Annexures
Pharmacokinetics and tissue distribution of a M1 muscarinic acetylcholine receptor positive allosteric potentiator, benzyl quinolone carboxylic acid

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A simple, sensitive and selective high performance liquid chromatography method has been developed and validated for the estimation of benzyl quinolone carboxylic acid (BQCA) in Sprague-Dawley (SD) rat plasma and tissue samples. Plasma and tissue samples were extracted by a protein precipitation technique using methanol as a precipitating agent and donepezil as the internal standard. Chromatographic separation was performed on a Hibar C18 column with a mobile phase of acetonitrile and potassium dihydrogen orthophosphate buffer (20 mM, pH 6.5) at a flow rate of 0.8 mL min\(^{-1}\). The lower limit of quantitation of the developed method was found to be 2.0 ng mL\(^{-1}\) and 5.0 ng g\(^{-1}\) for plasma and tissue samples, respectively. Following intraperitoneal (i.p.) administration, BQCA was remarkably absorbed into the systemic circulation with maximum concentration (\(C_{24}\)) of 8,000.0 ng mL\(^{-1}\) within 1.5 h. The order of the area under the curve results from the tissue distribution study was kidney > lung > liver > brain > spleen > heart. BQCA was rapidly taken up into the brain resulting in a maximal brain concentration after 1.5 h which was maintained for up to 3–4 h. The method was successfully applied in the analysis of BQCA in plasma and tissue samples following i.p. administration to SD rats at a dose of 10 mg kg\(^{-1}\).

1. Introduction

Acetylcholinesterase inhibitors (AChEIs) such as donepezil, galantamine, rivastigmine and tacrine are approved by the U.S. Food and Drug Administration (FDA) for the treatment of Alzheimer’s disease (AD). They act by limiting the degradation of synaptic acetylcholine (Ach) levels to activate cholinergic receptors.\(^1,2\) However, the efficacy of AChEIs in enhancing cognition has failed so far, in part because of central and peripheral adverse effects that are due to the activation of other subtypes of muscarinic Ach receptors (mAChRs) such as M2 to M5 and/or to direct agonism.\(^3\) Recent research suggests that selective stimulation of M1 receptors, but not of other subtypes, is a beneficial strategy for the treatment of AD and does not produce adverse effects.\(^4,5\)

There are five receptor subtypes in the muscarinic family (M1–M5) that differ in terms of their localization, signalling activity and presumed function.\(^6\) Among the five existing mAChR subtypes, M1 is predominant in the many memory related brain regions, including the cortex, hippocampus, and striatum.\(^7\) Thus drugs which selectively target the M1 receptor could play an important role in regulating higher cognitive functioning.\(^8,9\) There is difficulty in developing highly selective M1 receptor agonists due to the high sequence homology among the orthosteric binding sites of mAChR subtypes.\(^7\) The alternative novel approach to achieve high subtype selectivity is to target the allosteric binding sites that are distinct from the ACh binding sites. In this approach many G-protein-coupled receptors, including mAChRs,\(^10,11\) have allosteric binding sites bound by molecules that activate the receptor in the absence of ligand (allosteric agonist) or enhance the response to the native ligand (positive allosteric modulator).\(^12\)

Benzyl quinolone carboxylic acid (BQCA), [1-(4-methoxy-benzyl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid] is a novel highly selective potent positive allosteric modulator (PAM) of M1 receptor.\(^13\) It reduces the concentration of ACh required to activate M1 as well as having no effect on potentiation, agonism or antagonism activity on other subtypes of mAChRs and does not show unwanted peripheral cholinergic stimulation.\(^3,14\) BQCA enhances the memory and reverses the cognitive impairment in scopolamine-induced memory deficits,
suggested that the M1 receptor PAM BQCA has therapeutic potential for the treatment of AD. To date, there has been limited published data on pharmacokinetic and tissue distribution studies of BQCA. The main purpose of the present study is to report on the detailed pharmacokinetics and tissue distribution of BQCA in Sprague-Dawley (SD) rats following intraperitoneal (i.p.) administration at 10 mg kg⁻¹.

2. Experimental

2.1. Chemicals and reagents

BQCA (≥ 98%) as a gift sample was obtained from the Vanderbilt Center for Neuroscience Drug Discovery (Nashville, USA) and donepezil (internal standard, IS, ≥ 98%) was a gift from Dr Reddy's Laboratories Limited (Hyderabad, India). The corresponding structures are shown in Fig. 1. Acetonitrile (ACN) and methanol of HPLC grade were purchased from Merck (Mumbai, India), ortho-phosphoric acid and triethylamine of AR grade were procured from Qualigens Fine Chemicals (Mumbai, India), potassium dihydrogen orthophosphate was obtained from SD Fine Chemicals (Mumbai, India), polysorbate 80 was procured from Sigma Aldrich (St. Louis, USA) and HPLC grade water was obtained using a Milli-Q RO system (Millipore India, Bangalore, India).

2.2. Chromatographic conditions

The analysis of BQCA was performed using a Shimadzu Prominence HPLC system (Shimadzu Corporation, Kyoto, Japan). The HPLC instrument was equipped with a model series LC-20AT pump, a Rhodyne 7752i injector with a 20 µl loop and an SPD-20A UV/VIS detector. Separation was carried out on a Hibar C₁₈ column (250 x 4.6 mm i.d., 5 µm; Merck Limited, Mumbai). Spinchrom Chromatography station software was used for data acquisition. The mobile phase used was ACN-potassium dihydrogen orthophosphate buffer (20 mM, pH 6.5) at a ratio of 70:30 v/v with a flow rate of 0.8 mL min⁻¹, an injection volume of 20 µL and a detection wavelength of 215 nm. Prior to use, the mobile phase was filtered through a 0.22 µm hydrophilic membrane filter. All determinations were performed at room temperature (15-18 °C).

2.3. Preparation of calibration standards and quality control samples

Calibration standards were prepared by spiking the working standard solution into a pool of drug free rat plasma and processed tissue homogenate in order to obtain the following concentrations 2.0, 10.0, 50.0, 200.0, 1000.0, 5000.0, 20 000.0 and 5000.0 ng mL⁻¹ in plasma and 5.0, 50.0, 100.0, 500.0, 1000.0, 1500.0, 2000.0 and 5000.0 ng g⁻¹ in tissues. These solutions were labelled and stored at −70 ± 2 °C until analysis. Quality control samples (QC) at a minimum of three concentrations for BQCA were prepared by spiking the working standard solutions into a pool of drug free SD rat plasma and tissues to produce a concentration of 6.0, 20 000.0 and 32 000.0 ng mL⁻¹ and 5.0, 2500.0, and 4000.0 ng g⁻¹, respectively. These solutions were labelled and stored at −70 ± 2 °C until the analysis.

2.4. Extraction of plasma and tissue samples

The protein precipitation (PPT) technique was generally the preferred choice of extraction technique because it is simple, economical, and less cumbersome than other techniques. An aliquot of 500 µL of plasma, 500 µL of IS (100 µg mL⁻¹) and 500 µL of methanol was taken into a 2 mL Eppendorf tube and vortexed for 30 s then centrifuged (Remi Instruments, Mumbai) at 10 000 rpm for 10 min. The clear supernatant solution was transferred into a vial and 20 µL was subjected to HPLC analysis. Tissue samples were weighed accurately and homogenized using a glass tissue homogenizer after addition of 1 mL of physiological saline. Tissue homogenates were processed in a similar manner to the plasma samples and were analyzed by HPLC.

2.5. Method validation

The method for the determination of BQCA in SD rat plasma and tissue was validated according to the USFDA guidelines. The assay was validated for specificity, extraction recovery, linearity, sensitivity, accuracy, precision, and stability.

2.5.1. Specificity. The specificity was established by the lack of interference peaks at the retention time of BQCA and the IS. Recovery was determined by comparing the mean peak area obtained from either the extracted plasma or tissue samples with the peak area obtained by the direct injection of the corresponding spiked standard solutions.

2.5.2. Extraction recovery. Recovery was determined by comparing the mean peak area obtained from either the extracted plasma or tissue samples with the peak area obtained by the direct injection of the corresponding spiked standard solutions. Different concentrations of BQCA (6.0, 20 000.0, and 32 000.0 ng mL⁻¹ in plasma and 15.0, 2500.0, and 4000.0 ng g⁻¹ in tissue samples) were measured.

2.5.3. Linearity and sensitivity. The linearity was tested over the concentration range of 2.0–40 000.0 ng mL⁻¹ in plasma and 10.0–5000.0 ng g⁻¹ in tissues. The calibration curves were established by plotting the peak area ratio of BQCA to IS versus the BQCA concentration. The regression parameters of the slope, intercept and correlation coefficient were calculated by linear regression equation. The lowest limit of quantification (LLOQ) was set as the lowest amount of analyte in a sample that could be quantitatively determined with acceptable precision and accuracy (i.e., 20% coefficient of variation (CV) and ±20% nominal concentration in these assays, respectively).

2.5.4. Accuracy and precision. The intra- and inter-day precision and accuracy in plasma and tissue samples were evaluated at three different QC levels in six replicates on the
same day and on three different days, respectively. Acceptable deviation was set within 15% of the nominal concentration for accuracy and within 15% of the CV for precision.

2.5.5. Stability. The stability of BQCA in plasma and brain tissue were determined by the analysis of QCs \((n = 6)\) subjected to different storage conditions such as freeze thaw (3 cycles), short-term, long-term and stock solution stability. For freeze-thaw (3 cycles) stability, the spiked plasma and tissue samples were frozen at \(-70^\circ\text{C}\) for 24 h and thawed at room temperature. When completely thawed, the samples were refrozen for 12–24 h under the same conditions, at the end of each cycle samples were analysed and compared with the freshly prepared QCs \((n = 6)\) in plasma and tissue. For the short-term and stock solution stability study, plasma and tissue QCs were kept at 25 °C for 6 h and samples were processed, analysed and compared with the freshly prepared QCs. The long-term stability was evaluated by analyzing plasma and tissue samples that had been frozen at \(-70 ± 2^\circ\text{C}\) for 30 days. The samples were considered to be stable when the deviation from the nominal values were within ±15%.

2.6. Pharmacokinetics and tissue distribution study

The pharmacokinetic and tissue distribution studies were performed on male SD rats weighing 180–220 g. The animal house was well ventilated and the animals were maintained on a 12 : 12 h light/dark cycle in large spacious cages throughout the experimental period. The animals were provided with food and water ad libitum and fasted for 12 h prior to the commencement of the experiment. The Institutional Animal Ethical Committee (IAEC) of the JSS College of Pharmacy, Udagamandalam, India, approved the study protocol (JSSCP/IAEC/PH.D/PH.BIO-TECH/01/2012-13). Forty eight SD rats were randomly assigned into eight groups, each group contained six rats. BQCA was dissolved in normal saline (containing 1% polysorbate 80) and filtered through a 0.22 μm hydrophilic membrane filter and then administrated to SD rats at a dose of 10 mg kg\(^{-1}\) by means of i.p. administration. Aliquots of approximately 0.3 mL of blood samples were collected via heart puncture at time intervals of 0 min (pre-dose), 0.5, 1.0, 1.5, 2.0, 4.0, 6.0, and 8.0 h post-dose. The organs (heart, liver, spleen, lung, kidney and brain) of interest were collected immediately after cervical dislocation at the above described time points and were weighed rapidly. Tissues were kept in normal saline solution to remove blood or content and were blotted dry with tissue paper. Blood and tissue samples were centrifuged at 10 000 rpm for 10 min and the supernatant solutions were collected and stored at \(-70 ± 2^\circ\text{C}\) until analysis.

![Representative chromatograms of (a) blank plasma; (b) plasma spiked with BQCA and IS; (c) plasma sample after i.p. administration of BQCA; (d) blank tissue of brain; (e) brain tissue spiked with BQCA and IS, and (f) brain tissue sample after i.p. administration of BQCA.](image)
The pharmacokinetic parameters were calculated by non-compartmental analysis of individual concentration–time data using Phoenix WinNonlin® v 6.3 software (Pharsight Corporation, Mountain view, CA, USA). The pharmacokinetic parameters such as maximum plasma concentration ($C_{\text{max}}$) and the time to reach $C_{\text{max}}$ ($T_{\text{max}}$) were obtained directly from the plasma concentration–time curve. The elimination rate constant ($K_{\text{e}}$) was obtained from the least-squares fitted terminal log-linear portion of the plasma concentration–time profile, elimination half-life ($T_{1/2}$) was calculated as $0.693/K_{\text{e}}$, area under the plasma concentration time curve from 0 to 8 h ($\text{AUC}_{0-8h}$) was calculated by the linear trapezoidal rule, and area under the curve from 0 h extrapolated to infinity ($\text{AUC}_{0-\infty}$) was calculated as $\text{AUC}_{0-8h} + C_t/K_{\text{e}}$ where $C_t$ represents the observed plasma concentration at the last measurable sampling time. The apparent clearance ($CL/F$) was calculated as the drug dose divided by $\text{AUC}_{0-8h}$ and the apparent volume of distribution ($V/F$) was calculated as $CL/F$ divided by $K_{\text{e}}$. All the values are expressed as means ± standard deviation except for the $T_{\text{max}}$, which is expressed as the median.

3. Results and discussion

3.1. HPLC analysis

As the molecular weight of the BQCA is less than 2000 Da and it is extremely polar in nature, the reverse phase mode was used for analysis with a C18 column. The best resolution factor was 15 resulting in a run time of 10 min with retention times of 4.9 and 7.6 min for BQCA and IS, respectively. No attempt was made to further reduce the mobile phase flow rate as this may have increased the analysis run time. BQCA is highly sensitive to a wavelength of 215 nm. During the process of validation, solid phase extraction (SPE), liquid–liquid extraction (LLE) and PPT techniques were applied to determine the limit of detection for BQCA. A limit of detection of 0.002 μg mL$^{-1}$ was obtained when SPE was used and the same limit was obtained with PPT was used with methanol as the protein precipitation agent. A much higher limit of detection for BQCA was obtained with LLE. As SPE is time consuming and involves multiple purification steps, further validation of the method was carried out using the PPT technique.

3.2. Method validation

3.2.1. Specificity. The specificity of a method can be defined as the extent to which the analyte can be estimated without the interference of other components. BQCA and IS were very well resolved under the proposed chromatographic conditions. None of the drug free plasma and tissue samples studied in this assay yielded endogenous interference at the retention time observed for drug. Fig. 2 represents the standard HPLC chromatogram of blank plasma, tissue and spiked plasma, tissue.

3.2.2. Extraction recovery. The percentage mean recovery of BQCA in plasma ranged from 94.43–95.69% and in brain ranged from 91.57–96.52%. The recovery of IS at 100 μg mL$^{-1}$ was found to be 94.15 and 92.76% in plasma and tissue.

Table 1 Recovery, accuracy and precision for determination of BQCA in plasma and tissues ($n=6$)

<table>
<thead>
<tr>
<th>Biological sample</th>
<th>QC (ng mL$^{-1}$ or g$^{-1}$)</th>
<th>Concentration found (ng mL$^{-1}$ or g$^{-1}$)</th>
<th>Recovery (%)</th>
<th>Intra-day</th>
<th>Inter-day</th>
</tr>
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<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Accuracy (%)</td>
<td>Precision (%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>nominal</td>
<td>CV</td>
</tr>
<tr>
<td>Plasma</td>
<td>6.0</td>
<td>5.7 ± 0.24</td>
<td>94.43</td>
<td>95.81</td>
<td>4.2</td>
</tr>
<tr>
<td></td>
<td>20 000.0</td>
<td>19 138 ± 880.35</td>
<td>95.69</td>
<td>94.54</td>
<td>4.6</td>
</tr>
<tr>
<td></td>
<td>32 000.0</td>
<td>30 406.4 ± 1489.91</td>
<td>95.02</td>
<td>95.07</td>
<td>4.9</td>
</tr>
<tr>
<td>Brain</td>
<td>15.0</td>
<td>13.73 ± 0.92</td>
<td>91.57</td>
<td>94.43</td>
<td>6.7</td>
</tr>
<tr>
<td></td>
<td>2500.0</td>
<td>2391.75 ± 138.72</td>
<td>95.67</td>
<td>95.84</td>
<td>5.8</td>
</tr>
<tr>
<td></td>
<td>4000.0</td>
<td>3860.8 ± 216.21</td>
<td>96.52</td>
<td>95.32</td>
<td>5.6</td>
</tr>
<tr>
<td>Liver</td>
<td>15.0</td>
<td>13.94 ± 0.86</td>
<td>92.91</td>
<td>95.14</td>
<td>6.2</td>
</tr>
<tr>
<td></td>
<td>2500.0</td>
<td>2371.25 ± 144.65</td>
<td>94.85</td>
<td>94.86</td>
<td>6.1</td>
</tr>
<tr>
<td></td>
<td>4000.0</td>
<td>3786.4 ± 215.82</td>
<td>94.66</td>
<td>96.47</td>
<td>5.7</td>
</tr>
<tr>
<td>Lung</td>
<td>15.0</td>
<td>13.97 ± 0.95</td>
<td>93.14</td>
<td>93.24</td>
<td>6.8</td>
</tr>
<tr>
<td></td>
<td>2500.0</td>
<td>2344.5 ± 107.85</td>
<td>93.78</td>
<td>95.62</td>
<td>4.6</td>
</tr>
<tr>
<td></td>
<td>4000.0</td>
<td>3832.8 ± 206.97</td>
<td>95.82</td>
<td>94.08</td>
<td>5.4</td>
</tr>
<tr>
<td>Kidney</td>
<td>15.0</td>
<td>14.05 ± 0.83</td>
<td>92.69</td>
<td>93.68</td>
<td>5.9</td>
</tr>
<tr>
<td></td>
<td>2500.0</td>
<td>2335.25 ± 121.43</td>
<td>93.41</td>
<td>95.71</td>
<td>5.2</td>
</tr>
<tr>
<td></td>
<td>4000.0</td>
<td>3669.2 ± 168.78</td>
<td>91.73</td>
<td>96.22</td>
<td>4.6</td>
</tr>
<tr>
<td>Spleen</td>
<td>15.0</td>
<td>13.55 ± 0.93</td>
<td>90.32</td>
<td>93.91</td>
<td>6.7</td>
</tr>
<tr>
<td></td>
<td>2500.0</td>
<td>2287 ± 118.92</td>
<td>91.48</td>
<td>94.97</td>
<td>5.2</td>
</tr>
<tr>
<td></td>
<td>4000.0</td>
<td>3652.8 ± 211.86</td>
<td>91.32</td>
<td>94.83</td>
<td>5.8</td>
</tr>
<tr>
<td>Heart</td>
<td>15.0</td>
<td>13.79 ± 0.86</td>
<td>91.93</td>
<td>95.36</td>
<td>6.2</td>
</tr>
<tr>
<td></td>
<td>2500.0</td>
<td>2316 ± 111.17</td>
<td>92.64</td>
<td>95.39</td>
<td>4.8</td>
</tr>
<tr>
<td></td>
<td>4000.0</td>
<td>3715.2 ± 170.9</td>
<td>92.88</td>
<td>94.25</td>
<td>4.6</td>
</tr>
</tbody>
</table>
respectively. Table 1 summarizes the extraction recoveries for various plasma and tissue samples.

3.2.3. Linearity and sensitivity. The linearity of each calibration curve was determined by plotting the response factors versus the concentrations of the standard solutions. Linear calibration curves for BQCA in plasma and tissue were observed at 2.0–40 000.0 ng mL\(^{-1}\) and 5.0–5000.0 ng g\(^{-1}\), respectively. The results of the linear regression analysis are listed in Table 2 and showed that the correlation coefficients of the calibration curves for all the sample types were greater than 0.99. The LLOQ in plasma and tissues were determined by testing different levels ranging from 2.0 to 100.0 ng mL\(^{-1}\) or g\(^{-1}\) and were found to be 2.0 ng mL\(^{-1}\) and 5.0 ng g\(^{-1}\), respectively, with an accuracy of 92.43% with 5.8% of precision in plasma and 90.78% of accuracy with 6.7% of precision in tissues. The results indicated that the LLOQ of BQCA was within the acceptable precision and accuracy range.

3.2.4. Accuracy and precision. The accuracy and precision for the intra-and inter-day studies of three different QC standards of BQCA in plasma and tissues were found to be within the acceptable limits. The results indicated that the assay method was accurate and precise for replicate analysis of BQCA in plasma and tissues and the results are summarized in Table 1.

Table 2: Equation of linear regression analysis

<table>
<thead>
<tr>
<th>Biological sample</th>
<th>Concentration range (ng mL(^{-1}) or g(^{-1}))</th>
<th>Equation</th>
<th>(R^2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma</td>
<td>2.0–40 000.0</td>
<td>(y = 0.00002x + 0.01075)</td>
<td>0.999</td>
</tr>
<tr>
<td>Brain</td>
<td>5.0–5000.0</td>
<td>(y = 0.1174x + 0.0128)</td>
<td>0.998</td>
</tr>
<tr>
<td>Liver</td>
<td>5.0–5000.0</td>
<td>(y = 0.1338x + 0.0153)</td>
<td>0.998</td>
</tr>
<tr>
<td>Lung</td>
<td>5.0–5000.0</td>
<td>(y = 0.1289x + 0.0123)</td>
<td>0.999</td>
</tr>
<tr>
<td>Kidney</td>
<td>5.0–5000.0</td>
<td>(y = 0.0899x + 0.0071)</td>
<td>0.999</td>
</tr>
<tr>
<td>Spleen</td>
<td>5.0–5000.0</td>
<td>(y = 0.0962x + 0.0048)</td>
<td>0.999</td>
</tr>
<tr>
<td>Heart</td>
<td>5.0–5000.0</td>
<td>(y = 0.0716x + 0.0024)</td>
<td>0.998</td>
</tr>
</tbody>
</table>

Table 3: Summary of stability studies of BQCA in rat plasma under various storage conditions (\(n = 6\))

<table>
<thead>
<tr>
<th>Stability test</th>
<th>Biological sample</th>
<th>Plasma</th>
<th>QCs (ng mL(^{-1}))</th>
<th>Mean ± SD (ng mL(^{-1}))</th>
<th>Accuracy (% nominal)</th>
<th>Precision (%CV)</th>
<th>Brain</th>
<th>QCs (ng g(^{-1}))</th>
<th>Mean ± SD (ng g(^{-1}))</th>
<th>Accuracy (% nominal)</th>
<th>Precision (%CV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Freeze-thaw</td>
<td></td>
<td>Plasma</td>
<td>6.0</td>
<td>5.38 ± 0.33</td>
<td>89.68</td>
<td>6.2</td>
<td></td>
<td></td>
<td></td>
<td>15.0</td>
<td>13.52 ± 0.85</td>
</tr>
<tr>
<td>(3 cycles at −70 ± 2 °C)</td>
<td></td>
<td></td>
<td>20 000.0</td>
<td>18 092 ± 1031.24</td>
<td>90.46</td>
<td>5.7</td>
<td></td>
<td>2500.0</td>
<td>2281 ± 139.14</td>
<td>91.24</td>
<td>6.1</td>
</tr>
<tr>
<td>Short-term</td>
<td></td>
<td>Plasma</td>
<td>6.0</td>
<td>5.55 ± 0.34</td>
<td>92.45</td>
<td>6.1</td>
<td></td>
<td>4000.0</td>
<td>3656.8 ± 215.75</td>
<td>91.42</td>
<td>5.9</td>
</tr>
<tr>
<td>(at 25 °C for 6h)</td>
<td></td>
<td></td>
<td>32 000.0</td>
<td>29 372.8 ± 1703.62</td>
<td>91.79</td>
<td>5.8</td>
<td></td>
<td>2500.0</td>
<td>2289.5 ± 132.79</td>
<td>91.58</td>
<td>5.8</td>
</tr>
<tr>
<td>Long-term</td>
<td></td>
<td>Plasma</td>
<td>6.0</td>
<td>5.32 ± 0.36</td>
<td>88.68</td>
<td>6.8</td>
<td></td>
<td>4000.0</td>
<td>3735.6 ± 209.19</td>
<td>93.39</td>
<td>5.6</td>
</tr>
<tr>
<td>(at −70 ± 2 °C for 1 month)</td>
<td></td>
<td></td>
<td>32 000.0</td>
<td>29 852.8 ± 1761.32</td>
<td>93.29</td>
<td>5.9</td>
<td></td>
<td>2500.0</td>
<td>2201 ± 136.46</td>
<td>88.04</td>
<td>6.2</td>
</tr>
<tr>
<td>Stock solution</td>
<td></td>
<td>Plasma</td>
<td>6.0</td>
<td>5.87 ± 0.27</td>
<td>97.89</td>
<td>4.6</td>
<td></td>
<td>4000.0</td>
<td>3835.6 ± 180.27</td>
<td>95.89</td>
<td>4.7</td>
</tr>
<tr>
<td>(at 25 °C for 6h)</td>
<td></td>
<td></td>
<td>32 000.0</td>
<td>30 966.4 ± 1269.61</td>
<td>96.77</td>
<td>4.1</td>
<td></td>
<td>2500.0</td>
<td>2417.75 ± 108.79</td>
<td>96.71</td>
<td>4.5</td>
</tr>
</tbody>
</table>

Fig. 3 Concentration–time curves for (a) plasma and (b) tissues after i.p. administration of rats with 10 mg kg\(^{-1}\) of BQCA (mean ± SD).
Table 4  Mean pharmacokinetic parameters for BQCA in plasma and brain following i.p. administration at 10 mg kg⁻¹.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Plasma</th>
<th>Brain</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cmax (ng mL⁻¹ or g⁻¹)</td>
<td>7574.21 ± 82.01</td>
<td>808.57 ± 32.11</td>
</tr>
<tr>
<td>AUC₀–₈h (h ng mL⁻¹ or g⁻¹)</td>
<td>36 610.64 ± 293.15</td>
<td>3412.93 ± 114.74</td>
</tr>
<tr>
<td>AUC₀–∞ (h ng mL⁻¹ or g⁻¹)</td>
<td>45 876.05 ± 787.02</td>
<td>3660.01 ± 140.76</td>
</tr>
<tr>
<td>CL/F (mL or g h⁻¹ kg⁻¹)</td>
<td>218.03 ± 3.7</td>
<td>2735.56 ± 103.54</td>
</tr>
<tr>
<td>Ka (h⁻¹)</td>
<td>0.16 ± 0.01</td>
<td>0.31 ± 0.02</td>
</tr>
<tr>
<td>Vz/F (mL or g kg⁻¹)</td>
<td>1324.19 ± 25.36</td>
<td>8883.73 ± 527.12</td>
</tr>
<tr>
<td>T₁/₂ (h)</td>
<td>4.21 ± 0.07</td>
<td>2.25 ± 0.11</td>
</tr>
<tr>
<td>MRT (h)</td>
<td>3.42 ± 0.02</td>
<td>2.99 ± 0.04</td>
</tr>
</tbody>
</table>

3.2.5. Stability. The stability of BQCA in plasma and brain was evaluated by measuring the concentration change in QCs (n = 3) under different storage conditions, the results indicated that BQCA was stable under all the tested stability conditions. Table 3 depicts the percentage changes in the mean concentration of BQCA under all the conditions tested.

3.3. Pharmacokinetic study

A validated method was applied in the quantitative estimation of BQCA in plasma samples following i.p. administration at a dose of 10 mg kg⁻¹. The mean plasma concentration–time profile for BQCA is shown in Fig. 3a, and the pharmacokinetic parameters are listed in Table 4. BQCA was rapidly absorbed into the systemic circulation, the maximum concentration (∼8000.0 ng mL⁻¹) being achieved 1.5 h after i.p. administration. The mean peak plasma concentration of BQCA was 7574.21 ± 82.01 ng mL⁻¹ and its plasma concentration after four half-lives was ∼473.39 ng mL⁻¹. Since the method LLOQ was 2.0 ng mL⁻¹ for BQCA, its sensitivity was adequate for bioavailability studies. The pharmacokinetic parameters in plasma of Cmax, AUC₀–₈h, AUC₀–∞, CL/F, Ka, Vz/F, Tmax, T₁/₂ and MRT were found to be 7574.21 ± 82.01 ng mL⁻¹, 3 6610.64 ± 293.15 h ng mL⁻¹, 45 876.05 ± 787.02 h ng mL⁻¹, 218.03 ± 3.7 mL h⁻¹ kg⁻¹, 0.16 ± 0.01 h⁻¹, 1324.19 ± 25.36 mL kg⁻¹, 1.5 h, 4.21 ± 0.07 h and 3.42 ± 0.02 h, respectively.

3.4. Tissue distribution study

Concentrations of BQCA were determined in various tissues of SD rat such as brain, heart, lung, liver, spleen, and kidney. Fig. 3b shows the concentration–time curve of BQCA in various tissues following i.p. administration at 10 mg kg⁻¹ in SD rats. BQCA is rapidly taken up into the brain reaching a maximum concentration after 1.5 h, this concentration is then maintained for up to 3–4 h. The pharmacokinetic parameters in brain of Cmax, AUC₀–₈h, AUC₀–∞, CL/F, Ka, Vz/F, Tmax, T₁/₂ and MRT were found to be 808.57 ± 32.11 ng g⁻¹, 3412.93 ± 114.74 ng mL⁻¹, 3660.01 ± 140.76 h ng mL⁻¹, 2735.56 ± 103.54 g h⁻¹ kg⁻¹, 0.31 ± 0.02 h⁻¹, 8883.73 ± 527.12 g kg⁻¹, 1.5 h, 2.25 ± 0.11 h and 2.9 ± 0.04 h, respectively. The pharmacokinetic tissue distribution of BQCA was analysed by a non-compartment model and the parameters are presented in Table 5. The concentration of BQCA was low in all the collected tissues at 8 h. The order of AUC was kidney > lung > liver > brain > spleen > heart. The order of the maximum BQCA concentration was kidney > lung > liver > spleen > brain > heart. The maximum concentration (1951.8 ng g⁻¹) was found in the kidney suggesting that renal excretion may be the main BQCA elimination pathway due to the increased drug exposure.

4. Conclusion

A sensitive, rapid HPLC method was developed and validated for the estimation of BQCA in SD rat plasma. A simple PPT technique was employed to analyse the plasma and tissue samples. High sample throughput and good sensitivity (2.0 ng mL⁻¹ in plasma and 5.0 ng g⁻¹ in tissues) was achieved using the presented method. This work provides the data for plasma pharmacokinetics and tissue distribution of BQCA in SD rats. The achieved pharmacokinetics and tissue distribution results may be useful for further studies of the bioactive mechanism of BQCA. The method was successfully applied in the analysis of BQCA in plasma and tissue samples following i.p. administration at 10 mg kg⁻¹.

Acknowledgements

Mr Rizwan Basha Khatwal wished to express his gratitude to the Council of Scientific and Industrial Research (CSIR), New Delhi, India for the award of a Senior Research Fellowship (File no: 08/484(0010)/2013-EMR-I). The authors are thankful to Dr Jeffrey Conn, Director; Dr Craig W. Lindsley, Director of Medicinal Chemistry and Dr Thomas A. Ekman, Project Consultant, Vanderbilt Center for Neuroscience Drug Discovery, USA for providing BQCA as a gift sample.

Table 5  The main pharmacokinetic parameters of BQCA in visceral organs following i.p. administration at 10 mg kg⁻¹.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Heart</th>
<th>Kidney</th>
<th>Liver</th>
<th>Lung</th>
<th>Spleen</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cmax (ng g⁻¹)</td>
<td>398.56 ± 15.75</td>
<td>1951.8 ± 49.44</td>
<td>1234.25 ± 37.08</td>
<td>1883.25 ± 36.98</td>
<td>1239.50 ± 37.36</td>
</tr>
<tr>
<td>AUC₀–₈h (h ng g⁻¹)</td>
<td>1419.36 ± 26.80</td>
<td>7373.93 ± 236.50</td>
<td>4251.01 ± 102.82</td>
<td>5989.59 ± 59.04</td>
<td>2774.81 ± 79.13</td>
</tr>
<tr>
<td>AUC₀–∞ (h ng g⁻¹)</td>
<td>1701.51 ± 59.50</td>
<td>8038.46 ± 440.21</td>
<td>4543.22 ± 117.88</td>
<td>6621.28 ± 86.87</td>
<td>2910.92 ± 98.50</td>
</tr>
<tr>
<td>CL/F (g h⁻¹ kg⁻¹)</td>
<td>5883.289 ± 211.19</td>
<td>1247.11 ± 67.84</td>
<td>2202.33 ± 57.45</td>
<td>1510.50 ± 19.80</td>
<td>3438.69 ± 118.61</td>
</tr>
<tr>
<td>Ka (h⁻¹)</td>
<td>0.28 ± 0.02</td>
<td>0.24 ± 0.01</td>
<td>0.33 ± 0.02</td>
<td>0.23 ± 0.02</td>
<td>0.33 ± 0.03</td>
</tr>
<tr>
<td>Vz/F (g kg⁻¹)</td>
<td>21 025.23 ± 1176.04</td>
<td>5161.03 ± 245.53</td>
<td>6656.71 ± 382.91</td>
<td>6703.33 ± 515.54</td>
<td>10 366.41 ± 1233.78</td>
</tr>
<tr>
<td>Tmax (h)</td>
<td>1.5</td>
<td>1</td>
<td>1</td>
<td>1.5</td>
<td>1</td>
</tr>
<tr>
<td>T₁/₂ (h)</td>
<td>2.48 ± 0.16</td>
<td>2.87 ± 0.17</td>
<td>2.09 ± 0.17</td>
<td>3.08 ± 0.26</td>
<td>2.09 ± 0.19</td>
</tr>
<tr>
<td>MRT (h)</td>
<td>3.28 ± 0.06</td>
<td>2.9 ± 0.07</td>
<td>2.83 ± 0.06</td>
<td>3.28 ± 0.05</td>
<td>2.59 ± 0.04</td>
</tr>
</tbody>
</table>
References


# Packing List

**Shipped From**

Thomas A. Ekman, Ph.D.  
Project Consultant  
*On behalf of Craig Lindsley, Ph.D.*  
Vanderbilt Center for Neuroscience Drug Discovery  
Vanderbilt University Medical Center  
1215B Light Hall (MRB IV)  
2215-B Garland Avenue  
Nashville, TN 37232-6600  
USA  
615-936-6427 - Phone  
615-343-9332 - Fax  
E-mail: tom.ekman@vanderbilt.edu

**Shipped to**

Dr. Malay K Samanta  
Professor and Head  
Department of Pharmaceutical Biotechnology  
JSS College of Pharmacy  
Rocklands  
Ooty, Tamilnadu 643001  
INDIA  
Phone: +91-9443032280 or +91-9003721177  
Fax: +91-423-2442937  
E-mail: dr.mksamanta07@gmail.com or dr.mksamanta@yahoo.co.in  
FedEx account #: 364407386

<table>
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<th>Vial #</th>
<th>Amount Shipped (g)</th>
<th>MW (free base)</th>
<th>Salt MW</th>
<th>Salt Form</th>
<th>Country of Origin</th>
<th>Value</th>
<th>HTS #</th>
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</thead>
<tbody>
<tr>
<td>1 Vial 1-(4-methoxybenzyl)-4-oxo-1.4-dihydroquinoline-3-carboxylic acid, known as VU0238386 or &quot;BQCA&quot;</td>
<td>VU0238 386-8</td>
<td>5.00</td>
<td>309.32</td>
<td>331.3</td>
<td>Na</td>
<td>United States</td>
<td>$5.00</td>
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*Unless otherwise specified, all goods are solid synthetic chemical compounds. Health and physical hazards have not been assessed, but there are no hazards expected for the quantities that are shipped.

**FREE OF CHARGE – NO COMMERCIAL VALUE - VALUE FOR CUSTOMS PURPOSES ONLY**  
**RECIPIENT IS RESPONSIBLE FOR ANY IMPORT DUTIES AND CUSTOMS CLEARANCE QUESTIONS**

Above compound(s) are shipped to the recipient for the purposes of *in vivo* and/or *in vitro* pharmacology research only. Not for use in humans.

Thomas A. Ekman, Ph.D.  
Project Consultant  

Date: 7/12/2011
RIZWAN BASHA KHATWAL
RESEARCH SCHOLAR, DEPARTMENT OF
PHARMACEUTICAL BIOTECHNOLOGY, JSS
COLLEGE OF PHARMACY, ROCKLANDS NA
OOTY-643001

Award Letter

Sir/Madam,

With reference to your application and subsequent interview, I am happy to inform you that you have been selected for the award as per terms stated above (in the Hindi Version). The award will be effective from the date mentioned above or from the date of joining research whichever is later. The duration of CSIR SRF and SRF (Extended) is as indicated above.

The duration of the CSIR Research Associateship is initially for one year and any further extension is at the discretion of CSIR, based on a three member Assessment Committee Report & Annual Progress Report. A copy of Terms & Conditions of CSIR Fellowship/ Associateship is available on HRDG website (http://www.cisirhrdg.res.in). In case, the terms & conditions are acceptable to you, you may join the Fellowship/Associateship within the validity period and intimate to us.

The Director General, CSIR has also been pleased to sanction the Stipend and Contingency as stated above. In addition to Stipend & Contingency, House Rent Allowance & Medical Benefits will be payable as per rules of the host Institute limited to Central Govt. rates.

Please note that the validity of the award is for six months only from the effective date of award.

The award of CSIR Fellowship / Associateship does not imply any assurance or guarantee to subsequent employment by CSIR.

Yours faithfully,

[Signature]

SECTION OFFICER

Copy to:

1. Registrar/Principal/Director, with the request to send the following documents to this office.
   (A) Joining Report in the enclosed prescribed form.
   (B) Undertaking in the enclosed prescribed form and consolidated bill claiming grants in respect of new awardees showing their names, fellowship letter number, date of joining and the amount admissible, in triplicate, as per enclosed bill form.
2. Sr. F&AO (EMR). The expenditure will be debitable to the Budget Head 'P-81-101'.
4. Office Copy.
आवार्ड पत्र

फैलोशिप का नाम: SRF  [Initially for the period of ............yrs]
आर्थिक वृत्त: Rs. 18000 /-
लागू होने की तारीख: 1st April 2013
भाषा: PHARMACEUTICAL BIOTECHNOLOGY, JSS COLLEGE OF PHARMACY, OOTY
कुल उपम एवं आकृतिक भर्ती राशि: Stipend 216000 + Contigency 20000 = Total 236000 /-

महीना/महीज, अंक 31, 2013

आपके आवेदन पत्र तथा तत्प्राप्त हुए साक्षात्कार के संदर्भ में आपको सूचित करते हुए मुझे प्रसन्नता हो रही है कि उपर्युक्त निवेदन के अनुसार आवार्ड के लिए आपका यथार्थ आवेदन किया गया है। यह आवार्ड उपर दी गई तारीख अथवा अनुमानित कार्य प्रारंभ करने की तारीख से, जो भी बाद का होगा, सामर्थ्य होगा। सीएसआईआर एससीआर एवं एससीआर(विशेषता) की अवधि उपर दी गई है।

सीएसआईआर अनुसंधान एससीएट यह अवधि एक वर्ष है। तथा इसमें किसी भी प्रकार का विस्तार तीन सदस्यीय मूल्यांकन समिति की रिपोर्ट के आधार पर, सीएसआईआर के विवेकनन्द संस्थान किया जाएगा। यदि वह निवेदन एवं शर्त आपकी स्वीकार हो तो आवर्ध उपर दी गई लागू होने की तारीख से वैधता अवधि के अनुदान फैलोशिप/एससीएटिशिप का प्रत्यय कर सकता है।

अनुवादक: वैज्ञानिक तथा औषधिय अनुसंधान परिषद्, भी उपर्युक्त तृतीय एवं आकृतिक राशि के लिए यू.सी.सी. भारत में आशियन के संस्थान के चिकित्सकों से संपर्क रखता है।

इस अवधि की वैधता, लागू होने की तारीख से सेवन पर: भारत के लिए है।

सीएसआईआर फैलोशिप/एससीएटिशिप प्रदान करने जा रहे का आवश्यक सीएसआईआर द्वारा राहत के लिए किसी प्रकार का आधारभूत अवधारणा अद्यतन देना नहीं है।

अनुबंध अधिकारी

प्रति:-

(1) कूल राशियों, उपभोग,(PHARMACEUTICAL BIOTECHNOLOGY, JSS COLLEGE OF PHARMACY, OOTY) - को अनुसूचित के साथ क्यों निवेदित करने को निर्देशित की जाये।
(2) संलग्न कपड़े और वस्तुओं का कार्यान्वयन को नेहनें।
(3) संलग्न निर्देशित मामलों में वस्तुमान दिन विंग अवधि के संदर्भ में संभवत अद्यतन दिन विंग, जिसमें उसके नाम, फैलोशिप पत्र संबंध, कार्यालय ग्‌रहण करने की तारीख और अनुमानित दिन विंग ग्‌रहण की दिन होगी, संलग्न कपड़े प्रत्यक्ष के अनुसार तीन दिन पहले ईमेल में भेजे। कार्यालय ग्‌रहण करने की तारीख से 31 मार्च, 2014 का अनुसूचित का दिन कार्यालय प्रारंभ करने के 15 दिन के भीतर कपड़े आम्ब्रा जाना चाहिए।
(4) बैलासंस (ई. एम. आर.)
(5) ईमेल प्रकार

2013-03-01

(2) वैज्ञानिक (ई. एम. आर.)
(3) ईमेल लाइन (ई. एम. आर.), यह व्यवस्था बाजट पी-81 01 101 से किया जाएगा।
(4) बैलासंस
(5) बैलासंस प्रकार
To,

Dr. Malay Kumar Samanta
Professor & Head,
Deptt. of Pharmaceutics,
J.S.S. College of Pharmacy
Rocklands, Guntur-644001

Subject: Award of Senior Research Fellowship to Mr. Razwanbasha Khatwal, SRF on the Research project entitled “Enhancement of neuroprotection by selective allosteric potentiators of M1 muscarinic receptor to restore reversal rearing management in Alzheimer’s disease”.

SIR/Madam,

The Council award to Mr. Razwanbasha Khatwal, SRF on a stipend of Rs. 18,000/- p.m. to carry out research on the project mentioned above, under your guidance. H.R.A. and Medical reimbursement will be paid as per rules of your University.

Comments of the Council have already been communicated to you. These comments should be taken into account while carrying out the research work on the project. The award of SRF will be subject to the following terms and condition:

**TENURE:** It will be tenable for one year only from the date of joining duty and will be on yearly basis subject to maximum of three years.

Its continuance will, however, depend on the satisfactory progress of work and can be terminated at any time on a one month’s notice, if the progress is not satisfactory, or on receiving an adverse report from the Guide. The Fellow will be required to work on the project for a period at least one year.

The event of his/her leaving before completing one year on the fellowship, he/she may be required to refund the stipend drawn by him/her from the date of joining to the date of leaving the fellowship.

**PRIVATE PRACTICE:** Private practice of any kind, or taking up any appointment even in an honorary capacity during the fellowship is not permitted.

**ADMINISTRATIVE CONTROL:** The candidate will be under the administrative control of the Institute where he/she works, and will also be subject to the rules and regulations of the Institute.

**LEAVE:** Leave will be admissible according to the rules of the Institution, however, in the case of female research fellows leave without stipend up to 3 months (in lieu of maternity leave) may be granted. No other kind of leave (such as sick leave) etc. will be admissible. Awardees are not entitled to vacation normally admissible to the staff of an Institution.

**HRA:** HRA will only be paid, if the fellow is not availing any hostel facility. A certificate to this effect should be sent along with joining report for payment of HRA.

**REPORTS:** The awardee shall submit 1st annual reports for the first 10 months, on the prescribed standard proforma.

The first annual report should be submitted after 10 months from the date of commencement of the fellowship giving complete factual details of the research work done through the Guide along with his/her appraisal. Subsequent annual report should be submitted through the Guide two months before the completion of fellowship tenure. Failure to submit reports in time may lead to termination of the award. Six copies of the final report in the prescribed form clearly shall be submitted one month before the date of termination of the award.

A list of the papers published or presented at Scientific Conferences during the tenure of the fellowship should also be furnished with the annual and final reports.
PUBLICATION OF PAPERS: Prior permission for publication of papers based on the research work done during the tenure of the award should be obtained from the Council. The paper should be sent to the Council through the Guide with his/her recommendations. Due acknowledgement to the Council should be made in these papers.

PAYMENT OF FUNDS: The stipend and the funds for contingencies shall be paid as per procedure laid down in the enclosed annexeure.

CONTINGENT EXPENDITURE: An annual contingent grant of Rs. 20000/- p.a. will be admissible for the financial year from 1st April to 31st March. In case a fellow join duty after 1st April, the contingent grant will be proportionate to the period of the award in that financial year. The contingent grant is given to meet petty expenditure for purchase of chemicals, reagents etc. No non-expenditure article or equipment can be purchased out of the grant.

TRAVEL: Traveling allowance will not be admissible for joining duty or on termination of the award.

The Council may approve tours of research fellows/associate for:-

1. Attending symposium/seminar/conference provided the fellow/associate is presenting a paper which has been accepted by the organizers of the symposium/seminar/conference.
2. Field work connected with research
3. TA/DA would be admissible as per the rules applicable to Central Government Officers with basic pay equivalent to the amount of the fellowship stipend.

NOTE: The expenditure on this account will be met

POST FELLOWSHIP CARRIER:
1. The Research Fellow can register himself/herself for postgraduate qualification and to utilize in his/her the work done by him/her during his/her fellowship tenure. A copy of these submitted for postgraduate degree will have to be sent to the Council for information and record from the contingent grant sanctioned to the fellow. Due acknowledgement to the IC MR should be made in the thesis by the research fellows.

2. The Research Fellow should also send to the Council for information a brief report on the postjob taken by him/her after the expiry of the fellowship.

The date indication forenoon/afternoon on which he/she the fellowship may please be intimated to this office. He/she may be asked to report for duty within a month from the date of issue of this letter failing which the award will be treated as canceled.

Yours faithfully,

(K. Kotnala)
Administrative Officer
For Director-General

Copy to:- (Head of the Institution) The Principal, J.S.S. College of Pharmacy, Gocamund-643001

Mr. Rizwanabasha Khatwal, SRF, Deptt. of Pharmaceutics, J.S.S. College of Pharmacy Rocklands, Gocamund-643001

2. Accounts Section – V. IC MR

3. IRIS Cell No. 2012-14280

Administrative Officer
For Director-General
DEPARTMENT OF HEALTH RESEARCH (MINISTRY OF HEALTH & FAMILY WELFARE)  
V. RAMALINGASWAMI BHAWAN, ANSARI NAGAR, NEW DELHI - 110 029

SANDHYA DIWAKAR  
Scientist- E  

Rizwan Basha Khatwal  
Research Scholar,  
Dept. of Pharmaceutical Biotechnology,  
JSS College of Pharmacy, Rockland, Ooty-643110


Dear Sir/Madam,

I am glad to inform you that Director General, ICMR, based on the recommendation of Expert Committee, has sanctioned a sum of ₹ 92,135/- (Rupees ninety two thousand one hundred thirty five only) to you towards air fare, visa fee and registration fee (The air tickets are to be booked in economic class in a National Carrier i.e. Air India) to attend international conference/workshop/training.

If, you are willing to avail the assistance, you may convey your acceptance within 15 days of issue of this communication, failing which it will be assumed that you are not interested to avail the grant. In the event of your not being able to utilize this amount for various reasons even after confirming your acceptance, please inform us immediately for necessary action at our end.

We have following comments to make:

The actual amount will be reimbursed after your return from the conference and receiving the required travel documents. Please find enclosed herewith accounts proforma in which you will have to submit your claim along with a copy of:

- Award letter
- Participation certificate and copy of presented paper in the proceedings/abstract book
- Participation report, air ticket showing air fare and boarding pass original copy only (from Air India only as per Government orders)
- Award letter from other agencies
- Any other relevant documents

You are requested to produce the original bills/vouchers. The claim should be forwarded to us through competent authority and should reach this office within one month after the completion of the scientific conference/workshop/training.

The financial assistance is governed by the terms and conditions as mentioned in enclosed form.

Yours faithfully,

Sandhya Diwakar  
For Director General ICMR  
sandhyadiwakar@yahoo.com  
011- 26589287 (o)

Copy to: Principal, JSS College of Pharmacy, Rockland, Ooty-643110

Note: Journey by other than National carrier is not permissible under the Govt. of India norms. For travel to stations not connected by Air India, Officials may travel by Air India to the point closest to their eventual destination beyond which they may utilize the services of another airline, which should also preferably be an alliance partner of the national carrier (Air India). A certificate to this office may be obtained from Air India.
H R Grover  
Scientist  

Mr Rizwan Basha Khatwal  
Dept. of Pharmaceutical Biotechnology  
JSS College of Pharmacy  
Rocklands  
Ootacamund - 643 001  

Ref No. TG/6300/11-HRD  
Oct 07, 2011  

SUBJECT: CSIR Foreign Travel Grant  

Dear Sir / Madam  

With reference to your application on the aforesaid subject, we are happy to inform that the Director General, CSIR has been pleased to sanction foreign travel grant to enable you to attend and present your paper at the 2nd Nano Today 2011, Hawaii, USA during 11 Dec 2011 to 15 Dec 2011 subject to the following conditions:-  

1. The CSIR Foreign Travel Grant is limited to HALF AIR FARE payable in Indian Rupees only. The journey should be strictly performed by the shortest route in excursion economy class by Air India only. Tickets should be purchased directly from Govt. booking office or through any IATA approved travel agent as warranted under Govt. of India orders in this subject. Travel by Air India is mandatory. Fare will not be reimbursed if you travel by other airlines. In case of deviation because of operational or other reasons or on account of non availability, relaxation/permission may be obtained from Ministry of Civil Aviation office (Contact Person - Shri SK Chhikara, Under Secretary Min of Civil Aviation, Rajiv Gandhi Bhawan, Safdarjung Airport, New Delhi 110003 ).  

2. The incumbent should submit a tour report in the prescribed proforma within one month of return from abroad forwarded through his/her Supervisor/Head of the Institution. One reprint of the research paper presented at the Conference/Symposium etc. should be sent to CSIR, invariably after its publication.  

3. The grant should be claimed by filling-in the tour report & Grant-in-Aid bill proforma (in duplicate) along with the counter foil of original boarding pass, original cash receipt/ e-ticket and certificate of attending the conference from the organizers.  

4. Please communicate your acceptance of this grant immediately by email only, failing which it will be presumed that you do not need support from CSIR.  

Yours sincerely,  

(H R Grover)  

Copy to:-  

i) Principal  
JSS College of Pharmacy  
Ootacamund - 643 001  

ii) Audit (EMR)  

* Note: Tour report & Grant-in-Aid Bill proforma may be downloaded from our website http://csirhrdg.res.in
To,
Sh. Rizwan Basha Khatwal  
D/o Pharmaceutical Biotechnology  
JSS College of Pharmacy  
Rocklands, Ooty - 643001 (T.N)

Sub.: Financial Assistance to Sh. Rizwan Basha Khatwal for participating in 16th Canadian Society for Pharmaceutical Sciences Annual Symposium to be held from 11/06/2013 to 14/06/2013 in Canada

Sir / Madam

We are happy to inform you that your application seeking financial grant to attend the above mentioned international scientific event has been recommended for support by the Science and Engineering Research Board (SERB). We will provide to and fro economic class air-fare by the shortest route, airport tax, visa fees and registration fees. We hope this support will provide you an opportunity to interact with leading international experts in the area. The support, however, is subject to the following conditions:

1. You should not have received financial support during last three years under this scheme. The air tickets are to be booked in economic class by the shortest route in a National Carrier, i.e., Air India. For Travel to station not connected by Air India, you may travel by Air India to the hub/point closest to their eventual destination, beyond which you may utilize the services of another airline which should also preferably be an alliance partner of Air India. If you are traveling by Private Airline because of non-availability of tickets or any other reason, you are requested to seek relaxation from the Ministry of Civil Aviation. The contact details for obtaining relaxation are:

   (1) Shri Dinesh Sharma  
   Ministry of Civil Aviation  
   Rajiv Gandhi Bhawan, New Delhi  
   FAX: 24632950/2873, Tel Fax: 24651132

   (2) Shri S.K. Chhikara, Under Secretary  
   Ministry of Civil Aviation  
   Rajiv Gandhi Bhawan, New Delhi  
   FAX. 24651132, E-mail : chhikara.sk@nic.in

You are advised to attach a copy of permission letter from Ministry of Civil Aviation for travel by private airlines while claiming the reimbursement. Without this permission letter, it will not be possible to reimburse the travel grant.

2. E-ticket is acceptable provided the amount of the fare is clearly reflected on the ticket.
3. You will submit tour report and other documents in the enclosed proforma within 30 days of your return to India.
4. The claim sheet along with all documents must be tagged/stapled properly before sending it to the Board. Institute/University Accounts Details should be signed by the competitive Authority of the Institute/ University and Certified by Authorized Official of the Bank.
5. We will reimburse the grant after deducting the support received from any other sources, if any.
6. All other expenses such as per diem, taxi fare, bus fare etc. will not be reimbursed by the Department.
7. You have to make your own arrangements for foreign exchange required for the purpose.
8. You will not be treated as a delegate sponsored by the Government of India.

Based on this offer letter, your Institute may consider advancing necessary funds to enable you to attend the above event. We request you to intimate us within two weeks, if you are not availing this offer.

With kind regards,

Your's Faithfully,

(Dr. Virod Kumar)  
Scientist – F

Encl: Claim Sheet
RizwanBasha Khatwal  
CSIR Senior Research Fellow  
Department of Pharmaceutical Biotechnology  
JSS College of Pharmacy  
Ooty- 643001  
Tamilnadu, India

Dear RizwanBasha Khatwal:
On behalf of the 2013 Alzheimer’s Association International Conference (AAIC) Travel Fellowship Committee, I am pleased to inform you that your application for a Travel Fellowship has been approved as noted below. AAIC will be held in in Boston, Massachusetts, United States on July 13-18, 2013.

Your fellowship includes:
- Complimentary AAIC conference registration
- One economy-class, non-refundable, non-upgradeable airfare ticket
- Four (4) nights hotel accommodations (at a hotel determined by Association staff)

Note: Additional payment is required if you intend to register for any pre-conference events or seek continuing education credits.

You must confirm your acceptance of the travel fellowship by April 5, 2013 by sending an e-mail to nsanders@alz.org. You will forfeit the award if we do not receive your reply by the deadline. Once you have accepted, you will receive detailed instructions on how to register, book air travel and hotel accommodations. Please do not register or book any airfare or hotel prior to receiving the instructions.

Note: Poster notifications will be sent by April 3, 2013.

Once again, congratulations and I look forward to receiving your confirmation.

Questions: +1.312.335.5897 or nsanders@alz.org.

Sincerely,

Nicole Sanders  
Senior Specialist, Membership & Conference Programming  
Alzheimer’s Association  
nsanders@alz.org
Dear Rizwan Khatwal,

COUNTRY OF ORIGIN: INDIA

On behalf of the Scientific Committee of the XX World Congress on Parkinson's Disease and Related Disorders, I am happy to inform you that, despite the large number of applicants, you have been selected as one of the winners for a travel grant in the category:

"Awards for investigators from developing countries in the amount of USD 1000 & free congress registration fee."

Please take a note of the following:

1. Kindly proceed to your free congress registration via this special link as soon as possible:
   http://www2.kenes.com/parkinson/registration/Pages/Reginv.htm

2. The payment of your travel grant will be paid out to you by bank transfer after the congress. Please note that in case you do not attend the congress you will not be paid any travel reimbursement whatsoever.

Instructions on how to fill in your bank details into our online payment system will be sent to you closer to the event.

Please let me know in case you need anything else at this stage.

With kindest regards,

Mrs. Raya van Hugten, MSc
Congress Secretariat
XX World Congress on Parkinson’s Disease and Related Disorders
De Ruyterkade 7 (12th Floor)
1013 AA, Amsterdam, The Netherlands
Tel: +31 20 763 0517 I Fax: +31 20 763 0511
E-mail address: parkinson@kenes.com
JSS College of Pharmacy
Udhagamandalam – 643001
The Nilgiris, Tamil Nadu
Ph: +91-423-2443393  Fax:+91-423-2442937

JSSCP/IPA/005/Ooty

09.11.2013.

To
Mr. Rizwan Basha Khatwal
Research Scholar
Department of Pharmaceutical Biotechnology
JSS College of Pharmacy
Ootacamund

Sir,

Ref: Your request letter dated 09.10.2013

Greetings. The Indian Pharmaceutical Association, Nilgiris Local Branch has received your application cited in the reference for financial assistance towards your travel to Switzerland to attend and present a paper at ‘XX World Congress on Parkinson’s Disease and Related Disorders’ to be held during 08-11, December, 2013.

I am happy to inform you that, the executive committee has approved your application and Rs. 20000/- (Twenty Thousand Only) has been sanctioned as ‘International Travel Grant’. Hence you are hereby directed to submit a brief report along with participation certificate after your return to India to avail this grant.

Wish you all the best.

Yours sincerely

Dr. Arun K.P.
Secretary
Monday, December 02, 2013

Dear Rizwan Basha Khatwal:

On behalf of the Society for Laboratory Automation and Screening's Program Committee, thank you for your abstract submission and Tony B. Academic Travel Award application. This year's Tony B. was highly competitive due to the record number of applications received. SLAS is pleased to inform you that you have been selected to receive a travel award to present at the Third Annual SLAS Conference and Exhibition (SLAS2014) in San Diego, CA, USA. The conference will be held January 18-22, 2014 at the San Diego Convention Center.

Your abstract has been scheduled for presentation as part of the poster program at SLAS2014. Important details and deadlines regarding your poster presentation are included within this confirmation. Your poster presentation is scheduled for Monday, January 20, 2014 from 1:00-3:00pm (local time). Your presentation details are also included below (see poster assignment #):

**Number:** 60  
**Date:** Monday, January 20, 2014  
**Time:** 1:00 PM – 3:00 PM  
**Significant Delivery of M1 Acetylcholine Receptor Selective Allosteric Potentiator BQCA to Brain using Polysorbate-80 coated PLGA Nanoparticles for Management of Alzheimer’s Disease**

Complete guidelines regarding poster presentations at SLAS2014 are located online at [http://www.slas2014.org/presenters/posterguidelines.cfm](http://www.slas2014.org/presenters/posterguidelines.cfm). Please read these guidelines carefully.

If you submitted additional abstracts, you will be contacted separately regarding those abstract submissions at a later date.

**CONFERENCE LOCATION:**  
San Diego Convention Center  
111 W Harbor Dr.,  
San Diego, CA 92101

**SPEAKER RESOURCE CENTER (SRC):**  
All podium presenters are asked to visit the SRC as soon as possible. This resource will serve as your single reference for all details and requirements regarding your presentation at
SLAS2014 including:

1. Conference Participation Form/Agreement to Participate;
2. Key Logistical Dates/Presentation Details/Program Details;
3. Copyright Waiver and Speaker Agreement;
4. Conference Registration and Registration Waiver Request;
5. Housing/Travel Details (including hotel check-in/out dates);
6. Student Poster Competition Details.

To access the SRC, please click on the link below as soon as possible. There are various deadlines and important dates listed within this email and in the SRC, please review these deadlines closely.

**SPEAKER RESOURCE CENTER (SRC):**


**Username:** rizwanbasha07@gmail.com

**Password (Access Key):** NWZAYQUJ

**TRAVEL:**
As a Tony B. Awardee, your airfare expense will be covered in coach class up to $500USD (domestic) or $1200USD (international). Travel expenses are nontransferable.

- You must book your travel by December 13, 2013. Fares booked after this deadline may not be covered by SLAS.
- Should you drive, rather than fly, your automobile mileage will be reimbursed at the current (2013) United States Internal Revenue Service rate.

**IMPORTANT NOTE:** The travel agent has set up online authorizations based on your name as indicated in this confirmation letter. All airline tickets must be booked with the name that matches your identification (passport, etc.). Should your official identification name be different from the name on this confirmation, please indicate so when booking your travel.

Please contact National Travel directly to book at:

**Email:** vipservices@nationaltravel.com
**US Toll Free:** 1-800-557-0842
**International:** 1-304-357-0808

**SHARED ACCOMMODATIONS:**
• Shared accommodations (nontransferable) at an official SLAS hotel are provided you as a Tony B. Awardee by SLAS for a maximum of (four) 4 nights.
• It is required that you use the Speaker Resource Center (SRC) to provide details regarding your accommodations. Please visit the Speaker Resource Center as soon as possible.
• You may extend your stay beyond this by indicating this within the SRC; however you will be expected to pay for additional nights upon check-out from the hotel. In order to have the cost of your hotel nights covered, you must stay at an official SLAS hotel.
• Please book your hotel by November 4, 2013.
• Although your room/tax for your approved number of covered nights will be direct billed to SLAS, you will be required to provide the hotel with a credit card number for incidental charges when you check in to the hotel
• To assist you in making roommate requests, a complete list of awardees is available using the SRC and will be finalized in October 2013.
• If you do not select a room mate, one will be chosen for you. If you do not wish to share a room, only half of your hotel cost will be reimbursed. Please indicate as such within the SRC.
• A confirmation of your reservation will be sent directly to you via email after December 13, 2013. This confirmation will also include information on how to make future changes to your reservation.

Please note that the SLAS travel policy does not include ground transportation, rental cars, or any food or beverage reimbursement.

CONFERENCE REGISTRATION:

As a Tony B. Awardee, registration to attend SLAS2014 is complimentary for you. No action is needed on your part to register for the conference. You will receive a confirmation from SLAS2014 Registration by December 2013 and should plan to pick up your credentials onsite at SLAS2014 in San Diego. See below regarding short course registration.

STUDENT POSTER COMPETITION:

The award consists of cash awards of $500 each for the Top 3 student poster competition winners. In addition, each of the Top 3 is invited to participate in an interview with the SLAS Lab Man just after the awards ceremony at SLAS2014. The poster award ceremony and podcasts are to take place on Monday, January 20th at 5PM (local time) in the SLAS Member Center.

For complete details regarding the Student Poster Competition including submission and judging, please visit http://www.slas2014.org/awards/posterCompetition.cfm.

Note: in order to be considered for the poster award, you are required to submit the poster to the SRC by November 18, 2013.

AWARDEE GIFT:
Please contact me via email regarding your awardee gift. Please include shirt size (S, M, L, XL) and fit (male or female) preference as well as your name listed as you would like for it to appear on your plaque by **November 4, 2013.**

**COMPLIMENTARY SHORT COURSE:**

As a Tony B. Awardee, you are eligible to receive complimentary registration to one (1) 1-Day short course at SLAS2014. Please visit the SRC to make your short course requests by **November 4, 2013.**

We will make every attempt to register you in your first choice, but space is limited so please do respond as soon as possible.

**IMPORTANT DEADLINES:**

We request that you please access the Speaker Resource Center as soon as possible to accommodate the following deadlines:

**November 4, 2013:**

- Housing and Travel Booking
- Speaker Agreement and Copyright Waiver
- Presentation Drop-Off and Format Agreement
- Short Course Registration Request
- Awardee Gift and Room Mate Details Due

**November 18, 2013:**

- Student Poster Competition Poster Image Upload Deadline

**December 13, 2013:**

- Airfare Booking Deadline

Please contact me directly if you have any questions.

Best,

Amy McGorry
Manager, Events and Education
Society for Laboratory Automation and Screening
amcgorry@slas.org
P: 630.256.7527 ext 101
F: 630.741.7527
SLAS.org

*Join us in San Diego, CA, USA for SLAS2014: January 18-22, 2014*
Dr. (Mrs) A. AMUDESWARI  
Director

DOILr.\TF-IV\2013-14  
24 January 2014

Mr. Rizwan Basha Khatwal  
Res. Scholar  
Dept. of Pharmaceutical Biotech.  
JSS College of Pharmacy, Rocklands  
Ooty 643 001 (Tamilnadu)

Dear Mr. Rizwan Basha Khatwal,

Sub: Travel support to attend SLAS 2014 at San Diego, USA during 18-22 Jan.’14

We are extremely pleased to inform you that CICS will provide financial assistance of Rs.25,000/- which is subject to actual expenditures and receipts from all other sources whichever is less, towards partial travel / registration / accommodation for attending the above meeting/conference. Please confirm your acceptance of this offer within 15 days from the date of this letter, failing which the award will be forfeited.

In the event you are not able to utilise this grant for various reasons even after confirming your acceptance, please do inform us immediately so that the money can be transferred to waitlisted awardees.

The actual amount will be paid after your return from the Conference. Please find enclosed herewith a Proforma in which you will have to submit your claim along with a copy of

- award letter  
- participation certificate  
- participation report & air ticket  
- visa page  
- passport (first two pages)  
- passport size photograph  
- documents as specified in the terms & conditions  
- Award letter from other agencies

Your claim must be endorsed by the Head of the Department / Institution and should reach our office within one month after the completion of the scientific meeting / training. The payment will be made in favour of Head of organisation / Finance Controller of your organization.

The grant is however subject to the consideration that you have not availed such offer from CICS (CCSTDS) during the last three years.

The grant is governed by Terms and Conditions (enclosed). The admissibility of the claim will be as per INSA/CICS norms.

In order to avoid delay in reimbursement of your claim, please do ensure that the terms and conditions are strictly adhered to.

With kind regards,

Yours sincerely,

(A. AMUDESWARI)

Encls: As above

2, Gandhi Mandapam Road, Chennai - 600 025, India  
Phone: 0091 – 44 – 24430228 (Direct), 24419466, 24901367 ; Fax: 0091 – 44 – 24914543  
Email: dircics@gmail.com  
Website: www.cics.tn.nic.in
Dear Dr Rizwan Basha Khatwal,

Further to our email on 3 Jan about the approval of your scholarship application of the 14th Asian Oceanian Congress of Neurology (AOCN 2014), we would like to supplement the following information for your kind attention:

**Complimentary Congress Registration**

Kindly please remind that the complimentary congress registration offered doesn’t include the participation to the Pre-Congress Workshop/ Teaching Course on 2 March and Gala Dinner on 4 March. If you wish to attend these additional programme, please let us know and you are required to settle the payment separately. Registration confirmation will be emailed to you after processing.

**Hotel Accommodation**

Hotel accommodation will be arranged by Congress Secretariat on 2, 3 and 4 March (3 nights) for you. Hotel confirmation for check-in purpose will be emailed to you in due course.

**Scholarship**

As mentioned, the scholarship will be up to the maximum amount of US$850 which are used to cover your travel cost from your residing country to Macao. Please be reminded that you are required to arrange your own flight to attend the Congress in Macao and send us the required documents (i.e. invoice/ receipt of your air ticket) **by 20 January 2014** as the supporting documents in order to finalize the amount of your scholarship. Documents received after the said deadline will NOT be considered. Kindly be reminded that the scholarship will be collected during the Congress in Macao.

We look forward to meeting you at the Congress. Should you have any enquiries, please do not hesitate to contact us.

Best regards,

Po LI

AOCN 2014 Congress Secretariat
Tel: (852) 2559 9973