UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL
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Gabriel Oliveira de Azambuja

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Orientadora

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"A melhor maneira de prever o futuro é criá-lo"

Peter Drucker

Desde a descoberta de Monastrol em 1999 como o primeiro inibidor de Eg5, diidropirimidinonas / thiones funcionalizadas (DHPMs) emergiram como protótipos para o desenvolvimento de drogas em diferentes alvos biológicos. O presente trabalho teve como objetivo avaliar a atividade antifúngica de DHPMs que foram obtidas empregando a reação de Biginelli. Suas atividades antifúngicas foram avaliadas contra *C. neoformans* e *C. albicans*. Os compostos **1-i** e **1-k** inibiram moderadamente o crescimento fúngico de *C. neoformans*, já o composto **2-k** apresentou valores de MIC_{80} de 62,5-125 $\mu g.mL^{-1}$. Considerando a atividade contra *C. albicans*, os compostos **1-i** e **1-n** apresentaram um valor MIC_{50} de 125-250 $\mu g.mL^{-1}$. As mudanças realizadas no núcleo das DHPMs parecem ser valiosas para gerar compostos com potencial efeito antifúngico.

Este manuscrito foi elaborado segundo as normas do *"Letters in Drug Design & Discovery"* apresentadas em anexo.

In vitro antifungal activity of dihydropyrimidinones/thiones against *Candida albicans* and *Cryptococcus neoformans*

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Abstract: Since the Monastrol discovery in 1999 as the first inhibitor of Eg5, functionalized dihydropyrimidinones/thiones (DHPMs) have emerged as prototypes for drug design in different targets. The present work aimed to evaluate the antifungal activity of a chemical library of DHPMs which were obtained employing the Biginelli reaction. Their antifungal activities were assessed against *C. neoformans* and *C. albicans*. The compounds **1-i** and **1-k** inhibited moderately the fungal growth of *C. neoformans*, with compound **2-k** presenting MIC_{80} values of 62.5-125 $\mu g \cdot mL^{-1}$. Considering activity against *C. albicans*, the compounds **1-i** and **1-n** present a MIC_{50} value of 125-250 $\mu g \cdot mL^{-1}$. The changes performed in DHPM scaffold appear to be valuable for generating compounds with potential antifungal effect.

Keywords: Biginelli reaction, *Cryptococcus neoformans*, *Candida albicans*, antifungal activity, structure activity relationship, multicomponent reaction.

1. Introduction

Dihydropyrimidinones/thiones (DHPMs) are a class of heterocyclic compounds with several pharmacological activities, being considered privileged structures due to its ability of interaction with different biological targets [1]. Among their pharmacological effects, the anticancer, antihypertensive, anti-inflammatory, antimicrobial and antioxidant activities were previously described [2]. In relation to their antifungal properties, the importance of the DHPMs' scaffold was pointed out in several investigations [3-5]. This scaffold is obtained by a simple and straightforward procedure, identified as Biginelli reaction, which consists on a three-component condensation of an aldehyde, a β-ketoester and a (thio)urea. A large diversity of building blocks can be used in Biginelli reaction, such as 1,3-dicarbonyl compounds, substituted aldehydes and (thio)ureas [6-8].

Nowadays, fungal infections have emerged as a big problem for the public health [9-10]. Their incidence has increased dramatically in recent years and in spite of the several available antifungal drugs, they are not completely effective for their eradication [11]. In addition, they all possess a certain degree of toxicity and quickly develop resistance due to the large-scale use [12]. In this context, there is a special interest in screening different molecules for their antifungal effect in order to find new antifungal chemical structures alternatives to the existing ones.

In the present study, we have synthesized 32 DHPMs which were evaluated against *Candida albicans* and *Cryptococcus neoformans*, two clinically important fungi that produce severe, and many times fatal, fungal infections [13-14].

2. Results and Discussion

2.1. Synthesis of dihydropyrimidinones/thiones

The DHPMs **1a-p** and **2a-p** were prepared using a previously reported methodology (**Scheme 1)** and were obtained in good yields as shown in Table **1** [15].

2.2. Spectral analysis

In ^{1}H-NMR spectrum, one downfield broad singlet or duplet of the NH-1 hydrogen of the DHPM skeleton appeared around 7.70-9.35 or 9.10-10.55 ppm, and for NH-3 appeared around 5.50-7.95 or 7.60-9.80 ppm in $CDCl_3$ and DMSO-d_6, respectively. Downfield signals of aromatic hydrogens produced the next group of signals. The benzylic hydrogen produced a signal among 5.00-5.40 ppm, while the singlet of allylic CH_3 appeared at 2.20 ppm. The signals of system H_3CCH_2 system appeared as a triplet and quartet, respectively at 1.10 and 4.00 ppm.

In ^{13}C-NMR decoupled spectrum, the most representative signals were the methyl carbons at 14 and 18 ppm, the methyne carbon at 54 ppm, and the methylene carbon at 60 ppm. The two carbon vinylic of DHPM ring appeared at about 105 and 146 ppm. The ester carbonylic carbon, produced a signal at 165 ppm, while the most downfield signals were appeared at 178 ppm corresponds to the quaternary carbon of C=S bond thionyl and near of 152 ppm corresponds to the quaternary carbon of C=O, which are situated between the two nitrogen. These assignments are represented in **Figure 1**. The spectra data of the most active compound (**2-k**) are described below, and the spectral characterization of others compounds are listed in supplementary material.

Ethyl 6-methyl-4-(4-nitrophenyl)-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (**2-k**): yield 76%, mp 210-212°C. ^{1}H NMR (300 MHz, DMSO-d_6): 1.11 (t, 3H, J = 7.1 Hz), 2.29 (s, 3H), 4.01 (q, 2H, J = 7.0 Hz), 5.30 (d, 1H, J = 3.1 Hz), 7.53 (d, 2H, J = 8.6 Hz), 7.92 (s, 1H, NH), 8.23 (d, 2H, J = 8.7 Hz), 9.39 (s, 1H, NH). ^{13}C NMR (75 MHz, DMSO-d_6): 14.1, 17.9, 53.7, 59.4, 98.2, 123.9, 127.7, 146.7, 149.4, 151.8, 152.0, 165.1.

2.3. Antifungal activity

In order to have a look into the pharmaceutical application of DHPMs as prototypes for the development of new antifungal drugs, the antifungal properties of 32 DHPMs (Table **1**) against *C. albicans* and *C. neoformans* were explored. The selection of *C. neoformans* was due to the fact that this opportunistic fungus is the main cause of cryptococcal meningoencephalitis, which has a high incidence among HIV patients with impaired defenses [16]. Even though new antifungal drugs have been developed in recent years, the availability of antifungal agents with anticryptococcal activity is still limited and sometimes the strains develop quickly resistance [12-13, 17]. This scenario has motivated the search of new compounds that have antifungal properties against this fungus [18].

In turn, *C. albicans* is the fourth leading cause of nosocomial bloodstream infection in intensive care units, causing fatal invasive candidiasis in a high percentage of patients. As a consequence, new anti-*Candida* structures are highly needed [14].

Results were expressed as the percentage of inhibitions of the fungus displayed by each compound in the range 250–3.9 $\mu g \cdot mL^{-1}$.They were determined by using the standardized microbroth dilution method M-27A3 of the Clinical and Laboratory Standards Institute [19], that assures confident and reproducible results. Also the minimum concentration that inhibits either 80% (MIC_{80}) or 50% of the fungal growth (MIC_{50}) was assessed for each compound.

For a better comprehension of the antifungal results, we grouped the compounds into two series: (I) dihydropyrimidin-2-thiones derivatives (**1-a** - **1-p**) and (II) dihydropyrimidin-2-one derivatives (**2-a** - **2-p**) that differ one each other in that (I) are thio-ketones and (2) are ketones that bear the same substituents in the aromatic ring for comparative purposes. They were tested against *C. albicans* ATCC 10231 and *C. neoformans* ATCC 32264 at concentrations from 250 to 3.9 $\mu g.mL^{-1}$ and the percentages of inhibition of each compound at all concentrations were determined. These inhibition percentages can be seen in full in the Supplementary Table 1 (Table **S1**). For the sake of clarity, Table **S1** was summarized in Table **2** by recording the MICs at different endpoints such as MIC_{80} and MIC_{50} (minimum concentration at which each compound that inhibits 80% or 50% of the fungal cell growth that have showed to consistently represent the *in vitro* activity of compounds [20].

From the analysis of Table **2**, it is clear that all compounds displayed some degree of activity (MICs ≤ 250 µg/mL) against *C. albicans* (lines in white) and *C. neoformans* (lines highlighted in grey). However, *C. neoformans* showed to be more sensitive to the whole series than *C. albicans*, since ten compounds (**1c, 1d, 1h-1l, 1n, 2h** and **2k**) showed $MIC_{50} \leq 250\ \mu g \cdot mL^{-1}$ against *C. neoformans* while only tow (**1n** and **1i**) showed marginal activity against *C. albicans*. The same analysis can be performed with MIC_{80}; for this

breakpoint, four compounds (**1i**, **1l**, **1n** and **2k**) showed activity against *C. neoformans* while none showed activity against *C. albicans*.

Regarding the difference in activity between series I and II against *C. neoformans*, Table **2** clearly shows that compounds of the series I, with a thio-ketone in their structures, displayed better activities than those of series II with a ketone, showing that the sulfur atom can have an important role in the activity. This is evidenced by the fact that eight compounds of series I (**1c, 1d, 1h-1l** and **1n**) showed MIC_{50} values ≤ 250 $\mu g \cdot mL^{-1}$ against *C. neoformans*, while among the compounds of series II, just two of them (**2-h** and **2-k**) inhibited more than 50% of the *C. neoformans* growth. The same analysis can be made with MIC_{80}: five compounds (**1i-1l** and **1n**) showed antifungal activities in the series I while only one (**2k**) showed activity in the series II. **Figures 2** and **3** clearly show the differences in activity of series I and II against *C. neoformans.*

Within series I, it is worth to observe that most of active compounds against *C. neoformans* (**1-h-1n, Figure 2**) possess a substituent in the *p*-position of the aromatic ring and, the most active compound was **1h** that possesses a CN- substituent in the *p*-position.

To deepen the analysis of the compounds with antifungal activities, the Log*P* of each compound (**1a-1p**; **2a-2p**) was analyzed. It is known that Log*P* (logarithm of the partition coefficient in a biphasic system, e.g. *n*-octanol/water) describes the macroscopic hydrophobicity of a molecule which is a factor that many times determines its ability to penetrate the membranes of fungal cells and to reach the interacting sites, thus influencing the antifungal activity of compounds [21].

In Table **3**, the Log*P* were recorded and in **Figure 4** the correlation with the antifungal activity can be observed. The rule-of-five comprises four physicochemical parameters that can predict whether the molecules will be orally active or not. It is worth to take into account that 90% of orally active medicines, which are on clinical phase II, follow this rule. The four physicochemical parameters are Log $P \leq 5$, H-bond acceptors ≤ 10, molecular weight ≤ 500 and H-bond donors ≤ 5 [22]. The 32 DHPMs tested against *C. neoformans* and *C. albicans* yeasts fit on these four parameters (Table **3**) presenting a high probability of these compounds to be developed as a drug.

In **Figure 4**, it can be observed that the most active compounds [those with inhibition percentages of ≥ 60 % (right and up quarter)] have Log*P* higher than 1 and, mostly, higher than 1.5. Instead, most compounds that have low (< 50%) growth inhibition percentages (left and down quarter) belong to series II, with Log*P* values were lower than 1. Thus, in the results reported here, were identified that the *para*-substituent in the aromatic ring with a Log*P* value higher than 1.00 can improve the activity.

It is important to consider the relevance of the toxicity of these molecules to be a promising candidate for pharmaceutical development, because the compound not only should be effective against fungi but also should have good pharmacokinetics and not be toxic for healthy cells. Considering this point, a pre-clinical study with **1-e** compound, with *in vitro* effect against glioma cell line [23], was performed in rats and it demonstrated that it has appropriate bioavailability, linear pharmacokinetics and no acute toxicological effects after oral administration [24], showing that DHPMs could be the prototype of a new medicine.

There are previous promising studies approaching DHPMs derivatives against *C. albicans* showing MIC values of 50 $\mu g \cdot mL^{-1}$ [3] and 32 $\mu g \cdot mL^{-1}$ [5], and zones of inhibition of 17 mm [4]. However, the data reported in this paper are the first pharmacological screening of DPHM against *C. neoformans*.

3. Materials and Methods

3.1. Chemistry

The compounds **1-a**-**1-p** and **2-a**-**2-p** were synthetized through a Biginelli reaction promoted by triethylorthoformate as described elsewhere [15]. A mixture of ethyl acetoacetate (1eq), aromatic aldehyde (1eq), (thio)ureas (2eq), citric acid (10 mol %) and triethylorthoformate (2eq) were placed in a round bottom flask and heated under stirring in a pre-heated oil batch (100 °C). The reactions were monitored by TLC, and stopped by addition of water and the crude mixture was cooled in an ice-bath under vigorous stirrer. The solid that was formed was filtered, washed with small portions of cold ethanol and then, dried under vacuum to afford the desired product with good purity grade. The Log*P* value was calculated by ChemDraw Ultra 12.0 (2010, CambridgeSoft).

Nuclear magnetic resonance spectra (^{1}HNMR and ^{13}C NMR) were recorded in an Bruker Ascend, Varian INOVA-300 spectrometer or Brucker Avance DPX-250 NMR spectrometer with standard pulse sequences operating at 400 MHz, 300 MHz or 250 MHz for 1H NMR and 100MHz, 75 MHz or 62.5 MHz for 13C NMR, respectively, using DMSO-d6 or $CDCl_3$ as solvent. Chemical shifts are reported as d values (ppm) relative to TMS (0.0 ppm). The NMR multiplicities br s, s, d, t, q, and m stand for broad singlet, singlet, doublet, triplet, quartet and multiplet, respectively. TLC analyses were performed on Merck´s silica plates 60 F254. Melting-points (mp) were determined on a System Kofler type WME apparatus and are uncorrected. The term room temperature means 20-30ºC. All products were identified through their spectroscopic dada and the melting-points which were confirmed by comparison with those reported in the literature.

3.2. Antifungal assays

3.2.1. Microorganisms and media

For the antifungal evaluation, strains from the American Type Culture Collection (ATCC), Rockville, MD, USA, *C. albicans* ATCC 10231 and *C. neoformans* ATCC 32264 were used, Strains were grown on Sabouraud-chloramphenicol agar slants for 48 h at 30 °C, maintained on slopes of Sabouraud-dextrose agar (SDA, Oxoid) and sub-cultured every 15 days to prevent pleomorphic transformations. Inocula were obtained according to reported procedures [19] and adjusted to $1\text{-}5\cdot10^3$ cells with colony forming units (CFU)/mL.

3.2.2. Fungal Growth Inhibition Percentage Determination.

Yeasts broth microdilution techniques M27-A3 of CLSI [19], were performed in 96-well microplates. For the assay, compound test-wells (CTWs) were prepared with stock solutions of each compound in DMSO (maximum concentration ≤ 1%), diluted with RPMI-1640, to final concentrations of 250-3.9 $\mu g\cdot mL^{-1}$. An inoculum suspension (100 µL) was added to each well (final volume in the well = 200 µL). A growth control well (GCW) (containing medium, inoculum, and the same amount of DMSO used in a CTW, but compound-free) and a sterility control-well (SCW) (sample, medium, and sterile water instead of inoculum) were included for each fungus tested. Microtiter trays were incubated in a moist, dark chamber at 30 °C for 48 h for both yeasts. Microplates were read in a VERSA Max microplate reader (Molecular Devices, Sunnyvale, CA, USA). Amphotericin B (Sigma Aldrich, St Louis, MO, USA) was used as positive control. Tests were performed in triplicate. Reduction of growth for each compound concentration was calculated as follows: % of inhibition = 100 - (OD 405 CTW − OD 405 SCW)/(OD 405 GCW − OD 405 SCW). The means ± SD (standard deviations) were used for constructing the dose-response curves representing % inhibition *vs* concentration of each compound.

3.2.3. MIC_{80} and MIC_{50} determinations

Two endpoints were defined from the dose-response curves. Minimum inhibitory concentrations resulting in 80 or 50 % of the fungal growth respectively were named MIC_{80} and MIC_{50}.

3.2.4. Statistical Analysis.

Statistical calculations were done by using GraphPad Prisma 6.0 software.

4. Conclusion

Two series of DHPMs were prepared and their activities against *C. albicans* and *C. neoformans* were assessed. Furthermore, compounds with a substituent in the *p*-position of the aromatic ring showed promising activities mainly against *C. neoformans*. The data reported here, showed that the presence of thio-ketone in dihydropirymidinic ring can be an important role in antifungal effect. Moreover, the most active structures possess Log*P* between 1 and 2.5, while the less active molecules possess Log*P* values lower than 1.

In addition, the values for the four physicochemical parameters Log*P* ≤ 5, molecular weight ≤ 500, H-bond acceptors and H-bond donors ≤ 10, and ≤ 5 that can predict if the molecules will be orally active, were fulfilled for the active molecules, thus appearing DHPMs good candidates for pharmaceutical development.

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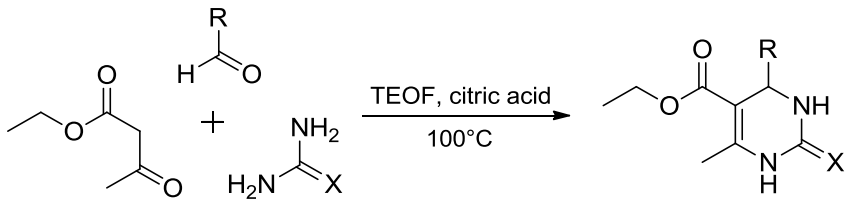
Figures:



Scheme 1 – Synthesis of DHPM's

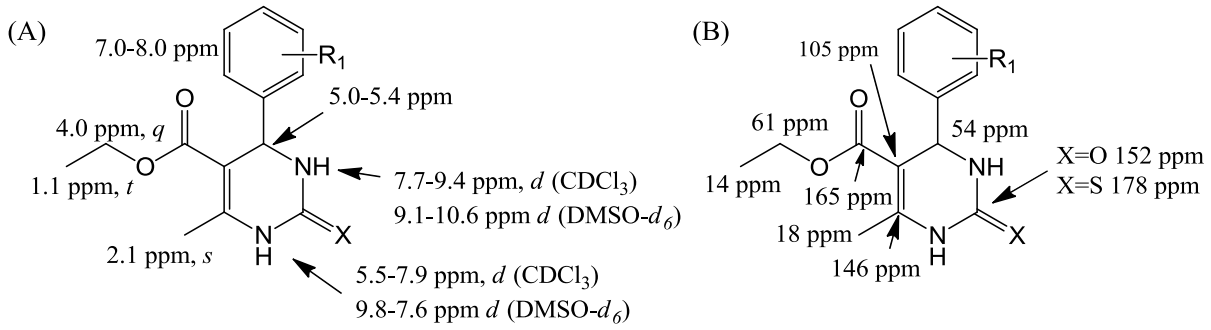


Fig1- General features of ^{1}H NMR (**A**) and ^{13}C NMR (**B**) of synthetized DHPM's.

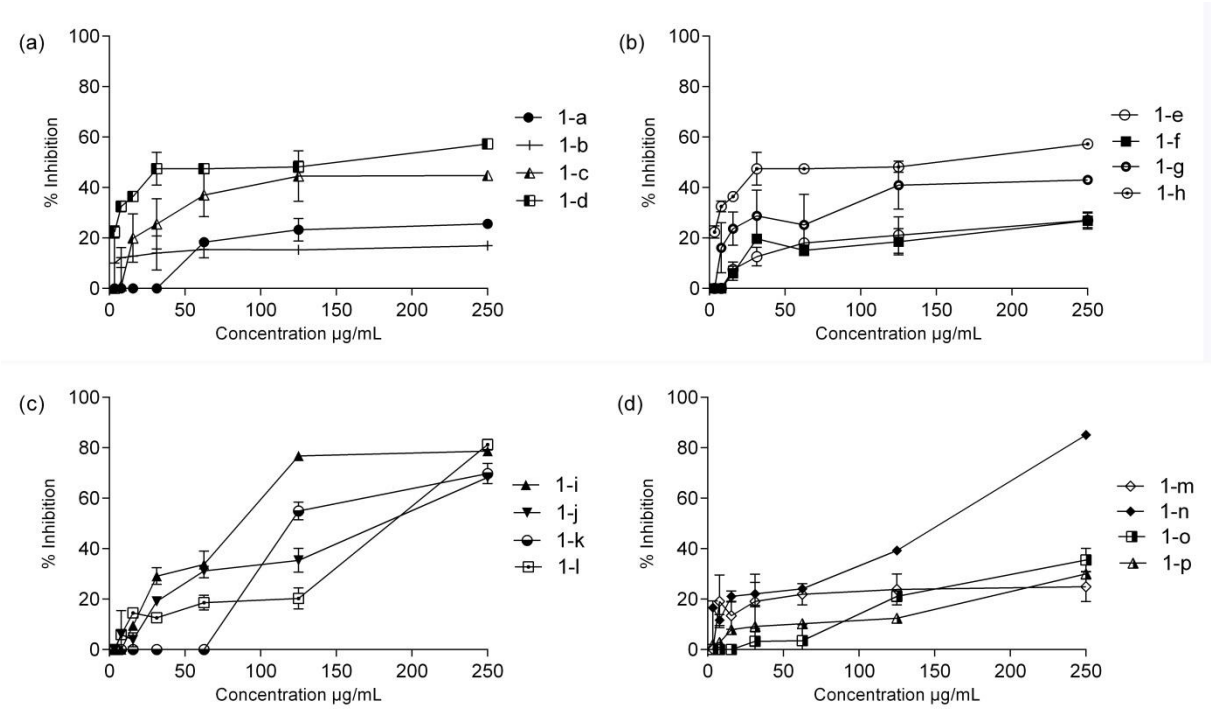


Fig2- Antifungal activity of dihydropyrimidin-2-thiones **1-a**-**1-p** (series I) against *C. neoformans.*

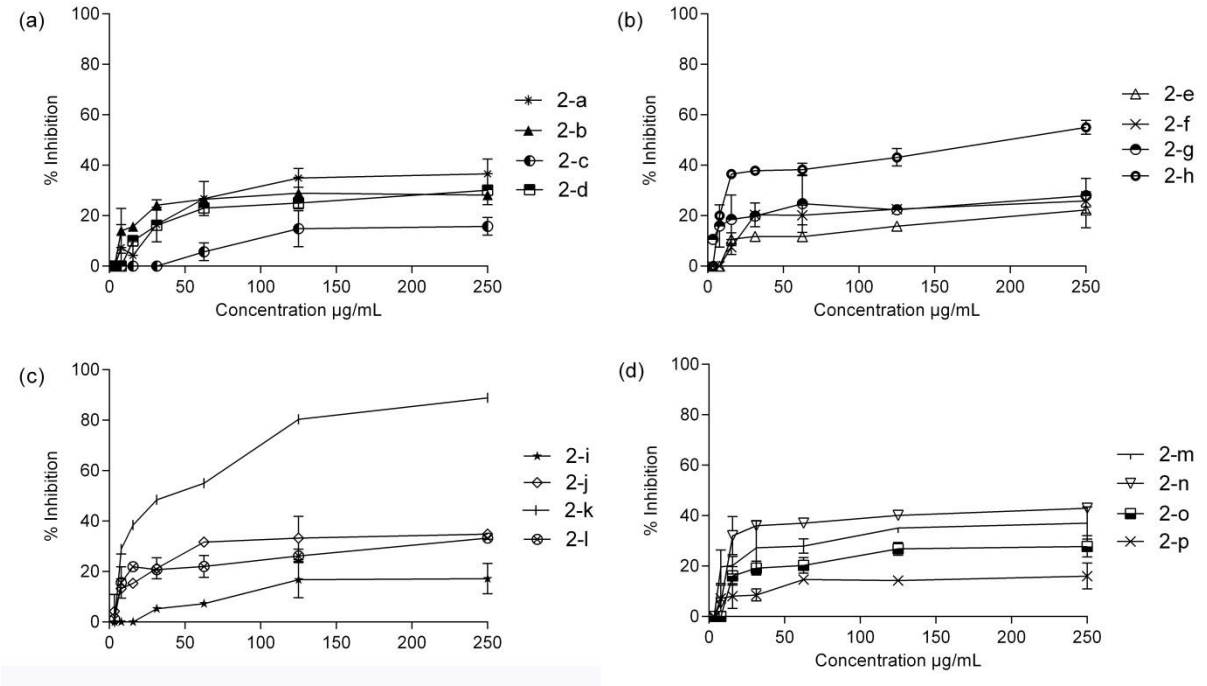


Fig3- Antifungal activity of dihydropyrimidin-2-ones **2-a**-**2-p** (series II) against *C. neoformans.*

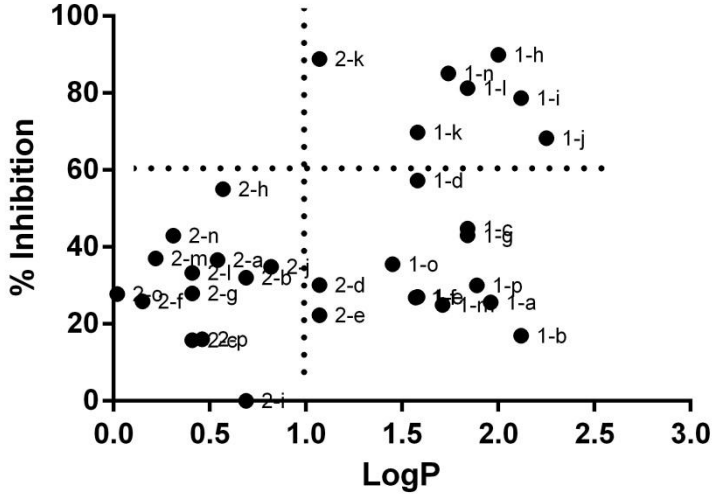


Fig4- Relationship between Log*P* and % inhibition at 250 µg·mL^{-1} of **1-a**-**1-p** and **2-a**-**2-p** against *C. neoformans*.

Tables:

Table 1 – Dihydropirimidin-2-ones/thiones synthesized.

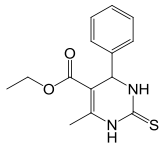
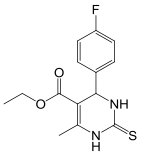
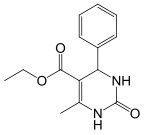
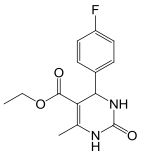
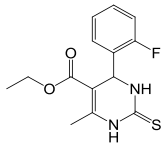
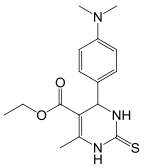
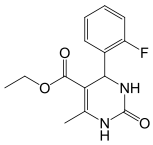
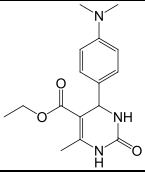
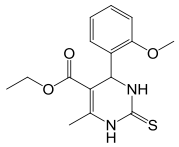
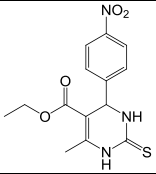
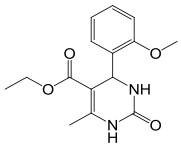
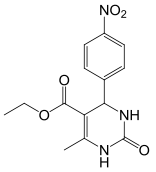
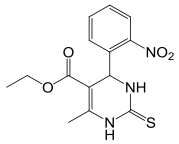
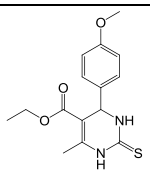
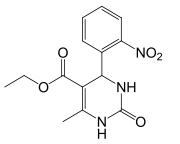
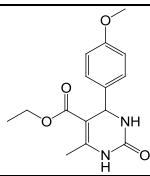
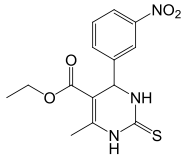
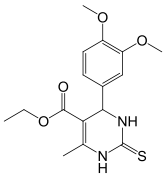
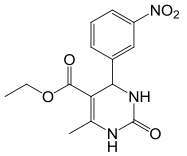
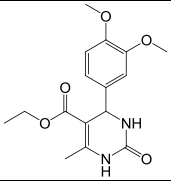
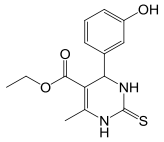
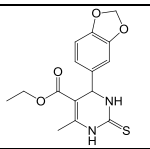
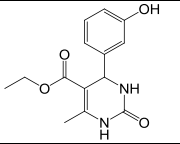
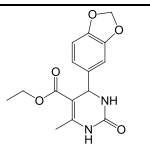
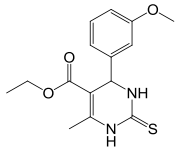
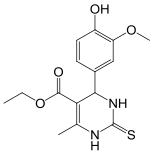
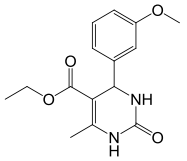
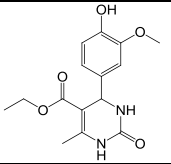
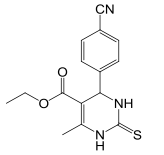
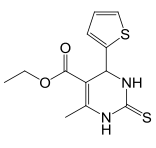
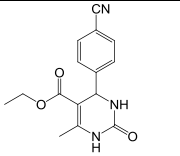
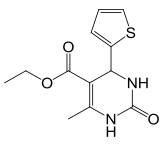
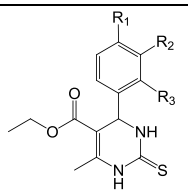
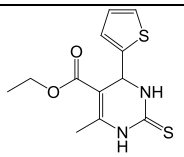
1-a		1-i		2-a		2-i	
yield	92%		82%		93%		89%
1-b		1-j		2-b		2-j	
yield	77%		82%		74%		75%
1-c		1-k		2-c		2-k	
yield	54%		74%		61%		76%
1-d		1-l		2-d		2-l	
yield	63%		85%		68%		91%
1-e		1-m		2-e		2-m	
yield	97%		92%		92%		83%
1-f		1-n		2-f		2-n	
yield	88%		84%		87%		81%
1-g		1-o		2-g		2-o	
yield	93%		54%		93%		58%
1-h		1-p		2-h		2-p	
yield	80%		51%		86%		59%

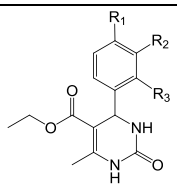
Table 2: The 80% and 50% inhibitory concentrations (MIC_{80} and MIC_{50}) of **1a-p** and **2a-p** against *Candida albicans (Ca)* ATCC 10231 and *Cryptococcus neoformans (Cn)* ATCC 32264. The whole inhibition percentages' data can be seen in the Supplementary Table **S1**



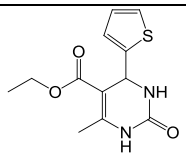
A



B



C



D

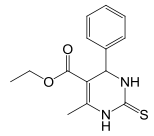
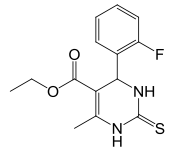
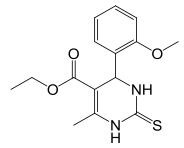
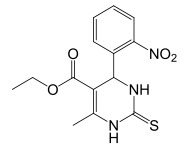
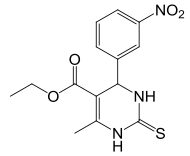
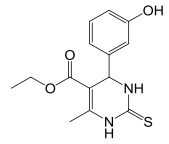
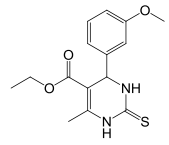
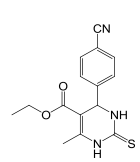
N°	type	R_1	R_2	R_3	fungi	MIC_{80}	MIC_{50}	N°	type	R_1	R_2	R_3	fungi	MIC_{80}	MIC_{50}
1-a	A	H	H	H	*Ca*	>250	>250	**2-a**	C	H	H	H	*Ca*	>250	>250
					Cn	>250	>250						*Cn*	>250	>250
1-b	A	H	H	F	*Ca*	>250	>250	**2-b**	C	H	H	F	*Ca*	>250	>250
					Cn	>250	>250						*Cn*	>250	>250
1-c	A	H	H	OCH_3	*Ca*	>250	>250	**2-c**	C	H	H	OCH_3	*Ca*	>250	>250
					Cn	>250	**250**						*Cn*	>250	>250
1-d	A	H	H	NO_2	*Ca*	>250	>250	**2-d**	C	H	H	NO_2	*Ca*	>250	>250
					Cn	>250	**125**						*Cn*	>250	>250
1-e	A	H	NO_2	H	*Ca*	>250	>250	**2-e**	C	H	NO_2	H	*Ca*	>250	>250
					Cn	>250	>250						*Cn*	>250	>250
1-f	A	H	OH	H	*Ca*	>250	>250	**2-f**	C	H	OH	H	*Ca*	>250	>250
					Cn	>250	>250						*Cn*	>250	>250
1-g	A	H	OCH_3	H	*Ca*	>250	>250	**2-g**	C	H	OCH_3	H	*Ca*	>250	>250
					Cn	>250	>250						*Cn*	>250	>250
1-h	A	CN	H	H	*Ca*	>250	>250	**2-h**	C	CN	H	H	*Ca*	>250	>250
					Cn	>250	**31.2**						*Cn*	>250	**250**
1-i	A	F	H	H	*Ca*	>250	**250**	**2-i**	C	F	H	H	*Ca*	>250	>250
					Cn	**125**	**125**						*Cn*	>250	>250
1-j	A	$N(CH_3)_2$	H	H	*Ca*	>250	>250	**2-j**	C	$N(CH_3)_2$	H	H	*Ca*	>250	>250
					Cn	>250	**250**						*Cn*	>250	>250
1-k	A	NO_2	H	H	*Ca*	>250	>250	**2-k**	C	NO_2	H	H	*Ca*	>250	>250
					Cn	>250	**125**						*Cn*	**125**	**31.2**
1-l	A	OCH_3	H	H	*Ca*	>250	>250	**2-l**	C	OCH_3	H	H	*Ca*	>250	>250
					Cn	**250**	**250**						*Cn*	>250	>250
1-m	A	OCH_3	OCH_3	H	*Ca*	>250	>250	**2-m**	C	OCH_3	OCH_3	H	*Ca*	>250	>250
					Cn	>250	>250						*Cn*	>250	>250
1-n	A	$-OCH_2O-$		H	*Ca*	>250	**250**	**2-n**	C	$-OCH_2O-$		H	*Ca*	>250	>250
					Cn	**250**	**250**						*Cn*	>250	>250
1-o	A	OH	OCH_3	H	*Ca*	>250	>250	**2-o**	C	OH	OCH_3	H	*Ca*	>250	>250
					Cn	>250	>250						*Cn*	>250	>250
1-p	B				*Ca*	>250	>250	**2-p**	D				*Ca*	>250	>250
					Cn	>250	>250						*Cn*	>250	>250
Amphotericin B					*Ca*	1.00	0.78								
					Cn	0.50	0.25								

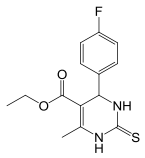
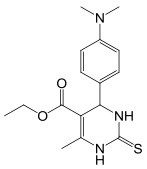
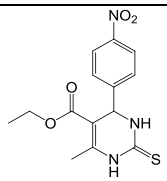
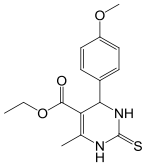
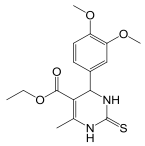
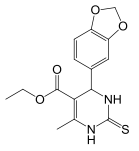
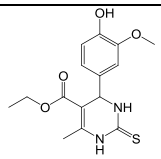
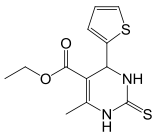
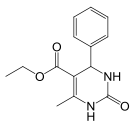
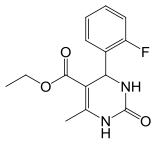
Table 3 - Chemical features of compounds

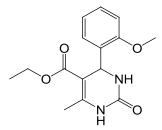
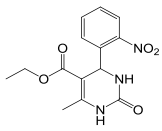
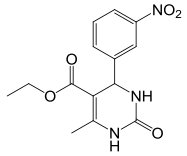
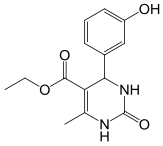
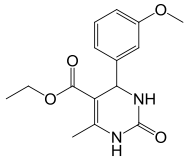
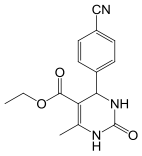
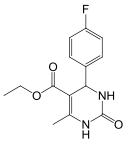
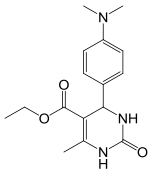
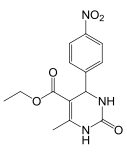
	M.W.	Donor	Acceptor	Log*P*		M.W.	Donor	Acceptor	Log*P*
1-a	276.09	2	1	1.96	2-a	260.29	2	2	0.54
1-b	294.43	2	1	2.12	2-b	278.28	2	2	0.69
1-c	306.38	2	2	1.84	2-c	290.31	2	3	0.41
1-d	321.35	2	3	1.58	2-d	305.29	2	4	1.07
1-e	321.35	2	3	1.58	2-e	305.29	2	4	1.07
1-f	292.35	3	2	1.57	2-f	276.29	3	3	0.15
1-g	306.38	2	2	1.84	2-g	290.31	2	4	0.41
1-h	301.36	2	2	2.00	2-h	285.30	2	3	0.57
1-i	294.43	2	1	2.12	2-i	278.28	2	2	0.69
1-j	319.42	2	2	2.25	2-j	303.16	2	3	0.82
1-k	321.35	2	3	1.58	2-k	305.29	2	4	1.07
1-l	306.38	2	2	1.84	2-l	290.31	2	3	0.41
1-m	336.41	2	3	1.71	2-m	320.34	2	4	0.22
1-n	320.36	2	3	1.74	2-n	304.30	2	4	0.31
1-o	322.38	3	3	1.45	2-o	306.31	3	4	0.02
1-p	282.38	2	1	1.89	2-p	266.32	2	2	0.46

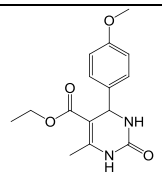
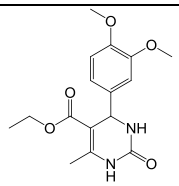
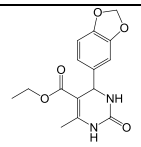
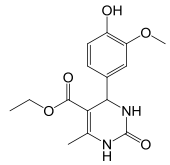
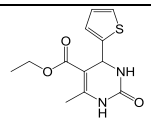
Supplementary material (Antifungal Assays)

.Table 1S: Inhibition percentages (%) of *Candida albicans* (Ca) ATCC 10231 and *Cryptococcus .neoformans (Cn) ATCC 32264* by the compounds **1-a**-**1-p** and **2-a**-**2-p**.

Comp	Structure	Fungi	250 μg/mL	125 μg/mL	62.5 μg.mL	31.2 μg.mL	15.6 μg.mL	7.81μg/mL	3.9 μg/mL
1-a		*Ca*	0	0	0	0	0	0	0
		Cn	25.59±0.68	23.25±4.45	20.58±3.06	0	0	0	0
1-b		*Ca*	0	0	0	0	0	0	0
		Cn	16.93±1.03	15.33±1.80	15.47±3.38	14.04±6.79	12.76±1.41	12.24±3.98	10.02±1.27
1-c		*Ca*	0	0	0	0	0	0	0
		Cn	44.82±1.37	44.53±1.03	37.00±8.57	25.56±9.95	19.93±9.56	0	0
1-d		*Ca*	6.53±0.67	6.40±1.73	5.49±1.03	4.30±1.14	2.23±0.15	3.54±1.56	1.33±0.25
		Cn	57.28±1.10	48.24±2.20	47.44±1.34	47.45±6.53	36.49±0.46	32.51±2.12	22.37±2.41
1-e		*Ca*	27.00±2.88	21.16±7.23	18.00±0.70	12.60±3.67	7.54±2.90	0	0
		Cn	27.00±2.88	21.16±7.23	18.00±0.70	12.60±3.67	7.54±2.90	0	0
1-f		*Ca*	0	0	0	0	0	0	0
		Cn	26.91±3.36	18.50±5.42	15.14±2.08	19.70±0.67	6.00±2.76	0	0
1-g		*Ca*	2.27±1.46	1.30±0.34	0	0	0	0	0
		Cn	43.01±1.69	40.96±9.52	25.17±12.14	28.75±10.22	23.72±6.61	16.11±9.93	0
1-h		*Ca*	19.96±1.04	1.78±0.95	1.27±0.40	0	0	0	0
		Cn	57.28±1.10	48.24±2.20	47.44±1.34	47.45±6.53	36.49±0.46	32.51±2.12	22.37±2.41

1-i		*Ca*	51.23±1.60	19.78±1.32	6.32±1.03	0.77±0.29	0	0	0
		Cn	78.72±0.72	76.81±1.72	33.76±5.32	29.16±3.27	9.51±0.18	0	0
1-j		*Ca*	41.85±2.62	37.28±1.09	6.80±3.07	1.92±0.45	0	0	0
		Cn	68.33±1.83	35.39±4.70	31.20±0.43	19.09±0.67	3.89±2.76	6.03±0.94	0
1-k		*Ca*	39.84±3.57	9.11±2.81	4.05±2.22	3.84±0.38	2.21±0.29	0	0
		Cn	69.77±7.69	54.64±3.36	0	0	0	0	0
1-l		*Ca*	42.13±5.24	16.61±0.11	0	0	0	0	0
		Cn	81.25±2.13	20.29±4.19	18.63±2.93	12.64±1.14	14.58±0.80	5.93±0.72	0
1-m		*Ca*	0	0	0	0	0	0	0
		Cn	24.92±5.87	23.84±6.15	21.92±4.23	19.15±1.07	13.42±5.42	19.13±1.04	0
1-n		*Ca*	51.56±5.79	30.09±1.29	6.22±1.10	2.81±1.01	0	0	0
		Cn	85.13±1.63	39.30±1.68	24.10±1.64	22.10±4.55	21.13±2.04	16.73±1.06	11.77±2.14
1-o		*Ca*	0	0	0	0	0	0	0
		Cn	35.52±4.58	21.16±2.12	3.50±0.63	3.20±0.37	0	0	0
1-p		*Ca*	7.10±5.10	4.33±2.68	3.10±0.35	2.58±0.58	1.86±0.89	0	0
		Cn	30.01±2.06	12.47±1.10	10.27±0.04	9.20±0.77	7.96±0.95	2.84±0.32	2.02±1.38
2-a		*Ca*	2.29±0.35	0	0	0	0	0	0
		Cn	36.63±5.82	34.99±3.72	26.82±0.67	16.52±0.69	4.27±0.54	7.41±0.90	0
2-b		*Ca*	0	0	0	0	0	0	0
		Cn	28.18±3.84	28.96±6.26	26.52±0.80	24.23±2.03	15.73±1.06	14.01±8.90	0

2-c		*Ca*	0	0	0	0	0	0	0
		Cn	15.80±3.53	14.84±7.10	5.69±3.50	0	0	0	0
2-d		*Ca*	5.29±2.97	4.21±0.01	3.65±2.81	0	0	0	0
		Cn	30.11±0.74	25.02±2.23	23.01±0.76	16.18±0.82	10.03±2.08	0	0
2-e		*Ca*	3.32±0.49	2.12±0.49	1.52±0.83	1.47±0.25	0	0	0
		Cn	22.33±7.12	15.91±1.69	11.73±0.80	11.77±1.13	10.67±2.57	0	0
2-f		*Ca*	2.01±0.29	0	0	0	0	0	0
		Cn	25.86±2.37	22.63±1.93	20.24±3.85	20.35±4.69	7.58±2.94	0	0
2-g		*Ca*	3.26±1.27	3.02±1.72	2.79±0.12	2.57±0.48	1.47±1.02	0	0
		Cn	27.92±6.85	22.36±0.99	24.75±0.99	19.86±0.74	18.58±0.96	15.92±0.83	10.52±0.39
2-h		*Ca*	0	0	0	0	0	0	0
		Cn	55.05±2.73	43.16±3.44	38.33±2.43	37.88±1.75	36.56±1.70	20.03±0.47	0
2-i		*Ca*	0	0	0	0	0	0	0
		Cn	17.23±5.95	16.66±7.41	7.26±0.85	5.22±0.87	0	0	0
2-j		*Ca*	15.06±2.22	9.74±2.85	3.93±0.49	3.45±0.64	1.16±0.36	0	0
		Cn	34.85±2.04	33.27±0.86	31.69±1.47	21.32±4.16	15.41±1.62	13.30±3.85	4.32±0.65
2-k		*Ca*	4.86±0.57	1.42±0.22	1.38±1.24	0	0	0	0
		Cn	88.90±0.90	80.34±0.15	55.03±0.96	48.47±0.85	38.43±1.91	28.92±1.99	0

2-l		*Ca*	0	0	0	0	0	0	0
		Cn	33.33±1.66	26.23±2.68	22.02±4.35	20.75±0.25	21.94±1.49	15.66±6.21	1.24±0.97
2-m		*Ca*	0	0	0	0	0	0	0
		Cn	37.06±6.36	35.14±0.30	27.96±2.83	27.29±0.62	20.31±3.46	19.73±6.63	0
2-n		*Ca*	5.80±0.48	4.36±0.56	2.22±0.54	2.02±0.42	1.53±0.31	0	0
		Cn	42.99±0.61	40.14±1.96	37.00±1.25	36.06±0.68	32.07±7.57	5.40±1.46	0
2-o		*Ca*	3.31±0.88	3.24±1.83	1.39±0.99	0	0	0	0
		Cn	27.82±0.72	26.85±2.52	20.30±3.11	19.20±2.73	16.11±3.65	0	0
2-p		*Ca*	7.31±0.53	7.75±2.95	2.64±0.27	0	0	0	0
		Cn	16.08±5.12	14.32±1.69	14.72±0.01	8.58±2.41	8.14±4.93	7.18±5.26	0
Amphotericin B		*Ca*	100	100	100	100	100	100	100
		Cn	100	100	100	100	100	100	100

Supplementary material (Spectra Data)

Ethyl 6-methyl-4-phenyl-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (**1-a**): yield 92%; mp 204-207 ºC. ^{1}H NMR (300 MHz, DMSO-d_6): 1.09 (t, 3H, *J*=7.0 Hz), 2.29 (s, 3H), 4.00 (q, 2H, J 7.0 Hz), 5.17 (d, 1H, J 3.7 Hz), 7.23-7.37 (m, 5H), 9.65 (d, 1H, J 3.8 Hz, NH), 10.34 (br s, 1H, NH). ^{13}C NMR (75 MHz, DMSO-d_6): 14.1, 17.2, 54.1, 59.9, 100.7, 126.4, 127.7, 128.6, 143.5, 145.1, 165.1, 174.2.

Ethyl 4-(2-fluorophenyl)-6-methyl-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (**1-b**): yield 77%; mp 140-143 ºC. ^{1}H NMR (300 MHz, DMSO-d_6): 1.03 (t, 3H, J 7.0 Hz), 2.30 (s, 3H), 3.93 (q, 2H, J 7.0 Hz), 5.45 (d, 1H, J 3.1 Hz), 7.13-7.34 (m, 4H), 9.59 (s, 1H, NH), 10.37 (s, 1H, NH). ^{13}C NMR (75 MHz, DMSO-d_6): 14.5, 17.8, 49.4, 60.2, 100.0, 116.1, 116.4, 125.3, 129.9, 130.5, 131.27, 146.1, 165.5, 174.7.

Ethyl 6-methyl-4-(3-nitrophenyl)-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (**1-e**): yield 97%; mp 206-209 ºC. ^{1}H NMR (250 MHz, DMSO-d_6): 1.13 (t, 3H, J 7.1 Hz), 2.34 (s, 3H), 4.05 (q, 2H, J 7.1 Hz), 5.36 (d, 1H, J 3.6 Hz), 7.70-7.72 (m, 2H), 8.10- 8.11 (m, 1H), 8.17-8.20 (m, 1H), 9.81 (br s, 1H, NH), 10.55 (br s, 1H, NH). ^{13}C NMR (62.5 MHz, DMSO-d_6): 14.0, 17.3, 53.5, 59.8, 99.8, 121.2, 122.8,130.5, 133.0, 145.5, 146.0, 147.8, 164.9, 174.5.

Ethyl 4-(3-hydroxyphenyl)-6-methyl-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (**1-f**): yield 88%; mp 184-187 ºC. ^{1}H NMR (250 MHz, DMSO-d_6): 1.14 (t, 3H, J 7.1 Hz), 2.30 (s, 3H), 4.04 (q, 2H, J 7.1 Hz), 5.11 (d, 1H, J 3.7 Hz), 6.65-6.69 (m, 3H), 7.10-7.18 (m, 1H), 9.46 (s, 1H, OH), 9.62 (br s, 1H, NH), 10.31 (br s, 1H, NH); ^{13}C NMR (62.5 MHz, DMSO-d_6): 14.1, 17.2, 53.9, 59.6, 100.8, 113.2, 114.6, 117.0, 129.5, 144.8, 144.9, 157.5, 165.2, 174.2.

Ethyl 4-(3-methoxyphenyl)-6-methyl-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (**1-g**): yield 93%; ^{1}H NMR (400 MHz, $CDCl_3$): 1.17 (t, 3H, J 7.1 Hz), 2.34 (s, 3H), 3.78 (s, 3H), 4.08 (m, 2H), 5.37 (d, 1H, J 2.6 Hz), 5.89 (br s, 1H, NH), 6.80 (m, 1H), 6.86 (m, 1H), 6.91 (d, 1H, J 7.8 Hz), 7.24 (m, 1H), 8.14 (br s, 1H, NH). ^{13}C NMR (100 MHz, $CDCl_3$): 14.1, 18.2, 55.3, 56.0, 60.4, 102.7, 112.7, 112.7, 113.4, 119.0, 143.1, 143.8, 159.9, 165.3, 174.4.

Ethyl 4-(4-cyanophenyl)-6-methyl-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (**1-h**): yield 80%; mp 130-133 °C. ^{1}H NMR (300 MHz, DMSO-d_6): 1.09 (t, 3H, J 7.0 Hz), 2.30 (s, 3H), 4.00 (q, 2H, J 7.0 Hz), 5.24 (d, 1H, J 3.5 Hz), 7.40 (d, 2H, J 8.3 Hz), 7.83 (d, 2H, J 8.3 Hz), 9.74 (br s, 1H, NH), 10.47 (br s, 1H, NH). ^{13}C NMR (75 MHz, DMSO-d_6): 14.0, 17.2, 53.8, 59.8, 99.8, 127.5, 129.9, 132.7, 133.2, 138.8, 145.9, 148.5, 164.9, 174.5.

Ethyl 4-(4-fluorophenyl)-6-methyl-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (**1-i**): yield 82%; ^{1}H NMR (400 MHz, $CDCl_3$): 1.31 (t, 3H, J 7.0 Hz), 2.32 (s, 3H), 4.05 (m, 2H), 5.33 (s, 1H), 6.95 (d, 2H, J 8.3 Hz), 7.22 (d, 2H, J 6.2 Hz), 7.63 (br s, 1H, NH), 8.16 (br s, 1H, NH). ^{13}C NMR (100 MHz, $CDCl_3$): 14.1, 18.3, 55.5, 60.5, 102.9, 115.7 (d, ^{2}J 21.6Hz), 128.6 (d, ^{3}J 8.3Hz), 138.3 (d, ^{4}J 3.0Hz), 142.8, 162.6 (d, ^{1}J 247.8), 165.1, 174.4.

Ethyl 4-(4-(dimethylamino)phenyl)-6-methyl-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (**1-j**): yield 82%; mp 206-208 ºC. ^{1}H NMR (300 MHz, DMSO-d_6): 1.11 (t, 3H, J 7.0 Hz), 2.28 (s, 3H), 2.85 (s, 6H), 3.97 (q, 2H, J 7.0 Hz), 5.04 (d, 1H, J 3.2 Hz), 6.66 (d, 2H, J 8.5 Hz), 7.01 (d, 2H, J 8.5 Hz), 9.55 (br s, 1H, NH), 10.24 (br s, 1H, NH). ^{13}C NMR (75 MHz, DMSO-d_6): 14.1, 17.1, 40.1, 53.5, 59.5, 101.3, 112.2, 127.1, 131.2, 144.3, 150.0, 165.3, 173.8.

Ethyl 6-methyl-4-(4-nitrophenyl)-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (**1-k**): yield 74%. ^{1}H NMR (400 MHz, $CDCl_3$): 1.17 (t, 3H, J 7.1 Hz), 2.35 (s, 3H), 4.10 (q, 2H, J 7.1 Hz), 5.50 (s, 1H), 7.24 (br s, 1H, NH), 7.45 (d, 2H, J 8.6 Hz), 7.76 (br s, 1H, NH), 8.15 (d, 2H, J 6.41Hz). ^{13}C NMR (100 MHz, $CDCl_3$): 14.2, 18.5, 53.4, 60.9, 102.1, 124.2, 127.7, 143.6, 147.7, 148.9, 164.9, 174.9.

Ethyl 4-(4-methoxyphenyl)-6-methyl-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (**1-l**): yield 85%. ^{1}H NMR (400 MHz, $CDCl_3$): 1.16 (t, 3H, J 7.1 Hz), 2.34 (s, 3H), 3.77 (s, 3H), 4.08 (m, 2H), 5.33 (d, 1H, 3.0 Hz), 6.83 (m, 2H), 7.20 (m, 2H), 7.71 (br s, 1H, NH), 8.33 (br s, 1H, NH). ^{13}C NMR (100 MHz, $CDCl_3$): 14.1, 18.1, 55.3, 55.6, 60.3, 103.2, 114.2, 128.0, 134.9, 142.5, 159.6, 165.3, 174.3.

Ethyl 4-(3,4-dimethoxyphenyl)-6-methyl-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (**1-m**): yield 92%. ^{1}H NMR (400 MHz, $CDCl_3$): 1.13 (t, 3H, J 7.1 Hz), 2.31 (s, 3H), 3.80 (s, 6H), 4.05 (m, 2H), 5.30 (d, 1H, 2.9Hz), 6.76 (m, 3H), 7.76 (br s, 1H, NH), 8.32 (br s, 1H, NH). ^{13}C NMR (100 MHz, $CDCl_3$): 14.2, 18.2, 55.8, 55.9, 56.0, 60.4, 103.1, 110.0, 111.3, 119.0, 135.1, 142.6, 149.0, 149.1, 165.4, 174.2.

Ethyl 4-(benzo[d][1,3]dioxol-5-yl)-6-methyl-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (**1-n**): yield 84%; mp 156-159 ºC. ^{1}H NMR (250 MHz, DMSO-d_6): 1.11 (t, 3H, J 7.0 Hz), 2.28 (s, 3H), 3.99 (q, 2H, J 7.0 Hz), 5.08 (d, 1H, J 3.8 Hz), 5.99 (s, 2H), 6.64-6.72 (m, 1H), 6.87 (m, 2H), 9.61 (br s, 1H, NH), 10.33 (br s, 1H, NH). ^{13}C NMR (62.5 MHz, DMSO-d_6): 14.1, 17.2, 53.6, 59.6, 100.6, 101.0, 106.7, 108.1, 119.6, 137.4, 145.0, 146.7, 147.3, 165.0, 173.9.

Ethyl 4-(4-hydroxy-3-methoxyphenyl)-6-methyl-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (**1-o**): yield 54%. ^{1}H NMR (400 MHz, $CDCl_3$): 1.19 (t, 3H, J 7.1 Hz), 2.36 (s,

3H), 3.87 (s, 3H), 4.11 (m, 2H), 5.36 (d, 1H, 2.9 Hz), 6.79 (m, 2H), 6.85 (m, 1H), 7.19 (br s, 1H, NH), 7.73 (br s, 1H, NH). ^{13}C NMR (100 MHz, $CDCl_3$): 14.2, 18.5, 56.0, 56.1, 60.5, 103.2, 109.2, 114.6, 119.8, 134.5, 142.1, 145.8, 146.7, 165.3, 174.6.

Ethyl 6-methyl-4-(thiophen-2-yl)-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (**1-p**): yield 51%. ^{1}H NMR (400 MHz, $CDCl_3$): 1.25 (t, 3H, J 7.1 Hz), 2.36 (s, 3H), 4.18 (m, 2H), 5.69 (d, 1H, J 3.4Hz), 6.92 (m, 1H), 6.98 (m, 1H), 7.21 (m, 1H), 7.70 (br s, 1H, NH), 8.22 (br s, 1H, NH). ^{13}C NMR (100 MHz, $CDCl_3$): 14.2, 18.3, 51.0, 60.6, 103.2, 124.8, 125.6, 126.9, 143.3, 145.6, 165.0, 175.1.

Ethyl 6-methyl-2-oxo-4-phenyl-1,2,3,4-tetrahydropyrimidine-5-carboxylate (**2-a**): yield 93%; mp 210-212 ºC. ^{1}H NMR (300 MHz, DMSO-d_6): 1.09 (t, 3H, J 7.1 Hz), 2.25 (s, 3H), 3.98 (q, 2H, J 7.1 Hz), 5.15 (d, 1H, J 2.9 Hz), 7.22-7.32 (m, 5H), 7.74 (br s, 1H, NH), 9.20 (br s, 1H, NH). ^{13}C NMR (75 MHz, DMSO-d_6): 12.2, 16.0, 52.1, 57.3, 97.4, 124.4, 125.3, 126.4, 143.1, 145.2, 150.2, 163.3.

Ethyl 4-(2-fluorophenyl)-6-methyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (**2-b**): yield 74%; mp 235-237 ºC. ^{1}H NMR (300 MHz, DMSO-d_6): 1.20 (t, 3H, J 7.1 Hz), 2.44 (s, 3H), 4.07 (q, 2H, J 7.1 Hz), 5.62 (s, 1H), 7.28-7.48 (m, 4H), 7.87 (br s, 1H, NH), 9.43 (br s, 1H, NH). ^{13}C NMR (75 MHz, DMSO-d_6): 13.8, 17.5, 54.5, 61.1, 115.2, 122.4, 124.0, 127.8, 129.3, 135.8, 154.7, 157.2, 158.3, 160.3.

Ethyl 4-(2-methoxyphenyl)-6-methyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (**2-c**): yield 61%. ^{1}H NMR (400 MHz, $CDCl_3$): 1.09 (t, 3H, J 7.1 Hz), 2.43 (s, 3H), 3.88 (s, 3H), 4.06 (m, 2H), 5.76 (d, 1H, 3.0 Hz), 6.89 (m, 2H), 7.03 (m, 1H), 7.27 (m, 1H), 7.43 (br s, 1H, NH), 8.22 (br s, 1H, NH). ^{13}C NMR (100 MHz, $CDCl_3$): 14.2, 18.5, 50.0, 55.3, 59.9, 98.4, 110.5, 120.6, 126.5, 129.1, 129.7, 148.2, 153.6, 156.8, 165.9.

Ethyl 6-methyl-4-(3-nitrophenyl)-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (**2-e**): yield 92%; mp 225-227 ºC. ^{1}H NMR (250 MHz, DMSO-d_6): 1.08 (t, 3H, J 6.9 Hz), 2.26 (s, 3H), 3.98 (q, 2H, J 6.9 Hz), 5.29 (s, 1H), 7.64-8.10 (m, 4H), 8.90 (s, 1H, NH), 9.37 (s, 1H, NH). ^{13}C NMR (62.5 MHz, DMSO-d_6): 14.0, 17.9, 53.6, 59.4, 98.4, 121.2, 122.3, 130.0, 133.0, 147.0, 147.8, 149.3, 151.9, 165.0.

Ethyl 4-(3-hydroxyphenyl)-6-methyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (**2-f**): yield 87%; mp 163-166 ºC. ^{1}H NMR (300 MHz, DMSO-d_6): 1.11 (t, 3H, J 7.0 Hz), 2.22 (s, 3H), 3.98 (q, 2H, J 7.0 Hz), 5.04 (s, 1H), 6.64-7.09 (m, 4H), 7.64 (s, 1H, NH), 9.15 (s, 1H, NH). ^{13}C NMR (75 MHz, DMSO-d_6): 14.2, 17.6, 52.9, 59.2, 100.1, 113.2, 114.9, 116.7, 129.4, 147.0, 148.5, 152.2, 157.3, 165.9.

Ethyl 4-(3-methoxyphenyl)-6-methyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (**2-g**): yield 93%; ^{1}H NMR (400 MHz, $CDCl_3$): 1.18 (t, 3H, J 7.1 Hz), 2.35 (s, 3H), 3.79 (s, 3H), 4.09 (m, 2H), 5.38 (s, 1H), 5.52 (br s, 1H, NH), 6.81 (dd, 1H, J 8.1, 1.9 Hz), 6.86 (s, 1H), 6.91 (d, 1H, J 7.5 Hz), 7.23 (d, 1H, J 7.9 Hz), 7.35 (br s, 1H, NH). ^{13}C NMR (100 MHz, $CDCl_3$): 14.2, 18.7, 55.2, 56.6, 60.1, 101.2, 112.6, 112.9, 118.9, 129.8, 145.2, 146.4, 153.4, 159.8, 165.6.

Ethyl 4-(4-cyanophenyl)-6-methyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (**2-h**): yield 86%; mp 130-133 °C. ^{1}H NMR (300 MHz, DMSO-d_6): 1.07 (t, 3H, J 7.1 Hz), 2.25 (s, 3H), 3.97 (q, 2H, J 7.1 Hz), 5.21 (s, 1H), 7.42 (d, 2H, J 8.1 Hz), 7.80 (d, 2H, J 8.1 Hz), 7.88 (s, 1H, NH), 9.33 (s, 1H, NH). ^{13}C NMR (75 MHz, DMSO-d_6): 14.5, 18.3, 54.3, 59.8, 98.7, 110.5, 119.2, 127.8, 133.0, 149.8, 150.5, 152.3, 165.6.

Ethyl 4-(4-fluorophenyl)-6-methyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (**2-i**): yield 89%; ^{1}H NMR (400 MHz, $CDCl_3$): 1.16 (t, 3H, J 7.1 Hz), 2.31 (s, 3H), 4.07 (m, 2H), 5.36 (d, 2H, J 2.72 Hz), 6.29 (br s, 1H, NH), 6.98 (m, 2H), 7.26 (m, 2H), 8.65 (br s, 1H, NH). ^{13}C NMR (100 MHz, $CDCl_3$): 14.2, 18.6, 54.9, 60.1, 101.2, 115.5 (d, ^{2}J 21.6Hz), 128.3 (d, ^{3}J 8.1Hz), 139.7 (d, ^{4}J 3.3Hz), 146.5, 153.8, 162.3 (d, ^{1}J 246.5), 165.6.

Ethyl 4-(4-(dimethylamino)phenyl)-6-methyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (**2-j**): yield 75%; mp 257-259 ºC. ^{1}H NMR (300 MHz, DMSO-d_6): 1.05 (t, 3H, J 7.0 Hz), 2.23 (s, 3H), 2.84 (s, 6H), 4.00 (q, 2H, J 7.0 Hz), 5.03 (d, 1H, J 3.1 Hz), 6.64 (d, 2H, J 8.5 Hz), 7.04 (d, 2H, J 8.5 Hz), 7.59 (br s, 1H, NH), 9.09 (br s, 1H, NH). ^{13}C NMR (75 MHz, DMSO-d_6): 14.2, 17.7, 40.2, 53.3, 59.1, 99.9, 112.2, 126.9, 132.7, 147.6, 149.8, 152.3, 165.5.

Ethyl 6-methyl-4-(4-nitrophenyl)-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (**2-k**): yield 76%, mp 210-212°C. ^{1}H NMR (300 MHz, DMSO-d_6): 1.11 (t, 3H, J 7.1 Hz), 2.29 (s, 3H), 4.01 (q, 2H, J 7.0 Hz), 5.30 (d, 1H, J 3.1 Hz), 7.53 (d, 2H, J 8.6 Hz), 7.92 (s, 1H, NH), 8.23 (d, 2H, J 8.7 Hz), 9.39 (s, 1H, NH). ^{13}C NMR (75 MHz, DMSO-d_6): 14.1, 17.9, 53.7, 59.4, 98.2, 123.9, 127.7, 146.7, 149.4, 151.8, 152.0, 165.1.

Ethyl 4-(4-methoxyphenyl)-6-methyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (**2-l**): yield 91%. ^{1}H NMR (400 MHz, $CDCl_3$): 1.17 (t, 3H, J 7.1 Hz), 2.31 (s, 3H), 3.77 (s, 3H), 4.07 (m, 2H), 5.33 (d, 1H, 1.6 Hz), 6.13 (br s, 1H, NH), 6.82 (m, 2H), 7.22 (m, 2H), 8.64 (br s, 1H, NH). ^{13}C NMR (100 MHz, $CDCl_3$): 14.2, 18.5, 55.0, 55.3, 60.0, 101.5, 114.0, 127.8, 136.2, 146.2, 153.8, 159.2, 165.8.

Ethyl 4-(3,4-dimethoxyphenyl)-6-methyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (**2-m**): yield 83%. ^{1}H NMR (400 MHz, $CDCl_3$): 1.17 (t, 3H, J 7.1 Hz), 2.33 (s, 3H), 3.83 (s, 3H), 3.84 (s, 3H), 4.09 (m, 2H), 5.35 (d, 1H, 2.6Hz), 6.02 (br s, 1H, NH), 6.79 (d, 1H, J 8.8Hz), 6.85 (m, 2H), 8.35 (br s, 1H, NH). ^{13}C NMR (100 MHz, $CDCl_3$): 14.2, 18.5, 55.3, 55.9, 60.0, 101.6, 110.2, 111.4, 118.7, 136.5, 146.0, 148.9, 149.2, 153.6, 165.7.

Ethyl 4-(benzo[d][1,3]dioxol-5-yl)-6-methyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (**2-n**): yield 81%; mp 188-190 ºC. ^{1}H NMR (300 MHz, DMSO-d_6): 1.11 (t, 3H, J 7.0 Hz), 2.25 (s, 3H), 3.99 (q, 2H, J 7.0 Hz), 5.08 (d, 1H, J 2.6 Hz), 5.98 (s, 2H), 6.69-6.75 (m, 2H), 6.84- 6.86 (m, 1H), 7.71 (br s, 1H, NH), 9.20 (br s, 1H, NH). ^{13}C NMR (75 MHz, DMSO-d_6): 14.1, 17.8, 53.7, 59.2, 99.3, 101.0, 106.7, 108.0, 119.4, 138.9, 146.4, 147.3, 148.3, 152.1, 165.4.

Ethyl 6-methyl-2-oxo-4-(thiophen-2-yl)-1,2,3,4-tetrahydropyrimidine-5-carboxylate (**2-p**): yield 59%. ^{1}H NMR (400 MHz, $CDCl_3$): 1.17 (t, 3H, J 7.1 Hz), 2.23 (s, 3H), 4.07 (m, 2H), 5.43 (s, 1H), 6.91 (m, 1H), 6.94 (m, 1H), 7.35 (m, 1H), 7.92 (br s, 1H, NH), 9.32 (br s, 1H, NH). ^{13}C NMR (100 MHz, $CDCl_3$): 14.6, 18.1, 49.8, 59.8, 100.3, 124.0, 125.1, 127.1, 149.1, 149.2, 152.7, 165.5.

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- [2] Zhang, W.; Brombosz, S.M.; Mendoza, J.L.; Moore, J.S. A high-yield, one-step synthesis of o-phenylene ethynylene cyclic trimer via precipitation-driven alkyne metathesis. *J. Org. Chem*., **2005**, *70*, 10198-10201.

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- [3] Crabtree, R.H. *The Organometallic Chemistry of the Transition Metals*, 3rd ed.; Wiley & Sons: New York, **2001**.

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- [4] Wheeler, D.M.S.; Wheeler, M.M. In: *Studies in Natural Products Chemistry*; Atta-ur-Rahman, Ed.; Elsevier Science B. V: Amsterdam, **1994**; Vol. *14*, pp. 3-46.

Conference Proceedings:

- [5] Jakeman, D.L.; Withers, S.G.E. In: *Carbohydrate Bioengineering: Interdisciplinary Approaches*, Proceedings of the 4th Carbohydrate Bioengineering Meeting, Stockholm, Sweden, June 10-13, 2001; Teeri, T.T.; Svensson, B.; Gilbert, H.J.; Feizi, T., Eds.; Royal Society of Chemistry: Cambridge, UK, **2002**; pp. 3-8.

URL(WebPage):

- [6] National Library of Medicine. Specialized Information Services: Toxicology and Environmental Health. sis.nlm.nih.gov/Tox/ToxMain.html (Accessed May 23, **2004**).

Patent:

- [7] Hoch, J.A.; Huang, S. Screening methods for the identification of novel antibiotics. U.S. Patent 6,043,045, March 28, **2000**.

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