Research Journal of Chemical Sciences _____ ISSN 2231-606X
Vol. **2(9),** 63-71, Sept. **(2012)** *Res.J.Chem.Sci.*

Synthesis and Biological Evaluation of 3-(4-aminophenyl)-2-(1H-indol-3-yl) Propanamide derivatives as novel PTP1B Inhibitors

Arun P.C.*[1], Badiger E. Sangamesh[1], Dubey P.K.[2], Panigrahi Sunil Kumar[1] and Sekhar Reddy M.V.V.V.[1]
[1]Medicinal Chemistry, Aurigene Discovery Technologies Ltd, Hosur Road, Bangalore – 560100, INDIA
[2]Department of Chemistry, JNTUH College of Engineering, Hyderabad - 500085, INDIA

Available online at: www.isca.in
Received 11th June 2012, revised 14th June 2012, accepted 18th June 2012

Abstract

Indole scaffold represents one of the most important subunit explored in medicinal chemistry. We explored the possibilities of using the 3-(4-aminophenyl)-2-(1H-indol-3-yl)propanamide derivatives as PTP1B inhibitors. These inhibitors were designed based on molecular docking studies. Among the prioritized molecule 2-((4-(3-amino-2-(1H-indol-3-yl)-3-oxopropyl) phenyl) amino)-2-oxoacetic acid and their derivatives found to show reasonable potency for PTP1B. These compounds were examined for PTP1B inhibition through in-vitro assay. Best compound 15a showed Ki of 52 µM.

Keywords: Indole, PTP1B, Diabetes, oxamic acids.

Introduction

Protein tyrosine phosphatases (PTP's) constitute a large family of signaling enzymes that control several fundamental cellular functions via phosphorylation and dephosphorylation reactions[1-2]. Deregulation of these PTP's activity can lead to number of diseases including cancer and diabetes[3-4]. Protein tyrosine phosphatase 1B (PTP1B) enzyme is an effective target for the treatment of both type 2 diabetes and obesity. Inhibition of PTP1B should therefore increase insulin sensitivity and responsiveness. PTP1B knockout mice showed increased insulin sensitivity, improved glucose tolerance and resistance to diet-induced obesity, thus, making it a validated target for the treatment of both T2DM and obesity[5-6]. Efforts were made to use the pTyr substrate 1 meimetics difluoromethyl phophonates (DFMP) 2, carboxymethyl salicylic acids (CMS) 3, and oxalylaminobenzoic acids (OBA) 4 as PTP1B inhibitors shown in figure 1.

Compound **5** reported earlier has shown very good inhibitory potency against PTP1B (Ki = 18 nM) but as it had a diaryl oxamic acid benzoic acid, a catalytic site binding pharmacophore it showed very less cell permeability[7]. Latter synthesized compound **6** (with R = Boc, Ki=8.8 µM and with R = Ac, Ki=6.1 µM) by the same group showed moderate inhibitory activity towards PTP1B which had only one carboxylic group[8].

Indole[9-11] is one of the highly explored scaffolds in the field of drug discovery, but not many compounds were reported for the PTP1B inhibition. We explored the possibilities of using indole-3-acetic acid to mimic compound 6, keeping the oxamic acid at para position as it is and making the different substituted amides. Based on compound 6 we designed several molecules based on the computational docking study. X-ray structure of one of the potent inhibitor in complex with PTP1B (PDB ID: 2VEU) was taken as reference for docking and prioritization. The prioritized compounds were later synthesized and biochemical potency was determined using in-vitro assay. PTP1B inhibitory activity and the in-vitro results are summarized in table 1. Eight compounds were synthesized. The biochemical potency of these compounds ranges from 52.5 to 230 µM. These compounds were also selective against TCPTP.

Material and Methods

All chemicals and solvents were of reagent grade (Sigma – Aldrich, Spectrochem, Merck) and used without further purification. All melting points (°C) were determined by the open tube capillary method and quoted uncorrected. IR spectra were prepared on a FT-IR spectrophotometer using KBr discs. The 1H NMR and 13CNMR spectra of the compounds were measured in CDCl3 or DMSO-d6 solution 300MHz or 400MHz spectrometer (varian) using TMS as internal standard. All intermediates characterized and confirmed by HNMR and all the final compounds were characterized and confirmed by LCMS, 1HNMR, 13CNMR and IR.

Synthesis of compound 15 is as illustrated in the scheme 1. Indole-3-acetic acid is converted into its corresponding methyl ester followed by protection of indole NH with benzene sulfonyl chloride yielded compound 9. This was further reacted with 4-Nitrobenzyl bromide yield the alkylated product 10. The acid obtained by the hydrolysis of compound 10 was reacted with amines to get amides 12. The nitro and olefin groups of compound 12 was reduced by using hydrogen in presence of 10% Pd/C and reacted with ethyl oxalyl chloride and further hydrolysis and deprotection of compound 14 in one pot in presence of cesium carbonate yielded compound 15.

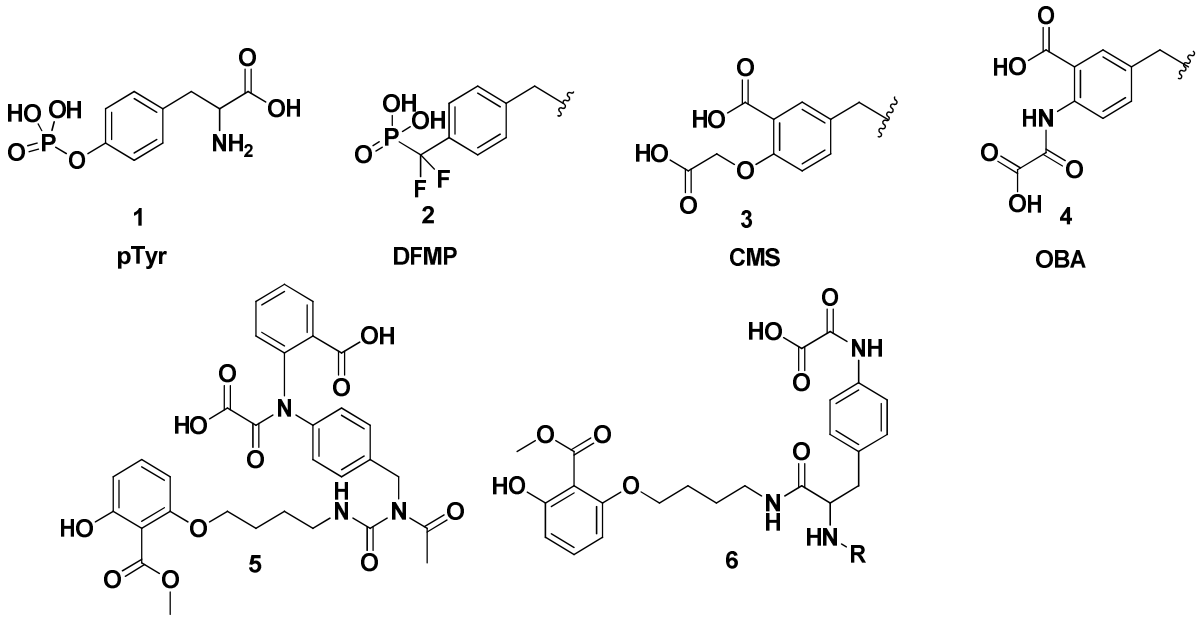
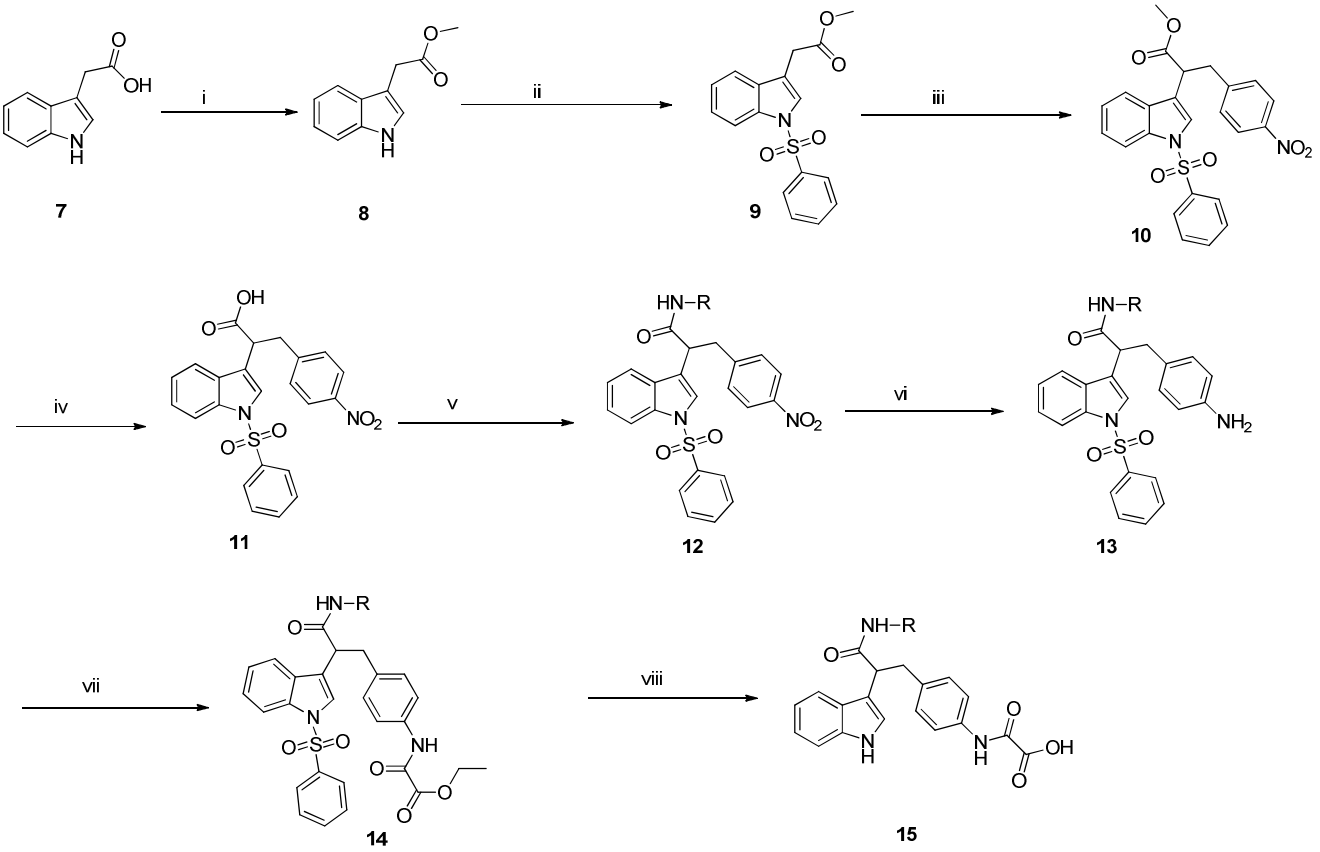


Figure-1
PTP1B inhibitors from literature



Scheme - 1

i) MeOH, $SOCl_2$,RT, 4 h; ii) Benzene sulfonyl chloride, Powdered NaOH, DCM; iii) 4-Nitrobenzyl bromide,K_2CO_3, DMF, RT 8 h; iv) KOH, THF, Water, 4 h; v) RNH_2, EDCI, HOBt,Et_3N, DMF; vi) Pd/C, H_2, MeOH; vii) Ethyl oxalylchloride, DIPEA, DCM; viii) Cs_2CO_3,MeOH,Reflux, 16 h

Methyl 2-(1H-indol-3-yl)acetate (8) : Indole-3-acetic acid (25 g, 0.142 mol) was taken in 250 mL dry methanol and added thionyl chloride (25.2 g , 0.21 mol) slowly over a period of 10 min with cooling. The reaction mixture was allowed to stir for 5 h. The reaction mixture was evaporated to 50 mL volume added into crushed ice and extracted with ethyl acetate. The organic layer washed with saturated bicarbonate solution followed by brine, dried over sodium sulfate and concentrated (yield= 22 g, 81%).

Methyl 2-(1-(phenylsulfonyl)-1H-indol-3-yl) acetate (9): Methyl 2-(1H-indol-3-yl)acetate (10 g, 0.053 mol) was taken in 100 mL of dichloromethane and added powdered sodium hydroxide (8.46 g , 0.21 mol) followed by benzene sulfonyl chloride (18.6 g, 0.105 mol) at 0°C for 10 min and the reaction mixture was allowed stir over night at room temperature. The reaction mixture was added water, DCM layer separated, washed with saturated bicarbonate solution, brine dried over sodium sulfate and concentrated (yield = 13 g, 74.7 %).

Methyl 3-(4-nitrophenyl)-2-(1-(phenylsulfonyl)-1H-indol-3-yl)propanoate (10): Methyl 2-(1-(phenylsulfonyl)-1H-indol-3-yl) acetate (13 g, 0.039 mol) was taken in 70 mL dry DMF and added dry potassium carbonate (16.5 g, 0.118 mol) at RT and stirred for 30 min. Added 4-nitrobrnzyl bromide (10.2 g, 0.047 mol) in dry DMF (30 mL) over a period of 5 min at RT. The reaction mixture is allowed to stir at room temperature for 16 h. The reaction mixture was added ice water followed by extraction with ethyl acetate. The ethyl acetate layer washed with brine, dried over sodium sulfate and concentrated. The residue obtained was chromatographed over silica gel (60 – 120 mesh) with 10 % ethyl acetate / hexane (yield = 12 g, 65.5%).

3 - (4-nitrophenyl) – 2 - (1- (phenylsulfonyl) - 1H -indol-3-yl) propanoic acid (11): Methyl 3-(4-nitrophenyl)-2-(1-(phenylsulfonyl)-1H-indol-3-yl) propanoate (12 g, 0.025 mol) was dissolved in 36 mL THF and added aqueous solution of potassium hydroxide (5.06 g in 100 mL water) and the mixture was allowed to stir at RT for 3 h. The reaction mixture was added ice water acidified with 3N HCl to pH~ 3 and extracted with ethyl acetate. The ethyl acetate layer washed with brine, dried over sodium sulfate and concentrated (yield = 9 g, 77.5%).

General procedure for synthesis of amides (12 a-h) N-(4-fluorophenyl)-3-(4-nitrophenyl)-2-(1-(phenylsulfonyl)-1H-indol-3-yl)propanamide (12a) : 3-(4-nitrophenyl)-2-(1-(phenylsulfonyl)-1H-indol-3-yl)propanoic acid (1.8 g, 4 mmol) was taken in dry DMF (5 mL), added 4-fluoroaniline (488 mg, 4.4 mmol), HATU (1.67 g, 4.4 mmol) followed by diisopropylethyl amine (1.54 g, 1.2 mmol) and the reaction mixture was stirred for 16 h at RT. The reaction mixture was added ice water (25 mL) and extracted with ethyl acetate. Ethyl acetate layer washed with brine solution, dried over sodium sulfate and concentrated. The residue obtained was chromatographed over silica gel (60 – 120 mesh) with 10 % ethyl acetate / hexane (yield = 1.5 g, 71.4 %).

General procedure for synthesis of amines (13 a-h) 3-(4-aminophenyl)-N-(4-fluorophenyl)-2-(1-(phenylsulfonyl)-1H-indol-3-yl)propanamide (13a): N-(4-fluorophenyl)-3-(4-nitrophenyl)-2-(1-(phenylsulfonyl)-1H-indol-3-yl)propanamide (1.5 g, 2.7 mmol) was taken in methanol (50 mL) and added 10% Pd/C (300 mg) and hydrogenated under hydrogen bladder at atmospheric pressure for 20 h. The reaction mixture is filtered through celite, concentrated and purified by column chromatography, silica, 25 % ethyl acetate / hexane (yield = 1.05 g, 74.4 %).

General procedures for synthesis of Ethyl oxamates (14a-h) Ethyl 2-((4-(3-((2,6-dimethoxyphenyl)amino)-3-oxo-2-(1-(phenylsulfonyl)-1H-indol-3-yl)propyl) phenyl) amino)-2-oxoacetate (14d): 3-(4-aminophenyl)-N-(2,4-dimethoxyphenyl)-2-(1-(phenylsulfonyl)-1H-indol-3-yl)propanamide (500 mg, 0.9 mmol) was taken in dry dichloromethane (5 mL) added N,N-dimethylpyridin-4-amine (330 mg, 2.7 mmol) followed by ethyl oxalyl chloride (245 mg, 1.8 mmol) at RT and stirred for 6 h. The reaction mixture was quenched in water; DCM layer was separated and washed with satuarted bicarbonate solution, brine, dried over sodium sulfate and concentrated. The residue obtained was purified by column chromatography using 15 % ethyl acetate / hexane (Yield = 200 mg, 33.8%).

General procedures for synthesis of oxamic acids (15a-h) 2-((4- (3 - ((2, 4 - dimethoxyphenyl) amino) -3-oxo-2-(1-(phenylsulfonyl) -1H-indol-3-yl) propyl) phenyl) amino) -2-oxoacetic acid (15d): Ethyl 2-((4-(3-((2,4-dimethoxyphenyl) amino) – 3 – oxo – 2 - (1-(phenylsulfonyl) -1H-indol-3-yl) propyl) phenyl) amino) -2-oxoacetate (200 mg, 0.30 mmol) was taken in dry methanol (3 mL) added cesium carbonate (319 mg, 0.978 mmol) and refluxed for 8 h. The reaction mixture was added ice water (25 mL) and made pH~3 by adding 3N HCl. The precipitate obtained was filtered and washed with water and dried to yield **15 d:** (yield = 50 mg, 33.5 %).

Spectral analysis of compounds synthesized Methyl 2-(1H-indol-3-yl) acetate (8): HNMR: HNMR (400 MHz, $CDCl_3$): 3.72 (s, CH_3, 3H), 3.80 (s, CH_2, 2H), 7.14-7.178 (t, ArH, 1H), 7.178 (s, ArH, 1H), 7.20-7.27 (t,ArH, 1H), 7.36-7.38 (d,ArH, 1H), 7.62-7.64 (d, ArH, 1H), 8.1 (brs, NH,1H).

Methyl 2-(1-(phenylsulfonyl)-1H-indol-3-yl) acetate (9): HNMR (400 MHz, $CDCl_3$): 3.71 (s, 5H, CH_2 and CH_3), 7.24 – 7.28 (m, ArH, 1H,), 7.31-7.36 (m, ArH , 1H), 7.41-7.45 (m, ArH, 2H), 7.49-7.55 (m, ArH, 2H), 7.59 (s, ArH, 1H), 7.88-7.90 (m, ArH, 2H), 7.98- 8.00(d, ArH, 1H).

Methyl 3-(4-nitrophenyl)-2-(1-(phenylsulfonyl)-1H-indol-3-yl)propanoate (10): **HNMR** (400MHz, $CDCl_3$): 3.24-3.29 (dd,CH_2,1H), 3.47-3.52 (dd, CH_2, 1H),3.62 (s, CH_3,3H), 4.03-4.07 (t, CH, 1H), 7.12-7.14(d, ArH,2H), 7.22-26 (t,ArH, 1H), 7.32-7.35 (t, ArH, 1H), 7.39-7.43 (m, ArH, 3H), 7.53-7.57 (m,

ArH, 2H),7.68-7.79 (m, ArH, 2H), 7.95-8.00(m, ArH, 2H), 8.20-8.22 (d, ArH, 1H).

3- (4-nitrophenyl) – 2 - (1- (phenylsulfonyl) - 1H – indol – 3 - yl)propanoic acid (11): **HNMR** (400 MHz, $CDCl_3$): 3.26-3.32 (dd,CH_2,1H), 3.48-3.54 (dd, CH_2, 1H), 4.27-4.31 (t, CH, 1H), 7.13-7.17(d, ArH,1H), 7.22-26 (m,ArH, 1H), 7.31-7.44 (t, ArH, 4H), 7.48-7.58 (m, ArH, 3H), 7.61-7.78 (m, ArH, 1H),7.85-7.88 (m, ArH, 1H), 7.93-8.00(m, ArH, 2H), 8.18-8.02 (d, ArH, 1H).

N-(4-fluorophenyl)-3-(4-nitrophenyl)-2-(1-(phenylsulfonyl)-1H-indol-3-yl)propanamide (12a) : **HNMR** (400 MHz, CDCl3): 3.25-3.32 (dd, CH_2, 1H), 3.65-3.7 (dd,CH_2,1H), 4.0-4.05 (m, CH,1H), 6.93-6.97 (m, ArH, 2H), 7.08-7.10 (m, ArH, 2H), 7.27-7.35 (m, ArH, 2H), 7.36-7.45 (m,ArH,4H), 7.56-7.58 (d, ArH, 1H), 7.63-7.65(d,ArH,1H), 7.70 (s,ArH,1H), 7.74-7.76 (d,ArH,2H), 7.78-7.91 (m,ArH,2H), 8.02-8.04 (d,ArH,2H).

3-(4-aminophenyl)-N-(4-fluorophenyl)-2-(1-(phenylsulfonyl) – 1 H-indol-3-yl)propanamide (13a): HNMR (400 Mhz, DMSO-d6): 2.95-3.0 (dd, CH_2, 1H), 3.2-3.25 (dd, CH_2, 1H), 4.08-4.11(t, CH, 1H), 4.85 (Br s, NH2, 2H), 6.38-6.4 (d, ArH, 2H), 6.84-6.86 (d, ArH, 2H), 7.08 – 7.12 (t, ArH, 2H), 7.22-7.34 (m,ArH, 2H), 7.5 – 7.58 (m, ArH, 4H), 7.64-7.68(m, ArH, 2H), 7.72-7.75(d, ArH, 1H), 7.84-7.88(m, ArH, 3H),10.16 (s, NH, 1H).

Ethyl 2-((4-(3-((2,4-dimethoxyphenyl)amino)-3-oxo-2-(1-(phenylsulfonyl)-1H-indol-3-yl)propyl) phenyl) amino)-2-oxoacetate (14d): HNMR (400 MHz, DMSO-d6), 1.30 (t, CH_3, 3H), 3.15-3.17 (dd, CH_2, 1H), 3.3 – 3.35 (dd, CH_2,1H), 3.65 (s, OCH3, 3H), 3.66 (s, OCH3, 3H), 4.28 (q, CH2,2H), 4.59 (t, CH, 1H), 6.58-6.61 (dd, ArH, 1H), 6.88-6.91 (d, ArH,1H), 7.2-7.34 (m, ArH, 4H), 7.52-7.58 (m, ArH, 5 H), 7.65 (t, ArH, 1H), 7.73 (s,ArH,1H), 7.81-7.88(m, ArH, 4H), 9.35 (brS, NH,1H),10.7 (BrS,NH,1H).

2-((4-(3-((4-fluorophenyl) amino) – 2 - (1H-indol-3-yl) – 3 - oxopropyl) phenyl)amino) – 2 - oxoacetic acid (15a): (yield=38.5 %). MP = 151 – 153°C; IR (KBr, cm^{-1}): 1743.6, 1689.6, 1529.5, 1408.0, 1213.2, 833.2, 744.5; HNMR(400 MHz, DMSO): 3.05 (dd, CH_2, 1H), 3.3 (dd, CH_2,1zH),4.2 (t, CH,1H), 6.55 (m, ArH, 2H), 7.0 (t, ArH,1H), 7.2 (m, ArH, 4H), 7.2-7.35(m, ArH, 4 H), 7.5-7.65 (m, ArH, 4H), 7.8 (d,ArH,1H), 10.03 (brS, NH,1H),10.6 (BrS,NH,1H),10.95 (BrS,NH,1H); 13CNMR:37.78 46.03, 111.23, 113.24, 114.67, 114.96, 118.23, 118.70, 119.95, 120.78, 122.77, 126.14, 128.76, 135.33, 136.03,136.30, 136.44, 136.49, 161.87, 171.34; m/z (M-1) = 444.1.

2-((4-(2-(1H-indol-3-yl)-3-((3-methoxy-5-trifluoromethyl) phenyl) amino) -3-oxopropyl)phenyl)amino)-2-oxoacetic acid (15b): (Yield=33 %). MP = 173 – 175°C; IR (KBr, cm^{-1}): 1743.6, 1687.6, 1664.5, 1608.6, 1537.2, 1355.9, 1174.6, 1126.4, 1053.1, 856.3, 817.8, 746.4; HNMR(400 MHz, DMSO): 3.05-3.10 (dd, CH_2, 1H), 3.42-3.45 (dd, CH_2,1H),3.76 (s, CH_3, 3H), 4.18-4.20 (m, CH,1H), 6.86 (m, ArH, 1H), 6.97-7.06 (m, ArH,2H), 7.21-7.23 (m, ArH, 2H), 7.28 (d, ArH, 1 H),7.33-7.35(d,ArH,1H), 7.39 (s, ArH, 1H), 7.56 (s,ArH,1H),7.61-7.63 (d,ArH, 2H), 7.54-7.77(d,ArH, 1H), 10.3 (brS, NH,1H),10.6 (BrS,NH,1H),10.99 (BrS,NH,1H); 13CNMR: 38.1, 46.45, 55.51,105.2,107.75, 107.93, 111.49, 112.89, 118.53, 118.83, 120.02, 121.03, 123.14, 126.16, 128.9, 135.76, 136.11, 141.11, 159.89, 162.13, 172.27; m/z (M+1) = 525.8.

2-((4-(3-((2,5-dimethoxyphenyl)amino)-2-(1H-indol-3-yl)-3-oxopropyl)phenyl)amino)-2-oxoacetic acid (15c): (Yield = 38.5 %). MP = 146 – 148°C; IR (KBr, cm^{-1}): 1741.7, 1691.5, 1597.0, 1531.5, 1483.2, 1427.1, 1219.0, 1043.0, 817.8, 744.5; HNMR(400 MHz, DMSO): 3.05 – 3.09 (dd, CH_2, 1H), 3.36-3.42 (dd, CH_2, 1H), 3.67 (s, CH_3, 3H), 3.69 (s, CH_3, 3H), 4.4 (t, CH,1H), 6.40-6.42 (m, ArH, 1H), 6.52 (d, ArH,1H), 6.97-7.08 (m, ArH, 3H), 7.23-7.34(m, ArH, 5 H), 7.59-7.63 (m, ArH, 4H), 7.79-7.81 (d,ArH,1H), 8.95 (brS, NH,1H),10.6 (BrS,NH,1H),10.95 (BrS,NH,1H); 13CNMR: 37.95, 45.79, 55.69, 56.65, 107.95, 108.13, 111.87, 112.33, 113.60, 118.88, 119.61, 120.41, 121.48, 123.72, 126.78, 128.65, 120.54, 136.02, 136.58, 136.89, 140.53, 153.30, 156.98, 162.59, 172.38; m/z (M+1) = 487.8.

2 - ((4- (3- ((2, 4 - dimethoxyphenyl) amino) -3-oxo-2-(1-(phenylsulfonyl) - 1H – indol – 3 - yl)propyl)phenyl)amino)-2-oxoacetic acid (15d): MP = 133 – 135°C; IR (KBr, cm^{-1}): 1681.9, 1529.5, 1462.0, 1413.8, 1209.3, 1159.2, 1126.4, 833.2, 769.6, 742.59; HNMR (400 MHz, DMSO): 3.06 – 3.14 (dd, CH_2, 1H), 3.38-3.42 (dd, CH_2,1H),3.61 (s, CH_3, 3H), 3.64 (s, CH_3, 3H), 4.53 (t, CH,1H), 6.53-6.56 (m, ArH, 1H), 6.84-6.86 (d, ArH,1H), 6.64-7.06 (m, ArH, 2H), 7.23-7.35(m, ArH, 4 H), 7.60-7.62 (m, ArH, 2H), 7.65-7.66 (d,ArH,1H), 7.78-7.80(d, ArH, 1H), 9.0 (brS, NH,1H),10.6 (BrS,NH,1H),10.95 (BrS,NH,1H); 13CNMR: 37.75, 45.25, 55.22, 55.60, 98.75, 103.97, 111.34, 113.54, 118.32, 119.20, 119.96, 120.46, 120.94, 123.11, 126.40, 129.07, 135.53, 136.13, 136.55, 151.11, 156.53, 156.66, 162.14, 171.46; m/z (M+1) = 487.8.

2- ((4- (2- (1H- indol – 3 - yl) – 3 – oxo – 3 - (pyridin-3-ylamino) propyl) phenyl) amino)- 2-oxoacetic acid (15e): (Yield = 23 %). MP = 215 – 217°C; IR (KBr, cm^{-1}): 1681.9, 1527.6, 1460.1, 1381.0, 1309.6, 1188.1, 802.3, 746.4; HNMR(400 MHz, DMSO): 3.08 – 3.13 (dd, CH_2, 1H), 3.40-3.46 (dd, CH_2,1H), 4.23 – 4.26 (m, CH,1H), 6.98-7.09 (m, ArH, 2H), 7.23-7.25 (d, ArH,2H), 7.29-7.39 (m, ArH, 3H), 7.61-7.63 (d, ArH, 2 H), 7.77-7.79 (d, ArH, 1H), 8.02-8.04 (d,ArH,1H), 8.25 (s, ArH, 1H), 8.74(s, ArH, 1H), 10.34 (brS, NH,1H),10.62 (BrS,NH,1H),11.0 (BrS,NH,1H); 13CNMR: 37.76, 46.29, 11.57, 112.94, 118.60, 118.91, 120.16, 121.11, 123.22, 124.14, 126.23, 126.15, 129.08, 135.76, 136.16, 136.28, 139.59, 143.13, 156.72, 162.17, 172.41; m/z (M+1) = 428.8.

2-((4-(3-(cyclohexylamino)-2-(1H-indol-3-yl)-3-oxopropyl) phenyl) amino)-2-oxoacetic acid (15f): (Yield = 44 %). MP =

149 – 151°C; IR (KBr, cm^{-1}): 1691.5, 1535.3, 1450.4, 1348.2, 1172.7, 1095.5, 821.6, 742.5; HNMR(400 MHz, DMSO): 0.93-0.98 (m, CH_2, 2H), 1.03-1.23(m,CH_2, 4H), 1.46-1.58(m, CH_2, 4H), 2.94-2.99 (dd, CH_2, 1H), 3.28-3.31 (dd, CH_2,1H), 3.42 – 3.45 (m, CH,1H),3.92-3.96(m,CH,1H), 6.94-6.97 (t, ArH, 1H), 7.03-7.06 (t, ArH,1H), 7.19-7.20 (m, ArH, 3H), 7.31-7.33 (d, ArH, 1 H), 7.61-7.63 (d, ArH, 2H), 7.69-7.71(d,ArH,1H),7.76-7.78(d,ArH,1H), 10.62 (BrS, NH, 1H),10.85 (BrS,NH,1H); 13CNMR: 24.46, 25.18, 25.46, 32.22, 32.37, 38.05, 45.10, 47.31, 111.29, 114.18, 118.19, 118.98, 119.96, 120.84, 122.58, 126.47, 129.04, 135.53, 136.04, 136.69, 156.62, 162.18, 171.50; m/z (M+1) = 433.9.

2 - ((4- (3- (benzylamino) – 2 - (1H-indol-3-yl)-3-oxopropyl) phenyl) amino) – 2 -oxoacetic acid (15g): (Yield = 35 %). MP = 201 – 203°C; IR (KBr, cm^{-1}): 1658.7,1529.5, 1454.3, 1369.4,823.6, 742.5; HNMR(400 MHz, DMSO): 2.97-3.20 (dd, CH_2, 1H), 3.36-3.37 (dd, CH_2,1H), 3.97 – 4.02 (m, CH_2,1H),4.07-4.08(m,CH_2,1H), 4.27-4.32(m,CH,1H), 6.86-6.87 (m, ArH, 2H), 6.94-6.97 (t, ArH,1H), 7.03-7.07 (m, ArH, 1H), 7.12-7.24 (m, ArH, 6 H), 7.32-7.34 (d, ArH, 1H), 7.70-7.75(m,ArH,3H),8.42(m,NH,1H), 10.35 (BrS,NH,1H),10.9 (BrS,NH,1H); 13CNMR: 38.04, 41.84, 45.28, 111.29, 113.98, 118.23, 119.01, 119.29, 120.87, 122.70, 125.32, 126.41, 126.80, 127.94, 129.09, 135.88, 136.00, 136.7, 139.24, 156.5, 162.28, 172.58; m/z (M+1) = 441.8

2- ((4- (2- (1H- indol - 3 - yl) - 3- oxo - 3- (phenethylamino) propyl) phenyl) amino)-2-oxoacetic acid (15h): (Yield = 38 %). MP = 214 – 215°C; IR (KBr, cm^{-1}): 1691.5, 1525.5, 1452.4,1342.4, 12444.09, 817.8, 742.5; HNMR(400 MHz, DMSO): 2.96-3.01 (dd, CH_2, 1H),3.096-3.12(m, CH_2,1H), 3.16-3.21 (m, CH_2,1H), 3.28-3.33(m,CH_2,1H), 3.72-3.75 (m,CH2,2H), 3.94-3.98(m,CH,1H), 6.95-6.98 (m, ArH, 3H), 7.05(t, ArH,1H), 7.14-7.20 (m, ArH, 6H), 7.32-7.34 (d, ArH, 1 H), 7.62-7.64 (m,ArH,2H), 7.68-7.70 (d, ArH, 1H), 7.96-7.99(t,NH,1H), 10.61 (BrS,NH,1H),10.87 (BrS,NH,1H); 13CNMR: 35.14, 37.99, 40.41, 45.30, 111.41, 113.90, 118.38, 119.13, 120.06, 120.99, 122.92, 125.97, 126.53, 128.24, 128.65, 129.15, 135.70, 136.13, 136.73, 139.52, 157.05, 162.32, 172.78; m/z (M+1) = 455.9

Results and Discussion

Molecular modeling study: Based on compound 6, figure 1, several designs were proposed and prioritized. Molecular docking was carried out in PTP1B (PDB ID: 2VEU) using GOLD software (version 5.1). Docking model of compound 15a is shown in figure 2. Site-A, amino-2-oxoacetic acid of compound 15a occupies pTyr binding site. It interacts with residues Gly220, Arg221, Asp181, Phe182 of PTP-loop. The *para* fluoro phenyl group is extended towards secondary site (Site-B) with additional interactions with residue Asp-48 through amide NH.

Enzymatic assay: Para-nitro phenyl phosphate (pNPP) is a chromogenic substrate for most of the phosphatases which upon hydrolyzed to p-Nitro phenol and phosphate by protein phosphatase. This end product under alkaline conditions is intense yellowish color monitored at 405nm using spectrophotometer.

All assays were carried out with buffer having 25mM Tris, pH:7.5, 75 mM NaCl,1mM DTT and 0.1% BSA. The IC_{50} values of all the compounds were determined and the Ki values were deduced[12] and shown in below[13] table 1.

Table–1
Structures of compound 15 and Ki values for PTP1B and TCPTP

Entry	R	PTP1B Ki (µM)	TCPTP Ki (µM)
15a	4-F Ph	52.5	10
15b	3-CF3, 5-OMe Ph	83	31
15c	2,5-Di-OMe Ph	220	115.5
15d	2,4-Di-OMe Ph	140	39
15e	3-Py	182	80.5
15f	Cyclohexyl	230	103
15g	Ch2 Ph	91	24
15h	CH2,CH2 Ph	55	28

Conclusion

Based on computational docking studies we designed novel indole-3-acetamide derivatives of phenyl oxamic acids which target, site A and site B of PTP1B. Selected 8 compounds were synthesized and screened by in-vitro assay method. These molecules have shown PTP1B inhibitory activity and Ki values between ranges between 52 – 210 µM. Further studies to improve the activity selectivity towards the PTP1B are under progress.

Acknowledgement

I am thankful to Aurigene Discovery Technologies, Bangalore for funding and facilitating this research.

References

1. Andres Alonso, Joanna Sasin, Nunzio Bottini, Ilan Friedberg, Iddo Friedberg, Andrei Osterman, Adam Godzik, Tony Hunter, Jack Dixon and Tomas Mustelin, Protein Tyrosine Phosphatases in the Human Genome., *Cell.,* **117,** 699-711 **(2004)**
2. Katherine R. Schiller and Laura J. Mauro, Tyrosine phosphatases as regulators of skeletal development and metabolism, *J. Cell Biochem*. **96,** 262-277 **(2005)**
3. Tony Hunter, Signaling—2000 and Beyond**,** *Cell*, **100**, 113-127 **(2000)**
4. Jacqueline Montalibet and Brian P. Kennedy, Therapeutic strategies for targeting PTP1B in diabetes, *Drug Discovery Today Therap. Strateg.,* **2,** 129-135 **(2005)**

5. Mounib Elchebly, Paul Payette, Eva Michaliszyn, Wanda Cromlish, Susan Collins, Ailsa Lee Loy, Denis Normandin, Alan Cheng, Jean Himms-Hagen, Chi-Chung Chan, Chidambaram Ramachandran, Michael J. Gresser, Michel L. Tremblay, and Brian P. Kennedy., Increased Insulin Sensitivity and Obesity Resistance in Mice Lacking the Protein Tyrosine Phosphatase-1B Gene., *Science.,* **283,** 1544-1548 **(1999)**

6. Lori D. Klaman, Olivier Boss, Odile D. Peroni, Jason K. Kim, Jennifer L. Martino, Janice M. Zabolotny, Nadeem Moghal, Margaret Lubkin, Young-Bum Kim, Arlene H. Sharpe, Alain Stricker-Krongrad, Gerald I. Shulman, Benjamin G. Neel, and Barbara B. Kahn., Increased Energy Expenditure, Decreased Adiposity, and Tissue-Specific Insulin Sensitivity in Protein-Tyrosine Phosphatase 1B-Deficient Mice., *Mol. Cell Biol*. **20,** 5479-5489 **(2000)**

7. Gang Liu, Zhili Xin, Heng Liang, Cele Abad-Zapatero, Philip J. Hajduk, David A. Janowick, Bruce G. Szczepankiewicz, Zhonghua Pei, Charles W. Hutchins, Stephen J. Ballaron, Michael A. Stashko,Thomas H. Lubben, Cathy E. Berg, Cristina M. Rondinone, James M. Trevillyan, and Michael R. Jirousek., Selective Protein Tyrosine Phosphatase 1B Inhibitors: Targeting the Second Phosphotyrosine Binding Site with Non-Carboxylic Acid-Containing Ligands., *J. Med. Chem.*, **46**, 3437-3440 **(2003)**

8. Zhili Xin, Gang Liu, Cele Abad-Zapatero, Zhonghua Pei, Bruce G. Szczepankiewicz, Xiaofeng Li, Tianyuan Zhang, Charles W. Hutchins,Philip J. Hajduk, Stephen J. Ballaron, Michael A. Stashko, Thomas H. Lubben,James M. Trevillyana and Michael R. Jirousek.., Identification of a monoacid-Based, cell permeable, selective inhibitor of protein tyrosine phosphatase 1B., *Bioorganic and Medicinal Chemistry Letters.,* **13,** 3947–3950 **(2003)**

9. Matthew E. Welsch, Scott A. Snyder, and Brent R Stockwell., Privileged Scaffolds for Library Design and Drug Discovery., *Curr Opin Chem Biol.* **14(3)**, 347–361 **(2010)**

10. Fernando Rodrigues de Sa Alves, Eliezer J. Barreiro and Carlos Alberto Manssour Fraga., From Nature to Drug Discovery: The Indole Scaffold as a 'Privileged Structure, *Mini Reviews in Medicinal Chemistry;* **9(7)**, 782-793 **(2009)**

11. Prajapati Ajaypal., Synthesis, Antimicrobial and Insecticidal Activity Studies of 5-Nitro N'-[Arylidenhydrazidomethyl Indole] 2-(Substituted Aryl)-3-(N'-Indolyl Acetamiddyl)-4-Oxothiazolidines., *Research Journal of Recent Sciences,* **1,** 99-104 **(2012)**

12. Douglas P. Wilson, Zhao-Kui Wan, Wei-Xin Xu, Steven J. Kirincich, Bruce C. Follows, Diane Joseph-McCarthy, Kenneth Foreman, Alessandro Moretto, Junjun Wu, Min Zhu, Eva Binnun, Yan-Ling Zhang,| May Tam, David V. Erbe, James Tobin, Xin Xu, Louis Leung, Adam Shilling, Steve Y. Tam, Tarek S. Mansour, and Jinbo Lee.; Structure-Based Optimization of Protein Tyrosine Phosphatase 1B Inhibitors: From the Active Site to the Second Phosphotyrosine Binding Site., *J. Med. Chem.,* **50**, 4681 **(2007)**

13. M.V.V.V.Sekhar Reddy, G.Chakshusmathi, P.C.Arun, M. Lakshmi Narasu.; Developing selective inhibitors of PTP1B over TCPTP., *Journal of Global Trends in Pharmaceutical Sciences,* **3(1)**, 558-563, **(2012)**

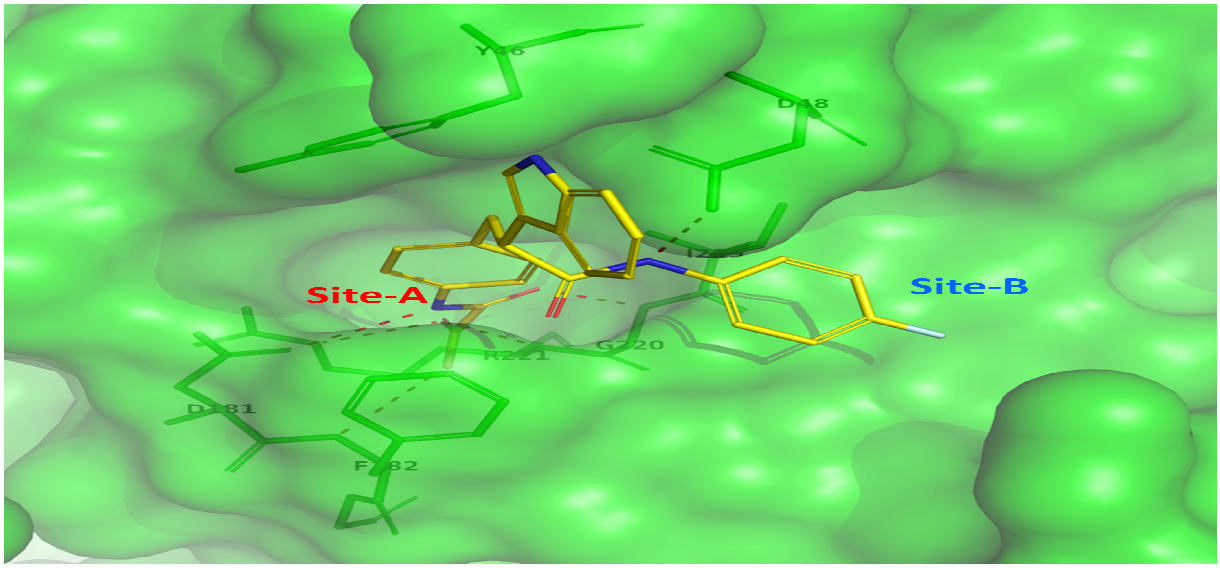


Figure-2
Docking model of 15a shown in PTP1B X-ray structure

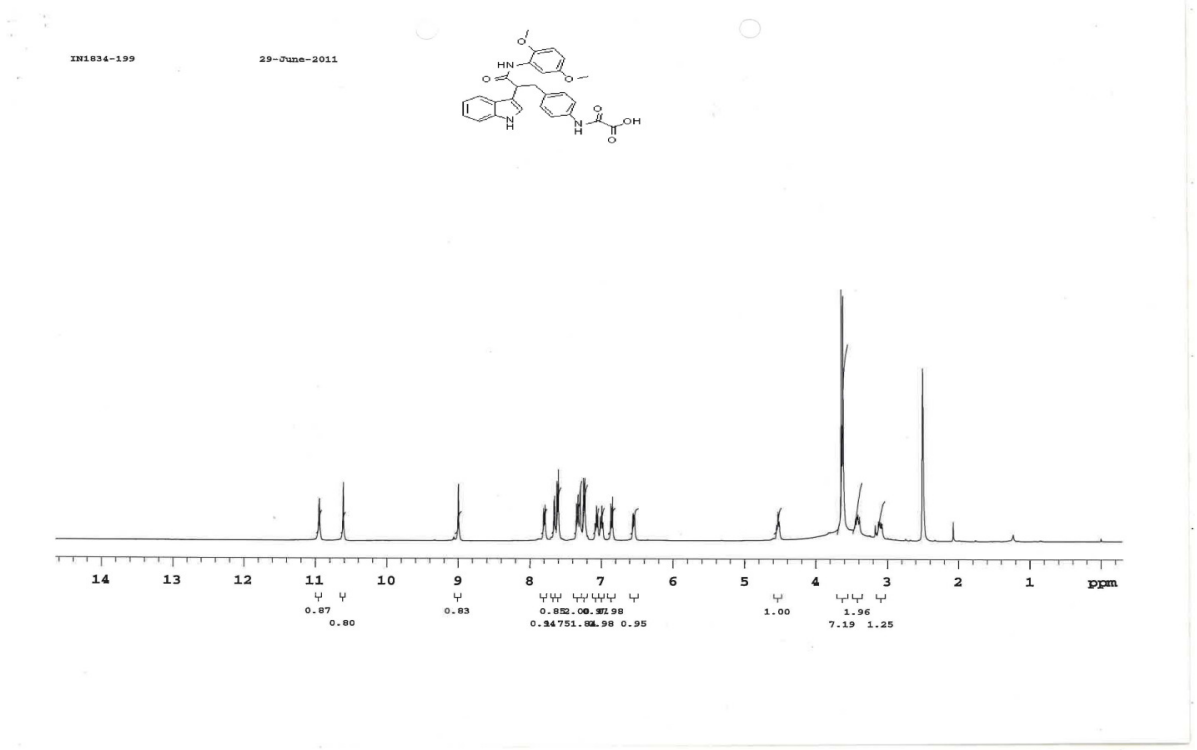


Figure-3
HNMR spectra for the compound 15c

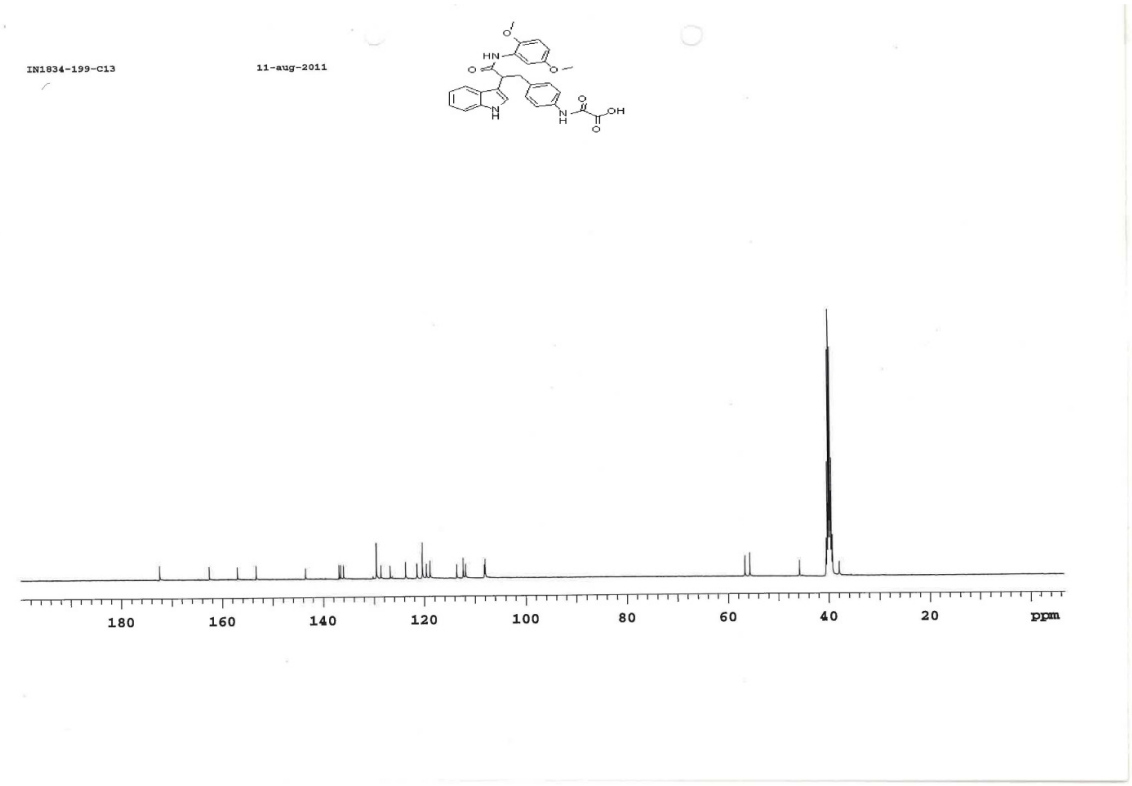
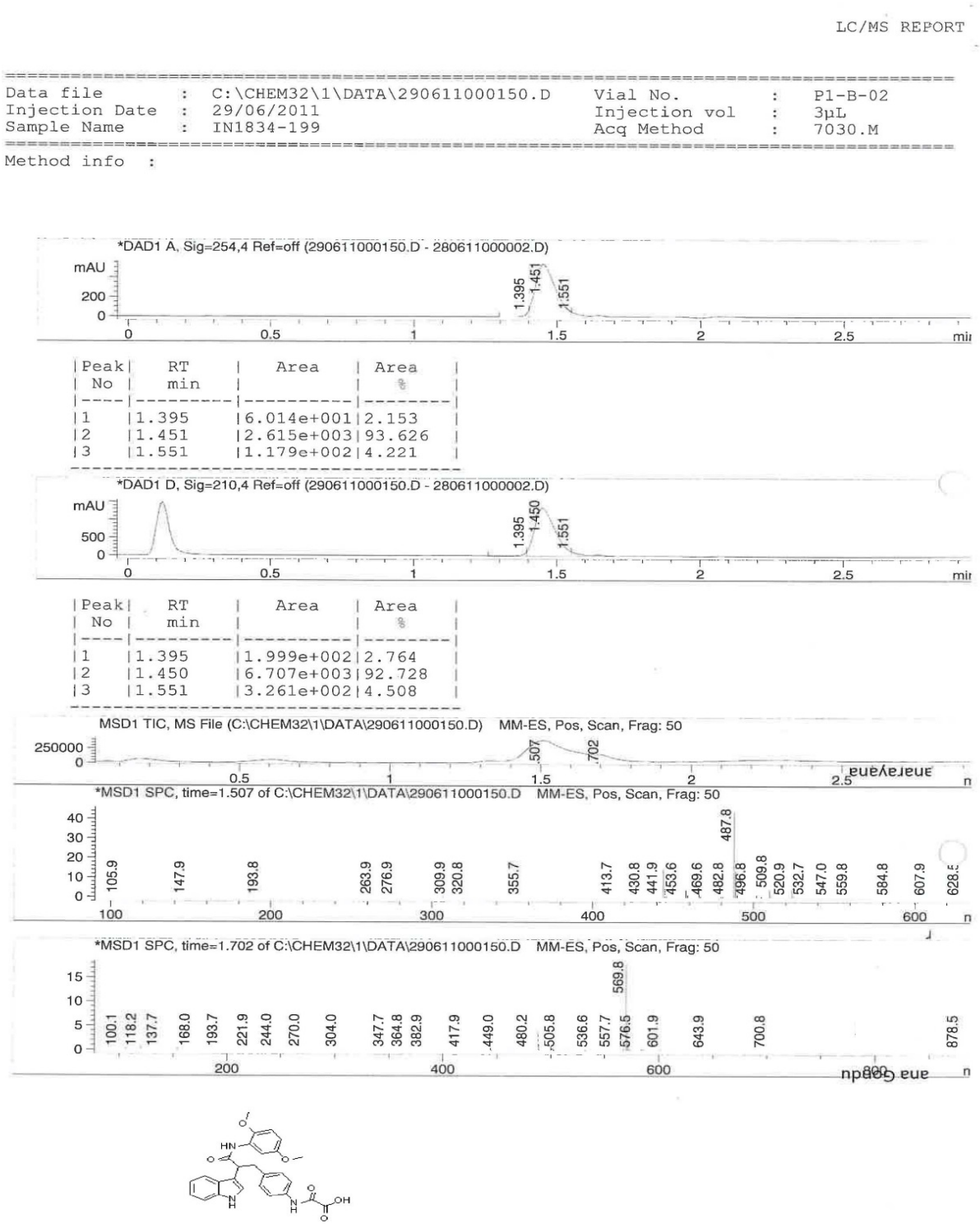
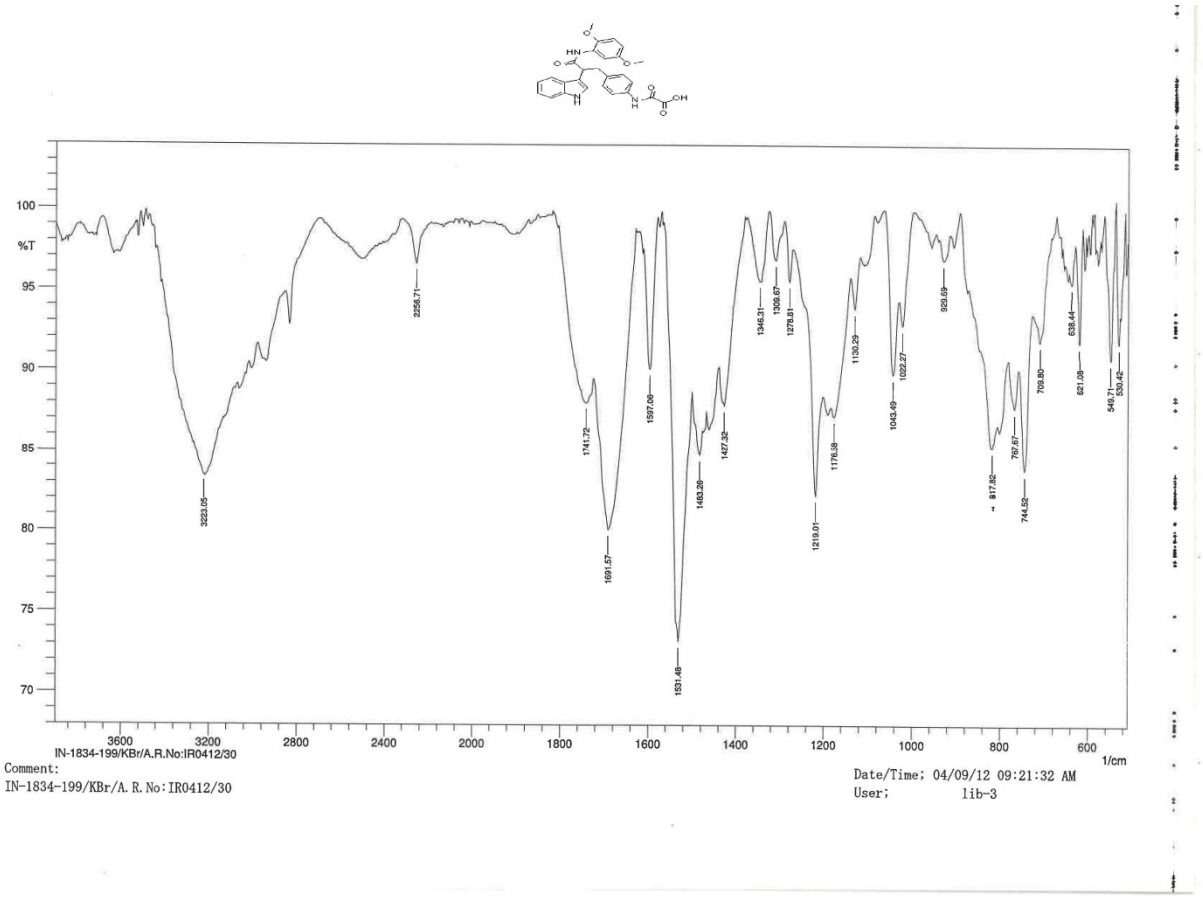


Figure-4
13CNMR spectra for the compound 15c



Figure–5
LCMS spectra for the compound 15c



Figure–6
FTIR spectra for the compound 15c