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SYNTHESIS AND CHARACTERIZATION OF 1,2,3-TRIAZOLINE FROM CREATININE AND STUDY OF THEIR BIOLOGICAL ACTIVITY

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ABSTRACT : In this paper, we prepared 2-N-arylidene amino creatinine from reaction of 2-N-chloro acetamido creatinine with sodium azide and then synthesized new 1,2,3-triazolines from reaction of 2-N-arylidene amino creatinine with (maliec anhydride, maleimide derivative, cinnamic acid and cinnamaldehyde). And studied anti-bacterial, anti-fungal and antioxidant activity of synthesis compounds. The prepared compound were characterized by FT-IR and some of them by ^{1}HNMR. All physical properties are listed in Table 1.

Key words : 2-N-arylidene creatinine, 1,2,3-triazolidine derivatives, biological activity.

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INTRODUCTION

Triazoline and its derivatives are class of heterocyclic compounds they have interesting biological application. These compounds can be used in medicine as antibacterial, antiviral, anticancerous antiasthmatic, analgesic and antiinflammatory drugs because of their pharmaceutical properties (Goswami *et al*, 1984; Hamadouche *et al*, 2010). Further, triazolines Interest in 1,3-dipolar cycloadditions involving olefins as dipolarophiles and azides as 1,3 dipoles originates from the synthetic potential of these reactions which lead to the formation of five membered nitrogen containing heterocycles like1,2,3-triazolines. The first involves the isomerisation of arylazoaziridines (Scheiner, 1968).

MATERIAL AND METHODS

Commercial reagents and solvents were used without further purification. Melting points of the synthesized compounds were determined in open-glass capillaries and were uncorrected. FT-IR spectra (KBr disc) were recorded Affinity-1 Shimadzu as FT-IR spectrometer using KBr pellets. 1HNMR spectra were scanned on Bruker spectro spin ultrashield magnets 300 MHz instrument, using DMSO-d_6 solvent and TMS as internal reference used to identification the organic inhibitor.

Synthesis of 2-N-chloro acetamido creatinine (Vikrishchuk *et al*, 1995) (1a) : A mixture of (1g, 0.008mml) creatinine, (0.03 mml) chloro acetyl chloride,(0.04 ml) of Et3N in (20ml) dry benzene was heated for 1 hour and then the mixture was cooled for 2 hr in ice bath then filtrated the solvent was evaporate and recrystallized from ethanol.

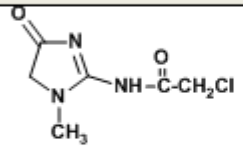
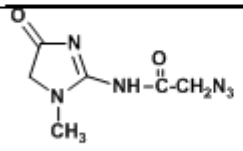
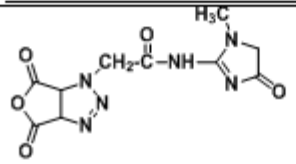
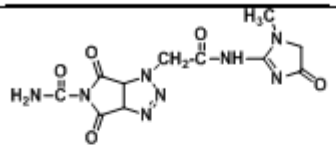
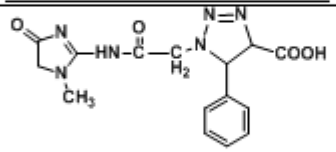
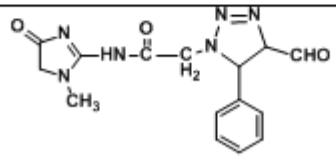
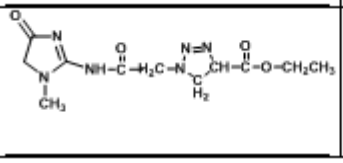
Synthesis of 2-N-arylidene amino creatinine (Ralph *et al*, 1980) (2a) : Added (0.52 g, 0.008 mol) of Sodium azide to (1g, 0.004 mol) of compound (1a) in 15ml absolute ethanol. The mixture was refluxed for (8 h) with stirring.The solvent was evaporated then light brown precipitate was washed with cold water, filtrated and recrystallized with ethanol.

Synthesis of new 1,2,3-triazoline derivatives (Al-Ajely *et al*, 2008) (3a, 3b, 3c, 3d, 3e) : (0.01g, 0.001 mol) of different α, β-unsaturated compounds were added to the solution of compound (2a) (0.25g, 0.001 mol) in absolute ethanol (15 ml). The mixture was refluxed for (24 hr.). The solvent was evaporated and the residue was washed with diethyl ether.

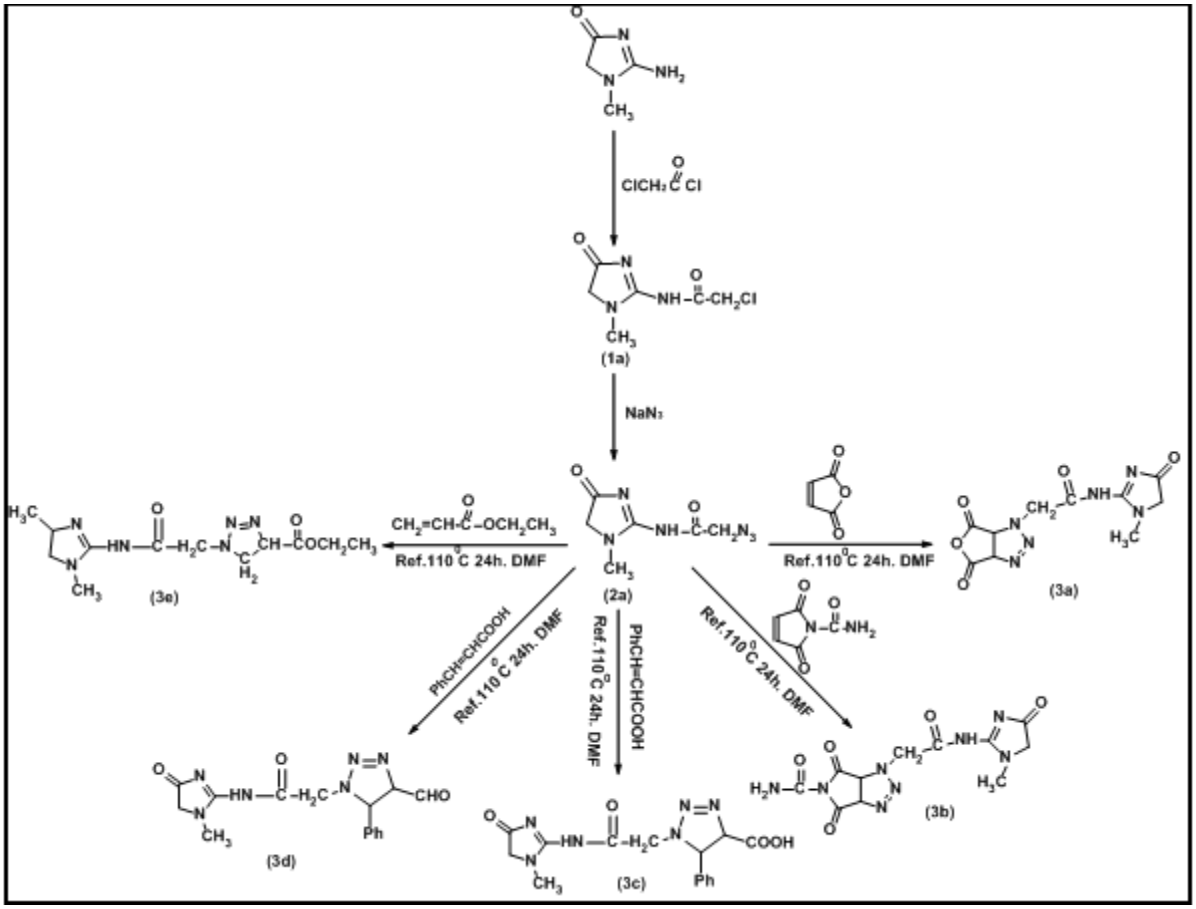
Biological activity (Vandepitte *et al*, 2003)

The test was performed according to the disk diffusion method. The prepared compounds were tested against

Table 1 : Some physical properties and FT-IR spectral data $cm^{-1}$of compounds (1a-3e).

Code	Structure	m.p.° C	Color	Yield %	Major FT-IR Absorption Cm^{-1}				
					ν(C-H) aliphatic	ν(C=O) amide	ν(C=O) anhyd.	ν(C=N) ν(C-N)	Other bands
1a		142-144	Pale yellow	80	2800 2939	1697 1770	--	1640 1435	ν (C-Cl) 646 ν (NH) 3257
2a		170-172	Off white	75	2814 2976	1697 1772	--	1660 1398	ν(N₃) 2106 1440 ν (NH) 3255
3a		110-112	Yellow	60	2800 2939	1700 1732	1789	1620 1398	ν (N=N) 1435 ν(C-O-C) 1244 ν (NH) 3190
3b		145-147	Yellow	70	2880 2985	1622 1670 1716	--	1550 1396	ν (N=N) 1448 ν (NH_2) 3446 ν (NH) 3205
3c		150-152	White	80	2812 2929	1695 1760 1720	--	1631 1400	ν (N=N) 1450 ν (C=C)Arom. 1564 ν (NH) 3190 ν (OH acid) 2682-3300 ν (C-H) Arom. 3026
3d		160-162	yellow	75	2818 2950	1700 1676		2516 1419	ν (N=N) 1450 ν (C=C) Arom. 1496 ν (NH) 3150 ν (CHO) 1725 ν (C-H) Arom. 3062
3e		185-187	yellow	65	2877 2939	1739 1728	--	1624 1398	ν (N=N) 1446 ν (N-H) 3200 ν (C-C-O ester) 1033

one strain of Gram positive bacteria (*Staphylococcus aureus*) and one Gram negative bacteria (*Salmonella*) and two fungi (*Aspergillus flavus* and *Cryptococcus*). Prepared agar and Petri dishes were sterilized by autoclaving for (15min) at 121^{0}C. The agar plates were surface inoculated uniformly from the broth culture of the tested microorganisms. In the solidified medium suitably spaced apart holes were made all (6mm) in diameter, were filled with 100µl of the prepared compounds (0.025mg of the compound dissolved in 1ml of DMSO solvent). These plates were incubated at (37^{0}C) for 24 hrs. The inhibition zones caused by the various compounds on the bacteria were examined. The results of the preliminary screening test are listed in Table 3.



Scheme 1 : Syntheses new 1,2,3-triazoline from creatinine.

Determination of antioxidant capacity (Blois *et al*, 2005)

DPPH (1, 1-Diphenyl-2-Picrylhydrazyl) Free Radical Scavenging Activity Assay : The assay was carried out according to the modified method of Blois (1958). 1ml of 0.1mM solution of DPPH (Alfa Aesar, Japan) in methanol was mixed with 2ml of the aqueous extracts at different concentrations (250-1000 PPM). The mixture was then incubated at room temperature for 30 min in the dark. The control was prepared by mixing 1 ml of DPPH solution with double distilled water. The absorbance was measured against a blank at 517 nm using a spectrophotometer. Lower absorbance of the reaction mixture indicates higher DPPH free radical scavenging activity. Ascorbic acid (Merck, India) was used as the standard. Samples were prepared and measured in triplicates. The percentage of scavenging activity of each extract on DPPH radical was calculated as % inhibition

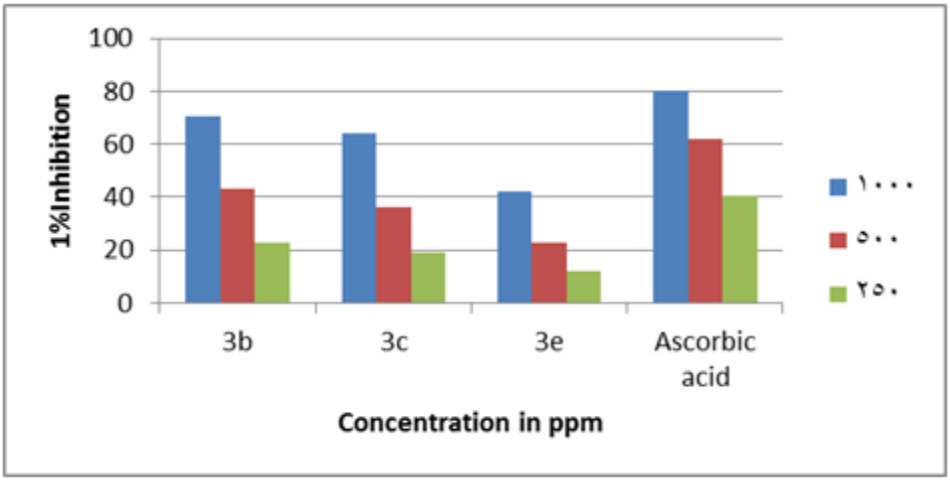


Fig. 1 : Antioxidant activity of some synthesized derivatives.

of DPPH (1%) using the following equation:

$1\% = [(Ao-As)/Ao] \times 100.$

RESULTS AND DISCUSSION

Scheme (1) illustrated of 1,2,3-triazoline that prepared from reaction of 2-N-arylidene creatinine with sodium azide. All the derivatives characterization by FT-IR and some of derivatives by ^{1}H-NMR.

The 2-N-arylidene creatinine was prepared from reaction of chloroactyl chloride with creatinine and then prepared 1,2,3-triazoline derivatives from reaction of 2-N-arylidene creatinine with different α, β unsaturated compounds the synthesized compounds were identified by melting points FT-IR and ^{1}HNMR.

for C-Cl. ^{1}HNMR spectrum showed singlet signal at δ = (2.94) ppm due to (N-C<u>H</u>$_3$) protons, singlet signal at δ = (3.40) ppm due to (C<u>H</u>$_2$-CO) protons (3.69) ppm due to (C<u>H</u>$_2$-Cl) protons. And single signal at δ = (7.53) ppm due to N<u>H</u>proton and in the Table 2 are listed ^{1}HNMR spectral data for some synthesized 1,2,3-triazoline compounds.

CONCLUSION

The synthesized compounds were confirmed by using spectroscopic techniques (FT-IR and ^{1}HNMR). The microbial activity of the new 1,2,3-triazoline derivatives caused activator effects on two types of bacteria *Staphylococcus aureus* and *Salmonella*. And two fungal *Cryptococcus* and *Asp. flavus*. The organisms bacteria *Staphylococcus aureus* and *Salmonella* show moderately active of compounds (3a,3c) and compounds (3b,3c) have very high activity against *Cryptococcus* and *Asp. flavus*.

Table 2 : 1HNMR spectral data for some synthesized derivatives.

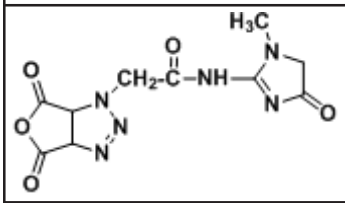
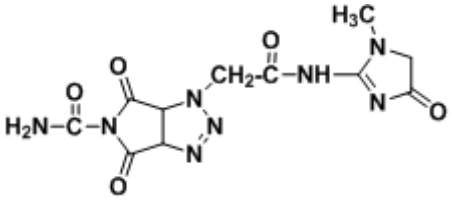
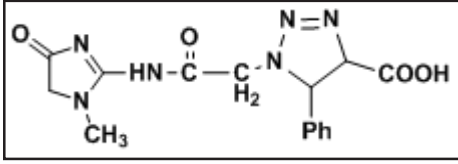
Structure	^{1}HNMR signals data,δ (ppm)
	ä 1.25 ppm (s,3H,C<u>H</u>$_3$); ä 2.10ppm (s, 2H, C<u>H</u>$_2$-CO), ä 4.09 ppm (s,2H,C<u>H</u>$_2$-CO.), ä 5.61.6.31 ppm (dd, 2H, CO-C<u>H</u>-N.) and 7.74 ppm (s,1H,N<u>H</u>).
	ä 2.94 ppm (s,3H,C<u>H</u>$_3$); ä 2.63ppm (s, 2H, C<u>H</u>$_2$-CO),ä 3.37 ppm (s,2H,N<u>H</u>$_2$),ä 4.23 ppm (s,2H,C<u>H</u>$_2$-CO.), ä 5.52.6.06 ppm (2d, 2H,CO-C<u>H</u>-N.) and 7.91 ppm (s,1H,N<u>H</u>).
	ä 2.10 ppm (s,3H,C<u>H</u>$_3$); ä 2.95ppm (s, 2H, C<u>H</u>$_2$-CO) , ä 3.71 ppm (s,2H,C<u>H</u>$_2$-CO.) , ä 6.59 ppm (s,1H,N<u>H</u>)., ä 7.42-7.71ppm(m,5H,Ar-H) and ä 8.5ppm(s,1H,OH)

Table 3 : Biological activity of compounds (3a-3d) against selected bacteria and fungi.

Comp. code	*Cryptococcus*	*Asp. flavus*	*St.aureas*	*Salmonella*
3a	-	-	-	++
3b	-	++++	-	-
3c	+++	-	++	-
3d	-	-	-	-
DMSO	-	-	-	-

Key to symbols = Inactive = (-) inhibition Zone < 6 mm
Slightly active = (+) = inhibition Zone 6-9mm
Moderately active = (++)inhibition Zone 9-12mm
Highly active = (+++) inhibition Zone 13-17 mm
Very high activity = (++++) inhibition zone < 17 mm Conc. =10^{-3}.

The structure of compound (1a) was confirmed by physical properties which are listed in Table 1. FTIR spectrum showing the absorpte at 2800-2939cm^{-1} for C-H aliph. 1697-1770cm^{-1} for C=O (amide) and 758cm^{-1}

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