SYNTHESIS AND BIOLOGICAL EVALUATION OF SOME DIPEPTIDE DERIVATIVES AND THEIR HETEROCYCLIC COMPOUNDS

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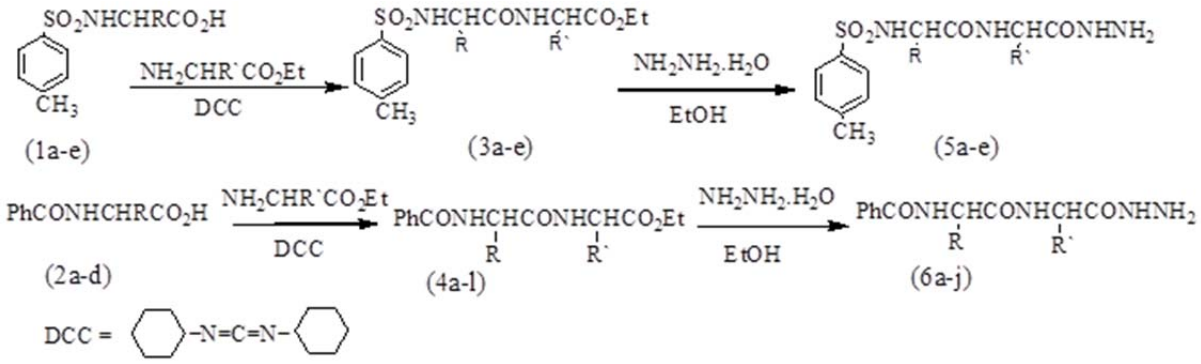
Abstract:

The protected dipeptide esters (3a-e, 4a-l) is prepared by the reaction of compounds (1a-e, 2a-d) with dicyclohexylcarbodiamide (as coupling reagent) and amino acid esters .Thereafter hydrazides (5a-e, 6a-j) are obtained by the reaction of corresponding esters with hydrazine hydrate. Hydrazones (7a-e, 8a-e) are synthesized by the reaction of the above hydrazides with p-nitro benzaldehyde , which was cyclized to 2,5-disubstituted 1,3,4- oxadiazole (9a-e, 10a-e) through lead oxide and to phthalazines (13a-b) through hydrochloric acid. 1,3,4- oxadiazole -2- thione (11a-b, 12a-e) were prepared by the reaction of the corresponding hydrazides with carbon disulfide in alcoholic potassium hydroxide. Hydrazides were reacted with ammonium thiocyanate to afford thiosemicarbazide (14a-c) which were cyclized to 1,2,4-triazole -3- thione (15a-b) in sodium hydroxide medium. The structures of the synthesized compounds were confirmed by physical and spectral methods. The antibacterial activity of the prepared compounds (5d, 7a, 9e, 10d, 11a, 12e, 13a, 14b) against the gram +ve and –gram –ve Bactria (*Staphylococcus aureus, Bacillus subtilis, Escherichia coli* and *Proteus mirabilis*) were studied and discussed.

Introduction

Oxadiazole derivatives displayed wide spectrum of activities such as antibacterial(Salimon et al 2011; Jumat et al 2010), antimicrobial (Karthikeyan et al 2008; Gaonkar and Rai 2006), anti-fungal (Kanthiah et al 2011; Srivastava et al 2010) analgesic (Amir and Kumar 2007), as anticonvulsant (Zargahi et al 2005; Rastogi et al 2006), antitumor (Bezerra et al 2005), anti-tubercular (Dhoel et al 2005), antihypoglycemic (Goankar et al 2006) and anti-hepatitis B viral activities (Tan et al 2006). Compounds bearing 1,3,4- Oxadiazole nucleus are known to exhibit unique anti-edema and anti-inflammatory activity (Husain and Ajmal 2009; Franski 2005; Amir and Kumar 2007). Some of 2,5-disubstituted-1,3,4-oxadiazole derivatives used against 60 tumor cell lines derived from nine cancer cell type, anti-tumor activity against leukemia, colon and breast cancer (Wagle et al 2008; Aboraia et al 2006) While compounds having 1,2,4-triazole nucleus are very important in the field of medicinal chemistry, as fungicidal (Royr et al 2005), antibacterial (Singh and Singh 2009), antimicrobial (Hussain et al 2008), antimycotic (Zamani et al 2004), antidepressive (Clerici et al 2001), cardiotonic (Onkol et al 2004) and anticonvulsant (Parmar et al 1974) activites. Triazole ring derivatives are known to possess anti-inflammatory (Moise et al 2009), analgesic (Shenone et al 2001) antihaemostatic activity (Kamble and Sudha 2006) and for anti-HIV-1 activity by examination of their inhibition of HIV-1-induced cytopathogenicity in MT-4 cells and by determination of their inhibitory effect on HIV-1 reverse transcriptase (Wu et al 2007) On the other hand, heterocycles containing the phthalazine moiety are of interest due to their pharmacological and biological activities (Jain and Vederas 2004). Some of the phthalazinone derivatives have found application in clinical medicine due to interesting vasodialator (Demirayak et al 2004), anticonvulsant (Zhang et al 2009), antidiabatic (Boland et al 1993), antiallergic (Hamamoto et al 1993), and antiasthmatic (Yamaguchi et al 1993). Phthalazinone nucleus has been proved to be a versatile system in medicinal chemistry such as, aldose reductase inhibitor activities (Kashima et al 1998). According to the above and due to the significance of peptides in antibiotic activity; we made a combination of peptide and heterocycles nucleus in a goal to increase the biological effects.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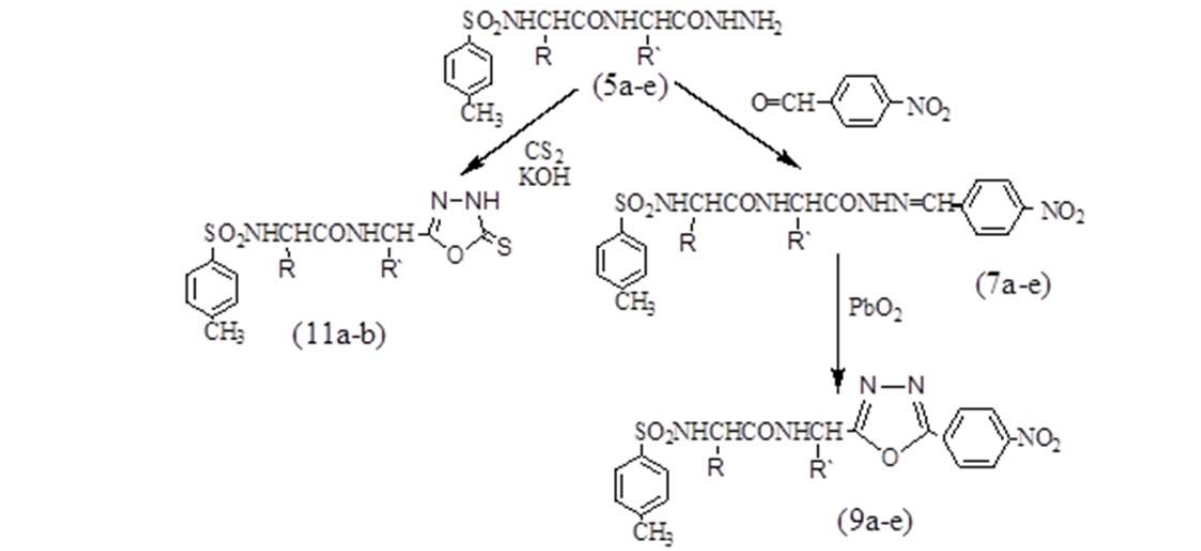
NH2CHR`CO2Et
DCC
SO2NHCHCONHCHCO2Et
NH2NH2.H2O
EtOH
SO2NHCHCONHCHCONHNH2
(1a-e)
(3a-e)
(5a-e)
PhCONHCHRCO2H
PhCONHCHCONHCHCO2Et
PhCONHCHCONHCHCONHNH2
(2a-d)
(4a-l)
(6a-j)
DCC =
-N=C=N-
R,R` = residue of amino acid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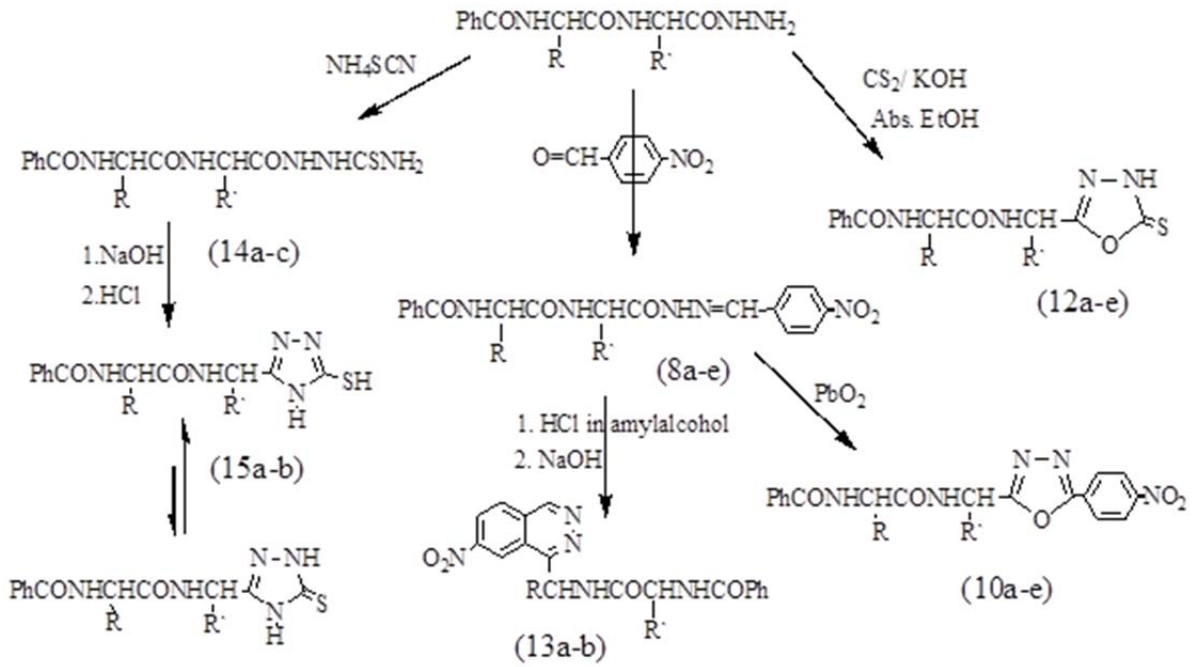
CS2
KOH
O=CH-NO2
(11a-b)
(7a-e)
PbO2
(9a-e)


PhCONHCHCONHCHCONHNH2
NH4SCN
CS2/ KOH
Abs. EtOH
O=CH-NO2
PhCONHCHCONHCHCONHNHCSNH2
(14a-c)
1.NaOH
2.HCl
(12a-e)
PhCONHCHCONHCHCONHN=CH-NO2
(8a-e)
PbO2
1. HCl in amylalcohol
2. NaOH
(15a-b)
(10a-e)
RCHNHCOCHNHCOPh
(13a-b)

Experimental

Uncorrected melting points were determined by using bibby scientific limited stone, Staffordshire, ST 15 OSA, UK., IR spectra were recorded as KBr disc in the (400-4000 cm^{-1}) range by using (spectrum one B FT-IR spectrometer), ^1H NMR spectra in δ units (ppm) relative to an internal standard of tetramethyl silane on ^1H NMR (NMR: BRUKER400 MHz Ultrashield^{TM}) in DMSO-d6, In Department of chemistry, faculty of Science, Diçle University, in Turkey. Amino acids esters and benzoyl amino acids were prepared according to literature procedure (Basheer 2000), p-toluene sulfonyl amino acids were prepared following literature reported procedure (Qreenstien and winitz 1961).

Synthesis of protected dipeptide ester (3a-e, 4a-l)

To a solution of 0.01 mole protected amino acid and 0.01mole of amino acid ester in 50 ml. of dichloromethane is added 0.01 mole N,N'-dicyclohexylcarbodiimide the mixture is allowed stirring over night at room temperature ,The precipitated dicyclohexylurea is removed by filtration and the filtrate washed with water , diluted hydrochloric acid , water , half saturated sodium bicarbonate solution and water, and finally dried over anhydrous sodium sulfate. Evaporation of the solution gives residual mixture of crystals and oil. This is treated with a small amount of diethylether and filtrate; although the material is quite soluble in diethylether and hence is lost in appreciable amount when this solvent is employed, the white precipitate is recrystallized from acetone – petroleum ether.

Synthesis of protected dipeptide hydrazide (5a-e, 6a-j)

A mixture of protected dipeptide ester (0.01mole) and hydrazine hydrate (0.2mole) in absolute ethanol (70 ml) was refluxed for (3) hours. The solvent was evaporated under reduced pressure and the residue was crystallized from methanol.

Synthesis of 4-nitrobenzaldehyde protected dipeptide hydrazone (7a-e,8a-e)

A mixture of p-Nitrobenzaldehyde (0.01mole) and (0.01mole) of protected dipeptide hydrazide (5a-e,6a-e) in (20 ml) ethanol was refluxed for 2 hrs. The solvent was concentrated and the precipitate was filtered and recrystallized from benzene.

Synthesis of 2-protected dipeptide residue, 5-(4´-nitrophenyl) -1, 3, 4-oxadia-zole (9a-e, 10a-e)

To a homogenous solution of hydrazone (7a-e,8a-e) (0.01mole) in 20 ml glacial acetic acid, lead oxide (pbO_2) (0.01mole) was added, the mixture was stirred at 25 C° for 1 hr. The reaction mixture was diluted with ice- water and left to stand for 24 hrs. The precipitate was filtered off and recrystallized from benzene.

Synthesis of 5-protected dipeptide residue -1,3,4-oxadiazole -2-thione(11a-b, 12a-e)

(0.05mole) of the protected dipeptide hydrazide (5a-b,6a-e) was dissolved separately in (70 ml) 0.5% ethanolic potassium hydroxide. (0.1 mole, 6 ml) of carbon disulfide was added gradually and the resulted mixture was refluxed for 16 hours until the evolution of hydrogen sulfide was ceased (checked by filter paper moister with lead acetate). The solvent was evaporated under reduced pressure and the residue was poured on crushed ice, diluted with ice-water, acidifies with diluted HCl, filtered and dried recrystallized from chloroform.

Synthesis of 1-(benzoyl dipeptide residue)-7-nitrophthalazine (13a-b)

(0.002mole) of the hydrazones (8a-b) in (10 ml) of amyl alcohol (saturated with HCl gas) was heated on steam bath for (1.5 hrs.) and then reflexed for (1hr.). The reaction mixture was cooled, washed with (10 ml) of (20%) sodium hydroxide and then with water until neutralized. Evaporation of the solvent and recrystallization from ethanol afforded the product (13a-b).

Synthesis Substituted thiosemicarbazide (14a-c)

A mixture of (0.003mole) benzoyl dipeptide hydrazide (6a-c), (0.009mole) of ammonium thiocyanate, (4 ml) hydrochloric acid in (25 ml) absolute ethanol, was reflexed for 22 hrs. The solvent was evaporated and the residue poured on crushed ice. The precipitated product was filtered, dried and recrystallized from ethanol.

Synthesis 5-substituted-1,2,4-triazole-3-thione (15a-b)

A mixture of substituted thiosemicarbazide (14a-b) (0.0012mole) and (7.5 ml) 1% aqueous sodium hydroxide solution was refluxed for 3 hrs. , the mixture was treated with charcoal and

the charcoal then removed by hot filtration. The solution was acidified by 10% hydrochloric acid with cooling; the precipitate was filtered, and recrystallized from methanol.

Antibacterial assay

Discs of filter paper (6mm diameter) were sterilized at 140 Cº for 1hr. and impregnated with 1ml. of stock solution (10mg. /ml, 1mg. / ml, 0.1mg. /ml, and 0.01mg. /ml) of each compound and then dried-DMSO (dimethyl sulfoxide) was used as a solvent for compounds (5d, 7a, 9e, 10d, 11a, 12e, 13a, 14d). The same solvent was used for antibiotics. Blank paper of DMSO was used as control. The inoculated plates were incubated at 37 Cº for 24 hrs. And the inhibition zones (mm) were measured. In all experiments, the mean of each triplicate was measured (Garrod et al 1981).

Results & Discussion

Protected dipeptide esters were prepared from the reaction of p-toluene sulfonyl amino acid with amino acid ester by using N,N-dicyclohexylcarbodiimide (DCC) as coupling group. The synthesized compounds (3a-e,4a-l) were characterized by their IR and ^{1}H NMR spectra, the IR spectra data provide evidence in support of structures (3a-e,4a-l) for these series of compounds in which characteristic bands at 3373 -3268cm^{-1} for N-H stretching, 1651-1610 cm^{-1} for C=O (phCONH) stretching, 1660-1626cm^{-1} for C=O amide stretching, 1751-1721cm^{-1} for C=O ester stretching, as illustrated in Table (1). The ^{1}H NMR spectra (Table 4) of the compounds (3a,e 4c,j) indicated the presence of the ethyl group resonating triplet signals at the region of 1.3 -0.7ppm for -CH_3 group and quartet for -CH_2- group at the region ٤-3.1 ppm, aromatic protons appeared in the expected range δ 7.9-7.0 ppm, finally the two amide protons occurred at the relatively downfield positions of 8.9- 8.1. Protected dipeptide hydrazides were prepared by the reaction of their corresponding ester with hydrazine hydrate in absolute ethanol. The structures of hydrazide compounds (5a-e, 6a-j) are confirmed on the basis of the following evidence. The IR showed the characteristic absorption bands as follow 3253-3325 cm^{-1} (NH), 1629-1603cm^{-1} C=O (phCO-) and 1656-1605 cm^{-1} (C=O amide). In addition of absence of band for C=O of ester, as illustrated in Table (2).The ^{1}HNMR spectrum of the compounds (6e,6j) showed a signal at 4.5ppm indicating the presence of NH<u>NH_2</u> protons, absence the protons of the ethyl group and other signals were observed at appropriate places, as illustrated in Table (4).

Hydrazone compounds (7a-e, 8a-e) were prepared by the condensation reaction of protected dipeptide hydrazide with 4-nitrobenzaldehyde. Hydrazone compounds (7a-e,8a-e) were confirmed by the IR spectroscopy which showed the absorption bands of (C=N, C=C), amide, C=O (phCO-) and amine groups appeared stretching vibration at (1600-1530cm^{-1}), (1690-1629cm^{-1}) (1625-1600cm^{-1}), and (3467-3251cm^{-1}), respectively. While at stretching vibration (1538-1513cm^{-1}) Asymmetric stretching of aromatic NO_2, and (1345 - 1326 cm^{-1}) Symmetric stretching of aromatic NO_2, as shown in Table (2). The ^{1}H NMR spectrum of the compounds (7d,e, 8c,e) showed a signal at (δ 7.3- 7.1ppm) (parikh 1974) indicating the presence of CH=N proton and other signals were observed at appropriate places, Table (4).

The hydrazone were cyclized to 2,5-disubstituted- 1,3,4- Oxadiazole (9a-e,10a-e) by their reaction with lead oxide. The IR characterization absorption bands of the oxadiazoles (9a-e,10a-e) were given in Table (3).The main absorption bands for imine and amide groups appeared at (1600-1561cm^{-1}) ,(1656 -1600 cm^{-1}) and (1686-1633cm^{-1}) for(C=N, C=C),C=O (phCONH)and C=O, while at (1525-1519 cm^{-1}) (1346-1340cm^{-1}) represent NO_2 asymmetric, symmetric stretching, respectively. The N-H groups of these compounds appeared at (3435- 3258cm^{-1}) as broad bands.

^{1}H NMR spectral analyses of compounds (9a,c-d,10b-d) exhibited aromatic protons in the expected range (8.4-7.1ppm), hiding peak due to (CH=N) protons, and other signals were observed at appropriate places, as illustrated in Table(5).

Oxadiazole -2-thione (11a-b,12a-e) synthesis was performed by the reaction of hydrazides and carbon disulfide in alkaline medium. The mechanism of the reaction is accomplished by nucleophilic attack of the enol hydrazide form at the carbon atom of carbon disulfide. The formed xanthat salts underwent intra nucleophilic attack followed by hydrogen sulfide elimination. The IR spectra of the compounds (11a-b,12a-e) showed absorption bands at (1266-1154cm^{-1}) corresponds to the thione stretching vibration, and others gave the following vibrational

absorption bands (1597-1529cm^{-1}) , (1667-1632cm^{-1}) and (3430- 3246cm^{-1}) which were assigned to (C=C, C=N), (C=O amide), and (N-H) respectively as illustrated in Table (3). The ^{1}H NMR spectrum of compounds (11a,12b,e) revealed the resonance peaks that appeared at 7.1-8.2 ppm could be assigned to the contribution of aromatic protons, and the remaining signals has appeared in the expectation of places, as illustrated in Table (5).

Phthalazine compounds (13a-b) were prepared through ring closure of hydrazone by using hydrochloric acid in amyl alcohol. The main absorption bands of substituted Phthalazine compounds which includes stretching vibrations of C=N, C=C, C=O amide at (1605-1585cm^{-1}), and (1630, 1629 cm^{-1}) respectively. While the absorption bands at (3435, 3327 cm^{-1}) were assigned to (N-H) stretching vibrations, as shown in Table (3).

Hydrazides were converted to thiosemicarbazide (14a-c) by its reaction with ammonium thiocyanate / hydrochloric acidic. The structure of compounds (14a-c) was confirmed by IR in which strong bands for C=S, C=O, C=O (phCO) and N-H stretching were observed at (1225-1085), (1631–1630), (1615-1610) and (3152–3114) cm^{-1}, respectively Table (3).

Cyclocondensation of the thiosemicarbarbazide with aqueous sodium hydroxide afford 5-substituted-1,2,4-triazole-3-thione. The IR spectra of the synthesized compound (15a-b) showed the presence of N-H stretching bond at(3326-3127cm^{-1}) and detection of C=N stretching bond at (1582 -1577 cm^{-1}) for evidence of ring closure of triazole ring Table (3).

The antibacterial activity of the compounds (5d, 7a, 9e, 10d, 11a, 12e, 13a, 14b) were evaluated using various species of bacteria, *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli* and *Proteus mirabilis*. The result showed that the compounds (7a-12e) were active in inhibiting the growth of nearly all organisms used as indicated from the diameter of inhibition zone Table (6). Blank discs DMSO did not show any activity. According to the data (Table 6), it was evident that the activity of tested compounds decreased considerably at lower concentration (0.01mg/ml).

However, compounds (7a, 9e, 11a and 12e) showed the higher antibacterial effect on *Bacillus subtilis*, *Proteus mirabilis* then *Staphylococcus aureus* and *Escherichia coli*, as indicated from the diameter of inhibition zone.

Table(1): Physical properties and spectral data of compounds (3a-e, 4a-l)

Comp No.	R,R´	M. p. ℃	Yield %	IR (KBr) υ cm^{-1}				UV DMSO max (nm)
				C=C	C=O amide	C=O ester	NH (OH)	
3a	H , H	78-79	65	1597m	1655s	1730s	3373b	264
3b	$-CH(CH_3)_2$, H	113-115	80	1535m	1632s	1741s	3277b	265
3c	$-CH_3$,$-CH_2CH(CH_3)_2$	98-100	63	1598m	1655s	1716s	3268b	264
3d	$-CH_2CH(CH_3)_2$, H	127-128	64	1539m	1647s	1736s	3303b	265
3e	$-CH(CH_3)CH_2CH_3$, H	98-100	77	1555S	1647S	1744s	3340b	265
4a	H , $-CH_2C_6H_5$	102-103	64	1545b	1650w,1642s	1739s	3295b	263
4b	H , H	140-141	43	1573m	1626s	1744b	3327s	266
4c	H ,$-CH_2C_6H_4OH$	120-122	55	1555s	1647s,1632 m	1744b	3339s (3389)	266
4d	$-CH(CH_3)_2$,H	82-83	45	1580w,1546m	1660w,1651s	1751s	3299b	267
4e	$CH(CH_3)CH_2CH_3$, $-CH(CH_3)_2$	135-137	44	1578w,1536s	1635s,1620w	1742s	3323b	264
4f	$-CH(CH_3)_2$,- $CH(CH_3)_2$	139-140	57	1578w,1537s	1633s	1742s	3276b	262
4g	$-CH(CH_3)_2$,- $CH_2C_6H_5$	155-156	76	1578w,1548m	1631b	1723s	3303b	261
4h	$-CH_3$,$-CH_2CH(CH_3)_2$	113-115	50	1536b	1655w,1633s	1743s	3279b	265
4i	$-CH_3$, H	133-134	55	1577w,1534m	1640m,1631 s	1721s	3325b	262
4j	$-CH_3$, $-CH_2C_6H_5$	97-98	72	1577w,1535s	1656m,1632 s	1736s	3302b	268
4k	$-CH_3$, $-CH_2C_6H_4OH$	80-81	60	1532b	1640s,1610w	1739s	3324b (3400)	263
4l	$-CH(CH_3)CHCH_3$, $- CH_2C_6H_5$	127-128	69	1578w,1530m	1660m,1631 s	1740s	3284s	264

Table(2): Physical properties and spectral data of compounds (5a-e,6a-j, 7a-e, 8a-e)

Comp No.	R,R´	M. p. ˚C	Yield %	IR (KBr) υ cm^{-1}				UV DMSO max nm
				C=C	C=O amide	NO_2	NH (OH)	
5a	H,H	116-117	91	1542m	1636s	-	3307b	264
5b	-$CH(CH_3)_2$, H	133-135	67	1545m	1639s	-	3309b	264
5c	-CH_3, $CH_2CH(CH_3)_2$	112-113	85	1533m	1639s	-	3283b	270
5d	$CH_2CH(CH_3)_2$, H	102-103	66	1533m	1651s,1605m	-	3336b	264
5e	$CH(CH_3)CH_2CH_3$,H	188-189	60	1555m	1634s,1656w	-	3253b	264
6a	H , -$CH_2C_6H_5$	180-181	68	1576m1 546w	1645s,1603w	-	3284b	265
6b	H , $CH_2C_6H_4OH$	116-117	94	1577w 1542m	1636s,1610w	-	3307b 3410b	264
6c	-$CH(CH_3)_2$,H	140-142	94	1578w	1640s,1629m	-	3287b	262
6d	-$CH(CH_3)_2$, -$CH(CH_3)_2$	152-153	96	1530w	1629b	-	3287b	262
6e	-$CH(CH_3)_2$, -$CH_2C_6H_5$	204-205	94	1532m	1630b	-	3283b	263
6f	-CH_3 , $CH_2CH(CH_3)_2$	102-104	90	1578w 1532m	1628b	-	3323b	263
6g	-CH_3 , H	102-103	96	1541m	1628s 1610w	-	3325b	263
6h	CH_3 , -$CH_2C_6H_5$	163-164	97	1538m	1633s	-	3267b	267
6i	-CH_3 , - $CH_2C_6H_4OH$	126-127	98	1540m	1640s 1628w	-	3284b	266
6j	$CH(CH_3)CHCH_3$ $CH_2C_6H_5$	201-202	94	1578w 1533m	1631b	-	3281b	267
7a	H,H	167-169	78	1597m 1540m	1688m 1644s	1519s 1343s	3353b	341 262
7b	-$CH(CH_3)_2$, H	205-206	52	1599m 1545w	1656b	1522s 1345s	3433b	337 260
7c	-CH_3, $CH_2CH(CH_3)_2$	161-163	81	1600m 1535w	1686m 1649s	1522s 1345s	3467b	337 260
7d	$CH_2CH(CH_3)_2$, H	183-185	92	1595m 1586w	1686s 1656s	1520s 1344s	3394b	335 264
7e	$CH(CH_3)CH_2CH_3$,H	180-182	93	1597m	1690m 1646m	1520s 1345s	3251b	336 264
8a	-H,$CH_2C_6H_5$	170-171	74	1587m 1560w	1656b 1610w	1522s 1345s	3279b	335 262
8b	-$CH(CH_3)_2$, -$CH_2C_6H_5$	180-181	82	1590m	1630b	1533m 1342m	3288b	371 262
8c	-CH_3, -$CH_2C_6H_5$	149-150	84	1578m	1632b	1538s 1328m	3271s	371 262
8d	-CH_3, -$CH_2C_6H_4OH$	117-119	79	1578m 1530w	1635s 1625m	1513m 1326s	3324b 3424b	371 261
8e	-$CH(CH_3)CH_2CH_3$, - $CH_2C_6H_5$	181-183	92	1599m 1578w	1629s 1600m	1525s 1345s	3282b	345 262

Table (3): Physical properties and spectral data of compounds (9a-e, 10a-e, 11a-b, 12a-e, 12a-c, 13a-b, 14a-c, 15a-b)

Com No.	R R'	M. p. C°	Yield %	IR (KBr) υ cm^{-1}						UV DMSO max (nm)
				C-O-C	NO_2	C=C C=N	C=O amide	NH	C=S	
9a	-H,H	201-203	70	1153m	1519s 1344s	1596m 1561w	1648s	3364b	-	341 264
9b	$-CH(CH_3)_2$ -H	248-249	81	1162m	1520s 1345m	1597s	1656m	3433b	-	335 261
9c	$-CH_3$ $CH_2CH(CH_3)_2$	197-198	94	1162s	1519s 1344s	1593s 1570w	1649s	3258b	-	338 263
9d	$CH_2CH(CH_3)_2$ -H	194-195	92	1162b	1520m, 1340s	1592m 1586m	1648m	3435b	-	339 263
9e	$-\dot{C}HCH_2CH_3$ $\dot{C}H_3$ -H	195-196	79	1160m	1521s 1343s	1600w 1596m	1643s	3298b	-	335 259
10a	-H , $-CH_2C_6H_5$	179-181	66	1106w	1522s 1345s	1587s	1647s 1600w	3421b	-	338 261
10b	$-CH(CH_3)_2$, $CH_2C_6H_5$	268-271	43	1106w	1525s 1346s	1597m	1633b	3434b	-	337 263
10c	$-CH_3$, $-CH_2C_6H_5$	230-231	61	1162w	1520s 1342s	1595w 1586w	1686s 1656s	3394b	-	334 260
10d	$-CH_3$, $CH_2C_6H_4OH$	205-207	45	1153m	1525s 1346s	1597m 1570w	1681s 1629m	3430b	-	340 265
10e	$-\dot{C}HCH_2CH_3$ $\dot{C}H_3$ $CH_2C_6H_5$	228-230	64	1125w	1524s 1345s	1595m 1580w	1633b 1605w	3277b	-	335 262
11a	$CH(CH_3)_2$, H	205	64	1093m	-	1575m 1548m	1656s	3323s 3246s	1161s	344 274
11b	$CH_2CH(CH_3)_2$,H	161-162	71	1092m	-	1595m 1542m	1667s	3363s 3255s	1163s	327 268
12a	-H , $-CH_2C_6H_5$	75-76	79	1065m	-	1590w 1531m	1643s	3411b	1154m	350 275
12b	$-CH(CH_3)_2$, $-CH_2C_6H_5$	82-83	68	1058m	-	1597m 1540w	1637s	3273b	1157m	335 270
12c	$-CH_3$, -H	163-164	45	1188m	-	1577m 1535m	1632s	3326b	1266m	345 271
12d	$-CH_3$, $-CH_2C_6H_5$	150-151	60	1059w	-	1585m 1529w	1635s	3430b	1158m	348 274
12e	$CH(CH_3)CH_2CH_3$ $-CH_2C_6H_5$	93-95	85	1059w	-	1577w 1529s	1632s	3287b	1155m	350 270
13a	$-CH_3$,- $CH_2C_6H_4OH$	292-293	48	-	-	1605s 1595s	1628m	3435b	1519s 1343s	334 260
13b	$CH(CH_3)CH_2CH_3$ $-CH_2C_6H_5$	>300	43	-	-	1596s 1585s	1630m	3327b	1519s 1345s	340 265
14a	$-CH(CH_3)_2$,-H	212-213	65	-	-	1596m	1630s 1610m	3152b	1085m	261
14b	$-CH(CH_3)_2$, $-CH_2C_6H_5$	122-123	73	-	-	1600m	1631s 1615m	3138b	1225m	276
14c	$CH(CH_3)CH_2CH_3$- $CH_2C_6H_5$	212-214	68	-	-	1610m	1631s 1612m	3114b	1096m	275
15a	$-CH(CH_3)_2$,-H	214-215	85	-	-	1582m	1625m16 05m	3127b	1094s	335 263
15b	$-CH(CH_3)_2$, $-CH_2C_6H_5$	152-153	65	-	-	1577m	1629b	3326b	1216s	338 260

Table (4): 1H NMR spectral data of compounds (3a,e, 4c,j, 6e,j, 7d,e, 8c,e)

Com. No.	R , R'	^{1}H NMR (400 MHz, DMSO-d6, δ, ppm)
3a	-H , -H	1.2-1.3 (t, 3H) CH_3CH_2; 2.5 (s, 3H) CH_3ph; 3.3, 3.5 (s, 4H) $2CH_2CO$; 3.6-3.7(q, 2H) -CH_2CH_3 7.4-7.8(m, 4H) ArH; 8.9(s, 1H) NH; 8.1(s, 1H) NH.
3e	-CHCH$_2$CH$_3$ (CH$_3$) -H	0.7-0.9 (m, 6H) $2CH_3$; 1.2-1.3 (t, 3H) CH_3CH_2; 2.5(s, 3H) CH_3ph; 1.0-1.1 (m, 2H) $CHCH_2CH_3$; 3.5-3.6 (m, 2H) CH_2CO; 4.0-4.1 (quartet, 2H) $COCH_2CH_3$; 1.5-1.7(m, 2H) 2CH; 7.3-7.8(m, 4H) ArH; 8.2-8.3(s, s, 2H) 2NH.
4c	-H -$CH_2C_6H_4OH$	1.0(t, 3H) CH_3CH_2; 3.1(q, 2H) $COCH_2CH_3$; 3.4(d, 2H) phCH_2CH, 3.9(s, 2H) CH_2CO; 4.2(d, 1H) COCH CH_2ph; 7.0-7.9(m, 9H) 2ArH; 8.4(s, 1H) NH; 8.7 (s, H) NH; 8.9(s, 1H) OH.
4j	-CH_3,-$CH_2C_6H_5$	1.1(t, 3H) CH_2CH_3; 1.3(d, 3H) $CHCH_3$; 2.9-3.3 (m, 2H) 2CHCO; 3.9 (q, 2H) $COCH_2CH_3$; 4.5 (d, 2H) phCH_2CH; 7.2-7.9(m, 10H) 2ArH; 8.3 (d, 1H) NH; 8.5(d, 1H) NH.
6e	-$CH(CH_3)_2$ $CH_2C_6H_5$	0.7(d,6H)(CH_3)$_2$CH; 1.0(m,1H)CH(CH_3)$_2$; 2.1(d,2H) phCH_2CH; 2.8(m,2H)2CHCO; 4.5(b, 2H)NH_2; 7.2-8.2 (m, 10H) 2ArH; 8.5(d, H) NH; 9.3 (s, 2H)2NH.
6j	-CHCH$_2$CH$_3$ (CH$_3$) -$CH_2C_6H_5$	0.7(m, 6H) $2CH_3$; 1.2(m, 2H)CH_2CH_3; 1.7(m, 1H) CH_2CHCH_3; 1.9(d,2H)phCH_2CH, 2.9(m,2H)2CHCO; 4.5 (d,2H) NH_2;7.1-8.0(m, 10H) 2ArH; 8.2(s, 1H) NH; 8.9(s, 1H)NH; 9.1(s, 1H) NH.
7d	$CH_2CH(CH_3)_2$ -H	0.8(m,6H)$2CH_3$; 1.2(m, 1H) CH(CH_3)$_2$; 1.4(m, 2H) CH_2CH; 2.5(s, 3H) CH_3ph; 4.2(t, 1H) $COCHCH_2$; 5.1(q,2H)CH_2CO; 7.2(s,1H) CH=N; 7.1,7.8 (dd,4H) ArH; 7.4(s,1H)NH; 7.5,8.2(d,d, H)ArH; 8.8(s,1H) NH; 8.9(s,1H) NH.
7e	-CHCH$_2$CH$_3$ (CH$_3$) -H	0.9(m, 6H)$2CH_3$; 1.2(m, 1H)CH; 1.5(m, 2H) CH_2CH_3;2.3(s, 3H) CH_3ph; 3.6(m, 1H)CHCO; 3.9(d, 2H) CH_2CO; 7.3(s, 1H) CH=N; 7.4(s, 1H) NH; 7.7, 8.1(dd, 4H) ArH; 7.8(s,1H)NH; 7.9,8.1 (dd, 4H)ArH 8.4(s,1H)NH; 9.0(s, 1H) NH.
8c	-CH_3 -$CH_2C_6H_5$	1.1(d, 3H) CH_3CH; 2.4(d, 2H) CH_2CHph; 2.8(m, 1H) $COCHCH_3$; 3.0(t, 1H) $COCHCH_2$; 7.1(s, H) CH=N; 7.3-8.3 (m, 14H) 3ArH; 4.2, 4.5(b, 2H)2NH; 9.1(s, H) NH.
8e	-CHCH$_2$CH$_3$ (CH$_3$) $CH_2C_6H_5$	0.8(m, 6H) $2CH_3$; 1.3(m, 2H) CH_2CH; 1.9(m, H) $CHCH_3$; 2.8 (d, 2H) phCH_2CH; 4.3(m, 2H) 2COCH; 7.1(s, 1H)CH=N; 7.4-8.1(m,14H) 3ArH; 4.5(s, 1H) NH 8.4(s, 1H) NH; 9.1(s, 1H)NH.

Table (5): 1H NMR spectral data of compounds (9a,c-d, 10b-d, 11a, 12b,e)

Com. No.	R , R'	^{1}H NMR (400 MHz, DMSO-d6, δ, ppm)
9a	-H,H	2.4(s, 3H)$\underline{CH_3}$ph; 3.9(d, 2H)NH$\underline{CH_2}$; 4.3(s, 2H) CH_2CO; 7.4,7.7(dd,4H)ArH; 7.9,8.2 (dd,4H) ArH; 8.0(s,2H)2NH.
9c	-CH_3, $CH_2CH(CH_3)_2$	0.9(d,6H)($\underline{CH_3}$)$_2$CH; 1.2(m,H)$\underline{CH}(CH_3)_2$; 1.5(d, 3H) $\underline{CH_3}$CH; 1.7(m, 2H) $\underline{CH_2}(CH)_2$; 2.5(s, 3H) $\underline{CH_3}$ph;2.8(t,H)NH$\underline{CH}CH_2$; 7.1-7.9(m, 8H) 2ArH; 8.9(s, 1H)NH;8.5(s, 1H) NH.
9d	-$CH_2CH(CH_3)_2$, -H	0.8(d,6H)($\underline{CH_3}$)$_2$CH; 1.3(d,2H)$\underline{CH_2}$CH;1.7 (m,1H) $\underline{CH}(CH_3)_2$; 2.4(s,3H) $\underline{CH_3}$ph; 3.7 (t,2H) $\underline{CH_2}$NH; 4.1(m,1H) CO$\underline{CH}CH_2$; 7.3,7.9(dd,4H) ArH; 7.6-8.3 (dd,4H) ArH; 8.5(s, 2H)2NH.
10b	-$CH(CH_3)_2$, $CH_2C_6H_5$	0.9(d, 6H)($\underline{CH_3}$)$_2$CH; 1.8(d, 2H)ph$\underline{CH_2}$CH; 1.4(m, 1H)$\underline{CH}(CH_3)_2$; 2.1(t, 1H)CO$\underline{CH}CH_2$ph; 2.8 (d,1H) CO$\underline{CH}$CH; 4.5(s,1H)NH; 5.6(s,1H)NH; 7.1-8.2(m, 14H)3ArH.
10c	-CH_3 , $CH_2C_6H_5$	1.1(d, 3H) $\underline{CH_3}$CH; 1.6(d, 2H) ph$\underline{CH_2}$CH; 2.0(m,1H) CO$\underline{CH}CH_3$; 2.3(t,1H) $\underline{CH}CH_2$ph; 4.5(s,1H)NH; 5.7 (s,1H)NH; 7.2-8.2(m, 14H)3ArH.
10d	-CH_3 , $CH_2C_6H_4OH$	0.8(d, 3H) $\underline{CH_3}$CH; 1.0(d, 2H) ph$\underline{CH_2}$CH; 1.4 (t, 1H) $\underline{CH}CH_2$ph; 2.1(q, H) CO$\underline{CH}CH_3$; 4.5(s, 1H) NH; 5.6(s,1H)$\underline{NH}$;7.4-8(m,5H)ArH;8.1,8.4 (dd,8H)2ArH; 8.9(s, 1H)OH.
11a	$CH(CH_3)_2$, H	0.9(d, 6H) ($\underline{CH_3}$)$_2$CH; 1.3(m, 1H) $\underline{CH}(CH_3)_2$; 2.5(s, 3H) $\underline{CH_3}$ph; 2.7 (d, 2H) $\underline{CH_2}$NH;4.1(d,1H)CO$\underline{CH}$CH;5.6(b,1H)NH; 7.2,7.5(dd,4H) Ar$\underline{H}$;7.4(s,H)NH;8.6(s, 1H)N$\underline{H}$.
12b	$CH(CH_3)_2$, $CH_2C_6H_5$	0.7(d, 6H)($\underline{CH_3}$)$_2$CH; 1.3(m, H)$\underline{CH}(CH_3)_2$; 1.8 (t,1H) NH$\underline{CH}CH_2$;2.3 (d,2H)$\underline{CH_2}$CH; 3.6(d,1H) $\underline{CH}$CO; 4.2 (b,1H) NH, 7.2(d, 1H)NH; 8.4 (s,1H) NH; 7.6-8.2(m, 10H)2ArH.
12e	-$\underset{CH_3}{\overset{|}{C}}HCH_2CH_3$ $CH_2C_6H_5$	0.9 (m, 6H) 2$\underline{CH_3}$; 1.2(m, 2H) $\underline{CH_2}CH_3$; 1.6 (m,2H) 2$\underline{CH}$; 2.1(d,2H) $\underline{CH_2}$Ph; 4.3(d,1H) CHCO; 5.2(b, 1H) NH; 7.1-7.8(m, 10H) 2ArH; 8.2 (s, 1H)NH, 8.8(s, H) NH.

Table (6): the antibacterial activity of compounds (5d, 7a, 9e, 10d, 11a, 12e, 13a, 14d); and diameter of inhibition zone(cm).

Comp. No.	Mg/ml	5d	7a	9e	10d	11a	12e	13a	14b	DMSO control
Staph. Aureus	10	-	1.2	1.8	-	1.8	0.9	-	-	-
	1	-	-	1.3	-	1.0	-	-	-	-
	0.1	-	-	0.9	-	-	-	-	-	-
	0.01	-	-	0.6	-	-	-	-	-	-
Bacillus subtilis	10	1.5	1.6	2.1	2.0	1.6	2.2	1.4	-	-
	1	0.7	1.2	1.4	1.2	1.2	1.4	1.0	-	-
	0.1	-	0.7	1.0	0.6	0.7	0.7	0.7	-	-
	0.01	-	-	0.6	-	0.5	-	-	-	-
Escherichia coli	10	-	0.6	1.2	-	-	0.8	0.6	-	-
	1	-	-	0.8	-	-	-	-	-	-
	0.1	-	-	0.5	-	-	-	-	-	-
	0.01	-	-	-	-	-	-	-	-	-
Proteus mirabilis	10	1.2	0.8	1.4	-	1.2	1.4	-	-	-
	1	0.7	-	1.0	-	0.8	0.8	-	-	-
	0.1	-	-	0.8	-	-	-	-	-	-
	0.01	-	-	0.5	-	-	-	-	-	-

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تحضير بعض المركبات الحلقية من المشتقات ثنائية الببتيد ودراسة فعاليتها البايولوجية

الخلاصة:

تم تحضير استرات الببتيدات الثنائية المحمية (3a-e, 4a-l) من تفاعل المركبات (1a-e, 2a-d) مع استرات الاحماض الامينية وبوجود ثنائي هكسيل الحلقى كاربو ثنائي اميد كعامل ازدواج ، ثم تم تحضير الهيدرازيدات (5a-e, 6a-j) وذلك بتفاعل الاسترات المناظرة مع الهيدرازين المائي. كما تم تحضير الهيدرازونات (7a-e, 8a-e)من تفاعل الهيدرازيدات مع البارانايتروبنزالديهايد. ومن ثم حولقة الهيدرازونات الناتجة الى ٥,٢– ثنائي المعوضة ٤,٣,١– اوكسادايازول (9a-e, 10a-e) بوجود اوكسيد الرصاص والى الفثالازين (13a-b) بوجود حامض الهيدروكلوريك ، كما تم تحضير ٤,٣,١– اوكسادايازول –٢– ثايون (11a-b)وذلك بتفاعل الهيدرازيدات المقابلة مع كاربون ثنائي السلفايد في وسط هيدروكسيد البوتاسيوم الكحولي . اما عند تفاعل الهيدرازيد مع ثايوسيانات الامونيوم فقد تم الحصول على الثايوسيمى كاربازايد (14a-c) والذى تحولق الى ٤,٢,١–ترايازول –٣–ثايول (15a-b) في وسط من هيدروكسيد الصوديوم. شخصت المركبات المحضرة بالطرق الفيزياوية والطيفية. كما تم دراسة الفعالية البايولوجية ضد بعض انواع البكتريا الموجبة والسالبة لصبغة كرام (*Staphylococcus aureus*, *Bacillus subtilis*,_*Escherichia coli* and *Proteus mirabilis*) للمركبات (5d, 7a, 9e, 10d, 11a, 12e, 13a, 14b)

ئامادەکرنا هەندیك ئاویتێن ئەلقەیی ژ دەتاشراوێن دووەمی پیتیداتێ وخاندنا کارێگەریا بایولوجی

پوختـــــــــە:

ئامادەکرنا ئەستەراتێن پیتیداتێ دووەمی یێ پاراستی (3a-e,4a-1) ژ کارلێکا پێکهاتییێن (1a-e,2a-d) دگەل ئەستەراتێن ترشێن ئەمینی و ب هەبونا دووەم هکسیلی ئەلقەیی کار بو دووەمین ئەمید وەك ڤاکتەرەکێ تێکەل ، و لدیڤدا هایدرازیدات (5a-e,6a-j) ئامادەکر ب رێکا کارلێکرنا ئەستەراییێن وەك ئێك دگەل هایدرازینێن ئاڤێ . ولدیڤدا هێدرازونات (7a-e,8a-e) هاتە ئامادەکرن ب کارلێکرنا هیدرازیداتێن دگەل پارانایتر و بەنزالدیهاد ولدیڤدا بازنەیا هێدرازونات کو هاتیە دروستکرن بو 5,2 – دووانیێ قەرەبوکری 4,3,1 – ئوکسادایازول (9a-e,10a-e) ب هەبونا ئوکسیدێ قورقوشمی و هەر وەسا بو فسالازین (13a-b) ب هەبونا ترشێ هایدرو کلوریك، دیسان ئامادەکرنا 4,3, 1- ئوکسادایازول - 2 - سایون (15a-b) بریکا کارلێکرنا هێدرازیداتێن بەرامبەر دگەل کاربونیێ دووەم سلڤاید دناڤ هێدروکسیێ پوتاسیوم کهولی .بەلێ دەمێ کارلێکرنا هێدرازید دگەل سایوسیا ناتیێ ئەمونیوم ، دئەنجامدا سایوسیمی کاربازاید (12a-c) دەست ب مەڤە هات کو دهێتە گوهورین بو بێکهاتیێ بازنەیێ 4,2,1 - ترازایول -3- سایول (15a – b) دناڤ هێدروکسیدێ پوتاسیوم . بێکهاتییێن مە ئامادەکرین مە دەست نیشانکرن بریکێن فیزیایی و سبێکتروسکوبی ، هەروەسا کارێگەریا بایولوجی یا ڤان بێکهاتیا دژی هندەك بەکتریاییێن پوسوتیڤ و نیکەتیڤ . (*Staphylococcus _aureus* , *Bacillus Subtilis* and *Escherichia Coli* and *Proteus Mirabilis*) . بو پێکهاتییێن (5d,7a,9e,10d,11a,12e,13a,14b)