Bioorg. Med. Chem. Lett. 37 (2021) 127843

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Promising hit compounds against resistant trichomoniasis: Synthesis and antiparasitic activity of 3-(ω-aminoalkoxy)-1-benzyl-5-nitroindazoles

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ARTICLE INFO

Keywords:
Antiprotozoal agents
Indazole
Nitrogen heterocycles
Trichomonas vaginalis
Resistance

ABSTRACT

A series of 11 3-(ω-aminoalkoxy)-1-benzyl-5-nitroindazoles (**2**–**12**) has been prepared starting from 1-benzyl-5-nitroindazol-3-ol **13**, and evaluated against sensitive and resistant isolates of the sexually transmitted protozoan *Trichomonas vaginalis*. Compounds **2**, **3**, **6**, **9**, **10** and **11** showed trichomonacidal profiles with IC_{50} < 20 μM against the metronidazole-sensitive isolate. Moreover, all these compounds submitted to cytotoxicity assays against mammalian cells exhibited low non-specific cytotoxic effects, except compounds **3** and **9** which displayed moderate cytotoxicity (CC_{50} = 74.7 and 59.1 μM, respectively). Those compounds with trichomonacidal effect were also evaluated against a metronidazole-resistant culture. Special mention deserve compounds **6** and **10**, which displayed better IC_{50} values (1.3 and 0.5 μM respectively) than that of the reference drug (IC_{50} MTZ = 3.0 μM). The high activity of these compounds against the resistant isolate reinforces the absence of cross-resistance with the reference drug. The remarkable trichomonacidal results against resistant *T. vaginalis* isolates suggest the interest of 3-(ω-aminoalkoxy)-1-benzyl-5-nitroindazoles to be considered as good prototypes to continue in the development of new drugs with enhanced trichomonacidal activity, aiming to increase the non-existent drugs to face clinical resistance efficiently for those patients in whom therapy with 5-nitroimidazoles is contraindicated.

Parasitic diseases make a larger contribution to the global burden of human infections, taking special consideration the neglected infections of poverty (NIPs)[1,2] and the neglected tropical diseases (NTDs).[3] Among the former, trichomoniasis is one of the main sexually transmitted infections (STI) worldwide, caused by the protozoan parasite *Trichomonas vaginalis*. This cosmopolitan STI presents special epidemiological prevalence in low-income countries, with an estimated prevalence of 42.8 million cases in Africa, 28.7 million in South-East Asian Region or 57.8 million in America.[4] However, the current epidemiology of trichomoniasis, calculated in 156 million cases,[5] is probably underestimated because it is not a disease subject to compulsory declaration and there are no surveillance programs. Of special relevance is the fact that *T. vaginalis* infection is associated with 1.52–2.74-fold increased risk of HIV infection.[6,7] Some researches have estimated that almost 20% of HIV infections could be attributed to *T. vaginalis* infection due to the greater susceptibility of HIV shedding in patients with trichomoniasis.[8]

On the other hand, the main risks of trichomoniasis in the developed countries are the high relationship of this STI and the development of cervical and prostate neoplasias,[9,10,11] reproductive outcomes including association with other STI, pelvic inflammatory disease and pregnancy complications.[12]

Nevertheless, and despite the aforementioned drawbacks, trichomoniasis has been considered as a neglected disease highly associated with low-income social groups[1,2] and has been included in the group of the five CDCs neglected parasitic infections (NPIs) due to the little attention that has been devoted by public health organisms.[13]

Metronidazole (MTZ) has been the treatment of choice for *T. vaginalis* in the last six decades. This 5-nitroimidazole and its related compound tinidazole are the only two drugs approved by the FDA for trichomoniasis treatment.[14] The most common adverse reactions are gastrointestinal side effects including nausea, dry mouth, metallic taste, etc. Hypersensitivity to metronidazole is also reported with common allergic signs, like urticaria, facial edema, flushing or anaphylactic shock in the worst cases.[15] For refractory episodes and adverse reactions, the

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https://doi.org/10.1016/j.bmcl.2021.127843
Received 2 December 2020; Received in revised form 16 January 2021; Accepted 26 January 2021
Available online 5 February 2021

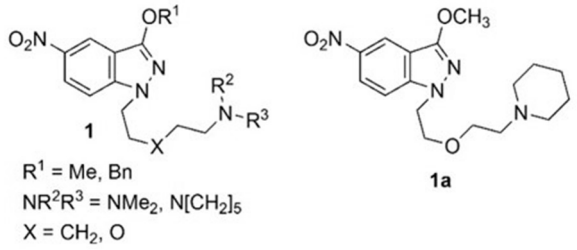
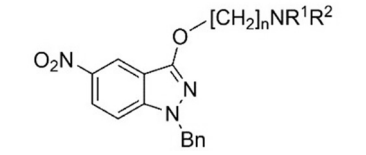


Fig. 1. Structures of the previously studied 3-alkoxy-1-(5-aminopentyl and 5-amino-3-oxapentyl)-5-nitroindazoles **1**.[18]



n = 2	NR^1R^2	n = 5	NR^1R^2
2	NH_2	**9**	NH_2
3	NHMe	**10**	NHMe
4	NMe_2	**11**	NMe_2
5	$N[CH_2]_5$	**12**	$N[CH_2]_5$

n = 3	NR^1R^2
6	NH_2
7	NMe_2
8	$N[CH_2]_5$

Fig. 2. Structures of 3-(*ω*-aminoalkoxy)-1-benzyl-5-nitroindazoles prepared and studied in this work.

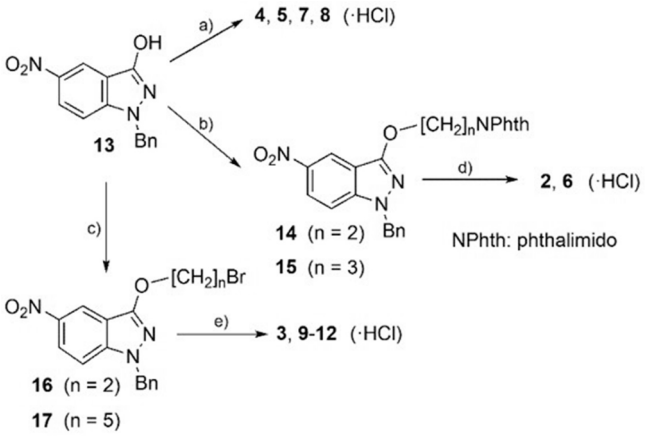
European guidelines cite other 5-nitroimidazoles, nonoxynol-9, furazolidone or paromomycin as possible alternatives;[16] however, their antiparasitic efficacy is remarkably lower. For those cases of hypersensitivity, desensitization is proposed, but patients must be carefully monitored and cure rates are low.[12,15]

Therefore, no effective alternative drugs are currently on the market for clinical failure, iatrogenic effects or hypersensitivity to 5-nitroimidazoles. Taking into account that the clinical resistance cases are near 10%,[12] the research on new trichomonacidal compounds is mandatory.[17]

In this sense, our research group has synthesized and tested a large number of nitroheterocycles against this neglected STI. In connection with the current article, the trichomonacidal activity of some 3-alkoxy-1-(*ω*-aminoalkyl and *ω*-aminooxaalkyl)-5-nitroindazoles **1**, e.g., **1a** (IC_{50} ca. 2.60 μM against *T. vaginalis* trophozoites) (Figure 1) has been reported by our group.[18] Additionally, other 5-nitroindazole derivatives such as 1-substituted indazol-3-ols,[19,20] 2-substituted indazolinones,[19] 3-alkoxyindazoles,[19] and 1,2-, 1,3- and 2,3-disubstituted indazoles,[21–23] carrying different moieties at the mentioned positions, have also been studied.

In this article we describe the synthesis and activity against *T. vaginalis* trophozoites of a series of 3-(*ω*-aminoalkoxy)-1-benzyl-5-nitroindazoles **2**–**12** (Fig. 2), position isomers of some of the mentioned compounds **1**. Amines **2**–**6** and **8**–**12** are new; compound **7** has previously been reported[24] but full analytical and spectral data were not given in the corresponding article. All compounds were isolated as the corresponding hydrochlorides.

Final 3-(*ω*-aminoalkoxy)-1-benzyl-5-nitroindazoles **2**–**12** have been prepared starting from 1-benzyl-5-nitroindazol-3-ol **13**[25] through alkylation reactions. Treatment of this compound with functionalized



Scheme 1. Synthesis of final indazole-derived amines **2**–**12** and intermediates **14**–**17**. *Reagents and conditions*: a) $Cl[CH_2]_nNR_2$, K_2CO_3, KI, butanone, reflux overnight, 68–85%; b) $Br[CH_2]_nNPhth$, K_2CO_3, butanone, reflux overnight, 90%; c) $Br[CH_2]_nBr$, K_2CO_3, butanone, reflux overnight, 78–87%; d) $MeNH_2/H_2O$, 80 °C, 4 h, 97–98%; e) for **3**, **10** and **11**: $MeNH_2$/EtOH or Me_2NH/EtOH, rt overnight, 93–96%; for **9**: NH_3/MeOH, 70 °C (autoclave), 24 h, 96%; for **12**, piperidine, EtOH, reflux overnight, 97%. The initially obtained free amines were converted into the corresponding hydrochlorides by treatment with dil. HCl; in these cases, given yields correspond to those of the respective salts.

alkyl halides leads to a mixture of *N*-2 and 3-*O* alkyl derivatives but the latter is always the main product when the reaction is carried out, as in the current study, in the presence of a base such as K_2CO_3.[25–27]

Thus, direct alkylation of 1-benzylindazolol **13** with the required *N*-(*ω*-chloroalkyl)amines yielded the expected *ω*-(dimethylamino)- (**4**, **7**) and *ω*-piperidinoalkoxy (**5**, **8**) derivatives (Scheme 1). Other amines were prepared through the required precursors. Thus, alkylation of indazolol **13** with *N*-(2-bromoethyl)- or *N*-(3-bromopropyl)phthalimide afforded the corresponding 3-(*ω*-phthalimidoalkoxy)indazoles **14** and **15**, respectively. Removal of *N*-protecting phthaloyl group of these compounds with $MeNH_2/H_2O$[28,29] afforded final 3-(*ω*-aminoalkoxy) indazoles **2** and **6**, respectively.

On the other hand, treatment of compound **13** with an excess of 1,2-dibromoethane or 1,5-dibromopentane afforded 3-(*ω*-bromoalkoxy) indazoles **16** and **17**, respectively. These compounds, treated with ammonia or the required amines yielded the desired primary (**9**), secondary (**3**, **10**) and tertiary (**11**, **12**) amines.

The activity against *T. vaginalis* of amines **2**–**12** as well as that of reaction intermediates **14**–**17** was initially determined on trophozoites of the metronidazole-sensitive JH31A#4 isolate, as reported previously;[30,31] the obtained activity values are shown in Table 1.

Primary amines **2**, **6** and **9**, secondary amines **3** and **10** and tertiary amine **11** were able to overcome the first screening filter, displaying antiparasitic values of $IC_{50} < 20$ μM; derivatives **9**–**11**, containing the lipophilic *ω*-substituted 3-pentyloxy chain, although less active than metronidazole ($IC_{50} = 1.7$ μM), showed to be the most active compounds with $IC_{50} \leq 6.5$ μM. In many cases, a direct comparison with our formerly studied compounds is difficult owing to the different methodologies used to define trichomonacidal activity (growth inhibition at 100, 10 and 1 μg/mL, IC_{50} (μM) and SI not determined, etc.)[18–21]; nevertheless, among 5-nitroindazole derivatives, only some 1-benzylindazoles of structure **1**,[18] as well as some 2-benzylindazoles (3-hydroxypropoxy,[22] 3-alkylamino[23] and 3-aminopropoxy[23] derivatives) exhibited similar activity ($IC_{50} \leq 10$ μM).

The most interesting compounds, with $IC_{50} < 20$ μM (**2**, **3**, **6**, **9**, **10** and **11**), were then assayed on trophozoites of the metronidazole-resistant isolate IR78 following the same procedure;[30,31] the obtained results are also included in Table 1. The activity of some compounds (**2**, **3**, **9** and **11**) against this isolate was lower, but other derivatives (**6** and

Table 1
In vitro activities against *T. vaginalis* trophozoites (IC_{50}), non-specific cytotoxicity against Vero cells (CC_{50}) and selectivity indexes (SI) of compounds **2**–**12** and **14**–**17**.

Compounds	*T. vaginalis* JH31A#4		*T. vaginalis* IR78		Vero cells CCL-81
	IC_{50} (μM)[a]	SI[b]	IC_{50} (μM)[a]	SI[b]	CC_{50} (μM)[a]
2	10.5 [7.0–14.2]	> 28.6	14.3 [5.5–26.5]	> 21.0	> 300
3	16.3 [11.5–22.7]	10.5	74.7 [59.8–98.7]	2.3	171.3 [157.6–186.9]
4	207.3 [177.6–249.0]	–	–	–	–
5	265.1 [201.4–409.2]	–		–	–
6	19.2 [15.7–22.7]	2.4	1.3 [0.8–1.9]	35.6	46.3 [27.9–76.6]
7	63.7 [53.7–76.0]	–	–	–	–
8	104.0 [91.1–118.3]	–	–	–	–
9	4.2 [3.4–5.2]	13.7	59.1 [39.3–81.5]	1.0	57.7 [34.9–82.3]
10	2.5 [1.8–3.4]	9.6	0.5 [0.3–0.7]	48.0	24.0 [10.6–44.4]
11	6.5 [3.9–9.6]	8.0	55.9 [37.6–84.4]	0.94	52.3 [21.0–110.1]
12	69.0 [46.1–108.7]	–	–		–
14	281.5 [114.5–626.6]	–	–	–	–
15	70.3 [57.5–89.1]	–	–	–	–
16	> 300	–	–	–	–
17	> 300	–	–	–	–
MTZ	1.7 [1.5–1.8]	> 176	3.0 [2.4–3.5]	> 100	> 300

[a] Mean values; in square brackets, 95% confidence intervals.
[b] Selectivity indexes (SI = CC_{50} Vero cells/IC_{50} trophozoites).

especially **10**), showed an improved activity against the resistant isolate, better than the one obtained with the reference drug (IC_{50} = 3.0 μM).

According to the step-wise workflow screening procedure,[31] the above mentioned compounds with IC_{50} < 20 μM were then subjected to a study of non-specific cytotoxicity against the Vero CCL-81 cell line as a model of mammalian cell; the obtained results (CC_{50} values), as well as the corresponding selectivity indexes (SI) are included in Table 1. Considering remarkable values for those compounds with SI > 10, and in relation to JH31A#4 isolate, SI were moderate to high (SI = 8.0 – > 28.6) except for compound **6** (SI = 2.4) which was active but also very toxic. In fact, the toxicity of compound **7** in mice, leading to cyanosis and a complex symptomatology characterized by convulsive and depressive phenomena, has been previously reported.[24] On the other hand, better SI were obtained in the case of IR78 isolate, especially for the most active compounds **6** and **10** (SI = 35.6 and 48.0, respectively).

The remarkable activity detected in these two nitroindazoles, agrees with the high trichomonacidal profile published for a derivative with a 2-benzyl-3-(3-aminopropoxy)indazole structure, which exhibited IC_{50} values of 5.6 μM and 8.5 μM in both metronidazole-sensitive and resistant isolates, respectively.[23] In relation to cytotoxicity, the mentioned 3-(3-aminopropoxy) derivative showed low non-specific cytotoxicity on Vero cells (CC_{50} = 104.1 μM),[23] which is in agreement with the low cytotoxic profile obtained for the compounds evaluated in the current study.

Apart from the good in vitro results against sensitive and resistant isolates, these six compounds (**2**, **3**, **6**, **9**, **10** and **11**) could be considered interesting for the development of new analogs as they satisfy the physicochemical properties suggested by Lipinski and colleages[32] as shows Table 2. This rule-of-thumb states that most molecules with high oral bioavailability comply the following criteria: a molecular weight (MW) below 500 Da, a calculated octanol–water coefficient of partition (CLogP) lower than 5 and no more than 5 hydrogen bond donors (Hd) and 10 hydrogen bond acceptors (Ha) ("rule of five"). Beyond these parameters other studies have detected that excellent permeability and oral bioavailability are correlated with compounds with a polar surface area (TPSA) below 140 $Å^2$ and <12 hydrogen bond donors and acceptors (Ha + Hd) or a reduced number of rotatable bonds (Rotb ≤ 10) which reflects the molecular flexibility of the compound.[33] For an accurate estimation of the octanol/water partition coefficient at physiological pH of 7.4 in these ionizable molecules, the distribution coefficient (cLogD) has been also calculated.

Table 2
"Rule of five" and other physicochemical descriptors found for compounds **2**, **3**, **6**, **9**, **10** and **11** (free amines).

Comp.	MW	CLogP	CLogD	Hd	Ha	Lipinskís violation	TPSA	Rotb
2	312.33	2.72	0.87	2	7	0	98.89	6
3	326.36	3.16	1.22	1	7	0	84.91	7
6	326.36	2.78	0.37	2	7	0	98.89	7
9	354.41	3.75	1.14	2	7	0	98.89	9
10	368.44	4.18	1.29	7	1	0	84.91	10
11	382.46	4.56	2.20	7	0	0	76.11	10
MTZ	171.15	−0.46	–	6	1	0	83.88	3

As achieves Table 2, the six compounds present a hydrophilic profile as shows CLogD and all meet excellent molecular properties considered to influence in an adequate permeation. In this sense, our candidates present excellent physicochemical properties in terms of bioavailability and moderate to high selectivity index in both isolates. These molecules meet the criteria of other groups that consider that high quality lead compounds must comply the rule-of-five, but also present an adequate activity against their targets.[34] Additionally, according to Pink and colleages[35] indazoles **6** and **10** meet all the criteria to be classified as antiparasitic hit compounds, due to their remarkable trichomonacidal activities in vitro (IC_{50} ≤ 1 μg/mL) as well as for their excellent selectivity indexes (SI ≥ 10).

We have not been able to establish a general structure–activity relationship; however, it is clear that the length of $[CH_2]_n$ chain of ω-aminoalkoxy substituents (**2**, **3** with n = 2; **6** with n = 3 and **9**, **10** with n = 5) is not relevant for activity against both sensitive and resistant parasites. Neither the fact that primary (**2**, **6**, **9**), secondary (**3**, **10**) and tertiary amines (**11**) have presented excellent antiparasitic activity. On the contrary, compounds **3** and **9**, which showed the best activity against the resistant isolate, supported the same NHMe substituent. The unexpected heterogeneous response to one compound and not to another with similar structure is a hallmark detected in previous works.[21,22] Collectively, these findings seem to support the extent heterogenicity observed in clinical isolates in terms of biological and genetic features.[36–38] All of this explains, to a certain extent, the difficulties to face a rational drug design to find pharmacologically effective alternatives for trichomoniasis.

To our knowledge this is the first time that the activity of 1-alkyl-3-(ω-aminoalkoxy)indazoles against *T. vaginalis* has been reported. The remarkable results obtained for amines **2**, **3**, **6** and **9**–**11** make this type of compounds worthy of further research, in order to increase its trichomonacidal activity and decrease its non-specific cytotoxicity. The

interesting activity of these compounds reinforces the potential trichomonacidal effect of the 5-nitroindazole scaffold detected in previous works. In fact, the preparation of new indazole-derived amines for the treatment of metronidazole-sensitive and resistant isolates of *T. vaginalis*, based on these scaffolds, is in progress.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

This work was partly supported by the Spanish Ministry of Science, Innovation and Universities (MICIU, ref. RTI2018-093940-BI00), and by the UCM Research Group (ref. 911120: Diagnosis, Epidemiology and Antiparasitic Therapy).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bmcl.2021.127843.

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