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새로운 Tetrahydroquinazoline의 합성, 구조 결정 및 생물학적 평가

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Synthesis, Characterization and Bioevaluation of New Tetrahydroquinazolines

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요 약. 새로운 다섯개의 tetrahydroquinazolines 의 합성과 구조 결정 및 생물학적 평가는 보고 되었다. 최초에 준비된 cyclohexanones는 목표분자를 얻기 위한 synthon으로 사용되어졌다. 이들은 치환된 벤즈알데히드와 결합되어졌고, 얻어진 chalcone은 구아니딘 염산염으로 처리했다. 모든 분자들은 인간세포에 무독하며 상당한 항균성을 보여줬다.

주제어: Tetrahydroquinazolines, cyclohexanones, 무독성 항균병원체들

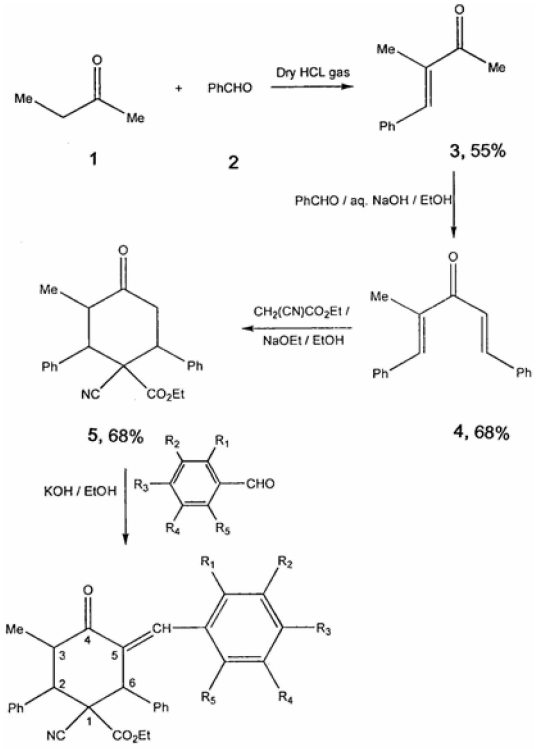
ABSTRACT. The synthesis, characterization and bioevaluation of five new tetrahydroquinazolines was reported. Initially cyclohexanones were prepared and they were used as synthons to get the target molecules. These were condensed with substituted benzaldehydes and the resulting chalcones were treated with guanidine hydrochloride . All the molecules were non-toxic to human cells and showed significant antibacterial activity.

Keywords: Tetrahydroquinazolines, Cyclohexanones, Non-toxic Antibacterial Agents

INTRODUCTION

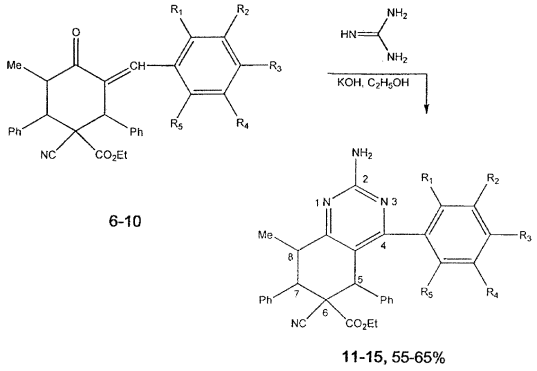
A number of plants belonging to the families Acanthaceae, Cruciferae, Malvoceae and Rutaceae are known to contain quinazoline alkaloids.[1] Quinazoline derivatives[2] possess a broad spectrum of biological activities such as antidiabetic,[3] anti-convulsant,[4] analgesic,[5] antibacterial,[6] protein tyrosine kinase inhibitors,[7] EGFR inhibitors,[8] PDGFR phosphorylation inhibitors,[9] CNS depressants[10] and antitumor activity.[11] Furthermore, the heterocyclic core constitutes more than 40 alkaloids[12] isolated from natural products and some show interesting biological profiles such as antimalarial[13] and diuretic[14] properties. Keeping this in view it was proposed to synthesize new tetrahydroquinazoline derivatives and study the influence of substituents on biological activities. A simple and convenient synthesis of five new quinazolines **11-15** have been reported (*Schemes* 1 and 2).

2 - Butanone **1** was condensed with benzaldehyde **2** in presence of dry HCl gas , yielded pure compound **3** which was again condensed with benzaldehyde in presence of 20% aq. NaOH at 5 °C. The compound **4**, thus formed was crystallized from methanol and its structure was confirmed by



6. R_1=R_4=R_5=H, R_2=R_3=OCH_3
7. R_1=R_2=R_4=R_5=H, R_3=$N(CH_3)_2$
8. R_1=R_2=R_4=R_5=H, R_3=OH
9. R_1=R_4=R_5=H,R_2=OCH_3, R_3=OH
10. R_1=R_2=R_4=R_5=H, R_3=Cl

Scheme 1



6&11. R_1=R_4=R_5=H, R_2=R_3=OCH_3
7&12. R_1=R_2=R_4=R_5=H, R_3=$N(CH_3)_2$
8&13. R_1=R_2=R_4=R_5=H, R_3=OH
9&14. R_1=R_4=R_5=H,R_2=OCH_3, R_3=OH
10&15. R_1=R_2=R_4=R_5=H, R_3=Cl

Scheme 2

physical and spectral data.[15] Cycloaddition of compound **4** to ethyl cyanoacetate in presence of sodium ethoxide resulted compound **5** as white coloured crystals. The I.R. spectra exhibited bands at 1705-1720 (cyclic C=O), 1725-1755 (C=O ester), 2235 and 2260 cm^{-1} (CN). The ^{1}HNMR spectra can be rationalized by presuming that the two aryl groups at 2 and 6 positions are in cis-1,3-di-equatorial arrangement in the preferred rigid chair conformation of cyclohexanone ring, although a number of dynamic forms do exist.[15] Thus, the ^{1}HNMR spectral data is assigned as, δ 0.60-0.75 (t, 3H, **CH_3** CH_2-O),0.9 (d, 3H, CH_3), 2.72-2.83 (dd,1H, H_{c-eq}), 3.18-3.46 (m, 2H, H_c−H_{c-ax}), 3.65-3.84 (m, 4H, H_b+H_f+OCH_2), 7.2-7.4 (m, 10H, aromatic protons).The formation of compound **5** is further proved by its ^{13}C NMR data viz., δ11.994 (1C, CH_3), 13.527 (1C, OCH_2**CH_3**), 43.956 (1C, C_e), 45.718 (1C, C_f), 49.401(1C, C_c), 56.280 (1C, C_a), 60.238 (1C, C_b), 62.633 (1C, OCH_2), 116.780 (1C, CN), 128.212, 128.667, 128.815, 128.967, 135.861, 136.624 (12C, Ar-C's), 166.634 (1C, COO), 206.939 (1C, C_d).

Compound **5** on condensation with substituted benzaldehydes, yielded chalcones **6-10** which were crystallized from methanol as yellow needles. The thin layer chromatography of these chalcones showed characteristic colour spots with methanol – sulphuric acid (9 : 1) as spraying reagent. They also exhibited the characteristic colour test with antimony trichloride.[16] Further the formation of chalcones is conformed by their spectral data. The synthesis of tetrahydroquinazolines **11-15** was accomplished by condensing chalcones **6-10** with guanidine hydrochloride in alkaline medium (*Scheme* 2).

Compound **11** analyzed for $C_{31}H_{32}N_4O_4$, was well supported by its spectral data. The I.R. spectrum showed the absorption at 1685 and 1597 cm^{-1} characteristic[17] of the C=N and C=C stretch of the pyrimidine system and a sharp peak at 3492 cm^{-1} indicating the NH stretchings of the amino group. The characteristic out of plane CH bending[18] was observed at 1002 and 805 cm^{-1}.The formation of compound **11** is further confirmed by its FAB mass spectrum. Mass spectrum showed the characteristic $[M]^+$ ion at m/z 548(5%). The other fragment ions

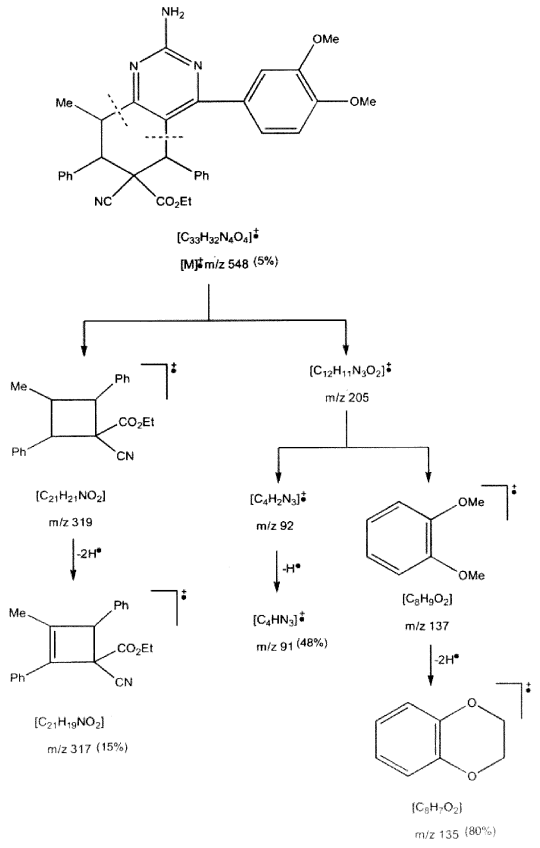


Chart 1

of m/z 307(16%), 240(21%), 209(18%), 195(58%), 165(16%), 154(100%, base peak), 135(80%), 120(18%), 107(36%), 91(48%), 69(38%), 55(44%) were observed. The fragmentation pattern was presented in the *Chart*-1. These fragmentations were characteristic for tetrahydroquinazolines.[19-24] The ^{1}H and ^{13}C NMR data is in accordance with the structure. The ^{13}C NMR data was explained as 16.622 (1C, CH_3), 37.499 (1C, C_e), 37.763 (1C, C_h), 44.015(1C, C_g), 50.319 (1C, C_i), 53.510 (1C, OCH_3), 67.476 (1C, OCH_2), 116.704 (1C, CN), 156.238-129.520 (22C, Ar-C's), 167.369 (1C, C=O). Thus, the formation of compound **11** was characterized.

RESULTS AND DISCUSSION

Tetrahydroquinazolines **11-15** were tested for cytotoxic activity at NFMC, Bharathidasan University, Tiruchirapally, India. Studies were performed by using Dye exclusion method. All the compounds **11-15** are non-toxic to PBMC and two different cancer cell lines (Jurkat & Raji) from 5 μg to 800 μg level (*Table* 1).

Table 1. Cytotoxic Evaluation of compounds **11-15***

Entry No.	Compound	Result
01.	11	Non Toxic (5 μg – 800 μg)
02.	12	Non Toxic (5 μg – 700 μg)
03.	13	Non Toxic (5 μg – 800 μg)
04.	14	Non Toxic (5 μg – 600 μg)
05.	15	Non Toxic (5 μg – 800 μg)

*Anticancer Activity Testing was performed by Dye exclution method. Bioactivity assay was performed in a 96 Well tissue culture plate (Greiner, Germany). A constant number of cancerous (Jurkat, Raji & PBMC) cell suspension and desired concentrations of the compounds and media were added into each well. The plate was incubated at 37 °C in CO_2 incubator with 5% CO_2. The number of live cells were counted after every 12 hrs by using inverted phase contrast microscope (Nikon TM) and the Newbauer's counting chamber by dye exclution method.

The minimum inhibitory concentrations (MIC) of quinazoline derivatives **11-15** were obtained against five representative Gram-positive organisms and four Gram-negative organisms. It has been observed that all the derivatives exhibited interesting biological activity however, with a degree of variation. The potencies of these molecules varied somewhat, depending upon the nature of their substituent(s) on the phenyl moiety in the 4-position. The methoxy analogue **11** displayed good zone of inhibition for B. subtilis and moderately active on B.pumilus and E.faecalis whereas it is inactive against S.faecalis and M.luteus (*Table* 2, entry 1) at MIC 20 μg/ml and it showed good activity, almost equal to that of Benzyl Penicillin against B. subtilis at 200 μg/ml concentration (*Table* 2, entry 4). Surprisingly and in contrast to compound **11**, the N,N-dimethyl amino derivative **12** is inactive against B. subtilis, B. pumilus and E.faecalis whereas it is active against S.faecalis and M.luteus (*Table* 2, entry 5) at MIC 20 μg/ml and it is equally active as Benzyl Penicillin against S.faecalis and M.luteus at 200 μg/ml dose (*Table* 2, entry 8). Substitution of both methoxy and hydroxyl groups on phenyl ring **(14)** increases the activity of

Table 2. Antibacterial Activity Inhibition Zones (mm)*# Gram-Positive Bacteria

Compd. No.	Entry No.	Conc. μg/ml	B.subtilis	B.pumilus	E.faecalis	S.faecalis	M.luteus
11	1	20	12	10	11	-	-
	2	50	14	13	13	11	11
	3	100	18	14	15	13	14
	4	200	21	18	17	17	19
12	5	20	-	-	-	11	12
	6	50	-	11	12	15	14
	7	100	14	15	15	18	18
	8	200	17	18	19	20	20
13	9	20	-	10	12	10	10
	10	50	12	12	13	12	13
	11	100	15	16	17	15	17
	12	200	19	18	20	18	19
14	13	20	-	13	11	12	10
	14	50	13	16	14	13	11
	15	100	16	19	17	15	18
	16	200	18	21	20	19	20
15	17	20	11	12	-	-	10
	18	50	12	15	14	12	11
	19	100	16	17	16	13	14
	20	200	19	20	19	17	16
Benzyl Penicillin	21	200	25	24	22	21	22

*Negative Control DMSO, no activity, #Solutions of different concentrations of compounds **11-15** were prepared in DMSO and tested against Gram-positive bacteria. The antibacterial activity of the test compounds **11-15** was compared with Benzyl Penicillin. All the compounds are non-toxic to human cells at the above dose.

the molecule and this is evidenced by the fact that it inhibited all Gram-positive organisms except B. subtilis at MIC 20 μg/ml (*Table* 2, entry 13) and at 200 μg/ml concentration (*Table* 2, entry 16) it exhibits inhibitory activity, similar to Benzyl Penicillin against all Gram-positive organisms except B. subtilis . All these interpretations were better visualized in *Figs*. 1 and 2.

The level of activity of tetrahydroquinazolines **11-15** towards Gram-negative organisms is slightly less than to that observed with Gram-positive organisms (*Figs* 1-4). Compound **12**, which contain N,N-dimethyl amino substituent, displayed good antibacterial activity against E.Coli, P.vulgaris and K. pneumoniae at all concentrations (*Table* 3, entry 5-8). The methoxy analogue **11** exhibits inhibitory zones against P.marginalis and E.Coli but it is inactive against P.vulgaris and K.pneumoniae at MIC 20 μg/ml (*Table* 3, entry 1). Quinazoline **14** was inactive against all Gram-negative organisms at MIC 20 μg/ml (*Table* 3, entry 13) and less potent than other

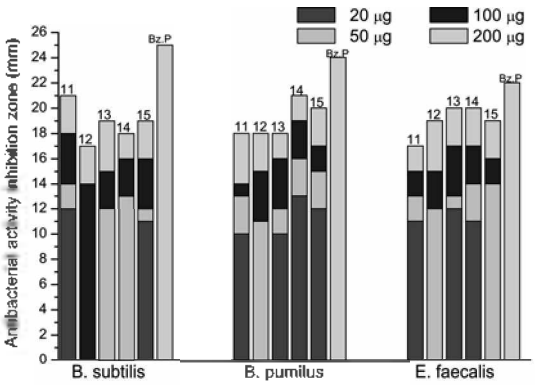


Fig. 1. Effect of quinazolines **11-15** against B. subtilis, B. pumilus and E. faecalis at 20 μg-200 μg concentration. Benzyl Penicillin is used as standard reference to compare the activity. Compounds **11-15** were non-toxic to normal human PBMC and Jurkat and Raji (cancer cell lines) from 5 μg-800μg level.

molecules at remaining concentrations (*Table* 3, entry 14-16). Moreover, this finding is also in sharp contrast with the findings of Gram-positive organisms

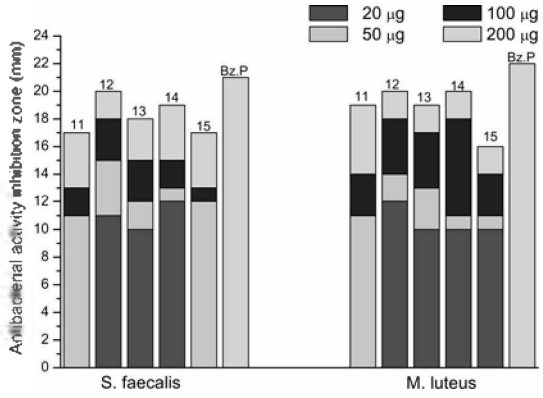


Fig. 2. Effect of quinazolines **11-15** against S. faecalis and M. luteus at 20 μg-200 μg concentration. Benzyl Penicillin is used as standard reference to compare the activity. Compounds **11-15** were non-toxic to normal human PBMC and Jurkat and Raji (cancer cell lines) from 5 μg-800 μg level.

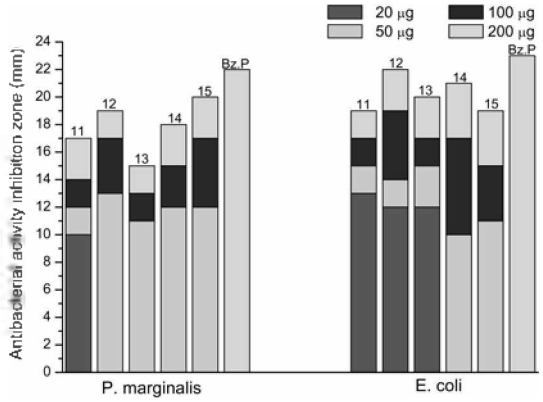


Fig. 3. Effect of quinazolines **11-15** against P. marginalis and E. coli at 20 μg-200 μg concentration. Benzyl Penicillin is used as standard reference to compare the activity. Compounds **11-15** were non-toxic to normal human PBMC and Jurkat and Raji (cancer cell lines) from 5 μg-800 μg level.

for **14** (*Table* 2, entry 13-16). The Chloro substituted analogue **15** displayed MIC 50 μg/ml against all Gram-negative organisms except K.pneumoniae (*Table* 3, entry 17) and it was slightly more potent than other molecules at 100 μg/ml and 200 μg/ml (*Table* 3, entry 19, 20), concentrations against P.marginalis.

CONCLUSIONS

We have herein reported the activity of new tetrahydroquinazoline derivatives **11-15** to inhibit the bacterial activity of the Gram-positive organisms and Gram-negative organisms along with their cytotoxic profile. We have synthesized and tested a set of variously substituted quinazolines and the best results were obtained with compound **14** in case of Gram-positive bacteria and compound **12** is more potent in case of Gram-negative bacteria. All compounds **11-15** are non-toxic from 5 μg to 800 μg level and showed moderate to good antibacterial activity. The difference in potency of the compounds, was most probably the result of substituent differences on the phenyl moiety. This is in good agreement with previous findings that a quinazoline ring with appropriate substituents are the dominant factors for multiple biological activities. So we conclude that, the newly synthesized tetrahydro quinazolines are non-toxic antibacterial agents. Actually our aim is to synthesize anticancer agents and the studies are in progress. The present compounds possess non-toxic nature with antibacterial properties.

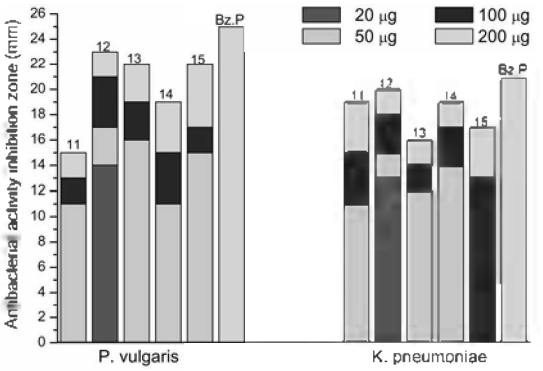


Fig. 4. Effect of quinazolines **11-15** against P. vulgaris and K. pneumoniae at 20 μg-200 μg concentration. Benzyl Penicillin is used as standard reference to compare the activity. Compounds **11-15** were non-toxic to normal human PBMC and Jurkat and Raji (cancer cell lines) from 5 μg-800 μg level.

EXPERIMENTAL

Melting points of the compounds were recorded on an electro-thermal apparatus and were uncorrected. IR-spectra in KBr were recorded in NICO-

Table 3. Antibacterial Activity Inhibition Zones (mm)*# Gram-Negative Bacteria

Compd. No.	Entry No.	Conc. μg/ml	P.marginalis	E.coli	P.vulgaris	K.pneumoniae
11	1	20	10	13	-	-
	2	50	12	15	11	11
	3	100	14	17	13	15
	4	200	17	19	15	19
12	5	20	-	12	14	13
	6	50	13	14	17	15
	7	100	17	19	21	18
	8	200	19	22	23	20
13	9	20	-	12	-	-
	10	50	11	15	16	12
	11	100	13	17	19	14
	12	200	15	20	22	16
14	13	20	-	-	-	-
	14	50	12	10	11	14
	15	100	15	17	15	17
	16	200	18	21	19	19
15	17	20	-	-	-	-
	18	50	12	11	15	-
	19	100	17	15	17	13
	20	200	20	19	22	17
Benzyl Penicillin	21	200	22	23	25	21

*Negative Control DMSO, no activity, # Solutions of different concentrations of compounds **11-15** were prepared in DMSO and tested against Gram-negative bacteria. The antibacterial activity of the test compounds **11-15** was compared with Benzyl Penicillin. All the compounds are non-toxic to human cells at the above dose.

LET AVATAR-320-FT-IR spectrophotometer. Elemental analysis was carried out on CHNS OEA 1108 elemental analyzer. ^{1}H NMR spectra were recorded on BRUKER AMX-400 spectrometer operating at 400 MHz. ^{13}C NMR spectra were recorded on BRUKER AMX-400 spectrometer at operating frequency 100 MHz. Mass spectra were recorded on either FINNIGAN MAT 1020B or MICRO MASS VG 70-70H spectrometer operating at 70 ev using direct inlet system. The GCMS spectra were recorded on SHIMADZU-QP-5050A instrument. The purity of the compounds were checked on TLC and HPLC (SHIMADZU-LC 6A) using Shimpack CLC-Sil column and Shimpack CLC-ODS column using UV detector. Starting materials and solvents were purchased from Acros, Merck or Aldrich.

Synthesis of 3-methyl-4-phenylbut-3-en-2-one (3)

In a 100 ml two - necked round - bottomed flask benzaldehyde (0.10 mmol) and 2-butanone (0.02 mmol) were taken. The contents were cooled to 0-5 °C and then dry HCl gas was passed until it was saturated and turns to red colour. The reaction mixture was stirred for 8 hrs and the layer formed at the bottom was separated and rejected. The crude product was dilute with benzene, washed with $NaHSO_3$ solution followed by water. The organic layer was separated, dried with anhydrous Na_2SO_4 and evaporated. The residue when distilled under reduced pressure yielded pure compound, which was solidified on keeping in a refrigerator for 2-3 days. B.p. 100-103 °C/5 mm (lit[17], b.p., 127-130 °C/12 mm, m.p. 38 °C), yield 55%. HPLC purity : 100%, GCMS purity: 96.96%; ^{1}HNMR ($CDCl_3$/TMS): δ2.0 (s, 3H, H_c), 2.4 (s, 3H, H_a), 7.2-7.4 (m, 5H, Ar-H's), 7.5 (s, 1H, H_b). GC.M.S (%):160 [M$^-$] (65%), 159 [M-H]$^+$ (85), 145(25), 115(100%, base peak), 91(40), 63(13), 51(23), 43(80).

Synthesis of 2-methyl-1,5-diphenylpenta-1,4-dien-3-one (4)

A solution of 20% aq. NaOH (50 ml) was added drop wise to an ice-cold solution of compound **3** (0.10 mmol) and benzaldehyde (0.12 mmol) in ethanol stirred for 1-2 hrs. Then the reaction mixture

was brought to room temperature and the stirring was continued for 5-7 hrs. The contents were poured into cold water and acidified with acetic acid and extracted with ether. The ethereal layer was washed with $NaHCO_3$ solution followed by water and dried with anhydrous Na_2SO_4. Then the solvent was evaporated and the residue was distilled under reduced pressure, which was solidified on keeping at room temperature for 12 hrs.B.p. 192-198 °C/5 mm (lit[15]. 180-182 °C/0.45 mm), yield 68%. HPLC purity : 99%, GCMS purity : 96.12%; ^{1}HNMR ($CDCl_3$/TMS): δ2.2 (s, 3H, H_b), 7.3-7.6 (m, 10H, Ar-H's), 7.6-7.8 (dd, 2H, H_d+H_e), 7.85 (s, 1H, H_a). GCMS(%): 248 $[M]^+$ (60%), 247 $[M-H]^+$(35), 205 (18%), 144(10), 131(30), 116(100%, basepeak), 103(40), 77(35), 51(20).

Synthesis of ethyl 1-cyano-3-methyl-4-oxo-2,6-diphenylcyclohexanecarboxylate (5)

Compound **4** (0.05 mmol), ethyl cyanoacetate (0.05 mmol) and absolute ethanol (50 ml) were taken in a 100 ml round bottomed flask. To this 15 ml of 10% NaOEt solution was added and refluxed for 5 hrs. The contents were concentrated and poured in ice cold water, acidified with acetic acid and extracted with ether. The ethereal layer was washed with $NaHCO_3$ solution followed by water and dried over anhydrous $MgSO_4$ and the solvent was stripped off by distillation. The crude product was chromatographed over silicagel with n-hexane and ethyl acelate (95:5) as eluent. Further, the product was recrystallised from methanol, which yielded white coloured crystals. M.p.: 128 °C, yield 68%, molecular formula: $C_{23}H_{23}NO_3$. Elemental analysis: Found (%): C, 76.32; H, 6.35; N 3.82, Required (%): C, 76.43; H, 6.41; N, 3.88. ^{1}H NMR ($CDCl_3$/TMS): δ0.60-0.75 (t, 3H, $\mathbf{CH_3}CH_2O$), 0.9 (d, 3H, CH_3), 2.72-2.83 (dd, 1H, H_{e-eq}), 3.18-3.46 (m, 2H, H_c+ H_{e-ax}), 3.65-3.84 (m, 4H, H_b+H_f+OCH_2), 7.2-7.4 (m, 10H, Ar-H's).^{13}C NMR : 11.994 (1C, CH_3), 13.527 (1C, $OCH_2\mathbf{CH_3}$), 43.956 (1C, C_c), 45.718 (1C, C_f), 49.401 (1C, C_e), 56.280 (1C, C_a), 60.238 (1C, C_b), 62.633 (1C, OCH_2), 116.780 (1C, CN), 128.212, 128.667, 128.815, 128.967, 135.861, 136.624 (12C, Ar-C's) 166.634 (1C, COO), 206.939 (1C, C_d).I.R (ν_{max}): 1708, 1728, 2247, 960, 785 cm^{-1}.

Synthesis of Chalcones-General Procedure

A mixture of compound **5** (0.01 mmol), substituted benzaldehydes (0.01 mmol) in ethanol (25 ml) and aqueous potassium hydroxide (15 g in 15 ml of water) was stirred for 2 hrs and kept at room temperature for 24 hours. The reaction mixture was neutralized with acetic acid and diluted with water. The solid thus obtained was filtered, washed with water and crystallized from appropriate solvent and characterized by using spectral data and elemental analysis. Compound **5** was condensed with veratraldehyde, 4-(dimethylamino) benzaldehyde, 4-hydroxy benzaldehyde, vanillin, 4-chlorobenzaldehyde to furnish the respective chalcones **6-10**.

Synthesis of Ethyl 2-amino-6-cyano-4-(3,4-dimethoxyphenyl)-8-methyl-5, 7-diphenyl- 5,6,7,8-tetrahydroquinazoline-6-carboxylate (11)

Compound **6** (1 mmol) and guanidine hydrochloride (1 mmol) were refluxed together in 10% ethanolic potassium hydroxide (12 ml) for 5 hrs, progress of the reaction was monitored by TLC. The excess of the solvent was removed in vaccum, extracted with chloroform and washed with water. The combined organic layers were dried over anhydrous Na_2SO_4, filtered and distilled under reduced pressure to give a gummy product, which was chromatographed over silicagel column and recrystallised from chloroform / methanol as yellow needles, m.p.: 238 °C, yield 61%, molecular formula : $C_{33}H_{32}N_4O_4$. Elemental Analysis : Found (%) : C, 71.96; H, 5.69; N, 10.14%, Required (%) : C, 72.26; H, 5.84; N, 10.22%. ^{1}HNMR (d^6-acetone/TMS): δ0.76-0.88 (m,6H,$\mathbf{CH_3}$+$OCH_2\mathbf{CH_3}$), 2.41-2.56 (m,1H, H_h), 3.05(br.s, 2H, NH_2), 3.6(d,1H, $\mathbf{H_g}$), 3.8 (s, 6H, 2 x OCH_3), 3.9-4.0 (m, 2H, -$\mathbf{OCH_2}CH_3$), 4.2 (s, 1H, H_e), 7.25-7.43 (m, 13H, Ar-H's).I.R. (ν_{max}): 3492, 2972, 2646, 2253, 1715, 1597, 1506, 1271, 1228, 1131, 1002, 921, 765, 701, 601, 515 cm^{-1}.

Synthesis of Ethyl 2-amino-6-cyano-8- methyl-4-[4-(dimethylamino) phenyl]-5,7-diphenyl-5,6,7,8-tetrahydroquinazoline-6-carboxylate (12)

M.p.: 238 °C, yield 59%, molecular formula : $C_{33}H_{33}N_5O_2$ Elemental Analysis : Found (%) : C, 74.33; H, 6.18; N, 13.08%, Required (%) : C, 74.57; H, 6.22; N, 13.18%. ^{1}HNMR (d^6-acetone/TMS): δ 0.74-

0.86 (m, 6H, CH_3+OCH_2 CH_3), 2.40-2.58 (m, 1H, H_h), 3.0 (br. s, 2H, NH_2), 3.15 (s, 6H, NMe_2), 3.6 (d, 1H, H_g), 3.8-4.0 (m, 2H, -OCH_2CH_3), 4.0 (s,1H, H_e), 7.2-7.5 (m, 14H, Ar-H's).I.R. (ν_{max}): 3384, 3165, 2965, 2915, 1694, 1626, 1438, 1359, 1190, 1106, 1049, 968, 881 cm^{-1}.

Synthesis of Ethyl 2–amino-6-cyano-4-(4-hydroxy phenyl)-8-methyl-5,7-diphenyl-5,6,7,8-tetrahydroquinazoline-6-carboxylate (13)

M.p.: 198 °C, yield 63%, molecular formula : $C_{31}H_{28}N_4O_3$. Elemental Analysis : Found (%) : C, 72.75; H, 5.51; N, 11.02%, Required (%) : C, 73.81; H, 5.56; N, 11.12%. ^{1}HNMR (d^6-acetone/TMS): δ0.56 (t, 3H, CH_3CH_2O-), 0.7 (d, 3H, CH_3), 2.31-2.59 (m, 1H, H_h), 2.9 (s, 2H, NH_2), 3.38 (d, 1H, H_g), 3.65-3.85 (m, 2H, -OCH_2CH_3), 4.1 (s, 1H, H_e), 7.08-7.8 (m, 14H, Ar-H's), 9.87 (s, 1H, -OH). I.R. (ν_{max}): 3441, 3356, 3206, 1680, 1608, 1494, 1290, 1174, 1068, 970, 885, 784 cm^{-1}.

Synthesis of Ethyl 2-amino-6-cyano-4-(4-hydroxy-3-methoxy phenyl)-8-methyl-5,7-diphenyl-5,6,7,8-tetrahydroquinazoline-6-carboxylate(14)

M.p: 256°C, yield 57%, molecular formula : $C_{32}H_{31}N_4O_4$ Elemental Analysis :Found (%) : C, 71.46; H, 5.72; N, 10.36% , Required (%) : C, 71.77; H, 5.79; N, 10.48%. ^{1}HNMR (d^6-acetone/TMS): δ0.74-0.86 (m, 6H, CH_3+OCH_2CH_3), 2.40-2.56 (m, 1H, H_g), 3.0 (br.s, 2H, NH_2), 3.6 (d, 1H, H_g), 3.81 (s, 3H, -OCH_3), 3.85-4.0 (m, 2H, -OCH_2CH_3), 4.05 (s, 1H, H_e), 7.41-7.6 (m, 13H, Ar-H's), 9.6 (s, 1H, -OH). I.R. (ν_{max}): 3448, 2968, 2670, 2284, 1724, 1579, 1498, 1290, 1246, 1180, 1056, 980, 764, 710, 615, 540 cm^{-1}.

Synthesis of Ethyl 2-amino-4-(4-chlorophenyl)-6-cyano-8-methyl-5,7-diphenyl-5,6,7,8-tetrahydroquinazoline-6-carboxylate (15)

M.p.: 208 °C, yield 64%, molecular formula : $C_{31}H_{27}N_4O_2Cl$. Elemental Analysis: Found (%) : C, 71.16; H, 5.09; N, 10.46; Cl, 6.65 %, Required (%) : C, 71.27; H, 5.18; N, 10.73, Cl, 6.71%. ^{1}HNMR (d^6-acetone/TMS): δ0.68 (t, 3H, CH_3CH_2O-), 0.8 (d, 3H, CH_3), 2.34-2.62 (m, 1H, H_h), 2.85 (s, 2H, NH_2), 3.36 (d, 1H, H_g), 3.68-3.82 (m, 2H, -OCH_2CH_3), 4.0 (s, 1H, H_e), 7.28-7.46 (m,14H, Ar- H's). I.R. (ν_{max}): 3368, 3340, 1684, 1605, 1524, 1349, 1268, 1026, 956, 849, 715 cm^{-1}.

Cytotoxic studies - Dye exclution method

The experiments were carried out in the laboratory of Prof. T. Tirunala Sundari, Dept. of Microbiology, Bharathidasan University, Tiruchirapally, India. Anticancer Activity Testing was performed by Dye exclution method. Bioactivity assay was performed in a 96 Well tissue culture plate (Greiner, Germany). Various concentrations of the compound ranging from 5 μg to 800 μg were made. A constant number of cancerous (Jurkat, Raji & PBMC) cell suspension and a constant volume of the complete media were added into each well. Desired concentrations of the compounds were added into each well and the volume was made constant, using complete media. Negative (solvent used for dissolving the compound) and positive (known immunomodulator) controls were also maintained. The plate was incubated at 37 °C in CO_2 incubator with 5% CO_2. The number of live cells were counted after every 12 hours by using inverted phase contrast microscope (Nikon TM) and the Newbauer's counting chamber by dye exclution method.

Antimicrobial activity studies-Cup Plate Method

Sterilized molten nutrient agar medium was inoculated with 50 μL of the test organism aseptically. The temperature of the molten medium should be below 45 °C. The inoculated medium was poured in the assay – plate and kept on a horizontal surface to avoid non-uniform solidification of the medium. Cups of 8 mm diameter were made at equidistance with bore-puncher. Solutions of different concentrations of compounds **11-15** were prepared in DMSO. 50 μL of test solutions were introduced into the cups using micropipette. These plates were covered and kept at 5-10 °C for 3 hours for better diffusion of the solution into the medium. They were then incubated at 37 °C for 18 hrs in an incubator and the diameters of inhibition zones were measured in mms (*Tables* 2 & 3). DMSO alone was kept as control, which did not have any inhibition zone. The antibacterial activity of the test compounds **11-15** was compared with Benzyl Penicillin.

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REFERENCES

1. Johne, S. *The Alkaloids, Chemistry and Pharmacology*; Brossi, A., Ed.; Academic Press: New York, U.S.A., **1986**, *29*, 99.
2. (a) Armarego, W.L.F. *Adv. Heterocycl. Chem.* **1963**, *1*, 253. (b) Armarego, W.L.F. *Adv. Heterocycl. Chem.* **1979**, *24*, 1. (c) Ellis, G.P. *Synthesis of Fused Heterocycles*; John Wiley & Sons Ltd.: Chichester, 1987; Vol. 47.
3. Malamas, M.S.; Millen J. *J. Med. Chem.* **1991**, *34*, 1492.
4. (a) Mannschreck, A; Koller, H.; Stuhler, G; Davies, M.A.; Traber, J. *Eur. J. Med. Chem.* **1984**, *19*, 381. (b) Gupta, C.M.; Bhaduri, A.P.; Khanna, N.M. *J. Med. Chem.* **1968**, *11*, 392.
5. Fisnerova, L.; Brunova, B.; Kocfeldova, Z.; Tikalova, J.; Maturova, E.; Grimova, J. *Collect. Czech. Chem. Commun.* **1991**, *56*, 2373.
6. Kung, P.P.; Casper, M.D.; Cook, K.L.; Wilson, Lingard, L.; Risen L.M.; Vickers, T.A.; Ranken, R.; Blyn, L.B.; Wyatt, J.R.; Cook, P.D.; Ecker, D.J. *J. Med. Chem.* **1999**, *42*, 4705.
7. (a) Sharma, S.D.; Kaur, V. *Synthesis* **1989**, 677. (b) Palmer, B.D.; Trumpp - Kallmeyer, S.; Fry, D.W.; Denny, W.A. *J. Med. Chem.* **1997**, *40*, 1519.
8. Tsou, H.R., Mamuya, N.; Johnson. B.D.; Reich, M.F.; Gruber, B.C.; Wissner, A. *J. Med. Chem.* **2001**, *44*, 2719.
9. Matsuno, K.; Ichimura, M.; Nakajima, T.; Oda, S.; Namoto, Y. *J. Med. Chem.* **2002**, *45*, 3057.
10. Srivastava, B., Shukla, J.S. *Indian J. Chem., Sect. B.* **1991**, *30B*, 332.
11. Fetter. J.; Czuppo, T.; Hornyak, G; Feller, A. *Tetrahedron* **1991**, *47*, 9393.
12. (a) Katritzky, A.R.; Rees, C.W. *Comprehensive Heterocyclic Chemistry- The Structure Reaction Synthesis and uses of Heterocyclic compounds*; Pergamon Press: New York, U.S.A., 1984; Vol. 3., part 2B. (b) Pelletier, S.W. *Alkaloids : Chemical and Biological Prospective*; John Wiley & Son's Ltd.: New York, U.S.A., 1983; Vol.1. (c) Johne, S. *Alkaloids* **1986**, *29*, 99.
13. Abdel Rahman, M.M.; Mangoura, S.A.; El-Bitar, H.L. *Bull. Pharm. Sci.* **1990**, *13*, 137.
14. He, F.; Snider, B.B. *J. Org. Chem.* **1999**, *64*, 1397.
15. Bhaskar Reddy, D.; Padmavathi, V. ; Ramana Reddy, P.V. *Indian J.Chem.* **1992**, *31 B*, 774 and references cited therein.
16. Marin bettolo ; Ballio, A. *Gazz. Chim. Ital.* **1946**, *76*, 410.
17. Al- Hajjar, F.H.; Sabri, S.S. *J. Het. Chem.* **1982**, *19*, 1087.
18. Katritzky, A.R.; Ambler, A.P. *Physical Methods in Heterocyclic Chemistry*, 1963, Vol. 2, p292.
19. Beynon, J.H. *Mass Spectrometry and Its Application to Organic Chemistry* ; Elsevier: Amsterdam, 1960; p368.
20. (a) Colotter, J.L. *Org. Mass Spectrum.* **1972**, *5*, 345. (b) Porter, Q.N. *Mass Spectrometry of Heterocyclic compounds* (2nd edition).; John Wiley & Sons.: New York, U.S.A.,1985; p329 and references cited therein.
21. Brown, R.F.C.; Eastwood, F.W.; Mc Mullen, G.L. Aust. J. Chem. **1977**, *30*, 179.
22. Grunstein, J.F.; Dizalso, P.; Richard, M.; Braun, J.P. *Org. Mass Spectrum.* **1974**, *9*, 1166.
23. Nishiwaki, T. *Tetrahedron* **1966**, *22*, 3117.
24. Spitller, G. *Physical Methods in Heterocyclic Chemistry*; Katritzky, A.R., Ed.; Academic press: New York, U.S.A., 1971; Vol.3, p286.