SYNTHESIS, BIOEVALUATION AND MOLECULAR PROPERTIES OF SOME TRIAZINE DERIVATIVES

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Abstract

A series of 1,2,4-triazines have been synthesized and characterized by means of TLC, melting point, IR spectral data and elemental analysis. The synthesized compounds were screened for their antibacterial activity against Gram positive and Gram negative bacteria and *Candida* sp. Screening of antioxidative capacity was based on ability to scavenge free radicals by DPPH (2,2-diphenyl-1-picryl-hydrazyl) method and by reducing ability. Both antimicrobial and antioxidant activities depended on some structural characteristics of the synthesized compounds. The compounds II and III were subjected to molecular properties prediction, lipophilicity.

Rezumat

Au fost sintetizaţi derivaţi de 1,2,4-triazină care au fost caracterizaţi prin punct de topire, spectre IR şi analiza elementală calitativă. S-a evaluat activitatea antimicrobiană faţă de bacterii Gram pozitive, Gram negative şi *Candida* sp. Capacitatea antioxidantă a derivaţilor investigaţi a fost stabilită faţă de radicalul DPPH (2,2-difenil-1-picril-hidrazil) şi prin determinarea capacităţii reducătoare. Atât activitatea antimicrobiană, cât şi cea antioxidantă sunt în corelaţie cu caracteristicile structurale. Pentru derivaţii obţinuţi s-a determinat lipofilia şi alţi parametri moleculari.

Keywords: triazine derivatives, antimicrobial activity, antioxidant activity

Introduction

Suitable functionalized isatins are versatile molecules for obtaining polycyclic fused heteroaromatics, compounds well known for their biological activities [4,9]. 1,2,4-Triazine nucleus is a prominent structural core system present in numerous active compounds, possessing a wide

range of biological activities. Triazine derivatives have been also introduced as anti-inflammatory, free radicals scavenger and antifungal agents [3].

Resistance to antimicrobial agents by pathogenic bacteria towards available substances is rapidly becoming a major worldwide problem; thus, the discovery of novel potent substances is very important taking into account the resistance phenomenon of the microorganisms.

The presence of free radicals in the biological system is very harmful, these species being responsible for the damage of biomolecules such as nucleic acids, proteins, lipids and carbohydrates and this fact may cause many diseases such as cancer, atherosclerosis, aging, hair loss, inflammation, immunosupresssion, diabetes and neurodegeneration associated disorders [1,11].

In continuation of our work on synthesis of various derivatives of isatins, we decided to prepare some new heterocyclic compounds starting from isatins thiosemicarbazones and to investigate their antibacterial and antioxidative properties.

Materials and Methods

Melting points were determined using an electro thermal Melting Point apparatus and were uncorrected. Elemental analysis was performed on Elemental Exeter Analytical CE 440 Analyzer. The IR (infrared) spectra were recorded on a FTIR Shimadzu Prestige 8400s spectrophotometer. The synthesis of the compounds II and III was carried out according to figure 1.

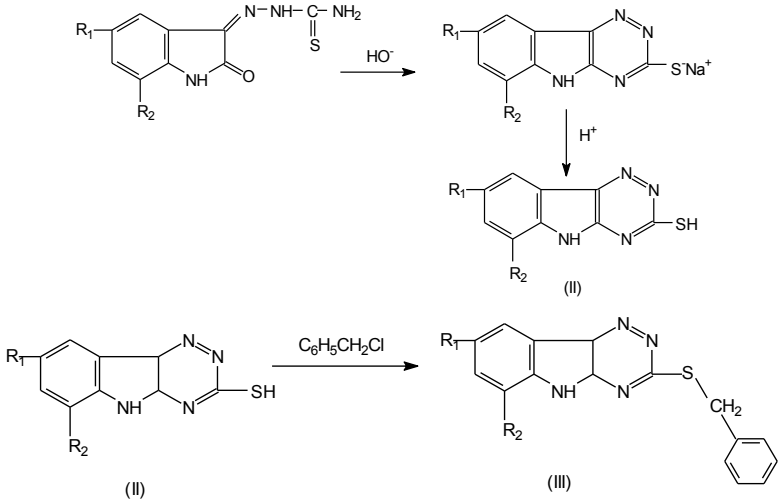

Figure 1

Synthetic route for obtaining the derivatives II and III
(R_1 = H, CH_3, Cl, NO_2 and R_2= H, CH_3)

Thiosemicarbazone derivatives were put in an aqueous NaOH solution and heated 15 h. After cooling, the mixture was acidified with HCl (pH=3). The resulting precipitates were filtred and then purified by recristallization, compounds II being obtained. Benzyl derivatives (III) were synthesized by alkylating the compounds II with benzyl chloride in aqueous sodium hydroxide. Crude products were isolated and recrystallized from suitable solvents to yield target compounds III.

The disk diffusion test was performed using Mueller Hinton (Oxoid) for bacteria and Sabouraud agar for fungal strains. Microorganisms test: *Staphylococcus aureus* ATCC 25923, *Sarcina lutea* ATCC 9341, *Bacillus cereus* ATCC 14579, *Bacillus subtilis*, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa*, *Candida albicans* ATCC 10231. The bacterial and fungal strains were incubated over night at 30 ^{0}C and from each microbial culture it was prepared a suspension with the same density as the 0.5 standard from the Mac Farland turbidimetric scale. The suspensions of microorganisms were incorporated in Muller-Hinton medium, melted and cooled afterwards to 50 ^{0}C in a 1/10 ratio. After homogenization, we placed 25 mL of this mixture in Petri plates with a diameter of 9 cm. On the surface of each plate, we put (after solidification) paper circles impregnated with 10 µL from the DMF (dimethyl formamide) solutions (100 mg/mL) of the tested compounds. Chloramphenicol, ampicillin and nystatin were used as standards for antibacterial and antifungal activity [8].

In vitro antioxidant activity of the investigated derivatives was evaluated by the DPPH (2,2-diphenyl-1-picryl-hydrazyl) radical-scavenging method [2,6]. The principle of this assay is based on the measurement of the scavenging ability of the antioxidant toward the stable radical. The free radical DPPH is reduced to the corresponding hydrazine when it reacts with hydrogen donors, and its stability is evaluated by the discolouring assay, which evaluates the decrease in absorbance at 517 nm produced by the addition of the antioxidant to a DPPH solution in ethanol. Ascorbic acid was used as standard.

The analysis was performed using the folloing equation:

Scavenging effect (%) = (Absorbance of control-Absorbance of test)/Absorbance of control) x 100

The reducing ability is an important parameter which serves for appreciating the antioxidant activity. The reducing ability was determined according to the method of Oyaizu using Fe^{3+} to Fe^{2+} reduction assay, the Fe^{2+} iron can be monitored by measurement of the formation of a blue colour at 700 nm. The activity was compared with ascorbic acid, which was

used as reference. An increase in the absorbance of the reaction mixture indicates a higher reducing ability [7].

The lipophilicity and the theoretical physico-chemical parameters of the synthesized derivatives were determined using the Molinspiration software.

Results and Discussion

All the synthesized derivatives structures were confirmed by IR spectral analysis and elemental analysis (Table I). The IR spectrum of the target compounds showed an absorption peak at about 1550-1600 cm^{-1} due to the stretching of C=N. The S-H stretching vibration appeared at 2500-2620 cm^{-1}. The absorption at 570-710 cm^{-1} was obtained due to C-S stretching vibration. For the C-N bond the peak was at about 808-820 cm^{-1}.

Table I

The structure of the synthesized derivatives II and III (e=experimentally; t=theoretically)

Comp.	R1	R2	Name of compound	MW	M.p. (°C)	%C(e/t)	(e/t)H	(e/t)N	(e/t)S	Cl(e/t)
IIa	H	H	5H-[1,2,4]-triazino-[5,6-b]-indol-3-yl-mercaptan	202	360-3	53.2 53.4	2.89 2.99	27.3 27.7	15.75 15.86	
IIb	CH_3	H	8-methyl- 5H-[1,2,4]-triazino-[5,6-b]-indol-3-yl-mercaptan	216	375-8	55.2 55.54	3.68 3.73	25.82 25.91	14.78 14.83	
IIc	Cl	H	8-chloro-5H-[1,2,4]-triazino-[5,6-b]-indol-3-yl-mercaptan	236.5	>400	45.5 45.67	2.08 2.13	23.5 23.67	13.48 13.55	14.84 14.98
IId	NO_2	H	8-nitro-5H-[1,2,4]-triazino-[5,6-b]-indol-3-yl-mercaptan	247	>400	43.02 43.72	1.98 2.04	28.01 28.33	12.81 12.97	
IIe	H	CH_3	6-methyl-5H-[1,2,4]-triazino-[5,6-b]-indol-3-yl-mercaptan	216	367-8	55.28 55.54	3.7 3.73	25.82 25.91	14.80 14.83	
IIIa	H	H	3-benzylmercapto-5H-[1,2,4]-triazino-[5,6-b]-indole	292	262-4	65.68 65.73	4.1 4.14	19.01 19.16	10.78 10.97	
IIIb	CH_3	H	3-benzylmercapto-8-methyl-5H-[1,2,4]-triazino-[5,6-b]-indole	306	280	66.51 66.64	4.54 4.61	18.1 18.24	10.38 10.47	
IIIc	Cl	H	3-benzylmercapto-8-chloro-5H-[1,2,4]-triazino-[5,6-b]-indole	326.5	283-4	58.1 58.8	3.35 3.39	17.02 17.14	9.75 9.81	10.78 10.85
IIId	NO_2	H	3-benzylmercapto-8-nitro-5H-[1,2,4]-triazino-[5,6-b]-indole	337	270-2	56.79 56.96	3.11 3.29	20.58 20.76	9.34 9.5	
IIIe	H	CH_3	3-benzylmercapto-6-methyl-5H-[1,2,4]-triazino-[5,6-b]-indole	306	226-8	66.58 66.64	4.52 4.61	18.18 18.24	10.35 10.47	

Mw – molecular weight; Mp – melting point

The antimicrobial activities of the synthesized compounds against target strains were examined qualitatively by the diameter of the inhibition zones. Results given in Table II showed a good antibacterial activity for all the tested bacteria and fungus, except *B. subtillis.* No significant

improvement in the antimicrobial activity was observed with the incorporation of the benzyl group by substitution of the thiol group.

Table II

Antimicrobial activity of the target compounds against the investigated strains

Compound	*S. aureus* ATCC 25923	*Sarcina lutea ATCC 9341*	*Bacillus cereus ATCC 14579*	*B. subtilis*	*E. coli* ATCC 25922	*Candida albicans* ATCC 25922	*Candida sake*	*Candida glabrata*
IIa	18	20	13	0	11	18	16	23
IIb	21	21	15	0	12	22	19	24
IIc	18	21	15	0	12	20	20	25
IId	21	20	12	0	11	19	18	24
IIe	20	23	16	0	12	20	20	24
IIIa	17	20	11	0	10	17	17	20
IIIb	21	20	15	0	15	20	17	22
IIIc	18	13	11	0	15	18	15	20
IIId	20	13	10	0	14	19	13	20
IIIe	20	12	12	0	12	18	16	20
Chloramphenicol 30 µg	20	25	21	21	22	-	-	-
Ampicillin 25 µg	20	28	0	21	11	-	-	-
Nystatin 100 µg	-	-	-	-	-	20	19	24

The DPPH radical scavenging activity in terms of percentage inhibition exhibited by the title compounds summarized in Table III. The ability to release a hydrogen atom or an electron is lower when compared to the control, 0.1 mM ascorbic acid, compounds IIc and IIIe showing the highest activity. The better radical scavenging capacity of compounds without benzyl was due to the presence of acidic proton in the thiol group.

Table III

DPPH radical scavenging activities of tested compounds in a dose of 2mM

Comp.	**Percentage inhibition (±SD)**
IIa	31.82± 0.02
IIb	40.35± 0.03
IIc	89.43± 0.015
IId	41.76± 0.03
IIe	45.35± 0.02
IIIa	31.87± 0.02
IIIb	18.82± 0.04
IIIc	31.07± 0.021
IIId	40.91± 0.03
IIIe	93±0.04
Ascorbic acid	95.32 ± 0.01

Assayed compounds were able to reduce the ferric ions to corresponding ferrous ions; they have some degrees of reducing ability, parameter that was inferior to ascorbic acid, which is known to be a strong reducing agent. Compounds IIc, IId, IIIc and IIId show the best activity, this could be attributed to electron withdrawing natures of substituents (Table IV).

Table IV

Equivalence in reducing ability of the investigated derivatives compared to ascorbic acid

Comp. (2mM)	Ascorbic acid (mmols)
IIa	0.1328
IIb	0.1332
IIc	0.1402
IId	0.1395
IIe	0.1382
IIIa	0.1257
IIIb	0.1371
IIIc	0.138
IIId	0.1399
IIIe	0.1374

According to Lipinski's rule-of-five, molecules should have log P values ≤ 5 in order to be readily bioavailable [5]. All the investigated compounds obey this rule. Furthermore, Lipinski's rule says that the molecular weight should be below 500, the number of hydrogen bond acceptors should not be more than 5 and the number of hydrogen bond donors should not be more than 5. These numbers are the upper limits for drugs to be able to penetrate through biomembranes. All the synthesized compounds have the molecular weight within the acceptable range 202-337. The analysed compounds possess an adequate number of proton acceptors and proton donors group to ensure efficient interaction with the hydrogen-bonding groups of the receptors. According to their predictive TPSA (topological polar surface area) data, the compounds could have a good capacity for penetrating cell membranes (Table V) [10].

Table V

Structural properties of the investigated compounds

Compound	miLogP	nrotb	nON	nOHNH	VM	TPSA
IIa	2.23	0	4	1	162.2	54.4
IIb	2.66	0	4	1	178.7	54.4
IIc	2.88	0	4	1	175.7	54.4
IId	2.17	1	7	1	185.5	100.2
IIe	2.63	0	4	1	178.7	54.4
IIIa	4.03	3	4	1	250.8	54.4
IIIb	4.45	3	4	1	267.4	54.4
IIIc	4.68	3	4	1	264.4	54.4
IIId	3.96	4	7	1	274.2	100.2
IIIe	4.43	3	4	1	267.4	54.4

nrotb- number of rotatable bonds; nON- number of hydrogen acceptors; nOHNH- number of hydrogen bond donors; TPSA- topological polar surface area; VM-molecular volume

Conclusions

New 1,2,4- triazine derivatives were synthesized starting from isatin-thiosemicarbazones; all compounds were characterized by IR and elemental analysis. The synthesized compounds were evaluated for antimicrobial inhibition activity which demonstrated a good activity. Also, the antioxidant ability of these compounds was evaluated. Some of the compounds were found to be significant scavengers of free radicals. The compounds IIc and IIId are potential candidates with respect to antioxidative activity whereas compounds IIb and IIIb show a good antimicrobial activity.

References

1. Florence TM, The role of free radicals in disease, *Aust NZJ Ophthalmol*. 1995, 23(1):3-7
2. Gupta M, Mukhtar HM, Ahmad S, In vitro evaluation of antioxidant activity and total phenolic content of Tylophora Indica (Burm F.) Merill, *IJPSR*, 2011, 2(1): 121-126
3. Irannejad H, Amini M, Khodagholi F, Ansari N, Tusi SK, Sharifzadeh M, Shafiee A, Synthesis and *in vitro* evaluation of novel 1,2,4-triazine derivatives as neuroprotective agents, *Bioorg Med Chem.,* 2010, 18(12): 4224-30
4. Ivachtchenko AV, Il'yin AP, Kobak VV, Zolotarev DA, Boksha LV, Trifilenkov AS, Ugoleva DM, New scaffolds for combinatorial synthesis. 1.5-sulfamoylisatins and their reactions with 1,2-diamines, *J. Comb. Chem,* 2002: 419-428
5. Lipinski CA, Lombardo F, Dominy B W, Feeney P J, Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. *Adv. Drug Delivery Rev.* 1997, 23: 4-25
6. Onita N, Sisu I, Penescu M, Purcarea VL, Kurunczi L, Synthesis, characterization and biological activity of some a-aminophosphonates, *Farmacia*, 2010, 58(5): 531-545
7. Oyaizu M, Studies on product of browning reaction prepared from glucose amine. *Japanese Journal of Nutrition,*1986, 44: 307-315

8. Nitulescu GM, Draghici C, Chifiriuc MC, Missir AV, Synthesis of isomeric N-(1-methyl-1H-pyrazole-4-carbonyl)-N'-(XYLYL)-thiourea and their antimicrobial evaluation, *Farmacia*, 2009, 57(5): 527-533
9. Tătărîngă G, Zbancioc AM, Rotariu A, Tuchiluş C, Synthesis, characterization and antimicrobial activity of some new semi- and thiosemicarbazones of isatin, *Eur. J. Drug Metab*., 2009, 34(1). Proceedings at the 14 Panthellenic Pharmaceutical Congress, Athens, Greece
10. Veber D. et al, Molecular properties that influence the oral bioavailability of drug candidates. *J. Med. Chem*., 2002, 45: 2615– 2623.
11. Wu WM, Lu L, Long Y, Wang T, Liu L, Chen Q, Wang R, Free radical scavenging and antioxidative activities of caffeic acid phenethyl ester (CAPE) and its related compounds in solution and membranes: a structure-activity insight, *Food Chem*, 2007, 105: 107-115

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