Synthesis and Structure–Activity Relationships of Imidazopyridine/Pyrindine- and Furopyridine-Based Anti-infective Agents against Trypanosomiases

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Neglected tropical diseases remain among the most critical public health concerns in Africa and South America. The drug treatments for these diseases are limited, which invariably leads to fatal cases. Hence, there is an urgent need for new antitrypanosomal drugs. To address this issue, a large number of diverse heterocyclic compounds were prepared. Straightforward synthetic approaches tolerated pre-functionalized structures, giving rise to a structurally diverse set of analogs. We report on a set of 57 heterocyclic compounds with selective activity potential against kinetoplastid parasites. In general, 29 and 19 compounds of the total set could be defined as active against Trypanosoma cruzi and T. brucei brucei, respectively (antitrypanosomal activities < 10 μM). The present work discusses the structure–activity relationships of new fused-ring scaffolds based on imidazopyridine/pyrimidine and furopyridine cores. This library of compounds shows significant potential for anti-trypanosomiases drug discovery.

Introduction

Trypanosomiases caused by the unicellular protozoan parasites Trypanosoma brucei and T. cruzi are economically significant obstacles to human welfare. Human African Trypanosomiases

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Introduction

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Drug discovery and development is a complex endeavor since new candidates need to meet acceptable pharmacological endpoints combined with favorable safety profiles. Moving from target identification to lead generation is laborious, but our compound D may represent a potential hit for trypanosomiases,[13] and was therefore studied further in the present work.

Imidazopyridine/pyrimidine fused rings have been employed in drugs such as antipsychotics, anxiolytics, analgesics, and migraine therapeutics, demonstrating drug-like features associated with the core structures.[10,15–17] The furo[2,3-b]-pyridine has recently received extensive attention as a useful pharmacophore in different therapeutic areas.[18–21] We also identified promising selective bioactive compounds containing furopyridine as a central core against different drug-resistant strains of mycobacteria for tuberculosis.[22] However, this class of compound has not yet been employed for antitrypanosomal agents.

To enlarge the chemical space of heterocycles as potential anti-infective agents against trypanosomiases, we aimed to explore, develop, and modify the central core of the hit compound D (bottom of Figure 1) and perform SAR analysis based on biological assays against T. cruzi, T. brucei, and mammalian cells.

Results and Discussion

Designed library and synthesis of heterocycles

The biochemical targets or mechanisms of action of the hit compound D are unknown. A new set of compounds was designed by dividing the general scaffold (Figure 1) into regions and specific insertion of modifications at the positions V–Z and in regions R1–R3 on the central core of the fused ring. The modifications to the general scaffold are shown in Schemes 1 and 2.

A relatively simple synthetic pathway (low cost, few steps and good yields) was amenable to many functional groups, giving rise to a structurally diverse set of analogs (Scheme 1). Synthesis of the compounds 1–5, 7 and 16–19 started with a condensation reaction of the appropriate amino pyrimidine/pyridine and bromoacetophenone, which resulted in the intermediate endowed with the imidazopyrimidine or imidazopyridine cores (I). Following the reaction with triphosgene, a corresponding isocyanate was obtained, which provided a way for the formation of urea derivatives D and 15 (II). The reaction with the appropriate acyl chloride provided the amide derivatives 8–14 (II). Upon Mannich reaction, we explored aminoalkylations in region R2 of the imidazopyrimidin/pyridine scaffold III (analogos 6, 20–40, 56 and 57).

Compounds 1–3 are imidazopyrimidine fragment-like containing a free amine group (R1) and bearing different phenyl groups at the 2-position of the imidazole portion of the backbone. Compound 4 is an analog of compound 2 without the free amine and the pyrimidine nitrogen at the position W. Compounds 5–7 are imidazopyridine fragment-like bearing 3,4-
difluorophenyl (5 and 6) and phenyl groups (7) at the 2-position of the imidazole region of the backbone (R²). In addition, compound 6 has a (dimethylamine)methyl in region R² and compound 7 has a methyl group at the position R¹ and X.

Analogs 8–14 are imidazopyrimidine amides retaining a phenyl substituent on the same imidazole portion mentioned before and showing modifications in region R³. Compound D and 15 have an electron withdrawing substituents on the imidazopyrimidine system (R³) with a 3-fluoropyrrolidinyl urea in region R³.

Compounds 16–19 have a 6-methyl-2-phenylimidazo[1,2-a]pyridine as the central core and diverse phenyl groups in region R³. Additionally, a set of 20 alkylamine-type compounds in the

Scheme 1. Synthetic routes and sets of modifications into the imidazopyridine/pyrimidine I (~ 21–92 %), II (~ 54–86 %) and Mannich-type scaffolds III (~ 38–97 %). Reagents and conditions: a) appropriate bromoacetophenone, NaHCO₃, MeOH, reflux, 12 h; b) triphosgene, Et₃N, CH₂Cl₂, 0 °C and then appropriate 2 °amine, 0 °C to 25 °C, 15 h or c) appropriate acyl chloride, Et₃N, CH₂Cl₂, 0 °C to 25 °C, 16 h; d) appropriate amine, formalin, acetic acid, CH₂Cl₂, 18 h.

Scheme 2. Synthetic routes and sets of modifications into the furopyridine scaffold (V: ~ 14–41 %; VI: 18–67 %; VII: 87 %; VIII: 44 %). Reagents and conditions: e) appropriated acyl chloride, DBU, DMAP, CH₂Cl₂, RT, overnight; f) mCPBA, CH₂Cl₂, RT, 48 h; g) appropriate 2 °amine, PyBroP, DIPEA, MeCN, RT, 18 h; h) LiOH, THF/EtOH/H₂O, 55 °C, 24 h; i) MeNH·HCl, EDCI, HOBt, DMF, Et₃N, 25 °C, 4 h.

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side chain of the imidazo[1,2-al]pyridine core ring was synthesized. The synthetic route provided efficient ways to introduce substituents via the Mannich reaction at region R² (analogs 21–40). The hit compound D embedded with the 4-fluorophenylpiperazinyl group resulted in compound 56. Compounds 20 and 57 are side products of the Mannich reaction, and we isolated and purified these compounds as well.

We took the opportunity to employ robust synthetic routes to further alter the internal portion of the imidazopyridine core and introduce different functional groups at different positions of the general scaffold, which led to the fused ring furopyridine derivatives 41–55 (Scheme 2). Recently our group reported a concise strategy to synthesize and decorate this core through C–H activation reaction. The synthesis of furo[2,3-b]pyridines started from pyridine-N-oxide derivative (IV). Under mild, metal-free conditions, we synthesized the furopyridines derivatives 41–44 with appropriated acyl chlorides (V). In addition, we functionalized this heteroaromatic core through C–H amination (VI, compounds 45–53). Carboxylic acid derivative 54 (VII) was obtained through ester hydrolysis. The following coupling reaction afforded the amide derivative 55 (VII). Analogs 41–45 are furopyridines bearing different phenyl groups in region R¹ of the backbone (2-position) and an ethyl ester group in the side chain in region R² of the furan moiety (3-position). Compounds 46–55 have a methyl or isopropyl group in region R² and N-cycloalkyl groups at different positions (R³, V or X).

Antitrypanosomal activities and cytotoxicity

Compounds 1–55 were tested in vitro against T. cruzi and T. brucei using EC₅₀ and EC₉₀ protocols.[25] Assay results are presented in Table 1. Additionally, we retested the hit compound D and the new compounds 56 and 57 using IC₅₀ protocols against T. cruzi and T. brucei (Table 1). Experimental details are provided in Supporting Information and in the literature.[24–26]

Fast analysis of the antitrypanosomal activities reveals that 14 compounds are totally inactive against both parasites (EC₅₀ values of T. cruzi and T. brucei > 20.0 μM). Additional 17 compounds are also inactive against T. brucei, but some of them displayed high potencies against T. cruzi. Notably, this set of compounds exhibited better T. cruzi than T. brucei activities. Twenty-nine compounds showed anti-T. cruzi potency and 19 compounds showed anti-T. brucei potency lower than 10 μM. It suggests that a more detailed analysis of the underlying structure–activity relationships would be of interest.

Furthermore, cytotoxicity was evaluated in mammalian cells (lymphocytic cells – CRL-8155 and hepatocellular cells – HepG2) and selectivity index (SI) calculated for selected compounds and compared to the published results for compound D.[15] (Table 2).

Only compounds 28 and 52 exhibited low toxicity to the CRL-8155 cell line, at 33.0 and 23.70 μM, respectively. The remaining compounds exhibited no considerable or detectable toxicity to either cell line.

The selectivity ratio of compounds for T. cruzi and T. brucei parasites over each of the two cell lines was calculated. As an example, the most active compound 15 exhibited a selectivity index (SI) > 625 for T. cruzi and > 1667 for T. brucei against either cell line, comparable to the hit compound D.

Compounds D, 56 and 57 were also screened against T. b. rhodesiense, Leishmania infantum and cytotoxicity for MCR-5 and PMM cells. Besides the already known biological activity of the compound D against T. cruzi and T. brucei,[15] this compound also exhibited high biological activity against T. b. rhodesiense (IC₅₀ of 0.11 μM). Compounds 56 and 57 showed anti-T. b. rhodesiense activities of 0.90 μM and 1.06 μM, respectively. However, these three compounds were not potent against L. infantum. Marginal cytotoxicity was observed only for compound D against PMM cells (IC₅₀ of 48.0 μM) and no cytotoxicity against MRC-5 cell line.

Structure–activity relationships (SAR)

We discussed the SAR of heterocyclic compounds based on the wide range of biological activity obtained against T. cruzi and T. brucei for compounds D and 1–57. In order to accomplish this in a feasible way, we divided and discussed the substitutions systematically introduced into specific regions (R¹–R³) and positions (V–Z) of 3 distinct general scaffolds: imidazopyrimidine (15 analogs), imidazopyridine (28 analogs) and furopyridine (15 analogs). The following, we discuss the most representatives SARs established in this work.

The attempts to optimize the imidazopyrimidine scaffold is shown in Figure 2. The different regions of the molecule (R¹–R³) were explored with 14 variants. At the left of Figure 2 the SAR analysis for the region R¹ is presented. At the bottom and top right the SAR analysis for the region R² and variants for the region R³, respectively. Analogs 1–15 were assayed against T. cruzi (highlighted in green), T. brucei (highlighted in orange) and the results are expressed as EC₅₀ (μM).

Compounds 1 and 8–14 bear a phenyl group in region R² and hydrogen atom in region R³. Thus, the evaluation of the SAR was based on replacements in region R¹. The addition of a phenyl carbamate group (1 vs 8) or even the introduction of a nitro group at para-position of the phenyl moiety (8 vs 9) had no effect in the potency against both parasites. However, moving the nitro group to meta-position enhanced the potency against T. brucei by more than 5-fold (9 vs 10). Compound 10 showed an EC₅₀ value against T. brucei of 3.88 μM. Replacing the oxygen atom in the phenyl carbamate group for a methylene linker (8 vs 11) resulted in low anti-T. cruzi activity (11, EC₅₀ T. cruzi of 14.20 μM). It appears that the cyclic aliphatic amide is better than an aromatic group in the region R¹ for achieving potency against T. cruzi (8 vs 12). Cyclohexylacetamide derivative 12 had an EC₅₀ value against T. cruzi of 6.27 μM.

T. cruzi activity was not affected by the exploration of the substitution pattern on the aromatic ring. Compounds 13 (R¹ = 4-cyanophenyl) and 14 (R¹ = 3,4-difluoro phenyl) showed an EC₅₀ value against T. cruzi around 10.0 μM, although the effect of the cyano group was more pronounced against T. brucei (EC₅₀ T. brucei of 4.45 μM) than against T. cruzi (EC₅₀ T. cruzi of 10.33 μM).
Table 1. Antitrypanosomal activities of heterocyclic compounds

| Compd. | R^1 | R^2 | R^3 | V, W, X = CH unless otherwise noted | T. cruzi | T. brucei |
|--------|-----|-----|-----|-----------------------------------|----------|----------|
|        |     |     |     |                                   | EC_{50} | EC_{50}  |
|        |     |     |     |                                   | [μM]    | [μM]    |
| 1      | NH_2 | H   | phenyl | V = N                            | >20.0   | >20.0   |
| 2      | NH_2 | H   | 3,4-difluorophenyl | V = N                        | >20.0   | >20.0   |
| 3      | NH_2 | H   | 3-trifluoromethylphenyl | V = N          | >20.0   | >20.0   |
| 4      | H    | H   | 3,4-difluorophenyl | W = N                        | >20.0   | >20.0   |
| 5      | H    | H   | 3,4-difluorophenyl | –                          | >20.0   | >20.0   |
| 6      | H    | CH_N(CH_3)_2 | 3,4-difluorophenyl | –                       | >20.0   | >20.0   |
| 7      | CH_3 | H   | phenyl | X = CH                           | 7.2     | 13.4    |
| 8      | phenoxy | H | phenyl | –                                 | >20.0   | >20.0   |
| 9      | 4-nitrophenox | y H | phenyl | –                                 | >20.0   | >20.0   |
| 10     | 3-nitrophenox | y H | phenyl | –                                 | 9.8     | 3.9     |
| 11     | benzyl | H | phenyl | –                                 | 14.2    | 20.0    |
| 12     | cyclohexylmethyl | H | phenyl | –                                 | 6.3     | 12.3    |
| 13     | 4-cyanophenyl | H | phenyl | –                                 | 10.3    | 4.4     |
| 14     | 3,4-difluoro | H | phenyl | –                                 | 10.5    | >20.0   |
| 15     | (3S)-3-fluoropyrrolidin-1-yl | H | 3-trifluoromethylphenyl | –                    | 0.08    | 0.09    |
| 16     | –    | H   | phenyl | –                                 | >20.0   | >20.0   |
| 17     | –    | H   | 4-methylphenyl | –                         | >5.0    | >5.0    |
| 18     | –    | H   | 4-chlorophenyl | –                        | >5.0    | >10.4   |
| 19     | –    | H   | 3,4-difluorophenyl | –                      | >5.0    | >12.3   |
| 20     | –    | CH_2OH | 3,4-difluorophenyl | –                     | >5.0    | >20.0   |
| 21     | –    | CH_3-N(CH_3)_2 | phenyl | –                                | >20.0   | >20.0   |
| 22     | –    | 3-(trifluoromethyl)phenyl | phenyl | –                               | >20.0   | >20.0   |
| 23     | –    | pyridin-1-ylmethyl | phenyl | –                               | >20.0   | >20.0   |
| 24     | –    | piperidin-1-ylmethyl | phenyl | –                              | 15.0    | >20.0   |
| 25     | –    | morpholin-4-ylmethyl | phenyl | –                             | 13.4    | >20.0   |
| 26     | –    | (3S)-3-fluoropyrrolidin-1-ylmethyl | phenyl | –                         | 8.6     | >20.0   |
| 27     | –    | 4-phenylpiperazin-1-ylmethyl | phenyl | –                        | 5.2     | 9.7     |
| 28     | –    | 4-(2,3-dichlorophenyl)piperazin-1-ylmethyl | phenyl | –                      | 3.3     | 6.5     |
| 29     | –    | 4-(4-chlorophenyl)piperazin-1-ylmethyl | phenyl | –                      | 1.9     | 6.5     |
| 30     | –    | 4-phenylpiperazin-1-ylmethyl | 3,4-difluorophenyl | –                     | 1.6     | 3.6     |
| 31     | –    | 4-(4-fluorophenyl)piperazin-1-ylmethyl | 3,4-difluorophenyl | –                   | 1.0     | 3.6     |
| 32     | –    | 4-[3-(trifluoromethyl)phenyl]piperazin-1-ylmethyl | 3,4-difluorophenyl | –                 | 1.6     | 4.1     |
| 33     | –    | 4-(4-fluorophenyl)piperazin-1-ylmethyl | 4-chlorophenyl | –                | 1.1     | 3.3     |
| 34     | –    | 4-[3-(trifluoromethyl)phenyl]piperazin-1-ylmethyl | 4-chlorophenyl | –            | 1.7     | >5.0    |
| 35     | –    | 4-(4-fluorophenyl)piperazin-1-ylmethyl | 4-methylphenyl | –               | 1.0     | 3.4     |
| 36     | –    | 4-[3-(trifluoromethyl)phenyl]piperazin-1-ylmethyl | 4-methylphenyl | –          | 1.5     | >5.0    |
| 37     | –    | 4-cyclohexylpiperazin-1-ylmethyl | 3,4-difluorophenyl | –     | 2.6     | >5.0    |
| 38     | –    | 4-[2-(pyridin-3-yl)ethyl]piperazin-1-ylmethyl | 3,4-difluorophenyl | –         | 1.8     | >5.0    |
| 39     | –    | 4-benzylpiperidin-1-ylmethyl | 3,4-difluorophenyl | –             | 1.6     | 4.7     |
Table 1. continued

| Compd. | R\(^1\) | R\(^2\) | R\(^3\) | T. cruzi \(\text{EC}_{50}\) [\(\mu M\)]* | T. brucei \(\text{EC}_{50}\) [\(\mu M\)]*** |
| --- | --- | --- | --- | --- | --- |
| 40 | – | 4-phenylpiperidin-1-yl | methyl | 3,4-difluorophenyl | – | 1.1 | >5.0 | 7.6 | 9.6 |
| 41 | H | COOCH\(_3\), CH\(_3\), | phenyl | – | 14.7 | >20.0 | >20.0 | >20.0 |
| 42 | H | COOCH\(_3\), CH\(_3\), | 4-fluoro-phenyl | – | 13.8 | >20.0 | 18.9 | >20.0 |
| 43 | H | COOCH\(_3\), CH\(_3\), | 3,4-difluorophenyl | – | 10.9 | >20.0 | 10.3 | >20.0 |
| 44 | H | COOCH\(_3\), CH\(_3\), | 4-methylphenyl | – | 8.6 | 18.9 | 17.5 | >20.0 |
| 45 | (3S)-3-fluoropyrrolidin-1-yl | COOCH\(_3\), CH\(_3\), | 4-methylphenyl | – | 10.9 | >20.0 | >20.0 | >20.0 |
| 46 | (3S)-3-fluoropyrrolidin-1-yl | COOCH\(_3\), CH\(_3\), | CH\(_3\) | – | 17.3 | >20.0 | >20.0 | >20.0 |
| 47 | H | COOCH\(_3\), CH\(_3\), | CH\(_3\) | V = C-(3S)-3-fluoropyrrolidin-1-yl | 18.9 | >20.0 | 14.5 | >20.0 |
| 48 | morpholin-4-yl | COOCH\(_3\), CH\(_3\), | CH\(_3\) | – | 17.4 | >20.0 | >20.0 | >20.0 |
| 49 | morpholin-4-yl | COOCH\(_3\), CH\(_3\), | C(CH\(_2\))\(_2\) | – | 4.8 | 18.4 | >20.0 | >20.0 |
| 50 | 4-phenylpiperazin-1-yl | COOCH\(_3\), CH\(_3\), | CH\(_3\) | – | 3.4 | 6.4 | >20.0 | >20.0 |
| 51 | 4-phenylpiperazin-1-yl | COOCH\(_3\), CH\(_3\), | C(CH\(_2\))\(_2\) | – | 11.0 | >20.0 | >20.0 | >20.0 |
| 52 | H | COOCH\(_3\), CH\(_3\), | C(CH\(_2\))\(_2\) | X = C-4-phenylpiperazin-1-yl | 7.1 | 9.2 | 9.8 | 13.8 |
| 53 | pyrrolidin-1-yl | COOCH\(_3\), CH\(_3\), | C(CH\(_2\))\(_2\) | – | 4.5 | >20.0 | >20.0 | >20.0 |
| 54 | pyrrolidin-1-yl | COOH | C(CH\(_2\))\(_2\) | – | >20.0 | >20.0 | >20.0 | >20.0 |
| 55 | pyrrolidin-1-yl | CONHCH\(_3\) | C(CH\(_2\))\(_2\) | – | >20.0 | >20.0 | >20.0 | >20.0 |
| ***56 | (3S)-3-fluoropyrrolidin-1-yl | 4-(4-fluorophenyl)piperazin-1-yl | 3,4-difluorophenyl | – | 10.4 | >20.0 | >20.0 | >20.0 |
| ***57 | (3S)-3-fluoropyrrolidin-1-yl | CH\(_3\)OH | 3,4-difluorophenyl | – | 18.9 | >20.0 | >20.0 | >20.0 |
| ***D | (3S)-3-fluoropyrrolidin-1-yl | H | 3,4-difluorophenyl | – | 0.73 | <0.1 | – | – |

Compounds 1–55. * the values are averages of triplicate data. Benznidazole was used as a control compound for the T. cruzi assay, with EC\(_{50}\) and EC\(_{90}\) values (average ± SEM) of 0.65 ± 0.10 μM (n = 5) and 1.64 ± 0.09 μM (n = 5). ** Pentamidine was used as a control compound for the T. brucei assays, with EC\(_{50}\) and EC\(_{90}\) values (average ± SEM) of 1.24 ± 0.23 nM (n = 5) and 6.20 ± 2.12 nM (n = 5). Compounds 56, 57 and D: ***activity is expressed as IC\(_{50}\) values in μM concentrations. The values of T. cruzi and T. brucei activities are averages of duplicate data (average ± SD). Benznidazole (IC\(_{50}\) of 2.43 ± 0.45 μM) and Suramine (IC\(_{50}\) of 0.06 ± 0.01 μM) were used as reference drugs to measure T. cruzi and T. brucei activities, respectively.

Compounds 3 and 15 are analogs bearing 3-trifluoromethylphenyl substituent in region R\(^1\) and hydrogen atom in region R\(^2\). Substantial enhancement of activity was achieved by the introduction of a 3-fluoropyrrolidinyl urea group in region R\(^3\) of the imidazopyrimidine system. This substitution enhanced the potency of compound 15 more than 250-fold against T. cruzi and more than 660-fold against T. brucei, when compared to compound 3 (R\(^1\) = –NH\(_2\)). Compound 15 exhibited an EC\(_{50}\) value against T. cruzi of 0.08 μM and against T. brucei of 0.03 μM (Figure 2).

The same pattern can be observed for the amide derivatives compared to the urea derivatives D and 15, the presence of the urea group attached to the region R\(^3\) of the fused ring system proved to be essential for bioactivity. Compounds D and 15 are nanomolar inhibitors of T. cruzi and T. brucei, while some of the amide derivatives showed moderate activity only against one of these parasites.

According to the literature compounds bearing methyl-NN-substituted amines (phenylpiperazines) could be an attractive group to increment potency for anti-infective agents against trypanosomiasis.\(^{27–29}\) We then used the strategy of combining chemical substructures found in the literature, with the highly active compound D to derive new compounds, but keeping the skeleton unaltered. Embedding the 4-
All the results are expressed as EC₅₀.

T. cruzi and T. brucei activities, respectively. Green and orange dashed arrows represent the loss of the fluorophenylpiperazinylmethyl (R⁴) into the compound D with the combination of 3-fluoropyrrolidinyl urea group in region R¹ and 3,4-difluorophenyl group in region R² did not improve the antitrypanosomal activities (D vs 56). The activity of compound 56 decreased more than 4-fold against both parasites.
The incorporation of the hydroxymethyl functional group (–CH₂OH) in region R₁ (57) resulted in similar antitrypanosomal activities as compound 56. However, hydroxymethyl group can be a versatile point of diversification to access other target compounds; to alter physicochemical properties such as lipophilicity (logP), solubility and through hydrogen-bonding interactions, the mode of binding of the pharmacophore.²³ In these two compounds the combination of the best moiety present in region R₁ and R₃ of analog D, plus the alteration of the imidazo portion has not shown to be synergistic, i.e., compounds 56 and 57 risen from this approach have shown bioactivities lower than D. Aiming to explore and evaluate the SAR for modifications only in region R₃, a neutral scaffold was chosen. That means an absence of substituents in region R₃ and an exchange of a basic nitrogen atom for a methyl group at the 6-position of the imidazopyrimidine core (Figure 2); which turned into a new scaffold 6-methylimidazo[1,2-a]pyridine (Figure 3).

The introduction of dimethylmethanamine (21), 3-(trifluoromethyl)phenylaminemethyl (22) and pyrrolidinylmethyl (23) as substituents in region R₂, resulted in inactive compounds (Figure 3). Replacements with piperidinylmethyl (24), EC₅₀ of 15.04 μM, morpholinylmethyl (25, EC₅₀ of 13.40 μM) and (35)-3-fluoropyrrolidinylmethyl groups (26, EC₅₀ of 8.61 μM) showed only low potency against T. cruzi (Figure 3, highlighted in green).

However, the insertion of a phenylpiperazinylmethyl moiety in region R₂ of the imidazole portion (Figure 3, bottom right) showed to be promising to enhance antitrypanosomal activity (16 vs 28 and 29). The introduction of chloride atoms at the phenyl group attached at the piperazine at the 4- and 2,3-positions enhanced only Anti-T. cruzi potency. Phenylpiperazines derivatives 28 and 29 exhibited EC₅₀ values of 3.31 and 1.90 μM, respectively. In fact, upon embedding phenylpiperazinylmethyl in region R₂ of the imidazole portion an enhancement in potency for the T. brucei compared to T. cruzi potencies was not observed. While some compounds showed EC₅₀ about 1.0 μM against T. cruzi, most of the compounds showed EC₅₀ above to 5.0 μM against T. brucei.

Bulky aminoalkyl groups in the side chain of imidazopyridine derivatives are clearly required for better anti-T. cruzi activity. About 11 compounds bearing bulky aminoalkyl groups achieved potency lower than 2 μM only against T. cruzi.

Moreover, inserting electron-withdrawing groups (31 and 32) or exchanging the aromatic portion of the piperazine (37–39) did not modify the potency against T. cruzi (Figure 3, bottom left). At the same time, the activities of compounds 37–39 reveal that the presence of more flexible groups in region R₂ did not contribute to enhancing the potency against T. brucei. The compounds display above 7 times better T. cruzi potency than T. brucei potency.

Furthermore, the SAR were explored diversifying phenyl groups in region R₁ of the imidazole portion. The methylation...
(17), chlorination (18) or difluorination (19) conferred a slight improvement in potencies compared to 16 (Figure 3, top right). A similar pattern was observed for the pairs of compounds containing replacements of the aromatic ring attached in region $R^1$ (pairs: 31 vs 33; 32 vs 34; 31 vs 35; 32 vs 36, Table 1).

Subsequently, altering the core ring system from an imidazopyridine/pyrimidine to a furo[2,3-b]pyridine allowed us to expand the chemical space of new heterocyclic compounds as potential antitrypanosomal agents. We selected substituents from our previous SAR and we also introduced new modifications into the core ring of furopyridines, such as aliphatic groups in region $R^2$ or functional groups in region $R^3$. Figure 4 shown the selected SAR analysis for each region.

Fluorination or methylation (41 vs 42–44) of the aromatic ring attached to the 2-position of furopyridine ($R^3$) had no impact on the potency of this class of compounds. Despite that, isopropyl and methyl groups appeared to be more promising impact on the potency of this class of compounds. Despite that, the absence of the functional urea group in this furopyridine class of compounds could be an explanation for no enhancement in the potency. In other words, the imidazopyrimidine series of compounds required a functional group containing a carbonyl group bonded to two nitrogen atoms. For example, compounds D and 15 achieved nanomolar potency against both parasites. In summary, our findings in the SAR analysis can guide further studies with this class of compounds.

**Conclusion**

The imidazopyridines/pyrimidines and furopyridines planned and obtained herein allowed us to build a SAR study by means of cell assays against *T. cruzi*, *T. brucei*, and mammalian cells. By exploring the chemical diversity of three different heteroaromatic cores and introducing various groups at eight different positions of the general scaffold within this study, we were able to enlarge the chemical space of heterocycles as potential antityranosomal agents. The central core was embedded with urea, amide, ester, and organic acid functions. However, the presence of the urea group attached to the 7-position ($R^1$) of the fused ring system proved to be essential for bioactivity (D and 15 are nanomolar inhibitors of *T. cruzi* and *T. brucei*).

We introduced the furopyridine derivatives as a new core ring to be developed for antityranosomal agents. We explored aromatic and aliphatic replacements and employed different organic functions at the 3-position of the furan ring portion, respectively. In addition, we embedded four different groups at three

![Figure 4](https://www.chemmedchem.org)  
Figure 4. Modifications of the furo[2,3-b]pyridine core ring. Compounds assayed against *T. cruzi* (highlighted in green)/*T. brucei* (highlighted in orange). Results are expressed as EC$_{50}$ (μM). Green solid or dashed arrows represent the improvement or the loss of the *T. cruzi* activities, respectively. Gray arrow represents no improvement of the antityranosomal activities.
positions of the pyridine portion. However, we still need to combine promising substituents found through SAR in order to improve the potency of furopyridines as anttrypanosomal agents. The best replacements identified in this SAR studies, will guide the design and selection of the novel compounds that can be transitioned further into drug development for these parasitic infections.

Author contributions

The manuscript was written through the contributions of all authors. All authors have given approval to the final version of the manuscript. D.G.S. planned and synthesized 47 compounds. S.M.G.M. and F.F. planned and synthesized 10 compounds. Biological assays were performed by J.R.B., N.M., and A.M. Discussions to develop this work. Open access funding enabled University of Münster (WWU) in Germany and also for helpful

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Conflict of Interest

The authors declare no conflict of interest.

Keywords: Anti-infectives · heterocyclic · T. brucei · T. cruzi · in vitro

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