Synthesis, Characterization and Pharmacological Studies of Some New Furosemide Derivatives

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Abstract

Furosemideis used as synthetic intermediates for the preparation of new heterocyclic derivatives biomolecules that possesses a pharmacological activity such as pyrazole, pyrrole and pyrimidine. The new derivatives synthesized compounds were confirmed using spectroscopic methods (FTIR, 1H-NMR spectral) and the physicochemical studies were investigated. The activity of these compounds was then screened as potential antibacterial against different types of pathogenic bacterial isolates and also as acute toxicity. The results displayed that some of prepared compounds were the most or equal powerful compared with Amoxicillin and Cephalexin as references drugs.

Key Words: Furosemide, Synthesis, Characterization, Pharmacological Studies.

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تحضير وتشخيص والدراسة الدوائية لبعض مشتقات فوروسيميد الجديدة

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الخلاصة:

تماستخدام مركب فوروسيميد كوسيط رئيسي لتحضير مشتقات جديدة تحمل حلقات غير متجانسة نشطة بيولوجيا مثل البيرازول ، البيرول والبيريميدين. تم فحص المركبات المحضره حديثًا من خلال FTIR, 1H-NMRالدراسات الطيفية والفيزيائية والكيميائية ومضادات للجراثيم المحتملة ضد أنواع مختلفة من المضادات البكتيرية المسببة للأمراض وكذلك السمية الحادة. أظهرت النتائج أن بعض المركبات المحضرة كانت الأكثر قوة أو متساوية مقارنة مع الأموكسيسيلين والسيفاليكسين كمراجع للأدوية

الكلمات المفتاحية: فوروسيميد ، تحضير ، تشخيص ، الدراسات الدوائية

Introduction

Sulfonamides bioactive compounds include the subclass of drugs with various important directions in medical applications such as antimicrobial agents, [1] diuretics,[2]antidepressants,[3,4]inhibitors for carbonic anhydrase, [5,6]anti-viral [7] and anti-cancer agents.[8] Furosemide, 4-chloro-2-(furan-2-ylmethylamino)-5-sulfamoylbenzoic acid, a drug of the sulfonamide type used to treatment of edema cases of liver,[9] heart failure[10] and kidney diseases [11] origin. This effective drug can be used also to treat hypertension on its own or in conjunction with other factors for high blood pressure.[12]

Since the detection of E7010 in the early 1990s,[13] many classes of sulfonamide derivatives have been notified as potential anticancer drug candidates. These compounds proved various cellular mechanisms. According to the importance of these compounds to identify new molecules and due to greet attention in heterocyclic rings which show various biological and pharmaceuticals activities, the present study will describe synthesis a new series of heterogeneous cycles containing furosemide shipment starting with 4-chloro-2-((furan-2-ylmethyl)amino)-5-sulfamoylbenzoic acid for its anti-bacterial and acute toxicity assessments.

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Experimental Part

The starting key reagent (Furosemide) was supplied by Sigma-Aldrich. Other solvents and reagents used in this research are purchased from commercial suppliers and they were used without further purification. The melting points are identified on the digital STUART fusion point device and have not been corrected. FTIR spectra were registered on the FTIR-8400 infrared spectrometer using KBr disks in the spectral range (500-4000) cm^{-1}. ^{1}H-NMR spectra were recorded on a 400MHz Bruker instrument using DMSO-d6 as solvent and Tetra methyl saline as internal standard reference. The anti-bacterial and acute toxicity evaluation was conducted in the department of Biology, college of women, University of Baghdad.

Synthesis of 4-chloro-5-(N-(2,2-dicyanovinyl)sulfamoyl)-2-(furan-2-ylmethylamino)benzoic acid (1):

A mixture of 4-chloro-2-(furan-2-ylmethylamino)-5-sulfamoylbenzoic acid (Furosemide) (0.01 mol.), malononitrile (0.01mol.), triethylorthoformate (0.01mol.) and acetic acid (1ml.) in methanol (25 ml.) was refluxed for (6hrs.). The resulting reaction mixture was cooled to room temperature. And the formedsolid product was filtrated and then it was recrystallized from ethanol to afford compound (1).

Synthesis of 4-chloro-5-(N-((3,5-diamino-1-phenyl-1,5-dihydro-4H-pyrazol-4-ylidene)methyl)sulfamoyl)-2-(furan-2-ylmethylamino)benzoic acid (2):

To compound (1) 4-chloro-5-(N-(2,2-dicyanovinyl)sulfamoyl)-2-(furan-2-ylmethylamino) benzoic acid(0.01mol.),phenyl hydrazine (0.01mol.) was added in absolute dioxane (25 ml.) The obtained mixture was refluxed for (7 hrs.), cooled at room temperature, and then it was poured over crushed ice water. The solid product was filtered and recrystallized from methanol to afford compound (2).

Synthesis of 5-((3-substituted phenyl-5-cyano-4-oxo-2-thioxo-3,4-dihydropyrimidin-1(2H)-yl)sulfonyl)-4-chloro-2-(furan-2-ylmethylamino)benzoic acid (3-7)

The compound (1)(0.01mol.) was mixed with aryl isothiocyanate (0.01mol.) in ethanol containing sodium ethoxide (0.01mol.)and refluxed for (12hrs.). After completion of the reaction, the mixture was allowed to cool to room temperature, poured over crushed ice, and then some drops of diluted hydrochloric acid were added to acidify the product. The solid product formed was filtered and recrystallization from methanol was done to get the desired compounds (3-7) respectively.

Synthesis of 5-(N-(2-acetyl-3-oxobut-1-en-1-yl)sulfamoyl)-4-chloro-2-(furan-2-ylmethylamino)benzoic acid (8)

A mixture of 4-chloro-2-(furan-2-ylmethylamino)-5-sulfamoylbenzoic acid (Furosemide) (0.01 mol.), acetylacetone (0.01mol.), triethylorthoformate (0.01mol.) and acetic acid (1 ml.) in methanol (25 ml.) was refluxed for (7hrs.). The obtained solid product was filtered and then recrystallized from ethanol to get the desired compound (8).

Synthesis of 5-((3-amino-2,4-dicyano-1H-pyrrol-1-yl)sulfonyl)-4-chloro-2-(furan-2-ylmethylamino)benzoic acid (9)

Compound (1) 4-chloro-5-(N-(2,2-dicyanovinyl)sulfamoyl)-2-(furan-2-ylmethylamino) benzoic acid (0.01mol.) was mixed with 2-chloroacetonitrile (0.01mol.) in dioxane (25ml.) containing few drops of triethylamine and refluxed for (7hrs.). The resulting mixture was left to cool to room temperature, poured over crushed ice water. The solid products formed were filtered and recrystallized from methanol to get compound (9).

Synthesis of 4-chloro-5-(N-((4,6-diamino-2-thioxopyrimidin-5(2H)-ylidene)methyl)sulfamoyl)-2-(furan-2-ylmethylamino)benzoic acid (10)

A mixture of compound (1) 4-chloro-5-(N-(2,2-dicyanovinyl)sulfamoyl)-2-(furan-2-ylmethylamino) benzoic acid (0.01mol.) and thiourea (0.01mol.) in ethanol containing sodium ethoxide (0.01 mol.) was refluxed for (7hrs.) The mixture was left to cool, poured over crushed ice bath, acidified with dilutehydrochloric acid. The formed precipitate was filtered and recrystallized from methanol to afford compound(10).

General Procedure for Synthesis of 4-chloro-5-((5-cyano-4-oxo-3-phenyl-2-(2-phenylhydrazineylidene)-3,4-dihydropyrimidin-1(2H)-yl)sulfonyl)-2-(furan-2-ylmethylamino)benzoic acid (11)and 4-chloro-5-((5-cyano-2-hydrazineylidene-4-oxo-3-phenyl-3,4-dihydro pyrimidin-1(2H)-yl)sulfonyl)-2-(furan-2-ylmethylamino)benzoic acid (12)

Compound (3) 4-chloro-5-((5-cyano-4-oxo-3-

phenyl-2-thioxo-3,4-dihydropyrimidin-1(2H)-yl)sulfonyl)-2-(furan-2-ylmethylamino)benzoic acid (0.01 mol.) and either hydrazine hydrate or phenyl hydrazine (0.01mol.) were mixed in dioxane (25ml.) and refluxed for (8hrs.). The resulting products was left to cool, and then poured onto ice bath. The obtained solid products were filtered and recrystallized from methanol to afford compounds (11) and (12) respectively.

In-vitro Pharmacological Screening

Antibacterial Activity Assay

Evaluation of antibacterial activity of some newly studied compounds were determined by agar diffusion method at concentration (1mg), DMSO served as control due to this there was no visible change in bacterial growth. Amoxicillin and Cephalexin were used a standard drug and the zones of inhibition were recorded in millimeters after incubation of plates for 24hours at 37°C .

Acute Toxicity

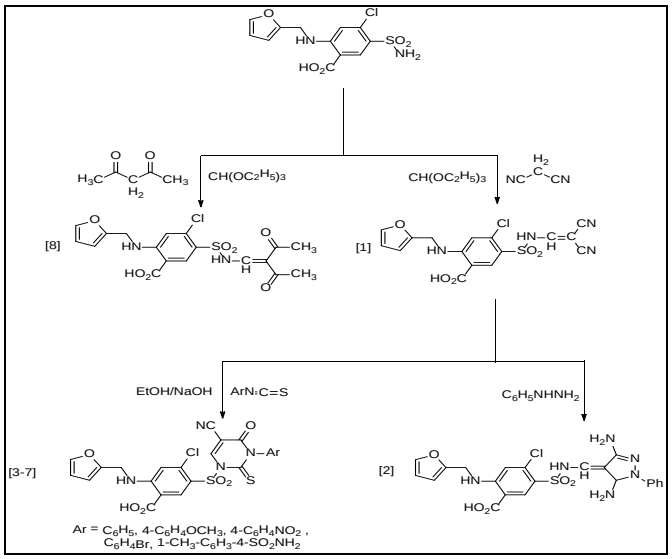
Six-groups of six mice/group, one dose was given to each group, after were monitored for 24hrs give the result that listed in table that show some prepared compounds did not kill the same number of mice as lethal dose for standard drugs that indicate these compound have higher lethal dose than drugs (Amoxicillin and Cephalexin). Therefore these selected prepared compounds more safety (low toxic) than drugs that derived from.

Result and Discussion

Chemistry

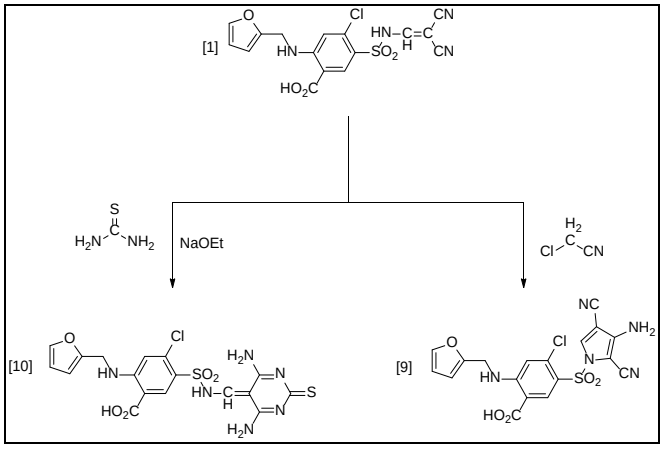
In this research, the starting material Furosemide, with active methylene containing compounds (acetylacetone, malononitrile and 2-choloroacetontrile) was considered. The synthetic approach assumes by adding triethylorthoformate in methanol and the presence of amount of acetic acid as catalytic was succeeding the reaction condition. [16]

The new derivatives obtained of pyrazole, pyrrole and pyrimidine were synthesized due to the high importance biologically of these heterocyclic rings as anti-bacterial agents and acute toxicity. [17-19]The furosemide derivative (1) was reacted with different nucleophiles to obtain pyrazole (2), pyrrole (9) and pyrimidine (3-7 and 10) derivatives. Also, the reaction of compound (1) with phenyl hydrazine affords the corresponding pyrazole derivative (2). The reactivity of compound (1) towards different aryl isothiocyanates in presence of basic media NaOH was done and the reaction progress via adding reactionmixture onto the isothiocyanato group. After that intramolecular cyclization to produce the pyrimidine ring was followed as shown in compounds (3-7). The synthetic pathways for compounds (1-8) are presented in Scheme (1).



Scheme (1)

Further, the reaction of compound (1) with 2-chloroacetonitrile in dioxanein presence of a catalytic amount of triethylamine produced the analogicalpyrrole derivatives (9). In another hand, the interaction of compound (1)with Thiourea and refluxed with mixture of ethanol and sodium ethoxide afforded the corresponding pyrimidine derivative (10). Synthetic pathways for compounds (9,10) are displayed in scheme (2).

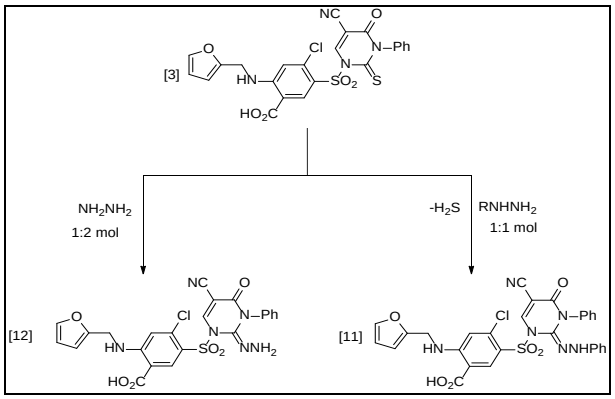


Scheme (2)

The reaction of compound (3) with hydrazine hydrate afforded compound (11) where its reaction with phenyl hydrazine afforded the compound (12)

which were the hydrazono pyrimidine derivatives. These reactions proceeded by elimination of hydrogen sulfide and using a lead acetate paper to identify this gas. The synthetic pathways for compounds (11,12) are shown in scheme (3).

The structures of all compounds were proved by physicochemical and spectral data (FTIR, ^{1}H-NMR) as shown in Tables (1,2 and 3) respectively were consistent with the proposed structures.



Scheme (3)

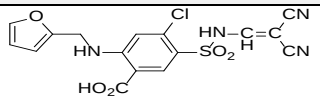
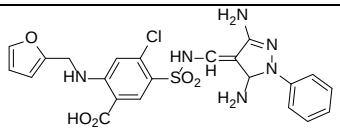
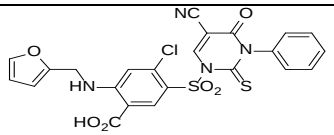
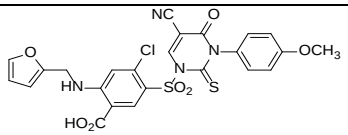
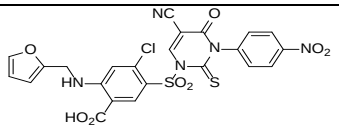
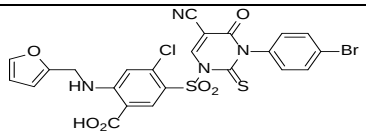
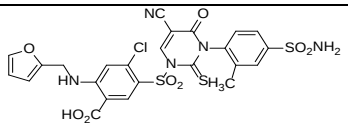
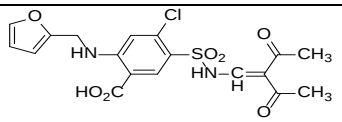
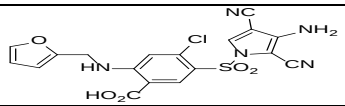
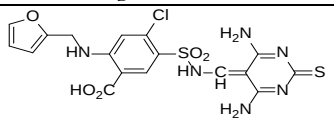
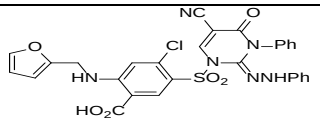
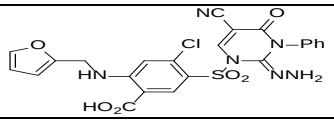
Table 1. Physico-chemical properties of synthesized compounds (1-12)

Comp. no.	Mol. formulas	Yield (%)	m. p. °C.	Color	Solvent Recryst.
1	$C_{16}H_{11}ClN_4O_5S$	78	147-148	Off white	Ethanol
2	$C_{22}H_{21}ClN_6O_5S$	65	179-180	White	Ethanol
3	$C_{23}H_{15}ClN_4O_6S_2$	71	166-168	Brown	Ethanol-water (1:1)
4	$C_{24}H_{17}ClN_4O_7S_2$	58	155-157	White	Ethanol
5	$C_{23}H_{14}ClN_5O_8S_2$	67	160-161	Light yellow	Methanol
6	$C_{23}H_{14}BrClN_4O_6S_2$	73	190-192	Brown	Methanol
7	$C_{24}H_{18}ClN_5O_8S_3$	76	185-186	Off white	Methanol
8	$C_{18}H_{17}ClN_2O_7S$	60	201-203	Light Brown	Methanol
9	$C_{18}H_{12}ClN_5O_5S$	63	173-174	Off white	Methanol
10	$C_{17}H_{15}ClN_6O_5S_2$	69	159-161	Brown	Methanol
11	$C_{29}H_{21}ClN_6O_6S$	75	194-196	White	Ethanol-water (1:1)
12	$C_{23}H_{17}ClN_6O_6S$	72	188-190	White	Ethanol-water (1:1)

Table 2: FTIR ν(cm^{-1}) spectral data of synthesized compounds (1-12)

Comp No.	ν(N-H)	ν(O-H)	ν(C-H) Ar.	ν(C=O) carboxyl	ν(C=N)	ν(C=C) Ar.	ν(SO_2) Asym.	Others
1	3321	3244	3065	1736	-	1551	1373	ν(CN) 2252
2	3328	3238	3055	1738	1608	1561	1332	ν(NH_2) 3422
3	3329	3257	3056	1731	1599	1538	1367	ν(CN) 2248
4	3325	3252	3071	1726	1598	1539	1339	ν(CN) 2245
5	3331	3239	3045	1728	1596	1529	1344	ν(CN) 2249
6	3330	3249	3078	1735	1605	1533	1371	ν(CN) 2251
7	3318	3261	3062	1739	1602	1542	1363	ν(CN) 2255
8	3322	3242	3043	1734	1603	1547	1351	-
9	3321	3262	3072	1729	1598	1552	1336	ν(CN) 2234
10	3327	3238	3051	1722	1613	1537	1346	-
11	3323	3258	3044	1727	1597	1528	1342	ν(CN) 2257
12	3319	3251	3038	1734	1612	1536	1338	ν(CN) 2259

Table 3. ^{1}H-NMR spectral data (δppm) of synthesized compounds (1-12)

Comp. No.	Comp. Structure	^{1}H-NMR parameters (δppm)
1		4.16 (s,2H, CH$_2$-NH, methylene), 6.21 (s, 1H, NH, N-sulfonamide), 6.88-7.79 m (2H, Ar-H), (3H, furan ring), (1H, NH-CH=C-), 8.44 (s, 1H, NH sec. amine), 12.63 (s, 1H, OH).
2		4.27 (s,2H, CH$_2$-NH, methylene), 6.28 (s, 1H, NH, N-sulfonamide), 6.85-7.82 m (7H, Ar-H), (3H, furan ring), (1H, NH-CH=C-), 8.33 (s, 1H, NH sec. amine), 8.71(s, 2H, NH$_2$ primary amine),12.52 (s, 1H, OH).
3		4.38 (s,2H, CH$_2$-NH, methylene), 6.16 (s, 1H, NH, N-sulfonamide), 6.93-7.88 m (7H, Ar-H), (3H, furan ring), (1H, pyrimidine ring), 8.37 (s, 1H, NH sec. amine), 12.21 (s, 1H, OH).
4		3.66 (s, 3H, OCH$_3$,methoxy), 4.22 (s,2H, CH$_2$-NH, methylene), 6.18 (s, 1H, NH, N-sulfonamide), 6.85-7.92m (6H, Ar-H), (3H, furan ring), (1H, pyrimidine ring), 8.41 (s, 1H, NH sec. amine), 12.35 (s, 1H, OH).
5		4.40 (s,2H, CH$_2$-NH, methylene), 6.23 (s, 1H, NH, N-sulfonamide), 6.74-7.93 m (6H, Ar-H), (3H, furan ring), (1H, pyrimidine ring), 8.46 (s, 1H, NH sec. amine), 12.37 (s, 1H, OH).
6		4.24 (s,2H, CH$_2$-NH, methylene), 6.29 (s, 1H, NH, N-sulfonamide), 6.78-7.89 m (6H, Ar-H), (3H, furan ring), (1H, pyrimidine ring), 8.44 (s, 1H, NH sec. amine), 12.52 (s, 1H, OH).
7		3.21 (s, 3H, CH$_3$, methyl), 4.31 (s,2H, CH$_2$-NH, methylene), 6.37 (s, 1H, NH, N-sulfonamide), 6.55 (s, 2H, NH$_2$, sulfonamide), 6.71-7.95 m (5H, Ar-H), (3H, furan ring), (1H, pyrimidine ring), 8.49 (s, 1H, NH sec. amine), 12.57 (s, 1H, OH).
8		2.96 (s, 6H, COCH$_3$),4.36 (s,2H, CH$_2$-NH, methylene), 6.41 (s, 1H, NH, N-sulfonamide), 6.87-7.59 m (2H, Ar-H), (3H, furan ring), (1H, NH-CH=C-), 8.59 (s, 1H, NH sec. amine), 12.61 (s, 1H, OH).
9		4.45 (s,2H, CH$_2$-NH, methylene),6.17 (s, 2H, NH$_2$) 6.81-7.83 m (2H, Ar-H), (3H, furan ring), (s, 1H, pyrrole ring), 8.52 (s, 1H, NH sec. amine), 12.39 (s, 1H, OH).
10		4.47 (s,2H, CH$_2$-NH, methylene), 6.11 (s, 4H, NH$_2$), 6.44 (s, 1H, NH, N-sulfonamide), 6.75-7.94 m (2H, Ar-H), (3H, furan ring), (1H, NH-CH=C-), 8.61 (s, 1H, NH sec. amine), 12.75 (s, 1H, OH).
11		4.41 (s,2H, CH$_2$-NH, methylene), 6.82-7.91 m (12H, Ar-H), (3H, furan ring), (1H, pyrimidine ring), 8.73 (s, 1H, NH sec. amine), 12.81 (s, 1H, OH).
12		4.37 (s,2H, CH$_2$-NH, methylene), 6.14 (s, 2H, NH$_2$), 6.63-7.90 m (7H, Ar-H), (3H, furan ring), (1H, pyrimidine ring), 8.58 (s, 1H, NH sec. amine), 12.63 (s, 1H, OH).

In-vitro Pharmacological Activity

Antibacterial Screening

Some of newly prepared compounds were evaluated for antibacterial activity against different bacterial strain i.e. gram positive bacteria: Bacillus subtitles, Staphylococcus aurous and gram negative bacteria: Escherichia coli, Pseudomonas aeruginosa at concentration (1mg/mL).

Inhibition zones caused by these compounds are determined and listed in Table (4).

Table 4: Antibacterial activity of some prepared compounds

Comp. No.	*Inhibition zone diameter(mm)*			
	Staphylococcus aurous	*Bacillus subtitles'*	*Escherichia coli*	*Pseudomonas Aeruginosa*
2	15	11	9	7
3	14	12	8	-
5	15	11	9	8
6	14	12	8	-
7	16	13	-	7
8	13	12	6	-
9	14	12	8	-
10	14	11	7	7
11	13	10	7	-
12	15	11	9	-
Amoxicillin [A]	14	11	8	-
Cephalexin [C]	12	9	8	-
DMSO	-	-	-	-

[Conc.]: 1mg/ml
Zone inhibition: (-) no inhibition zone

In general the result showed that some synthesized compounds exhibited antibacterial activity against *Pseudomonas aeruginosa* compared with standard drugs used. The compounds[2,5,7 and 12] exhibited antibacterial activity more than standard drugs. Figures [1-4] show effect of selected compounds on some types of these bacterial isolates.

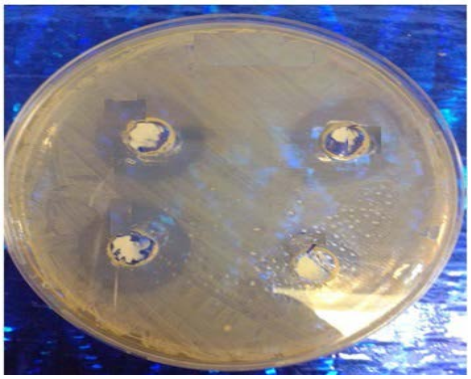


Figure 1. Effect of compounds on Staphylococcus aureus

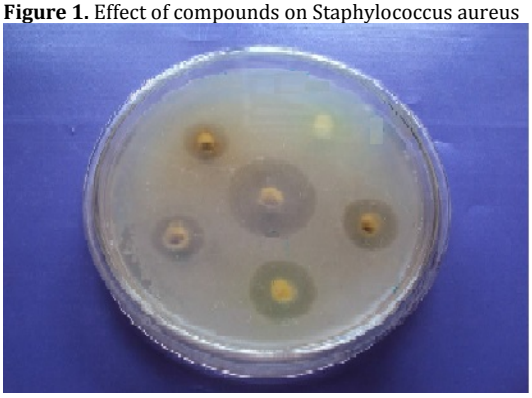


Figure 2. Effect of compounds on Bacillus subtitles

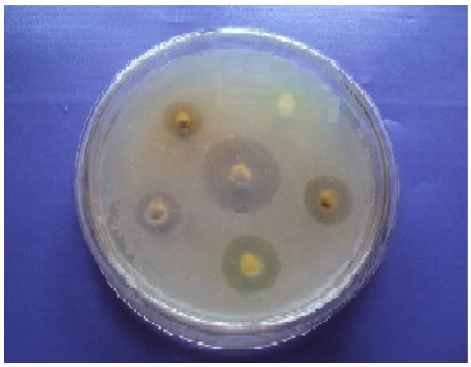


Figure 3. Effect of compounds on *Escherichia coli*

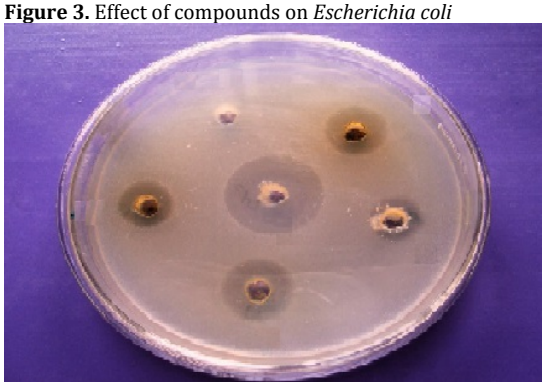


Figure 4. Effect of compounds on *Pseudomonas Aeruginosa*

Acute Toxicity Screening

In this research the Amoxicillin and Cephalexin was used as a positive controls, most of the common medications. The connection between surviving fraction and drug concentration was represented. IC_{50} value indicates the calculated response variables, which equals to the compound concentration give rise to 50% mortality in net cells and the results are given in Table (5).

Table 5. *In-vitro* acute toxicity investigation of selected compounds

Comp. No.	IC_{50}(µg/mL)	No. of animals	No. of animals dead
2	0.625	6	3
3	0.0374	6	2
8	0.625	6	2
9	0.0374	6	1
10	0.625	6	2
11	0.0374	6	3

Above table observed that some of the evaluated newly compounds were improve to be having moderate potencies with IC_{50} values extent from 0.374 to 0.625.65 µg/ml differentiated with standard drug.

Conclusions

In this study we have prepared successfully new derivatives from furosemide drug as starting material. The new derivatives based on pyrazole, pyrrole, and pyrimidine, which are biologically active heterocyclic compounds, which were

characterized spectroscopically. The results of antimicrobial activity demonstrate that some synthesized compounds exhibited equal or higher antibacterial activity against *Pseudomonas aeruginosa* compared with standard drugs used.

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