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# Compounds containing 2-substituted imidazole ring for treatment against human African trypanosomiasis

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ABSTRACT

A series of compounds containing 2-substituted imidazoles has been synthesized from imidazole and tested for its biological activity against human African trypanosomiasis (HAT). The 2-substituted 5-nitroimidazoles such as fexinidazole (**7a**) and 1-[4-(1-methyl-5-nitro-1*H*-imidazol-2-ylmethoxy)-pyridin-2-yl-piperazine (**9e**) exhibited potent activity against *T. brucei* in vitro with low cytotoxicity and good solubility. The presence of the $NO_2$ group at the 5-position of the imidazole ring in 2-substituted imidazoles is the crucial factor to inhibit *T. brucei*.

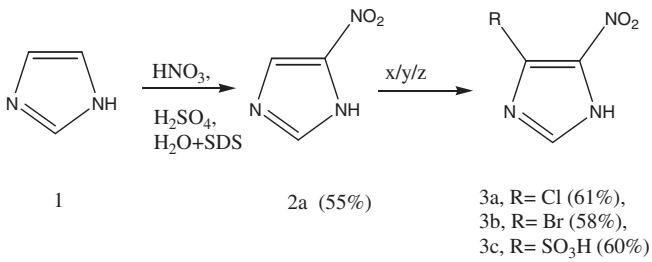


Human African trypanosomiasis (HAT), commonly known as sleeping sickness, is one of the most neglected diseases in the Tropics.[1] HAT is a vector-borne disease caused by the parasitic protozoa belonging to the Genus *Trypanosoma*.[2–4] The protozoan is transmitted to humans by bites of blood sucking tsetse flies (*Glossina* Genus) infected from biting human beings or animals harboring the human pathogenic parasites.[5] Sleeping sickness is a threat to millions of people around 36 countries of sub-Saharan Africa.

The drugs used for the treatment of HAT have various drawbacks; for example, an intravenous drug melarsoprol causes a life-threatening syndrome.[6,7] Hence, an oral drug without harmful side effects, acting against acute and chronic stages of the disease and which can be synthesized easily and cost effectively is always appealing. In this respect, the members of azole class of compounds like 2-substituted-5-nitroimidiazoles (e.g., fexinidazole) qualify as promising drug candidates. Their advantages, especially of fexinidazole, include oral administration and curing of chronic sleeping sickness in the brain within two weeks. The phase I human trials of fexinidazole began in 2009.[8,9] The synthesis processes in the literature for various 2-substituted-5-nitroimidiazoles describe the use of either costly starting materials,[10,11] catalysts,[12] and reaction conditions,[13] or compounds that are not easily available. Thus, there is a demand for a synthesis process, which uses simple, inexpensive reaction conditions, and starting compounds.

As a part of our ongoing research on novel chemical entities with antitrypanosomal activities, we hereby like to introduce a synthesis of different derivatives of 2-substituted 5-nitroimidazoles from easily available, economically and ecologically viable starting compounds (i.e., imidazole), and evaluate their biological activity against HAT. This series of compounds contain 2-substituted imidazole ring as a backbone structure; by substituting different functional groups on this backbone, we studied structural–activity relationship with respect to their ability to fight against HAT.

Regioselective nitration at 5-position of imidazole (**1**), using nitrating mixture (nitric acid and sulfuric acid) in an aqueous surfactant solution to form 5-nitroimidazole (**2a**) as a product (Scheme 1) was the first step of the synthesis process.[14,15] The spatial orientation of substrate **1** in micelles increased the selectivity towards 5-nitroimidazole. The use of surfactant as a catalyst in the reaction media to increase the reaction rate towards particular




**Scheme 1.** Synthesis of 4-substituted-5-nitro-1*H*-imidazole. [x]Oxychlorination; [y]oxybromination; [z]sulfonation. Values in brackets indicate isolated yields.

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product is well known.[16] Nitration reaction of **1** in pure water was carried out to form 4-nitroimidazole (**2b**); in order to substitute a $NO_2$ group at the 4-position of imidazole (to obtain 1-methyl-2-(4-methylsulfanyl-phenoxymethyl-)-4-nitro-1*H*-imidazole **18** as the final product of total synthesis).

Different substitution reactions on 5-nitroimidazole (**2**) were carried out as exemplified in Scheme 1. A cost effective oxyhalogenation method was applied to obtain 4-chloro-5-nitro-1*H*-imidazole (**3a**) and 4-bromo-5-nitro-1*H*-imidazole (**3b**) by using hydrogen peroxide and corresponding acid.[16] Hydrochloric acid-hydrogen peroxide and sulfuric acid-hydrogen peroxide-sodium bromide systems were used for chlorination and bromination, respectively. Oxidation of hydrochloric acid by hydrogen peroxide produced hypochlorous acid (HOCl), which then formed the electrophilic $Cl^+$ species in the reaction mixture. In the case of bromination, hydrogen peroxide and sodium bromide formed hypobromous acid (HOBr), which ultimately gave $Br^+$ species and NaOH. The generated $Br^+$ species on reaction with aromatic compound formed brominated aromatic compound, while sulfuric acid neutralized NaOH. The oxyhalogenation reaction conditions were simple, with no hazardous side products, and with satisfactory yields (61% for **3a** and 58% for **3b**). Sulfonation of **2** by Sulfuric acid formed 5-nitro-4-sulfoimidazole (**3c**) with satisfactory yield (60%).

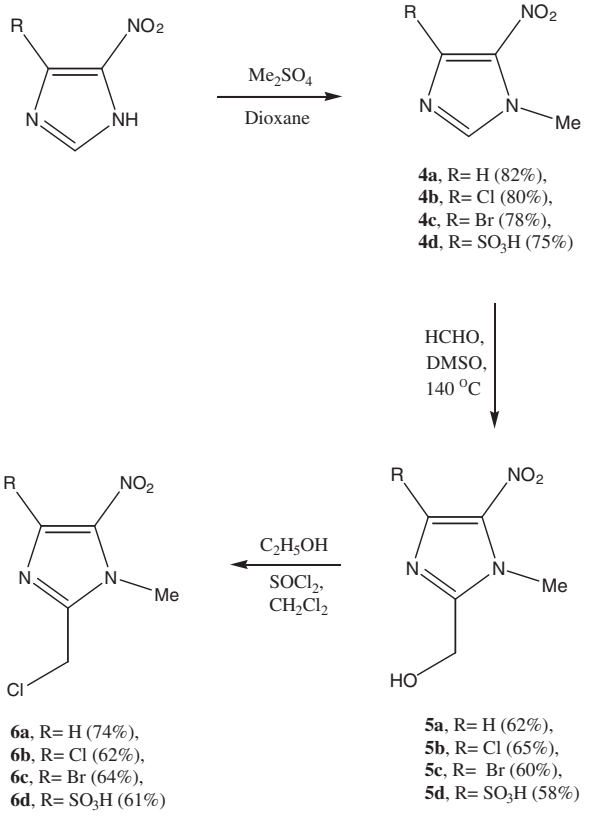
Methylation of these substituted imidazoles (**2** and **3a–c)**, by the action of dimethyl sulfate in dioxane, was carried out to obtain 80% average yield of methylated products **4a–d** (Scheme 2). These methylated derivatives of substituted imidazoles (**4a–d**), on reaction with formaldehyde in DMSO, gave the corresponding methylated imidazolyl methanol derivatives (**5a–d**) as products in excellent yield. This reaction provided substitution on the 2-position of the imidazole ring.

Compounds **5a**–**d** reacted with thionyl chloride in chloroform to give corresponding 2-chloromethyl-1-methyl-5-substituted imidazole (**6a**–**d**) as product. For this reaction, a solution of compound **5** in dichloromethane was added drop wise into pre cooled (0 °C) mixture of thionyl chloride and dichloromethane. This mixture was then heated to reflux for half an hour. The concentrate, which was obtained after evaporation of solvent, was dissolved in ethanol and again refluxed for 15 min to afford product **6**. Williamson ether synthesis reaction was used to generate fexinidazole (**7a**), its derivatives (**7b**–**d**), and other 2-substituted 5-nitro-imidazoles (**9a**–**c**) by the condensation of compound **6** with 4-(methylmercapto)-phenol or (*p*-hydroxyphenyl methyl sulfide) (Table 1); and the compound **6a** with corresponding substituted alcohols (Table 2), respectively.[17]

Three different methods such as Zn metal catalysis reaction in the microwave, in normal oil bath, and using $Cs_2CO_3$ and KI as the catalysts were used to synthesize the final 2-substituted 5-nitro-imidazoles (**7a–d** and **9a–e**). Though, the percentage yield for microwave method is less than oil bath method, the reaction time for microwave is shorter (1.45 h) compared to other methods. Lower yield in the microwave method was due to the formation of impurities in the reaction media. There was no significant improvement in the product yield after the change in temperature, or reduction in time for the microwave reaction. We also synthesized the analogues of fexinidazole utilizing the same protocol to study the changes in the biological activity against HAT, after replacing $NO_2$ group with other functional groups (Me, Cl, Br and $SO_3H$).

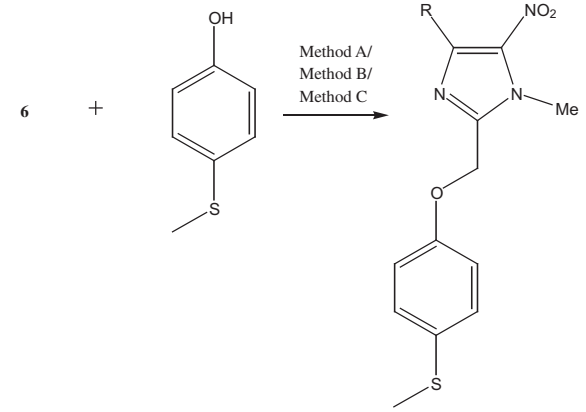
The series of 2-substituted imidazoles has been tested for its biological activity against HAT. These compounds were evaluated on the basis of their ability to inhibit cell proliferation of *T. brucei rhodesiense* in culture. The growth inhibitory activity against L-6 rat skeletal muscle myoblast cells was determined to establish a cellular therapeutic index. The solubility of each compound at acidic and basic pH was determined.

Fexinidazole (**7a**) showed effective *T. brucei rhodesiense* inhibitory activity and low cytotoxicity (Table 3, entry 1). The *T. brucei* cell proliferation inhibition ability by three derivatives of fexinidazole was also tested. 4-Chloro-1-methyl-2-(4-methylsulfanyl-phenoxymethyl)-5-nitro-1*H*-imidazole (**7b**), 4-bromo-1-methyl-2-(4-methylsulfanyl-phenoxymethyl)-5-nitro-1*H*-imidazole (**7c**), and 1-methyl-2-(4-methylsulfanyl-phenoxymethyl)-5-nitro-1*H*-imidazole-4-sulfonic acid (**7d**) showed higher inhibition activity with higher solubility than fexinidazole; however, their cytotoxicity was also high. The presence of an additional electron



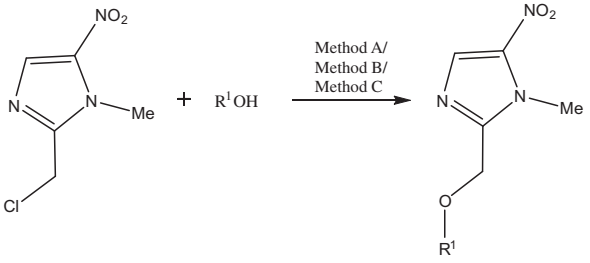

**Scheme 2.** Synthesis of 4-substituted 2-chloromethyl-1-methyl-5-nitro-1*H*-imidazoles (**6a**–**d**). Values in brackets indicate isolated yields.

**Table 1**
Isolated yields of fexinidazole and its derivatives by three different methods[17]




| Entry | Product | Method A (%) | Method B (%) | Method C (%) |
|---|---|---|---|---|
| 1 | **7a**, R = H | 25 | 55 | 60 |
| 2 | **7b**, R = Cl | 20 | 52 | 55 |
| 3 | **7c**, R = Br | 17 | 49 | 57 |
| 4 | **7d**, R = $SO_3H$ | 19 | 47 | 52 |

**Table 2**
Isolated yields of various 2-substituted 5-nitro-imidazoles (**9a–e**) by three different methods[17]




6a 9

| Entry | Product | Method A (%) | Method B (%) | Method C (%) |
|---|---|---|---|---|
| 1 | **9a**, $R^1$ = Ph | 33 | 62 | 72 |
| 2 | **9b**, $R^1$ = Pyridine | 28 | 57 | 61 |
| 3 | **9c**, $R^1$ = 2-Methyl-pyridine | 22 | 52 | 58 |
| 4 | **9d**, $R^1$ = 2-Ethyl-pyridine | 25 | 55 | 57 |
| 5 | **9e**, $R^1$ = 1-Pyridine-2-yl-piperizine | 24 | 56 | 55 |

withdrawing group (like Cl, Br and $SO_3H$) on the imidazole ring increased the *T. brucei* inhibitory activity. On comparing the *T. brucei* inhibitory activities of the compounds **7b–d** (Table 3, entries 2–4), it was observed that compound **7c** showed less potent *T. brucei* inhibitory activity than compounds **7b** and **7d**. This may be due to the steric and less electronegative nature of Br (which is present on the imidazole ring in compound **7c**) than Cl and $SO_3H$. However, there is no significant difference between cytotoxicity and solubility of the compounds **7b–d**. The substitution of Cl, Br and $SO_3H$ groups along with the presence of the $NO_2$ moiety on the imidazole ring causes an increase in the electrophilicity of this ring and confers activity against *T. brucei*.

To evaluate the importance of the $NO_2$ group on an imidazole ring with respect to the inhibition of *T. brucei* cell proliferation, we compared the activities of compounds **7a** and **8**. Compound **8** had weak *T. brucei* inhibitory activity (Table 3, entry 5) indicating an important factor with respect to the required position of $NO_2$ group on the imidazole ring. The only difference between the structures of compounds **7a** and **8** is the position of $NO_2$ group on an imidazole ring. However, there is a remarkable difference in their *T. brucei* inhibitory activities. This proves that an electron withdrawing $NO_2$ group on the imidazole ring is an essential factor to inhibit the *T. brucei* and should be present at the 5-position of the imidazole ring.

The importance of $NO_2$ group position may be related to the structural arrangement of the five-member imidazole ring with the carbon atom containing $NO_2$ (5-position), which is adjacent to the electron donating tertiary amine group (1-position) as in compound **7a**. This distinctive feature in the structure may be the cause of *T. brucei* inhibitory activity. This is in accordance with an earlier study of 2-substituted nitro imidazoles for their biological activity against HAT.[18,19]

Further, to study the effect of an electron donating group at 2-position of imidazole on *T. brucei* inhibitory activity of 2-substituted 5-nitro imidazoles, we analyzed compounds **9a–e**. We observed that *T. brucei* inhibitory activity increased from compound **9a** to **9e** and surprisingly **9e** showed higher *T. brucei* inhibitory activity than fexinidazole (**7a**). The compound **9e** also had low cytotoxicity and good solubility. The reason for this enhancement in *T. brucei* inhibitory activity may be the balance between the electron donating group at the 2-position and strong electron withdrawing group at 4-position ($NO_2$) of the imidazole ring in **9e**.

Concurrently, we replaced $NO_2$ with other electron withdrawing groups (Cl, Br and $SO_3H$) in fexinidazole and also substituted two halo groups on 4- and 5-positions of an imidazole ring to test the effect of different and/or more number of electronegative groups on *T. brucei* inhibitory activity (compounds **11–17**, Supplementary data Table S1). However, these attempts failed to give any enhancement in *T. brucei* inhibitory activity for these compounds. Only the solubility of these compounds increased effectively (~9-fold) due to the presence of halogen functional groups in the structure.

The exact mechanism of action for this series of compounds is yet to be elucidated. However, the above results indicate that the compounds with 2-substituted-5-nitroimidiazole as the backbone structure have an effective *T. brucei* inhibitory activity. This might be due to their electrophilic center and the presence of a phenyl ring, which is considered to be a good source of electron density, and can react with nucleophile (i.e., cysteine and lysine) favorably in the target. The substitution of Cl, Br and $SO_3H$ groups along with the presence of the $NO_2$ moiety on the imidazole ring causes an increase in electrophilicity of this ring. These groups also act as good leaving groups in the binding step of the cysteine thereby, conferring activity against *T. brucei*. It seems that these compounds act as a suicide inhibitor of the enzyme which regulates cell division by catalysing the first step in polyamine biosynthesis. As the inhibitor has short half-life in humans, it is rapidly degraded while the parasite cannot metabolise it quick enough to survive.

In summary; a series of compounds containing 2-substituted imidazole ring has been synthesized from imidazole and tested for its biological activity against HAT including its cytotoxicity and solubility. Compound **9e** with an electron donating group at

**Table 3**
Pharmacological properties of 2-substituted imidazoles

| Entry | Compound | $IC_{50}$ versus *T. brucei*[a] (μM) | $IC_{50}$ versus L-6 rat skeletal myoblast cells[a] (μM) | Aq solution solubility[b] (μM) | |
|---|---|---|---|---|---|
| | | | | pH 1.5 | pH 7.5 |
| 1 | **7a** | 0.70 ± 0.1 | 377 ± 25 | 5.5 ± 0.4 | 2.5 ± 0.1 |
| 2 | **7b** | 0.25 ± 0.1 | 0.2 ± 0.05 | 17.1 ± 0.8 | 5.9 ± 0.2 |
| 3 | **7c** | 0.55 ± 0.1 | 0.8 ± 0.1 | 16.7 ± 0.5 | 5.8 ± 0.2 |
| 4 | **7d** | 0.37 ± 0.1 | 0.2 ± 0.1 | 22.8 ± 1.5 | 7.9 ± 0.5 |
| 5 | **8** | >30 | >400 | 5.1 ± 0.5 | 2.5 ± 0.1 |
| 6 | **9a** | >30 | >400 | 5.7 ± 0.5 | 2.1 ± 0.1 |
| 7 | **9b** | >30 | >400 | 4.8 ± 0.5 | 2.7 ± 0.1 |
| 8 | **9c** | 27 ± 3 | 286 ± 15 | 4.3 ± 0.5 | 2.2 ± 0.1 |
| 9 | **9d** | 25 ± 3 | 243 ± 10 | 5.9 ± 0.5 | 3.1 ± 0.1 |
| 10 | **9e** | 0.67 ± 0.1 | 255 ± 10 | 5.6 ± 0.5 | 3.5 ± 0.1 |

[a] $IC_{50}$ values are means standard deviations; three independent experiments were done with replicates of three.
[b] Solubility of compounds was determined at 25 °C.

the 2-position, and the strong electron withdrawing group at 4-position ($NO_2$) seems to be the crucial factor to show an effective *T. brucei* inhibitory activity along with low cytotoxicity and good solubility. Substituting other electron withdrawing groups Cl, Br, and $SO_3H$ on 4-position of imidazole in fexinidazole seems to increase *T. brucei* inhibitory activity and solubility; however, cytotoxicity also increased for these compounds (**7b–d**). Although an exact mechanism of action is still unknown for this series of compounds, it is observed that the presence of $NO_2$ group at 5-position of an imidazole ring is an essential factor to illustrate *T. brucei* inhibitory activity.

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## Supplementary data

Supplementary data (detail synthesis process and characterization of all synthesized products (**2**–**10**), detail procedure for adaptation of bloodstream trypanosomes to liquid in vitro culture, trypanosoma brucei proliferation assay, general cytotoxicity assay and pharmacological properties of compounds **11**–**17** (Table S1)) associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.12.040.

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17. *Method A*: *p*-Hydroxyphenyl methyl sulfide (0.8 g, 5.7 mmol), 2-chloromethyl-1-methyl-5-substituted imidazoles (**6a**–**d**) (1 g, 5.7 mmol, **6a**), Zn powder (0.09 g, 1.42 mmol) in DMF (10 mL), microwave 150 °C, 1.45 h; Method B: *p*-Hydroxyphenyl methyl sulfide (0.8 g, 5.7 mmol), 2-chloromethyl-1-methyl-5-substituted imidazoles (**6a**–**d**) (1 g, 5.7 mmol, **6a**), THF (5 mL), 55 °C, 14 h; Method C: 2-Chloromethyl-1-methyl-5- substituted imidazoles (**6a**–**d**) (1 g, 5.7 mmol, **6a**), *p*-hydroxyphenyl methyl sulfide (0.8 g, 5.7 mmol), $Cs_2CO_3$ (6.6 g, 34.2 mmol), KI (0.095 g, 0.57 mmol) in MeCN under inert atmosphere at 80 °C reflux for overnight. Please refer supporting information for detail procedures and analytical spectroscopic information of products.
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