Journal of Advanced Zoology
ISSN: 0253-7214
Volume 45 Issue S-2 Year 2024 Page 168-175

"Design, Synthesis And Microbicidal Activity Of Some New N^2, N^4-Bis{2-(Substitutedphenyl)-3-Phenyl-1,3-Thiazolidin-4-One}-N^6-(4-Nitrophenyl)-1,3,5-Triazine-2,4,6-Triamine Derivatives"

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Abstract:

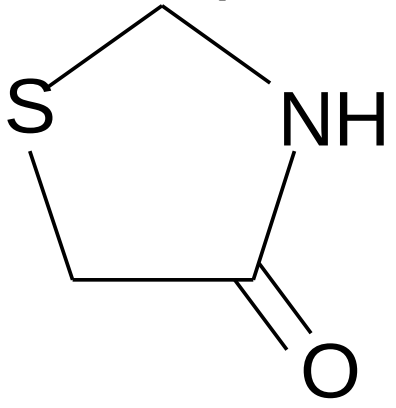
Saturated thiazole thiazolidinone, which has a carbonyl group on its fourth carbon, is regarded as a magic moiety that possesses nearly every kind of biological activity. It is a member of a significant class of heterocyclic compounds that have nitrogen and sulfur arranged in a five-member ring. Although there are several heterocyclic five-membered rings accessible, thiazolidinone has a wide range of biological functions. The current review covers all of the pharmacological activity associated with thiazolidinone, as well as future prospects. Using these reviews, we created a novel series of N^2,N^4-bis{2-(substitutedphenyl)-3-phenyl-1,3-thiazolidin-4-one}-N^6-(4-nitrophenyl)-1,3,5-triazine-2,4,6-triamine derivatives generated by the reaction between N^2,N^4-bis(4-{[(substitutedphenyl)methylidene]amino} phenyl)-N^6-(4-nitrophenyl)-1,3,5-triazine-2,4,6-triamine with thioglycolic acid and anhydrous zinc chloride in absolute ethanol. IR and NMR spectrum data, as well as element analysis, were used to describe the title compounds. Using the Cup-Borer technique, the antimicrobial properties of each chemical were examined.

Key Words: 4-Thiazolidinone, IR, NMR, MTCC, Muller-Hinton agar (MHA)

INTRODUCTION

Heterocyclic compounds are significant in several forms of therapy, thiazolidin-4-one has been suggested as a desirable scaffold for the establishment of novel molecules in medicinal chemistry. It is possible to modify the thiazolidin-4-one ring at positions 2, 3, and 5. These changes make it easier to find novel compounds that have the needed properties. According to published research, thiazolidin-4-one is a significant scaffold with potential applications in treatments. When substituted with alternative substituents, it exhibits a broad spectrum of pharmacological activity, including antidiabetic [1], antioxidant [2], antitubercular [3], antimicrobial [4,5,6,7], anticonvulsant [8] anticancer [9,10,11], antiprotozoal [12,13] and anti-inflammatory activities [14,15]. Furthermore, thiazolidine-2,4-diones are a popular class of antidiabetic medications that have affinity for PPARγ, including pioglitazone and rosiglitazone [16,17]. Because they have been shown to be helpful intermediates for the synthesis of several heterocyclic compounds, thiazolidinone derivatives are of great

interest [18]. Over the last ten years, advancements in combinatorial chemistry have made chemical libraries based on privy structures accessible [19]. The moiety's 2, 3, and 5 positions can all be substituted, however the group bonded to the carbon atom in the 2-position exerts the most structural and functional variation. The moiety's carbonyl group is incredibly unreactive. The tetrahydro derivative of thiazole is called thiazolidine, while its oxo counterpart is called thizolidinone.



4-Thiazolidinone

EXPERIMENTAL

The products were all synthesized and their spectrum analyses were performed to describe them, all of the reagents were of analytical reagent quality and were used without additional purification. M.P. in an open capillary tube were measured. Tetramethyl silane was utilized as an internal standard for the NMR spectroscopy Brooker apparatus, which operated at 400 MHz, and the Bruker Model Alpha, Laser Class1 manufactured in Germany were utilized to capture the IR spectra. utilized DMSO as a solvent. TLC was used to examine the compounds' purity on silica-G plates. Using the Cup-Borer technique, the anti-microbicidal properties of each chemical were evaluated.

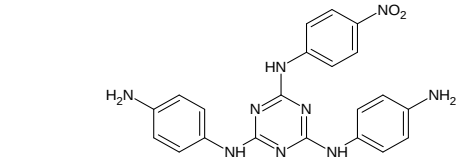
Preparation of N^2,N^4-bis(4-{[(substitutedphenyl)methylidene]amino}phenyl)-N^6-(4-nitro phenyl) -1,3,5-triazine-2,4,6-triamine (1a-1j)

To N^2-(4-methylphenyl)-N^4,N^6-bis(4-aminophenyl)-1,3,5-triazine-2,4,6-triamine (0.01M) in absolute ethanol (60 ml), 2-chlorobenzaldehyde (0.02M) and After adding a few drops of glacial acetic acid, the mixture refluxed for ten hours. After cooling, it was concentrated, added to crushed ice, and filtered. The resultant product was refined using recrystallization from methanol. **IR, cm^{-1}(3f):** 3460 (-OH), 3320 (>NH-), 3030 (=C-H), 2970 (-C-H), 1680 (>C=N- Stretch), 1530 (>C=C< Aromatic), 1450 ($-CH_3$), 1220 (C-N), 1110 (C-O-C). **(3h):** 3410 (>NH-), 3025 (=C-H), 2970 (-C-H), 1660 (>C=N- Stretch), 1475 (>C=C< Aromatic), 1420 ($-CH_3$), 1180 (C-N), 1120 (C-O-C). **(^{1}H-NMR-3f: $C_{38}H_{34}N_8O_4$ -DMSO, δ, ppm):** 3.365 (6H, s, $-OCH_3$), 2.504 (3H, s, $-CH_3$), 8.171 (1H, s, =CH-), 9.773 (1H, s, -OH), 6.610 (1H, s, -NH<), 6.954-7.430 (18H, m, Ar-H). **(3g: $C_{36}H_{30}N_8O_2$ -DMSO, δ, ppm):** 2.504 (3H, s, $-CH_3$), 8.112 (2H, s, =CH-), 9.788 (2H, s, -OH), 6.605 (3H, s, -NH<), 6.838-7.770 (20H, m, Ar-H).

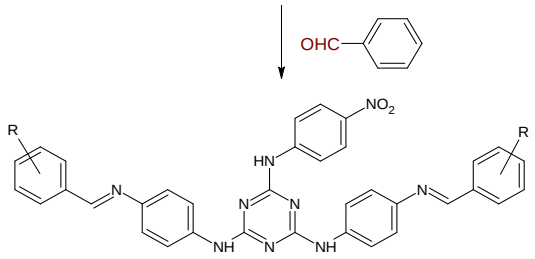
Preparation of N^2,N^4-bis{2-(substitutedphenyl)-3-phenyl-1,3-thiazolidin-4-one}-N^6-(4-nitro phenyl)-1,3,5-triazine-2,4,6-triamine (2a-2j)

solution of compound N^2,N^4-bis(4-{[(substitutedphenyl)methylidene]amino} phenyl)-N^6-(4-nitrophenyl)-1,3,5-triazine-2,4,6-triamine (0.01M), thioglycolic acid (0.01M) and anhydrous zinc chloride(2g) in absolute ethanol (60 ml)was refluxed for 8 hours, concentrated, chilled, and then poured over crushed ice before being filtered. The resultant product was further processed by recrystallization using acetone. **IR, cm^{-1}(2a):** 3370 (>NH-), 3030 (=C-H), 2950 (-C-H stretching), 1740 (>C=O stretching), 1680 (>C=N- Stretching), 1570 (N=O), 1510 (>C=C< Aromatic), 1460 ($-CH_2$- bend), 1200 (C-N), 800 (-C-S-C), 670 (C-Cl). **(2j):** 3420 (>NH-), 3025 (=C-H), 2970 (-C-H stretching), 1725 (>C=O stretching), 1680 (>C=N- Stretching), 1560 (N=O), 1500 (>C=C< Aromatic), 1460 ($-CH_2$- bend), 1150 (C-N), 820 (-C-S-C). **(^{1}H-NMR-2b: $C_{39}H_{29}Cl_2N_9O_4S_2$ -DMSO, δ, ppm):** 6.609 (3H, s, Ar-NH-), 3.365 (4H, s, $>CH_2$), 5.914 (2H, s, >CH-), 7.664-8.127 (20H, m, Ar-H). **(2d: $C_{39}H_{31}N_9O_4S_2$ -DMSO, δ, ppm):** 6.607 (3H, s, Ar-NH-), 3.360 (4H, s, $-CH_2$-), 5.912 (2H, s, >CH-), 7.379-8.136 (22H, m, Ar-H)

Reaction Scheme

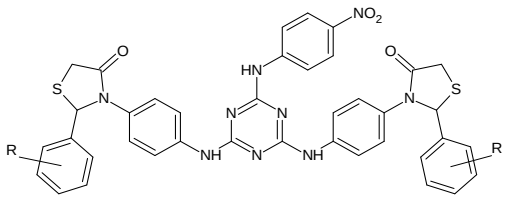


N^2-(4-nitrophenyl)-N^4,N^6-bis(4-aminophenyl)-1,3,5-triazine-2,4,6-triamine



(1a-1j)

N^2,N^4-bis(4-{[(substitutedphenyl)methylidene]amino}phenyl)-N^6-(4-nitrophenyl)-1,3,5-triazine-2,4,6-triamine



(2a-2j)

N^2,N^4-bis{2-(substitutedphenyl)-3-phenyl-1,3-thiazolidin-4-one}-N^6-(4-nitrophenyl)-1,3,5-triazine-2,4,6-triamine

MICROBICIDAL ACTIVITY

AMR-related illnesses claimed the lives of an estimated 4.95 million individuals in 2019, per a recent study[20,21]. The most popular approaches for assessing antimicrobial activity are conventional technologies[22]. To address some of the shortcomings of traditional approaches, including their low repeatability and long processing times, new methodologies for evaluation have been created[23,24]. However, these systems are not frequently analysed because to their high prices and restricted accessibility, particularly in areas with poor resources[25]. This study discusses the most frequent in vitro assays used to determine the antibiotic, antifungal, antiparasitic, and antiviral properties of promising natural substances. Furthermore, each part discusses the benefits and drawbacks of the methodology under consideration, as well as emerging methods and future trends in the field.

Details of bacteria used for antimicrobial activity: [26-33]

1.Escherichia coli NCIM 2066:

Warm-blooded organisms typically have the rod-shaped, gram-negative bacteria Escherichia coli in their lower intestines. Although the majority of E. coli strains are safe, some serotypes can occasionally result in product recalls because of food contamination and cause serious food poisoning in humans. E. Coli is an opportunistic pathogen that can potentially infect hosts with weakened defences.

2.Staphylococcus aureus MTCC 737:

This Gram-positive coccal bacteria is facultatively anaerobic. It is often found on the skin and nasal passages as a typical component of the skin flora. From minor skin infections like impetigo, boils (furuncles), cellulitis folliculitis, carbuncles, scalded skin syndrome, and abscesses to potentially fatal conditions like pneumonia, meningitis, osteomyelitis, endocarditis, toxic shock syndrome (TSS), bacteraemia, and sepsis, S. aureus can cause a wide range of illnesses. Skin, soft tissue, respiratory, bone, joint, endovascular, and wound infections are among the areas where it occurs frequently.

3.Bacillus subtilis MTCC 441:

B. subtilis is a Gram-positive bacillus. It is only known to cause illness in patients with extreme immunocompromised states, but in healthy people, it can be used as a probiotic. Food poisoning seldom results from it.

4.Pseudomonas aeruginosa MTCC 1688:
Pseudomonas aeruginosa is Gram negative bacteria. a nosocomial, opportunistic pathogen that targets immunocompromised people. P. aeruginosa commonly causes blood infections in addition to infections of the burns, wounds, urinary tract, and pulmonary system.

5.Salmonella Para typhi A MTCC 735:
It is Gram negative bacteria. Enteric fevers, often known as paratyphoid fevers, are a class of enteric disorders brought on by serotypic strains of the Salmonella bacteria (S. Para typhi).

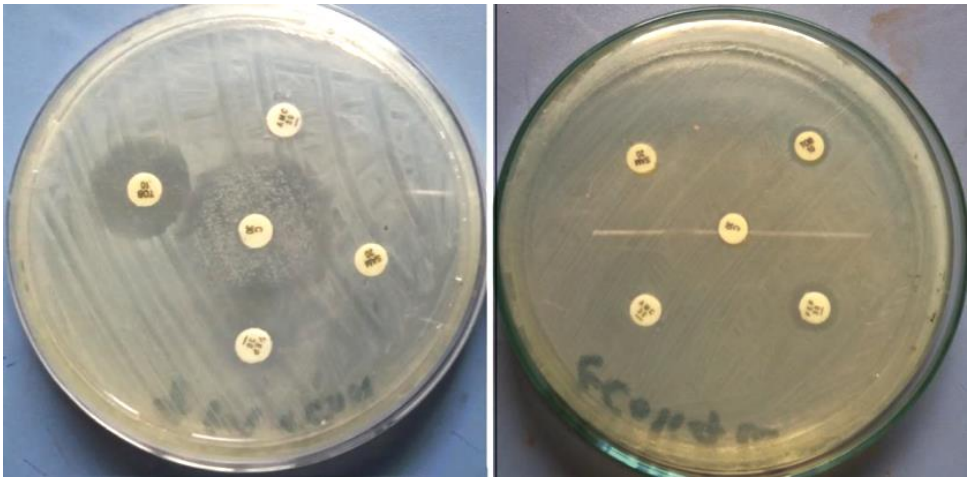
6.Bacillus pumilus MTCC 1607:
It is a spore-forming, aerobic, gram-positive bacillus that is frequently found in soil.

7.Klebsiella pneumoniae MTCC 432:
The typical flora of the mouth, skin, and intestines contain the rod-shaped, facultatively anaerobic, non-motile, encapsulated, lactose-fermenting bacteria Klebsiella pneumoniae. Pneumonia is the most typical infection caused by Klebsiella bacteria that occurs outside of hospitals, more frequently as bronchitis and bronchopneumonia. Pleural adhesions, empyema, lung abscesses, and cavitation are more common in these patients. Infections in the lower biliary tract, urinary tract, and surgical incision sites can also be brought on by Klebsiella.

8.Candida albicans MTCC 227:
The fungus Candida albicans is diploid and can grow as filamentous cells or yeast. It is the cause of opportunistic infections of the genitalia and mouth in humans.
Sample preparation: Sterile distilled water is used to make an antibiotic solution.
Name of antibiotic: Penicillin: 12 units, Chloramphenicol: 30 µg/ml, Streptomycin: 30 µg/ml, Tetracycline: 30 µg/ml, Fluconazole: 30 µg/ml.

Culture activation:
For bacteria and yeast, the culture was activated in nutritional broth and potato dextrose broth, respectively. Each organism was infected with one colony, which was then incubated for 24 hours at 37^0C for bacteria and 28^0C for yeast. For bacteria and yeast, 200 µl of active culture was injected in 25 ml of molten nutrient agar and yeast peptone agar, respectively. The culture was properly mixed and then transferred onto a sterile 100 mm Petri dish. 6mm well was prepared with help of sterile stainless steel cup borer after solidification of agar.

Sample loading: 100 µl in each well of 6mm diameter with sterile micropipette tip.



Physical constant of 2a-2j

Table: 1

Sr.No	Sub. No.	R	M.F.	Mol.Wt (g/m)	Yield %	M.P. °C	% Carbon		% Hydrogen		% Nitrogen	
							Found	Calcd	Found	Calcd	Found	Calcd
1	2a	-2-Cl	$C_{39}H_{29}Cl_2N_9O_4S_2$	822.74	77	152	56.92	56.93	3.53	3.55	15.30	15.32
2	2b	-4-Cl	$C_{39}H_{29}Cl_2N_9O_4S_2$	822.74	72	147	56.91	56.93	3.54	3.55	15.30	15.32
3	2c	-3,4-OCH_3	$C_{43}H_{39}N_9O_8S_2$	873.95	76	158	59.08	59.09	4.49	4.50	14.40	14.42
4	2d	-H	$C_{39}H_{31}N_9O_4S_2$	753.85	79	117	62.12	62.14	4.12	4.14	16.71	16.72
5	2e	-2-OH	$C_{39}H_{31}N_9O_6S_2$	785.85	80	131	59.59	59.61	3.97	3.98	16.02	16.04
6	2f	-4-OH-3-OCH_3	$C_{41}H_{35}N_9O_8S_2$	845.90	71	155	58.19	58.21	4.15	4.17	14.89	14.90
7	2g	-4-OH	$C_{39}H_{31}N_9O_6S_2$	785.85	81	127	59.60	59.61	3.96	3.98	16.02	16.04
8	2h	-2,4,6-OCH_3	$C_{45}H_{43}N_9O_{10}S_2$	934.00	73	176	57.85	57.87	4.63	4.64	13.49	13.50
9	2i	-4-OCH_3	$C_{41}H_{35}N_9O_6S_2$	813.90	75	132	60.49	60.50	4.32	4.33	15.47	15.49
10	2j	-3-NO_2	$C_{39}H_{29}N_{11}O_8S_2$	843.84	69	163	55.49	55.51	3.44	3.46	18.24	18.26

Antimicrobial activities of 2a-2j

Table: 2

Sr. No.	Comp. No.	R	Microorganisms							Yeast
			E.coli NCIM 2066	S.aureus MTCC 737	B.subtilis MTCC 441	P.aeruginosa MTCC 1688	S.paratyphi A MTCC 735	B.pumillus MTCC 1607	K.pneumoniae MTCC 432	C.albicans MTCC 227
1	2a	-2-Cl	11	21	22	10	22	23	22	19
2	2b	-4-Cl	21	15	18	9	18	19	23	18
3	2c	-3,4-OCH_3	20	25	20	10	21	21	NA	22
4	2d	-H	15	14	24	12	19	NA	22	25
5	2e	-2-OH	19	18	NA	13	22	25	19	21
6	2f	-4-OH-3-OCH_3	14	22	26	15	16	22	14	20
7	2g	-4-OH	19	16	23	14	20	20	16	23
8	2h	-2,4,6-OCH_3	22	20	19	NA	14	12	21	18
9	2i	-4-OCH_3	NA	26	24	12	20	21	15	22
10	2j	-3-NO_2	20	21	18	10	13	26	17	23

Note: The digits in above cell indicates diameter for the zone of inhibition in millimeter (mm)

1. The Standard Drugs minimum inhibition concentration

Table: 3

Sr. No.	Antibiotic Organism	SD1-Penicillin	SD2-Chloramphenlcol	SD3-Streptomycin	SD4-Tetracyclin	SD5-Fluconazole
1	Escherichia coli NCIM 2066		12	18	17	
2	Staphylococcus aureus MTCC 737	22	16	16	22	
3	Bacillus subtilis MTCC 441	23		21	22	
4	Pseudomonas aeruginosa MTCC 1688			12		

5	Salmonella paratyphi A MTCC 735	19	18	12	18	
6	Bacillus pumilus MTCC 1607	15		16	22	
7	Klebsiella pneumoniae MTCC 432		19	13	17	
8	Candida albicans MTCC 227					21

Note: The digits in above cell is indicates diameter for the zone of inhibition in milimeter (mm)

CONCLUSION

The main focus of this research work was to synthesize, characterize and evaluate antimicrobial activities of the newly synthesized N^2,N^4-bis{2-(substitutedphenyl)-3-phenyl-1,3-thiazolidin-4-one}-N^6-(4-nitrophenyl)-1,3,5-triazine-2,4,6-triamine derivatives, structures of synthesized compounds were confirmed and characterized with the help of analytical data's such as IR and ^{1}H-NMR. In summary, we have described the synthesis and antimicrobial activity of N^2,N^4-bis{2-(substitutedphenyl)-3-phenyl-1,3-thiazolidin-4-one}-N^6-(4-nitrophenyl)-1,3,5-triazine-2,4,6-triamine has shown good activity against the bacterial strains.

ACKNOWLEDGEMENT

The authors are thankful to the Principal Dr. J. R. Shukla of Sheth L. H. Science College, Mansa and Management of Sarvodaya Higher Education Society, Mansa for providing research Facilities.

REFERENCES

1. Kumar A., Chawla A., Jain S., Kumar P., Kumar S. (2011) 3-Aryl-2-{4-[4-(2,4-dioxothiazolidin-5-ylmethyl)phenoxy]-phenyl}-acrylic acid alkyl ester: synthesis and antihyperglycemic evaluation. Med. Chem. Res. **20**, 678–686.https://doi.org/10.1007/s00044-010-9369-3
2. Djukic M., Fesatidou M., Xenikakis I., Geronikaki A., Angelova V.T., Savic V., Pasic M., Krilovic B., Djukic D., Gobeljic B., et al. (2018) In vitro antioxidant activity of thiazolidinone derivatives of 1,3-thiazole and 1,3,4-thiadiazole. Chem. Biol. Interact. **286**, 119–131.https://doi.org/10.1016/j.cbi.2018.03.013
3. Trotsko N. (2021) Antitubercular properties of thiazolidin-4-ones – A review. Eur. J. Med. Chem. **215**, 113266.https://doi.org/10.1016/j.ejmech.2021.113266
4. Trotsko N., Kosikowska U., Paneth A., Wujec M., Malm A. (2018) Synthesis and antibacterial activity of new (2,4-dioxothiazolidin-5-yl/ylidene)aceticacid derivatives with thiazolidine-2,4-dione, rhodanine and 2-thiohydantoin moieties. Saudi Pharm. J. **26**(4), 568–577.https://doi.org/10.1016/j.jsps.2018.01.016
5. Marc G., Ionuț I., Pîrnău A., Vlase L., Vodnar D.C., Duma M., Tiperciuc B., Oniga O. (2017) Microwave Assisted Synthesis of 3,5-Disubstitutedthiazolidine-2,4-Diones with Antifungal Activity. Design, Synthesis, Virtual And In Vitro Antifungal Screening. Farmacia **65**(3), 414–422.https://farmaciajournal.com/wp-content/uploads/2017-03-art-15-Marc_Ionut_Oniga_414-422.pdf
6. Aneja D.K., Lohan P., Arora S., Sharma C., Aneja K.R., Prakash O. (2011) Synthesis of new pyrazolyl-2, 4-thiazolidinediones as antibacterial and antifungal agents. Org. Med. Chem. Lett. **1**, 15.https://doi.org/10.1186/2191-2858-1-15
7. Trotsko N., Kosikowska U., Paneth A., Plech T., Malm A., Wujec M. (2018) Synthesis of new pyrazolyl-2, 4-thiazolidinediones as antibacterial and antifungal agents. Molecules **23**(5), 1023.https://doi.org/10.3390/molecules23051023
8. Sachin Saini A. (2019) Synthesis and Anticonvulsant Studies of Thiazolidinone and Azetidinone Derivatives from Indole Moiety. Drug Res. **69**(8), 445–450.https://www.thieme-connect.com/products/ejournals/pdf/10.1055/a-0809-5098.pdf[**Google Scholar**]

9. Asati V., Mahapatra D.K., Bharti S.K. (2014) Thiazolidine-2,4-diones as multi-targeted scaffold in medicinal chemistry: Potential anticancer agents. Eur. J. Med. Chem. **87**, 814–833.https://doi.org/10.1016/j.ejmech.2014.10.025
10. Asati V., Bharti S.K. (2018) Design, synthesis and molecular modeling studies of novel thiazolidine-2,4-dione derivatives as potential anti-cancer agents. J. Mol. Struct. **1154**, 406–417.https://doi.org/10.1016/j.molstruc.2017.10.077
11. Trotsko N., Przekora A., Zalewska J., Ginalska G., Paneth A., Wujec M. (2018) Synthesis and in vitro antiproliferative and antibacterial activity of new thiazolidine-2,4-dione derivatives. J. Enzym. Inhib. Med. Chem. **33**(1), 17–24.https://doi.org/10.1080/14756366.2017.1387543
12. Trotsko N., Bekier A., Paneth A., Wujec M., Dzitko K. (2019) Synthesis and In Vitro Anti-Toxoplasma gondii Activity of Novel Thiazolidin-4-one Derivatives. Molecules **24**(17), 3029.https://doi.org/10.3390/molecules24173029
13. Kryshchyshyn A., Kaminskyy D., Karpenko O., Gzella A., Grellier P., Lesyk R. (2019) Thiazolidinone/thiazole based hybrids – New class of antitrypanosomal agents. Eur. J. Med. Chem. **174**, 292–308.https://doi.org/10.1016/j.ejmech.2019.04.052
14. Barros C.D., Amato A.A., De Oliveira T.B., Iannini K.B.R., Da Silva A.L., Da Silva T.G., Leite E.S., Hernandes M.Z., De Lima M.C.A., Galdino S.L., et al. (2010) Synthesis and anti-inflammatory activity of new arylidene-thiazolidine-2,4-diones as PPARγ ligands. Bioorg. Med. Chem. **18**(11), 3805–3811.https://doi.org/10.1016/j.bmc.2010.04.045
15. Ghafoori H., Rezaei M., Mohammadi A. (2017) Anti-inflammatory Effects of Novel Thiazolidinone Derivatives as Bioactive Heterocycles on RAW264.7 Cells. Iran. J. Allergy Asthma Immunol. **16**(1), 28–38. http://www.ncbi.nlm.nih.gov/pubmed/28417622
16. Nazreen S., Alam M.S., Hamid H., Yar M.S., Dhulap A., Alam P., Pasha M.A.Q., Bano S., Alam M.M., Haider S., et al. (2014) Thiazolidine-2,4-diones derivatives as PPAR-γ agonists: Synthesis, molecular docking, in vitro and in vivo antidiabetic activity with hepatotoxicity risk evaluation and effect on PPAR-γ gene expression. Bioorg. Med. Chem. Lett. **24**(14), 3034–3042.https://doi.org/10.1016/j.bmcl.2014.05.034
17. Devchand P.R., Liu T., Altman R.B., FitzGerald G.A., Schadt E.E. (2018) The Pioglitazone Trek via Human PPAR Gamma: From Discovery to a Medicine at the FDA and Beyond. Front. Pharmacol. **9**, 1093.https://doi.org/10.3389/fphar.2018.01093
18. S. P. Singh, S. S. Parmar, K. Raman, V. I. Stenberg (1981) Chem. Rev. 175
19. A. Verma, Shailendra K. Saraf (2008) Eur. J. Med. Chem. **43**, 897-905
20. Laxminarayan R. (2022) The overlooked pandemic of antimicrobial resistance. Lancet **399**, 606–07.https://doi.org/10.1016/S0140-6736(22)00087-3
21. Nadimpalli M.L., Chan C.W., Doron S. (2021) Antibiotic resistance: a call to action to prevent the next epidemic of inequality. Nat. Med. **27**, 187–188.https://doi.org/10.1038/s41591-020-01201-9
22. Balouiri M., Sadiki M., Ibnsouda S.K. (2016) Methods for in vitro evaluating antimicrobial activity: A review. J. Pharm. Anal. **6**(2), 71–79.https://doi.org/10.1016/j.jpha.2015.11.005
23. Thomford N.E., Senthebane D.A., Rowe A., Munro D., Seele P., Maroyi A., Dzobo K. (2018) Natural Products for Drug Discovery in the 21st Century: Innovations for Novel Drug Discovery. Int. J. Mol. Sci. **19**(6), 1578.https://doi.org/10.3390/ijms19061578
24. Masota N.E., Vogg G., Ohlsen K., Holzgrabe U. (2021) Reproducibility challenges in the search for antibacterial compounds from nature. PLoS ONE **16**, e0255437.https://doi.org/10.1371/journal.pone.0255437
25. Khan Z.A., Siddiqui M.F., Park S. (2019) Current and Emerging Methods of Antibiotic Susceptibility Testing. Diagnostics **9**(2), 49.https://doi.org/10.3390/diagnostics9020049
26. "Escherichia coli O157:H7". CDC Division of Bacterial and Mycotic Diseases. (2011), 04-19
27. Vogt RL, Dippold L, "Escherichia coli O157:H7 outbreak associated with consumption of ground beef, June-July 2002", Public Health Rep120 (2)174-8 PMC 1497708.PMID 15842119. (2005)Bentley R, Meganathan R, Microbiol. Rev,46 (3), 241-80. PMC 281544. PMID 6127606 (1982)
28. Ryan KJ, Ray CG (editors), Sherris Medical Microbiology (4^{th}ed.). Mc Graw Hill. (2004) ISBN 0-8385-8529-9.
29. Todar's , Online Textbook of Bacteriology. Textbookofbacteriology.net (2004-06-04). Retrieved on (2011-10-09).
30. Ryan KJ, Ray CG(editors), Sherris Medical Microbiology (4^{th} ed.). McGraw Hill. (2004) ISBN 0-8385-8529-9.

31. Ryan KJ, Ray CG (editors), Sherris Medical Microbiology (4th ed.). McGraw Hill. (2004) ISBN 0-8385-8529-9.
32. dEnfert C, Hube B, Candida: Comparative and Functional Genomics. Caister Academic Press (2007) ISBN 978-1-904455-13-4.
33. Bergey's Mannual of Systematic Bactriology, second edition, volume 2, Springer. (2005)