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SYNTHESIS OF NOVEL 3,6-DICHLOROBENZO[*b*]THIOPHENE-2-CARBONYLAMINO ACID DERIVATIVES AS POTENTIAL ANTIMICROBIAL AGENTS

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Abstract

Some new 3,6-dichlorobenzo[*b*]thiophene-2-carbonylamino acid ester and their corresponding hydrazide, hydrazone and peptide derivatives (II-XXXI) have been prepared. All the newly synthesized compounds are characterized by elemental analysis and spectral studies, and evaluated for antimicrobial activity.

Key words: 3,6-dichlorobenzo[*b*]thiophene-2-carbonyl chloride, amino acid derivatives. , antimicrobial activity.

Introduction

Several derivatives of benzo[*b*]thiophene including chlorobenzo[*b*]thiophene-2-carboxylic acid have been found to possess a wide range of pharmacological properties[1-10]. Combination of amino acids with many substituted heterocyclic compounds afforded derivatives of interesting biological activities [!1-15]. Prompted by these reports and in continuation of our work on structure-activity relationship (SAR)[16-18], the synthesis of new titled compounds was undertaken in which 3,6-dichlorobenzo[*b*]thiophene-2-carboxylic acid was incorporated with amino acid ester, dipeptide , hydrazide , and hydrazone residues and their antimicrobial properties were evaluated. The structures of the synthesized derivatives were assigned on the basis of their elemental analysis and IR, ^{1}H-NMR and MS spectral data.

For preparation of 3,6-dichlorobenzo[*b*]thiophene-2-carbonylamino acid methyl ester derivatives (II-IV) , 3,6-dichlorobenzo[*b*]thiophene-2-carbonyl chloride (I) was coupled with some amino acid methyl ester hydrochlorides, previously treated with triethylamine to liberate the free amino acid ester, in presence of dioxane – Et_3N medium. All the products were isolated, purified and obtained in 60-66% yield (cf.Table 1).

Treatment of 3,6-dichlorobenzo[*b*]thiophene-2-carbonylamino acid metyl ester derivatives (II-IV) with an alcoholic solution of hydrazine hydrate for 2h under

conditions of reflux afforded the corresponding hydrazide derivatives (V-VII) which were successively isolated , purified and obtained in 79-86 % yields (cf.Table 1).

Elongation of the amino acid derivatives (II-IV) to produce the corresponding dipeptide derivatives (VIII-X) was carried out using the azide method[19] in which 3,6-dichlorobenzo[*b*]thiophene-2-carbonylamino acid azides previously prepared from the reaction of respective amino acid hydrazide derivatives (V-VII) with cold solution of nitrous acid; was treated with freshly prepared solution of free amino acid methyl ester in tetrahydrofuran. The resulting dipeptides were easily isolated, purified and obtained in 53- 64 % yield (cf.Table 1).

Condensation of 3,6-dichlorobenzo[*b*]thiophene-2-carbonylamino acid hydrazide derivatives (V-VII) with *p*-substituted benzaldehyde in absolute ethanol resulted in the formation of 3,6-dichlorobenzo[*b*]thiophene-2-carbonylamino acyl hydrazone derivatives (XI-XXXI) . The precipitated hydrazones were filtered, dried and purified by recrystallization from the proper solvent and obtained in 66-96 % yield(cf.Table 1).

All the systhesized derivatives (II-XXXI) were found to be chromatographically homogeneous when detected under UV-lamp and their structures were assigned on the basis of their elemental analysis, spots reactions, IR, ^{1}H-NMR and MS spectra.

Experimental

Melting points were uncorrected and meassured on electric melting point apparatus SMP1. Thin layer chromatography (tlc, R_f) was run on plastic sheets coated with silica gel-60 (Merck) and developed with *n*-butanol- acetic acid- water (4:1:1 , v/v) and detected under UV light and also using iodine / KI (20%) solution as spraying agent. The infrared spectra (ν_{max} in cm^{-1}) were taken in KBr discs using FTIR-2000 instrument. ^{1}H-NMR spectra were measured in DMSO-d_6 or $CDCl_3$ using FX90Q Fourier Transform NMR spectromrter. The mass spectra were performed using Shimadzu-GC-MS-QP 100 Ex by the direct inlet system. Elemental analysis were carried out at Microanalytical Uint, Faculty of Science, Cairo University, Cairo, Egypt.

Synthesis of 3,6-dichlorobenzo[*b*]thiophene-2-carbonyl chloride (I).

The titled compound was prepared according to the procedure described earlier[20,21].

General procedure for the synthesis of 3,6-dichlorobenzo[*b*]thiophene-2-carbonylamino acid methyl ester derivatives (II-IV).

An amino acid methyl ester hydrochloride (0.026 mol) was suspended in 50 ml of dioxane containing triethylamine (0.055 mol) and stirred for 30 min. The precipitated triethylamine hydrochloride was filtered off and the filtrate was added to a solution of 3,6-dichlorobenzo[*b*]thiophene-2-carbonyl chloride (I) in 50 ml of dioxane and the reaction was stirred for 3 h at room temperature and then left overnight.The second portion of the precipitated triethylamine hydrochloride was filtered off and the filtrate was evaporated under reduced pressure . The residual product was purified by recrystallization from the proper solvent.The IR spectrum of 3,6-dichlorobenzo[*b*]thiophene-2-carbonylglycine methyl ester (II) showed characteristic bands (ν_{max} in cm^{-1}) at : 3380 (NH), 3039,1607 (CH and C=C , aromatic), 2955, 2854 (CH, aliphatic), 1723 (C=O of ester), 1643, 1548 (amide I and II) and 695 (C-Cl). For 3,6-dichlorobenzo[*b*]thiophene-2-carbonyl-Dl-phenylalanine methyl ester (IV) , its IR bands were noticed at : **3**396 (NH), 3081, 3033, 1607 (CH and C=C , aromatic), 2951, 2846 (CH, aliphatic), 1741 (C=O of ester), 1652 (amide I) and 692 (C-Cl). ^{1}H-NMR spectrum of 3,6-dichlorobenzo[*b*]thiophene-2-carbonyl-β-alanine methyl ester (III) exhibited chemical shifts (δ in ppm) at : 2.8 (t, 2H, CH_2), 3.75 (s, 3H, OCH_3), 3.8 (t, 2H, CH_2COO), 7.2 – 7.7 (m, 3H, Ar-H), 9.2 (s, 1H, NH, cancelled with D_2O) and other signals assignable to the proposed structure.

General procedure for the synthesis of 3,6-dichlorobenzo[*b*]thiophene-2-carbonylamino acid hydrazide derivatives (V-VII).

The methyl ester (II- IV, 0.01 mol) was dissolved in abs. ethanol and hydrazine hydrate (85%, 0.03 mol) was then added. The reaction mixture was stirred for 2 h at room temperature and then left overnight. The reaction mixture was evaporated under reduced pressure and the crude solid product was purified by recrystalization from the proper solvent. The IR spectrum of 3,6-dichlorobenzo[*b*]thiophene-2-carbonylglycine hydrazide (V) gave bands (ν_{max} in cm^{-1}) at : 3378, 3285 (NH_2), 3126 (NH) , 3066 (CH , aromatic), 2953 (CH, aliphatic), 1661, 1557 (amide I and II) and 695 (C-Cl) and for 3,6-dichlorobenzo[*b*]thiophene-2-carbonyl-Dl-phenylalanine hydrazide (VII) , its IR spectrum contains the following characteristic bands : 3386 (NH_2), 3217 (NH), 3078 (CH , aromatic), 2916 (CH, aliphatic), 1689 , 1537 (amide I and amide II).

General procedure for the synthesis of 3,6-dichlorobenzo[*b*]thiophene-2-carbonyldipeptide methyl ester derivatives (VIII-X).

3,6-Dichlorobenzo[*b*]thiophene-2-carbonylamino acid hydrazide derivatives (V-VII , 0.001 mol) was dissolved in a mixture of 8 ml of acetic acid, 2 ml of 5*N* HCl and 10 ml of water and the solution was cooled to -5° C. On adding , in one portion, with shaking, a cold concentrated aqueous solution of $NaNO_2$ (0.002 mol) , the azide precipitated as a syrup and was taken up in cold ether. The etheral layer was kept cold while washing successively with ice – cold water , 3 % $NaHCO_3$ solution , and again with water, and dried over anhydrous Na_2SO_4. The azide solution was added to a clear solution of free an amino acid methyl ester (0.001mol) in tetrahydrofuran with stirring for 3h at -5 °C and then left overnight at room temperature. The reaction mixture was filtered and the filtrate was washed successively with 0.5 *N* HCl, cold water , 3 % $NaHCO_3$ solution , and again with water, and dried over anhydrous Na_2SO_4. The crude product which obtained after complete evaporation in vaccuo was purified by recrystalization from the proper solvent. The IR spectrum of 3,6-dichlorobenzo[*b*]thiophene-2-carbonylglycylglycine methyl ester (VIII) displayed principle IR bands at : 3354, 3193 (NH), 3072 (CH, aromatic), 2952, 2854 (CH , aliphattic), 1744 (C=O, ester), 1690, 1554 (amide I and II), and 699 (C-Cl) while IR bands of 3,6-dichlorobenzo[*b*]thiophene-2-carbonyl-β-alanine-β-alanyl methyl ester (IX) were noticed at : 3316 (NH), 3024 (CH, aromatic), 2965, 2853 (CH , aliphattic), 1744 (C=O, ester), 1654 (amide I), and 706 (C-Cl). . ^{1}H-NMR spectrum of compound (VIII) exhibited signals (δ in ppm) at : 3.76 (s, 3H, OCH_3), 3.91 (s, 2H, CH_2), 4.52 (s, 2H, CH_2COO), 7.76- 7.92 (m, 3H, Ar-H), and other signals in support of its proposed structure.

General procedure for the synthesis of 3,6-dichlorobenzo[*b*]thiophene-2-carbonylamino acid (*p*-substituted benzylidene) hydrazone derivatives (XI-XXXI).

A mixture of the hydrazide compound (V-VII, 0.001 mol), *p*-substituted benzaldehyde (0.001 mol) in 30 ml of abs. ethanol was refluxed for 3h. The precipitated product was filtered, washed with a few ml. of cold ether, dried, and then recrystalized from the proper solvent. The IR spectrum of 3,6-dichlorobenzo[*b*]thiophene-2-carbonylglycyl (*p*-chlorobenzylidene) hydrazone (XII) displayed principle IR bands at : 3357, 3185 (NH), 3081 (CH, aromatic), 2984, 2864 (CH , aliphatic), 1680, 1590 (amide I and II), 1625 (CH=N), 802 (*p*-disubstituted benzene) and 711 (C-Cl) while 3,6-dichlorobenzo[*b*]thiophene-2-carbonylglycyl (*p*-

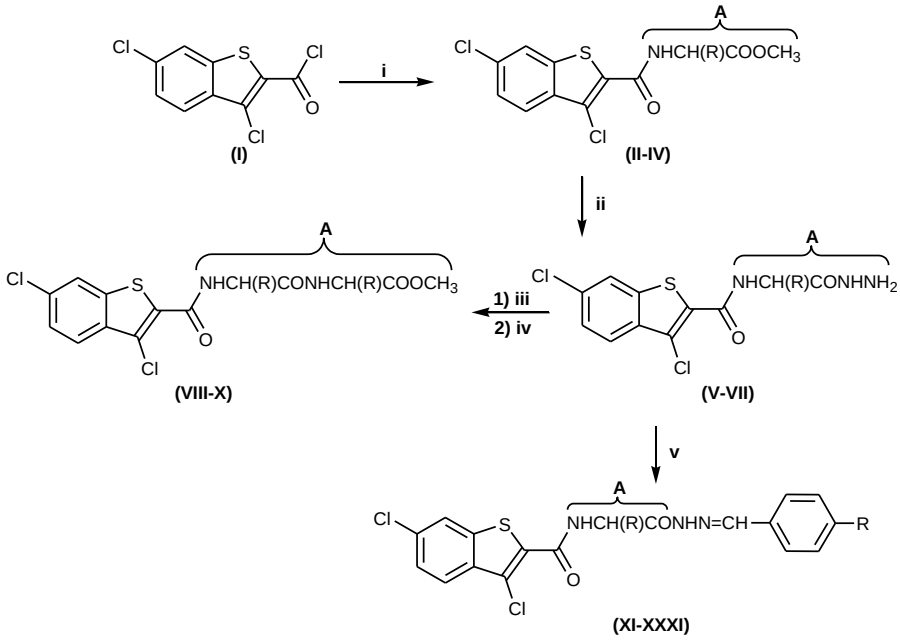
nitrobenzylidene) hydrazone (XIV) showed the following characteristic bands : 3392, 3191 (NH), 3079, 1612 (CH and C=C aromatic), 2973 (CH , aliphatic), 1687, 1576 (amide I and II), 1623 (CH=N). The IR bands of 3,6-dichlorobenzo[*b*]thiophene-2-carbonyl-β-alanyl (benzylidene) hydrazone (XVIII) were noticed at : 3363, 3205 (NH), 3072, 1604 (CH and C=C aromatic), 2983 (CH, aliphatic), 1686 (amide I), 1630 (CH=N) and for 3,6-dichlorobenzo[*b*]thiophene-2-carbonyl-β-alanyl (*p*-methylbenzylidene) hydrazone (XXII) the IR bands appeared at: 3364, 3201 (NH), 3057 (CH and C=C aromatic), 2983 (CH, aliphatic), 1684, 1586 (amide I and II), 1633 (CH=N), 809 (*p*-disubstituted benzene) and 703 (C-Cl). On the other hand, 3,6-dichlorobenzo[*b*]thiophene-2-carbonyl-Dl-phenylalanyl (*p*-bromobenzylidene) hydrazone (XXVII) showed the following characteristic IR bands: 3336, 3194 (NH), 3050, 1605 (CH and C=C aromatic), 2961, 2834 (CH, aliphatic), 1676, 1579 (amide I and II), 1624 (CH=N), 807 (*p*-disubstituted benzene) and 711(C-Cl) while the IR spectrum of 3,6-dichlorobenzo[*b*]thiophene-2-carbonyl-Dl-phenylalanyl [*p*-(*N,N*-dimethyl)aminobenzylidene] hydrazone (XXXI) revealed the following characteristic bands: 3291, 3161 (NH), 3055 (CH, aromatic), 2917, 2854 (CH, aliphatic), 1652, 1548 (amide I and II), 1619 (CH=N), 804 (*p*-disubstituted benzene) and 712 (C-Cl). ^{1}H-NMR spectrum of compound (XIII) exhibited signals (δ in ppm) at: 4.50 (s, 2H, CH_2), 7.6-7.95 (m, 7H, Ar-H), 8.21 (s, 1H, CH=N), 8.50 and 11.6 (s, 2H, 2NH, cancelled with D_2O). For compound (XXIII) the signals appeared at: 2.55 (t, 2H, CH_2), 2.98 (t, 2H, CH_2), 3.8 (s, 3H, OCH_3) 7.0- 8.1 (m, 7H, Ar-H), 8.26 (s, 1H, CH=N), 11.24 and 11.60 (s, 2H, 2NH, cancelled with D_2O). For compound (XXIX) the NMR signals noticed at: 2.5 (s, 3H, CH_3), 4.71 (d, 2H, CH_2), 5.4 (t, 1H, CH), 7.23- 8.15 (m, 12H, Ar-H), 8.3 (s, 1H, CH=N), 11.68 and 11.9 (s, 2H, 2NH, cancelled with D_2O). The mass spectrum of (XVII), (XX) and (XXX) gave molecular ion peaks m/z (% abundance) at : 449.5 (M^+, 2.03 %), 497.5 (M^{+2}, 0.76) and 526 (M^+, 29.32) respectively which were compatible with their proposed molecular formulas.

The IR , ^{1}H-NMR and mass spectra of the remaining derivatives (II-XXXI) displayed analogous bands and peaks comfirming their structures.

Antimicrobial screening results:

The compounds (II-XXXI) were screened for their antibacterial activity using the hole plate and filter disc methods[23-25] at 150 μgml^{-1} concentration against gram positive: Bacillus subtilis, and Staphylococcus aureus, and gram negative: Escherichia coli, Moraxella Lachunata, Pseudomonas aeruginosa, Salmonlla sp. and

Sarcina sp. The activity of the compounds was compared with 3,6-dichlorobenzo[*b*]thiophene-2-carbonyl chloride (II) at the same concentration. All compounds were biologically inactive against all the tested bacteria except (VI), (VII), (VIII), (XVIII) and (XXVIII) which were moderately active against some strains of bacteria. The results are summarized in table (2).



Scheme (1)

Reagents : i) amino acid methyl ester / dioxane-TEA
ii) hydrazine hydrate / abs.ethanol
iii) a mixture of AcOH: 5NHCl : H_2O (4:1:5)
iv) amino acid methyl ester / tetrahydrofuran-TEA
v) substituted aro.aldehyde / ethanol

Table (1) : Physical data of 3,6-dichlorobenzo[b]thiophene-2-carbonylamino acid, hydrazide, dipeptide and hydrazone derivatives (II-XXXI)

Compd No	A	R	Cryst. Solv*	M.P.	Yield %	R_f	Mol.Formula	Elemental analysis **		
								C%	H%	N%
II	Gly.OMe	---	A	152-154	66	0.65	$C_{12}H_9Cl_2NO_3S$	45.28 45.20	2.83 2.71	4.40 4.63
III	β-Ala.OMe	---	A	98-100	62	0.62	$C_{13}H_{11}Cl_2NO_3S$	46.99 46.93	3.31 3.52	4.21 4.08
IV	Dl-Phe.OMe	---	A	133-135	60	0.70	$C_{19}H_{15}Cl_2NO_3S$	55.88 55.81	3.68 3.72	3.43 3.22
V	Gly.N_2H_3	---	B	207-208	86	0.78	$C_{11}H_9Cl_2N_3O_2S$	41.51 41.31	2.83 3.21	13.21 13.11
VI	β-Ala. N_2H_3	---	B	184-185	79	0.76	$C_{12}H_{11}Cl_2N_3O_2S$	43.37 43.65	3.31 3.09	12.65 12.96
VII	Dl-Phe. N_2H_3	---	B	120-122	82	0.81	$C_{18}H_{15}Cl_2N_3O_2S$	52.94 53.00	3.68 3.27	10.29 10.33
VIII	Gly.Gly.OMe	---	A	159-160	64	0.85	$C_{14}H_{12}Cl_2N_2O_4S$	44.80 44.90	3.20 3.45	7.46 7.50
IX	β-Ala.β-la.OMe	---	A	160-162	57	0.79	$C_{16}H_{16}Cl_2N_2O_4S$	47.64 47.59	3.97 4.01	6.94 7.00
X	Dl-Phe.Dl-Phe.OMe	---	A	193-195	53	0.72	$C_{28}H_{24}Cl_2N_2O_4S$	60.45 60.53	4.32 4.30	5.04 5.10
XI	Gly	H	C	250-251	66	0.69	$C_{18}H_{13}Cl_2N_3O_2S$	53.20 53.00	3.20 3.11	10.34 10.21
XII	Gly	Cl	A	269-270	89	0.65	$C_{18}H_{12}Cl_3N_3O_2S$	49.03 48.99	2.72 2.69	9.53 9.35
XIII	Gly	Br	C	271-273	83	0.71	$C_{18}H_{12}BrCl_2N_3O_2S$	44.53 44.62	2.47 2.51	8.66 8.54
XIV	Gly	NO_2	C	255-257	92	0.90	$C_{18}H_{12}Cl_2N_4O_2S$	47.89 48.01	2.66 2.87	12.41 12.33
XV	Gly	CH_3	A	261-262	67	0.80	$C_{19}H_{15}Cl_2N_3O_2S$	54.28 54.61	3.57 3.51	10.00 10.11
XVI	Gly	OCH_3	B	212-215	69	0.92	$C_{19}H_{15}Cl_2N_3O_3S$	52.29 52.33	3.44 3.40	9.63 9.70
XVII	Gly	$N(CH_3)_2$	C	240-242	88	0.73	$C_{20}H_{18}Cl_2N_4O_2S$	53.45 53.31	4.00 4.23	12.47 12.58
XVIII	β-Ala	H	B	214-215	73	0.87	$C_{19}H_{15}Cl_2N_3O_2S$	54.28 54.31	3.57 3.49	10.00 9.79
XIX	β-Ala	Cl	B	222-225	96	0.78	$C_{19}H_{14}Cl_3N_3O_2S$	50.16 50.55	3.08 3.12	9.24 9.67
XX	β-Ala	Br	C	211-213	94	0.75	$C_{19}H_{14}BrCl_2N_3O_2S$	45.69 45.50	2.80 3.00	8.41 8.12

Cont. Table (1)

XXI	β-Ala	NO_2	C	212-214	92	0.85	$C_{19}H_{14}Cl_2N_4O_4S$	49.03 48.93	3.01 3.22	12.04 12.14
XXII	β-Ala	CH_3	C	206-208	77	0.97	$C_{20}H_{17}Cl_2N_3O_2S$	55.29 55.38	3.92 4.01	9.67 9.77
XXIII	β-Ala	OCH_3	A	210-211	75	0.89	$C_{20}H_{17}Cl_2N_3O_3S$	53.33 53.24	3.77 3.81	9.33 9.55
XXIV	β-Ala	$N(CH_3)_2$	B	219	93	0.80	$C_{21}H_{20}Cl_2N_4O_2S$	54.42 54.53	4.31 4.39	12.09 12.21
XXV	Dl-Phe	H	B	220-221	73	0.72	$C_{25}H_{19}Cl_2N_3O_2S$	60.48 60.41	3.83 3.72	8.46 8.53
XXVI	Dl-Phe	Cl	C	250-251	96	0.85	$C_{25}H_{18}Cl_3N_3O_2S$	56.55 56.61	3.39 3.48	7.91 8.11
XXVII	Dl-Phe	Br	B	263-265	95	0.82	$C_{25}H_{18}BrCl_2N_3O_2S$	52.17 51.91	3.13 3.23	7.30 7.44
XXVIII	Dl-Phe	NO_2	B	197-199	93	0.84	$C_{25}H_{18}Cl_2N_4O_4S$	55.45 55.51	3.33 3.11	10.35 10.41
XXIX	Dl-Phe	CH_3	C	271	82	0.89	$C_{26}H_{21}Cl_2N_3O_2S$	61.17 61.21	4.12 4.00	8.24 8.19
XXX	Dl-Phe	OCH_3	C	276-279	93	0.80	$C_{26}H_{21}Cl_2N_3O_3S$	59.31 59.60	3.99 4.08	7.98 8.11
XXXI	Dl-Phe	$N(CH_3)_2$	B	239-241	92	0.77	$C_{27}H_{24}Cl_2N_4O_2S$	60.11 60.23	4.45 4.61	10.39 10.31

*Cryst.solv. : A= Methanol, B = Ethanol, C = Acetic acid – water
** Calculated / Found

Table (2): Antibacterial activity data of biologically active compounds (II-XXXI)

Compd. .no.	Bacillus subtilis	Escherichia coli	Moraxella lachunta	Psedomonas aeruginosa	Salmonella sp.	Sarcina sp	Staphococcus aureus
II	+	++	-	+++	+	-	+
VI	-	-	-	-	-	+	-
VII	+	-	++	+	++	-	++
VIII	-	-	-	-	-	+	-
XVIII	-	++	-	-	-	-	-
XXVIII	-	-	-	-	-	+	-

References

1. P.GRUNDT, E.E.CARLSON, C.J. BENNETT, AND A.H.NEWMAN, J.Med. Chem.; 2005, **48 (3)**, 839.
2. P.JANICE, E.D.SUSAN, L.O.JOHN, AND T.C.FRED, J.Med. Chem.; 1988, **31 (10)**, 1993.
3. K.MATSUDA, H.TOYODA, H.NISHIO, T.NISHIDA, M. DOHOGA, M. BINGO, AND S.YOSHIDA, j.Agric.Food.Chem.; (Article); 1988. **46 (10)** ;4416.
4. M.H.JUDY, C.M.LEE GARY, W. MERCY, W.ROBERT, AND A.W.LARRY, J.Med. Chem.; 1994, **37 (11)**. 1646.
5. J.K.RAY S.GUPTA, G.K. KAR, B.C.ROY, J. LIN, AND S. AMIN, J.Org.Chem.; (Article); 2000, **65 (24)**; 8134.
6. H.J.KNOLKER, AND K.R. REDDY, Chem.Rev.; 2002, **102 (11)** ; 4303.
7. K.J.DOGAN, M.GRDISA, B. ZAMOLA, K. PAVELIC, AND G.KAMINSIKI, J.Med. Chem.; (Article) ; 2003; **46 (21)**; 4516.
8. K JOHN, K.HAROLD, F. HAROLD, G. LAVERENE, AND N. ROGER, j. Agric.Food.Chem.; 1969; **17(1)**; 91
9. E.F.DIMAURO,AND J.R.VITULLO, J.Org.Chem.; (Note); 2006;**71 (10)**;3959.
10. I.JARAK, M.KARLJ, L.SUMAN, G. PAVLOVIC, J. DOGAN, M. ZINIC, AND G.KARMINSKI, J.Med. Chem.; 2005, **48 (7)**; 2346.
11. K.STJEPAN,E.D.SUSAN, J.G.BERNARD, C.H.DAVID, L.P.JANICE, D.G.ROBIN, L.O.JOHN, AND T.C.FRED, J.Med. Chem.; 1985; **28 (12)** ; 1896.
12. N.J.HRIB, I.G.JURCAK, D.E. BERGNA H.B.HARTMAN, S. KAFKA, L.L. KERMAN, AND R.CORBETT, J.Med. Chem.; (Article); 1996; **39 (20)**; 4044.
13. I.LAU, S.C. BERNER, N.G. SIDEMANN, B.LUNDT, C. SAMS, L. PRIDAL, A.LING, D.KIEL, S. SHI, AND P. MADSEN, J. Med. Chem.; (Article); 2007; **50 (1)**; 113.
14. F.A.KORA, N.S. KHALAF, S.A. ABDEL-GHAFFAR, AND H.M.HASSAN, Indian J.Hetero.Chem.; 1992; **2 (1)** ; 29.
15. S.A. ABDEL-GHAFFAR, AND Y.A.ABBAS, Acta Pharm.; 1993; **(43)** ; 27.
16. H.M. HASSAN,F.A..KORA, A.F.EL-HADDAD, A.M.EL-NAGGAR, AND M.ABDEL-KADER, Acta Pharm.; 1997; **(47)** ; 159.
17. H. M. HASSAN, M. A. EL-NAWAWY, Z. H. ABDEL-WAHB, AND S. A. M. SHEDID, Indian J. Hetero. Chem.; 1997; **7 (1)** ; 35.
18. H.M.HASSAN, J.Serb.Chem.Soc.; 1998; **63 (2)** ; 117-122
19. T.CURTIUS; Ber.;1902; **(35)**; 3226.

20. W.B.WRIGHT; J.Heterocycl.Chem.; 1972; **(9)**; 879.

21. A.J.KRUPSACK, AND T.HIGA, Tetrahedron Lett.; 1968; **(49)**; 515.

22. J.G.VINCENT, AND H.W.VINCENT, Proc.Expt.Biol.Med.; 1944; (55); 162.

23. J.A.EPSTEIN, AND S.W.LEE, J.Lab Clin.Med.; 1944; **(29)**; 319

24. R.S.VERMA AND IMAM, Indian J.Microbiol.; 1973, **(13)**, 45.