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Research Article

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Synthesis, characterization, antimicrobial evaluation and docking studies of novel compounds 1-(5-(4-(morpholinomethyl)-3-(4-(trifluoromethyl) phenyl)-3, 4-dihydroimidazo [4,5-b] indol-2-yl)-2-oxidobenzo[d] [1,3,2] dioxaphosphol-2-yl)-3 phenyl urea-Mannich bases

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ABSTRACT

New novel derivatives of 1-(4-subtetude methyl/methoxy /chloro/bromo/nitro/phenyl)-3-(5-((3-(4-trifluromethyl phenyl)-4-(thiomorpholine/4-methyl piperazine / morpholin -4-yl) methyl)-3,4dihydroimidazo (4, 5-b) indol -2yl) methyl)-2-oxo-2H-1, 3, 2-benzo Dioxaphosphol-2-yl) urea. 9(a-l) as depicted in scheme: I.1 were prepared by condensation reaction between 4-(4-(thiomorpholino / morpholinomethyl / 4 - methylpiperazin) -3-(4-(trifluoromethyl) phenyl)-3,4-dihydroimidazo[4,5-b] indol-2-yl) benzene-1, 2-diol 7(a-c) and Phenyl corbomylphosphoramidic dichloride 8(a-f). The synthon 7(a-c)was obtained by mannich reaction of 4-(3-(4-(trifluoro methyl) phenyl) -3,4-dihydroimidazo[4,5-b]indol-2-yl)benzene-1,2-diol (5) with different secondary amines having hetero atomin cyclic ring and HCHO 6(a-c) in presence of DMF. The synthon (5) was obtained by condensation reaction between 4-(((4-(trifluoromethyl) phenyl)imino)methyl)benzene-1,2-diol(3) and Isanti (4). The Synthon (3) was synthesized by reaction between 3, 4-dihydroxy benzaldehyde and 4-(trifluorometyl) aniline.The procedure was characterized by IR,^{1}H-NMR, ^{13}C-NMR, ^{31}P-NMR and elemental analysis. The newly synthesized compounds were subjected to various biological activities and docking studies.

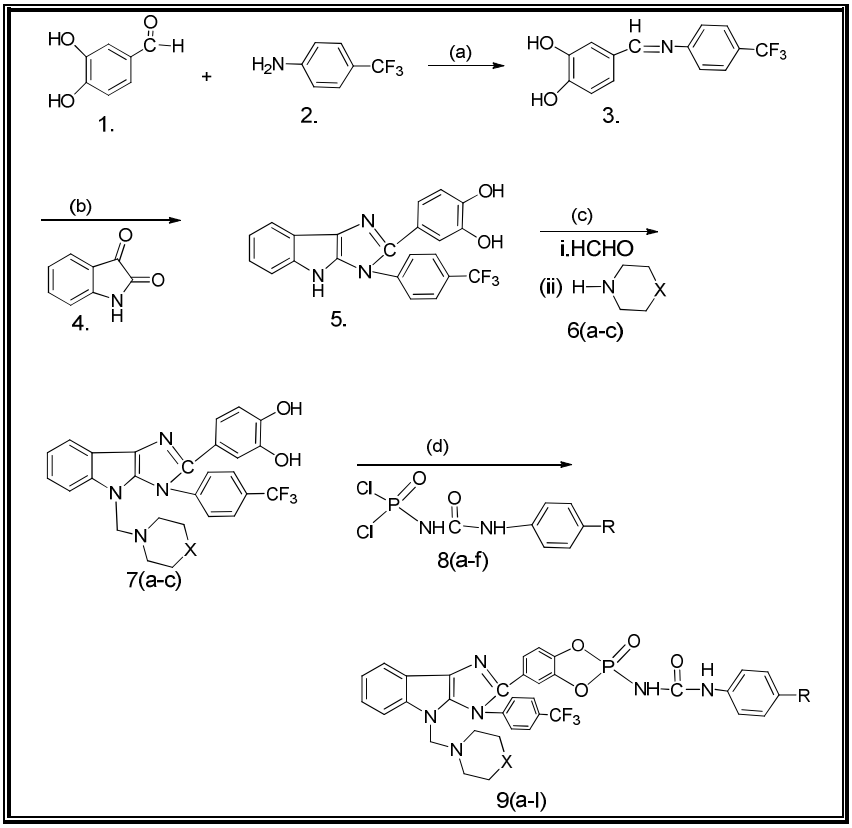
Key words: mannich bases, Phenyl corbomylphosphoramidic dichloride, antimicrobial, docking studies.

INTRODUCTION

Mannich bases and its derivatives have many attractive applications in paint and polymer chemistry as hardeners, cross linkers, reaction accelerators[1,2]etc.However,the most important applications are in the field of pharmaceutical products[3, 4]. These can widely use as anti-neoplastic drugs, analgesic drug, antibiotic drugs in pharmaceuticalfield[5-9]. Including labeled molecules [10-12] have received particular attention in the recent past. Mannich bases can either directly be employed or used as intermediates in chemicals synthesis.

Organo phosphorus heterocyclic urea derivatives widely used as anti- infective agents, anti- tumor agents, anti cancer agents, antibacterial agents, herbicides, insecticides, pesticides, anti-HIV, anti inflammatory activity[13-15].

A good deal of importance was given to dioxaphospholane derivatives in the good field of organophosphorus heterocyclic chemistry due to their unique stereo chemical features and diverse potential biological applications[16].In view of the numerous commercial applications of organophosphorus compounds. It appeared of interest to synthesize mannich bases possessing moiety dioxaphospholanes.



Scheme-l.1: 1-(4-subtetude methyl/methoxy /chloro/bromo/ nitro/phenyl)-3-(5-((3-(4-trifluromethyl phenyl)-4-(thiomorpholine/4-methyl piperazine / morpholin -4-yl) methyl)-3, 4dihydroimidazo (4, 5-b) indol -2yl) methyl)-2-oxo-2H-1, 3, 2-benzo Dioxaphosphol-2-yl) urea9(a-l)

Comp	7a	7b	7c
X	-O-	-S-	-N-CH_3

Comp	9a	9b	9c	9d	9e	9f	9g	9h	9i	9j	9k	9l
X	-O-	-O-	-O-	-O	-O-	-O-	N-CH_3	- S-	-S-	S-	-S-	-S-
R	-H	CH_3	OCH_3	Cl	Br	NO_2	H	H	CH_3	OCH_3	Cl	NO_2

EXPERIMENTAL SECTION

All the chemicals used in the present investigation were purchased from Sigma-Aldrich Chemicals company, Inc USA , and used without further purification. TLC was performed on aluminium sheet of silica gel 60F254. E-mark Germany using iodine as visualizing agent. Melting points were determined in open capillary tubes on Mel-Temp apparatus and uncorrected. Column chromatography was performed on silica gel with different solvent systems as eluents to afford the pure compound. The IR Spectra were recorded as KBR pellets on Perkin-Elmer 1000 units, instruments. All ^{1}H and ^{13}C-NMR spectra were recorded on a Varin XL-300 spectrometer operating at 400MHz for ^{1}H-NMR and 75 MHz for ^{13}C-NMR,^{31}P-NMR spectra were recorded on a Varin XL-spectrometer operating at 161.89MHz .The compounds were dissolved in DEMSO-d_6 and chemical shifts were referenced to TMS (^{1}H-NMR and ^{13}C-NMR) and 85% H_3PO_4 (^{31}P-NMR). Mass spectral data was recorded on FAB-MS instrument at 70ev with direct inlet system. Elemental analyses were recorded on a CarioErba 1108 elemental analyser. Central Drug Research Institute, Lucknow, India. Docking studies were carried out using GOLD soft ware in Bio-chemistry applications institute, Hyderabad.

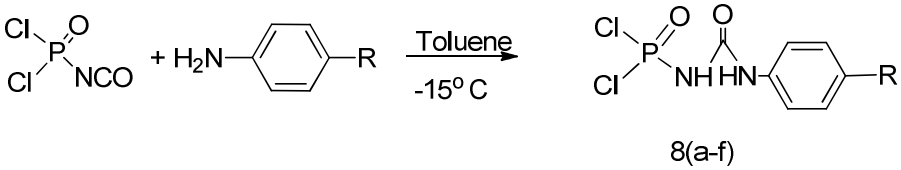
Preparation of Intermidiates:

Isatin:

Conc. H_2SO_4 is wormed to 50^{0}C in a round-bottomed flask fitted with an efficient mechanical stirrer, and to this, 75 gm. (0.46 mol) of dry Isonitosoacetanilideis added at such a rate as to keep the temperature between65^{0}C and 70^{0}C.The solution is heated to 83^0 C and kept at this temperature for about ten minutes to complete the reaction. Then the reaction mixture is cooled to room temperature and poured upon ten to twelve times its volume of cracked ice. After one hour, the isatin is filtered and suction. The compound is washed with cold water and then dried in the air. The yield is 65-67gm, melting point at 192^{0}C.

(phenylcarbamoyl)phosphoramidic dichloride[17,18]

A solution of aniline (0.51g, 0.004mole) in dry toluene (25ml) was added drop wise to phosphide oxide (6, 0.64g, 0.004mole) in dry toluene (30ml). After the addition, the temperature of the reaction mixture was maintained between -15 to -5^{0}C for 30 minutes. Later the temperature of the mixture was raised to room temperature, with stirring for 30 minutes. Phenyl carbomido phosphoric acid dichloride being insoluble in toluene was separated out. It was collected by filtration and dried under reduced pressure.



8(a-f)

Scheme1.1: (phenylcarbamoyl)phosphoramidic dichloride

Table-I.1: Physical Characteristics of Phenylcarbamoyl)phosphor amidicdichlorides

Comp	Name of the dichloridate	Reaction Time (min)	Mp (^{0}c /mm)	Yield (%)
8a	(Phenyl carbamoyl) phosphoramidic dichloride	80	125-127	70
8a	((4-methylPhenyl) carbamoyl) phosphoramidic dichloride	80	130-135	60
8c	((4-methoxyPhenyl) carbamoyl) phosphoramidic dichloride	80	138-140	65
8d	((4-chloroPhenyl)carbamoyl) phosphoramidic dichloride	100	153-155	75
8e	((4-bromoPhenyl) carbamoyl) phosphoramidic dichloride	100	170-173	70
8f	((4-bromoPhenyl) carbamoyl) phosphoramidic dichloride	100	143-145	63

RESULTS AND DISCUSSION

Typical Procedure for Synthesis of 4-(((4-(trifluoromethyl)phenyl)imino)methyl)benzene-1,2-diol (3):

3, 4-dihydroxybenzaldehyde (6.1018g, 50mmol) was dissolved in ethanol (12.181g, 15.433mL) in an Erlenmeyer flask. 4-(trifluorometyl) aniline (4.6529g, 50mmol) was added slowly to the solution. Stirred at R.T. for 21 hours. Cooled over iceand filtered. The progress of the reaction was monitored by TLC with hexane and ethylacetate (7:3) as mobile phase. The solid product was dried in a desiccators under high vacuum, the compound was recrystalized with ethanol to afford a compound 4-(((4-(trifluoromethyl) phenyl)imino)methyl)benzene-1,2-diol. The yield of compound was 64% with melting point 148-149^0 C. The compound was characterized by spectral data (IR, ^{1}H-NMR, ^{13}C-NMR and mass data) and elemental analysis.

Typical Procedure for Synthesis of 4-(3-(4-(trifluoromethyl) phenyl)-3,4-dihydroimidazo [4,5-b]indol-2-yl) benzene-1,2-diol (5)

In a typical procedure, (Schiff bases) 4-(((4-(trifluoromethyl) phenyl)imino)methyl)benzene-1,2-dio on heating with isatin (0.02mol) were refluxed in ethanol (15 mL) for 4 hours. The progress of the reaction was monitored by TLC with hexane and ethyl acetate (7:3) as mobile phase .The reaction mixture was poured on to 50 mL of ice-cold water to remove excess of NH_4OAc, and then it was filtered and recrystalized from ethanol to afford a compound in good yield. The name of the compound is 4-(3-(4-trifluorometyl)phenyl)-3,4-dihydroimidazo[4,5-b]indol-2-yl)-benzene-1,2-diol.The yield of compound was 74% with melting point 163-169^{0}C. The compound was characterized by spectral data (IR, ^{1}H-NMR, ^{13}C-NMR and mass data) and elemental analysis.

Typical Procedure for Synthesis of 4-(4-(thiomorpholino / 4 - methylpiperazin)/morpholinomethyl) -3-(4-(trifluoromethyl) phenyl)-3,4-dihydroimidazo[4,5-b]indol-2-yl)benzene-1,2-diol7(a-c)

A mixture of 4-(3-(4-trifluorometyl)phenyl)-3,4-dihydroimidazo[4,5-b]indol-2-yl)-benzene-1,2-diol, Piperdine and water stirred to obtain a clear solution. That, the solution of HCHO and DMF were added in ice-cold condition and stirred for 6 hours in an ice-bath and left overnight at room temperature. The progress of the reaction was monitored by TLC with hexane and ethyl acetate (7:3) as mobile phase. The obtained solid was isolated and recrystalized with ethanol to give afford 4-(4-morpholinomethyl)-3-(4-trifluoromethyl)phenyl)-3,4dihydroimidazo [4,5-b]indol-2yl)benzene-1,2-diol .The yield of compound was 70% with melting point 154-156^0 C. The compound was characterized by spectral data (IR, ^{1}H-NMR, ^{13}C-NMR and mass data) and elemental analysis.

Typical Procedure for Synthesis of 1-(4-subtetude methyl/methoxy /chloro/bromo/nitro/phenyl)-3-(5-((3- (4-trifluro methyl phenyl)-4-(thiomorpholine/4-methylpiperazine/morpholin -4-yl) methyl)-3, 4dihydroimidazo (4, 5-b) indol -2yl) methyl)-2-oxo-2H-1, 3, 2-benzo Dioxaphosphol-2-yl) urea 9(a-l).

A solution of (phenyl carbomyl) phosphoramidic dichloride (2mmol) in 25 ml of dry toluene was added drop wise over a period of20 minutes to a stirred solution of 4-(4-morpholinomethyl)-3-(4-trifluoromethyl)phenyl)-3,4-dihydroimidazo[4,5-b]indol-2yl) benze-1,2-diol (7a) and triethyl- amine in 30ml of dry toluene and 10ml of

tetrahydrofuran at 5^0C.After completion of the addition, the temperature of the reaction mixture was slowly raised to room temperature and stirred for 24 hours later the reaction mixture was heated to 60-65^0C and maintained for 5 hours with stirring. The completion of the reaction was monitored by TLC analyser.Tri ethylamine and hydrochloric acid was filtered from mixture and solvent was removed under reduced pressure. The residue was washed with water and then recrystalized with aqueous 2-propanal to get pure compound 1-(5-(4-(morpholinomethyl)-3-(4-(trifluoromethyl)phenyl)-3,4-dihydroimidazo[4,5-b]indol-2-yl)-2-oxidobenzo[d] [1,3,2] dioxaphosphol-2-yl)-3 phenyl urea**9(a),** the compound of the yield 63%,melting point at 151^0C.The structures of compounds were established by IR, ^{1}H-NMR,^{13}C-NMR,^{31}P-NMR, Mass data and elemental analysis.

The similar procedure was adopted to synthesis by the reaction between 4-(4-thiomorpholine/methylpiperazine morpholine/)-(3-(4-trifluorometyl) phenyl)-3,4-dihydro imidazo[4,5-b]indol-2-yl)-benzene-1,2-diol**7(b-c)** with (phenyl carbomyl) phosphoramidic dichloride**8(a-f)** were prepared by the condition respectively. The structures of newly synthesized compounds **9(b-l)** were established by IR,^{1}H-NMR,^{13}C-NMR,^{31}P-NMR,Mass data and elemental analysis.

Spectral, Physical and analytical data for the compounds:

Table 1.2: The IR (KBR) Spectra of 1-(4-subtetude methyl/methoxy /chloro/bromo/nitro/phenyl)-3-(5-((3-(4-trifluro methyl phenyl)-4-(thiomorpholine/4-methyl piperazine / morpholin -4-yl) methyl)-3, 4dihydroimidazo (4, 5-b) indol -2yl) methyl)-2-oxo-2H-1, 3, 2-benzo Dioxaphosphol-2-yl) urea 9(a-l) ν/ δ, cm^{-1}

Comp	X	R	P-NH	Ar-H	C=O (Urea)	C=N	C-N	P=O	P-O-$C_{(-Ar)}$
9a	O	H	3416-3382	3040	1675	1618	1416	1250	950
9b	O	-CH_3	3420-3382	3040	1675	1618	1416	1250	950
9c	O	OCH_3	3416-3385	3040	1675	1618	1416	1250	950
9d	O	Cl	3414-3383	3040	1675	1616	1416	1250	950
9e	O	Br	3416-3385	3027	1670	1620	1416	1250	950
9f	O	NO_2	3020-3382	3027	1680	1618	1416	1250	950
9g	N-CH_3	H	3416-3382	3032	1678	1616	1416	1250	950
9h	S	H	3425-3384	3025	1670	1618	1416	1250	950
9i	S	CH_3	3420-3386	3027	1675	1620	1416	1250	950
9j	S	OCH_3	3420-3382	3027	1680	1622	1416	1250	950
9k	S	Cl	3430-3386	3040	1675	1622	1416	1250	950
9l	S	NO_2	3425-3384	3040	1675	1618	1416	1250	950

Table 1.3: The ^{1}H- NMR (400MHz) spectral data1-(4-subtetude methyl/methoxy /chloro/bromo/nitro/phenyl)-3-(5-((3-(4-trifluro methyl phenyl)-4-(thiomorpholine/4-methyl piperazine / morpholin -4-yl) methyl)-3, 4dihydroimidazo (4, 5-b) indol -2yl) methyl)-2-oxo-2H-1, 3, 2-benzo Dioxaphosphol-2-yl) urea 9(a-l)δ, ppm

Comp	X	R	^{1}H – NMR (DMSO – d6)(δPPM)
9a	O	H	2.50 (t,4H-CH_2-N-CH_2 of morpholine ring J=7.1 Mz ,H-3',H-2'), 3.65 (t,4H-CH_2-O-CH_2 of morpholine ring), 4.92(s,2H,N-CH_2 attached to indol ring), 6.1(s,1H,NH of Urea), 6.3 (s,1H, NH group attached to phosphorus moiety), 6.84-7.47 (m,3H- Phenyl attached to phosphorus moiety), 7.29-7.62 (m,4H ,Ar-CF_3 attached to imidazole ring), 7.42-8.36(m,4H,Indol benzene ring).
9b	O	-CH_3	2.50 (t,4H-CH_2-N-CH_2 of morpholine ring J=7.1 Mz ,H-3',H-2'), 2.34 (s,3H, CH_3-Ar), 3.65 (t,4H-CH_2-O-CH_2 of morpholine ring), 4.92(s,2H,N-CH_2 attached to indol ring), 6.1(s,1H,NH of Urea), 6.3 (s,1H, NH group attached to phosphorus moiety), 6.84-7.47(m,3H- Phenyl attached to phosphorus moiety), 7.29-7.62 (m,4H ,Ar-CF_3 attached to imidazole ring), 7.42-8.36(m,4H,Indol benzene ring),7.43-7.61(m,5H,NH-Ar)
9c	O	OCH_3	2.50 (t,4H-CH_2-N-CH_2 of morpholine ring J=7.1 Mz ,H-3',H-2'), 3.65 (t,4H-CH_2-O-CH_2 of morpholine ring), 3.84(s,3H,OCH_3-Ar), 4.92 (s,2H ,N-CH_2 attached to indol ring), 6.1(s,1H,NH of Urea), 6.3 (s,1H, NH group attached to phosphorus moiety),6.71-7.54 (m,4H,Indol benzene ring), 6.84-7.47(m,3H- Phenyl attached to phosphorus moiety), 7.29 -7.62 (m,4H ,Ar-CF_3 attached to imidazole ring),7.43-7.61(m,5H,NHAr)
9d	O	Cl	2.50 (t,4H-CH_2-N-CH_2 of morpholine ring J=7.1 Mz ,H-3',H-2'), 3.65 (t,4H-CH_2-O-CH_2 of morpholine ring), 4.92 (s,2H,N-CH_2 attached to indol ring), 6.1(s.1H,NH of Urea), 6.3 (s,1H, NH group attached to phosphorus moiety),6.71-7.54 (m,4H,Indol benzene ring), 6.84-7.47(m,3H- Phenyl attached to phosphorus moiety), 7.29 7.62 (m,4H ,Ar-CF_3 attached to imidazole ring),7.43-7.71(m,5H,Cl-Ar)
9e	O	Br	2.50 (t,4H-CH_2-N-CH_2 of morpholine ring J=7.1 Mz ,H-3',H-2'), 3.65 (t,4H-CH_2-O-CH_2 of morpholine ring), 4.92(s,2H,N-CH_2 attached to indol ring), 6.1(s.1H,NH of Urea), 6.3 (s,1H, NH group attached to phosphorus moiety),6.71-7.54 (m,4H,Indol benzene ring), 6.84-7.47(m,3H- Phenyl attached to phosphorus moiety), 7.29- 7.62 (m,4H ,Ar-CF_3 attached to imidazole ring),7.53-7.71(m,5H,Br-Ar)
9f	O	NO_2	2.50 (t,4H-CH_2-N-CH_2 of morpholine ring J=7.1 Mz ,H-3',H-2'), 3.65 (t,4H-CH_2-O-CH_2 of morpholine ring), 4.92(s,2H,N-CH_2 attached to indol ring), 6.1(s,1H,NH of Urea), 6.3 (s,1H, NH group attached to phosphorus moiety),6.71-7.54 (m,4H,Indol benzene ring), 6.84-7.47(m,3H- Phenyl attached to phosphorus moiety), 7.29- 7.62 (m,4H, Ar-CF_3 attached to imidazole ring),7.83-8.21(m,5H,NH-Ar)
9g	N-CH_3	H	2.24(s,3H, CH_3 attached to piperdine 2.50 (t,4H-CH_2-N-CH_2 of morpholine ring J=7.1 Mz ,H-3',H-2'), 3.65 (t,4H-CH_2-O-CH_2 of morpholine ring), 4.92(s,2H,N-CH_2 attached to indol ring), 6.1(s.1H,NH of Urea), 6.3 (s,1H, NH group attached to phosphorus moiety),6.71-7.54 (m,4H,Indol benzene ring), 6.84-7.47(m,3H- Phenyl attached to phosphorus moiety), 7.29 -7.62 (m,4H ,Ar-CF_3 attached to imidazole ring),7.19-7.61(m,5H,Urea-Ar).
9h	S	H	2.50 (t,4H-CH_2-N-CH_2 of morpholine ring J=7.1 Mz ,H-3',H-2'), 2.45 (t,4H-CH_2-S-CH_2 of morpholine ring), 4.92(s,2H,N-CH_2 attached to indol ring), 6.1(s,1H,NH of Urea), 6.3 (s,1H, NH group attached to phosphorus moiety), 6.84-7.47(m,3H- Phenyl attached to phosphorus moiety), 7.29 7.62 (m,4H,Ar-CF_3 attached to imidazole ring), 7.42-8.36(m,4H,Indol benzene ring) 7.19-7.61(m,5H,Urea-Ar).
9i	S	CH_3	2.30(s,3H Me group attached to phenyl ring), 2.50 (t,4H-CH_2-N-CH_2 of morpholine ring J=7.1 Mz ,H-3',H-2'), 2.45 (t,4H-CH_2-S-CH_2 of morpholine ring), 4.92(s,2H,N-CH_2 attached to indol ring), 6.1(s.1H,NH of Urea), 6.3 (s,1H, NH group attached to phosphorus moiety), 6.84-7.47(m,3H- Phenyl attached to phosphorus moiety), 7.29-7.62 (m,4H,Ar-CF_3 attached to imidazole ring), 7.42-8.36(m,4H,Indol benzene ring),7.21-7.56 (m,5H,Urea-Ar).
9j	S	OCH_3	3.83 (s,3H OMe group attached to phenyl ring), 2.50 (t,4H-CH_2-N-CH_2 of morpholine ring J=7.1 Mz ,H-3',H-2'), 2.45 (t,4H-CH_2-S-CH_2 of morpholine ring), 4.92(s,2H,N-CH_2 attached to indol ring), 6.1(s.1H,NH of Urea), 6.3 (s,1H, NH group attached to phosphorus moiety), 6.84-7.47(m,3H- Phenyl attached to phosphorus moiety), 7.29-7.62 (m,4H ,Ar-CF_3 attached to imidazole ring), 7.42-8.36(m,4H,Indol benzene ring), 6.93-7.51 (m,5H,Urea-Ar).
9k	S	Cl	2.50 (t,4H-CH_2-N-CH_2 of morpholine ring J=7.1 Mz ,H-3',H-2'), 2.25 (t,4H-CH_2-S-CH_2 of morpholine ring), 4.92(s,2H,N-CH_2 attached to indol ring), 6.1(s,1H,NH of Urea), 6.3 (s,1H, NH group attached to phosphorus moiety),6.71-7.54 (m,4H,Indol benzene ring), 6.84-7.47(m,3H- Phenyl attached to phosphorus moiety), 7.29- 7.62 (m,4H ,Ar-CF_3 attached to imidazole ring),7.43-7.71(m,5H,Cl-Ar)
9l	S	NO_2	2.50 (t,4H-CH_2-N-CH_2 of morpholine ring J=7.1 Mz ,H-3',H-2'),2 .45 (t,4H-CH_2-S-CH_2 of morpholine ring), 4.92(s,2H,N-CH_2 attached to indol ring), 6.1(s,1H,NH of Urea), 6.3 (s,1H, NH group attached to phosphorus moiety),6.71-7.54 (m,4H,Indol benzene ring), 6.84-7.47(m,3H- Phenyl attached to phosphorus moiety), 7.29- 7.62 (m,4H ,Ar-CF_3 attached to imidazole ring),7.83-8.21(m,5H,NH-Ar)

Table 1.4: The ^{13}C-NMR (75 MHz) Spectral data of 1-(4-subtetude methyl/methoxy /chloro/bromo/nitro/phenyl)-3-(5-((3-(4-trifluro methyl phenyl)-4-(thio/4-methyl piperazine / morpholin -4-yl) methyl)-3, 4dihydroimidazo (4, 5-b) indol -2yl) methyl)-2-oxo-2H-1, 3, 2-benzo Dioxaphosphol-2-yl) urea 9(a-l)δPPM

Comp	X	R	^{13}C-NMR (75 MHz),NMR(DMSO d6)(δ_PPM)
9a	O	H	117.8, 123.8, 124.6, 114.3, 145.7, 145.2, 123.9, 141.5, 14.1.4, 124.0, 130. 5, 124.1, 124.0, 123.9, 122.2, 119.8, 121.7, 109.6, 127.7, 136.7, 136.5, 120.2, 84.2, 53.8, 64.4, 64.4, 53.8, 152.0, 139.4, 121.6, 128.0, 128.0, 128.9 and 121.6. Corresponding to C_1, C_2, C_3, C_4, C_5, C_6, C_7, C_8, C_9, C_{10}, C_{11}, C_{12}, C_{13}, C_{14},C_{15},C_{16}, C_{17}, C_{18}, C_{19}, C_{20}, C_{21}, C_{22}, C_{23}, C_{24}, C_{25}, C_{26}, C_{27}, C_{28}, C_{29}, C_{30}, C_{31}, C_{32}, C_{33}, and C_{34} respectively.
9b	O	-CH_3	117.8, 123.8, 124.6, 114.3, 145.7, 145.2, 123.9, 141.5, 14.1.4, 124.0, 130. 5, 124.1, 124.0, 123.9, 122.2, 119.8, 121.7, 109.6, 127.7, 136.7, 136.5, 120.2, 84.2, 53.8, 64.4, 64.4, 53.8, 152.0,131.7, 119.8, 114.5, 158.9, 114.5, 119.8 and 55.8. Corresponding to C_1, C_2, C_3, C_4, C_5, C_6, C_7, C_8, C_9, C_{10}, C_{11}, C_{12}, C_{13}, C_{14} ,C_{15} ,C_{16}, C_{17}, C_{18}, C_{19}, C_{20} , C_{21}, C_{22}, C_{23}, C_{24}, C_{25}, C_{26}, C_{27}, C_{28}, C_{29}, C_{30}, C_{31}, C_{32}, C_{33}, C_{34} andC_{35}respectively
9c	O	OCH_3	117.8, 123.8, 124.6, 114.3, 145.7, 145.2, 123.9, 141.5, 14.1.4, 124.0, 130. 5, 124.1, 124.0, 123.9, 122.2, 119.8, 121.7, 109.6, 127.7, 136.7, 136.5, 120.2, 84.2, 53.8, 64.4, 64.4, 53.8, 152.0,136.4, 121.5, 129.2,136.8, 129.2, 121.5 and 21.3. Corresponding to C_1, C_2, C_3, C_4, C_5, C_6, C_7, C_8, C_9, C_{10}, C_{11}, C_{12}, C_{13}, C_{14} ,C_{15} ,C_{16}, C_{17}, C_{18}, C_{19}, C_{20} , C_{21}, C_{22}, C_{23}, C_{24}, C_{25}, C_{26}, C_{27}, C_{28}, C_{29}, C_{30}, C_{31}, C_{32}, C_{33}, C_{34} andC_{35}respectively
9d	O	Cl	117.8, 123.8, 124.6, 114.3, 145.7, 145.2, 123.9, 141.5, 14.1.4, 124.0, 130. 5, 124.1, 124.0, 123.9, 122.2, 119.8, 121.7, 109.6, 127.7, 136.7, 136.5, 120.2, 84.2, 53.8, 64.4, 64.4, 53.8, 152.0, 137.5, 120.8, 129.0, 133.3, 129.0, and 120.8. Corresponding to C_1, C_2, C_3, C_4, C_5, C_6, C_7, C_8, C_9, C_{10}, C_{11}, C_{12}, C_{13}, $C_{14,}$ $C_{15,}$ C_{16}, C_{17}, C_{18}, C_{19}, $C_{20,}$ C_{21}, C_{22}, C_{23}, C_{24}, C_{25}, C_{26}, C_{27}, C_{28}, C_{29}, C_{30}, C_{31}, C_{32}, C_{33}, and C_{34} respectively.
9e	O	Br	117.8, 123.8, 124.6, 114.3, 145.7, 145.2, 123.9, 141.5, 14.1.4, 124.0, 130. 5, 124.1, 124.0, 123.9, 122.2, 119.8, 121.7, 109.6, 127.7, 136.7, 136.5, 120.2, 84.2, 53.8, 64.4, 64.4, 53.8, 152.0, 138.4, 121.9, 131.8, 123.3, 131.8, and 121.9. Corresponding to C_1, C_2, C_3, C_4, C_5, C_6, C_7, C_8, C_9, C_{10}, C_{11}, C_{12}, C_{13}, $C_{14,}$ $C_{15,}$ C_{16}, C_{17}, C_{18}, C_{19}, $C_{20,}$ C_{21}, C_{22}, C_{23}, C_{24}, C_{25}, C_{26}, C_{27}, C_{28}, C_{29}, C_{30}, C_{31}, C_{32}, C_{33}, and C_{34} respectively.
9f	O	NO_2	117.8, 123.8, 124.6, 114.3, 145.7, 145.2, 123.9, 141.5, 14.1.4, 124.0, 130. 5, 124.1, 124.0, 123.9, 122.2, 119.8, 121.7, 109.6, 127.7, 136.7, 136.5, 120.2, 84.2, 53.8, 64.4, 64.4, 53.8, 152.0, 145.5, 119.9, 124.1, 143.5, 124.1, and 119.9. Corresponding to C_1, C_2, C_3, C_4, C_5, C_6, C_7, C_8, C_9, C_{10}, C_{11}, C_{12}, C_{13}, $C_{14,}$ $C_{15,}$ C_{16}, C_{17}, C_{18}, C_{19}, $C_{20,}$ C_{21}, C_{22}, C_{23}, C_{24}, C_{25}, C_{26}, C_{27}, C_{28}, C_{29}, C_{30}, C_{31}, C_{32}, C_{33}, and C_{34} respectively.
9g	N-CH_3	H	117.8, 123.8, 124.6, 114.3, 145.7, 145.2, 123.9, 141.5, 14.1.4, 124.0, 130. 5, 124.1, 124.0, 123.9, 122.2, 119.8, 121.7, 109.6, 127.7, 136.7, 136.5, 120.2, 84.2, 52.8, 57.3, 57.3, 52.8 , 152.0, 139.4, 121.6, 128.0, 128.0, 128.9, 121.6 and 46.6 . corresponding to C_1, C_2, C_3, C_4, C_5, C_6, C_7, C_8, C_9, C_{10}, C_{11}, C_{12}, C_{13}, C_{14} ,C_{15} ,C_{16}, C_{17}, C_{18}, C_{19}, C_{20} , C_{21}, C_{22}, C_{23}, C_{24}, C_{25}, C_{26}, C_{27}, C_{28}, C_{29}, C_{30}, C_{31}, C_{32}, C_{33}, C_{34} andC_{35}respectively
9h	S	H	117.8, 123.8, 124.6, 114.3, 145.7, 145.2, 123.9, 141.5, 14.1.4, 124.0, 130. 5, 124.1, 124.0, 123.9, 122.2, 119.8, 121.7, 109.6, 127.7, 136.7, 136.5, 120.2, 84.2, 53.3, 28.0, 28.0, 58.3, 152.0, 139.4, 121.6, 128.0, 128.0, 128.9 and 121.6. Corresponding to C_1, C_2, C_3, C_4, C_5, C_6, C_7, C_8, C_9, C_{10}, C_{11}, C_{12}, C_{13}, $C_{14,}$ C_{15}, C_{16}, C_{17}, C_{18}, C_{19}, $C_{20,}$ C_{21}, C_{22}, C_{23}, C_{24}, C_{25}, C_{26}, C_{27}, C_{28}, C_{29}, C_{30}, C_{31}, C_{32}, C_{33}, and C_{34} respectively.
9i	S	CH_3	117.8, 123.8, 124.6, 114.3, 145.7, 145.2, 123.9, 141.5, 14.1.4, 124.0, 130. 5, 124.1, 124.0, 123.9, 122.2, 119.8, 121.7, 109.6, 127.7, 136.7, 136.5, 120.2, 84.2, 53.3, 28.0, 28.0, 58.3, 152.0,136.4, 121.5, 129.2, 136.8, 129.2, 121.5, and 21.3. Corresponding to C_1, C_2, C_3, C_4, C_5, C_6, C_7, C_8, C_9, C_{10}, C_{11}, C_{12}, C_{13}, $C_{14,}$ C_{15}, C_{16}, C_{17}, C_{18}, C_{19}, $C_{20,}$ C_{21}, C_{22}, C_{23}, C_{24}, C_{25}, C_{26}, C_{27}, C_{28}, C_{29}, C_{30}, C_{31}, C_{32}, C_{33}, $C_{34,}$ and C_{35}respectively.
9j	S	OCH_3	117.8, 123.8, 124.6, 114.3, 145.7, 145.2, 123.9, 141.5, 14.1.4, 124.0, 130. 5, 124.1, 124.0, 123.9, 122.2, 119.8, 121.7, 109.6, 127.7, 136.7, 136.5, 120.2, 84.2, 53.3, 28.0, 28.0, 58.3, 152.0, 131.7, 119.8, 114.5, 158.9, 114.5, 119.8 and 55.8. Corresponding to C_1, C_2, C_3, C_4, C_5, C_6, C_7, C_8, C_9, C_{10}, C_{11}, C_{12}, C_{13}, $C_{14,}$ C_{15}, C_{16}, C_{17}, C_{18}, C_{19}, $C_{20,}$ C_{21}, C_{22}, C_{23}, C_{24}, C_{25}, C_{26}, C_{27}, C_{28}, C_{29}, C_{30}, C_{31}, C_{32}, C_{33}, $C_{34,}$ and C_{35}respectively.
9k	S	Cl	117.8, 123.8, 124.6, 114.3, 145.7, 145.2, 123.9, 141.5, 14.1.4, 124.0, 130. 5, 124.1, 124.0, 123.9, 122.2, 119.8, 121.7, 109.6, 127.7, 136.7, 136.5, 120.2, 84.2, 53.3, 28.0, 28.0, 58.3, 152.0, 137.5, 120.8, 129.0, 133.3, 129.0 and 120.8. Corresponding to C_1, C_2, C_3, C_4, C_5, C_6, C_7, C_8, C_9, C_{10}, C_{11}, C_{12}, C_{13}, $C_{14,}$ C_{15}, C_{16}, C_{17}, C_{18}, C_{19}, $C_{20,}$ C_{21}, C_{22}, C_{23}, C_{24}, C_{25}, C_{26}, C_{27}, C_{28}, C_{29}, C_{30}, C_{31}, C_{32}, C_{33}, and C_{34} respectively.
9l	S	NO_2	117.8, 123.8, 124.6, 114.3, 145.7, 145.2, 123.9, 141.5, 14.1.4, 124.0, 130. 5, 124.1, 124.0, 123.9, 122.2, 119.8, 121.7, 109.6, 127.7, 136.7, 136.5, 120.2, 84.2, 53.3, 28.0, 28.0, 58.3, 152.0, 145.5, 119.9, 124.1, 143.5, 124.1, and 119.9. Corresponding to C_1, C_2, C_3, C_4, C_5, C_6, C_7, C_8, C_9, C_{10}, C_{11}, C_{12}, C_{13}, $C_{14,}$ C_{15}, C_{16}, C_{17}, C_{18}, C_{19}, $C_{20,}$ C_{21}, C_{22}, C_{23}, C_{24}, C_{25}, C_{26}, C_{27}, C_{28}, C_{29}, C_{30}, C_{31}, C_{32}, C_{33}, and C_{34} respectively.

Table 1.5: The ^{31}P-NMR (161.89 MHz) spectra of 1-(4-substituted/phenyl)-3-(5-((3-(4-trifluro methyl phenyl)-4-(thio/morpholin-4-yl) methyl)-3,4dihydroimidazo (4,5-b)indol -2yl)methyl)-2-oxo-2H-1,3,2-benzo Dioxaphosphol-2-yl)urea 9(a-l)(δ, ppm)

Comp	X	R	^{31}P – NMR (DMSO – d6) δ (PPM)
9a	O	H	6.5-6.7
9b	O	-CH_3	7.2-7.4
9c	O	OCH_3	7.6
9d	O	Cl	8.0-8.4
9e	O	Br	7.9
9f	O	NO_2	11.0-11.3
9g	N-CH_3	H	8.4-8.5
9h	S	H	7.30
9i	S	CH_3	**7**.85-7.89
9j	S	OCH_3	8.0-8.3
9k	S	Cl	9.4-9.6
9l	S	NO_2	10.7-10.9

Table 1.6: Physical and Analytical data of 1-(4-subtetude methyl/methoxy /chloro/bromo/nitro/phenyl)-3-(5-((3-(4-trifluro methyl phenyl)-4-(thio/4-methyl piperazine / morpholin -4-yl) methyl)-3, 4dihydroimidazo (4, 5-b) indol -2yl) methyl)-2-oxo-2H-1, 3, 2-benzo Dioxaphosphol-2-yl) urea 9(a-l)

COMP	MOLECULAR FORMULA	MP (^{0}C)	YIELD (%)	ELEMENTAL ANALYSIS (%)	
				FOUND	CLAC
9a	$C_{34}H_{28}F_3N_6O_5P$	151-153	63	C:58.50,H:3.60, F:7.38,N:11.60, O:10.72,P:3.60	C,59.30,H:4.10, F,8.28, N,12.20, O, 11.62, P,4.50
9b	$C_{35}H_{30}F_3N_6O_5P$	143-145	65	C:59.03,H:3.70, F:7.31,N:11.56, O:10.59, P:3.81	C:59.83,H:4.30, F:8.11,N:11.96, O:11.39, P:4.41
9c	$C_{35}H_{30}F_3N_6O_6P$	153-155	67	C:57.70,H:3.71, F:7.13,N:11.19, O:12.66,P:3.61	C:58.50,H:4.21, F:7.93,N:11.69, O:13.36, P:4.31
9d	$C_{34}H_{27}ClF_3N_6O_5P$	168-170	65	C:54.67,H:3.21, Cl:4.10, F:6.97, N:11.03,O:10.26, P:3.58	C:56.48, H;3.76 , Cl:4.90, F:7.88, N:11.62,O:11.06, P: 4.28
9e	$C_{34}H_{27}BrF_3N_6O_7P$	156-158	70	C:50.28,H:2.80, Br:9.49, F:6.53, N:9.81,O:13.21, P:3.27	C:51.08,H:3.40, Br:9.99, F:7.33 N:10.51,O:14.01, P, 3.87
9f	$C_{34}H_{27}ClF_3N_6O_5P$	116-118	73	C:55.58,H:3.26, Cl:4.20,F:7.18, N:11.02,O:10.26, P:3.58	C:56.48,H:3.76, Cl: 4.90, F:7.88, N:11.62,O:11.06, P:4.28
9g	$C_{34}H_{31}F_3N_7O_4P$	134-136	70	C:58.42,H:4.13, F:7.46,N:13.72, O:8.58,P:3.79	C:59.22,H:4.53, F:8.26,N:14.22, O: 9.28, P: 4.49
9h	$C_{34}H_{28}F_3N_6O_4PS$	163-165	65	C:57.35,H:3.51, F:7.29,N:11.43, O:8.28,P:3.70, S:3.95	C:57.95,H:4.01, F:8.09,N:11.93, O:9.08,P:4.40, S: 4.55
9i	$C_{35}H_{30}F_3N_6O_4PS$	171-173	68	C:57.69,H:3.71, F:7.13,N:11.09, O:8.10,P:3.61, S:3.96	C:58.49,H:4.21, F:7.93,N:11.69, O:8.90,P:4.31, S:4.46
9j	$C_{35}H_{30}F_3N_6O_5PS$	146-148	75	C:56.72, H:3.62, F:6.96,N:10.94, O:10.09,P:3.62, S:3.47	C:57.22,H:4.12, F:7.76,N:11.44, O:10.89,P:4.22, S: 4.36
9k	$C_{34}H_{27}ClF_3N_6O_4PS$	163-165	70	C:54.45,H:3.18, Cl:4.00,F:6.91, N:10.77,O:7.86, P:2.39, S:3.74	C:55.25,H:3.68, Cl: 4.80, F:7.71, N:11.37,O:8.66, P,:4.19, S: 4.34
9l	$C_{34}H_{27}F_3N_7O_6PS$	140-142	67	C:53.67,H:3.03, F:6.90, N:12.50, O:12.01,P:3.53, S:3.58	C:54.47,H:3.63, F:7.60,N:13.08, O:12.81,P:4.13, S: 4.28

Biological activity

The antimicrobial activity[19] of these newly synthesized compounds was performed according to disc diffusion method as recommended by the National Committee for Clinical Laboratory[20]. The synthesized compounds were used as the concentration of 250µg/ml DMF as a solvent[21].

Antibacterial activity

The antibacterial activity[22] of mannich bases containing Dioxaphospholanes 9(a-l) were screened against the *Staphyloccus aureus* and *Bacillus cerus* (gram positive) and *Escherichia coli*, *Pseudomonas aeruginosa* (gram negative) organisms. Most of the compounds exhibited good antibacterial activity against both bacteria. Here Amoxicillin is tested as reference compound to compare the activity. The anti-bacterial activity was shown in the table: 1.7

Table 1.7: Anti-bacterial activity (Diameter zone of Inhibition in mm) of compounds of 9(a-l)

S.NO	COMP	Zone of inhibition(mm)			
		Staphylococcus aureus NCCS 2079 250 µg/disc	***Bacillus cereus NCCS 2106 250 µg/disc***	***Escherichia coli NCCS2065 250µg/disc***	***Pseudomonas aeruginosa NCCS 2200 250µg/disc***
1	9a	11	05	08	10
2	9b	12	06	09	11
3	9c	13	07	10	12
4	9d	15	08	11	13
5	9e	16	10	13	14
6	9f	18	11	15	16
7	9g	09	04	07	08
8	9h	10	05	08	09
9	9i	12	06	09	10
10	9j	13	07	10	11
11	9k	14	09	11	13
12	9l	16	10	12	14
Amoxicillin		21	27	24	22

Anti-fungal activity

Anti fungal activity of mannich bases containing Dioxaphospholanes 9(a-l) were screened against Aspergillus Niger and Candida albicans. Most of the compounds exhibit well anti fungal activity against both fungi. The most of the compounds exhibit good antifungal activity against both fungai. Here Ketoconazole is tested as reference[23, 24]compound to compare the activity. The anti-fungal activity was shown in the table: 1.8

Table 1.8: Anti-fungal activity (Diameter zone of inhibition in mm) of compounds of 9(a-l) (250µg/ml)

S.NO	COMP	Zone of inhibition (mm)	
		Aspergillus niger NCCS 1196 250 µg/disc	***Candida albicans NCCS 3471 250 µg/disc***
1	9a	13	09
2	9b	14	10
3	9c	15	11
4	9d	16	13
5	9e	17	14
6	9f	19	16
7	9g	11	08
8	9h	12	09
9	9i	13	10
10	9j	14	11
11	9k	15	12
12	9l	17	14
Ketoconazole		22	25

Docking studies:

Computational methodologies have become a crucial module of many new drug discovery programs, from his identification to lead optimization and beyond[25]and approaches such as ligand[26] or structure based virtual screening techniques[27] are widely used in many discovery efforts. One key methodology is docking of a small molecule to protein binding site was pioneered during the early 1980s, and became a highly active area of drug research.[15]Furthermore, docking can also contribute to the analysis of drug metabolism using structures such as cytochrome P450 isoforms.

Docking was carried out using GOLD (Genetic Optimization of Ligand Docking) which is based on genetic algorithm (G. A) .This method allows as partial flexibility of protein and full flexibility of ligand. The compounds are docked to the active site of proteins. After Docking, The individual binding possess of each ligand were observed and their interactions with the protein were studied.

The synthesis of Organo phosphorus mannich base 9(a-l).The Docking studies of 9a, 9b, 9g, 9k and 9(l) were carried out as model compounds on Phospholipases A2 (PLA2S) protein to study the anti-microbial activity of mannich base.

Fifure-1: Docking images of compounds 1(a-e) with Phospholipases A2

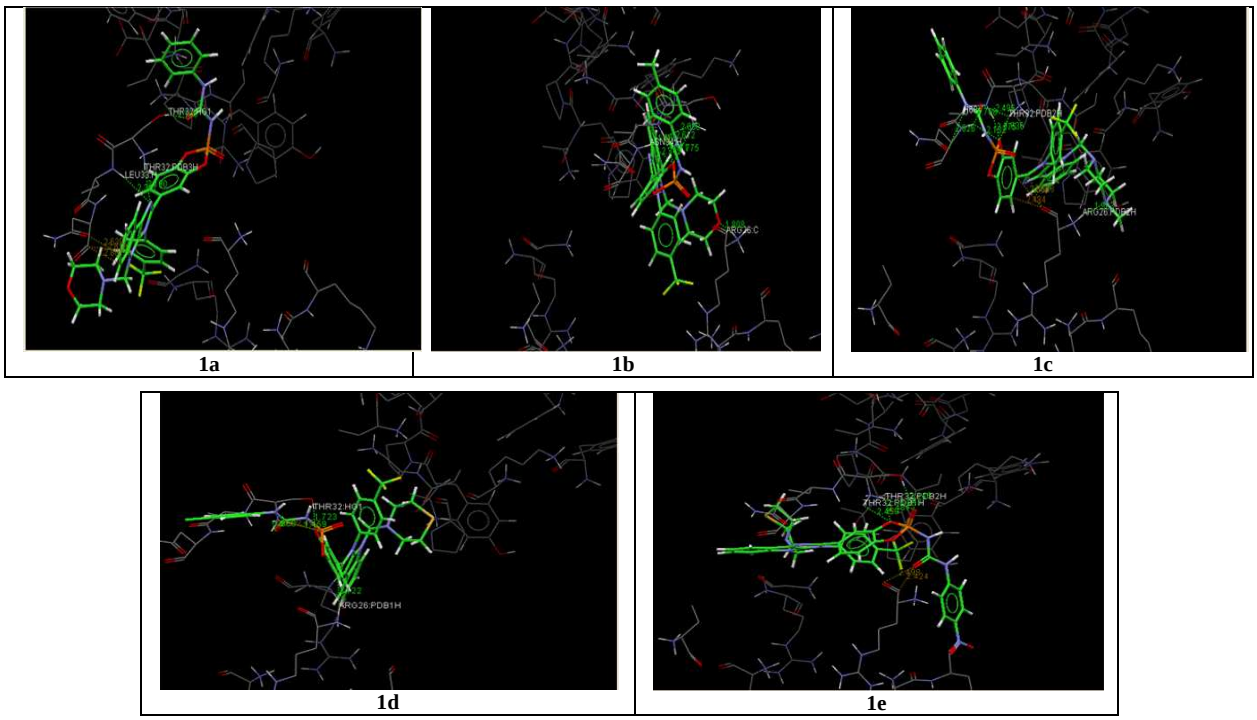


Table 1.9: Docking results of 1(a-e) on Phospholipases A2 (PLA2s)

Comp	X	R	Fitness	S(Hb_ext)	S(vdw_ext)	S(Hb_int)	S(vdw_int)
1a	O	H	28.34	8.00	23.29	0.00	-11.69
1b	O	CH_3	37.59	12.15	24.63	0.00	-8.43
1c	N-CH3	H	28.17	10.06	27.03	0.00	-19.06
1d	S	Cl	23.91	15.01	12.61	0.00	-8.43

Table 1.10: Hydrogen bonding interactions of compounds 1(a-e) with Phospholipases A2

Comp	R	x	No of Hydrogen bonds	Atom		Bond Length (A^0)	Fitness
				Protein	Comp		
2a	O	H	3	THR32:HG1		2.186	28.34
				THR32:PDB3H,		2.180	
				LEU33H		2.33	
2b	O	CH_3	2	ARG:26C,		1.808	37.59
				ASN:4H		2.343	
2c	N-CH_3		2	ARG26:PDB2H	H:66	1.903	28.17
				THR32:PDB2H		2.496	
2d	S	Cl	2	THR32:HO1		1.723	23.92
				ARG26:PDB1H		2.122	
2e	S	NO_2	2	THR32:PDB2H,		2.118	31.96
				THR32:PDB4H		2.459	

Based on protein–ligand interaction gold score fitness was evaluated and the mannich base having high gold score fitness exhibits high anti-microbial activity based on Docking studies is 9b>9l>9a>9g>9k.

CONCLUSION

The newly synthesis compounds mannich bases containing Dioxaphopolanes derivatives 9(a-l)were found to be active in the study of anti-bacterial, anti-fungal and Docking studies. It can be concluded that this class of compounds certainly holds great promise towards the pursuit to discover novel classes of antimicrobial agents

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