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Approach to the synthesis of a new analogue of sildenafil through palladium-catalyzed 3-methylsulfanyl-1,2,4-triazine - boronic acid cross coupling

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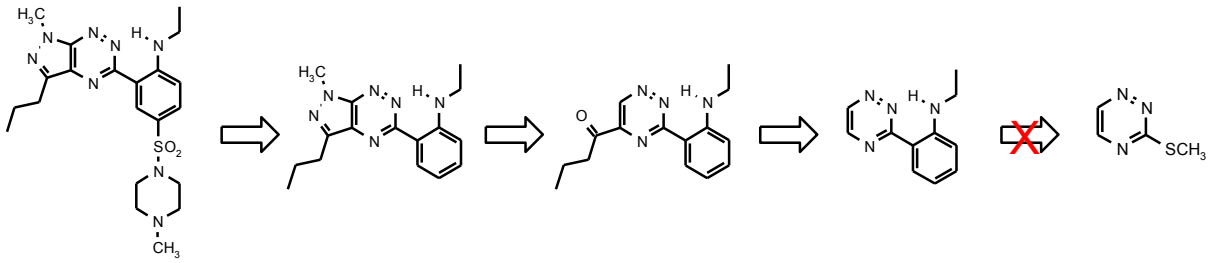
Graphical Abstract:



Abstract: In the search for more potent and selective PDE5 inhibitors, we designed an approach to synthesize a new sildenafil analogue **2**, characterized by the presence of the pyrazolo[4,3-*e*][1,2,4]triazine core and ethylamino group in the 2' position on the phenyl ring.

Keywords: *sulfonamides, 1H-pyrazolo[4,3-e][1,2,4]triazine, biological activity, PDE5 inhibitor*

Introduction

Sildenafil citrate (Viagra, Figure 1)[1] is the first orally effective phosphodiesterase type 5 (PDE5) inhibitor available for the treatment of male erectile dysfunction (MED), a common and important medical problem. Despite of the efficacy of sildenafil as a treatment for MED, clinically significant adverse side effects have been noted, such as nausea, headache, facial flushing, and visual disturbances, all of which are associated with sildenfil's non-specific inhibition of other PDE isozymes, most notably PDE1 and PDE6.[2,3] Therefore, the discovery of new and more specific PDE5 inhibitors is of great medicinal and commercial interest. The search for new PDE5 inhibitors with improved therapeutic efficacy is based on earlier work[4] on the structure of sildenafil **1**, which suggested that the 5'-sulfonamide moiety of the phenyl ring might reproduce the role of the cyclic phosphate of cGMP in binding the enzyme active site, thus leading to efficient and selective inhibition of PDE5. Moreover, intramolecular hydrogen bonding between the pyrimidinone NH and oxygen lone pair of the alkoxy group has been shown to play an important role in maintaining co-planarity between the phenyl and purine ring in the sildenafil analogues.[4] In addition it was suggested that the 2'-alkoxy moiety of the phenyl ring serves as a requirement for a small lipophilic substituent.[4] Thus, this work aims to identify more selective PDE5 inhibitors that lack sildenafil's side effects. Here we describe the synthesis of a new sildenafil *aza*-analogue **2** (Figure 1, right) belonging to the pyrazolo[4,3-*e*][1,2,4]triazine series, in which triazine ring nitrogen N1 replaces the C=O group present in the pyrimidinone moiety of sildenafil. Furthermore, the ethylamino group in position 2' of phenyl ring in analogue **2** allows for the formation of an intramolecular hydrogen bond between the phenyl ring and the pyrazolotriazine ring system (Figure 1).

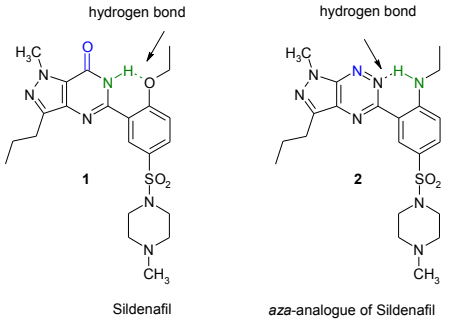


Figure 1: Sildenafil and *aza*-analogue of sildenafil.

Results & Discussion

Based on our previous study of the synthesis of sildenafil analogues[5] we propose a pathway (Figure 2) for the preparation of sildenafil analogue **2** with a 2-ethylamino substituent on the phenyl ring. It is clear that the preparation of 3-(2-ethylaminophenyl)-1,2,4-triazine (**7**) is a crucial step in this multistep synthesis.

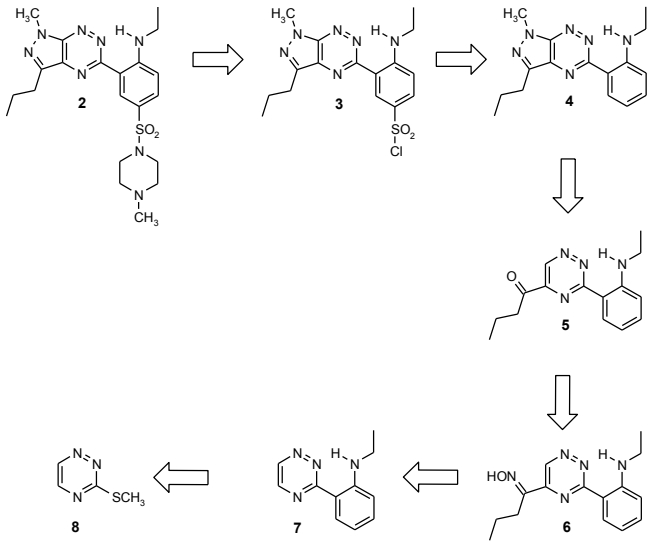


Figure 2: Retrosynthesis of sildenafil analogue **2**.

Moreover, the structure of 3-(2-ethylaminophenyl)-1,2,4-triazine (**7**) is useful to establish the place of the intramolecular hydrogen bond in the aza-analogue of sildenafil **2** because, as shown in Figure 3, the intramolecular hydrogen bond in analogue **2** can be observed between the hydrogen atom of the amino group and either nitrogen atom N2 (structure **2a**) or N4 (structure **2b**) in the triazine ring. Accordingly, the aim of this work is to optimize a synthetic route for the construction of 3-(2-ethylaminophenyl)-1,2,4-triazine (**7**) as a valuable intermediate for the synthesis of sildenafil analogue **2**.

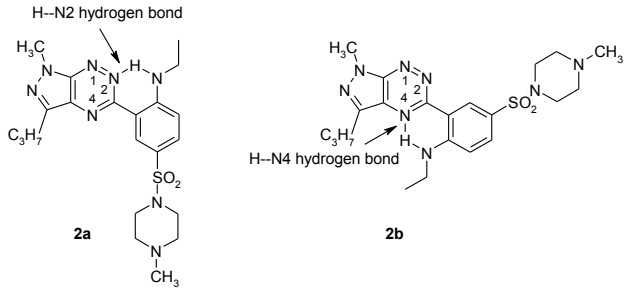


Figure 3: Theoretically possible location of hydrogen bonds in **2**.

Guillaumet et. al indicated that the palladium-catalyzed coupling reaction of 3-methylsulfanyl-1,2,4-triazine with different organoboron compounds in the presence of copper (I) 3-methylsalicylate (MeSalCu) produces the corresponding 3-substituted-1,2,4-triazines with good yield.[6] Thus, we studied whether a palladium-catalyzed replacement of the 3-methylthio group on *as*-triazine by 2-amino- or 2-ethylaminoboronic acid or its pinacol esters is possible for the synthesis a new *aza*-analogue of sildenafil. According to the literature[6], 3-methylsulfanyl-1,2,4-triazine (**8**) was treated with 2.2 equiv of 2-aminophenylboronic acid pinacol ester (**10**) in the presence of $Pd(PPh_3)_4$ (5% mol) and 2.2 equiv of the readily accessible CuMeSal[7] in anhydrous THF at 50°C for 24 hrs (Figure 4, conditions d). At this point, only starting triazine was observed in the reaction mixture. The failure of this experiment prompted us to investigate this process under different reaction conditions (Figure 4). However, no expected product **9** was observed in these experiments. Only starting triazine **8** was recovered.

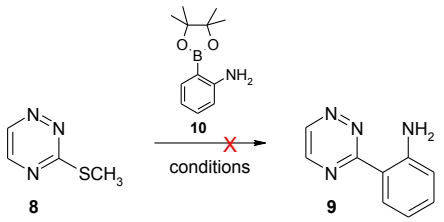


Figure 4: Conditions: (a) $[PdCl_2(dppf)]CH_2Cl_2$, CH_3COOK, THF, reflux; (b) $PdCl_2(dppf)$, CH_2Cl_2, K_2CO_3, 1,2-dioxane, DMSO, 100°C; (c) $Pd(PPh_3)_4$, K_2CO_3, 1,4-dioxane-H_2O (4:1), 70°C; (d) CuMeSal, $Pd(PPh_3)_4$, THF, reflux.

Additionally we have applied the reaction conditions presented in Figure 4 to study the reactions of triazine **8** with 2-aminophenylboronic acid (**11**) and 2-ethylaminophenyl boronic acid (**12**) (Figure 5). Monitoring the reaction by TLC showed exclusively starting material for all the conditions studied.

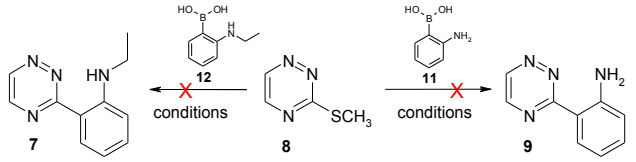


Figure 5: Conditions: (a) [$PdCl_2$(dppf)], CH_2Cl_2, CH_3COOK, THF, reflux; (b) [$PdCl_2$(dppf)], CH_2Cl_2, K_2CO_3, 1,4-dioxane, DMSO, 100^{o}C; (c) $Pd(PPh_3)_4$, K_2CO_3, 1,4-dioxane-H_2O (4:1), 70^{o}C; (d) CuMeSal, $Pd(PPh_3)_4$, THF, reflux;

It is noteworthy, that 2-aminophenylboronic acid (**11**) and 2-ethylaminophenylboronic acid (**12**) were prepared according to protocols described in the literature, starting from the appropriate commercially available pinacol ester **10** using BCl_3 in CH_2Cl_2.[8]

These unsuccessful attempts to synthesize compounds **9** and **7,** precursors for construction of analogue of sildenafil **2,** prompted us to investigate a Suzuki reaction using 3-halo-1,2,4-triazine. For this study we have chosen readily-available 3-bromo- and 3-chloro-5,6-diphenyl-1,2,4-triazine as the model substrates and different boronic compounds (Figure 6 and Table 1). Initially, the Suzuki reaction of the substrate **13a** with 2-aminophenylboronic acid pinacol ester (**10**) was investigated using protocols described in the literature[9] and conditions ($PdCl_2(PPh_3)_2/Na_2CO_3$/DMF/80^{o}C) to produce derivative **14** in low yield (8%). Next, the Suzuki reaction between 3-halo-1,2,4-triazines **13ab** and boronic compounds was carried out under a variety of reaction conditions (Table 1), and the best result was obtained when the reaction was carried out between **13b** and 2-aminophenylboronic acid pinacol ester **10** using $Pd(PPh_3)_4$, K_2CO_3, 1,4-dioxane/H_2O (4:1) reaction conditions.

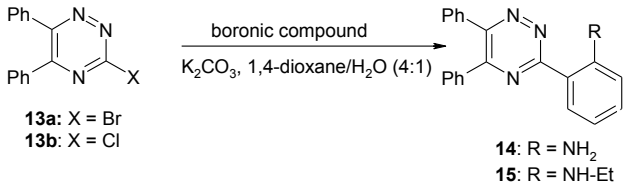


Figure 6: Suzuki coupling.

The lack of the reactivity of 2-aminophenylboronic acid (**11**) with 3-methylsulfanyl-1,2,4-triazine (**8**) and 3-halo-1,2,4-triazine under anhydrous conditions (in anhydrous THF or dioxane) could be due the specific nature of this boronic acid. Namely, Robinson et. al reported that 2-aminophenylboronic acid exists as a monomer in $(CD_3)_2SO/D_2O$ solution but as an asymmetric didehydro dimer both in anhydrous aprotic solution and in the solid state.[10]

Table 1. Optimization of reaction conditions for the conversion of **13a-b** into **14-15**.

X	Boronic comp.	Conditions	Product	Yield (%)
X=Br	**10**	($PdCl_2(PPh_3)_2$, Na_2CO_3,DMF, 80^{o}C	**14**	8
X=Cl	**11**	$Pd(PPh_3)_4$, K_2CO_3, 1,4-dioxane/H_2O (4:1), 70^{o}C.	**14**	58
X=Cl	**10**	$Pd(PPh_3)_4$, K_2CO_3, 1,4-dioxane/H_2O (4:1), 70^{o}C.	**14**	74
X=Br	**10**	$Pd(PPh_3)_4$, K_2CO_3, 1,4-dioxane/H_2O (4:1), 70^{o}C.	**14**	31
X=Cl	**10**	$Pd(PPh_3)_4$, 1,4-dioxane, MeSalCu, 100^{o}C.	**14**	0
X=Br	**12**	$Pd(PPh_3)_4$, K_2CO_3, 1,4-dioxane/H_2O (4:1), 70^{o}C.	**15**	54

Next, in an attempt to synthesize triazine derivative **9**, we incorporated a nitro group on the phenyl ring as a source for amino group (Figure 7).

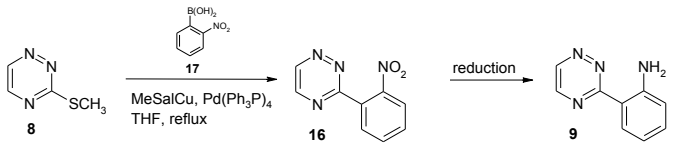


Figure 7: Attempt to the synthesize of 3-(2-aminophenylo)-1,2,4-triazine **9**.

Synthesis of the compound **16** was carried out by treating **8** with 2-nitrophenylboronic acid (**17**) according to procedure described in the literature.[6] The crude reaction mixture obtained after workup was purified by silica gel column chromatography using EtOAc/hexane as an eluent, furnishing target product **16** in less than 10% isolated yield. Due to the very low yield of derivative **16** we have not investigated transformations of **16** into **9**. In order to establish the position of intramolecular hydrogen bond in planar structure **2**, we used the previously-determined crystal structure of model compound **14**, shown in Figure 8.[11]

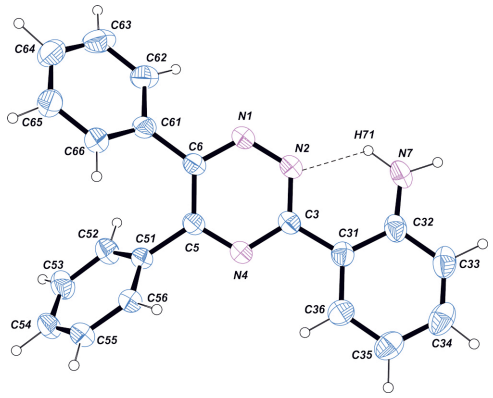


Figure 8: X-ray structure of **14** with the atomic labeling.[11]

One can see that the 3-aminophenyl-1,2,4-triazin system exists in the crystal in the *cis* conformation with the torsion angle N2–C3–C31–C32 of -7.9(2)°. This conformation is forced by the strong N7–H71…N2 intramolecular hydrogen bond [N7–H71 = 0.98(3), H71…N2 = 1.99(3), N7…N2 = 2.701(2) Å, N7–H71…N2 = 128(2)°].

The analysis of x-ray diffraction of suitable crystals of compound **14** confirmed the possibility of forming the N7–H…N2 intramolecular hydrogen bond and thereby stabilizing its *cis* conformation in the crystalline state, analogous to the active conformation of the sildenafil molecule.

Conclusion

In conclusion, here we have proposed a multistep methodology for the synthesis of a new sildenafil analogue **2,** in which the crucial step includes the Suzuki cross coupling reaction. However, our study has shown that a Suzuki coupling reaction between readily-available 3-methylsulfanyl-1,2,4-triazine (**8**), as well as 2-aminophenylboronic acid (**11**) and its derivatives, is impossible under tested conditions. The synthesis of 3-(2-aminophenyl)- or 3-(2-ethylaminophenyl)-1,2,4-triazine derivatives is possible using 3-halo-5,6-disubstituted-1,2,4-triazines and typical Suzuki cross coupling conditions. Applying this standard procedure, we have previously performed X-ray analysis on compound **14** to establish the position of the intramolecular hydrogen bond (N2-H71, Fig.8), which is of crucial relevance for the sildenafil and its analogues.[11]

Experimental procedures

Chemicals

Chemicals used in the synthesis of the compounds described were purchased from Sigma Aldrich in high purity.

Instruments

All melting points are uncorrected and were determined using a Boetius melting point apparatus. Nuclear magnetic resonance (^{1}H-NMR, ^{13}C-NMR) spectra were recorded on a Varian spectrometer at 400MHz (400 and 100 MHz, respectively) in a suitable deutered solvent using TMS as the internal standard. Elemental compositions are within ±0.4% of the calculated values.

Chemical Synthesis

General procedure for the synthesis of 3-(2-aminophenyl)-5,6-diphenyl-1,2,4-triazine (**14**) and 3-(2-ethylaminophenyl)-5,6-diphenyl-1,2,4-triazine (**15**):

Boronic compound (0.22 mmol) in 1,4-dioxane/water mixture (4:1) (2.5 mL), solution of K_2CO_3 (0.6 mmol) in 1 mL, and $Pd(PPh_3)_4$ (23 mg, 0.02 mmol) were added to a solution of 3-halo-5,6-diphenyl-1,2,4-triazine (0.2 mmol). The reaction mixture was stirred at 70°C for 12h. After that time, the solution was diluted with H_2O (1.5 mL), and then the product was extracted three times with CH_2Cl_2 (3x2mL). The combined organic layer was dried over Na_2SO_4 and the solvent was removed *in vacuo*. The crude product was then subjected to column chromatography using CH_2Cl_2:hexane (4:1) as the eluent.

14: mp 161.°C. ^{1}H-NMR (400 mHz, DMSO) δ: 6.85 (d, J = 7.2 Hz, 1H), 7.31 (t, J = 8.0 Hz, 1H), 7.35-7.45 (m, 6H), 7.60 (d, J = 8.0 Hz, 2H), 7.65 (d, J = 8.0 Hz, 2H), 8.61 (d, J = 8.0 Hz, 1H). ^{1}C-NMR (100 MHz, DMSO) δ:167.6, 164.3, 162.9, 155.2, 153.9, 147.7, 135.9, 135.4, 132.7, 130.8, 130.7, 130.7, 129.8, 129.5, 129.3, 128.8, 128.6, 128.5, 117.9.

15: Light yellow oil. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 1.40 (t, J = 7.2 Hz, 3H), 3.39 (q, J = 7.2 Hz, 2H), 6.79 (t, J = 7.2 Hz, 1H), 6.84 (d, J = 8.4 Hz, 1H), 7.17-7.19 (m, 1H), 7.25-7.28 (m, 1H), 7.37-7.46 (m, 5H), 7.61 (d, J = 6.4 Hz, 2H), 7.67 (d, J = 7.2 Hz, 2H), 8.73 (d, J = 8.0 Hz, 1H), 8.91 (bs, 1H, NH). ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 163.1, 154.6, 153.4, 149.8, 136.0, 135.6, 133.1, 131.0, 130.5, 129.7, 129.3, 129.2, 129.1, 128.9, 128.5, 128.4, 128.1, 125.2, 115.0, 114.6, 111.4, 37.7, 14.6.

Synthesis of 3-(2-nitrophenyl)-1,2,4-triazine (**16**):

To a solution of 3-methylsulfanyl-1,2,4-triazine (64 mg, 0.5 mmol), MeSalCu (269 mg, 1.25 mmol) and 2-nitrophenylboronic acid (208 mg, 1.25 mmol) in dry THF (5 mL) under argon atmosphere, solid $Pd(PPh_3)_4$ (58 mg, mmol) was added. The reaction mixture was stirred overnight at reflux, then wasquenched with a Na_2CO_3 saturated solution and extracted with dichloromethane. The combined organic phases were dried over $MgSO_4$ and concentrated *in vacuo*. After purification by column chromatography on silica gel, (hexane : CH_2Cl_2, 5:1), the desired product was obtained as a

yellow powder. Yield was 8%. Light yellow oil. ^{1}H-NMR (400 MHz, CDCl3) δ: 7.71 (d, J = 6.4 Hz, 1H), 7.78 (t, J = 7.6 Hz, 1H), 7.98 (d, J = 8.0 Hz, 1H), 8.68 (d, J = 7.6 Hz, 1H), 8.70 (d, J = 2.4 Hz, 1H), 9.27 (d, J = 2.4 Hz, 1H). Anal. Calcd. for $C_9H_6N_4O_2$ (202.17): C, 53.47; H, 2.99; N, 27.71. Found: C, 53.37, H, 3.03; N, 27.68.

Acknowledgments

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