Biological Activity of New Heterocyclic Systems Containing Thiazolic Ring

CRISTIANA RADULESCU[1]*, CLAUDIA STIHI[1]
[1]Valahia University of Targoviste, Faculty of Sciences and Arts, Sciences Department, 18-22 Unirii Bdvl., 130082, Targoviste, Romania

In this study several tests were performed to determine the toxicity and biological activity of some new compact condensed systems with thiazolic ring, obtained by original syntheses which are published in many articles. The presence of N-C-S linkage in the heterocyclic systems, as thiazolic ring, has been determined antimicrobial and antifungal activity. So, these new heterocyclic systems, obtained for the first time by authors, have been screened in vitro for antimicrobial activity determination against various strains of bacteria and fungi. Most of the compounds have shown significant antifungal activity while few have shown excellent antimicrobial activity. An attempt is made to study the compounds and biological activity relationship.

Keywords: compact condensed system, thiazolic ring, acute toxicity, antimicrobial activity, antifungal activity

The compact condensed heterocyclic systems with thiazolic ring are solid substances with high melting points, having the tendency to decompose before reaching them. Their great stability of the compact condensed systems with thiazolic ring, doubled by a remarkable biological activity, lead to their application in many fields of interest.

It is well known that a great number of compact condensed systems derived of pyridine exert microbistatic and microbicide effects in small concentrations and others are used as antiseptics for skin and mucous membranes [1, 2].

Nowadays, many heterocyclic systems have been used for the preparation of selective growth environment necessary to isolate certain bacteria, just because they inhibit the development of some species and stimulate the development of others.

It has known from literature the fact that fungi are microorganisms of eukaryote type, monocellulars or pluricellulars, morphologically different and reproducing by spores [6]. These microorganisms have spread in all natural habitats, due to their special capacity to adapt well to the most different environmental conditions. They determine the decay of food products and textile fibres or rubber, etc., because they have the capacity to produce induced enzymes related to the nature of their support. The moulds in the form of spores or *hife* are very resistant to dryness (the minimum humidity they can develop is 15%) and they are able to survive in a latent state for a long time [1, 3].

With their capacity of decaying organic dead matter, fungi produce the change of some organic compounds (cellulose, hemicelluloses, and pectic substances) into small and simpler compounds without biological activity. That is why they have been considered as decaying agents.

The compact condensed systems with thiazolic ring obtained by original synthesis [1, 2, 4-11] performed several preliminary pharmacological tests as follows: acute toxicity, antimicrobial activity and antifungal tests. These tests were applied starting from the specific literature data, where a lot of information about the compact condensed compounds and their biological activity has been found. The presence of N-C-S linkage in the heterocyclic systems, as thiazolic ring, has shown antimicrobial and antifungal activity. All heterocyclic systems obtained by synthesis have been screened *in vitro* for their antimicrobial activity determination against different strains of bacteria and fungi.

The microbiological studies performed with solutions of new compact condensed systems consist in tests, which follow to show their antimicrobial potential activity, by monitoring the growth of a *Penicillium* stem and some variety of bacterial strains. This method is the one using the germs incorporation in nutritious environments.

Apart from their chemical interest, these compounds could also be a subject of research studies as pharmacological agents.

Experimental part

Materials and methods

Compact condensed systems obtained by original syntheses [2, 4-11] such as:

2-aminothiazolo[4,5-*b*]pyridine, 2-aminothiazolo[5,4-*c*]pyridine, 2-aminothiazolo[4,5-*b*] quinoxaline-6-carboxylic acid, 2-aminothiazolo[5,4-*b*] quinoxaline-7-carboxylic acid, 2-aminothiazolo[4,5-*f*]indazole, 2-aminothiazolo[5,4-*f*]indazole. These heterocyclic systems (Table 1) are synthesized for the first time by authors and the results of spectral analyses (UV-VIS, IR, NMR) and elemental analysis have been published in many articles and studies [1, 2, 4-11, 13-15]. The heterocyclic systems are purified and separated [4-12] by thin-layer chromatography (TLC) and high performance liquid chromatography (HPLC) and then the biological activity is tested for each system.

DMSO (Merck), alcohol p.a. (Aldrich); antibiotics such as streptomycin and gentamycin, used as standard drugs showed zones of inhibition.

Bacterial strains such as Gram positive bacteria *Bacillus substilis*, *Staphylococcus aures*, and Gram negative bacteria *Escherichia coli*, *Pseudomonas auriginosa* and *Penicillium glaucum* stem as inferior fungus.

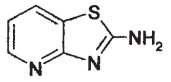
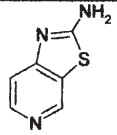
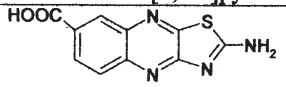
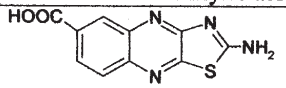
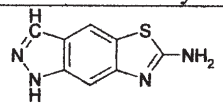
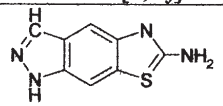
Acute toxicity

White mice of *Swiss* race weighing 20+/-2g and white rats of *Wistar* race weighing 140+/-20g were used in batches of 20 animals (10 males and 10 females) and the dose to be tested.

The solutions of synthesized heterocyclic systems with thiazolic ring (table 1) were injected as alcoholic-solutions, in a unique dose, according to the data presented in table 2.

* *Tel.: 0729851455*

Table 1
NEW COMPACT CONDENSED SYSTEMS OBTAINED BY ORIGINAL SYNTHESIS

Compact condensed systems	Colour	Wavelength, λ_{max}, [nm] (absorbance)	Melting point [^{0}C]
 2-aminothiazolo[4,5-*b*]pyridine	Yellow	275.5(2.257)	238-240^{0}C
 2-aminothiazolo[5,4-*c*]pyridine	Brown-red	276.2(2.109)	251-253^{0}C
 2-aminothiazolo[4,5-*b*] quinoxaline-6-carboxylic acid	Yellow-brown	327.5(1.765)	256-258^{0}C
 2-aminothiazolo[5,4-*b*] quinoxaline-7-carboxylic acid	Yellow-brown	326.2(1.662)	257-259^{0}C
 2-aminothiazolo[4,5-*f*]indazole	Brown light	378.2(1.620)	218-219^{0}C
 2-aminothiazolo[5,4-*f*]indazole	Brown dark	376.9(1.578)	221-222^{0}C

The animals were supervised for 14 days and the modifications of their behaviour and the mortality have been registered.

At the end of the testing period, there were performed biochemical determinations and anatomico-pathological exams of the rats in order to disclose possible damages of their principal internal organs.

Antimicrobial activity

The antimicrobial activity was assayed by using the cup-plate agar diffusion method [3, 16] by measuring the inhibition zone in mm. The standard nutrient agar medium contain: extract (bacteriological) 1.0%, peptone 1.0%, sodium chloride 0.5%, agar 2.0% and water 100 mL. All the compact condensed systems, 2-aminothiazolopyridine,2-aminothiazoloindazole, 2-aminothiazoloquinoxaline-carboxylic acid, were screened *in vitro* for their antimicrobial activity determination towards variety of bacterial strains such as *Bacillus substilis*, *Staphylococcus aures*, *Escherichia coli* and *Pseudomonas auriginosa*. DMSO was used as solvent control. Known antibiotics such as streptomycin and gentamycin, used as standard drugs showed zones of inhibition.

All the synthesized heterocyclic systems were dissolved in aqueous ethanol at 50 μg/mL. The toxicity of compounds was determined via pipette additions into the wells of multi-well slides, followed by 25 μL of culture medium. The culture plates were dried in the incubator with the lid until its surface was free from visible moisture without further delay. Then, the known concentration of drug was applied as discs [prepared by uniformly punching out 6 mm disc from Whatmann filter paper (no.41)] and impregnating with drug (100 discs in 1 mL), with adequate spacing to the surface of the culture plates, with sterile fine pointed forceps and pressed gently to ensure full contact with the medium. The inoculated slides were then incubated at 37^{0}C until short germ tubes appeared (24 hours). At the end of 24 h the diameter of zone inhibition produced was measured.

Antifungal activity

A stem of *Penicillium glaucum* was used to test the antifungal activity of some compact condensed systems with the structures presented in table 1. This type of *Penicillium glaucum* is very wide spread in the exterior environment and is of the most resistant to the action of external factors [17-19]. The mould has been isolated and obtained in pure culture; it was prepared a suspension of spores in sterile water with $4 \cdot 10^6$ spores/mL, by using the Thorna counter camera.

After several dilutions in decimal system were made inseminations with the same inocul in Petri dishes with fluidized and cooled at around 45^{0}C Czapek medium, in the alternative of witness specimen without heterocyclic systems added, used in relation to the growth medium, in a dose of 0.01 and 0.02%.

The above samples were optimally maintained at 25^{0}C for 15 days. Afterward, the colonies developed were counted and the antifungal effect of each compact condensed systems has been assessed.

Results and discussion

Acute toxicity

For the maximum tolerated dose administrated p.o. and i.p., neither particular clinical phenomena occurred and

Animal	Dose	Concentration of compact condensed systems [%]	Compact condensed systems
White rats of *Wistar*	2 mL/100g p.o.	0.01	2-aminothiazolo[4,5-*b*]pyridine
	1 mL/100g i.p.	0.01	2-aminothiazolo[4,5-*b*]pyridine
	2 mL/100g p.o.	0.01	2-aminothiazolo[5,4-*c*]pyridine
	1 mL/100g i.p.	0.01	2-aminothiazolo[5,4-*c*]pyridine
	2 mL/100g p.o.	0.01	2-aminothiazolo[4,5-*b*]quinoxaline-6-carboxylic acid
	1 mL/100g i.p.	0.01	2-aminothiazolo[4,5-*b*]quinoxaline-6-carboxylic acid
	2 mL/100g p.o.	0.01	2-aminothiazolo[5,4-*b*]quinoxaline-7-carboxylic acid
	1 mL/100g i.p.	0.01	2-aminothiazolo[5,4-*b*]quinoxaline-7-carboxylic acid
	2 mL/100g p.o.	0.01	2-aminothiazolo[4,5-*f*]indazole
	1 mL/100g i.p.	0.01	2-aminothiazolo[4,5-*f*]indazole
	2 mL/100g p.o.	0.01	2-aminothiazolo[5,4-*f*]indazole
	1 mL/100g i.p.	0.01	2-aminothiazolo[5,4-*f*]indazole
White mice of *Swiss*	0.5 ml/20 p.o.	0.01	2-aminothiazolo[4,5-*b*]pyridine
	0.5 ml/20g i.p.	0.01	2-aminothiazolo[4,5-*b*]pyridine
	0.5 ml/20 p.o.	0.01	2-aminothiazolo[5,4-*c*]pyridine
	0.5 ml/20g i.p.	0.01	2-aminothiazolo[5,4-*c*]pyridine
	0.5 ml/20 p.o.	0.01	2-aminothiazolo[4,5-*b*]quinoxaline-6-carboxylic acid
	0.5 ml/20g i.p.	0.01	2-aminothiazolo[4,5-*b*]quinoxaline-6-carboxylic acid
	0.5 ml/20 p.o.	0.01	2-aminothiazolo[5,4-*b*]quinoxaline-7-carboxylic acid
	0.5ml/20g i.p.	0.01	2-aminothiazolo[5,4-*b*]quinoxaline-7-carboxylic acid
	0.5 ml/20 p.o.	0.01	2-aminothiazolo[4,5-*f*]indazole
	0.5 ml/20g i.p.	0.01	2-aminothiazolo[4,5-*f*]indazole
	0.5 ml/20 p.o.	0.01	2-aminothiazolo[5,4-*f*]indazole
	0.5 ml/20g i.p.	0.01	2-aminothiazolo[5,4-*f*]indazole

Table 2
TESTING THE TOXICITY OF COMPACT CONDENSED SYSTEMS

Table 3
ANTIMICROBIAL ACTIVITIES OF TESTED HETEROCYCLIC SYSTEMS

Heterocyclic system	Antimicrobial activity (% relative inhibition) con. in 50 μg/mL (zone of inhibition, mm)							
	Bacillus substilis		*Staphylococcus aures*		*Escherichia coli*		*Pseudomonas auriginosa*	
	a	b	a	b	a	b	a	b
2-aminothiazolo[4,5-*b*] pyridine	36 (8)	33 (9)	30 (10)	27 (9)	34 (13)	32 (11)	30 (10)	30 (12)
2-aminothiazolo[5,4-*c*] pyridine	35 (6)	32 (7)	30 (12)	28 (10)	33 (10)	32 (8)	31 (11)	20 (11)
2-aminothiazolo[4,5-*b*] quinoxaline-6-carboxylic acid	25 (8)	22 (6)	28 (11)	26 (12)	31 (9)	28 (8)	29 (8)	29 (7)
2-aminothiazolo[5,4-*b*] quinoxaline-7-carboxylic acid	20 (7)	18 (10)	24 (9)	22 (9)	29 (7)	26 (8)	29 (10)	28 (11)
2-aminothiazolo[4,5-*f*] indazole	09 (6)	08 (6)	10 (5)	10 (5)	12 (6)	09 (7)	18 (7)	15 (6)
2-aminothiazolo[5,4-*f*] indazole	06 (5)	05 (4)	09 (3)	08 (4)	10 (5)	06 (5)	16 (6)	15 (8)

a – values in comparison with streptomycin; b – values in comparison with gentamycin.

$$\%\ relative\ inhibition = \frac{inhibition\ of\ the\ test\ compound}{inhibition\ of\ the\ standard\ drug} \times 100$$

nor mortality. The biochemical tests applied did not show any modification comparing to the witnesses.

The anatomico-pathological exam of the rat, at the end of the testing period does not show modifications of the principal internal organs for any dose of systems used.

The studies of acute toxicity applied on 2 species and using 2 ways of administration, with solutions 0.01% of heterocyclic systems proved no toxicity for both p.o. and i.p. administration. Therefore, the DL_{50} could not be calculated but it was determined the maximum dose.

Antimicrobial activity

The results presented in table 3 clearly revealed that all tested heterocyclic systems were active against Gram positive bacteria *Bacillus substilis* and *Staphylococcus aures*; also, these systems are active against Gram negative bacteria *Escherichia coli* and *Pseudomonas auriginosa* (relative inhibition 30-35%).

The systems 2-aminothiazolo[4,5-*b*]pyridine and 2-aminothiazolo[5,4-*c*]pyridine showed good antimicrobial activity against Gram positive bacteria *Bacillus substilis* and *Staphylococcus aures* and Gram negative bacteria *Escherichia coli* and *Pseudomonas auriginosa*.

The systems 2-aminothiazolo[4,5-*b*]quinoxaline-6-carboxylic acid and 2-aminothiazolo[5,4-*b*]quinoxaline-7-carboxylic showed a moderate antimicrobial activity against Gram positive bacteria *Bacillus substilis* and *Staphylococcus aures* and Gram negative bacteria *Escherichia coli* and *Pseudomonas auriginosa*.

On the other hand 2-aminothiazolo[4,5-*f*]indazole and 2-aminothiazolo[5,4-*f*]indazole showed lower antimicrobial activities against Gram positive bacteria *Bacillus*

Table 4
ANTIFUNGAL ACTIVITY OF COMPACT CONDENSED SYSTEMS

Sample		Number of colonies/plate	Inhibition of the growth [%]	Observations
Witness		165	-	Colonies in diameter 2-4 mm, pigmented in light green, characteristic the stem of *Penicillium glaucum.*
2-aminothiazolo [4,5-*b*] pyridine	0.01%	36	61.2	Colonies in diameter 1-2 mm, white colour.
	0.02%	10	96.4	
Witness		162	-	Colonies in diameter 2-4 mm, pigmented in light green, characteristic the stem of *Penicillium glaucum*
2-aminothiazolo [5,4-*c*] pyridine	0.01%	38	60.5	Colonies in diameter 1-2 mm, white colour.
	0.02%	9	95.56	
Witness		171	-	Colonies in diameter 2-4 mm, pigmented in light green, characteristic the stem of *Penicillium glaucum.*
2-aminothiazolo [4,5-*b*]quinoxaline-6-carboxylic acid	0.01%	149	12.05	The development is more delayed, *Penicillium* were point colonies, white colour
	0.02%	120	27.50	
Witness		174	-	Colonies in diameter 2-4 mm, pigmented in light green, characteristic the stem of *Penicillium glaucum.*
2-aminothiazolo [5,4-*b*]quinoxaline-7-carboxylic acid	0.01%	151	11.63	The development is more delayed, *Penicillium* were point colonies, white colour.
	0.02%	132	21.51	
Witness		180	-	Colonies in diameter 2-4 mm, pigmented in light green, characteristic the stem of *Penicillium glaucum*
2-aminothiazolo [4,5-*f*]indazole	0.01%	136	4.9	Reduction un-significant face to witness sample.
	0.02%	106	23.36	Colonies in diameter 1-3 mm; one part is un-pigmented, with negative influence upon the sizes and the dynamic of growth.
Witness		172	-	Colonies in diameter 2-4 mm, pigmented in light green, characteristic the stem of *Penicillium glaucum*
2-aminothiazolo [5,4-*f*]indazole	0.01%	156	6.1	Reduction un-significant face to witness sample
	0.02%	119	21.71	Colonies in diameter 1-3 mm; one part is un-pigmented, with negative influence upon the sizes and the dynamic of growth.

substilis and *Staphylococcus aures*, but the antimicrobial activity increase against Gram negative bacteria *Escherichia coli* and *Pseudomonas auriginosa*.

Antifungal activity

The investigation of fungicidal screening data of compact condensed systems (table 4) revealed that all compounds showed variable activities towards the *Penicillium glaucum*, which showed that these compounds are biologically active due to the presence of thiazolic ring condensed with different heterocycles (indazole, quinoxaline and pyridine).

The systems 2-aminothiazolo[4,5-*b*]pyridine and 2-aminothiazolo[5,4-*c*]pyridine showed very high antifungal activity against *Penicillium glaucum* stem.

The systems 2-aminothiazolo[4,5-*b*]quinoxaline-6-carboxylic acid and 2-aminothiazolo[5,4-*b*]quinoxaline-7-carboxylic showed a moderate antifungal activity against *Penicillium glaucum* stem and 2-aminothiazolo[4,5-*f*]indazole and 2-aminothiazolo[5,4-*f*]indazole systems showed lower activity against *Penicillium glaucum*.

2-Aminothiazolo-pyridine compounds which failed to show significant antibacterial activity however showed excellent antifungal activity, too. Perhaps, the pyridinic ring condensed with thiazolic ring may be responsible for the enhanced antifungal activity of these systems.

Conclusions

The studies of acute toxicity applied on 2 species and using 2 ways of administration, with solutions 0.01% of heterocyclic systems proved no toxicity for both p.o. and i.p. administration.

The results concerning antimicrobial activity clearly revealed that all six heterocyclic systems with thiazolic ring were active against Gram positive bacteria *Bacillus substilis* and *Staphylococcus aures* and also, are active

against Gram negative bacteria *Escherichia coli* and *Pseudomonas auriginosa* (relative inhibition 30-35%).

The strongest inhibitive effects of all tested heterocyclic systems (>90%) over the stem of *Penicillium* were noticed for the compact condensed systems of 2-aminothiazolo[4,5-*b*]pyridine and 2-aminothiazolo[5,4-*c*]pyridine, in both concentrations (0.01% and 0.02% respectively). The same behaviour is valid for all the other agents belonging to the same class; they have a strong antifungal activity.

In the case of the compact condensed systems of 2-aminothiazolo[4,5-*f*]indazole and 2-aminothiazolo[5,4-*f*]indazole, is necessary a dose of minimum 0.02% for the inhibition of the *Penicillium* stem.

By doubling the concentration of the compact condensed systems of 2-aminothiazolo[4,5-*b*]quinoxaline-6-carboxylic and 2-aminothiazolo[5,4-*b*]quinoxaline-7-carboxylic acids, the percent of the inhibiting effect over the growth of *Penicillium* stem increased by 2.5 times approximately. The antifungal effect of these systems became obvious regarding also the dimensions of the colonies developed, they being much smaller (not reaching the maturity), by comparison with the tests performed with the aminothiazolo-pyridinic and aminothiazolo-quinoxaline compounds.

All compact condensed systems inhibit the development of *Penicillium* in 4.05 – 95.56% proportion depending on the structure of the heterocyclic compound and the dose used.

The structure-biological activity relationship suggested that the presence of thiazolic ring in tested heterocyclic systems may be responsible for the good antibacterial and antifungal activities of these compounds. Looking to the structure activity relationship it can be concluded that remarkable inhibition was observed at heterocyclic systems 2-aminothiazolo-pyridines.

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