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Synthesis of atorvastatin substituted diamine and their corresponding isatin schiff bases as antibacterial and cytotoxic agent

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Abstract: Two substituted diamines (**2-3**) and their corresponding Schiff bases (**4-5**) have been synthesized by amination of Atorvastatin an acid with two diamines with different carbon chain followed by condensation with Isatin. Spectroscopic characterization and study of antibacterial activity as well as cytotoxicity of the synthesized compound have been made to establish the Structure Activity Relation (SAR).

Keywords: Atorvastatin Substituted amide, Schiff base, Isatin, Antibacterial, Cytotoxicity.

Introduction

Atorvastatin [1-2], an acid having a penta substituted pyrrole ring is reported for its well-known anti-lipid property as this is competitive inhibitor of HMG-CoA reductase, the rate limiting enzyme that converts 3-hydroxy-3-methylglutaryl-coenzyme A to mevalonate, a precursor of sterols, including cholesterol. Cholesterol and triglycerides circulate in the bloodstream as part of lipoprotein complexes [3-4]. On the other hand Schiff bases [5] have antibacterial as well as cytotoxic property [6-9] and that's why Schiff base derivatives of Atorvastatin should posses antibacterial and Cytotoxic [10] property. Keeping this in mind we have synthesized the title compounds (Schemes 1,2) in order to study antibacterial activity and cytotoxicity.

Result and disscussion

The IR and NMR spectrum were recorded for the starting Atorvastatin **1** (Scheme **1**), and then the change of the spectrum for the imparted chemical changes were marked.

The IR and NMR spectrum were recorded for the starting Atorvastatin **1** (Scheme **1**), and then the change of the spectrum for the imparted chemical changes were marked.

Conversion of **1** into amide **2** (Scheme **2**) is appeared in the IR spectrum by disappearance of the wide acid band and formation of N-H coupled bands at 3318 and 3220 for presence of NH_2. NMR spectrum shows a wide peak at 3.11ppm that was absent in precursor acid's spectrum. The formation of Schiff base **4** (Scheme 2) gives rise to an additional carbonyl band at 1731 for the lactum C=O that was previously absent in case of amide and one N-H band at 3360 rather than N-H coupled bands. The aromatic region of NMR spectrum of the **4** shows clear peaks for 18 aromatic protons, among them two doublets appears at 7.36 & 6.86ppm and those for the protons of lactum ring (H_d&$H_{d'}$). The formation of Schiff base is further confirmed by absence of NH_2 peaks.

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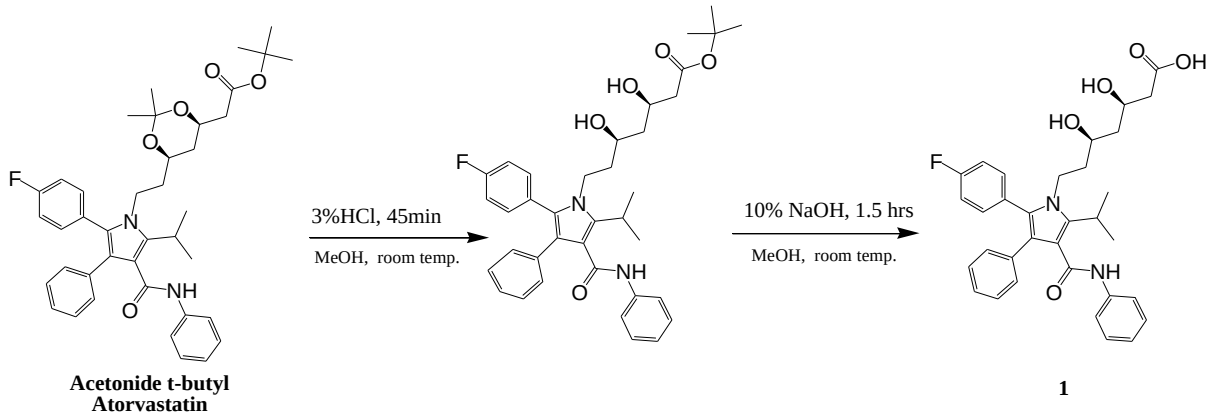
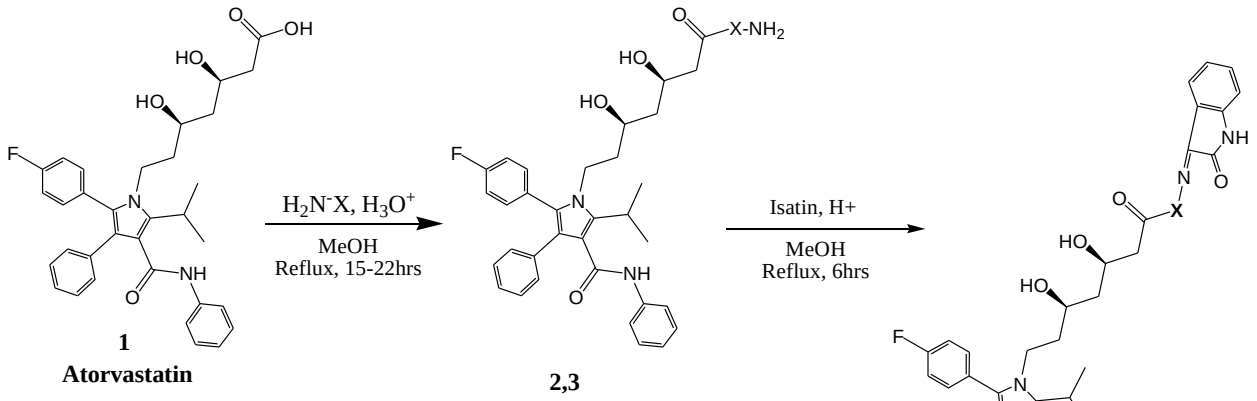


Scheme 1: Synthesis of Atorvastatin, **1** from Acetonide tertiarybutyl atorvastatin**.**



	X	Yield
2	NH	82.0%
3	$NHCH_2CH_2CH_2$	73.2%
4	NH	88.1%
5	$NHCH_2CH_2CH_2$	75.4%

Scheme 2: Synthesis of Schiff bases from Atorvastatin substituted diamides.

Similarly formation of amide **3** (Scheme 2) results removal of the acid band and appearance of NH_2 coupled bands at 3345 & 3251 in the IR spectrum. In NMR spectrum NH_2 protons are appeared as wide peak at 3.2ppm and additional three methelene signal are found as multiplet at 3.09ppm ($H_{1'''}$&$H'_{1'''}$), triplet at 2.58 ($H_{3'''}$&$H'_{3'''}$), multiplet at 1.5 ($H_{2'''}$&$H'_{2'''}$) and these are actually for the propylene chain. The formation of Schiff base **5** (Scheme 2) was marked like previous, lactam C=O band at 1730 of IR spectrum and 18 aromatic protons in NMR spectrum with two doublets at 7.36 & 6.88ppm (lactum ring H_d&$H_{d'}$ protons).

Conclusion

Two new Schiff bases were successfully synthesized and in all cases Methanol was used as solvent due to better solubility of the precursor. Yields in case of hydrazine products **2** and **4** were found higher than the propylene diamine products **3** and **5**. The biological

study shows significant cytotoxicity for compound **3** which shows strong antibacterial property too.

Experimental Section

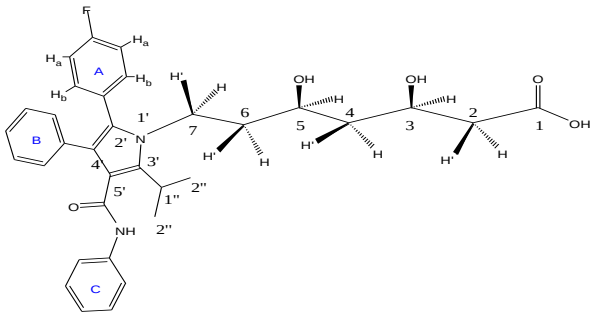
General Method and Procedure

Fisher-John's electro thermal melting point apparatus was used for recording the melting temperature (T_m) of all the synthesized compounds by thin disc method. The analytical thin layer chromatography (ATLC) was used to monitor the progress of the reaction and to check the purity of the synthesized products. The material used for ATLC was silica gel, 60 GF_{254} (Merck). Solvents that were used during present investigation were purified by distillation at the boiling point of the respective solvents. Traditional oil bath [maximum temperature 160^{o}C] was used for heating the reaction mixture. Solvents were removed from reaction mixture with the help of vacuum evaporator under the reduced pressure at a temperature bellow 50 ^{o}C. Column chromatography technique was extensively used for the separation of pure product from a reaction mixture. Infrared spectra were recorded on Fourier Transform Infra-red Spectrophotometer (FTIR-8300, SHIMADZU) as KBr disc. ^{1}H-NMR spectra and ^{13}C-NMR were in $CDCl_3$, DMSO-d6 using 400 and 100 MHz NMR Spectrophotometer respectively and Microanalysis was carried out by vario EL cube, Elementar, Germany. Synthesis of all the compounds was accomplished from Acetonide t-butyl Atorvastatin first. All the compounds were structurally characterized by spectroscopic technique.

Synthesis of Atorvastatin

The methanolic solution of acetonide tertiarybutyl atorvastatin, **1** (0.5g, 0.76 mmol) was stirred with 5 drops of 3% methanolic HCl (45 mins) for deprotection of the acetonide group, the reaction progress was monitored with the help of TLC (EA:PE=1:8). After completion of the deprotection the mixture was stirred with 5 mL 10% methanolic solution of NaOH for 1.5 hour at room temperature for ester hydrolysis. The product was obtained after evaporation of solvent by vacuum evaporator was white powder (0.435 g, **95.4** % yields, m.p. 113^{o}C-117^{o}C) and found to be pure on TLC after recrystalyzation of the crude product by using methanol as a solvent. R_f value of the product was 0.28 (ethyl acetate: chloroform =1:5).

Compound **1** was characterized spectroscopically; IR, υ_{max} KBr (cm^{-1}): 3650-2600 (υO-H, carboxylic acid); 2967 (υC-H); 1655 (υC=O); 1597 (υN-H); 1560, 1509 (υC=C aromatic); 1316 (υC-H, bending); 1221 (υC-N); 1111 (υC-O); 1157(υC-F); 841,690 (phenyl ring). ^{1}H-NMR ($CDCl_3$): δ_H(ppm) 7.51 (d, 2H, H_b); 7.0 (d, 2H, H_a); 7.35-6.98 (2m, 10H, aromatic ring B & C); 3.71 (m, 1H, H_3); 3.51 (m, 1H, H_5); 3.9 & 3.81 (m, 2H, H_7&H´$_7$); 3.11 (m, 1H, $H_{1''}$); 1.98&1.75 (dd, 2H, H_2&H´$_2$); 1.6 &1.53 (m, 2H, H_6 &H´$_6$); 1.39 (d, 6H, $H_{2''}$); 1.19 (m,2H, H_4 &H´$_4$); 9.8 (s, 1H, NH) ; 5.09 (s, 2H, 2×OH); 10.8(s, H, -COOH). ^{13}CNMR (DMSO): δ_H(ppm); 200.9, 166.6 (C=O), 137.2-123.8 (aromatic CH), 65.1, 62.2, 19.2 (CH), 45.5, 43.5, 37.6, 34.7 (-CH_2-), 25.8 (-CH_3).

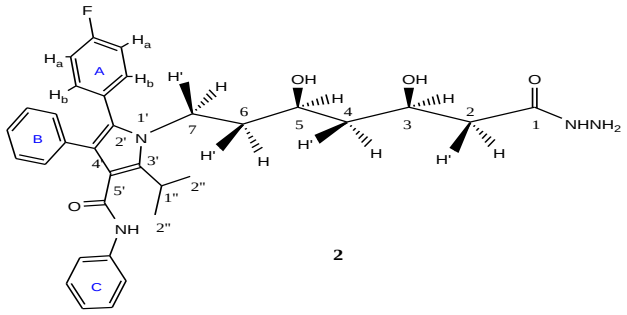


1

3.2 Synthesis of 5-(4-Fluoro-phenyl)-1-(6-hydrazinocarbonyl-3,5-dihydroxy-hexyl)-2-isopropyl-4-phenyl-1H-pyrrole-3-acidphenylamide, 2

Amination of methanolic solution of Atorvastatin, 1 (0.4 g, 0.72mmol) with hydrazine hydride (1eqv, 23 mg, 0.72 mmol) in presence of dilute 3% HCl (2drops) at 100°C for 30 hours afforded the product 2 in 82% yields. The usual work-up gave the off white solid, 2 and found to be pure on TLC after recrystalyzation of the crude product when methanol was used as solvent. R_f value of the product was 0.40 in ethyl acetate and melting point 123-125 °C.

IR, υ_{max} KBr (cm^{-1}): 3413 (υO-H); 3318, 3220 (υN-H); 3058 (υC-H, aromatic); 2960&2930 (υC-H, asymmetric stretching); 2875(υC-H, symmetric stretching); 1650 (υC=O, amide); 1596 (υN-H, bending); 1560, 1509 (υC=C aromatic); 1437 (υC-H, aliphatic bending); 1222 (υC-N stretching); 1157 (υC-F stretching); 842 (para-disubstituted phenyl ring); 693 (monosubstituted phenyl ring).

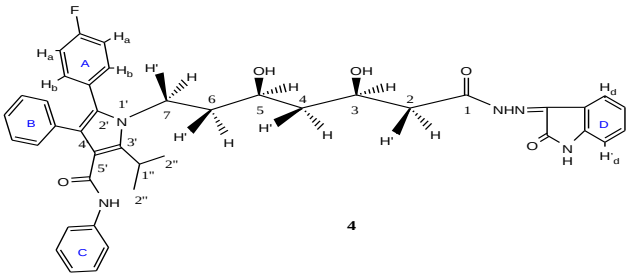


2

^{1}H-NMR ($CDCl_3$): δ_H(ppm) 7.54 (d, 2H, H_b); 7.08 (d, 2H, H_a); 7.3-7.01 (2m, 10H, aromatic ring B & C); 3.67 (m, 1H, H_3); 3.54 (m, 1H, H_5); 3.94 & 3.79 (m, 2H, H_7&H'_7); 3.12 (m, 1H, $H_{1''}$); 1.99&1.84 (dd, 2H, H_2&H'_2); 1.61&1.53 (m, 2H, H_6 &H'_6); 1.37 (d, 6H, $H_{2''}$); 1.19 (m,2H, H_4 &H'_4); 9.84 (s, 1H, NH) ; 3.11 (s, 2H, NH_2); 5.09 (s, 2H, 2×OH).^{13}C-NMR ($CDCl_3$): δ_C (ppm); δ_C 173 and 165.7 (C=O), δ_C 138.6-120.5(aromatic CH), δ_C 160.7 (CH-F), δ_C 65.7, 60.7 & 21.2(CH), δ_C 45, 41.4, 35.9 & 31.1 (-CH_2-), δ_C 25.67(-CH_3).

3.3 Synthesis of Schiff base 1-(3,5-Dihydroxy-6-methylenehydrazinocarbonyl-hexyl)-5-(4-fluoro-phenyl)-2-isopropyl-4-phenyl-1H-ple-3-carboxylic acid phenylamide; compound with 1,3-dihydro-indol-2-one, 4

15 mL methanolic solution of amide 2 (0.3g, 0.52mmol) was added dropwise to the boiling solution of isatin (0.772 g, 0.52mmol). Then the reaction mixture was refluxed for 9 hours at 100^{o}C in presence of 2drops of dilute 3%HCl. Solvent was evaporated by rotary evaporator, the crude product was recrystalized using ethyl acetate to get pure product 4, which was reddish yellow solid (m.p. 74-75^{0}C) having a yield of 0.331g (88.1% yields). This product was found homogeneous on TLC plate with R_f value 0.16 (EA: Chloroform= 1:5).

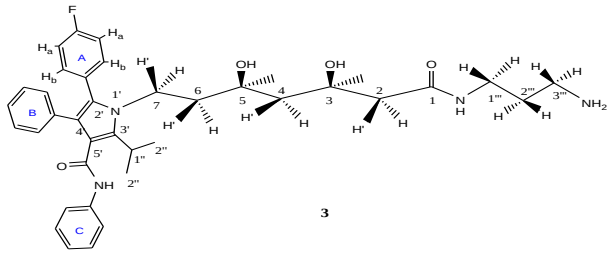


4

IR, υ_{max} KBr (cm^{-1}): 3415 (υO-H); 3360 (υN-H); 3064 (υC-H, aromatic); 2957 & 2926 (υC-H, asymmetric stretching); 2875 (υC-H, symmetric stretching); 1731 (υC=O, lactam); 1655 (υC=O, amide); 1685 (υC=N, imine); 1595 (υN-H, bending); 1554 & 1509 (υC=C aromatic); 1314, 1437 (υC-H, aliphatic bending).^{1}H-NMR ($CDCl_3$): δ_H(ppm) 7.36 (d, 1H, H_d); 6.86 (d, 1H, $H_{d'}$); 7.51 (d, 2H, H_b); 7.08 (d, 2H, H_a); 7.32-6.96 (2m, 12H, aromatic ring B, C & D); 4.0 (m, 1H, H_3); 3.95 (m, 1H, H_5); 3.86&3.78 (m, 2H, H_7&H'_7); 3.24 (m, 1H, $H_{1''}$); 2.37&2.30 (dd, 2H, H_2&H'_2); 1.62 (m, 2H, $H_{2''}$); 1.31&1.20 (m, 2H, H_4&H'_4); 1.38 (d, 6H, $H_{2''}$); 9.76 (s, 2H, 2×NH) ; 10.68 (s, H, lactam ring NH); 4.73&4.62 (s, 2H, 2×OH).

3.4 Synthesis of 1-[6-(3-Amino-propylcarbamoyl)-3,5-dihydroxy-hexyl]-5-(4-fluoro-phenyl)-2-isopropyl-4-phenyl-1H-pyoxylic acid phenylamide, 3

This compound was prepared by using same procedure as used for 2. Propylenediamine (53.04 mg, 0. 17 mmol) was used instead of Hydrazine hydride. IR, υ_{max} KBr (cm^{-1}): 3416 (υO-H, hydrogen bond); 2959 & 2927 (υC-H, aliphatic); 1653 (υC=O, amide); 1559 (υN-H, bending); 1559 & 1507 (υC=C aromatic); 1437 & 1490 (υC-H, aliphatic bending). ^{1}H-NMR (DMSO-d6): δ_H(ppm) 7.52 (d, 2H, H_b); 7.08 (d, 2H, H_a); 7.25-6.99 (2m, 10H, aromatic ring B & C); 3.84 (m, 1H, H_3); 3.77 (m, 1H, H_5); 3.95&3.92 (m, 2H, H_7&H'_7); 3.25 (m, 1H, $H_{1''}$); 3.09 (m, 2H, $H_{1'''}$&$H'_{1'''}$); 2.58 (t, 2H, $H_{3'''}$&$H'_{3'''}$); 2.1&1.8 (dd, 2H, H_2&H'_2); 1.53 (m, 2H, H_6); 1.5 (m, 2H, $H_{2'''}$&$H'_{2'''}$); 1.38&1.22 (m, 2H, H_4&H'_4); 1.39 (d, 6H, $H_{2''}$); 1.19 (m, 2H, CH_2); 3.2 (s, 2H, NH_2); 9.84&9.79 (s, 2H, 2×NH) ; 4.74&4.61 (s, 2H, 2×OH).



3

3.5 Synthesis of Schiff base 1-[3,5-Dihydroxy-6-(3-methyleneamino-propylcarbamoyl)-hexyl]-5-(4-fluoro-phenyl)-2-isopropyl-4-phenyrole-3-carboxylic acid phenylamide; compound with 1,3-dihydro-indol-2-one, 5

This compound was prepared by using same procedure as used for 4. Substituted amide, 3 (0.4 g, 0.65mmol), isatin (95.7 mg, 0.65mmol) were used in this case. IR, υ_{max} KBr (cm^{-1}): 3415 (υO-H, hydrogen bond); 3050 (υC-H, aromatic); 2960 & 2940 (υC-H,

aliphatic); 1730 (υC=O, lactam); 1653 (υC=O, amide); 1623 (υC=N, imine).^{1}H-NMR ($CDCl_3$): δ_H(ppm) 7.36 (d, 1H, H_d); 6.88 (d, 1H, $H_{d'}$); 7.53 (d, 2H, H_b); 7.08 (d, 2H, H_a); 7.32-7.0 (2m, 12H, aromatic ring B, C & D); 3.78 (m, 1H, H_3); 3.51 (m, 1H, H_5); 3.95 (m, 2H, H_7&H'_7); 3.23 (m, 1H, $H_{1'''}$); 3.1 (m, 2H, $H_{1'''}$&$H'_{1'''}$); 2.58 (t, 2H, $H_{3'''}$&$H'_{3'''}$); 2.37&2.25 (dd, 2H, H_2&H'_2); 1.53 (m, 2H, $H_{2'''}$&$H'_{2'''}$); 1.5 (m, 2H, H_6); 1.32&1.22 (m, 2H, H_4&H'_4); 1.39 (d, 6H, $H_{2''}$); 9.84(s, 1H, lactam NH); 9.79 (s, 1H, NH) ; 4.74&4.61 (s, 2H, 2×OH).

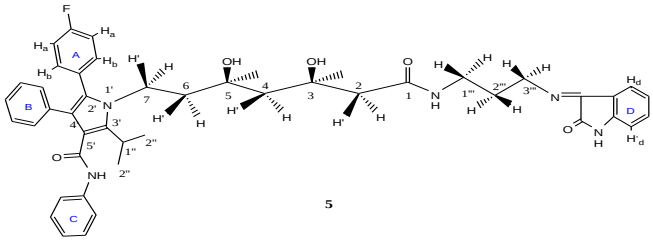


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Table 1: Reaction condition and yields of the products.

Compound	Reaction Condition			Yields
	Temperature	Solvent	Duration	
2	100 °C	Methanol	30 hours	82.0 %
3	100 °C	Methanol	15 hours	73.2 %
4	90 °C	Methanol	9 hours	88.1 %
5	90 °C	Methanol	6 hours	75.4 %

Biological Study

The synthesized products were allowed to do antibacterial test, brine shrimp lethality test and found like following;

Antibacterial study

Bacterial strains and constructs were used in this study are pathogens of plants, human and environmental bacteria of both gram positive and gram negative bacteria. Bacteria were grown in LB NY (5gL yeast extract; 4g/L nutrient broth), and TSB media at 28 ºC or 37 °C. Solid media was solidified with 1.5% (w/v) agar.

Brine Shrimp lethality test

The toxic activity of the prepared compounds was tested quantitatively by determination of LD_{50} values (The minimum amount of sample required for the mortality of half of the total test population is termed as Lethal Dose 50, shortly LD_{50}) by brine shrimp lethality test [11-12](Table -2). The toxicity regularly increases in conversion of the acid into amide and amide into Schiff base.

Table 2: Comparative cytotoxicity of prepared compounds [LD_{50} value against Brine shrimp]

Atorvastatin		Substituted Diamines		Schiff bases	
Compounds	LD_{50}	Compounds	LD_{50}	Compounds	LD_{50}
1	1.74	2	1.73	4	1.59
		3	1.54	5	1.80

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