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RESEARCH ARTICLE

Synthesis of some new benzimidazol-2-yl-(1,1,4,1'') terphenyl-2,4-dicarbonitrile and related heterocyclic systems of expected biological activities

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Abstract

The synthesized benzimidazoles compounds were prepared from the condensation reaction between o-phenylene diamine and carbonyl compound in 4N HCl to form (1),through nucleophilic substitution and decarboxylation to give (2) which is reacted with malononitrile and morpholine in ethanol to give (3). Their cyclization with formamide, sodium nitrite and triethylorthoformate afforded a series of polycyclic heterocycles containing condensed quinazoline, triazine rings and formimidic acid ethylester derivatives (4-6). Also condensation of compound (3) with acetic anhydride, phthalic anhydride and anisaldehyde to give condensed quinazoline, isoindolo and benzylidene rings (7-9). The compound (9) reacted with mercaptoacetic acid in $zncl_2$ to form thiazolidin ring (10) which reacted with 2-chlorobenzaldehyde to give compound (11), also compound (3) fused with ethylcyanoacetate to form compound (12). **(Scheme1)**

Chloroderivatives (5) reacted with nucleophilic reagents such as hydrazine hydrate to give the hydrazino derivative (13) which, in turn, proved to be a useful intermediate.

In fact the triazolo derivatives (14) and (15) were produced from the reaction of (13) with formic acid and carbon disulfide respectively in addition, treatment of (13) in acetic acid and an aqueous solution of sodium nitrite at room temperature, gave the azido derivative (16). **(Scheme2)**

INTRODUCTION

Benzimidazole derivatives are of wide interest because of their diverse biological activity and clinical application **(1)**. This ring system is present in numerous anti-parasitic, fungicidal, anti-inflamatory drugs(2) ,anti-hypertensive, anti-viral, anti-tumour, anti-helminthic , anti-microbial,anti-oxidant, anti-ulcer, anti-amoebic, anti-histaminic activity, anti-bacterial, antipsychotic, antiprotozoal, antineoplastic, analgesic, anti dopaminergic, anti-hepatitis B virus, anticonvulsant, CNS depressant, anti-parkinson.

In addition, benzimidazoles are very important precursors in organic synthesis as (vitamin B12). Constituents a milestone in the chemistry of benzimidazoles and antidiabetic activity **(3-26)**.

Synthesis of benzimidazolyl chalcones which have been used as intermediate for the synthesis of bioactive heterocyclic compounds vis, quinazoline,triazine,ethyloxy-methylene and azido derivatives, which have high biological activity as antimicrobial and anticancer activity[27,28,29].

Result and discussion:

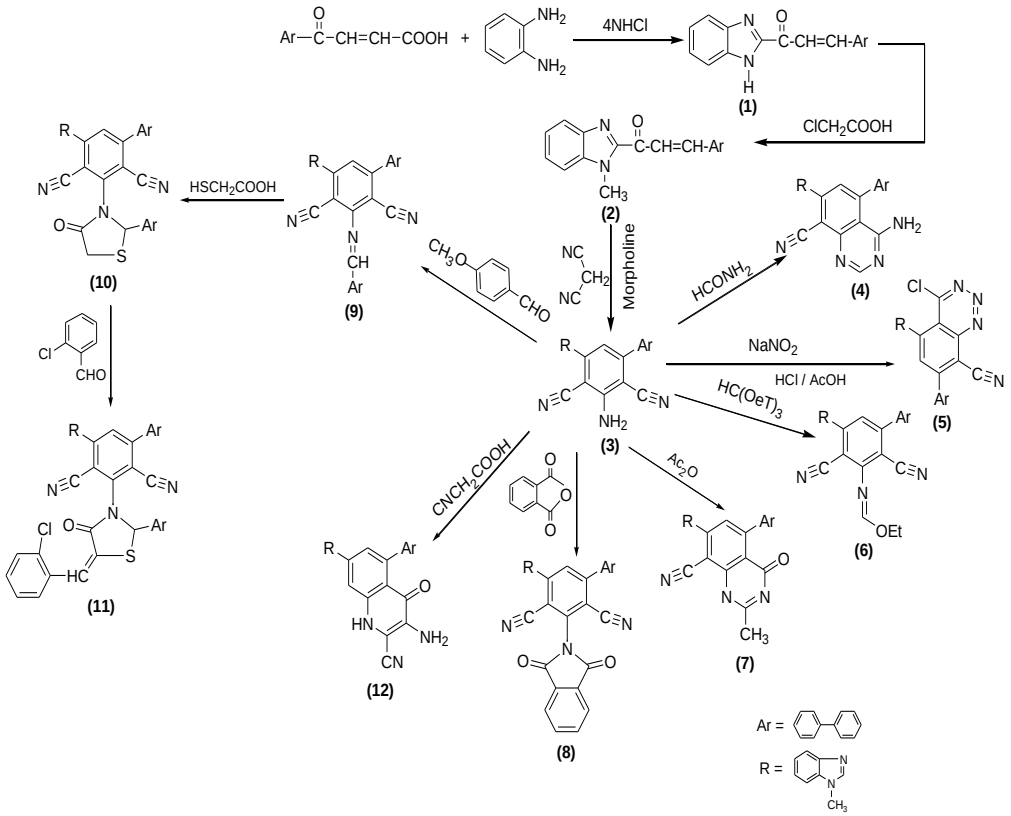
The new derivatives were prepared according to the reaction sequences depicted in scheme (1,2). Reaction of equimolar amounts of o-phenylene diamine with 4-Biphenyl-4-yl-4-oxo-but-2-enoic acid in 4N HCl afforded (1) , through alkylation and decarboxylation to give (2) which on treatment with malononitrile in presence of catalytic amount of morpholine afforded desired product (3).

When treatment with formamide gives quinazoline derivative (4),traizine derivative formed when diazodization of compound (3) with sodium nitrite in a mixture of hydrochloric acid and acetic acid formed (5), formimidic acid ethyl ester derivative formed when compound (3) reacted with triethylorthoformate gives (6),also gives quinazoline derivative , when fused with acetic anhydride results (7) , on the other hand formed isoindolo derivative when reacted with phthalic anhydride in glacial acetic acid result (8),finally, benzylidene derivative formed when compound (3) condensed with anisaldehyde in methanol with (4-5) drops of glacial acetic acid gives (9).In turn ,the compound (9) when reacted with mercaptoacetic acid with a pinch of anhydrous zncl$_2$ in methanol result thiazolidin derivative (10) which reacted with 2-chlorobenzadehyde in ethanol in the presence of sodium hydroxide result thiazolidin derivative (11) , also compound (3) reacted with ethylcyanoacetate afforded (12). **(scheme 1)**

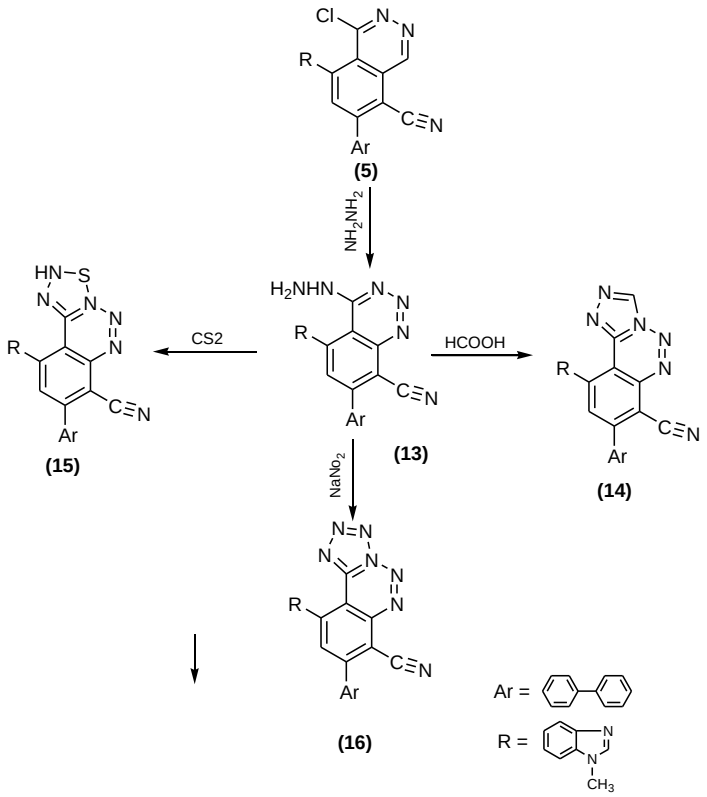
The chlorine atom reactivity of (5) was highlighted by its easy displacement with nucleophilic reagents such as hydrazine hydrate to give the hydrazine derivative (13) which, in turn, proved to be a useful intermediate.

In fact, the triazine derivatives (14 and 15) were produced from the reaction of (13) with formic acid and carbon disulfide, receptively.

In addition, treatment of (13) in glacial acetic acid and an aqueous solution of sodium nitrite at room temperature, gave the azido derivative (16). **(Scheme 2)**.



Scheme (1)



Scheme (2)

Experimental:

Melting points were determined in open capillary tubes on an electrothermal 9100 digital melting point apparatus (Buchi, stritzerland) Elmer 2400 analyzer (USA).

IR spectra were recorded on a Perkin-Elmer 160 FTIR (VSA) as KBr. The ^{1}H-NMR and ^{13}C-NMR spectra were measured on Brüker DRX 500 and 125 MHZ at Max-Plank Institute, Germany in DMSO-d_6.

The elemental analysis were carried out at microanalytical, faculty of science, Cairo university by using Perkin-Elmer 2400 C,H,N elemental analyzer.

Synthesis of 1-(1H-benzimidazol-2-yl)-3-biphenyl-4-yl propenone (1)

To a solution of o-phenylene diamine (0.01 mol), 4-Biphenyl-4-yl-4-oxo-but-2-enoic acid (0.01 mol) in 4N HCl (100 ml). the reaction mixture was refluxed for 6 h. , then the reaction mixture was cooled and basified with conc. Ammonia solution. The precipitate so obtained was filtered, dried and recrystallized with benzene , yield: 75% , M.P190°C, FT-IR (KBr, υ,cm^{-1}) 1604 (C=O),3431(NH),3030 (Ar-CH) , ^{1}H-NMR (DMSO-d_6) 1.3 (S,H,NH),2.2 (dd,2H,CH=CH),7.5-6.9 (m,14H,Ar-H),^{13}C-NMR, 129.47-127 (Ar-C),40.59-40.17 (CH), Anal. Calcd. for $C_{22}H_{16}N_2O$: C,81.46 ; H,4.97 ; N,8.64 Found: C,81.1; H,4.8 ; N,8.3 %

Synthesis of 3-biphenyl-4-yl-1-(1-methyl-1H-benzimidazol-2-yl)-propenone (2)

To a solution of (1) in methanol (20 ml) was treated with methanolic sodium hydroxide solution (25 ml; 20%) and then chloroacetic acid (0.01 mol) was added dropwise with continuous shaking .the reaction mixture was refluxed for 2h. treated with dilute HCl and the solid obtained was recrystallized from benzene to give (2) yield: 70% , M..P. 170 °C, FT-IR (KBr, υ,cm^{-1}) 1621(C=O),1601 (C=N),1314 (CH_3),^{1}H-NMR (DMSO-d_6), 3.3

(s,3H,CH_3),2.2 (dd,2H,CH=CH),7.8-6.8 (m,14H,Ar-H), Anal Calcd for $C_{23}H_{18}N_2O$: C, 81.36 ; H,5.36 ;N,8.28 ; O,4.73, Found: C, 81.33; H,5.11 ;N,8.18.16 %..

Synthesis of 3-Amino-5-(1-methyl-1H-bezimidazol-2-yl)-[1,1';4',1''] terphenyl-2,4-dicarbonitrile (3)

A mixture of chalcone (2) (0.01mol), malononitrile (0.02 mol) and heterocyclic secondary amine (morpholine) (0.01 mol) dissolved in ethanol (15 ml) was refluxed for (9-10) h. in around bottom flask on completion of reaction. The reaction mixture was concentrated .the solid obtained was filtered, washed with methanol and recrystallized from benzene. Yield: 65%, M.P. 140 °C,

FT-IR (KBr, υ,cm^{-1}) 3342 (NH_2),1657 (C=O),2207 (C=N),1515 (C=C),1302 (CH_3), 3197 (Ar-CH),^{1}H-NMR (DMSO-d_6) 3.8 (S,3H,CH_3),4.1(S,H,CH),7.8-7.1 (m,14H,Ar-H),8.8 (S,2H,NH_2). ^{13}C-NMR, 165.23-114.71 (Ar-C), 26.31(CH_3), 66.48-66.242 (2 CN), 63.75 (C=N) 49.25 (CH). Anal. Calcd. for C_{28} H_{19} $N_{5:}$ C, 79.04; H, 4.50; N, 16.46,Found: C, 49.1; H, 4.4; N, 16.13 %.

Synthesis of 4-Amino-5-biphenyl-4-yl-7-(1-methyl-1H benzimidazol-2-yl)-quinazoline-8-carbonitrile (4)

A mixture of (3) (0.01 mol) and formamide (15 ml) was refluxed for 2h., the solid product thus formed after cooling was filtered off and recrystallized from benzene , yield: 75%, M.P. 215 °C , FT-IR (KBr, υ,cm^{-1}) 2198 (CN) , 1628 (C=N), 1405 (CH_3),3152 (NH_2)., ^{1}H- NMR(DMSO-d_6) ,1.2 (S,3H,CH_3),3.5 (S,2H,NH_2),7.7-6.9 (m,4H,Ar-H). Anal. Calcd. C_{29} H_{20} N_6: C, 76.97; H, 4.45; N, 18.57 Found: C,76.18; H,4.44; N, 8.55 %

Synthesis of 7-Biphenyl-4-yl-4-chloro-5-(1-methyl-1H-benzimidazol-2-yl)-benzo[d][1,2,3]triazine-8-carbonitrile (5)

To an ice-cold solution of (3) (0.01mol) in a mixture of acetic acid (20 ml) and hydrochloric acid (10ml) and sodium nitrite (0.01mol in 10 ml H_2o) was added with Stirring for 30 min.) and the stirring was continued for 3h., The product was collected and recrystallized from benzene. yield: 60 % MP 200 °C , FT-IR (KBr, υ,cm^{-1}) 2213 (CN),2609 (C=N),1789 (C-Cl), ^{1}H- NMR (DMSO-d_6) 3.4 (S,3H,CH_3),8.1-6.8 (m,15H,CH,Ar-H), Anal. Calcd.: C_{28} H_{17} N_6CL : C,71.11; H,3.62 ; Cl,7.50 N,17.77 , Found :C,71;H,3.15;CL,7.04; N,17.1 %.

Synthesis of N-[2,4-Dicyano-5-(1-methyl-1H-benzoimid-azol-2-yl)-)-[1,1';4',1'']terphenyl-3-yl]-formimidic acid ethyl ester (6)

A mixture of (3) and triethylorthoformate (3ml)in acetic acid (20ml) was refluxed for 6h. the reaction mixture was cooled ,diluted with cold water and the solid product was collected and recrystallized from pet. ether (60-80)%. Yield 55% ,M.P 160°C , FT-IR (KBr, υ,cm^{-1}) ,2192 (CN),1373 (CH_3),1605 (C=N),1252 (C-O), ^{1}H-NMR (DMSO-d_6), 7.8-6.8 (m,14H,Ar-H),3.3 (S,3H,CH_3),2.1(q,2H,CH_2),2.3 (T,3H,CH_3), 8.1(S,2H,2CH). ^{13}C-NMR 129.55-127.36 (Ar-C), 39.50 (CH_3), 40.60 (CH), Anal. Calcd. for $C_{21}H_{23}N_5O$: C, 77.32; H, 4.81; N, 14.54 Found: C, 77.11; H 4.21; N, 14.31%.

Synthesis of 5-Biphenyl-4-yl-2-methyl-7-(1-methyl-1H-benzimidazol-2-yl)-4-oxo-1,4-dihydro-quinazoline-8-carbonitrile (7):

Treatment of compound (3) (0.01mol) with acetic anhydride ,the solution was refluxed for 2h.. The crude product was separated, filtered and recrystallized from pet. ether (60-80)% ,yield 70%, MP 185°C , FT-IR (KBr, υ,cm^{-1}), 1506 (C=N), 2195 (CN), 3431 (NH), 1618 (C=O),1414 (CH_3),^{1}H-NMR (DMSO-d_6), 7.8-6.9 (m,14H,Ar-H),1.2 (S,3H,CH_3) ,3.2 (S,3H,CH_3),3.6 (S,H,NH), 3.7 (S,H,CH) Anal. Calcd. For: $C_{30}H_{21}N_5O$, C, 77.1; H, 4.22; N, 14.60; ,Found: C, 77.1 ; H, 4.22;N,14.60 %.

Synthesis of 3-(1,3-Dioxo-1,3-dihydro-isoindolo-2-yl)-5-(1-methyl-1H-benzimidazol-2-yl)-[1,1';4',1'']terphenyl-2,4-dicarbonitrile (8)

A mixture of (3) and phthalic anhydride was dissolved in glacial acetic acid. The solution was refluxed for 6h. the crude product was separated, filtered and recrystallized from ethanol, yield 65% ,M.P 170°C, FT-IR (KBr, υ,cm^{-1}),1372 (CH_3),1672 (C=O), 1606 (C=N), 2195 (CN), ^{1}H-NMR (DMSO-d_6) 8.1-6.8 (m,19H,CH,Ar-H),3.4 (S,3H,CH_3), Anal. Calcd for: $C_{36}H_{21}N_5O_2$ C,77.83;H,3.81;N,12.61;Found: C,77.71; H, 3.22; N,12.11%.

Synthesis of 3-[(4-Methoxy-benzylidene)-amino]-5-(1-methyl-1H-benzimidazol-2-yl)-[1,1';4',1'']terphenyl-2,4-dicarbonitrile (9)

To a solution of compound (3) (0.01 mol) with in methanol (20 ml) with (4-5) drops of glacial acetic acid was refluxed for 6h. resulted (9) and recrystallized from benzene ,yield 70 %, M.P 160 °C , FT-IR (KBr, υ,cm^{-1}),1173 (OCH_3),1308 (CH_3),1626 (C=N), 2205 (CN), ^{1}H NMR (DMSO-d_6) 8.1-7.1(m,18H,Ar-H),3.3 (S,3H,CH_3),3.8 (s,3H, OCH_3),7.9 (S,1H,CH=N),4.1(S,H,CH),^{13}C-NMR,166.45-101.53 (Ar-C),26.31(CH_3), 3.30 (CH_3),40.58 (CH),63,74 (CN) Anal. Calcd.: $C_{36}H_{25}N_5O$, C, 79.54 ; H,4.64 ;N, 12.88 Found : C,79.51 ;H,4.52, N,12.11 %.

Synthesis of 3-[2-(4-Methoxy-phenyl)-4-oxo-thiazolidin-3-yl]-5-(1-methyl-1H-benzimidazol-2-yl)-[1,1';4',1'']terphenyl-2,4-dicarbonitrile (10)

When the equimolar solution of compound (9) (0.01 mol) and mercaptoacetic acid (0.01 mol) with a pinch of anhydrous $Zncl_2$ in methanol (30 ml) was refluxed for 6h. to give compound (10) and recrystallized from benzene, yield: 65%, M..P. 165 °C , FT-IR (KBr, υ,cm^{-1}) 1606 (C=O), 1398 (CH_3),1117(OCH_3) ,2207 (CN) ,^{1}H-NMR (DMSO-d_6), 8.1- 6.8 (m,19H,CH,Ar-H), 3.3 (S,3H,CH_3),3.5 (S,3H, OCH_3),2.8 (S,2H,CH_2),8.2(S,H,CH) Anal Calcd, $C_{38}H_{27}N_5O_2S$. C,73.89 ;H,4.41 ; N,11.34 Found : C,73.81 ; H,4.30 ; N, 11.30 %

Synthesis of 3-[5-(2-chloro-benzylidene)-2-(4-methoxy-phenyl)-4-oxo-thiazolidin-3-yl]-5-(1-methyl-1H-benzimidazol-2-yl-[1,1;4,1] terphenyl-2,4-dicarbonitrile (11)

The equimolar solution of compound (10) (0.01 mol) and 2-chloro benzaldehyde (0.01 mol) in methanol (10 ml) in the presence of sodium ethoxide with stirring for 3h. resulted compound (11) and recrystallized from benzene ,yield 65% M.P. 250 °C, FT-IR (KBr, υ,cm^{-1}), 1114 (OCH_3),2205 (CN),1619 (C=O),1398 (CH_3)^{1}H-NMR (DMSO-d_6)8.3-6.7 (m,25H,3H,Ar-H),3.2 (S,3H,CH_3),3.6 (S,3H,OCH_3). Anal. Calcd.: $C_{45}H_{30}ClN_5O_2S$ C, 73.01 ; H,4.08 , N,9,46 Found : C,73 ;H,4 ,N,9.42 %

Synthesis of 3-Amino-5-biphenyl-4-yl-7-(1-methyl-1H-benzimidazol-2-yl)-4-oxo-1,4-dihydro-quinoline-2-carbonitrile :(12)

A mixture of **(3)** (0.01 mol) and ethyl cyanoacetate (0.01 mol) was fused at 180–200°C for 2 h. The reaction mixture was allowed to cool and the solid product was collected and recrystallized from Benzene Yield 55% ,M.P 190 °C , FT-IR (KBr, υ,cm^{-1}), 1657(C=O) , 2105 (CN), ^{1}H-NMR (DMSO-d_6), 8.1-6.5 (m, 15H, CH, Ar-H), 3.3-3.2 (S, 3H, NH, NH_2). Anal. Calcd. for $C_{30}H_{21}N_5O$, C, 77.07; H, 4.53 ; N14.98 Found :C,77.01 ; H,44.50 ; N , 14.78 % .

Synthesis of 5-Biphenyl-4-yl-4-hydrazino-7-(1-methyl-1H-benzimidazol-4-yl)-benzo[d][1,2,3]triazine-8-carbonitrile (13):

A mixture of (5) (0.01mol) and hydrazine hydrate 98% (0,01 mol) in ethanol (30 ml) was heated under reflux for 6h. the product obtained after cooling was filtered off ,washed with water and recrystallized from benzene yield 65% ,M.P 170 °C , FT-IR (KBr, υ,cm^{-1}) 3431-3031(NH/NH_2),1365(CH_3),1603(C=N),^{1}H-NMR(DMSO-d_6),1.7(s,H, NH),1.4(s,3H,CH_3),3.9(S,2H,NH_2),8.1(S,H,CH),^{13}C-NMR,139.99-114.54 (Ar-C),26.29 (CH_3),40.50 (CH) Anal Calcd for $C_{28}H_{20}N_8$, C,71.78 ;H,4.30 ;N,23.92 Found : C, 71.70 ; H, 4.30 ; N,23.8 %

Synthesis of phenyl -4-yl-3-methyl-5-(1-methyl -1H-benzimidazole-2-yl)-4-(methylene-azono)-3,4-dihydro-benzo[d][1,2,3]triazine-8-carbonitrile (14)

A mixture of (13) (0.01 mol) in formic acid (20 ml) was heated under reflux for 6h. the reaction mixture was concentrated and the solid product was collected and recrystallized from benzene to give compound (14) yield: 70%, M.P. 180 °C FT-IR (KBr, υ,cm^{-1})1315 (CH_3),1627 (C=N),2036 (CN),1508 (C=C),^{1} H-NMR (DMSO-d_6),8.1-7.1(m,5H,CH,Ar-H),3.1(S,3H,CH_3) Anal Calcd for: $C_{29}H_{18}N_8$, C 72.8; H,3.76;N,23.43. Found: C,72.3 , H,3.66; N,23.43%.

Synthesis of 7-Biphenyl -4-yl-9-(1-methyl-1H-benzimidazol-2-yl)-2H-3-thia-1,2,3a,4,5-Pentaaza-cyclopentana(a-naphthaline-6-carbonitrle (15)

A mixture of (13) (0.01 mol) carbon disulfide (5 ml) in ethanol (50 ml) and two pellets of potassium hydroxide was heated under reflux on a water bath for 5h. The solid product obtained acidified with acetic acid ,recrystallized from pet. ether (60-80)%, yield 70 %,M.P 145 °C

FT-IR (KBr, υ, cm^{-1}) 2627 (C=N), 1361(NH), 1311(CH_3), 2036 (CN),

^{1}H-NMR(DMSO-d_6),9.1(s,H,NH),3.2(s,3H,CH_3),7.5-6.8(m,15H, CH,Ar-H) Anal Calcd for: $C_{28}H_{18}N_8S$, C, 67.4 ;H,3.61 ;N,22.48 Found:C,67.1 ; H ,3.51 , N.22.4 %.

Synthesis of 3-Amino-7-biphenyl-4-yl-5(1-methyl-1H-benzimidazol-2-yl)-4(methylene-hydrazino-3,4-dihydro-benzo[d] [1,2,3] triazine-8-carbonitrile (16)

To a well stirred solution of (13) (0.01 mol) in glacial acetic acid (50 ml) ,a solution of sodium nitrite (1 gm in 10 ml of water) was added at r.t and stirring was continued for 1h. the solid obtained was filtered off ,washed with water and recrystallized from benzene to give compound (16) yield 65%, M.P. 165 °C. FT-IR (KBr, υ, cm^{-1})1635 (C=N), 2125 (CN), 1330 (CH_3), 1527 (C=C) ^{1}H-NMR DMSO-d_6), 8.1-7.3 (m, 14 H, Ar-H), 8.4 (S, H, CH), 3.2 (S, 3H,CH_3) Anal Calcd $C_{28}H_{17}N_9$, C,70.14;H,3.54;N22.54. Found: C,70 ; H, 3.44 ; N, 22.50 %.

Biological activity

I- Antimicrobial activity

All the synthesized compounds were screened for their antibacterial and antifungal activity in vitro by disc diffusion method [31] using nutrient agar medium against following microorganisms, *Staphylococcus aureus* [ATCC 29213] *Bacillus Subtilis (Gram positive bacteria), Escherichia coli* [ATCC 27853] ,*Pseudomonas aeuroginosa* [ATCC 25922] *(Gram negative bacteria)* and fungal species like Candida albicans, Aspergillus flavin organisms.

Ampicillin was used as a standard drug for antibacterial screening and used as a Amphotericin B was used as a standard for antifungal screening. DMSO was diluents which not effected the growth of microbes (negative control). The tested compounds were dissolved in *Dimethylsulfoxide (DMSO)*. The sterile nutrient agar medium for antibacterial study was melted and inoculated with 16-18 hours old broth culture at 1% level. The aseptic conditions used for the inoculation. After the inoculated medium was transferred to sterile Petri dishes it was evenly distributed and allowed for solidification, the cups (8 mm diameter) were made by punching into the agar surface into each of these cups 50 L of the test compound to get a solution of 1% concentration. Filter paper discs (Whatman, 5 mm diameter) were saturated with this former solution for bacterial test. The saturated filter paper discs were placed on the surface of solidified Czapek's Dox agar dishes seeded by the test fungi. The inhibition zones were measured in mm at the end of an incubation period of 48 h at 28°C. The screening results have been tabulated in table 1.and represented in figure (1,2). zone of inhibition (mm) of test compounds .

Thus , the above data revealed that all compounds showed good to moderate antimicrobial activity as compared to standard drug (ampicillin as antibacterial drug and Amphotericin B as antifungal drug), so , the result shows that:-

-All compounds showed good activity against *B.subtilis* except (3,11,14) compounds showed good activity against *S.aureus* except (6,14).

- All compounds showed good activity against *E.coli* except (2,6,9, 10,11,12,16).
- Compounds showed good activity against *P.aeruginosa* except (2, 9, 11,12,16)
- All compounds showed good activity against *A.flavin* except (2,4,7,10,14,16)
- All compounds showed good activity against *C.albicans* finally, all these compounds exhibited significant activity specially against Gram positive bacteria and candida albicans (fungal).

Table 1- Antimicrobial study of synthesized compounds(1-16):

Comp. No.	Antibacterial activity				Antifungal Activity	
	Gram positive		Gram negative			
	B.subtilis	*S.aureus*	*E.coli*	*P.aeruginosa*	*A. flavin*	*C.albicans*
1	12	9	13	9	12	13
2	11	8	-	-	-	--
3	--	10	16	10	10	12
4	14	6	11	7	-	11
5	10	8	18	13	9	-
6	13	-	-	10	13	15
7	12	11	10	14	-	-
8	15	9	18	10	8	10
9	13	8	-	-	12	13
10	11	8	-	13	-	-
11	-	7	-	-	14	17
12	16	9	-	-	15	17
13	12	10	17	12	12	-
14	-	-	16	15	-	16
15	17	12	19	14	13	16
16	16	13	-	-	11	-
Ampicillin (antibacterial drug)	20	18	22	17	-	-
Amphotericin B (antifungal drug)	-	-	-	-	17	19

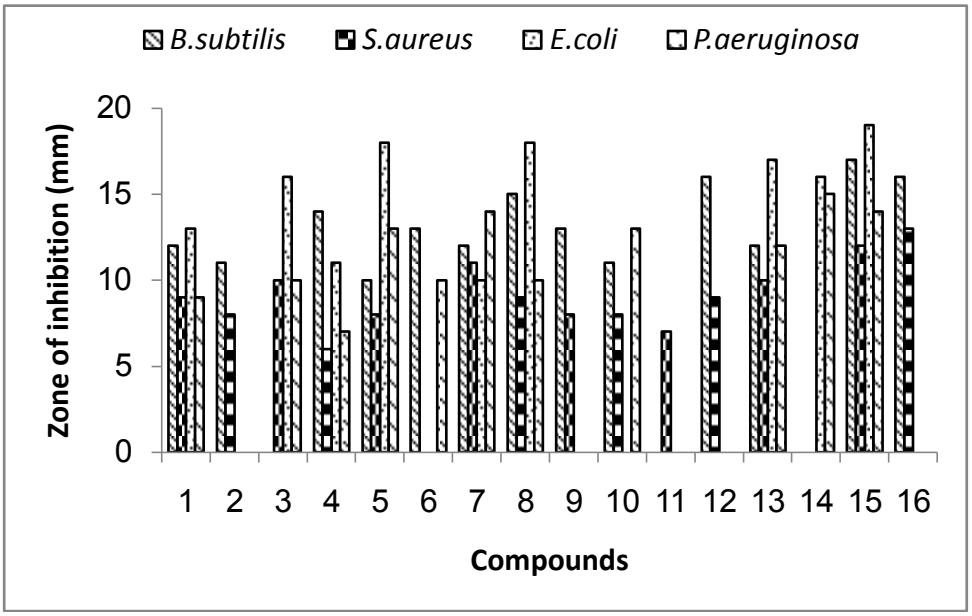


Fig. (1)

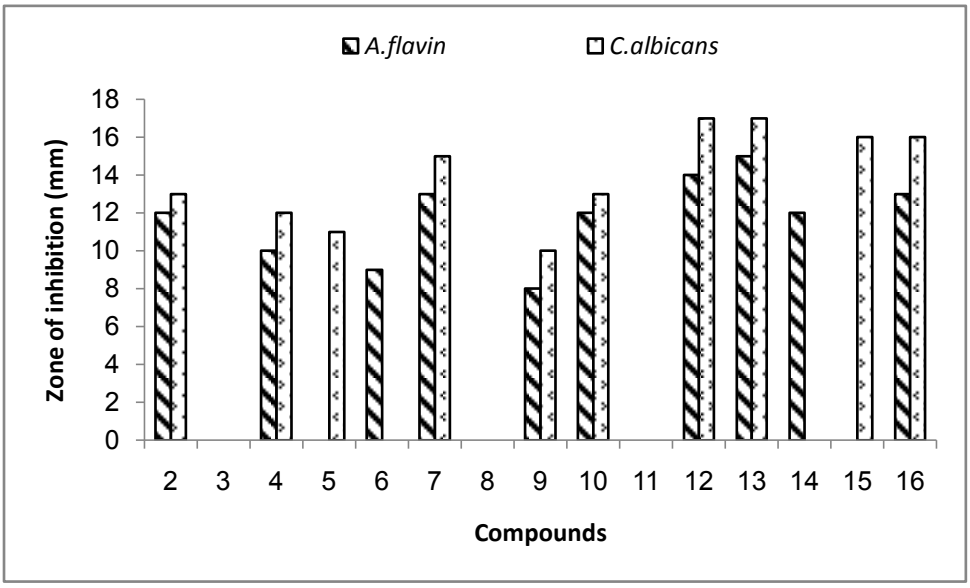


Fig. (2)

Antiproliferative activity:-

Cytotoxicity assay

All the newly synthesized compounds were tested for antiproliferative activity in vitro against different types of cancer cell lines as (HEPG2) human liver carcinoma cell line, (MCF-7) human breast cancer cell line and (HCT-116) human colon cancer cell line at different concentrations (100,50,25,12.5,6,25.0)ug/ml, and the Doxorubicin , one of the most effective anticancer agents ,was used as a reference drug . these cell lines were grown in RPMI -1640 medium supplemented with 2mg/ml sodium bicarbonate,4.5 mg/ml glucose, 100 ug/ml streptomycin sulphate ,40ug/ml gentamycin,100 U/ml pencillin, as well as 10% (vol/vol) foetal bovine serum (FBS). All cell lines incubated at 37 C in humidified air containing 5 % CO_2. Cytotoxicity was determined by MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) is a water soluble tetrazolium salt, which is converted to an insoluble purple formazan by cleavage of the tetrazolium ring by succinate dehydrogenase within the mitochondria. The formazan product is impermeable to the cell membranes and therefore it accumulates in healthy cells. (Mossmann, 1983) [(32)].

Breifly, the Plate cells (10^4 –10^6 cells) in 200 µl PBS in 96-well (flat bottom). Add 20 ul of MTT solution, mix well, incubate for 4h in 37C in dark, Remove aliquot for analysis; add 200 l acidic isopropanol and mix well and incubate additional 1h in 37C in dark. Then read plate in ELISA Reader – measure OD in 570 nm (background wavelength is 570 nm). IC_{50}% (µg/ml) i.e (50% inhibition concentration) was calculated for the tested compounds by using (sigmaplot software). The results of the IC50% of the compounds was tabulated (table 2) which represented in figure (3)

Antiproliferative activity of the newly synthezid compounds against human carcinoma cell lines

Table 2. IC_{50}% (µg/ml) of newly synthesid compounds on human liver carcinoma cell line (HEPG2), human breast cancer cell line (MCF-7) and human colon cancer cell line (HCT-116).

Inhibition concentration (50%) (IC_{50}) µg/mL			
Compound	**HEPG-II**	**MCF-7**	**HCT-116**
1	24.4	53.2	37.7
2	2.3	49.7	24.9
3	28.7	26. 6	67.3
4	12.03	12.9	119.9
5	43.7	162.5	32.8
6	3.30	108.7	170.7
7	80. 9	40.2	31.6
8	29.8	21.5	37.4
9	38.3	6.9	79.3
10	25.1	125.5	111.5
11	8.4	85.5	106.3
12	17.4	61.4	98.5
13	9.8	40.0	18.1
14	2.8	78.03	73.9
15	53.1	60.5	69.7
16	49.7	17.4	26. 6

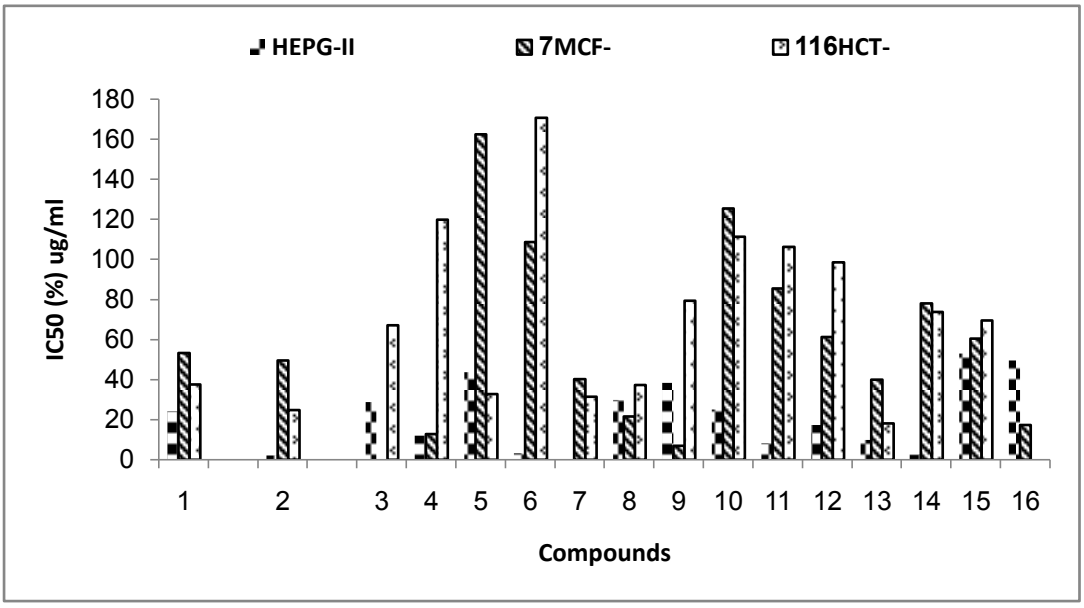


Fig. (3)

Thus, the above data revealed that, the compounds (2,14,6,11,13,4) showed to be the most activity which have IC50% values (2.3,2.7,3.30,8.4,9.8,12.03) µg/ml against HEPG-II cell line, while the compounds (1,10,3,8) showed to be moderate activity which IC50% values (24.4, 25.1, 28.7, 29.8) µg/ml while the other compounds less activity. In case of (MCF-7) cell line , the compounds no. (9,4,16,8) revealed highly activity than other compounds which IC50% values (6.9, ,12.9, 17.4, 21.5) µg/ml

Whereas in case of (HCT-116) cell line the compounds (13,2,16) which their IC50% (18.1, 24.9, 26.6) µg/ml

Found to be the most activity than others.

Overall, the compound (2) revealed the best activity against HEPG-II and HCT-116 cell lines which IC50% (2.3 and 24.9) µg/ml respectively.

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