



Synthesis and Antifungal Activity of Some 6H-thiochromeno[4,3-b]quinolines

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Abstract

A series of some 6H-thiochromeno[4,3-b]quinoline derivatives were prepared by the reaction of 4-chloro-2H-thiochromene-3-carbaldehydes with 2-aminophenols in DMF and characterized by analytical and spectral methods. All the compounds under test were found to be active against *M.gypseum* and *E.floccosum* higher than that of the standard fluconazole.

Keywords: 6H-thiochromeno[4,3-b]quinolines, Synthesis, Antifungal activity

1. Introduction

Over the last three decades there has been a dramatic increase in the incidence of fungal infections. Discovery of new drugs for the treatment of systemic mycoses is a major challenge in infectious disease research. There is an urgent need for new antifungal remedies with novel modes of action due to a decreased antifungal susceptibility of newly emerging fungi in growing setting of the immunocompromised patients (e.g., HIV-positive and neutropenic patients), the development of resistance to the present azole therapies, and high toxicity of polyenes (Rees, J. R. et. al 1998, Polak, A. 1999, Fostel, J. M., Lartey, P. 2000). Due to these undesirable side effects, a polynuclear heterocyclic framework such as 8-hydroxyquinoline (I, Figure 1) and its derivatives has been designed and identified as an effective pharmacophore for the development of better antifungal agents (Gershon, H. et. al 2004, Dardari, Z. et. al 2004). A series of compounds derived from 8-hydroxyquinoline as potential HIV-1 integrase inhibitors were synthesized recently (Majerz-Maniecka, K. et. al 2005). These compounds show a significant similarity to some novel antifungal agents, homoallyl amines, which possess potent antifungal activity (Vargas, L. Y. et. al 2003). Based on the report that this framework can be utilized for the development of potential drugs, we became interested in the synthesis of substituted 6H-thiochromeno[4,3-b]quinolines derived from I. The thiochromenoquinolines II were initially designed via connecting quinoline with thiochromene moiety.

Herein, we report a very simple and single pot synthesis of hydroxy derivatives of various 6H-thiochromeno[4,3-b]quinolines via construction of the fused quinoline moiety using 4-chloro-2H-thiochromene-3-carbaldehydes and 2-aminophenol as synthetic precursors, and screened for their antifungal activity.

2. Synthesis

The synthetic pathway for the preparation of the title compounds is depicted in Figure 2. Substituted **1** with 3-chloropropanoic acid gave **2**, which was ring-closed in concentrated sulfuric acid to yield the known thiochroman-4-one derivatives **3** (Li J. T. *et al* 2004, Krollpfeiffer, F., Schultze, H. 1923). 4-chloro-2H-thiochromene-3-carbaldehydes **4** were obtained from the **3** by a Vilsmeier-Haack-Arnold reaction. These initially prepared 4-chloro-2H-thiochromene-3-carbaldehydes **4** with 2-aminophenol **5** in DMF gave 6H-thiochromeno[4,3-b]quinolines **6**.

6H-thiochromeno[4,3-b]quinoline derivatives (6a-f) were synthesized (Table 1) in good yields ranging from 65% to 79%. As evident from Table 1, the 2-aminophenols participated in this reaction smoothly and the yields of the products were remarkably similar irrespective of the presence of electron withdrawing group (-NO₂) on the aniline ring (entry 1, 3, 5 vs entries 2, 4, 6, Table 1).

Mechanistically, the final step seems to proceed via generation of a 4-chloroimine in situ at low temperature in the initial step, followed by the construction of fused quinoline ring at elevated temperature in the next step to afford the observed product **6**. A plausible mechanism showing the formation of compound **6** from a 4-chloroimine is depicted in Figure 3. The reaction may proceed via protonated imine intermediate, which then reacts with a second molecule of the 2-aminophenol by addition-elimination of HCl to form the N-C bond (Balasubramanian, K. K. *et al* 1977). The beta-amino imine, when protonated, would be able to undergo ringclosure and then subsequent aromatization to give compound **6** with regeneration of the 'second' 2-aminophenol molecule. To prove the intermediacy of 4-chloroimine, the reaction of **4a** with **5a** was carried out in DMF at 25 °C for 1.0 h where the corresponding 4-chloroimine **7** was isolated in 76% yield (Figure 4).

3. Antifungal activity

Six prepared compounds were tested for their in vitro antifungal activity. All experiments were performed in comparison with fluconazole, a known antifungal agent (Odds, F. C. *et al* 1986, Hoban, D. J. *et al* 1999). All evaluated compounds showed medium or high antifungal activity against all evaluated fungal strains. Their MICs ranged from 2 to 64 mg/L. The activities of the compounds are shown in Table 2. It is clear that All the compounds under test were found to be active against *M.gypseum* and *E.floccosum* higher than that of the standard fluconazole. And compounds **6a-6b** showed high in vitro antifungal activity.

4. Experimental

4.1 General

Unless stated otherwise, reactions were monitored by thin layer chromatography (TLC) on silica gel plates (60 F₂₅₄), visualizing with ultraviolet light. Flash chromatography was performed on silica gel (60-120 mesh) using distilled petroleum ether and ethyl acetate. ¹H NMR spectra were determined in CDCl₃ solution using Bruker 400 MHz spectrometer (Model RX-400, Switzerland). Proton chemical shifts (δ) are relative to tetramethylsilane (TMS, δ=0.0) as internal standard and expressed in parts per million. Spin multiplicities are given as s (singlet), d (doublet), t (triplet), and m (multiplet). Melting points were determined using a SPSIC melting point X-4 apparatus and are uncorrected. MS spectra were obtained on a Agilent 1100 Series Liquid Chromatograph/Mass Selective Detector (LC/MSD). All the reagents used are commercially available.

4.2 Synthesis of 4-chloro-2H-thiochromene-3-carbaldehydes (4a-c): general procedure

To a solution of the benzenethiol **1** (50 mmol) in 2M NaOH aqueous solution (20 mL) was added 3-chloropropanoic acid (8.1g, 75 mmol) at 25°C. The resulting solution was subjected to microwave irradiation for 5 min. After cooling to room temperature, the reaction mixture was neutralized with HCl, and poured into cold water to give the 3-(phenylthio)propanoic acid **2**, which was ring-closed in concentrated sulfuric acid to yield the thiochroman-4-one derivatives **3**. Phosphorus oxychloride (8.4mL) was added dropwise to DMF (27mL) over 50 min while the reaction temperature was kept below 16°C in an ice-bath. The mixture was stirred for further 30 min then a solution of thiochroman-4-ones **3** (36mmol) in DMF (8mL) was added dropwise and stirred for 18h at ambient temperature. The reaction mixture was poured into a mixture of sodium acetate (37g), water (140mL), and ethyl acetate (7mL) below -30°C, and stirred for 1h. The precipitate was collected by filtration and dried to give the 4-chloro-2H-thiochromene-3-carbaldehydes **4**.

4.2.1 4-chloro-6-methyl-2H-thiochromene-3-carbaldehyde (4a)

Light yellow solid; R_f 0.80 (20% ethyl acetate/ petroleum ether); mp 90-91 °C; IR (KBr): 2871.8, 1660.6, 1575.7, 1544.9, 1299.9, 1244.0; 1211.2, 1176.5; ¹H NMR (CDCl₃, 400 MHz) δ 10.32 (s, 1H, -CHO), 7.72 (s, 1H), 7.25-7.27 (m, 1H), 7.14 (d, J_{HH}=7.2 Hz, 1H), 3.68 (s, 2H), 2.38 (s, 3H); m/z (ES mass) 225.7 (M+1, 100%).

4.2.2 4-chloro-7-fluoro-6-methyl-2H-thiochromene-3-carbaldehyde (4b)

Deep yellow solid; R_f 0.80 (20% ethyl acetate/ petroleum ether); mp 53-55 °C; IR (KBr): 2873.7, 1661.6, 1541.0, 1475.4, 1307.7, 1272.9, 1218.9, 1139.9; ^1H NMR (CDCl_3 , 400 MHz) δ 10.30 (s, 1H, -CHO), 7.58 (d, $J_{\text{HH}}=10.6$ Hz, 1H), 7.19 (d, $J_{\text{HH}}=7.1$ Hz, 1H), 3.69 (s, 2H), 2.30 (s, 3H); m/z (ES mass) 243.7 (M+1, 100%).

4.2.3 4,6-dichloro-2H-thiochromene-3-carbaldehyde (4c)

Light yellow solid; R_f 0.80 (20% ethyl acetate/ petroleum ether); mp 84-85 °C; IR (KBr): 2864.1, 1658.7, 1577.7, 1533.3, 1450.4, 1299.9, 1224.7, 1099.4; ^1H NMR (CDCl_3 , 400 MHz) δ 10.31 (s, 1H, -CHO), 7.89 (d, $J_{\text{HH}}=1.7$ Hz, 1H), 7.30 (dd, $J_{\text{HH}}=11.5$, 3.3 Hz, 2H), 3.70 (s, 2H); m/z (ES mass) 246.1 (M+1, 100%).

4.3 Synthesis of 6H-thiochromeno[4,3-b]quinolines (6a-f): general procedure

To a solution of the 4-chloro-2H-thiochromene-3-carbaldehydes **4** (2.57 mmol) in DMF (5 mL) was added 2-aminophenol (280 mg, 2.57 mmol) at 25 °C under a nitrogen atmosphere. The resulting solution was stirred at 25 °C for 1.0 h under a nitrogen atmosphere. The resulting reaction mixture was then stirred at 120 °C for 2-4 h. After completion of the reaction (as indicated by TLC, mobile phase: 20% ethyl acetate/petroleum ether), solvent was removed under reduced pressure and the residue was purified by column chromatography using light petroleum ether/ethyl acetate to furnish the desired product.

4.3.1 2-methyl-6H-thiochromeno[4,3-b]quinolin-11-ol (6a)

Light yellow solid; R_f 0.50 (20% ethyl acetate/ petroleum ether); mp 143-145 °C; IR (KBr): 3369.4, 2889.2, 1496.7, 1477.4, 1334.7, 1240.1, 1226.7 cm^{-1} ; ^1H NMR (CDCl_3 , 400 MHz) δ 8.34 (s, 1H, -OH), 8.27 (s, 1H), 8.00 (s, 1H), 7.43 (t, $J_{\text{HH}}=15.8$ Hz, 1H), 7.30-7.34 (m, 2H), 7.16-7.19 (m, 2H), 4.10 (s, 2H), 2.48 (s, 3H); m/z (ES mass) 280.4 (M+1, 100%). Anal. calcd for $\text{C}_{17}\text{H}_{13}\text{NOS}$: C, 73.09; H, 4.69; N, 5.01. Found: C, 73.12; H, 4.71; N, 4.95.

4.3.2 2-methyl-9-nitro-6H-thiochromeno[4,3-b]quinolin-11-ol (6b)

Light yellow solid; R_f 0.56 (20% ethyl acetate/ petroleum ether); mp 274-276 °C; IR (KBr): 3332.1, 2887.8, 1493.5, 1334.9, 1229.2, 1121.1 cm^{-1} ; ^1H NMR (CDCl_3 , 400 MHz) δ 9.14 (s, 1H, -OH), 8.42 (d, $J_{\text{HH}}=2.4$ Hz, 1H), 8.30 (s, 1H), 8.27 (s, 1H), 8.02 (dd, $J_{\text{HH}}=7.8$, 1.6 Hz, 2H), 7.40 (d, $J_{\text{HH}}=2.4$ Hz, 1H), 4.16 (s, 2H), 2.52 (s, 3H); m/z (ES mass) 325.1 (M+1, 100%). Anal. calcd for $\text{C}_{17}\text{H}_{12}\text{N}_2\text{O}_3\text{S}$: C, 62.95; H, 3.73; N, 8.64. Found: C, 63.11; H, 3.78; N, 8.58.

4.3.3 3-fluoro-2-methyl-6H-thiochromeno[4,3-b]quinolin-11-ol (6c)

Light yellow solid; R_f 0.52 (20% ethyl acetate/ petroleum ether); mp 179-180 °C; IR (KBr): 3375.2, 2918.1, 1487.0, 1404.1, 1332.7, 1236.3, 1180.4, 1151.4 cm^{-1} ; ^1H NMR (CDCl_3 , 400 MHz) δ 8.16 (s, 1H, -OH), 8.00 (s, 1H), 7.44 (t, $J_{\text{HH}}=7.8$ Hz, 1H), 7.30 (d, $J_{\text{HH}}=8.2$ Hz, 1H), 7.23-7.26 (m, 2H), 7.17 (d, $J_{\text{HH}}=7.5$ Hz, 1H), 4.10 (s, 2H), 2.33 (s, 3H); m/z (ES mass) 298.3 (M+1, 100%). Anal. calcd for $\text{C}_{17}\text{H}_{12}\text{FNOS}$: C, 68.67; H, 4.07; N, 4.71. Found: C, 68.70; H, 4.18; N, 4.68.

4.3.4 3-fluoro-2-methyl-9-nitro-6H-thiochromeno[4,3-b]quinolin-11-ol (6d)

Light yellow solid; R_f 0.56 (20% ethyl acetate/ petroleum ether); mp 339-341 °C; IR (KBr): 3337.2, 2921.2, 1489.4, 1404.3, 1334.8, 1237.9, 1163.4, 1137.6 cm^{-1} ; ^1H NMR (CDCl_3 , 400 MHz) δ 9.04 (s, 1H, -OH), 8.43 (d, $J=2.4$ Hz, 1H), 8.02 (s, 1H), 7.42 (d, $J_{\text{HH}}=2.4$ Hz, 1H), 7.30-7.33 (m, 1H), 7.22 (d, $J_{\text{HH}}=8.1$ Hz, 1H), 4.12 (s, 2H), 2.35 (s, 3H); m/z (ES mass) 343.0 (M+1, 100%). Anal. calcd for $\text{C}_{17}\text{H}_{11}\text{FN}_2\text{O}_3\text{S}$: C, 59.64; H, 3.24; N, 8.18. Found: C, 59.84; H, 3.32; N, 8.09.

4.3.5 2-chloro-6H-thiochromeno[4,3-b]quinolin-11-ol (6e)

Light yellow solid; R_f 0.53 (20% ethyl acetate/ petroleum ether); mp 175-177 °C; IR (KBr): 3359.8, 2908.5, 1492.8, 1473.5, 1336.6, 1240.1, 1095.5 cm^{-1} ; ^1H NMR (CDCl_3 , 400 MHz) δ 8.50 (s, 1H, -OH), 8.15 (s, 1H), 7.99 (s, 1H), 7.46 (t, $J_{\text{HH}}=15.8$ Hz, 1H), 7.36 (d, $J_{\text{HH}}=8.3$ Hz, 1H), 7.31 (dd, $J_{\text{HH}}=8.3$, 3.2 Hz, 2H), 7.18 (dd, $J_{\text{HH}}=7.6$, 0.9 Hz, 1H), 4.12 (s, 2H); m/z (ES mass) 300.8 (M+1, 100%). Anal. calcd for $\text{C}_{16}\text{H}_{10}\text{ClNOS}$: C, 64.11; H, 3.36; N, 4.67. Found: C, 64.14; H, 3.38; N, 4.42.

4.3.6 2-chloro-9-nitro-6H-thiochromeno[4,3-b]quinolin-11-ol (6f)

Light yellow solid; R_f 0.57 (20% ethyl acetate/ petroleum ether); mp 340-342 °C; IR (KBr): 3322.6, 2908.6, 1492.2, 1468.7, 1335.6, 1109.4 cm^{-1} ; ^1H NMR (CDCl_3 , 400 MHz) δ 9.25 (s, 1H, -OH), 8.41 (d, $J_{\text{HH}}=3.1$ Hz, 1H), 8.16 (s, 1H), 8.02 (s, 1H), 7.44 (d, $J_{\text{HH}}=3.2$ Hz, 1H), 7.37 (dd, $J_{\text{HH}}=8.1$, 1.1 Hz, 2H), 4.12 (s, 2H); m/z (ES mass) 300.8 (M+1, 100%). Anal. calcd for $\text{C}_{16}\text{H}_9\text{ClN}_2\text{O}_3\text{S}$: C, 55.74; H, 2.63; N, 8.13. Found: C, 55.75; H, 2.88; N, 7.96.

4.4 Antifungal Activity

In vitro antifungal activities were measured by means of the minimal inhibitory concentrations (MIC) by consecutive double dilution method. The MIC means the lowest concentration of an antimicrobial agent that prevents visible growth of a microorganism in broth dilution susceptibility test (Marcelo C. Murguía *et al* 2008). The MIC was determined

according to the national committee for clinical laboratory standards (NCCLS) recommendations. Ten human opportunistic pathogenic fungi were tested, Fluconazole was taken as the reference drug for positive control. The compounds were dissolved in dimethyl sulfoxide (DMSO) (1 mL), further progressive dilutions by RPMI 1640 gave the required concentrations (64, 32, 16, 8, 4, 2, 1, 0.5, 0.25, 0.125 µg/mL); the fungi were prepared and adjusted to a final concentration of 0.5×10^4 – 2.5×10^4 CFU /mL. MIC values were determined by visual observation after 2-7d of incubation.

5. Conclusion

In conclusion, the reaction of 4-chloro-2H-thiochromene-3-carbaldehydes with 2-aminophenols in DMF was investigated, which yielded 6H-thiochromeno[4,3-b]quinolines thereby providing an easy method for the synthesis of these compounds. Therefore, the process may prove to be a powerful tool in the direct synthesis of thiochromenoquinolines based agents of potential pharmacological interest, preparation of which via other route may require lengthy synthetic procedure. Moreover, all the 6H-thiochromeno[4,3-b]quinolines were found to be active against *M. gypseum* and *E. floccosum* higher than that of the standard fluconazole.

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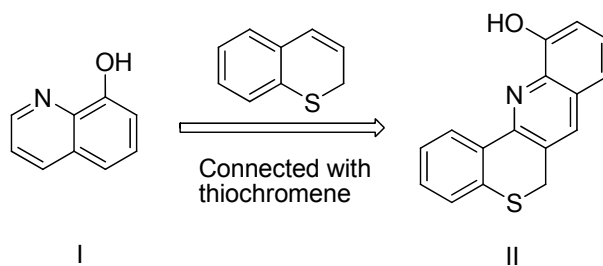


Figure 1. Design of the thiochromenoquinolines

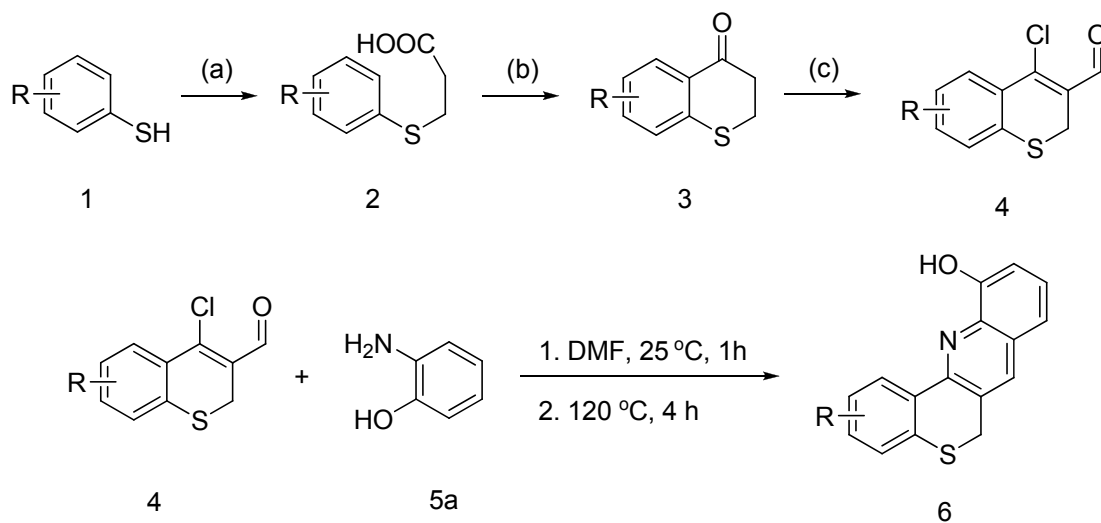
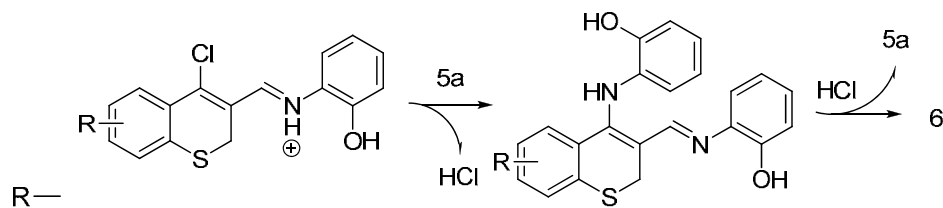
Figure 2. Preparation of 6H-thiochromeno[4,3-b]quinolines. Reagents and conditions: (a) 3-chloropropanoic acid, NaOH/H₂O, MW, 10min; (b) H₂SO₄, rt, 16 h; (c) DMF/POCl₃, 20h

Figure 3. Proposed reaction mechanism

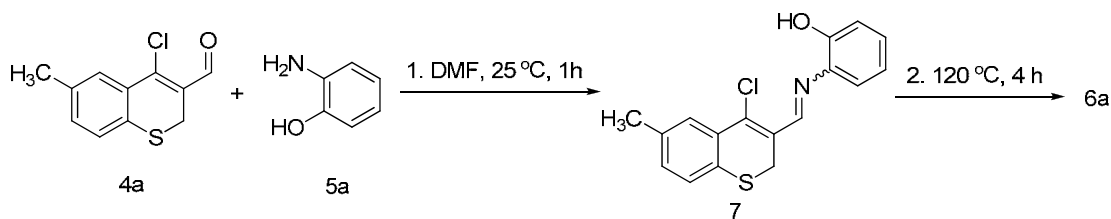


Figure 4. Preparation of 4-chloroimine (7) followed by 6a

Table 1. Synthesis of 6H-thiochromeno[4,3-b]quinolines

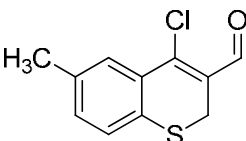
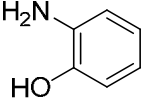
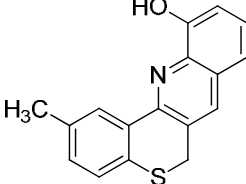
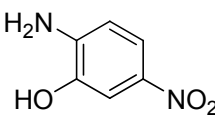
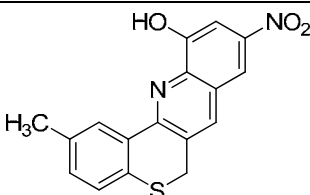
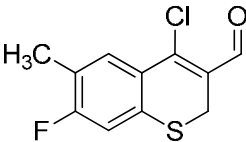
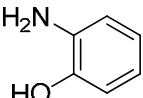
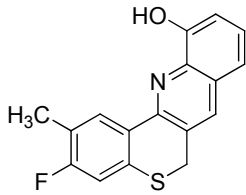
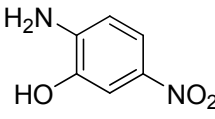
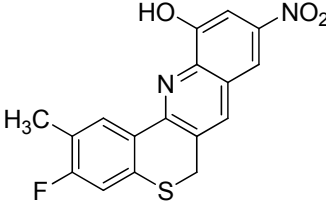
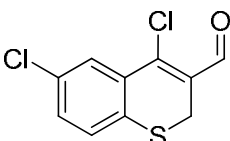
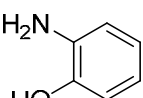
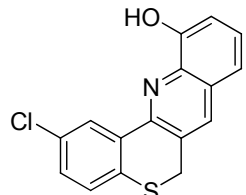
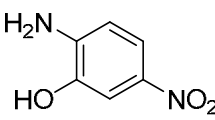
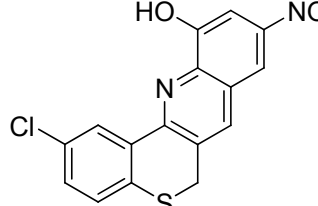
Entry	4-chloro-2H-thiochromene-3-carbaldehydes (4)	Aminophenols (5)	Products (6)	Yield (%)
1	 4a	 5a	 6a	67
2		 5b	 6b	72
3	 4b	 5a	 6c	79
4		 5b	 6d	65
5	 4c	 5a	 6e	71
6		 5b	 6f	74

Table 2. The capitals, assets and revenue in listed banks

Compound	MIC (mg/L)					
	C.albicas	C.neoformans	C. krusei	M.gypseum	E.floccosum	C. glabrata
6a	16	2	>64	4	4	>64
6b	32	8	32	16	8	8
6c	32	8	>64	8	16	>64
6d	32	4	32	8	16	8
6e	32	64	>64	16	32	64
6f	16	32	>64	8	4	16
FLU	0.5	4	64	64	>64	16