# Evaluation of $ED_{50}$ and 5α-reductase inhibitor activities of some thiopyrimidine, pyrane, pyrazoline and thiazolopyrimidine derivatives

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**Abstract**: In this work, twenty-one thiopyrimidine (1-21) candidates containing a pyrane, pyrazoline and thiazolopyrimidine ring screened for their $ED_{50}$ and 5α-reductase inhibitors comparable to that of Anastrozole as positive drug. Some of the tested product showed moderate 5α-reductase inhibitors with lower toxicity. The detailed $ED_{50}$ and 5α-reductase inhibitor activities of the synthesized compounds were studied.

**Keywords**: Citrazinic acid, pyrimidinone, thiazolopyrimidine, anstrazole, 5α-reductase inhibitors.

## INTRODUCTION

5α-Reductase inhibitors are drugs with anti androgen effects, used mainly in the treatment of prostatic hyperplasia via inhibiting the 5α-reductase enzyme and consequently inhibit the conversion of testosterone to dihydrotestosterone. Pyridine and pyrimidine derivatives are very important for anticancer activities (Shao *et al.,* 2014), antitumor (Shi *et al*., 2015), anticonvulsant (El-Sawy *et al*., 2014), antiviral (Naidu *et al*., 2015), antibacterial (Aggarwal *et al*., 2014), antimicrobial (Gupta *et al*., 2013), and fungicidal (Mo *et al*., 2008) activities. On the other hand, some of pyrazoline derivatives have been interesting group of products, many of which possess wide-spread as analgesic, antidepressant, antipyretic and antirheumatic activities (Jung *et al*., 2005; Palaska *et al*., 2001) and are also well known as anti-inflammatory agents (Bansal *et al.,* 2001) and they are used as antidiabetic agents (Ahn *et al*., 2004; Villhauer *et al*., 2002). Recently, several candidates containing heterocyclic moieties were designed, which had potent anticancer activity as potential telomerase inhibitors (Liu *et al*., 2011, 2010), antitubercular (Siddiqui *et al.,* 2014), anticancer (Kandeel *et al*., 2015), and antibacterial and antifungal (Aggarwal *et al*., 2014) agents. In view of these literary survey and in continuation of previous work in thienopyridine chemistry, in this study, some newly compounds containing pyridine, pyimidine, thiopyrimidine, thiazolopyrimidine substituted with thiophene ring were evaluated for theire 5α-reductase inhibitors compared to anastrozole as a referenece drug.

## MATERIALS AND METHODS

### *Chemistry*

All the tested compounds were confirmed by physical and spectroscopic evidences according to the previously literatures (Amr *et al.,* 2007, 2008).

### *Biological assay*

*Experimental animals*

All animals were obtained from National Research Center, Cairo, Egypt, Giza, Egypt and were acclimatized for 10 days under standard housing conditions (24°±1°C; 45-55% RH with 12:12h light/dark cycle). The animals had free access to rat food (Lipton Gold Mohr, India) and water. The animals were habituated to laboratory conditions for 48h prior to the experimental protocol to minimize any nonspecific stress. The experimental protocol was approved by the Institutional Animal Ethics Committee by Government College of Pharmacy, Karad, India and animals were maintained under standard conditions in the animal house approved by Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA).

### *Animals*

Male Sprague-Dawley rats weighting 150-200gm were obtained from National Research Center, Cairo, Egypt, Giza, Egypt and were acclimatized for 2 days under standard housing conditions (24°±1°C; 45-55% RH with 12:12h light/dark cycle). The experimental protocol was approved by the Institutional Animal Ethics Committee by Government College of Pharmacy, Karad, India and animals were maintained under standard conditions in the animal house approved by Committee for the Purpose of Control and Supervision on Experiments on Animals.

### *Treatment of animals*

23 groups, each group maily composed of 12 male Sprague-Dawley rats in the postnatal third days.

The first group act as negative standard and received only 5%. Tween 80 in water beginning on the postnatal third day until the age of seven weeks.

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The second group acts as positive reference standard and revived the standard anstrazole. Each of the remaining 21 groups received individually and separately subcutaneously one the newly tested compounds as 5α-reductase inhibitor. The tested compounds were dissolved in 5% Tween 80 in water. After scarifying, blood was withdrawn for testosterone and dihydrotestosterone determination (George *et al.,* 1989). Intraprostatic concentrations of testosterone and DHT were determined according to (Di Salle *et al*., 1993).

***Determination of acute toxicity***

The $LD_{90}$ was determined by using rats. It has been performed following current OECD guideline (table 2).

## RESULTS

In continuation of previous study, some of heterocyclic systems substituted with a thiophene moiety, such as pyrimidine, pyridine, thiazolopyrimidine and thiazolopyridine derivatives 1-21 (figs. 1 and 2) were produced in advance and evaluated as analgesic, anti-Parkinsonian and anti-inflammatory agents (Amr *et al.,* 2007, 2008). Herein, due to aspects of streochemical and conformational similarity of tested compounds and some clinical used 5α-reductase inhibitors these compounds were evaluated for their 5α-reductase inhibitors activities.

All tested products were evaluated for their 5α-reductase inhibitor activity *in vivo* via determining their $ED_{50}$, and for their acute toxicity via determining their $LD_{90}$ data and given in tables 1 and 2 respectively. Some of the tested products showed 5α-reductase inhibitor activities with good $ED_{50}$ in the range of 1.68-5.45mg $kg^{-1}$. $LD_{90}$ for all products were highly enough to provide good therapeutic windows and softy profile margin. The relative potency was calculated by dividing the $ED_{50}$ of Anastrazole $^{R}$ by that of a tested compound**.**

Compounds showed 5α-reductase inhibitor activities descending order of activity was 11, 12, 8, 21, 1 and 5.

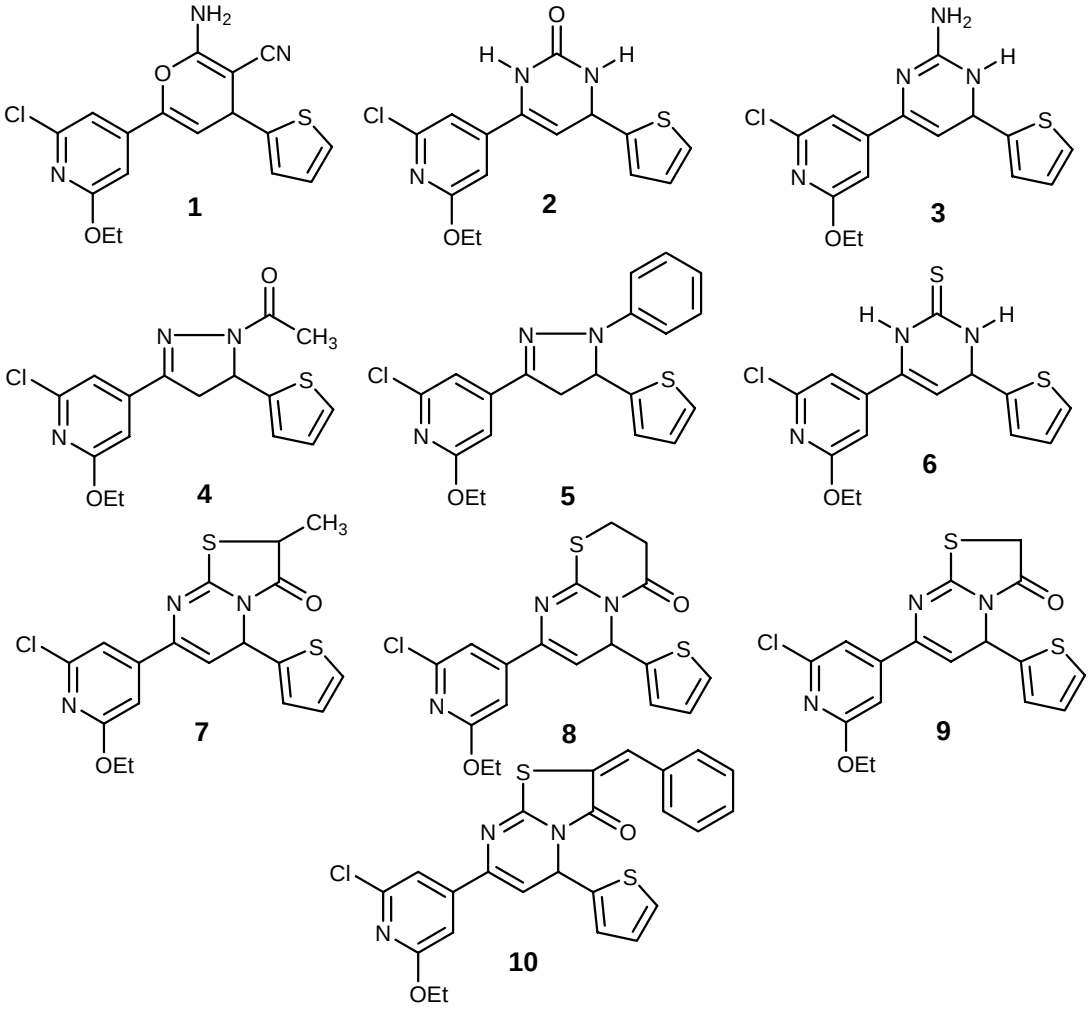


**Fig. 1**: Chemical structure for compounds 1-10

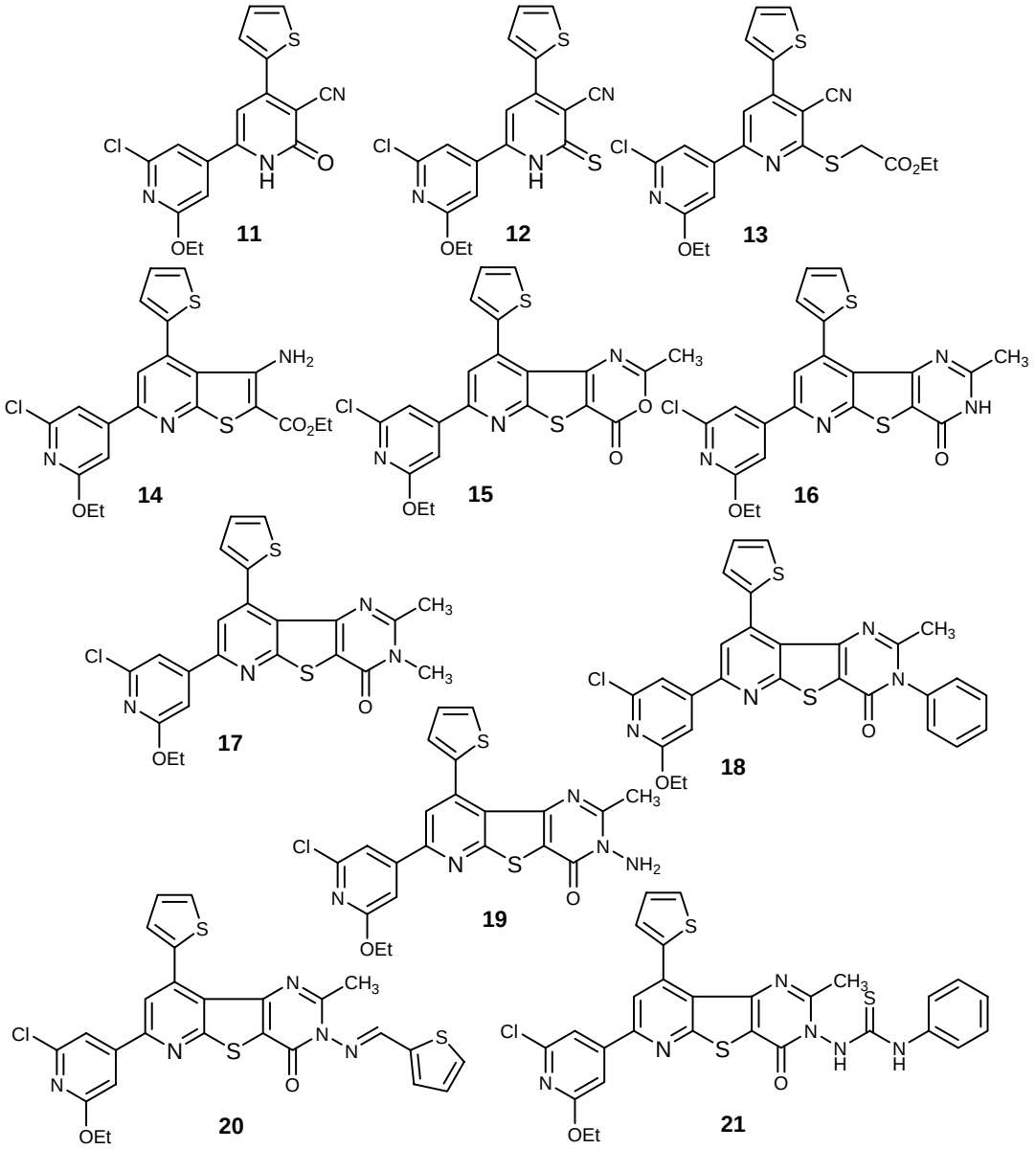


**Fig. 2**: Chemical structure for compounds 11-21

The following tested compounds showed potent α-reductase inhibitor activities compound 1; (ED $ED_{50}$ 4.34 mg $kg^{-1}$), compound 2; (ED $ED_{50}$ 1.72mg $kg^{-1}$), compound 3; (ED $ED_{50}$ 4.75 mg $kg^{-1}$), compound 4; (ED $ED_{50}$ 2.06 mg $kg^{-1}$). Compound 5; (ED $ED_{50}$ 4.56 mg $kg^{-1}$), Compound 6; (ED $ED_{50}$ 1.84mg $kg^{-1}$), compound 7; (ED $ED_{50}$ 1.92 mg $kg^{-1}$), Compound 8; (ED $ED_{50}$ 3.85 mg $kg^{-1}$), compound 9; (ED $ED_{50}$ 4.65mg $kg^{-1}$), compound 10; (ED $ED_{50}$ 1.85 mg $kg^{-1}$), compound 11; (ED $ED_{50}$ 2.08 mg $kg^{-1}$), compound 12; (ED $ED_{50}$ 3.75mg $kg^{-1}$), compound 13; (ED $ED_{50}$ 5.45 mg $kg^{-1}$), compound 14; (ED $ED_{50}$ 1.72 mg $kg^{-1}$), compound 15; (ED $ED_{50}$ 2.05 mg $kg^{-1}$), compound 16; (ED $ED_{50}$ 1.89 mg $kg^{-1}$), compound 17; (ED $ED_{50}$ 4.89 mg $kg^{-1}$), compound 18; (ED $ED_{50}$ 2.05 mg $kg^{-1}$), compound 19; (ED $ED_{50}$ 1.74 mg $kg^{-1}$), compound 20; (ED $ED_{50}$ 1.72 mg $kg^{-1}$), compound 21; (ED $ED_{50}$ 4.12 mg $kg^{-1}$), Anastrazole®; (ED $ED_{50}$ 1.09 mg $kg^{-1}$).

The following tested compounds showed good safety margines compound 1; ($LD_{90}$ 712 mg $kg^{-1}$), compound 2; ($LD_{90}$ 926mg $kg^{-1}$), compound 3; ($LD_{90}$ 832mg $kg^{-1}$), compound 4; ($LD_{90}$ 980 mg $kg^{-1}$), compound 5; ($LD_{90}$ 658 mg $kg^{-1}$), compound 6; ($LD_{90}$ 810 mg $kg^{-1}$), compound 7; ($LD_{90}$ 820 mg $kg^{-1}$), compound 9; ($LD_{90}$ 992mg $kg^{-1}$), compound 10; ($LD_{90}$ 895 mg $kg^{-1}$), compound 11; ($LD_{90}$ 914mg $kg^{-1}$), compound 12; ($LD_{90}$ 790mg $kg^{-1}$), compound 13; ($LD_{90}$ 953 mg $kg^{-1}$), compound 14; ($LD_{90}$ 922mg $kg^{-1}$), compound 15; ($LD_{90}$ 743mg $kg^{-1}$), compound 16; ($LD_{90}$ 818 mg $kg^{-1}$), compound 17; ($LD_{90}$ 1010mg $kg^{-1}$), compound 18; ($LD_{90}$ 955mg $kg^{-1}$), compound 19; ($LD_{90}$ 915 mg $kg^{-1}$), compound 20; ($LD_{90}$ 658 mg $kg^{-1}$), compound 21; ($LD_{90}$ 730 mg $kg^{-1}$).

**Table 1**: Evaluation of $ED_{50}$ and 5α-reductase inhibitor activities of the tested compounds (1-21) relative to Anastrazole ®

| Comp. No | $ED_{50}$[a] (mg $kg^{-1}$) | Potency relative to Anastrazole ® |
|---|---|---|
| 1 | 4.34 | 0.25 |
| 2 | 1.72 | 0.63 |
| 3 | 4.75 | 0.23 |
| 4 | 2.06 | 0.53 |
| 5 | 4.56 | 0.24 |
| 6 | 1.84 | 0.59 |
| 7 | 1.92 | 0.56 |
| 8 | 3.85 | 0.28 |
| 9 | 4.65 | 0.23 |
| 10 | 1.85 | 0.59 |
| 11 | 2.08 | 0.52 |
| 12 | 3.75 | 0.29 |
| 13 | 5.45 | 0.20 |
| 14 | 1.72 | 0.63 |
| 15 | 2.05 | 0.53 |
| 16 | 1.89 | 0.58 |
| 17 | 4.89 | 0.22 |
| 18 | 2.05 | 0.53 |
| 19 | 1.74 | 0.63 |
| 20 | 1.72 | 0.63 |
| 21 | 4.12 | 0.26 |
| Anastrazole® | 1.09 | 1.00 |

[a]$ED_{50}$: Dose cause 50% of pharmacological response in test.

Compounds 2, 14, 19 and 20 have nearly the same 5α-reductase inhibitor potencies and were the most potent in this study and the safety of compounds 2, 14 1nd 19 nearly the same while compound 20 is the lowest safe one. Also compounds 6 and 10 have equal 5α-reductase inhibitor potencies but compound 10 is safer. Compound 16 and 7 nearly have equal safety but compound 16 more potent as reductase inhibitor. Compounds 4, 15 and 18 have equal 5α-reductase inhibitor potencies and the safety descending order was compounds 4, 18 and 15. Compounds 1, 5, 8, 11, 12 and 21 showed moderate 5α-reductase inhibitor potencies and the descending order of activity was 11, 12, 8, 21, 1 and 5, their descending safety order was 8, 11, 12, 21, 1 and 5. Compounds 3, 9, 13 and 17 showed low 5α-reductase inhibitor potencies, their descending order of activity was (3 & 9), 17 and 13. Compound 3 and showed the same 5α-reductase inhibitor potencies while compounds 13 where the least potent one but having good safety. The obtained results are summarized in table 1.

## DISCUSSION

Analysis and correlations between the streochemical, chemical and conformational atructural aspects of the tested compounds and their 5α-reductase inhibitor potencies leaded to the following structural activity relationships (SAR) (Ahmed and Denison, 1998; Liu, *et al*., 2006; Bruno *et al*., 2002; and Salem *et al*., 2006).

***Structure activity relationship***

Substituted ethoxy chloropyridine linked to thiophens (bioisoster for the triazole moiety of anstrazole) via a propene brige (similar to propenenitril in anstrazole) essential for the 5α-reductase inhibitor activitis. Incorporating the propene brigge in heterocyclic ring system greatly affected the 5α-reductase inhibitor activity is due to remote effects of the cage strain as follow:

1. Pyrimidone and pyridine heterocyclic ring sysems fused to the thiophene nucleus provided the most active ones (compounds 2, 14, 19 & 20) more than pyrimidinoethione or pyrimidine thione heterocycles involved the heterocyclic ring system (compounds 6 & 10).
2. Further fusion of heterocyclic ring system for the pyridine or pyrimidine nucleus greatly reduces the 5α-reductase inhibitor activities as in compounds 15, 16, 17 and 18). Also the same thing occu wred when the propene bridge incorporated within five membered heterocyclic ring system as in compounds 4 and 5 but compound 4 more active due its capability of hydrogen bond formation with the receptor.
3. The ketonic derivatives having heigher 5α-reductase inhibitor activity is than the thione ones due its capability of hydrogen bond formation with the receptor as in compounds 8, 11 and 12.
4. Cage strains induced by either five ring heterocyclic as in compounds 5 and 9 or the remote cage strain induced by polycyclic ring system as in compounds 17 and 21.

## CONCLOUSION

A series of heterocyclic thiopyrimidine, pyrane, pyrazoline and thiazolopyrimidine derivatives substituted with thiophene ring were screened as 5α-reductase inhibitors. These compounds showed potent 5α-reductase inhibitors activities and the descending order of potency was: (2, 14, 19 & 20), (6 & 10), 16, 7, (4, 15 & 18), 11, 12, 8, 21, 1, 5, (3 & 9), 17 and 13.

**Table 2**: Acute toxicity $LD_{90}$ of all tested compounds (1-21)

| Comp. No | $LD_{90}$[a] (mg $kg^{-1}$) |
|---|---|
| 1 | 712 |
| 2 | 926 |
| 3 | 832 |
| 4 | 980 |
| 5 | 658 |
| 6 | 810 |
| 7 | 820 |
| 8 | 930 |
| 9 | 992 |
| 10 | 895 |
| 11 | 914 |
| 12 | 790 |
| 13 | 953 |
| 14 | 922 |
| 15 | 743 |
| 16 | 818 |
| 17 | 1010 |
| 18 | 955 |
| 19 | 915 |
| 20 | 658 |
| 21 | 730 |

[a]$LD_{90}$: Dose kill 90% of the tested animals.

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