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SYNTHESIS AND ANTIMICROBIAL ACTIVITY OF NEW SUBSTITUTED DIHYDROPYRIMIDINE DERIVATIVES

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تم في هذا البحث تحضير مجموعة جديدة من مشتقات الهيدروبيريميدين عن طريق التفاعل المختصر الواحد للألدهيدات العطرية والاسيتو خلات الايثيل مع الثيويوريا في وجود حمض الخليك طبقا لتفاعل بيجنيللي. ثم تفاعل هذه المركبات الوسيطة مع بروميدات الفيناسيل لتحضير مشتقات الفيناسيل ثيو ثنائى الهيدروبيريميدين. وقد تم التحقق من التركيب البنائى ودرجة النقاوة للمركبات المحضرة بواسطة التحاليل الدقيقة للعناصر والقياسات الطيفية مثل الاشعة تحت الحمراء، الرنين النووى المغناطيسى و كذلك مطياف الكتلة. تم اختبار فاعلية المركبات الجديدة المحضرة كمضادات للبكتريا بالمقارنة بعقار الكلورامفنيكول كعقار مرجعى. وقد تم ايضا تحديد اقل تركيز تثبيطى للمركبات ذات الفاعلية العالية. ايضا تم اختبار المركبات المستهدفة كمضادات للفطريات بالمقارنة بكلوتريمازول كعقار مرجعى. واظهرت النتائج ان المركبات **2b, 2e, 2k, 2l, 2m, 2n, 2o, 2p, 2q, 2r** و **2x** هى الاكثر فاعلية كمضادات للبكتريا و الفطريات مقارنة بالأدوية المرجعية.

*A new series of ethyl 6-methyl-4-(substituted)phenyl-2-(substituted)-phenacyl-thio-1,4-dihydropyrimidine-5-carboxylate **(2a-x)** was prepared by reaction of ethyl 1,2,3,4-tetrahydro-6-methyl-4-(substituted)phenyl-2-thioxopyrimidine-5-carboxy-late **1(a–d)** with phenacyl bromides. Compounds **1(a–d)** were synthesized using the principle of Bignelli condensation by one pot reaction of the appropriate araldehyde, ethyl acetoacetate and thiourea in acidic medium. Confirmation of the chemical structure of the synthesized compounds **(2a-x)** was substintiated by different spectral data IR, ^{1}H-NMR, MS in addition to their microanalyses. The newly synthesized compounds were evaluated for their antimicrobial activities. The antibacterial and antifungal testing identified compounds **2b, 2e, 2k, 2l, 2m, 2n, 2o, 2p, 2q, 2r** and **2x** as the most effective agents in comparison to Chloramphenicol and Clotrimazole as reference antibacterial and antifungal drugs respectively.*

INTRODUCTION

Bacterial infections are increasingly complicated by the ability to develop resistance to antimicrobial agents. Bacteria may be intrinsically resistant to ≥1 class of antimicrobial agents or may acquire resistance by de novo mutation or via the acquisition of resistant genes from other organisms.[1] The antimicrobial resistance is a global problem, probably due to the indiscriminate and irrational use of antibiotics, prescriptions for incorrect medicines or incorrect determinations of dose, route and/or duration. Another consideration is the uncertainty of patients receiving antibiotics about whether the quality of a generic medicine is equal to, greater than or less than its equivalent brand-name drug. The antimicrobial agent must be evaluated *in-vitro* and *in-vivo* in order to confirm their suitability for therapeutic use.[2]

Recently much interest has been focused on the chemistry of 2-thioxotetrahydro-pyrimidine-5-carboxylate and their derivatives,

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known as Bigenelli compounds, owing to their diverse range of biological properties such as antimicrobial,[3-6] antitumor,[5-8] anti-inflammatory and/or analgesic,[9&10] antioxidant,[11] calcium channel blocker[12-15] activities.

Motivated by the above documents, we tried to prepare the new derivatives of ethyl 6-methyl-4-(substituted)-phenyl-2-(substituted)-phenacylthio-1,4-dihydropyrimidine-5-carboxylate **(2a-x),** aiming at the development of new antimicrobial agents.

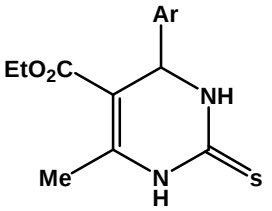


Fig. 1: compound **I**

EXPERIMENTAL

Melting points were determined on an electrothermal melting point apparatus (Stuart Scientific, Model SMP1, UK) and were uncorrected. TLC was carried out using silica gel 60 F_{254} precoated sheets (E. Merk, Darmstadt, Germany) and was visualized by UV lamp (Spectroline Model CM 10, USA), and/or iodine stains.

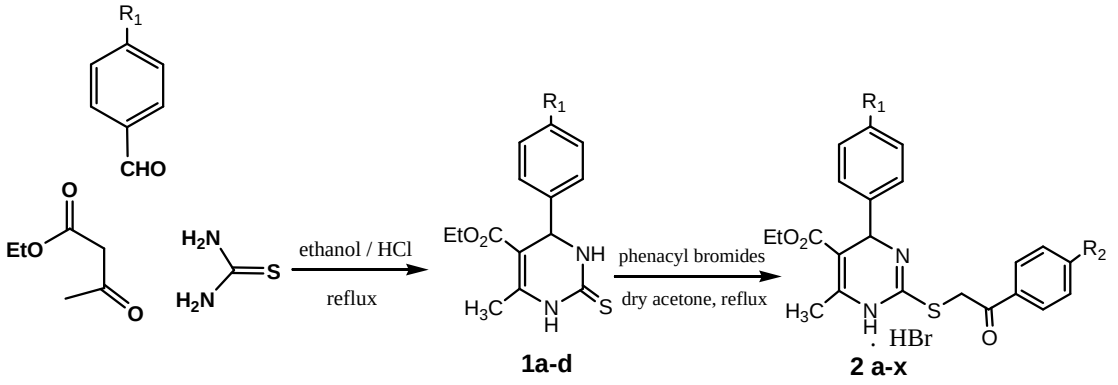
IR spectra (KBr discs) were recorded on a Shimadzu IR-470 spectrometer (Shimadzu, Japan). ^{1}H-NMR spectra were scanned on a Varian EM-360 L NMR spectrometer (60 MHz), (Varian, USA). Chemical shifts are expressed in δ values (ppm) relative to tetramethylsilane (TMS) as an internal standard, using DMSO-d_6 as a solvent. Elemental analyses were performed at the Micro Analytical Center, Cairo University, Cairo, Egypt. Mass spectra were recorded on a JEOL JMS 600 mass spectrometer (JEOL, Japan) at the Micro Analytical Center, Faculty of Science, Cairo University, Cairo and Central Lab., Assiut University, Assiut, Egypt.

Most of chemicals used were of commercial grade: *p*-bromobenzaldehyde, *p*-chlorobenzaldehyde, *p*-methylbenzaldehyde, *p*-methylacetophenone (Riedel-de Haën, Germany), benzaldehyde, thiourea (El Nasr Pharm. Co. Egypt), ethyl acetoacetate, aceto-phenone, *p*-bromoacetophenone, *p*-chloroaceto-phenone, *p*-methoxyacetophenone, bromine (Aldrich, Germany) and *p*-nitroacetophenone (MERCK-Schuchardt, Germany).

The key intermediates 2-thioxo-dihydropyrimidines **(1a-d)** were prepared by one pot reaction of the appropriate aldehyde, ethyl acetoacetate and thiourea in acidic medium according to Bignelli reaction.

General method for preparation of ethyl 6-methyl-4-(substituted)phenyl-2-(substituted) phenacylthio-1,4-dihydropyrimidine-5-carboxylate hydrobromide (2a-x)

A mixture of ethyl 6-methyl-4-(substituted-phenyl)-2-thioxo-1,4dihydro-pyrimidine-5-carboxylate (**1a-d**) (1.0 mmol), appropriate phenacyl bromide (1.1 mmol) in anhydrous acetone (25 ml) was refluxed for 30 min, the formed precipitate was filtered, dried and crystallized from ethanol (Scheme 1, Table I).



R_1 = H, Br, Cl, and CH_3 R_2 = H, Br, Cl, and OCH_3, CH_3, and NO_2

Scheme 1: Synthetic route for compounds **(2a-x)**.

Ethyl 6-methyl-4-phenyl-2-phenacylthio-1,4-dihydropyrimidine-5-carboxylate hydro bromide (2a)

^{1}H-NMR (DMSO-d_6) δ: 1.15 (3H, t, $CH_2\underline{CH_3}$); 2.58 (3H, s, CH_3); 3.80-4.30 (4H, m, SCH_2, $\underline{CH_2}CH_3$); 5.55 (1H, s, pyr. C4); 7.66-8.0 (10H, m, Ar-H); 12.00 (1H, b s, exchangeable NH). IR (KBr) cm^{-1}: 3475 (NH stretching), 1700 (carbonyl group of the ester), 1649 (carbonyl group of phenacyl moiety) and 1514 (C=N stretching vibration). MS (70 ev, EI): m/z (%) (M. Wt 394.49): M^+ (394.74, 0.2%) and (198.71, 100%).

Ethyl 6-methyl-4-phenyl-2-(4-bromo-phenacylthio)-1,4-dihydropyrimidine-5-carboxyla-te hydrobromide (2b)

^{1}H-NMR (DMSO-d_6) δ: 1.10 (3H, t, $CH_2\underline{CH_3}$); 2.60 (3H, s, CH_3); 3.80-4.40 (4H, m, SCH_2, $\underline{CH_2}CH_3$); 5.60 (1H, s, pyr. C4); 6.66 - 7.50 (9H, m, Ar-H); 13.20 (1H, b s, exchangeable NH). IR (KBr) cm^{-1}: 3480 (NH stretching), 1694 (carbonyl group of the ester), 1644 (carbonyl group of phenacyl moiety) and 1517 (C=N stretching vibration). MS (70 ev, EI): m/z (%) (M. Wt 473.38): M^+ (473.60, 8.9%), M^++2 (475.50, 6.9%) and (139,00, 100%).

Ethyl 6-methyl-4-phenyl-2-(4-chloro-phenacylthio)-1,4-dihydropyrimidine-5-carboxylate hydrobromide (2c)

^{1}H-NMR (DMSO-d_6) δ: 1.15 (3H, t, $CH_2\underline{CH_3}$); 2.60 (3H, s, CH_3); 3.90-4.40 (4H, m, SCH_2, $\underline{CH_2}CH_3$); 5.69 (1H, s, pyr. C4); 6.70-7.70 (9H, m, Ar-H); 12.33 (1H, b s, exchangeable NH). IR (KBr) cm^{-1}: 3425 (NH stretching), 1696 (carbonyl group of the ester), 1645 (carbonyl group of phenacyl moiety) and 1518 (C=N stretching vibration). MS (70 ev, EI): m/z (%) (M. Wt 428.10): M^+ (427.84, 0.6%), M^++2 (429.87, 0.1%), M^+- 18 (409.90, 9.3%) and (139.00, 100%).

Ethyl 6-methyl-4-phenyl-2-(4-methoxy-phenacylthio)-1,4-dihydropyrimidine-5-carboxyl-ate hydrobromide (2d)

^{1}H-NMR (DMSO-d_6) δ: 1.10 (3H, t, $CH_2\underline{CH_3}$); 2.50 (3H, s, CH_3); 3.97 (3H, s, OCH_3); 3.80-4.33 (4H, m, SCH_2, $\underline{CH_2}CH_3$); 5.83 (1H, s, pyr. C4); 7.00-8.30 (9H, m, Ar-H); 11.97 (1H, b s, exchangeable NH). IR (KBr) cm^{-1}: 3460 (NH stretching), 1697 (carbonyl group of the ester), 1648 (carbonyl group of phenacyl moiety) and 1504 (C=N stretching vibration).

Ethyl 6-methyl-4-phenyl-2-(4-methyl-phenacylthio)-1,4-dihydropyrimidine-5-carboxylate hydrobromide (2e)

^{1}H-NMR (DMSO-d_6) δ: 1.10 (3H, t, $CH_2\underline{CH_3}$); 2.30 (3H, s, CH_3-C_6H_4); 2.50 (3H, s, CH_3); 3.80-4.33 (4H, m, SCH_2, $\underline{CH_2}CH_3$); 5.50 (1H, s, pyr. C4); 6.90 -7.50 (9H, m, Ar-H); 6.00 (1H, b s, exchangeable NH). IR (KBr) cm^{-1}: 3310 (NH stretching), 1691 (carbonyl group of the ester), 1666 (carbonyl group of phenacyl moiety) and 1512 (C=N stretching vibration).

Ethyl 6-methyl-4-phenyl-2-(4-nitrophenacyl-thio)-1,4-dihydropyrimidine-5-carboxylate hydrobromide (2f)

^{1}H-NMR (DMSO-d_6) δ: 1.10 (3H, t, $CH_2\underline{CH_3}$); 2.36 (3H, s, CH_3); 3.60-4.15 (4H, m, SCH_2, $\underline{CH_2}CH_3$); 5.30 (1H, s, pyr. C4); 6.80-8.10 (9H, m, Ar-H); 11.00 (1H, b s, exchangeable NH). IR (KBr) cm^{-1}: 3455 (NH stretching), 1696 (carbonyl group of the ester), 1647 (carbonyl group of phenacyl moiety) and 1514 (C=N stretching vibration).

Ethyl 6-methyl-4-(4-bromophenyl)-2-phenacylthio-1,4-dihydropyrimidine-5-carboxylate hydrobromide (2g)

^{1}H-NMR (DMSO-d_6) δ: 1.10 (3H, t, $CH_2\underline{CH_3}$); 2.43 (3H, s, CH_3); 3.66-4.20 (4H, m, SCH_2, $\underline{CH_2}CH_3$); 5.50 (1H, s, pyr. C4); 6.66-8.00 (9H, m, Ar-H); 12.00 (1H, b s, exchangeable NH). IR (KBr) cm^{-1}: 3460 (NH stretching), 1701 (carbonyl group of the ester), 1644 (carbonyl group of phenacyl moiety) and 1513 (C=N stretching vibration).

Ethyl 6-methyl-4-(p-bromophenyl)-2-(4-bromophenacylthio)-1,4-dihydropyrimidine-5-carboxylate hydrobromide (2h)

^{1}H-NMR (DMSO-d_6) δ: 1.10 (3H, t, $CH_2\underline{CH_3}$); 2.43 (3H, s, CH_3); 3.80-4.30 (4H, m, SCH_2, $\underline{CH_2}CH_3$); 5.50 (1H, s, pyr. C4); 7.00-8.00 (8H, m, Ar-H); 11.00 (1H, b s, exchangeable NH). IR (KBr) cm^{-1}: 3435 (NH stretching), 1707 (carbonyl group of the ester), 1648 (carbonyl group of phenacyl moiety) and 1514 (C=N stretching vibration).

Ethyl 6-methyl-4-(4-bromophenyl)-2-(4-chlorophenacylthio)-1,4-dihydropyrimidine-5-carboxylate hydrobromide (2i)

^{1}H-NMR (DMSO-d_6) δ: 1.10 (3H, t, $CH_2\underline{CH_3}$); 2.40 (3H, s, CH_3); 3.80-4.30 (4H, m, SCH_2, $\underline{CH_2}CH_3$); 5.66 (1H, s, pyr. C4); 6.50-8.20 (8H, m, Ar-H); 12.30 (1H, b s, exchangeable NH). IR (KBr) cm^{-1}: 3440 (NH stretching), 1707 (carbonyl group of the ester), 1649 (carbonyl group of phenacyl moiety) and 1515 (C=N stretching vibration). MS (70 ev, EI): m/z (%) (M. Wt 507.83): M^+ (508.20, 2.5%), and (138.80, 100%).

Ethyl 6-methyl-4-(4-bromophenyl)-2-(4-methoxyphenacylthio)-1,4-dihydro-pyrimidine-5-carboxylate hydrobromide (2j)

^{1}H-NMR (DMSO-d_6) δ: 1.10 (3H, t, $CH_2\underline{CH_3}$); 2.40 (3H, s, CH_3); 3.73 (3H, s, OCH_3); 3.66-4.30 (4H, m, SCH_2, $\underline{CH_2}CH_3$); 5.60 (1H, s, pyr. C4); 6.33-8.20 (8H, m, Ar-H); 11.90 (1H, b s, exchangeable NH). IR (KBr) cm^{-1}: 3395 (NH stretching), 1693 (carbonyl group of the ester), 1643 (carbonyl group of phenacyl moiety) and 1498 (C=N stretching vibration).

Ethyl 6-methyl-4-(4-bromophenyl)-2-(4-methylphenacylthio)-1,4-dihydropyrimi-dine-5-carboxylate hydrobromide (2k)

^{1}H-NMR (DMSO-d_6) δ: 1.30 (3H, t, $CH_2\underline{CH_3}$); 2.40 (6H, s, $2CH_3$); 3.80-4.30 (4H, m, SCH_2, $\underline{CH_2}CH_3$); 5.66 (1H, s, pyr. C4); 6.80-8.10 (8H, m, Ar-H); 12.50 (1H, b s, exchangeable NH). IR (KBr) cm^{-1}: 3420 (NH stretching), 1707 (carbonyl group of the ester), 1648 (carbonyl group of phenacyl moiety) and 1512 (C=N stretching vibration).

Ethyl 6-methyl-4-(4-bromophenyl)-2-(4-nitrophenacylthio)-1,4-dihydropyrimidine-5-carboxylate hydrobromide (2l)

^{1}H-NMR (DMSO-d_6) δ: 1.50 (3H, t, $CH_2\underline{CH_3}$); 2.86 (3H, s, CH_3); 4.00-5.00 (4H, m, SCH_2, $\underline{CH_2}CH_3$); 5.96 (1H, s, pyr. C4); 6.80-9.00 (8H, m, Ar-H); 11.50 (1H, b s exchangeable NH). IR (KBr) cm^{-1}: 3450 (NH stretching), 1707 (carbonyl group of the ester), 1654 (carbonyl group of phenacyl moiety) and 1515 (C=N stretching vibration).

Ethyl 6-methyl-4-(4-chlorophenyl)-2-phenacylthio-1,4-dihydropyrimidine-5-carboxylate hydrobromide (2m)

^{1}H-NMR (DMSO-d_6) δ: 1.18 (3H, t, $CH_2\underline{CH_3}$); 2.51 (3H, s, CH_3); 3.80-4.36 (4H, m, SCH_2, $\underline{CH_2}CH_3$); 5.66 (1H, s, pyr. C4); 6.66-7.66 (9H, m, Ar-H); 12.39 (1H, b s, exchangeable NH). IR (KBr) cm^{-1}: 3450 (NH stretching), 1705 (carbonyl group of the ester), 1650 (carbonyl group of phenacyl moiety) and 1513 (C=N stretching vibration).

Ethyl 6-methyl-4-(4-chlorophenyl)-2-(4-bromophenacylthio)-1,4-dihydropyrimidine-5-carboxylate hydrobromide (2n)

^{1}H-NMR (DMSO-d_6) δ: 1.10 (3H, t, $CH_2\underline{CH_3}$); 2.50 (3H, s, CH_3); 3.80-4.33 (4H, m, SCH_2, $\underline{CH_2}CH_3$); 5.60 (1H, s, pyr. C4); 6.70-8.00 (8H, m, Ar-H); 12.00 (1H, b s, exchangeable NH). IR (KBr) cm^{-1}: 3500 (NH stretching), 1706 (carbonyl group of the ester), 1649 (carbonyl group of phenacyl moiety) and 1513 (C=N stretching vibration). MS (FAB): m/z (%) (M. Wt 507.83): $(M+H)^+$ (508.20, 0.3%), $(M+H)^+$+2 (510.02, 0.5%) and (475.30, 100%).

Ethyl 6-methyl-4-(4-chlorophenyl)-2-(4-chlorophenacylthio)-1,4-dihydropyrimidine-5-carboxylate hydrobromide (2o)

^{1}H-NMR (DMSO-d_6) δ: 1.10 (3H, t, $CH_2\underline{CH_3}$); 2.50 (3H, s, CH_3); 3.80-4.23 (4H, m, SCH_2, $\underline{CH_2}CH_3$); 5.46 (1H, s, pyr. C4); 6.80-7.80 (8H, m, Ar-H); 11.59 (1H, b s, exchangeable NH). IR (KBr) cm^{-1}: 3395 (NH stretching), 1707 (carbonyl group of the ester), 1649 (carbonyl group of phenacyl moiety) and 1514 (C=N stretching vibration). MS (70 ev, EI): m/z (%) (M. Wt 462.06): $M^{+\cdot}$ (462.00, 5.0%) and (139.00, 100%).

Ethyl 6-methyl-4-(4-chlorophenyl)-2-(4-methoxyphenacylthio)-1,4-dihydropyrimi-dine-5-carboxylate hydrobromide (2p)

^{1}H-NMR (DMSO-d_6) δ: 1.10 (3H, t, $CH_2\underline{CH_3}$); 2.43 (3H, s, CH_3); 3.80 (3H, s, OCH_3); 3.66-4.23 (4H, m, SCH_2, $\underline{CH_2}CH_3$); 5.46 (1H, s, pyr. C4); 6.80-7.90 (8H, m, Ar-H); 12.59 (1H, b s, exchangeable NH). IR (KBr) cm^{-1}: 3450 (NH stretching), 1695 (carbonyl group of the ester), 1643 (carbonyl group of phenacyl moiety) and 1499 (C=N stretching vibration).

Ethyl 6-methyl-4-(4-chlorophenyl)-2-(4-methylphenacylthio)-1,4-dihydropyrimidine-5-carboxylate hydrobromide (2q)

^{1}H-NMR (DMSO-d_6) δ: 1.10 (3H, t, $CH_2\underline{CH_3}$); 2.33 (3H, s, CH_3-C_6H_4-); 2.45 (3H, s, CH_3); 3.66-4.40 (4H, m, SCH_2, $\underline{CH_2}CH_3$); 5.43 (1H, s, pyr. C4); 6.80-7.50 (8H, m, Ar-H); 11.00 (1H, b s, exchangeable NH). IR (KBr) cm^{-1}: 3440 (NH stretching), 1706 (carbonyl group of the ester), 1648 (carbonyl group of phenacyl moiety) and 1512 (C=N stretching vibration).

Ethyl 6-methyl-4-(4-chlorophenyl)-2-(4-nitrophenacylthio)-1,4-dihydropyrimidine-5-carboxylate hydrobromide (2r)

^{1}H-NMR (DMSO-d_6) δ: 1.00 (3H, t, $CH_2\underline{CH_3}$); 2.33(3H, s, CH_3); 3.66-4.66(4H, m, SCH_2, $\underline{CH_2}CH_3$); 5.40(1H, s, pyr. C4); 6.66-8.33 (8H, m, Ar-H); 11.30 (1H, b s, exchangeable NH). IR (KBr) cm^{-1}: 3440 (NH stretching), 1707 (carbonyl group of the ester), 1652 (carbonyl group of phenacyl moiety) and 1515 (C=N stretching vibration).

Ethy 6-methyl-4-(4-methylphenyl)-2-phenacylthio-1,4-dihydropyrimidine-5-carboxylate hydrobromide (2s)

^{1}H-NMR (DMSO-d_6) δ: 1.16 (3H, t, $CH_2\underline{CH_3}$); 2.30 (3H, s, CH_3-C_6H_4-); 2.50 (3H, s, CH_3); 3.80-4.40 (4H, m, SCH_2, $\underline{CH_2}CH_3$); 5.50 (1H, s, pyr. C4); 6.60-8.00 (9H, m, Ar-H); 12.39 (1H, b s, exchangeable NH). IR (KBr) cm^{-1}: 3405 (NH stretching), 1702 (carbonyl group of the ester), 1657 (carbonyl group of phenacyl moiety) and 1519 (C=N stretching vibration).

Ethyl 6-methyl-4-(4-methylphenyl)-2-(4-bromophenacylthio)-1,4-dihydropyrimidine-5-carboxylate hydrobromide (2t)

^{1}H-NMR (DMSO-d_6) δ: 1.16 (3H, t, $CH_2\underline{CH_3}$); 2.30 (3H, s, CH_3-C_6H_4-); 2.50 (3H, s, CH_3); 3.86-4.33 (4H, m, SCH_2, $\underline{CH_2}CH_3$); 5.50 (1H, s, pyr. C4); 6.60-8.00 (8H, m, Ar-H); 12.00 (1H, b s exchangeable NH). IR (KBr) cm^{-1}: 3430 (NH stretching), 1707 (carbonyl group of the ester), 1647 (carbonyl group of phenacyl moiety) and 1515 (C=N stretching vibration).

Ethyl 6-methyl-4-(4-methylphenyl)-2-(4-chlorophenacylthio)-1,4-dihydropyrimidine-5-carboxylate hydrobromide (2u)

^{1}H-NMR (DMSO-d_6) δ: 1.10 (3H, t, $CH_2\underline{CH_3}$); 2.26 (3H, s, CH_3-C_6H_4-); 2.46 (3H, s, CH_3); 3.60-4.33 (4H, m, SCH_2, $\underline{CH_2}CH_3$); 5.43 (1H, s, pyr. C4); 6.50-8.33 (8H, m, Ar-H); 12.45 (1H, b s, N_1H exchangeable NH). IR (KBr) cm^{-1}: 3390 (NH stretching), 1707 (carbonyl group of the ester), 1648 (carbonyl group of phenacyl moiety) and 1514 (C=N stretching vibration).

Ethyl 6-methyl-4-(4-methylphenyl)-2-(4-methoxyphenacylthio)-1,4-dihydropyrimidine-5-carboxylate hydrobromide (2v)

^{1}H-NMR (DMSO-d_6) δ: 1.16 (3H, t, $CH_2\underline{CH_3}$); 2.30 (3H, s, CH_3-C_6H_4-); 2.50 (3H, s, CH_3); 3.83 (3H, s, OCH_3); 3.60-4.33 (4H, m, SCH_2, $\underline{CH_2}CH_3$); 5.50 (1H, s, pyr. C4); 6.60-8.20 (8H, m, Ar-H); 11.55 (1H, b s, exchangeable NH). IR (KBr) cm^{-1}: 3410 (NH stretching), 1695 (carbonyl group of the ester), 1643 (carbonyl group of phenacyl moiety) and 1499 (C=N stretching vibration).

Ethyl 6-methyl-4-(4-methylphenyl)-2-(4-methylphenacylthio)-1,4-dihydropyrimidine-5-carboxylate hydrobromide (2w)

^{1}H-NMR (DMSO-d_6) δ: 1.20 (3H, t, $CH_2\underline{CH_3}$); 2.30 (6H, s, $2CH_3$-C_6H_4-); 2.50 (3H, s, CH_3); 3.90-4.40 (4H, m, SCH_2, $\underline{CH_2}CH_3$); 5.80 (1H, s, pyr. C4); 7.00-8.20 (8H, m, Ar-H); 11.20 (1H, b s, exchangeable NH). IR (KBr) cm^{-1}: 3390 (NH stretching), 1698 (carbonyl group of the ester), 1662 (carbonyl group of phenacyl moiety) and 1500 (C=N stretching vibration).

Ethyl 6-methyl-4-(4-methylphenyl)-2-(4-nitrophenacylthio)-1,4-dihydropyrimidine-5-carboxylate hydrobromide (2x)

^{1}H-NMR (DMSO-d_6) δ: 1.10 (3H, t, $CH_2\underline{CH_3}$); 2.20 (3H, s, CH_3-C_6H_4-); 2.43 (3H, s, CH_3); 3.80-4.30 (4H, m, SCH_2, $\underline{CH_2}CH_3$); 5.43 (1H, s, pyr. C4); 6.50-8.00 (8H, m, Ar-H); 12.69 (1H, b s, exchangeable NH). IR (KBr) cm^{-1}: 3425 (NH stretching), 1707 (carbonyl group of the ester), 1651 (carbonyl group of phenacyl moiety) and 1514 (C=N stretching vibration).

Antimicrobial screening

Antibacterial activity
Organisms

Five bacterial species representing both Gram-positive and Gram-negative strains were used to test the antibacterial activities of the new compounds: *Staphylococcus aureus* (AUMC No. B-54) and *Bacillus cereus* (AUMC NoB-5) as representatives of Gram-positive strains, and *Escherichia coli* (AUMC No.B-53), *Pseudomonas aeruginosa* (AUMC No. B-73), as well as *Serratia marcescens* (AUMC No. B-55) as representatives of Gram-negative strains.

Materials and methods[2&16]

To prepare inocula for bioassay, bacterial strains were individually cultured for 24 hrs in 100 ml conical flasks containing 30 ml nutrient broth medium. Bioassay was done in 10 cm sterile plastic Petri plates in which bacterial suspension (1 m/plate) and 15 ml Nutrient agar medium (15 ml/plate) were poured. After solidification of the media, 5 mm diameter cavities were cut in the solidified agar (4 cavities/plate) using sterile cork borer. Tested compounds dissolved in dimethyl sulfoxide (DMSO) at 100 µmol/ml were pipetted in the cavities (20 µl/cavity). Cultures were then incubated at 28°C for 48 hrs. Results were read as the diameter (in mm) of inhibition zone around cavities.

Antifungal activity
Organisms

Six pathogenic, phytogenic, or food-poisning fungal species were used in the present study: *Candida albicans* (AUMC No. 418), *Geotrichum candidum* (AUMC No. 226), *Aspergillus flavus* (AUMC No. 1276), *Trichophyton rubrum* (AUMC No. 1804), *Scopulariopsis brevicaulis* (AUMC No. 729), *Fusarium oxysporum* (AUMC No. 5119).

Materials and method[2&16]

To prepare inocula for bioassay, Fungi were grown for 7 days in 100 ml conical flasks containing 30 ml sabouraud`s dextrose broth. Bioassay was done in 10 cm sterile plastic Petri plates in which fungal suspension (1 m/plate) and 15 ml sabouraud`s dextrose agar medium (15 ml/plate) were poured. After solidification of the media, 5 mm diameter cavities were cut in the solidified agar (4 cavities/plate) using sterile cork borer. Tested compounds dissolved in dimethyl sulfoxide (DMSO) at 100 µmol/ml were pipetted in the cavities (20 µl/cavity). Cultures were then incubated at 28°C up to 7 days. Results were read as the diameter (in mm) of inhibition zone around cavities.

For determination of the minimum inhibitory concentrations (MICs), tested compounds giving positive results were diluted with DMSO to prepare a series of descending concentrations down to 0.39 µmol/ml. diluted compounds were similarly assayed as mentioned before and the least concentration (below which no activity) was recorded as the MIC.

RESULTS AND DISCUSSION

Chemistry

Ethyl 6-methyl-4-(substituted)phenyl-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxy-lates (**1a-d**) were synthesized according to a reported procedure through reaction of the appropriate aldehyde with thiourea and ethylacetoacetate[17-19]. S-alkylation of com-pounds (**1a-d**) with phenacyl bromides in dry acetone afforded compounds (**2a-x**) as their hydrobromide salts in an excellent yields (85-93%). It was reported that, S-alkylation supercsedes the N-alkylation due to the difference in nucleophilicity between sulfur and nitrogen atoms[7&8]. Structures of compounds (**1a-d**) were confirmed by comparison with the reported data[17-19]. Structures of the synthesized compounds (**2a-x**) were verified by IR, ^{1}H-NMR, mass spectra in addition to elemental microanalyses. IR spectra of compounds (**2a-x**) revealed absorption bands at 3505-3310 cm^{-1} indicating NH stretching, 1707-1691 cm^{-1} and 1666-1643 cm^{-1} for the carbonyl groups of the ester and phenacyl moieties, respectively. In addition to a strong absorption band at 1519-1498 cm^{-1} indicating C=N stretching vibration. ^{1}H-NMR revealed a triplet signal of CH_3 of the ethyl group at 1.00-1.50 ppm, quartet signal of CH_2 of the ethyl group at 3.60-4.00 ppm, singlet signal of SCH_2 of the phenacyl moiety at 3.80-4.15 ppm, multiplet at 6.30-8.30 ppm indicating aromatic protons and broad singlet signal at 11.97-13.20 ppm attributed to NH group. Moreover, mass spectra (EI) of compounds **2a, 2b, 2c, 2i** and **2o**

revealed a molecular ion peaks (M^+) at 394.74, 473.60, 427.84, 508.20 and 462.00 m/z corresponding to their molecular weights, respectively. Compounds **2b** and **2c** showed M^++2 at 475.50 and 429.87 m/z, respectively. Compound **2a** showed a base peak at 198.71 m/z while compounds **2b, 2c, 2i** and **2o** showed a base peak at 139.00 m/z. On the other hand, mass spectra (FAB) of compound **2n** showed $(M+H)^+$ at 508.20, $(M+H)^+$+2 at 510.02 and base peak at 475.30 m/z. physicochemical data of compounds (**2a-x**) are shown in table I and spectral data in the experimental section.

Antimicrobial activities

Antibacterial activity

The newly synthesized compounds (**2a-x**) were tested for their *in-vitro* antibacterial activity against *Staphylococcus aureus* (AUMC No. B-54) and *Bacillus cereus* (AUMC No. B-5) as representatives of Gram-positive strains and *Escherichia coli* (AUMC No. B-53), *Pseudomonas aeruginosa* (AUMC No. B-73) *and Serratia marcescens* (AUMC No. B-55) as representatives of Gram-negative ones.

The test compounds (**2a-x**) were assayed using the standard agar cup diffusion method[16] at a concentration of 100 µmol/mL and those giving positive results were diluted with DMSO to prepare a series of descending concentrations down to 0.39 µmol/mL and were similarly assayed and the test concentration (below which no activity) was recorded as the MIC.

Results of the antibacterial activity, table II, indicated that at a concentration of 100 µmol/mL most of the test compounds were active against most of the used bacterial strains. Compounds **2a, 2s** and **2v** were completely inactive against all used organisms. Compounds **2b, 2m** and **2w** were active only against *Staphylococcus aureus* and compounds **2c, 2f** and **2g** were active only against *Bacillus cereus.* In addition, the active compounds (**2a-x**) showed 51.9-85.2% antibacterial activity of that of choramphenicol against *Staphylococcus aureus*, 40.6-78.1% against *Bacillus cereus*, 53.3-76.7% against *Escherichia coli*, 66.7-83.3% against *Pseudomonas aeruginosa* and 31.7-48.8% against *Serratia marcescens*. Moreover, the variation of the antibacterial activity with concentrations was indicated in table III. It was noted that, the most sensitive organisms to the test compounds were *Staphylococcus aureus* and *Pseudomonas aeruginosa.*

Again, from tables III and IV, compounds **2n, 2o** and **2r** have a wide spectrum of antibacterial activity being able to inhibit all test bacterial organisms with MICs ranging from 100 to 0.39 µmol/mL. Compounds **2i, 2k** and **2l** were effective against four out of five bacterial strains with MICs ranging from 100 up to 6.25 µmol/mL. It is noteworthy to mention that, the most active compounds comprise in their structures an electron withdrawing group (R_1=Cl; R_2= Br, Cl, or NO_2) while the least active compounds comprise in their structures an electron donating group (R_1=H, CH_3; R_2= OCH_3, CH_3).

Antifungal activity

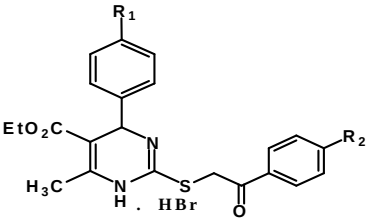
Compounds (**2a-x**) were tested for their *in-vitro* antifungal activity using the standard agar disc diffusion method against *Candida albicans* (AUMC No. 418), *Geotrichum candidum* (AUMC No. 226), *Fusarium oxysporum* (AUMC No. 5119), *Aspergillus flavus* (AUMC No. 1276), *Trichophyton rubrum* (AUMC No. 1804) and *Scopulariopsis brevicaulis* (AUMC No. 729). The results of the antifungal activity are given in table V and expressed as inhibition zones in mm using Clotrimazole as a reference drug.

Results of the antifungal activity, table V, indicated that at a concentration of 100 µmol/mL all compounds were completely inactive against *Aspergillus flavus* and *Scopulariopsis brevicaulis* except **2f**. Compound **2w** was active only against *Trichophyton rubrum.* Compounds **2a** and **2c** were possessed antifungal activity equal to that of the reference drug against *Fusarium oxysporum* and *Geotrichum candidum*, respectively. In addition, the active compounds (**2a-x**) showed 53.3-80.0% antifungal activity of that of Clotrimazole against *Candida albicans* with MICs ranged from 6.25-100 µmol/mL, 54.2-100% against *Geotrichum candidum*, 36.4-100% against *Fusarium oxysporum* and 34.3-97.1% against *Trichophyton rubrum.*

Also, the results indicated that compound **2f** has a wide spectrum of antifungal activity being able to inhibit all test fungal organisms with MICs ranging from 50 to 12.5 µmol/mL. Compounds **2b** and **2q** were effective against four out of six fungal strains with MICs ranging from 100 to 6.25 µmol/mL. Again, it is noteworthy to mention that the most active

compounds comprise in their structures an electron withdrawing group (R_1= H, Cl; R_2= Br, NO_2) while the least active compounds comprise in their structures an electron donating group (R_1= CH_3; R_2= CH_3, OCH_3).

Table I: The physicochemical data of compounds **(2a-x)**.



No.	R_1	R_2	M.p. (°C)	Yield (%)	R*f**	C log p**	Mol. Formula (Mol. Wt.)	Microanalyses Calcd/ Found		
								C%	C%	C%
2a	H	H	172-3	93	0.38	6.13	$C_{22}H_{23}BrN_2O_3S$ (475.4)	55.58 55.79	4.88 4.70	5.89 5.84
2b	H	Br	175	92	0.33	8.36	$C_{22}H_{22}Br_2N_2O_3S$ (554.29)	47.67 47.41	4.00 3.94	5.05 4.70
2c	H	Cl	174-6	90	0.31	8.21	$C_{22}H_{22}BrClN_2O_3S$ (509.84)	51.83 52.10	4.35 4.54	5.49 5.51
2d	H	OCH_3	219	91	0.48	7.64	$C_{23}H_{25}BrN_2O_4S$ (505.42)	54.66 55.00	4.99 5.18	5.54 5.50
2e	H	CH_3	156	92	0.42	7.92	$C_{23}H_{25}BrN_2O_3S$ (489.43)	56.44 56.70	5.15 5.02	5.72 5.60
2f	H	NO_2	136-8	92	0.21	7.32	$C_{22}H_{22}BrN_3O_5S$ (520.4)	50.78 50.80	4.26 4.30	8.07 7.97
2g	Br	H	185	93	0.39	6.99	$C_{22}H_{22}Br_2N_2O_3S$ (554.29)	47.67 47.57	4.00 3.82	5.05 5.10
2h	Br	Br	210	91	0.36	9.22	$C_{22}H_{21}Br_3N_2O_3S$ (633.19)	41.73 41.93	3.34 3.29	4.42 4.21
2i	Br	Cl	200	90	0.36	9.07	$C_{22}H_{21}Br_2ClN_2O_3S$ (588.74)	44.88 45.10	3.60 3.71	4.76 4.69
2j	Br	OCH_3	209	93	0.43	8.50	$C_{23}H_{24}Br_2N_2O_4S$ (584.32)	47.28 47.58	4.14 3.80	4.79 4.68
2k	Br	CH_3	186-7	90	0.43	8.78	$C_{23}H_{24}Br_2N_2O_3S$ (568.32)	48.61 48.95	4.26 4.00	4.93 5.14
2l	Br	NO_2	208	92	0.25	8.18	$C_{22}H_{21}Br_2N_3O_5S$ (599.29)	44.09 44.38	3.53 3.19	7.01 7.23
2m	Cl	H	175	91	0.40	8.13	$C_{22}H_{22}BrClN_2O_3S$ (509.84)	51.83 51.52	4.35 4.18	5.49 5.59
2n	Cl	Br	197	90	0.36	9.07	$C_{22}H_{21}Br_2ClN_2O_3S$ (588.74)	44.88 44.61	3.60 3.58	4.76 4.81
2o	Cl	Cl	199	92	0.34	8.92	$C_{22}H_{21}BrCl_2N_2O_3S$ (544.29)	48.55 48.60	3.89 3.90	5.15 5.10
2p	Cl	OCH_3	197	88	0.45	8.35	$C_{23}H_{24}BrClN_2O_4S$ (539.87)	51.17 50.93	4.48 4.40	5.19 5.31
2q	Cl	CH_3	180	89	0.48	8.63	$C_{23}H_{24}BrClN_2O_3S$ (523.87)	52.73 52.59	4.62 4.30	5.35 5.24
2r	Cl	NO_2	199	93	0.36	8.04	$C_{22}H_{21}BrClN_3O_5S$ (554.84)	47.62 47.29	3.81 4.00	7.57 7.53
2s	CH_3	H	186-7	91	0.32	7.92	$C_{23}H_{25}BrN_2O_3S$ (489.43)	56.44 56.70	5.15 5.30	5.72 5.77
2t	CH_3	Br	188	90	0.30	8.85	$C_{23}H_{24}Br_2N_2O_3S$ (568.32)	48.61 48.82	4.26 4.37	4.93 4.88
2u	CH_3	Cl	187-8	92	0.30	8.70	$C_{23}H_{24}BrClN_2O_3S$ (523.87)	52.73 53.00	4.62 4.60	5.35 5.14
2v	CH_3	OCH_3	198	90	0.38	8.14	$C_{24}H_{27}BrN_2O_4S$ (519.45)	55.49 55.81	5.24 5.42	5.39 5.53
2w	CH_3	CH_3	197	88	0.36	8.42	$C_{24}H_{27}BrN_2O_3S$ (503.45)	57.26 57.52	5.41 5.63	5.56 5.67
2x	CH_3	NO_2	198-9	85	0.21	7.82	$C_{23}H_{24}BrN_3O_5S$ (534.42)	51.69 52.00	4.53 4.61	7.86 8.00

* = 10% Acetone/$CHCl_3$ **= Calculated logarithm partition coefficient

Table II: The antibacterial activity [zones of inhibition (mm) at concentration 100 µmol/mL] of compounds **(2a-x)** and Chloramphenicol.

No.	*Staphylococcus aureus*	*Bacillus cereus*	*Escherichia coli*	*Pseudomonas aeruginosa*	*Serratia marcescens*
2a	-	-	-	-	-
2b	21	-	-	-	-
2c	-	16	-	-	-
2d	-	-	-	-	18
2e	22	-	-	20	19
2f	-	19	-	-	-
g	-	14	-	-	-
2h	-	13	21	-	-
2i	-	15	21	17 pi	20
2j	-	22	22	20	17
2k	18	14	18	19	-
2l	21	25	21	20	-
2m	14	-	-	-	-
2n	23	16	22	21	18
2o	19	18	21	20	17
2p	16	23	-	16	13
2q	21	23	-	-	17
2r	21	18	20	21	14
2s	-	-	-	-	-
2t	-	14	23	-	-
2u	-	14	16	-	-
2v	-	-	-	-	-
2w	14	-	-	-	-
2x	22	-	-	20	14
CHL	27	32	30	24	41

Pi = partial inhibition - = no inhibition
CHL= Chloramphenicol AUMC = Assiut University Mycological Center

Table III: The antibacterial activity [zones of inhibition (mm) and a serial dilution (µmol)] of compounds **(2a-x)** and Chloramphenicol.

No.	Conc. µmol/mL	*Staphylococcus aureus*	*Bacillus cereus*	*Escherichia coli*	*Pseudomonas aeruginosa*	*Serratia marcescens*
2b	50	16	0	0	0	0
	25	14	-	-	-	-
	12.5	12	-	-	-	-
	6.25	0	-	-	-	-
2c	50	0	0	0	0	0
2d	50	-	-	-	-	21
	25	-	-	-	-	18
	12.5	-	-	-	-	13
	6.25	-	-	-	-	12
	3.125	-	-	-	-	0
2e	50	17	0	0	20	13
	25	13	-	-	17	10
	12.5	12	-	-	12	8
	6.25	10	-	-	10	0
	3.125	8	-	-	0	-
	1.56	0	-	-	-	-
2f	50	0	18	0	0	0
	25	-	14	-	-	-
	12.5	-	12	-	-	-
	6.25	-	0	-	-	-
2g	50	0	0	0	0	0
2h	50	-	0	18	-	-
	25	-	-	16	-	-
	12.5	-	-	`13	-	-
	6.25	-	-	10	-	-
	3.125	-	-	0	-	-
2i	50	0	0	18	0	12
	25	-	-	15		0
	12.5	-	-	13	-	-
	6.25	-	-	12	-	-
	3.125	-	-	0	-	-
2j	50	0	23	23	18	14
	25	-	18	18	15	12
	12.5	-	16	16	12	0
	6.25	-	0	0	0	-
2k	50	22	14	19	16	0
	25	17	0	16	15	-
	12.5	14	-	14	13	-
	6.25	12	-	12	0	-
	3.125	11	-	0	-	-
	1.56	10	-	-	-	-
	0.78	0	-	-	-	-
2l	50	17	22	21	19	0
	25	14	18	16	17	-
	12.5	12	14	12	13	-
	6.25	9	10	10	10	-
	3.125	0	0	0	0	-
2m	50	0	-	-	-	-
2n	50	20	`10	18	22	17
	25	15	0	15	18	12
	12.5	13	-	12	15	10
	6.25	10	-	10	13	0
	3.125	10	-	0	0	-
	1.56	10	-	-	-	-
	0.78	8	-	-	-	-
	0.39	8	-	-	-	-
	0.19	0	-	-	-	-

Table III: Continued.

No.	Conc. µmol/mL	*Staphylococcus aureus*	*Bacillus cereus*	*Escherichia coli*	*Pseudomonas aeruginosa*	*Serratia marcescens*
2o	50	17	0	17	21	16
	25	14	-	14	16	12
	12.5	12	-	12	14	10
	6.25	10	-	0	11	0
	3.125	0	-	-	0	-
2p	50	14	22	0	15	0
	25	12	18	-	13	-
	12.5	10	14	-	10	-
	6.25	10	12	-	10	-
	3.125	8	0	-	0	-
	1.56	8	-	-	-	-
	0.78	0	-	-	-	-
2q	50	18	18	0	0	17
	25	13	13	-	-	12
	12.5	12	0	-	-	10
	6.25	11	-	-	-	0
	3.125	11	-	-	-	-
	1.56	10	-	-	-	-
	0.78	10	-	-	-	-
	0.39	0	-	-	-	-
2r	50	18	14	18	20	13
	25	14	0	17	16	12
	12.5	12	-	12	14	0
	6.25	11	-	10	12	-
	3.125	10	-	0	0	-
	1.56	10	-	-	-	-
	0.78	0	-	-	-	-
2t	50	0	0	22	0	0
	25	-	-	15	-	-
	12.5	-	-	13	-	-
	6.25	-	-	12	-	-
	3.125	-	-	0	-	-
2u	50	0	12	17	0	0
	25	-	0	12	-	-
	12.5	-	-	10	-	-
	6.25	-	-	0	-	-
2w	50	12	0	0	0	0
	25	8	-	-	-	-
	12.5	0	-	-	-	-
2x	50	17	0	0	15	16
	25	14	-	-	12	10
	12.5	12	-	-	8	0
	6.25	10	-	-	0	-
	3.125	8	-	-	-	-
	1.56	0	-	-	-	-
CHL	10	17	32	26	16	40
	5	17	32	26	14	38
	2.5	15	30	20	12	34
	1.25	13	28	16	12	28
	0.6	12	25	14	10	26
	0.3	10	18	12	10	20
	0.15	10	16	0	10	-
	0.08	0	0	-	-	-

Table IV: Antibacterial activity [inhibition zone in mm and MICs (in µmol) given in brackets] of compounds **(2a-x)** and Chloramphenicol.

No.	*Staphylococcus aureus*	*Bacillus cereus*	*Escherichia coli*	*Pseudomonas aeruginosa*	*Serratia marcescens*
2a	-	-	-	-	-
2b	12(12.5)	-	-	-	-
2c	-	16(100)	-	-	-
2d	-	-	-	-	12(6.25)
2e	8(3.125)	-	-	10(6.25)	8(12.5)
2f	-	12(12.5)	-	-	-
2g	-	14(100)	-	-	-
2h	-	13(100)	10(6.25)	-	-
2i	-	15(100)	12(6.25)	17p.i(100)	12(50)
2j	-	16(12.5)	12(12.5)	12(25)	-
2k	10(1.56)	14(50)	12(6.25)	13(12.5)	-
2l	9(6.25)	10(6.25)	10(6.25)	10(6.25)	-
2m	14(100)	-	-	-	-
2n	8(0.39)	10(50)	10(6.25)	13(6.25)	10(12.5)
2o	10(6.25)	18(100)	12(12.5)	11(6.25)	10(12.5)
2p	8(1.56)	12(6.25)	-	10(6.25)	13(100)
2q	10(0.78)	13(25)	-	-	10(12.5)
2r	10(1.56)	14(50)	10(6.25)	12(6.25)	12(25)
2s	-	-	-	-	-
2t	-	14(100)	12(6.25)	-	-
2u	-	12(50)	10(12.5)	-	-
2v	-	-	-	-	-
2w	8(25)	-	-	-	-
2x	8(3.125)	-	-	8(12.5)	10(25)
CHL	10(0.15)	16(0.15)	12(0.3)	10(0.15)	20(0.3)

Table V: The antifungal zones of inhibition (mm) of compounds **(2a-x)** and Clotrimazole.

No.	*Candida albicans*	*Geotrichum candidum*	*Fusarium oxysporum*	*Aspergillus flavus*	*Trichophyton rubrum*	*Scopulariopsis brevicaulis*
2a	-	14	22	-	12	-
2b	22	22	20	-	32	-
2c	22	24	-	-	32	-
2d	20	19	-	-	16	-
2e	16	13	-	-	18	-
2f	19	14	12	15	17	16
2g	-	20	8	-	24	-
2h	24	20	-	-	34	-
2i	21	20	-	-	32	-
2j	19	10	-	-	20	-
2k	20	18	-	-	25	-
2l	-	14	-	-	20	-
2m	18	18	-	-	23	-
2n	22	23	-	-	32	-
2o	12	22	-	-	14	-
2p	19	-	-	-	25	-
2q	16	17	8	-	23	-
2r	-	17	8	-	20	-
2s	-	18	-	-	25	-
2t	18	23	-	-	33	-
2u	-	14	-	-	31	-
2v	-	16	-	-	16	-
2w	-	-	-	-	25	-
2x	-	17	-	-	16	-
CLO	30	24	22	27	35	26

CLO = Clotrimazole

Table VI: The antifungal activity [zones of inhibition (mm) and a series of descending concentrations (µmol)] of compounds **(2a-x)** and clotrimazole.

No.	Conc. µmol/mL	*Candida albicans*	*Geotrichum candidum*	*Fusarium oxysporum*	*Aspergillus flavus*	*Trichophyton rubrum*	*Scopulariopsis brevicaulis*
2a	50	0	11	17	0	16	0
	25	-	11	16	-	12	-
	12.5	-	0	12	-	0	-
	6.25	-	-	10	-	-	-
	3.125	-	-	0	-	-	-
2b	50	0	16	13	0	26	0
	25	-	12	12	-	22	-
	12.5	-	10	8	-	18	-
	6.25	-	10	0	-	14	-
	3.125	-	0	-	-	0	-
2c	50	23	14	0	0	28	0
	25	18	13	-	-	23	-
	12.5	12	12	-	-	17	-
	6.25	0	12	-	-	-	-
	3.125	-	0	-	-	-	-
2d	50	17	16	0	0	15	0
	25	12	13	-	-	10	-
	12.5	10	11	-	-	0	-
	6.25	0	10	-	-	-	-
	3.125	-	10	-	-	-	-
	1.56	-	8	-	-	-	-
	0.78	-	0	-	-	-	-
2e	50	0	14	0	0	17	0
	25	-	12	-	-	11	-
	12.5	-	11	-	-	0	-
	6.25	-	0	-	-	-	-
2f	50	16	14	10	12	14	17
	25	14	13	0	0	10	13
	12.5	0	10	-	-	0	0
	6.25	--	0	-	-	-	-
	3.125	-	-	-	-	-	-
2g	50	0	15	0	0	22	0
	25	-	13	-	-	18	-
	12.5	-	11	-	-	12	-
	6.25	-	11	-	-	10	-
	3.125	-	10	-	-	0	-
	1.56	-	0	-	-		-
2h	50	20	18	0	0	30	0
	25	16	`15	-	-	28	-
	12.5	10	12	-	-	20	-
	6.25	8	10	-	-	14	-
	3.125	0	9	-	-	0	-
	1.56	-	0	-	-		-
2i	50	18	16	0	0	28	0
	25	14	13	-	-	22	-
	12.5	13	13	-	-	15	-
	6.25	0	12	-	-	13	-
	3.125	-	0	-	-	0	-
2j	50	16	14	0	0	20	0
	25	14	12	-	-	18	-
	12.5	10	10	-	-	14	-
	6.25	0	8	-	-	10	-
	3.125		0	-	-	0	-
2k	50	16	16	0	0	26	0
	25	16	14	-	-	22	-
	12.5	12	13	-	-	12	-

Table VI: Continued.

No.	Conc. µmol/mL	*Candida albicans*	*Geotrichum candidum*	*Fusarium oxysporum*	*Aspergillus flavus*	*Trichophyton rubrum*	*Scopulariopsis brevicaulis*
	6.25	0	12	-	-	8	-
	3.125	-	0	-	-	0	-
2l	50	0	14	0	0	16	0
	25	-	12	-	-	13	-
	12.5	-	10	-	-	0	-
	6.25	-	0	-	-		-
2m	50	20	14	0	0	22	0
	25	18	14	-	-	20	-
	12.5	12	12	-	-	14	-
	6.25	0	10	-	-	10	-
	3.125	-	10	-	-	0	-
	1.56	-	0	-	-		-
2n	50	22	18	0	0	33	0
	25	18	16	-	-	30	-
	12.5	16	12	-	-	22	-
	6.25	12	12	-	-	20	-
	3.125	0	0	-	-	18	-
	1.56	-	-	-	-	0	-
2o	50	0	16	0	0	0	0
	25	-	12	-	-	-	-
	12.5	-	12	-	-	-	-
	6.25	-	0	-	-	-	-
2p	50	18	0	0	0	30	0
	25	16	-	-	-	25	-
	12.5	10	-	-	-	18	-
	6.25	0	-	-	-	13	-
	3.125	-	-	-	-	0	-
2q	50	17	16	0	0	24	0
	25	14	13	-	-	20	-
	12.5	0	13	-	-	14	-
	6.25	-	12	-	-	0	-
	3.125	-	0	-	-	-	-
2r	50	0	14	0	0	14	0
	25	-	12	-	-	10	-
	12.5	-	11	-	-	0	-
	6.25	-	8	-	-	-	-
	3.125	-	0	-	-	-	-
2s	50	0	14	0	0	25	0
	25	-	12	-	-	17	-
	12.5	-	11	-	-	10	-
	6.25	-	10	-	-	0	-
	3.125	-	0	-	-	-	-
2t	50	16	16	0	0	28	0
	25	12	13	-	-	20	-
	12.5	10	12	-	-	18	-
	6.25	0	12	-	-	14	-
	3.125	-	12	-	-	10	-
	1.56	-	10	-	-	0	-
	0.78	-	8	-	-	-	-
	0.39	-	0	-	-	-	-
2u	50	0	0	0	0	25	0
	25	-		-	-	20	-
	12.5	-	-	-	-	15	-
	6.25	-	-	-	-	10	-
	3.125	-	-	-	-	0	-
2v	50	0	14	0	0	14	0
	25	-	13	-	-	10	-
	12.5	-	12	-	-	0	-
	6.25	-	12	-	-	-	-

Table VI: Continued.

No.	Conc. µmol/mL	*Candida albicans*	*Geotrichum candidum*	*Fusarium oxysporum*	*Aspergillus flavus*	*Trichophyton rubrum*	*Scopulariopsis brevicaulis*
	3.125	-	11	-	-	-	-
	1.56	-	11	-	-	-	-
	0.78	-	0	-	-	-	-
2w	50	0	0	0	0	23	0
	25	-	-	-	-	12	-
	12.5	-	-	-	-	0	-
2x	50	0	16	0	0	16	0
	25	-	14	-	-	0	-
	12.5	-	13	-	-	-	-
	6.25	-	12	-	-	-	-
	3.125	-	0	-	-	-	-
CLO	10	30	22	22	27	34	24
	5	30	22	22	27	34	23
	2.5	26	22	22	25	34	23
	1.25	26	22	18	25	34	20
	0.6	26	22	18	25	34	20
	0.3	26	22	18	25	34	20

Table VII: Antifungal activity [inhibition zone (mm) and MICs (µmol) given in brackets] of compounds **(2a-x)** and Clotrimazole.

No.	*Candida albicans*	*Geotrichum candidum*	*Fusarium oxysporum*	*Aspergillus flavus*	*Trichophyton rubrum*	*Scopulariopsis brevicaulis*
2a	-	11(25)	6(25)	-	12(25)	-
2b	22(100)	10(6.25)	8(12.5)	-	14(6.25)	-
2c	12(12.5)	12(6.25)	-	-	17(12.5)	-
2d	10(12.5)	8(1.56)	-	-	10(25)	-
2e	16(100)	11(12.5)	-	-	11(25)	-
2f	14(25)	10(12.5)	10(50)	12(50)	10(25)	13(25)
2g	-	10(3.125)	8(100)	-	10(6.25)	-
2h	8(6.25)	9(3.125)	-	-	14(6.25)	-
2i	13(12.5)	12(6.25)	-	-	13(6.25)	-
2j	10(12.5)	8(6.25)	-	-	10(6.25)	-
2k	12(12.5)	12(6.25)	-	-	8(6.25)	-
2l	-	10(12.5)	-	-	13(25)	-
2m	12(12.5)	10(3.125)	-	-	10(6.25)	-
2n	12(6.25)	12(6.25)	-	-	18(3.125)	-
2o	12(100)	12(12.5)	-	-	14(100)	-
2p	10(12.5)	-	-	-	13(6.25)	-
2q	14(25)	12(6.25)	8(100)	-	14(12.5)	-
2r	-	8(6.25)	8(100)	-	10(25)	-
2s	-	10(6.25)	-	-	10(6.25)	-
2t	10(12.5)	8(0.78)	-	-	10(12.5)	-
2u	-	14(100)	-	-	10(6.25)	-
2v	-	11(1.56)	-	-	10(25)	-
2w	-	-	-	-	12(25)	-
2x	-	12(6.25)	-	-	16(50)	-
CLO	26(0.3)	22(0.3)	8(0.3)	25(0.3)	34(0.3)	20(0.3)

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