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Synthesis, Characterization and Biological Activity of Some New 4-Substituted-phenyl-3-chloro-1-{5-[(3, 5-dimethyl-1H-pyrazol-1-yl) methyl]-1, 3, 4-thiadiazol-2-yl} azetidin-2-one

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الخلاصة:

تم تحضير سلسلة جديدة من مركبات حلقية غير متجانسة (1–15) مشتقة من 5,3–داي مثيل–1–هايدرو–بايرازول تحوي على ذرات مختلفة من النيتروجين والكبريت وذلك عند معاملته مع اثيل كلورواسيتيت بوجود كاربونات البوتاسيوم لتكوين المركب اثيل (3, 5–داي مثيل–1هايدرو–بايرازول–1–يل) اسيتيت(1). تفاعل المركب (1) مع الثايوسمي كاربزايد في الميثانول لتحضير المركب 2–[(5,3–ثنائي مثيل– 1هيدرو–بايرازول–1–يل)استيل] هيدرازينكاربثايوامايد(2) .

حضر المركب 5–[(5,3– ثنائي مثيل– 1هيدرو–بايرازول–1–يل)مثيل] 4,3,1– ثاياديازول–2–امين (3) عن طريق غلق حلقي للمركب (2) بواسطة الحامض المعدني H_2SO_4 . حضرت قواعد شيف (4–9) الجديدة عن طريق تكثيف المركب 5–[(5,3–ثنائي مثيل–1هيدرو–بايرازول–1–يل) مثيل] 4,3,1– ثاياديازول–2–امين (3) مع الألدهيدات الاريل الارماتية المختلفة. ستة مركبات جديدة (10–15) قد تم تحضيرها من خلال تكثيف مختلف قواعد شيف (4–9) مع كلورواسيتايل كلورايد بوجود ثلاثي اثيل الامين. تم تشخيص المركبات الجديدة (1–15) عن طريق تحليل العناصروالبيانات الطيفية .

دُرست بعض من المركبات الجديدة (10–15) لبيان نشاطهم المضاد للبكتيريا ضد أربعة أنواع منها، وجدت أن لها فعالية بايولوجية جيدة.

Abstract:

A new series of 3,5-dimethyl-1H-pyrazole derivatives (1-15) incorporated into fused to different nitrogen and sulphur containing heterocyclic were prepared from 3,5-dimethyl-1H-pyrazole when treated with ethylchloroacetate in the present of potassium carbonate to offer Ethyl (3, 5-dimethyl-1*H*-pyrazol-1-yl) acetate (1). Compound (1) converted to 2-[(3,5-dimethyl -1H-pyrazol -1-yl) acetyl] hydrazinecarbothioamide (2) by reaction with thiosemicarbazide in methanol. Cyclocondensation (2) was reacted with mineral acid H_2SO_4 to produce 5-[(3, 5-dimethyl-1H-pyrazol-1-yl) methyl]-1, 3, 4-thiadiazol-2-amine (3). Various new Schiff bases (4-9) were synthesized by the condensation of 5-[(3, 5-dimethyl-1H-pyrazol-1-yl) methyl]-1, 3, 4-thiadiazol-2-amine (3) with various aryl aromatic aldehydes.A series of six new 4-substituted-phenyl-3-chloro-1-{5-[(3,5-dimethyl-1H-pyrazol-1-yl)methyl]-1,3,4-thiadiazol-2-yl}azetidin -2-one (10-15) have been synthesized by

condensation of various Schiff bases (4-9) with chloroacetyl chloride in presence of triethylamine. The structures of the new compounds (1-15) have been characterized by elemental analysis and spectral data. The newly synthesized compounds (10-15) were screened for their antibacterial activity against four bacterial species. They were found to exhibit good antibacterial activity.
Key words: Pyrazole, Thiadiazole, Azetidinone, Antibacterial activity.

Introduction:

Pyrazole derivatives are well established in the literature as important biologically active heterocyclic compounds. These derivatives were the subject of many research studies due to their widespread potential biological activities such as anti-inflammatory[1], antimicrobial [2],antitumor[3] ,antihistaminic[4] . 2-Azetidinone skeleton is well stabilized as the key pharmacophore of β-lactam antibiotics, the most widely employed class of antibacterial agents[5].They are the carbonyl derivatives of azetidines containing carbonyl group at the position-2. These are also known as 2-azetidinones or more commonly β-lactam. Cycloaddition of monochloroacetylchloride with imine(schiff base) result in formation of 2-azetidinone (β-lactam). The reaction involves direct acylation of imine with monochloroacetylchloride. The reaction is carried out with base as triethylamine gives β-lactams[6]. A number of 1, 3, 4-thiadiazoles and azetidinone derivatives have been reported to possess antibacterial activity.

Recently, some pyrazole types of biological activity besides the antibacterial activity have been reported in compounds such as containing benzimidazole ring [7].

The goal of this study is to synthesize some new pyrazoles derivative with acyclic substituents 2-azetidinone ring for the purpose of synergism and/or increasing the expected biological effects.

Materials and Methods:

Apparatus and Chemicals:

Reagents and solvents used are commercially available, Merck, Fluka and BDH. The melting points were determined in open capillaries tube on Stuart SMP10 Melting point apparatus. The purity of the compounds was confirmed by TLC using silica gel (0.5mm thickness, Merk) and visualized in iodine. The IR spectra were recorded in potassium bromide on Shimadzu FTIR-8400S. The elemental analyses (C, H, and N) were performed using Perkin-Elmer 240C analyzer. Their results were found to be in good agreement with calculated values. 1HNMR spectra was carried out by: Bruker, model: ultra shield 300 MHz, origin: Switzerland and are reported in ppm (δ) DMSO was used as a solvent with TMS as an internal standard. Measurements were made in Al-albyat University, Jordon.

Experimental:

3, 5-dimethyl-1H-pyrazole [8]

Dissolve (0.5mole) of hydrazine sulphate in 400ml of 2.5M sodium hydroxide solution and immerse the flask in ice bath and when the temperature reached 15^{o}C, (0.5mole) of acetyl acetone was added dropwise, with stirring while maintain the temperature at 15^{o}C. When addition was completed (after about 30minutes), stir for1 hour at 15^{o}C; the product separated at this period. 200ml of water was added, to dissolve inorganic salts, separated the aqueous layer with four 40ml portion of ether. Wash the combined ethereal extracts with brine solution, dry over anhydrous potassium carbonate and remove the ether on a rotary evaporator. The yield of pale yellow solid, m.p.107-108^{o}C. Recrystallise from light petroleum (80 -100 ^{o}C).

Ethyl (3, 5-dimethyl-1*H*-pyrazol-1-yl) acetate (1) [7]

A mixture of 3,5-dimethyl-1H-pyrazole (0.3mole), ethylchloroacetate (0.3mole) and potassium carbonate (6.168g) in methanol (250ml) was kept overnight at room temperature. The mixture was refluxed on a steam bath for about 3hrs. It was cooled, filtered and solvent distilled off under reduced pressure. The crude ester thus obtained was purified by recrystallisation from ethanol.

2-[(3, 5-dimethyl-1H-pyrazol-1-yl) acetyl] hydrazinecarbothioamide (2) [9]

The compound **1** (0.15 mole) and thiosemicarbazide (0.15 mole) in methanol (200 ml) was refluxed on a steam bath for about 8 hrs. It was then cooled, filtered and excess of solvent was removed which gave a product. Product was recrystallised with ethanol to give compound 2.

5-[(3, 5-dimethyl-1H-pyrazol-1-yl)methyl]-1,3,4-thiadiazol-2-amine (3) [9]

Equimolar solution of compound **2** (0.1mole) and concentrated H_2SO_4 (0.1mole, 9.80g, ARgrade) in methanol (150ml) was kept overnight at room temperature. The mixture was refluxed on a steam bath for about 10 hrs. After cooling the solution was neutralized with concentrated liq. Ammonia and solid formed was filtered. Product was recrystallised with ethanol to give compound**3**.

Synthesis of Schiff bases (4-9) [9]

Equimolar mixture of compound **3** (0.01mole) and benzaldehyde (0.01mole) in methanol (100ml) with 9-10 drops glacial acetic acid was refluxed on a water bath for about 2hr. The solvent was distilled off under reduced pressure and the solid thus obtained was recrystallised with ethanol to give crystals of compound 4 .Other compounds (**5-9)** were synthesized in the similar manner using compound 3 and various selected aromatic aldehydes.

Synthesis of 2- Azetidinones (10-15) [10, 11]

A mixture of Schiff bases **(4-9)** (0.001mole) and triethyl amines (0.002mole) were dissolved in 1, 2-dioxane (25ml) cooled and stirred. To this well-stirred cooled solution chloroacetyl chloride (0.004mole) was added drop by drop for a period of 30 min at low temperature. The reaction mixture was stirred for additional 3-6 hrs, and left at room temperature for 48hrs. The

resultant mixture were concentrated, cooled, poured in to ice, filtered and then dried to give a product which was recrystallised from suitable solvent.

Table-1 shows the physical properties and element analyze.

Comp No.	Molecular Formula	M.P/°C (Recryst. solvent)	Color	Yield %	Calc. (Found) %		
					C	H	N
1	$C_9H_{14}N_2O_2$	145-48 (Ethanol)	Off-white	85	59.32 (59.25)	7.74 (7.70)	15.37 (15.40)
2	$C_8H_{13}N_5OS$	128-30 (Ethanol)	Pale-Yellow	72	42.28 (42.22)	5.77 (5.75)	30.81 (30.89)
3	$C_8H_{11}N_5S$	162-64 (Ethanol)	Yellow	66	45.91 (45.87)	5.30 (5.28)	33.47 (33.51)
4	$C_{15}H_{15}N_5S$	133-36 (Ethanol)	Yellow	73	60.58 (60.51)	5.08 (5.05)	23.55 (23.59)
5	$C_{15}H_{14}ClN_5S$	139-42 (Ethanol)	Pale-Orange	70	54.29 (54.26)	4.25 (4.22)	21.11 (21.16)
6	$C_{15}H_{14}BrN_5S$	195-97 (Ethanol)	Orange	68	47.88 (47.85)	3.75 (3.71)	18.61 (18.65)
7	$C_{16}H_{17}N_5OS$	111-17 (Ethanol)	Green	64	58.70 (58.72)	5.23 (5.21)	21.39 (21.44)
8	$C_{17}H_{20}N_6S$	123-26 (Ethanol)	Greenish - yellow	61	59.97 (59.94)	5.92 (5.89)	24.69 (24.73)
9	$C_{15}H_{14}N_6O_2S$	170-74 (Ethanol)	Yellow	58	52.62 (52.64)	4.12 (4.10)	24.55 (24.58)
10	$C_{17}H_{16}ClN_5OS$	144-45 (Ethanol)	Off-white	48	54.61 (54.63)	4.31 (4.33)	18.73 (18.77)
11	$C_{17}H_{15}Cl_2N_5OS$	160-63 (Ethanol)	Pale-Brown	55	50.01 (50.04)	3.70 (3.72)	17.15 (17.20)
12	$C_{17}H_{15}BrClN_5OS$	169-72 (Acetone)	Pale-Brown	41	45.10 (45.14)	3.34 (3.32)	17.15 (17.19)
13	$C_{18}H_{18}ClN_5O_2S$	Oily $CHCl_3$/pet. ether	Yellow	38	53.53 (53.50)	4.49 (4.47)	17.34 (17.39)
14	$C_{19}H_{21}ClN_6OS$	Oily ($CHCl_3$/pet. Ether)	Pale-Brown	29	54.73 (54.69)	5.08 (5.05)	20.16 (20.21)
15	$C_{17}H_{15}ClN_6O_3S$	147-49 (Acetone)	Yellow	31	48.75 (48.77)	3.61 (3.60)	20.06 (20.11)

Table-1: Physical and analytical data of newly synthesized compounds (1-15)

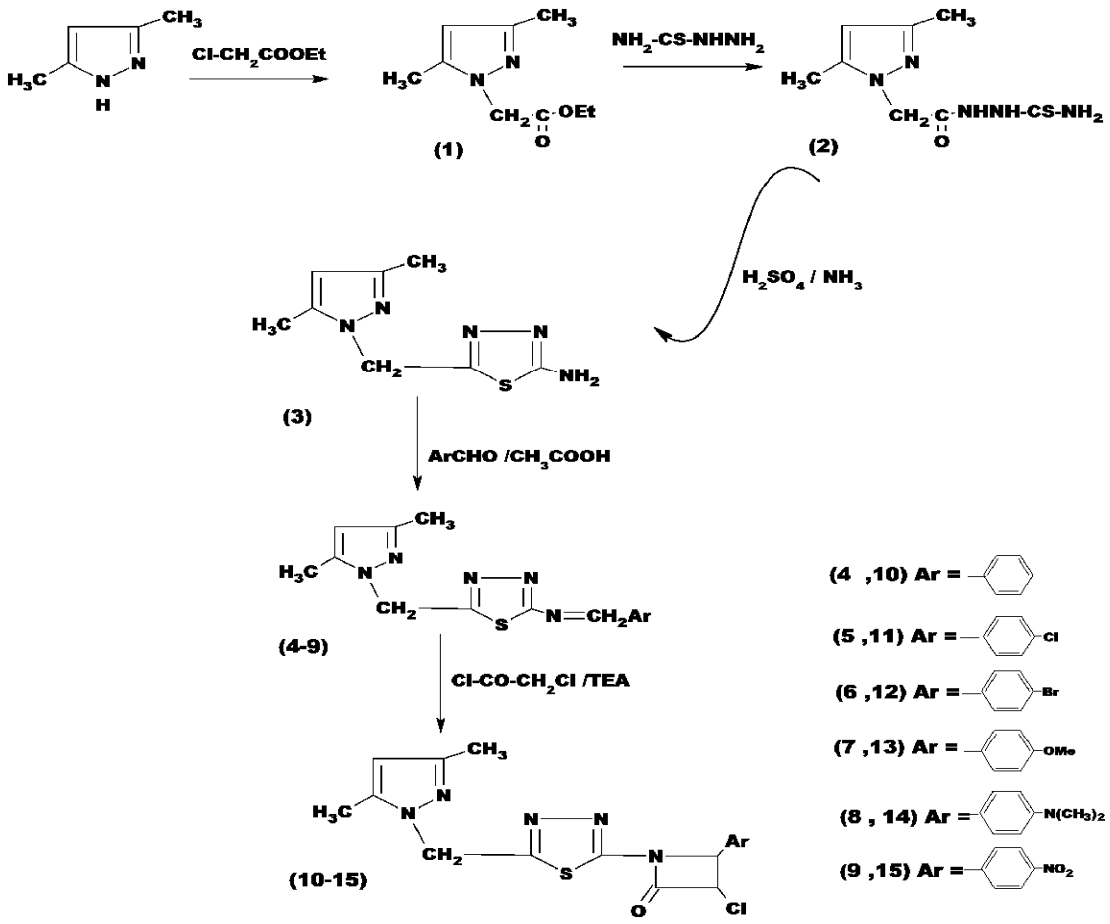
Biological Screening:

Compounds **(10-15)** were screened for their antibacterial activity against bacterial species *K.pneumoniae, E. Coli, S.aureus and B. subtilis* by agar well-diffusion method [12, 13] using Mueller Hinton agar medium to assess the activity

of the chosen synthesized compounds. The drug Streptomycin was tested under similar conditions for comparison. Wells were made (by scooping out medium with sterilized cork borer (6mm) in each plate which was streaked with test bacterial. Uniform volume of different concentration 250 µg/ml of the test synthesized compounds was placed in each of the cavity, after overnight incubation at 37 °C. The diameter of inhibition zone formed around the well was measured in mm. DMSO was used as a solvent for these compounds, which did not show any inhibition against test bacteria.

Results and discussions:

3,5-dimethyl-1H-pyrazole was reacted successfully with ethyl chloro acetate in refluxing to give **(1)** was used as a key compound for this study and for further syntheses of other fused heterocyclic Synthetic route of outlined in Scheme-1 .

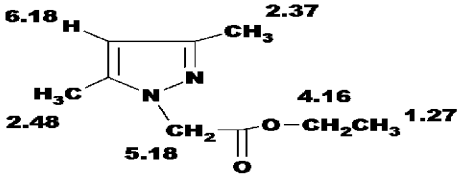


Scheme-1

The FTIR spectra of compound **(1)** showed two sharp absorption bands, one of which appearing at 1724.01cm^{-1} was attributed to carbonyl frequency corresponding to acyl carbonyl, and the other observed at 1595.18 cm^{-1} was

assigned to (C=N) stretching frequency. In addition the absence of stretching frequency of the (N-H) band at 3132.50 cm^{-1} in starting material.

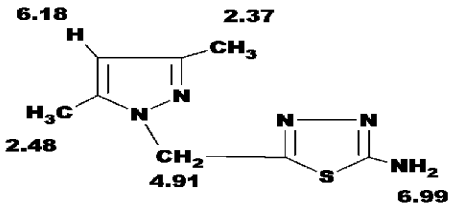
The ^{1}H-NMR spectrum of compound **(1)**, showed signals at δ (6.18 ppm) integrated for one proton of 1- Pyrazole ring. A signal at δ (5.18 ppm) integrated for two protons of methylene group. Multiple signal for the methylene protons appeared at δ (4.16 ppm). A signal at δ (2.37-2.48 ppm) integrated attributed for the six protons of the methyl group. Finally triplet signal at δ (1.27 ppm) integrated for three protons of methyl group.



Compound **(2)** was obtained in good yield when the compound **(1)** was allowed to condense with thiosemicarbazide in methanol. The characteristic bands in FTIR spectrum of compound **(2)** showed the important strong band at 3410.88, 3253.54 cm^{-1} attributed to the (NH_2) group, 3361.32 cm^{-1} attributed to (NH) ,band at 1599.04 due to (C=N) stretching vibration ,band at 1647.28 cm^{-1}(C=O) and band at 1107.18cm^{-1} for(C=S) stretching vibration.

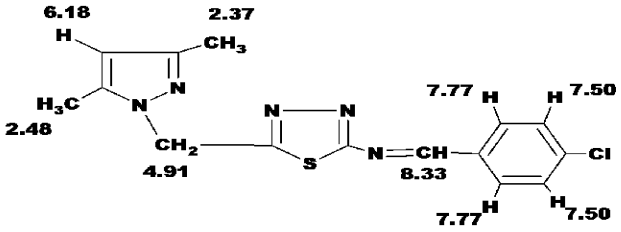
Dehydrative annulations of the compound **(2)** by mineral acid afforded compound **(3),** The FTIR spectrum of this compound, showed a moderately strong band at 3394.29 cm^{-1} due to (NH_2) stretching vibrations, at 1638.13 ,1126.14, 1070.01 cm^{-1} for (thiadiazole ring) [14] and 1626.54 cm^{-1} for (C=N) stretching vibration. Disappearance of the stretching vibration of (C=O) which is a good indication for successful condensation.

The ^{1}H-NMR spectrum of compound **(3)** showed singlet at δ (2.35 and 2.45 ppm) integrated for six protons attributed to the two methyl groups. The spectrum also shows signal at δ (4.91) ppm integrated for two protons assigned for the protons of the methylene group. The spectrum also shows signal at δ (6.18) ppm integrated for one proton assigned for the proton of the 1-pyrazole ring. Protons of the two terminals (NH_2) groups showed signal at δ (6.99) ppm integrated.

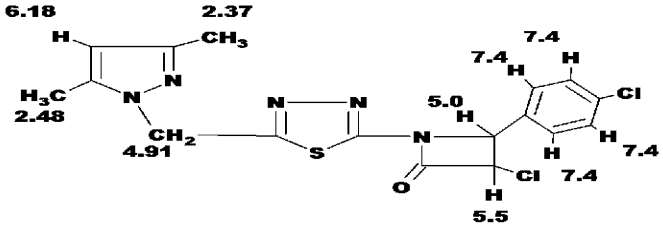


The FTIR spectrum of synthesized Schiff's bases **(4-9),** showed the disappearance of absorption band of (NH_2) group in compound **(3)** and appearance of a band at (1570.08-1546.39 cm^{-1}) due to (C=NH) stretching vibration. The ^{1}H-NMR spectrum of compound **(5)**, showed a signal at δ (8.33 ppm) integrated for one proton of (N=CH) group. Multiple signals for the

aromatic protons appeared as AB quartet at δ (7.50-7.77ppm) integrated for four protons.



Finally on reaction the synthesized Schiff's bases **(4-9),** with chloroacetyl chloride in the presence of triethyl amine afforded the product **(10-15)** , after isolation showed an FTIR spectrum which exhibited bands at 1740.30-1761.33 cm^{-1} (C=O of the ring), band at 700.03 - 806.19 cm^{-1} stretching vibration for(CH-Cl). The ^{1}H-NMR spectrum of compound **(11)**, Aromatic protons appeared as AB quartet at (7.0 and 7.5 ppm) for p-substituted ring integrated for four protons. A douplet at δ (5.0 and 5.5) ppm, integrated for one proton, which may be attributed to the proton of each propiolacton ring.



Spectral data for new compounds (1-15) shown in table-2.

Antibacterial Activity:

Some of synthesized compounds **(10-15)** were tested for antibacterial activity aganist bacterial species *K.pneumoniae*, *E. coli*, *S.aureus and B. subtilis*. DMSO was used as diluents to get desired concentration (250 µg/ml) of Streptomycin drugs to test upon standard bacterial species. The zone inhibition was measured in mm. The standard drug used was Streptomycin. The results of activity summarized in Table 3. From the table 3 it's clear that antibacterial results were found uneven. The compounds **(12- 15),** showed good activity against all bacterial species. The compounds **(10, 11)** exhibited moderate antibacterial activity against (*K.pneumoniae, B. Subtilis)*. The antibiotic Streptomycin shows 100% inhibition against all tested bacteria. Antibacterial activity of synthesized compounds (10-15) shown in table-3.

Comp. No.	*v* (C=N) cm^{-1}	*v* (C=O) cm^{-1}	*v* (C-H)al. *v* (C-H)ar. cm^{-1}	Others cm^{-1}
1	1595.18	1724.01	2881.75 -------	1446.66 $(C\text{-}CH_3)_{st}$ 1278.85 $(N\text{-}CH_2)_{st}$
2	1599.04	1647.28	2850.34 ------	3410.88,3253.54 $(NH_2)_{st}$ 3361.32 $(N\text{-}H)_{st}$ 1107.18 $(C{=}S)_{st}$
3	1638.13(thiadiazole ring) 1626.54	--------	2969.29 -----	3394.29 $(NH_2)_{st}$ 1126.14 $(N\text{-}CH_2)_{st}$ 1070.01 $(C\text{-}S)_{st}$
4	1632.99(thiadiazole ring) 1576.11	--------	2880.88 3077.12	1160.10 $(N\text{-}CH_2)_{st}$ 1068.55 $(C\text{-}S)_{st}$
5	1640.37(thiadiazole ring) 1559.01	-------	2880.88 3077.12	720.20 $(C\text{-}Cl)_{st}$
6	1642.00(thiadiazole ring) 1566.90	--------	2872.39 3091.00	607.79 $(C\text{-}Br)_{st}$
7	1628.22(thiadiazole ring) 1601.37	--------	2880.66 3061.07	1263.42 $(OCH_3)_{st}$
8	1638.98(thiadiazole ring) 1544.30	--------	2855.34 3043.07	1437.66$(N\text{-}CH_3)_{st}$
9	1644.11(thiadiazole ring) 1610.02	-------	2891.05 3090.22	1580.26, 1265.35 $(NO_2)_{st}$
10	1636.49(thiadiazole ring) 1576.11	1740.30	2881.00 3070.17	700.033 $(C\text{-}Cl)_{st}$
11	1646.01(thiadiazole ring) 1559.55	1748.99	2907.22 3070.00	806.19 $(C\text{-}Cl)_{st}$
12	1650.10(thiadiazole ring) 1569.73	1752.49	2870.84 3086.39	777.21 $(C\text{-}Cl)_{st}$ 616.11 $(C\text{-}Br)_{st}$
13	1629.71(thiadiazole ring) 1609.39	1744.81	2977.29 3065.05	714.033 $(C\text{-}Cl)_{st}$ 620.966 $(C\text{-}S\text{-}C)_{st}$ 1169.78 $(OCH_3)_{st}$
14	1648.00(thiadiazole ring) 1540.30	1749.06	2904.33 3041.79	779.00 $(C\text{-}Cl)_{st}$ 1437.66 $(N\text{-}CH_3)_{st}$
15	1641.71(thiadiazole ring) 1620.11	1761.33	2901.11 3086.64	770.99 $(C\text{-}Cl)_{st}$ 1580.26,1265.35 $(NO_2)_{st}$

Table-2: Spectral data for new compounds (1-15)

Compound No.	*K.pneumoniae*	*E.coli*	*S.aureus*	*B. subtilis*
10*	6	10	11	6
11*	8	9	10	7
12*	14	11	16	10
13*	16	10	10	10
14*	17	11	11	12
15*	15	12	10	10
Streptomycin *	24	21	26	27

Table-3: Antibacterial activity of synthesized compounds (10-15) (Zone of inhibition in mm). *250 µg/ml

Reference:

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