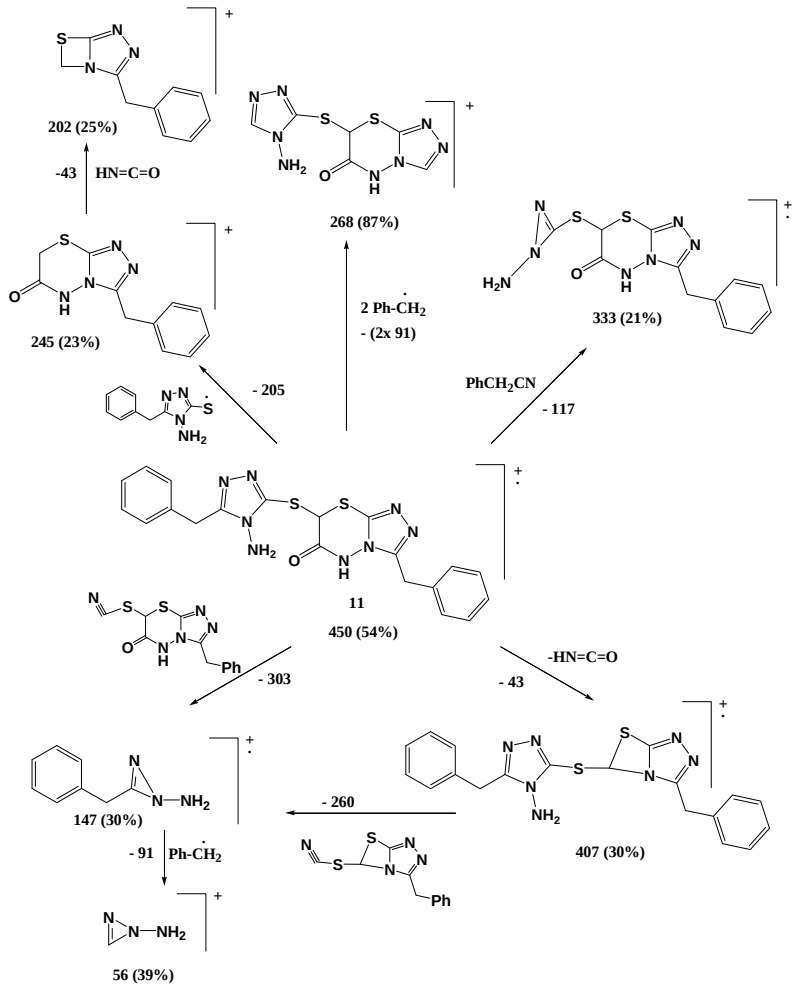
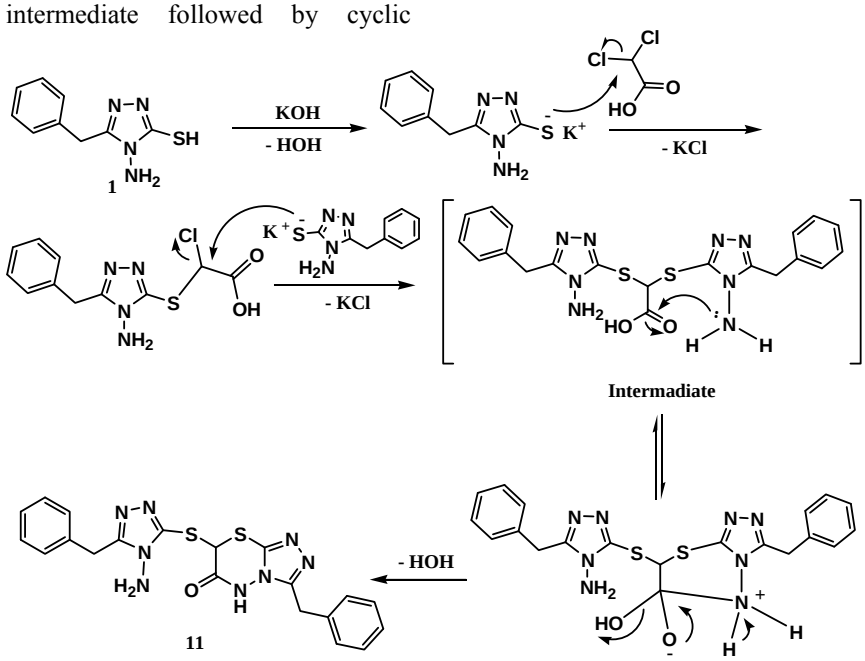


Scheme 5

Scheme 5: Mass fragmentation pattern of compound **11**.

Formation of compound **11** may proceed via reaction of two molecules of **1** with one molecule of dichloroacetic acid to give an unstable intermediate followed by cyclic condensation through losing of water according to the proposed mechanism (Scheme 6).



Scheme 6

Scheme 6: The suggested reaction mechanism of **1** to give compound **11**

3.1 Antimicrobial Activity

Bacterial source and culture condition:

The used Bacterial strains were Gram negative bacteria including *E. Coli* (ATCC 25922) and Gram positive bacteria *Enterococcus faecalis* (ATCC 29212). Mueller-Hinton Agar was used as culture media (gl^{-1}) [29], Beef extract, 3.0; Peptone, 17.5; Starch, 1.5; Agar, 17, pH= 7.3 ± 0.1. The plates were incubated at 37ºC for 24 – 48 hrs.

Paper disc technique: Antibacterial activity was determined against the above strains using the paper disc assay method [30]. Whatman number 1 filter paper disc of 6.0 mm diameter was sterilized by autoclaving for 20 min at 121 °C. The sterile discs were impregnated with the spaced apart and plates were incubated at 37°C for 24- 48 hrs [31]. Chloramphenicol 30 µg/disc was used as a positive control. Diameter of the growth inhibition halos caused by the tested compounds were measured and expressed in millimeter. All the assays were carried out in triplicate.

Table 1: Effect of the synthesized compounds (**1- 11**) on bacterial growth (mm).

Sample No.	Bacterial growth inhibition zone diameter (mm)	
	Gram (-ve) Bacteria	Gram (+ve) Bacteria
	E. Coli	*Enterococcus faecalis*
1	**7**	**5**
2a	**6**	-----
2b	**6**	-----
3a	**8**	**7**
3b	**6**	-----
4a	**9**	**7**
4b	-----	-----
5	**9**	-----
6	**9**	**7**
7	**9**	-----
8	**7**	**7**
9	**7**	**7**
10	**7**	**6**
11	-----	-----
***Choramphenicol* 30 µg (Control)**	**18**	**18**

E. coli (Escherichia coli) is the name of a germ, or bacterium that lives in the digestive tracts of humans and animals. Many types of *E. coli* can cause bloody diarrhea and urinary tract infections. Some strains of E. coli bacteria may also cause severe anemia or kidney failure [32]. Also, *Enterococci* are Gram-positive *cocci* that often occur in pairs (diplococci) or short chains. The important clinical infections caused by *Enterococcus* include urinary tract infections, bacteremia, bacterial endocarditis, diverticulitis, and meningitis [33].

The antibacterial activity of the synthesized compounds **1-11** were carried out on the growth of two pathogenic bacteria (*E. Coli* and *Enterococcus faecalis*). The data obtained in Table (1) indicate that 12/14 of these compounds have effects on *E. Coli* bacteria where the great inhibition (9 mm) was observed by the Mannich base **4a**, benzenesulfonamide **5**, acetamide **6** and the triazolothiadiazine **7**, but the low inhibition (6 mm) was appeared by Schiff bases **2a**, **b** and the thiazolidinone **3b**. The triazole **1**, thiazolidinone **3a**, Phenyl-thioacetic acid ester **8**, thiadiazinone **9** and thiadiazinedione **10** showed moderate inhibition (7- 8 mm), while the Mannich base **4b** and the triazolothiadiazinone **11** showed no activity.

The inhibition effect was decreased on *Enterococcus faecalis* and also 7/14 only of the tested compounds showed moderate and low inhibition effect (5- 7 mm).

4. Conclusion

In summary, 4-amino-5-benzyl-4*H*-[1,2,4]triazole-3-thiol (**1**) has been utilized as a key starting material in the synthesis of many novel heterocyclic compounds **2- 11**. The constitution of these compounds assigned on the basis of IR, ^{1}H ^{13}C NMR, mass spectra and elemental analyses were found to be in correlation with the desired structure. The antimicrobial activity screening revealed that the compounds **1- 10** have significant antimicrobial activity. Compounds **4a**, **5**, **6** and **7** have high biological activity against gram (-ve) bacteria, and compounds **1**, **3a**, **8, 9** and **10** showed moderate inhibition effect.

On the other hand, compounds **1**, **3a**, **4a**, **6**, **8**, **9** and **10** showed moderate inhibitions effect against gram (+ve) bacteria, while compounds **2a**, **2b**, **3b**, **4b**, **5**, **7** and **11** showed no activity. The results are promising and show that the fine tuning of the structures **4a**, **5**, **6** and **7** can lead to some new antimicrobial agents in treating microbial infections.

5. Acknowledgements

The author is grateful to Prof. Dr. Fawzy Attaby, chemistry department, faculty of science, Cairo University, Egypt for his help and support during the course of this work.

References

[1] Gabriela L.A., Stefania F.B., E. Almajan, Draghici C., Saramet G., Synthesi, characterization and antibacterial activity of some triazole Mannich bases carrying diphenylsulfone moieties, Eur. J. Med Chem. 2009; 44: 3083–3089.
[2] Plech T., Wujec M., Siwek A., Kosikowska U., Malm A., Synthesis and antimicrobial activity of thiosemicarbazides, s-triazoles and their Mannich bases bearing 3-chlorophenyl moiety, Eur. J. Med Chem. 2011; 46: 241-248.
[3] Plech T., Wujec M., Kosikowska ., Malm A., Rajtar B., Synthesis and in vitro activity of 1,2,4-triazole-ciprofloxacin hybrids against drug-susceptible and drug-resistant bacteria, Eur. J. Med Chem. 2013; 60:128-134.
[4] Aggarwal N., Rajesh Kumar, Dureja P., Khurana J.M., Synthesis, antimicrobial evaluation and QSAR analysis of novel nalidixic acid based 1,2,4-triazole derivatives, Eur. J. Med Chem. 2011; 46 :4089-4099.

[5] Zou Y., Zhao Q., Liao J., Chai X., Mingjuan X., New triazole derivatives as antifungal agents: Synthesis via click reaction, in vitro evaluation and molecular docking studies, Bioorg. Med. Chem. Lett. 2012; 22: 2959– 2962.
[6] Zoumpoulakis P., Camoutsis C., Pairas G., Pitsas A., Synthesis of novel sulfonamide-1,2,4-triazoles, 1,3,4-thiadiazoles and 1,3,4-oxadiazoles, as potential antibacterial and antifungal agents. Biological evaluation and conformational analysis studies, Bioorg. Med. Chem. 2012; 20: 1569– 1583.
[7] Rezaei Z., Khabnadideh S, Pakshir K., Hossaini Z., Amiri F., Assadpour E., Design, synthesis, and antifungal activity of triazole and benzotriazole derivatives, Eur. J. Med Chem. 2009; 44: 3064–3067.
[8] Harish K., Neena, Synthesis and Characterization of 1,2,4-triazole and their diazotized compound as Bio-active agents, J. Chem. & Chem. Sci. 2010;1: 1-19.
[9] Farghalya A.R., Clercq E.D., and El- Kashef H., Synthesis and antiviral activity of novel [1,2,4]triazolo[3,4-b][1,3,4]thiadiazoles, [1,2,4] triazolo[3,4-b][1,3,4] thiadiazines and [1,3,4] triazolo [3,4-b][1,3,4]thiadiazepines, ARKIVOC 2006; 10: 137-151.
[10] Suresh Kumar G.V., Rajendra Prasad Y., Mallikarjuna B.P., Chandrashekar S.M., Synthesis and pharmacological evaluation of clubbed isopropylthiazole derived triazolothiadiazoles, triazolothiadiazines and mannich bases as potential antimicrobial and antitubercular agents, Eur. J. Med Chem. 2010; 45: 5120-5129.
[11] Pattan S.R., Gadhave P., Tambe V., Dengale S., Thakur D., Synthesis and evaluation of some novel 1,2,4-triazole derivatives for antimicrobial, anti-tubercular, anti-inflammatory activities, Ind. J. Chem 2012; 51B: 297-301.
[12] Siddiqui N., Ahsan W., Triazole incorporated thiazoles as a new class of anticonvulsants: Design, synthesis and in vivo screening, Eur. J. Med Chem. 2010; 45: 1536–1543.
[13] Plech T., Luszczki J.J., Wujec M., Flieger J., Pizon M., Synthesis, characterization and preliminary anticonvulsant evaluation of some 4-alkyl-1,2,4-triazoles, Eur. J. Med Chem. 2013; 60: 208-215.
[14] Sambrekar S.N., Patil S.A., Synthesis of 3-Substituted-4-Amino-5-Mercapto-4H-1,2, 4- Triazole and Screening for Anti-Convulsant Activity, Inter. J. Res. Pharm. Biomed. Sciences. 2011; 2: 520-524.
[15] Diwedi R., Alexander S., Chandrasekar M. J. N., Rapid and efficient synthesis of microwave assisted some bis-1,2,4-triazole derivatives and their antioxidant and anti-inflammatory evaluation, RJPBCS 2011; 2(1): 194-204.
[16] Mosaad S.M. Abdalla, Hegab M.I., Nageh A. Abo Taleb, Sherifa M. Hasabelnaby, A. Goudah. Synthesis and anti-inflammatory evaluation of some condensed [4-(3,4-dimethylphenyl)-1(2H)-oxo-phthalazin-2-yl]acetic acid hydrazide, Eur. J. Med Chem. 2010; 45: 1267–1277.
[17] Moise M., Sunel V., Profire L., Popa M. and Peptu C., Synthesis and Biological Activity of Some New 1,3,4-Thiadiazole and 1,2,4-Triazole Compounds Containing a Phenylalanine Moiety, Molecules 2009,14: 2621-2631.
[18] Gowda J., Khader A.M.A., Kalluraya B., Padma Shree, Shabaraya A.R., Synthesis, characterization and pharmacological activity of 4-{[1-substituted aminomethyl- 4-arylideneamino-5-sulfanyl-4,5-dihydro-1H-1,2,4-triazol-3-yl]methyl}-2H-1,4-benzothiazin-3(4H)-ones, Eur. J. Med Chem. 2011; 46: 4100-4106.
[19] Bhat K.S., Poojary B., D. Jagadeesh Prasad, Naik P., Holla B.S., Synthesis and antitumor activity studies of some new fused 1,2,4-triazole derivatives carrying 2,4-dichloro-5-fluorophenyl moiety, Eur .J. Med Chem. 2009; 44: 5066–5070.
[20] Zhao P.L., Ma W.F., Duan A.N., Yan Y., Wu S.G., One-pot synthesis of novel isoindoline-1,3-dione derivatives bearing 1,2,4-triazole moiety and their preliminary biological evaluation, Eur. J. Med Chem . 2012; 54: 813-822.
[21] Goyal P.K., Bhandari A., Rana A.C., Jain C.B., Synthesis, Characterization and analgesic Activity of some 4H-1, 2, 4-Triazole Derivatives, Inter. J. Chem. Tech. Res. 2010;2: 1992-1997.
[22] Desai S.R., Laddi U., Bennur R.S., Patil P.A. and Bennur S., Synthesis and Pharmacological Activities of Some New 3- Substituted-4-Amino-5-Mercapto-1,2,4-Triazoles, Ind. J. Pharm. Sci., 2011; 73: 115-120.
[23] Vijesh A.M., Isloor A.M., Shetty P., Sundershan S., Hoong Kun Fun., New pyrazole derivatives containing 1,2,4-triazoles and benzoxazoles as potent antimicrobial and analgesic agents, Eur. J. Med Chem. 2013; 62: 410-415.
[24] Zafer A.K., Ahmet O., Gulhan T.Z., Mehlika D.A., Ozgur D.C., New pyrazoline derivatives and their antidepressant activity, Eur. J. Med Chem. 2010; 45: 4383-4387.
[25] Puthiyapurayil P., Poojary B., Chikkanna C., Buridipad S.K., Synthesis, spectral characterization and biological evaluation of a novel series of 6-arylsubstituted-3-[2,4-substitutedphenyl)propan-2yl]-7H-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazines, Eur. J. Med Chem. 2012; 57: 407-416.
[26] Zhang Li-xue, Zhang An-jiang, Chen Guang, Chen Fei-yu, Jiang Yi-ping, Zhang Zi-yi, Preparation and Spectral Characterization of 3-Benzyl-6-aryl-1,2,4-triazolo[3, 4-b][1, 3, 4]thiadiazoles, CHEM . RES . CHINESE U., 2002; 18(3): 280- 283.
[27] Cansiz A, Koparir M., Demirdag A., Synthesis of Some New 4,5-Substituted-4H-1,2,4-triazole-3-thiol Derivatives, Molecules. 2004, 9: 204- 212.
[28] Shakirullah, Syntheses and characterization of optically active small aza-heterocyclic scaffolds: 1,2,4-triazolo[3,4-b]thiadiazoles, M.Sc thesis, Faculty of Sciences, Allama Iqbal Open University, Islamadad, V867291, 4th Feb, 2010, 14- 17.
[29] Mueller J. H. and Hinton, A protein-free medium for primary isolation of gonococcus and meningococcus, J. Proc. Soc. Exp. Biol. Med. 1941; 48: 3330- 3333.
[30] Omenka C. A., Osuoha J. O., Antimicrobial potency of Grapefruit seed extract on five selected pathogens, Nigerian Journal of Microbiology. 2000; 14 (2):39- 42.

[31] Bauer R. W., Kirby M. D., Sherris J C, Turck M, Antibiotic susceptibility testing by a standardized single disk method, Amer. Clin. Pathol. 1966; 45: 493-496.
[32] Bad Bug Book, "Foodborne Pathogenic Microorganisms and Natural Toxins: Pathogenic Escherichia coli Group, Enterococcus," Center for Food Safety and Applied Nutrition (FDA). 2nd Edition. 2012, 68-115.
[33] Fisher K., Phillips C., The ecology, epidemiology and virulence of Enterococcus, Microbiology. 2009; 155: 1749–57.

Author Profile

Dr. Ahmed M. Abo-Bakr B.Sc, M.Sc and PhD degrees in Organic Chemistry from Faculty of Science, South Valley University 1996- 2006. During 1996-2014, he is having 18 years of teaching experience at chemistry department, Faculty of science, South Valley University, Ministry of Higher Education, Egypt. His career is "synthesis of new biologically active heterocyclic compounds".