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Design, Synthesis and characterization of t-butyl 4-(5-amino-6-methoxy-2H-indazol-2-yl) piperidine-1-carboxylate and it's derivatives as an antiprotozoal activity

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ABSTRACT
*In vitro antiprotozoal activity of the synthesized derivatives of t-butyl 4-(5-amino-6- methoxy-2H-indazol-2-yl) piperidine-1-carboxylate (**6a-j**) were evaluated and assays against E. histolytica, G. intestinalis, and T. vaginalispathogens. Metronidazole and albendazole used as for the drugs references. There are three compounds **6a**, **6e** and **6d** showed moderate to high activity against the pathogens*

KEYWORDS: *Antiprotozoal activity,Albendazole,E. histolytica, 2H-indazol, G. intestinalistert-butyl 4-(5-amino-6-methoxy-2H-indazol-2-yl) piperidine-1-carboxylate, T. vaginalis.*

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I. INTRODUCTION

Indazole and its derivatives form an important class of heterocyclic compounds that can combine with various pharmacological actions in the composition of many substances: antihistamines [2,4], anti- Viral [1,5], Anti-microbial [15, 12], cytostatic [7-12], anti-inflammatory [4], analgesic [18], anti-psychotic [6,4], anti-arrhythmia [12], anti-HIV [14], anti-malaria [5] and anti-fungal. A number of drugs also have antagonists of neuronal inhibitors [12] as well as glucocorticoid receptors [20]. Infectious diseases originated by protozoa, bacteria, and yeasts have a major impact on human health. Enteric pathogenic protozoa and bacteria are a frequent cause of intestinal disease which, in turn, is an important cause of morbidity and mortality around the world. Indazole is considered a very important scaffold in medicinal chemistry. Two important etiological agents of intestinal parasitic diseases are the protozoa *Giardia intestinalis* and *Entamoebahistolytica*, which have been estimated to affect 280 million and 50 million people worldwide each year, respectively. On the other hand, *Trichomonasvaginalis* and *Candida albicans* are two of the major etiological agents of vaginitis. According to the World Health Organization (WHO), 276 million new cases of trichomoniasis have been estimated [6]. Infection by *T. vaginalis* can cause severe inflammation of the genital tract, which has been associated with preterm labor, low-birth weight, sterility, cervical cancer, and predisposition to HIV infection [5,6,7]. In addition, it has been reported that 75% of women have at least one vaginal yeast infection during their lifespan [8]. Infections by *Candida* usually cause swelling, itching, and irritation and can turn into a very serious problem for pregnant and immuno compromised womenIndazole is considered a very important scaffold in medicinal chemistry, It is commonly found in compounds with diverse biological activities, e.g., antimicrobial and antiprotozoal activity against E. Histolytica and T.vaginalis. Infectious and parasitic diseases (e.g., amebiosis and trichomonosis), an inflammatory reaction is generally initiate. Apart from these its core nucleus are very useful for making different type of drugs many of the commercially available drugs based on 1 H, 2 H and 3 H indazole derivatives as shown the figure -**2**.

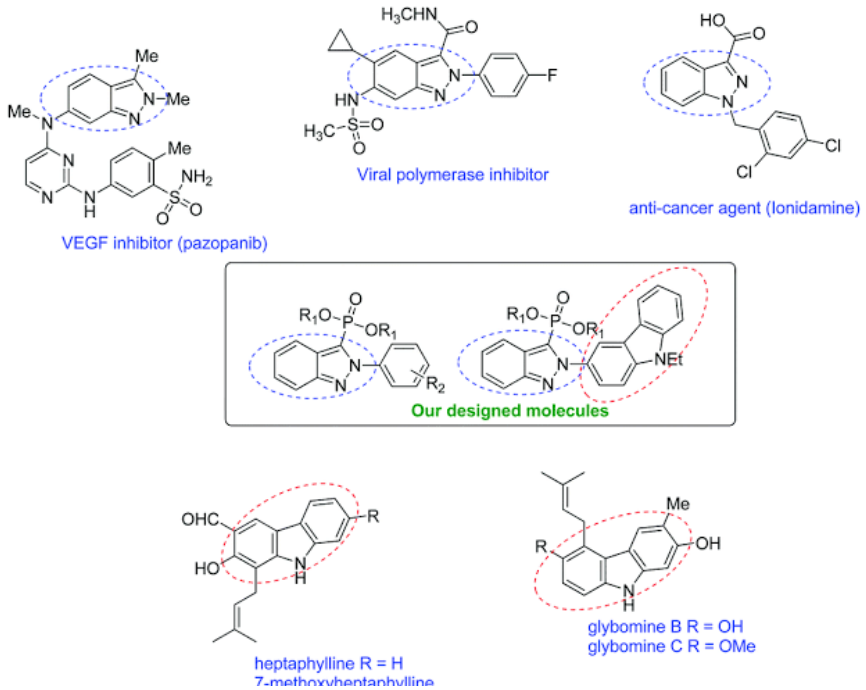
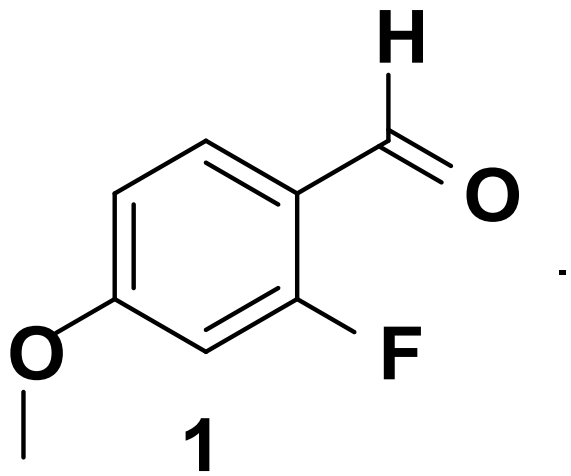


Figure-1: pharmacological Inhibitors actions of Indazol

II. RESULT AND DISCUSSION

I. Reaction scheme:



1

A peculiar compound (**5**) precursor for diversity were prepared from Nitration of 2-Fluro-4-methoxy-benzaldehyde(**1**) by treatment with sulfuric and nitric acid provided corresponding nitro compound (**2**) with 65-70% yield, IR of this compound confirmed the presence of nitro group ,aldehyde and other group. This compound also confirmed by ^{1}H NMR and LCMS with characteristic aldehydic peak present at 9-10 ppm. To this compound (**2**) on reaction with sodium azide provided corresponding azide derivative (**3**). Reduction of azide**(3**) and followed by cyclization facilitates compound (**4**). This cyclization of compound was confirmed by ^{1}H NMR data and LCMS. Hydrogenation of compound (**4**) in presence of Pd catalyst at 14 Psi pressure in ethyl acetate gave corresponding amine derivatives of compound (**5**). A strategy of final amidation derivatives of

compound (**5**) with various commercially available acid provided compound **6a-i** as a target compounds for biological testing's. The synthesized derivatives were confirmed by ^{1}H NMR, ^{13}C NMR, LCMS and elemental analysis.

IIBiology:

IIA. **Antiprotozoal Activity**: In vitro antiprotozoal assays against E. histolytica, G. intestinalis, and T. vaginalis of the synthesize derivatives of 2H-Indazol were acknowledged out following the procedure previously noticeable. Tert-butyl 4-(5-amino-6-methoxy-2H-indazol-2-yl) piperidine-1-carboxylate derivatives **6a-i** were evaluated and the results are shown in Table **1** as IC50 values. Metronidazole and Albendazole were used as for the drugs references. The novel active synthesized derivatives contrary to the three protozoa were compounds **6a,6e** and **6d**, are tert-butyl 4-(6-methoxy-5-(pyrimidine-4-carboxamido)-2H-indazol-2-yl)piperidine-1-carboxylate and tert-butyl 4-(5-(3,3a-dihydropyrazolo[1,5-a]pyrimidine-3-carboxamido)-6-methoxy-2H-indazol-2-yl)piperidine-1-carboxylate. Consistently, compound **6a** (tert-butyl 4-(6-methoxy-5-(5-methyl-3-phenylisoxazole-4-carboxamido)-2H-indazol-2-yl) piperidine-1-carboxylate), has good activity against the three protozoa, position third inactivity for T.vaginalis and forth against G.intestinalis and E.histolytica, at slightest for two parasites evaluated (G.intestinalis and T.vaginalis). As these results, Tert-butyl 4-(5-amino-6-methoxy-2H-indazol-2-yl) piperidine-1-carboxylate derivatives **6b, 6c, 6f, 6g,** and **6i** were selected to be tested for their antiprotozoal activity. That persuaded the moderate to high response in Tert-butyl 4-(5-amino-6-methoxy-2H-indazol-2-yl) piperidine-1-carboxylate derivatives. The 6 h exhibited a very poor response against all protozoa. However, all tested compounds exhibit potency as antiprotozoal agents, with metronidazole being superior to the drug of choice in almost all cases.

Table 1. Antiprotozoal activity of Tert-butyl 4-(5-amino-6-methoxy-2H-indazol-2-yl) piperidine-1-carboxylate derivatives.

Entry	Compound structure	IUPAC name of Compounds	**IC50 (_M) G. intestinalis**	**IC50 (_M) E. histolytica**	**IC50 (_M) T. vaginalis**
6a		tert-butyl 4-(6-methoxy-5-(5-methyl-3-phenylisoxazole-4-carboxamido)-2H-indazol-2-yl)piperidine-1-carboxylate	0.1133±0.0218	0.0798 ± 0.0036	0.1184 ±0.0218
6b		tert-butyl 4-(6-methoxy-5-(1-methyl-1H-pyrazole-4-carboxamido)-2H-indazol-2-yl)piperidine-1-carboxylate	0.1062 ±0.0081	0.0459 ±0.0081	0.1062 ± 0.0081
6c		tert-butyl 4-(5-(6-chloropyrazine-2-carboxamido)-6-methoxy-2H-indazol-2-yl)piperidine-1-carboxylate	0.1209 ± 0.0090	0.0509 ± 0.0000	0.2402 ± 0.0067
6d		tert-butyl 4-(6-methoxy-5-(pyrimidine-4-carboxamido)-2H-indazol-2-yl)piperidine-1-carboxylate	0.0634 ± 0.0031	0.0415±0.0031	0.1071±0.0031
6e		tert-butyl 4-(5-(3,3a-dihydropyrazolo[1,5-a]pyrimidine-3-carboxamido)-6-methoxy-2H-indazol-2-yl)piperidine-1-carboxylate	0.0634 ±0.0056	0.0218±0.0028	0.1070±0.0056
6f		tert-butyl 4-(5-(3-bromo-5-fluorobenzamido)-6-methoxy-2H-indazol-2-yl)piperidine-1-carboxylate	0.0518 ± 0.0052	0.3033 ± 0.0105	0.0573 ± 0.0026
6g		tert-butyl 4-(5-(1-(tert-butoxycarbonyl)-5,5-difluoropyrrolidine-3-carboxamido)-6-methoxy-2H-indazol-2-yl)piperidine-1-carboxylate	0.0795 ±0.0045	0.0445 ±0.0045	0.1113 ±0.0180
6h		tert-butyl 4-(6-methoxy-5-(thiazole-4-carboxamido)-2H-indazol-2-yl)piperidine-1-carboxylate	0.1188 ± 0.0086	0.0731 ± 0.0086	0.1431 ± 0.0043

6i		tert-butyl 4-(6-methoxy-5-(2-(pyridin-2-yl)thiazole-4-carboxamido)-2H-indazol-2-yl)piperidine-1-carboxylate	0.0959 ± 0.0022	0.0502 ± 0.0022	0.1020 ± 0.0151
Metronidazole			1.2260 ± 0.1250	0.3798 ± 0.1461	0.2360 ±0.0160
ABZ			0.0370 _ 0.0030	56.5334±18.8445	1.5905 ±0.0113

II B. Antibacterial and Anticandidal Assays: The liability assays against E. coli 933, E. coli 042, S. entericaserovarTyphi, C. albicans, and C.glabrata were accepted out using the disk diffusion test, in conflict of method outlined by The Clinical and Laboratory Standards Institute (CLSI).[l.] An assortment of compounds based on the results from the antiprotozoal assays were tested at 5mg/mL, though, they were inactive or poorly active even at high concentration against the bacterial strains tested. However, compounds **6e** showed a distinguished inhibition zone against C. albicans. Furthermore, these identical compound showed activity against C. glabrata, which is frequently less sensitive to the marketable antimycotics. Established on these interpretations, the minimum inhibitory concentration (MIC) against C. albicans and C. glabrata was intended for compounds **6e**.

Sl No:	**structure**	**IUPAC name of compounds**	**MIC (mM) C. albicans**	**MIC (mM) C. glabrata**
6e		tert-butyl 4-(5-(3,3a-dihydropyrazolo[1,5-a]pyrimidine-3-carboxamido)-6-methoxy-2H-indazol-2-yl)piperidine-1-carboxylate	3.807	15.227
Ketoconazole			0.045	0.079

III. EXPERIMENTAL

III A. Materials and Methods

Chemicals and Instruments Generally chemicals and starting materials were acquired from Sigma-Aldrich (Toluca, MC, and Mexico). Progress of reaction was monitored by TLC on 0.2 mm silica gel 60 F254 plates (Merck, Darmstadt, Germany) and envisaged by irradiation with a UV lamp. Silica gel (100–230 mesh) was used for column chromatography. ^{1}H-NMR and ^{13}C-NMR spectra were measured with an Agilent spectrometer at 400 MHz for ^{1}H and ^{13}C, respectively. Parts per million relative to tetramethylsilane Chemical shifts are given in (Me4Si, $\delta = 0$); J values are given in Hz. Splitting patterns are expressed as follow: s, singlet; d, doublet; q, quartet; dd, doublet of doublet; t, triplet; m, multiplet; bs, broad singlet. Mass spectra were recorded on a waters LCMS, MicroTOF-II-Focus spectrometer (Billerica, MA, USA) by electrospray ionization (ESI). All compounds were named using the automatic name generator tool implemented in ChemBioDraw Ultra 13.0 software (PerkinElmer, Waltham, MA, USA), according IUPAC rules.
Experimental procedure:

Table-5;structure, IUPAC name and melting point.

Entry	**Compound structure**	**IUPAC name of Compounds**	**m.p.(0 °C**
6a		tert-butyl 4-(6-methoxy-5-(5-methyl-3-phenylisoxazole-4-carboxamido)-2H-indazol-2-yl)piperidine-1-carboxylate	229 °C
6b		tert-butyl 4-(6-methoxy-5-(1-methyl-1H-pyrazole-4-carboxamido)-2H-indazol-2-yl)piperidine-1-carboxylate	220°C
6c		tert-butyl 4-(5-(6-chloropyrazine-2-carboxamido)-6-methoxy-2H-indazol-2-yl)piperidine-1-carboxylate	218 °C
6d		tert-butyl 4-(6-methoxy-5-(pyrimidine-4-carboxamido)-2H-indazol-2-yl)piperidine-1-carboxylate	205°C

6e		tert-butyl 4-(5-(3,3a-dihydropyrazolo[1,5-a]pyrimidine-3-carboxamido)-6-methoxy-2H-indazol-2-yl)piperidine-1-carboxylate	209^{o}C
6f		tert-butyl 4-(5-(3-bromo-5-fluorobenzamido)-6-methoxy-2H-indazol-2-yl)piperidine-1-carboxylate	222 ^{o}C
6g		tert-butyl 4-(5-(1-(tert-butoxycarbonyl)-5,5-difluoropyrrolidine-3-carboxamido)-6-methoxy-2H-indazol-2-yl)piperidine-1-carboxylate	223 ^{0}C
6h		tert-butyl 4-(6-methoxy-5-(thiazole-4-carboxamido)-2H-indazol-2-yl)piperidine-1-carboxylate	232^{o}C
6i		tert-butyl 4-(6-methoxy-5-(2-(pyridin-2-yl)thiazole-4-carboxamido)-2H-indazol-2-yl)piperidine-1-carboxylate	235^{o}C

Synthesis of 2-fluoro-4-methoxy-5-nitrobenzaldehyde (**2**);
To a stirred solution of compound **1** (1g, 6.49 mmol) was dissolved in sulfuric acid (10 mL) at 0 ^{o} C and added nitric acid drop wise(1.5eq).The reaction mixture was stirred for 1h, then poured in ice water, precipitate was formed, filtered and dry to afford 2-fluoro-4-methoxy-5-nitrobenzaldehyde(**2**) (0.8 g, 62.10%) as yellow solid.
^{1} H NMR (400 MHz, DMSO-d6) δ 10.36(s, 1H), 8.40(s, 1H), 7.80(s, 1H).4.02(s, 3H).
Synthesis of2-azido-4-methoxy-5-nitrobenzaldehyde (3)
To a stirred solution of 2-fluoro-4-methoxy-5-nitrobenzaldehyde (**2**) (1.29 g, 6.49 mmol) in DMSO(5 mL) was added sodium azide (632 mg, 1.5 eq, 9.2 mmol).The reaction mixture was heated at 80 ^{o}C for 2h. Reaction mixture was extracted with EtOAc by diluting with water. Combined organic extract were washed with water and brine, dried over anhydrous sodium sulfate and concentrated under reduced pressure to afford **2-azido-4-methoxy-5-nitrobenzaldehyde(3)** (0.9 g, 62.93 % crude) as white solid. Residue was used as such for the next without further purification.
^{1} H NMR (400 MHz, DMSO-d6) δ 10.36(s, 1H), 8.42(s, 1H), 7.22(s, 1H), 4.02(s, 3H)
Synthesis of tert-butyl 4-(6-methoxy-5-nitro-2H-indazol-2-yl) piperidine-1-carboxylate (**4**);
To a stirred solution of **2-azido-4-methoxy-5-nitrobenzaldehyde (3)** (0.9 g, 62.93 % crude) in toluene (20 mL) was added tert-butyl 4-aminopiperidine-1-carboxylate (**A**) was heated at 130 ^{o}C for 6h. Reaction mixture was evaporated and the residue was purified through column chromatography (100-200 mesh size silica gel, 20-30% EtOAc in hexane) to afford **tert-butyl 4-(6-methoxy-5-nitro-2H-indazol-2-yl) piperidine-1-carboxylate** (**4**) (1.0 g, 65.72%) as yellow solid.
^{1} H NMR (400 MHz, DMSO-d6) δ 8.78 (s, 1H), 8.02(s, 1H), 7.48(s, 1H), 4.02(s, 3H), 3.75(m, 1H), 3.49-3.59(m, 4H), 1.96-2.21(m, 4H), 1.42(s, 9H).
MS (ESI) + for m/z =377
Synthesis of tert-butyl 4-(5-amino-6-methoxy-2H-indazol-2-yl) piperidine-1-carboxylate (5)
To a stirred solution of compound **tert-butyl 4-(6-methoxy-5-nitro-2H-indazol-2-yl) piperidine-1-carboxylate** (**4**) (1.0 g, 65.72%) in ethyl acetate (20 mL) was added 10 mol percentage palladium on carbon and hydrogenated under atm pressure for 6 h. Filtered through celite pad and concentrated under reduced pressure to afford as **tert-butyl 4-(5-amino-6-methoxy-2H-indazol-2-yl)piperidine-1-carboxylate**(**5**) white solid(750 mg, 81 %) white solid.
^{1} H NMR (400 MHz, DMSO-d6) δ 8.02(s, 1H), 7.25(s, 1H), 7.07(s, 1H), 5.02(brs, 2H), 4.02(s, 3H), 3.75(m, 1H), 3.49-3.59(m, 4H), 1.96-2.21(m, 4H), 1.42(s, 9H).
General procedure for synthesis of compound (6a-i).
To a stirred solution of **tert-butyl 4-(5-amino-6-methoxy-2H-indazol-2-yl) piperidine-1-carboxylate** (**5**) (100 mg, 282 mmol) in DMF (1 mL) was added different acids (a-j, 1.0 eq) followed by addition of HATU (1.5eq) and DIPEA (2.5 eq).The resulting reaction mixture was stirred for overnight. Reaction mixture was extracted with EtOAc by diluting with water. Combined organic extract were washed with water and brine, dried over

anhydrous sodium sulfate and concentrated under reduced pressure. The residue was purified by column chromatography (100-200 mesh size silica gel, 10% -50% EtOAc in hexane) to afford compound (**6a-j).**

6a) tert-butyl4-(6-methoxy-5-(5-methyl-3-phenylisoxazole-4-carboxamido)-2H-indazol-2-yl) piperidine-1-carboxylate as off white solid; Yield = 64.56%;**Chemical Formula**: $C_{29}H_{33}N_5O_5$,**Elemental Analysis calc**: C, 65.52; H, 6.26; N, 13.17; O, 15.05; Elemental Analysis obs; C, 64.52; H, 6.36; N, 14.17; O, 13.05; **HPLC purity**: 98.99% (λ =220 nm).**[1] H NMR (400 MHz, DMSO-d6)** δ9.11(s, 1H), 8.31(s, 1H), 8.23(s, 1H), 7.72(s, 2H), 7.55(d, J =6.76Hz, 3H), 7.00(s, 1H), 4.59(t, J =10.72Hz, 1H), 4.07(d, J= 10.84Hz, 2H), 3.69(s, 3H),2.94(s, 2H), 2.68(s, 3H), 1.86-2.08(m, 4H),1.42(s, 9H),**[13] C NMR (400 MHz, DMSO-d6)**δ12.1, 27.1, 28.5, 44.2, 55.8, 63.3, 79.8, 107.2, 111.6, 115.6, 122.8, 123.6, 127.5, 128.2, 129.8σσ, 144.5, 151.2, 159.6, 162.8, 164.7, 175.2.**MS (ESI) + for m/z** =532.

6b) tert-butyl 4-(6-methoxy-5-(1-methyl-1H-pyrazole-4-carboxamido)-2H-indazol-2-yl) piperidine-1-carboxylate as yellow solid: Yield= 51.26%, **Chemical Formula:** $C_{23}H_{30}N_6O_4$,

Elemental Analysis calc: C, 60.78; H, 6.65; N, 18.49; O, 14.08 Elemental Analysis obs: C, 61.28; H, 5.65; N, 17.49; O, 15.26. **HPLC purity**: 99.37% (λ =220 nm)**[1]H NMR (400 MHz, DMSO-d6)** δ 9.40(brs, 1H), 9.19(brs, 1H), 8.31(s, 1H), 8.10(s, 1H), 7.52(s, 1H), 7.08(d,J=10.36Hz, 1H), 4.76(bs, 1H), 4.08(s, 3H), 3.86(s, 3H), 3.10(d,J=8.8Hz, 2H), 2.88(s, 2H), 2.28(bs, 4H),1.42(s, 9H),**[13] C NMR (400 MHz, DMSO-d6)**δ 27.1, 28.5, 39.8, 44.2, 55.8, 63.3, 79.8, 107.2, 111.6, 115.6, 122.8, 123.0, 131.2, 139.6, 144.5, 151.2, 159.6, 164.2. **MS (ESI)** + for m/z=455.

6c)Tert-butyl4-(5-(6-chloropyrazine-2-carboxamido)-6-methoxy-2H-indazol-2-yl) piperidine-1-carboxylate as white solid: Yield= 35.25%, **Chemical Formula**: $C_{23}H_{27}ClN_6O_4$**Elemental Analysis calc**: C, 56.73; H, 5.59; Cl, 7.28; N, 17.26; O, 13.14; Elemental Analysis obs: C, 57.73; H, 4.59; Cl, 6.28; N, 16.26; O, 14.14.**HPLC purity**: 97.55% (λ =220 nm)**[1] H NMR (400MHz, DMSO-d6)**δ 10.19(s, 1H), 9.24(s, 1H), 8.78-8.84(m, 3H), 8.65(s, 1H), 7.67-7.71(m, 1H), 7.17(m, 1H), 4.60(t, J=11.64Hz, 1H), 4.03-4.08(t, J=10.52Hz, 2H), 4.00(s, 3H), 2.95(bs, 2H), 1.87-2.10(m, 2H), 1.42(s, 9H).**[13] C NMR (400 MHz, DMSO-d6)**δ 27.1, 28.5, 39.8, 44.2, 55.8, 63.3, 79.8, 107.2, 111.6, 115.6, 122.8, 123.0, 143.5, 144.2, 145.2, 148.6, 151.2, 159.2, 162.5; **MS (ESI) +** for m/z = 487

6d) tert-butyl 4-(6-methoxy-5-(pyrimidine-4-carboxamido)-2H-indazol-2-yl)piperidine-1-carboxylate as off white solid; Yield 38.26%**. Chemical Formula**: $C_{23}H_{28}N_6O_4$, **HPLC purity**: 99.99% (λ =220 nm)**Elemental Analysis calc**: C, 61.05; H, 6.24; N, 18.57; O, 14.14; Elemental Analysis obs: C, 63.05; H, 5.24; N, 17.57; O, 14.04;**[1] H NMR (400MHz, DMSO-d6)**δ 10.51(s, 1H), 9.42(s, 1H), 9.16(d, J=5.4Hz, 1H), 8.70(s, 1H), 8.38(s, 1H), 8.17(d, J= 4.96Hz, 2H), 4.60-4.64(m, 1H), 3.99-4.09(m, 2H), 3.90(s, 3H), 2.96(bs, 2H), 1.88-2.10(m, 4H), 1.43(s, 9H).**[13] C NMR (400 MHz, DMSO-d6)**δ 27.1, 28.5, 39.8, 44.2, 55.8, 63.3, 79.8, 107.2, 111.6, 115.6, 118.6, 122.8, 123.0, 144.2, 151.2, 156.2, 157.8, 158.2, 159.2, 162.5. **MS (ESI) +** for m/z=454

6e) tert-butyl 4-(5-(3, 3a-dihydropyrazolo [1, 5-a] pyrimidine-3-carboxamido)-6-methoxy-2H-indazol-2-yl) piperidine-1-carboxylate as white solid: Yield= 22.22%;

Chemical Formula: $C_{25}H_{31}N_7O_4$.**Elemental Analysis calc:** C, 60.84; H, 6.33; N, 19.87; O, 12.97; Elemental Analysis obs: C, 60.34; H, 6.53; N, 19.67; O, 12.57.**HPLC purity**: 99.21% (λ =220 nm)**[1] H NMR (400MHz, DMSO-d6)** δ 10.54(s, 1H), 9.51(d, J=8.48Hz, 1H), 8.96(bs, 1H), 8.76(d, J=7.92Hz, 2H), 8.32(s, 1H), 7.36(s, 1H),7.10(s, 1H), 4.60-4.64(m, 2H), 3.99-4.09(m, 2H), 3.90(s, 3H), 2.96(bs, 3H), 1.88-2.10(m, 4H), 1.43(s, 9H).**[13] C NMR (400 MHz, DMSO-d6)**δ 27.1, 28.5, 39.8, 44.2, 55.8, 63.3, 79.8, 107.2, 111.6, 115.6, 118.6, 122.8, 123.0, 144.2, 145.0, 149.0, 151.2, 159.2, 163.5, 172. **MS (ESI)** + for m/z= 494.5.

6f) Tert-butyl4-(5-(3-bromo-5-fluorobenzamido)-6-methoxy-2H-indazol-2-yl) piperidine -1-carboxylate as off white solid; Yield= 76.25%**, Chemical Formula**: $C_{25}H_{28}BrFN_4O_4$, **HPLC purity**: 98.59% (λ =220 nm).**Elemental Analysis calc**: C, 54.85; H, 5.16; Br, 14.60; F, 3.47; N, 10.23; O, 11.69; Elemental Analysis obs: C, 53.85; H, 6.16; Br, 15.60; F, 2.47; N, 11.23; O, 10.69.**[1] H NMR (400MHz, DMSO-d6)**δ 9.70(s, 1H), 8.34(s, 1H), 7.99(d, J=17.56Hz, 2H), 7.80(t, J=6.16Hz, 2H), 7.07(s, 1H), 4.62 (t, J=11.36Hz, 1H), 4.08(d,J=9.48Hz, 2H), 3.90(s, 3H), 2.96(bs, 2H), 1.88-2.10(m, 4H), 1.43(s, 9H).**[13] C NMR (400 MHz, DMSO-d6)**δ 27.1, 28.5, 39.8, 44.2, 55.8, 63.3, 79.8, 107.2, 111.6, 115.6, , 122.8, 123.0,124.2, 124.8, 125.2, 138.0, 144.2, 151.2, 159.2, 164.5, 165.8.**MS (ESI) +** for m/z= 547.2

6g) Tert-butyl 4-(5-(1-(tert-butoxycarbonyl)-5, 5-difluoropyrrolidine-3-carboxamido)-6-methoxy-2H-indazol-2-yl) piperidine-1-carboxylate as off white solid; Yield=15.25%;

Chemical Formula: $C_{28}H_{39}F_2N_5O_6$; **Elemental Analysis calc**: C, 58.02; H, 6.78; F, 6.56; N, 12.08; O, 16.56; Elemental Analysis obs: C, 59.02; H, 5.78; F, 5.66; N, 13.88; O, 16.56; **HPLC purity**: 99.33% (λ =260 nm).**[1]H NMR (400MHz, DMSO-d6)**δ 8.87(s, 1H), 8.26(d, J=9.16Hz, 2H), 7.09(s, 1H), 4.59(bs, 1H),4.62 (t, J=11.36Hz, 1H), 4.01-4.08(m, 3H), 3.90(s, 3H), 2.96(bs, 3H), 1.88-2.10(m, 5H), 1.43(s, 18H);**[13]C NMR (400 MHz, DMSO-d6)**δ 25.2 27.1, 28.5, 34.9, 38.2, 44.2, 55.8, 63.3, 79.8, 107.2, 111.6, 115.6, 118.2, 122.8, 123.0,144.2, 151.2, 152.5 157.5, 159.2, 174.2; **MS (ESI) +** for m/z= 580.2

6h) Tert-butyl 4-(6-methoxy-5-(thiazole-4-carboxamido)-2H-indazol-2-yl) piperidine-1-carboxylate as yellow solid; Yield= 59.25%; **Chemical Formula**: $C_{22}H_{27}N_5O_4S$. **Elemental Analysis calc**: C, 57.75; H, 5.95; N, 15.31; O, 13.99; S, 7.01 Elemental Analysis obs: C, 58.75; H, 5.95; N, 16.31; O, 12.99; S, 7.10; **HPLC purity**: 98.75% (λ =220 nm).**^{1}H NMR (400MHz, DMSO-d6)**δ 9.93(s, 1H), 9.28(s, 1H), 8.64(s, 1H), 8.52(s, 1H), 8.35(s, 1H), 7.14(s, 1H), 4.62 (t, J=11.36Hz, 1H), 4.08(d,J=9.48Hz, 2H), 3.97(s, 3H), 2.96(bs, 2H), 1.88-2.10(m, 4H), 1.43(s, 9H);**^{13}C NMR (400 MHz, DMSO-d6)**δ 27.1, 28.5, 39.8, 44.2, 55.8, 63.3, 79.8, 107.2, 111.6, 115.6,118.2 , 122.8, 123.0,128.2, 144.2, 151.2,152.2 159.2, 162.5, **MS (ESI)+** for m/z= 458.5

6i) tert-butyl 4-(6-methoxy-5-(2-(pyridin-2-yl) thiazole-4-carboxamido)-2H-indazol-2-yl) piperidine-1-carboxylate as white solid; Yield: 27.22%, **Chemical Formula**: $C_{27}H_{30}N_6O_4S$;

Elemental Analysis calc: C, 60.66; H, 5.66; N, 15.72; O, 11.97; S, 6.00; Elemental Analysis obsd: C, 61.66; H, 4.66; N, 14.72; O, 12.97; S, 6.12;**HPLC purity**: 99.12% (λ =220 nm).

^{1}H NMR (400MHz, DMSO-d6)δ 10.01(s, 1H), 9.25(s, 1H), 8.74(t, J=1.74Hz, 1H), 8.59(s, 1H), 8.35(s, 2H), 8.42(d, J=7.96Hz, 1H), 8.36(s, 1H),7.61-7.64(m, 1H),7.15(s, 1H), 4.62 (t, J=11.36Hz, 1H), 4.08(d, J= 9.48Hz, 2H), 4.01(s, 3H), 2.96(bs, 1H), 1.88-2.10(m, 4H), 1.43(s, 9H);**^{13}C NMR (400 MHz, DMSO-d6)**δ 27.1, 28.5, 39.8, 44.2, 55.8, 63.3, 79.8, 107.2, 111.6, 115.6, 122.8, 123.0, 124.2, 127.2, 137.7 144.6, 149.7, 151.2, 157.1, 159.7, 162.5, **MS (ESI)** + for m/z= 535.2

Biological assay methods: This antiprotozoal action accesses Trichomonasvaginalis strain GT3, Giardia intestinal isolates, and Entamoebahistolytica strain HM1-IMSS were used. Intestinal trophozoites were preserved in a TII-S-33 medium with 10% calf serum and bovine bile. E. Histolytica and T. Vaginalistrophozoites were preserved in TYI-S-33 medium with 10% bovine serum. With different concentrations of the compound to be tested, each additional as a solution in DMSO. As an undesirable control, parasitic cultures established only an equal amount of DMSO, whereas albendazole and metronidazole were included as positive controls. Assuming the duration of treatment, the cells were washed and subcultured for another 48 h in a fresh medium to which no drugs were added. Trophozoites were calculated with a hemocytometer and a 50% inhibitory concentration (IC50), with a 95% con dence limit calculated by probate analysis

IV. CONCLUSION:

In summary, **9** novel compounds were synthesized of derivative of compound of Tert-butyl 4-(5-amino-6-methoxy-2H-indazol-2-yl) piperidine-1-carboxylate. Three compounds resulted in new structures (**6a**, **6d**, and **6e**). Biological valuations shown this compounds active against the three protozoa were compounds **6e** and **6d**, are tert-butyl 4-(6-methoxy-5-(pyrimidine-4-carboxamido)-2H-indazol-2-yl)piperidine-1-carboxylate and tert-butyl4-(5-(3,3a-dihydropyrazolo[1,5-a]pyrimidine-3-carboxamido)-6-methoxy-2H-indazol-2-yl) piperidine-1-carboxylate, amebicidal, giardicidal, and trichomonicidal activity minor than one micromolar and, in maximum cases, are advance potent than the drug of choice metronidazole. While the compounds are mostly inactive against the used bacterial strains, a main finding was that most of the compounds are discriminatory antiprotozoal agents. In accumulation of, compounds **6e** inhibit in vitro growth of C. glabrata and C. albicans. Are encouraging scaffolds for the design of new compounds against intestinal and vaginal pathogens, such as protozoa and yeasts? The mechanisms of action of synthesized indazol derivatives in this effort as antiprotozoal and anticandidal agents are still unfamiliar and establishes a further research topic to be addressed in forthcoming research.

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