

REVIEW

Functionalized formazans: A review on recent progress in their pharmacological activities



Ahmad S. Shawali ^{a,*}, Nevien A. Samy ^b

^a Department of Chemistry, Faculty of Science, University of Cairo, Giza, Egypt

^b Department of Dermatology, National Institute of Laser Enhanced Sciences, University of Cairo, Giza, Egypt

GRAPHICAL ABSTRACT

$R-N=N-C(R')=N-NH-R''$ → Pharmaceutical activities

Functionalized Formazans

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ABSTRACT

This review provides an up to date information about the diverse pharmaceutical activities of formazans. The bibliography includes 97 references which have been published during the period from 1980 to 2013. The covered biological activities of the title compounds include antioxidant, anticonvulsant, therapeutic, anthelmintic, anti-tubercular, antiviral, anti-inflammatory, anticancer, anti-HIV, antimicrobial, antiparkinsonian, cardiovascular and antiproliferative activities.

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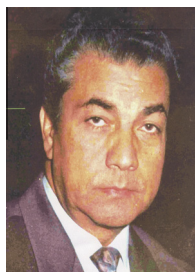
* Corresponding author. Tel.: +20 25084164.

E-mail address: as_shawali@mail.com (A.S. Shawali).

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Ahmad S. Shawali is presently Emeritus Professor of Physical Organic Chemistry, Department of Chemistry, Faculty of Science, University of Cairo, Giza, Egypt. He graduated with B.Sc. from the University of Cairo in 1958. He received his M.Sc. and Ph.D. degrees in 1962 and 1966, respectively, from Lowell Technological Institute, presently the University of Lowell, Massachusetts, USA. He was awarded the degree of Doctor of Science (D.Sc.) from the university of Cairo

after recommendation from British committee from the Royal Chemical Society in 1995. Prof. Shawali has been the recipient of the state award and Egypt State Medal of Science and Arts in 1977. He holds several national and international certificates of merit for his distinguished services. He was appointed Vice-Dean for student affairs in 1989 and he was elected Dean of the Faculty of Science in 1991. He was visiting professor at the university of Texas at el Paso, Texas, USA from 1979 to 1980, University of Kuwait from 1973 to 1977 and King Abdulaziz University, Jeddah, Saudi Arabia from 1982 to 1988. He has published 231 scientific papers and 17 review articles all in international journals. At present there are more than 1950 citations of his work from 1970 till mid 2006 (i.e. about 50 citations/year or 8 citations/paper). He supervised till now 45 graduate M.Sc. and 15 Ph.D. theses. He was invited to present plenary lectures in 23 conferences. His research interests are in the fields of reaction mechanisms, applications of LFERs, Chemistry of hydrazoneic acid derivatives, 1,3-dipolar cycloadditions and 1,5-electrocyclizations.



Nevien A. Samy is presently Associate Professor of Dermatology, Department of Medical Applications of Laser, National Institute of Laser Enhanced Sciences, University of Cairo, Giza, Egypt. She graduated with B.Sc. from the University of Cairo in 1985 from faculty of Medicine. She received her M.Sc. and Ph.D. degrees in 1997 and 2003, respectively, from the University of Cairo. She has published 13 scientific papers and 2 review articles all in international journals.

She supervised till now 3 M.Sc. and 20 Ph.D. theses. She was invited to present plenary lectures in 12 conferences. Her research interests are in the field of Laser applications in Dermatology.

Introduction

Formazans, of the general formula **1** (Chart 1), are an important and distinct class of organic compounds. Their chemistry has attracted the interest of many research groups due to their wide biological and industrial applications as well as their utility in analytical chemistry and synthesis of heterocyclic compounds. At present, there are several review articles and books devoted to the synthesis, physical properties and chemical reactions of formazans [1–16].

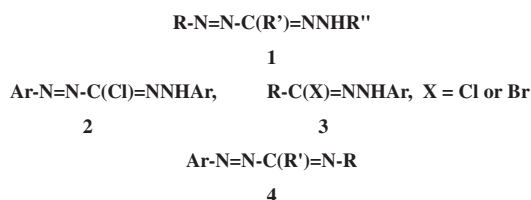
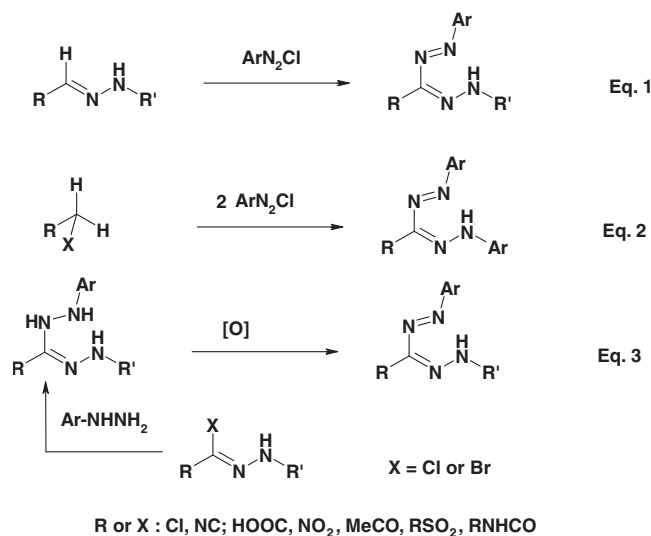


Chart 1



Scheme 1

In contrast to these comprehensive literature reports on the chemistry of formazans, there has been no survey of their biological activities hitherto although they have been found to possess wide spectrum of biological activities such as antiviral, antimicrobial, anti-inflammatory, antifungal, anticancer, anti HIV. Based on these findings and in continuation of our studies of the chemistry of both 3-chloro-1, 5-diarylformazans **2** [17–21] and the related hydrazoneyl halides **3** [22–35] (Chart 1), it was thought necessary to present this review. Our objective is to shed light on the recent developments in biological activities of various functionalized formazans **1** (Chart 1). Only recent reports that have been published during the period from 1980 to 2013 are covered. Compounds of type **4** (Chart 1), which were erroneously named, in some articles [36–40] as formazans, will not be included in this review as they are amidrazone derivatives and not formazans.

At present there are three synthetic strategies for the synthesis of formazans (Scheme 1) [2,3]. The first one involves coupling of aldehyde hydrazones with diazonium salts (Eq. (1)). This strategy has the advantage of being able to synthesize symmetrical and asymmetrical formazans. The second strategy depends on coupling of active methylene compounds each with two molar equivalents of diazonium salts (Eq. (2)). This method proved useful for the synthesis of only symmetrical formazans. The third method involves oxidation of the corresponding hydrazidines (Eq. (3)) are usually prepared *via* reaction of hydrazoneyl halides with the appropriate hydrazine derivatives. Most of the formazans, whose biological activities are covered in this review, were prepared by the first method.

The general structural formulas of the various formazans covered in this review together with their biological activities are indicated in Table 1.

Pharmacological activities

A survey of literature reveals that many formazans have been reported to possess wide spectrum of biological activities. In the following, a complete coverage of the recently reported pharmacological applications of various formazan derivatives

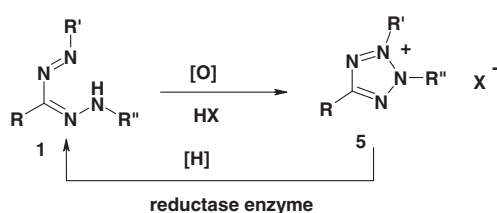
Table 1 The general structural formulas of the various formazans covered in this review together with their biological activities which have been evaluated.

No.	General structure	Their screened biological activities
I	$\text{Ar}-\text{C}(=\text{NNHAr}')-\text{N}=\text{N}-\text{Ar}''$	Anti-oxidant, anticonvulsant, antiviral, anti-inflammatory, Antimicrobial, antiparkinsonian, analgesic
II	$\text{Ar}-\text{C}(=\text{NNHAr}')-\text{N}=\text{N}-\text{Het}$	Anticonvulsant, antiviral, antimicrobial, antiparkinsonian, antiproliferative
III	$\text{Het}-\text{C}(=\text{NNHAr}')-\text{N}=\text{N}-\text{Ar}''$	Antiviral
IV	$\text{Ar}-\text{C}(=\text{NNH}-\text{CO}-\text{Ar}')-\text{N}=\text{N}-\text{Ar}''$	Anthelmintic, anti-inflammatory, anticancer, anti-HIV, antimicrobial, analgesic
V	$\text{Ar}-\text{C}(=\text{NNH}-\text{CO}-\text{Het})-\text{N}=\text{N}-\text{Ar}''$	Anti-oxidant, Antitubercular, Antimicrobial
VI	$\text{Het}-\text{C}(=\text{NNH}-\text{CO}-\text{Ar}')-\text{N}=\text{N}-\text{Ar}''$	Antiviral, anti-inflammatory
VII	$\text{Het}-\text{C}(=\text{NNH}-\text{CO}-\text{Ar}')-\text{N}=\text{N}-\text{Het}'$	Antimicrobial
VIII	$\text{Ar}-\text{C}(=\text{NNH}-\text{R})-\text{N}=\text{N}-\text{Ar}''$	Anti-inflammatory, antimicrobial, antiparkinsonian, cardiovascular
IX	$\text{Ar}-\text{C}(=\text{NN}-\text{Het})-\text{N}=\text{N}-\text{Ar}''$	Antiviral, anti-inflammatory, antimicrobial

is presented. In such a coverage, the formazans, that have been screened for some biological activities and found to be inactive, are also pointed out.

Markers of cell vitality and antioxidant activity

Formazans are colored compounds because of the $\pi-\pi^*$ and $n-\pi^*$ electronic transitions of the azo-hydrazone chromophore. Oxidation of such compounds results in their conversion into colorless tetrazolium salts **5** (Scheme 2) [41]. Among the various oxidants used for the oxidation of formazans **1** to the tetrazolium salts **5** are mercuric oxide, nitric acid, isoamyl nitrite, N-bromo succinimide, potassium permanganate, lead tetraacetate and t-butyl hypochlorite. When a tetrazolium salt is given to a living organism, it is reduced back to the corresponding colored formazan by the reductase enzyme in the living cells and stains tissues depending upon the viability of the organism. Accordingly, tetrazolium-formazan systems are classified as markers of vitality [42,43].



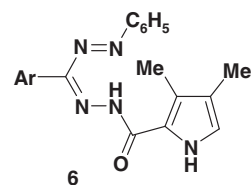
Scheme 2

Formazans are used for screening of anticancer drugs, determination of activity of tumor cell and sperm viability [42–47]. They are also used in Brucella-ring test in milk [48] and to investigate dehydrogenase activity inhibition of a soil bacterium caused by soil contaminated with Cu, Pb and As [49].

At present, the reduction of tetrazolium salts to formazans is widely exploited as indicator of reducing systems with applications in chemistry and biology [36,50]. For example, it is applied to natural products drug discovery for *Leishmania* [51], the assay of lactate dehydrogenase [52] and as indicator of viability in respiring bacteria [53]. Also, they have been used in developing method of yeast cells permeabilization in succinate dehydrogenase activity assay in situ which can be used for different industrial *Saccharomyces cerevisiae* strains [54].

Recently, a rapid method was reported for detection of reduction of 2-hydroxy ketones. This method is based on enzymatic reduction of colorless 2,3,5-triphenyltetrazolium chloride to a red colored 1,3,5-triphenylformazan via ω -transaminases [55]. The enzymatic reductive amination of a wide range of various aliphatic, aliphatic-aromatic and aromatic-aromatic 2-hydroxy ketones was determined spectrophotometrically at λ 510 nm by the decrease in the red coloration due to substrate consumption.

Also, the evaluation of the antioxidant effect of formazans **6a–j** (Chart 2) was recently reported [56]. In that study, the formazans, as antioxidants, each reacts with the stable free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) and converts it to 2,2-diphenyl-1-picrylhydrazine. The degree of decoloration indicates the scavenging potential of the compound. The results showed that compound **6g**, while it shows good activity comparable with the standard compound namely ascorbic acid, the other compounds exhibited moderate to good activities.



6a–d, g–j, Ar = $\text{X}_n\text{C}_6\text{H}_{5-n}$ **6e**, 2-furyl; **6f**, PhCH_2CH_2

X : a, H; b, 4-MeO; c, 2-Cl; d, 4-Me₂N;

g, 4-HO; h, 3,4-(MeO)₂; i, 2-HO;

j, 3,4-CH₂O₂

Chart 2

Anticonvulsant activity

Several formazans were reported to show promising anticonvulsant and therapeutic activities [57,58]. For example, Mariappan et al. [58] reported the results of screening of the formazan derivatives **7a–d** and **8** (Chart 3a) for their anticonvulsant activity by maximum electroshock induced seizures (MES) method using diazepam, as a standard. Compound **8** was the most active among the studied compounds. The other compounds **7a–d** were found to be moderate or less active. None of the studied compounds showed neurotoxicity [58].

corresponding aldehyde N-(2-carboxyphenyl)hydrazone, was reported to exhibit 100% protection against the Ranikhet disease virus [62].

Misra and Dhar [63] synthesized the two formazan derivatives **13** and **14** (Chart 9) by coupling of the corresponding hydrazones each with benzene diazonium salt and screened them for their antiviral activity against vaccinia virus and Ranikhet disease virus in a stationary culture of chorioallantoic membrane of chick embryo. The results revealed that the two compounds were found to exhibit significant activity 87% and 83%, respectively against the Ranikhet disease virus. However no activity against vaccinia virus could be observed [63].

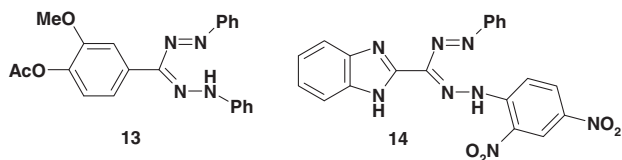
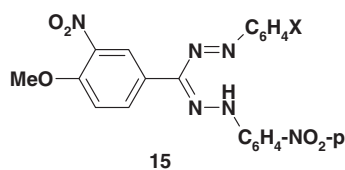


Chart 9

Also, Mukerjee et al. [64] prepared the formazan derivatives **15a–o** (Chart 10) by coupling of 3-nitro-anisaldehyde N-4-nitrophenylhydrazone with the appropriate aryldiazonium salts and evaluated their antiviral activity against Ranikhet disease virus (RDV) in chick embryos (WLH). The results of the study of structure activity relationship (SAR) revealed that structure variations in the aryl moiety have a high effect on their activity. For example, compound **15c** with p-bromo substituent showed 50% protection against RDV as compared with compound **15a** with the unsubstituted ring, whereas 4-nitro derivative **15e** was found to be inactive. Also, introduction of carboxyl group in 2- or 4-position as in formazans **15f** and **15h**, respectively enhances the antiviral activity where the corresponding formazan esters **15i–l** exhibit no marked antiviral activity.

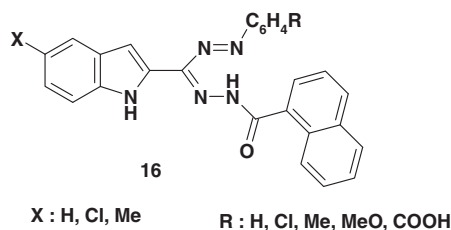


X: a, H; b, 4-Cl; c, 4-Br; d, 4-I; e, 4-O₂N; f, 2-HOOC;
g, 3-HOOC; h, 4-HOOC; i, 4-MeOOC; j, 4-EtOOC;
k, 4-C₃H₇OOC; l, 4-BuOOC; m, 2-MeO; n, 3-MeO;
o, 4-MeO

Chart 10

Tiwari et al. [65] screened the formazan derivatives **16** (Chart 11) for their antiviral activity against Ranikhet disease and vaccinia viruses. The results indicated that some of such formazans showed promising activity against one or both viruses *in vitro* [65].

In addition, Pandey and Negi [66] reported the synthesis of a series of 1-(2'-aryl-4'-oxo(3H)quinazoly)-3-aryl-5-phenyl-formazans **17** (Chart 12) and indicated that they exhibit mild activity against Vaccinia virus.



X : H, Cl, Me

R : H, Cl, Me, MeO, COOH

Chart 11

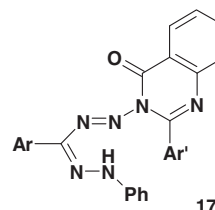


Chart 12

Recently, the formazan derivatives **7** and **8** (Chart 3b) were tested for their antiviral activity against Japanese encephalitis virus [58]. The results showed that none of them was found effective.

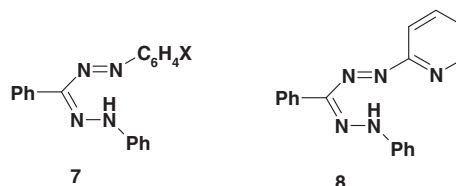
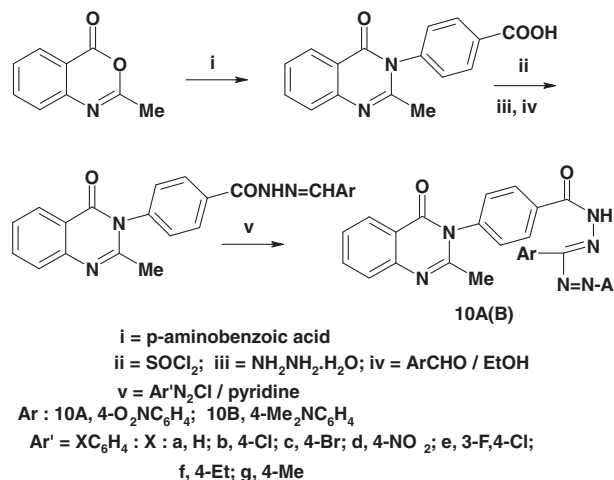
X : a, 4-NO₂; b, 4-Cl; c, 2,3-Cl₂; d, 3-Cl

Chart 3b

Anti-inflammatory activity

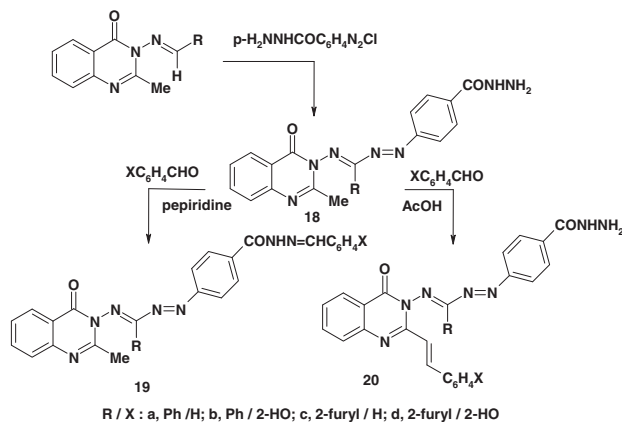
Several formazans have been found to exhibit anti-inflammatory activity [67–69]. For example, in a recent article, Babu and Naderndia [70] reported the synthesis of two series of formazans **10A** and **10B** (Scheme 3) and the results of their



Scheme 3

anti-inflammatory screening. The results revealed that the compounds **10Ad**, **10Ae**, **10Ae** and **10Bf** afforded 67% protection against *carrageenan* induced edema equally with the standard drug *diclofenac* sodium under similar conditions while compounds **10Ab**, **10Ac**, **10Af**, **10Ag**, **10Bd** and **10Bg** exhibited 33% protection.

The substituted quinazolonoformazans **18–20** were also synthesized as depicted in **Scheme 4** and were screened for their anti-inflammatory activity [68]. The results indicated that all compounds except **20a**, possessed anti-inflammatory activity. The degree of protection provided by these compounds (100 mg/kg, po) against *carrageenan*-induced edema in rat paw ranged from 26% to 57%. Such protection was comparable to that of 51% observed with phenylbutazone (100 mg/kg, po) which was used as a standard reference drug. Regarding the structure–activity relationship, the results showed that compounds **19** possessed higher anti-inflammatory activity than that of **20** with the exception of **20d**. The authors [68], in an attempt to gain some idea of their possible analgesic effectiveness, they screened also compounds **19a, b** and **20b, d** for their antiwrithmogenic activity. The results showed that three of such compounds namely **19a, b** and **20b** showed 10–50% protection against aconitine-induced writhing response in mice, whereas compound **20d** exhibited 80% protection similar to that of acetylsalicylic acid.



Scheme 4

Furthermore, Kumar et al. [71] synthesized the formazans **21** (**Chart 13**) and tested them for their anti-inflammatory activity in *albino* rats. The results indicated that they exhibit only moderate activity.

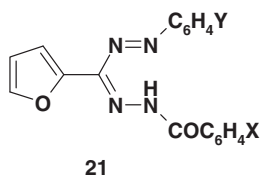
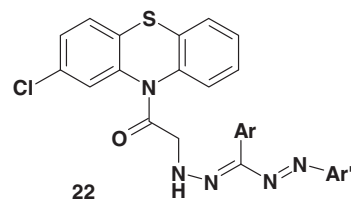


Chart 13

Formazans **22** (**Chart 14**) were synthesized by Kumar et al. [72] and their anti-inflammatory activity was screened against *carrageenan*-induced edema in *albino* rats. On the basis of the results of such screening, the authors concluded that such formazans exhibit promising anti-inflammatory activity.

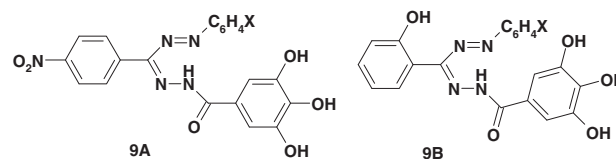


Ar = X_nC₆H_{5-n}; X : 2-Cl; 4-F; 2,6-Cl₂; 2-Me; 4-Me

Ar' = YC₆H₄; Y : 2-Me; 4-Me

Chart 14

Very recently, Kumara Prasad et al. [59] reported the results of screening of compounds **9A** and **9B** (**Chart 4b**) for their anti-inflammatory activity by *carrageenan* induced rat paw edema inhibition method. The reported results indicate that compounds **9Ac** and **9Bc** are the most active among the tested compounds. The other compounds were found less active.



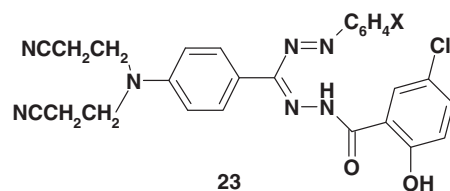
X : a, H; b, 4-COOH; c, 4-OH; d, 4-NO₂

Chart 4b

Anticancer and anti-HIV activities

Some formazans were tested for their anticancer and anti-HIV activities [73–75]. The results showed that most of the tested formazans were inactive.

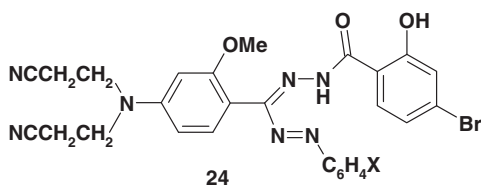
Bhardwaj and Jolly [76] synthesized the formazan derivatives **23** (**Chart 15**) and evaluated them for anticancer and anti-HIV activities. The results revealed that the studied compounds did not show any significant anticancer or anti-HIV activity [76].



X : a, 4-Me; b, 4-Cl; c, 4-MeO; d, 4-NO₂; e, 4-HOOC

Chart 15

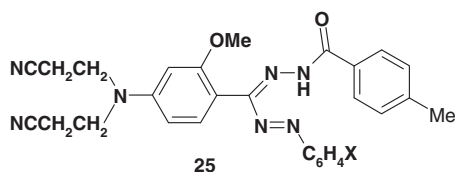
In another report, Bhardwaj [77] prepared also a similar series of formazans **24** (**Chart 16**) and the results of testing them for anticancer activity. The results revealed that none of the compounds was found active at the dose level tested. Also, the compounds did not show significant anti-HIV activity.



X : a, H; b, 2-Me; c, 3-Me; d, 4-Me; e, 2-Cl; f, 3-Cl; 4-Cl;
g, 2-COOH; h, 4-COOH; i, 2-MeO; j, 3-MeO; k, 4-MeO;
l, 2-O₂N; m, 3-O₂N; n, 4-O₂N

Chart 16

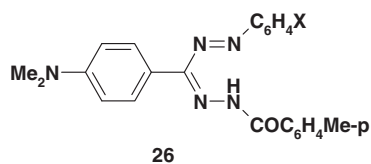
Also, the formazans **25** (Chart 17) were prepared and tested for anticancer and anti-HIV activities [78]. However, none of such compounds was found active at the dose level tested.



X : a, H; b, 2-Me; c, 3-Me; d, 4-Me; e, 2-Cl; f, 3-Cl; 4-Cl;
g, 2-COOH; h, 4-COOH; i, 2-MeO; j, 3-MeO; k, 4-MeO;
l, 2-O₂N; m, 3-O₂N; n, 4-O₂N

Chart 17

Furthermore, the formazans **26a–o** (Chart 18) were prepared and screened for their anti-cancer and anti-HIV activities [79]. The results of such a study revealed that the compounds **26b, d, e, f, i, j, k, l, o** did not show any significant anticancer activity or anti-HIV activity.

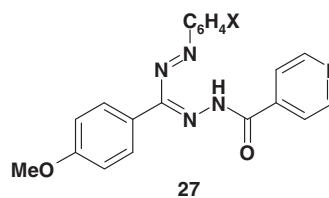


X : a, H; b, 2-Me; c, 3-Me; d, 4-Me; e, 2-Cl; f, 3-Cl
g, 4-Cl; h, 2-MeO; i, 3-MeO; j, 4-MeO; k, 2-NO₂;
l, 3-NO₂; m, 4-NO₂; n, 2-HOOC; o, 3-HOOC

Chart 18

Antimicrobial activities (Antibacterial and antifungal activities)

Several formazan derivatives have shown a broad spectrum of activity against pathogens [49,69,73,80,81]. Raval et al. [82] synthesized the formazans **27a–m** (Chart 19a) and screened them for their antibacterial activity against representative Gram-positive organisms viz. *Bacillus subtilis* (MTCC 121), *Micrococcus luteus* (MTCC 106), *Bacillus sphaericus* (MTCC 11) *Staphylococcus aureus* (MTCC 96) and Gram-negative organisms viz. *Chromobacterium violaceum* (MTCC 2656), *Klebsiella aerogenes* (MTCC 39), *Pseudomonas*

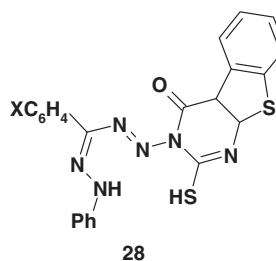


X : a, H; b, 2-MeO; c, 3-MeO; d, 4-MeO; e, 2-NO₂; f, 3-NO₂;
g, 4-NO₂; h, 2-Cl; i, 3-Cl; j, 4-Cl; k, 2-Me; l, 3-Me; m, 4-Me

Chart 19a

aeruginosa (NTCC 791), *Escherichia coli* (MTCC 443), *Klebsiella pneumoniae* (MTCC 109), *Salmonella paratyphi A* (MTCC 735) using standard antibacterial agents like penicillin and streptomycin under identical conditions for comparison [82]. The results showed that the tested compounds **27a, d, h, k** possessed the highest degree of inhibition against all tested Gram +ve organisms, while compounds **27c** and **27h** showed highest degree of inhibition against all tested Gram –ve organisms. Compounds **27c, d, h, k** showed highest degree of inhibition against *Escherichia coli*, *Klebsiella pneumoniae*, *Salmonella paratyphi A*, while compound **27k** showed highest inhibition against *Chromobacterium violaceum*, *Klebsiella aerogenes*, *Pseudomonas aeruginosa*, *Escherichia coli* respectively [82].

Lakshmi et al. [83] prepared the formazans **28a–i** (Chart 20) and evaluated their antimicrobial activity by the cup–plate agar diffusion method. All the synthesized compounds were tested *in vitro* for their antibacterial activity against various microbes such as *Bacillus subtilis* NCIM 2063, *Escherichia coli* NCIM 2118, *Pseudomonas aeruginosa* NCIM 2036 and antifungal activity against *Candida albicans* NCIM 3102. Ciprofloxacin (10 µg/disk) and Fluconazole (2 µg/disk) were used as standard. The results showed that compounds **28a, c, g, h, i** were active against *Bacillus subtilis* and **28b, d, e, h, i** showed significant activity against *Escherichia coli*. Compounds **28a, c, d, e, g, h, i** showed activity against *Pseudomonas aeruginosa*. The compounds **28a, b, c, f, g, h, i** were found active against *Candida albicans*. The other compounds were resistant to the selected microbial strains [83].



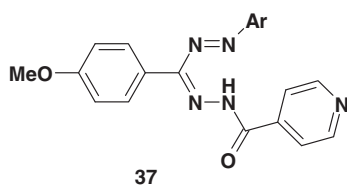
X : a, H; b, 2-Cl; c, 4-NO₂; d, 3,4,5-(MeO)₃; e, 3-NO₂; f, 4-Me²N;
g, 3,4-(MeO)₂; h, 4-MeO; i, 4-Me

Chart 20

In another report Revanasiddappa and Subrahmanyam [84] synthesized the formazans **29a–l** (Chart 21) by coupling the corresponding hydrazones with the appropriate diazonium salts and assayed them *in vitro* for antibacterial activity against *S. aureus*, *P. aeruginosa*, *E. coli* and *B. subtilis* and antifungal activity against *C. albicans* and *A. niger* by the cup–plate method using DMF as solvent. The activity was compared

460 radiometric system. The results showed that 3-(4-methoxyphenyl)-5-(2,4-dichlorophenyl)-1-isonicotinoylformazan (**36n**) and 3-(4-methoxyphenyl)-5-(2,6-dichlorophenyl)-1-isonicotinoyl-formazan (**36p**) produced highest efficacy and exhibited >90% inhibition at a concentration of 0.0179 μM and 0.0149 μM whereas 3-(4-methoxyphenyl)-5-(2-methoxyphenyl)-1-isonicotinoylformazan (**36b**), 3-(4-methoxyphenyl)-5-(4-methoxyphenyl)-1-isonicotinoylformazan (**36d**) and 3-(4-methoxyphenyl)-5-(2,5-dichlorophenyl)-1-isonicotinoylformazan (**36o**) showed moderate inhibitory activity with 0.0277 μM , 0.0203 μM , 0.0292 μM respectively. This finding was taken as evidence that the 2,4-dichloro and 2,6-dichloro groups substitution derivatives displayed relatively higher inhibitory activity in general. However, the electron rich groups such as, 2,4-dichloro, 2,5-dichloro, 2,6-dichloro, 2-methoxy, 3-methoxy and 4-methoxy substituted analogs produced significant increase in inhibitory activity against *M. tuberculosis* H37Rv [82]. On the other hand, analogs with methyl group substitution (**36k–m**) and phenyl substitution (**36a**) were reported to show relatively low inhibitory activity against *M. tuberculosis* H37Rv. Instead (OH) group, (CH₃) group and (NO₂) group substitution at phenyl ring in formazan analogs worsens the anti-mycobacterial activity in comparison with (Cl) group analogs.

In another report, Raval et al. [61] indicated that the results of screening the antimicrobial activities of formazans **37a–m** (Chart 19b) revealed that compounds **37a**, **d**, **h** and **37k** exhibited the highest degree of inhibition against all tested Gram +ve organisms, while compounds **37c**, **h** showed highest degree of inhibition against all tested Gram –ve organisms compared to standard antibiotic drugs. Compounds **37c**, **d**, **h** and **37k** showed highest degree of inhibition against *Escherichia coli*, *Klebsiella pneumoniae*, *Salmonella paratyphi A*, while compound **37k** showed highest inhibition against *Chromobacterium violaceum*, *Klebsiella aerogenes*, *Pseudomonas aeruginosa*, *Escherichia coli*, respectively [61].



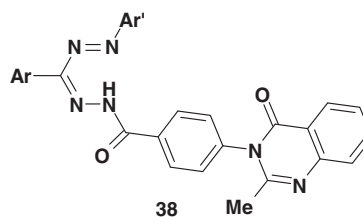
Ar = XC₆H₅

X : a, H; b, 2-MeO; c, 3-MeO; d, 4-MeO; e, 2-NO₂; f, 3-NO₂; g, 4-NO₂; h, 2-Cl; i, 3-Cl; j, 4-Cl; k, 2-Me; l, 3-Me; m, 4-Me

Chart 19b

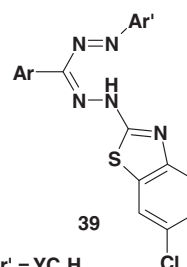
Nadendla et al. [60] synthesized a series of quinazolinone formazans **38a–m** (Chart 25) and screened them for antibacterial activity against six bacterial strains namely *Bacillus subtilis*, *Bacillus cereus*, *Escherichia coli*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Pseudomonas aeruginosa* and antifungal activity against four fungal strains: *Candida albicans*, *Candida glabrata*, *Aspergillus niger* and *Saccharomyces cerevisiae* by paper diffusion method. Of the compounds studied only compounds **38f**, **g**, **h** and **38k** showed moderate to good inhibition.

Various (6-chloro-benzothiazol-2-yl)formazans **39** (Chart 26) were synthesized by Basavaraja et al. [86] and



Ar / Ar' : a, Ph / 4-CIPh; b, Ph / 4-BrPh; c, 4-CIPh / 4-O₂NPh; d, 4-CIPh / 4-EtPh; e, 4-O₂NPh / 4-CIPh; f, 4-O₂NPh / 4-BrPh; g, 4-O₂NPh / 3-F,4-CIPh; h, 4-Me₂NPh / 4-CIPh; i, 4-Me₂NPh / 3-F,4-CIPh; j, 2-HO,4-MeOPh / 4-CIPh; k, 2-HO,4-MeOPh / 3-F,4-CIPh; l, 2-pyridyl / 4-CIPh; m, 2-pyridyl / 4-BrPh

Chart 25



Ar = XC₆H₄ Ar' = YC₆H₄

X / Y : a, H / H; b, H / 4-Cl; c, H / 4-F; d, H / 4-Me; e, H / 4-Br; f, 2-HO / H; g, 2-HO / 4-F; h, 2-HO / 4-Cl; i, 4-MeO / 4-Me; j, 4-MeO / 4-Cl; k, 4-MeO / H; l, 4-MeO / 4-F; m, 2-NO₂ / 4-F; n, 2-NO₂ / 4-Cl; o, 2-NO₂ / 4-Me

Chart 26

evaluated for their antimicrobial activity against *E. coli*, *Pseudomonas*, *Staphylococcus aureus*, *Bacillus subtilis* and antifungal activity against *Aspergillus*, *Flavus* and *Candida albicans* using DMF as solvent at 50 and 100 $\mu\text{g}/\text{ml}$ concentration by using cup-plate method. The activities were compared with known reference drugs like *procaine penicillin*, *Streptomycin* and *Griseofulvin* [86]. The results showed that compounds **39l–n** are active against *S. aerus* whereas compounds **39h**, **j** are active against *B. subtilis*. In addition, while the formazans **39j**, **n**, **m** exhibited activities against *E. coli* and *Pseudo Mona* comparable to that of the standards used. Furthermore, compounds **39c**, **h**, **l** and **39d**, **n** possessed activities close to that of *Griseofulvin* against *Candida albicans* and *Aspergillus Flavus*, respectively [86].

Recently, Uraz et al. [80] reported the synthesis of the formazans **40–42** (Chart 27) with various substituents on 1,3,5-sites and their antimicrobial activity against some selected microorganism namely, *Staphylococcus aureus*, *S. epidermidis*, *S. saprophyticus*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Klebsiella pneumoniae*. Moreover, the antifungal effects of these formazans were tested on the *Candida kefir*, *C. glabrata*, *C. tropicalis*, *Cryptococcus neoformans*, and *Saccharomyces cerevisiae*. The results of that study indicated that the studied formazans were very active against *Candida kefir*, *C. tropicalis*, *Cryptococcus neoformans* and *Saccharomyces cerevisiae* [80].

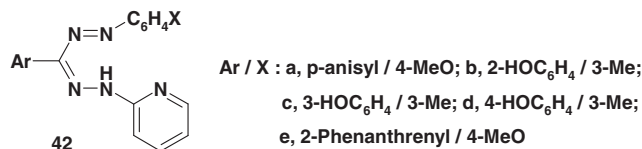
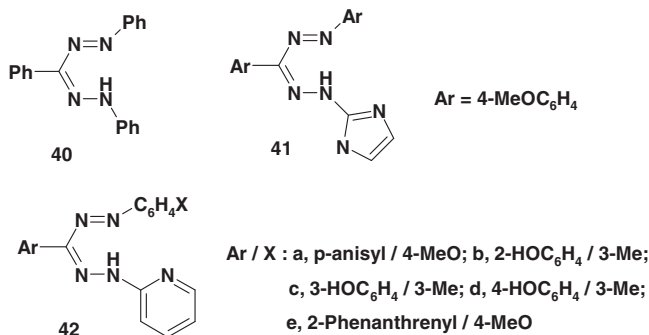
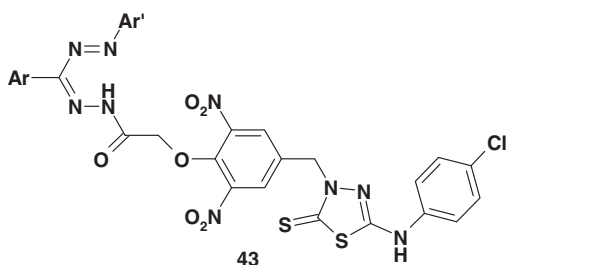


Chart 27

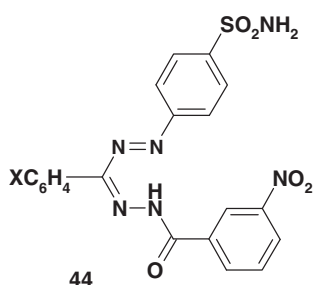
Furthermore, Sah et al. [87] reported the synthesis of the formazans **43a–g** (Chart 28) containing 1,3,4-thiadiazole ring residue and their *in vitro* antibacterial activity against *Escherichia coli* and *Salmonella typhi* and antifungal activity against *Aspergillus* sp. and *Candida albicans*. The results revealed that all of the studied compounds showed moderate activities.



Ar / Ar' : a, 2-ClPh / 4-O₂NPh; b, 2-ClPh / 2-Me,4-NO₂C₆H₃;
c, 2-ClPh / 4-HO₂SC₆H₄; d, 2-ClPh / 4-H₂NSO₂C₆H₄;
e, 2-ClPh / 4-H₂NC(=NH)NH-SO₂C₆H₄;
f, 3-MeO,4-HOC₆H₃ / 2-pyrimidinyl-SO₂C₆H₄;
g, 3-MeO,4-HOC₆H₃ / 4,6-Me₂-pyrimidin-2-yl-SO₂C₆H₄

Chart 28

Other formazans **44a–e** (Chart 29) were synthesized by Desai et al. [88] and were assayed for their *in vitro* antibacterial activity against *E. coli*, *P. fluorescens*, *B. mega*, *B. subtilis*, and antifungal activity against *Aspergillus awamori* using DMF as solvent at 50 µg concentration by cup-plate method. The activity was compared with the known antibiotic namely ampicillin, chloramphenicol, norfloxacin, and griseofulvin at the same concentration. The results showed that compounds **44b–d** exhibit antibacterial activity similar to that of ampicillin and

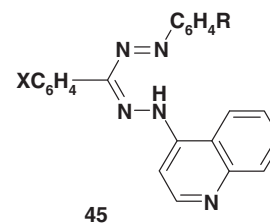


X : a, H; b, 4-Cl; c, 4-EtO; d, 4-MeO; e, 2-NO₂

Chart 29

compound **44e** showed comparable antifungal activity as that of griseofulvin.

Also, Tandel et al. [89] reported the synthesis of formazans **45** (Chart 30) and indicated that they exhibited potent activity against *E. coli*, *B. Subtilis*, *S. aureus*, and *S. paratyphi B* bacteria.

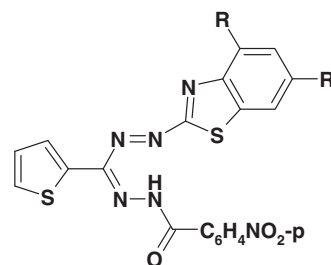


R : 4-MeO; 2-Cl

X : 2-MeO; 4-MeO; 4-EtO; 4-HO; 3-HO

Chart 30

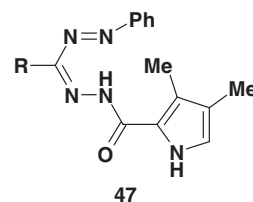
The formazans **46a–j** (Chart 31) have been prepared by Desai et al. [90] and screened them for antifungal activity against *Candida albicans*, *Candida krusei*, and *Candida parapsilosis* and antibacterial activity against *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Bacillus subtilis*.



46

Chart 31

Very recently, the synthesis of the formazans **47a–j** (Chart 32) and the results of screening of their antibacterial and antifungal activities were reported by Bhosale et al. [56]. The results indicated that all compounds **47a–j** showed good activities against all strains of bacteria. For example, compound **47i** showed excellent activities against the species *Pseudomonas aeruginosa*, *Klebsiella pneumonia* and *Bacillus subtilis* comparable with the standard antibacterial agent



47

R : a, Ph; b, 4-MeOC₆H₄; c, 2-ClC₆H₄; d, 4-Me₂NC₆H₄; e, 2-furyl;

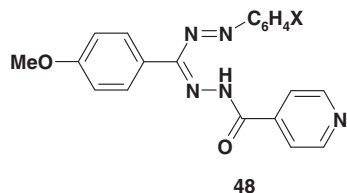
f, PhCH₂CH₂-; g, 4-HOC₆H₄; h, 3,4-(MeO)₂C₆H₃;

i, 2-HOC₆H₄; j, 3,4-CH₂O₂C₆H₃

Chart 32

tetracycline. Compound **47g** showed good activity among all the compounds studied against *Salmonella typhi*. In antifungal assay, while compounds **47a–i** demonstrated moderate results against the fungi *Aspergillus niger*, compounds **47a–j** displayed good antifungal activities against *Aspergillus flavus*.

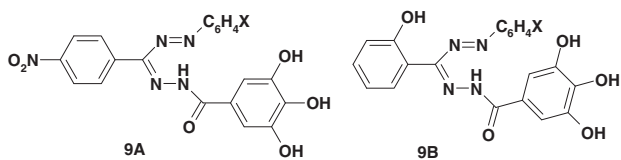
Also, Rajput [91] synthesized a series of 1-isonicotinoyl-3-(4-methoxyphenyl)-5-substituted phenylformazans **48a–n** (Chart 19c) and screened them for their antimicrobial activity against *Escherichia coli*, *Bacillus*, *Salmonella typhimurium* and *A. niger* using DMF as solvent and nutrient agar was employed as culture media. The results showed that the studied compounds were only active against *E. coli*.



48
X : a, H; b, 2-Cl; c, 3-Cl; d, 4-Cl; e, 2-NO₂;
f, 3-NO₂; g, 4-NO₂; h, 2-Me; i, 4-Me; j, 2-MeO;
k, 4-MeO; l, 4-SO₃H; m, 2-COOH; n, 2-SO₃H

Chart 19c

Very recently, Kumara Prasad et al. [59] reported the results of assaying the formazan derivatives **9A(B)** (Chart 4c) for antibacterial activity against *Staphylococcus aureus* and *Escherichia coli* and anti fungal activity against *Aspergillus Niger* in comparison with ofloxacin and fluconazole as standard. The reported results indicate that compounds with electron withdrawing substituents namely **9Ab** and **9Ad** are the most active among the tested compounds. The other compounds were found less active.



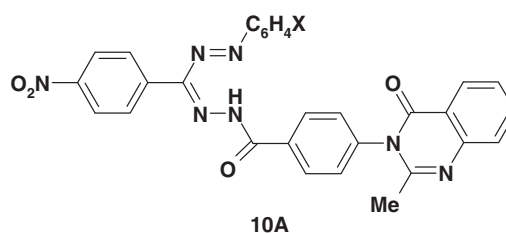
X : a, H; b, 4-COOH; c, 4-OH; d, 4-NO₂

Chart 4c

Analgesic activity

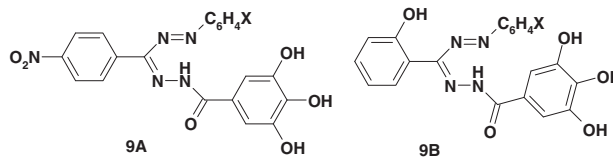
Nadendla and Babu [92] prepared a series of 1-substituted phenyl-3-(4-nitrophenyl)-4-[benzamido-(2-methyl-3-quinazolin)-4-one]formazans **10Aa–h** (Chart 6b) and screened them for analgesic activities. The reported results revealed that only compounds **10d, e, f** exhibit significant analgesic activity compared to the standard namely *paracetamol*.

Very recently, Kumara Prasad et al. [59] synthesized the two series of formazan derivatives **9A, B** (Chart 4d) and screened them for their analgesic activity using Eddy's Hot Plate Method. The reported results indicate that compounds **9Ac** and **9Bc** are the most active among the tested compounds. The other compounds were found less active.



10A
X : a, H; b, 4-Cl; c, 3-Cl; d, 4-Br; e, 4-NO₂; f, 3-F,4-Cl;
g, 4-Et; h, 4-Me

Chart 6b

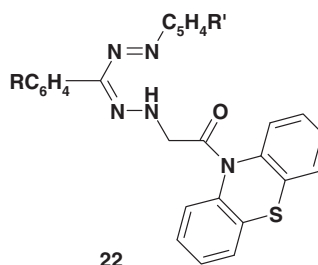


X : a, H; b, 4-COOH; c, 4-OH; d, 4-NO₂

Chart 4d

Antiparkinsonian activity

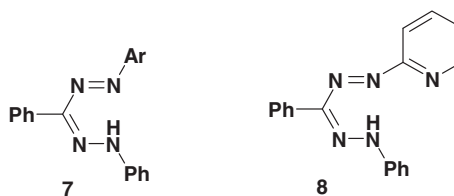
The formazans **22a–c** (Chart 33a) were prepared by coupling diazotized anilines with aldehyde N-(10-phenothiazinyl)acetylhydrazones. Compounds **22a–c** were reported to exhibit prominent antiparkinsonian activity better than *l-dopa* and *bromocriptine* in mice/rats (*in vivo*) with no toxicity effects with doses ≤ 1000 mg/kg [93,94].



R / R' : a, H / 2-Me; b, H / 2,5-Cl₂; c, 3,4-(MeO)₂ / 2,5-Cl₂

Chart 33a

Very recently, Mariappan et al. [58] reported that the formazan derivatives **7–8** (Chart 3d) showed significant



Ar = X_nC₆H_{5-n}

X : a, 4-NO₂; b, 4-Cl; c, 2,3-Cl₂; d, 3-Cl

Chart 3d

anticonvulsant effect at 100 mg/kg po and the experimental data were statistically significant at $P < 0.001$ level.

Cardiovascular activity

Three 1,3-disubstituted phenyl-5[(2-acetyl-phenothiazin-10-yl)-2-oxo-ethyl]formazans **22** (Chart 33b) have been reported and were evaluated for their cardiovascular activity [95].

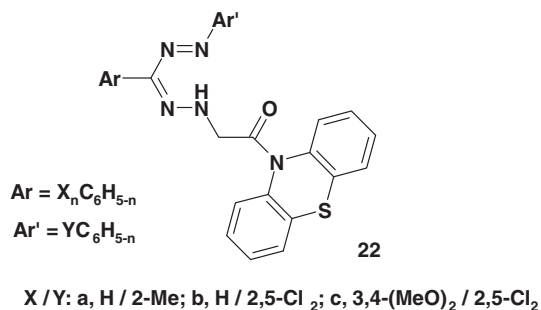


Chart 33b

Anti-proliferative activity

As prolactin and other lactogenic hormones are mitogenic for the rat T-cell lymphoma line Nb2 and glucocorticoids have antiproliferative effects on these cells, Alder et al. used the tetrazolium salt **49** (MTT) (Chart 34) for proliferation assay for the Nb2 cell line. Reduction of the yellow MTT to form the corresponding purple formazan **50** increases with cellular metabolic activity due to elevated NAD(P)H flux [96]. Resting cells such as thymocytes and splenocytes that are viable but metabolically quiet reduce very little MTT. In contrast, rapidly dividing cells exhibit high rates of MTT reduction.

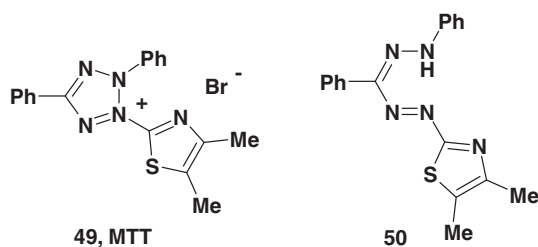
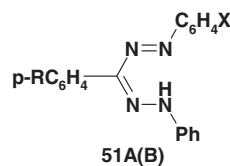


Chart 34

Recently, Yaglioglu and Senöz [97] reported the results of antiproliferation activity of 1,3,5-triphenylformazan **51Aa** and 1-substituted phenyl-3-(4-methoxycarbonyl)-phenyl-5-phenylformazans **51Ba-m** (Chart 35) against HeLa an C6 cell line using BrdU cell proliferation ELISA assay. Cisplatin and 5-fluorouracil were used as standards. The activities of the studied compounds and standards were investigated on eight concentrations. The results revealed that compounds with 2-OCH₃, 3-CH₃ and 4-I substituents displayed maximum activity against HeLa while those having 2-NO₂, 3-I and also 4-I groups were active against C6.



R: A, H; B, MeOOC

X: a, H; b, 2-NO₂; c, 3-NO₂; d, 4-NO₂; e, 2-Me; f, 3-Me; g, 4-Me; h, 2-MeO; i, 3-MeO; j, 4-MeO; k, 2-I; l, 3-I; m, 4-I

Chart 35

Conclusions

This review presents an up-to-date survey of the various biological activities of formazans. The literature reports covered in this review indicate that formazans exhibit versatile biological activities. It is hoped that this review will capture the attention of other research groups to develop newer formazans that could be better agents in terms of efficacy and safety.

Conflict of Interest

The authors have declared no conflict of interest.

Compliance with Ethics Requirements

This article does not contain any studies with human or animal subjects.

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