

Bio- Chemical Studying of Chemical Compounds

Wisam Hassan Ali¹ , Manar Ghyath Al-Mosawy² , Muna Abbas Hadi³

A.Ch. Chemists, Kufa University , Iraq¹.

Imam Jafar Al-Sadiq University , Najaf, Iraq².

College of Education , Chemistry Department , Kufa University , Iraq³.

Abstract

Four chemical compounds were synthesized , identified , azo compounds have a wide applications in bio-activity field which act starting material for many bio-molecules and anti-active compounds that tested against types of bacteria .

Keywords: types , materials .

Introduction

Industrial dyes are found in large quantities are extensively used in textile dyeing, paper printing, color photography, pharmaceutical, food, cosmetics with other industries, among them the textile industry is a major consumer. Industrial effluents involving azo dyes⁽¹⁻⁵⁾ are potential health hazards as they may be converted to toxic and/or carcinogenic products under anaerobic conditions. The most importantly these undergo reductive cleavage, leading to the formation of aromatic amines of which have known mutagenic and/or carcinogenic properties. Therefore the use of certain azodyes⁽⁶⁻¹³⁾ is prohibited all over the world. General route for the synthesis of azo dyes involves diazotization of primary aromatic amine followed by coupling with one or more nucleophiles, amino or any other groups are used in coupling components⁽¹⁴⁻²²⁾ .

Azo derivatives are known to be involved in a many of biological in many reactions such as inhibition of RNA, DNA, protein production, carcinogenesis and nitrogen (N) fixation[8]. Evans blue and Congo Red are being considered as HIV inhibitors. This effect is supposed to be resulted by binding of azo dyes to both reverse transcriptase and protease of this virus⁽²³⁻³⁰⁾ The existence of an azo moiety in different types of compounds has caused them to explain pesticidal actions and antibacterial⁽³¹⁻⁴²⁾ It has been found that the activity of azo compound increases the incorporation of proper heterocyclic moiety.

Drug resistance refers to a situation in which the drugs that usually destroy the bacteria no longer do so. In different organisms, multi-drug resistance mechanisms are established differently. Bacterial resistance⁽⁴³⁻⁵¹⁾ to antibiotics can be divided as natural or acquired resistance. In the former, the bacteria are "Intrinsically" resistant, while the latter refers to bacteria that are susceptible to develop resistance to antibiotics despite the fact that they are usually sensitive. Mutations in chromosomal genes, acquisition of mobile genetic elements like plasmids and transposons, which are carriers of antibiotic resistance genes are frequent causes of acquired resistance. The development of microbial resistance to chemotherapeutic agents usually results from their wide-spread exploitation in the treatment of infectious diseases. The emergence of resistance to the major classes of antibacterial drugs is recognized as a serious health concern. The study⁽⁵²⁻⁶⁰⁾ for new antibacterial agents with different action modes has always remained paramount. Azo compounds, with wide range of biological properties are very promising to this effect. The specific pathway of metabolism generally attributes this activity to azo colorants. *In vivo*, there is an enzyme-catalyzed reduction of the azo bond. The azoreductase activity was found in liver, in digestive tract bacteria of mammals, as well as in the skin of bacteria like *Staphylococcus aureus*. This reaction results in the azo-bond cleavage and the release of the corresponding aromatic amines originating from the azo dye. The products can be more or less toxic than the parent molecules and thus this process can decrease or increase any toxic or carcinogenic effects of the dyes⁽⁶¹⁻⁶⁵⁾.

Experimental & Materials :

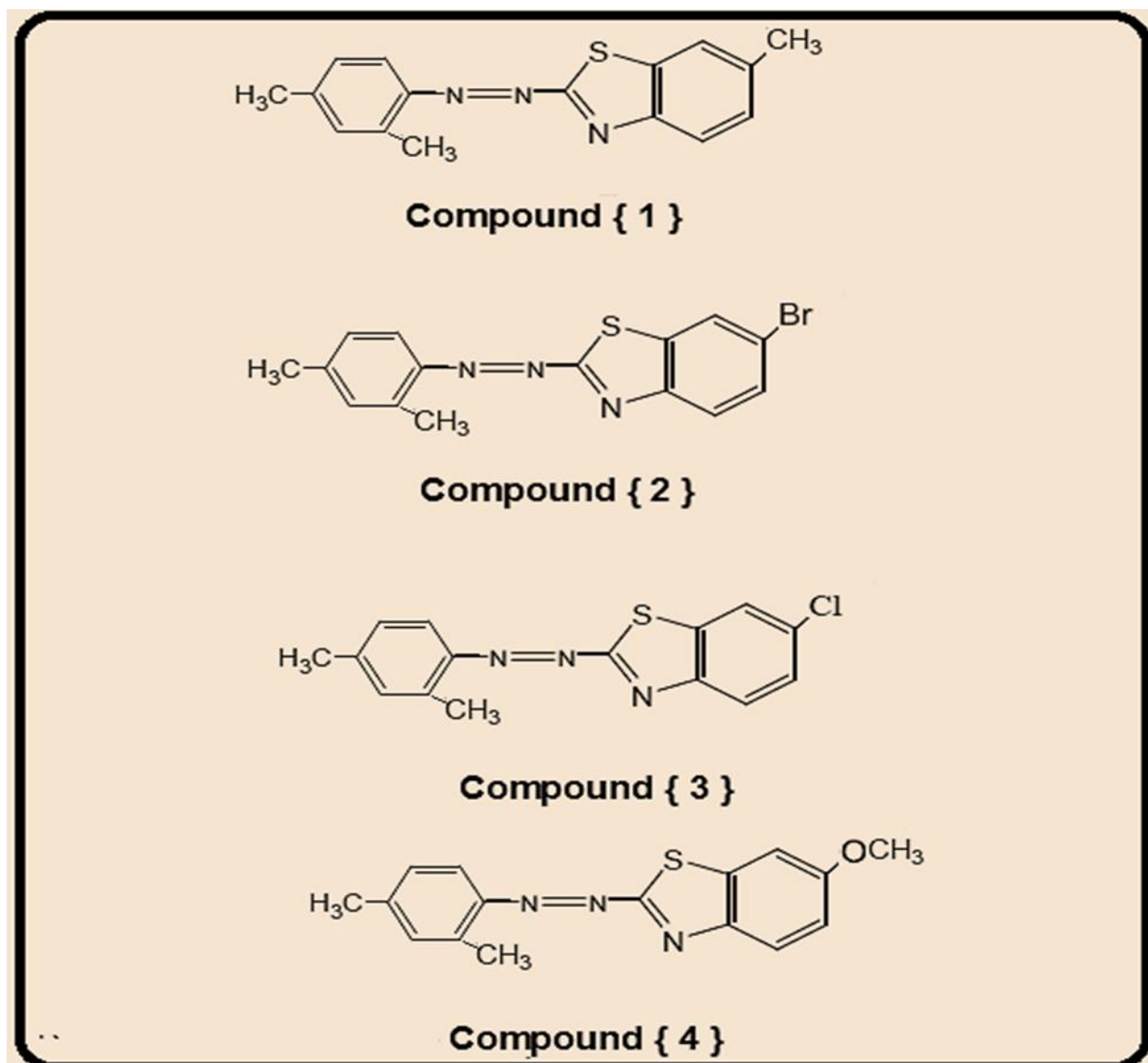
Biological studying carried out in Bio – lab in biological department.,Bio- Chemical Studying carried out in Bio-Lab.

EXPERIMENTAL PROCEDURES

The in vitro biological testing effects of the identified compounds were tested against selected types of bacteria which include (*Klebsiella* spp., *E. coli* and *B. subtilis*) through using the Well Diffusion Method⁽³¹⁾ using agar nutrient as the medium. Stock solutions (10^{-3} M) were prepared by dissolving the compounds in DMSO solution. In a typical procedure, a well was made on the agar medium inoculated with microorganisms. The well was filled with the test solution using a micropipette and the plat was incubated at ((35°C)) for((72)) hrs. During this period, the test solution diffused and the growth of the inoculated microorganisms was affected

Synthesized Compounds In Schemes:

In our schemes , we prepared iminecompounds , but now we will study the biological activity for them in this work :



Scheme (1): Synthesized Compounds

RESULTS AND DISCUSSION

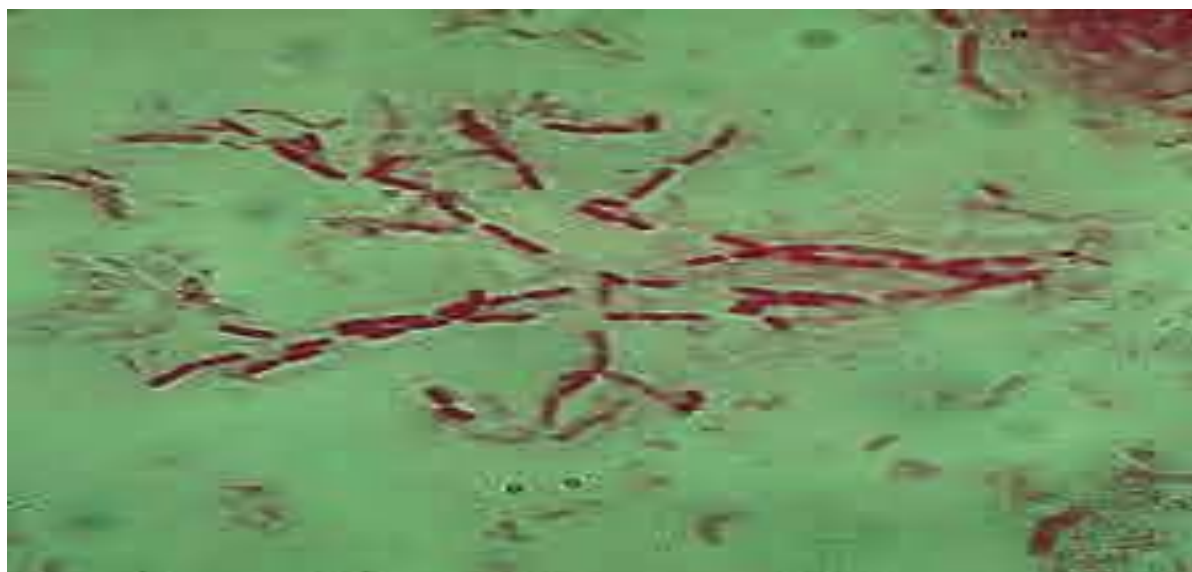
The synthesized compounds screened for Biological Activity against three types of bacteria .

Biological Tests⁽⁶¹⁻⁷⁰⁾ :

The azo dyes containing heterocyclic rings involved in the biological reactions have continued to attract more attention as potential drugs for therapeutic intervention in various diseases. In the present study, the results of the newly synthesized azo compounds tested for their antibacterial activity against pathogenic strains (*Klebsiella* spp., *E. coli* and *B. subtilis*). The test of the sensitivity of the bacteria, which included work on three types of bacteria to measure the biological activity of certain compounds which bacteria positive for the dye Cram (bacteria) and

negative gram , Table (1) shows the diameter of inhibition zone for vehicles chemical measured in mm towards the bacteria.

B. subtilis is a Gram-positive, aerobic bacterium. It is rod-shaped and catalase-positive. *B. subtilis* is found in soil and the gastrointestinal tract of ruminants and humans.



B. subtilis

Klebsiellaspp is a genus of nonmotile, Gram-negative, oxidase-negative, rod-shaped bacteria with a prominent polysaccharide-based capsule. *Klebsiella* species are found everywhere in nature



Klebsiellaspp

*E. coli*s bacteria found in the environment, foods, and intestines of people and animals. *E. coli* are a large and diverse group of bacteria. Although most strains of *E. coli* are harmless, others can make you sick. Some kinds of *E. coli* can cause diarrhea.



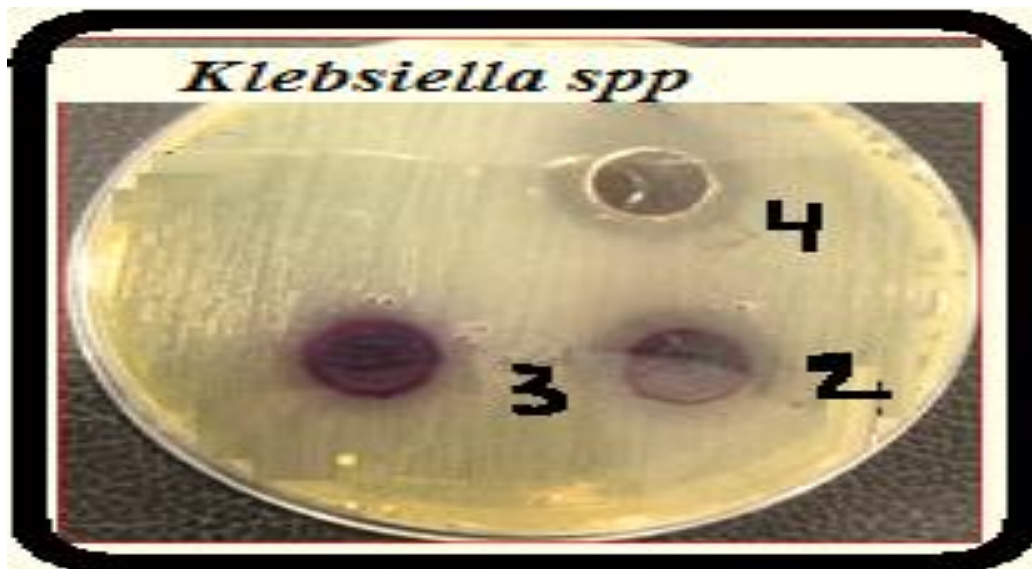
E- Coli

Table.1:Biological Activity (Inhibition Zone in (mm)) of Compounds in Concentration (1 X 10⁻³ M).

Comp. No.	<i>B. subtilis</i>	<i>Klebsiellaspp</i>	<i>E - Coli</i>
[1]	4	6	< 4
[2]	10	8	6
[3]	10	10	6

[4]	6	6	4
-----	---	---	---

The results showed the Biological Activity for compounds (3, 2) the effectiveness of anti-resistant bacteria is much higher than other compounds in the inhibition of bacteria, ((Cl and Br)), which gave vital to the effectiveness of many of the bacteria, and the following photos show the following:



Picture(1).The amount of inhibition of the compounds on *Klebsiella spp*



Picture(2).The amount of inhibition of the compounds on *E. Coli*

REFERENCES

1. Sack, D.A., C. Lyke, C. McLaughlin and V. Suwanvanichkij, 2001. Antimicrobial resistance in shigellosis, cholera and campylobacteriosis. WHO/CDS/CSR/DRS/2001.8, World Health Organization, Rome, Italy, pp: 1-51.
2. Robens, J.F., G.S. Dill, J.M. Ward, J.R. Joiner, R.A. Griesemer and J.F. Douglas, 1980. Thirteen-week subchronic toxicity studies of direct blue 6, direct black 38 and direct brown 95 dyes. *Toxicol. Applied Pharmacol.*, 54: 431-442
3. Martin, C.N. and J.C. Kennelly, 1981. Rat liver microsomal azoreductase activity on four azo dyes derived from benzidine, 3,3'-dimethoxybenzidine or 3,3'-dimethoxybenzidine. *Carcinogenesis*, 2: 307-312
4. Chung, K.T., G.E. Fulk and M. Egan, 1978. Reduction of azo dyes by intestinal anaerobes. *Applied Environ. Microbiol.*, 35: 558-562
5. Bos, R.P., W. van der Krieken, L. Smeijsters, J.P. Koopman, H.R. de Jonge, J.L.G. Theuws and P.T. Henderson, 1986. Internal exposure of rats to benzidine derived from orally administered benzidine-based dyes after intestinal azo reduction. *Toxicology*, 40: 207-213
6. Platzek, T., C. Lang, G. Grohmann, U.S. Gi and W. Baltes, 1999. Formation of a carcinogenic aromatic amine from an azo dye by human skin bacteria in vitro. *Hum. Exp. Toxicol.*, 18: 552-559
7. Martin, C.N. and J.C. Kennelly, 1985. Metabolism, mutagenicity and DNA binding of biphenyl-based azodyes. *Drug Metab. Rev.*, 16: 89-117
8. Levine, W.G., 1991. Metabolism of azo dyes: Implication for detoxication and activation. *Drug Metab. Rev.*, 23: 253-309
9. Collier, S.W., J.E. Storm and R.L. Bronaugh, 1993. Reduction of azo dyes during in vitro percutaneous absorption. *Toxicol. Applied Pharmacol.*, 118: 73-79
10. Shafeeque, S., S. Mohan and K.S. Manjunatha, 1999. Synthesis, analgesic and antiinflammatory activity of some 2-substituted amino-3-(Np-tolylcarboxamido)-4, 5-dimethyl thiophenes. *Indian J. Heterocyclic Chem.*, 8: 297-300.
11. Dzhurayev, A.D., K.M. Karimkulov, A.G. Makhsumov and N. Amanov, 1992. Antibacterial activity of new thiophene derivatives. *Khimiko-FarmatsevticheskiZhurnal*, 26: 73-75.
12. Singh, D., S. Mohan, P.C. Sharma and J. Sarvanan, 2007. Synthesis and evaluation of some novel piperidinothiophenes as potential antioxidant and anti-inflammatory agents. *Acta Pharm. Sci.*, 49: 29-38.
13. Govindaswamy, P., S. Mohan and P.G. Rao, 1998. Synthesis and antifungal activity of some 2-substituted 5, 6 dimethyl thieno (2, 3-d) 3, 1-oxazin-4-ones. *Indian J. Heterocycl. Chem.*, 7: 205-208.
14. Ferreira, I.C.F.R., M.J.R.P. Queiroz, M. Vilas-Boas, L.M. Estevinho, A. Begouin and G. Kirsch, 2006. Evaluation of the antioxidant properties of diarylamines in the benzo[b]thiophene series by free radical scavenging activity and reducing power. *Bioorg. Med. Chem. Lett.*, 16: 1384-1387
15. Gadad, A.K., H. Kumar, C.J. Shishoo, I.M. Khazi and C.S. Mahajanshetti, 1994. Synthesis of some 2-aminoacetyl-amino-3-carbethoxy/anilido-4,5,6,7-tetrahydrobenzo[b] thiophenes for local anesthetic activity. *Indian J. Chem. Soc.*, 33: 298-301.
16. Gillespie, E., K.W. Dungan, A.W. Gomoll and R.J. Seidehamel, 1985. Selected immunologic properties of tiprinast, a non-steroidal antiallergy agent. *Int. J. Immunopharmacol.*, 7: 655-660
17. Elslager, E.F., P. Jacob and L.M. Werbel, 1972. Folate antagonists. 6. Synthesis and antimalarial effects of fused 2,4-diaminothieno[2,3-d]pyrimidines. *J. Heterocycl. Chem.*, 9: 775-782
18. Mishra, R., K.K. Jha, S. Kumar and I. Tomer, 2011. Synthesis, properties and biological activity of thiophene: A review. *Der PharmaChemica*, 3: 38-54
19. Mishra, R., I. Tomer and S. Kumar, 2012. Synthesis and antimicrobial evaluation of novel thiophene derivatives. *Der Pharmacia Sinica*, 3: 332-336

20. Santagati, A., M. Modica, M. Santagati, A. Caruso and V. Cutuli, 1994. Synthesis of 2,3-dihydro-3-amino-6-phenyl-2-thioxothieno [2,3-d] pyrimidin-4 (1H)-one and of potential antiinflammatory agents 2-aryl-7-phenyl-3H, 9H-pyrimido [2,1-b]thieno-[2',3':4,5]]1,3,4] thiadiazin-9-ones. *Pharmazie*, 49: 64-65
21. Egan, D., R. O'Kennedy, E. Moran, D. Cox, E. Prosser and R.D. Thornes, 1990. The pharmacology, metabolism, analysis and applications of coumarin and coumarin-related compounds. *Drug Metab. Rev.*, 22: 503-529
22. Borges, F., F. Roleira, N. Milhazes, L. Santana and E. Uriarte, 2005. Simple coumarins and analogues in medicinal chemistry: Occurrence, synthesis and biological activity. *Curr. Med. Chem.*, 12: 887-916
23. Harvey, R.G., C. Cortez, T.P. Ananthanarayan and S. Schmolka, 1988. A new coumarin synthesis and its utilization for the synthesis of polycyclic coumarin compounds with anticarcinogenic properties. *J. Organ. Chem.*, 53: 3936-3943
24. Kostova I., S. Raleva, P. Genova and R. Argirova, 2006. Structure-activity relationships of synthetic coumarins as HIV-1 inhibitors. *Bioinorg. Chem. Applied*, 10.1155/BCA/2006/68274
25. Moffett, R.S., 1964. Central nervous system depressants. VII.1 Pyridylcoumarins. *J. Med. Chem.*, 7: 446-449
26. Musicki, B., A.M. Periers, P. Laurin, D. Ferroud and Y. Benedetti et al., 2000. Improved antibacterial activities of coumarin antibiotics bearing 5',5'-dialkylribose: biological activity of RU79115. *Bioorg. Med. Chem. Lett.*, 10: 1695-1699
27. Al-Haiza, M.A., M.S. Mostafa and M.Y. El-Kady, 2003. Synthesis and biological evaluation of some new coumarin derivatives. *Molecules*, 8: 275-286
28. Fylaktakidou, K.C., D.J. Hadjipavlou-Litina, E.K. Litinas and D.N. Nicolaides, 2004. Natural and synthetic coumarin derivatives with anti-inflammatory/antioxidant activities. *Curr. Pharm. Des.*, 10: 3813-3833
29. Bucolo, C., K.W. Ward, E. Mazzon, S. Cuzzocrea and F. Drago, 2009. Protective effects of a coumarin derivative in diabetic rats. *Invest. Ophthalmol. Vis. Sci.*, 50: 3846-3852
30. Paya, M., B. Halliwell and J.R.S. Houlst, 1992. Interactions of a series of coumarins with reactive oxygen species: Scavenging of superoxide, hypochlorous acid and hydroxyl radicals. *Biochem. Pharmacol.*, 44: 205-214
31. Marshall, M.E., J.L. Mohler, K. Edmonds, B. Williams and K. Butler et al., 1994. An updated review of the clinical development of coumarin (1,2-benzopyrone) and 7-hydroxycoumarin. *J. Cancer Res. Clin. Oncol.*, 120: S39-S42
32. Stanchev, S., G. Momekov, F. Jensen and I. Manolov, 2008. Synthesis, computational study and cytotoxic activity of new 4-hydroxycoumarin derivatives. *Eur. J. Med. Chem.*, 43: 694-706
33. Fondjo, S.E., J. Tsemeugne, B.L. Sondengam, T. Oppenlaender and K.H. Wabo et al., 2011. Coupling of two diazotized 3-aminothieno[3,4-c]coumarins with aromatic amines. *J. Hetero. Chem.*, 48: 1295-1301
34. Fogue, P.S., P.K. Lunga, E.S. Fondjo, J.D.D. Tamokou and B. Thaddee et al., 2012. Substituted 2-aminothiophenes: Antifungal activities and effect on *Microsporiumgypseum* protein profile. *Mycoses*, 55: 310-317
35. Fondjo, E.S., J. Tsemeugne, J.D.D. Tamokou, A.N. Djintchui, J.R. Kuate and B.L. Sondengam, 2013. Synthesis and antimicrobial activities of some novel thiophene containing azo compounds. *Heterocyclic Commun.*, 19: 253-259
36. Gewald, K., 1965. Heterocyclen aus CH-acidennitrilen, VII. 2-Amino-thiophene aus α -Oxo-mercaptanen und methylenaktivennitrilen. *Chemische Berichte*, 98: 3571-3577
37. Ried, W. and E. Nyiondi-Bonguen, 1973. Über die gemeinsame einwirkung von schwefel und methylenaktivennitrilen oder ammoniak auf 2-hydroxyacetophenon. *Justus Liebigs Annalen der Chemie*, 1973: 134-140
38. Fondjo, E.S., D. Dopp and G. Henkel, 2006. Reactions of some anellated 2-aminothiophenes with electron poor acetylenes. *Tetrahedron*, 62: 7121-7131

39. Al-Saleh, B., M.M. Abdelkhalik, M.A. El-Asasery and M.H. Elnagdi, 2005. Studies with condensed thiophenes: Reactivity of condensed aminothiophenes toward carbon and nitrogen electrophiles. *J. Chem. Res.*, 2005: 23-26
40. NCCLS., 1997. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically-fourth edition; approved standard. NCCLS Document M7-A4, National Committee for Clinical Laboratory Standards, Wayne, PA., USA., January 1997.
41. NCCLS., 1999. Methods for determining bactericidal activity of antimicrobial agents; approved guideline. CLSI Document M26-A, Vol. 19, No. 18, Clinical and Laboratory Standards Institute, Wayne, PA., USA., September 1999.
42. Avila, J., J.G. de Liverant, A. Martinez, G. Martinze, J.L. Munoz, A. Arciniegas and A.R. de Vivar, 1999. Mode of action of Buddlejacobdata verbascoside against *Staphylococcus aureus*. *J. Ethnopharmacol.*, 66: 75-78
43. Mosmann, T., 1983. Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. *J. Immunol. Methods*, 65: 55-63
44. Situ, H. and L.A. Bobek, 2000. In vitro assessment of antifungal therapeutic potential of salivary histatin-5, two variants of histatin-5 and salivary mucin (MUC7) domain 1. *Antimicrob. Agents Chemother.*, 44: 1485-1493
45. Tamokou, J.D., D.J.S. Mpetga, P.K. Lungu, M. Tene, P. Tane and J.R. Kuate, 2012. Antioxidant and antimicrobial activities of ethyl acetate extract, fractions and compounds from stem bark of *Albizia adianthifolia* (Mimosoideae). *BMC Complement. Altern. Med.*, Vol. 12. 10.1186/1472-6882-12-99
46. Tanamatayarat, P., P.N. Limtrakul, S. Chunsakaow and C. Duangrat, 2003. Screening of some rubiaceae plants for cytotoxic activity against Cervix carcinoma (KB-3-1) cell line. *Thai J. Pharm. Sci.*, 27: 167-172
47. Caamal-Fuentes, E., L.W. Torres-Tapia, P. Sima-Polanco, S.R. Peraza-Sanchez and R. Moo-Puc, 2011. Screening of plants used in Mayan traditional medicine to treat cancer-like symptoms. *J. Ethnopharmacol.*, 135: 719-724
48. PA Insel. Analgesic-antipyretic and anti-inflammatory agents and drugs employed in the treatment of gout. In: JG Hardman, LE Limbird, PB Molinoff, RW Ruddon, GA Gilman. *The pharmacological Basics of Therapeutics*, 9th edition. McGraw Hill, New York, 1996, 617-657.
49. N. Bramhananda Reddy, VenkataramuduBurra, L. K. Ravindranath, S. A. Aleem and N. S. Narendra, *Der PharmaChemica*, 8(4):101-112, 2016.
50. Subbiah Ramasamy, Tanmoy Guria, Tanushree Singha, Puspita Roy, Benu P Sahu, Jayatri Naskar, Avijit Das and Tapan K Maity., *Der Pharma Chemica*, 8,4,446-452, 2016.
51. Sahar B Aand Ammar A. Razzak Mahmood Kubba., *Der PharmaChemica*, 8,4, 63-66. 2016.
52. Y. Filali Baba, H. Elmsellem, Y. Kandri Rodi, H. Steli, C. AD5, Y. Ouzidan, F. Ouazzani Chahdi, N. K. Sebbar, E. M. Essassi and B. Hammouti., *Der PharmaChemica*, 8,4,159-169, 2016.
53. Kiran M. Kulkarni, Sagar A. Jadhav, Pramod B. Patil, Vikas R. Dhole and Shitalkumar S. Patil, *Der PharmaChemica*, 8,4,1-5, 2016.
50. Chao jun-shu, Huia ping-xin, Liashuo, "Synthesis and Antibacterial Activities of Novel Biphenyltetrazole Derivatives Bearing 1,3,4- Oxadiazole." *Journal of the Chinese Chemical Society*, 2005, 52, 539-544
51. Srinivas K, Srinivas U, Bhanuprakash K, Harakishore K. "Synthesis and antibacterial activity of various substituted s-triazines". *Eur J Med Chem* 2006; 41: 1240-1246.
52. KD Tripathi. *Essentials of medical pharmacology*. Jaypee Brothers Medical Publishers Ltd, New Delhi, India, 2008, 189.
53. Aatesh Eznur, Kocobalkanli AysEe, Cesur Nesrin, "Synthesis and antimicrobial activity of some 5-aryl-2-[(N,N-disubstitutedthiocarbamoylthio) acylamino]-1,3,4-oxadiazoles" , *Farmaco* , 53 (1998) 541-544.

54. Montalbetti, Christian A. G. N.; Falque, Virginie, (2005). "Amide bond formation and peptide coupling". *Tetrahedron*. 61 (46): 10827–10852., doi:10.1016/j.tet. 2005.08.031
55. Valeur, Eric; Bradley, Mark (2009). "Amide bond formation: beyond the myth of coupling reagents". *Chem. Soc. Rev.*, 38: 606–631. doi:10.1039/B701677H.
56. Nanjunda S, Swamy S, Basppa, PriyaBs, Prabhuswamy B, Doreswamy BH (2006). "Crystal Structure of Novel 2-butyl-4-chloro-1H-imidazolyl-5-Carboxaldehyde". *European Journal of Medicinal Chemistry*, 41: 531-538.3.
57. MiesadMohamd , NaghamMahmoodAljamali , WassanAlaShubber , Sabreen Ali Abdalrahman .," New Azomethine- Azo Heterocyclic Ligands Via Cyclization of Ester "., *Research J. Pharm. and Tech.*, 11, 6 ,2018 ., DOI: 10.5958/0974-360X.2018.00472.9 .
58. Intisar Obaid A , Eman HS , NaghamMahmoodAljamali ., "Synthesis of (Tetrazole, Oxazepine, Azo, Imine) Ligands and Studying of Their (Organic Identification, Chromatography, Solubility, Physical, Thermal Analysis, Bio-Study) " ., *Research J. Pharm. and Tech* ,2018; 11,7,: 2821-2828., DOI: 10.5958/0974-360X.2018.00521.8 .
59. S. Sreedaran, K. S. Bharathi, A. K. Rahiman et al., "Synthesis, electrochemical, catalytic and antimicrobial activities of novel unsymmetrical macrocyclic dicompartmental binuclear nickel(II) complexes," *Polyhedron*, vol. 27, no. 7, pp. 1867–1874, 2008.
60. SaherMahmoodJwad ., *Research Journal of Pharmaceutical, Biological and Chemical Sciences* ., 8, 3, 2017, 549–563.
61. Shivi, B. and Monika, G. 2011. Oxadiazole as antimicrobial agents: An overview. *J. Chem. Pharm. Res.*, 3(3):137-147.
62. Fatehia, K. M. 2010. Synthesis, reactions and antimicrobial activity on some novel phthalazinones derivatives. *PRLDCS*; 1 (1): 20-31.
63. Intisar ObaidAlfatlawi , Nuha Salman S, ZainabMahmood J , NaghamMahmoodAljamali , " Synthesis of New Organic Compounds Via Three Components Reaction with Studying of (Identification ,Thermal Behavior, Bioactivity on Bacteria of Teeth) "., *Journal of Global Pharma Technology*. 2017; 11, 9 ,157-164.
64. Eman H. S ., NaghamMahmoodAljamali .,"New Azo-Thiadiazole Ligands (Preparation, Spectral, Thermal, Biochemical, Physical properties) - Studying " ., *Journal of Global Pharma Technology*. 2017; 11, 9 ,165.
65. Peng. F.; Zhi, Z.; Xin, P. H.; Zi, Y. Z.; and Rong, L. Z. 2004. Synthesis of Triazoles, Oxadiazoles and Condensed Heterocyclic Compounds Containing Cinchopheny and Studies on Biological Activity of Representative Compounds. *JCC S*; 51(2): 315-319.
66. NaghamMahmoodAljamali .,"Synthesis and Chemical Identification of Macro Compounds of (Thiazol and Imidazol)"., *Research J. Pharm. and Tech*, 2015, 8,1, 78-84., DOI : 10.5958/0974-360X.2015.00016.5 .
67. Scott B.T.&Bailey,P.E.,*Diagnostic Microbiology*. John-Weily Inc.,1999;3rdEd., Mosby Baily,London.
68. Awetz,J. and Delbrgs,A.,*MedicalMicrobiology*.John-WeilyInc.,2007;4thEd., MC GrawHil-US.

Copyrights

Copyright for this article is retained by the author(s), with first publication rights granted to the journal.

This is an open-access article distributed under the terms and conditions of the Creative Commons Attribution license (<http://creativecommons.org/licenses/by/4.0/>)