Design, Synthesis and In Vitro Biological Evaluation Of N-(2-Aminophenyl)-3-Quinolin-4-yl -Prop-2-enamide Derivatives As Novel Colon Anticancer Agents

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Abstract: Acetylation and deacetylation of histone proteins is regulated through two enzymes; acetylation is mediated through histone acetyl transferase (HAT) whereas deacetylation is mediated through histone deacetylase (HDAC). Histone deacetylase removes acetyl group of lysine residue on histone proteins this makes negatively charged histone able to bind with DNA containing positive centers. In this way DNA remain in compact form with histone proteins. Therefore the transcription or replication enzymes and cofactors not able to bind such compact DNA structure. Histone deacetylase inhibition results into inhibition of various transcription activities which may get accelerated during cancer development. The aim of proposed study is to develop effective isoform selective Histone deacetylase inhibitor. Synthesis of N-(2-Aminophenyl)-3-Quinolin-4-y1-Prop-2-Enamide derivatives from isatin and its derivatives was achieved. Synthesis was carried out in two steps with good yield. 21 Compounds were synthesized and found to be effective with IC₅₀ values in between 3.694 - 38.4µmol in human HCT-116, COLO 205 and COLO 320 DM colon cancer cells in vitro. For the cytotoxicity activity study MTT assay method was adopted. Docking studies were performed initially with HDAC8 enzyme using V-life MDS software for synthesized compounds and had selected best fit molecules for synthesis. The synthesized molecules are effective as colon anticancer agents.

Keywords: HDAC, Colon Cancer, MTT assay, Docking.
In protein biosynthesis acetylation is one type of post translational modification. For histone proteins the acetylation and deacetylation is regulated by two functionally opposite enzymes histone deacetylase (HDAC) a zinc dependent metalloenzyme and Histone acetyl transferase (HAT). HDACs catalyses deacetylation of lysine residues at N-terminal of histone proteins. The deacetylation of histone proteins increases positive charge on the N-terminal of histone protein. The positively charged DNA interact, leading to tight histone-DNA binding, which limits the access of transcription factors and finally leads to transcriptional gene silencing. On other hand HAT causes acetylation of amino group of lysine residues present on N-termini of core histones. The acetylated neutral lysine doesn’t have any interaction with negatively charged DNA. This results into histone-DNA loose binding that is more relaxed chromatin state and gene-transcription activation. In addition histone acetylation has been associated with other genome functions such as chromatin assembly, DNA repair and recombination.

Histone acetylation and deacetylation play significant role in the regulation of proliferation, cell differentiation, apoptosis and many other biological processes. Silencing of tumor suppressor genes associated with increased histone deacetylase which results into various cancers. In certain cancer overexpression of specific isoform of histone deacetylase is observed. In recent years HDAC inhibitors were developed and are in clinical testing against different cancers. US Food and Drug Administration (FDA) approved SAHA (ievorinostat) in 2006 for treatment of cutaneous T-cell lymphoma (CTCL). HDAC is metalo-enzyme which contains Zn⁺ in its catalytic site. Therefore classic inhibitors designed were having zinc binding group and cap group connected through hydrophobic linker. Some of the hydroxamic acid derivatives such as Panobinostat, Belinostat, ITF2357 are in phase-II development. Several other inhibitor benzamidem (MS-275, MGCD0103), cyclic peptide (Romedepsin), valproic acid and butyrate are also in active development.

Very few literatures have reported selective HDAC8 inhibitors such as Alex A Tabackman and coworkers have conformed the formation of isoform-specific subpocket in HDAC 8 to bind linkerless hydroxamic acid derivatives. Keris Krenn Hrubee and coworkers proposed subpocket formation in HDAC8. Here we have designed isoform selective HDAC inhibitor. We have designed and synthesized 21 molecules as selective HDAC8 inhibitor.

**Experimental:**

**Synthesis of compounds:**

**Step 1- Synthesis of quinoline 4-carboxylic acid derivatives**: Potassium hydroxide (0.01mol) solution was prepared in absolute ethanol:distilled water (4:1) to this solution isatin (0.01mol) was added, resulting reaction mixture was refluxed for 1 hr. Then appropriate ketone was added to the mixture and continued refluxing. After refluxing solution was poured on crush-ice and conc.HCl was added till pH became between 1-2. The excess of solvent were evaporated until the solid brown precipitate appeared. The crude quinoline 4-carboxylic acid derivative was recrystallized from ethanol.

**Step 2-Synthesis of quinoline 4-aldehyde derivatives**: Synthesized quinoline-4-carboxylic acid derivatives were reduced to respective aldehyde using lithium tri-tert-butoxyaluminum hydride in presence of tetrahydrofuran.

**Step 3-Synthesis of (2E)-3-(quinolin-4-yl) prop-2-enio acid derivatives**: Triethylphosphonaacetate was treated with methyl magnesium bromide at room temperature. Then to the reaction mixture above aldehyde were added and refluxed for about 2.5 hr.

**Step 4-Synthesis of n-(2-aminophenyl)-3-quinolin-4-yl prop-2-enamide derivatives**: 1, 2diphenylamine was treated with Boc2O and DMAP in tetra hydro furan (THF). Then this resulting product was treated with carboxylic acid which was synthesized in above step. After amidation product was treated by trifluoroacetic acid in DCM for removal of protecting group.
Preparation of Protein and Ligands for Molecular Docking Studies

Trichostatin A complexed in human HDAC8 having PDB code 1T64 was downloaded from the Protein Data Bank (PDB) and was used for validation of the docking protocol. All water molecules and metals were deleted except zinc metal and hydrogens were added corresponding to pH 6.8. The protein was prepared using the VLifeMDS®-4.6, force field to obtain a minimized structure of protein. Proposed inhibitor structures were first energy-minimized and converted to .pdb files using Chem3D Pro 12.0 and ChemBioDraw Ultra 12.0 (CambridgeSoft). The binding cavity for catalytic site was defined as a sphere of 10 Å radiuses around the ligand Trichostatin A.

Docking studies using VLife MDS

The docking studies were carried out using VLifeMDS®-4.6.

In the docking runs the ligands dihedral angles, ring flipping and geometries of the ligand, hydroxyl (OH) and amino (NH2) group’s dihedral angles, hydrogen bonds mappings between enzymes and ligand were allowed to change. All enlisted variables were randomized at start of a docking run. Best fit 21 molecules were selected for synthesis.

Anticancer activity study:

For determination of anticancer activity of synthesized molecules cell lines HCT-116, COLO 205 and COLO 320 DM were procured from National center for cell science (NCCS), Pune. For cytotoxicity study MTT assay was applied. MTT assay was performed in triplicates. The cell cytotoxicity assay observations were analyzed for IC50 determination using Graph Pad Prism 8.2 software.

Result:

i. \((2E)\)-N-(2-aminophenyl)-3-(2-methylquinolin-4-yl)prop-2-enamide (II1a): C-N(1425), NH(1671), C=O(1662), Ar-H(3050), Ar-C-H (2876), O-H (3330), C=C(970), 7.5(4H), 7.1(2H), 7.2(1H), 6.8 (1H), 4.7(2H), 6.5(1H), 7.8(2H) 1.2(3H), 9.2(1H) 304.19 C(75.27%) H(5.62%) N(13.86%) O(4.90%)

ii. \((2E)\)-N-(2-aminophenyl)-3-(2,3-dimethylquinolin-4-yl)prop-2-enamide (II1b): C-N(1425), NH(1671), C=O(1662), Ar-H(3050), Ar-C-H (2876), O-H (3330), C=C(970), 7.5(4H), 7.1(2H), 6.8 (1H), 4.7(2H), 6.5(1H), 7.8(2H) 1.2(6H), 9.2(1H) 318.17 C(75.71%) H(6.04%) N(13.27%) O(5.05%)

iii. \((2E)\)-N-(2-aminophenyl)-3-(3-ethyl-2-methylquinolin-4-yl)prop-2-enamide (III1c): C-N(1425), NH(1671), C=O(1662), Ar-H(3050), Ar-C-H (2876), O-H (3330), C=C(970), (1670), 7.5(4H), 7.1(2H), 6.8 (1H), 4.7(2H), 6.5(1H), 7.8(2H) 1.2(6H), 1.6(2H), 9.2(1H) 332.18 C(76.16%) H(6.36%) N(12.71%) O(4.90%)

iv. \((2E)\)-N-(2-aminophenyl)-3-(3-ethyl-3-methylquinolin-4-yl)prop-2-enamide (III1d): C-N(1425), NH(1671), C=O(1662), Ar-H(3050), Ar-C-H (2876), O-H (3330), C=C(970), (1670), 7.5(4H), 7.1(2H), 6.8 (1H), 4.7(2H), 6.5(1H), 7.8(2H) 1.2(6H), 1.6(2H), 9.2(1H) [M+H]+: 332.18 C(76.16%) H(6.36%) N(12.71%) O(4.90%)

v. \((2E)\)-N-(2-aminophenyl)-3-(2-phenylquinolin-4-yl)prop-2-enamide (III1e): C-N(1425), NH(1671), C=O(1662), Ar-H(3050), O-H (3330), C=C(970), (1670), 7.5(9H), 7.1(2H), 7.2 (1H), 6.8(1H), 4.7(2H), 6.5(1H), 7.8(2H), 9.2(1H) 366.16 C(78.91%) H(5.21%) N(11.45%) O(4.43%)

vi. \((2E)\)-N-(2-aminophenyl)-3-(3-ethyl-2-phenylquinolin-4-yl)prop-2-enamide (III1f): C-N(1425), NH(1671), C=O(1662), Ar-H(3050), Ar-C-H (2876), O-H (3330), C=C(970), (1670), 7.5(9H), 7.1(2H), 6.8(1H), 4.7(2H), 6.5(1H), 7.8(2H), 1.2(3H), 9.2(1H) 380.19 C(79.23%) H(5.53%) N(11.01%) O(4.23%)

vii. \((2E)\)-N-(2-aminophenyl)-3-(3-ethyl-2-phenylquinolin-4-yl)prop-2-enamide (II2g): C-N(1425), NH(1671), C=O(1662), Ar-H(3050), Ar-C-H (2876), O-H (3330), C=C(970), (1670) 366.160 7.5(9H), 7.1(2H), 6.8(1H), 4.7(2H), 6.5(1H), 1.2(3H), 1.6(2H), 7.8(2H), 9.2(1H) 366.19 C(79.38%) H(5.87%) N(10.70%) O(4.68%)

viii. \((2E)\)-N-(2-aminophenyl)-3-(6-chloro-2-methylquinolin-4-yl)prop-2-enamide (II2a): C-N(1425), NH(1671), C=O(1663), Ar-H(3050), Ar-C-H (2876), O-H (3332), C-Cl (780) C=C(970), (1670)
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7.3(3H),7.1(2H), 7.2(1H), 6.8 (1H), 4.7(2H), 6.5(1H), 7.7(2H) 1.2(3H), 9.2(1H) 338.18 C(67.66%) 
H(4.73%) Cl(10.46%) N(12.39%) O(4.76%)

ix. (2E)-N-(2-aminophenyl)-3-(6-chloro-2,3-dimethylquinolin-4-yl)prop-2-enamide(II2b): C-N(1425), NH(1671),C=O(1663), Ar-H(3050),Ar-C-H (2876), O-H (3332), C-Cl (780) C=C(970), (1670)
7.3(3H),7.1(2H), 6.8 (1H), 4.7(2H), 6.5(1H), 7.7(2H) 1.2(6H), .9.2(1H), 352.18 C(68.26%) H(5.15%)
Cl(10.15%) N(11.92%) O(4.57%)

x. (2E)-N-(2-aminophenyl)-3-(6-chloro-3-ethyl-2-methylquinolin-4-yl)prop-2-enamide(II2c): C-N(1425), 
NH(1671),C=O(1663), Ar-H(3050),Ar-C-H (2876), O-H (3332), C-Cl (780) C=C(970), (1670),
7.3(3H),7.1(2H), 6.8 (1H), 4.7(2H), 6.5(1H), 7.7(2H) 1.2(6H),1.6(2H),9.2(1H) 366.15 C(69.01%)
H(5.53%) Cl(9.66%) N(11.47%) O(4.34%)

xi. (2E)-N-(2-aminophenyl)-3-(6-chloro-2-ethyl-3-methylquinolin-4-yl)prop-2-enamide(II2d): C-N(1425), 
NH(1671),C=O(1663), Ar-H(3050),Ar-C-H (2876), O-H (3332), C-Cl (780) C=C(970), (1670),
7.3(3H),7.1(2H), 6.8 (1H), 4.7(2H), 6.5(1H), 7.7(2H) 1.2(6H),1.6(2H),9.2(1H) 366.16 C(68.97%)
H(5.48%) Cl(9.71%) N(11.51%) O(4.39%)

xii. (2E)-N-(2-aminophenyl)-3-(6-chloro-2-phenylquinolin-4-yl)prop-2-enamide(II2e): C-N(1425), 
NH(1671),C=O(1663), Ar-H(3050), O-H (3332), C-Cl (780) C=C(970), (1670), 400.18 C(72.05%)
H(4.56%) Cl(8.85%) N(10.46%) O(4.05%)

xiii. (2E)-N-(2-aminophenyl)-3-(6-chloro-3-methyl-2-phenylquinolin-4-yl)prop-2-enamide(II2f): C- 
N(1425), NH(1671),C=O(1663), Ar-H(3050),Ar-C-H (2876), O-H (3332), C-Cl (780) C=C(970), 
(1670), 414.16 C(72.51%) H(4.85%) Cl(8.55%) N(10.10%) O(3.92%)

xiv. (2E)-N-(2-aminophenyl)-3-(6-chloro-3-ethyl-2-phenylquinolin-4-yl)prop-2-enamide(II2g): C-N(1425), 
NH(1671),C=O(1663), Ar-H(3050),Ar-C-H (2876), O-H (3332), C-Cl (780) C=C(970), (1670)
C(72.97%) H(5.18%) Cl(8.85%) N(9.82%) O(3.74%)

xv. (2E)-N-(2-aminophenyl)-3-(2-methyl-6-nitroquinolin-4-yl)prop-2-enamide(II3a): C-N(1425), 
NH(1670),C=O(1664), Ar-H(3051),Ar-C-H (2876), O-H (3332), NO2(1510) C=C(970), (1670), 
7.6(3H),7.0(2H), 7.3(1H), 6.8 (1H), 4.5(2H), 6.5(1H), 7.6(2H) 1.2(3H), 9.2(1H) 349.13 C(65.56%)
H(4.59%) N(16.02%) O(13.83%)

xvi. (2E)-N-(2-aminophenyl)-3-(2,3-dimethyl-6-nitroquinolin-4-yl)prop-2-enamide(II3b): C-N(1425), 
NH(1670),C=O(1664), Ar-H(3051), Ar-C-H (2876), O-H (3332), NO2(1510) C=C(970), (1670)
7.6(3H),7.0(2H), 6.8 (1H), 4.5(2H), 6.5(1H), 7.6(2H) 1.2(6H), 9.2(1H) 363.16 C(66.31%) H(4.96%)
N(15.43%) O(13.30%)

xvii. (2E)-N-(2-aminophenyl)-3-(3-ethyl-2-methyl-6-nitroquinolin-4-yl)prop-2-enamide(II3c): C-N(1425), 
NH(1670),C=O(1664), Ar-H(3051), Ar-C-H (2876), O-H (3332), NO2(1510) C=C(970), (1670), 
7.6(3H),7.0(2H), 6.8 (1H), 4.5(2H), 6.5(1H), 7.6(2H) 1.2(6H),1.4(2H),9.2(1H) 377.18 C(67.01%)
H(5.36%) N(14.88%) O(12.75%)

xviii. (2E)-N-(2-aminophenyl)-3-(2-ethyl-3-methyl-6-nitroquinolin-4-yl)prop-2-enamide(II3d): C-N(1425), 
NH(1670),C=O(1664), Ar-H(3051), Ar-C-H (2876), O-H (3332), NO2(1510) C=C(970), (1670)
7.6(3H),7.0(2H), 6.8 (1H), 4.5(2H), 6.5(1H), 7.6(2H) 1.2(6H),1.4(2H),9.2(1H) C(67.01%) H(5.36%)
N(14.88%) O(12.75%)

xix. (2E)-N-(2-aminophenyl)-3-(6-nitro-2-phenylquinolin-4-yl)prop-2-enamide(II3e): C-N(1425), 
NH(1670),C=O(1664), Ar-H(3051), Ar-C-H (2876), O-H (3332), NO2(1510) C=C(970), (1670)
C(70.23%) H(4.42%) N(13.65%) O(11.69%)

xx. (2E)-N-(2-aminophenyl)-3-(3-methyl-6-nitro-2-phenylquinolin-4-yl)prop-2-enamide(II3f): C-N(1425), 
NH(1670),C=O(1664), Ar-H(3051), Ar-C-H (2876), O-H (3332), NO2(1510) C=C(970), (1670)
C(70.74%) H(4.75%) N(13.20%) O(11.31%)

xxi. (2E)-N-(2-aminophenyl)-3-(3-ethyl-6-nitro-2-phenylquinolin-4-yl)prop-2-enamide(II3g): C-N(1425), 
NH(1670),C=O(1664), Ar-H(3051), Ar-C-H (2876), O-H (3332), NO2(1510) C=C(970), (1670)
C(71.22%) H(5.06%) N(12.78%) O(10.95%)
Table 1: Indicating structure % yield and melting point of synthesized compounds.

| Compound Code | R     | R1 | R2 | % yield | M.P.   |
|---------------|-------|----|----|---------|--------|
| II1a          | CH3   | H  | H  | 54.54   | 301.0  |
| II1b          | CH3   | CH3| H  | 63.55   | 308.2  |
| II1c          | CH3   | C2H5| H  | 66.87   | 312.3  |
| II1d          | C2H5  | CH3| H  | 63.56   | 296.5  |
| II1e          | C6H5  | H  | H  | 65.23   | 286.6  |
| II1f          | C6H5  | CH3| H  | 68.23   | 296.4  |
| II1g          | C6H5  | C2H5| H  | 65.43   | 297.8  |
| II2a          | CH3   | H  | Cl | 54.23   | 312.2  |
| II2b          | CH3   | CH3| Cl | 59.12   | 299.7  |
| II2c          | CH3   | C2H5| Cl | 69.08   | 289.3  |
| II2d          | C2H5  | CH3| Cl | 61.45   | 311.4  |
| II2e          | C6H5  | H  | Cl | 64.65   | 307.9  |
| II2f          | C6H5  | CH3| Cl | 56.87   | 312.0  |
| II2g          | C6H5  | C2H5| Cl | 69.25   | 289.5  |
| II3a          | CH3   | H  | NO2| 51.34   | 310.6  |
| II3b          | CH3   | CH3| NO2| 68.15   | 316.4  |
| II3c          | CH3   | C2H5| NO2| 79.07   | 317.7  |
| II3d          | C2H5  | CH3| NO2| 63.03   | 318.2  |
| II3e          | C6H5  | H  | NO2| 68.54   | 313.3  |
| II3f          | C6H5  | CH3| NO2| 67.32   | 314.4  |
| II3g          | C6H5  | C2H5| NO2| 57.05   | 315.5  |
Table 2: Indicating results of cell line study for synthesized molecules

| Cell Line Compounds | HCT-116 IC₅₀(in µmol) | COLO 205 IC₅₀(in µmol) | COLO320 DM IC₅₀(in µmol) |
|---------------------|------------------------|------------------------|--------------------------|
| II1a                | 10.26                  | 13.16                  | 21.09                    |
| II1b                | 12.75                  | 17.4                   | 17.31                    |
| II1c                | 25.36                  | 12.36                  | 12.21                    |
| II1d                | 23.54                  | 20.53                  | 14.36                    |
| II1e                | 17.42                  | 16.19                  | 27.25                    |
| II1f                | 18.67                  | 17.35                  | 10.94                    |
| II1g                | 21.22                  | 10.54                  | 14.97                    |
| II2a                | 24.47                  | 11.45                  | 9.504                    |
| II2b                | 19.92                  | 13.19                  | 19.87                    |
| II2c                | 25.93                  | 9.896                  | 14.99                    |
| II2d                | 26.39                  | 14.95                  | 18.13                    |
| II2e                | 18.97                  | 10.95                  | 12.98                    |
| II2f                | 21.61                  | 16.87                  | 22.57                    |
| II2g                | 19.47                  | 12.24                  | 19.04                    |
| II3a                | 21.65                  | 11.99                  | 26.52                    |
| II3b                | 23.23                  | 12.77                  | 22.11                    |
| II3c                | 19.92                  | 12.41                  | 17.62                    |
| II3d                | 25.93                  | 11.29                  | 19.6                     |
| II3e                | 42.79                  | 11.58                  | 14.8                     |
| II3f                | 18.51                  | 11.97                  | 15.56                    |
| II3g                | 20.25                  | 10.85                  | 14.8                     |

Figure 1: General synthesis scheme.
Discussion:

Figure 2 and Figure 3 shows the interactions of protein -Trichostatin A and protein - synthesized molecule II1a respectively. From docking studies resulting 21 best fit molecules were found. The best fit molecules (Table.1) were synthesized in four steps as described above. Synthesized molecules were tested for in vitro anticancer activity using MTT assay on cell line HCT-116, COLO 320DM and COLO 205. For cell line HCT-116, COLO 205 and COLO 320DM inhibition IC₅₀ showed in range 6.915 – 38.4µmol, 3.694 – 28.74µmol and 8.911 – 28.34µmol, respectively(Table no.2). The minimum inhibition concentration was 3.694µmol shown by compound 7 against cell line COLO 205. For Cell line HCT-116 lowest IC₅₀ observed was 6.915µmol by compound 8. 8.911 µmol IC₅₀ was showed by compound against cell line COLO 320DM (Table no.2).
References:

1. M. Antonello, M. Silvio, R. Rino, E. Monica, S. Gianluca, N. Giuseppina, S. Roberto, J. Florian, L. Peter, B. Gerald, Binding mode analysis of 3-(4-benzoyl-1- methyl-1H-2-pyrrolyl)-N-hydroxy-2-propenamide: A new synthetic histone deacetylase inhibitor inducing histone hyperacetylation, growth inhibition, and terminal cell differentiation, J. Med. Chem. 45 (2002) 1778–1784.

2. S. Vadivelan, B.N. Sinhab, G. Rambabu, B. Kiran, A.R.P.J. Sarma, Pharmacophore modeling and virtual screening studies to design some potential histone deacetylase inhibitors as new leads, J. Mol. Graph. Model. 26 (2008) 935–946.

3. De Ruijter, A.J., van Gennip, A.H., Caron, H.N., Kemp, S., van Kuilenburg, A.B.; 2003. Histone deacetylases (HDACs): characterization of the classical HDAC family. Biochem. J. 370, 737–749.

4. Emanuele, S., Lauricella, M., Tesoriere, G., 2008. Histone deacetylase inhibitors: apoptotic effects and clinical implications. Int. J. Oncol. 33, 637–646.

5. Glozak, M.A., Seto, E., 2007. Histone deacetylases and cancer. Oncogene 26, 5420–5432.

6. P. Gallinari, S. DiMarco, P. Jones, M. Pallaro, C. Steinkuhler, HDACs, histone deacetylation and gene transcription: from molecular biology to cancer therapeutics, Cell Res. 17 (2007) 195–211.

7. K. Garber, HDAC inhibitors overcome first hurdle, Nat. Biotechnol. 25 (2007) 17–19.

8. M. Paris, M. Porcelloni, M. Binaschi, D. Fattori, Histone deacetylase inhibitors: from bench to clinic, J. Med. Chem. 51 (2008) 1505–1529.

9. M. Dokmanovic, C. Clarke, P.A. Marks, Histone deacetylase inhibitors: overview and perspectives, Mol. Cancer Res. 5 (2007) 981–989.

10. Duvic, J. Vu, Vorinostat: a new oral histone deacetylase inhibitor approved for cutaneous T-cell lymphoma, Expert Opin. Investig. Drugs 16 (2007) 1111–1120.

11. P.A. Marks, W.S. Xu, Histone deacetylase inhibitors: Potential in cancer therapy, J. Cell. Biochem. 107 (2009) 600–608.

12. L. Stimson, V. Wood, O. Khan, S. Fotheringham, N.B. La Thangue, HDAC inhibitor-based therapies and haematological malignancy, Ann. Oncol. 20 (2009) 1293–1302.

13. Finn, Paul W. et al preparation of carbamic acid compounds comprising a bicyclic heteroaryl group as histone deacetylase inhibitors from PCT Int Appl., 2004076386.

14. Karen Lackey, Daniel D Sternbach, Synthesis of substituted quinoline-4-carboxylic acids; synthesis, October 1993 pg no 995-997

15. Tamotsu Fujisawa,* Toshiki Mori, Shoichi Tsuge, and Toshio Sato direct and chemoselective conversion of carboxylic acids into aldehydes Tetrahedron Letters, Vol.24, No.14, pp 1543-1546,1983

16. Herbert C. Brown,* Jin Soon Cha, Nung Min Yoon,lb and Behrooz Nazer Selective Reductions. 39. Partial reduction of carboxylic acids with thexylchloroborane-methyl sulfide. A direct and simple aldehyde synthesis J. Org. Chem. 1987, 52, 5400-5406

17. Timothy D. W. Claridge et al. Highly (E)-Selective Wadsworth-Emmons Reactions Promoted by Methylmagnesium Bromide. Org. Lett., Vol. 10, No. 23, 2008.

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