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Synthesis And Biological Activity Of Fluoro Pyrrolo Benzoxazepinyl Oxazolidinone

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ABSTRACT

The synthesis and *in vitro* antibacterial activity of fluoro anologue of pyrrolo benzoxazepinyl oxazolidinone is reported. The synthetic route involves synthesis of fluoro isatoicanhydride followed by construction of pyrrole, oxazepine and oxazolidinone ring.

KEYWORDS

Oxazolidinone;
Linezolid;
Fluorine atom;
Clauson-Kasan reaction;
Fluoro isatoicanydride.

INTRODUCTION

Oxazolidinones[1] are novel class of synthetic antibiotics highly active against gram-positive organisms such as Methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant enterococci (VRE) and glycopeptides-resistant *Staphylococcus aureus* (GISA). Oxazolidnones inhibits the bacterial protein synthesis at an early stage by binding selectively to 50S ribosomal subunit[2]. Linezolid **(1)**[3], was the first member of this series introduced in the market by Pharmacia in 2000. According to the recent reports, even linezolid has acquired bacterial resistance and due to myelosuppression, linezolid is not suitable for long-duration therapy[4]. Therefore, there is a need to pursue development of second generation oxazolidinones.

In our earlier communication, we discussed about

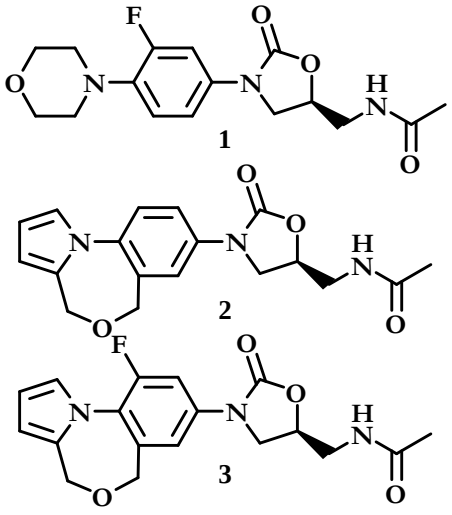
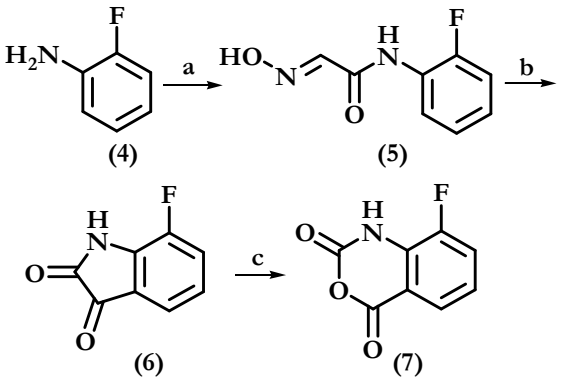


Figure 1



SCHEME 1: Reagents and conditions:(a) $CCl_3CH(OH)_2$, $NH_2OH.HCl$, Na_2SO_4, HCl, relux; (b) conc.H_2SO_4, 70^0C; (c) Cr_2O_3, Ac_2O, AcOH, 90^0C

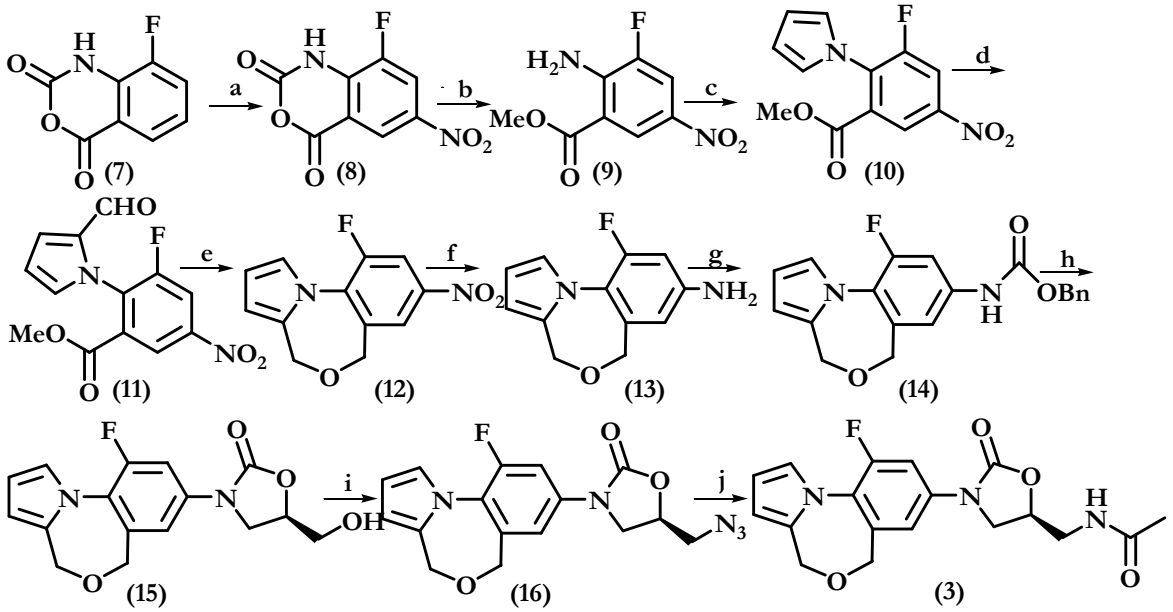
the confirmationally constrained and rigid tricyclic pyrrolo benzoxazepinyl oxazolidinone **(2)** and its SAR study[5]. To improve its pharmacokinetic profile and make it more bioavailable, we incorporate 'F' atom in the compound **(2)**. It is well known from the literature, incorporation of fluorine atom into bioactive compounds to create molecules with improved biochemical, physiological and pharmaceutical properties and thus to upgrade their values for medicinal chemistry[6]. The incorporation of fluorine in to potent molecules can, however, tremendously effect their bioavailability by enhancing pharmacokinetical properties, such as membrane binding and permeation. The increase in lipophilicity that is obtained by fluoro substitution can be further applied in order to increase the binding properties of drug molecules to hydrophobic regions of its target molecule in the body. As it is evident in the linezolid, 'F' plays vital role in improving its biological properties. The synthetic routes are depicted in SCHEMES 1 and 2.

RESULTS AND DISCUSSION

Synthesis of fluoro isatoicanhydride(7)

The hydroximinoacetamide**(5)** was obtained by the reaction of 2-fluoro aniline with chloral hydrate and hydroxylamine hydrochloride in acidic medium[7]. On addition of compound**(5)** to conc. H_2SO_4 at 70^0C, indoledione **(6)**[7,8] was obtained. The insertion of oxygen in between α-diketone utilizing Ac_2O and Cr_2O_3 resulted, 8-fluoro-1H-benzo[d][1,3]oxazine-2,4-dione**(7)**(fluoro isatoicanhydride)[9](SCHEME 1).

Synthesis of compound (3)



SCHEME 2 : Reagents and conditions: (a)KNO_3, H_2SO_4, 0^0C; (b) MeOH, NaOH, reflux; (c) 2,5-dimethoxytetrahydrofuran, AcOH. reflux; (d) DMF, $POCl_3$, 0^0C tort; (e) $NaBH_4$, MeOH, HCl, rt; (f) ammonium formate, 10% Pd-C, THF-MeOH, 0^0C to rt; (g) CBzCl, $NaHCO_3$, 0^0C to rt; (h) n-BuLi, (R)-glycidy butyrate, THF, -78^0C to rt; (i) MsCl/TEA, 0^0C and NaN_3/DMF, 80^0C; (j) thioacetic acid, rt.

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TABLE 1 : *In vitro* potency of compound (3) in comparison with compound (2) and linezolid (1)

Compd	SA 33591	SA 49951	SA 29213	EF 29212	EF 12201	EFe 12202
3	4	4	4	4	> 32	>32
2	2	2	2	2	2	2
1	2	2	2	2	2	4

Nitro fluoro isatoicanhydride **(8)** was obtained by the nitration of fluoro isatoic anhydride **(7)** (SCHEME 2) and subsequently subjected to methanolysis resulted amino-ester **(9)**. The pyrrole ring **(10)** was constructed via Clauson-Kass reaction by refluxing amino ester and 2,5-dimethoxytetrahydrofuran in glacial acetic acid. The aldehyde **(11)** was obtained by the Vilsmeier-Hack formylation of the compound **(10)**. Reduction of compound **(11)** followed by acidification of the resulting diol afforded benz-azulene compound **(12)**. The nitro compound **(12)** was reduced to amine **(13)** and protected with CBzCl subsequently to obtain **(14)**. The carbamate **(14)** was treated with (R)-glycidyl butyrate in the presence of n-BuLi to afford 5-(R)-(hydroxymethyl) oxazolidinone**(15)**. The alcohol**(15)** was converted azide **(16)**via mesylation. The azide **(16)** was treacted with thioacetic acid to obtain (S)-N-[3-(10-Fluoro-4H,6H-5-oxa-10b-aza-benzo[e]azulen-8yl)-2-oxo-oxazolidin-5ylmethyl] acetamide **(3)**.

The compound**(3)** synthesized above was screened for *in vitro* antibacterial activity against panel of gram-positive bacteria including both sensitive and resistant strains: *S.aureus* ATCC 29213 (MSSA), *S.aureus* ATCC 49951(Smith), *S.aureus* ATCC 33591 (MRSA), *E.faecalis* ATCC 29212, *E. faecalis* NCTC 12201(VREf) *E.faecium* NCTC 12202(VR Efm) and compared it with compound **(2)** and linozelid **(1)**. The fluoro analogue **(3)** of compound **(2)** has shown activity of MIC 4μg/mL in four strains. Infact, the fluoro substitution in compound **(2)** has reduced the *in vitro* activity by two fold (TABLE 1).

In summary, we have synthesized fluoro analogue **(3)** of compound **(2)** in an efficient manner and its *in vitro* antibacterial activity was examined. The SAR study of compound **(3)**along with its activity studies, currently undergoing in our laboratory.

EXPERIMENTAL

General methods

Infrared spectra were recorded on a FT-IR spectrometer and the absoption bands are noted in cm^{-1}. ^1H NMR spectra are determined in $CDCl_3$ solution on 200 MHz spectrometers, respectively. Proton chemical shifts(δ) are relatively to tetramethylsilane (TMS, δ = 0.00) as internal standard and expressed in ppm. While the spin multiplicities are given as a s (singlet), d (doublet), t(triplet), m (multiplet)as well as bs(broad singlet), the coupling constants(J) are given Hz. Coupling constants were corrected to the nearest value after decimal. MS spectra were obtained on a HP-5989A mass spectrometer. Column chromatography carried out with sillica gel, grade 100-200, 230-400 mesh. Reactions were monitored by thin layer chromatography(TLC) on sillica gel plates(60 F254), visualizing with ultraviolet light or alkaline $KMnO_4$ stain. Unless stated otherwise reactions were performed under nitrogen atmosphere. All other reagents were purchased from Aldrich at the highest commercial quality and used without further purification.

N-(2-Fluoro-phenyl)-2-hydroxyimino-acetamide (5)

To a solution of chloral hydrate (17.8g, 90.0 mmol) and Na_2SO_4 (127.8g, 900.0mmol) in water (340mL), 2-fluoro aniline (10g, 900.0mmol) in conc. HCl (21.9g, 0.32mol) in water(55mL) was added at rt. The resulting solution was refluxed for 15min and it was allowed to reach rt. The precipitate formed was collected onto buchner funnel and it was thoroughly washed with water and dried to afford **(5)** as white solid.

Yield: 15.8g (91%).

IR(KBr): 3393, 3062, 3010, 1660, 1617, 1543, 14591261, 1020, 758, 654cm^{-1}.

^{1}H-NMR(200 MHz, $CDCl_3$): δ8.56(bs, 1H), 8.39-8.28 (m, 2H), 7.60 (s, 1H), 7.20-7.07 (m, 3H).

EI-MS: 182 [M^+], 137, 111.

7-Fluoro-1H-indole-2,3-dione (6)

N-(2-Fluoro-phenyl)-2-hydroxyimino-acetamide **(5)**(14.9g, 81.8mmol) was added in small batches to

conc. H_2SO_4(45mL) at 70^{0}C under N_2 atmosphere and stirred for 1h. The mixture was poured onto ice flakes and solid formed was filtered off. The filtrate was extracted with EtOAc and washed with water and brine successively. It was dried over Na_2SO_4 and concentrated in vacuum to afford **(6)** as orange colour solid.

Yield: 5.4 g (40%).

IR (KBr): 3197, 1742, 1639, 1500, 1326, 1260, 779, 706cm^{-1}.

^{1}H-NMR(200MHz, $CDCl_3$+DMSO-d_6): δ11.33 (bs, 1H), 7.46-7.29(m, 2H), 7.09-6.99(m, 1H).

EI-MS: 165[M$^+$], 137, 109, 94.

8-Fluoro-1H-benzo[d][1,3]oxazine-2,4-dione (7)

To a solution of 7-Fluoro-1*H*-indole-2,3-dione **(6)**(3.5g, 21.2 mmol) in acetic anhydride(20mL) and acetic acid(20mL), Cr_2O_3(4.8g, 31.5mmol) was added at 90^{0}C and stirred at the same temperature for 15min. The mixture was poured onto ice flakes and solid formed was filtered, thoroughly washed with water and dried to afford **(7)** as a orange colour solid.

Yield: 2.8 g (73%).

IR (KBr): 3200, 3120, 1710, 1009, 743, 538 cm^{-1}.

^{1}H-NMR(200MHz, $CDCl_3$+DMSO-d_6): 11.83 (bs, 1H), 7.83 (d, J=8.3Hz, 1 H), 7.48 (t, J= 9.0 Hz, 1H), 7.43-7.15 (m, 1H).

EI-MS: 181[M$^+$], 137, 109.

8-Fluoro-6-nitro-1H-benzo[d][1,3]oxazine-2,4-dione(8)

To a solution of 8-Fluoro-1H-benzo[d][1,3] oxazine -2,4-dione **(7)**(2.5g, 13.8 mmol) in conc.H_2SO_4 (10mL), KNO_3 (1.39g, 13.8mmol) was added in small batches at 0 ^{o}C and stirred for 15 min. The mixture was poured onto ice flakes and solid formed was filtered, thoroughly washed with water and dried to afford **(8)** as a yellow solid.

Yield: 2.18 g (70%).

IR(KBr,): 3120, 1782, 1761, 1356, 1339, 1032cm^{-1}.

^{1}H-NMR(200MHz, $CDCl_3$): δ10.76,(s, 1H), 8.31-8.21(m, 2H).

EI-MS: 227[M$^+$+1], 182, 164.

2-Amino-3- fluoro-5-nitro benoic acid methyl ester (9)

To a suspension of 8-fluoro-6-nitro-1H-benzo [d][1,3]oxazine-2,4-dione 8(2.15g, 9.5mmol) in methanol was added NaOH (38mg, 0.95 mmol) and heated to reflux for 15 min. The reaction was cooled and diluted with EtOAc, water and brine washing followed. It was dried over Na_2SO_4 and concentrated in vacuum to afford **(9)** as a yellow solid.

Yield: 1.57g (77.5%).

IR(KBr): 3487, 3359, 1702, 1625, 1518, 1329, 1284, 1206, 1076cm^{-1}.

^{1}H-NMR(200MHz, $CDCl_3$: δ 8.70(s, 1H), 8.03 (dd, J=8.0, 2.4Hz, 1H), 6.52(bs, 2H), 3.97(s, 3H).

EI-MS: 214[M$^+$], 182, 152, 124, 108, 97.

3-Fluoro-5-nitro-2-pyrrol-1-yl benzoic acid methyl ester (10)

To a solution of 2-amino-3- fluoro-5-nitro-benoic acid methyl ester **(9)**(1.5g, 7.0 mmol) in AcOH (25mL) was added 2,5-dimethoxy tetrahydrofuran (1.11g. 8.41mmol) and heated to reflux for 2h. The reaction was cooled to rt, poured into water and extracted with EtOAc. The organic layer was washed with saturated $NaHCO_3$ solution, water and brine successively. It was then dried over Na_2SO_4 and concentrated in vacuum to afford 10 as a thick liquid.

Yield: 1. 33g(72%).

IR (Neat): 1736, 1529, 1502, 1351, 1301, 1308, 1222, 1083, 1002, 890, 745, 732cm^{-1}.

^{1}H-NMR(200MHz, $CDCl_3$): δ8.50(d, J=2.4Hz, 1H), 8.28 (dd, J=9.3, 2.4Hz, 1H), 6.81 (t, J=2.0 Hz, 2H), 6.43 (t, J=2.2Hz, 2H), 3.79 (s, 3H).

EI-MS: 264[M$^+$], 206.

3-Fluoro-2-(2-formyl-pyrro-1-yl)-5-nitro-benzoic acid methyl ester (11)

$POCl_3$(811mg, 5.28mmol) was added dropwise to DMF(7mL) under N_2 at 0^{0}C to form a viscous yellow complex. To this reaction mixture was added a solution of 3-Fluoro-5-nitro-2-pyrrol-1-yl benzoic acid methyl ester **(10)**(1.33g, 5.28mmol) in DMF(5 mL). The reaction mixture was brought to rt and stirred for 2h. It was then poured in to water which was basified with Na_2CO_3. The precipitate formed was filtered, washed with water, and dried under vacuum to afford the compound **(11)** as a white solid.

Yield: 882 mg (60%).

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IR (KBr): 3444, 3094, 1733, 1663, 1539, 1497, 1351, 1299, 1011, 773cm^{-1}.

^{1}H-NMR(200MHz, CDCl$_3$): δ9.54(s, 1H), 8.70 (s, 1H), 8.25 (dd, J=8.8, 2.4Hz, 1H), 7.18 (t, J=2.0 Hz, 1H), 6.95 (s, 1H), 6.55 (t, J = 3.2 Hz, 1H), 3.76 (s, 3H).

EI-MS: 292[M$^+$].

10-Fluoro-8-nitro-4H,6H-5-oxa-10b-aza-benzo [e]azulene (12)

To a solution of 3-fluoro-2-(2-formyl-pyrro-1-yl)-5-nitro-benzoate methyl ester **(11)**(880mg, 3.01 mmol) in MeOH(10mL)was added $NaBH_4$(137mg, 3.6 mmol) at rt. After stirring for 1h, con. HCl(2mL) was added and was diluted with water (10mL). After stirring for a further 10min, the reaction mixture was extracted with EtOAc. The organic layer was washed with water, brine, dried over Na_2SO_4 and concentrated to afford compound **(12)**as a yellow solid.

Yield: 373 mg (50%).

IR(KBr,): 1520, 1492, 1330cm^{-1}.

^{1}H-NMR(200MHz, CDCl$_3$): δ 8.18(t, J=4.2Hz, 2H), 7.22(d, J=1.5Hz, 1H), 6.42(d, J=3.4Hz, 2H), 4.54(s, 2H), 4.51 (s, 2H).

EI-MS: 248[M$^+$], 219, 201, 172, 146, 107.

10-Fluoro-4H,6H-5-oxa-10b-aza-benzo[e]azulen -8-ylamine (13)

To a solution of 10-fluoro-8-nitro-4H,6H-5-oxa-10b-aza-benzo[e]azulene **(12)**(370mg, 1.49 mmol) and ammonium formate(376mg, 5.96mmol) in THF:MeOH(1:4, 5mL) at 0^{0}C was added 10% Pd-C(10mol%) and stirred at rt under N_2 for 1h. The reaction mixture was filtered over a celite pad and filterate was concentrated. The residue was diluted with EtOAc, washed with water and brine successively. It was then dried over Na_2SO_4 and concentrated to afford the amine **(13)**as white solid.

Yield: 195mg (60%).

IR (KBr): 3350, 2852, 1665, 1620, 1510, 1310, 1064cm^{-1}.

^{1}H-NMR(200MHz, CDCl$_3$): δ6.60-6.16(m, 5H), 4.46(s, 2H), 3.98(s, 2H), 3.44(bs, 2H).

EI-MS: 218 [M$^+$], 189, 172, 148, 133.

(10-Fluoro-4H,6H-5-oxa-10b-aza-benzo[e]azulen -8-yl)-carbamicacid benzyl ester (14)

To a solution of amine **(13)**(190mg, 0.87mmol) in acetone(5mL) was added a solution of $NaHCO_3$ (146mg, 1.7mmol) in water(2.5mL). Benzylchloro formate(50%, 163mg, 0.9mmol) was added dropwise to reaction at 0^{0}C. The reaction was broght to rt and stirred for 1h. The reaction was poured into water, the precipitate formed was fitered and dried under vacuum to afford the compound **(14)** as white solid.

Yield: 211 mg (69%).

IR (KBr): 3246, 1724, 1562, 1508, 1314, 1230cm^{-1}.

^{1}H-NMR(200MHz, CDCl$_3$): δ 7.59 (d, J=12.2Hz, 1H), 7.50-7.40(m, 5H), 7.12(s, 2H), 6.85(s, 1H), 6.33(s, 2H). 5.23(s, 2H), 4.49(s, 2H), 4.36(s, 2H).

EI-MS: 352[M$^+$], 244, 215, 187, 108.

(R)-5-Hydroxmethyl -3-(10-fluoro-4H,6H-5-oxa-10b-aza-benzo[e]azulen-8yl)-oxazolidin-2-one (15)

To a solution of carbamate **(14)**(200mg, 0.6 mmol) in dry THF(5mL) was added n-BuLi (15% solution in hexane, o.36 mL, 0.8 mmol) at -78^{0}C and allowed to stir at the same temperature for 30 min. R-(-)-Glycidyl butyrate(82mg, 0.6mmol) was added to the reaction and then allowed to come to rt and stirred for additional 4h. The reaction was then diluted with EtOAc and water after quenching with saturated NH_4Cl solution. The organic layer was separated and aqueous layer was further extracted with EtOAc. The combined organic layers were dried over Na_2SO_4 and concentrated in vacuum. The residue was purified by column chromatography to afford alcohol **(15)** as white solid.

Yield: 150mg(83.3%).

IR (KBr): 3401, 1740, 1505, 1088cm^{-1}.

^{1}H-NMR (200MHz, CDCl$_3$): δ7.59 (d, J=2.7Hz, 1H), 7.50(d, J=2.6Hz, 1H), 6.35(dd, J=11.4, 2.6 Hz, 1H), 6.24-6.16(m, 2H), 5.42(bs, 1H), 4.74-4.61(m, 1H), 4.46(s, 1H), 3.98(s, 2H), 4.08-3.78(m, 4H).

EI-MS: 318[M$^+$], 172.

(R)-5-Azidomethyl-3-(10-fluoro-4H,6H-5-oxa-10b-aza-benzo[e]azulen-8yl)-oxazolidin-2-one (16)

To a solution of alcohol **(15)**(150mg, 0.4mmol) in CH_2Cl_2(5mL) at 0^{0}C was added triethylamine (95mg, 0.9mmol) followed by the addition of

methanesul fonylchloride(80mg, 0.7mmol) and stirred for 1h. The reaction quenched by addition of water. The layers were separated and the aqueous layer was further extracted with CH_2Cl_2. The combined organic layers were dried over Na_2SO_4 and concentrated in vacuum to afford mesylate 168 mg (90%). To solution of mesylate in DMF (3 mL) was added NaN_3 (90mg, 1.3mmol) and heated to 80^0C for 1h. The reaction was extracted with EtOAc after quenching it with the water. It was dried over Na_2SO_4, concentrated in vacuum and column purified to afford azide **(16)** as a off-white solid.

Yield: 116 mg (80%).

IR (KBr): 2106, 1748cm^{-1}.

^{1}H-NMR(200MHz, $CDCl_3$): δ 7.71-7.55(m, 2H), 7.01(s, 1H), 6.31(s, 2H), 4.79-4.83(m, 1H), 4.50-4.47(m, 4H), 4.11 (t, J=8.8Hz, 1H), 3.96-3.90(m, 1H), 3.75-3.59 (m, 2H)

EI-MS: 343[M^+]

(S)-N-[3-(10-Fluoro-4H,6H-5-oxa-10b-aza-benzo[e]azulen-8yl)-2-oxo-oxazolidin-5ylmethyl]-acetamide(3)

(R)-5-Azidomethyl-3-(10-fluoro-4H,6H-5-oxa-10b-aza-benzo[e]azulene-8yl)-oxazolidin-2-one **(16)**(100mg, 0.29mmol) was treated with thioacetic acid (1 mL) and stirred at rt for 16h. The reaction was directly loaded onto the column and eluted(3% MeOH/$CHCl_3$) to afford acetamide **(3)** as a white solid.

Yield: 78mg(75%).

IR (KBr): 1678, 1510, 1321, 736cm^{-1}.

^{1}H-NMR(200MHz, $CDCl_3$): δ7.70-7.40(m, 2H), 7.05(s, 1H), 6.31-6.38(m, 2H), 6.17-6.12(m, 1H), 4.81-4.75(m, 1H), 4.51-4.48(m, 4H), 3.65-4.17(m, 4H), 2.05(s, 3H)

EI-MS: 359[M^+], 330.

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