxymethasoline), Nifecard 30 mg tabl. (nifedipine), Norvir 100 mg caps.; 80 mg/ml solut. (ritonavir), Olfen 37.5 mg/ml amp. (diclofenac), Omepral 20 mg amp.; caps. (omeprasole), Oxazepam 10 mg tabl. (oxazepam), Penester 1 mg tabl. (finasteride), Pentasa 10 mg/ml rectal susp. (mesalasine), Pentilin 600 mg tabl. (pentoxyphiline), Posorutin 50 mg/ml eye dr. (troxerutin), Prazol 10 mg caps. (omeprasole), Prinivil 20 mg tabl. (lisinopril), Pritor 20 mg tabl. (telmisartan), Pyrantelum 50 mg/ml (pirantel), Ranigasan 150 mg tabl. (ranitidine), Ratiopharm Nasen Spray K (xylometazoline), Rebetol 5 mg tabl. (ribavirin), Relanium 0.4 mg/ml; Relset 2 mg/ml susp. (diazepam), Reumax 600 mg tabl. (oxaprosine), Risperidol 1 mg/ml solut. (risperidol), Robitussin expectorans 20 mg/ml syrup (guaiaphenesin), Robitussin Junior 1 mg/ml syrup (dextrometorphan), Sandimmun Neoral 50 mg caps. (ciclosporine), Sapoven T 150 mg caps. (*Aesculus hippocastanum*), Selegeline 10 mg tabl. (selegiline), Sepatrem 10 mg tabl. (selegiline), Sevredol 20 mg tabl. (morphine), Simredin; Simvor 40 mg tabl. (simvastatin), Sindronat 400 mg caps. (clodronate), Sirupus Sulfoguaicoli 6% (sulfoguajacole), Sonata 5 mg caps. (zaleplon), Spasmex 15 mg tabl. (trospium), Tantum Verde 50 mg tabl. (benzidamine), Tauredon 20 mg amp. (aurothiomalate), Tebonin forte 40 mg tabl. (*Gingco biloba*), Temodal 100 mg caps. (temosolomide), Theophyllinum 100 syrup (teofiline), Thiopenthal 500 mg amp. (thiopenthal), Ticlid 250 mg tabl. (ticlopidine), Tramadol 100; 50 mg/ml dr. (tramazoline), Tramundin retard 100 mg tabl. (tramadol), Tropicamidum 1% eye dr. (tropicamide), Trusopt 2% eye dr. (dorsolamidum), Tuss Drill 2 mg/ml syrup; Tussal 40 mg/ml drops (dextromethorphan), Venoruton forte 300 mg tabl. (troxerutin), Vessel due F 250 LSU caps. (sulodexide), Vibrocil 2.5; 0.25 mg/ml dr. (phenylephrine, dimetinolene maleate), Yasmin 30 mg tabl. (drospirenone), Zaditen 0.2 mg/ml syrup (ketotifen), Zelmac 50 tabl. (tegaserol), Zofran 4 mg/2 ml amp. (andansetrone), Zovirax 200 mg tabl. (acyclovir).

**Initial screening of antimicrobial activity**

The sterile blotting–paper disks were soaked with 10% (v/v or w/v) solution of tested drug in 0.08 M phosphate buffer, pH=7, and placed onto Mueller–Hinton 2 Agar (BioMerieux). Plates were inoculated with standardised suspension 0.5 unit (Mc Farland scale) of tested strain. The inhibition of bacterial growth was seen as a halo around the disk containing the tested compound. Size of inhibition zone was correlated with the antimicrobial activity of the drug.

**Test of minimal inhibitory concentration (MIC)**

Appropriate dilution of the drug in 0.08 M phosphate buffer, pH=7.0 was mixed with 19 ml of a Mueller–Hinton 2 Agar, cooled to 45°C. The suspension of particular strain of density 0.5 unit (Mc Farland scale) – 2 µl was applied on the agar surface. The lowest concentration of the tested drug, which totally inhibited growth of the examined strain was chosen as a MIC.

## RESULTS AND DISCUSSION

It was shown that the drugs listed below inhibited growth of at least one of the examined strains: acyclovir (Awirol 5%, cream), alendronate (Alenato 5 mg, tabl.), alverine (Meteospasmyl 20 mg, caps.), butorphanole (Butamidor 10 mg/ml, amp.), clodronate (Sindronat 400 mg, caps), diclofenac (Olfen 75 mg, amp.), emadastine (Emadine 0.05%, eye dr.), etodolac (Febret 200 mg, caps.), fluvastatine (Lescol 40 mg, tabl.), ketamine (ketamidor 10%, amp.), levocabastine (Histimet 0.5 mg/ml, eye dr.), losartan (Lorista 50 mg, tabl.), matipranolol (Betaman 0.3% eye dr.), mesalazine (Pentasa 1%, susp.), naproxen (Nalgesin 550 mg, tabl.), oxaprosine (Reumax 600 mg, tabl.), oxymethazoline (Nasivin 0.025%, nose dr.), proxymetacaine (Alcaine 0.5%, eye dr.), ribavirin (Rebetol 200 mg, caps.), rutoside with ascorbic acid (Cerutin 20+200 mg, tabl.), sulodexide (Vessel due F, 250 LSU, caps.), tegaserole (Zelmac 50 mg, tabl.), telmisartan (Pritor 20 mg, tabl.), temosolomide (Temodal 100 mg, caps.), ticlopidine (Ticlid 250 mg, tabl.), tolfenamic acid (Migea rapid 200 mg, tabl.), tramadole (Tramundin 100 mg, tabl.), tropicamide (Tropicamidum 1%, eye dr.), (Table 1).

*Staphylococcus aureus* was susceptible to most of the drugs listed above. Levocabastine and emadastine (antihistamine drugs) inhibited growth of this microorganism at a concentration of 0.02 mg/ml and tegaserole at a concentration of 0.08 mg/ml. Ticlopidine showed activity against *S. aureus*, *E. coli* and *C. albicans* (MICs equal 0.45; 0.45; 0.65 mg/ml, respectively). Fluvastatine inhibited growth of *S. aureus* and *C. albicans* at a concentration of 0.4 mg/ml. Oxymetazoline showed quite high activity against *S. aureus* and *E. coli* (MICs: 5; 25 µg/ml, respectively). The growth of *E. coli* was also inhibited by butorphanole (MIC: 0.9 mg/ml). *Pseudomonas aeruginosa* strain was sensitive to some analysed drugs, but at much higher concentration. Alendronate, clondroniate, oxaprozine, ribavirin and tramadole inhibited its growth at a concent-

rations 10, 63, 60, 3 and 43 mg/ml respectively. Moreover, alendronate at a concentration of 5.5 mg/ml inhibited growth of *E. coli* and *C. albicans*.

Kristiansen et al. (1,2,9) confirmed that non–antibiotic compounds also enhance the *in vitro* activity of certain antibiotics against specific bacteria, so our further investigations will focus on this type of activity. Having in mind the rapid growth of microbial resistance mechanisms against frequently used antibiotics, a synergistic interaction might be a solution in fight with infection diseases.

Moreover, the antimicrobial activity of such non–antibiotic drugs emphasises a necessity of the neutralisation of their activity during the microbial purity tests of pharmaceutical products (10,11).

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# ANTIMYCOBACTERIAL ACTIVITY OF SOME PYRIDO–1,2–THIAZINE DERIVATIVES


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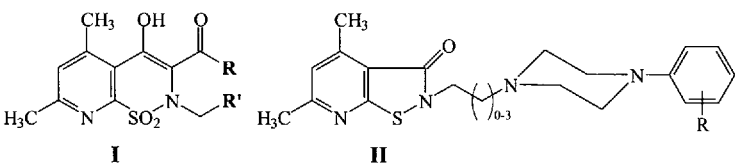
**Abstract:** The 3–benzoylpyrido–1,2–thiazine–1,1–dioxides **1** and the related pyrazolopyrido–1,2–thiazine–5,5–dioxides **2** with a 4–arylpiperazin–1–ylpropyl side chained by the nitrogen atom of the thiazine ring were evaluated *in vitro* against *Mycobacterium tuberculosis* $H_{37}Rv$. Some of the tested compounds proved to be potent antimycobacterial agents and for the most active of them (**1a,b**) minimum inhibitory concentrations (MIC=3.13 and 6.25 μg/ml, respectively) were determined. The correlation between mycobacterium growth inhibition and the lipophilicity ($logP_{calc.}$) within the series of derivatives **1** and **2** was studied.


**Keywords:** antimycobacterial agents

During the pharmacological screening of 2–substituted–3–acetyl(benzoyl)–4–hydroxy–5,7–dimethyl–2H–pyrido[3,2–e]–1,2–thiazine–1,1–dioxides **I** (Figure 1) synthesized in connection with another project (1,2), a weak antimycobacterial effect was observed (MIC>12.5 μg/ml; ~10–40% inhibition of *Mycobacterium tuberculosis* $H_{37}Rv$) (3). On the other hand, some of the recently investigated representatives of our isothiazolopyridines, especially those bearing a 4–(substituted–phenyl)–piperazin–1–ylalkyl chained by the nitrogen of the isothiazole ring (**II**, Figure 1), showed a significant effect of inhibition of *M. tuberculosis*




**I** **II**

**R** = $CH_3$, $C_6H_5$, **R'** = H, COR"
(R" = $OC_2H_5$, $NHC_4H_9$, $NHC_6H_{11}$, $C_6H_5$)

R = *o*-$CH_3$, *m*-$CF_3$, *m*-Cl

Figure 1.