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Synthesis, Characterization and Antimicrobial Activity of N-(3-Phthalidyl)amines

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Abstract: In this research a new series of 3-(alkylanilino) phthalides are synthesized by reacting *o*-phthalaldehydic acid with substituted aniline in a single step and good yields. The reaction occurred selectivity at carbon no.3 of the cyclic form of the acid through the displacement of water molecules by the amine through nucleophilic substitution reaction(S_N^2). None of Phthalimidine product of type (**C**) and the Schiff bases of type (**B**) has been formed. All these compounds were characterized by means of their ^{1}H-NMR, ^{13}C-NMR, FT-IR and mass spectrometry. The synthesized products were evaluated for their antimicrobial activity. The compounds were tested for their antibacterial and antifungal activities by the ditch-plate technique.

Keywords: syntheses, phthalides, *o*-phthalaldehydic acid, Antimicrobial Activity.

Introduction

The chemistry of phthalides (Isobenzofuranones) are an important class of synthetic and naturally occurring products exhibiting diverse biological and pharmacological properties. In particular, 3-substituted phthalide moieties are embodied in numerous natural products. For examples, Fuscinarin[1] isolated from soil fungas was found to compete effectively with macrophage inflammatory protein (MIP)-1α for binding to human CCR5, an important anti HIV-1 target that interferes with HIV entry into cells; ;3-butylphthalides[2] isolated from the basidiomycete phanerochaete velutina CL6387 appear to be specific for Helicobacter pylori and Cytosporone[3] E has antifungal activities; (-)-Alcyopterosin[4] E which contains phthalides bone shows mild cytotoxicity toward Hep-2(human larynx carcinoma) cell line; Typhaphthalide[5] isolated from Typha capensis. Furthermore, it is well known that are more than 180 naturally occurring phthalides appear in the literature. Most of these (~137) are extracted from 202 diverse species of plants; as a result, phthalide-containing plants were long used as herbal medicines.[6] Most naturally occurring phthalides are obtained from two plant species, Ligusticum and Angelica, in the Umbelliferae family some of the isoquinoline type phthalides such as noscapine and bicuculine are isolated from the poppy family. From the genus Ligusticum, more than 53 naturally occurring phthalides have been isolated from Ligusticum, and 38 biologically phthalides have been isolated from Angelica.[7, 8]. On the other hand, N-(3-Phthalidyl) amines can possess some biological

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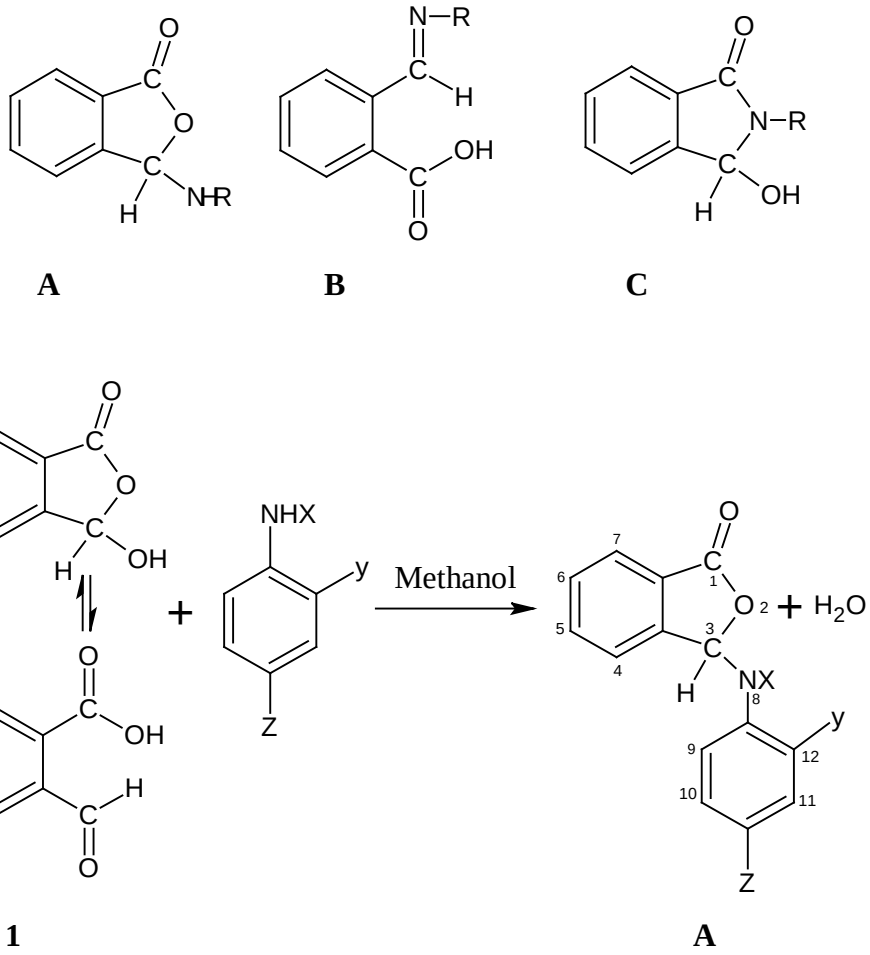
activity; 3-(N-dimethylamino) phthalide[9] can be used as a toxicant in fungicidal, germicidal and herbicidal composition; 3(2,4,5-trichloroanilino) phthalide[10] can be used also as herbicides as well as for the growth and germination of seeds. The reaction of *o*-phthalaldehydic acid with primary and secondary amines have been explained[11-13]. The reaction mechanism **(Scheme-2)** occurs through bimolecular nucleophilic substitution reaction affording N-(3-phthalidyl) amines type (**A).** Other authors[14-17] shows that some secondary aromatic and aliphatic amines afforded a Schiff bases of type **(B),** resulted from the reaction of the amine with formyl group of the acid, and the phthalimidine compounds of type (**C**) resulted from the nucleophilic attack of the amine at the carbonyl group of the cyclic form of *o*-phthalaldehydic acid **(Scheme-1)**.

Results and Discussion

The potentional biological activities of phthalides and substituted alkyl anilines motivated us to synthesis of an interesting type of heterocyclic compounds via condensation of 2-formylbenzoic acid with substituted alkyl anilines was conducted in methanol, thus with; 2-methylaniline, 2-iso propyl aniline, 4-isopropyl aniline and N-benzoyl-aniline, a solid product was obtained in each case **(Table-2)**. The IR (KBr) spectra of each the isolated products, from these reactions (**Fig. 2, 5, 9,** and **11**) and **(Table-4)**, show a characteristic peak at 1730, 1755, 1741 and 1773 cm^{-1} respectively. Indicating the presence of lactonic group and the absence of OH group and appearance of NH group in the region 3342-3380 cm^{-1}. while the ^{1}H-NMR spectra in (d_6-DMSO) of these products(**Fig. 1, 4, 7,** and **10**) and **(Table-4)** show a characteristic peak as one proton a doublet at δ 6.46, 6.57, 6.89 and 7.09 ppm respectively (**fig. 5-8**), assigned for H-3 in products of type (A), and one proton broad singlet or doublet with J= 6.4 - 9.5 Hz at δ 6.83, 7.06 and 7.12 ppm respectively assigned for NH, for the rest of spectra see table (5). Moreover, ^{13}C-NMR spectra in (d_6-DMSO) of compounds [A1-A3] shows number of resolved carbon signals, the carbonyl carbon signal was observed at δ 169, 170.00 and 169.89 respectively and the aniline carbon (C-NH) signal was observed at δ 145.77, 147.16 and 146.66 respectively, (**Fig. 3, 6,** and **8**), and the rest of the resolved carbon signals are corresponding to the aromatic carbon atoms of the product. Mass spectrum of the Compound [A2] as example clearly show a peak at 237.8 (M-30) due to loss of two $CH_3^{\cdot}$ and a peak at 239.1 (M-28) due to loss of $CO^{\cdot}$ and a peak at 223 (M-44) due to loss of $CO_2^{\cdot}$, and a peak at 134.1 due to phthalidyl cation (M-133) for the rest of spectrum (**Fig. 12**). These data ruled out the possibilities of either structure (**B**) and (**C**) and confirmed the structure to be type (A).Therefor the structure of product will be the corresponding N-(3-phthalidyl) amino derivatives [A1], [A2] and [A3] respectively. After that we thought of reaction o-phthalaldehydic acid with secondary aromatic amines namely N-benzoyl aniline as weak base and more steric effect afforded a solid product **(Table-2)** whose IR (KBr) spectrum (**Fig. 11**) shows two strong absorptions at 1653 cm^{-1} due to benzoyl group and at 1773 cm^{-1} due to lactonic group. Its ^{1}H-NMR spectra in (d_6-DMSO) (**Fig. 10**) show (H-3) at δ 7.10 ppm the highly deshielding (0.53 ppm) compared with those in compound [A1], [A2] and [A3], can be attributed to the presence of second phenyl group which through space cause a strong anistropic effect leading to lower chemical shift of this protons these data confirmed that the structure of the product will be N-(3- phthalidyl) amine[A4].

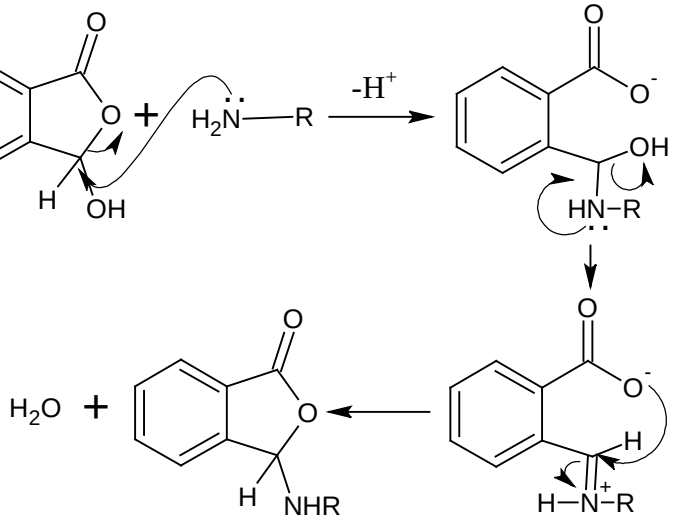
Scheme-1



N-(2-methylanilino) phthalide **(A1):** (X = H, Y= CH_3, Z= H)
N-(2-isopropylanilino) phthalide **(A2):** (X = H, Y= CH $(CH_3)_2$, Z=CH)
N-(4-isopropylanilino) phthalide **(A3):** (X = H, Y= H, Z= CH $(CH_3)_2$)
N-(benzoyl anilino) phthalide **(A4):** (X = Benzoyl, X=Y= H)

Scheme-2

The possible reaction mechanism involved in the formation of N-(3-Phthalidyl) amines (A1-A4) from the 3-hydroxyphthalide



Experimental

All melting points were measured on electrotherrmal melting point and were uncorrected, infrared (IR) spectra were measured using a Shimadzu DR-8001 spectrophotometer as a potassium bromide disc and FTIR. The ^{1}H-NMR and ^{13}C-NMR spectra were recorded on a Bruker at 400 MHz and 100 MHz using TMS as the internal standard in DMSO-d^6. The chemical shifts values (δ) were recorded in ppm spectrometer. The Mass Spectrum was recorded on VG 7070 E.G.C mass spectrometer. The purity of the compounds was checked by TLC-using Silica gel-G (Merck). Thin layer chromatography was performed using silica gel 60 F254 pre coated aluminium sheets, the progress of the reactions was monitored on silica gel plates using iodine vapours as visualizing agent or by UV light. Reagents and solvents used were purchased from Aldrich Chemicals.

Reaction of *o*-phthalaldehydic acid with Aniline derivatives.

To a solution of *o*-phthalaldehydic acid (**1**) (1.5g, 0.01mole) in methanol (20ml) was added alkyl aniline (0.01mole). The mixture was refluxed for about (3-6 hrs.), then cooled. The product which separated out on concentration was collected by filtration, dried and recrystallized from methanol.

Antimicrobial activity

Materials and Methods

The antibiotics used as positive controls in the disk diffusion assay include: nalidixic acid (30 µg), miconazole (10 µg), and DMSO (10 % v/v) as the constituting solvent and negative control. Test bacteria include Gram-positive (*Staphylococcus aureus* ATCC 11632) and Gram-negative bacteria (*Escherichia coli* ATCC 10536). And tested fungal include (Candida *albicans* P225).

The antimicrobial activities of synthesized compound**s** N-(3-phthalidyl) Amines **A1-A4** Was screened *in vitro* for their antibacterial activity against *Escherichia coli* (gram negative) and *Staphylococcus aureus* (gram positive), also tested for antifungal activity against *Candida albicans* by the ditch-plate technique[18]. Using concentrations of 5 mg/ml for each compound. The microorganisms were grown over night in Nutrient broth (NB) at 37 ^{o}C and a compared to 0.5 Mackferland solution before being used. Mueller-Hinton Agar (MHA) was employed as culture media and Dimethyl sulfoxide (DMSO), was used as solvent.As positive controls nalidixic acid as antibacterial drug and miconazole as antifungal drug were used.

Biological Results

The results of antimicrobial activity showed clearly that all tested compound exhibited antifungal activity against yeast-like fungi, the antibacterial activity of the compound showed a good activity against four used strains (**Table-1**).

According to the results, we founded that compound A1 have no inhibition power on E. coli, but it has more powerful inhibition on C. albicans and S. aureus. Compounds A2, A**3** and **A4** have inhibition effect on the three strains. We can deduce that N-(3-phthalidyl) amines (conc.5mg/ml) have more powerful inhibition effect on *S. aureus* compared to *E. coli* and *C. albicans*.

Table - 1

Antibacterial and Antifungal activities of N-(3-phthalidyl) Amines (conc.5mg/ml) for compounds A1-A4

(Zone on inhibition in mm)

Phthalides	***E. coli***	***S. aureus***	***C. albicans***
N-(4-methylanilino) phthalide **(A1)**	-	**48**	**28**
N-(2-isopropylanilino) phthalide **(A2)**	**24**	46	**34**
N-(4-isopropylanilino) phthalide **(A3)**	**26**	48	**30**
3-{[2-(propan-2-yl)phenyl]amino}-2-benzofuran-1(3*H*)-one **(A4)**	**24**	**42**	**26**

Table-2

Analytical data of Phthalides (A1-A4)

Phthalide	**Molecular Formula**	**Color**	**Melting Point (°C)**	**Yield (%)**	**Solvent for Crystallization**
N-(4-methylanilino) phthalide Phthalide**(A1)**	$C_{15}H_{13}NO_2$	White	162-163	92	Methanol
N-(2-isopropylanilino) phthalide **(A2)**	$C_{17}H_{17}NO_2$	White	152-153	61	Methanol
N-(4-isopropylanilino) phthalide **(A3)**	$C_{17}H_{17}NO_2$	White	142-143	62	Methanol
3-{[2-(propan-2-yl)phenyl]amino}-2-benzofuran-1(3*H*)-one **(A4)**	$C_{21}H_{15}NO_3$	White	163-164	50	Methanol

Table-3

Spectral data (^{1}H -NMR and ^{13}C-NMR) of Phthalides (A1-A4)

Phthalide	^{1}H-NMR (400MHz) d_6-DMSO δ (ppm)	^{13}C-NMR (100MHz) d_6-DMSO
N-(2-methylanilino) phthalide **(A1)**	2.17(s, 3H, CH_3, H-13), 6.46-6.49 (d, 1H, CH, H-3, J=10.3 H_Z), 6.81-6.83 (d, 1H, NH, H-8, J=6.4 H_Z), 7.06 - 7.89 (m, 8H, Ar-H).	17.78 (C-aliphatic,CH_3), 87.89 (C-3), 113.55-142.06(C-aromatic), 145.77(C=C- N) 169.77(C-1, C=O)
N-(2-isopropylanilino) phthalide **(A2)**	1.17-1.19(d, 6H, $2CH_3$), 3.19-3.29 (m,1H, CH), 6.57-6.59 (d,1H, H-3, J=8.9 H_Z), 7.04-7.06 (d, 1H, NH, J=8.9 H_Z), 6.91-8.15 (m,8H, Ar-H)	23.52 (C-aliphatic,$2CH_3$), 26.62 (CH, tertiary carbon), 90.07 (C-3), 115.22-142.49(aromatic carbons), 147.16 (C=C-N, 170.00(C-1,C=O).
N-(4-isopropylanilino) phthalide **(A3)**	1.8-1.20(d, 6H, $2CH_3$), 2.79-2.85 (m, 1H, CH), 6.89-6.90 (d, 1H, H-3, J=6.8 H_Z), 7.10-7.12(d, 1H, NH, J=6.2 H_Z), 7.68-7.89 (m, 8H, Ar-H).	24.94 (CH, tertiary carbon), 33.41(C-aliphatic,$2CH_3$), 89.28 (C-3), 115.12 143.67(aromatic carbons), 146.66 (=C-N) and 169.89(C-1,C=O).
3-{[2-(propan-2-yl)phenyl]amino}-2-benzofuran-1(3*H*)-one **(A4)**	7.09-7.11(d,1H, H-3, J=7.3 H_Z) 7.11-7.99 (m, 14H, Ar-H).	

Table-4

Spectral data (IR) of Phthalides (A1-A4)

Phthalide	IR (KBr) ν cm^{-1}
N-(4-methylanilino) phthalide **(A1)**	1730(C=O), 2850-2920 (CH- aliphatic), 3000-3050 (CH-aromatic), 3380(NH).
N-(2-isopropylanilino) phthalide **(A2)**	1755(C=O), 2960-2980 (CH-aliphatic), 3000-3025 (CH-aromatic), 3411 (NH).
N-(4-isopropylanilino) phthalide **(A3)**	1741(C=O), 2960-2970 (CH-aliphatic), 3020-3060 (CH-aromatic), 3342 (NH).
3-{[2-(propan-2-yl)phenyl]amino}-2-benzofuran-1(3*H*)-one **(A4)**	1653($C=O_{benzoyl}$), 1773 (C=O), 2960-2970(CH-aliphatic), 3020-3060(CH-aromatic).

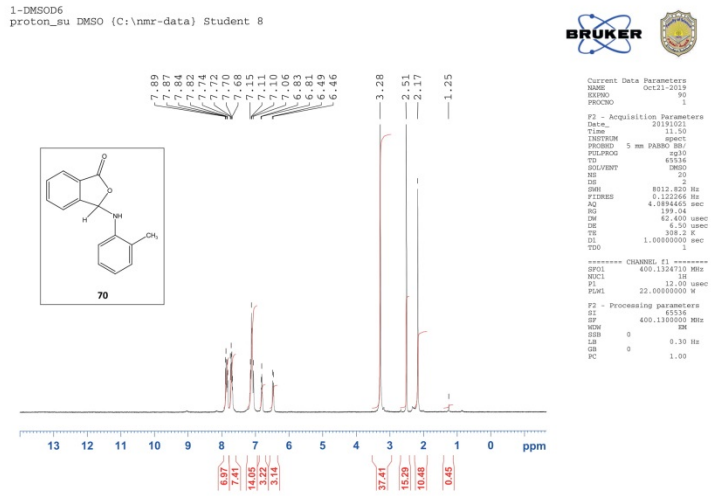


Fig.1. The ^{1}H-NMR spectrum of N-(2-methylanilino) phthalide(A1) in d^{6}-DMSO

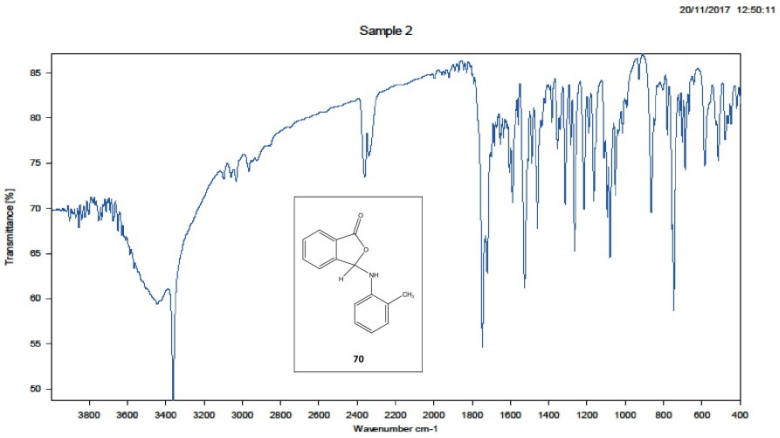


Fig.2. The I.R Spectrum of N-(2-methylanilino) phthalide(A1).

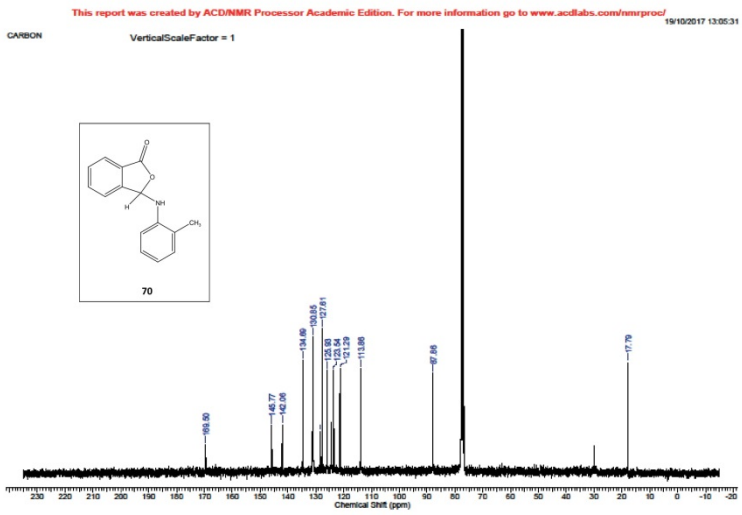


Fig.3. The ^{13}C-NMR spectrum of N-(2-methylanilino) phthalide(A1) in d^{6}-DMSO.

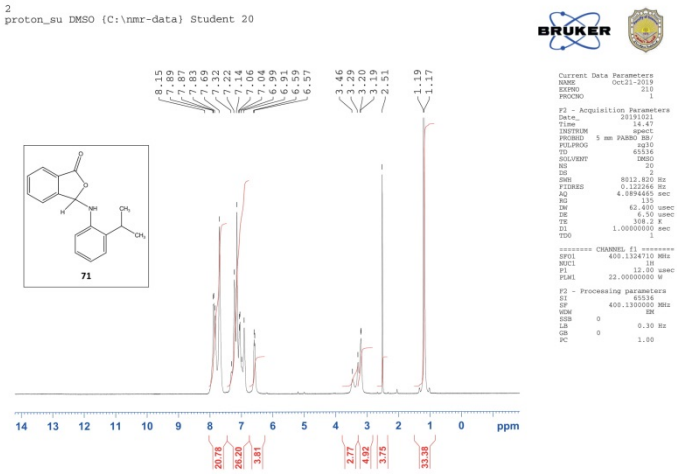


Fig.4. The ^{1}H-NMR spectrum of N-(2-isopropylanilino) phthalide(A2) in d^{6}-DMSO

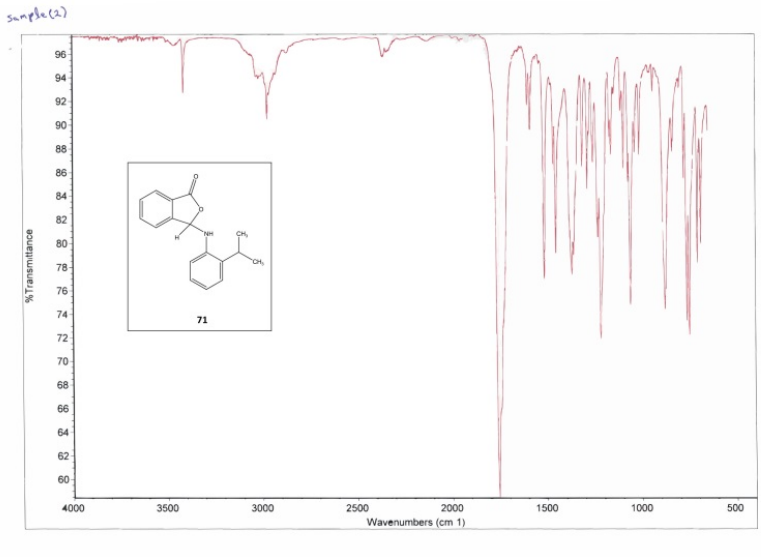


Fig.5. The I.R Spectrum of N-(2-isopropylanilino) phthalide(A2).

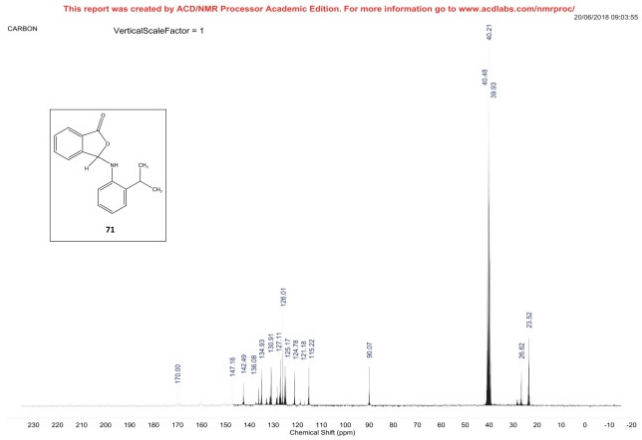


Fig.6. The ^{13}C-NMR spectrum of N-(2-isoproylanilino) phthalide(A2) in d^{6}-DMSO

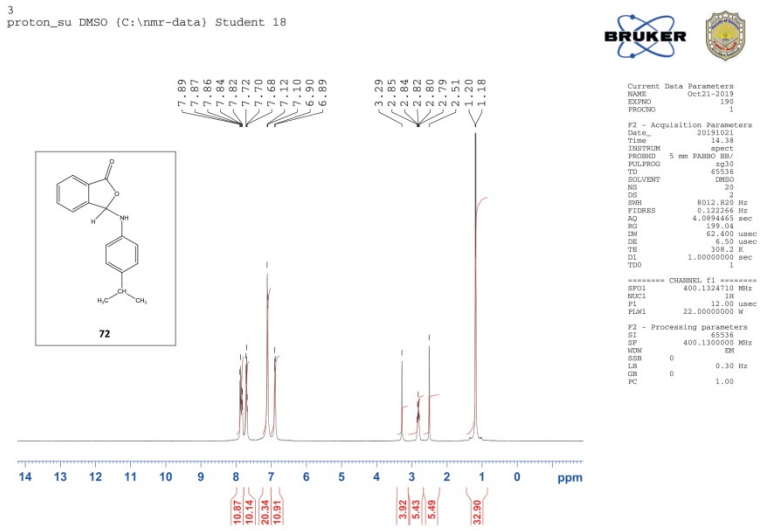


Fig.7. The ^{1}H-NMR spectrum of N-(4-isopropylanilino) phthalide(A3) in d^{6}-DMSO

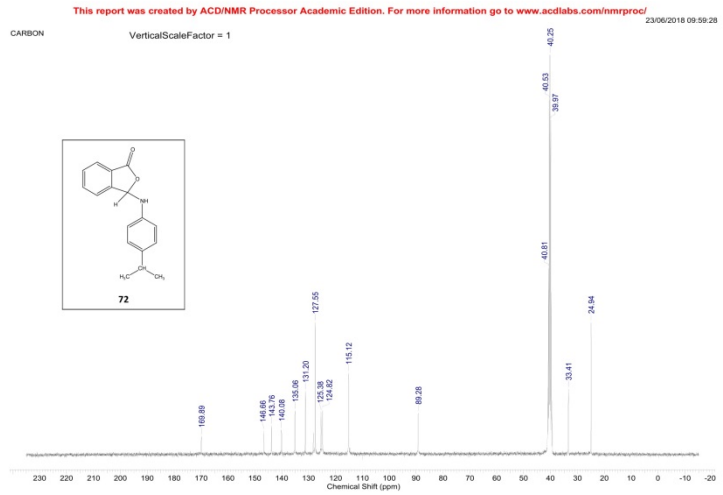


Fig.8. The ^{13}C-NMR spectrum of N-(4-isopropylanilino) phthalide(A3) in d^{6}-DMSO

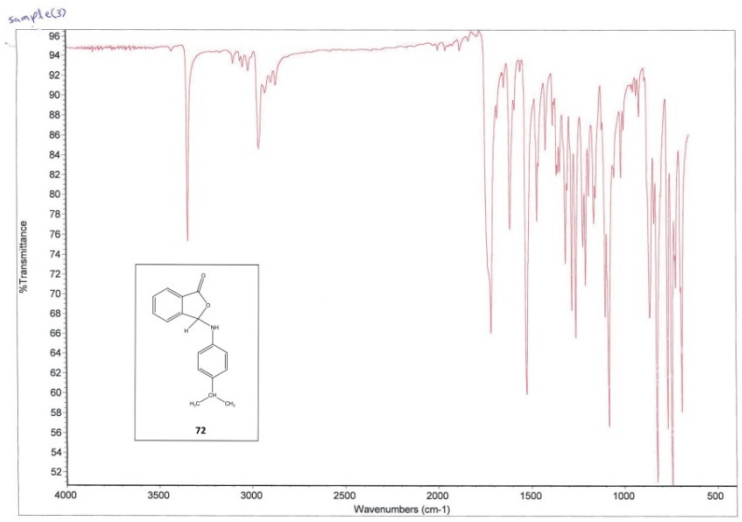


Fig.9. The I.R Spectrum of N-(4-isopropylanilino) phthalide(A3)

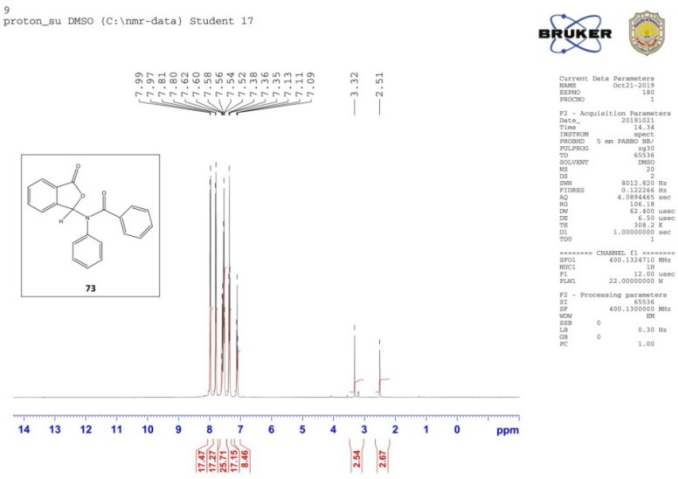


Fig.10. The ^{13}C-NMR spectrum of 3-{[2-(propan-2-yl)phenyl]amino}-2-benzofuran-1(3*H*)-one (A4) in d^{6}-DMSO

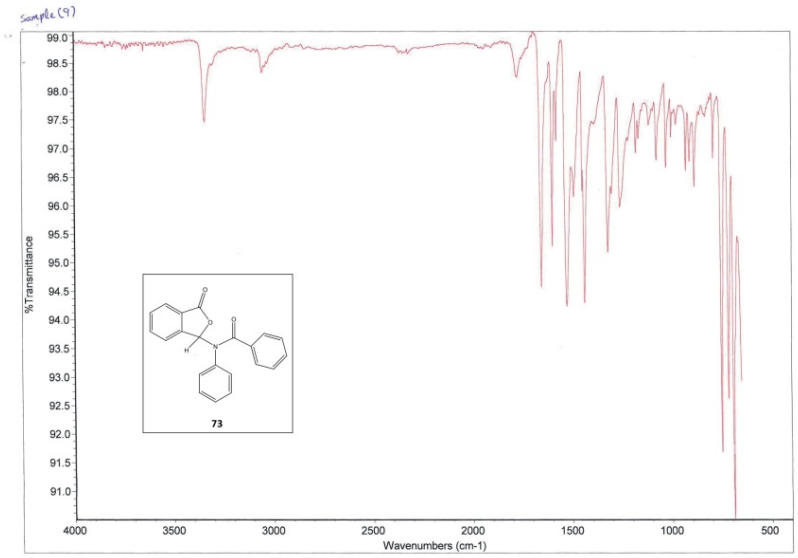


Fig.11. The I.R Spectrum of 3-{[2-(propan-2-yl)phenyl]amino}-2-benzofuran-1(3*H*)-one(A4).

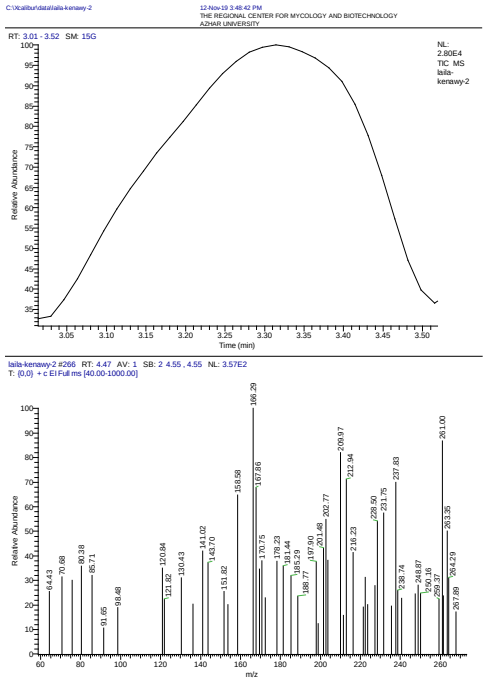


Fig.12. The Mass Spectrum of N-(2-isopropylanilino) phthalide(A2).

Acknowledgements

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Refrences

[1]. K. Yoganathan, C. Rossant, S. Ng, Y. Huang, M. S. Butler and A. D. Buss, Journal of natural products, (2003), 66, 1116-1117.

[2]. K. A. Dekker, T. Inagaki, T. D. Gootz, K. Kanede, E. Nomura, T. Sakakibara, S. Sakemi, Y. Sugie, Y. Yamau-chi, N. Yoshikawa and N. Kojima, "CJ-12,954 and Its Congeners, New Anti-Helicobacter Pylori Compounds Produced by Phanerochaete Velutina: Fermentation, Iso- lation, Structural Elucidation and Biological Activities," Journal of Antibiotics, Vol. 50, 1997, pp. 833-839. doi:10.7164/antibiotics.50.833

[3]. X. W. Wang, 3-n-Butylphthalide. Cerebral Antiis- chemic, Drugs of the Future, Vol. 25, No. 1, 2000, pp. 16-29. doi:10.1358/dof.2000.025.01.562281

[4]. B. Witulski, A. Zimmermann and N. D. Gowans, "First total Synthesis of the Marine Illudalane Sesquiterpenoid Alcyopterosin E," Chemical Communications, No. 24, 2002, pp. 2984-2985. doi:10.1039/b209573d

[5]. F. O. Shode, A. S. Mahomed and C. B. Rogers, Phytochemistry, (2002), 61, 955-957.

[6]. G. Lin, S. S.-K. Chan, H.-S. Chung and S.-L. Li, Studies in natural products chemistry, 2005, 32, 611-669.

[7]. J. Taunton, J. L. Wood and S. L. Schreiber, Journal of the American Chemical Society, 1993, 115, 10378-10379.

[8]. J. J. Beck and S.-C. Chou, Journal of natural products, 2007, 70, 891-900.

[9]. D. D. Wheeler, D.C.Yong; Pat, U. S, No 2, 997, 481, (1958). C A. 56, 1400, (1961).

[10]. D. D. Wheeler, D.C.Yong; Pat, U. S, No 2, 945, 865, (1960). C A. 55, 257, (1961).

[11]. Abdulhakem Betrow, et al. Z. kristallogr. NCS 229 (2014)/ DOl:10.1515/ ncrs-2014-0204.

[12]. A. R. Betrow, Kh. M. Hamza and R. M. Tarhoun, Zawia University Bulletin , 31-38, ISSUE No.15 Vol.3 (2013).

[13]. A. R. Betrow, Kh. M. Hamza and R. M. Tarhoun, Zawia University Bulletin , 31-38, ISSUE No.13 Vol.3 (2011).

[14]. K. Sempuk, Pat: Jpn. 58, 41, 865, 1981, C.A. 99. 122300n, 1983.

[15]. Nakgawa. A, Pat: Jpn. 61, 41, 271, C.A. 106. 138252X, 1987.

[16]. Y. Kubota, T. Tatsuno; Chem.Pharm. Bull; 19(6), 1226,1971.

[17]. Mario. Ghelordoni; Ann. Chim.(Rome). 64(5), 421, 1974.

[18]. C.H. Collins and P.M. Lyne, Microbiological Methods, Butterworths, London, edn. 3,p. 424, (1970).