EXECUTIVE SUMMARY OF UGC-MAJOR RESEARCH PROJECT
No. F.41-576/2012(SR)

Principal Investigator: Dr. J. Shankar
Co-Principal Investigator: Dr. R. A. Vasantha
Duration: 3 Years (2012-2015)
Grant Amount: Rs. 14,88,300-00

"SYNTHESIS AND EVALUATION OF NOVEL QUINAZOLINE ANALOGUES AS TYROSINE KINASE INHIBITORS: ROLE IN ANGIOGENIC PREVENTION"

ANNEXURE – IX

12. Summary of the Findings:

The key intermediate compound 6,7-dimethoxy-quinazolin-4(3*H*)-one **1** was prepared by using the earlier reported procedure (Chandregowda *et al.*, 2007) . Compound **1** was heated with thionylchloride and few drops of DMF to get 4-chloroquinazoline **2** and which was then coupled with *m*-phenylenediammine in isopropyl alocohol at 80 oC to get 3-aminoanilinoquinazoline derivative **3**. Structure of the compound **3** was confirmed by the appearance of –NH2 in the 1H NMR spectrum around δ 9.5 and a peak at δ 10.5 corresponding to –NH. The key intermediate **3** was reacted with different acid chlorides in presence of triethylamine in dichloromethane to get products **RB 1-11.** Similarly, reaction of **3** with various sulfonyl chlorides furnished a new series of quinazoline derivatives **4 A-J** and reaction with isothiocyanates afforded another series of compounds **PR 1-6 (Scheme 1)**. All synthesized compounds were characterized by IR, 1H NMR, 13C NMR and mass spectroscopy. Structures and yields of compounds are tabulated.

In order to evaluate the cytotoxic effects, synthesized compounds were screened against four different cancer cell lines- HCT116, K562, SKBR3 and EAC using MTT, trypan blue dye exclusion and LDH leak assays. All the cell lines were exposed to different concentrations of compounds (0-100 μM in DMSO and vehicle alone) for 24 h. In the first series of compounds, 4-chlorobenzoyl derivative of anilinoquinazoline **RB 1** emerged as the most potent compound with IC50 value of 18-19.5 μM on HCT 116, 14.15-15.3 μM on K562, 19.2-21.2 μM on SKB3, 12.6-13.5 μM on EAC cell line in all three assays. In the second series, 4-nitrophenylsulfonyl derivative of quinazoline **4G** showed profound activity with IC50 value of 12.1-10.9 μM on HCT 116, 10.8-11.8 μM on K562, 11.28-12.8 μM on SKBR3 and 10.08-10.29 μM on EAC

cell lines in all three assays. In the third series, urea derivative of quinazoline **PR 6** showed the highest activity with IC50 value of 10.4-11.34 μM on HCT 116, 10.9-11.24 μM on K562, 11.12-10.9 μM on SKBR3 and 10.08-10.48 μM on EAC cell lines in all three assays, in comparison with reference cisplatin and doxorubicin. The preliminary screening of all the synthesized compounds was useful in selecting the compounds which shows highest cytotoxic effect. Thus the quinazolines **RB 1**, **4G** and **PR 6** emerged as potent cytotoxic agents, which were selected as lead compounds from each series for further analysis. It is clear from the collected data that amide, sulfonyl and urea derivatives of anilinoquinazoline compounds were active. Synthesized novel quinazoline derivatives have shown anticancer potential against EAC cells. Compound **4G, RB-1 and PR-6** possess the capability of inducing mitochondrial pathway in EAC cells, which is well regulated by caspase enzymes. Moreover, the active role of mitochondrial dependent pathway has been studied and was further confirmed by increasing BAX pro-apoptotic protein level, activation of caspase-3 and cleavage of PARP protein by caspase-3 proteins. Compounds **4G, RB-1 and PR-6** inhibits the growth of solid EAC cells *in vivo*. This was evident from reduced tumor size and enhanced life span of that study group. The treatment with **4G, RB-1 and PR-6** restored the deviated haematological and biochemical parameters to the normal range. It was demonstrated to be an effective anticancer and antineoplastic agent with less toxic effects. Compound **4G, RB-1 and PR-6** is a very potent anti-angiogenic compounds which inhibit the growth of EAC cells *in vivo*. These inhibitory effects may be related to inhibition of transcription factor HIF-1α nuclear translocation. HIF-1α is responsible for inhibition of hypoxic up regulation of VEGF gene expression, resulting in decreased ascites volume and micro vessel density and thereby inhibiting the tumor growth which is angiogenesis dependent and also inhibiting the downstream signalling protein such as ERK ½, p38 and JNK of the MAPK pathway. Our findings strongly suggest that the anticancer, Anti-angiogenic and Pro-apoptotic potential of compound **4G, RB-1 and PR-6** can be used as lead for developing therapeutic agent for treating cancer.

Signature of the Co-PI

Head of the Department of Chemistry
Teresian College, Mysore-11

Signature of the PI

Dr. J. Shanker, M.Sc., Ph.D.,
Principal Investigator
UGC-Major Research Project
P.G. Dept. of Biotechnology
Teresian College, Mysore-11