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SCREENING OF NOVEL ISOINDOLE LEAD COMPOUNDS AND DETERMINATION MECHANISM OF ACTION OF MOST ACTIVE ONES ON ISOLATED RABBIT INTESTINE

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ABSTRACT

Background: Isoindole derivatives were rich source for numerous useful drugs and attract new drug discovery seekers. **Objectives:** The purpose of the study was to investigate the contracting effect of three newly synthesized isoindole derivatives (TIND-1, 10, and 11) on isolated rabbit intestine preparations, in vitro, moreover elucidating of the possible mechanism of action for the most active compound was carried out. **Methods:** The three tested compounds and standards agonists; acetylcholine, 5HT and barium chloride in concentration range of (10^{-5}-10^{-10}M) were used during the experiments, whilst standard antagonists; atropine and cyproheptadine were used only in two concentrations low (10^{-10}M) and high (10^{-8}M). Using organ bath, dose response curves of tested compounds alone and in the presence of their blockers in comparison to standards were constructed using cumulative dose cycle procedure on isolated rabbit intestine, in vitro. **Results:** The synthetic tested compounds TIND-1, TIND-10, TIND-11 demonstrated contracting effect on isolated rabbit intestine smooth muscle and TIND-1 showed the best profile. On other hand, TIND-1 had low efficacy upon comparison with standard spasmogen. Atropine in two different doses shifted the TIND-1 dose–response curve to right and downward, whilst cyproheptadine was without effect on TIND-1 dose-response curve. **Conclusion:** All the three tested compounds produced contracting effects on isolated rabbit intestine preparation and TIND-1 was the most promising one. TIND-1 mediates its contraction via muscurinic receptors. Further investigations are required to demonstrate other mechanistic role(s) involved in this contracting effect such as ion channels or prostaglandin receptors.

KEYWORDS: TIND-1, TIND-10, TIND-11, Isoindole, Rabbit intestine, Madani.

INTRODUCTION

Isoindole derivatives recently becomes a promising target for synthesis of new chemical entities to serve as lead compound for anti-inflammatory[1], anticancer[2,3] and antimicrobial.[4] Famous isoindole derivatives thalidomide was withdrawn from market at late 1961 as result of fetal malformation.[5] Thalidomide again reintroduce to the market for treatment of many disorders with certain safety requirements.[6,7] Teratogenicity the most hazardous side effect is controlled by education[8,9]; however the most common side effect associated with use of thalidomide and its analogues[3,10] still should be monitored such as gastrointestinal symptoms including diarrhea, constipation and nausea.[6]

Smooth muscle contraction activity plays important role in regulation of gastrointestinal [11] and urinary tracts functions[12]; and malfunction of this contraction effect causes many clinical disorders. Peppermint oil has been shown to relax isolated gastrointestinal smooth muscle and used treatment of relief of flatulence and colon pain[13] and irritable bowel syndrome.[14] *Fumaria parviflora* extract cause contraction of smooth muscle and used for treatment of GIT malfunction such as constipation and indigestion.[15] Bethanechol stimulates muscarinic receptors of urinary tract causing contraction of detrusor muscle of the bladder whereas the trigone and sphincter muscle are relaxed therefore, bethanecol is used therapeutically for treatment of urinary retention particularly postoperative.[16] Recently set of three synthetic isoindole derivatives TIND-1, TIND-10 and TIND-11 were designed with hope to have promising anti-inflammatory activity.[17] This study carried out to study effect of these

compounds on isolated rabbit intestine and to identify mechanism of action (s) of the most active compound.

2. MATERIALS AND METHODS

2.1. Animals housing

Local breed rabbits of either sexes weighted 1-3 Kg, purchased from local market. Rabbits were housed in standard cages at animal house of Omdurman Islamic University (OIU) under controlled environment (23-25C^{o}) and fed with carrot heads and grass, whilst as routine rabbit had access to continuous source of purified water.

2.2. Ethical consideration

The experiments were carried out after study proposal approved by researches committee at faculty of pharmacy, OIU and obtaining of ethical clearance permission from institutional animal committee, pharmacology department, under the serial no (OIU/I.A.E.C./Exp.Ph., TOX./2015/0.01).

2.3. Preparation of tested compounds, standard agonists and their antagonists

All the three tested compounds and the standard agonists; acetylcholine, 5-HT and barium chloride and their relevant blockers; atropine and cyproheptadine powders were dissolved in distilled water with gentle heat aid and shaked well specially for the synthetic compounds. Solution for either each tested compound or standard agonists prepared by serial dilution in cumulative manner to give six different concentrations ranged (10^{-5}-10^{-10} M), whilst for blockers only two different concentrations were used; low (10^{-10} M) and high (10^{-8}M).

2.4. Experimental Design

Rabbit was fasted overnight and then killed by decapitation. Abdomen was opened and suitable length (1-1.5 inch) of jejunum part of small intestine was isolated and preserved in tyrode's solution and completely cleared from attached tissues and any food content (if present) using tyrode's solution. Intestine then fixed in inner organ path to tissue holder and simple writing recording lever (Harvard-USA). Tissue was kept for 30 minutes to be adapted; meanwhile tyrode solution was changed every 10 minutes. Preparation was balanced using one gram piece of plasticine to fix base line of spontaneous smooth muscle activity. Base line was set as zero at first, and then six doses of different concentration for either the tested compounds or standard spasmogen agonist was added in fixed cumulative dose cycles and kept in contact with tissue for one minute. On other hand, standard blockers kept for one minute before recording the response on the trace. The Kymograph with its specific graph papers (Harvard-USA) was used to obtain uniform response reading of each dose in 30 seconds. All these steps were performed under presence of tyrode's solution, continuous aeration supply and fixed temperature (37C^{o}). The traces of repeated six experiments were measured after calibration of whole responses using different standard weights. All results were introduced into Microsoft excel program and the response percentages were calculated using $((D_1/D_2)*100)$ equation. Finally, dose response curve was constructed between Log M doses concentrations versus their response percentage values using GraphPad software non-linear regression facility and main pharmacological parameters such as; E_{max}, EC_{50} and pA_2 or pD_2 were calculated.

2.5. Statistical analysis

The Prism 5.0 for windows computer program version 5.01 (USA) was used for statistical calculations. Data were represented as mean ± standard error of mean (SEM). Unless, indicated all results were analyzed using One-way Analysis of Variance (1W-ANOVA) followed by Dunnett's PostHoc test to estimate statistical differences among groups compared to standard and the difference considered significant at $p \leq 0.05$.

3. RESULTS

The novel three synthetic compounds exhibited various contractions on isolated rabbit intestine as shown in **Fig 1**.

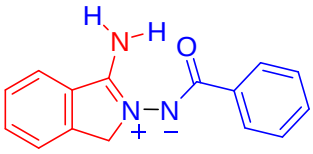
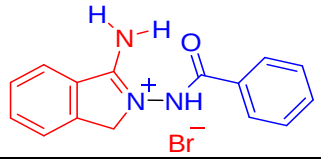
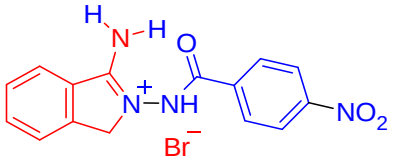
Compound TIND-1 showed higher efficacy [Emax=117.3 ± 7.9] upon comparison with TIND-10 [E max=77.6 ± 7.8] and TIND-11 [E max= 51.89 ± 11.2], whilst TIND-1 showed low value upon comparison with standard spasmogens Acetylcholine (Ach) [205.9 ± 17.1], serotonin (5HT) [239.6 ± 19.02] and barium chloride ($BaCl_2$) [328.8 ± 6.7], see **Fig 2**.

The muscarinic antagonist; atropine in both low (10^{-10}M) and high dose (10^{-8}M) shifted the dose response curve of the tested compound TIND-1 to the right and downward in dose-dependent manner (**Fig. 3**). The low dose shifted TIND-1 dose response curvet to [E max = 57.10 ± 13.4], whilst the high dose showed [E max = 48.00 ± 7.7]. Additionally, the relevant pharmacological parameters (EC_{50} and pA_2) of both TIND-1 and ACh were represented in **Table 2**.

The non-selective serotonergic antagonist; cyproheptadine in low dose (10^{-10}M) and high dose (10^{-8}M) shifted the dose response curve of 5-HT to the right in dose-dependent manner, whilst it did not affect the curve of synthetic compound TIND-1, see **Fig 4**.

Figures + Tables

Table 1: Chemical Structure and Molecular Weight of Three Tested Synthetic Isoindole Derivatives.

Code	Chemical Structure	Molecular weight
TIND-1		252
TIND-10		332
TIND-11		378

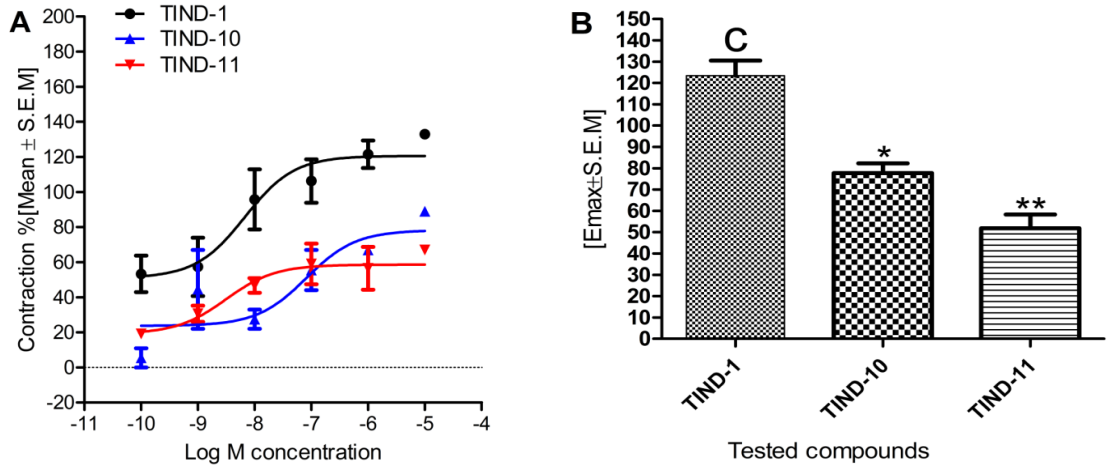


Fig. 1: Cumulative dose-response curve of compounds; TIND-1, TIND-10 and TIND-11 on isolated rabbit intestine (A), whilst the main pharmacological parameters, E max was shown in (B). Values presented are means of 6-8 observations, vertical bars denote standard errors of mean (S.E.M).

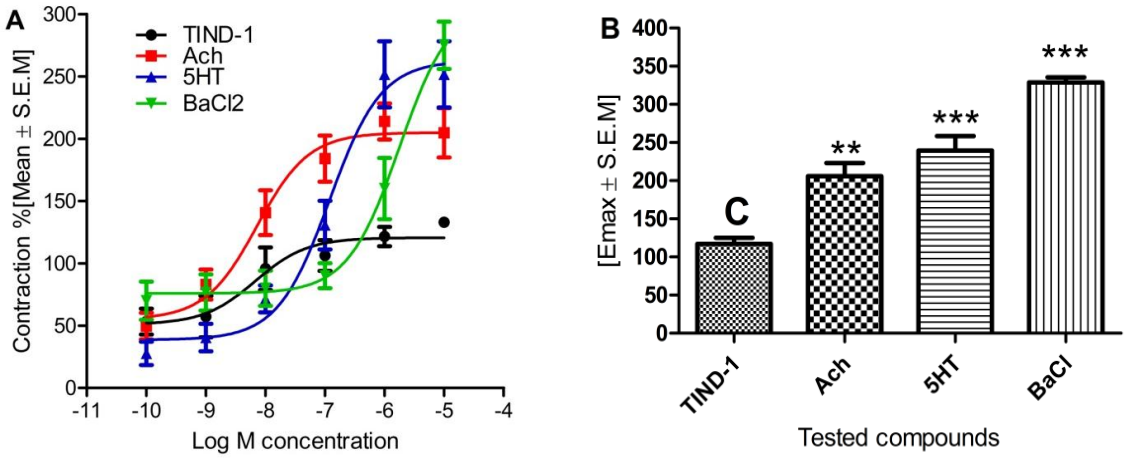


Fig. 2: Cumulative Dose-Response Curve Demonstrate Contracting Effects of The Tested Compound Tind-1 In Comparison With The Three Contracting Standards (Ach, 5HT and bacl2) On Isolated Rabbit Intestine (A), Whilst The Main Pharmacological Parameters E Max was shown in (B). Values presented Are Means of 6-8 Observations, Vertical Bars Denote Standard Errors Of Mean (S.E.M).

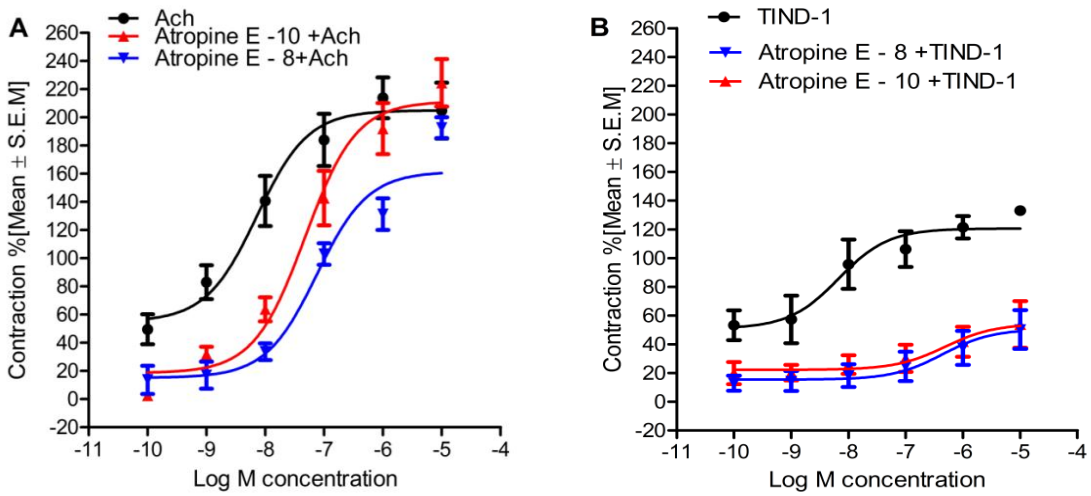


Fig 3: Cumulative Dose-Response Curve Demonstrate Contracting Effects of Acetylcholine (A), and The Tested Compound TIND-1 (B), Both In The Presence of Atropine In two different Doses (10^{-10} and 10^{-8}M) on Isolated Rabbit Intestine. Values Presented are Means of 6-8 Observations, Vertical Bars Denote Standard Errors of Mean (S.E.M).

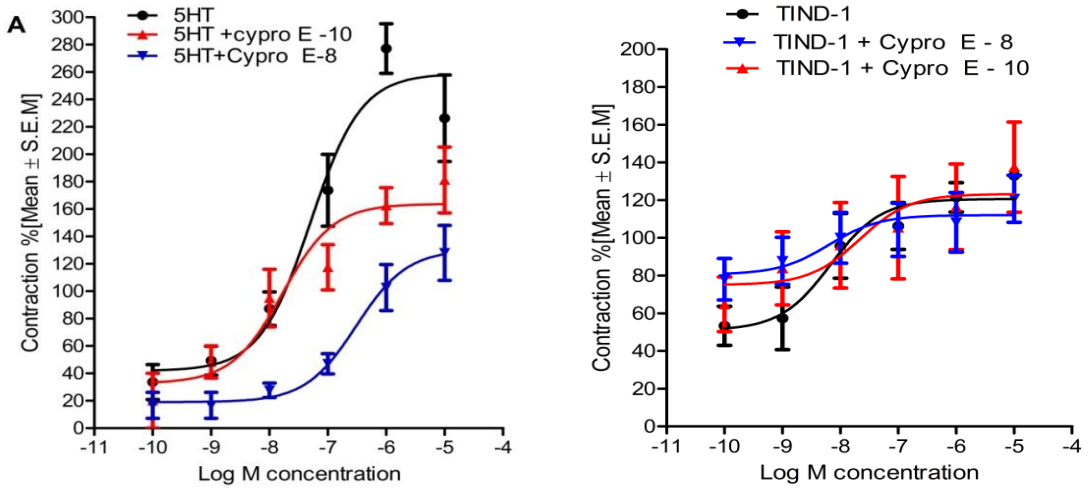


Fig. 4 Cumulative dose-response curve demonstrate contracting effects of 5HT (A), and the selected active compound TIND-1 (B), all were tested in presence of cyproheptadine in doses of 10^{-8} and 10^{-10} on isolated rabbit intestine. Values presented are means of 6-8 observations, vertical bars denote standard errors of mean (S.E.M).

Table 2: The Main Pharmacological Parameters EC50 and pA2 of TIND-1, and ACh in Presence of Atropine at Doses of 10-10 M and 10-8 M.

Tested compounds	EC_{50} Alone	pA_2/pD_2
ACh	[4.2 ± 2.1] x 10^{-8} **C**	----------
Atropine 10^{-10}+ACh	[10.1 ± 4.4] x10^{-7}ns	6.0 ± 0.3 ns
Atropine 10^{-8}+ACh	[9.9 ± 4.4] x10^{-8} ns	6.1 ±0.13 ns
TIND-1	[1.4 ± 0.7] x10^{-8} **C**	----------
TIND-1 + Atropine E-10	[1.3 ± 0.6] x10^{-7}ns	7.1 ± 0.2 *
TIND-1 + Atropine E-8	[1.5±0.9] x10^{-6} ns	4.7 ± 0.7 *

Statistical method, t-test used to calculate p-value between pA2/pD2 values. P ≤ 0.05; ** p≤ 0.01; ***p ≤ 0.001; C, control, ns, Not significant statistically.

DISCUSSION

The three synthetic isoindole derivatives were synthesized and proved to serve as lead compounds for the discovery of new anti-inflammatory drugs[18], whilst their spasmogeic activity in this study was carried out on isolated rabbit intestine.

The study revealed that all the three tested synthetic compounds TIND-1, TIND-10 and TIND-11 exhibited various dose dependent contraction effect on the smooth muscle of isolated rabbit intestine, in vitro, this indicates their spasmogenic activity, and contradict with famous thalidomide spasmolytic activity on rabbit intestine reported by Somers.[19]

On the other hand, the three synthetic compounds showed different EC50 values TIND-1 [1.4 ± 0.7] $x10^{-8}$, TIND-10 [1.4 ± 0.5] $x10^{-7}$, TIND-11 [3.8 ± 1.9] $x10^{-10}$, with significant difference among them (p = 0.0187), and this means that the compounds have different potencies.

TIND-1 showed significant higher efficacy when compared with TIND-10 and TIND-11 (p = 0.0094) and this exhibited that it the most active one, so TIND-1 nominated for the next step in this study.

TIND-1 showed significant low efficacy (p= < 0.0001) upon comparison with standard spasmogens, and its dose response curve did not behave like barium chloride and this proved that TIND-1 contracting effect was not mediated through direct smooth muscle action. The standard muscarinic antagonist atropine shifted the curve of TIND-1 to the right (p value = 0.3426) and downward (p value = 0.0008) in dose-dependent manner and these findings proved that TIND-1 interactes irreversibly with atropine at the same muscarinic cholinergic receptors or seems likely to exerts its activity by binding allosterically, thus both pA_2 and pD_2 values can be used to express its antagonistic feature. The contraction of intestine that used for treatment of many gastro intestinal disorders was reported by many studies such as Mehmood and his colleagues who reported that crude extract of Fumaria produced spasmogenic effect, which is blocked by atropine.[20]

The overall findings exhibited that TIND-1 mediated its contraction effects on rabbit intestine through activation of acetylcholine receptor, however this effect is less than the effect of Acetylcholine so TIND-1 considered as partial chlolinoceptor agonist.

CONCLUSION AND RECOMMENDATION

All three tested synthetic compounds exhibited spasmogenic effects on rabbit intestine smooth muscle, TIND-1 was the most active one and exerts its effect via muscurinic receptors. Other investigations are recommended to demonstrate other mechanisms such as interaction with ion channels or prostaglandin receptors that may have role in this spasmogenic effect.

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